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# Essentials of Biochemistry

Pankaja Naik

2nd Edition

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# BIOCHEMISTRY

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# ESSENTIALS OF BIOCHEMISTRY

## Second Edition

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# Preface

This second edition of **Essentials of Biochemistry** is a clear, concise, comprehensive and in full color, exam oriented ideal textbook for medical, dental, physiotherapy, occupational therapy, nursing and other health sciences students. This book is revised and updated keeping in view all categories of students and it addresses their needs in a simple manner. Essentials of Biochemistry has been streamlined to focus on only the most essential biochemical concepts and avoids details not required by undergraduate students. Additional information has been given in the form of diagrams, tables, and flowcharts to understand the topics and its proper perspective.

The text has been so presented that the students would find it easy to attempt any question in the form of objective type or essay type after going through the text. To facilitate the student learning, I have added new features:

- Exam questions in the form of long answer questions (LAQs), short answer questions (SAQs), multiple choice questions (MCQs), and clinical case studies.
- A glossary with precise definitions of the most common words of biochemical sciences.
- A list of reference/normal values for selected biochemical laboratory tests.

After reading this book, reader suggestion and healthy criticism will be of great help in the future improvement of the book. I would like you to send me your feedback at pankajanaik@hotmail.com/ pankajanaik@gmail.com

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CHAPTER

# 1 Cell and Membrane Transport

**Chapter outline**

- Types of Living Cell
- Structure and Functions of a Cell and its Subcellular Components
- Cytoskeleton
- Membrane Transport
- Cell Fractionation
- Marker Enzymes

## INTRODUCTION

'Cell' means a small room or chamber; cells are the structural and functional units of all living organisms. The major parts of a cell are the **nucleus** and the **cytoplasm.**

The electron microscope allowed classification of cells into two major groups, **prokaryotes** and **eukaryotes,** based on the presence and absence of the true nucleus.

## TYPES OF LIVING CELL

The electron microscope allowed classification of cells into two major groups, **prokaryotes** and **eukaryotes** based on the presence and absence of the true nucleus.

- **Eukaryotes** (Greek: Eue = true, karyon = nucleus), which have a membrane enclosed nucleus encapsulating their DNA, (deoxyribonucleic acid). Animals, plants and fungi belong to the eukaryotes. Eukaryotic cells are much larger than prokaryotes. Eukaryotes may be multicellular as well as unicellular, are far more complex than prokaryotes and are characterized by having numerous membrane enclosed **organelles** (subcellular elements) in their cytoplasm, including:
  - Mitochondria
  - Lysosomes
  - Endoplasmic reticulum
  - Golgi complexes
- **Prokaryotes** have no typical nucleus (Greek: Pro = before) instead consists of **nucleoid** in which the genome, the complete set of genes, composed of DNA is replicated and stored with its associated proteins. The nucleoid, in bacteria and archaea, is not separated from the cytoplasm by a membrane **(Fig. 1.1)**. Bacteria and blue green algae belong to the prokaryotes. Prokaryotes lack membrane enclosed organelles (subcellular elements) in their cytoplasm. Prokaryotes; which comprise the various types of bacteria, have relatively small structures and are invariably unicellular.

**Table 1.1** and **Figure 1.1** describe some of the major structural features of the prokaryote and eukaryote cells.

**Viruses**, it should be noted, are not living organism in the sense that cells are. They are incapable of replication themselves outside their host cells and have virtually no biochemical activities of their own. However, they possess genomes and have the ability to enter living cells, where they cause the host's molecular biology machinery to replicate them as a result of which they cause disease.

## STRUCTURE AND FUNCTIONS OF A CELL AND ITS SUBCELLULAR COMPONENTS

A cell has three major components:

1. Plasma membrane (Cell membrane)
2. Cytoplasm with its organelles:
   - Endoplasmic reticulum
   - Golgi apparatus
   - Mitochondria
   - Lysosomes
   - Peroxisomes
3. Nucleus.

**Fig. 1.1:** Cell structure of eukaryotic and prokaryotic cell.

**Table 1.1:** Structural features of prokaryotes and eukaryotes.

| *Organelle* | *Eukaryotes* | *Prokaryotes* |
|---|---|---|
| Nucleus | Present | No define nucleus. DNA present but not separated from rest cell |
| Plasma membrane | Present | Present |
| Mitochondria | Present | Absent. Enzymes for oxidation reactions located on plasma membrane |
| Endoplasmic reticulum | Present | Absent |
| Ribosomes | Present | Present |
| Chromosomes | Linear | Circular |
| Cytoplasm | Contains various membrane bound organelles, such as mitochondria, lysosomes, peroxisomes and Golgi apparatus | Undifferentiated |
| Reproduction | Mitosis | By binary division |

**Table 1.2:** Biochemical functions of subcellular organelles of the eukaryotic cell.

| *Subcellular organelles* | *Function* |
|---|---|
| Plasma membrane | Transport of molecules in and out of cell, receptors for hormones and neurotransmitters |
| Lysosome | Intracellular digestion of macromolecules and hydrolysis of nucleic acid, protein, glycosaminoglycans, glycolipids, sphingolipids |
| Golgi apparatus | Post-transcriptional modification and sorting of proteins and export of proteins |
| Rough endoplasmic reticulum | Biosynthesis of protein and secretion |
| Smooth endoplasmic reticulum | Biosynthesis of steroid hormones and phospholipids, metabolism of foreign compounds |
| Nucleus | Storage of DNA, replication and repair of DNA, transcription and post-transcriptional processing |
| Peroxisomes | Metabolism of hydrogen peroxide and oxidation of long-chain fatty acids |
| Nucleolus | Synthesis of rRNA and formation of ribosomes |
| Mitochondrion | ATP synthesis, site for tricarboxylic acid cycle, fatty acid oxidation, oxidative phosphorylation, part of urea cycle and part of heme synthesis |
| Cytosol | Site for glycolysis, pentose phosphate pathway, part of gluconeogenesis, urea cycle and heme synthesis, purine and pyrimidine nucleotide synthesis |

**Table 1.2** shows biochemical functions of subcellular organelles of the eukaryotic cell.

## Plasma Membrane

- The cell is enveloped by a thin membrane called **cell membrane** or **plasma membrane.**
- Plasma membranes mainly consist of **lipids, proteins** and smaller proportion of **carbohydrates** that are linked to lipids and proteins. The basic organization of biologic membranes is illustrated in **Figure 1.2.**

**Fig. 1.2:** The basic organization of biological membrane.

- The Plasma membrane is an organized structure consisting of a **lipid bilayer** primarily of **phospholipids** and penetrated **protein** molecules forming a **mosaic-like pattern (Fig. 1.3).**

### *Membrane Lipids*

- The major classes of membrane lipids are:
  - Phospholipids
  - Glycolipids
  - Cholesterol.

  They all are **amphipathic** molecules, i.e. they have both hydrophobic and hydrophilic ends.
- Membrane lipids spontaneously form bilayer in aqueous medium, burying their hydrophobic tails and leaving their hydrophilic ends exposed to the water **(Fig. 1.2).**

### *Membrane Proteins*

- Proteins of the membrane are classified into two major categories:
  - Integral proteins or intrinsic proteins or trans-membrane proteins and
  - Peripheral or extrinsic proteins.
- **Integral proteins** are either partially or totally immersed in the lipid bilayer. Many integral membrane proteins span the lipid bilayer from one side to the other and are called **transmembrane protein** whereas others are partly embedded in either the outer or inner leaflet of the lipid bilayer **(Fig. 1.2). Transmembrane proteins** act as **enzymes** and **transport carriers** for ions as well as water soluble substances, such as glucose.
- **Peripheral proteins** are attached to the surface of the lipid bilayer by electrostatic and hydrogen bonds. They bound loosely to the polar head groups of the membrane phospholipid bilayer **(Fig. 1.2)**. Peripheral proteins function almost entirely as **enzymes** and **receptors.**

**Fig. 1.3:** The fluid mosaic model of cell membrane.

### *Membrane Carbohydrates*

- Membrane carbohydrate is not free. It occurs in combination with proteins or lipids in the form of **glycoproteins** or **glycolipids**. Most of the integral proteins are glycoproteins and about one-tenth of the membrane lipid molecules are glycolipids. The carbohydrate portion of these molecules protrudes to the outside of the cell, dangling outward from the cell surface **(Fig. 1.2).**
- Many of the carbohydrates act as **receptor** for hormones. Some carbohydrate moieties function in **antibody** processing.

### *Functions of Cell Membrane*

- The plasma membrane maintains the physical integrity of the cell by preventing the contents of the cell from leaking into the outside fluid environment and at the same time facilitating the entry of nutrients, inorganic ions and most other charged or polar compounds from the outside. It permits only some substances to pass in either direction, and it forms a barrier for other substances.
- The cell membrane protects the cytoplasm and the organelles of the cytoplasm.
- It m**aintenance of shape and size of the cell.**

## The Fluid Mosaic Model of Cell Membrane

- In 1972, Singer and Nicolson postulated a theory of membrane structure called the **fluid mosaic model**, which is now widely accepted.
- A mosaic is a structure made up of many different parts. Likewise, the plasma membrane is composed of

A Rough or granular endoplasmic reticulum B Smooth or agranular endoplasmic reticulum

**Figs. 1.4A and B:** Structure of endoplasmic reticulum.

different kinds of macromolecules like **phospholipid, integral proteins, peripheral proteins, glycoproteins, glycolipids** and **cholesterol.**

- According to this model, the membrane structure is a **lipid bilayer** made of phospholipids.
- The bilayer is fluid because the hydrophobic tails of phospholipids consist of an appropriate mixture of saturated and unsaturated fatty acids that is fluid at normal temperature of the cell.
- Proteins are interspersed in the lipid bilayer, of the plasma membrane, producing a mosaic effect **(*see* Fig. 1.3).**
- The peripheral proteins literally float on the surface of 'sea' of the phospholipid molecules, whereas the integral proteins are like icebergs, almost completely submerged in the hydrophobic region.
- There are no covalent bonds between lipid molecules of the bilayer or between the protein components and the lipids.
- Thus, there is a mosaic pattern of membrane proteins in the fluid lipid bilayer.
- Fluid mosaic model allows the membrane proteins to move around laterally in two dimensions and that they are free to diffuse from place to place within the plane of the bilayer. Whereas, they cannot outflow from one side of the lipid bilayer to the other.
- **The Singer-Nicolson model can explain many of the physical, chemical and biological properties of membranes and has been widely accepted as the most probable molecular arrangement of lipids and proteins of membranes.**

## Cytoplasm and its Organelles

Cytoplasm is the internal volume bounded by the plasma membrane. The clear fluid portion of the cytoplasm in which the particles are suspended is called **cytosol**.

Six important organelles that are suspended in the cytoplasm are:

1. Endoplasmic reticulum
2. Golgi apparatus
3. Lysosomes
4. Peroxisomes
5. Mitochondria
6. Nucleus.

### *Endoplasmic Reticulum (ER)*

- Endoplasmic reticulum is the interconnected network of tubular and flat vesicular structures in the cytoplasm **(Figs. 1.4A and B)**.
- Endoplasmic reticulum forms the link between nucleus and cell membrane by connecting the cell membrane at one end and the outer membrane of the nucleus at the other end **(*see* Fig. 1.1).**
- A large number of minute granular particles called **ribosomes** are attached to the outer surface of many parts of the endoplasmic reticulum, this part of the ER is known as **rough** or **granular ER.**
- During the process of cell fractionation, rough ER is disrupted to form small vesicles known as **microsomes**. It may be noted that microsomes as such do not occur in the cell.
- Part of the ER, which has no attached ribosomes, is known as **smooth endoplasmic reticulum.**

#### *Functions of the ER*

- Rough ER functions in the biosynthesis of protein.
- The smooth endoplasmic reticulum functions in the synthesis of steroid hormones and cholesterol.
- Smooth endoplasmic reticulum is the site of the metabolism of certain drugs, toxic compounds and carcinogens (cancer producing substances).

## Golgi Apparatus

Golgi apparatus is present in all cells **except in red blood cells**. It is situated near the nucleus and is closely related to the endoplasmic reticulum. It consists of four or more membranous sacs. This apparatus is prominent in secretory cells.

### *Functions of Golgi apparatus*

The Golgi apparatus functions in association with the endoplasmic reticulum:

- Proteins synthesized in the ER are transported to the Golgi apparatus where these are processed by addition of carbohydrate, lipid or sulfate moieties. These chemical modifications are necessary for the transport of proteins across the plasma membrane.
- Golgi apparatus are also involved in the synthesis of intracellular organelles, e.g. lysosomes and peroxisomes.

## Lysosomes

- Lysosomes are vesicular organelles formed from Golgi apparatus and dispersed throughout the cytoplasm.
- **Among the organelles of the cytoplasm, the lysosomes have the thickest covering membrane to prevent the enclosed hydrolytic enzymes from coming in contact with other substances in the cell and therefore, prevent their digestive actions.**
- Many small granules are present in the lysosome. The granules contain more than 40 different hydroxylases (hydrolytic enzymes). All the enzymes are collectively called **lysozymes**.

### *Functions of lysosomes*

Lysozymes present in lysosomes digest proteins, carbohydrates, lipids and nucleic acids. Apart from the digestive functions, the enzymes in the lysosomes are responsible for the following activities in the cell:

- Destruction of bacteria and other foreign bodies.
- Removal of excessive secretory products in the cells of the glands.
- Removal of unwanted cells in embryo.

## Peroxisomes

- These organelles resemble the lysosomes in their appearance, but they differ both in function and in their synthesis.
- They do not arise from Golgi membranes, but rather from the division of pre-existing peroxisomes or perhaps through budding off from the smooth endoplasmic reticulum.

### *Functions of peroxisomes*

- Peroxisomes contain enzymes **peroxidases** and **catalase** which are concerned with the metabolism of peroxide. Thus, the peroxisomes are involved in the detoxification of peroxide.
- Peroxisomes are also capable of carrying out β-oxidation of fatty acid.

## Mitochondria (Powerhouse of Cell)

- Mitochondria are called "**Power Plant**" of the cell since they convert energy to form ATP that can be used by cell.
- A mitochondrion is a double-membrane organelle **(Fig. 1.5)** that is fundamentally different in composition and function:
  - **The outer membrane** forms a smooth envelope. It is freely permeable for most metabolites.
  - **The inner membrane** is folded to form **cristae**, which give it a large surface area and are the site of **oxidative phosphorylation**. The components of the electron transport chain are located on the inner membrane.
- The space within the inner membrane is called the **mitochondrial matrix**. It contains the enzymes of the:
  - Citric acid cycle
  - β-oxidation of fatty acid
  - Some other degradative enzymes.

### *Functions of mitochondria*

- The mitochondrial matrix is the site of most of the reactions of the **citric acid cycle** and **fatty acid oxidation**. In contrast oxidative phosphorylation takes place in the inner mitochondrial membrane.
- The outer membrane is permeable to most small molecules and ions because it contains many **mitochondrial**

**Fig. 1.5:** Structure of mitochondria.

**porin** (pore forming protein) also known as **voltage-dependent anion channel (VDAC)** that permit access to most molecules. In contrast inner membrane is impermeable to nearly all ions and polar molecules. Many transporters shuttles metabolites such as ATP, pyruvate, and citrate across the inner mitochondrial membrane.

- Mitochondria contain their own DNA (mtDNA) which encodes a few polypeptides involved in oxidative phosphorylation.
- It is worth noting that sperms contribute no mitochondria to the fertilized egg, so that mitochondrial DNA is inherited exclusively through the female line. Thus, mitochondria are maternally inherited.

## Nucleus

The cells with nucleus are called **eukaryotes** and those without nucleus are known as **prokaryotes**. Most of the cells have only one nucleus but cells of **skeletal muscles have many nuclei**. **The matured red blood cell contains no nucleus**.

### *Structure of Nucleus*

- The nucleus is spherical in shape and situated near the center of the cell. The nucleus is surrounded by the nuclear envelope.
- The space enclosed by the nuclear envelope is called **nucleoplasm**; within this the **nucleolus** is present. Nucleolus is an organized structure of DNA, RNA and protein that is involved in the synthesis of ribosomal RNA. The remaining nuclear DNA is dispersed throughout the nucleoplasm in the form of **chromatin fibers**. At mitosis, chromatin is condensed into discrete structures called **chromosomes**.

### *Functions of Nucleus*

The major functional role of the nucleus is that of:

- **Replication:** Synthesis of new DNA.
- **Transcription:** The synthesis of the three major types of RNA:

1. Ribosomal RNA (rRNA)
2. Messenger RNA (mRNA)
3. Transfer RNA (tRNA).

## CYTOSKELETON

- The cytoplasm of most eukaryotic cells contains network of protein filaments that interact extensively with each other and with the component of the plasma membrane. Such an extensive intracellular network of protein has been called **cytoskeleton.** The plasma membrane is anchored to the cytoskeleton. The cytoskeleton is not a rigid permanent framework of the cell but is a dynamic, changing structure.
- The cytoskeleton consists of three primary protein filaments:
  1. Microfilaments
  2. Microtubules
  3. Intermediate filaments.

1. **Microfilaments** are about 5 nm in diameter. They are made up of protein **actin.** Actin filaments form a meshwork just underlying the plasma membrane of cells and are referred to as **cell cortex,** which is labile. They disappear as cell motility increases or upon malignant transformation of cells. The function of microfilaments is:
   - To help muscle contraction
   - To maintain the shape of the cell
   - To help cellular movement.
2. **Microtubules** are cylindrical tubes, 20 to 25 nm in diameter. They are made up of protein **tubulin.** Microtubules are necessary for the formation and function of mitotic spindle. They provide stability to the cell. They prevent tubules of ER from collapsing. These are the major components of **axons** and **dendrites.**
3. **Intermediate filaments** are so called as their diameter (10 nm) is intermediate between that of microfilaments (5 nm) and of microtubules (25 nm):
   - Intermediate filaments are formed from fibrous protein which varies with different tissue type.
   - They play role in cell-to-cell attachment and help to stabilize the epithelium. They provide strength and rigidity to axons.

## Functions of Cytoskeleton

- The cytoskeleton gives cells their characteristic shape and form, provides attachment points for organelles, fixing their location in cells and also makes communication between parts of the cell possible.
- It is also responsible for the separation of chromosomes during cell division.
- The internal movement of the cell organelles as well as cell locomotion and muscle fiber contraction could not take place without the cytoskeleton. It acts as **"track"**

**Fig. 1.6:** Types of membrane transport mechanism.

**Fig. 1.7:** Uniport, symport and antiport transport of substance across the cell membrane.

on which cells can move organelles, chromosomes and other things.

## MEMBRANE TRANSPORT

- One of the functions of the plasma membrane is to regulate the passage of a variety of small molecules across it.
- Biological membranes are **semipermeable** membranes through which certain molecules freely diffuse across membranes but the movement of the others is restricted because of **size**, **charge** or **solubility.**
- The two types of transport mechanisms are **(Fig. 1.6):**
  1. Passive transport or passive diffusion
  2. Active transport.

### Passive Transport or Passive Diffusion

- Passive transport is the process by which molecules move across a membrane without energy (ATP).
- The direction of passive transport is always from a **region of higher concentration to one of lower concentration.**
- There are two types of passive transport as follows:
  1. Simple diffusion
  2. Facilitated diffusion.

#### *Simple Diffusion*

- Lipid soluble, i.e. lipophilic molecules can pass through cell membrane, without any interaction with carrier proteins in the membrane. Such molecules will pass through membrane along the concentration gradient, i.e. from a region of higher concentration to one of lower concentration. This process is called **simple diffusion.**

#### *Facilitated Diffusion*

- The movement of water soluble molecules and ions across the membrane requires specific transport system. They pass through specific **carrier proteins.** A carrier protein binds to a specific molecule on one side of the membrane and releases it on the other side. This type of crossing the membrane is called **facilitated diffusion** or **carrier-mediated diffusion.**
- An example of facilitated diffusion is the movement of glucose and most of the amino acids across the plasma membrane.
- These diffusion processes are not coupled to the movement of other ions, they are known as **uniport transport processes (Fig. 1.7).**

### Active Transport

- If a molecule moves against a concentration gradient, an external energy source is required; this movement is referred to as **active transport**.

**Fig. 1.8:** Mechanism of sodium-potassium pump (primary active transport).

- Substances that are actively transported through cell membranes include, **$Na^+$, $K^+$, $Ca^{++}$, $H^+$, $Cl^-$,** several different **sugars** and most of the **amino acids.**
- Active transport is classified into two types according to the source of energy used as follows:
  i. Primary active transport
  ii. Secondary active transport.
- In both instances, transport depends on the **carrier proteins;** like facilitated diffusion. However, in active transport, the carrier proteins function differently from the carrier in facilitated diffusion. **Carrier protein for active transport is capable of transporting substance against the concentration gradient**.

## *Primary Active Transport*

- In primary active transport, the energy is derived **directly** from **hydrolysis of ATP.**
- Sodium, potassium, calcium, hydrogen and chloride ions are transported by primary active transport.

### *Primary active transport of $Na^+$ and $K^+$ (sodium-potassium pump)*

- $Na^+$-$K^+$ Pump, a primary active transport process that pumps $Na^+$ ions out of the cell and at the same time pumps $K^+$ ions from outside to the inside generating an electrochemical gradient.
- Carrier protein of $Na^+$-$K^+$ pump has three receptor sites for binding sodium ions on the inside of the cell and two receptor sites for potassium ions on the outside. The inside portion of this protein has **ATPase activity (Fig. 1.8).**
- The pump is called **$Na^+$-$K^+$ ATPase** because the hydrolysis of ATP occurs only when three $Na^+$ ions bind on the inside and two $K^+$ ions bind on the outside of the carrier proteins. The energy liberated by the hydrolysis of ATP leads to conformational change in the carrier protein molecule, extruding the three $Na^+$ ions to the outside and the two $K^+$ ions to the inside.

### *Physiological importance of $Na^+$-$K^+$ pump*

- The active transport of $Na^+$ and $K^+$ is of great physiological significance. The $Na^+$ – $K^+$ gradient created by this pump in the cells, **controls cell volume.**
- It carries the active transport of sugars and amino acids.

## *Secondary Active Transport*

Secondary active transport uses an energy generated by an electrochemical gradient. It is not directly coupled with hydrolysis of ATP. Secondary active transport is classified into two types:

1. **Cotransport** or **symport**, in which both substances move simultaneously across the membrane in the same direction **(*see* Fig. 1.7)**, e.g. transport of $Na^+$ and glucose to the intestinal mucosal cells from the gut.
2. **Counter transport** or **antiport**, in which both substances move simultaneously in opposite direction **(*see* Fig. 1.7)**, e.g. transport of $Na^+$ and $H^+$ occurs in the renal proximal tubules and exchange of $Cl^-$ and $HCO3^-$ in the erythrocytes.

## Transport of Macromolecules Across the Plasma Membrane

- The process by which cells take up large molecules is called **endocytosis (Fig. 1.9)** and the process by which cells release large molecules from the cells to the outside is called **exocytosis (Fig. 1.10)**.

## *Endocytosis*

- There are two types of endocytosis:
  – Pinocytosis (cellular drinking)
  – Phagocytosis (cellular eating).

## *Pinocytosis*

- Pinocytosis is the cellular uptake of fluid and fluid contents and is a cellular drinking process.
- Pinocytosis is the only process by which most macromolecules, such as most proteins, polysaccharides and polynucleotides can enter cells **(Fig. 1.9)**.
- These molecules first attach to specific receptors on the surface of the membrane.
- The receptors are generally concentrated in small pits on the outer surface of the cell membrane. These receptors

**Fig. 1.9:** Three stages of the absorption of macromolecules by endocytosis.

are coated on the cytoplasmic side with a fibrillar protein called **clathrin** and contractile filaments of **actin** and **myosin**.

- Once the macromolecules (which are to be absorbed) have bound with the receptors, the entire pit invaginates inward, and the fibrillar protein by surrounding the invaginating pit causes it to close over the attached macromolecule along with a small amount of extracellular fluid.
- Then immediately, the invaginated portion of the membrane breaks away from the surface of the cell forming **endocyte vesicle** inside the cytoplasm of the cell.

## *Phagocytosis*

- Phagocytosis involves the ingestion of large particles such as viruses, bacteria, cells, tissue debris or a dead cell.
- It occurs only in specialized cells such as **macrophages** and some of the **white blood cells.**

**Fig. 1.10:** Stages in exocytosis.

- Phagocytosis occurs in much the same way as pinocytosis.

## *Exocytosis*

- Most of the endocytic vesicles formed from pinocytosis fuse with lysosomes. Lysosomes empty their acid hydrolases to the inside of the vesicle and begin hydrolyzing the proteins, carbohydrate, lipids and other substances in the vesicle.
- The macromolecular contents are digested to yield amino acids, simple sugars or nucleotides and they diffuse out of the vesicle and reused in the cytoplasm.
- Undigestible substances called residual body is finally excreted through the cell membrane by a process called **exocytosis**, opposite to endocytosis **(Fig. 1.10)**.
- The undigestible substances produced within the cytoplasm may be enclosed in membranes to form vesicles called **exocytic vesicles.**
- These cytoplasmic exocytic vesicles fuse with the internal surface of the plasma membrane.
- The vesicle then ruptures releasing their contents into the extracellular space and their membranes are retrieved (left behind) and reused.

**Fig. 1.11:** Subcellular fractionation of cell by differential centrifugation.

## CELL FRACTIONATION

- To obtain purified preparations of organelles, the tissue is first carefully broken up in a homogenizing apparatus using isotonic 0.25 M sucrose solution.
- Sucrose solution is used because it is not metabolized in most tissues and it does not pass through membranes readily and thus, does not cause inter organelles to swell.
- Then homogenate is centrifuged at a series of increasing centrifugal force **(Fig. 1.11)**.
- The subcellular organelles, which differ in size and specific gravity, sediment at different rates and can be isolated from homogenate by differential centrifugation.
- The dense nuclei are sediment first, followed by the mitochondria, and finally the microsomal fraction at the highest forces. After all the particulate matter has been removed, the soluble remnant is the cytosol.
- Organelles of similar sedimentation coefficient obviously cannot be separated by differential centrifugation. For example, mitochondria isolated in this way are contaminated with lysosome and peroxisomes. These may be separated by **isopycnic centrifugation technique**.

## MARKER ENZYMES

- The purity of isolated subcellular fraction is assessed by the analysis of **marker enzymes**.
- Marker enzymes are the enzymes that are located exclusively in a particular fraction and thus become characteristic of that fraction.
- Analysis of marker enzymes confirms the identity of the isolated fraction and indicates the degree of contamination with other organelles. For example, isolated mitochondria have a high specific activity of cytochrome oxidase but low catalase and acid phosphatase, the catalase and acid phosphatase activities being due to contamination with peroxisomes and lysosomes respectively.
- Some typical subcellular markers are given in **Table 1.3.**

**Table 1.3:** Marker enzymes of subcellular fractions

| *Fraction* | *Enzymes* |
|---|---|
| Plasma membrane | 5 Nucleotidase, $Na^+$-$K^+$-ATPase |
| Nucleus | DNA polymerase<br>RNA polymerase |
| Endoplasmic reticulum<br>Golgi bodies | Glucose-6-phosphatase<br>Galactosyl transferase |
| Lysosomes | Acid phosphatase<br>β-glucuronidase |
| Mitochondria | Succinate dehydrogenase<br>Cytochrome C-oxidase |
| Peroxisomes | Catalase |
| Cytosol | Lactate dehydrogenase<br>Glucose-6-phosphate dehydrogenase |

## EXAM QUESTIONS

### Short Notes

1. Diagrammatic representation of cell with functions of the subcellular organelles.
2. Give structure and function of:
   i. Mitochondria
   ii. Endoplasmic reticulum
   iii. Golgi apparatus
   iv. Plasma membrane
   v. Nucleolus
   vi. Lysosomes
   vii. Peroxisomes.

### Multiple Choice Questions (MCQs)

**1. The following is the metabolic function of ER:**
a. RNA processing
b. Fatty acid oxidation
c. Synthesis of plasma protein
d. ATP-synthesis

**2. In biologic membranes, integral proteins and lipids interact mainly by:**
a. Covalent bond
b. Both hydrophobic and covalent bond
c. hydrogen and electrostatic bond
d. None of the above

**3. Plasma membrane is:**
a. Composed entirely of lipids
b. Mainly made up of proteins
c. Mainly made up of lipid and protein
d. Composed of only carbohydrates and lipids

**4. Select the subcellular component involved in the formation of ATP:**
a. Nucleus b. Plasma membrane
c. Mitochondria d. Golgi apparatus

**5. Mitochondrial DNA is:**
a. Maternal inherited
b. Paternal inherited
c. Maternal and paternal inherited
d. None of the above

**6. All of the following statements about the nucleus are true, *except*:**
a. Outer nuclear membrane is connected to ER
b. It is the site of storage of genetic material
c. Nucleolus is surrounded by a bilayer membrane
d. Outer and inner membranes of nucleus are connected at nuclear pores

**7. Golgi apparatus is present in all of the following, *except*:**
a. RBC b. Parenchymal cells
c. Skeletal muscle cells d. Pancreatic cell

**8. Peroxisomes arise from:**
a. Golgi membrane
b. Lysosomes
c. Mitochondria
d. Pre-existing peroxisomes and budding off from the smooth ER

**9. $Na^+ - K^+$ ATPase is the marker enzyme of:**
a. Nucleus b. Plasma membrane
c. Golgi bodies d. Cytosol

**10. Exocytosis:**
a. Is always employed by cells for secretion
b. Is used to deliver material into the extracellular space
c. Take up large molecules from the extracellular space
d. Allows the salvage of elements of the plasma membrane

**11. The approximate number of cells in a normal human body is:**
a. 10 b. 100
c. 1014 d. 10144

**12. The largest cell in the human body is:**
a. Nerve cell b. Muscle cell
c. Liver cell d. Kidney cell

**13. Which one of the following eukaryotic cell structures does not contain DNA?**
a. Nucleus
b. Mitochondrion
c. The endoplasmic reticulum
d. Chloroplast

**14. The cytoskeleton includes all of the following, *except*:**
a. Microtubules
b. Intermediate filaments
c. Myosin filaments
d. Action filaments

**15. Ribosomes are found:**
a. Only in the nucleus
b. In the cytoplasm
c. Attached to the smooth endoplasmic reticulum
d. Both b and c

**16. The Golgi apparatus is involved in:**
a. Packaging proteins into vesicles
b. Altering or modifying proteins
c. Producing lysosomes
d. All of the above

**17. All of the following are functions of the cell membrane, *except*:**
a. Participating in chemical reactions
b. Participating in energy transfer
c. Being freely permeable to all substances
d. Regulating the passage of materials

**18. Who proposed the fluid mosaic model of cell membrane structure in 1972?**
a. Davidson and Singer
b. Frye and Edidin
c. Brown and Goldstein
d. Singer and Nicholson

**19. Which of the following are involved with the movement or transport of materials or organelles throughout the cell?**
a. Rough endoplasmic reticulum
b. Cytoskeleton
c. Smooth endoplasmic reticulum
d. All of the choices are true

**20. Lysosomes are produced by the:**
a. Nucleus b. Mitochondria
c. Golgi apparatus d. Ribosomes

**21. Glucose-6-phosphatase is the marker enzyme of:**
a. Nucleus b. Plasma membrane
c. Golgi bodies d. Endoplasmic reticulum

**22. Galactosyltransferase is the marker enzyme of:**
a. Nucleus b. Peroxisomes
c. Golgi bodies d. Cytosol

**23. Acid phosphatase is the marker enzyme of:**
a. Mitochondria b. Lysosomes
c. Golgi bodies d. Cytosol

**24. Succinate dehydrogenase is the marker enzyme of:**
a. Mitochondria
b. Plasma membrane
c. Golgi bodies
d. Endoplasmic reticulum

**25. Lactate dehydrogenase and Glucose-6-phosphate dehydrogenase are the marker enzymes of:**
a. Nucleus b. Peroxisomes
c. Golgi bodies d. Cytosol

## Answers for MCQs

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **1.** c | **2.** c | **3.** c | **4.** c | **5.** a | **6.** c | **7.** a | **8.** d |
| **9.** b | **10.** b | **11.** c | **12.** a | **13.** c | **14.** c | **15.** d | **16.** d |
| **17.** c | **18.** d | **19.** d | **20.** c | **21.** d | **22.** c | **23.** b | **24.** a |
| **25.** d | | | | | | | |

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CHAPTER

# 2 Carbohydrate Chemistry

**Chapter outline**


- Definition, Classification, and Functions of Carbohydrates
- Structure of Glucose
- Isomerism
- Mutarotation
- Chemical Properties of Monosaccharides
- Glycoside Formation
- Derivatives of Monosaccharides
- Disaccharides
- Polysaccharides (Glycans)
- Glycoproteins


## INTRODUCTION

The carbohydrates are widely distributed both in animal and plant tissues. Chemically, they contain the elements carbon, hydrogen, and oxygen. The empirical formula of many simple carbohydrates is $\mathbf{[CH_2O]_n}$. Hence, the name ***"carbohydrate",*** i.e. hydrated carbon. They are also called ***"saccharides".*** In Greek, saccharon means sugar.

Although many common carbohydrates confirm the empirical formula $[CH_2O]_n$, others like deoxyribose, rhamnohexos do not. Some carbohydrates also contain ***nitrogen, phosphorus,*** or ***sulfur.***

## DEFINITION, CLASSIFICATION, AND FUNCTIONS OF CARBOHYDRATES

Carbohydrates may be defined chemically as ***aldehyde*** or ***ketone*** derivatives of polyhydroxy (more than one hydroxy group) alcohols or as compounds that yield these derivatives on hydrolysis.

### Functions of Carbohydrates

Carbohydrates have a wide range of functions. The following are few of them:

- Source of energy for living beings, e.g. ***glucose***
- Storage form of energy, e.g. ***glycogen*** in animal tissue and ***starch*** in plants
- Serve as structural component, e.g. ***glycosaminoglycans*** in humans, ***cellulose*** in plants and ***chitin*** in insects
- Non-digestible carbohydrates like cellulose, serve as dietary fibers
- Constituent of nucleic acids RNA and DNA, e.g. ***ribose*** and ***deoxyribose*** sugar
- Play a role in lubrication, cellular intercommunication and immunity
- Carbohydrates are also involved in detoxification, e.g. ***glucuronic acid***.

### Classification of Carbohydrates

Carbohydrates are classified into three groups:

1. Monosaccharides
2. Oligosaccharides
3. Polysaccharides.

#### *Monosaccharides (Greek: Mono = one)*

Monosaccharides are also called ***simple sugars.*** The term sugar is applied to carbohydrates that are soluble in water and sweet to taste. They consist of a single polyhydroxy aldehyde or ketone unit, and thus cannot be hydrolyzed into a simpler form. They may be subdivided into two groups as follows:

1. Depending upon the number of carbon atoms they possess, e.g.

- Trioses
- Tetroses
- Pentoses
- Hexoses
- Heptoses.

2. Depending upon the functional aldehyde (CHO) or ketone (C=O) group present:
   - Aldoses
   - Ketoses.

Classification of monosaccharides based on the number of carbon and the type of functional group present with examples is given in **Table 2.1.** ***The most abundant monosaccharide in nature is six carbon sugar D-glucose.*** Biologically important monosaccharides are listed in **Table 2.2.**

## Oligosaccharides (Greek: oligo = few)

Oligosaccharides consist of a short chain of monosaccharide units (2 to 10 units), joined together by a characteristic bond called ***glycosidic bond*** which, on hydrolysis, gives two to ten molecules of simple sugar (monosaccharide) units. Oligosaccharides are subdivided into different groups based on the number of monosaccharide units present **(Table 2.3).**

***The disaccharides which have two monosaccharide units are the most abundant in nature.*** Oligosaccharides with more than three subunits are usually found in glycoproteins; such as blood group antigens.

## Polysaccharides (Greek: Poly = many) or Glycans

Polysaccharides are polymers consisting of hundreds or thousands of monosaccharide units. They are also called

**Table 2.1:** Classification of monosaccharides and their examples.

| *No. of carbon* | *Empirical formula* | *Type of sugar* | *Aldoses* | *Ketoses* |
|---|---|---|---|---|
| 3 | $C_3H_6O_3$ | Trioses | Glyceraldehyde | Dihydroxyacetone |
| 4 | $C_4H_8O_4$ | Tetroses | Erythrose | Erythrulose |
| 5 | $C_5H_{10}O_5$ | Pentoses | Ribose, Xylose | Ribulose, Xylulose |
| 6 | $C_6H_{12}O_6$ | Hexoses | Glucose, Galactose, and Mannose | Fructose |
| 7 | $C_7H_{14}O_7$ | Heptoses | Glucoheptose | Sedoheptulose |

**Table 2.2:** Biologically important monosaccharides.

| *Type of monosaccharide* | *Example* | *Importance* |
|---|---|---|
| Trioses | Glyceraldehyde and Dihydroxyacetone | • Intermediates in the glycolysis<br>• Precursor of glycerol which is required for the formation of triacylglycerol and phospholipid |
| Tetroses | D-Erythrose | • Intermediate product of carbohydrate metabolism (Hexose monophosphate pathway) |
| Pentoses | D-Ribose | • Constituent of nucleic acid RNA and coenzymes, e.g. ATP, NAD, NADP, and FAD<br>• Intermediate product of pentose phosphate pathway |
| | D-Ribulose | • Intermediate product of pentose phosphate pathway |
| | D-Xylulose | • Constituent of proteoglycans and glycoproteins |
| | L-Xylulose | • An intermediate in uronic acid pathway |
| Hexoses | D-Glucose | • The main sugar of the body which is utilized by the tissue for energy purposes |
| | D-Fructose | • Can be converted to glucose in the liver and so used in the body for energy purpose |
| | D-Galactose | • Can be converted to glucose in the liver and metabolized<br>• Synthesized in mammary gland to make the lactose of milk<br>• A constituent of glycolipids, proteoglycans, and glycoproteins |
| | D-Mannose | • A constituent of glycoprotein, glycolipids and blood group substances |
| Heptoses | Sedoheptulose | • An intermediate in the pentose phosphate pathway |

**Table 2.3:** Classification of oligosaccharides and their examples.

| *Type of oligosaccharide* | *No. of monosaccharide* | *Example* | *Type of monosaccharide present* |
|---|---|---|---|
| Disaccharide | Two | Maltose<br>Lactose<br>Sucrose | Glucose + Glucose<br>Glucose + Galactose<br>Glucose + Fructose |
| Trisaccharide | Three | Raffinose | Glucose + Galactose + Fructose |
| Tetrasaccharide | Four | Stachyose | 2 Molecules of<br>Galactose + Glucose + Fructose |
| Pentasaccharide | Five | Verbascose | 3 Molecules of<br>Galactose + Glucose + Fructose |

**Table 2.4:** Classification of polysaccharides.

| *Polysaccharides* | |
|---|---|
| Homopolysaccharides or Homoglycans, e.g.<br>Starch<br>Dextrin<br>Glycogen<br>Cellulose<br>Inulin | Heteropolysaccharides or<br>Heteroglycans, e.g.<br>Glycosaminoglycans or<br>Mucopolysaccharides |

***glycans*** or ***complex carbohydrates.*** They may be either ***linear*** (e.g. cellulose), or ***branched*** (e.g. glycogen), in structure.

Polysaccharides have high molecular weight and are only sparingly soluble in water. They are not sweetish and do not exhibit any of the properties of aldehyde or ketone group. Polysaccharides are of two types **(Table 2.4).**

i. Homopolysaccharides (homoglycans)
ii. Heteropolysaccharides (heteroglycans).

*Homopolysaccharides (Homoglycans)*

- When a polysaccharide is made up of several units of one and the same type of monosaccharide unit, it is called homopolysaccharide.
- The most common homoglycans are:
  - Starch
  - Dextrins
  - Glycogen
  - Inulin
  - Cellulose.
- Some homopolysaccharides serve as a storage form of monosaccharides used as fuel, e.g. starch and glycogen, while others serve as structural elements in plants, e.g. cellulose.

*Heteropolysaccharides (Heteroglycans)*

- They contain two or more different types of monosaccharide units or their derivatives.
- Heteropolysaccharide present in human beings is glycosaminoglycans (mucopolysaccharides), e.g.
  - Heparin
  - Chondritin sulfate
  - Hyaluronic acid
  - Dermatan sulfate
  - Keratan sulfate
  - Blood group polysaccharides.

## STRUCTURE OF GLUCOSE

Physiologically and biomedically, glucose is the most important monosaccharide. The structure of glucose can be represented in the following ways **(Fig. 2.1)**:

1. The straight chain structural formula (Fisher projection).
2. Cyclic formula (Ring structure or Haworth projection)
   - Monosaccharide in solution is mainly present in ring form. In solution, aldehyde (CHO) or ketone (C=O) group of monosaccharide react with a hydroxy (OH) group of the same molecule forming a bond ***hemiacetal*** or ***hemiketal*** respectively.
   - The aldehyde group of glucose at C-1 reacts with alcohol (OH) group of C-5 or C-4 to form either six membered ring called **glucopyranose** or five membered ring called glucofuranose, respectively **(Fig. 2.1).**
   - However, in case of glucose, the six membered **glucopyranose** is much more stable than the glucofuranose ring. In the case of fructose, the more stable form is **fructofuranose.**

**Straight chain structure of D-Glucose (Fisher projection formula)**

**Ring structure or Haworth projection formula of glucose**

**Fig. 2.1:** Structure of D-Glucose.

## ISOMERISM

The compounds possessing identical molecular formula but different structures are referred to as ***isomers.*** The phenomenon of existence of isomers is called ***isomerism.*** (Greek "isos" means *equal,* "meros" means parts). The five types of isomerism exhibited by sugar are as follows:

1. Ketose-aldose isomerism
2. D and L isomerism
3. Optical isomerism
4. Epimerism
5. Anomerism.

### Ketose-aldose Isomerism

Glucose and fructose are isomers of each other having the same chemical (molecular) formula $C_6H_{12}O_6$, but they differ in structural formula with respect to their functional groups. There is a ***keto*** group in position two of fructose and an ***aldehyde*** group in position one of glucose **(Fig. 2.2).** This type of isomerism is known as ***ketose-aldose isomerism.***

### D and L Isomerism

- D and L isomerism depends on the orientation of the H and OH groups around the asymmetric carbon atom adjacent to the terminal primary alcohol carbon, e.g. carbon atom number 5 in glucose determines whether the sugar belongs to D or L isomer.

**Fig. 2.2:** Ketose-Aldose isomerism.

**Fig. 2.3:** D and L isomers (enantiomeric pairs) of glyceraldehyde and glucose.

- When OH group on this carbon atom is on the right, it belongs to **D-series,** when it is on the left; it is the member of the **L-series.** The structures of D and L-glucose based on the reference monosaccharide, D and L glyceraldehyde, a three carbon sugar **(Fig. 2.3).**
- D and L isomers are mirror images of each other. These two forms are called **enantiomers.**
- Most of the monosaccharides in the living beings belong to the D-series.

### Optical Isomerism

- The presence of **asymmetric carbon** atoms exhibits **optical activity** on the compound. Optical activity is the capacity of a substance to rotate the plane polarized light passing through it.

- When a beam of plane-polarized light is passed through a solution of an optical isomer, it will be rotated either to the right and is said to be **dextrorotatory (d)** or (+) or to the left and is said to be, **levorotatory (l)** or (-).
- When equal amount of D and L isomers are present, the resulting mixture has no optical activity. Since the activity of each isomer cancel one another, such a mixture is said to be a ***racemic*** or ***dl mixture.***

## Epimerism

When two monosaccharides differ from each other in their configuration around a single **asymmetric carbon** (other than anomeric carbon) atom, they are referred to as **epimers** of each other.

For example, galactose and mannose are two epimers of glucose **(Fig. 2.4)**. They differ from glucose in the configuration of groups (H and OH) around C-4 and C-2 respectively. Galactose and mannose are not epimers of each other as they differ in configuration at two asymmetric carbon atoms around C-2 and C-4.

**Fig. 2.4:** Epimers of glucose.

## Anomerism

### *α and β Anomerism*

The predominant form of glucose and fructose in a solution are not an open chain. Rather, the open chain form of this sugar in solution cyclize into rings. An additional asymmetric center is created when glucose cyclizes. Carbon-1 of glucose in the open chain form becomes an asymmetric carbon in the ring form **(Fig. 2.5)** and two ring structures can be formed. These are:

- α-D-glucose
- β-D-glucose

The designation α means that the hydroxyl group attached to C-1 is below the plane of the ring, β means that it is above the plane of the ring. The C-1 carbon is called the ***anomeric carbon atom*** and so, α and β forms are **anomers.**

**Fig. 2.5:** Formation of α and β anomers.

## MUTAROTATION

- Mutarotation is defined as the change in the **specific optical rotation** by the interconversion of α and β forms of D-glucose to an equilibrium mixture.
- In water, α-D-glucopyranose and β-D-glucopyranose interconvert through the open chain form of the sugar. This interconversion was detected by optical rotation.
- The specific rotation $[\alpha]_D$, of the α and β anomers of D-glucose are +112° and +18.7°. When crystalline sample of either anomers is dissolved in water, specific rotation $[\alpha]_D$ changes with time until an equilibrium value of + 52.7° is attained **(Fig. 2.6)**. This change called ***mutarotation,*** results from the formation of an equilibrium mixture containing about **one-third α-anomers** and **two-thirds β-anomers.** Very little of the open chain form of glucose is present (<1%).
- Non-reducing sugar cannot show mutarotation due to the absence of the free anomeric OH group.

## CHEMICAL PROPERTIES OF MONOSACCHARIDES

Some of the important chemical properties of monosaccharides are:

1. Action of strong acids: Furfural formation
2. Action of alkalies: Enolization
3. Oxidation: Sugar acid formation
4. Reduction: Sugar alcohol formation
5. Action of phenylhydrazine: Osazone formation.

### Action of Strong Acids (Furfural Formation)

On heating a sugar with mineral acids ($H_2SO_4$ or HCl), the sugar loses water and forms ***furfural derivatives.*** These may condense with α-naphthol, thymol, or resorcinol to produce colored complexes. This is the basis of the:

- Molisch's test
- Seliwanoff's test
- Bial's test
- Tollen's-phloroglucinol-HCl test.

### Action of Alkalies (Enolization)

- On treatment with dilute aqueous alkalies, both aldoses and ketoses are changed to **enediols.** Enediol is the enol form of sugar because two OH groups are attached to the double bonded carbon **(Fig. 2.7).**
- Enediols are good reducing agents and form basis of the **Benedict's** test and **Fehling's test**.
- Thus, alkali enolizes the sugar and thereby causes them to be strong reducing agents.
- Through the formation of a common 1, 2-enediol, glucose, fructose, and mannose may isomerize into each other in a dilute alkaline solution **(Fig. 2.7)**.

### Oxidation (Sugar Acid Formation)

- When aldoses oxidize under proper conditions they may form:
  - Aldonic acid
  - Saccharic acids
  - Uronic acid.
- Oxidation of an aldose with hypobromous acid (HOBr), which acts as an oxidizing agent gives **aldonic acid.** Thus, glucose is oxidized to gluconic acid **(Fig. 2.8)**.
- Oxidation of aldoses with nitric acid under proper conditions converts both aldehyde and terminal primary alcohol groups to carboxyl groups, forming **saccharic acid.**

**Fig. 2.6:** Mutarotation of glucose.

**Fig. 2.7:** Action of alkali on reducing sugar.

**Fig. 2.8:** Sugar acids produced by oxidation of glucose.

**Fig. 2.9:** Reduction of sugar to form alcohol.

- When an aldose is oxidized in such a way that the terminal primary alcohol group is converted to carboxyl without oxidation of the aldehyde group (usually by specific enzymes), uronic acid is formed **(Fig. 2.8)**.

## Reduction to Form Sugar Alcohol

Both aldoses and ketoses may be reduced by enzymes or non-enzymatically to the corresponding polyhydroxy alcohols. The alcohols formed from glucose, mannose, fructose and galactose are given in **Figure 2.9**.

- Manitol, the sugar alcohol derived from mannose, is frequently used medically as an osmotic diuretic to reduce cerebral edema.
- Sorbitol, the sugar alcohol derived from glucose, often accumulates in the lenses of diabetics and produces cataracts.

## Action of Phenylhydrazine (Osazone Formation)

Osazones are yellow or orange crystalline derivatives of reducing sugars with phenylhydrazine and have a characteristic

**Fig. 2.10:** Formation of glucosazone.

crystal structure, which can be used for identification and characterization of different sugars having closely similar properties (like maltose and lactose).

The reactions of glucose with phenylhydrazone are shown in **Figure 2.10**.

- Osazone formed from glucose, mannose, and fructose are identical because these are identical in the lower four carbon atoms.
- The osazone crystals of glucose and of the reducing disaccharides, lactose and maltose differ in forms **(Fig. 2.11)**:
  - Glucosazone is needle shaped
  - Lactosazone is powder puff or tennis ball shaped
  - Maltosazone is sunflower shaped.
- Non-reducing sugars like the disaccharide sucrose cannot form osazone due to the absence of a free carbonyl (CHO or C = O) group in them.

**Fig. 2.11:** Structure of different osazones.

## GLYCOSIDE FORMATION

- Glycosides are formed when the hydroxyl group of anomeric carbon of a monosaccharide reacts with **OH** or **NH** group of second compound that may or may not be a carbohydrate. The bond so formed is known as **glycosidic** or **glycosyl bond.**
- The monosaccharides are joined by glycosidic bonds to form **disaccharides, oligosaccharides**, and **polysaccharides.**

- In disaccharides, the glycosidic linkage may be either α or β depending on the configuration of the atom attached to the anomeric carbon of the sugar **(Fig. 2.16).**

**Therapeutic importance of glycosides**

- Glycosides are found in many drugs, e.g. in antibiotic ***streptomycin.***
- Cardiac glycosides such as ***Ouabain*** and ***digoxin*** increase heart muscle contraction and are used for treatment of congestive heart failure.
- ***Anthracycline*** glycosides (daunorubicin and doxirubicin), ***Daunorubicin*** is used to treat leukemia. ***Doxirubicin*** is used to treat a wide range of cancers.

## DERIVATIVES OF MONOSACCHARIDES

Some important sugar derivatives of monosaccharides are:

- Phosphoric acid ester of monosaccharides
- Amino sugar
- Deoxy sugars
- Sugar acids
- Sugar alcohols
- Neuraminic acid
- Sialic acid.

### Phosphoric Acid Ester of Monosaccharides

These are formed from the reaction of phosphoric acid with hydroxyl group of the sugar, e.g. glucose-1-phosphate or glucose-6-phosphate **(Fig. 2.12).**

#### *Importance*

- Phosphorylation of sugar within cells is essential to prevent the diffusion of the sugar out of the cell.
- Nucleic acids (RNA and DNA) of cell nuclei also contain sugar phosphates of ribose and deoxyribose.

### Amino Sugar

Amino sugars have a hydroxyl group replaced by an amino or an acetylated amino (acetyl amino) group. For example, glucosamine, N-acetyl glucosamine **(Fig. 2.13),** galactosamine, and mannosamine.

#### *Importance of Amino Sugar*

- Amino sugars are components of glycolipid (ganglioside), glycoprotein, and proteoglycans (glycosaminoglycans).
- Several antibiotics, e.g. erythromycin, carbomycin contain amino sugar.

**Fig. 2.12:** Phosphoric acid ester of glucose.

**Fig. 2.13:** Structure of amino sugars.

### Deoxy Sugars

Deoxy sugars possess a hydrogen atom in place of one of their hydroxy groups **(Fig. 2.14)**, e.g. 2-deoxyribose found in nucleic acid DNA.

**Fig. 2.14:** Structure of 2-deoxyribose sugar.

## Sugar Acids

Sugar acids are produced by oxidation of the monosaccharides, for example:

- Ascorbic acid or vitamin C (not synthesized by human beings) is required for the synthesis of collagen. It acts as water soluble antioxidant.
- Glucuronic acid (uronic acid) (*see properties of monosaccharide: oxidation*).

## Sugar Alcohols

Sugar alcohols are discussed in chemical properties of monosaccharide. They are not metabolically very active but have some medical importance in that they are used as non-glucose forming sweeteners in food stuffs for diabetics, ***sorbitol*** and ***xylitol*** are the most commonly used.

## Neuraminic Acid

Neuraminic acid is a nine carbon sugar derived from mannosamine (an epimer of glucosamine) and pyruvate.

Mannosamine + Pyruvate $\longrightarrow$ Neuraminic acid

## Sialic Acid

Sialic acids are acetylated derivatives of neuraminic acid in which amino ($NH_2$) or hydroxy (OH) group is acetylated **(Fig. 2.15)**.

### *Importance*

Sialic acids are constituents of both glycoproteins and glycolipids (ganglioside).

Sialic acid or N-Acetylneuraminic acid (NANA)

**Fig. 2.15:** Structure of sialic acid or NANA.

# DISACCHARIDES

- Disaccharides consist of two monosaccharide units.
- They are crystalline, water soluble, and sweet to taste.
- They are subclassified on the basis of the presence or absence of free reducing (aldehyde or ketone) group **(Table 2.5).**
  - Reducing disaccharides with free aldehyde or keto group, e.g. maltose, lactose
  - Non-reducing disaccharides with no free aldehyde or keto group, e.g. sucrose.

## Maltose

- Maltose contains two glucose residues, joined by glycosidic linkage between C-1 (the anomeric carbon) of one glucose residue and C-4 of the other, leaving one free anomeric carbon of the second glucose residue, which can act as a reducing agent. Thus, maltose is a ***reducing disaccharide***.
- The numerical description like ($1 \rightarrow 4$) of glycosidic bond represents the number of carbon atoms that connect the two sugars as shown in **Figure 2.16**. The sugar contributing anomeric carbon is written first.
- Maltose is produced as an intermediate product in the digestion of starch and glycogen by the action of the enzyme $\alpha$-amylase.

**Table 2.5:** Classification of disaccharides.

| Disaccharides | | | |
|---|---|---|---|
| *Reducing (with free aldehyde or ketone group)* | | *Non-reducing (absence of free aldehyde or ketone group)* | |
| *Example* | *Constituent* | *Example* | *Constituent* |
| Maltose | Glucose + Glucose | Sucrose | Glucose + Fructose |
| Lactose | Galactose + Glucose | Trehalose | Glucose + Glucose |
| Isomaltose | Glucose + Glucose | | |

**Fig. 2.16:** Structure of disaccharides.

### Isomaltose

- It consists of two glucose molecules linked by an (α-1 → 6) glycosidic bond.
- Isomaltose is a disaccharide derived from the digestion of starch or glycogen. It is hydrolyzed to glucose in the intestinal tract by an enzyme called ***isomaltase.***

### Lactose (Milk Sugar)

- It is present in milk. Lactose contains one unit of β-**galactose** and one unit of β-**glucose** that are linked by a β **(1 → 4) glycosidic linkage (Fig. 2.16).**
- The anomeric carbon of the glucose unit is available for oxidation and thus lactose is a reducing disaccharide.
- Lactose is hydrolyzed to glucose and galactose by ***lactase*** enzyme in human beings.

### Sucrose (Common Table Sugar)

- Sucrose is a disaccharide of glucose and fructose. It is formed by plant but not by human beings. Sucrose is an intermediate product of photosynthesis. Sucrose is the commonly used **table sugar.**
- In contrast to maltose and lactose, sucrose contains no free anomeric carbon atom. The anomeric carbon of both glucose and fructose are involved in the glycosidic bond **(Fig. 2.16).** Sucrose is therefore, a non-reducing sugar.
- Sucrose is hydrolyzed to fructose and glucose by an enzyme ***sucrase*** which is also called ***invertase.***

## POLYSACCHARIDES (GLYCANS)

Carbohydrates composed of ten or more monosaccharide units or their derivatives (such as amino sugars and uronic acids) are generally classified as **polysaccharides.** Polysaccharides are colloidal in size. In polysaccharides, monosaccharide units are joined together by glycosidic linkages. Another term for polysaccharides is a ***"glycans".*** Polysaccharides are subclassified in two groups **(Table 2.4).**

1. Homopolysaccharides (Homoglycans): When a polysaccharide is made up of several units of one and the same type of monosaccharide unit only, it is called homopolysaccharide.
2. Heteropolysaccharides (Heteroglycans): They contain two or more different types of monosaccharide units or their derivatives.

### Homopolysaccharides or Homoglycans

#### *Starch*

It is the storage form of glucose in plants, e.g. in potato, in grains and seeds and in many fruits. Starch is composed of two constituent viz. ***amylose*** and ***amylopectin.***

*Amylose*

Amylose is a linear polymer of D-glucose units joined by α-1→4 glycosidic linkages **(Fig. 2.17)**.

*Amylopectin*

Amylopectin is structurally identical to those of amylose (α-1→4 glycosidic linkages) but with side chains joining them by α-1→6 linkages **(Fig. 2.18)**.

Thus, amylopectin is a branched polymer having both α-(1→4) and α-(1→6) linkages. The branch points in amylopectin are created by α-1→ 6 bonds and occur at an interval of 20 to 30 units of glucose. **Figures 2.17** and **2.18** represent diagrammatically the difference in the amylose and amylopectin molecules.

**Fig. 2.17:** Structure of amylose.

**Fig. 2.18:** Structure of amylopectin.

### Dextrin

Partial hydrolysis of starch by acids or α-amylase (enzyme) produces substances known as dextrins. These also occur in honey. All dextrins have few free aldehyde groups and can show mild reducing property. They are not fermented by yeast.

### Glycogen (Animal Starch)

Glycogen is the major storage form of carbohydrate (glucose) in animals, found mostly in liver and muscle. It is often called ***animal starch***.

The structure of glycogen is similar to that of amylopectin, except that it is more highly branched **(Fig. 2.19)**, having α-(1 → 6) linkages at intervals of about 8 to 10 glucose units.

#### Function

- The function of muscle glycogen is to act as a readily available source of glucose for energy within muscle itself.
- Liver glycogen is concerned with storage and maintenance of the blood glucose.

### Cellulose

Cellulose is the chief constituent of cell wall of plants. It is an ***unbranched polymer*** of glucose and consists of long straight chains which are linked by **β-(1→4) glycosidic linkages** and not α-(1→4) as in amylose **(Fig. 2.20)**.

Since humans lack an enzyme **cellulase** that can hydrolyse the β-(1→4) glycosidic linkages, ***cellulose cannot be digested and absorbed*** and has no food value unlike starch. However, the ruminants can utilize cellulose because they have in their digestive tract microorganisms whose enzymes hydrolyse cellulose.

**Fig. 2.19:** Diagrammatic representation of glycogen molecule.

**Fig. 2.20:** Structure of cellulose.

**Importance of cellulose**

For human cellulose has nutritional significance.

- Cellulose is a component of fiber in the diet.
- Although there is no known metabolic requirement for fiber, yet high fiber diet is associated with reduced incidence of a number of diseases like:
  - Cardiovascular disease
  - Colon cancer
  - Diabetes
  - Diverticulosis.
- Cellulose is present in unrefined cereals. It increases bulk of stool, aids intestinal motility, acts as a stool softener and prevents constipation.

### Inulin

Inulin is a polymer of ***D-Fructose (Fructosans)*** linked together by β-(1 → 2) glycosidic linkage. It occurs in the tubers of some plants, e.g. chicory, bulb of onion and garlic. Inulin is not hydrolyzed by α-amylase but is hydrolyzed by ***inulinase,*** which is not present in the humans and so it is not utilized as food.

**Clinical importance of inulin**

Inulin has clinical importance as it is used in the studies of ***glomerular filtration rates (kidney function test).***

## Heteropolysaccharides or Heteroglycans

### Glycosaminoglycans (GAGs) or Mucopolysaccharides (Table 2.6)

#### Structure of GAG

- A GAG is an unbranched heteropolysaccharide, made up of **repeating disaccharides.**
  - One component of which is always an **amino sugar** (hence the name glycosaminoglycans), either **D-glucosamine** or **D-galactosamine.**
  - The other component of the repeating disaccharide (except in the case of keratan sulfate) is a **uronic acid,** either **L-glucuronic acid** or its epimer **L-iduronic acid.**
- Thus, GAG is a polymer of **[uronic acid-amino sugar]$_n$**

**Table 2.6:** Structure and functions of glycosaminoglycans (GAGs).

| GAG | Disaccharide unit | Function |
|---|---|---|
| Hyaluronic acid | N-Acetyl glucosamine-Glucuronic acid | Serves as lubricant and shock absorber, facilitates cell migration in embryogenesis, morphogenesis, wound healing |
| Chondroitin sulfate | N-Acetyl-galactosamine-Glucuronic acid | Provides an endoskeleton structure helping to maintain their shape. Has a role in compressibility of cartilage in weight bearing |
| Keratan sulfate | N-Acetyl-glucosamine-Galactose (no uronic acid) | Transparency of cornea |
| Dermatan sulfate | N-Acetyl-galactosamine-L-iduronic acid | Transparency of cornea and maintains the overall shape of the eye |
| Heparin | Glucosamine-Glucuronic acid or Iduronic acid | Serves as an anticoagulant, causes release of lipoprotein lipase from capillary walls |
| Heparan sulfate | Same as heparin except that some glucosamine are acetylated | Component of plasma membrane where it may act as receptor and may also participate in the mediation of cell growth, cell-to-cell communication |

**Fig. 2.21:** Bottle brush structure of proteoglycan monomer.

**Fig. 2.22:** Proteoglycan aggregate.

- This polymer is attached covalently to extracellular proteins called ***core protein*** (except hyaluronic acid) to form ***proteoglycans.***
- A resulting structure resembles a ***"bottle brush"*** **(Fig. 2.21).**
- The proteoglycan monomer associates with a molecule of hyaluronic acid to form ***proteoglycan aggregates*** **(Fig. 2.22).**
- The association is stabilized by additional small proteins called ***link proteins.***
- With the exception of hyaluronic acid, all the GAGs contain **sulfate** group.
- The amount of carbohydrates in proteoglycans is usually much greater than is found in a glycoprotein and may comprise upto 95% of its weight.

## Occurrence of GAGs

Glycosaminoglycans are found in the:

- Synovial fluid of joints
- Vitreous humor of the eye
- Arterial walls
- Bones
- Cartilage.

## Functions of GAGs

- They are major components of the extracellular matrix or ground substance.
- GAGs carry sulfate and carboxyl groups which give them a negative charge and have special ability to bind large amounts of water, thereby producing a gel-like matrix which functions as a cushion against mechanical shocks.
- They act as ***"molecular sieves"***, determining which substances enter and leave cells.

- They also give resilience (elasticity) to cartilage, permitting compression and re-expansion.
- They lubricate joints both at the surface of cartilage and in synovial fluid.
- The viscous lubricating properties of mucous secretions are also due to the presence of glycosaminoglycans, which led to the original naming of these compounds as ***mucopolysaccharides.***

### *Types of GAGs*

- The different types of GAGs are:
  - Hyaluronic acid
  - Chondroitin sulfate
  - Keratan sulfate
  - Dermatan sulfate
  - Heparin
  - Heparin sulfate.
- The different types of GAGs differ from each other in the following properties:
  - Uronic acid composition
  - Amino sugar composition
  - Linkage between amino sugar and uronic acid
  - Chain length of the disaccharide polymer
  - Presence or absence of sulfate groups and their position of attachment to the sugar
  - The nature of the core protein to which they are attached
  - Their tissue and subcellular distribution and their biological function.

**Table 2.6** describes structure and functions of different types of glycosaminoglycans.

## GLYCOPROTEINS

- Glycoproteins are proteins to which oligosaccharides are covalently attached to their polypeptide chain.
- Glycoproteins contain much shorter carbohydrate chain than proteoglycans.
- The distinction between glycoproteins and proteoglycans may be based on the amount of carbohydrate.
  - Glycoproteins contain less than 4 percent carbohydrate in the molecule.
  - Proteoglycans contain more than 4 percent carbohydrate.

### Functions of Glycoproteins

- Almost all the plasma proteins of humans are glycoproteins, except albumin.
- Many integral membrane proteins are glycoproteins.
- Most proteins that are secreted, such as antibodies, hormones and coagulation factors are glycoproteins.
- Glycoproteins serve as **lubricant** and **protective agent**, e.g. mucin of gastrointestinal and urogenital tracts.
- Glycoproteins also serve as transport molecules, such as **transferrin** and **ceruloplasmin.**

## EXAM QUESTIONS

### Long Answer Question (LAQ)

1. Define carbohydrates; classify carbohydrates with examples and their functions.

### Short Notes

1. Glycogen or animal starch
2. Glycosaminoglycans
3. Glycoproteins
4. Disaccharide
5. Clinically important carbohydrates
6. Write functions of carbohydrates
7. Biologically important sugar derivatives of monosaccharide
8. Glycosides
9. Chemical properties of monosaccharide
10. Various types of isomerism exhibited by sugars.

### Multiple Choice Questions (MCQs)

**1. All the following are composed exclusively of glucose, *except*:**

a. Glycogen b. Starch
c. Lactose d. Maltose

**2. The epimer of glucose is:**

a. Galactose b. Fructose
c. Arabinose d. Xylose

**3. The carbohydrate serves as an anticoagulant:**

a. Sucrose b. Heparin
c. Arabinose d. Maltose

**4. A sugar alcohol is:**
a. Mannitol b. Trehalose
c. Xylulose d. Arabinose

**5. Which of the following carbohydrate is dietary fiber?**
a. Cellulose b. Starch
c. Glycogen d. Inulin

**6. Glycosaminoglycans are:**
a. Disaccharide b. Homoglycans
c. Heteroglycans d. None of the above

**7. Which of the following glycosaminoglycans is unsulfated?**
a. Chondroitin sulfate
b. Heparin
c. Hyaluronic acid
d. Keratan sulfate

**8. All of the following GAGs contain uronic acid *except*:**
a. Hyaluronic acid
b. Keratan sulfate
c. Heparin
d. Heparan sulfate

**9. Which of the following is a non-reducing sugar?**
a. Sucrose b. Trehalose
c. Lactose d. a and b

**10. Which of the following is not hydrolyzed by α-amylase?**
a. Starch b. Glycogen
c. Cellulose d. Dextrin

**11. The sugar found in DNA is:**
a. Xylose b. Ribose
c. Deoxyribose d. Ribulose

**12. Which of the following is not applicable to homoglycans?**
a. These are carbohydrates
b. These are polymer of monosaccharide
c. Sweet to test
d. Animal starch is homoglycans

**13. Two sugars which differ from one another only in configuration around a single carbon atom are termed:**
a. Epimers b. Anomers
c. Optical isomers d. Stereoisomers

**14. A polysaccharide which is called animal starch is:**
a. Glycogen b. Starch
c. Inulin d. Dextrin

**15. The homopolysaccharide used for intravenous infusion as plasma substitute is:**
a. Agar b. Inulin
c. Dextrans d. Starch

**16. The polysaccharide used in assessing the glomerular filtration rate (GFR) is:**
a. Glycogen b. Agar
c. Inulin d. Hyaluronic acid

**17. The constituent unit of inulin is:**
a. Glucose b. Fructose
c. Mannose d. Galactose

**18. Which of the following is a heteroglycans?**
a. Dextrins b. Agar
c. Inulin d. Chitin

**19. The glycosaminoglycan which does not contain uronic acid is:**
a. Dermatan sulfate
b. Chondroitin sulfate
c. Keratan sulfate
d. Heparan sulfate

**20. The glycosaminoglycan which is sulfated:**
a. Keratan sulfate b. Heparin
c. Chondroitin sulfate d. All of the above

**21. Keratan sulfate is found in abundance in:**
a. Heart muscle b. Liver
c. Adrenal cortex d. Cornea

**22. Glucose on oxidation gives which of the following, *except*:**
a. Mucic acid
b. Glucosaccharic acid
c. Gluconic acid
d. Glucuronic acid

**23. A positive Benedict's test is not given by:**
a. Sucrose b. Lactose
c. Maltose d. Glucose

**24. A positive Seliwanoff's test is obtained with:**
a. Glucose b. Fructose
c. Lactose d. Maltose

**25. Osazones are not formed with the:**
a. Glucose b. Fructose
c. Sucrose d. Lactose

**26. Carbohydrates play which of the following roles:**
a. Constituent of nucleic acid
b. Structural components
c. Energy storage
d. All of the above

**27. What monosaccharides make up a sucrose (table sugar) molecule?**
a. Galactose and fructose
b. Galactose and maltose
c. Lactose and fructose
d. Glucose and fructose

**28. Glucose is a building block for, *except*:**
a. Cellulose b. Glycogen
c. Inulin d. Starch

**29. Carbohydrate found abundantly in semen is:**
a. Glucose b. Fructose
c. Mannose d. Galactose

**30. The sugar found in milk is:**
a. Galactose b. Glucose
c. Fructose d. Lactose

**31. Invert sugar is:**
a. Lactose b. Sucrose
c. Maltose d. Fructose

**32. An L-isomer of monosaccharide formed in human body is:**
a. L-fructose b. L-erythrose
c. L-erythrulose d. L-xylulose

**33. Which of the following is an epimeric pair?**
a. Glucose and galactose
b. Galactose and mannose
c. Fructose and galactose
d. Lactose and maltose

**34. N–Acetylglucosamine is present in:**
a. Keratan sulfate
b. Chondroitin sulfate
c. Heparin
d. All of these

**35. Amylose is a constituent of:**
a. Cellulose b. Starch
c. Glycogen d. All of the above

**36. Amylopectin is a constituent of:**
a. Starch only b. Glycogen only
c. Inulin d. Glycogen and starch

**37. Synovial fluid contains which of the following GAGs:**
a. Heparin
b. Hyaluronic acid
c. Chondroitin sulfate
d. Keratin sulfate

**38. The carbohydrate of the blood group substances is:**
a. Deoxyribose b. Fucose
c. Arabinose d. Ribose

**39. Which of the following glycosaminoglycans is intracellular?**
a. Heparin b. Hyaluronic acid
c. Chondroitin sulfate d. Keratin sulfate

**40. Following glycosaminoglycans are extracellular, *except*:**
a. Heparin b. Hyaluronic acid
c. Chondroitin sulfate d. Keratin sulfate

## Answers for MCQs

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **1.** c | **2.** a | **3.** b | **4.** a | **5.** a | **6.** c | **7.** c | **8.** b |
| **9.** d | **10.** c | **11.** c | **12.** c | **13.** a | **14.** a | **15.** c | **16.** c |
| **17.** b | **18.** b | **19.** c | **20.** d | **21.** d | **22.** a | **23.** a | **24.** b |
| **25.** c | **26.** d | **27.** d | **28.** c | **29.** b | **30.** d | **31.** b | **32.** d |
| **33.** a | **34.** a | **35.** b | **36.** d | **37.** b | **38.** b | **39.** a | **40.** a |

CHAPTER

3

# Chemistry of Lipids

**Chapter outline**


- Definition, Classification and Functions of Lipids
- Fatty Acids
- Essential Fatty Acids
- Reactions of Lipids
- Characterization of Fat (Tests for Purity of Fat)
- Triacylglycerols or Triacylglycerides or Neutral Fat
- Phospholipids
- Glycolipids (Glycosphingolipids)
- Cholesterol (Animal sterol)
- Lipoproteins
- Eicosanoids
- Micelles, Lipid Bilayer, and Liposomes
- Detergents


## INTRODUCTION

Lipids are a major source of energy for the body besides their various other biochemical function and their role in cellular structure. Lipids are a heterogenous group of water insoluble (hydrophobic) organic molecules. Lipids include fats, oils, steroids, waxes and related compounds.

This chapter introduces the chemistry and functions of lipids.

## DEFINITION, CLASSIFICATION AND FUNCTIONS OF LIPIDS

### Definition of Lipids

Lipids may be defined as organic substances insoluble in water but soluble in organic solvents like chloroform, ether and benzene. **They are esters of fatty acids with alcohol esters and are utilizable by the living organism.**

### Classification of Lipids

There are many different methods of classifying lipids. The most commonly used classification of lipids is modified from Bloor as follows:

1. Simple lipids
2. Complex or compound lipids
3. Derived lipids.

#### *Simple Lipids*

These are esters of fatty acids with various alcohols. Depending on the type of alcohols, these are subclassified as:

1. Neutral fats or triacylglycerol or triglycerides
2. Waxes.

*Neutral fats or triacylglycerol or triglycerides*

These are esters of fatty acids with alcohol ***glycerol,*** e.g. tripalmitin. Because they are ***uncharged,*** they are termed as ***neutral fat.*** The fat we eat are mostly triglycerides. A fat in liquid state is called an **oil**, e.g. vegetable oils like groundnut oil, mustard oil, corn oil, etc.

*Waxes*

***True waxes***

These are esters of fatty acids with higher molecular weight ***monohydric long chain alcohols.*** These compounds have no importance as far as human metabolism is concerned. For example,

- Lanolin (from lamb's wool)
- Bees-wax
- Spermaceti oil (from whales)

These are widely used in pharmaceutical, cosmetic and other industries in the manufacture of lotions, ointments and polishes.

***Other waxes***

These are esters of fatty acid with alcohol, e.g.

- Cholesterol forms cholesterol ester
- Retinol (vitamin A) forms vitamin A ester
- Cholecalciferol (vitamin D) forms vitamin D ester.

## Complex or Compound Lipids

These are esters of fatty acids, with alcohol containing additional (prosthetic) groups. These are subclassified according to the type of prosthetic group present in the lipid as follows:

1. Phospholipids
2. Glycolipids
3. Lipoproteins

### *Phospholipids*

Phospholipids are lipids containing in addition to fatty acids and an alcohol, a ***phosphoric acid*** residue. They also have nitrogen containing bases and other substituents. Phospholipids may be classified on the basis of the type of alcohol present in them as:

- Glycerophospholipids
- Sphingophospholipids

***Glycerophospholipids***

The alcohol present is ***glycerol***. Examples of glycerophospholipids are:

- Phosphatidyl choline (lecithin)
- Phosphatidyl ethanolamine (cephalin)
- Phosphatidyl serine
- Phosphatidyl inositol
- Lysophospholipid
- Plasmalogens
- Cardiolipins

***Sphingophospholipids***

The alcohol present is **sphingosine**, e.g.

- Sphingomyelins.

### *Glycolispids*

Lipids containing fatty acid, alcohol **sphingosine** and additional residue are ***carbohydrates*** with ***nitrogen base.*** They do not contain phosphate group. These sugar containing sphingolipids are also called ***glycosphingolipids.*** For example:

- Cerebrosides
- Gangliosides

### *Lipoproteins*

Lipoproteins are formed by combination of lipid with a prosthetic group protein, e.g. serum lipoproteins like:

- Chylomicrons
- Very low density lipoprotein (VLDL)
- Low density lipoprotein (LDL)
- High density lipoprotein (HDL)

## Derived Lipids

Derived lipids include the products obtained after the hydrolysis of simple and compound lipids which possess the characteristics of lipids, e.g.

- Fatty acids
- Steroids
- Cholesterol
- Lipid soluble vitamins and hormones
- Ketone bodies

## Functions of Lipids

Lipids serve as:

- **Storage form of energy:** The fats and oils are used almost universally as stored forms of energy in living organisms.
- **Structural lipids:** Lipids are major structural components of membranes, e.g. phospholipids, glycolipids and sterols.
- **Cholesterol,** a sterol, is a precursor of many **steroid hormones, vitamin D** and is also an important component of plasma membrane.
- Lipid acts as a **thermal insulator** in the subcutaneous tissues and around certain organs.
- Nonpolar lipids act as **electrical insulators** in neurons.
- Lipids are important dietary constituents because of the fat soluble vitamins and essential fatty acids which are present in the fat of natural foods.
- Lipids help in absorption of fat soluble vitamins (A, D, E and K). They act as a solvent for the transport of fat soluble vitamins.

# FATTY ACIDS

Fatty acids are carboxylic acids with hydrocarbon chains ($-CH_2-CH_2-CH_2-$) and represented by a chemical formula **R-COOH,** where R stands for hydrocarbon chain. The fatty acids are ***amphipathic*** in nature, i.e. each has hydrophilic (COOH) and hydrophobic (hydrocarbon chain) groups in the structure.

**Table 3.1:** Some naturally occurring fatty acids.

| Common name | Carbon atoms | Double bonds | Position of double bond | Unsaturated fatty acid class |
|---|---|---|---|---|
| **Saturated fatty acids** | | | | |
| Acetic acid | 2 | 0 | - | - |
| Propionic acid | 3 | 0 | | |
| n-butyric acid | 4 | 0 | | |
| Valeric acid | 5 | 0 | | |
| Lauric acid | 12 | 0 | | |
| Myristic acid | 14 | 0 | | |
| Palmitic acid | 16 | 0 | | |
| Stearic acid | 18 | 0 | | |
| Arachidic acid | 20 | 0 | | |
| Behenic acid | 22 | 0 | | |
| Lignoceric acid | 24 | 0 | | |
| **Unsaturated fatty acid** | | | | |
| • **Monoenoic acid** (one double bond) Palmitoleic acid | 16 | 1 | 9 | $\omega$-7 |
| Oleic acid | 18 | 1 | 9 | $\omega$-9 |
| Nervonic acid | 24 | 1 | 15 | $\omega$-9 |
| • **Dienoic acids** (two double bonds) Linoleic acid | 18 | 2 | 9, 12 | $\omega$-6 |
| • **Trienoic acid** (three double bonds) Linolenic acid | 18 | 3 | 9, 12, 15 | $\omega$-3 |
| • **Tetraenoic acid** (four double bonds) Arachidonic acid | 20 | 4 | 5, 8, 11, 14 | $\omega$-6 |
| • **Pentaenoic acid** (five double bonds) Clupandonic acid | 22 | 5 | 7, 10,13, 16, 19 | $\omega$-3 |
| • **Docosa hexaenoic acid** (DHA) Cervonic acid | 22 | 6 | 4,7,10,13,16,19 | $\omega$-3 |

Some naturally occurring fatty acids are given in **Table 3.1.**

## Classification of Fatty Acids

Fatty acids are classified into four major classes **(Fig. 3.1)**.

1. Straight chain fatty acids
2. Branched chain fatty acids
3. Substituted fatty acids
4. Cyclic fatty acids

### *Straight Chain Fatty Acids*

Fatty acids, in which the carbons are arranged linearly, are subclassified into two classes:

i. Saturated fatty acids
ii. Unsaturated fatty acids

#### *Saturated fatty acids*

There is no double bond in the hydrocarbon chain of these fatty acids. Saturated fatty acids are subclassified into two classes:

a. **Even carbon acids** carry even number of carbons, e.g. palmitic acid and stearic acid.
b. **Odd carbon acids** carry odd number of carbons, e.g. propionic acid.

#### *Unsaturated fatty acids*

These contain double bonds in their hydrocarbon chains.

These are subclassified according to the number of double bonds present in the structure as follows:

a. Monoenoic or monounsaturated fatty acid
b. Polyenoic or polyunsaturated fatty acid.

- **Monoenoic** or monounsaturated fatty acids carry a single double bond in the molecule, e.g. oleic acid.

**Fig. 3.1:** Classification of fatty acids.

- **Polyenoic** or polyunsaturated fatty acids contain two or more double bonds; for example:
  - **Dienoic acids** have two double bonds, e.g. linoleic acid present in soybean, sunflower, saffola and groundnut oil.
  - **Trienoic acids** have three double bonds, e.g. Linolenic acid present in poppy seed oil, linseed oil.
  - **Tetraenoic acid** with four double bonds, e.g. arachidonic acid present in groundnuts.

### *Branched Chain Fatty Acids*

These are less abundant than straight chain acids in animals and plants, e.g.

- Isovaleric acid
- Isobutyric acid.

### *Substituted Fatty Acids*

In substituted fatty acids one or more hydrogen atoms have been replaced by another group, e.g.

- Lactic acid of blood
- Cerebronic acid and oxynervonic acids of brain glycolipids
- Ricinoleic acid of castor oil.

### *Cyclic Fatty Acids*

Fatty acids bearing cyclic groups are present in some bacteria and seed lipids, e.g. hydnocarpic acid (Chaulmoogra acid) of chaulmoogra seed.

## Functions of Fatty Acids

Fatty acids have three major physiological functions.

1. They serve as building blocks of ***phospholipids*** and ***glycolipids.*** These amphipathic molecules are important components of biological membranes.
2. Fatty acid derivatives serve as ***hormones,*** e.g. ***prostaglandins***.
3. Fatty acids serve as a major fuel for most cells.

## Numbering of Fatty Acid Carbon Atoms (Fig. 3.2)

- Fatty acid carbon atoms are numbered starting at the carboxyl terminus.

**Palmitic acid**

**Fig. 3.2:** Numbering of fatty acid carbon.

- Carbon atoms 2 and 3 are often referred to as α and β respectively.
- The methyl carbon atom at the distal end of the chain is called omega (ω) carbon.

## Representation of Double Bonds of Fatty Acids

Two systems are used to designate the position of double bond:

- C-system
- ω-or n-system

### *C-System*

In C-system (i.e. $C_1$ being the carboxyl carbon) the position of double bond is represented by the symbol Δ (delta), followed by a superscript number.

For example, oleic acid is a $C_{18}$ fatty acid with one double bond between carbon number 9 and 10 is represented as C: 18:1: $\Delta^9$ **(Fig. 3.3)**.

### *ω– or n-System*

In this system, *'ω'* or *'n'* refers to the carbon of their terminal methyl group in a fatty acid. In *ω*-system or n-system, the oleic acid is denoted as C: 18:1: *ω*-9 **(Fig. 3.3)** to indicate that:

- *ω*-9 represents the double bond position which is found between 9th and 10th carbon atoms, the first carbon atom being that of the terminal methyl group. This method is widely used by nutritionists.
- Naturally occurring unsaturated fatty acids belong to ***ω-9, ω-6*** and ***ω-3*** series. For example,
  - *ω*-9 : Oleic acid (C:18:1:*ω*-9)
  - *ω*-6 : Linoleic acid (C:18:2:*ω*-6)
  - Arachidonic acid (C:20:4:*ω*-6)
  - *ω*-3 : Linolenic acid (C:18:3:*ω*-3)

## ESSENTIAL FATTY ACIDS

Fatty acids that are required for optimal health and cannot be synthesized by the body and should be supplied in the diet are called ***essential fatty acids.***

They are polyunsaturated fatty acids, namely **linoleic acid** and **linolenic acid. Arachidonic acid** can be synthesized from linoleic acid. Therefore, in deficiency of linoleic acid, arachidonic acid also becomes essential fatty acids.

Humans lack the enzymes to introduce double bonds at carbon atoms beyond $C_9$ in the fatty acid chain. Hence, humans cannot synthesize linoleic acid and linolenic acid having double bonds beyond $C_9$. And thus, linoleic and linolenic are the essential fatty acids.

## Functions of Essential Fatty Acids (EFA)

Essential fatty acids are involved in the following:

### *Synthesis of Eicosanoids*

Linoleic acid and linolenic acid supplied by the diet are the precursors for the synthesis of a variety of other unsaturated fatty acids. Arachidonic acid, a fatty acid derived from linoleic acid is an essential precursor of eicosanoids, which include:

- Prostaglandins
- Thromboxanes
- Prostacyclin
- Leukotrienes.

### *Maintenance of Structural Integrity*

EFAs are required for membrane structure and function. These fatty acids are important constituents of phospholipids in cell membrane and help to maintain the structural integrity of the membrane.

EFAs are constituent of **structural lipids** of the cell and are concerned with the structural integrity of the mitochondrial membrane.

### *Development of Retina and Brain*

Docosahexaenoic acid (DHA: *ω*-3) (cervonic acid), which is synthesized from linolenic acid is present in high

**Fig. 3.3:** Representation of double bonds of unsaturated fatty acid.

concentration in retina, cerebral cortex, testes and sperm. DHA is particularly needed for development of the brain and retina during the neonatal period. Therefore, a small daily intake of EFA is now recommended. This may be especially important when the nervous system is developing.

### *Skin Protector*

It covalently binds another fatty acid attached to cerebrosides in the skin forming an unusual lipid **(acylglucosylceramide)** that helps to make the skin impermeable to water. The red **scaly dermatitis** and other skin problems associated with a dietary deficiency of essential free fatty acids.

### *Antiatherogenic Effect*

Essential fatty acids increase esterification and excretion of cholesterol, thereby lowering the serum cholesterol level. Thus, essential fatty acids help to prevent the atherosclerosis.

**Essential Fatty Acid Deficiency**

- Deficiency of EFAs is characterized by scaly skin, eczema (in children), loss of hair and poor wound healing.
- Impaired lipid transport and fatty liver may occur due to deficiency of EFAs.
- EFAs deficiency decreases efficiency of biological oxidation.

## REACTIONS OF LIPIDS

### Saponification

Hydrolysis of a fat by ***alkali*** is called ***saponification***. The products are glycerol and the alkali salts of the fatty acids, which are called **soaps (Fig. 3.4)**. Acid hydrolysis of a fat yields the free fatty acids and glycerol.

### Hydrogenation

Hydrogenation of unsaturated fats in the presence of a catalyst (nickel) is known as ***"hardening".*** It is commercially valuable as a method of converting these liquid fats, usually of plant origin into solid fats as margarines, vegetable *ghee*, etc.

**Fig. 3.4:** Saponification of fat.

### Peroxidation

Peroxidation (auto-oxidation) of lipids exposed to oxygen is responsible not only for deterioration of foods (rancidity) but also for damage to tissues *in vivo*, where it may be a cause of **cancer.** Lipid peroxidation is a chain reaction generating free radicals that initiate further peroxidation. To control and reduce peroxidation, humans make use of ***antioxidants.*** Naturally occurring antioxidants include vitamin E (tocopherol) and β-carotene (provitamin A), which are lipid soluble and vitamin C, which is water soluble.

### Rancidity

The unpleasant odor and taste, developed by natural fats upon aging, is referred to as ***"rancidity".*** Rancidity may be due to ***hydrolysis*** or ***oxidation*** of fat.

- **Rancidity due to hydrolysis:** Naturally occurring fats, particularly those from animal sources, are contaminated with enzyme **lipase.** The action of lipase brings about partial hydrolysis of glycerides of fat.
- **Rancidity may also be caused by various oxidative processes.** For example, oxidation at the double bonds of unsaturated fatty acids of glycerides may form peroxides, which then decompose to form aldehydes of unpleasant odor and taste, this process is increased by exposure to light or heat.

Many natural vegetable fats and oils may contain antioxidants like vitamin E which prevent onset of rancidity. Therefore, vegetable fats can be preserved for a longer time than animal fats.

## CHARACTERIZATION OF FAT (TESTS FOR PURITY OF FAT)

Fats are characterized and their purity assayed by the following tests:

### Saponification Number

It is defined as, number of mgs of KOH required to saponify one gm of fat. It is inversely proportional to the molecular weight of fat. This value is high in fats containing a short chain fatty acids. For example, the saponification number of:

- Butter = 220
- Coconut oil = 260

## Iodine Number

The number of gms of iodine required to saturate 100 gms of a given fat is known as iodine number. Since iodine is taken up by the double bonds, a high iodine number indicates a high degree of unsaturation of the fatty acids in fat, e.g.

- Butter fat = 27
- Coconut oil = 8
- Linseed oil = 200

Iodine number is important in the identification of the fat or oil as well as is used for identification of adulteration of oils.

## Acid Number

Number of mg of KOH required to neutralize the free fatty acids present in one gm of fat is known as acid number. The acid number indicates the degree of rancidity of the given fat. Acid number is directly proportional to the rancidity. The edibility of a fat is inversely proportional to the acid number.

Refined oil should not contain free fatty acids. The presence of free fatty acids in any oil indicates that it is not pure.

## Reichert Meissl Number

The number of mL of 0.1 N alkalis, required to neutralize the volatile fatty acids distilled from 5 gm of fat, e.g. the Reichert Meissl value for:

- Butter = 26
- Coconut oil = 7.

It is less than one for other edible oils. The admixture of certain fats may be used to prepare synthetic butter which may simulate butter in most of the constants except RM value and hence, can be detected.

# TRIACYLGLYCEROLS OR TRIACYLGLYCERIDES OR NEUTRAL FAT

These are esters of fatty acids with glycerol. Triacylglycerol consists of three fatty acids, which are esterified through their carboxyl groups, resulting in a loss of negative charge and formation of neutral fat **(Fig. 3.5)**.

- Triacylglycerols containing the same kind of fatty acid in all three positions are called ***simple triacylglycerols.***
- ***Mixed triacylglycerols*** contain two or more different fatty acids. The fatty acid on carbon 1 is usually saturated. That on carbon 2 is usually unsaturated and that on carbon 3 can be either of the two. The stereospecific numbering (sn) of the glycerol carbon atom is shown in **Figure 3.5**.

**Fig. 3.5:** Glycerol and triacylglycerol.

- As the polar hydroxyl groups of glycerol and polar carboxyl groups of the fatty acids are bound in ester linkages, triacylglycerols are nonpolar, hydrophobic and neutral (in charges) molecules, essentially insoluble in water.
- The presence of the unsaturated fatty acid(s) in triacylglycerol decreases the melting temperature of the lipid and remains in liquid form (oil).
- Vegetable oils such as corn and olive oil are composed largely of triacylglycerols with unsaturated fatty acids and thus are liquids at room temperature.
- Triacylglycerols containing only saturated fatty acids, such as beef fat, are white greasy solids at room temperature.

## Functions of Triacylglycerols

Triacylglycerols are highly concentrated stores of metabolic energy.

- Triacylglycerols have two significant advantages over other forms of metabolic fuel, polysaccharides such as glycogen. The carbon atoms of fatty acids are more reduced than those of sugars and oxidation of triacylglycerides yields twice the energy as that of carbohydrates.
- Furthermore, triacylglycerol are very non-polar, hydrophobic, so they are stored in a nearly anhydrous form, they no need to carry the extra weight of water of hydration whereas, proteins and carbohydrates are much more polar and therefore, more highly hydrated and have to carry extra weight of water.
- In fact, a gram of dry glycogen binds about two grams of water. Consequently, a gram of nearly anhydrous fat stores more than six times as much energy as a gram of hydrated glycogen.
- The glycogen and glucose stores provide enough energy to sustain physiological function for about 24 hours; whereas the triacylglycerol stores allow survival for several weeks.

- Obese persons may have 15 or 20 kg of triacylglycerol deposited in their adipocytes, sufficient to supply energy needs for months. In contrast, the human body can store less than a day's energy supply in the form of glycogen.
- Carbohydrates (glucose and glycogen) do offer certain advantages as quick sources of metabolic energy, one of which is their ready solubility in water.

## PHOSPHOLIPIDS

- These are made up of ***fatty acid, glycerol*** or other alcohol, ***phosphoric acid*** and ***nitrogenous base***.
- Phospholipids are the major lipid constituents of cell membranes.
- Like fatty acids, phospholipids are ***amphipathic*** in nature, i.e. each has a hydrophilic or polar head (phosphate group) and a long hydrophobic tail (containing two fatty acid chains) **(Fig. 3.6)**.

**Fig. 3.6:** Diagrammatic representation of amphipathic phospholipid.

### Classification of Phospholipids

There are two classes of phospholipids **(Fig. 3.7)**:

1. Glycerophospholipids or phosphoglycerides that contain glycerol as the alcohol.
2. Sphingophospholipids that contain sphingosine as the alcohol.

#### *Glycerophospholipids or Phosphoglycerides*

- Phospholipids derived from glycerol are called **phosphoglycerides** or **glycerophospholipids.**
- In glycerophospholipid, the hydroxyl groups at $C_1$ and $C_2$ of glycerol are esterified with two fatty acids. The $C_3$ hydroxyl group of the glycerol is esterified to phosphoric acid and resulting compound called, ***phosphatidic acid*** **(Fig. 3.8)**.
- Phosphatidic acid is a key intermediate in the biosynthesis of other glycerophospholipids.
- In glycerophospholipid, phosphate group of phosphatidic acid becomes esterified with the hydroxyl group of one of the several nitrogen base or other groups. Different types of glycerophospholipids are discussed below.

*Phosphatidylcholine (lecithin)*

- These are glycerophospholipids containing choline **(Fig. 3.9)**. These are most abundant phospholipids of

**Fig. 3.7:** Classification of phospholipids.

**Fig. 3.8:** Structure of glycerol and phosphatidic acid.

the cell membrane having both structural and metabolic functions.

- Dipalmitoyl lecithin is an important phosphatidylcholine found in lungs, secreted by **pulmonary type II epithelial** cell. It acts as a ***lung surfactant*** and is necessary for normal lung function. It reduces surface tension in the alveoli, thereby prevents alveolar collapse (adherence of the inner surfaces of the lungs).

**Acute Pulmonary Respiratory Distress Syndrome (RDS)**

- RDS in infants is associated with insufficient ***surfactant dipalmitoyl phosphatidylcholine*** production.
- The lungs of immature infants do not have enough type II epithelial cells to synthesize sufficient amounts of dipalmitoyl phosphatidylcholine (DPPC).
- In its absence, the lungs tend to collapse and this condition is known as ***respiratory distress syndrome.***

**Fig. 3.9:** Structure of different phospholipids.

*Phosphatidylethanolanine (Cephalin)*

- They differ from lecithin in having nitrogenous base ethanolamine in place of choline **(Fig. 3.9)**.
- ***Thromboplastin*** (coagulation factor III), which is needed to initiate the clotting process, is composed mainly of cephalin.

*Phosphatidylserine*

It contains the amino acid serine rather than ethanolamine and is found in most tissues **(Fig. 3.9)**.

*Phosphatidylinositol*

- In phosphatidylinositol, inositol is present as the stereoisomer ***myoinositol*** **(Fig. 3.9)**.
- Phosphatidylinositol is a **second messenger** for the action of hormones like oxytocin and vasopressin.

*Plasmalogens*

- Plasmalogens are generally similar to other phospholipids but the fatty acid at $C_1$ of glycerol is linked through an **ether**, rather than an ester bond **(Fig. 3.9)**.
- There are three major classes of plasmalogens:
  i. Phosphatid**al**cholines
  ii. Phosphatid**al**ethanolamines
  iii. Phosphatid**al**serines.
- These are found in myelin and in cardiac muscle.
- Plasmalogen is a platelet activating factor (PAF) and involved in platelet aggregation and degranulation.

*Lysophospholipids*

Lysophospholipids are produced when one of the two fatty acids is removed from glycerophospholipid. The most common of these are lysophosphatidylcholine (lysolecithin) **(Fig. 3.9)** and lysophosphatidylethanolamine.

*Cardiolipin (Diphosphatidylglycerol)*

- Cardiolipin is composed of two molecules of phosphatidic acid connected by a molecule of glycerol.
- Two molecules of phosphatidic acid esterified through their phosphate groups with a molecule of glycerol are called cardiolipin **(Fig. 3.9).**
- Cardiolipin is a major lipid of mitochondrial membrane and is necessary for optimum function of the electron transport process.
- This is only human glycerophospholipid that possess ***antigenic properties.***

## Sphingophospholipids

Phospholipids derived from alcohol ***sphingosine*** instead of glycerol are called sphingophospholipids, e.g. sphingomyelin.

**Fig. 3.10:** Structure of sphingomyelin.

*Sphingomyelin*

- Sphingomyelin is the only phospholipid in membranes that is not derived from glycerol. Instead, the alcohol in sphingomyelin is ***sphingosine,*** an amino alcohol.
- In sphingomyelin, the amino group of the sphingosine is linked to a fatty acid to yield ***ceramide*** (sphingosine-fatty acid complex).
- In addition, the primary hydroxy group of sphingosine is esterified with phosphorylcholine **(Fig. 3.10)**.
- Sphingomyelin is one of the principal structural lipids of membranes of nerve tissue.

## Functions of Phospholipids

- Phospholipids are the major lipid constituents of cell membranes.
- They regulate ***permeability of membranes*** as well as activation of some membrane bound enzymes.
- Phospholipids are of importance in ***insulating the nerve impulse*** (like the plastic or rubber covering around an electric wire) from the surrounding structures, e.g. sphingomyelins act as electrical insulators in the myelin sheath around nerve fibers.
- Phospholipids are important constituents of lipoproteins.
- Phospholipids act as a ***lipotropic factor.*** Lipotropic factor is the component that prevents ***fatty liver,*** i.e. accumulation of fat in the liver.
- These are good ***emulsifying agents*** that help in intestinal absorption of lipids.
- ***Thromboplastin*** (coagulation factor III), which is needed to initiate the clotting process, is composed mainly of ***cephalin***.

- Phospholipid (lecithin) acts as ***lung surfactant,*** which prevents alveolar collapse.
- Lecithin represents a storage form of lipotropic factor **choline.**
- Phosphatidylinositol acts as a **second messenger** for the activity of certain hormones.
- In mitochondria, cardiolipin is necessary for optimum functions of the **electron transport process**.
- Plasmalogens (platelet activating factor) involved in **platelet aggregation** and **degranulation**.

## GLYCOLIPIDS (GLYCOSPHINGOLIPIDS)

- Glycolipids as their name implies, are sugar containing lipids. Glycolipids consist of alcohol **sphingosine**.
- The amino group of sphingosine is esterified by a fatty acid and one or more sugar units are attached to the hydroxyl group of sphingosine.
- Glycolipids are widely distributed in every tissue of the body, particularly in nervous tissue such as brain.

### Classification of Glycolipids

Four classes of glycolipids have been distinguished:
1. Cerebrosides
2. Sulfatides
3. Globosides
4. Gangliosides.

#### *Cerebrosides (Ceramide + Monosaccharides)*

- Cerebroside is the simplest glycolipid in which there is only one sugar residue, either ***glucose*** or ***galactose*** linked to ceramide and named as ***glucocerebroside*** and ***galactocerebroside*** respectively.
- Galactocerebroside is found in nerve tissue membrane whereas; glucocerebroside is the predominant glycolipid of extraneural (non-neural) tissues, where it acts as a precursor for the synthesis of more complex glycolipids, e.g. gangliosides.

**Cerebroside**

#### *Sulfatides (Ceramide + Monosaccharide + Sulfate)*

- Sulfatides are cerebrosides in which the xmonosaccharide contains a sulfate ester.

**Sulfatide**

#### *Globosides (Ceramide + Oligosaccharide)*

- Globosides contain two or more sugar molecules attached to ceramide.
- These glycolipids are important constituents of the RBC-membrane and are the determinants of the A, B, O blood group system.

**Globoside**

#### *Gangliosides: (Cerebroside + Oligosaccharides + N-acetylneuraminic acid, NANA)*

- Gangliosides are complex glycolipids, derived from glucocerebroside.
- Ganglioside contains oligosaccharides and one or more molecules of ***sialic acid,*** which is usually ***N-acetylneuraminic acid (NANA)*** attached to ceramide.
- Several types of gangliosides such as **$GM_1$, $GM_2$, $GM_3$,** etc. have been isolated from brain and other tissues. The simplest ganglioside found in tissues is $GM_3$. G represents Ganglioside; M represents mono which indicates presence of one residue of NANA and subscript number assigned on the basis of chromatographic migration of ganglioside.

**$GM_3$ - Ganglioside**

- $GM_1$ is a more complex ganglioside derived from $GM_3$.

## Functions of Glycolipids

- Glycolipids are important constituents of the nervous tissue, such as brain and outer leaflet of all cell membrane.
- They play a role in the regulation of cellular interactions, growth and development.
- Glycolipids serve as cell **surface receptors** for certain hormones and a number of drugs. They also serve as receptors for cholera and tetanus toxins.
- Glycolipids are antigenic and they have been identified as a source of blood group antigens.

## CHOLESTEROL (ANIMAL STEROL)

- Cholesterol is the major sterol in animal tissues. Sterols are a class of steroids containing hydroxyl group.
- It consists of steroid nucleus namely ***phenanthrene*** containing 19-carbon atoms **(Fig. 3.11)**.
- It consists of methyl side chains at position $C_{10}$ and $C_{13}$ which are shown as single bonds.
- Cholesterol, a 27-carbon compound, has an 8-carbon side chain attached to the D ring at $C_{17}$ and a hydroxyl group attached to $C_3$ of the A ring, with one double bond between carbon atoms 5 and 6 **(Fig. 3.12)**.
- Cholesterol is ***amphipathic,*** with a ***polar*** head the hydroxyl group at $C_3$ and a ***nonpolar,*** the steroid nucleus and hydrocarbon side chain at $C_{17}$.

**Fig. 3.11:** The steroid nucleus, phenanthrene (ring A, B and C), to which cyclopentane D ring is attached.

**Fig. 3.12:** The structure of cholesterol.

- Most of the cholesterol in the body exists as a cholesterol ester, with a fatty acid attached to the hydroxyl group at $C_3$.
- Cholesterol is widely distributed in all the cells of the body but particularly in nervous tissue.
- ***It occurs in animal fats but not in the plant fats.***

## Functions of Cholesterol

- It is a major structural constituent of the cell membranes and plasma lipoproteins.
- Cholesterol serves as the precursor for a variety of biologically important products, including:
  1. **Steroid hormones**: Cholesterol is the precursor of the five steroid hormones, e.g.
     i. Progesterone
     ii. Glucocorticoids
     iii. Mineralocorticoids
     iv. Androgens (male sex hormones)
     vi. Estrogen (female sex hormones).
  2. **Bile acids:** Bile acids, derived from cholesterol, act as a detergent in the intestine, emulsifying dietary fats to make them readily accessible to digestive enzyme lipase.
  3. **Vitamin D:** It is derived from cholesterol and is essential in calcium and phosphate metabolism.

## LIPOPROTEINS

- Lipoproteins are large water soluble complexes formed by a combination of lipid and protein that transport insoluble lipids through the blood between different organs and tissues.
- Lipoproteins consist of a lipid core containing nonpolar ***triacylglycerol*** and ***cholesterol ester*** surrounded by a single layer of ***amphipathic phospholipids*** and ***free***

**Fig. 3.13:** Structure of lipoprotein.
(TG: Triacylglycerol, CE: Cholesterol ester)

***cholesterol*** molecules with some ***proteins***, (apoprotein) **(Fig. 3.13)**.

- The protein components are referred to as an ***apoprotein*** or ***apolipoprotein.*** There are four major types of apolipoproteins designated by letters A, B, C and E with subgroups given in Roman numerals I, II, III, etc.

## Classes of Lipoproteins

Lipoproteins have been categorized into four major classes according to their physical and chemical properties **(Table 3.2).** These are :

1. Chylomicrons
2. Very low density lipoproteins (VLDL)
3. Low density lipoprotein (LDL)
4. High density lipoprotein (HDL).

- These lipoprotein complexes contain different proportions of lipids and proteins **(Table 3.2)**. The density of these lipoproteins is inversely proportional to triacylglycerol content. As the density increases, the diameter of the particle decreases as shown in **Figure 3.14**.

**Fig. 3.14:** Diagrammatic representation of lipoprotein with increasing densities.

- Chylomicrons containing about 1 percent protein and 99 percent triacylglycerol have the lowest density.
- While HDL containing 50 percent of protein and 50 percent of lipid have the highest density.
- Triacylglycerol is the predominant lipid in chylomicrons and VLDL. Cholesterol is the predominant lipid in LDL, whereas phospholipid is the predominant lipid in HDL.
- Percentage of three major lipid classes, i.e. triacylglycerol, cholesterol and phospholipids present in lipoproteins are shown in **Table 3.2**.

## Site of Synthesis and Functions of Lipoproteins

The site of synthesis of the four main lipoproteins and their functions are summarized in **Table 3.3**.

# EICOSANOIDS

- **Prostaglandins** and the related compounds **thromboxanes** and **leukotrienes,** are collectively known as **eicosanoids**.

**Table 3.2:** Characteristics of human plasma lipoproteins.

| *Variable* | *Chylomicron* | *VLDL* | *LDL* | *HDL* |
|---|---|---|---|---|
| Diameter (nm) | 70 to 1200 | 25 to 70 | 20 to 25 | 5 to 10 |
| Density (g/mL) | < 0.95 | 0.95 to 1.006 | 1.02 to 1.06 | 1.06 to 1.21 |
| Lipid-Protein ratio | 99:1 | 90:10 | 80:20 | 50:50 |
| Major lipids | Triacylglycerol | Triacylglycerol | Cholesterol | Phospholipids |
| Apolipoproteins | B-48 | B-100 | B-100 | A, C and E |
| Lipid components (%) | | | | |
| Triacylglycerol | 85 | 50 | 10 | 5 |
| Cholesterol (free and ester) | 5 | 20 | 50 | 15 |
| Phospholipid | 9 | 18 | 20 | 25 |
| Protein composition (%) | 1 | 10 | 20 | 55 |

**Table 3.3:** The four main lipoproteins and their site of synthesis and function.

| *Lipoprotein* | *Site of synthesis* | *Function* |
|---|---|---|
| Chylomicrons | Intestine | Transport of dietary lipids from intestine to peripheral tissues |
| VLDL | Liver | Transport of triacylglycerol from liver to peripheral tissues |
| LDL | Plasma VLDL | Transport of cholesterol from liver to peripheral tissues |
| HDL | Liver and intestine | Transport of free cholesterol from peripheral tissues to the liver (Reverse cholesterol transport) |

- Eicosanoids are synthesized from **arachidonic acid.** A polyunsaturated fatty acid containing 20-carbon atoms from which they take their general name (Greek: eikosi means twenty).

## Prostaglandins

- Prostaglandins are a group of 20-carbon compounds derived from **arachidonic acid (Fig. 3.15)**.
- They derive their name from the tissue in which they were first recognized (the prostate gland) but they are now known to be present in almost all tissues.
- Chemically, the prostaglandins are derivatives of the hypothetical parent compound **prostanoic acid**, having cyclopentane (5 carbon) ring and two aliphatic side chains $R_1$ and $R_2$ **(Fig. 3.15)**.
- Prostanoic acid does not occur naturally but is regarded as the parent compound of the prostaglandins and thromboxanes for the purpose of classification and carbon numbering.
- In addition to cyclopentane ring, each of the biologically active prostaglandin has a hydroxyl group at carbon 15, a double bond between carbons 13 and 14, and various substituents on the ring.

### *Classification of Prostaglandins*

- By convention, prostaglandins are abbreviated as PG.
- They are classified into groups designated by a capital letter (A, B, C, D, E, F, G, H and I) depending on the substituents on the cyclopentane ring.
- These are subclassified by a subscript number 1, 2, or 3 corresponding to the number of double bonds in the side chains but not in the cyclopentane ring.
- Sixteen naturally occurring prostaglandins have been described but only seven are found commonly throughout the body. These are $PGE_1$, $PGE_2$, $PGF_{1\alpha}$, $PGF_{2\alpha}$, $PGG_2$, $PGH_2$, $PGI_2$.
- Prostaglandins are not stored; instead the precursor $C_{20}$ arachidonic acids are stored in tissues.

**Fig. 3.15:** The structure of arachidonic acid, prostanoic acid, common prostaglandin ($PGE_2$), thromboxane ($TXA_2$) and leukotrienes ($LTA_4$).

### *Functions of Prostaglandins*

- Prostaglandins and other eicosanoids have hormone like actions.
- Prostaglandins in many tissues act by regulating the synthesis of cyclic AMP (cAMP). As cAMP mediates the action of many hormones, the prostaglandins affect a wide range of cellular and tissue functions. Some of these are:
  - **Smooth muscle contraction and relaxation:** For example, in pregnancy $PGF_{2\alpha}$ are produced in response to oxytocin and act to promote uterine contraction. Because of this effect, they have been used to terminate unwanted pregnancies. $PGE_2$ are involved in relaxation of bronchial smooth muscle.

- **Inflammatory response:** PGs are involved in inflammatory response causing pain, edema, swelling and prolonged erythema (abnormal flushing of skin) by increasing capillary permeability.
- **Platelet aggregation:** Prostaglandins have an effect on platelet aggregation. $PGE_2$ promote aggregation and are thus, involved in the blood clotting.
- **Regulation of Blood pressure:** $PGE_2$ decrease blood pressure. It can lower systemic arterial pressure through their vasodilator effect.
- **Body temperature:** Prostaglandins elevate body temperature producing fever and cause inflammation, resulting in pain.
- **Gastric secretion:** $PGE_2$ suppress gastric secretion.
- PGs are involved in **$Na^+$** and ***water retention*** by kidney tubules.

## Thromboxanes

Thromboxanes were first isolated from blood platelets, thrombocytes—hence the name. They have six membered oxane ring **(Fig. 3.15)** that includes an oxygen atom.

### *Nomenclature of Thromboxanes*

- Thromboxanes are abbreviated as TX. Different capital letters are used to designate different substituents of the ring (like prostaglandins).
- A subscript, if present, denotes the number of unsaturated bonds (double bonds), e.g. the most common thromboxane $TXA_2$ having two double bonds.

### *Functions of Thromboxanes*

- $TXA_2$ is produced by platelets, promotes platelets aggregation. Platelet aggregation initiates thrombus formation at sites of vascular injury.
- $TXA_2$ causes contractions of the smooth muscles of the arterial wall and therefore, raises blood pressure.

## Leukotrienes (LT)

Leukotrienes were so named because they were initially described in leucocytes and are characterized by a conjugated **triene** system but no such ring structure that is found in prostaglandins and thromboxanes.

### *Nomenclature of Leukotrienes*

- All leukotrienes are abbreviated as LT.
- These are grouped into five classes (A to E) based on the type of substituents attached to the parent compound.
- The LTs found in humans have a subscript four to denote that they contain four double bonds **(Fig. 3.15)**.

### *Functions of Leukotrienes*

- The LTs facilitate chemotaxis, inflammation and allergic reactions.
- $LTC_4$, $LTD_4$ induce contraction of muscle of the lung and constrict pulmonary airways. Overproduction of LT causes asthmatic attacks.
- $LTD_4$ has been identified as the ***slow reacting substance of anaphylaxis (SRS-A)*** which causes smooth muscle contraction.
- $LTB_4$ attracts neutrophils and eosinophils to sites of inflammation.

# MICELLES, LIPID BILAYER AND LIPOSOMES

## Micelles

The polar lipid like phospholipid is amphipathic in nature. It has a hydrophilic or polar head (phosphate group attached to choline, ethanolamine, inositol, etc.) and a long hydrophobic tail containing two fatty acid chains **(Fig. 3.16)**.

**Fig. 3.16:** Formation of micelle, a phospholipid bilayer and liposome.

In aqueous systems the polar phospholipid spontaneously disperse to form micelles, in which the hydrocarbon tails of the phospholipid are hidden from the aqueous environment and electrically charged hydrophilic heads are exposed on the surface facing the aqueous medium **(Fig. 3.16)**.

### Lipid Bilayer

Phospholipids also readily and spontaneously form a very thin bilayer separating two aqueous compartments. In these structures, the hydrocarbon tails of the phospholipid molecules extend inward from the two surfaces to form a continuous inner hydrocarbon core and the hydrophilic heads face outwards extending into the aqueous phase **(Fig. 3.16).**

### Liposome (Artificially Formed Phospholipid Vesicles)

- Liposomes are artificially formed aqueous vesicles enclosed by a lipid bilayer.
- In the laboratory, liposomes (lipid bilayer) are formed by suspending phospholipid in an aqueous medium and then sonicating, i.e. agitating by high frequency sound waves to give dispersed closed vesicles. Vesicles formed by these methods are nearly spherical in shape **(Fig. 3.16)** and have a diameter of about 5 nm.
- Liposomes can be used to study membrane permeability or to deliver drugs to cells.

## DETERGENTS

- Detergents are amphipathic molecules and are not natural membrane constituents. In aqueous solutions, they form micelles, with the hydrophilic regions on the outside, interacting with the water, and the hydrophobic regions inside.
- The bile acids and bile salts are powerful naturally occurring detergents which emulsify fats in the digestive tract; they can also be used to solubilize membrane proteins.
- Lysophospholipids also have detergent like action.

## EXAM QUESTIONS

### Long Answer Questions (LAQs)

1. Define lipids, classify lipids with suitable examples and mention their functions.
2. Give definition, classification and functions of phospholipids.
3. Define fatty acids, classify fatty acids with suitable examples and mention their functions.
4. Define glycolipids, classify glycolipids and mention their functions.
5. Define lipoproteins, classify lipoproteins and mention their functions.

### Short Notes

1. Cholesterol
2. Prostaglandins
3. Essential fatty acids
4. Functions of phospholipids
5. Lecithin
6. Lipoproteins
7. Glycolipids
8. Eicosanoids
9. Triacylglycerol.
10. Test for purity of fats

### Multiple Choice Questions (MCQs)

1. **The precursor for vitamin D is:**
   a. Cholesterol b. Arachidonic acid
   c. Triacylglycerol d. Phospholipids
2. **Which of the following lipids is deficient in infants with respiratory distress syndrome?**
   a. Sphingomyelins
   b. Cardiolipins
   c. Leukotrienes
   d. Dipalmitoyl phosphatidylcholine
3. **Which of the following carbohydrates distinguishes a ganglioside from a globoside?**
   a. Glucose
   b. N-acetylneuraminic acid
   c. N-acetylgalactosamine
   d. Galactose
4. **Methylated form of phosphatidyl ethanolamine is known as:**
   a. Phosphatidylinositol
   b. Phosphatidylserine
   c. Phosphatidylcholine
   d. Lysophosphatidylcholine

**5. Which ring of the cholesterol molecule contains a double bond?**
a. A-ring b. B-ring
c. C- ring d. D-ring

**6. All of the following statements are true for phosphoglycerides, *except*:**
a. They are major storage of metabolic energy
b. They are found in cell membrane
c. They are amphipathic
d. They are derived from glycerol

**7. All of the following are sphingolipids, *except*:**
a. Sphingomyelin
b. Cerebroside
c. Phosphatidylinositol
d. Ganglioside

**8. The lipoprotein particles that have the highest concentration of triacylglycerol are:**
a. VLDL b. HDL
c. LDL d. Chylomicrons

**9. All of the following statements about prostaglandins are true, *except*:**
a. They are derived from arachidonic acids
b. They are potent biologic effectors
c. They were first observed to cause uterine contraction
d. They are synthesized only in prostate gland

**10. Glycerol is the backbone of:**
a. Glycerophospholipid b. Sphingophospholipid
c. Glycolipids d. Cholesterol esters

**11. All of the following statements about lipids are true, *except*:**
a. They are esters of fatty acids
b. They have poor solubility in water
c. They are a source of energy
d. They are polyhydroxy aldehydes

**12. Which of the following statements about cholesterol is true?**
a. It is saturated
b. It contains 27-carbon atom
c. It is a major sterol of plants
d. It contains four hexane rings fused together

**13. Which of the following phospholipids has an antigenic activity?**
a. Lecithin b. Cardiolipin
c. Sphingomyelin d. Cephalin

**14. Which of the following glycolipids is known to be the receptor in human intestine for cholera toxin?**
a. GM1 b. GM3
c. Globoside d. Cerebroside

**15. Which of the following is the major storage and transport form of fatty acids?**
a. Cholesterol b. Triacylglycerol
c. Albumin d. Phospholipid

**16. Triglycrides are:**
a. Esters of higher fatty acids with acetyl alcohol
b. Esters of fatty with cholesterol
c. Esters of stearic acid with retinol
d. Esters of fatty acids with glycerol

**17. Which of the following is a phospholipid?**
a. Cerebroside b. Lecithin
c. Gangliosides d. Kerasin

**18. Which of the following is a saturated fatty acids?**
a. Palmitic acid b. Oleic acid
c. Linoleic acid d. Linolenic acid

**19. Arachidonic acid is formed from:**
a. Stearic acid b. Palmitic acid
c. Linoleic acid d. Oleic acid

**20. Skin lesion and hyper keratosis are due to deficiency of:**
a. Essential fatty acids
b. Saturated fatty acids
c. Branched chain fatty acids
d. Cholesterol.

**21. Cyclopentanoperhydrophenanthrene nucleus is found in all of the following, *except*:**
a. Steroids b. Ergosterol
c. Waxes d. Cholesterol.

**22. Which of the following comprise an important part of lung surfactant?**
a. Lecithin b. Cephalins
c. Cholesterol d. Plasmalogens

**23. Niemann-Pick is an inherited disorder due to deficiency of:**
a. Lecihtinase b. Hexosaminidase A
c. Sphingomyelinase d. Phospholipase B

**24. Cerebrosides and Gangliosides have the following similarities, *except*:**
a. Both are compound lipids
b. Both contain sphingol
c. Both are found in brain and nervous tissues
d. Both are degraded by the enzyme glucocerebrosidase

**25. Thromboxanes:**
a. Inhibit platelet aggregation
b. Produces vasodilation
c. Enhances platelet aggregation
d. Relaxes smooth muscles.

**26. In addition to alcohol and fatty acids, some of the lipids may contain:**
a. Phosporic acid b. Nitrogen base
c. Carbohydrates d. All of the above

**27. According to Bloor, lipids are compound having the following characteristics:**
a. They are insoluble in water
b. Solubility in one or more organic solvents
c. Some relationship to the fatty acids as esters
d. All of the above

**28. Which are the examples of phospolipidses, *except*:**
a. Lecithin b. Cephalin
c. Sphingosine d. Sphingomyelins

**29. Glycolipids contain a special alcohol called:**
a. Sphingosine b. Cholesterol
c. Cephalin d. All of the above

**30. Chaulmoogra acid is an example of:**
a. Substituted fatty acids
b. Cyclic fatty acids
c. Branched chain FA
d. Unbranched fatty acids

**31. From which EFA, prostaglandins and leukotrienes are synthesized in the body:**
a. Linoleic acid b. Linolenic acid
c. Arachidonic acids d. All of the above

**32. Docosahexaenoic acid is formed from dietary:**
a. Linoleic acid b. Linolenic acid
c. Arachidonic acid d. Palmitic acid

**33. Which are the examples of glycerophosphatides?**
a. Cephalin b. Lecithin
c. Phosphatidyl serine d. All of the above

**34. Which of the following does not contain 'Glycerol'?**
a. Cephalin b. Lecithin
c. Plasmalogen d. Sphingomyelins

**35. The simplest and common ganglioside found in tissue is:**
a. GM-1 b. GM-2
c. GM-3 d. GD-3

**36. Ganglioside type GM-3 contains:**
a. Ceramide b. Glucose
c. Neuraminic acid d. All of the above.

**37. All naturally occurring PGs are 20–C fatty acids containing:**
a. Cyclopentane ring
b. Cyclopentane hydroxy ring
c. Cyclopentanoperhydrophenanthrene ring
d. None of the above

**38. Eicosanoids include the:**
a. Prostaglandins b. Leukotrienes
c. Thromboxane d. All of the above

**39. The lipid that mainly functions are fuel reserve in animals is:**
a. Triglycerides b. Cholesterol
c. Phospholipid d. Lipoprotein

**40. The phospholipid that produces second messenger in hormonal action is:**
a. Phosphatidyl lecithin
b. Plasmalogen
c. Phosphatidyl inositol
d. Phosphatidyl ethanolamine

## Answers for MCQs

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **1.** a | **2.** d | **3.** b | **4.** c | **5.** b | **6.** a | **7.** c | **8.** d |
| **9.** d | **10.** a | **11.** d | **12.** b | **13.** b | **14.** a | **15.** b | **16.** d |
| **17.** b | **18.** a | **19.** c | **20.** a | **21.** c | **22.** a | **23.** c | **24.** d |
| **25.** c | **26.** d | **27.** d | **28.** c | **29.** a | **30.** b | **31.** c | **32.** b |
| **33.** d | **34.** d | **35.** c | **36.** d | **37.** a | **38.** d | **39.** a | **40.** c |

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CHAPTER

# 4

# Chemistry of Proteins

**Chapter outline**


- General Nature of Amino Acids
- Classification of Amino Acids
- Modified or Nonstandard Amino Acids
- Properties of Amino Acids
- Biologically Important Peptides
- Definition, Classification and Functions of Proteins
- Structure of Proteins
- Properties of Proteins
- Denaturation of Proteins


## INTRODUCTION

Proteins are the most abundant macromolecules in living cells. The term 'protein' was first used by Berzelius in 1838 and was derived from the Greek word **"protos"** which means **primary** or **holding first place.** As the name indicates, protein is the most important of cell constituents. They are responsible for almost every function that occurs in the body. Protein is the important source of nitrogen for the body.

Proteins are linear chains of amino acids that are linked together by covalent, ***peptide bonds.*** Each protein has specific and unique sequence of amino acids that defines both its three-dimensional structure and its biologic function.

## GENERAL NATURE OF AMINO ACIDS

- There are approximately 300 amino acids present in various animal, plant and microbial systems, but only ***20 amino acids are involved in the formation of proteins.***
- All the 20 amino acids found in proteins **(Table 4.1)** have a carboxyl group (-COOH) and an amino acid group (-$NH_2$) bound to the same carbon atom called the α-***carbon*** **(Fig. 4.1).**
- Amino acids differ from each other in their **side chains** or **R-groups,** attached to the α-carbon.
- The 20 amino acids of proteins are often referred to as the ***standard*** or ***primary*** or ***normal amino acids***.
- The standard amino acids have been assigned three letters abbreviations and one letter symbol, e.g. amino acid **glycine** has abbreviated name ***Gly*** and symbol letter ***G***.
- All the amino acids found in proteins are exclusively of the ***L-configuration.***

**Fig. 4.1:** General structure of α-amino acid found in protein.

## CLASSIFICATION OF AMINO ACIDS

There are five ways of classifying amino acids depending on the:

1. Chemical nature of the amino acid in the solution
2. Structure of the side chain of the amino acids
3. Nutritional requirement of amino acids
4. Metabolic product of amino acids
5. Nature or polarity of the side chain of the amino acids.

### Classification Based on Chemical Nature of the Amino Acid in Solution

According to this type of classification, amino acids are classified as follows:

i. Neutral amino acids
ii. Acidic amino acids
iii. Basic amino acids.

**Table 4.1:** The 20, L-α-amino acids (standard amino acids) found in proteins.

| *Name* | *Symbol* | *Structural formula* |
|---|---|---|
| **Aliphatic side chain** | | |
| Glycine | Gly (G) | $H—CH(NH_3^+)—COO^-$ |
| Alanine | Ala (A) | $CH_3—CH(NH_3^+)—COO^-$ |
| Valine | Val (V) | $(CH_3)_2CH—CH(NH_3^+)—COO^-$ |
| Leucine | Leu (L) | $(H_3C)_2CH—CH_2—CH(NH_3^+)—COO^-$ |
| Isoleucine | Ile (I) | $CH_3—CH_2—CH(CH_3)—CH(NH_3^+)—COO^-$ |
| **Hydroxylic (OH) group containing side chains** | | |
| Serine | Ser (S) | $CH_2(OH)—CH(NH_3^+)—COO^-$ |
| Threonine | Thr (T) | $CH_3—CH(OH)—CH(NH_3^+)—COO^-$ |
| Tyrosine | Tyr (Y) | See aromatic group containing side chain amino acids |
| **Sulfur containing side chains** | | |
| Cysteine | Cys (C) | $CH_2(SH)—CH(NH_3^+)—COO^-$ |
| Methionine | Met (M) | $CH_2—CH_2—S—CH_3—CH(NH_3^+)—COO^-$ |
| **Side chains containing acidic groups (–COOH) and their amides** | | |
| Aspartic acid | Asp (D) | $COO^-—CH_2—CH(NH_3^+)—COO^-$ |
| Aspargine | Asn (N) | $H_2N—C(=O)—CH_2—CH(NH_3^+)—COO^-$ |
| Glutamic acid | Glu (E) | $^-OOC—CH_2—CH_2—CH(NH_3^+)—COO^-$ |

*Contd...*

*Contd...*

| *Name* | *Symbol* | *Structural formula* |
|---|---|---|
| Glutamine | Gln (Q) | |
| **Basic groups containing side chains** | | |
| Arginine | Arg (R) | |
| Lysine | Lys (K) | |
| Histidine | His (H) | |
| **Aromatic group containing side chains** | | |
| Histidine | His (H) | See above |
| Phenylalanine | Phe (F) | |
| Tyrosine | Tyr (Y) | |
| Tryptophan | Trp (W) | |
| **Imino acids** | | |
| Proline | Pro (P) | |

## Neutral Amino Acids

The amino acids which are neutral in solution are monoamino-monocarboxylic acids (i.e. having one amino group and one carboxylic group), e.g.

| | | |
|---|---|---|
| Glycine | Serine | Phenylalanine |
| Alanine | Threonine | Tyrosine |
| Valine | Cysteine | Tryptophan |
| Leucine | Methionine | Aspargine |
| Isoleucine | Proline | Glutamine |

## Acidic Amino Acid

These are acidic in solution and are monoamino dicarboxylic acids, e.g.

- Aspartic acid
- Glutamic acid.

## Basic Amino Acid

These are basic in solution and are diamino-monocarboxylic acids, e.g.

- Lysine
- Arginine
- Histidine.

## Classification Based on Chemical Structure of Side Chain of the Amino Acid

According to this type of classification, amino acids are classified as:

1. Aliphatic amino acids
2. Hydroxy amino acids
3. Sulfur containing amino acids
4. Dicarboxylic acid and their amides
5. Diamino acids
6. Aromatic amino acids
7. Imino acids or heterocyclic amino acids.

### *Aliphatic Amino Acids*

Amino acids having aliphatic side chain, e.g.

- Glycine
- Alanine
- Valine
- Leucine
- Isoleucine.

### *Hydroxy Amino Acids*

Amino acids having hydroxy group in the side chain, e.g.

- Threonine
- Serine
- Tyrosine.

### *Sulfur Containing Amino Acids*

Amino acids having sulfur in the side chain, e.g.

- Cysteine
- Methionine.

### *Dicarboxylic Acid and their Amides*

Amino acids having carboxylic group in their side chain, e.g.

- Glutamic acid
- Glutamine (amide of glutamic acid)
- Aspartic acid
- Aspargine (amide of aspartic acid).

### *Diamino Acids*

Amino acids having amino group ($-NH_2$) in the side chain, e.g.

- Lysine
- Arginine
- Histidine.

### *Aromatic Amino Acids*

Amino acids containing aromatic ring in the side chain, e.g.

- Phenylalanine
- Tyrosine
- Tryptophan.

### *Imino Acids or Heterocyclic Amino Acids*

- One of the 20 amino acids, ***proline*** is an ***imino (-NH) acid*** not an amino ($-NH_2$) acid as are other 19. The side chains of proline and its α-amino group form a ring structure and thus proline differs from other amino acids, in that it contains an imino group, rather than an amino group.

**Structure of proline**

## Nutritional Classification of Amino Acids

On the basis of nutritional requirement, amino acids are classified into two groups:

i. Essential or indispensable amino acids
ii. Nonessential or dispensable amino acids.

### *Essential Amino Acids (Also Refer Chapter 11)*

Essential amino acids cannot be synthesized by the body and must, therefore, be essentially supplied through the diet. Ten amino acids, essential for humans include:

- Phenylalanine
- Valine
- Threonine
- Tryptophan
- Isoleucine
- Methionine
- Histidine
- Arginine
- Lysine
- Leucine.

- The mnemonics often used by students are **PVT. TIM. HALL** or **L.VITTHAL (MP)**.
- Among the ten essential amino acids; **arginine** and **histidine** are known as **semi-essential** amino acids since these amino acids are synthesized partially in human body and inadequate to support growth of children. Arginine and histidine become essential in diet during periods of rapid growth as in childhood and pregnancy.
- A deficiency of an essential amino acid impairs protein synthesis and leads to a negative nitrogen balance (nitrogen excretion exceeds nitrogen intake).

### *Nonessential Amino Acids*

Nonessential amino acids can be synthesized in human body and are not required in diet, e.g.

- Glycine
- Proline
- Serine
- Glutamic acid
- Glutamine
- Alanine
- Tyrosine
- Cysteine
- Aspartic acid
- Aspargine

## Metabolic Classification of Amino Acids

On the basis of their catabolic end products, the twenty standard amino acids are divided in three groups **(Table 4.2).**

i. **Glucogenic amino acids:** Those which can be converted into glucose. Fourteen out of the twenty standard amino acids are glucogenic amino acids **(Table 4.2).**

ii. **Ketogenic amino acids:** Those which can be converted to ketone bodies. Two amino acids **leucine** and **lysine** are exclusively ketogenic.

iii. **Both glucogenic and ketogenic:** Those which can be converted to both glucose and ketone bodies. Four amino acids **isoleucine, phenylalanine, tryptophan** and **tyrosine** are glucogenic and ketogenic.

## Classification Based on Nature or Polarity of Side Chain of Amino Acid

According to this type of classification, amino acids are classified into two major classes **(Fig. 4.2):**

i. Hydrophilic or polar amino acids

ii. Hydrophobic or nonpolar amino acids.

## Importance of Amino Acids

- **Formation of proteins:** Amino acids are joined to each other by peptide bonds to form proteins and peptides.
- **Formation of glucose:** Glucogenic amino acids are converted to glucose in the body.
- **Enzyme activity:** The thiol (-SH) group of cysteine has an important role in certain enzyme activity.
- **Transport and storage form of ammonia:** Amino acid glutamine plays an important role in transport and storage of amino nitrogen in the form of ammonia.
- **As a buffer**: Both free amino acids and some amino acids present in protein can potentially act as buffer, e.g. histidine can serve as the best buffer at physiological pH.
- **Detoxification reactions:** Glycine, cysteine and methionine are involved in the detoxification of toxic substances.
- **Formation of biologically important compounds:** Specific amino acids can give rise to specific biologically important compounds in the body **(Table 4.3)**.

**Table 4.2:** Metabolic classification of amino acids

| *Glucogenic* | *Ketogenic* | *Both ketogenic and glucogenic* |
|---|---|---|
| Glycine, alanine, serine, cysteine, aspartic acid, aspargine, glutamic acid, glutamine, proline, histidine, arginine, methionine, threonine, valine | Leucine<br>Lysine | Isoleucine<br>Phenylalanine<br>Tyrosine<br>Tryptophan |

**21st and 22nd Amino acid**

There are actually 22 rather than 20 amino acids specified by the known genetic code. The two extra ones are **selenocysteine** and **pyrrolysine** each found in very few proteins.

**21st Amino acid**

***Selenocysteine***

- Selenocystein is the 21st amino acid. It contains **selenium rather than the sulfur** of the cysteine.
- It is encoded by **UGA codon**, which is normally a stop codon.
- Selenocystein is a constituent of several human proteins and enzymes. It is located in the active site of enzymes that participate in oxidation reduction reactions. These include; **glutathione peroxidase**, **thioredoxin reductase** and **iodothyronine deiodinase**.

**22nd Amino acid**

- Pyrrolysine a 22nd amino acid was discovered in 2002 at the active site of methyl-transferase enzyme from methane-producing bacteria. This amino acid is encoded by UAG (normally a stop codon).
- It is similar to lysine, but with an added pyrroline ring linked to the end of the lysine side chain.

# MODIFIED OR NONSTANDARD AMINO ACIDS

In addition to the standard amino acids, a small number of modified amino acids are found in proteins. These amino acids are formed by the modification of one of the 20 standard amino acids, after incorporation into the protein. The following modified amino acids are of particular significance.

**Fig. 4.2:** Classification of amino acids based on polarity.

**Fig. 4.3:** Formation of disulfide bond of cysteine.

## Cystine

Cystine is formed by linkage of two cysteine side chains through a disulfide bond **(Fig. 4.3)**. This modified amino acid is found in many proteins, where it provides stability to the three-dimensional structure.

## Hydroxyproline and Hydroxylysine

The hydroxylation of selected lysine and proline residues is found primarily in the formation of collagen of connective tissue.

## Desmosine and Isodesmosine

These two amino acids are formed by the oxidation and crosslinking of four lysine side chains. These are found in connective tissue protein, *elastin.*

## Gamma Carboxyglutamate

Carboxylation of glutamic acid side chains occurs in many of the clotting proteins, e.g. in prothrombin.

**Table 4.3:** Biologically important compounds formed by amino acids

| *Amino acid* | *Biologically important compound* |
|---|---|
| Tyrosine | Hormone, e.g. adrenaline and thyroxine, skin pigment, e.g. melanin |
| Tryptophan | Vitamin, e.g. niacin |
| Glycine, arginine and methionine | Creatine |
| Glycine and cysteine | Bile salts |
| Glycine | Heme |
| Aspartic acid and glutamic acid | Pyrimidine bases |
| Glycine, aspartic acid and glutamine | Purine bases |
| β-alanine | Coenzyme-A |

# PROPERTIES OF AMINO ACIDS

## Physical Properties

They are colorless, crystalline substances generally soluble in water.

## Stereoisomerism

- For all the slandered or common amino acids except **glycine,** α- carbon is bonded to four different groups: a carboxyl group, an amino group, an R group, and a hydrogen atom. The- carbon is thus a chiral center.
- Because of the tetrahedral arrangement around the α-carbon atom the four different groups can occupy two unique spatial arrangements, and thus amino acids have

**Fig. 4.4:** D and L forms of amino acids

two possible stereoisomers, **L-isomer** and **D- isomer (Fig. 4.4).** *Only L- amino acids are constituent of proteins*

- These stereoisomers are mirror images of each other **(Fig. 4.4)** and called enantiomers.

## Optical Activity

All amino acids with chiral center are also **optically active,** that is they rotate plane polarized light.

**Glycine** is **optically inactive**: In glycine the R group is another hydrogen atom and does not have chiral carbon.

### *Ionization of Amino Acids*

- All amino acids have at least one **carboxyl** and one **amino** group. Both of these groups are **ionisable**.
- Due to ionizing property of amino acids, amino acids exert:
  - Acid base behavior
  - Amphoteric properties
  - Zwitterion formation
  - Buffering activity.

#### *Acid base behavior*

- The **carboxyl** and **amino** groups of amino acids, along with the **ionizable R** groups of some amino acids, function as weak bases and acids.
- The **carboxyl** (-COOH) group of an amino acid can donate proton ($H^+$) and behave as an acid (proton donor), forming a negatively charged **anion**.
- **Amino** group ($-NH_2$) of an amino acid can accept the proton ($H^+$) which behave as a base (proton acceptor), forming positively charged **cation (Fig. 4.5)**.

#### *Amphoteric properties*

- As amino acids are capable of acting as both an **acid** (i.e. proton donor) and a **base** (i.e. proton acceptor) these are regarded as ampholytes. Substances having a dual nature are called **amphoteric** (Greek word

**Fig. 4.5:** Ionization of amino acid.

**ampho** means **both**) and are often called **ampholytes** (amphoteric electrolytes).

- Amino acids behavior as acid or base and ionization state varies with pH.
  - At acidic pH, amino acid is **positively charged** because at acidic pH the amino group is ionized, protonated ($-NH_3^+$) and the carboxyl group is not ionized (COOH).
  - As pH is raised i.e. at alkaline pH amino acid is **negatively charged** as the carboxylic group is ionized to give up a proton ($COO^-$) and amino group is remained in unionized ($NH_2$) form**.**
- Between these pH ranges, there is a certain pH at which both carboxyl and amino groups are ionised and that pH is called **isoelectric pH** or **isoelectric point (PI).**
- **A**t isoelectric pH though both carboxyl and amino groups are ionized, the molecule as a whole is electrically neutral i. e. amino acid bears no net charge (zwitterion) and therefore does not move in an electric field.
- **Isoelectric pH or isoelectric point (PI)** is constant for every amino acid. The solubility of the amino acid is least at its isoelectric pH.
- Non-ionic form does not occur in significant amounts in aqueous solutions.

**Fig. 4.6:** Nonionic and zwitterion forms of amino acids. Zwitterion can act either as an acid (proton donor) or base (proton acceptor).

**Fig. 4.7:** Ionic forms of amino acid in acidic, basic and isoelectric pH.

*Zwitterion formation*

- When an amino acid lacking ionizable R group, is dissolved in water at neutral pH, it exists in solution as the **dipolar ion**, or **zwitterion** (hybrid ion) which means that they have both positive and negative charges on the same amino acids and the overall molecule is **electrically neutral**
  - The α-***COOH*** group is ionized and becomes negatively charged anion ($COO^-$) and
  - The α-$NH_2$ group is protonated to form a positively charged cation ($NH_3^+$).
- The molecular species which contain an equal number of ionizable groups of opposite charge and as a result bear no net charge are called ***zwitterions***. A zwitterion can act as either an acid (proton donor) or a base (proton acceptor) **(Fig. 4.6)**.
- When the pH of the surrounding medium is lowered below the pI value **(pH < pI)** of an amino acid its carboxyl group accepts a proton to form protonated (COOH) form. This form of amino acid carries net **positive charge**. Conversely When the pH of the surrounding medium is above the pI value **(pH > pI)** of an amino acid its NH3+ group loses its proton and becomes uncharged. This form of amino acid carries net **negative charge (Fig. 4.7)**.

## Buffering Activity

- Due to the acid base behavior of amino acids, both free amino acids and some amino acids present in proteins can potentially act as buffers.

> - ***Among the 20 standard amino acids, histidine serves as the best buffer at physiological pH.*** Because the side chain of histidine has a pKa of 6.0 and can serve as the best buffer at physiological pH. All other amino acids have pKa value, too far away from pH 7 to be an effective physiological buffer. ***Maximum buffer capacity occurs at pH equal to the pKa.***

**Fig. 4.8:** Formation of peptide bond.

## BIOLOGICALLY IMPORTANT PEPTIDES

- Peptides are chains (polymer) of amino acids. Two amino acid molecules can be covalently joined through a **peptide bond,** to yield **dipeptide.** Peptide linkage is formed by the removal of a molecule of water from the α-carboxyl group of one amino acid and the α-amino group of another **(Fig. 4.8).**
- When many amino acids are joined, the product is called **polypeptide.** Proteins are polypeptides with thousands of amino acids. Polypeptides generally have molecular weights below 10,000, and those called proteins have high molecular weights.
- There are many naturally occurring small polypeptides some of which have important biological activities and are called **biologically important peptides.** A few of them are given below.

## Glutathione (GSH)

- GSH is a tripeptide (γ glutamyl – cysteinyl – glycine) containing **glutamate**, **cysteine** and **glycine**.
- Glutathione is found in all mammalian cells **except** the **neurons.**

**Fig. 4.9:** Reduced and oxidized glutathione.

- Glutathione may exist as the reduced (GSH) or oxidized (G-S-S-G) form **(Fig. 4.9)** and can thus play a role in some oxidation–reduction reactions.
- In oxidized form, two molecules of glutathione are linked by disulfide bond.
- The sulfhydryl (-SH-) is the functional group primarily responsible for the properties of glutathione.

### *Functions of Glutathione*

- The reduced form of glutathione with a free sulfhydryl (-SH-) group serves as a **redox buffer** regulating the redox state of the cell.
- It helps in keeping the enzymes in an active state by preventing the oxidation of sulfhydryl (-SH-) group of enzyme to disulfide (-S-S-) group.
- Reduced glutathione is essential for maintaining the normal structure of red blood cells and for keeping **hemoglobin in the ferrous state**. Cells with lowered level of reduced glutathione are more susceptible to hemolysis.
- Glutathione plays a key role in **detoxification** by reducing $H_2O_2$ the harmful byproduct of metabolism.

$$2GSH + H_2O_2 \xrightarrow[\text{Peroxidase}]{\text{Glutathione}} G\text{-}S\text{-}S\text{-}G + 2H_2O$$

- Glutathione is involved in **transport of amino acids** across the cell membrane of the kidney and intestine.

## Thyrotropin Releasing Hormone (TRH)

TRH is a hypothalamic hormone of three amino acid residues. It stimulates the release of hormone thyrotropin, from the anterior pituitary gland.

## Oxytocin

This is a 9-amino acid residue hormone secreted by posterior pituitary and stimulates uterine contractions.

## Vasopressin

This is a 9-amino acid residue hormone secreted by posterior pituitary and it increases blood pressure and has an antidiuretic action.

## Gastrin

This is a local hormone produced by stomach. It stimulates the production of gastric juice.

## Angiotensin

Angiotensin II is a vasoconstrictor and elevates the arterial pressure and also promotes the synthesis of a steroid hormone called aldosterone that promotes sodium retention.

## Bradykinin

It contains, 9-amino acid residue. It is a powerful vasodilator and causes contraction of smooth muscle and is mainly responsible for causing intense peripheral and visceral pain by stimulating the pain receptors.

## Insulin

A pancreatic hormone contains two polypeptide chains: one having 30-amino acid residues and the other 21. Insulin regulates the glucose concentration in blood.

## Glucagon

This is a pancreatic hormone of 29-residues that opposes the action of insulin.

# DEFINITION, CLASSIFICATION AND FUNCTIONS OF PROTEINS

## Definition

Proteins are linear chains of amino acids that are linked together by covalent, **peptide bonds.** Each protein has specific and unique sequence of amino acids that defines both its three-dimensional structure and its biologic function.

## Classification of Proteins

Proteins have been classified in several ways. They are most conveniently classified on the basis of their:

- Function
- Molecular shape
- Composition

## Classification of Proteins Based on Functions

In a functional classification, they are grouped according to their biological role. Some functions that proteins serve and examples of specific functional proteins are as follows:

### *Catalytic Proteins or Enzymes*

These proteins act as enzymes, e.g.

- Glucokinase
- Dehydrogenases
- Transaminases
- Hydrolytic enzymes, pepsin, trypsin, etc.

### *Transport Proteins*

These proteins are involved in the process of transportation, e.g.

- Hemoglobin transports oxygen
- Transferrin transports iron
- Albumin carries fatty acids and bilirubin

### *Storage Proteins*

Many proteins serve as storage form, e.g.

- Apoferritin stores iron in the form of ferritin
- Myoglobin stores oxygen in muscles

### *Contractile Proteins*

Some proteins have the ability to contract and function in the contractile system of skeletal muscle, e.g.

- Actin
- Myosin

### *Structural Proteins*

Many proteins serve as supporting framework of cells to give biological structure, strength or protection, e.g.

- Collagen in bone
- Cartilage, elastin of ligaments
- Keratin of hair, nail

**Fig. 4.10:** Diagrammatic representation of fibrous and globular proteins $l$ = length, $b$ = breadth.

### *Defence Proteins*

Many proteins involved in defence mechanism against invasion of foreign substances such as viruses, bacteria and cells. Examples of defence proteins are:

- Immunoglobulins or antibodies
- Fibrinogen and thrombin are blood clotting proteins that prevent loss of blood when the vascular system is injured.

### *Regulatory Proteins*

Some proteins regulate cellular or physiological activity, e.g.

- Many hormones, e.g. **insulin,** regulate sugar metabolism; **growth hormone** of pituitary gland regulates growth of the cells.

## Classification Based on Molecular Shape of the Proteins

In this type of classification proteins are classified as follows:

### *Fibrous Proteins*

Fibrous proteins are also called **scleroproteins.** They are insoluble, high molecular weight fibers. The fibers are long and thin. These have axial ratio (length/breadth), greater than 10 **(Fig. 4.10)**. Examples of fibrous proteins are:

- Collagen found in cartilage and tendons.
- Elastin found in elastic tissue such as tendon and arteries
- Keratin of hair, skin and nail.

Keratins, those in mammals are called ***-keratins,*** being almost entirely α-helical in structure in contrast to ***-keratin*** which are made up of β-sheets and occurs in feathers.

### *Globular Protein*

They are also called **spheroproteins,** on account of their spherical or ovoid shape. In globular proteins the polypeptide chain is compactly folded and coiled and have axial ratio (length/breadth) of less than 10 **(Fig. 4.10)**. These are

soluble proteins of relatively low molecular weight. This group includes mainly:

- Albumin
- Globulin
- Many enzymes
- Histones
- Protamine
- Actin, troponin, etc.

## Classification of Proteins Based on Composition of Protein

According to the joint committee of the **American Society of Biological Chemists and American Physiological Society,** proteins are classified into three main groups as follows:

1. Simple proteins
2. Conjugated proteins
3. Derived proteins

### Simple Proteins

Simple proteins are defined as those proteins that upon hydrolysis yield only amino acids or their derivatives. They are subclassified according to their solubility and heat coagulability as follows:

#### *Albumins*

The albumins are **soluble in water, coagulated by heat.** It is deficient in glycine, e.g.

- Egg albumin
- Serum albumin
- Lactalbumin of milk

#### *Globulins*

The globulins are insoluble in water, but they are **soluble in dilute neutral salt solution and are heat coagulable, e.g.**

- Ovoglobulin of egg yolk
- Serum globulin
- Myosin of muscle

#### *Glutelins*

The glutelins are **soluble in dilute acids and alkalies** but they are **insoluble in neutral solvents.** They are plant proteins, e.g.

- Glutelin of wheat
- Oryzenin of rice

#### *Prolamins or alcohol soluble proteins*

The prolamins are soluble in **70 to 80% alcohol,** but they are **insoluble in water, neutral solvent** or **absolute alcohol.** The prolamins are rich in proline but are **deficient in lysine.** They are plant proteins, e.g.

- Zein of corn
- Gliadin of wheat

#### *Histones*

The histones are **soluble in water, but are not coagulated by heat.** Histones are basic proteins as they are rich in basic amino acids. The histones, being basic, usually occur in tissues in salt combinations with acidic substances, such as nucleic acids (RNA and DNA).

#### *Protamines*

They are strongly basic and rich in basic amino acid arginine. The protamines are ***soluble in water but are not heat coagulable.*** Like histones, they occur in tissues with nucleic acids.

#### *Scleroproteins (fibrous proteins)*

Fibrous proteins are also called **sclero *proteins.*** They are insoluble, (in all common solvents like water, neutral salt solution, organic solvents, dilute acid and alkali) high molecular weight fibers. Examples of sclero proteins are:

- Collagen found in cartilage and tendons
- Elastin found in tendon and arteries
- Keratin of hair, skin and nail

### Conjugated Proteins

Conjugated proteins are composed of simple protein combined with some non-protein substance. The non-protein group is referred to as the **prosthetic** (additional) group. Following are the examples of conjugated proteins:

#### *Nucleoproteins*

The nucleoproteins are composed of simple basic proteins (histones or protamines) with **nucleic acids (RNA and DNA) as the prosthetic groups.**

#### *Glycoproteins and proteoglycans or mucoproteins*

These consist of simple protein and **carbohydrate as a prosthetic group.**

- When carbohydrate content is less than 4% of protein it is called **glycoprotein**, e.g.
  - Mucin of saliva
  - Immunoglobulins
- When carbohydrate content is more than 4%, it is called **mucoprotein** or **proteoglycans,** e.g. glycosaminoglycans.

#### *Chromoproteins*

Chromoproteins are composed of simple proteins with a **colored prosthetic group**, e.g.

- Hemoglobin
- Cytochromes
- Catalase
- Peroxidase

  In all these chromo proteins, prosthetic group is heme.

*Phosphoproteins*

The phosphoproteins are formed by a combination of protein with **prosthetic group phosphoric acid,** e.g.

- Casein of milk
- Vitellin of egg yolk

*Lipoproteins*

The lipoproteins are formed by a combination of protein with a **prosthetic group lipid**, e.g.

- Serum lipoproteins like:
  - Chylomicrons
  - Very low density lipoprotein (VLDL)
  - Low density lipoprotein (LDL) and
  - High density lipoproteins (HDL)

*Metaloproteins*

**The prosthetic group is metallic elements** such as: Fe, Co, Mn, Zn, Cu, Mg, etc., for example;

- Ceruloplasmin is a copper containing protein.
- Carbonic anhydrase, carboxypeptidase and DNA polymerase are zinc containing proteins.

## Derived Proteins

This class of proteins as the name implies, includes those substances formed from simple and conjugated proteins. Derived proteins are subdivided into:

- Primary derived proteins (denatured proteins)
- Secondary derived proteins.

*Primary derived proteins (denatured proteins)*

These protein derivatives are formed by agents, such as heat, acids, alkalies, etc. which cause only slight changes in the protein molecule and its properties without hydrolytic cleavage of peptide bond. These are synonymous with denatured proteins **(Fig. 4.11)**, e.g.

**Fig. 4.11:** Formation of primary and secondary derived proteins.

- Proteans
- Metaproteins

*Proteans:* These are the earliest product of protein hydrolysis by action of dilute acids or enzymes, e.g. **myosan** from myosin and fibrin from fibrinogen.

*Metaproteins:* The metaproteins are formed by further action of acids and alkalies on proteans.

*Secondary derived proteins*

These substances are formed in the progressive hydrolytic cleavage of the peptide bonds of metaproteins (coagulated proteins) into progressive smaller molecules, e.g.

- Proteoses
- Peptones
- Peptides **(Fig. 4.11)**

**Fig. 4.12:** Polypeptide chain showing N-terminal and C-terminal.

**Fig. 4.13:** Schematic diagram for the primary structure of protein.

**Fig. 4.14:** Formation of hydrogen bond in α-helix.

## STRUCTURE OF PROTEINS

Every protein in its native state has a unique three-dimensional structure which is referred to as its ***conformation*** and made up of only 20 different amino acids. The number and sequence of these amino acids are different in different proteins. Protein structure can be classified into four levels of organization:

1. Primary structure
2. Secondary structure
3. Tertiary structure
4. Quaternary structure

### Primary Structure of Proteins

- The sequence of amino acids forming the backbone of proteins and location of any disulfide bond in a protein is called, the primary structure of the protein **(Figs 4.12 and 4.13)**.
- In proteins, amino acids are joined covalently by **peptide bonds,** which are formed between α-carboxyl group of one amino acid and α-amino group of another with the elimination of a water molecule **(Fig. 4.8)**.
- Linkage of many amino acids through peptide bonds results in an unbranched chain called a **polypeptide (Fig. 4.12)**. Each amino acid in a polypeptide is called a **residue** or **moiety.** Each polypeptide chain is having free amino group at one end called N-terminal and free carboxyl group at another end, called C-terminal.
- Proteins have unique amino acid sequences that are specified by genes. This amino acid sequence of a protein is referred to as its primary structure.

**Clinical Importance of Primary Structure**

Understanding of primary structure of a protein is important because many genetic diseases result due to an abnormal amino acid sequences. If the primary structure of the normal and mutated proteins is known, this information may be used to diagnose or study the disease.

### Secondary Structure of Proteins

- For stability of primary structure, **hydrogen bonding** between the hydrogen of NH and oxygen of C=O groups of the polypeptide chain occurs, which gives rise to folding or twisting of the primary structure.

**Fig. 4.15:** Schematic diagram of α-helical structure of protein. C=O group of each amino acid is hydrogen bonded to the NH of the amino acid that is situated four residues ahead (Number indicates the amino acid residues).

- **Thus, regular folding and twisting of the polypeptide chain brought about by hydrogen bonding is called secondary structure of protein.** The most important kinds of secondary structure are:
  - α-Helix (helicoidal structure)
  - β-Pleated sheet (stretched structure)

#### *α-Helix*

It is called α because it was the first structure elucidated by Pauling and Corey. If a backbone of polypeptide chain is twisted by an equal amount about each α-carbon, it forms a coil or helix. The helix is a rod-like structure.

- The helix is stabilized by hydrogen bonds between the NH and CO groups of the same chain.

- These hydrogen bonds have essentially optimal nitrogen to oxygen (N-O) distance of 2.8 Å. Thus, CO group of each amino acid is hydrogen bonded to the -NH of the amino acid that is situated four residues ahead in the linear sequence **(Fig. 4.14)**.
- The axial distance between adjacent amino acids is 1.5Å and gives 3.6 amino acid residues per turn of helix **(Fig. 4.15).**

In some proteins, α-helices contribute significantly to the secondary structure, e.g. α-**keratin**, **myoglobin**, and **hemoglobin**, whereas in others their contribution may be small, e.g. chymotrypsin and cytochrome or absent, e.g. collagen and elastin.

*Helix destabilizing amino acids*

***Glycine*** and ***proline*** are the helix-destabilizing amino acids.

## β-Pleated Sheet Structure or Stretched State Structure

Pauling and Corey discovered another type of structure which they named β-**pleated sheet** (β because it was the second structure they elucidated). The surfaces of β-sheet appear "pleated" and these structures are therefore often called **"β-pleated sheet'.**

- A polypeptide chain in the β-pleated sheet is almost fully extended rather than being tightly coiled as in the α-helix.
- Unlike α-helix, β-pleated sheets are composed of two or more polypeptide chains.
- β-pleated sheet is stabilized by hydrogen bonds between NH and C=O groups in a different polypeptide chain whereas in α-helix, the hydrogen bonds are between NH and CO groups in the same polypeptide chain.
- In β-sheet, the hydrogen bonds are perpendicular to the polypeptide backbone rather than parallel as in the α-helixes.
- Like α-helix, β-sheets are found in both **fibrous** and **globular** proteins. An example of protein that contains primarily the β-structure is **transthyretin.**
- The arrangement of polypeptide chains in β-pleated sheet conformation can occur in two ways:
  - Parallel pleated sheet
  - Anti-parallel pleated sheet

**Figs 4.16A and B:** β-pleated sheet structure. (A) Anti-parallel; (B) Parallel.

**Fig. 4.17:** Tertiary structure of protein.

*Parallel pleated sheet*

In parallel pleated sheet:

- The polypeptide chains lie side-by-side and in the same direction (with respect to N- and C-terminal), so that their N-terminal residues are at the same end (N-terminal faces to N-terminal) and stabilized by hydrogen bonding.
- Here the hydrogen bonds are (interchain) formed between NH of a polypeptide in one chain and carbonyl (C=O) of a neighboring chain **(Fig. 4.16B)**.

*Anti-parallel pleated sheet*

In the anti-parallel pleated sheet:

- The polypeptide chains lie in opposite directions, i.e.-terminal end of one is next to the C-terminal of the other. (N-terminal faces to C-terminal) **(Fig. 4.16A).**
- It is stabilized by interchain hydrogen bonding.
- Antiparallel β-sheet conformation is less common in human proteins. The best example in nature is **silk fibiron**.

**Other Types of Secondary Structures**

Besides the α- and β-structures described above, the β-bends, loop regions and disordered regions, are also found in proteins.

## Tertiary Structure

- The peptide chain, with its secondary structure, may be further folded and twisted about itself forming three-dimensional arrangement of the polypeptide chain **(Fig. 4.17)**.
- Amino acid residues which are very distant from one another in the sequence can be brought very near due to the folding and thus form regions essential for the functioning of the protein, like **active site** or catalytic site of enzymes.
- Thus, the three-dimensional folded compact and biologically active conformation of a protein is referred to as its tertiary structure, e.g. myoglobin.

**Fig. 4.18:** Quaternary structure of polymeric protein hemoglobin.

### Tertiary Structure Stabilizing Forces

The three-dimensional tertiary structure of a protein is stabilized by:

- Hydrogen bonds
- Hydrophobic interactions
- Van der Waals forces
- Disulfide bond
- Ionic (electrostatic) bonds or salt bridges

## Quaternary Structure of Protein

- Only those proteins that have **more than one polypeptide chain (polymeric)** have a quaternary structure. Not all proteins are polymeric. Many proteins consist of a **single polypeptide chain and are called monomeric** proteins, e.g. myoglobin.
- The arrangement of these polymeric polypeptide subunits in three-dimensional complexes is called the quaternary structure of the protein **(Fig. 4.18)**.
- Examples of proteins having quaternary structure are:
  - Lactate dehydrogenase
  - Pyruvate dehydrogenase
  - Hemoglobin.

### Quaternary Structure Stabilizing Forces

The subunits of polymeric protein are held together by noncovalent interactions or forces such as:

- Hydrophobic interactions
- Hydrogen bond
- Ionic bonds

## Bonds Responsible for Protein Structure

Protein structure is stabilized by two types of bonds

1. Covalent bond, e.g.
   - Peptide bonds
   - Disulfide bond
2. Noncovalent bond, e.g.
   - Hydrogen bond
   - Hydrophobic bond or interaction
   - Electrostatic or ionic bond or salt bond or salt bridge
   - Van der Waals interactions

### *Covalent Bond*

*Peptide bonds (—CO-NH—)*

- A peptide bond is formed by the condensation of the amino group of one amino acid with the carboxyl group of another amino acid with a removal of a water molecule **(See Fig. 4.7)**.

*Disulfide bond (—S-S—)*

- A covalent bond formed between the sulfhydryl group (-SH) of side chain of cysteine residues in the same or different peptide chains.
- These disulfide bonds help to stabilize against denaturation and confer additional stability.

### *Noncovalent Bonds*

*Hydrogen bond*

- Bond formed between -NH and -CO groups of peptide bond by sharing single hydrogen.
- Hydrogen bond may occur within the same polypeptide chain (intrachain) or between different polypeptide chains (interchain).
- Side chains of 11 out of the 20 standard amino acids can also participate in hydrogen bonding.

*Hydrophobic bond or interaction*

These are formed by interaction between nonpolar hydrophobic R groups (side chain) of amino acids like alanine, valine, leucine, isoleucine, methionine, phenylalanine and tryptophan.

*Electrostatic or ionic bond or salt bond or salt bridge*

These are formed between oppositely charged groups when they are close, such as amino ($NH_3^+$) terminal and carboxyl ($COO^-$) terminal groups of the peptide and the oppositely charged R-groups of polar amino acid residues.

*Van der Waals interactions*

Van der Waals forces are extremely weak and act only on extremely short distances and include both an attractive and repulsive forces (between both polar and nonpolar side chain of amino acid residues).

# PROPERTIES OF PROTEINS

## Colloidal Nature

Protein molecules exist in the form of colloidal particle, 5–100 mμ in dimension, are heavier than water and sink.

## Colloidal Osmotic Pressure

- Colloidal protein molecules exert **osmotic pressure.** The osmotic pressure generated by plasma proteins is often called the ***colloidal osmotic pressure*** or ***oncotic pressure*** of plasma.
- The osmotic pressure of protein is proportional to its concentration, but inversely proportional to its molecular weight.
- In blood plasma, albumin contributes 75–80% of osmotic pressure (although it represents no more than half the plasma proteins), because its molecular weight is lower.
- Oncotic pressure exerted by protein is clinically important in maintaining blood volume.

## Molecular Weight

Proteins are macromolecules and have very high molecular weights with wide variations, e.g. molecular weight of:

- Albumin = 69,000
- γ-Globulin = 1, 60000.

## Solubility

In general, globular proteins, such as, albumin has higher solubility than elongated fibrous proteins. Moreover, smaller molecules are more soluble than larger molecules.

## Shape of the Protein

There is a wide variation in the protein shape.

- Scleroproteins like keratin, collagen is in the form of fibers.
- While soluble proteins tend to be of rounded shape and are called globular proteins.

## Hydration of Proteins

- Proteins, when brought into contact with water, absorb water and swell up.
- The polar groups like COOH, $NH_2$, and OH of protein bind to the molecules of water by hydrogen bonds to hold a considerable amount of water. Thus, a relatively immobile shell-like layer of water, called the **"solvation layer"** or **water envelope** is held around each protein particle in an aqueous medium.

## Amphoteric Nature and Isoelectric pH of the Proteins

The isoelectric pH of amino acid has been described previously. One end of the protein molecule has free amino group, while the other end has free COOH group.

- In acid solution, the $NH_2$ groups accept $H^+$ ion and present as $NH_3^+$ (cation). Therefore protein in acid solution will be positively charged.
- In alkaline pH, the COOH groups donate $H^+$ ion and are present as $COO^-$ (anion). Hence, proteins in alkaline solution are negatively charged.

So, proteins are **ampholytes** acting both as donors and acceptors of $H^+$ ion.

## Isoelectric pH of the Protein

- For every protein in solution, there is a particular pH at which the number of anions formed is exactly equal to the number of cations, and the solution is electrically neutral. That pH is called the **isoelectric pH (PI)** of that protein and the protein exists as **zwitterion.**
- At isoelectric pH:
  - Protein molecules do not migrate in an electric field.
  - Solubility, buffering capacity and viscosity is minimum and precipitability is maximum.
- PI of some proteins is given below:
  - Pepsin: 1.1
  - Casein: 4.6
  - Albumin: 4.7
  - Globulin: 6.4.
- At pH values above or below the isoelectric pH, they carry a net negative or positive charge and migrate to anode (+vely charged electrode) or cathode (–vely charged electrode).

## Precipitation of Proteins

The stability of protein in solution depends on the **charge** and **hydration** of the protein molecule.

- The factors which neutralize the charge or remove water of hydration will cause precipitation of proteins. The factors used for precipitation of proteins are:
  - Salting out
  - Isoelectric pH
  - Heavy positive or negative ions
  - Organic solvents

### *Salting Out Method*

When neutral salts such as ammonium sulfate or sodium sulfate are added to a protein solution, the addition may precipitate a protein from its solution.

- Mineral ions attract water molecules and consequently remove the shell of hydration (solvation layer) from around protein molecules.
- Since water layer around protein particles is removed, the protein is precipitated. This is called **salting out.**
- Higher molecular weight protein requires less salt to precipitate than low molecular weight protein. Thus, globulins are precipitated at half saturation of ammonium sulfate or 22% Na-sulfate, but albumin requires full saturation of ammonium sulfate or 28% Na-sulfate.

***Application:*** These methods are therefore useful in separating albumin and globulin from serum proteins.

### *Precipitation at Isoelectric pH*

All proteins are least soluble at their isoelectric pH (PI) and can then be precipitated.

## Precipitation by Heavy Positive or Negative Ions

- On the acidic side of its PI, a protein remains as cation (+vely charged) and is precipitated by neutralizing the charge on protein by adding anion (-vely charged) or alkaloidal reagents like **tungstate, trichloroacetate** or **picrate.**
- On the alkaline side of its PI, a protein exists as an anion and is precipitated as metal proteinates by heavy metal ions like $Zn^{2+}$, lead, mercury, etc.

**Fig. 4.19:** Denaturation of protein.

### Precipitation by Organic Solvents

Organic solvent like alcohol dehydrates and precipitates the proteins.

## DENATURATION OF PROTEINS

The three-dimensional conformation, the **primary, secondary, tertiary** and even in some cases **quaternary** structure is characteristic of a native protein. **Hydrogen bond, ionic bond** and **hydrophobic bond** stabilize the structure to maintain its conformation in space. This conformation can upset and disorganized **without breakage of any peptide linkage,** only by the rupture of ionic bond, hydrogen bonds and hydrophobic bond which stabilize the structure. This is called **denaturation (Fig. 4.19)**. Denaturation of proteins leads to:

- Unfolding of natural coils of native protein.
- Decrease in solubility and increase in precipitability.
- Loss of biological activities, (e.g. enzyme activity) and antigenic properties.
- Increased digestibility.

### Denaturing Agents

- Denaturation is brought about by certain:
  - Physical agents
  - Chemical agents
  - Mechanical means
- **Physical agents:** Heat, Ultraviolet rays and ionizing radiations can denature proteins.
- **Chemical agents:** Acids, alkalies and certain acid solutions of heavy metals, e.g. mercury, lead, detergents; organic solvents like alcohol, acetone, etc. denature proteins.
- **Mechanical means:** Vigorous shaking or grinding leads to denaturation of the protein.

### Examples of Denatured Protein

Cooked meat, boiled egg, milk paneer, etc.

### Significance of Denaturation

- Digestibility of native protein is increased on denaturation by gastric HCI or by heat on cooking. Denaturation causes unfolding of native polypeptide coil so that hidden peptide bonds are exposed to the action of proteolytic enzyme in the gut. It also increases reactivity of certain groups.
- Denaturation property of a protein is used in blood analysis to eliminate the proteins of the blood (deproteinization of blood).

### Coagulation

Denaturation may, in rare cases be reversible, in which case the protein refolds into its original native structure, when the denaturing agent is removed. However, most proteins, once denatured, remain permanently disordered and are called **irreversible denaturation** or **coagulation,** e.g. coagulated egg white of boiled egg.

## EXAM QUESTIONS

### Long Answer Questions (LAQS)

1. Define proteins. Classify proteins in various ways with suitable example and functions.
2. Define amino acids. Classify amino acids in various ways with suitable example and functions.
3. Describe structure of protein at different levels.

### Short Notes

1. Describe the secondary and tertiary levels of protein structure.
2. Functional classification of proteins.
3. Bonds responsible for the three-dimensional structure of proteins.
4. Denaturation of protein.
5. Biologically important peptides.
6. Essential amino acids.

### Multiple Choice Questions (MCQs)

**1. All amino acids found in proteins are optically active, except:**

a. Serine b. Glycine
c. Threonine d. Tyrosine

**2. Which of the following amino acid is not occurring in proteins?**

a. β-alanine
b. α-amino butyrate
c. Triiodothyronine
d. All of the above

**3. The greatest buffering capacity at physiological pH would be provided by a protein, rich in which of the following amino acids?**
a. Glycine b. Lysine
c. Histidine d. Valine

**4. All of the following are hemoproteins except:**
a. Myoglobin b. Cytochrome
c. Catalase d. Albumin

**5. Which of the following amino acid is exclusively ketogenic?**
a. Leucine b. Phenylalanine
c. Threonine d. Isoleucine

**6. In humans all of the following amino acids are essential, except:**
a. Valine b. Isoleucine
c. Glycine d. Phenylalanine

**7. During denaturation of protein the following bonds are disrupted, except:**
a. Hydrogen b. Hydrophobic
c. Peptide d. Sulfide

**8. The imino acid present in protein is:**
a. Phenylalanine b. Valine
c. Leucine d. Proline

**9. Glycine is used for synthesis of the following, except:**
a. Heme b. Serotonin
c. Purine d. Creatine

**10. Which class of amino acids contains only non-essential amino acids?**
a. Aromatic b. Acidic
c. Branched chain d. Basic

**11. Which of the following is a non-protein amino acid?**
a. Proline b. Histidine
c. Ornithine d. Asparagine

**12. Which of the following is a kind of secondary structure?**
a. α-helix b. β-bend
c. β-sheet d. All of the above

**13. Glutathione is found in all mammalian cells except:**
a. RBC b. Neurons
c. Skeletal muscle d. Argentaffin cells

**14. Albumin is deficient in which of the following amino acid?**
a. Glycine b. Tryptophan
c. Cystine d. Methionine

**15. Which of the following acts as a redox buffer?**
a. Insulin b. Glucagon
c. Glutathione d. Angiotensin

**16. The important source of nitrogen for the body is:**
a. Carbohydrate b. Lipids
c. Proteins d. All the above

**17. Which of the following type of amino acids are present in biological proteins?**
a. L-α b. D-α
c. L-β d. D-β

**18 In which of the following amino acid imidazole ring is present?**
a. Histidine b. Alanine
c. Tryptophan d. Valine

**19. Glucogenic amino acids are that:**
a. Which contains glucose in its structure
b. Acts as precursors for carbohydrates biosynthesis
c. On biological oxidation it gives energy equal to glucose
d. All of the above

**20. The pH at which the molecule act as Zwitterion is called as:**
a. Isoelectric pH b. Isoelectric focusing
c. Optimum pH d. Neutral pH

**21. The sequence of amino acids in a polypeptide is called the:**
a. Primary structure
b. Secondary structure
c. Tertiary structure
d. Quaternary structure

**22. The polypeptide responsible for uterine contraction is:**
a. Oxytocin b. Glutathione
c. Vasopressin d. Angiotensin

**23. The vasopressin is polypeptide which:**
a. Stimulate antidiuretic action
b. Stimulate kidney to loss water
c. Increases blood pressure d. a and c

**24. The polypeptide that protects RBC membrane is:**
a. Oxytocin b. Glutathione
c. Vasopressin d. Angiotensin

**25. Proteins are made up from how many standard amino acids:**
a. 15 b. 18
c. 20 d. 100

**26. Which of the following amino acids is hydroxy amino acid?**
a. Serine b. Isoleucine
c. Leucine d. All of the above,

**27. Which of the following amino acid is considered as basic amino acid?**
a. Tryptophan b. Histidine
c. Proline d. Hydroxyproline

**28. Which of the following is S-containing amino acid?**
a. Cysteine b. Cystine
c. Methionine d. All of the above

**29. Vitamin niacin is synthesized from:**
a. Tyrosine b. Tryptophan
c. Cystine d. Glycine

**30. Which of the following amino acid is involved in synthesis of creatine?**
a. Glycine b. Arginine
c. Methionine d. All of the above

**31. Glycine is used for the synthesis of:**
a. Heme b. Niacin
c. Serotonin d. Thyroxin

**32. Those amino acids can be synthesized by the body is called as:**
a. Essential amino acids
b. Nonessential amino acids
c. Standard amino acids
d. None of the above

**33. Proteins are linear chains of amino acids that are linked together by:**
a. Peptide bonds b. Hydrogen bonds
c. Hydrophobic bond d. Glycosidic bonds

**34. Which amino acids are involved in 'Detoxification' reaction?**
a. Glycine b. Cystine
c. Methionine d. All of the above

**35. Which of the following is fibrous protein?**
a. Collagen b. Elastin
c. Keratin d. All of the above

**36. Which of the following is true for α-helix structure of protein?**
a. There are 3.6 amino acids per turn
b. It is called α because it was the first structure elucidated by Pauling and Corey
c. The helix is stabilized by hydrogen bond
d. All of the above

**37. All of the following are glycoproteins except:**
a. Collagen b. Albumin
c. IgG d. Transferrin

**38. Which one of the following list is correct?**
a. Serine and tryptophan both have hydroxyl groups in their side chains
b. The most abundant amino acid in collagen is proline
c. Glutamine and asparagine have amide groups on their side chains
d. All amino acids found in proteins except proline, have a hydrogen atom attached to their α-carbon

**39. Which one of the following statement is correct concerning denaturation or hydrolysis of proteins?**
a. Denaturation alters the primary structure of a protein and destroys its biological activity
b. Proteins can be denatured by exposure to acids, alkalis or concentrated solutions of urea or by elevated temperatures
c. Most proteins become more soluble when denatured
d. Denaturation decreases digestibility

**40. Select the correct statement concerning the varying levels of structure found in proteins.**
a. The secondary and tertiary structures of protein depends on its amino acid sequence
b. The secondary structure of protein is the three-dimensional configuration
c. The primary, secondary and tertiary structures of a protein are destroyed when the protein is denatured
d. Protein secondary structures are stabilized by disulfide bonds

## Answers for MCQs

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **1.** b | **2.** d | **3.** c | **4.** d | **5.** a | **6.** c | **7.** c | **8.** d |
| **9.** b | **10.** b | **11.** c | **12.** d | **13.** b | **14.** a | **15.** c | **16.** c |
| **17.** a | **18.** a | **19.** b | **20.** a | **21.** a | **22.** a | **23.** d | **24.** b |
| **25.** c | **26.** a | **27.** b | **28.** d | **29.** b | **30.** d | **31.** a | **32.** b |
| **33.** a | **34.** d | **35.** d | **36.** d | **37.** b | **38.** c | **39.** b | **40.** a |

CHAPTER

# 5 Plasma Proteins and Immunoglobulins

**Chapter outline**

- Plasma Proteins
- Immunoglobulins

## INTRODUCTION

Plasma contains a variety of proteins with different functions. At present time over 100 different plasma proteins have been described. There are many proteins whose function remains to be determined. In this chapter, we shall see those plasma proteins which are present in significantly high concentrations, e.g. albumin, globulin and fibrinogen.

## PLASMA PROTEINS

- The plasma proteins are:
  1. Albumin
  2. Globulin
  3. Fibrinogen
- The normal value of plasma proteins are:
  - Total protein 6 to 8 gm %
  - Serum albumin 3.5 to 6 gm %
  - Serum globulin 2 to 3.5 gm %
  - Fibrinogen 200 to 400 mg %
  - A/G ratio 1.2:1 to 2.5:1

### Synthesis of Plasma Proteins

- All the ***albumin*** and ***fibrinogen*** are essentially synthesized by the **liver** only.
- Similarly, 50 to 80 percent of the globulin is formed in the liver. The remainder of the globulins are formed almost entirely in the **lymphoid tissues**. They are ***gamma globulins*** that constitute the ***antibodies*** used in the immune system.
- In severe liver disease, there is thus a lowered concentration of plasma albumin but globulin fraction may not show substantial fall, as gamma globulins are not synthesized by the liver. ***The A/G ratio therefore can be altered in the liver disease.***

### Separation of Plasma Proteins

- The plasma proteins can be separated by the following methods:
  1. Salting out
  2. Electrophoresis
  3. Ultracentrifugation
  4. Immunoelectrophoresis
- ***Electrophoresis:*** This is the most commonly employed analytical technique for the separation of plasma/serum proteins. The basic principles of electrophoresis are described in **Chapter 35**.
- Serum proteins separated on cellulose acetate membrane generally have five bands **(Figs. 5.1A and B):**
  1. Albumin
  2. $\alpha_1$-globulin
  3. $\alpha_2$-globulin
  4. $\beta$-globulin
  5. $\gamma$-globulin.

  These five bands appear as five peaks on the densitometer graph.
- Serum protein electrophoretic patterns provide useful diagnostic information.
- Different electrophoretic patterns of serum associated with various disorders, compared with normal serum are shown in **Figure 5.2**.

**Figs 5.1A and B:** Electrophoretic separation of serum proteins (A) Electrophoretogram of normal serum on cellulose acetate strip; (B) Densitometry scanning from cellulose acetate strip converts bands to characteristic peaks of albumin, $\alpha_1$-globulin, $\alpha_2$-globulin, β-globulin and γ-globulin.

**Fig. 5.2:** Different electrophoretic patterns of serum compared with normal serum.

- Changes in serum protein fractions associated with various conditions are given below:
  - Infective hepatitis shows slight decrease in albumin and significant increased γ-globulins.
  - Liver cirrhosis shows elevations in β and γ-globulins with a decrease in albumin.
  - Nephrosis shows low level of albumin, significantly elevated $\alpha_2$-globulin and elevated β-globulin.
  - Multiple myeloma shows marked increase in γ-globulin.

## Major Classes of Plasma Proteins (Table 5.1)

### *Albumin*

- Albumin is a globular protein consisting of single polypeptide chain with a molecular weight of about 69,000 in the humans. It comprises of some 585 amino acid residues.
- It is the most abundant protein found in plasma, accounting for approximately 50 percent of plasma protein mass.
- Albumin is exclusively synthesized by the liver for this reason; serum albumin levels are determined to assess liver function (synthesis decreased in liver diseases).
- The normal plasma **half-life** of albumin is **15** to **20 days.**

### *Functions of albumins*

- Albumin's primary function is the maintenance of **colloidal osmotic pressure.** Albumin makes the biggest contribution to the plasma oncotic pressure. Thus, albumin plays a predominant role in maintaining blood volume and body fluid distribution. Very low albumin concentration develops edema.
- A second important function of albumin is to bind and transport many metabolites which are poorly soluble in water such as:
  - Fatty acids
  - Bilirubin
  - Inorganic constituent of plasma like calcium
  - Certain steroid hormones and drugs.
- **Buffering function:** Among the plasma proteins, albumin has maximum buffering capacity due to its high concentration in blood.
- **Nutritive function:** Degradation of albumin provides essential amino acids during malnutrition.

**Table 5.1:** Major classes of plasma proteins, their functions and diagnostic importance.

| *Classes* | *Examples* | *Principal functions* |
|---|---|---|
| Albumin | | Exert colloidal osmotic pressure and transport function |
| Globulin | $\alpha_1$-protease inhibitor (API) or $\alpha_1$-antitrypsin (AAT) | Antiprotease, natural inhibitor of proteolytic enzyme elastase |
| | Ceruloplasmin | Transport of copper |
| | Haptoglobin | Conservation of iron by binding free hemoglobin |
| | $\alpha_2$-Macroglobulin (AMG) | Natural antiprotease, inhibits thrombin, trypsin and pepsin |
| | Hemopexin | Binds heme and prevents loss of iron |
| | Transferrin | Transport of iron |
| | C-reactive protein (CRP) | Body's defense mechanism |
| | $\beta_2$-microglobulin (BMG) | Body's defense mechanism |
| | Immunoglobulins IgG, IgA, IgM, IgD and IgE | Body's defense mechanisms |
| Fibrinogen | | Blood coagulation |

## Clinical Significance

Serum albumin measurements are used to assess the various clinical conditions.

### *Hypoalbuminemia*

Decreased level of plasma albumin is seen in:

- **Malnutrition**
  In malnutrition, due to insufficient intake of proteins, the availability of amino acid is reduced and so albumin synthesis is affected.
- **Nephrotic syndrome**
  In nephrotic syndrome, large amounts of protein are lost in urine (proteinuria).
- **Cirrhosis of liver**
  In cirrhosis, albumin synthesis is decreased and so blood level is lowered.

### *Hyperalbuminemia*

Increased levels of plasma albumin are present only in acute dehydration and have no clinical significance.

### *Analbuminemia*

Analbuminemia is a rare hereditary abnormality in which plasma albumin concentration is usually less than 1.0 gm/L (normal level is 3.5 to 6 gm/dl). However, there may be no symptoms or signs not even edema due to compensatory increase in plasma globulin concentration.

## Globulins

Globulins are bigger in size than albumins. Globulins constitute several fractions. These are:

- $\alpha_1$-globulin
- $\alpha_2$-globulin
- $\beta$-globulin
- $\gamma$-globulin

Biological functions of various globulins are summarized in Table 5.1, which are discussed below.

## *$\alpha_1$-Protease inhibitor (API) or also known as $\alpha_1$ antitrypsin (AAT)*

- API is one of the plasma proteins that inhibits activity of **proteases** particularly **elastase,** which degrades elastin, a protein that gives elasticity to the lungs.
- In a normal individual, the activity of elastase is regulated by API.
- A genetic deficiency in API can lead to **emphysema** (lung disorder). Excessive cigarette smoking also leads to emphysema as cigarette smoke inhibits the activity of API.

## Prothrombin

- It is synthesized by liver with the help of vitamin K and involved in **blood *clotting***.
- Liver damage causes lengthening of prothrombin time.

## Ceruloplasmin (Ferro-oxidase)

- This is a copper containing protein.
- It has oxidase activity.

- Ceruloplasmin is the major transport protein for copper, an essential trace element.
- It is also essential for the regulation of the oxidation-reduction, transport and utilization of iron.
- Plasma ceruloplasmin level is reduced in **Wilson's disease,** in patients with malnutrition and in the nephrotic syndrome.

### Haptoglobin

- It plays an important role in the conservation of iron by preventing its loss in the urine.
- Haptoglobin binds free hemoglobin to form a complex which is too large to be filtered by the kidney and thus prevents the loss of iron in the urine.

### $\alpha_2$-macroglobulin (AMG)

- This is major $\alpha_2$-globulin, which is a natural inhibitor of ***endopeptidase*** such as trypsin, chymotrypsin, plasmin, thrombin, etc.
- Plasma AMG is decreased in myeloma, peptic ulcer and increased in nephrotic syndrome, cirrhosis and collagen disorders.

### Hemopexin

- Like haptoglobin, hemopexin also plays an important role in the conservation of iron by preventing its loss in the urine.
- Hemopexin binds **free heme,** not hemoglobin.
- The complex hemopexin-heme is too large to be filtered by the kidney and thus prevents the loss of iron in the urine.

### Transferrin

- Transferrin is synthesized in liver.
- It transports iron (two molecules of $Fe^{3+}$ per molecule of transferrin) through blood to the sites where iron is required.
- Increased serum levels are seen in iron deficiency anemia and pregnancy.
- Low levels occur in chronic infections and in malnutrition.

### C-reactive Protein (CRP)

- CRP is named because it reacts with C-polysaccharides of the cell wall of pneumococci bacteria.
- CRP is involved in the body's defense mechanism.
- It is useful in differentiating bacterial infection from viral infections because the ***level of CRP is increased in bacterial infections only.***

### $\beta_2$-microglobulin

- This protein forms part of the ***human leucocyte antigen (HLA) system.***
- It is derived from myeloid and lymphoid cells and is normally synthesized at the constant rate.
- Plasma levels are increased whenever; there is malignant lymphoid or myeloid proliferation and renal failure.

### Immunoglobulins

(Discussed later separately)

### Fibrinogen (Blood Clotting Factor I)

- It is a glycoprotein and constitutes about 4 percent of total plasma proteins. It is synthesized in liver and secreted in blood where it is involved in **blood coagulation.**
- During blood coagulation, fibrinogen is converted to fibrin which polymerizes to form fibrin clot.
- Plasma level of fibrinogen decreases in severe hepatic diseases.

## IMMUNOGLOBULINS (IG)

- The immunoglobulins are $\gamma$-**globulins,** called **antibodies**. All antibodies are immunoglobulin but all immunoglobulins may not be antibodies.
- They constitute about 20 percent of all the plasma proteins.
- Immunoglobulins are produced by **plasma cells** and to some extent by **lymphocytes**.

### Structure of Immunoglobulins (Fig. 5.3)

- Immunoglobulins are **glycoproteins** made up of **light (L)** and **heavy (H)** polypeptide chains. The term **"light"** and **"heavy"** refer to **molecular weight.** Light chains have a molecular weight of 25,000 whereas heavy chains have a molecular weight of 50,000 to 70,000.
- All immunoglobulins have the same basic structure. The basic immunoglobulin is a **'Y'** shaped molecule and consists of four polypeptide chains, **two H** and **two L** chains.

**Fig. 5.3:** Schematic structure of IgG to show basic structure of immunoglobulin molecule.
VH: Variable heavy chain VL: Variable light chain
CH: Constant heavy chain CL: Constant light chain

**Table 5.2:** Different classes of immunoglobulins corresponding to the type of heavy chains.

| *Ig classes* | *Types of Heavy chains* |
|---|---|
| IgG | γ (gamma) |
| IgA | α (alpha) |
| IgM | μ (mu) |
| IgD | δ (delta) |
| IgE | ε (epsilon) |

- The four chains are linked by **disulfide** bonds.
- L chain may be either of two types, **Kappa (κ)** or **Lambda (λ)** but not both.

The heavy chains may be of five types and are designated by Greek letter:

1. Alpha (α)
2. Gamma (γ)
3. Delta (δ)
4. Mu (μ)
5. Epsilon (ε).

- Immunoglobulins are named as per their heavy chain type as **IgA, IgG, IgD, IgM** and **IgE (Table 5.2).**
- The L and H chains are subdivided into **variable** and **constant** regions.
- L chain consists of one **variable (VL)** and one **constant (CL)** domain or region.
- Most H-chains consist of one **variable (VH)** and three **constant (CH-1, CH-2, and CH-3)** domains.
- Each immunoglobulin molecule has **hinge region** between CH-1 and CH-2, which allows a better fit with the antigen surface.
- The variable regions of both the light and heavy chains form **antigen binding site.**
- Enzyme (papain) digestion splits the immunoglobulin molecule into two fragments named as **Fab (Fragment for antigen binding)** and **Fc (crystallizable fragment or fragment for complement binding (Fig. 5.3)**.

## Functions of Immunoglobulins (Antibody)

- The primary function of antibodies is to protect against infectious agents.
- In addition to these functions, antibodies can act as an enzyme to catalyze the synthesis of ozone ($O_3$) that has microbicidal activity.

## Immunoglobulin Classes

### *IgG (Heavy chain γ)*

- IgG is a smaller **monomeric** molecule with two antigen binding sites **(Fig. 5.3)**.
- IgG is the major class of immunoglobulin which accounts for 70 percent of the total serum immunoglobulins.
- IgG is the only antibody that **crosses the placenta** and is the **maternal antibody** that protects the fetus.
- It is produced mainly in the **secondary response** and provides defense against bacteria and viruses.

**Fig. 5.4:** Diagrammatic representation of monomeric, dimeric and pentameric forms of immunoglobulins.

### IgA (Heavy chain α)

- IgA is the **second most abundant class** constituting about 20 percent of serum immunoglobulins. It is **monomer** or **dimer (Fig. 5.4)**.
- Dimeric IgA molecule formed by two monomer units, joined together at their carboxy terminals by a protein termed **J-chains** (J for joining).
- IgA is found in external secretions such as **colostrum, saliva, tears** and **respiratory, intestinal** and **genital tract secretions**.
- It prevents attachment of microorganisms like bacteria and viruses to mucous surfaces and helps to protect mucous surfaces from antigenic attack and prevent access of foreign substances to the circulation.

### IgM (Heavy chain μ)

- It is a **pentamer** consisting of five identical immunoglobulin molecules, joined together by **disulfide bridges.** This pentamer is closed in a ring structure by J-chain **(Fig. 5.4).**
- IgM is the first antibody to be produced in response to an antigen and is important in defense against bacteria and viruses.
- IgM present on the surface of **B lymphocyte** is **monomer,** where it functions as an antigen binding **receptor**.

### IgD (Heavy chain δ)

- It is a **monomer** and resembles IgG structurally.
- IgD has no known antibody function but may function as an **antigen receptor.**
- Like IgM, it is present on the surface of many **B lymphocytes.**
- The circulating concentration of IgD in blood is very low.

### IgE (Heavy Chain ε)

- IgE is a **monomeric** molecule similar to IgG. It is sometimes called **reagin.**
- Although IgE is present in trace amounts, (approximately 0.004%) in normal persons with allergic activity have greatly increased amounts.
- IgE is responsible for **anaphylactic (immediate) type of hypersensitivity and allergy.** Its activity is mediated by histamine.
- IgE also participates in defense against certain **parasites,** e.g. **helminths** (worms).

**Table 5.3:** Structure, functions and characteristics of different types of immunoglobulins.

| *Types* | *Structure* | *Approximate percentage of the total immunoglobulin in serum* | *Characteristics* | *Functions* |
|---|---|---|---|---|
| IgG | Monomer | 70% | • It is the only immunoglobulin which crosses the placenta and is the only maternal antibody which protects the fetus | • Neutralizes bacterial toxins and binds to microorganisms making them easier to phagocytize |
| IgA | Monomer or Dimer | 20% | • Major component of colostrum<br>• Also occurs in saliva, tears and respiratory, intestinal and genital tract secretions | • Prevents attachment of bacteria and viruses to mucous membranes and helps protect mucous surface from antigenic attack |
| IgM | Pentamer | 8–10% | • Main antibody in the primary response to an antigen<br>• Produced by fetus | • Promotes phagocytosis and causes lysis of antigenic cells (bacteria)<br>• Antigen receptor on the surface of B lymphocytes |
| IgD | Monomer | Less than 1% | • Labile molecules | • May function as an antigen receptor. No known antibody function |
| IgE | Monomer | Trace amount 0.004% | • Activity is mediated by histamine | • Antiallergic and antiparasitic<br>• Mediates immediate hypersensitivity by causing release of histamine |

Structure, function and characteristics of different types of immunoglobulins are summarized in **Table 5.3.**

## Disorders of Immunoglobulins

Abnormally large amounts of certain immunoglobulins may be found in the plasma in several diseases of humans. As well as deficiency of γ-globulins is also found in rare hereditary disease.

### *Multiple Myeloma*

Multiple myeloma, a plasma cell cancer results due to abnormally high concentration of serum **immunoglobulins,** usually **IgG** or **IgA** (refer Bence Jones proteins also).

### *Amyloidosis*

Amyloidosis is the accumulation of immunoglobulin **light chain fragments** in the tissues of multiple myeloma patients.

### *Bence Jones Proteins*

- In multiple myeloma, excess **monoclonal light chains** are produced than heavy chains and enter the bloodstream, because they are of relatively low molecular weight, they pass through glomerular membrane and appear in the urine. These protein chains of low molecular weight are known as **Bence Jones Proteins.**
- Bence Jones proteins have the remarkable characteristic of precipitating on heating urine from 45° to 60°C and redissolve when the heating is continued above 80°C.
- Multiple myeloma with Bence Jones protein in the urine is called **"light chain disease."** The chain may be of either of the κ-(kappa) or the λ-(lambda) type.

- **Polyclonal antibodies** (mixture of antibodies, heterogenous) are those produced by many different B lymphocytes responding to one antigen.
- **Monoclonal antibodies** (homogenous), in contrast, are synthesized by identical B cells.

# EXAM QUESTIONS

## Long Answer Questions (LAQs)

1. Describe various types of immunoglobulins with their functions.
2. Describe various types of plasma proteins with their functions.

## Short Notes

1. Functions of plasma proteins.
2. Immunoglobulins.
3. Major classes of plasma proteins and their functions.
4. Disorders due to changes in immunoglobulins.
5. Albumin.

## Multiple Choice Questions (MCQs)

**1. In multiple myeloma which of the following plasma protein level is increased?**
a. Albumin b. Hemopexin
c. $\alpha_1$-fetoprotein d. γ-globulin

**2. Bence Jones proteins are:**
a. Monoclonal light chains
b. Monoclonal heavy chains
c. Intact γ-globulins
d. All of the above

**3. Cigarette smoke inhibits the activity of:**
a. $\alpha_1$-antitrypsin b. Ceruloplasmin
c. $\alpha_2$-macroglobulin d. C-reactive protein

**4. Emphysema is due to inhibition of:**
a. $\alpha_1$-antitrypsin b. Fibrinogen
c. Ceruloplasmin d. $\alpha_1$-fetoprotein

**5. Hypoalbuminemia may be due to:**
a. Reduced synthesis
b. Malnutrition
c. Excessive losses
d. All of the above

**6. The antibody class which can pass through the placenta to protect fetus is:**
a. IgA b. IgG
c. IgM d. IgD

**7. Plasma albumin performs the following function, *except*:**
a. Maintenance of osmotic pressure
b. Transport
c. Clotting of blood
d. Buffering action

**8. Following are pentameric immunoglobulins:**
a. IgG b. IgM
c. IgD d. IgE

**9. IgM present on the surface of B lymphocyte is:**
a. Monomer b. Dimer
c. Pentamer d. Tetramer

**10. Which of the following antibody is responsible for anaphylactic type of hypersensitivity and allergy?**
a. IgG b. IgM
c. IgE d. IgD

**11. Which of the following protein estimation is useful for the diagnosis of foetal neural tube defects?**
a. Ceruloplasmin
b. Transferrin
c. $\alpha_1$-fetoprotein
d. γ-globulin

**12. The immunoglobulin having least concentration in serum is:**
a. IgA b. IgM
c. IgD d. IgE

**13. The immunoglobulins are classified on the basis of:**
a. Light chains
b. Heavy chains
c. Carbohydrate content
d. Electrophoretic mobility

**14. All immunoglobulins contain:**
a. 4 L chains
b. 4 H chains
c. 3 L chains
d. 2 L chains and 2 H chains

**15. The number of types of H chains identified in human is:**
a. 2 b. 3
c. 4 d. 5

**16. The number of variable region in L chain is:**
a. 1 b. 2
c. 3 d. 4

**17. The number of variable region in H chain is:**
a. 1 b. 2
c. 3 d. 4

**18. Type γ heavy chain is present in:**
a. IgG b. IgA
c. IgM d. IgD

**19. Type α heavy chain is present in:**
a. IgE b. IgA
c. IgM d. IgD

**20. Type μ heavy chain is present in:**
a. IgG b. IgA
c. IgM d. IgD

**21. Type δ heavy chain is present in:**
a. IgG b. IgA
c. IgM d. IgD

**22. Type ε heavy chain is present in:**
a. IgA b. IgM
c. IgD d. IgE

**23. A 'J' chain is present in:**
a. IgD b. IgM
c. IgG d. IgE

**24. A pentamer immunoglobulin is:**
a. IgG b. IgA
c. IgM d. IgE

**25. The portion of the immunoglobulin molecule that binds the specific antigen is formed by:**
a. Variable regions of H and L chains
b. Constant region of H chain
c. Constant region of L chain
d. Hinge region

## Answers for MCQs

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **1.** d | **2.** a | **3.** a | **4.** a | **5.** d | **6.** b | **7.** c | **8.** b |
| **9.** a | **10.** c | **11.** c | **12.** d | **13.** b | **14.** d | **15.** d | **16.** a |
| **17.** a | **18.** a | **19.** b | **20.** c | **21.** d | **22.** d | **23.** b | **24.** c |
| **25.** a | | | | | | | |

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CHAPTER

6

# Enzymes

Chapter outline


- Definition of Enzyme
- Zymogen or Proenzyme
- Cofactors (Coenzyme and Activator)
- How Enzymes Work
- Mechanism of Enzyme Action
- Enzyme Classification
- Specificity of Enzyme Action
- Factors Affecting the Velocity of Enzyme Reaction
- Enzyme Kinetics
- Enzyme Inhibition
- Allosteric Enzyme
- Isoenzyme
- Clinical Significance of Enzymes


## INTRODUCTION

Enzymes are biological materials with catalytic properties, i.e. they increase the rate of chemical reactions in biological and *in vitro* (in the laboratory) systems, that otherwise proceed very slowly. The study of enzymes and of the changes in the enzyme activity that occur in body fluids has become a valuable diagnostic tool for the elucidation of various diseases and for testing organ function.

## DEFINITION OF ENZYME

Enzymes are biological catalyst produced by living tissues. They are proteins (except a small group of RNA acting as ribozyme) that have the property of accelerating specific chemical reactions without being consumed in the process.

## ZYMOGEN OR PROENZYME

- A number of proteolytic enzymes found in the blood or in the digestive tract are present in an inactive (precursor) form, called ***zymogen*** or **proenzymes**.
- For example, chymotrypsin is secreted by the pancreas as **chymotrypsinogen**. It is activated in the digestive tract by the proteolytic enzyme trypsin.
- Precursor proteins or inactive enzyme names have the prefix "***pro***" like prothrombin, proelastase, etc. or suffix "***ogen***" like chymotrypsinogen, trypsinogen, pepsinogen, etc.

## COFACTORS (COENZYME AND ACTIVATOR)

- Some enzymes require an additional non-protein component for its optimum activity. This additional component is called ***cofactor*** which may be either loosely or tightly bound to the protein portion of the enzyme.
- These cofactors may be:
  - Organic compounds, called ***coenzymes***.
  - Inorganic ions, called ***activators***.
- Enzymes without its cofactor are referred to as an **apoenzyme**; the complete catalytically active enzyme is called **holoenzyme**.

  Apoenzyme + cofactor = holoenzyme.
- Many vitamins function as coenzymes. Coenzymes derived from vitamins will be considered for more details in Chapter 7.
- The lists of coenzyme and activators are given in Tables 6.1 and 6.2, respectively.

## HOW ENZYMES WORK

### Energy Changes Occur during the Reaction

Virtually all chemical reactions have an energy barrier, separating the reactants and the products. This barrier, called the free energy of activation, is the energy difference between the energy of the reactant and high energy

**Table 6.1:** Some common coenzymes and their functions.

| *Vitamin* | *Coenzyme* | *Function as coenzyme* |
|---|---|---|
| Thiamine (Vit $B_1$) | TPP (Thiamine pyrophosphate) | Oxidative decarboxylation and transketolase reaction |
| Riboflavin (Vit $B_2$) reactions | FAD and FMN (Flavin Adenine Dinucleotide and Flavin Mononucleotide) | Oxidation and reduction |
| Niacin reactions | $NAD^+$ (Nicotinamide Adenine Dinucleotide), $NADP^+$ (Nicotinamide Adenine Dinucleotide Phosphate) | Oxidation and reduction |
| Pyridoxine (Vit $B_6$) decarboxylation | PLP (Pyridoxal phosphate) | Transamination, deamination reactions of amino acids |
| Biotin | Biocytin | Carboxylation reactions |
| Folic acid | THF (Tetrahydrofolate) | Carrier of one carbon group |
| Pantothenic acid | Coenzyme A | Acyl carrier |
| Cynocobalamin | Methylcobalamin and Deoxyadenosylcobalamin | Transfer of $CH_3$ group and isomerization |

intermediates that occurs during the formation of a product. **Figure 6.1** shows the changes in energy during the conversion of a molecule of reactant 'S' to product 'P' through the transition state S*.

**Fig. 6.1:** Comparison of the free energy of activation of a catalyzed and uncatalyzed reaction, S*: Transition state.

**Table 6.2:** Enzymes requiring or containing inorganic elements as cofactors (activators).

| *Enzyme* | *Cofactor (activator)* |
|---|---|
| Ferroxidase (ceruloplasmin), Ascorbic acid oxidase | Copper |
| Carbonic anhydrase, DNA-polymerase, Porphobilinogen synthase, Carboxypeptidase | Zinc |
| Cytochrome oxidase, Catalase | Iron |
| Glucose-6-Phosphatase, Hexokinase | Magnesium |
| Glutathione peroxidase | Selenium |
| Arginase, Pyruvate carboxylase | Manganese |
| Xanthine oxidase | Molybdenum |

$$\underset{\text{(Reactant)}}{S} \rightleftharpoons S^* \rightleftharpoons \underset{\text{(Product)}}{P}$$

The peak of free energy activation, represents the transition state, in which the high energy intermediates (S*) are formed during the conversion of a reactant to a product **(Fig. 6.1)**.

- An enzyme lowers the energy required for activation to the transition state.
- Without a catalyst, the reaction will occur only if enough heat energy is added to the reaction system.
- ***With an enzyme* as a *catalyst, the reaction may easily proceed at the normal physiological temperature*.**

## MECHANISM OF ENZYME ACTION

- Formation of an enzyme-substrate (ES) complex is the first step in enzymatic catalysis.
- Substrate is bound through multiple noncovalent interactions at the **active site** of the enzyme forming an **enzyme-substrate (ES)** complex which is subsequently converted to product and free enzyme.

$$E + S \rightleftharpoons ES \longrightarrow E + P$$

- The active site of an enzyme is the region that binds the substrate and which contains the specific amino acid residues.
- Two models for substrate binding to the active site of the enzyme have been proposed to explain the specificity that an enzyme has for its substrate:
  1. Lock and key model or rigid template model of Emil Fisher.

**Fig. 6.2:** Representation of formation of an ES-complex according to the Fisher's lock and key model. The active site of the enzyme is complementary in shape to that of substrate.

2. Induced fit model or hand-in-glove model of Daniel E Koshland.

## Lock and Key Model or Rigid Template Model of Emil Fisher

- This model was proposed by Emil Fisher in 1890.
- In this model, enzyme is preshaped and the active site has a rigid structure that is complementary to that of the substrate **(Fig. 6.2)**.
- This model is called lock and key model, because in this model the substrate fits into the active site in much the same way that a key fits into a lock.
- This model has been useful in understanding how some enzymes can bind only a specific substrate but will not bind another compound with an almost identical structure. For example, most enzymes in carbohydrate metabolism can bind the D-isomer of hexoses but cannot bind the corresponding L-isomer, which differs only in the configuration around a single carbon atom.
- This model explains all mechanisms but do not explain the changes in the enzyme activity in the presence of allosteric modulators.

## Induced Fit Model or Hand-in-glove Model of Daniel E Koshland (Fig. 6.3)

Fisher's model explained the specificity of enzyme substrate interaction but the implied rigidity of the enzymes active site failed to explain the dynamic changes that must take place during catalysis. A model that accounts for both of these aspects of enzyme catalysis is the **induced fit model of Daniel E Koshland**.

- In the Fisher's model the catalytic site is presumed to be preshaped to fit the substrate. However, Daniel E Koshland in 1958 postulated that the **enzymes are flexible and shapes of the active site can be modified by the binding of the substrate**.
- In the induced fit model, the substrate induces a conformational change in the enzyme, in the same manner in which placing a hand (substrate) into a glove (enzyme) induces changes in the glove's shape. Therefore, this model is also known as **hand-in-glove model**.
- Conformational change in enzyme in turn induces reciprocal changes in its bound substrate that alters their orientation and configuration and strains the structure of the bound substrate. Due to such changes energy is liberated, which is called **intrinsic binding energy (Fig. 6.3)**.
- This intrinsic binding energy due to the substrate-enzyme interaction is made available for the transformation of the substrate into product.
- This model is believed to describe more accurately the specificity of substrate binding than does lock and key model of E Fisher.

**Fig. 6.3:** Schematic representation of induced fit model of Koshland.

## ENZYME CLASSIFICATION

The classification of enzyme was described in 1961 by enzyme commission of the International Union of Biochemistry (IUB). According to this classification, each enzyme is characterized by a code number called **enzyme code number** or **"EC" number**, consisting of four digits.

According to the IUB system, enzymes are classified into six major classes as follows:

1. EC-1: Oxidoreductases
2. EC-2: Transferases
3. EC-3: Hydrolases
4. EC-4: Lyases
5. EC-5: Isomerases
6. EC-6: Ligases.

Some common examples of different classes of enzymes are given in **Table 6.3**.

## EC-1 Oxidoreductases

Those enzymes that catalyze oxidation-reduction reactions are included in this class which can be illustrated schematically as follows:

Enzymes in this category include:
- Dehydrogenases
- Reductases
- Oxidases
- Peroxidases.

### *Specific Example*

**Table 6.3:** Examples of enzymes with their main class.

| *Class* | *Example* |
|---|---|
| EC-1: Oxidoreductases | Lactate dehydrogenase (LDH) |
| | Glucose 6-phosphate dehydrogenase |
| | (G-6-PD) |
| | Cytochrome oxidase |
| EC-2: Transferases | Aspartate aminotransaminase (AST) |
| | Alanine aminotransaminase (ALT) |
| | Hexokinase |
| EC-3: Hydrolases | Lipase |
| | α-Amylase |
| | Trypsin |
| | Lactase |
| | Sucrase |
| | Pepsin |
| EC-4: Lyases | Aldolase |
| | Argininosuccinase |
| | Carbonic anhydrase |
| EC-5: Isomerases | Phosphoglucomutase |
| | Triphosphate isomerase |
| | Phosphohexose isomerase |
| EC-6: Ligases | Glutamine synthetase |
| | Pyruvate carboxylase |
| | DNA ligases |

## EC-2 Transferases

Those enzymes that catalyze the transfer of a group such as, amino, carboxyl, methyl or phosphoryl, etc., from one molecule to another are called transferases. These reactions can be illustrated as follows:

Some common enzymes in this category include:
- Amino transferase or transaminase
- Kinase
- Transcarboxylase.

### *Specific Example*

Where, ALT: Alanine aminotransferase
PLP: Pyridoxal phosphate (coenzyme).

## EC3 Hydrolases

Enzymes of this class catalyze the cleavage of **C-O**, **C-N**, **C-C**, and some other bonds with the addition of water. These can be illustrated as follows:

Some common enzymes in this category are:
- Acid phosphatase
- All digestive enzymes like α-amylase, pepsin, trypsin, chymotrypsin, etc.

### *Specific Example*

## EC-4 Lyases

Lyases catalyze the cleavage of **C-O**, **C-C**, and **C-N** bonds by means other than hydrolysis or oxidation, giving rise to compound with double bonds or catalyze the reverse reaction, by the addition of group to a double bond. In cases where reverse reaction is important, then synthase, (not synthetase of group EC-6) is used in the name. This type of reaction is illustrated as follows:

### Specific Example

## EC-5 Isomerases

Isomerases catalyze intramolecular structural rearrangement in a molecule. They are called **epimerases, isomerases** or **mutases,** depending on the type of isomerism involved. This reaction can be illustrated as follows:

### Specific Example

## EC-6 Ligases (Synthetases)

Ligases catalyze the joining of two molecules coupled with the hydrolysis of ATP. The reaction is illustrated as follows:

### Specific Example

# SPECIFICITY OF ENZYME ACTION

Specificity refers to the ability of an enzyme to discriminate between two competing substrates. Enzymes are highly specific both in the reaction catalyzed and in their choice of substrates. Specificity makes it possible for a number of enzymes to co-exist in the cell without interfering in each other's actions.

## Types of Specificity

The following types of specificity have been recognized:

1. Substrate specificity
2. Reaction specificity
3. Stereo specificity.

### Substrate Specificity

There are following types of substrate specificity:

i. Absolute substrate specificity
ii. Relative substrate specificity
iii. Broad substrate specificity.

#### *Absolute substrate specificity*

Certain enzymes will act on only one substrate and catalyze one reaction, e.g. glucokinase, lactase, urease, etc.

#### *Relative substrate specificity*

In this case, enzyme acts on more than one substrate. The relative substrate specificity is of two types:

a. Group specificity
b. Bond specificity.

- In **group specificity,** an enzyme acts on more than one substrate containing a particular group, e.g. chymotrypsin acts on several proteins by hydrolyzing peptide bonds attached to aromatic amino acids. Trypsin hydrolyzes peptide linkages involving arginine or lysine.
- In **bond specificity,** an enzyme acts on more than one substrate containing a particular kind of bond, e.g. salivary α-amylase cleaves α-(1→4) glycosidic bonds of carbohydrates, lipase hydrolyzes ester bonds of lipids.

#### *Broad substrate specificity*

In this case, an enzyme acts on more than one structurally related substrates, e.g. hexokinase catalyzes the phosphorylation of more than one kind of hexoses such as glucose, fructose, and mannose.

### Reaction Specificity

In this case, an enzyme is specific to a particular reaction but not to substrate(s) and catalyzes only one type of reaction. For example, pyruvate can undergo several reactions. Each reaction is catalyzed by a separate enzyme, which catalyzes only that reaction and none other as shown in **Figure 6.4**.

### Stereo Specificity

Many enzymes show specificity toward stereoisomers, i.e. they act on only one type of isomer. For example,

- L-lactate dehydrogenase will act only on L-lactic acid and not D-lactic acid.
- Likewise, L-amino acid oxidase and D-amino acid oxidase are distinct enzymes which act only on L and D-amino acids respectively.

**Fig. 6.4:** Example of reaction specificity.

- D-glucose oxidase can similarly act only on D-glucose and not on L-glucose.
- Salivary α-amylase acts on the α-1,4 glycoside linkage and is inactive on β-1,4 glycoside linkage.
- Isomerase and epimerase do not show stereospecificity.

## FACTORS AFFECTING THE VELOCITY OF ENZYME REACTION

Various factors that affect enzyme activity are:

- Substrate concentration
- Enzyme concentration
- pH, i.e. $H^+$ ion concentration
- Temperature
- Product concentration
- Activators and coenzymes
- Time
- Physical agents
- Inhibitors.

### Effect of Substrate Concentration

For a given quantity of enzyme, the velocity of the reaction increases as the concentration of the substrate is increased. At first, this relationship is almost linear but later, the reaction curve becomes hyperbolic in shape **(Fig. 6.5)**.

- At relatively low concentrations of substrate, V**0** increases almost linearly with an increase in substrate concentration [S], a condition known as **first order kinetics**.
- At higher substrate concentrations, $V_0$ increases by smaller amounts in response to increase in substrate concentration [S].
- Finally, a part is reached beyond which there are only vanishingly small increase in $V_0$ with increasing [S], a condition known as ***zero order kinetics*** and a plateau is called **maximum velocity**, $V_{max}$ **(Fig. 6.5)**. Under these conditions, all the free enzymes will have been converted into ES form so that any further increase in substrate concentration has no effect on the rate and the reaction achieves a steady state.

**Fig. 6.5:** Effect of substrate concentration [S] on enzyme activity keeping enzyme concentration constant.

Where, $V_0$ : initial velocity
$V_{max}$ : maximum velocity
Km : $1/2\ V_{max}$ = Michaelis-Menten constant
[S] : substrate concentration

### Effect of Enzyme Concentration

- The velocity of a reaction is directly proportional to the amount of enzyme present as long as the amount of substrate is not limiting.
- The substrate must be present at a concentration sufficient to ensure that all of the enzyme molecules have substrate bound to their active site **(Fig. 6.6)**.

### Effect of Hydrogen Ion Concentration pH

- Each enzyme has an ***optimum pH***, i.e. a pH at which the enzyme activity is maximum. Below or above this pH, enzyme activity is decreased. The optimum pH differs from enzyme to enzyme, e.g. optimum pH for:
  - Pepsin = 1.2
  - Trypsin = 8.0
- A bell-shaped curve is obtained when enzyme velocity is plotted against pH **(Fig. 6.7)**.
- Changes in pH can alter the following:
  - Ionization state of the amino acid residues present in the active site of the enzyme. Enzyme activity is related to the ionic state of active site of the enzyme.
  - The ionization state of the substrate. The active site of an enzyme may require particular ionic state of the substrate for optimum activity.
  - Drastic change in pH denatures the enzyme protein.

All these affect the enzyme substrate complex formation and decrease the rate of enzyme reaction. ***As pH***

**Fig. 6.6:** Effect of enzyme concentration on enzyme activity.

**Fig. 6.8:** Effect of temperature on enzyme activity.

***affects the enzyme activity, in enzyme studies, buffers are used to keep enzyme at an optimum or at least a favorable $H^+$ ion concentration.***

## Effect of Temperature

- Enzyme catalyzed reactions show an increase in rate with increasing temperature only within a relatively small and low temperature range.
- Each enzyme shows the highest activity at a particular temperature called ***optimum temperature***.
- The activity progressively declines both above and below this temperature.
- A bell-shaped curve is obtained when we plot the enzyme velocity vs temperature **(Fig. 6.8)**.
- Increase in velocity is due to the increase in the kinetic energy. Further elevation of the temperature results in a decrease in reaction velocity due to denaturation of the enzyme protein.
- Low temperature also decreases enzyme activity and enzymes may be completely inactive at temperature of 0°C and below.
- The inactivity at low temperature is reversible. So, many enzymes in tissues or extracts may be preserved for months by storing at –20°C or –70°C.
- The reaction velocity of most chemical reactions increases with temperature approximately doubles for each 10°C rise called temperature coefficient $Q_{10}$.
- Most of the body enzymes have the optimum temperature close to 37°C to 38°C and have progressively less activity as the temperature rises.

**Fig. 6.7:** Effect of pH on enzyme activity.

## Effect of Product

Accumulation of products of the reaction causes the inhibition of enzyme activity for some enzymatic reactions; this form of control will limit the rate of formation of the product when the product is under used. In biological systems, however, the product is usually removed as it becomes a substrate for a succeeding enzyme in a metabolic pathway.

## Effect of Activators and Coenzymes

The activity of many enzymes is dependent on the activators (metallic ions) like $Mg^{2+}$, $Mn^{2+}$, $Zn^{2+}$, $Ca^{2+}$, $Co^{2+}$, $Cu^{2+}$, etc. and coenzymes for their optimum activity. In absence of these activators and coenzymes, enzymes become functionally inactive.

## Effect of Time

Under optimum conditions of pH and temperature, time required for an enzyme reaction is less. The time required

for the completion of an enzyme reaction increases with changes in temperature and pH from its optimum.

### Effect of Physical Agents

Physical agent like, light rays can inhibit or accelerate certain enzyme reactions. For example, the activity of salivary amylase is increased by red and blue light. On the other hand, it is inhibited by ultraviolet rays.

### Effect of Inhibitors

The substances which stop the enzymatic reaction are called inhibitors. Presence of these substances in reaction medium decreases the rate of enzyme reaction. Different types of enzyme inhibitors are discussed separately later.

## ENZYME KINETICS

The study of enzyme reaction rates and how they change in response to changes in experimental parameters is known as ***kinetics***.

One of the key factors affecting the rate of a reaction catalyzed by an enzyme *in vitro* (in laboratory) is the amount of substrate present, [S]. The effect on $V_0$ (initial velocity) of varying substrate [S] concentration, when enzyme concentration is held constant, is shown in **Figure 6.5**.

### Michaelis-Menten Equation

Enzyme catalyzed reactions occur in two stages as shown in the following equations

$$E + S \underset{K_2}{\overset{K_1}{\rightleftharpoons}} ES \quad \text{.....1)}$$

$$ES \xrightarrow{K_3} E + P \quad \text{..... 2)}$$

$K_1$, $K_2$, and $K_3$ are rate constant. The Michaelis-Menten equation describes how reaction velocity varies with substrate concentration. The relationship between reaction rate and substrate concentration shown in **Figure 6.5** is described mathematically by the "Michaelis-Menten equation" as follows:

$$V_0 = \frac{V_{max}\,[S]}{K_m + [S]}$$

where,

$V_0$ = initial reaction velocity (is the rate of reaction as soon as enzyme and substrates are mixed).

$V_{max}$ = maximum velocity (is reached when all active sites on the enzyme are filled with substrate, i.e. enzyme is in the E-S complex).

$K_m$ = Michaelis-Menten constant [is the substrate concentration, at which the reaction rate is half of its maximum velocity ($V_{max}$)].

[S] = Substrate concentration.

Every enzyme has the characteristics $V_{max}$ and $K_m$ which are sensitive to change in pH, temperature and ionic strength.

#### *Significance of $K_m$ (Michaelis Constant)*

- $K_m$, the Michaelis constant is the substrate concentration at which the reaction rate is half of its maximal value. The $K_m$ value of an enzyme depends on the substrate and environmental conditions such as pH, temperature and ionic strength.
- The Michaelis constant, $K_m$ has two significances:
  1. $K_m$ provides a measure of the substrate concentration required for significant catalysis to occur.
  2. It is a measure of the **affinity of the enzyme for its substrate**, a high $K_m$ indicates low affinity and weak binding and a low $K_m$ indicates high affinity and strong binding with its substrate.

#### *Significance of $V_{max}$ (Maximal Velocity)*

- The $V_{max}$ of a reaction is an index of the catalytic efficiency of an enzyme. $V_{max}$ is reached when all active sites on the enzyme are filled with substrate, i.e. enzyme is in the E-S complex.
- The $V_{max}$ is useful in comparing the activity of one enzyme with that of another.

### Lineweaver-Burk Plot or Double-Reciprocal Plot

Lineweaver-Burk plots are used to obtain values for $V_{max}$ and $K_m$. A more accurate method of determining values for $V_{max}$ and $K_m$ uses Lineweaver-Burk equation shown below. This equation is obtained by taking the reciprocal of the Michaelis-Menten equation.

$$\frac{1}{V_0} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$$

- When $1/V_0$ is plotted against $1/[S]$, a straight line is obtained (**Fig. 6.9**), with a slope equal to $K_m/V_{max}$.

- The point, at which line intersects the y-axis, is numerically equal to $1/V_{max}$.
- The point at which the line intersects the x-axis is numerically equal to $-1/K_m$.
- This plot is useful to determine the mechanism of action of enzyme inhibitors.

## ENZYME INHIBITION

Any substance that can diminish the velocity of an enzyme catalyzed reaction is called inhibitor. Two general classes of inhibitors are recognized according to whether the inhibitor action is **reversible** or **irreversible (Fig. 6.10)**.

1. Reversible inhibitor
2. Irreversible inhibitor.

### Reversible Inhibitor

- Reversible inhibitors bind to enzymes through **non-covalent** bonds and the activity of the enzyme is restored fully when the inhibitor is removed from the system.
- Different types of reversible inhibitors are:
  i. Competitive or substrate analogue inhibitor
  ii. Non-competitive inhibitor
  iii. Uncompetitive inhibitor.

**Fig. 6.9:** Lineweaver-Burk plot (Double reciprocal plot).

### Competitive or Substrate Analogue Inhibitor

- A competitive inhibitor is usually a structurally similar (analogue) to the substrate.
- The chemical structure of the inhibitor (I) closely resembles that of the substrate (S) and binds to the enzyme at the **active site**, forming an EI complex rather than ES-complex **(Fig. 6.11)**.
- But because it is not identical with the substrate, breakdown into products does not take place.
- When both the substrate and this type of inhibitor are present, they compete for the same binding site on the enzyme.
- The competitive inhibition is reversed by increasing substrate concentration.
- In competitive inhibition, **$V_{max}$ remains unchanged** because inhibitor binding is **reversible** and can overcome by high concentrations of the substrate. However substrate binding to the enzyme is impaired and the binding affinity is decreased. Therefore competitive inhibitors increase $K_m$ **(Fig. 6.12)**.
- The classical example is the competitive inhibition of **succinate dehydrogenase** by the **malonate**.
- Succinate dehydrogenase is one of the enzymes of the citric acid cycle. Succinate dehydrogenase is inhibited by malonate which resembles succinate.
- Many drugs which act as competitive inhibitors are given below and some more are given in **Table 6.4**.
  - Sulfonamide (antibiotic)
  - Isoniazide [Isonicotinic acid hydrazine (INH)] (antituberculous drug)
  - Dicumarol (anticoagulant drug)
  - Physostigmine (to treat glaucoma and myasthenia gravis) inhibit **acetylcholinesterase**.

**Fig. 6.10:** Classes of enzyme inhibitors.

**Fig. 6.11:** Diagrammatic representation of competitive inhibition. (E: Enzyme; S: Substrate; I: Competitive inhibitor; P: Product)

**Fig. 6.12:** A double reciprocal plot of enzyme kinetics in presence and absence of competitive inhibitor, $V_{max}$ is unaltered whereas $K_m$ is increased.

## *Non-competitive Inhibitors*

- As the name implies, in this type of inhibition no competition occurs between substrate and inhibitor. Inhibitor is usually structurally different from the substrate.
- It binds at a site on the enzyme molecule other than the substrate-binding site and thus there is no competition between inhibitor and substrate.
- Since inhibitor and substrate may bind at different sites; formation of both EI and EIS-complexes is possible **(Fig. 6.13)**.
- They do not necessarily prevent substrate binding, but they block enzymatic catalysis. Thus it reduces $\mathbf{V_{max}}$ without changing $\mathbf{K_m}$ value **(Fig. 6.14)**. Inhibition cannot be overcome by increasing substrate concentration.
- Examples of non-competitive inhibitors are:
  - **Ethanol** or certain **narcotic drugs** are noncompetitive inhibitor of **acid phosphatase**.
  - **Trypsin inhibitors** occur in **soybean** and **raw egg white**, inhibit activity of trypsin non-competitively.
  - As well as ***Ascaris* parasites** (worm) contain **pepsin** and **trypsin inhibitors**, which inhibit noncompetitively action of pepsin and trypsin that is why Ascaris worm is not digested in human intestine.

## *Uncompetitive Inhibitor*

- Uncompetitive inhibitor can bind only to the enzyme-substrate (ES) complex but not to the free enzyme **(Fig. 6.15)**.
- It does not have affinity for free enzyme. Uncompetitive inhibitor **decreases both $\mathbf{V_{max}}$** and **$\mathbf{K_m}$ (Fig. 6.16)**.
- This form of inhibitor is rare with single substrate but more common in multiple substrate reaction.

## Irreversible Inhibitor

- An irreversible inhibitor binds with an enzyme tightly **covalently** and forms a stable complex.
- An irreversible inhibitor cannot be released by dilution or dialysis or simply by increasing the concentration of substrate.
- Irreversible inhibitors can be divided into three categories:

**Table 6.4:** Commonly used drugs that are enzyme inhibitors.

| *Drug* | *Type of inhibition* | *Target enzyme* | *Therapeutic use* |
|---|---|---|---|
| Mevinolin<br>Lovastatin | Competitive | HMG-CoA reductase (**3**-Hydroxy-**3**-Methyl-Glutaryl CoA-reductase) | Hypercholesterolemia |
| Captopril<br>Enalapril<br>Lisinopril | Competitive | Angiotensin-converting enzyme (ACE) | Antihypertensive |
| Allopurinol | Competitive | Xanthine oxidase | Gout |
| Methotrexate | Competitive | Dihydrofolate reductase | Cancer |
| 5-Fluorouracil | Suicide | Thymidylate synthase | Cancer |
| Aspirin | Suicide | Cyclooxygenase | Anti-inflammatory |
| Penicillin | Suicide | Bacterial transpeptidase | Antibacterial |
| Disulfiram | Suicide | Aldehyde dehydrogenase | Antabuse |

**Fig. 6.13:** Diagrammatic representation of noncompetitive inhibition. (E = Enzyme; S = Substrate; I = Non-competitive inhibitor; P = Product)

i. Group specific inhibitors
ii. Substrate analogue inhibitor or affinity labels
iii. Suicide inhibitor or mechanism based inactivation.

- In terms of enzyme kinetics, the effect of an irreversible inhibitor is like that of the **reversible non-competitive** inhibitors resulting in a **decreased in $V_{max}$** but having **no effect on the $K_m$ (Table 6.5)**.

**Fig. 6.14:** A double reciprocal plot of enzyme kinetics in presence and absence of non-competitive inhibitor; $K_m$ is unaltered by non-competitive inhibitor, whereas $V_{max}$ is decreased.

## *Group Specific Irreversible Inhibitor*

These inhibitors react with specific R-groups (side chain) of amino acid residues in the active site of enzyme. Examples of group specific irreversible inhibitors are:

- Diisopropylphosphofluoride (DIPF): DIPF inhibits **acetylcholine esterase**.
- *Iodoacetamide and heavy metals like, $Pb^{2+}$, $Ag^{+}$, $Hg^{2+}$, etc.*

## *Substrate Analogue Irreversible Inhibitor or Affinity Labels*

- Substrate analogues or affinity labels are molecules that are structurally similar to the substrate.
- These substrate analogues possess a highly reactive group which is not present in the natural substrate.
- The reactive group of substrate analogues covalently reacts with amino acid residues of the active site of the enzyme and permanently block the active site of the enzyme, e.g. 3-Bromoacetyl phosphate (BAP).

**Fig. 6.15:** Uncompetitive inhibitor binds only to the enzyme-substrate complex. (E = Enzyme; S = Substrate; I = Uncompetitive inhibitor; P = Product).

**Table 6.5:** Effect of inhibitors on kinetic properties of enzymes.

| *Type of inhibitor* | *$K_m$* | *$V_{max}$* |
|---|---|---|
| Irreversible | No effect | Decreased |
| Reversible competitive | Increased | No effect |
| Reversible non-competitive | No effect | Decreased |
| Reversible uncompetitive | Decreased | Decreased |

- BAP is a substrate analogue of the normal substrate dihydroxyacetone phosphate (DHAP) for the enzyme phosphotriose isomerase of glycolysis.

### *Suicide Inhibitor or Mechanism-based Inactivation*

- These compounds are relatively unreactive until they bind to the active site of a specific enzyme.
- On binding to the active site of the enzyme they carry out the first few catalytic activities of the normal enzyme reaction.
- Instead of being transformed into a normal product, however, the inhibitor is converted to a very reactive compound that combines irreversibly with the enzyme leading to its irreversible inhibition.

**Fig. 6.16:** Effect of uncompetitive inhibitor on the double reciprocal plot; it shows parallel lines with decrease in both $V_{max}$ and $K_m$.

- The enzyme literally commits suicide. These are also called **mechanism-based inactivation** because they utilize the normal enzyme reaction mechanism to inactivate the enzyme.
- These inhibitors act as drugs, for example
  **Penicillin**
  **Aspirin**
  **Disulfiram** (antabuse)

Disulfiram is a drug used in the treatment of alcoholism. It inhibits irreversibly **aldehyde dehydrogenase** enzyme resulting in accumulation of acetaldehyde in the body **(Fig. 6.17)**.

### *Clinical Application of Enzyme Inhibitor*

Enzyme inhibitors have therapeutic applications. Most antibiotics and anticancer drugs that are used therapeutically are either competitive inhibitor or mechanism based suicide inhibitor. Some commonly used drugs that exert their effects by inhibiting enzymes are described in **Table 6.4**.

## ALLOSTERIC ENZYME

Allosteric enzyme is a **regulatory enzyme**. The term allosteric derives from Greek word, **allo** means **other** and **steros** means **space** or **site**. ***Allosteric enzymes are those having other site***.

Like all enzymes, allosteric enzymes have active site for binding of the substrate but they also have one or more regulatory (or allosteric) sites for binding regulatory metabolites which is called **modulator**.

- Allosteric enzymes may be inhibited or stimulated by their modulators **(Table 6.6)**.
- Modulators that inhibit enzyme activity are termed ***negative modulators***. Whereas those that increase enzyme activity are called ***positive modulators***.

- Just as the active site of an enzyme is specific for its substrate, the allosteric site is specific for its modulators. Allosteric enzymes are generally larger and more complex than those of simple enzymes.

### Feedback Allosteric Inhibition

In some multienzyme systems, the first enzyme of the sequence is the regulatory enzyme and has distinctive characteristics.

- It is inhibited by the end product of the multienzyme system whenever the end product of such metabolic reaction produced in excess of the cell's needs. The end product of the pathway acts as a specific inhibitor of the first or regulatory enzyme in the pathway.
- The whole enzyme system thus slows down to bring the rate of production of its end product back into balance with the cell's needs.

This type of regulation is called ***feedback inhibition***. For example, first enzyme δ-**aminolevulinic acid synthase** is an allosteric enzyme in heme synthesis is inhibited by its end product heme **(Fig. 6.18)**.

## ISOENZYME

- Isoenzymes or isozymes are multiple forms (isomers) of the same enzyme that catalyze the same biochemical reaction. Isoenzymes show different chemical and physical properties like electrophoretic mobility and kinetic properties.
- Not all enzymes have isoenzymes. In fact, it was found that only those enzymes, which are active in polymeric form, demonstrate isoenzyme. For example:

**Fig. 6.17:** Irreversible suicide inhibitor or mechanism-based inactivation of **aldehyde dehydrogenase** enzyme by Disulfiram (antabuse).

1. Lactate dehydrogenase (LDH)
2. Creatine kinase (CK) (formerly called creatine phosphokinase (CPK).

- Some more examples of isoenzymes are given in **Table 6.7**.

### Lactate Dehydrogenase (LDH)

Lactate dehydrogenase is a tetrameric enzyme that catalyzes the oxidation of L-lactate to pyruvate.

- LDH has five isoenzymes:
  - $LDH_1$
  - $LDH_2$
  - $LDH_3$
  - $LDH_4$
  - $LDH_5$
- Since LDH is a **tetramer,** made up of two types of polypeptide **M (*muscle*)** type and **H (*heart*)** type, five combinations are possible with varying ratios of two kinds of polypeptides **(Table 6.8)**.
- Five isoenzymes of LDH can be detected by electrophoresis as they have different electrophoretic mobilities.
- **$LDH_1$** is the **fastest moving** fraction toward the anode and **$LDH_5$** is the **slowest moving** isoenzyme of LDH **(Fig. 6.19)**.
- $LDH_1$ predominates in cells of cardiac muscle, and erythrocytes and $LDH_5$ is the most abundant form in the liver and in skeletal muscle **(Table 6.8)**.

#### *Clinical Applications of LDH*

The LDH isoenzyme analysis may be useful in the following clinical situations **(Table 6.8)**:

- Significant elevation of $LDH_1$ and $LDH_2$ ($LDH_1$>$LDH_2$) occurs within 24 to 48 hours after myocardial infarction.
- Predominant elevation of $LDH_2$ and $LDH_3$ occur in leukemia. $LDH_3$ is the main isoenzyme elevated due to malignancy of many tissues.
- Elevation of $LDH_5$ occurs after damage to the liver or skeletal muscle.

**Table 6.6:** Allosteric enzymes and its modulators.

| *Pathway* | *Enzyme* | *Inhibitor* | *Activator* |
|---|---|---|---|
| Glycolysis | Phosphofructokinase-I | ATP | AMP |
| Pyruvate to acetyl-CoA | Pyruvate dehydrogenase | ATP | - |
| TCA cycle | Isocitrate dehydrogenase | ATP | ADP |
| Gluconeogenesis | Pyruvate carboxylase | - | Acetyl-CoA |
| Fatty acid synthesis | Acetyl-CoA carboxylase | - | Citrate |

## Creatine Kinase (CK)

- Creatine kinase isoenzymes are **dimer** that are made up of two types of polypeptide chains, which may be either **M *(muscle)*** type or **B *(brain)*** type, generating three isoenzymes **(Table 6.8)**.
  - $CK_1$ (BB): It is present in the brain

**Fig. 6.18:** Feedback inhibition.
First enzyme δ-ALA-synthase is an allosteric enzyme inhibited by its end product heme.

**Table 6.7:** Examples of isoenzymes.

| *Enzyme* | *Isoenzyme forms* |
|---|---|
| Acid phosphatase | Prostate, erythrocytes, platelets, liver, spleen, kidney, and bone marrow |
| Alkaline phosphatase | Bone, liver, placenta, intestine, and kidney |
| Amylase | Salivary and pancreatic |
| Hexokinase | Liver (glucokinase) and muscle |

  - $CK_2$ (MB): Cardiac tissue is the only tissue which has the mixed MB ($CK_2$) isoenzyme
  - $CK_3$ (MM): It is present in skeletal muscle.

### *Clinical Application*

- $CK_1$ may be elevated in neonates particularly in damaged brain or very low birth weight new-born.
- Increased level of **$CK_2$** in blood is characteristic of **damage of heart** tissue from myocardial infarction because cardiac tissue is the only tissue which has the mixed MB ($CK_2$) isoenzyme.
- Elevated levels of **$CK_3$** in serum occur in all types of **dystrophies** and **myopathies.**

## CLINICAL SIGNIFICANCE OF ENZYMES

Certain enzymes are used:
- For the diagnosis of the disease
- As therapeutic agents
- As analytical reagents.

### Diagnostic Use of Enzymes

Enzymes are known as marker of **cellular damage** and their measurement in plasma is used in the investigation of diseases of **liver, heart, skeletal muscle,** the **biliary tract,** and the **pancreas**. The enzymes that are found in plasma can be categorized into two major groups: The **plasma specific** enzyme and the **plasma nonspecific** enzyme.
- The **plasma specific** enzymes are present in higher concentration in plasma than in most tissues; e.g.

**Fig. 6.19:** Electrophoretic separation of LDH isoenzyme.

**Table 6.8:** Type, composition, location, and diagnostic importance of lactate dehydrogenase (LDH) and creatine kinase (CK) isoenzymes.

| *Type* | *Composition* | *Location* | *Diagnostic importance (cause of elevated level)* |
|---|---|---|---|
| $LDH_1$ | HHHH | Heart, RBC | Myocardial infarction |
| $LDH_2$ | HHHM | Heart, RBC | Megaloblastic anemia |
| $LDH_3$ | HHMM | Brain | Leukemia, malignancy |
| $LDH_4$ | HMMM | Lung, spleen | Pulmonary infarction |
| $LDH_5$ | MMMM | Liver, muscle | Liver diseases, muscle damage/diseases |
| $CK_1$ | BB | Brain | Neurological injury |
| $CK_2$ | BM | Heart | Myocardial infarction |
| $CK_3$ | MM | Skeletal muscle | Muscular dystrophies and myopathies |

- The enzymes involved in blood coagulation
- Ferroxidase
- Pseudocholinesterase
- Lipoprotein lipase.

- These enzymes are clinically of interest when their concentration decreases in plasma.
- The **plasma nonspecific** enzymes are present in very high concentration in tissues than in the plasma. Estimation of plasma nonspecific enzymes is very important for the diagnosis of several diseases.
- The important enzymes useful for the diagnosis of specific diseases are listed in **Table 6.9**. A brief account of selected diagnostic enzymes is discussed.

### Alanine Transaminase (ALT)

- Alanine transaminase was known formerly as glutamate pyruvate transaminase (GPT).
- The plasma ALT normal value for adult is 10 to 40 U/L.
- ALT level is elevated in **liver diseases** (viral or toxic hepatitis), **jaundice** and **cirrhosis** of liver.

### Aspartate Transaminase (AST)

- It was known formerly as glutamate oxaloacetate transaminase (GOT).
- The plasma AST normal value for adults is 10 to 30 U/L.
- Increased AST level occurs after **myocardial infarction**. The plasma AST level starts increasing after 6 to 8 hours after the onset of chest pain with peak values 18 to 24 hours and the values fall to normal level by the fourth or fifth day.
- It is moderately elevated in liver disease.

### Alkaline Phosphatase (ALP)

- ALP hydrolyzes organic phosphate at alkaline pH.
- Normal serum level for adults is 3–13 KA units/dL.
- It is elevated in certain **bone** and **liver disease**.
- Very high levels may be noticed in **obstructive jaundice, bone diseases** such as **Paget's disease**, rickets, osteomalacia, carcinoma of bone and hyperparathyroidism.

### Acid Phosphatase (ACP)

- It hydrolyzes phosphoric acid ester at pH 5 to 6.
- Normal serum value for ACP is 0.5 to 4 KA units/dL.
- Prostatic acid phosphatase enzyme is useful for the diagnosis and prognosis of **prostate cancer**. ACP is therefore an important **tumor marker**.

### Amylase

- It catalyzes hydrolysis of starch and glycogen.
- Normal serum value is 50–120 U/L.
- The activity of serum amylase is increased in **acute pancreatitis**.
- Elevated activity of amylase is also found in urine of the patient of acute pancreatitis.
- Increase in serum levels are also seen in **chronic pancreatitis**, **mumps**, and **obstruction of pancreatic duct**.

### Creatine Kinase (CK)

Refer isoenzyme.

### Lactate Dehydrogenase (LDH)

Refer isoenzyme.

### Prostate Specific Antigen (PSA Seminogelase)

- It is a glycoprotein with mild protease activity and involved in the liquification of the seminal coagulum formed after ejaculation.
- It is produced exclusively by prostate glands.
- Elevated blood levels of PSA occur in prostate cancer.

**Enzyme Assay in Myocardial Infarction**

The enzymes assay, which are most helpful in the diagnosis of myocardial infarction are: **Table 6.10 and Figure 6.20**

- Creatine kinase (CK)
- Lactate dehydrogenase (LD)
- Serum aspartate aminotransferase (AST)

- There is a rise in total CK activity following a myocardial infarction. The degree of increase varies with the extent of the tissue damage. CK is the first enzyme to appear in serum in higher concentration after myocardial infarction and is probably the first to return to normal levels if there is no further coronary damage. The CK-MB isoenzyme starts to increase within 3 to 10 hours after an acute myocardial infarction (AMI) and reaches a maximum within 24 hours and returns to normal after 2 to 3 days after the infarct.
- The serum activity of AST begins to rise about 6 to 12 hours after myocardial infarction and usually reaches its maximum value in about 24 to 48 hours and returns to normal after 4 to 6 days after the infarct.
- For patients having an AMI, serum LDH (heart specific) values become elevated about 8 to 16 hours after onset of symptoms, and usually reaches its maximum value in about 48 to 72 hours and returns to normal after 7 to 12 days after the infarct **(Fig. 6.20)**.

**Fig. 6.20:** Typical rise in serum enzyme activities following a myocardial infarction.

## Therapeutic Use of Enzymes

Some enzymes are used in the treatment of some diseases of human being. For example:

- **Bacterial asparginase** is used in the treatment of some types of leukemias. Asparginase hydrolyzes aspargine to aspartic acid. Aspargine is necessary for the formation of leukemic white cell.
- **Chymotrypsin:** Used for dissolving ligaments of the lens during the extraction of cataract.
- **Collagenase:** Used for debridement (cleaning of wound by removing dead tissue) of dermal ulcers and severe burns.
- **Fibrinolysin:** It is used in the venous thrombosis, pulmonary and arterial embolism (blood clot).
- **Hyaluronidase:** It is used to promote the rapid absorption of drugs injected subcutaneously. It acts by increasing tissue permeability. It is used in the treatment of traumatic or postoperative edema.
- **Lysozyme** (an antibiotic) found in human tears and egg white, is used in the infection of eye. It has antibacterial action, it acts on cellulose of bacteria.
- **Penicillinase** (bacterial enzyme) is used for the treatment of persons who are allergic to penicillin, penicillinase destroys penicillin.
- **Rennin** is used for the treatment of gastric achylia.
- **Streptokinase:** Streptokinase and urokinase are used in myocardial infarction to dissolve blood clot or purulent (containing pus) material. It causes fibrinolysis.
- **Trypsin:** It is a proteolytic enzyme and is used to clean the wounds by dissolving purulent material and in the treatment of acute thrombophlebitis to dissolve the blood clot.
- **Pepsin, trypsin peptidase, lipase, amylase elastase**, and **cellulase** are used in gastrointestinal tract (GIT) disorders and chronic pancreatitis.

**Table 6.9:** Enzymes of diagnostic importance.

| *Enzyme* | *Clinical application* |
|---|---|
| Acid phosphatase | Prostatic cancer |
| Alanine aminotransferase | Liver disease (viral or toxic hepatitis), jaundice, and liver cirrhosis |
| Aldolase | Muscle diseases |
| Alkaline phosphatase | Obstructive jaundice, bone diseases such as Paget's disease, rickets, osteomalacia, carcinoma of bone and hyperparathyroidism |
| Amylase | Acute pancreatitis, mumps, obstruction in pancreatic duct |
| Aspartate transaminase | Myocardial infarction, liver diseases |
| Cholinesterase | Organophosphorus insecticide poisoning, hepatic parenchymal diseases |
| Creatine kinase | Myocardial infarction, muscle diseases |
| γ-Glutamyl transferase | Hepatobiliary disease, alcoholism |
| Lactate dehydrogenase | Myocardial infarction, leukemia, muscular dystrophy, hepatic diseases |
| 5′-Nucleotidase | Hepatitis, obstructive jaundice |
| Prostate specific antigen | Prostate cancer |
| Trypsin | Pancreatic disease, cystic fibrosis |

## Analytical Use of Enzymes

- Enzymes can be used as **reagents** and **labels**.
- In addition to measurement of serum enzyme activity for the diagnosis and management of disease, enzymes are widely used in the clinical laboratory as reagents for the estimation of serum constituents. Some examples are given in **Table 6.11**.
- Many enzymes have been used as the label in various immunoassays (enzyme linked immunosorption assay, ELISA) for determining the serum concentration of drugs, hormones or other compounds of interest. Commonly used label enzymes are:
  - Glucose-6-phosphate dehydrogenase
  - Alkaline phosphatase
  - β-galactosidase
  - Peroxidase.

**Table 6.10:** Enzymes in diagnosis of myocardial infarction and time course of plasma enzyme activity changes after myocardial infarction.

| *Enzyme* | *Abnormal activity detectable (hours)* | *Time for maximum rise (hours)* | *Time for return to normal (days)* |
|---|---|---|---|
| $CK_2$ (MB-isoenzyme) | after 3–10 | after 12–24 | after 2–3 |
| AST | after 6–12 | after 24–48 | after 4–6 |
| LDH (heart specific) | after 8–16 | after 48–72 | after 7–12 |

**Table 6.11:** List of enzymes used in the clinical laboratory as reagents for assays.

| *Enzymes as reagents* | *Assays* |
|---|---|
| Alcohol dehydrogenase | Ethanol |
| Lactate dehydrogenase | Lactate |
| Glucose oxidase and peroxidase | Glucose |
| Hexokinase and glucose-6-phosphate dehydrogenase | Creatine kinase |
| Uricase | Uric acid |
| Urease | Urea |
| Cholesterol oxidase and peroxidase | Cholesterol |
| Lipase, glycerol kinase, glycerol phosphate dehydrogenase | Triacylglycerol |

# EXAM QUESTIONS

## Long Answer Questions (LAQs)

1. Explain with example various types of enzyme inhibitions.
2. Describe the role of enzymes and isoenzymes in various clinical disorders.
3. What are enzymes? Describe mechanism of action of enzymes and its importance in clinical medicine.
4. Define enzymes. Classify enzymes according to IUB system with suitable examples.
5. Name coenzymes of vitamin B complex with their corresponding vitamins and their role in metabolism with examples.

## Short Notes

1. State the enzymes, most commonly estimated after a myocardial infarct. Give details of the pattern shown by these enzymes after an infarct.
2. Mechanism of enzyme action.
3. Competitive inhibition and its importance in medicine.
4. Diagnostic use of enzymes and isoenzymes.
5. Factors affecting enzyme activity.
6. Suicide inhibitor.
7. Allosteric enzyme and feedback inhibition.
8. Therapeutic use of enzymes.
9. Use of enzymes as reagents and labels.
10. What is zymogen? Give examples of zymogen.
11. Name enzymes requiring metal elements as a cofactor.
12. Effect of different types of enzyme inhibition on the kinetic parameter.
13. Enzyme specificity.
14. Enzyme assay in myocardial infarction.

## Multiple Choice Questions (MCQs)

1. **Enzymes, which are produced in inactive form in the living cells, are called:**
   a. Papain b. Lysozymes
   c. Apoenzymes d. Proenzymes
2. **The Michaelis constant ($K_m$) is *except*:**
   a. Not changed by the presence of non-competitive inhibitor
   b. It is a measure of the affinity of the enzyme for its substrate

c. High $K_m$ indicates high affinity
d. The substrate concentration at 1/2 $V_{max}$

**3. Which of the following non-proteins can act as an enzyme?**
a. DNA b. RNA
c. Phospholipid d. Glycolipid

**4. The substrate concentration at which an enzyme exhibits half the maximum velocity is known as:**
a. $V_{max}$ b. [S]
c. $K_m$ d. Keq

**5. All of the following serum enzymes are elevated in myocardial infarction, *except*:**
a. Alkaline phosphatase
b. Lactate dehydrogenase
c. Aspartate transaminase
d. Creatine kinase

**6. Enzymes may be used as the following, *except*:**
a. Therapeutic agents
b. Nutrients
c. Diagnostic agents
d. Laboratory reagents

**7. Enzyme activity in biological systems may be affected by the following:**
a. Negative modifiers
b. Change in pH
c. Change in enzyme concentration
d. All of the above

**8. Isoenzymes can be characterized as:**
a. Non-protein part of enzyme
b. Enzymes with same quaternary structure
c. Similar enzymes that catalyze different reactions
d. Multiple forms of given enzyme that catalyze same type of reactions

**9. Transaminase enzymes belong to the class:**
a. Hydrolases b. Transferases
c. Oxidoreductases d. Isomerases

**10. Liver diagnostic enzyme is:**
a. Alkaline phosphatase
b. Acid phosphatase
c. Alanine transaminase
d. Amylase

**11. Which of the following enzyme levels increases in obstructive jaundice?**
a. Lipase b. Amylase
c. Acid phosphatase d. Alkaline phosphatase

**12. The digestive enzymes belong to:**
a. Isomerases b. Transferases
c. Hydrolases d. Ligases

**13. The enzyme useful in the treatment of leukemia is:**
a. α-chymotrypsin b. Hyaluronidase
c. Asparginase d. Streptokinase

**14. Earliest marker of myocardial infarction is:**
a. CK-1 b. CK-2
c. CK-3 d. AST

**15. Fischer's "lock and key" model of the enzyme action implies that:**
a. The active site is complementary in shape to that of substance only after interaction
b. The active site is complementary in shape to that of substrate
c. Substrates change conformation prior to active site interaction
d. The active site is flexible and adjusts to substrate

**16. In reversible non-competitive enzyme inhibition:**
a. Inhibitor bears structural resemblance to substrate
b. Inhibitor lowers the maximum velocity with a given amount of enzyme
c. $K_m$ is increased
d. $K_m$ is decreased

**17. In competitive enzyme inhibition:**
a. Apparent $K_m$ is decreased
b. Apparent $K_m$ is increased
c. $V_{max}$ is increased
d. $V_{max}$ is decreased

**18. Coenzymes are:**
a. Heat stable, dialyzable, non-protein organic molecules
b. Soluble, colloidal, protein molecules
c. Structural analogue of enzymes
d. Different forms of enzymes

**19. An example of hydrogen transferring coenzyme is:**
a. CoA b. $NAD^+$
c. Biotin d. TPP

**20. The isoenzymes of LDH:**
a. Differ only in a single amino acid
b. Differ in catalytic activity
c. Exist in 5 forms depending on M and H monomer contents
d. Occur as monomers

**21. The normal value of CK in serum varies between:**
a. 4–60 IU/L b. 60–250 IU/L
c. 4–17 IU/L d. > 350 IU/L

**22. Factors affecting enzyme activity:**
a. Concentration of substrate
b. pH
c. Temperature
d. All of these

**23. The normal serum GOT (AST) activity ranges from:**
a. 5–35 IU/L b. 4.0–17.0 IU/L
c. 4.0–60.0 IU/L d. 0.9–4.0 IU/L

**24. The normal GPT (ATT) activity ranges from:**
a. 60.0–250.0 IU/L b. 8.0–15.0 IU/L
c. 5–30 IU/L d. 0.1–14.0 IU/L

**25. The normal serum acid phosphatase activity ranges from:**
a. 5.0–13.0 KA units/100 mL
b. 1.0–5.0 KA units/100 mL
c. 13.0–18.0 KA units/100 mL
d. 0.2–0.8 KA units/100 mL

**26. The normal serum alkaline phosphatase activity ranges from:**
a. 1.0–5.0 KA units/100 mL
b. 5.0–13.0 KA units/100 mL
c. 0.8–2.3 KA units/100 mL
d. 13.0–21.0 KA units/100 mL

**27. In early stages of myocardial ischemia the most sensitive indicator is the measurement of the activity of:**
a. CPK b. SGPT
c. SGOT d. LDH

**28. Serum acid phosphatase level increases in:**
a. Metastatic carcinoma of prostate
b. Myocardial infarction
c. Wilson's disease
d. Liver diseases

**29. Serum alkaline phosphatase level increases in:**
a. Hypothyroidism
b. Carcinoma of prostate
c. Obstructive jaundice
d. Myocardial ischemia

**30. Serum lipase level increases in:**
a. Paget's disease
b. Gaucher's disease
c. Acute pancreatitis
d. Diabetes mellitus

**31. The isoenzymes $LDH_5$ is elevated in:**
a. Myocardial infarction
b. Peptic ulcer
c. Liver disease
d. Infectious diseases

**32. On the third day of onset of acute myocardial infarction the enzyme elevated is:**
a. Serum AST b. Serum CK
c. Serum LDH d. Serum ALT

**33. $LDH_1$ and $LDH_2$ are elevated in:**
a. Myocardial infarction
b. Liver disease
c. Kidney disease
d. Brain disease

**34. In acute pancreatitis, the enzyme raised is:**
a. Serum amylase
b. Serum lactic dehydrogenase
c. Aminotransferase
d. Pseudocholinesterase

**35. An example of functional plasma enzyme is:**
a. Lipoprotein lipase
b. Amylase
c. Aminotransferase
d. Lactate dehydrogenase

**36. A non-functional plasma enzyme is:**
a. Pseudocholinesterase
b. Lipoprotein lipase
c. Proenzyme of blood coagulation
d. Lipase

**37. Zymogen is a:**
a. Vitamin b. Enzyme precursor
c. Modulator d. Hormone

**38. Enzyme inhibition caused by a substance resembling substrate molecule is:**
a. Competitive inhibition
b. Non-competitive inhibition
c. Feedback inhibition
d. Allosteric inhibition

**39. Feedback inhibition of enzyme is influenced by:**
a. Enzyme b. External factors
c. End product d. Substrate

**40. If the amount of substrate is not limiting, the velocity of reaction is directly proportional to:**
a. pH
b. Temperature
c. Concentration of enzymes
d. Concentration of products

**41. Which one of the following statements concerning allosteric enzymes is correct?**
a. Allosteric effectors bind allosteric enzymes at their active sites
b. Allosteric enzymes obey the Michaelis Menten behavior
c. Allosteric control is irreversible
d. Allosteric control with negative feedback is found in many metabolic pathways

**42. Which of the following statement is not true about zymogen?**
a. Zymogen is inactive precursors of enzyme
b. Zymogens are activated by cleavage of specific peptide bonds
c. Pepsinogen is activated in stomach
d. Pepsinogen is synthesized in pancreas

**43. An example of enzyme as regents in the clinical laboratory is:**
a. Glucose oxidase
b. Urease
c. Cholesterol oxidase
d. All of the above

**44. Elevation of serum amylase and lipase is commonly seen in:**
a. Acute pancreatitis b. Liver cirrhosis
c. Bone disease d. Gallbladder disease

**45. Enzymes belong to which group of biomolecules:**
a. Carbohydrates b. Proteins
c. Lipids d. Phospholipids

**46. The enzymes with different structures but the some catalytic function are called:**
a. Allosteric enzymes
b. Proenzymes
c. Zymogens
d. Isoenzymes

**47. The model that explain that the active site is flexible and shapes of the active site can be modify by the binding of the substrate is called *except*:**
a. Induced fit model
b. Hand in gloves model
c. Koshland model
d. Lock and key model

**48. The inhibitor that binds reversibly to active site of the enzyme is known as:**
a. Allosteric inhibitor
b. Competitive inhibitor
c. Non-competitive inhibitor
d. Suicide inhibitor

**49. The site of the enzyme molecule into which the substrate binds is:**
a. Allosteric site b. Regulatory site
c. Active site d. None of the above

**50. Enzymes used as the treatment of some diseases of human being. Except:**
a. Asparginase
b. Penicillinase
c. Alcohol dehydrogenase
d. Hyaluronidase

## Answers for MCQs

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **1.** d | **2.** c | **3.** b | **4.** c | **5.** a | **6.** b | **7.** d | **8.** d |
| **9.** b | **10.** c | **11.** d | **12.** c | **13.** c | **14.** b | **15.** b | **16.** b |
| **17.** b | **18.** a | **19.** b | **20.** c | **21.** a | **22.** d | **23.** a | **24.** c |
| **25.** b | **26.** b | **27.** a | **28.** a | **29.** c | **30.** c | **31.** c | **32.** c |
| **33.** a | **34.** a | **35.** a | **36.** d | **37.** b | **38.** a | **39.** c | **40.** c |
| **41.** d | **42.** d | **43.** d | **44.** a | **45.** b | **46.** d | **47.** d | **48.** b |
| **49.** c | **50.** c | | | | | | |

CHAPTER

7

# Vitamins

**Chapter outline**

- Classification of Vitamins
- Difference Between Fat Soluble and Water Soluble Vitamins
- Water Soluble Vitamins
- Fat Soluble Vitamins

## INTRODUCTION

The name '***Vitamine***' was proposed in 1911 by Polish chemist Casimir Funk for the nutrient compound required to prevent the nutritional deficiency disease ***beriberi***, because of its vital (vita = life) need and because chemically it was found to be an ***amine***. Later, after a number of other essential organic nutrients were discovered, the "***e***" was dropped, when it was found that not all of them are ***amines***. The term "***Vitamin***" has now been adopted universally and applied to a group of biologically essential compounds that include 14 compounds which cannot be synthesized by human beings. They must, therefore, be supplied through food.

Since their chemical nature was unknown letter designations were applied for their nomenclature, e.g. vitamins A, B and C. Later, vitamin B was shown to consist of several substances and subscripts were added, i.e. vitamin $B_1$, $B_2$, $B_6$, etc. and collectively called ***vitamin B complex***.

## CLASSIFICATION OF VITAMINS

**Vitamins** are organic nutrients that are required in small quantities (in micrograms to milligram quantities per day) for a variety of biochemical functions and which generally cannot be synthesized by the body and must, therefore, be supplied by the diet. Some can be synthesized by intestinal microorganisms, but in quantities that are not sufficient to meet our needs. They may be water or fat soluble.

### Classification

The vitamins are grouped into two categories based on their solubility:

1. Water soluble vitamins
2. Fat soluble vitamins

- **Water soluble vitamins which include**
  - i. Vitamin B complex, e.g.
    - Thiamine (vitamin $B_1$)
    - Riboflavin (vitamin $B_2$)
    - Niacin (vitamin $B_3$)
    - Pantothenic acid (vitamin $B_5$)
    - Pyridoxine (vitamin $B_6$)
    - Biotin
    - Folic acid
    - Cobalamin (vitamin $B_{12}$)
  - ii. Vitamin C or ascorbic acid.
- **Fat soluble vitamins, which include**
  - Vitamin A or retinol
  - Vitamin D or cholecalciferol
  - Vitamin E or tocopherol
  - Vitamin K.

**Table 7.1** summarizes the best food sources, dietary allowances, the active coenzyme forms, the principal metabolic functions and the major clinical manifestations of deficiencies of the water soluble and fat soluble vitamins.

**Table 7.1:** Summary of the best food sources, dietary allowances, the active coenzyme forms, the principal metabolic functions and the major clinical manifestations of deficiencies of the water soluble and fat soluble vitamins.

| *Name* | *Active form* | *Sources* | *Daily requirements* | *Functions* | *Deficiency manifestations* |
|---|---|---|---|---|---|
| **WATER SOLUBLE VITAMINS** | | | | | |
| Thiamine (Vitamin $B_1$) | TPP | Cereals, meat, nuts green vegetables, eggs | 1.0–1.5 mg | Coenzyme for oxidative decarboxylation and transketolase reactions | Beriberi, Wernicke Korsakoff syndrome |
| Riboflavin (Vitamin $B_2$) | FMN, FAD | Yeast, germinating seeds, green leafy vegetables, milk, eggs, liver, meat | 1.3–1.7 mg | Coenzyme for oxidation-reduction reactions | Cheilosis, glossitis, dermatitis, vascularization of cornea |
| Niacin (Vitamin $B_3$) | $NAD^+$ and $NADP^+$ | Yeast, legumes, liver, meat | 15–20 mg | Coenzyme for oxidation reduction reactions | Pellagra |
| Pantothenic acid (Vitamin $B_5$) | Coenzyme A, (CoA-SH) | Wheat germs, cereals, yeast, liver, eggs | 5–10 mg | Acyl carrier | Burning feet syndrome |
| Pyridoxin (Vitamin $B_6$) | PLP | Yeast, unrefined cereals, pulses, vegetables, meat, fish, egg yolk | 1.6–2 mg | Coenzyme for transamination, decarboxylation, non-oxidative deamination, trans-sulfuration reactions | Epileptic convulsions, dermatitis, hypochromic microcytic anemia |
| Biotin (Vitamin $B_7$) | Biocytin (Enzyme bound biotin) | Liver, kidney, egg yolk, vegetables | 150–300 µg | Coenzyme for carboxylation reactions | Rare dermatitis |
| Folic acid (Vitamin $B_9$) | **THF** (Tetrahydrofolic acid) | Green leafy vegetables, liver, yeast | 200 µg | Carrier of one carbon unit. Synthesis of methionine, purines and pyrimidines | Megaloblastic anemia, Neural tube defects |
| Cynocobalamin (Vitamin $B_{12}$) | Methylcobalamin, Deoxyadenosyl-cobalamin | Only animal origin, meat, egg, liver, fish | 3 µg | Coenzyme for reactions: Homocysteine to Methionine. Methylmalonyl-CoA to succinyl-CoA | Pernicious anemia Megaloblastic anemia Neuropathy (dementia) Methylmalonic aciduria |
| Ascorbic acid (Vitamin C) | Ascorbic acid | Citrus fruits, Amla, leafy vegetables, tomatoes | 60–70 mg | Antioxidant, involved in hydroxylation reactions in the synthesis collagen, steroid hormones, adrenaline, etc. facilitates absorption of iron from intestine | Scurvy |
| **FAT SOLUBLE VITAMINS** | | | | | |
| Vitamin A | Retinol, Retinal, Retinoic acid | Fish liver oils, milk, milk products, Green leafy, Vegetables, carrots, yellow and red fruits | 800–1000 retinol equivalents; | Retinal and retinol are involved in vision. Retinoic acid regulates the expression of gene during growth and development. Antioxidant | Night blindness xerophthalmia formation of Bitot's spots, dry, rough and scaly skin. Retardation of growth in children |

*Contd...*

*Contd...*

| Name | Active form | Sources | Daily requirements | Functions | Deficiency manifestations |
|---|---|---|---|---|---|
| Vitamin D (Cholecalciferol) | 1, 25-Dihydroxy-cholecalciferol | Cod liver oil, sunlight induced synthesis of vitamin $D_3$ in skin, egg yolk | 200–400 IU | Regulation of the plasma level of calcium and phosphorus, calcification of bone | Rickets (in children) Osteomalacia (in adults) |
| Vitamin E ($\alpha$-Tocopherol) | $\alpha$-Tocopherol | Soya and corn oils, germ oil, fish oil, eggs, alfalfa | 8–10 mg | Natural antioxidant and acts as a scavenger of free radicals. Protects the RBCs from hemolysis Prevents peroxidation of PUFA in cell membrane | Hemolytic anemia. Retrolental fibroplasia (RLF) in premature infants |
| Vitamin K | Phylloquinone (Vitamin $K_1$) Menaquinone (Vitamin $K_2$) | Green leafy vegetables, tomatoes, cheese, meat, egg yolk | 70–140 $\mu$g | Required for activation of blood clotting factors. Required for $\gamma$ carboxylation of glutamic acid carboxylation of glutamic acid residue in clotting and osteocalcin proteins | Hemorrhagic disorder, increased clotting time |

## DIFFERENCE BETWEEN FAT SOLUBLE AND WATER SOLUBLE VITAMINS

- Water soluble vitamins function as precursor for coenzymes and antioxidants while fat soluble vitamins function as coenzymes, hormones and antioxidants.
- Water soluble vitamins are usually ***non-toxic*** since excess amounts of these vitamins are excreted in the urine, while fat soluble vitamins are ***toxic*** and even lethal when taken in excessive quantities.
- Water soluble vitamins are not stored extensively **except *vitamin $B_{12}$***, and so their intake has to be more frequent than that of other fat soluble vitamins which are stored.

## WATER SOLUBLE VITAMINS

### Thiamine (Vitamin $B_1$)

#### *Structure*

Thiamine consists of a pyrimidine ring attached to a thiazole ring **(Fig. 7.1)** by methylene bridge.

#### *Active Form of Thiamine*

Thiamine pyrophosphate (TPP) is an active coenzyme form of vitamin thiamine **(Fig. 7.2).**

#### *Sources*

- It is present in all natural foods but particularly good dietary sources are unrefined cereals, meat, nuts, green vegetables, eggs, etc.
- White bread and polished rice are very poor sources of the vitamin thiamine.

#### *Functions*

- Thiamine is required mainly for ***carbohydrate metabolism***
- Thiamine pyrophosphate (TPP) is a coenzyme involved in several enzymatic reactions mainly for ***oxidative decarboxylation*** and ***transketolase*** reactions as follows:
  1. TPP is a coenzyme for ***pyruvate dehydrogenase complex*** which catalyzes the conversion of pyruvate

**Fig. 7.1:** Structure of thiamine.

into acetyl CoA by oxidative decarboxylation **(Fig. 12.7).** Acetyl-CoA is a precursor for the synthesis of the **neurotransmitter acetylcholine** and also for the synthesis of myelin. ***Thus, thiamine is required for the normal functioning of the nervous system***.

2. TPP is a coenzyme for ***α-ketoglutarate dehydrogenase*** which catalyzes the conversion of α-ketoglutarate to succinyl-CoA in TCA cycle ***(see Fig. 12.8)***.
3. TPP is a coenzyme for the enzyme ***transketolase***, in the pentose phosphate pathway of glucose oxidation ***(see Fig. 12.17)***.

## Nutritional Requirements

- Nutritional Research Council recommends daily intake of ***1.0 to 1.5 mg*** of thiamine for adults which is increased with increased muscular activity, dietary carbohydrates and in pregnancy and lactation.

## Deficiency Manifestations

- The deficiency of vitamin $B_1$ results in a condition called **beriberi.** Deficiency of thiamine occurs in population who consume exclusively **polished rice** as staple food. Polishing of rice removes thiamine.
- The early symptoms of thiamine deficiency are anorexia, nausea, mental confusion, peripheral neuritis, muscle fatigue and irritability.
- Thiamine deficiency leads to three types of beriberi namely
  1. Dry beriberi
  2. Wet beriberi
  3. Infantile beriberi

### *Dry beriberi (neuritic beriberi)*

- It develops when the diet chronically contains slightly less than the thiamine requirements.

**Fig. 7.2:** Thiamine pyrophosphate, active coenzyme form of thiamine (OH-group of thiazole is replaced by pyrophosphate).

- This form of beriberi is characterized primarily by **peripheral neuritis,** severe muscular weakness and fatigue. Other symptoms of dry beriberi include dry skin, mental confusion and poor appetite.

### *Wet beriberi (cardiac beriberi)*

- It develops when the deficiency is more severe in which cardiovascular system is affected in addition to neurological symptoms.
- Wet beriberi is characterized primarily by **edema** of extremities, heart enlargement and cardiac insufficiency. Other symptoms include tachycardia or bradycardia and palpitation.
- Both forms of beriberi may overlap to a varying degree and patients of beriberi may die due to heart failure, if not treated.

### *Infantile beriberi*

- Infantile beriberi is observed in breast fed infants born to mother suffering from thiamine deficiency. The breast milk of these mothers is deficient in thiamine.
- It is characterized by cardiac dilation (enlargement of heart), tachycardia, convulsions, edema and GI disturbances such as vomiting, abdominal colic, etc. In acute condition, the infant may die due to cardiac failure.

### *Wernicke-Korsakoff syndrome*

- It is also known as **cerebral beriberi** and mostly seen in alcoholics.
- In chronic alcoholics, the nutritional deficiencies result from either poor intake of food or malabsorption of nutrients from intestine.
- Wernicke-Korsakoff syndrome is characterized by anorexia, nausea, vomiting, nystagmus, depression, ataxia, loss of memory, mental confusion, peripheral paralysis, muscular weakness, etc.

## Antimetabolites

Thiamine can be destroyed, if the diet contains ***thiaminase***. Thiaminase is present in raw fish and seafood.

### Thiamine Assay

Whole blood or erythrocyte ***transketolase*** *(requiring TPP as a coenzyme)* activity is used as a measure of thiamine deficiency.

## Riboflavin (Vitamin $B_2$)

**Riboflavin (Vitamin $B_2$)**

*Riboflavin (Vitamin $B_2$) works with other B vitamins to promote healthy growth and tissue repair, and helps release energy from carbohydrates*

RDA: 1.7 mg
Water soluble

### Structure

Riboflavin is a yellow compound (Flavus = ***yellow in Latin***) consisting of a ***isoalloxazine ring*** with a ribitol (sugar alcohol) side chain **(Fig. 7.3)**. Riboflavin is relatively ***heat stable*** but decomposes in the presence of visible light (photosensitive).

### Active Form of Riboflavin

The active or coenzyme forms of the riboflavin are:

- Flavin mononucleotide (FMN), and
- Flavin adenine dinucleotide (FAD).

### Sources

- The main dietary sources of riboflavin are yeast, germinating seeds, green leafy vegetables milk and milk products, eggs, liver and meat.
- Cereals are a poor source.

### Functions

- Riboflavin is a precursor of coenzymes ***FMN*** and **FAD,** which are required by several ***oxidation-reduction*** reactions in metabolism. FMN and FAD serve as coenzymes for *oxidoreductase enzymes* involved in carbohydrate, protein, lipid, nucleic acid metabolism

**Fig. 7.3:** Structure of riboflavin and its active coenzyme forms FMN and FAD.

and electron transport chain. Some examples are given in **Table 7.2.**

- It is needed for maintenance of mucosal epithelial and the ocular tissues.
- They are also involved in protection against peroxidation in ***metabolism of xenobiotics***.

### Nutritional Requirements

- The RDA for vitamin $B_2$ is **1.3 to 1.7 mg** for adults.
- It is related to protein use and increases during growth, pregnancy, lactation and wound healing.

### Deficiency Manifestations

- Riboflavin deficiency is quite rare as it has a wide distribution in food stuffs. It is usually seen along with deficiencies of other vitamins of B-complex group. It is most commonly seen in chronic alcoholics.
- The characteristic symptoms of riboflavin deficiency are:
  - *Cheilosis:* Fissures at the angles of the mouth,
  - *Glossitis:* Inflammation of the tongue that becomes swollen and magenta colored
  - *Dermatitis:* Rough and scaly skin
  - Vascularization (the development of blood vessels) of cornea, etc.

### Riboflavin Assay

A commonly used method for assessment of riboflavin status uses the determination of FAD dependent **glutathione reductase** activity in freshly lyzed erythrocytes.

## Niacin (Vitamin $B_3$)

### Structure

***Niacin*** is a general name for the ***nicotinic acid*** and ***nicotinamide***, either of which may act as a source of the vitamin in the diet. Niacin is a simple derivative of ***pyridine***.

**Table 7.2:** Examples of enzymes requiring FMN or FAD as a coenzyme and reaction where they are involved.

| *Flavoprotein enzyme* | *Pathway/Reaction* |
|---|---|
| Amino acid oxidase | Deamination of amino acids |
| Xanthine oxidase | Purine degradation |
| Succinate dehydrogenase | Citric acid cycle |
| Acyl-CoA dehydrogenase | Fatty acid oxidation |
| NADH dehydrogenase | Respiratory chain into mitochondria |
| Pyruvate dehydrogenase, and α-Ketoglutarate dehydrogenase | Oxidative decarboxylation of pyruvate and α-ketoglutarate |

### Active Form

Active forms of niacin are:

- Nicotinamide adenine dinucleotide ($NAD^+$)
- Nicotinamide adenine dinucleotide phosphate ($NADP^+$) **(Fig. 7.4).**

### Sources

- Yeast, liver, legumes and meats are major sources of niacin.
- Limited quantities of niacin can also be obtained from the metabolism of tryptophan. ***For every 60 mg of tryptophan, 1 mg equivalent of niacin can be generated***.

### Functions

- Niacin is a precursor of coenzymes, ***nicotinamide adenine dinucleotide ($NAD^+$)*** and ***nicotinamide adenine dinucleotide phosphate ($NADP^+$)***.
- $NAD^+$ and $NADP^+$ are involved in various ***oxidation*** and ***reduction reactions*** catalyzed by dehydrogenases in metabolism.
- They are, therefore involved in many metabolic pathways of carbohydrate, lipid and protein. Generally, ***$NAD^+$*** linked dehydrogenases catalyze oxidation-reduction reactions in ***oxidative pathways***, e.g. citric acid cycle and glycolysis.
- Whereas ***$NADP^+$*** linked dehydrogenases or reductases are often found in pathways concerned with ***reductive synthesis***, e.g. synthesis of cholesterol, fatty acid and pentose phosphate pathways.
- Selected examples of enzymes and the reactions they catalyze are given in **Table 7.3.**

**Fig. 7.4:** Structure and active coenzyme forms of niacin.

**Table 7.3:** Examples of enzymes requiring $NAD^+$ or $NADP^+$ or NADPH and reaction where they are involved.

| *Enzyme* | *Pathway/Reaction* |
|---|---|
| **NAD dependent** | |
| Glyceraldehyde-3-phosphate dehydrogenase | Glycolysis: Glyceraldehyde-3 phosphate to 1,3-bisphosphoglycerate |
| Pyruvate dehydrogenase | Oxidative decarboxylation of pyruvate to acetyl-CoA |
| α-Ketoglutarate dehydrogenase | TCA cycle: α-Ketoglutarate to Succinyl-CoA |
| β-Hydroxy acyl-CoA dehydrogenase | β-Oxidation of fatty acid: β-Hydroxy acyl-CoA to β-Keto acyl-CoA |
| **NADP dependent** | |
| Glucose-6-phosphate dehydrogenase gluconolactone | Pentose phosphate pathway: Glucose 6-phosphate to 6-phospho gluconolactone |
| Malic enzyme | Transfer of acetyl-CoA from mitochondria to cytosol |
| **NADPH dependent** | |
| 3-Ketoacyl reductase | Fatty acid synthesis: 3 Ketoacyl enzyme to 3-Hydroxyacyl enzyme |
| HMG CoA reductase | Cholesterol synthesis: HMG-CoA to Mevalonate |

### Nutritional Requirement

- The RDA for niacin is ***15*** to ***20 mg***.
- **Tryptophan** can only provide about 10% of the total ***niacin*** requirement.

### Deficiency Manifestation

#### *Pellagra*

- Deficiency of niacin in human causes pellagra, a disease involving the:
  - Skin
  - Gastrointestinal tract
  - Central nervous system.
- The symptoms of pellagra are characterized by **three 'Ds'**:
  1. Dermatitis
  2. Diarrhea
  3. Dementia and if not treated death.

***Dermatitis:*** Skin inflammation is seen in any area exposed to direct sunlight.

***Diarrhea:*** Frequent diarrhea nausea, vomiting, anorexia are the disorders of GI tract.

***Dementia:*** Dementia (loss of memory) is associated with degeneration of nervous tissues.

- To produce niacin deficiency, diet must be poor in both available niacin and tryptophan. Niacin deficiency occurs in:
  - Population dependent on maize (corn) or sorghum (*jowar*) as the staple food.
  - ***Deficiency of vitamin $B_6$*** (pyridoxal phosphate) leads to ***niacin deficiency*** as it is involved as a coenzyme in the pathway of synthesis of niacin from tryptophan.
  - **Malignant carcinoid syndrome** in which tryptophan metabolism is diverted to formation of serotonin.
  - In **Hartnup disease,** a genetic disorder in which tryptophan absorption and transportation is impaired.

### Therapeutic Uses of Niacin

Nicotinic acid (not nicotinamide), used at high doses (1–2 gm/day), has been shown to lower total cholesterol, LDL cholesterol and VLDL triglyceride in patients with hyperlipoproteinemias.

**Fig. 7.5:** Structure of pantothenic acid.

### Toxicity

High intake of the vitamin has undesirable side effects, mainly vasodilation and flushing and liver damage.

## Pantothenic Acid (Vitamin $B_5$)

**Pantothenic acid (Vitamin $B_5$)**

*Pantothenic acid is a component of coenzyme-A (CoA-SH) and acyl carrier protein (ACP). The RDA of pantothenic acid is not well established.*

RDA: 5–10 mg
Water soluble

The name pantothenic acid is derived from the Greek word "***pantethine***," meaning from "***everywhere***" and gives an indication of the wide distribution of the vitamin in foods.

### Structure

Pantothenic acid is formed by a combination of ***pantoic acid*** and ***β-alanine*** **(Fig. 7.5)**.

### Active form

Active forms of pantothenic acid are:

- Coenzyme-A (CoA-SH)
- Acyl carrier protein (ACP).

### Source

Eggs, liver, yeast, wheat germs, cereals, etc. are important sources of pantothenic acid, although the vitamin is widely distributed.

### Functions

- Pantothenic acid is a component of ***coenzyme-A (CoA-SH)*** and ***acyl carrier protein (ACP)***. Thiol (-SH) group of CoA-SH and ACP acts as a carrier of acyl groups.

**Fig. 7.6:** Structure of three different forms of vitamin $B_6$.

- Coenzyme-A participates in reactions concerned with:
  - Reactions of citric acid cycle
  - Fatty acid synthesis and oxidation
  - Synthesis of cholesterol
  - Utilization of ketone bodies.
- ACP participates in reactions concerned with fatty acid synthesis.

### *Nutritional Requirement*

The RDA of pantothenic acid is not well established. A daily intake of about 5–10 mg is advised for adults.

### *Deficiency Manifestations*

No clear cut case of pantothenic acid deficiency has been reported (because the substance is widely distributed in foods) except in malnourished prisoners of war in the far East in 1940s, where *neurological condition*, known as the ***burning feet syndrome***, was reported and ascribed to pantothenic acid deficiency. As these people were severely malnourished and were deficient in other vitamins as well, it is not possible to attribute this specific effect to pantothenic acid deficiency.

**Fig. 7.7:** Structure of pyridoxal phosphate: an active form of vitamin $B_6$.

Clinical signs observed in experimentally induced deficiencies are:

- Paraesthesia (abnormal tingling sensation)
- Headache
- Dizziness
- Gastrointestinal malfunction.

## Pyridoxine (Vitamin $B_6$)

**Pyridoxine (Vitamin $B_6$)**

*Vitamin $B_6$ (pyridoxine) is important for maintaining healthy brain function, the formation of red blood cells, the breakdown of protein and the synthesis of antibodies in support of the immune system*

Adult RDA: 2 mg
Water soluble

### *Structure*

Vitamin $B_6$ consists of a mixture of three different closely related pyridine derivatives **(Fig. 7.6)** namely:

1. Pyridoxine
2. Pyridoxal
3. Pyridoxamine

All the three have equal vitamin activity, as they can be interconverted in the body.

### *Active Form of Vitamin $B_6$*

**Pyridoxal phosphate (PLP)** is the active form of vitamin $B_6$ **(Fig. 7.7).** PLP is formed from phosphorylation of all three forms of vitamin $B_6$.

### *Sources*

- Pyridoxine occurs mainly in plants, whereas pyridoxal and pyridoxamine are present mainly in animal products.

- Major dietary sources of vitamin $B_6$ are yeast, unrefined cereals, pulses, meat, poultry fish, potatoes and vegetables.
- Dairy products and grains contribute lesser amounts.

## Functions

- Active form of vitamin $B_6$, pyridoxal phosphate (PLP) acts as coenzyme in large number of reactions of amino acid metabolism. For example:
  - Transamination
  - Decarboxylation
  - Nonoxidative deamination
  - Trans-sulfuration
  - Condensation reactions of amino acids.

**Transamination reactions:** Transamination reactions are catalyzed by transaminases and PLP acts as coenzyme converting amino acid to keto acid, e.g. aspartate transaminase (AST) and alanine transaminase (ALT) **(*see* Fig. 14.6).**

**Decarboxylation reaction:** PLP acts as coenzyme in decarboxylation of some amino acids. The amino acids are decarboxylated to corresponding amines. The important biogenic amines synthesized by PLP decarboxylation are given below **(*see* Fig. 14.47).**

- **γ-Amino butyric acid (GABA):** It is an inhibitory neurotransmitter derived from glutamate on decarboxylation hence in vitamin $B_6$ deficiency underproduction of GABA leads to convulsions (epileptic seizures) in infants and children.
- **Serotonin and melatonin:** These are produced from tryptophan. Serotonin is a neurotransmitter and stimulates the cerebral activity. Melatonin is a sleep inducing substance and is involved in regulation of **circadian rhythm** of body.
- **Histamine:** Histamine produced by decarboxylation of histidine. It is a vasodilator and lowers blood pressure. It is involved in allergic reactions.
- **Catecholamines** (dopamine, norepinephrine and epinephrine): Synthesis of catecholamines rom tyrosine requires PLP-dependent DOPA decarboxylase. Catecholamines are neurotransmitters and involved in metabolic and nervous regulation.

**Nonoxidative deamination:** Hydroxyl group containing amino acids (serine, threonine) are nonoxidatively deaminated to α-ketoacids and ammonia, which requires PLP **(*see* Fig. 14.9).**

**Trans-sulfurationreaction:** PLP is a coenzyme for **cystathionine synthase** involved in synthesis of cysteine from methionine **(*see* Fig. 14.27).** In these reactions transfer of sulfur from methionine to serine occurs to produce cysteine.

**Condensation reactions:** Pyridoxal phosphate is required for the condensation reaction of L-glycine and succinyl CoA to form δ-aminolevulinic acid, a precursor of heme **(*see* Fig. 18.2).**

**Other PLP-dependent reactions:**

- Pyridoxal phosphate is required for niacin coenzyme ($NAD^+/NADP^+$) synthesis from tryptophan **(*see* Fig. 14.24).**
- PLP is required for synthesis of serine from glycine **(*see* Fig. 14.14).**
- The enzyme glycogen phosphorylase contains covalently bound PLP.
- PLP is required for the synthesis of sphingosine, a component of sphingomyelin.

## Nutritional Requirement

The RDA for vitamin $B_6$ is ***1.6*** to ***2.0 mg***. Requirements increase during pregnancy and lactation.

## Deficiency Manifestations

As pyridoxine occurs in most foods, the dietary deficiency of vitamin $B_6$ is rare. The main clinical symptoms of deficiency are given below:

- Vitamin $B_6$ deficiency causes neurological disorders such as **depression, nervousness** and **irritability.** These symptoms are due to decreased production of neurotransmitters, catecholamines, GABA and serotonin.
- Severe deficiency of pyridoxine causes epileptic seizures (convulsions) in infants due to reduced production of GABA.
- Demyelination of nerves causes peripheral neuropathy. Since vitamin $B_6$ is required for synthesis of sphingolipids needed for myelin formation.
- Vitamin $B_6$ deficiency causes **hypochromic microcytic anemia** due to decreased heme synthesis. Since PLP is required for the synthesis of heme.
- The commonest cause of pyridoxine deficiency is:
  - ***Drug antagonism*, e.g. *Isoniazide (INH)***, used in the treatment of tuberculosis and ***penicillamide*** used in the treatment of Wilson's disease and rheumatoid arthritis can combine with pyridoxal phosphate forming an inactive derivative with pyridoxal phosphate.
  - ***Alcoholism*:** Alcoholics may be deficient owing to metabolism of ethanol to acetaldehyde, which

**Fig. 7.8:** Structure of biotin.

stimulates hydrolysis of the phosphate of the pyridoxal phosphate.

### Vitamin $B_6$ Assay

Activities of blood **transaminases** have been used frequently as indirect measurements of vitamin $B_6$ status. Erythrocyte levels of aspartate and alanine aminotransferase provide better information of vitamin $B_6$ status.

### Therapeutic Uses

Pyridoxine is used for the treatment of:

- **Seizures.**
- **Down's syndrome,** a state of mental subnormality (incomplete development of mind) due to chromosome defect.
- **Autism,** psychiatric disorder of childhood.
- **Premenstrual tension syndrome** (PMS).

### Toxicity

Pyridoxine seems to be safe at levels of 100–150 mg but taking 500–5000 mg per day, has shown peripheral neuropathy within 1–3 years.

## Biotin

Biotin was known formerly as vitamin H.

### Structure

Biotin is an imidazole derivative **(Fig. 7.8).** It consists of a **tetrahydrothiophene** ring bound to an imidazole ring and a **valeric acid** side chain.

### Sources

- It is widely distributed in foods.
- Liver, kidneys, vegetables and egg yolk are the important sources of biotin.
- Biotin is also synthesized by intestinal bacteria.

### Active Form of Biotin

Enzyme-bound biotin, **biocytin** is an active form of biotin. Biotin is covalently bound to ε-amino group of lysine of an enzyme to form **biocytin**.

### Functions

***Biotin is a coenzyme of carboxylase reactions***, where it is a carrier of $CO_2$. Four carboxylation reactions requiring biotin are given below.

- Conversion of acetyl-CoA into malonyl-CoA catalyzed by **acetyl-CoA carboxylase** in fatty acid synthesis **(*see* Fig. 13.17)**.
- Conversion of pyruvate into oxaloacetate, catalyzed by **pyruvate carboxylase** in gluconeogenesis **(*see* Fig. 12.10)**.
- Conversion of propionyl-CoA to D-methyl malonyl-CoA catalyzed by ***propionyl-CoA carboxylase*** in the pathway of conversion of propionate to succinate **(*see* Fig. 13.8)**.
- It is also involved in the catabolism of branched chain amino acid catalyzed by **β-methyl-crotonyl-CoA carboxylase (*see* Fig. 14.34)**.

**Biotin Independent Carboxylation Reaction**

There are few carboxylation reactions which do not require biotin. For example:

- Formation of carbamoyl phosphate by **carbamoyl phosphate synthetase** in urea cycle.
- Addition of $CO_2$ to form $C_6$ in purine ring.
- Conversion of pyruvate to malate by malic enzyme.

### Nutritional Requirements

A daily intake of about 150–300 µg is recommended for adults. Biotin is synthesized by intestinal microorganisms in large quantities so that a dietary source is probably not necessary.

### Deficiency Manifestation

- Since biotin is widely distributed in plant and animal foods and intestinal bacterial flora supply adequate amounts of biotin, the natural deficiency of biotin is not well characterized in humans.
- The experimentally induced symptoms of biotin deficiency are nausea, anorexia, glossitis, dermatitis, alopecia (loss of hair), depression and muscular pain.
- Deficiency of biotin occurs in:
  - The people with the unusual dietary habit of consuming large amounts of uncooked eggs. Egg white contains the glycoprotein ***avidin***, which binds the imidazole group of biotin and prevents biotin absorption.
  - Use of antibiotics, that inhibit the growth of intestinal bacteria, eliminates this source of biotin and leads to deficiency of biotin.

## Folic Acid (Vitamin $B_9$)

### Structure

Folic acid consists of three components, **pteridine ring, p-amino benzoic acid (PABA)** and **L-glutamic acid (Fig. 7.9).** In a folic acid molecule, the number of glutamic acid residues varies from one to seven. Folic acid usually has one glutamic acid residue.

### Active Form of Folic Acid

***Tetrahydrofolate (THF)*** is the active form of folic acid. Folate is enzymatically reduced in a two-stage process in tissues to yield the dihydro and then tetrahydrofolate, which requires vitamin C **(Fig. 7.10).**

### Source

Folic acid is found in green leafy vegetables, liver, yeast. The word folate is related to folium which means *leaf* in Latin.

### Functions

- THF acts as a carrier of one carbon units. The one carbon units are:

| | |
|---|---|
| Methyl | $CH_3$ |
| Methylene | $CH_2$ |
| Methenyl | CH |
| Formyl | CHO |
| Formimino | CH = NH |

- One carbon unit binds to THF through $N^5$ or $N^{10}$ or both $N^5, N^{10}$ position. The THF coenzymes serve as acceptors or donors of one carbon units in a variety of reactions involved in amino acid and nucleic acid metabolism. Five of the major reactions in which THF is involved are given below **(Fig. 7.11).**
  1. **Conversion of serine to glycine:** The conversion of serine to glycine is accompanied by the formation of $N^5$, $N^{10}$ -methylene THF.
  2. **Synthesis of thymidylate (pyrimidine nucleotide):** The enzyme ***thymidylate synthase*** that converts deoxyuridylate (dUMP) into thymidylate (TMP) uses $N^5$, $N^{10}$ -methylene THF as the methyl donor for this reaction. Thus, folate coenzyme plays a central role in the biosynthesis of **nucleic acids.**
  3. **Catabolism of histidine:** Histidine in the course of its catabolism is converted into ***formiminoglutamate (FIGLU)***. This molecule can donate the formimino group to THF to produce N-$^5$ formimino THF. In case of folic acid deficiency, FIGLU accumulates and is excreted in urine.
  4. **Synthesis of purine:** $N^5$-Formyl THF intermediate formed in histidine catabolism is used in the biosynthesis of purine and therefore in the formation of both DNA and RNA.

**Fig. 7.9:** The structure of folic acid.

**Fig. 7.10:** Formation of tetrahydrofolate from folic acid.

5. **Synthesis of methionine from homocysteine:** Homocysteine is converted to methionine in presence of $N^5$-methyl THF, and vitamin $B_{12}$.

In this reaction the methyl group bound to cobalamin (Vitamin $B_{12}$) is transferred to homocysteine to form methionine and the cobalamin then removes the methyl group from $N^5$-methyl THF to form THF **(Fig. 7.12)**.

This step is essential for the liberation of free THF and for its repeated use in one carbon metabolism. In $B_{12}$ deficiency, conversion of $N^5$-methyl THF to free THF is blocked.

## *Nutritional Requirements*

- The RDA of folate is **200 μg**.
- Requirements increase during pregnancy and lactation.

## *Deficiency Manifestations*

Folate deficiency frequently occurs particularly in pregnant women and in alcoholics. Clinical symptoms of folic acid deficiency include:

- **Megaloblastic or macrocytic anemia:** The deficiency of folic acid leads to impairment of synthesis of DNA. Impaired DNA synthesis, impairs the maturation of erythrocytes. Consequently, megaloblasts are formed instead of normoblast. These megaloblasts are accumulated in the bone marrow and leads to megaloblastic anemia.
- **Accumulation and excretion of FIGLU in the urine:** Folate deficiency blocks the last step of histidine catabolism, due to lack of THF. This results in accumulation of FIGLU in body, which leads to increased excretion of FIGLU in urine **(Fig. 7.13)**.
- **Hyperhomocysteinemia:** Due to folic acid deficiency the methylation of homocysteine to methionine is impaired which leads to hyperhomocysteinemia. Increased level of homocystein is a risk factor for cardiovascular disease.

**Fig. 7.11:** Role of folic acid in one carbon metabolism.
(DHF: Dihydrofolate; FIGLU: Formiminoglutamate; THF: Tetrahydrofolate; dUMP: Deoxyuridine monophosphate; TMP: Thymidine monophosphate; PLP: Pyridoxal phosphate)

**Fig. 7.12:** The combined roles of vitamin $B_{12}$ and folate in the synthesis of methionine.

**Fig. 7.13:** Excretion of FIGLU in folic acid deficiency.

- **Neural tube defect in fetus:**
  Since, folate is required for the formation of neural tube in early stage of gestation, the folate deficiency during early stage of pregnancy increases the risk of neural tube defect.

### *Therapeutic Uses*

$N^5$-methyl THF is called **folinic acid** or **citrovorum factor** is used as therapeutic drug to overcome the folate deficiency.

## Cobalamin (Vitamin $B_{12}$)

**Cynocobalamin (Vitamin$B_{12}$)**

*Vitamin $B_{12}$ is important for metabolism, the formation of red blood cells, and the maintenance of the central nervous system, which includes the brain and spinal cord*

Adult RDA: 3 μg
Water soluble

**Fig. 7.14:** Structure of cobalamin (vitamin $B_{12}$).
(R: Either methyl or deoxyadenosyl or hydroxy group)

## Structure

Vitamin $B_{12}$ bears a complex ***corrin ring*** (containing pyrrols similar to porphyrin), linked to a ***cobalt atom*** held in the center of the corrin ring, by four coordination bonds with the nitrogen of the pyrrole groups. The remaining coordination bonds of the cobalt are linked with the nitrogen of ***dimethylbenzimidazole nucleotide*** and sixth bond is linked to either ***methyl*** or ***5′-deoxyadenosyl*** or **hydroxy** group to form ***methylcobalamin, adenosylcobalamin*** or ***hydroxycobalamin*** respectively **(Fig. 7.14).**

Thus, cobalamin exists in three forms that differ in the nature of the chemical group attached to cobalt. ***Cynocobalamin is the commercial available form of vitamin $B_{12}$.***

## Active form of Vitamin $B_{12}$

The active coenzyme forms of vitamin $B_{12}$ are:

- Methylcobalamin
- Deoxyadenosylcobalamin.

## Sources

- ***Dietary sources of vitamin $B_{12}$ are of animal origin*** and include meat, eggs, milk, dairy products, fish, poultry, etc.
- ***Vitamin $B_{12}$ is absent in plant foods.***
- Humans obtain small amounts of vitamin $B_{12}$ from their intestinal flora.

## Absorption, Transport and Storage

- The intestinal absorption of vitamin $B_{12}$ requires an **intrinsic factor (IF),** a glycoprotein secreted by parietal cells of the stomach.
- In stomach IF binds the dietary vitamin $B_{12}$ to form **vitamin $B_{12}$-IF complex**. This complex binds to specific receptors on the surface of the mucosal cells of the ileum.
- After binding to the receptor, the bound vitamin $B_{12}$ is released from the complex and enters the illeal mucosal cells through a $Ca^{2+}$ dependent process.
- The vitamin in mucosal cell is converted into its main plasma transport form to **methylcobalamin**. It is then transported by a vitamin $B_{12}$ binding protein known as **transcobalamin (TC-I and TC-II)**.
- Methylcobalamin which is in excess is taken up by the liver, **stored in deoxyadenosyl $B_{12}$** form.
- Liver can store about 4-5 mg of vitamin $B_{12}$ in adults, an amount sufficient to meet the body requirements of vitamin $B_{12}$ for 3–6 years.
- Vitamin $B_{12}$ is the only water soluble vitamin that is stored in significant amounts in the liver.

## Functions

There are only two human enzyme systems that are known to require vitamin $B_{12}$ coenzyme.

1. **Isomerization of methylmalonyl-CoA to succinyl-CoA**
   - Propionyl-CoA-is produced as catabolic end product of some aliphatic amino acids and in β-oxidation of odd chain fatty acids. The propionyl-CoA is then converted to succinyl-CoA.
   - During conversion of propionyl-CoA to succinyl-CoA vitamin $B_{12}$ coenzyme, deoxyadenosyl cobalamin is required for the isomerization of L-methylmalonyl-CoA to succinyl-CoA.
   - In vitamin $B_{12}$ deficiency methylmalonyl CoA accumulates and is excreted in urine as methylmalonic acid **(Fig. 7.15).**
2. **Conversion of homocysteine to methionine**
   - Methylcobalamin is a coenzyme in the conversion of homocysteine to methionine, which joins the metabolic roles of vitamin $B_{12}$ and those of folic acid (for explanation see functions of folic acid and **Figure 7.12**). This is the only mammalian reaction known to require both vitamins.

## Nutritional Requirements

RDA for adult is **3 μg** with higher allowances for pregnancy and lactating women.

## Deficiency Manifestations

Deficiency may arise due to decreased absorption or decreased dietary intake. Dietary deficiency is seen in strict vegetarians, since the vitamin found only in foods of animal origin or in microorganisms. Deficiency of vitamin $B_{12}$ leads to:

- Pernicious anemia
- Megaloblastic anemia
- Methylmalonic aciduria
- Neuropathy.

1. **Pernicious anemia:** It is caused by a deficiency of **intrinsic factor** in the stomach, which leads to impaired absorption of vitamin $B_{12}$. It is characterized by megaloblastic anemia and **low hemoglobin** level with **neurological** disorders.

**Fig. 7.15:** Role of vitamin $B_{12}$ in the metabolism of propionyl-CoA.

2. **Megaloblastic anemia:** It occurs due to **functional folate deficiency.** The functional folate deficiency is seen in vitamin $B_{12}$ deficiency due to folate trap **(Fig. 7.12).**
3. **Methylmalonic aciduria:** Because vitamin $B_{12}$ is necessary for the conversion of methylmalonic acid to succinic acid, individuals deficient in vitamin $B_{12}$ excrete excess amounts of methylmalonic acid in the urine **(Fig. 7.15).**
4. **Neuropathy:** In vitamin $B_{12}$ deficiency, many neurological symptoms appear due to progressive degeneration of myelinated nerves. Degeneration of myelinated nerves is due to accumulation of L-methylmalonyl-CoA, which impairs the myelin sheath formation. The neurological symptoms include numbness and tingling of fingers and toes, mental confusion, poor muscular coordination and dementia.

**Folate Trap**

- Methylation of homocysteine to methionine depends on vitamin $B_{12}$ and $N^5$-methyl THF. When vitamin $B_{12}$ is deficient $N^5$-methyl THF cannot be converted to free THF. Thus, most of folic acid of the body is irreversibly "trapped" as its methyl derivative ($N^5$-methyl THF). There is functional folate deficiency due to the lack of free THF. This is called folate trap (**Fig. 7.12**).
- Folate trap creates folate deficiency and an adequate supply of free THF is not available for the synthesis of purine and pyrimidine bases. Thus, a $B_{12}$ deficiency can lead to a folate deficiency. Although the tissue folate levels are adequate or high, there is a functional folate deficiency due to the lack of THF.

L-Ascorbic acid

**Fig. 7.16:** Structure of ascorbic acid.

## Vitamin C (Ascorbic Acid)

**Ascorbic acid (Vitamin C)**

*Ascorbic acid is a water soluble antioxidant functions as a reducing agent. Required for normal collagen formation, as well as in bone and dentin formation and wound healing process. It prevents atherosclerosis and coronary heart disease by preventing oxidation of LDL. Deficiency of ascorbic acid causes scurvy.*

Adult RDA: 60-70 μg
Water soluble

### *Structure*

Vitamin C is also known as ***ascorbic acid***. It is a six-carbon sugar derivative **(Fig. 7.16).** Most animals can synthesize ascorbic acid. But humans cannot synthesize ascorbic acid, due to lack of the enzyme ***gluconolactone oxidase*** which is required for the synthesis of ascorbic acid. Thus, humans have a dietary requirement of ascorbic acid.

### *Active Form of Ascorbic Acid*

Ascorbic acid itself is an active form.

### *Sources*

- The main dietary sources of vitamin C are leafy vegetables and fruits, especially citrus fruits, strawberries, tomatoes, spinach and potatoes.
- Cereals contain no vitamin C.
- Animal tissues and dairy products are very poor sources.

### *Functions*

Ascorbic acid functions as a reducing agent in many metabolic processes as follows:

**Fig. 7.17:** Formation of bile acid.

- **Collagen biosynthesis:** Vitamin C is required for formation of collagen, where it is needed for the hydroxylation of ***proline*** and ***lysine*** residues, of protocollagen. Hydroxyproline and hydroxylysine are essential for the collagen cross-linking and collagen strength and stability. Since vitamin C is required for normal collagen formation, vitamin C is also involved in bone and dentin formation as well as wound healing process.
- **Steroid synthesis:** In adrenal cortex, vitamin C is involved in the hydroxylation reactions of steroids.
- **Adrenaline synthesis:** In adrenal medulla it serves as a reducing agent in hydroxylation reactions in the synthesis of adrenaline and noradrenaline from tyrosine.
- **Carnitine synthesis:** Vitamin C functions in the hydroxylation of γ-butyrobetaine to carnitine.
- **Bile acid formation:** Vitamin C is required for the hydroxylation of cholesterol in bile acid synthesis **(Fig. 7.17).**
- **Degradation of tyrosine:** The oxidation of P-hydroxy-phenylpyruvate to homogentisate requires vitamin C. The subsequent step is catalyzed by homogentisate oxidase, which is a ferrous ion containing enzyme that also requires vitamin C.
- **Folate metabolism:** Folic acid is converted to its active form tetrahydrofolate (THF) with the help of vitamin C **(*See* Fig. 7.10).**
- **Absorption of iron:** Ascorbic acid facilitates the absorption of iron from intestine by reducing it to the $Fe^{++}$ (ferrous) state.
- ***Ascorbic acid is a water soluble antioxidant:*** (Ascorbic acid is a strong reducing agent and acts as an antioxidant).
  - It reduces oxidized vitamin E (tocopherol) to regenerate functional vitamin E.
  - Vitamin C, thought to be involved in the prevention of atherosclerosis and coronary heart disease by preventing oxidation of LDL.
  - Antioxidant property of vitamin C is also associated with prevention of cancer by inhibiting nitrosamine formation from naturally occurring nitrates during digestion.

### Nutritional Requirements

The recommended daily allowance is about **60–70 mg.** Additional intakes are recommended for women during pregnancy and lactation.

### Deficiency Manifestation

Deficiency of ascorbic acid causes ***scurvy***. Symptoms of scurvy are related to ***deficient collagen formation*** (Refer functions of vitamin C). These include:

- Spongy, swollen, bleeding gums, loosening of teeth
- Abnormal bone development and osteoporosis
- Poor wound healing
- Anemia due to impaired erythropoiesis
- Easy bruising and bleeding due to fragile capillaries.

### Therapeutic Uses

Use of vitamin C in preventing cold and cancers has not been scientifically supported. Although the incidence of common cold is not reduced by vitamin C, the duration of cold episodes and severity of symptoms can be decreased. Vitamin C may act reacting with free radicals.

### Toxicity

Vitamin C can be taken in doses up to 2–3 g/day without undesirable effects. Above these levels, however, it cannot be absorbed from the intestine and can cause severe diarrhea and deposition of oxalate stones in kidneys.

## FAT SOLUBLE VITAMINS

### Vitamin A

### Structure

Vitamin A contains a single 6-membered ring to which is attached an 11-carbon side chain **(Fig. 7.18).** Vitamin A is an ***alcohol (retinol)***, but can be converted into an ***aldehyde (retinal)***, or an ***acid (retinoic acid)***.

**Fig. 7.18:** Structure of vitamin A, retinol.

### Active Form

Vitamin A consists of three biologically active molecules which are collectively known as **retinoids.**

1. *Retinol*: Primary alcohol ($CH_2OH$) containing form
2. *Retinal*: Aldehyde (CHO) containing form
3. *Retinoic acid*: Carboxyl (COOH) containing form
   - Each of these compounds is derived from the plant precursor molecule, β-***carotene*** (a member of a family of molecules known as carotenoids).
   - β-carotene which consists of two molecules of retinal linked at their aldehyde ends is also referred to as the ***provitamin*** form of vitamin A.
   - The retinol and retinal are interconverted by enzyme **retinal aldehyde reductase**. The retinoic acid is formed by oxidation of retinal. The retinoic acid cannot be reduced to either retinol or retinal **(Fig. 7.19)**.

### Absorption, Transport and Storage

- Ingested β-carotene is cleaved in the intestine by β-**carotene *dioxygenase*** to yield retinal. Retinal is reduced to retinol by ***retinaldehyde reductase***, an NADPH requiring enzyme within the intestine **(Fig. 7.19).**
- Retinol is esterified with palmitic acid incorporated into chylomicrons together with dietary lipid and delivered to the liver for storage.
- Transport of retinol from the liver to extrahepatic tissues, occurs by binding of retinol to ***retinol binding protein (RBP).***
- Transport of retinoic acid is accomplished by binding to albumin.

**Fig. 7.19:** Conversion of β-carotene (provitamin) to biologically active forms of vitamin A.

## Sources

- The richest dietary sources of vitamin A are fish liver oils (cod liver oil). Animal liver is also rich sources but meat is rather low in vitamin A.
- Other good sources are milk and dairy products, dark-green leaves, such as spinach and yellow and red fruits and vegetables, such as carrots, tomatoes, and peaches.

## Functions of Vitamin A

- Vitamin A is required for a variety of functions such as vision, cell differentiation and growth, mucus secretion, maintenance of epithelial cells, etc.
- The role of vitamin A in vision has been known through the studies of **G *Wald***, who received the Nobel Prize in 1943 for this work.
- Different forms of the vitamin have different functions.
  - Retinal and retinol are involved in vision.
  - Retinoic acid is involved in cellular differentiation and metabolic processes.
  - β-carotene is involved in antioxidant function.

### *Role of vitamin a in vision*

The cyclic events occur in the process of vision, known as ***rhodopsin cycle*** or ***Wald's visual cycle*** **(Fig. 7.20).** Both rod and cone cells of retina contain a photoreceptor pigment in their membrane and vitamin A is a component of these pigments. ***Rhodopsin*** or ***visual purple***, the visual pigment of rod cells in the retina consists of ***11-cis-retinal*** bound to protein ***opsin***.

- When rhodopsin absorbs light, the 11-cis-retinal is converted to all-trans retinal.
- The isomerization is associated with a conformational change in the protein opsin.

**Fig. 7.20:** Wald's visual cycle.

- Conformational changes in opsin generate a nerve impulse that is transmitted by the optic nerve to the brain.
- This is followed by dissociation of the all-trans retinal from opsin.
- The all-trans retinal is immediately isomerized by retinal isomerase to 11-cis-retinal.
- This combines with opsin to regenerate rhodopsin and complete the visual cycle.

The conversion of all-trans retinal to 11-cis-retinal is incomplete and therefore remaining all-trans retinal which is not converted to 11-cis-retinal is converted to all-trans retinol by ***alcohol dehydrogenase*** and is stored in the liver. When needed, retinol re-enters the circulation and is taken up by the retina, where it is converted back to 11-cis-retinal which combines with opsin again to form rhodopsin **(Fig. 7.20).**

**Dark adaptation time:** When a person enters from bright light to dark there is difficulty in seeing due to depletion of rhodopsin, but after few minutes the vision improves. During these few minutes, rhodopsin is resynthesized and vision is improved. The time taken for regeneration of rhodopsin is known as **dark adaptation time.** Dark adaptation time is increased in vitamin A deficient individuals.

### *Color vision*

- While vision in dim light is mediated by rhodopsin of the rod cells, color vision is mediated by three different

retinal containing pigments in the cone cells, the three pigments are called ***porphyropsin, iodopsin*** and ***cyanopsin*** and are sensitive to the three essential colors: **red, green** and ***blue*** respectively. All these pigments consist of **11-cis-retinal** bound to protein **opsin.**

- Thus, when light strikes the retina, it bleaches one or more of these pigments, depending on the color quality of the light. The pigments are converted to all-trans retinal, and the protein moiety opsin is released as in the case of rhodopsin. This reaction gives rise to the nerve impulse that is read out in the brain as color:
  - Red, if porphyropsin is split
  - Green, if iodopsin is split
  - Blue, if cyanopsin is split.
- If mixtures of the three are converted, the color read out in the brain depends on the proportions of the three split.

### *Cellular differentiation and metabolic effect*

- **Retinoic acid** is an important regulator of **gene expression** especially during growth and development. Retinoic acid is essential for normal gene expression during embryonic development such as cell differentiation in spermatogenesis and in the differentiation of epithelial cells.
- Retinoic acids exert a number of metabolic effects on tissues. These include:
  - Control of biosynthesis of membrane ***glycoproteins*** and ***glycosaminoglycans*** (mucopolysaccharide) necessary for mucus secretion. The normal mucus secretion maintains the epithelial surface moist and prevents keratinization of epithelial cell.
  - Control of biosynthesis of ***cholesterol***.

### *Antioxidant function*

- β-carotene is an antioxidant and may play a role in trapping peroxy free radicals in tissues.
- The antioxidant property of lipid soluble vitamin A may account for its possible anticancer activity.
- High levels of dietary carotenoids have been associated with a decreased risk of cardiovascular disease.

## Nutritional Requirements

The RDA of vitamin A for adults is **800–1000 retinol equivalents** (1 retinol equivalent = 1 μg retinol = 6 μg β-carotene).

## Deficiency Manifestation

Clinically, degenerative changes in eyes and skin are observed with vitamin A deficiency.

### *Effect on vision*

#### *Night blindness (nyctalopia)*

- Night blindness is one of the earliest symptoms of vitamin A deficiency. This is characterized by loss of vision in night (in dim or poor light) since **dark adaptation time** is increased. Prolonged deficiency of vitamin A leads to an irreversible loss of visual cells.
- Severe vitamin A deficiency causes dryness of cornea and conjunctiva, a clinical condition termed as **xerophthalmia** (dry eyes).
- If this situation prolongs, **keratinization** and **ulceration** of cornea takes place. This results in destruction of cornea. The cornea becomes totally opaque resulting in permanent loss of vision (blindness), a clinical condition termed as **keratomalacia.** Xerophthalmia and keratomalacia are commonly observed in children.
- White opaque spots develop on either side of cornea in vitamin A deficiency are known as **Bitot's spot.**

#### *Effect on Skin and Epithelial Cells*

- Vitamin A deficiency causes keratinization of epithelial cells of skin which leads to keratosis of hair follicles, and dry, rough and scaly skin.
- Keratinization of epithelial cells of respiratory, urinary tract makes them susceptible to infections.

#### *Other Symptoms of Vitamin A Deficiency*

- Failure of growth in children.
- Faulty bone modelling producing thick cancellous (spongy) bones instead of thinner and more compact ones.
- Abnormalities of reproduction, including degeneration of the testes, abortion or the production of malformed offspring.

## Therapeutic Use of Vitamin A

- The use of retinoic acid preparations, in the treatment of psoriasis, acne and several other skin diseases, is related to its involvement with epithelial cell differentiation and integrity.
- Some precancerous lesions seem to respond to treatment with carotenoids.

## Hypervitaminosis A

- The symptoms of hypervitaminosis A include nausea, vomiting, diarrhea, loss of hair (alopecia), scaly and rough skin, bone and joint pain, enlargement of liver, loss of weight, etc.

**Fig. 7.21:** Structure of 1,25-dihydroxycholecalciferol: an active form of vitamin $D_3$.

**Fig. 7.22:** The formation of vitamin $D_3$ in the body.

- In pregnant women, the hypervitaminosis A may cause congenital malformation in growing fetus (teratogenic effect).
- The excess intake of carotenoids is not toxic like vitamin A.

**Why Vitamin A is considered as a Hormone?**

- Within cells both retinol and retinoic acid function by binding to specific receptor proteins present in the nucleus of target tissues.
- Following binding, the receptor-vitamin complex interacts with several genes involved in growth and differentiation and affects expression of these genes.

In this capacity, retinol and retinoic acid are considered as hormones.

## Vitamin D (Cholecalciferol)

**Vitamin D (Cholecalciferol)**

*Regulates plasma level of calcium and phosphorus, calcification of bone. A deficiency of vitamin D lead to a condition called rickets, a weakening and softening of the bones brought on by extreme calcium loss*

Adult RDA: 400 IU
Fat soluble

Vitamin D is also known as **calciferol** because of its role in calcium metabolism and **antirachitic factor** because it prevents rickets.

**Vitamin D could be Thought of as a Hormone Rather Than a Vitamin Because**

- As it can be synthesized in the body
- It is released in the circulation
- Has distinct target organs
- Action of vitamin D is similar to steroid hormones. It binds to a receptor in the cytosol. Following binding, the receptor vitamin complex interacts with DNA to stimulate the synthesis of calcium binding protein.

### *Structure*

Vitamin D is a steroid compound. There are two forms of vitamin D.

1. The naturally produced $D_3$ or ***cholecalciferol*** **(Fig. 7.21)**, is the form obtained from animal sources in the diet, or made in the skin by the action of ultraviolet light from sunlight on ***7-dehydrocholesterol*** **(Fig. 7.22)**.
2. Artificially produced form $D_2$ or **ergocalciferol,** is the form made in the laboratory by irradiating the plant sterol, ***ergosterol***.

### *Absorption, Transport and Activation of Vitamin D*

Exogenous or dietary vitamin D is absorbed in the duodenum along with lipids. It is transported to the liver through chylomicron.

### *Active Form of Vitamin*

Cholecalciferol is an inactive form of vitamin D. It needs further metabolism to produce the active form of the vitamin. ***1, 25 dihydroxycholecalciferol*** also known as **calcitriol** is the active form of vitamin D. The steps involved in activation are:

1. The first step is the conversion of cholecalciferol to 25-hydroxycholecalciferol. The ***25-hydroxylation occurs in liver*** and is catalyzed by **hydroxylase**.
2. The 25-hydroxycholecalciferol formed transported to the kidney, where it is further hydroxylated by, **1, α-hydroxylase enzyme** in the 1-position to 1,25 dihydroxycholecalciferol **(Fig. 7.23).**

### *Sources*

- Best sources are cod liver oil and often fish oils and sunlight induced synthesis of vitamin $D_3$ in skin.
- Egg yolk and liver are good sources.

### *Functions*

Vitamin D (Calcitriol) plays an essential role in the regulation of **calcium** and **phosphorus** metabolism. It maintains the

**Fig. 7.23:** Activation of vitamin D.

normal plasma level of calcium and phosphorus by acting on **intestine**, **kidneys** and **bones (Fig. 7.24).**

*Action of calcitriol on intestine*

It increases the plasma calcium and phosphorus concentration by stimulating the absorption of calcium and phosphorus from the intestine by enhancing the synthesis of calcium binding proteins **calbindins.** This protein increases the calcium uptake by the intestine.

*Action of calcitriol on kidney*

It stimulates the reabsorption of calcium and phosphorus from the kidney and decreases their excretion.

*Action of calcitriol on bone*

It is believed that calcitriol has both **anabolic** and **catabolic** role on bone.

- Calcitriol promotes the mineralization of bones by deposition of calcium and phosphorus.
- Calcitriol along with PTH stimulates the mobilization of calcium and phosphorus from bone by stimulating the synthesis of **osteocalcin** (a calcium binding protein in bone). This causes elevation of plasma calcium and phosphorus levels.

## Nutritional Requirement

The daily requirement of vitamin D is **200–400 IU.**

## Deficiency Manifestation

Deficiency of vitamin D causes **rickets (rachitis)** in growing children and **osteomalacia** in adults.

*Rickets*

- Rickets is characterized by formation of soft and pliable bones due to poor mineralization and calcium deficiency. Due to softness, the weight bearing bones are bent and deformed.

**Fig. 7.24:** Metabolism and functions of Vitamin D.
(1,25 DHCC: 1,25-dihydroxycholecalciferol; PTH: Parathyroid hormone)

- The main features of the rickets are, a large head with protruding forehead, pigeon chest, bow legs, (curved legs) **(Fig. 7.25)**, knock knees and abnormal curvature of the spine (kyphosis).
- Rachitic children are usually anemic or prone to infections. Rickets can be fatal when severe.
- Rickets is characterized by low plasma levels of calcium and phosphorus and high alkaline phosphatase activity.

**Fig. 7.25:** Bowing of legs in rickets.

*Osteomalacia (adult rickets)*

- The deficiency of vitamin D in adults causes **osteomalacia.** This is a condition similar to that of rickets.
- Osteomalacia characterized by demineralization of previously formed bones, Demineralization of bones makes them soft and susceptible to fractures.

*Renal rickets (renal osteodystrophy)*

In chronic renal failure synthesis of calcitriol in kidney is impaired. As a result, the deficiency of calcitriol occurs which leads to hypocalcemia and hyperphosphatemia. It can be treated by oral or intravenous administration of calcitriol (active form of vitamin D).

*Vitamin D resistant rickets*

As the name implies, this is a disease which does not respond to treatment with vitamin D. There are various possible causes of this condition and all involve a defect in the metabolism or mechanism of action of 1,25 dihydroxycholecalciferol as follows:

- Due to **defective vitamin D receptor**
- Due to a ***defective 1, α-hydroxylase activity in kidney***
- Due to liver disease and kidney failure as the production of 25-hydroxycholecalciferol and 1,25 dihydroxycholecalciferol respectively will be inefficient in the damaged tissue.

### Hypervitaminosis D

High doses of vitamin D over a long period are toxic.

- The early symptoms of hypervitaminosis D include nausea, vomiting, anorexia, increased thirst, loss of weight, etc.

**Fig. 7.26:** Structure of α-tocopherol.

- Hypercalcemia is seen due to increased bone resorption and intestinal absorption of calcium.
- The prolonged hypercalcemia causes calcification of soft tissues and organs such as kidney and may lead to formation of stones in the kidneys.

### Therapeutic Use

Vitamin D analogues have been used in the treatment of psoriasis.

## Vitamin E (Tocopherol)

### Structure

Vitamin E consists of eight naturally occurring tocopherols, of which α-tocopherol is the most active form **(Fig. 7.26)**.

### Sources

The major dietary sources of vitamin E are fats and oils. The richest sources are germ oil, corn oil, fish oil, eggs, lettuce and alfalfa.

### Absorption, Transport and Storage

Vitamin E is absorbed from intestine together with dietary lipid. It is incorporated in chylomicrons. It is delivered to

the liver via chylomicron. The liver can export vitamin E into very low density lipoprotein (VLDL) to target cells. In cells, tocopherols are distributed where antioxidant activity is required. ***The major site of vitamin E storage is in the adipose tissue.***

### Functions

- Vitamin E acts as a natural antioxidant by scavenging free radicals and molecular oxygen.
- Vitamin E is important for preventing peroxidation of polyunsaturated fatty acids in cell membranes.
- Protection of erythrocyte membrane from oxidant is the major role of vitamin E in humans. It protects the RBCs from hemolysis.
- Vitamin E also helps to prevent oxidation of LDL. Oxidized LDL may be more atherogenic than native LDL and thus vitamin E may protect against atheromatous coronary heart disease.
- Whether vitamin E affects human fertility is unknown.
- In animals, vitamin E is required for normal reproduction and prevents sterility.

### Nutritional Requirements

A daily consumption of about:

- 10 mg (15 IU) of α-tocopherol for a man
- 8 mg (12 IU) for a woman is recommended. One mg of α-tocopherol is equal to 1.5 IU.

### Deficiency Manifestation

Vitamin E deficiency in humans is rare.

- The major symptom of vitamin E deficiency in human is ***hemolytic anemia*** due to increased red blood cell fragility.
- Another symptom of vitamin E deficiency is ***retrolental fibroplasia (RLF)*** observed in some premature infants of low birth weight. Children with this defect show ***neuropathy***.

### Hypervitaminosis E

Unlike other fat soluble vitamins such as A and D, vitamin E does not seem to have toxic effects.

## Vitamin K

**Vitamin K**

*Vitamin K plays an important role in blood coagulation and is associated with hemorrhagic disease. Required for the carboxylation of glutamic acid residues of osteocalcin, a $Ca^{2+}$ binding protein present in bone.*

RDA: 70–140 μg/day
Fat soluble

This vitamin is called an ***anti-hemorrhagic factor*** as its deficiency produced uncontrolled hemorrhages due to defect in blood coagulation.

In 1929, H Dam gave the name koagulation vitamin from the Danish word ***koagulation***. It is now called vitamin K.

### Structure (Fig. 7.27)

- There are two naturally occurring forms of vitamin K:
  i. **Vitamin $K_1$** or **phylloquinone** derived from plant.
  ii. **Vitamin $K_2$** or **menaquinones,** produced by microorganisms.

  Both these natural types have the same general activity.
- **Vitamin $K_3$ or menadione** is a synthetic product, which is an alkylated form of vitamin $K_2$.

### Sources

- Excellent sources are cabbage, cauliflower, spinach and other green vegetables.
- Good sources include tomatoes, cheese, dairy products, meat, egg yolk, etc.
- The vitamin is also synthesized by microorganisms in the intestinal tract.

### Absorption, Transport and Storage

The naturally occurring vitamin K derivatives are absorbed only in the **presence *of bile salts***, like other lipids. It is transported to the liver in the form of chylomicrons, where it is stored.

Menadione (synthetic vitamin K), being water soluble, is absorbed even in the absence of bile salt, passing directly into the hepatic portal vein.

**Fig. 7.27:** Structure of vitamin K.

## Functions of Vitamin K

- Vitamin K plays an important role in **blood coagulation.** Vitamin K is required for the activation of blood clotting factors, prothrombin (II), factor VII, IX and X. These blood clotting proteins are synthesized in liver in inactive form, and are converted to active form by **vitamin K dependent carboxylation** reaction. In this, vitamin K dependent carboxylase enzyme adds the extra carboxy group at χ-carbon of glutamic acid residues of inactive blood clotting factors.
- Vitamin K is also required for the carboxylation of glutamic acid residues of **osteocalcin,** a $Ca^{2+}$ binding protein present in bone.

Anticoagulants, **dicumarol** and **warfarin** are structurally similar to vitamin K and inhibit the action of vitamin K.

## Nutritional Requirements

The suggested intake for adults is **70–140 mg/day**.

## Deficiency Manifestation

- Vitamin K deficiency is associated with **hemorrhagic disease.** In vitamin K deficiency, clotting time of blood is increased. Uncontrolled hemorrhages occur on minor injuries as a result of reduction in prothrombin and other clotting factors.
- Vitamin K is widely distributed in nature and its production by the intestinal microflora ensures that dietary deficiency does not occur. Vitamin K deficiency, however, is found in:
  - **Patients with liver disease** and **biliary obstruction**. Biliary obstruction inhibits the entry of bile salts to the intestine.
  - **In new-born infants,** because the placenta does not pass the vitamin to the fetus efficiently, and the gut is sterile immediately after birth.
  - **Following antibiotic therapy** that sterilizes the gut.
  - **In fat malabsorption,** that impairs absorption of vitamin K.

## Hypervitaminosis K

Excessive doses of vitamin K produce a **hemolytic anemia** (due to increased breakdown of RBCs) and **jaundice** (in infants).

## Therapeutic Use

An important therapeutic use of vitamin K is an antidote (drug that counteracts the effects of a poison) to poisoning by dicumarol type drugs.

## EXAM QUESTIONS

### Long Answer Questions (LAQs)

1. Describe sources, functions, deficiency manifestations and daily requirements of vitamin A.
2. Describe sources, functions, and deficiency manifestations of vitamin D.
3. Describe sources, functions, and deficiency manifestations of ascorbic acid.
4. Describe sources, functions, and deficiency manifestations of vitamin thiamine or niacin or pyridoxine.
5. Describe sources, functions, and deficiency manifestations of vitamin $B_{12}$.
6. Describe sources, functions, and deficiency manifestations of vitamin folic acid.

### Short Notes

1. Metabolic role of ascorbic acid.
2. Vitamin $B_{12}$ functions and deficiency manifestation.
3. Vitamin D deficiency disorders.
4. Folate trap.
5. Beriberi.
6. Vitamin E. `
7. Vitamin K.
8. Role of vitamin A in the visual process.
9. Functions of folic acid.
10. Write coenzyme form of the following vitamins and reactions in which they participate:
    i. Pyridoxine
    ii. Thiamine
    iii. Niacin
    iv. Biotin
    v. Folic acid
    vi. Vitamin $B_{12}$
    vii. Riboflavin
    viii. Pantothenic acid.

### Solve

**Case History 1**

**A 15-year-old male has polished rice as a major component of his diet. He is hospitalized with symptoms of poor appetite, peripheral neuropathy and muscular weakness.**

**Questions**

a. Name the probable disorder and its different types.
b. Which factor is deficient in the diet?
c. Give the active form of the deficient factor.
d. Give any reaction where this factor is required.

**Case History 2**

**A male infant, 6 months of age, was admitted to the hospital in a coma. Blood investigation indicated that the child was anemic and that his vitamin $B_{12}$ level was very low. A urine sample contained increased amount of methylmalonate and homocysteine.**

**Questions**

a. Why was the infant anemic?
b. What are the sources of vitamin $B_{12}$ in the diet?
c. Explain the high level of methylmalonate and homocysteine in the infant's urine.
d. Give active form of vitamin $B_{12}$.

**Case History 3**

**A 42-year-old woman with a chronic inflammatory bowel disease was on intravenous feeding containing fat free and carbohydrate rich diet. After 3 months she began to complain of being unable to see appropriately in dim light.**

**Questions**

a. Name the probable disorder.
b. Which factor is deficient in the diet?
c. What is the daily requirement of this factor?
d. Name the rich sources of this factor.

**Case History 4**

**A 6-year-old girl is hospitalized with symptoms of digestive disorders, dermatitis, depression and dementia.**

**Questions**

a. Name the disorder.
b. Disorder is due to deficiency of which biomolecule?
c. Give active form of this biomolecule.
d. Give any reaction requiring the active form of this biomolecule.

**Case History 5**

**A 10-year-old boy presented with spongy bleeding gums with loose teeth.**

**Questions**

a. What is the disease he is suffering from?
b. What is the cause?
c. What is the biochemical basis of the disease?
d. Give RDA for the concerned biomolecule.

**Case History 6**

**A small 3-year-old child was brought with bow legs, protruding forehead, pigeon chest, depressed ribs and kyphosis.**

**Questions**

a. Name the disease.
b. Which biomolecule is deficient?
c. What are the functions of the concerned biomolecule?
d. RDA of the concerned biomolecule.

**Case History 7**

**A 65-year-old woman was admitted to the hospital with complaints of numbness, tingling in the calves and feet and weight loss. The physical examination revealed a slightly confused, depressed, pale woman. Her laboratory results showed decreased level of serum vitamin $B_{12}$.**

**Questions**

a. What is the biologically active form of vitamin $B_{12}$ in plasma?
b. Which factor is required for the absorption vitamin $B_{12}$ from intestine?
c. What is the biding protein for vitamin $B_{12}$?
d. List food substances that contain vitamin $B_{12}$.

## Multiple Choice Questions (MCQs)

1. **Which of the following coenzymes is not derived from vitamins?**
   a. CoASH
   b. TPP
   c. Pyridoxal phosphate (PLP)
   d. Coenzyme Q
2. **A deficiency of vitamin $B_{12}$ causes:**
   a. Scurvy
   b. Rickets
   c. Pernicious anemia
   d. Beriberi
3. **Rickets is due to deficiency of:**
   a. Vitamin D  b. Vitamin A
   c. Vitamin C  d. Vitamin $B_1$
4. **Which of the following vitamins would most likely become deficient in a person who developed a completely vegetarian lifestyle?**
   a. Vitamin C  b. Niacin
   c. Cobalamin  d. Vitamin E
5. **Pyridoxal phosphate is a coenzyme for the reactions, *except:***
   a. Transamination  b. Deamination
   c. Decarboxylation  d. Oxidation-reduction
6. **Both folic acid and methylcobalamin are required in:**
   a. Phosphorylation
   b. Deamination
   c. Methylation of homocysteine to methionine
   d. Conversion of pyruvate to acetyl-CoA
7. **Beriberi is caused by a deficiency of:**
   a. Thiamine  b. Thymine
   c. Threonine  d. Tyrosine
8. **Precursor of CoA is:**
   a. Folic acid  b. Thiamine
   c. Riboflavin  d. Pantothenic acid
9. **Biotin is involved in:**
   a. Oxidation-reduction
   b. Carboxylation
   c. Decarboxylation
   d. Dehydration
10. **Antihemorrhagic vitamin is:**
    a. Vitamin A  b. Vitamin E
    c. Vitamin K  d. Vitamin D
11. **Both Wernicke's disease and beriberi can be treated by administering vitamin:**
    a. Thiamine  b. Niacin
    c. Riboflavin  d. Ascorbic acid
12. **Pellagra occurs due to deficiency of:**
    a. Biotin
    b. Niacin
    c. Pantothenic acid
    d. Folic acid
13. **All of the following vitamins have antioxidant property, *except*:**
    a. β-carotene  b. Ascorbic acid
    c. Tocopherol  d. Cholecalciferol
14. **Increased prothrombin time is observed in the deficiency of:**
    a. Vitamin K  b. Vitamin B
    c. Vitamin A  d. Vitamin $B_{12}$

**15. Thiamine pyrophosphate is required for the following enzymatic activity:**
a. Hexokinase
b. Transketolase
c. Transaldolase
d. Glucose-6-phosphatase

**16. Excretion of FIGLU in urine occurs in deficiency of:**
a. Thiamine
b. Folic acid
c. Ascorbic acid
d. Nicotinic acid

**17. Which of the following vitamin is required for collagen synthesis?**
a. Ascorbic acid
b. Nicotinic acid
c. Pantothenic acid
d. Folic acid

**18. Functionally active form of vitamin D is:**
a. 1,25-dihydroxycholecalciferol
b. 24,25-dihydroxycholecalciferol
c. 25-hydroxycholecalciferol
d. 1,24-dihydroxycholecalciferol

**19. Which of the following enzyme is used as a measure of thiamine deficiency?**
a. Pyruvate dehydrogenase
b. Erythrocyte transketolase
c. α-ketoglutarate dehydrogenase
d. Decarboxylase

**20. Which of the following is used as an assay of riboflavin status?**
a. Transketolase
b. Glutathione reductase
c. FIGLU
d. Pyruvate dehydrogenase

**21. Which of the following trace elements is an integral part of the vitamin $B_{12}$ molecule?**
a. Zinc b. Iron
c. Copper d. Cobalt

**22. Deficiency of pyridoxine in the diet will impair the catalytic activity of:**
a. Glutamate dehydrogenase
b. Glutamine synthetase
c. Alanine aminotransferase
d. Glutaminase

**23. Identify the correct statement about the vitamin whose deficiency results in the disorder, pernicious anemia:**
a. It is absorbed in the gut as a combination with folic acid
b. Is the only water soluble vitamin can be stored in liver
c. Found in large amounts in the diets of vegans
d. It is synthesized in the parotid glands

**24. Which of the following vitamins can be synthesized in the liver by a pathway that involves pyridoxal phosphate and an aromatic amino acid?**
a. Vitamin D b. Thiamine
c. Folic acid d. Niacin

**25. Protein in raw egg white avidin prevents the absorption of which of the following vitamins?**
a. Pantothenic acid
b. Biotin
c. Niacin
d. Riboflavin

**26. Deficiency of which one of these vitamins is a major cause of blindness worldwide?**
a. Vitamin A b. Vitamin C
c. Vitamin D d. Vitamin K

**27. Deficiency of which one of these vitamins may lead to hemolytic anemia?**
a. Vitamin $B_3$ b. Vitamin E
c. Vitamin K d. Vitamin D

**28. Which of the following vitamins provides the cofactor for reduction reactions in fatty acid synthesis?**
a. Vitamin $B_6$ b. Thiamine
c. Niacin d. Riboflavin

**29. Which one of these vitamins may mask the anemia due to vitamin $B_{12}$ deficiency?**
a. Folic acid b. Riboflavin
c. Biotin d. Pyridoxin

**30. Which of the following vitamins provides the coenzyme for transamination of amino acids:**
a. Thiamine b. Vitamin $B_6$
c. Niacin d. Folic acid

**31. Deficiency of which of these vitamins lead to megaloblastic anemia?**
a. Vitamin D b. Vitamin $B_6$
c. Vitamin $B_{12}$ d. Vitamin E

**32. Which of the following vitamins acts as a coenzyme for transfer of one carbon units?**

a. Niacin b. Thiamine
c. Vitamin $B_6$ d. Folic acid

**33. For fatty acid synthesis which of the following vitamins as a coenzyme required?**

a. Biotin b. Riboflavin
c. Niacin d. Thiamine

**34. For calcium homeostasis which of the following vitamins is involved?**

a. Vitamin C b. Vitamin A
c. Vitamin D d. Vitamin E

**35. Vitamin involved in blood clotting is?**

a. Vitamin K b. Vitamin E
c. Vitamin C d. Vitamin D

## Answers for MCQs

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **1.** d | **2.** c | **3.** a | **4.** c | **5.** d | **6.** c | **7.** a | **8.** d |
| **9.** b | **10.** c | **11.** a | **12.** b | **13.** d | **14.** a | **15.** b | **16.** b |
| **17.** a | **18.** a | **19.** b | **20.** b | **21.** d | **22.** c | **23.** b | **24.** d |
| **25.** b | **26.** a | **27.** b | **28.** c | **29.** a | **30.** b | **31.** c | **32.** d |
| **33.** a | **34.** c | **35.** a | | | | | |

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CHAPTER

# 8

# Chemistry of Hemoglobin

**Chapter outline**

- Structure and Function of Hemoglobin
- Binding Sites for Oxygen, Hydrogen ($H^+$) and Carbon Dioxide ($CO_2$) with Hemoglobin
- Tense (T) and Relaxed (R) Forms of Hemoglobin
- Types of Normal and Abnormal Hemoglobin
- Derivatives of Hemoglobin

## INTRODUCTION

Hemoglobin is conjugated proteins, with a prosthetic group **heme.** Hemoglobin found in red blood cell, carries oxygen from lungs to the tissues and carbon dioxide from tissues to the lungs. The red color of blood is due to the hemoglobin content of the erythrocytes.

## STRUCTURE AND FUNCTION OF HEMOGLOBIN

Hemoglobin is a globular, oligomeric protein made up of ***heme*** and ***globin.***

Heme + globin → Hemoglobin

### *Heme*

- The heme is iron containing compound belonging to the class of compounds called ***protoporphyrin.***
- Protoporphyrin is composed of four **pyrrole rings** which are linked by methene (=CH) bridge to form **porphyrin ring (Fig. 8.1).**
- Four **methyl,** two **vinyl** and two **propionate** side chain groups are attached to the porphyrin ring. These substituents can be arranged in 15 different ways. But only one of this isomer called ***protoporphyrin IX*** is biologically active.
- The iron ($Fe^{2+}$) is held in the center of the protoporphyrin molecule by coordination bonds with the four nitrogen of the protoporphyrin ring **(Fig. 8.1)**

**Fig. 8.1:** Structure of heme. Side chains of the pyrrole rings are designated. (M: Methyl; V: Vinyl; P: Propionic acid).

- The iron atom has six coordination bonds.
  - Four bonds are formed between the **iron** and **nitrogen** atoms of the porphyrin ring system.
  - Fifth bond is formed between nitrogen atoms of histidine residue of the globin polypeptide chain, known as ***proximal histidine (F-8)***.
  - The sixth bond is formed with **oxygen.**
- The oxygenated form of hemoglobin is stabilized by the H-bond between oxygen and side chain of another histidine residue of the globin chain, known as ***distal histidine (F-7)*** **(Fig. 8.2)**

### *Globin*

- Globin molecule contains four polypeptide chains, ***two alpha (α)*** chains (141 amino acid residues each) and ***two beta (β)*** or ***two gamma (γ)*** or ***two delta (δ)*** as per the type of hemoglobin **(Table 8.1)**. The β, γ, and δ chains have 146 amino acid residues each.

**Fig. 8.2:** The coordination bonds of iron.

**Table 8.1:** Normal human hemoglobin.

| *Type* | *Composition* |
|---|---|
| $HbA_1$ (adult Hb) | $\alpha_2\beta_2$ |
| $HbA_2$ (minor adult Hb) | $\alpha_2\delta_2$ |
| HbF (Fetal Hb) | $\alpha_2\gamma_2$ |
| $HbA_{1c}$ (Glycosylated Hb) | $\alpha_2\beta_2$-glucose |

**Fig. 8.3:** Schematic representation of quaternary structure of hemoglobin.

- With each polypeptide chain, one molecule of heme is attached. A hemoglobin molecule therefore has four heme molecules.
- Four globin polypeptide chains with four heme molecules are held together in a definite arrangement or conformation to constitute a characteristic quaternary structure of hemoglobin, which is stabilized by:
  - Hydrogen bonds
  - Salt bridges
  - Van der Waals forces
- There is little contact between the two alpha or two beta chains. But there are many contact points between alpha and beta chains of dissimilar chain pair $\alpha_1\beta_1$ and $\alpha_2\beta_2$.
- There is a central open channel or cavity in hemoglobin molecule **(Fig. 8.3).**

### *Function of Globin*

The function of globin chain of hemoglobin is to form a protective hydrophobic pocket for binding of heme **(Fig. 8.3).** These pockets protect the reduced form of iron ($Fe^{2+}$) of heme from oxidizing to the ferric ($Fe^{3+}$) form from the aqueous environment and permits binding of oxygen with $Fe^{2+}$ ion of heme. Exposure of iron of heme to water results oxidation of $Fe^{2+}$ to $Fe^{3+}$ form and loss of oxygen binding capacity.

### *Functions of Hemoglobin*

- Transport of $O_2$ from lungs to tissues
- Transport of $CO_2$ and $H^+$ from tissues to lungs and kidney
- Acts as an intracellular buffer and is thus involved in acid-base balance.

## BINDING SITES FOR OXYGEN, HYDROGEN ($H^+$) AND CARBON DIOXIDE ($CO_2$) WITH HEMOGLOBIN

- **Oxygen** is bound to the ferrous ($Fe^{2+}$) atom of the heme **(Fig. 8.2)** to form oxyhemoglobin.
- **Hydrogen** is bound to R-groups (side chain) of histidine residues of α and β chains.
- **Carbon dioxide** is bound to N-terminal end of each of the polypeptide chains of hemoglobin to form carbaminohemoglobin **(Fig. 8.4).**

## TENSE (T) AND RELAXED (R) FORMS OF HEMOGLOBIN

### The Tense (T) Form

The deoxyhemoglobin is called the tense (T) or taut or a stressed form, in which the four subunits of Hb are held together by salt bonds, hydrogen bonds and Van der Waals forces **(Fig. 8.5).**

**Fig. 8.4:** Binding site for carbon dioxide with a globin chain of Hb.

**Fig. 8.5:** Schematic representation of tense (T) and relaxed (R) forms of hemoglobin.

## The Relaxed (R) Form

- The oxyhemoglobin is called the **relaxed (R)** form of hemoglobin.
- A molecule of $O_2$ bound first by the α-chain whose heme pockets are more readily accessible than those of the β-chains. Heme pockets of the ***β-chains are blocked by valine residue.***
- Binding of oxygen is accompanied by the rupture of salt bonds of all four subunits and protons are generated.
- These changes alter hemoglobin's secondary, tertiary and quaternary structures **(Fig. 8.5)** and lead to widening of heme pockets of the remaining subunits and facilitate the binding of $O_2$ to these subunits.
- As a result, a **stressed** or ***tense (T)*** or ***taut*** structure of the deoxyhemoglobin changes to ***relaxed (R)*** form in oxyhemoglobin **(Fig. 8.5).**

## Cooperative Oxygen Binding of Hemoglobin

- The binding of the first oxygen to heme of the hemoglobin enhances the binding of oxygen to the remaining heme of the same molecule of hemoglobin. This is called **cooperative oxygen binding** of hemoglobin. The cooperative binding of $O_2$ by hemoglobin enhances oxygen transport.
- The shape of $O_2$ binding curve of hemoglobin is ***sigmoidal (S-shaped)*** because oxygen binding is cooperative **(Fig. 8.6).** This shape indicates that the affinity of hemoglobin for binding the first molecule of oxygen is relatively very low but subsequent oxygen molecules are bound with a very much higher affinity (almost 500-fold) **(Fig. 8.7)** accounting for the **steeply rising portion of the S-shaped curve.**
- Because of cooperativity between $O_2$ binding sites, hemoglobin delivers more $O_2$ to tissues than would a noncooperative protein **myoglobin (Fig. 8.6)**

**Fig. 8.6:** Oxygen binding curves for hemoglobin and myoglobin. Note that the curve for hemoglobin is sigmoidal while that for myoglobin is hyperbolic.

## Effect of pH and Carbon Dioxide Concentration on Binding of Oxygen to Hemoglobin and Bohr Effect

- Hemoglobin also transports a significant amount (about 20%) of the total $H^+$ and $CO_2$ from tissues to the lungs and the kidney. The formation of $CO_2$ causes an increase in $H^+$ ion concentration (i.e. decrease in pH) in tissues.

**Fig. 8.7:** Cooperative binding of $O_2$ to hemoglobin.

- Binding of $H^+$ and $CO_2$ to hemoglobin decreases the binding of $O_2$. Thus, at a relatively low pH and high $CO_2$ concentration in the peripheral tissues, the affinity of hemoglobin for $O_2$ is decreased as $H^+$ and $CO_2$ are bound.
- Conversely, in the lung capillaries, as $CO_2$ is excreted and the blood pH consequently rises, the affinity of hemoglobin for $O_2$ is increased.

This effect of pH and $CO_2$ concentration on the binding and release of $O_2$ by hemoglobin is called ***Bohr* Effect,** after the Danish physiologist who discovered it. Thus, oxygen dissociation of hemoglobin is influenced by both **pH** and **$CO_2$ *concentration.*** This inverse relationship between the binding of oxygen on one hand and binding of $H^+$ and $CO_2$ on the other hand is advantageous to the organism.

- In the tissues, the low pH and high concentration of $CO_2$ tend to cause release of $O_2$ from hemoglobin
- In the lungs the higher concentration of $O_2$ tends to promote release of $H^+$ and $CO_2$

## Bohr Effect

- The $CO_2$ generated in peripheral tissues combines with water to form carbonic acid, which dissociates into protons and bicarbonate ions. The deoxygenated hemoglobin acts as a buffer by binding $H^+$ ions and delivering them to the lungs
- In the lungs, the binding of $O_2$ by hemoglobin releases $H^+$ ions that combine with bicarbonate ion, forming carbonic acid, which is dehydrated to $CO_2$, and exhaled from the lungs **(Fig. 8.8)**.

**Fig. 8.8:** Bohr effect.

### *Effect of 2-3 Bisphosphoglycerate (BPG) on Binding of Oxygen to Hemoglobin*

2-3 BPG is found in red blood cells at nearly the same concentration as hemoglobin. It is formed as an intermediate in glycolysis in RBC **(*see* Fig. 12.6).**

- 2-3 BPG regulates the binding of $O_2$ to hemoglobin.
- The presence of BPG significantly reduces the affinity of hemoglobin for oxygen.
- This reduced affinity releases oxygen efficiently in peripheral tissues.
- One molecule of 2-3 BPG binds in the central cavity of deoxyhemoglobin. It binds with β-chains through ionic bonds.
- These ionic bonds are formed between positively charged amino acids of β-chain with negatively charged phosphate groups of 2-3 BPG.
- In HbA, the binding site is made up of six +ve charges of amino acids of β-globin chains and five -ve charges of phosphate groups of 2-3 BPG **(Figs. 8.9A and B).**

### *Importance of 2-3 BPG*

- Without 2-3 BPG, hemoglobin would be an extremely **inefficient** oxygen transporter, releasing only 8 percent of its oxygen in the tissue and the oxygen saturation curve of hemoglobin would approach that of myoglobin.
- When there is a chronic deprivation of oxygen in tissue, the level of 2-3 BPG increases, such compensatory increase occurs in:
  - Individuals who live at high altitudes
  - Patients with chronic obstructive pulmonary disease (COPD) like emphysema
  - Anemia
  - Cardiac failure.

# TYPES OF NORMAL AND ABNORMAL HEMOGLOBIN

## Normal Hemoglobin

Several different forms of normal hemoglobin can be found in adult humans and during human development,

**Figs. 8.9A and B:** (A) Structure of 2,3-BPG; (B) Schematic representation of binding of 2,3-BPG to the hemoglobin.

each containing four polypeptide subunits. Most forms of hemoglobin contain **two α-chains** plus two other chains usually **β,** γ and **δ (*see* Table 8.1)**.

## *Adult Hemoglobin ($HbA_1$)*

It consists of **two alpha** and **two beta** chains and designated as $\alpha_2\beta_2$. Approximately 98 percent of the total hemoglobin of a normal adult is of this type.

## *Minor Component of Normal Adult Hemoglobin ($HbA_2$)*

It consists of **two alpha** and **two delta** chains and is designated as $\alpha_2\delta_2$. It is present usually to the extent of 2.5 percent of the total.

## *Fetal Hemoglobin (HbF)*

HbF is the major hemoglobin found in a fetus and a new born. It consists of **two alpha** and **two gamma** chains.

After birth, the gamma (γ) subunits are replaced by beta (β) chains and become $\alpha_2\beta_2$ ($HbA_1$).

## *Glycosylated Hemoglobin ($HbA_{1C}$)*

$HbA_1$ (adult Hb) reacts spontaneously with glucose to form a derivative, known as ***glycosylated hemoglobin ($HbA_{1C}$).***

**Fig. 8.10:** Formation of $HbA_{1C}$.

- The aldehyde group of glucose reacts nonenzymatically with N-terminal residue (valine) of β chains **(Fig. 8.10)**, when blood glucose enters the erythrocytes.
- Normally, the concentration of $HbA_{1C}$ in blood is very low to the extent of about 5 percent of the total hemoglobin.
- Formation of $HbA_{1C}$ is proportional to blood glucose concentration.
- Increased amounts of $HbA_{1C}$ are found in patients with diabetes mellitus, where the blood sugar level is high.
- Measurement of $HbA_{1C}$ provides useful information for the management of diabetes mellitus, since RBCs have a life span of about 120 days, the content of $HbA_{1C}$ is an indicator of how effectively blood glucose levels have been regulated over the previous 2 to 3 months.

## Abnormal Hemoglobin

Since α, β, γ and δ chains of the globin of hemoglobin are synthesized from amino acids under genetic control, mutations in the genes that code for globin chains can affect their formation and biological function of hemoglobin. Such hemoglobin is called ***abnormal hemoglobin.*** When biological function is altered due to a mutation in hemoglobin, the condition is called **hemoglobinopathy.** Some of the abnormal forms of hemoglobin are discussed below.

### *Thalassemia*

- Thalassemia is a group of genetically transmitted disorder of hemoglobin synthesis, due to **lack or decreased synthesis of α or β** globin chains. It is an **autosomal recessive** genetic disorder.
- Because the synthesis of one globin chain is reduced, there is a relative excess synthesis of the other globin chains. These globin chains may precipitate in the cell causing hemolysis, resulting in a ***hypochromic anemia***.
- The name of this group of diseases comes from the Greek word ***"thalasa",*** meaning **"sea",** because this disorder occurs more commonly among people living near the Mediterranean Sea.

### *Types of thalassemia*

Depending upon whether the genetic defect lies in synthesis of α or β globin chains, thalassemia are classified into **α-thalassemia** and **β-thalassemia** respectively.

#### *α-thalassemia*

In this condition, synthesis of α-globin chain is defective. α-globin chains are coded by **four copies of α-globin gene.** The α-thalassemia results from genetic defect in one or more copies of α-globin genes (located on chromosome 16) and is characterized by either decreased or total absence of synthesis of α-globin chains. The α-thalassemia is of four types. These are :

a. **Silent carrier type of α-thalassemia:** In this type of α-thalassemia, only one of the four copies of α-globin gene is mutated. Since the patients of this disorder can synthesize sufficient α-globin chains, they do not show any clinical symptoms of thalassemia. They are only carriers of α-thalassemia.

b. **α-thalassemia trait:** It this type of α-thalassemia, two of the four copies of α-globin genes are mutated. They usually have only mild anemia and is not fatal.

c. **Hemoglobin H disease:** In this type of α-thalassemia three of the four copies of α-globin genes are mutated. They have moderately severe hemolytic anemia.

d. **Hydrops fetalis:** In hydrops fetalis, all four copies of α-globin genes are mutated. This is a lethal condition. Most of the affected are stillborn (death before birth) or die soon after birth.

#### *β-thalassemia*

In β-thalassemia, synthesis of **β-globin chain** is impaired due to genetic defect in β-globin genes. β-globin chains are coded by **two copies of β-globin genes** and are characterized by decreased or total absence of synthesis of β-globin chains. The β-thalassemia is of two types. These are:

a. **β-thalassemia minor (also known as β-thalassemia trait):** In this condition, one of the two copies of β-globin genes is mutated. It is a heterozygous state. The presence of one normal gene in the heterozygous allows enough normal globin chain synthesis, so that affected individuals are usually asymptomatic. The individual may be completely normal or has a mild anemia.

b. **β-thalassemia major:** β-thalassemia major is homozygous state, carrying two mutated β-globin genes. Thalassemia major leads to **severe anemia.** They regularly need blood transfusion. Bone marrow transplant has recently been introduced as a remedy.

### *Sickle Anemia and Sickle Hemoglobin (Hbs)*

- Sickle cell anemia is a genetic disorder caused by production of abnormal hemoglobin, known as **sickle hemoglobin (HbS).**
- Production of HbS is due to **mutation in the β-globin gene** which codes for β-globin chain. The mutant β-globin chain of HbS has an altered amino acid sequence.
- **Glutamic acid** residue normally present in the sixth position of β-chain of HbA is replaced by a **valine** residue as a result of mutation in the β-globin chain.

  1 2 3 4 5 6 7 8
  Val—His—Leu—Thr—Pro—**Glu**—Glu—Lys—(HbA)
  Val—His—Leu—Thr—Pro—**Val**—Glu—Lys—(HbS)
- As polar (hydrophilic) glutamic acid residue is replaced by nonpolar (hydrophobic) valine, it generates hydrophobic contact point called, **"Sticky patch",** on the outer surface of the β-globin chain. This alteration markedly reduces the solubility of deoxygenated HbS.

**Fig. 8.11:** Schematic representation of polymerization of deoxy-Hb S molecules and formation of tubular fibrous structure.

- When HbS is deoxygenated, the sticky patch can bind to another deoxygenated HbS molecule. ***This binding causes polymerization of deoxy-HbS forming insoluble long tubular fiber*** **(Fig. 8.11).**
- The insoluble fibers of deoxygenated HbS are responsible for deforming the red blood cells, which look like the ***blade of a sickle*** **(Fig. 8.12)**. Hence, the disease is called ***sickle cell anemi.***
- The sickled red blood cells lose water, become fragile and have a much shorter lifespan than normal cells (17 days compared with 120 days), leading to lysis of the red blood cells and results in **hemolytic *anemia,*** called ***sickle cell anemia.***
- The more serious consequence is that, small blood capillaries in different organs become blocked by the long abnormally shaped red cell. This interrupts the supply of oxygen and leads to ***anoxia*** (oxygen deprivation) which causes pain and eventually death of the cell.

### *Sickle cell anemia and sickle cell trait*

- **Sickle cell anemia** is a homozygous disorder in which the individual has inherited two mutant globin genes one from each parent. It is characterized by chronic hemolytic anemia, tissue damage and pain and increased susceptibility to infections. Such patients usually die in their adult age.
- **Sickle cell trait** is a heterozygous state, in which individuals have received the abnormal mutated β-globin gene from only one parent and have one normal gene. They do not show any clinical symptoms and have normal lifespan.

**Fig. 8.12:** Sickle red blood cells.

- Persons with sickle cell trait are resistant to malaria caused by ***plasmodium falciparum.*** This parasite spends an obligatory part of its life-cycle in the red blood cell. Since the sickle red blood cells have a shorter lifespan than normal red blood cell, the parasite cannot complete its life cycle.

## *HbC or Cooley's Hemoglobin*

- In HbC, the glutamic acid at position 6 in the β-chain is replaced by a lysine residue.
- The red blood cells of people with HbC do not sickle; however, crystals of HbC may form within the cell.
- Both homozygous and heterozygous individuals of the disease are known. This disease is characterized

by a **mild hemolytic anemia.** Clinically, heterozygous individuals are asymptomatic.

### HbM

- These arise due to mutation in histidine residue of either α-or β-chains, which bound with the iron in the heme molecule.
- In HbM, ***histidine*** is replaced by ***tyrosine*** and iron is stabilized in the ferric ($Fe^{3+}$) instead of ferrous ($Fe^{2+}$) form which cannot bind oxygen and leads to cyanosis.
- The letter **'M'** of HbM signifies that the affected chains are in the ***methemoglobin*** (ferric hemoglobin) form.

## DERIVATIVES OF HEMOGLOBIN

- Hemoglobin readily combines with any gas or other substances to form some products which are called the ***derivatives of hemoglobin.*** These can be grouped into:
  1. Normal derivatives
  2. Abnormal derivatives.

The derivatives of hemoglobin give characteristic absorption bands (dark lines) in the solar spectrum when viewed through simple device "**spectroscope".** The bands arise due to absorption of light by hemoglobin derivatives. The absorption maxima of these bands differ from one hemoglobin derivative to another, which is used in the identification of these compounds.

### Normal Hemoglobin Derivatives

The normal hemoglobin derivatives are:
- Oxyhemoglobin
- Reduced hemoglobin
- Carbaminohemoglobin or carboxyhemoglobin.

### Oxyhemoglobin

This is formed by the combination of hemoglobin with $O_2$ by the process of oxygenations.

$$\underset{\text{Reduced Hb}}{Hb + 4O_2} \rightleftharpoons \underset{\text{Oxy Hb}}{Hb4O_2}$$

Oxyhemoglobin is an unstable compound and the combination is reversible, i.e. oxygen can be released from this compound. The iron remains in ferrous ($Fe^{2+}$) state in this compound.

### Reduced Hemoglobin

When oxygen is released from oxyhemoglobin, it is called reduced hemoglobin.

### Carbaminohemoglobin or Carboxyhemoglobin

It is a derivative of hemoglobin with carbon dioxide. Carbon dioxide can be released easily from this. The affinity of hemoglobin with $CO_2$ is 20 times more than for oxygen.

### Abnormal Hemoglobin Derivatives

- Abnormal hemoglobin derivatives reduce the oxygen carrying capacity of the blood.
- Abnormal hemoglobin derivatives are compounds of clinical importance. Measurement of these abnormal hemoglobin derivatives can be helpful in the diagnosing and monitoring exposure to the toxic compounds.

The abnormal hemoglobin derivatives can all be identified by means of their characteristic absorption spectra, in the solar spectrum by which they may be identified and it is possible to measure the various derivatives quantitatively if they are present in sufficient amounts.

- Abnormal hemoglobin derivatives are **(Table 8.2):**
  1. Methemoglobin
  2. Carboxyhemoglobin (COHb).
  3. Sulfhemoglobin

### Methemoglobin

- It is hemoglobin in which the iron is present in ferric ($Fe^{2+}$) state. This cannot transport oxygen.
- Only a very small amount of methemoglobin is present in normal erythrocytes (less than 0.5 g/dL), where it is formed during glycolysis and constantly reconverted to hemoglobin. The erythrocyte has enzyme mechanisms for keeping methemoglobin level below 1 percent of the total hemoglobin.
- Pathological increase in the amount of methemoglobin may be due to drugs or due to impaired reconversion of methemoglobin to hemoglobin.

**Table 8.2:** Abnormal hemoglobin derivatives.

| *Abnormal derivative* | *Description* |
|---|---|
| Methemoglobin | Ferrous ($Fe^{2+}$) is replaced by ferric ($Fe^{3+}$). Unable to transport $O_2$. Can be converted back to hemoglobin |
| Carboxyhemoglobin | Oxygen ($O_2$) is replaced by carbon monoxide (CO). Unable to transport $O_2$. Very stable compound. |
| Sulfhemoglobin | Contains one or more sulfur atom. Unable to transport $O_2$ nor can be converted back to hemoglobin |

- The ferrous of hemoglobin is oxidized to ferric state by **superoxide** by certain drugs and other oxidizing agents, forming methemoglobin.
- Increased amount of methemoglobin cause a shift of the oxygen dissociation curve to the left so that the tissues receive less oxygen.

### *Carboxyhemoglobin (COHb)*

- Carbon monoxide combines with the heme moiety in hemoglobin.
- It combines at the same position in the hemoglobin molecule as oxygen but with an affinity about 200 times greater than oxygen. As a result, even small quantities of CO in the inspired air cause the formation of relatively large amounts of COHb, with a corresponding reduction in the $O_2$ carrying capacity of the blood. Even as little as 1% CO in inspired air can be fatal in minutes.

### Sulfhemoglobin

- It contains one or more sulfur atom.
- It is formed by oxidation of hemoglobin in the presence of SH- containing compounds, such as hydrogen sulfide that may arise from bacterial action in the intestine.
- Sulfhemoglobin and methemoglobin are often present at the same time in these patients.
- Sulfhemoglobin cannot act as a carrier of oxygen nor can be converted back to hemoglobin.

## EXAM QUESTIONS

### Long Answer Question (LAQ)

Explain with example various types of abnormal hemoglobin.

### Short Notes

1. Thalassemia.
2. Normal forms of hemoglobin with their constituent polypeptide chains.
3. Sickle hemoglobin.
4. Role of 2, 3-BPG in transport of oxygen by hemoglobin.
5. Derivatives of hemoglobin.
6. A stressed or tense (T) or taut (deoxyhemoglobin) and relaxed (R) (oxyhemoglobin) forms of hemoglobin.
7. Binding sites for oxygen, hydrogen ($H^+$) and carbon dioxide ($CO_2$) with hemoglobin.
8. Cooperative oxygen binding of hemoglobin.
9. Effect of pH and carbon dioxide concentration on binding of oxygen to hemoglobin and Bohr effect.
10. Structure and function of hemoglobin.

### Solve

**Case History**

**A 20-year-old male complaining of severe back pain was hospitalized and sickle cell anemia was diagnosed.**

**Questions**

1. What is sickle cell anemia?
2. Give its biochemical cause.
3. Give cause for the sickling of RBC.
4. Why does a person with sickle cell trait show an increased resistance to malaria?

**Case History**

**An 8 years old girl was brought to the pediatrician for consultation. She was pale and her hemoglobin was 6 g/dl. Following further diagnostic studies, she was diagnosed with alpha thalassemia.**

1. What is thalassemia?
2. Give its biochemical cause.
3. Which are the different types of alpha thalassemia?
4. Give its type of inheritance pattern.

### Multiple Choice Questions (MCQs)

**1. 2, 3-BPG binds Hb by salt bonds by cross linking:**

a. $\beta_1, \beta_2$ b. $\alpha_1, \alpha_2$
c. $\beta_1, \alpha_1$ d. $\beta_2, \alpha_2$

**2. HbF has high affinity for $O_2$ than HbA due to presence of:**

a. β-chain b. γ-chain
c. δ-chain d. ε-chain

**3. The following are the normal forms of hemoglobin, *except*:**

a. $HbA_{1C}$ b. HbF
c. $HbA_1$ d. HbS

**4. Which of the following is not true for hydrops fetalis?**
a. It is β-thalassemia
b. It is α-thalassemia
c. Neither HbF nor HbA can be synthesized
d. Death occurs before birth

**5. Persons with sickle cell trait show an increased resistance to:**
a. Typhoid b. Cancer
c. Malaria d. Diabetes

**6. Hb Bart consists of:**
a. $\alpha_4$ b. $\beta_4$
c. $\gamma_4$ d. $\delta_4$

**7. Iron in heme is linked to globin through:**
a. Histidine b. Arginine
c. Glycine d. Cysteine

**8. Which of the following has a protective effect against malaria?**
a. HbF b. HbM
c. HbS d. Hb Bart

**9. Porphyrin is formed by joining pyrrole rings by:**
a. Methene bridge
b. Hydrogen bond
c. Phosphate bond
d. Glycosidic bond

**10. Normal adult hemoglobin ($HA_1$) has the following chains:**
a. $\alpha_2\beta_2$ b. $\alpha_2\delta_2$
c. $\alpha_2\gamma_2$ d. $\alpha_2\varepsilon_2$

**11. Hemoglobin is a:**
a. Monomeric protein b. Trimeric protein
c. Tetrameric protein d. Dimeric protein

**12. Which type of thalassemia results from mutation of three genes and produces a moderate hemolytic anemia:**
a. Silent carrier type α-thalassemia
b. Hemoglobin H disease
c. α-thalassemia trait
d. Hydrops fetalis

**13. In silent carrier type thalassemia, how many genes are mutated:**
a. 1 b. 2
c. 3 d. 4

**14. Which hemoglobin contains two alpha and two gamma chains and has high affinity for oxygen:**
a. HbF b. $HbA_2$
c. $HbA_{1C}$ d. Hb Bart's

**15. The oxygen binding curve of hemoglobin is sigmoidal because:**
a. Binding of the first oxygen molecule to heme increases the oxygen affinity of the other heme groups
b. The distal histidine allows the hemoglobin molecule to change its conformation in response to an elevated $CO_2$ concentration
c. The subunits are held in place by interchain disulfide bonds
d. The solubility of the hemoglobin molecule changes with its oxidation state

## Answers for MCQs

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **1.** a | **2.** b | **3.** d | **4.** a | **5.** c | **6.** c | **7.** a | **8.** c |
| **9.** a | **10.** a | **11.** c | **12.** b | **13.** a | **14.** a | **15.** a | |

CHAPTER

# 9 Chemistry of Nucleic Acids

**Chapter outline**

- Nucleic Acids
- Structure and Functions of Nucleotides
- Biologically Important Nucleotides
- Synthetic Analogues of Nucleotides or Antimetabolites
- DNA Structure and Function
- Organization of DNA
- RNA Structure and Function

## INTRODUCTION

Nucleic acids are macromolecules present in all living cells in combination with proteins to form ***nucleoproteins***. The protein is usually **protamines** and **histones**. Genetic information is encoded in a nucleic acid molecule.

## NUCLEIC ACIDS

- Nucleic acids are polymers of ***nucleotides***, linked by **phosphodiester** bond, they are therefore called **polynucleotides**.
- The nucleic acids are of two main types:
  - Deoxyribonucleic acid or DNA
  - Ribonucleic acid or RNA.
- DNA is present in nuclei and small amounts are also present in **mitochondria**, whereas 90% of the RNA is present in cell **cytoplasm** and 10% in the **nucleolus**.

## STRUCTURE AND FUNCTIONS OF NUCLEOTIDES

Nucleotides are the building block of **nucleic acid**. Nucleotides have three characteristic components:

1. Nitrogen containing base
2. Pentose sugar
3. One or more phosphates

The molecule without phosphate group is called **nucleoside**.

### Nitrogen Containing Bases of RNA and DNA

Two classes of nitrogen containing bases namely **purines** and **pyrimidines** are present in RNA and DNA.

#### *Purine Bases*

Two principal purine bases found in DNAs, as well as RNAs **(Fig. 9.1)** are:

i. Adenine (A)
ii. Guanine (G).

**Fig. 9.1:** Structure of purine ring and purine bases.

### Pyrimidine Bases

Three major pyrimidine bases **(Fig. 9.2)** are:

i. Cytosine (C)
ii. Uracil (U)
iii. Thymine (T).

- Cytosine and uracil are found in RNAs and cytosine and thymine in DNA.
- Both DNA and RNA contain the pyrimidine cytosine but they differ in their second pyrimidine base. DNA contains thymine whereas RNA contains uracil.

### Pentose Sugars Present in RNA and DNA

- Nucleic acids have two kinds of **pentoses,** either ***D-ribose*** or ***D-2-deoxyribose*** **(Fig. 9.3)**.
- DNA and RNA are distinguished on the basis of the pentose sugar present.
- DNA contains **D-2-deoxyribose** and RNA contains **D-ribose**.

**Fig. 9.2:** Structure of pyrimidine ring and pyrimidine bases.

- A pentose sugar (D-ribose or D-2-deoxyribose) is linked to a base (purine or pyrimidine) via covalent **N-glycosidic bond (Fig. 9.4)** and the structure formed is called **nucleoside**. The term **nucleoside** is used for structures containing only sugar and nitrogen base without phosphate a group.
- **N-glycosidic bond** joins **nitrogen-9** of the purine base or **nitrogen-1** of the pyrimidine base with carbon 1 of pentose sugar **(Fig. 9.4).**
- The atoms of the base in nucleosides are given ***cardinal*** numbers, whereas the carbon atoms of the sugars are given **primed** numbers as shown in **Figure 9.4** to distinguish sugar atoms from those of the nitrogen base.
- The nucleosides of A, G, C, T and U are named adenosine, guanosine, cytidine, thymidine and uridine respectively.
- If the sugar is ribose, ribonucleoside is produced; if the sugar is 2-deoxyribose, a deoxyribonucleoside is produced. The structures of these two types of nucleoside are illustrated by the examples in the **Figure 9.4**. **Table 9.1** indicates the bases and their corresponding nucleosides.

### Attachment of Phosphate to Pentose Sugar

- The phosphate group is attached by an ester linkage to **5′-carbon** of pentose sugar **(Fig. 9.5)**.
- Cells also contain nucleotides with phosphate groups in position other than on the 5′-carbon.

**Fig. 9.3:** Structure of sugars present in nucleic acid.

**Fig. 9.4:** Structures of nucleoside.

**Table 9.1:** Different major bases with their corresponding nucleosides and nucleotides.

| *Base* | *Ribonucleoside* | *Ribonucleotide* |
|---|---|---|
| Adenine (A) | Adenosine | Adenosine monophosphate (AMP) |
| Guanine (G) | Guanosine | Guanosine monophosphate (GMP) |
| Uracil (U) | Uridine | Uridine monophosphate (UMP) |
| Cytosine (C) | Cytidine | Cytidine monophosphate (CMP) |
| *Base* | *Deoxyribonucleoside* | *Deoxyribonucleotide* |
| Adenine | Deoxyadenosine | Deoxyadenosine monophosphate (dAMP) |
| Guanine | Deoxyguanosine | Deoxyguanosine monophosphate (dGMP) |
| Uracil | Deoxyuridine | Deoxyuridine monophosphate (dUMP) |
| Cytosine | Deoxycytidine | Deoxycytidine monophosphate (dCMP) |
| Thymine | Deoxythymidine | Deoxythymidine monophosphate (dTMP) |

**Fig. 9.5:** Structure of nucleotide.

- Mononucleotides are nucleosides in which single phosphate group is attached to hydroxyl group of the pentose sugar. For example, AMP (adenosine monophosphate) is adenine + ribose + phosphate.
- If an additional phosphate group is attached to the pre-existing phosphate of mononucleotide, a dinucleotide (e.g. ADP) or trinucleotide (e.g. ATP) results **(Fig. 9.6)**.
- The principle bases, their respective nucleosides and nucleotides found in the structure of nucleic acids are given in **Table 9.1.**

## *Functions of Nucleotides*

Nucleotides carry out various biological functions, some of these are discussed below:

- They are activated precursors of DNA and RNA.
- In addition to their roles as the subunit of nucleic acids nucleotides have a variety of other functions in every cell as:
  1. Energy carriers, e.g. ATP, GTP, CTP and UTP.
  2. Components of enzyme cofactors, e.g. adenine nucleotide is component of three major coenzymes **NAD**$^+$, FAD$^+$ and **CoA** and
  3. Chemical messengers, e.g. **cAMP**, and **cGMP**. One of the most common is adenosine 3', 5'–cyclic monophosphate (**cAMP**), serves regulatory functions in virtually every cell.
  4. Activator of metabolic intermediates: Nucleotides are required for activation of intermediates in many biosynthetic pathways, e.g. UDP-glucose and CDP-diacylglycerol are precursors for glycogen and phospholipid synthesis.

**Fig. 9.6:** Structure of ATP and its components.

## BIOLOGICALLY IMPORTANT NUCLEOTIDES

Besides being the structural components of nucleic acids, several nucleotides such as ATP, ADP, c-AMP, GTP, GDP, c-GMP, UDP, CTP, CDP, etc. participate in several biochemical and physiological functions. Such nucleotides are called **biologically important nucleotides**. Nucleotides involved in a various biochemical processes are discussed below.

### ATP

ATP serves as the main biological source of energy in the cell. ATP is required as a source of energy in several metabolic pathways, e.g. fatty acid synthesis, glycolysis, cholesterol synthesis, protein synthesis, gluconeogenesis, etc. and in physiologic functions such as muscle contraction, nerve impulse transmission, etc. ATP is a universal **currency of energy** in biological systems.

### AMP

AMP is the component of many coenzymes such as $NAD^+$, $NADP^+$, FAD, coenzyme A, etc. These coenzymes are essential for the metabolism of carbohydrate, lipid and protein.

### C-AMP (Cyclic Adenosine 3′, 5′-monophosphate) (Fig. 9.7)

- c-AMP is formed from ATP by the action of ***adenylate* cyclase**.
- c-AMP acts as a second messenger for many hormones, e.g. epinephrine, glucagon, etc. c-AMP affects a wide range of cellular processes by acting as a second messenger **(Table 9.2)**
- It enhances the degradation of storage fuels like fat and glycogen by stimulating lipolysis, glycogenolysis. cAMP serves regulatory functions in virtually every cell.
- It inhibits the aggregation of blood platelets.
- c-AMP increases the secretion of acid by the gastric mucosa.

Adenosine 3'-5' cyclic monophosphate (cyclic AMP; cAMP)

**Fig. 9.7:** Structure of c-AMP.

**Table 9.2:** Hormones using c-AMP as a second messenger.

| |
|---|
| • Calcitonin |
| • Chorionic gonadotropin |
| • Corticotropin |
| • Epinephrine |
| • Follicle stimulating hormone |
| • Glucagon |
| • Luteinizing hormone |
| • Melanocyte stimulating hormone |
| • Norepinephrine |
| • Parathyroid hormone |
| • Thyroid stimulating hormone |
| • Vasopressin |

### GDP and GTP

- These guanosine nucleotides participate in the conversion of **succinyl-CoA to succinate,** a reaction which is coupled to the substrate level phosphorylation of GDP to GTP in citric acid cycle.
- GTP is required for activation of adenylate cyclase by some hormones.
- GTP serves as an energy source for protein synthesis.

### C-GMP (Cyclic guanosine 3′, 5′-monophosphate)

- c-GMP is formed from GTP by ***guanylyl cyclase.***
- c-GMP is an intracellular signal or second messenger that can act antagonistically to c-AMP.
- c-GMP is involved in relaxation of smooth muscle and vasodilation.

### UDP (Uridine Diphosphate)

- UDP participates in glycogenesis.
- UDP-glucose and UDP-galactose take part in galactose metabolism and required for synthesis of lactose and cerebrosides.
- UDP-glucuronic acid is required in detoxification processes and for biosynthesis of mucopolysaccharides such as heparin, hyaluronic acid, etc.

### CTP (Cytidine Triphosphate) and CDP (Cytidine Diphosphate)

- CTP and CDP are required for the biosynthesis of some phospholipids. CDP-choline is involved in the synthesis of sphingomyelin.

## SYNTHETIC ANALOGUES OF NUCLEOTIDES OR ANTIMETABOLITES

Chemically, synthesized analogues of purines and pyrimidines, their nucleosides and their nucleotides have therapeutic applications in medicine. An analogue is prepared either by altering the heterocyclic ring or sugar moiety. These are used **chemotherapeutically** (treatment of disease by the use of chemical substances) to control **cancer** or **infections**.

Analogues of purines and pyrimidines used in the treatment of infections or in cancer chemotherapy are:

- The nucleoside ***cytarabine (arabinosyl cytosine; ara-c)*** in which arabinose replaces ribose, is used in the chemotherapy of cancer and viral infections.
- The purine analogue ***allopurinol*** used in treatment of hyperuricemia and gout.
- ***Azidothymidine (AZT)*** is a structural analogue of thymidine used in the treatment of acquired immunodeficiency syndrome (AIDS), a disease caused by the human immunodeficiency virus (HIV).
- ***Azathioprine,*** which is used during organ transplantation to suppress events involved in immunologic rejection.
- ***5-iododeoxyuridine,*** a nucleoside analogue has antiviral activity and is used in the treatment of herpetic keratitis, an infection of the cornea by *Herpes* virus.

## DNA STRUCTURE AND FUNCTION

DNA serves as the genetic material for cells both prokaryotes and eukaryotes. In eukaryotes, DNA is located in the nucleus separated from cytoplasm by the nuclear membrane. Because prokaryotes lack internal membrane systems, their DNA is not separated from the rest of the cellular contents. Eukaryotic DNA is bound to proteins, forming a complex known as chromatin.

DNA is also present in mitochondria (less than 0.1% of the total DNA) and in chloroplast of plants. Many viruses also contain DNA as their genetic material.

### Structure of DNA

- DNA is a very long, thread like macromolecule made up of a large number of **deoxyribonucleotides**. Deoxyribo-

**Fig. 9.8:** Structure of polynucleotide chain of DNA.

nucleotide is composed of a ***nitrogenous base, a sugar*** and ***phosphate group.***

- The bases of DNA molecule carry genetic information, whereas their sugar and phosphate groups perform a structural role.
- The sugar in a deoxyribonucleotide is ***deoxyribose.***
- The purine bases in DNA are **adenine (A),** and **guanine (G)**.
- Pyrimidine bases are **thymine (T)** and **cytosine (C)**.
- DNA is a polymer of many deoxyribonucleotides linked covalently by **3′, 5′ phosphodiester bonds**. The 3′-hydroxyl group of the sugar moiety of one deoxyribonucleotide is joined to the 5′-hydroxyl group of the adjacent sugar moiety of deoxyribonucleotide by a phosphodiester linkage **(Fig. 9.8)**.

## *The Watson-Crick DNA Double Helical Structure*

In 1953, James Watson and Francis Crick deduced the three-dimensional structure of DNA. The important features of their model of DNA are as follows:

- It consists of two helical polynucleotide chains wound around the same axis to form a right handed double helix.
- The chains run in opposite direction (antiparallel) **(Fig. 9.9)**.
- The purine and pyrimidine bases are on the inside of the helix, whereas the phosphate and deoxyribose units are on the outside.
- The diameter of the helix is 20 Å. Adjacent bases are separated by 3.4 Å along the helix axis and the helical structure repeats after ten residues on each chain, i.e. at intervals of 34 Å **(Fig. 9.10)**.
- The two chains are held together by hydrogen bonds between complementary pairs of bases:
  - ***Adenine is always paired with thymine by formation of two hydrogen bonds. Guanine is always paired with cytosine by formation of three hydrogen bonds*** **(Fig. 9.11)**.

**Fig. 9.9:** Antiparallel strands of DNA.

- The two strands are always complementary to each other. In a double stranded DNA molecule, the content of adenine equals to that of thymine and the contents of guanine equals to that of cytosine. The complementary base pairing proves the **Chargaff's rule** (discussed later).
- The model proposed by Watson and Crick **(Fig. 9.10)** is a **B form of DNA *(B-DNA)*** which is a right handed helix of 10 base pairs per turn, containing grooves of alternate size, known as major and minor grooves.

**Fig. 9.10:** A diagrammatic representation of the Watson and Crick model of the double-helical structure of the B form of DNA.

**Fig. 9.11:** Base pairing between adenine and thymine involves the formation of two hydrogen bonds and base pairing between cytosine and guanine involves the formation of three hydrogen bonds.

- Other forms of DNA may also occur, such as ***A-DNA*** and ***Z-DNA***. Under physiologic conditions, DNA is almost entirely in Watson-Crick B form.

### *Chargaff's Rule*

- Ervin Chargaff discovered that in DNA of all species, quantity of purines is the same as that of pyrimidines (A + G = T + C). He observed that in DNA, content of adenine equals that of thymine (A = T) and content of guanine equals that of cytosine (G = C).
- Watson and Crick deduced that adenine must pair with thymine and guanine with cytosine, because of stearic and hydrogen bonding factors. Adenine cannot pair with cytosine; guanine cannot pair with thymine.
- Thus, one member of a base pair in a DNA must always be a purine and the other a pyrimidine.
- This base pairing restriction explains that in a double stranded DNA molecule, the content of A equals that of T and the content of G equals that of C.

The ratio of purine to pyrimidine bases in the DNA is always one, i.e. G + A **:** T + C = 1. These quantitative relationships called **Chargaff's rule**.

## Functions of DNA

DNA is the store of genetic information. The genetic information stored in the DNA serves two functions.

1. It is the source of information for the synthesis of all protein molecules of the cell and
2. It provides the information inherited by daughter cells or offspring.

# ORGANIZATION OF DNA

## Prokaryotic DNA

A prokaryotic cell generally contains a single chromosome composed of double stranded circular DNA, which contains over $4 \times 10^6$ base pairs. Because DNA molecules are so large, they require special organization/packaging to enable them to reside within cells. In *E. coli*, the circular DNA is supercoiled and attached to an RNA-protein core.

## Eukaryotic DNA

- Eukaryotes contain over 1,000 times the amount of DNA found in prokaryotes. Consequently, their method of organizing or packing DNA is much more complex.
- A typical human cell contains 46 chromosomes, whose total DNA is approximately two meter in length.
- The packing of DNA in a chromosome represents a 10,000 fold shortening of its length from primary B-form DNA.
- In resting nondividing eukaryotic cells, the chromosomal material is called **chromatin**. Chromatin is made up of nucleosomes.

### *Different Levels of Organization of Eukaryotic DNA*

#### *Nucleosomes*

- Nucleosomes are primary structural units of chromatin; which consists of **DNA bound to protein histones**.
- There are five types of histones, designated **H1, H2A, H2B, H3** and **H4**. Two molecules of each H2A, H2B, H3 and H4 associate with one another to form a structural core called **histone octamer (Fig. 9.12)**.
- Around histone octamer a segment of the DNA double helix is wound nearly twice forming **nucleosome core** particles (a "bead").

#### *Chromatin fiber*

- Chromatin is made up of repeating units of nucleosomes in which one nucleosome core joins to the next to form chromatin fiber.

**Fig. 9.12:** Diagrammatic representation of nucleosome.

**Fig. 9.13:** Schematic representation of chromatin fiber (Beads on a string structure).

- Nucleosomes are separated by spacer DNA to which histone H1 is attached to stabilize the complex **(Fig. 9.13)**. This continuous string of nucleosomes representing beads on a string form of chromatin is termed as **10 nm fiber**.

*Solenoid structure*

In addition to the net shortening of a DNA strand produced by winding of it around the histones, additional shortening packaging of eukaryotic DNA is brought about by further coiling of 10 nm chromatin fiber to produce 30 nm fiber which has a **solenoid structure** with six nucleosomes per turn. The solenoids are further folded into supercoiled loops to produce 300 nm form **chromosomes**.

A schematic view of the different levels of organization of DNA in the chromosome is shown in **Figure 9.14**.

## RNA STRUCTURE AND FUNCTION

Unlike double stranded helical structure of DNA, the RNAs are single stranded. RNA is an unbranched linear polymer of ribonucleotides joined by 3', 5' phosphodiester bonds. The phosphodiester bonds join the 3'-OH group of ribose of one nucleotide unit to the 5'-OH group of ribose sugar of the next nucleotide.

### Differences between RNA and DNA

- In RNA, the sugar is **ribose** rather than **2'-deoxyribose** of DNA.
- RNA does not possess **thymine** except in the rare case. Instead of thymine, RNA contains **uracil**.
- In RNA, adenine pairs with uracil rather than thymine.
- Unlike DNA, RNA is a single stranded and does not exhibit the equivalence of adenine with uracil and cytosine with guanine.

### Types of RNA

Cell contains three major types of RNA:

1. Messenger RNA (mRNA)
2. Transfer RNA (tRNA)
3. Ribosomal RNA (rRNA).

All of these are involved in the process of protein biosynthesis. Each differs from the others by size and function.

**Fig. 9.14:** Packaging of DNA, arranged in increasing order of organization from top to bottom.

**Fig. 9.15:** Structure of mRNA.

## *Messenger RNA (mRNA)*

### *Structure of mRNA (Fig. 9.15)*

- The mRNA comprises only about 5-10% of total cellular RNA.
- mRNA is synthesized in the nucleus as heterogeneous RNA (hnRNA), which are processed into functional mRNA.
- The mRNA carries the genetic information in the form of codons. Codons are a group of three adjacent nucleotides that code for the amino acids of protein.
- In eukaryotes mRNAs have some unique characteristics, e.g. the 5' end of mRNA is ***"capped"*** by a ***7-methyl-guanosine triphosphate.***
- The cap is involved in the recognition of mRNA in protein biosynthesis and it helps to stabilize the mRNA by preventing attack of 5'-exonucleases.
- A poly (A) ***"tail"*** is attached to the other 3'-end of mRNA. This tail consists of series of **adenylate residues**, 20-250 nucleotides in length joined by 3' to 5' phosphodiester bonds.
- The function of poly A tail is not fully understood, but it seems that it helps to stabilize mRNA by preventing the attack of 3'-exonuclease.
- If the mRNA codes for only one peptide, the mRNA is **monocistronic**. If it codes for two or more different polypeptides, the mRNA is **polycistronic**. In eukaryotes most mRNA are monocistronic.

### *Function of mRNA*

mRNAs serve as template for protein biosynthesis and transfer genetic information from DNA to the site of protein synthesis, the **ribosome**.

## *Transfer RNA (tRNA)*

tRNA molecules vary in length from 74 to 95 nucleotides. In eukaryotic cells, 10-20% of the nucleotides of tRNA may be modified and known as unusual nucleotides **(Fig. 9.16)**, e.g.

- ***Dihydrouridine (D)***, in which one of the double bonds of the base is reduced.
- ***Ribothymidine (T),*** in which methyl group is added to uracil to form thymine.
- ***Pseudouridine (ψ)***, in which uracil is attached to ribose by a carbon-carbon bond rather than a nitrogen bond.

### *Structure of tRNA*

- All single stranded transfer RNA molecules get folded into a structure that appears like a **clover leaf**. All t-RNAs contain four main arms:
  1. The acceptor arm
  2. The D arm
  3. The anticodon arm
  4. The TψC arm.

**Fig. 9.16:** Three unusual (modified) nucleosides present in tRNA.

**Fig. 9.17:** Structure of clover leaf transfer RNA.

The arms have base paired stems and unpaired loops as shown in **Figure 9.17.** The structure of tRNA molecules is maintained by the base pairing in these arms or stem regions.

- The **acceptor arm** consists of a base paired stem that terminates in the sequence **CCA** at the 3' end. This is the attachment site for the amino acid.
- The **D arm** is named for the presence of the base dihydrouridine (D).
- **The anticodon arm** contains the **anticodon** that base pairs with the codon on mRNA. Anticodon has nucleotide sequence complementary to the codon of mRNA and is responsible for the specificity of the tRNA.
- The TψC arm contains both **ribothymidine (T)** and **pseudouridine (ψ, psi)**
- The extra arm is also known as variable arm because it varies in size, is found between the anticodon and TψC arms.

### *Function of tRNA*

tRNA carries amino acids in an activated form to the ribosome for the protein synthesis.

## *Ribosomal RNA (rRNA)*

The RNA of the ribosomes is called the rRNA.

- A ribosome is a cytoplasmic nucleoprotein that acts as machinery for the synthesis of proteins.
- The ribosome is a spheroidal particle and is composed of a large and a small nucleoprotein subunit.
- The eukaryotic ribosomes are composed of 60S and 40S subunits **(Fig. 9.17)**.
- Each subunit is composed of one or more strand of rRNA and numerous protein molecules **(Fig. 9.18)**.
- The 60S subunit contains 28S rRNA, 5S rRNA and 5.8S rRNA, while the 40S subunit contains 18S rRNA.

**Fig. 9.18:** The components of eukaryotic ribosomal subunits.

**Table 9.3:** Different types of cellular RNAs and their functions.

| *Types of RNA* | *Functions* |
|---|---|
| mRNA (messenger RNA) | Carries the genetic information from DNA to the cytosol, where it is used for protein synthesis |
| tRNA (transfer RNA) | Serves as an "adaptor" molecule that carries specific amino acid to the site of protein synthesis |
| rRNA (ribosomal RNA) | In association with protein serves as the sites for protein synthesis. Provides catalytic activities (peptidyl transferase activity) |
| hnRNA (heterogeneous nuclear RNA) | Serves as precursor for mRNA |
| scRNA (small nuclear cytoplasmic RNA) | Involved in recognition of signal sequence in protein synthesis on membrane bound ribosomes |
| snRNA (small nuclear RNA) | Involved in excising introns and splicing exons |

*Functions of ribosomal RNA*

The function of the ribosomal RNA molecules in the ribosomal particle are not fully understood, but they are:

- Necessary to maintain ribosomal structure and also participate in protein synthesis by binding of mRNA to ribosome.
- Recent studies suggest that ribosomal RNAs may also provide some of the catalytic activities and thus is an enzyme "a ***ribozyme".***

## Other Nuclear and Cytoplasmic RNAs

Besides mRNA, tRNA and rRNA, eukaryotes have some other RNAs. These are:

- Heterogeneous RNAs (hnRNAs)
- Small cytoplasmic RNAs (scRNAs)
- Small nuclear RNAs (snRNAs).

Various RNAs and their functions are given in **Table 9.3**.

# EXAM QUESTIONS

## Long Answer Questions (LAQs)

1. Write structure and function of DNA.
2. Write structure and function of RNA.
3. Give different levels of organization of eukaryotic DNA.

## Short Notes

1. cAMP.
2. Synthetic analogues of nucleotides.
3. Biologically important nucleotides.
4. Purine and pyrimidine bases of nucleic acids
5. Name nucleotides of purine and pyrimidine.
6. What is nucleotide? Give functions of nucleotides.
7. tRNA.
8. mRNA.

## Multiple Choice Questions (MCQs)

1. **Which of the following holds DNA strands together?**
   a. Phosphodiester bond
   b. Hydrogen bond
   c. Glycosidic bond
   d. Phosphate ester
2. **Which of the following sugar present in nucleic acid structure?**
   a. Pentose b. Hexose
   c. Tetrose d. Triose
3. **Thymine is present in which of the following:**
   a. Ribosomal RNA b. Messenger RNA
   c. Transfer RNA d. None of the above
4. **Unusual nucleotide pseudouridylic acid is present in:**
   a. mRNA b. tRNA
   c. rRNA d. hnRNA
5. **A nucleoside can be composed of, *except*:**
   a. Purine base b. Pentose sugar
   c. Phosphate group d. Pyrimidine base
6. **Nucleotides perform all of the following functions, *except:***
   a. Structural units of DNA and RNA
   b. Catalytic in nature
   c. Regulators of metabolic reactions
   d. Components of certain coenzymes

**7. Which of the following is not a feature of Watson-Crick Model of DNA?**
a. Helical
b. Two strands are held by hydrogen bond
c. A + T = C + G
d. Two strands are right handed

**8. The number of hydrogen bonds between guanosine and cytosine in DNA are:**
a. One b. Two
c. Three d. Four

**9. DNA is present in:**
a. Only nucleus
b. Only mitochondria
c. Both nucleus and mitochondria
d. Cytoplasm

**10. RNA is present in:**
a. Nucleus
b. Only cytoplasm
c. Mitochondria
d. Cytoplasm and nucleolus

**11. In a double stranded molecule of DNA the ratio of purines to pyrimidines is:**
a. 1:1 b. 1:2
c. 2:1 d. Variable

**12. In DNA base adenine is always paired with:**
a. Guanine b. Cytosine
c. Uracil d. Thymine

**13. Which of the following is a correct description of the chemistry of DNA and RNA?**
a. Uracil is a purine base that occurs in RNA but not in DNA
b. Cytosine is a pyrimidine base that occurs in both RNA and DNA
c. Ribose is a pentose sugar that occurs in DNA and RNA
d. Thymine is a pyrimidine base that occurs in RNA but not in DNA.

**14. Which of the following does not involved in the formation of DNA?**
a. Deoxyribose b. Pyrimidine
c. Phosphate d. Uracil

**15. Which of the following nitrogenous bases is found in DNA but is not found in RNA?**
a. Adenine b. Thymine
c. Cytosine d. Uracil

**16. In nucleic acids, the purine nitrogenous bases are:**
a. Guanine and thymine
b. Cytosine and guanine
c. Thymine and cytosine
d. Adenine and guanine

**17. Which element occurs in nucleic acids?**
a. Calcium b. Phosphorus
c. Manganese d. Sulphur

**18. The groups of molecules called nucleotides contain:**
a. Phosphate groups
b. Pyrimidine and purine
c. Pentose sugar
d. All of the above

**19. Nucleotides have a nitrogenous base attached to a sugar at the:**
a. 1′ carbon of sugar b. 2′ carbon of sugar
c. 3′ carbon of sugar d. 5′ carbon of sugar

**20. Which of the following nucleotide acts as chemical messenger?**
a. ATP b. cAMP
c. GTP d. UTP

## Answers for MCQs

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

CHAPTER

# 10

# Biological Oxidation

**Chapter outline**

- Free Energy (ΔG) and Redox Potential
- Enzymes, Coenzymes and Electron Carriers Involved in Biological Oxidation
- Electron Transport Chain
- Oxidative Phosphorylation
- Substrate Level Phosphorylation
- Inhibitors of Electron Transport Chain
- Shuttle Systems for Oxidation of Extramitochondrial NADH

## INTRODUCTION

Oxidation, which occurs in living systems, is called ***biological oxidation***. Biological oxidations are exergonic. During biological oxidations, the reacting chemical systems move from a higher energy level to a lower one and, therefore, there is liberation of energy. The energy released as heat is converted to chemical energy by formation of energy-rich compound ATP.

The formation of ATP from ADP and Pi is termed **phosphorylation,** as phosphorylation is coupled with biological oxidation; the process is called ***biological oxidative phosphorylation***.

## FREE ENERGY (ΔG) AND REDOX POTENTIAL

Free energy (ΔG) is that portion of total energy change in a system that is available for executing a task, i.e. it is the actual useful amount of energy, also known as the ***chemical potential***. In oxidation and reduction reactions, the **free energy change** is proportionate to the tendency of reactants to donate or accept electrons.

- If ΔG is negative in sign, it means that the products contain less free energy than the reactants and reaction proceeds spontaneously with loss of free energy and reaction is called ***exergonic***, e.g. catabolic reactions are exergonic.
- If ΔG is positive in sign, it means that the products of the reaction contain more free energy than the reactants and reaction proceeds only if free energy can be gained and this is known as ***endergonic reactions***.
- If ΔG is zero, it means that the system is at equilibrium and no net change takes place.

This free energy changes are expressed as **redox potential (Eo').** The redox potentials of some redox systems of electron transport chain are shown in **Table 10.1**. Increasing negative values of a system have an increasing tendency to lose electrons and increasingly positive values of a system have an increasing tendency to accept electrons. Thus, electrons tend to flow from redox couple to another

**Table 10.1:** The redox potential Eo' of redox couples of components of electron-transport chain.

| *Redox couple components of electron transport chain* | *Redox potential Eo' in volt* |
|---|---|
| $2H^+/H_2$ | – 0.41 |
| NAD/NADH | – 0.32 |
| $FMN/FMNH_2$ | – 0.30 |
| Ubiquinone (ox)/Ubiquinone (red) | + 0.045 |
| Cytochrome b (ox)/Cytochrome b (red) | + 0.077 |
| Cytochrome $c_1$ (ox)/Cytochrome $c_1$ (red) | + 0.22 |
| Cytochrome c (ox)/Cytochrome c (red) | + 0.25 |
| Cytochrome a (ox)/Cytochrome a (red) | + 0.29 |
| Cytochrome $a_3$ (ox)/Cytochrome $a_3$ (red) | + 0.35 |
| $1/2O_2 + 2H^+/H_2O$ | + 0.82 |

redox couple in the direction of the more positive system. The Eo' values of various redox couples allow us to predict the direction of flow of electrons from one redox couple to another.

## ENZYMES, COENZYMES AND ELECTRON CARRIERS INVOLVED IN BIOLOGICAL OXIDATION

Biological oxidation is brought about with different enzymes, coenzymes and electron carriers.

### Enzymes and Coenzymes

The enzymes involved in oxidation and reduction reactions are called **oxidoreductase** and are classified into four groups as follows:

1. **Oxidases:** They catalyze the removal of hydrogen from a substrate in the form of $H_2O$ or $H_2O_2$ (hydrogen peroxide), using oxygen as a hydrogen acceptor, e.g. cytochrome oxidase (cytochrome $aa_3$), L-amino acid oxidase, xanthine oxidase, etc.
2. **Dehydrogenases:** They catalyze the removal of hydrogen from a substrate but are not able to use oxygen as a hydrogen acceptor. These enzymes, therefore, require specific **coenzymes** as acceptor of hydrogen atoms. The coenzymes of dehydrogenases may be either nicotinamide coenzymes ($NAD^+$ or $NADP^+$) or flavin coenzymes (FMN or FAD).
3. **Hydroperoxidases:** They use hydrogen peroxide or organic peroxide as substrate. There are two types of hydroperoxidases, **peroxidases** and **catalases.**
4. **Oxygenases:** They catalyze the direct transfer and incorporation of oxygen into a substrate molecule. The two types of oxygenases are **dioxygenases** which catalyze the incorporation of both atoms of molecular oxygen into substrate and **mono-oxygenases**. This enzyme incorporates only one atom of molecular oxygen into the substrate in the form of hydroxyl group, while the other oxygen atom of $O_2$ is reduced to $H_2O_2$ by reducing equivalents donated by coenzymes.

### Electron Carriers

- The **cytochromes** are iron-containing electron transferring **hemoproteins** in which the **iron** atom oscillates between $\mathbf{Fe^{3+}}$ and $\mathbf{Fe^{2+}}$ during oxidation and reduction reactions.
- There are three classes of cytochromes ***a, b*** and ***c*** having differences in their light absorption spectra. In the respiratory chain, they are involved as carriers of electrons from flavoprotein to cytochrome oxidase.
- Several cytochromes occurring in the respiratory chain are **cytochrome b**, **cytochrome $c_1$**, **cytochrome c** and **cytochrome $aa_3$**. Cytochrome $aa_3$ are also called **cytochrome oxidases;** and are **copper-containing heme-proteins.**
- The cytochromes in the respiratory chain are arranged in the sequence $b \rightarrow c_1 \rightarrow c \rightarrow aa_3$.

- Of these, ***only cytochrome c is water-soluble and easily diffusible, whereas cytochromes b, $c_1$, and $aa_3$ are lipid-soluble and, therefore, are fixed components of the membrane.***

## ELECTRON TRANSPORT CHAIN

- The **electron transport chain** or **respiratory chain** is the final common pathway in aerobic cells by which electrons (reducing equivalents) derived from various substrates are transferred to oxygen. Electron transport chain is a series of highly organized oxidation reduction **enzymes**, **coenzymes** and **electron carrier cytochromes**.
- The electron transport chain is present in the ***inner mitochondrial membrane*** **(Fig. 10.1).** The enzymes of the electron transport chain are embedded in the inner membrane in association with the enzymes of oxidative phosphorylation.
- The energy liberated during oxidation of carbohydrates, fatty acids and amino acids is made available within the mitochondria as reducing equivalents ($H^+$ or $e^-$). The mitochondria which contain respiratory chain transport these reducing equivalents and hand them over to their final acceptor, oxygen to form water. Liberated free energy is trapped as high-energy phosphate **ATP** in the mitochondria by **oxidative phosphorylation**.

**Fig. 10.1:** Structural organization of components of respiratory chain and FoF1 ATPase in the mitochondrial membrane.

## Components and Structural Organization of the Respiratory Chain

The major components of the respiratory chain include **(Fig. 10.2)**:

- Nicotinamide adenine dinucleotide ($NAD^+$) of various dehydrogenases
- Flavin mononucleotide (FMN) of NADH dehydrogenase and Flavin adenine dinucleotide (FAD)
- Ubiquinone or coenzyme Q. [Except coenzyme Q, all members of this chain are proteins. Coenzyme Q (CoQ) is a fat-soluble quinone and is a constituent of mitochondrial lipids].
- Two different types of iron-containing proteins, the ***iron-sulfur (Fe-S) protein*** associated with **FMN** and **cytochrome b**. The iron takes part in the oxidation-reduction mechanism, which accepts only single electron.
- Cytochromes (heme-proteins), ***b, c,*** $c_1$ and $aa_3$.

## Structural Organization of Components of Respiratory Chain

Components of the respiratory chain are arranged in order of increasing redox potential **(Table 10.1)**. The reducing equivalents flow through the chain from the components of **more negative redox potential to the components of more positive redox potential.** The electron carriers of the respiratory chain are organized into **four enzyme complexes**, each capable of catalyzing electron transfer through a portion of the chain **(Fig. 10.2)**.

- **Complex I:** NADH–Q oxidoreductase (transfers electrons from NADH to Q).
- **Complex II:** Succinate–Q oxidoreductase (transfers electrons from $FADH_2$ to Q).
- **Complex III:** Q–Cytochrome c oxidoreductase (electrons are transferred from Q to cytochrome c).
- **Complex IV:** Cytochrome c oxidase–$O_2$ (Passing electrons from cytochrome c to oxygen and causing it be reduced to $H_2O$).

Complex II: Succinate – Q oxidoreductase in contrast with the other complexes does not pump protons. Complexes I, II and III are termed respirasome (super molecular complex); facilitate the rapid transfer of substrate and prevent the release of reaction intermediates.

## Reactions of Electron Transport (Respiratory) Chain

### *Complex I: NADH–Q Oxidoreductase*

NADH–Q oxidoreductase complex, also called **NADH dehydrogenate complex**, is a large enzyme consisting of **FMN** and **iron-sulfur (Fe-S) centers (proteins)**. Complex-I catalyzes two simultaneous and obligatory coupled processes **(Fig. 10.3).**

1. The transfer of two electrons and two protons from NADH + $H^+$ to FMN. FMN is then reduced to $FMNH_2$ from which two electrons are passed through Fe-S centers and finally to coenzyme Q to form $QH_2$.
2. The second process is the **transfer of four protons ($4H^+$) from the matrix to the intermembrane space**.

The flow of two electrons from NADH to coenzyme Q through **NADH–Q oxidoreductase** leads to the **pumping of four hydrogen ions** out of the matrix of the mitochondrion. The detailed mechanism that couples electron and proton transfer in complex-I is not yet known.

### *Complex II: Succinate–Q Oxidoreductase*

Complex II contains **succinate dehydrogenase** and its **iron-sulfur** centers. In complex-II, $FADH_2$ is formed during the conversion of **succinate** to **fumarate** in the citric acid cycle

**Fig. 10.2:** Organization of the components of the electron transport complexes within the inner mitochondrial membrane.

**Fig. 10.3:** Transfer of electrons through Complex I: NADH - Q oxidoreductase.

**Fig. 10.4:** Transfer of electrons through Complex II: Succinate - Q oxidoreductase.

and electrons are then passed via several Fe-S centers to coenzyme Q to form $QH_2$ **(Fig. 10.4)**. **Glycerol- 3-phosphate** (breakdown product of triacylglycerol and glycolysis) and **acyl CoA** also pass electrons to Q via $FADH_2$.

> Complex II in contrast with complex I **does not pump protons** from one side of the membrane to the other. Consequently, less ATP is formed from the oxidation of $FADH_2$ than from NADH.

## *Complex III: Q–Cytochrome C Oxidoreductase*

Complex III is also called **cytochrome $bc_1$ complex**. It contains **cytochrome b**, **cytochrome $c_1$** and **iron-sulfur center**. The function of Q–cytochrome c oxidoreductase is to catalyze the transfer of electrons from $QH_2$ to cytochrome c **(Fig. 10.5)** with **transfer of four protons ($4H^+$) from the matrix to the intermembrane space.**

## *Complex IV: Cytochrome c Oxidase–$O_2$*

Complex IV, also called **cytochrome c oxidase**, is the terminal component of the respiratory chain. It consists of four redox centers: **Cytochrome a**, **cytochrome $a_3$**, **$Cu_A$ center** and **$Cu_B$ center to heme**. Cu centers resemble the Fe-S center and like iron atoms, the copper ions functions as **one electron carrier.**

**Fig. 10.5:** Transfer of electrons through Complex III: CoQ-Cytochrome c reductase.

Cytochrome c oxidase catalyzes the transfer of electrons from reduced form of cytochrome c to cytochrome a, to cytochrome $a_3$ and finally to molecular oxygen, with the concomitant reduction of $O_2$ to two molecules of $H_2O$ **(Fig. 10.6).** For every pair of electrons passing down the chain from NADH or $FADH_2$, **$2H^+$ are pumped across the membrane by complex IV.**

The $O_2$ remains tightly bound to complex IV until it is fully reduced to water, and this minimizes the release of incompletely reduced potentially damaging intermediates such as hydrogen peroxide, or hydroxyl free radicals.

## OXIDATIVE PHOSPHORYLATION

During the transport of electrons through respiratory chain a portion of free energy liberated is conserved by an energy transducing system by which electrical energy is change to chemical energy. Since the energy is conserved in the form of ATP through phosphorylation of ADP to ATP by an enzyme **$F_oF_1$ ATPase**, the overall coupled process is known as **oxidative phosphorylation**.

### Sites of ATP Synthesis

There is **three ATP synthesizing sites** of the electron transport chain. These are ***complex I, complex III*** and ***complex IV*** that provide the energy required to make ATP from ADP and inorganic phosphate by an enzyme **$F_oF_1$ ATPase** in the process of oxidative phosphorylation.

- Electrons that enter the chain through NADH pass through all three energy conserving or coupling sites and thus yield **2.5 ATPs**.
- However, electrons that enter the chain through $FADH_2$ pass through only two energy conserving sites, as they bypass site-I, they yield **1.5 ATPs.**

**FoF1ATPase**

- The enzyme complex **$F_oF_1$ATPase** that synthesizes ATP is also called ***ATP synthase.*** **'F'** is for protein factor. $F_oF_1$ATPase is composed of two protein subunits, $F_O$ and $F_1$. *$F_O$ and $F_1$* are embedded in the inner membrane and extend across it **(Fig. 10.7).**
- The subscript **'O'** is not zero, but the letter 'O' to denote that it is the portion of ATP synthase that binds the toxic antibiotic ***oligomycin,*** an inhibitor of this enzyme and thus oxidative phosphorylation. $F_o$ unit forms a channel or path, through which hydrogen ions may pass across the membrane.
- $F_1$ is tightly bound to $F_o$ and protruding into the matrix from the inner mitochondrial membrane. It contains the catalytic site for ATP synthesis.

### Mechanism of Oxidative Phosphorylation

#### *Chemiosmotic Theory*

The **chemiosmotic coupling hypothesis** is the most accepted theory. The chemiosmotic theory which was proposed by the ***British biochemist Peter Mitchell*** explains the mechanism of oxidative phosphorylation. The theory states that the energy released from oxidation generates the ***electrochemical potential*** by the pumping of protons across the inner mitochondrial membrane and the energy in this electrochemical potential can be converted into high energy phosphate bond of ATP. There are three basic principles of the theory.

- The major electron carriers are organized into three complexes, complex I, III and IV **(Fig. 10.8)**, which span the inner mitochondrial membrane. This structural

**Fig. 10.6:** Transfer of electrons through Complex IV: Cytochrome c oxidase-$O_2$.

**Fig. 10.7:** FoF1ATPase (ATP synthase) showing catalytic site for ATP synthesis and $H^+$ channel.

feature of the electron transport chain allows proton to be pumped across the inner mitochondrial membrane from the matrix to the intermembrane space, while electrons are passed from one carrier to another.

- The inner mitochondrial membrane is impermeable to protons, so that their pumping results in the generation of the ***electrochemical potential.***
  - Protons once pumped to the cytosolic side of the membrane cannot diffuse back through the membrane into the matrix.
  - Thus, pumping of protons creates a much higher concentration of protons outside of the mitochondrion than inside, resulting in the generation of ***electrochemical potential gradient (proton motive force)*** across the inner mitochondrial membrane (This electrochemical potential would become zero if the protons could diffuse back across the membrane until their concentration is equal on both sides of the membrane).
- Due to this electrochemical potential or proton motive force, the $H^+$ ions ejected out by electron transport, flow back into the mitochondrial matrix down its electrochemical gradient through a specific $H^+$ channels or pore ($F_o$) of the $F_oF_1$ATPase molecule **(Fig. 10.8).** The free energy is released as $H^+$ ions flow back through the $F_oF_1$ATPase into the zone of lower $H^+$ concentration. The free energy released is coupled with the **phosphorylation of ADP** to ATP. The $F_1$ of $F_oF_1$ATPase catalyzes the addition of inorganic phosphate to ADP to form ATP.

Complexes I, III and IV acts as proton pumps generating electrochemical gradient. The proton motive force generated drives the synthesis of ATP by flowing protons back into the matrix through ATP synthase ($F_oF_1$ ATPase) enzyme

## P : O Ratios for Mitochondrial Electron Transport and Oxidative Phosphorylation

- The P :O ratio (ratio of phosphate incorporated into ATP to atoms of $O_2$ utilized) is a measure of the number of ATP molecules formed during the transfer of two electrons through the electron transport chain.

**Fig. 10.8:** Chemiosmotic theory of oxidative phosphorylation.

- The P : O ratio had been thought: 3 for transfer of two electrons from NADH-linked substrate to $O_2$, and 2 for succinate substrate to $O_2$. These P : O ratios suggested that one ATP was produced during electron transfer through each of the proton-pumping complexes I, III and IV.
- Currently, it is accepted that the transfer of two electrons from NADH to $O_2$ results in the translocation of 10 protons across the membrane, four each from complexes I and III and two from complex IV. **The most widely accepted value for number of protons required to drive the synthesis of an ATP molecule is 4**. If 10 protons are pumped out per NADH and 4 must flow in to produce one ATP, **the P : O ratio is 2.5 (10/4) for NADH as the electron donor and 1.5 (6/4) for $FADH_2$ as the electron donor.**

### Respiratory Control

Under physiological conditions, electron transport is tightly coupled to oxidative phosphorylation. Electrons do not usually flow through the electron transport chain to $O_2$ unless ADP is simultaneously phosphorylated to ATP. In the presence of excess substrate and oxygen, respiration continues until all ADP is converted to ATP. After that the respiration rate or utilization of oxygen decreases.

***This means that in the presence of adequate oxygen and substrate, ADP becomes rate-limiting; it exerts a control over the entire oxidative phosphorylation processes.*** The regulation of the rate of oxidative phosphorylation by ADP level is called ***respiratory control.*** The ADP level increases when ATP is consumed and so oxidation is coupled to the utilization of ATP.

## SUBSTRATE LEVEL PHOSPHORYLATION

The formation of ATP, directly coupled to metabolic process without involvement of electron transport chain and molecular $O_2$, is called the production of ATP at the substrate level.

- Examples of substrate level phosphorylation processes are the conversion of:
  - Phosphoenolpyruvate to pyruvate
  - 1,3 bisphosphoglycerate to 3-phosphoglycerate
  - Succinyl-CoA to succinate **(Fig. 10.9)**.

## INHIBITORS OF ELECTRON TRANSPORT CHAIN

Inhibitors of respiratory chain may be divided into three groups:

1. Inhibitors of the electron transport chain proper
2. Inhibitors of oxidative phosphorylation (F0F1 ATPase)
3. Uncouplers of oxidative phosphorylation.

**Fig. 10.9:** Substrate level phosphorylation processes. Reaction 1 and 2 are of glycolysis and 3 occurs in TCA cycle.

### Inhibitors of Electron Transport Chain Proper (Fig. 10.10)

Inhibitors of electron transport chain proper include inhibitors that inhibit the flow of electrons through the respiratory chain. These inhibitors block the respiratory chain at three sites:

1. **Complex I (NADH to CoQ),** inhibited by:
   - Barbiturates such as Amobarbital
   - An antibiotic piericidin A
   - The insecticide rotenone.

   These inhibitors prevent the oxidation of substrates by blocking the transfer of reducing equivalents from Fe-S protein to CoQ.
2. **Complex III (cytochrome b to cytochrome c1),** inhibited by:
   - Dimercaprol
   - Antimycin A (antibiotics)
   - British antilewisite (BAL), an antidote used against war gas.

   These inhibitors prevent the transfer of electrons from cytochrome b to cytochrome c1.

**Fig. 10.10:** Sites of action of various inhibitors of electron transport chain.

3. **Complex IV (cytochrome oxidase),** inhibited by:
   - Cyanide
   - Carbon monoxide
   - $H_2S$.

   These inhibitors prevent transfer of electrons from cyt $aa_3$ to molecular oxygen by inhibiting cytochrome oxidase and can, therefore, totally arrest respiration.

## Inhibitors of Oxidative Phosphorylation ($F_0F_1$ ATPase)

Another set of inhibitors do not inhibit special complexes. Instead, these compounds block phosphorylation directly by inhibiting ***$F_0F_1$ ATPase*** enzyme. For example, antibiotic ***oligomycin*** completely blocks oxidation and phosphorylation by inhibiting an enzyme F0F1 ATPase required for phosphorylation.

## Uncouplers of Oxidative Phosphorylation

- Uncouplers are chemical substances that allow electron transport chain in mitochondria but prevent the phosphorylation of ADP to ATP by uncoupling the essential linkage between electron transport and phosphorylation for the synthesis of ATP.
- Uncoupling agents are lipophilic (lipid-soluble) compounds, which readily diffuse through the mitochondrial membrane and are capable of binding $H^+$ ion.
- Uncouplers allow transport of $H^+$ ion across the membrane towards the side with the lower $H^+$ ion concentration, thus preventing the formation of proton gradient which is required for the formation of ATP.
- Thus, these compounds make the inner mitochondrial membrane abnormally permeable to protons. The energy produced by the transport of electrons is released as heat rather than being used for synthesis of ATP. Examples of uncouplers include:
  - *2,4-Dinitrophenol (DNP)*
  - Dicuomarol (an anticoagulant).
  - Salicylate, a metabolite of aspirin.

### *Physiological uncouplers*

Certain physiological substances act as uncouplers, e.g. thermogenin, thyroxine, bilirubin and free fatty acids. However, these compounds normally are not present in mitochondria in concentrations high enough to act as uncouplers.

## Ionophores

Ionophores mean ion carrier molecules. They are lipid-soluble substances, capable of binding and carrying specific cations (other than $H^+$) through the mitochondrial membrane. Oxidative phosphorylation can be prevented by certain ionophores. They differ from uncoupling agents in that they promote the transport of cations other than $H^+$ through the membrane and abolish the membrane potential and/or pH gradient across the membrane and phosphorylation is, therefore, completely inhibited. For example, antibiotic **valinomycin** and **gramicidin.**

**Thermogenin: A natural uncoupler**

- Brown adipose tissue is abundant in the newborn of mammals, including humans and hibernating animals. This specialized adipose tissue is brown due to high concentration of mitochondria, rich in cytoplasm.
- The mitochondria of brown fat have a unique protein in the inner membrane. ***Thermogenin,*** also called the ***uncoupling protein (UCP).*** This protein transports protons back into the matrix, without passing through the $F_oF_1$ATPase complex. As a result, the energy of oxidation is not conserved by ATP formation, but is dissipated as heat, which is used to maintain the body temperature.
- Until recently, adult humans were believed to lack brown fat tissue. However, new studies have established that adults, women especially, have brown adipose tissue in the neck and upper chest regions that is activated by cold. Obesity leads to a decrease in brown adipose tissue.

## SHUTTLE SYSTEMS FOR OXIDATION OF EXTRAMITOCHONDRIAL NADH

Most of the NADH and $FADH_2$ entering the mitochondrial electron transport chain arises from **Kreb's cycle** and **β-oxidation** of fatty acids, located in the mitochondria itself. Since the inner mitochondrial membrane is not permeable to cytoplasmic NADH, how can the NADH generated by glycolysis, which takes place outside of the mitochondria, be oxidized to NAD by respiratory chain located in mitochondria. Special shuttle systems carry **reducing equivalents** from **cytosolic NADH** into the mitochondria by an indirect route. This transport of reducing equivalents as NADH regardless of sources by enzymatic processes called **shuttle mechanisms.** Two such mechanisms that can lead to the transport of reducing equivalent from the cytoplasm into mitochondria are:

1. Malate-aspartate shuttle
2. Glycerol phosphate shuttle

### The Malate-Aspartate Shuttle System

Malate-aspartate shuttle is the most active **NADH shuttle** which functions in liver, kidney and heart mitochondria, which is mediated by **two membrane carriers** and **four enzymes (Fig. 10.11).**

- The reducing equivalents of cytosolic NADH are first transferred to cytosolic oxaloacetate to yield malate by **cytosolic malate dehydrogenase**.
- Malate, which carries the reducing equivalents, is transported across the inner membrane by **malate–α-ketoglutarate transport** system.
- The reducing equivalents carried by malate are then transferred to mitochondrial $NAD^+$ by **mitochondrial malate dehydrogenase** and malate itself gets reoxidized to oxaloacetate. The resulting mitochondrial NADH is oxidized by the mitochondrial electron transport chain, leading to formation of 2.5 molecules of ATP per electron pair.
- The oxaloacetate so formed cannot pass through the membrane from the mitochondrion back into cytosol, so it is converted to **aspartate.** Oxaloacetate in mitochondria undergoes transamination reaction, with glutamate, catalyzed by aspartate aminotransferase to yield α-ketoglutarate and amino acid aspartate.
- Aspartate is transported to the cytosolic side via amino acid transport system.

**Fig. 10.11:** Malate-aspartate shuttle.
(A: Malate/α-KG transporter; B: Aspartate/glutamate transporter; α-KG: α-ketoglutarate; ASP: Aspartate).

- In the cytosol, a reversal of the aspartate aminotransferase reaction gives rise to oxaloacetate and glutamate, thereby completing the 'shuttle like' process.

## Glycerol Phosphate Shuttle

Skeletal muscle and brain use a different NADH shuttle, the **glycerol-3-phosphate shuttle (Fig. 10.12).** It differs from the malate-aspartate shuttle in that it delivers the reducing equivalents from NADH to coenzyme Q and, thus, into complex III, not complex I synthesizing **1.5 ATP** molecules per pair of electrons.

**Fig. 10.12:** Glycerol phosphate shuttle. (DHAP = Dihydroxyacetone phosphate).

- The first step in this shuttle is the transfer of a pair of electrons from cytosolic NADH to dihydroxyacetone phosphate to form glycerol-3-phospate catalyzed by cytosolic glycerol-3-phosphate dehydrogenase enzyme.
- Glycerol-3-phosphate, in turn, diffuses through the outer mitochondrial membrane into the intermembrane space of the mitochondria.
- Here glycerol-3-phosphate is reoxidized to dihydroxy-acetone phosphate on the outer surface of the inner mitochondrial membrane by a membrane-bound FAD containing isoenzyme of glycerol-3-phosphate dehydro-genase. An electron pair from glycerol-3-phosphate is transferred to an FAD of the enzyme to form $FADH_2$. $FADH_2$ then transfers its electrons to the respiratory chain through $CoQH_2$. Since the mitochondrial enzyme is linked to the respiratory chain via FAD rather than NAD, only **1.5** molecules rather than **2.5** molecules of ATP are formed per atom of oxygen consumed.
- The dihydroxyacetone phosphate returns to the cytosol and can be reused for reduction of glycerol-3-phosphate.

## EXAM QUESTIONS

### Long Answer Question (LAQ)

1. Explain the electron transport chain in detail. Mention the ATP-synthesizing sites, the inhibitors and uncouplers of oxidative phosphorylation.

### Short Notes

1. Electron transport chain
2. Oxidative phosphorylation
3. Enzymes, coenzymes and electron carriers of biological oxidation
4. Chemiosmotic theory
5. Substrate level phosphorylation
6. P : O ratio and respiratory control
7. Inhibitors of ETC
8. Ionophores
9. Malate-aspartate shuttle
10. Glycerol phosphate shuttle
11. Uncouplers of oxidative phosphorylation.

### Multiple Choice Questions (MCQs)

1. **All of the following are true regarding mitochondrial cytochromes, *except*:**
   a. They all contain heme groups
   b. All are bound to protein components
   c. Iron must remain in the ferrous state to function in electron transport
   d. They accept or donate one electron at a time
2. **Which one of the following respiratory chain components reacts directly with molecular oxygen?**
   a. Coenzyme Q b. Cytochrome b
   c. Cytochrome $aa_3$ d. Cytochrome $C_1$
3. **In oxidative phosphorylation, the oxidation of one molecule of $FADH_2$ produces:**
   a. 3 ATP molecules b. 1.5 ATP molecules
   c. 1 ATP molecule d. No ATP at all
4. **All of the following statements are true regarding ETC, *except*:**
   a. Located in inner mitochondrial membrane
   b. Components are organized in decreasing order of redox potential
   c. Involved with ATP synthesis
   d. Cyanide inhibits electron flow
5. **The oxidation and phosphorylation of intact mitochondria are blocked by:**
   a. Puromycin b. Oligomycin
   c. Streptomycin d. Erythromycin
6. **Respiratory chain is found in:**
   a. Mitochondria
   b. Cytoplasm
   c. Nucleus
   d. Endoplasmic reticulum
7. **In oxidative phosphorylation, the oxidation of one molecule of NADH produces:**
   a. 2 ATP molecules b. 2.5 ATP molecules
   c. 4 ATP molecules d. 1 ATP molecule
8. **Which of the following vitamins is involved in ETC?**
   a. Thiamine b. Folic acid
   c. Riboflavin d. Cobalamin
9. **Which of the following trace elements has role in ETC?**
   a. Iron b. Iodine
   c. Zinc d. Fluoride
10. **An uncoupler of oxidative phosphorylation:**
    a. Inhibits electron transport and ATP synthesis
    b. Allows electron transport to occur without ATP formation
    c. Inhibits electron transport without impairment of ATP synthesis
    d. Inhibits transfer of electrons from cytochrome $aa_3$ to molecular oxygen
11. **Energy currency of the cell is:**
    a. High energy phosphate (~P)
    b. S-adenosyl methionine (SAM)
    c. Creatine phosphate
    d. Glucose-6-phosphate
12. **The enzyme that synthesizes ATP in oxidative phosphorylation is:**
    a. Hexokinase
    b. NADH dehydrogenase
    c. Cytochrome oxidase
    d. $F_0F_1$ ATPase
13. **A naturally occurring uncoupler is:**
    a. Dicumarol b. 2,4-DNP
    c. Thermogenin d. Salicylate

**14. A biochemical substance that can act as an uncoupler is:**
a. Bilirubin
b. Free fatty acid
c. Thyroxine
d. All of the above

**15. Which of the following is an ionophore?**
a. 2,4-dinitrophenol
b. Valinomycin
c. Thermogenin
d. Oligomycin

**16. Which of the following is not an ATP synthesizing site in respiratory chain?**
a. Complex I
b. Complex II
c. Complex III
d. Complex IV

**17. Which of the following is true during the transfer of electrons to $O_2$ via the electron transport chain?**
a. The energy is used directly in the addition of Pi to ADP to form ATP
b. The energy released is used to translocate protons across the inner membrane
c. A proton gradient is generated with the matrix now being more positive than the intermembrane space
d. Pumping of protons across the membrane occurs each time electrons are removed

**18. All of the following statements are correct for ATP synthase which consists of two domains $F_1$ and F0, *except:***
a. $F_1$ and $F_0$ are both associated with the outer mitochondrial membrane
b. $F_0$ unit forms a channel through which $[H^+]$ passes across the membrane
c. $F_1$ contains the catalytic site for ATP synthesis
d. Oligomycin inhibits this enzyme

**19. Which of the following statements is true for complex I?**
a. Is one of the ATP-synthesizing sites of respiratory chain
b. Transfers electrons directly from NADH to ubiquinone
c. Does not contain Fe-S center
d. Can transfer electrons from $FADH_2$ of succinate dehydrogenase as well as from NADH

**20. Cyanide inhibits the electron transport chain by preventing transfer of electrons from:**
a. Cyt $aa_3$ to molecular $O_2$
b. Cyt b to $Cytc_1$
c. From $FMNH_2$ to ubiquinone
d. From $FADH_2$ to ubiquinone

## Answers for MCQs

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

CHAPTER

# 11 Nutrients and their Role in Nutrition

**Chapter outline**

- Nutrients
- Role of Nutrients
- Nitrogen Balance
- Nutritional Quality of Proteins
- Recommended Daily Allowance
- Energy Requirements
- Basal Metabolic Rate
- The Thermogenic Effect (Specific Dynamic Action, SDA) of Food
- Balanced Diet
- Nutritional Disorders

## INTRODUCTION

Nutrition is the science of food and the nutrients and other substances contained in food. It is the study of their actions, interactions and balance in relation to health and disease. Thus, nutrition is concerned with the digestion, absorption, transport, metabolism and functions performed by the essential nutrients.

Nutrition is best defined as the composition and quantity of food intake and the utilization of the food by living organism. Study of human nutrition can be divided into three areas; **under nutrition, over nutrition** and **ideal nutrition.** Over nutrition is a particularly serious problem in developing countries, and thus is at risk for a number of series health consequences. There is increasing interest in the concept of ideal or optimal nutrition.

## NUTRIENTS

Nutrients are the necessary constituents of food required by organisms for growth and the maintenance of life. There are five classes of nutrients that contribute to an adequate diet **(Table 11.1)**. Each plays a special role. These may be divided into: ***Macronutrients*** and ***micronutrients.***

### Macronutrients

These are ***proteins, fats*** and ***carbohydrates.*** They form the main bulk of food. In the Indian dietary, they contribute to the total energy intake in the following proportions.

**Table 11.1:** Essential nutrients required by human beings.

| *Macronutrients* | *Micronutrients* |
|---|---|
| **Carbohydrates** | **Vitamins** |
| Glucose and | Fat soluble vitamins |
| Fiber | Retinol (A) |
| **Fat** | Cholecalciferol (D) |
| Essential fatty acids | α-tocopherol (E) |
| Linoleic acid | Phylloquinone (K) |
| Linolenic acid | Water soluble vitamins |
| **Protein** | Thiamine ($B_1$) |
| Essential amino acids | Riboflavin ($B_2$) |
| Phenylalanine | Niacin |
| Valine | Pyridoxine ($B_6$) |
| Threonine | Pantothenic acid |
| Tryptophan | Biotin |
| Isoleucine | Folic acid |
| Methionine | Cobalamin ($B_{12}$) |
| Histidine | **Mineral elements** |
| Arginine | Macrominerals |
| Leucine | $Na^+$, $K^+$, $Ca^+$, $Mg^{++}$, P, S and $Cl^-$ |
| Lysine | Microminerals |
| | Cr, Co, Cu, F, I, Fe, Mn, Mo, Se, Zn |

| | | |
|---|---|---|
| Proteins | : | 7 to 15% |
| Fats | : | 35 to 45% |
| Carbohydrates | : | 50 to 70% |

- Protein, fat and carbohydrate are sometimes referred to as **proximate principle**. They are oxidized in the body to yield energy, which the body needs.
- Although proteins provide energy their primary function is to provide **essential** and **nonessential amino acids** for building of body proteins.
- **Fats** particularly the vegetable oils, besides being concentrated source of energy, provide **essential fatty acids** which have a **vitamin** like function in the body.
- **Water** is the solvent of the body and transport vehicle for distributing nutrients to the tissues. Water, although not a nutrient by definition, is of course required to replace the water lost in the urine, breath and sweat.
- **Fiber** also is not a nutrient but is being considered as a necessary food component. In plant foods, fibers (dietary fiber) which have undigestible complex molecules also contribute to the bulk and have some useful functions in the digestive tract. Role of fiber is discussed later.

### Micronutrients

Vitamins and minerals are called micronutrients **(Table 11.1).** They are called micronutrients because they are required in small amounts which may vary from a microgram to several grams. Vitamins and minerals do not supply energy but they play an important role in the regulation of the metabolic activity in the body and help in the utilization of **proximate principles.** Minerals are also used for the formation of body structure and skeleton.

## ROLE OF NUTRIENTS

### Carbohydrate

Dietary carbohydrate is of two types:

1. Available or digestible carbohydrate.
2. Unavailable or undigestible carbohydrate (discussed later)

- The digestible carbohydrates are a major source of food energy, yielding 4 kcal/g and provide about 50 to 70% of the energy requirement. In addition, these carbohydrates have **protein sparing effect.**
- **Unavailable** or **undigestible carbohydrates** provide **dietary fiber**. Fiber is not digested by the digestive enzymes and does not serve as a source of energy. It is however a significant component of the diet.

### Carbohydrate Requirement

- The recommended intake of carbohydrate in balanced diet is placed so as to contribute between 50 and 70% of total energy intake.
- Most Indian diets contain amounts more than this, providing as much as 90% of total energy intake in some cases, which make the diet **imbalanced.**

### Fats

Dietary fats are high energy yielding nutrients that provide 35 to 45% of the caloric intake. Fat yields 9 kcal/gm. Besides satisfying metabolic energy needs, there are two essential functions of dietary fat, namely, to provide:

1. A vehicle for the absorption of the fat soluble vitamins (A, D, E and K).
2. To supply essential fatty acids, **linoleic acid** and **linolenic acid** to the body.

Dietary lipid also increases the **palatability** of food and produces a feeling of **satiety.**

#### *Fat Requirement*

- The daily requirement of fat is not known with certainty. During infancy, fats contribute to a little over 50% of the total energy intake. These scales get down to about 20% in adulthood.
- The ICMR Expert Group (1989) has recommended an intake of 20% of the total energy intake as fat of which at least 50% of fat intake should consist of vegetable oils rich in essential fatty acids.

### Protein and Amino Acids

- Proteins are vital to any living organism. Proteins are important constituent of tissues and cells of the body. They form the important component of muscle and other tissues and vital body fluids like blood.
- The proteins in the form of enzymes and hormones are concerned with wide range of vital metabolic processes in the body.
- Protein as antibodies helps the body to defend against infections.
- Proteins supply **essential** and **nonessential amino acids** for the synthesis of protein and nitrogen for the synthesis of several key compounds such as neurotransmitter and heme.

Thus, proteins are one of the most important nutrient required by the body and should be supplied in adequate amounts in the diet.

The amino acids, which are not used for protein synthesis, are broken down to provide energy, which is a wasteful way of using proteins (this is not their primary function). Hence, diet should contain adequate carbohydrate and fat to provide energy so that the proteins in the diet are most economically used for the formation of body proteins to fulfil other functions essential to life.

## Essential Amino Acids

- Any amino acid that humans either cannot synthesize or are unable to synthesize in adequate quantity is termed "essential" and rest of the amino acids are called **nonessential** as they can synthesized in the body.
- An essential amino acid must be provided in the diet. An absence of an essential amino acid from the diet impairs protein synthesis and generally causes negative nitrogen balance, i.e. the total nitrogen losses in the urine, feces and sweat exceed the dietary nitrogen intake.
- Ten of the twenty amino acids found in proteins are essential for humans **(Table 11.1)** (Refer Chapter 4 also).
- Of the 10 essential amino acids, 8 are essential at all times during life. The other two namely **histidine** and **arginine** are required in the diet during periods of rapid growth as in childhood and pregnancy and called semi essential.

### *Requirement of Essential Amino Acid*

- Each amino acid is required in differing amounts. Minimum requirements of essential amino acids are shown in **Table 11.2.**

## Dietary Fiber

Dietary fiber is the name given collectively to indigestible carbohydrates present in foods. These carbohydrates consist of **cellulose**, **pectin**, **gum** and **mucilage.** The dietary fiber is not digested by the enzyme of the human gastrointestinal tract, where most of the other carbohydrates like starch, sugars are digested and absorbed. Plant foods are the only sources of dietary fiber. It is found in vegetables, fruits, and grains.

**Table 11.2 :** Minimum requirement of essential amino acids

| *Essential amino acid* | *Requirement (mg/kg body weight/per day)* |
|---|---|
| Phenylalanine | 14 |
| Leucine | 11 |
| Lysine | 9 |
| Valine | 14 |
| Isoleucine | 10 |
| Threonine | 6 |
| Methionine | 14 |
| Tryptophan | 3 |

### *Importance of Fiber*

***Water holding capacity:*** The dietary fibers have a property of holding water and swell like sponge with a concomitant increase in viscosity. Thus, fiber adds bulk to the diet and increases transit time in the gut (gastric emptying time) due to high viscosity.

***Physiological effects:*** Dietary fiber exerts its influence along the entire gastrointestinal tract from ingestion to excretion **(Table 11.3).**

***Adsorption of organic molecules:*** The organic molecules like bile acids, neutral sterols, carcinogens, and toxic compounds can be adsorbed on dietary fiber and facilitates its excretion.

***It increases stool bulk:*** The fiber absorbs water and increases the bulk of the stool and helps to reduce the tendency towards constipation by increasing bowel movements.

***Hypoglycemic effect of fiber:*** Recent studies have shown that gum present in fenugreek seeds (it contains 40% gum) are most effective in reducing blood sugar and cholesterol levels.

***Hypocholesterolemic effects of fiber:*** Fiber has cholesterol lowering effect. Fiber binds **bile acids** and **cholesterol,** increasing their fecal exertion, and thus, decreasing plasma and tissue cholesterol level.

### *Significance of Dietary Fiber in Medicine*

High fiber diet is associated with reduced incidence of a number of diseases like:

- Coronary heart disease (CHD)
- Colon cancer

**Table 11.3:** Activities of dietary fiber along the entire GI-tract.

| *Site* | *Activity* |
|---|---|
| Mouth | Stimulates saliva secretion |
| Stomach | Delays gastric empting |
| Small intestine | Delays absorption |
| Large intestine | Traps water, binds cations |
| Stool | Softens, enlarges, prevents straining |

- Diabetes
- Diverticulosis
- Hemorrhoids (piles).

### *Adverse Effect of Dietary Fiber*

Dietary fiber also has some adverse effects on nutrition by binding some mineral elements and preventing their proper absorption. Thus, high dietary fiber intake may lead to deficiency of mineral elements.

## NITROGEN BALANCE

Catabolism of amino acids leads to a net loss of nitrogen from the body. This loss must be compensated by the diet in order to maintain a constant amount of body protein. ***Nitrogen balance studies evaluate the relationship between the nitrogen intake (in the form of protein) and nitrogen excretion.***

Three situations of nitrogen balance are possible as follows:

1. Nitrogen equilibrium
2. Positive nitrogen balance
3. Negative nitrogen balance.

### Nitrogen Equilibrium

In normal adults, nitrogen intake = nitrogen excretion. The subject is said to be in nitrogen equilibrium or balance. In this situation, the rate of body protein synthesis is equal to the rate of degradation.

### Positive Nitrogen Balance

In this, nitrogen intake > nitrogen excretion, i.e. intake of nitrogen is more than excretion. It shows that nitrogen is retained in the body, which means that protein is laid down. This occurs in growing ***infants*** and ***pregnant women.***

### Negative Nitrogen Balance

In this, nitrogen intake < nitrogen excretion, i.e. nitrogen output exceeds input, this occurs during ***serious illness*** and **major *injury*** and ***trauma,*** in ***advanced cancer*** and following failure to ingest adequate or sufficient high quality protein, e.g. in ***kwashiorkor*** and ***marasmus.*** If the situation is prolonged, it will ultimately lead to death.

## NUTRITIONAL QUALITY OF PROTEINS

In judging the adequacy of dietary proteins to meet the human needs, not only the quantity but the nutritional quality of the dietary proteins also matters. Proteins present in different foods vary in their nutritional quality because of the differences in their amino acid composition. **The quality of protein depends on the pattern of essential amino acids it supplies.**

The best quality protein is the one which provides essential amino acid pattern very close to the pattern of the tissue proteins. Egg proteins, human milk protein, satisfy these criteria and are classified as high quality proteins and serve as **reference protein** for defining the quality of other proteins.

### Assessment of Protein Quality

The quality of a protein is assessed by comparison to the **reference protein,** which is usually egg protein. Four methods of assessment of protein quality are:

1. Chemical score or amino acid score
2. Net protein utilization (NPU)
3. Protein efficiency ratio (PER)
4. Biological value (BV).

### *Chemical Score or Amino Acid Score*

It is a measure of the concentration of each essential amino acid in the test protein which is then compared with reference protein (usually egg protein). It is calculated by following formula:

$$\text{Amino acid score} = \frac{\text{Number of mg of one amino acid per gm of test protein}}{\text{Number of mg of the same amino acid per gm of egg protein}} \times 100$$

**This mode of chemical assessment does not take into account the digestibility of dietary proteins.** Hence, biological methods based on growth or nitrogen (N) retention are used to determine the overall quality of a protein.

### *Net Protein Utilization*

It is a product of digestibility coefficient and biological value divided by 100. Biological measures of net protein utilization (NPU) gives a more complete expression (both absorption and retention) of protein quality than the amino acid score as said above. It is calculated by the following formula:

$$\text{NPU} = \frac{\text{Nitrogen retained by the body}}{\text{Nitrogen intake}} \times 100$$

The protein requirement varies with the NPU of dietary protein. If the NPU is low, the protein requirement is high

and vice versa. The NPU of the protein of Indian diets varies between 50 and 60.

## Protein Efficiency Ratio

The overall quality, i.e. nutritive value of a food protein can be determined with laboratory animal like rat as follows.

The gain in weight of young animals per gram of protein consumed is measured and the value obtained is used to determine the protein efficiency ratio (PER) as follows:

$$\text{PER} = \frac{\text{Gain in body weight in gram}}{\text{Protein ingested in gram}}$$

## Biological Value

Biological value of protein is defined as the percentage of absorbed nitrogen retained by the body and is calculated by:

$$\text{Biological value (BV)} = \frac{\text{Nitrogen retained}}{\text{Nitrogen absorbed}} \times 100$$

- The amount of nitrogen in the diet eaten and in excreta of adult animals is measured and the percentage of nitrogen retained by animals from out of nitrogen absorbed from the diet is calculated. The value thus obtained is the **biological value** (BV) of the protein.
- This test also gives an estimate of digestibility of the protein. But it cannot take into account the nitrogen that might be lost during the digestion process. The chemical score, BV, NPU, PER and deficient amino acids of some important food proteins is given in **Table 11.4.**

## Mutual Supplementation of Proteins

It is seen that generally animal proteins are of higher biological value than proteins from plant foods. Plant proteins are of poorer quality since essential amino acids (EAAs) composition is not well balanced and a few EAAs deviate much from the optimal level present in egg, e.g.

- In comparison with egg protein, cereal proteins are poor in amino acid lysine.
- Pulses and oil seed proteins are rich in lysine but they are poor in sulfur containing amino acids.

Such proteins individually are therefore **incomplete proteins.** However, relative insufficiency of a particular amino acid of any vegetable food can be overcome by judicious combination with other vegetable foods, which may have adequate level of that limiting (deficient) amino acid. The amino acid composition of these proteins **complement** each other and the resulting mixture will have an amino acid pattern better than either of the constituent proteins of the mixture. This is the procedure normally used to improve quality of vegetable proteins. This phenomenon is called **mutual supplementation effect of amino acid.**

Thus, a protein of cereals, deficient in lysine and pulses with adequate lysine content have a mutually supplementary effect, a deficiency of an amino acid in one can be made good by an adequate level in another, if both are consumed together. Thus, the habitual diets of vegetarians in India based on cereal and pulse helps to overcome the deficiency of certain essential amino acids in one food.

**Table 11.4:** Nutritive value of proteins of some foodstuffs.

| *Protein source* | *Protein type* | *BV* | *Chemical score* | *NPU* | *PER* | *Limiting amino acid* |
|---|---|---|---|---|---|---|
| Animal protein | Egg | 96 | 100 | 96 | 3.8 | Nil |
| | Milk | 90 | 65 | 85 | 2.8 | S-containing amino acid |
| | Meat | 74 | 70 | 76 | 3.2 | S-containing amino acid |
| | Fish | 80 | 60 | 74 | 3.5 | Tryptophan |
| Vegetable proteins | Rice | 80 | 60 | 77 | 1.7 | Lysine, Threonine |
| Cereals | Wheat | 66 | 42 | 61 | 1.3 | Lysine, Threonine |
| Pulses | Bengal gram | 74 | 45 | 61 | 1.1 | S-containing amino acid |
| | Red gram | 72 | 45 | 54 | 1.7 | S-containing amino acid |
| Oil seeds | Ground nut | 55 | 44 | 45 | 1.7 | Lysine, Threonine<br>S-containing amino acid |
| | Soya bean | 62 | 55 | 55 | 2.1 | S-containing amino acid |

(BV: Biological value; NPU: Net Protein Utilization; PER: Protein energy ratio; S-containing: Sulphur containing)

### Protein Requirement

The requirement is dependent on the quality of dietary protein. The ICMR Expert Group, suggested an intake of one gram of protein per kg of body weight for adult males and females, assuming, an NPU of 65 for dietary protein. The requirement should be nearly double for growing children, pregnant and lactating women.

### Vitamins and Minerals

The nutritional aspects including metabolism, biochemical functions dietary sources, requirements and associated pathological conditions for vitamins (**chapter 7**) and for minerals (**chapter 17**) have been discussed in much detail.

## RECOMMENDED DAILY ALLOWANCE

The term ***'recommended daily allowance (RDA)'*** is defined as the amount of nutrient sufficient for the maintenance of health in nearly all individuals. Estimates of allowances are based on the defined minimum requirement plus a safety margin for most individuals. RDAs for selected nutrients for adults are shown in **Table 11.5.** Several terms have been used to define the amount of nutrients needed by the body, such as:

- Optimum requirements.
- Minimum requirements.
- Recommended daily allowances or intake, and
- Safe level of intake.

Of these the term ***recommended daily intake*** or ***allowance (RDA)*** has been widely accepted.

## ENERGY REQUIREMENTS

Energy is the principal requisite for body function and growth. The energy requirement of an individual is defined as the energy intake which will balance energy expenditure in an individual, whose body size and composition and level of physical activity are consistent with long-term good health.

- For children and for pregnant and lactating women, allowances are additionally made for growth of tissue and production of milk.
- Energy intake has to be adequate to meet energy expenditure, otherwise the body's reserves will be utilized without being properly replenished, resulting in loss of body weight, impairment of various body functions and finally death.
- If, on the other hand, the energy intake is excessive, compared to the energy expenditure, the body's fuel reserves will increase, resulting in obesity which is also a health risk.

Determinations of energy requirements depend, therefore, on measurements of energy expenditure.

### Measurement of Energy

The energy value of food has long been expressed in terms of the ***kilo-calorie (kcal),*** or abbreviated ***"Cal"*** with

**Table 11.5:** Recommended daily dietary allowances for Indians.

| Group | Particulars | Net energy kcal/d | Protein g/d | Fat g/d | Calcium mg/d | Iron mg/d | Vitamin A Retinol µg/d | Thiamine mg/d | Ribo-flavin mg/d | Nico-tinic acid mg/d | Pyridoxin mg/d | Ascorbic acid mg/d | Folic acid mg/d | Vitamin B12 µg/d |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Man | Sedentary work | 2425 | | | | | | 1.2 | 1.4 | 16 | | | | |
| | Moderate work | 2875 | 60 | 20 | 400 | 28 | 600 | 1.4 | 1.6 | 18 | 2.0 | 40 | 100 | 1 |
| | Heavy work | 3800 | | | | | | 1.6 | 1.9 | 21 | | | | |
| Woman | Sedentary work | 1875 | | | | | | 0.9 | 1.1 | 12 | | | | |
| | Moderate work | 2225 | 50 | 20 | 400 | 30 | 600 | 1.1 | 1.3 | 14 | 2.0 | 40 | 100 | 1 |
| | Heavy work | 2925 | | | | | | 1.2 | 1.5 | 16 | | | | |
| | Pregnant woman | + 300 | + 15 | 30 | 1000 | 38 | 600 | +0.2 | +0.2 | +2 | 2.5 | 40 | 400 | 1 |
| | Lactation | +550 | +25 | 45 | 1000 | 30 | 950 | +0.3 | +4 | +3 | 2.5 | 80 | 150 | 1.5 |

capital ***"C".*** This has been replaced by ***"joule"*** expressed as **J,** which has been accepted internationally, however, the use of kilocalorie for measuring energy still continues. The conversion factors are as follows:

1 kcal = 4184 J
1 kcal = 4.184 kJ
1000 kcal = 4.184 MJ.

All energy in the diet is provided by three nutrients:

- Carbohydrate
- Fat
- Proteins
- Ethanol if it is consumed.

They supply energy at the following rates:

| | |
|---|---|
| Protein | 4 kcal/g or 17 kJ |
| Fat | 9 kcal/g or 38 kJ |
| Carbohydrate | 4 kcal/g or 17 kJ |
| Ethanol | 7 kcal/g or 29 kJ |

The energy content of fat is more than twice that of carbohydrate or protein. If an adequate energy supply is not provided, some protein will be burnt to provide energy.

### Factors Affecting Energy Expenditure

The energy expended by an individual depends on four main factors:

1. The basal metabolic rate (BMR).
2. The thermogenic effect (specific dynamic actions, SDA) of food.
3. Physical activity, and
4. Environmental temperature.

Besides the above four factors extra provision of energy has to be made for growth, pregnancy and lactation.

## BASAL METABOLIC RATE

The basal metabolic rate (BMR) is the energy expenditure necessary to maintain basic physiologic functions, such as:

- The activity of the heart
- Respiration
- Conduction of nerve impulses
- Ion transport across membranes
- Reabsorption in the kidney
- Metabolic activity such as synthesis of macromolecules.

### Definition of BMR

It is defined as the energy expenditure at rest, awake (but not during sleep), in a thermoneutral (warm) environment 8 to 12 hours after the last meal and 8 to 12 hours after any significant physical activity.

### Measurement of Basal Metabolism

Basal metabolism can be measured by:

- Calorimeter directly by measuring the heat dissipated under basal condition
- Indirectly by measuring oxygen consumption.

### Factors Affecting BMR

The BMR differs among different individuals. It depends on many factors as follows:

***Gender or sex:*** The BMR of the males is slightly higher than that of females, partly due to:

- Women's lower percentage of muscle mass (lean body mass) and higher percentage of adipose tissue (that has lower rate of metabolism), when compared to men of the same body weight.
- The difference in sex hormone profile of the two genders.

***Age:*** Decline in BMR with increasing age is probably related to loss of muscle mass (lean body mass) and replacement of muscle with adipose tissue that has lower rate of metabolism.

***Nutritional state:*** BMR is low in starvation and under-nourishment as compared to well-fed state. Starvation leads to an adaptive decrease in BMR, which results from a decrease in lean body mass.

***Body size or surface area:*** The BMR is directly proportional to the surface area of the subject. Larger the surface area, greater will be the heat loss and equally higher will be the heat production and BMR.

***Body composition:*** The BMR is proportionate to lean body mass, (LBM). LBM is the body weight minus non-essential (storage triacylglycerol) weight. Adipose tissue is not as metabolically active as lean body mass. BMR is often expressed as per kilogram of lean body mass or fat-free mass. Therefore, higher the percentage of adipose tissue in the body, lower the BMR/kg body weight.

***Endocrinological or hormonal state:*** In hyperthyroidism, the BMR is increased and in hypothyroidism it may be decreased by up to 40%, leading to weight gain.

***Environmental temperature or climate:*** In colder climate, the BMR is higher and in tropical climates the BMR is proportionally low. Stress, anxiety and disease states, especially infections, fever, burns and cancer also increase the BMR.

***Drugs:*** Smoking (nicotine), coffee (caffeine) and tea (theophylline) increase the BMR, whereas β-blockers tend to decrease energy expenditure.

### Normal Values of BMR

- The BMR values are expressed as kcal per square meter of body surface per hour. In adults, BMR for:
  - Healthy males is 40 kcal/sqm/hour
  - Healthy females, it is 37 kcal/sqm/hour.
- This means that the total caloric expenditure in 24 hours to complete basal state is 1800 kcal for adult males and 1400 kcal for adult females, assuming that the total body surface areas are 1.8 sqm and 1.6 sqm respectively.

**Clinical application of BMR**

- Determination of BMR is useful for the diagnosis of disorders of thyroid. In hypothyroidism, BMR is low while in hyperthyroidism it is elevated.
- BMR is used in calculating caloric requirements of an individual and planning of diets.

## THERMOGENIC EFFECT (SPECIFIC DYNAMIC ACTION, SDA) OF FOOD

Another component of energy expenditure in man is diet induced thermogenesis, also known as ***postprandial thermogenesis.*** This is the energy expended in the digestion, absorption, storage and subsequent processing of food. This is called ***thermogenic effect of food*** because these processes require energy and generate heat. The thermogenic effect of food is equivalent to about 5 to 10% of total energy expenditure. This effect was originally attributed solely to the metabolic processing of protein and was termed ***specific dynamic action (SDA),*** but it is now recognized as an effect produced by the consumption of all dietary fuels.

The consumption of protein produces the greatest increase in energy loss compared to fat or carbohydrate. The thermogenic effect of food is given below:

- Protein 20 to 30% of intake
- Fat 2.5 to 4% of intake
- Carbohydrate 5 to 6% of intake.

It varies considerably from individual to individual. It has been suggested that in some people, a high degree of diet induced thermogenesis may be a factor which allows them to maintain their normal body weight even after overeating.

### Physical Activity

Physical activity is the largest variable affecting energy expenditure. For convenience, the activity level may be divided into three groups—**sedentary, moderate** and **heavy** with regard to their physical activity and requirement of energy. Additional calories are to be added for each group.

| | | |
|---|---|---|
| Sedentary work | — | + 30 to 40% of BMR |
| Moderate work | — | + 40 to 50% of BMR |
| Heavy work | — | + 50 to 60% of BMR |

**Table 11.6:** Energy expenditure during different types of activity for a 70 kg man.

| *Kind of activity* | *Calories/h* |
|---|---|
| Sleeping | 65 |
| Awake lying still | 77 |
| Sitting at rest | 100 |
| Standing relaxed | 105 |
| Walking slowly (2.6 miles/h) | 200 |
| Swimming | 500 |
| Running (5.3 miles/h) | 570 |
| Walking upstairs rapidly | 1100 |

**Table 11.6** shows energy expenditure during different types of physical activity for a 70 kg man.

### Environmental Temperature

Environmental temperature affects the metabolic rate. Low temperature increases energy expenditure by inducing ***shivering*** and ***non-shivering thermogenesis.***

- Shivering provides a regulated means of producing heat by increasing muscle activity in response to cold stress.
- Another mechanism nonshivering thermogenesis can also produce heat in response to cold stress. The site of nonshivering thermogenesis is the brown adipose tissue. Brown adipose tissue is important as a thermogenic tissue in hibernating animals and also in newborn human babies. This type of thermogenesis is stimulated by ***epinephrine*** and ***norepinephrine*** that in turn increase metabolic activity and heat generation. ***Nonshivering thermogenesis may also serve as a buffer against obesity.***
- High temperature also has an effect on energy expenditure by increasing heat loss through sweating.

## BALANCED DIET

### Definition

A balanced diet is defined as one which contains a variety of foods in such quantities and proportions that the need for energy, amino acids, vitamins, minerals, fats, carbohydrate and other nutrients is adequately met for maintaining health, vitality and general well-being and also makes

a small provision for extra nutrients to withstand short duration of illness.

### Balanced Diet Suggested by ICMR

The dietary pattern varies widely in different parts of the world. It is generally developed according to the:

- Kinds of food produced (which depends upon the climatic conditions of the region)
- Economic capacity
- Religion
- Customs
- Tasts and habits of the people.

ICMR has suggested balanced diet for different age groups, sex, and under various occupations for physical activity. These are given in **Table 11.7**. During pregnancy and lactation, additional food is required. This is shown in **Table 11.8**. For nonvegetarians, ICMR has recommended substitution of a part of pulses by animal food **(Table 11.9)**.

## NUTRITIONAL DISORDERS

When balanced diet is not consumed by a person for a sufficient length of time, it leads to nutritional deficiencies or disorders. This nutritional status is called ***malnutrition.*** The most common nutritional disorders are discussed hereunder:

### Protein Caloric Malnutrition (PCM) or Protein Energy Malnutrition (PEM)

The PEM is caused by protein and energy deficiency and has been identified as a major health and nutrition problem in India.

**Table 11.7:** Balanced diet suggested by ICMR.

| | *Adult man* | | *Adult woman* | | | | *Children* | | *Boys* | *Girls* |
|---|---|---|---|---|---|---|---|---|---|---|
| *Food item* | *Sedentary work* | *Moderate work* | *Heavy work* | *Sedentary work* | *Moderate work* | *Heavy work* | *1–3 yrs* | *4–6 yrs* | *10–12 yrs* | *10–12 yrs* |
| | *Quantity gram per day* | | *Quantity gram per day* | | | | *Quantity gram per day* | | | |
| Cereals | 460 | 520 | 670 | 410 | 440 | 575 | 175 | 270 | 420 | 380 |
| Pulses | 40 | 50 | 60 | 40 | 45 | 50 | 35 | 35 | 45 | 45 |
| Leafy vegetables | 40 | 40 | 40 | 100 | 100 | 100 | 40 | 50 | 50 | 50 |
| Other vegetables | 60 | 70 | 80 | 40 | 40 | 50 | 20 | 30 | 50 | 50 |
| Roots and tubers | 50 | 60 | 80 | 50 | 50 | 60 | 10 | 20 | 30 | 30 |
| Milk | 150 | 200 | 250 | 100 | 150 | 200 | 300 | 250 | 250 | 250 |
| Oil and fat | 40 | 45 | 65 | 20 | 25 | 40 | 15 | 25 | 40 | 35 |
| Sugar or jaggery | 30 | 35 | 55 | 20 | 20 | 40 | 30 | 40 | 45 | 45 |

**Table 11.8:** Additional allowances during pregnancy and lactation.

| *Food items* | *During pregnancy g/day* | *Calories (kcal)* | *During lactation g/day* | *Calories (kcal)* |
|---|---|---|---|---|
| Cereals | 35 | 118 | 60 | 203 |
| Pulses | 15 | 118 | 60 | 203 |
| Milk | 15 | 83 | 100 | 83 |
| Fat | – | – | 10 | 90 |
| Sugar | 10 | 40 | 10 | 90 |
| Total | – | 293 | – | 521 |

**Table 11.9:** Suggested substitution for nonvegetarians.

| *Food item which can be deleted in nonvegetarian diets* | *Substitution that can be suggested for deleted item or items* |
|---|---|
| 50% of pulses (20–30 g) | One egg or 30 g of meat or fish<br>Additional 5 g of fat or oil. |
| 100% of pulses (40–60 g) | Two eggs or 50 g of meat or one egg + 30 g meat or fish 10 g of fat or oil |

### Classification of PEM

The PEM can be classified as:
- Marasmus (Greek: "to waste") or nonedematous PEM
- Kwashiorkor or edematous PEM.

#### Marasmus or nonedematous PEM

- Marasmus is a chronic condition resulting from a deficiency of both protein and energy. Marasmus occurs in famine (extreme scarcity of food) areas when infants are weaned from breast milk and given inadequate bottle feedings of thin watery gruels (liquid food) of native cereals or other plant foods. These watery gruels are usually deficient in both calories and proteins.
- Marasmus is characterized by:
  - Growth retardation
  - Anemia
  - Fat and muscle wasting.
- Severe loss of body fat and muscle results in an ***emaciated*** appearance. Starvation adaptations cause serum protein and electrolyte concentrations to remain within their normal range and do not show edema.

#### Kwashiorkor or edematous PEM

- Kwashiorkor refers to conditions caused by severe protein deficiency in individuals with an adequate energy intake. Kwashiorkor is an African word that means ***weaning disease***. When children are weaned from protein rich breast milk, they receive insufficient protein. The clinical symptoms of kwashiorkor include:
  - Anorexia
  - Severe edema associated with hypoalbuminemia
  - Moon face
  - Depigmented hair and skin
  - Fatty liver
  - Distended abdomen (due to enlarged liver).
- The edema is due to low oncotic pressure in the plasma due to ***hypoalbuminemia***. Synthesis of plasma proteins by the liver is also decreased; this in turn impairs the export of triglycerides and other lipids from the liver, resulting in a ***fatty liver***.

The major differences between kwashiorkor and marasmus are given in **Table 11.10.**

**Table 11.10:** Differences between kwashiorkor and marasmus.

| *Features* | *Kwashiorkor* | *Marasmus* |
|---|---|---|
| *Age of onset* | *1–5 years* | *Below 1 year* |
| *Edema* | *Present* | *Absent* |
| Serum albumin | Hypoalbuminemia | Normal or slightly decreased |
| Fatty liver | Present | Absent |
| Muscle wasting | Absent or mild | Severe |
| Fat reserves | Normal to mildly diminished | Absent |

## Obesity

This is the pathological state resulting from the consumption of excessive quantity of food over an extended period of time. Obesity is defined as an accumulation of excess fat in the body.

The problem of obesity arises due to an ***imbalance of energy intake in relation to energy expenditure.*** The degree of obesity is commonly assessed by means of the ***body mass index (BMI)*** **(Table 11.11).**

$$\text{BMI} = \frac{\text{Body weight (Kg)}}{\text{Height (m}^2\text{)}}$$

### Causes of Obesity

Factors which influence the development of obesity are as follows:
- Metabolic
- Hormonal
- Genetic.

***Metabolic:*** Obesity may result due to accumulation of triacylglycerol. The triacylglycerol accumulates when:
- Caloric intake exceeds the amount needed for body function and the amount of work being done.
- Deficiency of the enzyme ATPase which impairs normal energy metabolism and gain more weight.

***Hormonal:*** Obesity may result from endocrine disorders like:
- Hypothyroidism
- Hypogonadism
- Hypopituitarism
- Cushing's syndrome.

***Genetic:*** Several genes have the potential to cause obesity in humans, e.g. mutation in **leptin gene (ob gene)** results in obesity. A peptide hormone **leptin** released from

**Table 11.11:** Relationship between BMI and degree of obesity.

| *Value of BMI* | *Degree of obesity* |
|---|---|
| 20–25 | Normal |
| 25–30 | Over weight or obesity grade I |
| 30–35 | Over obesity or grade II |
| Above 35 | Gross obesity or grade III |

adipocytes, is a product of gene **"ob gene".** Leptin leads to suppression of food intake. ***Grossly obese humans have a failure in production of leptin.***

### Obesity as a Health Risk

An obese person has the risk of:

- Hypertension.
- Coronary heart disease and stroke
- Insulin resistance diabetes mellitus
- Atherosclerosis
- Cholelithiasis (increased total body cholesterol)
- Osteoarthritis
- Hypoventilation syndrome
- Cancer.

## EXAM QUESTIONS

### Short Notes

1. Essential nutrients required by human beings
2. What is dietary fiber? Role of dietary fiber in the diet
3. Nutritional quality of protein.
4. Define BMR and state the factors affecting BMR
5. Balanced diet
6. Nutritional disorders
7. Protein energy malnutrition (PEM)
8. Obesity
9. Nitrogen balance
10. Essential amino acids

### SOLVE

**Case History 1**

**A 4-year-old child comes with retarded growth and pedal edema. She also has discoloration of skin and hair. On enquiring by the physician, mother told the physician that child was on breast milk up to one and a half years of age and for the past two and a half years she was being given rice and dal. The laboratory data of the child showed hypoalbuminemia.**

**Questions**

1. Name the probable condition.
2. What is the cause of edema?
3. What are the preventive measures?

**Case History 2**

**One-year-old female child comes to OPD with retarded growth and emaciated appearance (wasting of muscles). No edema is present. The condition is diagnosed by physician as marasmus.**

**Questions**

1. What is marasmus?
2. What is the difference between marasmus and kwashiorkor?
3. What is the cause of emaciated appearance?

### Multiple Choice Questions (MCQs)

**1. Dietary fiber has following effects, *except*:**
a. Increases stool bulk
b. Lowers cholesterol
c. Decreases risk of cardiovascular disease
d. Increases risk of diabetes mellitus.

**2. Which of the following dietary sources has the greatest thermogenic effect?**
a. Fat b. Protein
c. Carbohydrate d. Vitamins

**3. Following features are seen in marasmus, *except*:**
a. Edema b. Muscle wasting
c. Growth retardation d. Anemia

**4. The quality of a protein is assessed by:**
a. Protein efficiency ratio (PER)
b. Biological value (BV)
c. Net protein utilization (NPU)
d. All of the above

**5. An obese person has the health risk of:**
a. Atherosclerosis
b. Hypertension
c. Coronary heart disease and stroke
d. All of the above

**6. Which of the following statements is correct for BMR?**
a. Increases in response to starvation
b. Decreases in response to starvation
c. Is not responsive to changes in hormone levels
d. Increases with increasing age

**7. Intake of excess protein beyond the body's needs will cause:**
a. Excretion of the excess protein in the urine
b. An increase in the storage pool of protein
c. An increased synthesis of muscle protein
d. None of the above

**8. Which of the following statement is correct for obesity?**
a. Cause metabolic changes that are usually irreversible
b. Is caused solely by high caloric consumption
c. Several genes have the potential to cause obesity
d. Is associated with an increased sensitivity of insulin receptor

**9. Which of the following is not correct for dietary fat:**
a. If present excess stored as triacylglycerol in adi adipose tissue
b. If present excess stored as glycogen in liver
c. Should include linoleic and linolenic acids
d. High fat diets are associated with many health risks.

**10. The caloric value for a gram of fat is:**
a. 9 Kcal/g b. 6 Kcal/g
c. 4 Kcal/g d. 5 Kcal/g

**11. Protein quality is assessed by:**
a. Amino acid score
b. Net protein
c. Biological value
d. All of the above

**12. Marasmus is characterized by:**
a. Growth retardation
b. Anemia
c. Fat and muscle wasting
d. All of the above

**13. Kwashiorkor is characterized by:**
a. Severe edema
b. Fatty liver
c. Depigmented hair and skin
d. All of the above

**14. The value of BMI (body index mass) of 20-25 is considered as:**
a. Normal
b. Obesity grade I (Over weight)
c. Obesity grade II (Over obesity)
d. Obesity grade III (Gross obesity)

**15. Normal values of BMR for healthy male is:**
a. 80 Kcal/Sqm/hour
b. 40 Kcal/Sqm/hour
c. 60 Kcal/Sqm/hour
d. 100 Kcal/Sqm/hour

**16. Which of the following carbohydrate functions a dietary fiber:**
a. Cellulose b. Pectin
c. Gums d. All of the above

**17. Oxidation of which substance in the body yields the most calories:**
a. Glucose b. Glycogen
c. Protein d. Lipids

**18. The recommended energy intake for an adult sedentary Indian man is**
a. 1,900 kcal/day b. 2,400 kcal/day
c. 2,700 kcal/day d. 3,000 kcal/day

**19. The recommended energy intake for an adult sedentary Indian woman is:**
a. 1,900 kcal/day b. 2,200 kcal/day
c. 2,400 kcal/day d. 2,700 kcal/day

**20. During pregnancy, the following should be added to the calculated energy requirement:**
a. 300 kcal/day b. 500 kcal/day
c. 700 kcal/day d. 900 kcal/day

**21. The proximate principles of diet are:**
a. Vitamins and minerals
b. Proteins
c. Carbohydrates and fats
d. Carbohydrates, fats and proteins

**22. The limiting amino acid in wheat is:**
a. Leucine b. Lysine
c. Cysteine d. Methionine

**23. The limiting amino acid in pulses is:**
a. Leucine b. Lysine
c. Tryptophan d. Methionine

**24. Maize is poor in:**
a. Lysine b. Methionine
c. Tryptophan d. Lysine and tryptophan

**25. Net protein utilization depends upon:**
a. Protein efficiency ratio
b. Digestibility coefficient
c. Digestibility coefficient and protein efficiency ratio
d. Digestibility coefficient and biological value

**26. The following is considered as reference standard for comparing the nutritional quality of proteins:**
a. Milk proteins b. Egg proteins
c. Meat proteins d. Fish proteins

**27. The amino acids present in pulses can supplement the limiting amino acids of:**
a. Cereals b. Milk
c. Fish d. Nuts and beans

**28. Kwashiorkor occurs when the diet is severely deficient in:**
a. Iron b. Calories
c. Proteins d. Essential fatty acids

**29. Clinical features of Kwashiorkor include all of the following *except*:**
a. Mental retardation b. Muscle wasting
c. Edema d. Anemia

**30. Kwashiorkor usually occurs in:**
a. The post-weaning period
b. Pregnancy
c. Lactation
d. Old age

**31. Marasmus occurs from deficient intake of:**
a. Essential amino acids
b. Essential fatty acids
c. Calories
d. Zinc

**32. Marasmus differs from Kwashiorkor in which of these following respect:**
a. Mental retardation occurs in kwashiorkor but not in marasmus
b. Growth is retarded in kwashiorkor but not in marasmus
c. Muscle wasting occurs in marasmus but not kwashiorkor
d. Subcutaneous fat disappears in marasmus but not in kwashiorkor

**33. Energy reserves of an average well-fed adult man are about:**
a. 50,000 kcal
b. 100,000 kcal
c. 200,000 kcal
d. 300,000 kcal

**34. Obesity increases the risk of:**
a. Hypertension
b. Diabetes mellitus
c. Cardiovascular disease
d. All of these

## Answers for MCQs

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **1.** d | **2.** b | **3.** a | **4.** d | **5.** d | **6.** b | **7.** d | **8.** c |
| **9.** b | **10.** a | **11.** d | **12.** d | **13.** d | **14.** a | **15.** b | **16.** d |
| **17.** d | **18.** b | **19.** a | **20.** a | **21.** d | **22.** b | **23.** d | **24.** d |
| **25.** d | **26.** b | **27.** a | **28.** c | **29.** a | **30.** a | **31.** c | **32.** d |
| **33.** c | **34.** d | | | | | | |

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CHAPTER

# 12 Carbohydrate Metabolism

**Chapter outline**


- Digestion, Absorption and Transport of Carbohydrates
- Metabolic Fate of Carbohydrates
- Glycolysis
- Rapoport-Luebering Cycle
- Conversion of Pyruvate to Acetyl-CoA
- Citric Acid Cycle
- Gluconeogenesis
- Cori Cycle or Lactic Acid Cycle
- Glucose-alanine Cycle
- Glycogen Metabolism
- Pentose Phosphate Pathway
- Uronic Acid Pathway
- Galactose Metabolism and Galactosemia
- Metabolism of Fructose
- Blood Glucose Level and its Regulation
- Glycosuria
- Diabetes Mellitus
- Glucose Tolerance Test (GTT)


## INTRODUCTION

The major source of carbohydrates is found in plants. Glucose is the universal fuel for human cells. The glucose concentrations in the body are maintained within limits by various metabolic processes.

Carbohydrates are not essential to the diet because through gluconeogenesis the body can synthesize necessary carbohydrates from other metabolites. This chapter focuses on the chemical reactions involved in the:

- Digestion of dietary carbohydrate
- The storage of temporary excess of carbohydrate as glycogen
- Conversion of glucose to other molecules and control of these metabolic pathways
- Disorders related to glucose metabolism.

## DIGESTION, ABSORPTION AND TRANSPORT OF CARBOHYDRATES

### Digestion of Carbohydrates

The principal sites of carbohydrate digestion are the **mouth** and **small intestine.** The dietary carbohydrate consists of:

- **Polysaccharides:** Starch, glycogen and cellulose
- **Disaccharides:** Sucrose, maltose and lactose
- **Monosaccharides:** Mainly glucose and fructose.

Monosaccharides need no digestion prior to absorption, whereas disaccharides and polysaccharides must be hydrolyzed to simple sugars before their absorption **(Fig. 12.1).**

#### *Digestion in Mouth*

Digestion of carbohydrates begins in the mouth. Salivary glands secrete α-***amylase (ptylin)***, which initiates the hydrolysis of a starch. During mastication, salivary α-amylase acts briefly on dietary starch in random manner breaking some α-(1 → 4) bonds, α-amylase hydrolyzes starch into dextrins.

#### *Digestion in Stomach*

Carbohydrate digestion halts temporarily in the stomach because the high acidity inactivates the salivary α-amylase.

#### *Digestion in Intestine*

Further digestion of carbohydrates occurs in the small intestine by **pancreatic enzymes.** There are two phases of intestinal digestion.

1. Digestion due to pancreatic α-**amylase**
2. Digestion due to intestinal enzymes: **Sucrase, maltase, lactase, isomaltase.**

**Fig. 12.1:** Flow sheet of digestion of carbohydrates.

*Digestion due to pancreatic α-amylase*

- The function of pancreatic α-amylase is to degrade dextrins further into a mixture of maltose, isomaltose and α-limit dextrin.
- The α-limit dextrins are smaller oligosaccharides containing 3 to 5 glucose units.

*Digestion due to intestinal enzymes*

Enzymes responsible for the final phase of carbohydrate digestion are located in the brush-border membrane. The enzymes and the reactions they catalyze are as follows:

| | | |
|---|---|---|
| • Maltose | Maltase → | Glucose + Glucose |
| • Isomaltose | Isomaltase → | Glucose + Glucose |
| • Sucrose | Sucrase → | Glucose + Fructose |
| • Lactose | Lactase → | Glucose + Galactose |
| • α-Limit dextrin | Dextrinase → | Glucose + Maltose |

The end products of carbohydrate digestion are ***glucose, fructose*** and ***galactose*** which are readily absorbed through the intestinal mucosal cells into the bloodstream.

## Absorption of Carbohydrates

Carbohydrates are absorbed as monosaccharides from the intestinal lumen. Two mechanisms are responsible for the absorption of monosaccharides:

1. Active transport against a concentration gradient, i.e. from a low glucose concentration to a higher concetration.
2. Facilitative transport, with concentration gradient, i.e. from a higher concentration to a lower one.

### *Active Transport*

The transport of glucose and galactose across the brush border membrane of mucosal cells occurs by an *active transport*. Active transport is an energy requiring process that requires a specific transport protein and the presence of sodium ions **(Fig. 12.2).**

- A sodium dependent glucose transporter (SGLT-1) binds both glucose and $Na^+$ at separate sites and transports them both through the plasma membrane of the intestinal cell.
- The $Na^+$ is transported down its concentration gradient (higher concentration to lower concentration) and at the same time glucose is transported against its concentration gradient.

- The free energy required for this active transport is obtained from the hydrolysis of ATP linked to a **sodium pump** that expels $Na^+$ from the cell in exchange of $K^+$ **(Fig. 12.2)**.

### *Facilitative Transport*

- *Fructose* and *mannose* are transported across the brush border by a $Na^+$ independent facilitative diffusion process, requiring specific glucose transporter, GLUT-5.
- Movement of sugar in facilitative diffusion is strictly from a higher concentration to a lower one until it reaches an equilibrium.
- The same transport can also be used by glucose and galactose if the concentration gradient is favorable.

## Transport of Carbohydrates

The sodium independent transporter, GLUT-2 that facilitates transport of sugars out of the mucosal cells, thereby entering the portal circulation and being transported to the liver.

**Lactose Intolerance**

- Intolerance to lactose (the sugar of milk) not to milk. This is the most common disorder due to deficiency of enzyme ***lactase***.
- In this condition, lactose accumulates in the gut which undergoes bacterial fermentation in the large intestine with the production of $H_2$ and $CO_2$ gases and low molecular weight acids like acetic acid, propionic acid and butyric acid which are osmotically active.
- Abdominal cramps and flatulence results from the accumulation of gases and the osmotically active products that draw water from the intestinal cells into the lumen resulting in ***diarrhea*** and ***dehydration***.
- Treatment for this disorder is simply to remove lactose from the diet.

## Metabolic Fate of Carbohydrates

After being absorbed from the intestinal tract the monosaccharides are carried by the portal circulation directly to the liver. In the liver most of the entering free D-glucose is phosphorylated to ***glucose-6-phosphate*** and sugar is trapped within the cell and it cannot diffuse back out of the cell because its plasma membrane is impermeable to the glucose-6-phosphate. The remainder of the glucose passes into the systemic blood supply.

Other dietary monosaccharides **D-fructose** and **D-galactose** are phosphorylated and may be converted into glucose in the liver. Glucose-6-phosphate is an intermediate

**Fig. 12.2:** Transport of glucose, fructose, galactose and mannose.

in several metabolic pathways that uses glucose in the liver depending upon the supply and demand includes:

- Glycolysis
- Pentose phosphate pathway
- Glycogenesis and
- Glycogenolysis.

## GLYCOLYSIS

### *Definition*

Glycolysis is the sequence of reactions that converts glucose into pyruvate in the presence of oxygen (aerobic) or lactate in the absence of oxygen (anaerobic) with the production of ATP. This pathway is also called ***Embden Meyerhof pathway***.

It is a unique pathway since it can utilize oxygen if available, or it can function in the total absence of oxygen.

### *Location*

Glycolysis is the major pathway for the utilization of glucose and is found in **cytosol** of all cells.

### *Reactions of Glycolysis (Fig. 12.3)*

The breakdown of glucose (6-carbon compound) to two moles of pyruvate (3-carbon compound) is brought about by sequential action of ten enzymes which can be divided into two phases**.**

1. Ist phase: Energy requiring phase or preparative phase
2. IInd phase: Energy generating phase.

**Fig. 12.3:** The reactions of glycolysis.

*Ist phase: Energy requiring phase or preparative phase*

1. Glucose is phosphorylated to glucose-6-phosphate by **hexokinase** or **glucokinase** and ATP is required as a phosphate donor. Hexokinase and glucokinase are isoenzymes. The difference between glucokinase and hexokinase is given in **Table 12.1.** This is an **irreversible** reaction.
2. Conversion of glucose-6-phosphate to fructose-6-phosphate by an enzyme ***phosphohexose isomerase*** which involves isomerization and is freely **reversible** reaction.
3. Fructose-6-phosphate to fructose 1, 6-bisphosphate, second phosphorylation reaction requiring ATP catalyzed by an enzyme ***phosphofructokinase-I***. This step is irreversible under physiological conditions. **Phosphofructokinase-I** is ***regulatory enzyme of glycolysis***.
4. Fructose-1,6-bisphosphate is cleaved by aldolase to two three carbon compounds, glyceraldehyde-3-phosphate and dihydroxy acetone phosphate (DHAP).
5. DHAP is isomerized to glyceraldehyde-3-phosphate by the enzyme ***phosphotriose isomerase***, so that, 2-molecules of glyceraldehyde-3-phosphate are formed from one molecule of glucose.

*IInd phase: Energy generating phase*

6. Oxidation of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate by ***glyceraldehyde-3-phosphate dehydrogenase***, is a NAD dependent reversible reaction. The reducing equivalents NADH+ $H^+$ formed are reoxidized by electron transport chain, to generate 2.5 ATP molecules per NADH+$H^+$.
7. 1,3-bisphosphoglycerate to 3-phosphoglycerate is catalyzed by ***phosphoglycerate kinase***. This is the step in glycolysis that generates ATP at ***substrate level phosphorylation***. Since two molecules of glyceraldehyde-3-phosphate are formed per molecule of glucose undergoing glycolysis, two molecules of ATP are generated at this stage per molecule of glucose.
8. 3-phosphoglycerate to 2-phosphoglycerate is a reversible reaction catalyzed by ***phosphoglycerate mutase***.
9. 2-phosphoglycerate to phosphoenolpyruvate. This reaction is catalyzed by ***enolase***. ***Enolase is inhibited by fluoride, a property that can be used when it is required to prevent glycolysis in blood prior to the estimation of glucose.***
10. Phosphoenolpyruvate to pyruvate is an irreversible reaction catalyzed by **pyruvate kinase.** This is the second step in glycolysis that generates ATP at **sub strate level phosphorylation.** Enolpyruvate formed

**Table 12.1:** Difference between hexokinase and glucokinase.

| *Hexokinase* | *Glucokinase* |
|---|---|
| Present in extrahepatic tissue | Present in liver |
| High affinity for its substrate glucose (low $K_m$) | Low affinity for its substrate glucose (high $K_m$) |
| Inhibited by its product glucose-6-phosphate | No inhibition by its product glucose-6-phosphate |
| Its function is to ensure supply of glucose for the tissues, irrespective of blood glucose concentration | Its function is to remove glucose from the blood, when the blood glucose level increases (following meal) |
| Catalyze the phosphorylation of other hexoses like fructose, galactose, etc. | Specific for glucose |
| Its activity is not affected by insulin | It is an inducible enzyme that increases its synthesis in response to insulin |

in this reaction is converted spontaneously to the keto form of pyruvate.

Under aerobic condition, pyruvate is taken up into mitochondria and after conversion to acetyl-CoA is oxidized to $CO_2$ and $H_2O$ by citric acid cycle.

## Anaerobic Glycolysis

- In anaerobic conditions, the reoxidation of NADH (formed in the glyceraldehyde-3-phosphate step) by respiratory chain is prevented and gets reoxidized by conversion of pyruvate to lactate by **lactate dehydrogenase (Fig. 12.4).**
- Tissues that function under hypoxic conditions produce lactate, e.g. skeletal muscle, smooth muscle and erythrocytes.
- In erythrocytes even under aerobic conditions, glycolysis terminates in lactate because of absence of mitochondria.

**Fig. 12.4:** Anaerobic glycolysis. Conversion of pyruvate to lactose.

## Regulation of Glycolysis

Glycolysis is regulated at 3 steps which are ***irreversible (Fig. 12.5).*** These reactions are catalyzed by:

1. Hexokinase and glucokinase
2. Phosphofructokinase-I
3. Pyruvate kinase

### *Hexokinase and glucokinase*

- Hexokinase is an allosteric enzyme, which is inhibited by its product glucose-6-phosphate.
- Liver glucokinase is an inducible enzyme that increases its synthesis in response to insulin and decreases in response to glucagon.

### *Phosphofructokinase-l*

- Phosphofructokinase-l is activated by:
  - AMP (which signals low energy state)
  - Fructose-6-phosphate (substrate)
  - Fructose 2,6-bisphosphate
  - Insulin

**Fig. 12.5:** Regulation of glycolysis. (PFK: Phosphofructokinase)

- Phosphofructokinase-I is inhibited by:
  - ATP (which signals high energy state).
  - Citrate
  - Glucagon

*Pyruvate kinase*

- It is activated by:
  - Fructose-1, 6-bisphosphate feedforward stimulation.
  - Insulin
- Pyruvate kinase is inhibited by:
  - ATP
  - Glucagon

**Significance of Glycolysis**

- Glycolysis is the principal route for glucose metabolism for the production of ATP molecules.
- It also provide pathway for the metabolism of fructose and galactose derived from diet.
- Glycolysis has the ability to provide ATP in the absence of oxygen and allows tissues to survive anoxic episodes.
- It generates precursors for biosynthetic pathway, e.g.
  - Pyruvate may be transaminated to amino acid **alanine**. In the liver pyruvate provides substrate, acetyl-CoA for **fatty acid** biosynthesis.
  - Glycerol-3-phosphate derived from glycolytic pathway forms the backbone of **triacylglycerol**.
- In erythrocytes glycolysis supplies 2,3-BPG which is required for the hemoglobin function in the transport of oxygen.
- In mammals, glucose is the only fuel that the brain uses under non-starvation conditions and the only fuel that red blood cells can use at all.

### *Energetics of Glycolysis*

- The details of ATP generation in glycolysis are given in **Table 12.2.** Under aerobic conditions, 7 molecules of ATP are produced as per new concept.
- In anaerobic glycolysis, on the other hand, only 2 moles of ATP are produced per molecule of glucose.

## RAPOPORT-LUEBERING CYCLE

In Rapoport Luebering cycle, production of ATP by substrate phosphorylation from 1,3-BPG is bypassed in the erythrocyte by taking a diversion pathway **(Fig. 12.6).**

- In Rapoport Luebering cycle 1,3-BPG is converted to 2,3-BPG by an enzyme ***bisphosphoglycerate mutase***.
- Then 2,3-BPG is converted to 3-phosphoglycerate by ***2,3-bisphosphoglycerate phosphatase***, with a loss of high energy phosphate (energy is dissipated as heat) and there is no net production of ATP when glycolysis takes this route.

### *Significance of Rapoport Luebering Cycle*

- It supplies 2,3-BPG required for the transport of oxygen by hemoglobin. 2,3-BPG regulates the binding and release of oxygen from hemoglobin.
- 2, 3-BPG present in erythrocytes acts as a buffer.

## FATE OF PYRUVATE

- Pyruvate is converted to acetyl CoA by **oxidative decarboxylation.** This step occurs only in mitochondria. This is an irreversible reaction catalyzed by a multienzyme complex known as **pyruvate dehydrogenase complex (PDH) (Fig. 12.7).**
- The enzyme pyruvate dehydrogenase requires five coenzymes, namely thiamine pyrophosphate (TPP), lipoate, coenzyme-A, FAD and $NAD^+$.

*Energetics in conversion of pyruvate to Acetyl-CoA*

As a result of oxidation of pyruvate to acetyl-CoA catalyzed by pyruvate dehydrogenase, one molecule of NADH is

**Table 12.2:** Production of ATP in glycolysis aerobically.

| *Reaction* | *Reaction catalyzed by* | *Number of ATP formed or consumed/glucose molecule* |
|---|---|---|
| Glucose to glucose-6-phosphate | Hexokinase, glucokinase | – 1 |
| Fructose-6-phosphate to fructose 1,6-bisphosphate | Phosphofructokinase-I | – 1 |
| Glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate | Glyceraldehyde-3-phosphate dehydrogenase | + 5* |
| 1, 3-bisphosphoglycerate to 3-phosphoglycerate | Phosphoglycerate kinase | + 2 |
| Phosphoenolpyruvate to pyruvate | Pyruvate kinase | + 2 |

- Net production of ATP in aerobic glycolysis = Number of ATP produced minus number of ATPs consumed = 9 – 2 = 7
- *It is assumed that NADH formed in glycolysis uses malate shuttle to produce 5 ATPs
- Total ATP per molecule of glucose under anaerobic glycolysis = 2

**Fig. 12.6:** Rapoport Luebering cycle of erythrocytes shown in green color.

**Fig. 12.7:** Oxidative decarboxylation of pyruvate by the pyruvate dehydrogenase (PDH) complex.

produced for each molecule of pyruvate. Oxidation of NADH by electron transport chain results in synthesis of **2.5 ATP molecules.**

### *Significance*

- The conversion of pyruvate to acetyl-CoA is a central step, linking the glycolytic pathway with citric acid cycle.
- Acetyl-CoA is also an important precursor in fatty acid biosynthesis and cholesterol biosynthesis.

## CITRIC ACID CYCLE

Citric acid cycle is also called ***Krebs cycle*** or ***tricarboxylic acid (TCA) cycle***.

- It is called citric acid cycle because citrate was one of the first compounds known to participate.
- It is called Krebs cycle, because its reactions were formulated into a cycle by ***Sir Hans Krebs***.
- The most common name for this pathway is, the **tricarboxylic acid** or **TCA cycle,** due to involvement of the tricarboxylate, **citrate** and **isocitrate.**

### *Definition*

The citric acid cycle is a series of reactions in mitochondria that brings about the catabolism of acetyl-CoA to $CO_2$ and $H_2O$ with generation of ATP.

### *Location of Citric Acid Cycle*

The reactions of citric acid cycle are located in the **mitochondrial matrix.**

### *Reactions of Citric Acid Cycle (Fig. 12.8)*

1. First reaction of the citric acid cycle is the condensation of acetyl-CoA with oxaloacetate to yield citrate, catalyzed by ***citrate synthase***.
2. Citrate is converted to isocitrate by an enzyme ***aconitase***. This conversion takes place in two steps:
   - Dehydration to cis-aconitate
   - Rehydration to isocitrate.
3. Isocitrate undergoes dehydrogenation and decarboxylation in the presence of ***isocitrate dehydrogenase*** to form α-ketoglutarate. The formation of ***NADH*** and liberation of $\mathbf{CO_2}$ occurs at this stage.
4. Next α-ketoglutarate undergoes oxidative decarboxylation, catalyzed by a multi-enzyme complex, ***α-ketoglutarate dehydrogenase***, an α-ketoglutarate dehydrogenase complex requires thiamine pyrophosphate (TPP), Lipoate, NAD, FAD and coenzyme-A and results in the formation of succinyl-CoA, a high energy compound, this reaction is physiologically ***irreversible***. At this stage, ***second NADH is produced*** along with ***liberation of second $CO_2$ molecule***.
5. Succinyl-CoA is converted to succinate by the enzyme ***succinate thiokinase***. This reaction is coupled with the phosphorylation of GDP to GTP. This is a **substrate level phosphorylation.** This GTP is converted to ATP.
6. Succinate is oxidized further by ***succinate dehydrogenase*** to fumarate with the production of $FADH_2$.
7. Next, ***fumarase*** catalyzes the addition of water to fumarate to give malate.
8. Malate is converted to oxaloacetate by malate dehydrogenase, and requires $NAD^+$. ***The synthesis of third NADH occurs at this stage***. The oxaloacetate is regenerated which can combine with another molecule of acetyl-CoA and continue the cycle.

### *Energetics of Citric Acid Cycle*

- As a result of oxidation of acetyl-CoA to $H_2O$ and $CO_2$ by citric acid cycle, ***three molecules of NADH*** and ***one $FADH_2$*** are produced.
- Oxidation of 3 NADH by electron transport chain results in the synthesis of **3 × 2.5 = 7.5 ATP**, whereas $FADH_2$ generates **1.5 ATP** molecules.

- One molecule of ATP is generated at substrate level during the conversion of succinyl-CoA to succinate. Thus, a total of 10 ATP are generated from one molecule of acetyl-CoA **(Table 12.3).**

**Amphibolic Nature of Citric Acid Cycle**

Citric acid cycle has a dual function; it functions in both catabolism (of carbohydrates, fatty acids and amino acids) and anabolism **(Fig. 12.9).** It provides intermediates for the synthesis of many compound required for the body (refer significance of citric acid cycle) and hence the cycle is said to be an amphibolic (Greek amphi, "both").

**Significance of Citric Acid Cycle**

- The primary function of the citric acid cycle is to provide energy in the form of ATP.
- Citric acid cycle is the final common pathway for the oxidation of carbohydrates, lipids, and proteins as glucose, fatty acids and many amino acids are all metabolized to acetyl-CoA or intermediates of the cycle.
- Citric acid cycle is an **amphibolic process.** Citric acid cycle has a dual function; it functions in both catabolism (of carbohydrates, fatty acids and amino acids) and anabolism **(Fig. 12.9)**. Some metabolic pathways end in the constituent of the citric acid cycle while other pathways originate from the cycle, such as:
  - Gluconeogenesis
  - Transamination
  - Fatty acid synthesis
  - Heme synthesis.
- **Gluconeogenesis:** All major members of the citric acid cycle from citrate to oxaloacetate are glucogenic. They can give rise to glucose by gluconeogenesis.
- **Transamination:** Oxaloacetate and α-ketoglutarate respectively, serve as precursors for the synthesis of aspartate and glutamate by transamination which in turn is used for the synthesis of other nonessential amino acids, purines and pyrimidines.
- **Fatty acid synthesis:** Mitochondrial citrate is transported to the cytosol, where it is cleaved to provide acetyl-CoA for the biosynthesis of fatty acids and steroids.
- **Heme synthesis:** Succinyl-CoA (intermediate of TCA cycle) together with glycine is used for the synthesis of heme.

### *Regulation of Citric Acid Cycle*

- Citric acid cycle is regulated at three steps. These are catalyzed by:
  1. Citrate synthase
  2. Isocitrate dehydrogenase
  3. α-ketoglutarate dehydrogenase
- Activities of these enzymes are dependent on the energy status of the cycle.
- Excess of ATP, NADH and succinyl-CoA, which signals high energy status of the cell, inhibit these enzymes.
- High level of ADP which signals low energy status of the cell stimulates the operation of the cycle.

## GLUCONEOGENESIS

### Definition

The synthesis of glucose from **noncarbohydrate** precursors is called ***gluconeogenesis*** (i.e. synthesis of new glucose).

### *Precursors for Gluconeogenesis*

The major noncarbohydrate substrates for gluconeogenesis are:

- Lactate
- Glycerol
- Glucogenic amino acids
- Propionate
- Intermediates of the citric acid cycle.

1. **Lactate:** Lactate is formed from glucose by anaerobic glycolysis in the muscle. It is transported to the liver by **Cori's cycle** (discussed later) and is converted to glucose by gluconeogenesis.
2. **Glycerol:** Glycerol is formed in adipose tissue by hydrolysis of triacylglycerol. Glycerol cannot be utilized by adipose tissue due to poor content of enzyme **glycerol kinase.** Therefore, it is delivered to the liver where it is converted to glucose by gluconeogenesis.
3. **Glucogenic amino acids and intermediates of TCA cycle:** The carbon skeletons of glucogenic amino acids are converted to pyruvate or intermediates of TCA cycle, which are then converted to glucose by gluconeogenesis.
4. **Propionate:** Fatty acids with an odd number of carbons and carbon skeleton of some amino acids produce propionate. Propionate enters the gluconeogenic pathway via citric acid cycle after conversion of succinyl-CoA.

### *Location of Gluconeogenesis*

Gluconeogenesis occurs mainly in the cytosol although some precursors are produced in the mitochondria. **Liver** is the major tissue for gluconeogenesis. During starvation, the **kidney** is also capable of making glucose by gluconeogenesis. Certain enzymes required in gluconeogenesis are present only in these organs.

**Fig. 12.8:** Reactions of citric acid cycle.

**Fig. 12.9:** Anabolic role of the citric acid cycle.

**Table 12.3:** Production of ATP in citric acid cycle.

| *Reaction* | *Reaction catalyzed by* | *No. of ATP formed per acetyl-CoA molecule* |
|---|---|---|
| Isocitrate to α-ketoglutarate | Isocitrate dehydrogenase | + 2.5 |
| α-Ketoglutarate to succinyl-CoA | α-Ketoglutarate dehydrogenase | + 2.5 |
| Succinyl-CoA to succinate | Succinyl thiokinase | + 1 |
| Succinate to fumarate | Succinate dehydrogenase | + 1.5 |
| Malate to oxaloacetate | Malate dehydrogenase | + 2.5 |

Number of ATPs formed per acetyl-CoA molecule in citric acid cycle = 10

**Characteristics of Gluconeogenesis**

- Gluconeogenesis involves **glycolysis,** the **citric acid cycle** plus some **special reactions.**
- Glycolysis and gluconeogenesis share the same pathway but in opposite direction.
- Seven of the reactions of glycolysis are reversible and are used in the synthesis of glucose by gluconeogenesis.
- However, three of the reactions of glycolysis are irreversible and must be bypassed by four special reactions which are unique to gluconeogenesis and catalyzed by:
  1. Pyruvate carboxylase
  2. Phosphoenol pyruvate carboxykinase
  3. Fructose-1,6-bisphosphatase
  4. Glucose-6-phosphatase

### *Reactions of Gluconeogenesis (Fig. 12.10)*

1. **Pyruvate to oxaloacetate:** In gluconeogenesis, pyruvate is first carboxylated to oxaloacetate. ***Pyruvate carboxylase***, in presence of ATP, vitamin biotin and $CO_2$ converts pyruvate to oxaloacetate.
2. **Oxaloacetate to phosphoenol pyruvate (PEP):** Oxaloacetate is decarboxylated and phosphorylated by ***phosphoenol pyruvate carboxykinase***. High energy phosphate in the form of GTP is required in this reaction. PEP then enters the reversed reaction of glycolysis until it reaches fructose-1, 6-bisphosphate.
3. **Fructose-1,6-bisphosphate to fructose-6-phosphate:** Hydrolysis of fructose-1, 6-bisphosphate to fructose-6-phosphate by **fructose-1,6-bisphosphatase** bypasses the irreversible phosphofructokinase-I reaction of glycolysis. Fructose-6-phosphate is then converted to glucose-6-phosphate by reversed reaction of glycolysis.
4. **Glucose-6-phosphate to glucose:** Hydrolysis of gluose-6-phosphate to glucose by ***glucose-6-phosphatase*** bypasses the irreversible glucokinase and hexokinase reaction of glycolysis.

### *Regulation of Gluconeogenesis*

Gluconeogenesis is regulated by four key enzymes.

1. Pyruvate carboxylase
2. Phosphoenolpyruvate carboxykinase
3. Fructose-1,6-bisphosphatase
4. Glucose-6-phosphatase

- The hormones **glucagon** and **epinephrine** stimulate gluconeogenesis by inducing the synthesis of the key enzymes, while **insulin inhibits** the gluconeogenesis by repressing their synthesis.
- During starvation and in diabetes mellitus, a high level of glucagon stimulates gluconeogenesis. However in well-fed state, insulin suppresses the gluconeogenesis.
- Pyruvate carboxylase is an allosteric enzyme, which is stimulated by acetyl-CoA and inhibited by ADP.
- Fructose-1,6-bisphosphatase stimulated allosterically by c-AMP and inhibited by AMP.

***Significance of Gluconeogenesis***

- Gluconeogenesis maintains blood glucose level when carbohydrate is not available in sufficient amounts from the diet.
- During starvation when hepatic glycogen reserve is totally depleted, glucose is provided by gluconeogenesis to the brain and other tissues like erythrocytes, lens, cornea of the eye and kidney medulla. They require a continuous supply of glucose as a source of energy.
- Gluconeogenesis is used to clear the products of the metabolism of other tissues from the blood, for example,
  - Lactate, produced by muscle and erythrocytes
  - Glycerol produced by adipose tissue
  - Propionyl-CoA produced by oxidation of odd carbon number fatty acids and carbon skeleton of some amino acids.

## CORI CYCLE OR LACTIC ACID CYCLE

Lactate is produced in skeletal muscles during anaerobic oxidation of glucose. The lactate thus produced cannot be further metabolized in skeletal muscles. Through blood, lactate is transported to the liver where it is oxidized to pyruvate. Pyruvate so produced, is converted to glucose by gluconeogenesis, which is then transported to the muscle. The glucose thus reformed from lactate again becomes available for energy purpose in skeletal muscle.

**Fig. 12.10:** Pathway of gluconeogenesis compared with glycolysis.

This cycling of lactate between muscle and liver is known as the ***Cori Cycle*** or ***Lactic acid cycle*** **(Fig. 12.11).**

## GLUCOSE-ALANINE CYCLE

- Because muscle is incapable of synthesizing urea, most of the ammonia formed by protein catabolism is transferred to pyruvate to form **alanine** by transamination reaction.
- Alanine enters the blood and is taken up by the liver.
- In the liver, the amino group of alanine is removed to form urea, and the resulting pyruvate is converted to glucose by gluconeogenesis which is then transported to the muscle, where it is oxidized to pyruvate.
- The pyruvate acts again as the acceptor for another amino group.
- These reactions transport amino groups from muscle to the liver in the form of **alanine**. This cycle is called the **glucose-alanine cycle** or **the Cahill cycle (Fig. 12.11).**

Alanine is the predominant amino acid released from muscle to liver during fasting.

## GLYCOGEN METABOLISM

- Glycogen is the major storage form of glucose mainly in the liver and muscle.
- The concentration of liver glycogen (up to 6%) is greater than in muscle (1%) tissues. However, because muscle tissue comprises a large mass, its total capacity to storage is three to four times that of the liver.
- The synthesis, ***glycogenesis*** and degradation, **glycogenolysis** occurs via different pathways ***(Fig. 12.12)***. Glycogenesis and glycogenolysis are both cytosolic processes.

### Glycogenesis

#### *Definition*

Glycogenesis is the pathway for the formation of glycogen from glucose. This process requires energy, supplied by ATP and uridine triphosphate (UTP). It occurs in muscle and liver.

#### *Reactions of Glycogenesis*

1. Glucose is phosphorylated to glucose-6-phosphate catalyzed by ***hexokinase*** in muscle and ***glucokinase*** in liver.
2. Glucose-6-phosphate is converted to glucose-1-phosphate by the enzyme ***phosphoglucomutase***.
3. Glucose-1-phosphate reacts with uridine triphosphate (UTP) to form uridine diphosphate glucose (UDP-Glc). The reaction is catalyzed by the enzyme ***UDP-glucose pyrophosphorylase***.
4. By the action of the enzyme ***glycogen synthase***, the C1 of the glucose of UDP-Glc forms a glycosidic bond with $C_4$ of a terminal glucose residue of pre-existing

**Fig. 12.11:** Cori cycle or lactic acid cycle and glucose alanine cycle.
The pathway of Cori cycle is shown in green and glucose alanine cycle in blue.

**Fig. 12.12:** Pathway of glycogenesis and glycogenolysis in liver
Where, UTP = Uridine triphosphate, UDP = Uridine diphosphate, UDPGlc = Uridine diphosphate glucose.

**Fig. 12.13:** Schematic representation of glycogenesis (mechanism of branching).

glycogen molecule (glycogen primer), liberating uridine diphosphate (UDP). Thus, pre-existing glycogen molecule must be present to initiate this reaction.

5. In the above reaction, a new α-**1,4 linkage** is established between carbon atom 1 of incoming glucose and carbon 4 of the terminal glucose of a glycogen primer.
6. When the chain has been lengthened to a minimum of 11 residues, a second enzyme, the **branching enzyme,** transfers a part of the 1,4-chain (minimum length of 6-glucose residues) to a neighboring chain to form α-1,6-linkage, thus establishing a branching point in the molecule **(Fig. 12.13)**. The branches grow by further additions of glucose units and further branching.

## Glycogenolysis

### *Definition and Location*

Glycogenolysis is the degradation of glycogen to glucose-6-phosphate and glucose in muscle and liver respectively. Glycogenolysis is not the reverse of glycogenesis but is a separate pathway.

## Reactions of Glycogenolysis

1. Glycogenolysis occurs primarily by phosphorolytic breaking of α-1,4-glycosidic bonds of glycogen to yield glucose-1-phosphate and residual glycogen molecule. This process is catalyzed by the enzyme ***glycogen phosphorylase***. The glucose residues from outermost chain of the glycogen molecule are removed sequentially until approximately four glucose residues remain on either side of a branch point having α-1,6 linkage **(Fig. 12.14)**.
2. Phosphorolysis cannot continue until the branch is removed. This is accomplished by debranching enzyme. It has two catalytic activities—**glucan transferase** and **1,6-glucosidase.**
   - First, it acts as a glucan transferase and transfers three of the remaining residues from one branch to the other. This exposes the α-1,6 branch point.
   - In the second step, the hydrolytic splitting of the α-1,6 linkages occurs by the action of **1,6-glucosidase.** This step releases free glucose. Further splitting of the glycogen can then proceed by the actions of phosphorylase until another branch point is reached. The action of glucan transferase and 1,6-glucosidase are repeated.

   The combined action of ***phosphorylase*** and ***debranching enzyme*** leads to the complete breakdown of glycogen with the formation of glucose-1-phosphate and free glucose (from hydrolytic cleavage of the 1,6-glycosidic bond).
3. Next, glucose-1-phosphate is converted to glucose-6-phosphate by ***phosphoglucomutase***. This is a reversible reaction.
4. In the liver but ***not in the muscle***, there is a specific enzyme, ***glucose-6-phosphatase***, that cleaves glucose-6-phosphate to glucose and diffuse from the hepatic cell into the blood. As glucose-6-phosphatase is absent in muscle, free glucose cannot be produced from glucose-6-phosphate in muscle. Moreover, glucose-6-phosphate cannot diffuse out of the muscles. Therefore, muscle cannot provide glucose to maintain blood glucose level.

## Lysosomal Degradation of Glycogen

A small amount of glycogen is continuously degraded by the lysosomal enzyme α-***1,4-glucosidase (acid maltase).*** The significance of this pathway is unknown. However, a deficiency of this enzyme causes accumulation of glycogen in the cytosol resulting in ***glycogen storage disease type II Pompe's disease***.

**Significance of Glycogenolysis and Glycogenesis**

The functional role of glycogen differs considerably from tissue to tissue, as we can see in the case of liver and muscle.

**In liver**

Following a meal, excess glucose is removed from the portal circulation and stored as glycogen by glycogenesis. Conversely, between meals, blood glucose levels are maintained within the normal range by release of glucose from liver glycogen by glycogenolysis.

**In muscle**

The function of muscle glycogen is to act as a readily available source of glucose within the muscle itself during muscle contraction. ***The muscle cannot release glucose into the blood, because of the absence of glucose-6-phosphatase*** that hydrolyzes glucose 6-phosphate to glucose. Therefore, muscle glycogen stores are used exclusively by muscle.

## Regulation of Glycogenesis and Glycogenolysis

The principal enzymes controlling glycogen metabolism are ***glycogen phosphorylase*** and ***glycogen synthase*** which are regulated reciprocally by hormones.

**Fig. 12.14:** Schematic representation of glycogenolysis (mechanism of debranching).

- **Epinephrine** and **glucagon** regulate glycogen breakdown and glycogen synthesis.
- Epinephrine (in liver and muscle) and glucagon (in liver) stimulates glycogen breakdown (glycogenolysis) and inhibits glycogen synthesis (glycogenesis).

### *Regulation of Glycogenesis*

- **Glycogen synthase** is the regulatory enzyme of glycogenesis. It exists in two forms: **Glycogen synthase-a**, an active or dephosphorylated form and **Glycogen synthase-b**, an inactive or phosphorylated form.
- Glucagon in liver and epinephrine in liver and muscle activates **adenylate cyclase** enzyme that catalyzes the synthesis of **c-AMP.** c-AMP in turn activates **protein kinase** enzyme.
- *Protein kinase then **phosphorylates glycogen synthase** and thereby inactivates glycogen synthase and synthesis of glycogen is inhibited **(Fig. 12.15).***

### *Regulation of Glycogenolysis*

- **Glycogen phosphorylase** is the regulatory enzyme of glycogenolysis. It exists in two forms: **Glycogen phosphorylase-a,** an active or phosphorylated form and **Glycogen phosphorylase-b,** an inactive or dephosphorylated form.
- Degradation of glycogen is stimulated by epinephrine in the muscle and by glucagon in the liver via activation of ***adenylate cyclase*** that catalyzes the synthesis of c-AMP.
- The consequent increase in levels of c-AMP in turn activates **protein kinase.** Active protein kinase phosphorylates the inactive form of **phosphorylase kinase** to its active form.
- Active phosphorylase kinase eventually activates inactive form of glycogen phosphorylase to its active form. Active form of **glycogen phosphorylase** stimulates breakdown of glycogen to glucose-1-P **(Fig. 12.16).**

### Glycogen Storage Disease

This is a group of genetic diseases that result from a defect in an enzyme required for either glycogen synthesis or degradation and characterized by deposition of either normal or abnormal glycogen in the specific tissues. Some of the more common forms of these diseases and their characteristics are summarized in **Table 12.4.**

## PENTOSE PHOSPHATE PATHWAY

### *Definition*

The pentose phosphate pathway is an alternative route for the oxidation of glucose. It is the pathway for formation of pentose phosphate. It is also called ***hexose monophosphate shunt***.

### *Characteristics of Pentose Phosphate Pathway*

- It is a multicyclic process in which three molecules of glucose-6-phosphate give rise to three molecules of $CO_2$ and three molecules of 5-carbon sugars, (ribulose-5-phosphate).
- The three molecules of ribulose-5-phosphate are arranged to generate two molecules of fructose-6-phosphate and one molecule of glyceraldehyde-3-phosphate.
- It does not generate ATP.

The difference between glycolysis and pentose phosphate pathway is shown in **Table 12.5.**

**Fig. 12.15:** Hormonal regulation of glycogenesis.

**Fig. 12.16:** Hormonal regulation of glycogenolysis.

**Table 12.4:** Glycogen storage diseases.

| *Type* | *Name* | *Enzyme affected* | *Primary organ involved* | *Manifestations* |
|---|---|---|---|---|
| I | Von Gierke's disease | Deficiency of glucose-6-phosphatase | Liver or kidney | Hypoglycemia, lactic acidemia, hyperlipemia ketosis and hyperuricemia |
| II | Pompe's disease | Deficiency of lysosomal α-1, 4 glucosidase (acid maltase) | All organs with lysosomes | Infantile form, early death, cardiac failure, accumulation of glycogen in lysosomes |
| III | Limit dextrinosis, Forbe's or Cori's disease | Absence of debranching enzyme | Liver, skeletal muscle, heart | Accumulation of abnormal glycogen having short outer chains, hypoglycemia |
| IV | Amylopectinosis, Andersen's disease | Absence of branching enzyme | Liver | Accumulation of abnormal glycogen having few branches, early death due to cardiac or liver failure |
| V | Mc-Ardle syndrome | Absence of muscle glycogen phosphorylase | Skeletal muscle | Excessive induced muscular pain, cramps, decrease serum lactate after exercise |
| VI | Her's disease | Deficiency of liver glycogen phosphorylase | Liver | High content of liver glycogen, mild hypoglycemia and ketosis |
| VII | Taruis disease | Deficiency of phosphofructokinase in muscle and erythrocytes | Muscle & RBC | As in type V, in addition hemolytic anemia |

## *Location*

The enzymes of pentose phosphate pathway are present in cytosol. The pathway is found in all cells.

## *Reactions of the Pentose Phosphate Pathway*

The reactions of the pathway are divided into two phases: (Fig. 12.17)

**Table 12.5:** Difference between glycolysis and pentose phosphate pathway.

| *Glycolysis* | *Pentose phosphate pathway* |
|---|---|
| Oxidation occurs utilizing $NAD^+$ as an H-acceptor | Oxidation occurs utilizing NADP as an H-acceptor |
| Aerobic as well as anaerobic process | Anaerobic process |
| $CO_2$ is not produced at all | $CO_2$ is a characteristic product |
| ATP is generated, where it is a major function | ATP is not generated |
| Ribose phosphates are not generated | Ribose phosphates are generated |
| 80–90% of glucose oxidized by glycolysis | 10–20% glucose oxidized by pentose phosphate pathway |

**Fig. 12.17:** Pentose phosphate pathway
(TPP: Thiamine pyrophosphate)

1. Phase I: Oxidative irreversible phase
2. Phase II: Nonoxidative reversible phase

## Reactions of Phase I (Oxidative Irreversible Phase)

In the first phase, glucose-6-phosphate undergoes dehydrogenation and decarboxylation to give pentose, ribulose-5-phosphate with generation of NADPH.

1. Dehydrogenation of glucose-6-phosphate to 6-phosphogluconolactone, catalyzed by ***glucose- 6-phosphate dehydrogenase*** which is an NADP dependent enzyme.
2. 6-phosphogluconolactone is hydrolyzed by **6-phosphogluconolactone *hydrolase*** to 6-phosphogluconate.
3. The subsequent oxidative decarboxylation of 6-phosphogluconate is catalyzed by ***6-phosphogluconate* dehydrogenase,** which also requires NADP as hydrogen acceptor. This irreversible reaction produces ribulose-5-phosphate, $CO_2$ and second molecule of NADPH.

## Reactions of Phase II (Nonoxidative Reversible Phase)

In the second phase, ribulose-5-phosphate is converted to fructose-6-phosphate by a series of reactions.

4. Ribulose-5-phosphate formed in the phase I now serves as substrate for two different enzymes:
   i. **Ribulose-5-phosphate epimerase** catalyzes the epimerization of ribulose-5-phosphate to xylulose-5-phosphate.
   ii. **Ribulose-5-phosphate isomerase** catalyzes the isomerization of ribulose-5-phosphate to ribose-5-phosphate.
5. **Transketolase** catalyzes the transfer of two carbon units from xylulose-5-phosphate to ribose-5-phosphate, producing a 7-carbon, sedoheptulose-7-phosphate and glyceraldehyde-3-phosphate. The reaction requires coenzyme ***thiamine pyrophosphate (TPP)*** and $Mg^{2+}$ ions.
6. ***Transaldolase*** catalyzes the transfer of a three carbon dihydroxyacetone group from sedoheptulose-7-phosphate to glyceraldehyde-3-phosphate to form fructose-6-phosphate and the 4-carbon, erythrose-4-phosphate.
7. Further reaction again involves ***transketolase***, which catalyzes the transfer of the two carbon units from xylulose-5-phosphate to erythrose-4-phosphate producing fructose-6-phosphate and glyceraldehyde-3-phosphate.
8. Fructose-6-phosphate and glyceraldehyde-3-phosphate can be further catabolized through glycolysis and citric acid cycle.

**Significance of Pentose Phosphate Pathway**

- The pentoses (ribose-5-phosphate) required for the biosynthesis of nucleotide and nucleic acids (RNA and DNA) are provided by pentose phosphate pathway.
- It provides a route for the interconversion of pentoses and hexoses.
- It generates NADPH which plays important role in several other biological processes, as given below.
  - NADPH is required for the biosynthesis of fatty acids, cholesterol, steroid hormones and neurotransmitters.
  - It is required for oxidation-reduction reactions involved in detoxification, e.g. for detoxification of drugs by microsomal **cytochrome $P_{450}$ monooxygenase** and for reduction of oxidized glutathione.
  - In RBC, NADPH is required to maintain the level of **reduced glutathione.** The reduced glutathione protects the RBC membrane from toxic effect of $H_2O_2$ by reducing $H_2O_2$ to $H_2O$ **(Fig. 12.18).**
  - NADPH also keeps iron of hemoglobin in reduced ferrous ($Fe^{2+}$) state and prevents the formation of **methemoglobin.**
  - NADPH is necessary for phagocytosis carried out by white blood cell.

## Regulation of Pentose Phosphate Pathway

- The first step in the pathway, catalyzed by ***glucose-6-phosphate dehydrogenase (G-6-PD)*** is the rate limiting step.
- The activity of this enzyme is regulated by cellular concentration of NADPH. **NADPH is a competitive inhibitor of the enzyme G-6-PD.**
- An increased concentration of NADPH decreases the activity of G-6-PD, for example:
  - Under well-fed condition, the level of NADPH decreases and pentose phosphate pathway is stimulated.
  - However, in starvation and diabetes, the level of NADPH is high and inhibits the pathway.
- **Insulin** is also involved in the regulation of pentose phosphate pathway. It enhances the pathway by inducing the enzyme G-6-PD and 6-phosphogluconolactone dehydrogenase.

**Fig. 12.18:** Role of NADPH in disposal of $H_2O_2$.

## Disorders of Pentose Phosphate Pathway

### *Deficiency of Glucose-6-phosphate dehydrogenase (G-6-PD)*

Glucose 6-phosphate dehydrogenase deficiency is **X-linked** inherited disorder, characterized by **hemolytic anemia,** due to excessive hemolysis.

- This enzyme catalyzes the first step in the pentose phosphate pathway and is needed for the formation of NADPH.
- The NADPH is required for the detoxification of $H_2O_2$ in red blood cell **(Fig. 12.18).** In deficiency of G-6-PD, the production of NADPH is inadequate both to restore the reduced glutathione level and to maintain the RBC cell membrane. The consequence is destruction of the red blood cells and severe hemolytic anemia.
- Most of the patients of G-6-PD deficiency are asymptomatic and do not show hemolytic anemia under normal condition. However, they develop severe hemolytic anemia when they are exposed to certain antibiotic, antimalarial (primaquine) or antipyretic drugs.

**G-6-PD deficiency and resistance to malaria**

The malarial parasite, Plasmodium falciparum infects the red blood cell, where it depends on the reduced glutathione and the products of the pentose phosphate pathway for its optimum growth. Therefore, persons with G-6-PD deficiency cannot support growth of this parasite and thus are less prone to malaria than the normal person.

### *Wernicke-Korsakoff syndrome*

- This is a genetic disorder due to reduced activity of the **TPP-dependent transketolase** enzyme. The reduced activity of transketolase is due to reduced affinity for TPP, whereas the other TPP dependent enzymes are normal. Therefore, in the chronic thiamine deficiency the transketolase enzyme has a much reduced activity leading to the **Wernicke-Korsakoff syndrome.**
- The symptoms of Wernicke-Korsakoff syndrome include weakness, mental disorder, loss of memory, partial paralysis, etc.

# URONIC ACID PATHWAY (GLUCURONIC ACID CYCLE)

## Definition

A pathway in liver for the conversion of glucose to glucuronic acid, ascorbic acid (except in humans and other primates as well as in guinea pigs) and pentoses is referred to as the uronic acid pathway. It is also an alternative oxidative pathway for glucose but does not generate ATP.

## Reactions of Uronic Acid Pathway (Fig. 12.18)

1. Glucose-6-phosphate is converted to glucose-1-phosphate catalyzed by phosphoglucomutase.
2. Glucose-1-phosphate then reacts with uridine triphosphate (UTP) to form uridine diphosphate glucose (UDP-GLc). This reaction is catalyzed by the enzyme ***UDP-glucose pyrophosphorylase.***
3. UDP-Glucose is oxidized to glucuronate via UDP-glucuronate, catalyzed by an NAD dependent ***UDP-Glucose dehydrogenase.***
4. Glucuronate is reduced to L-gulonate by the NADPH dependent enzyme ***gulonate dehydrogenase.***
5. L-gulonate is the precursor of ascorbate (vitamin C) in those animals capable of synthesizing this vitamin. In humans and other primates, as well as in guinea pigs, ascorbic acid cannot be synthesized because of the **absence of the enzyme L-gulonolactone oxidase.**
6. L-gulonate is oxidized and decarboxylated to the pentose L-xylulose by the enzyme ***L-gulonate decarboxylase.***
7. L-xylulose is reduced to xylitol catalyzed by NADPH dependent ***L-xylulose dehydrogenase.***
8. Xylitol is oxidized to D-xylulose (isomer of L-xylulose) by an NAD dependent ***D-xylulose dehydrogenase enzyme.***
9. D-xylulose, in turn, is phosphorylated by ATP in the presence of ***xylulose kinase*** to yield xylulose-5-phosphate, which is further metabolized in pentose phosphate pathway and leads to formation of glucose.

**Significance of Uronic Acid Pathway**

- The uronic acid pathway is a source of UDP-glucuronate.
- UDP-glucuronate is a precursor in biosynthesis of proteoglycans (glycosaminoglycans) and glycoproteins.
- UDP-glucuronate is involved in detoxification reactions that occur in liver. Many naturally occurring waste substances (like bilirubin and steroid hormones) as well as many drugs (like morphine, methanol, salicylic acid, etc.) are eliminated from the body by conjugating with UDP-glucuronate **(Fig. 12.20).**
- The uronic acid pathway is a source of UDP-glucose, which is used for glycogen formation.
- The uronic acid pathway provides a mechanism by which dietary D-xylulose can enter the central metabolic pathway.

**Fig. 12.19:** Uronic acid pathway.

**Fig. 12.20:** Metabolic significance of UDP-glucuronate.

## *Disorder of Glucuronic Acid Pathway*

### *Essential pentosuria*

It is a benign (harmless) inborn error of metabolism in which the sugar L-xylulose is excreted in the urine in excess due to defect in $NADP^+$ linked ***L-xylulose dehydrogenase***, one of the enzymes in the glucuronic acid pathway **(Fig. 12.19)**. L-xylulose dehydrogenase is necessary to accomplish reduction of L-xylulose to xylitol.

# GALACTOSE METABOLISM AND GALACTOSEMIA

Galactose is derived from disaccharide, lactose (the milk sugar) of the diet. It is important for the formation of:

- Glycolipids
- Glycoproteins
- Proteoglycans
- Lactose during lactation

Galactose is readily converted in the liver to glucose. ***The ability of the liver to convert galactose to glucose is used as a liver function test (galactose tolerance test).***

### Reactions of the Pathway (Fig. 12.21)

1. The first reaction in galactose metabolism in the liver is phosphorylation of galactose to galactose-1-phosphate, by the enzyme galactokinase, using ATP as phosphate donor.
2. Galactose-1-phosphate reacts with UDP-glucose to form UDP-galactose and glucose-1-phosphate, catalyzed by ***galactose-1-phosphate uridyl transferase***. In this reaction, galactose displaces glucose of UDP-glucose.
3. The conversion of UDP-galactose to UDP-glucose is catalyzed by an ***UDP-galactose-4-epimerase.***
4. Finally, glucose is liberated from UDP-glucose via formation of glycogen by glycogenesis followed by glycogenolysis.

### Disorder of Galactose Metabolism

#### Galactosemia

- It is an inborn error of galactose metabolism, caused by deficiency of enzyme **galactose-1-phosphate uridyl transferase (Fig. 12.21).** The inherited deficiencies of **galactokinase** and **UDP-galactose-4-epimerase** also lead to minor types of galactosemia.
- They all interfere with the normal metabolism of galactose, causing a rise in blood and urine galactose.
- The commonest and most severe enzymatic defect is due to ***galactose-1-phosphate uridyl transferase*** deficiency which prevents conversion of galactose to glucose and leads to accumulation of galactose and galactose-1-phosphate in blood, liver, brain, kidney and eye lenses. In these organs, the galactose is reduced to galactitol (dulcitol) by the enzyme **aldose reductase.**

**Clinical findings:** The accumulation of galactitol and galactose-1-phosphate in liver, brain and eye lenses causes liver failure (hepatomegaly followed by cirrhosis), mental retardation and cataract formation respectively.

**Treatment:** Galactose in milk and milk products should be eliminated from the diet. Required galactose for the body can be synthesized endogenously as UDP-galactose.

## METABOLISM OF FRUCTOSE

Liver is the main site of fructose metabolism **(Fig. 12.22)**.

### Reactions of Fructose Metabolism

- Fructose is phosphorylated to fructose-1-phosphate by ***fructokinase*** in the liver.
- Fructose-1-phosphate is cleaved by liver ***aldolase (aldolase-B)*** to dihydroxyacetone phosphate (DHAP) and D-glyceraldehyde.

**Fig. 12.21:** Pathway for conversion of galactose to glucose in the liver.

**Fig. 12.22:** Metabolic pathway of fructose.

- D-glyceraldehyde is phosphorylated by glyceraldehyde kinase (or triokinase) to D-glyceraldehyde-3-phosphate. These two triose phosphates (DHAP and glyceraldhyde-3-phosphate) then may enter the glycolytic pathway, gluconeogenesis or glycogenesis according to the metabolic status of the tissue.

## *Sorbitol or Polyol Pathway for Formation of Fructose*

The sorbitol (polyol) pathway is for formation of fructose from glucose **(Fig. 12.23).**

- ***Aldose reductase*** reduces glucose to sorbitol (glucitol).
- In the liver, ovaries and sperm cells, there is a second enzyme, ***sorbitol dehydrogenase*** that can oxidize the sorbitol to fructose.
- The pathway from sorbitol to fructose in the liver provides a mechanism by which dietary sorbitol is converted into fructose.

### *The Effect of Hyperglycemia on Sorbitol Metabolism*

Because insulin is not required for entry of glucose into the cells such as retina, lens and nerve, large amounts of glucose may enter these cells during hyperglycemia, e.g. in uncontrolled diabetes.

- Elevated intracellular glucose concentrations cause increase in the amount of sorbitol and therefore accumulates inside the cell.
- This causes osmotic damage, leading to cataract formation, peripheral neuropathy, retinopathy and nephropathy.

Drugs inhibiting aldose reductase improve the peripheral nerve function in diabetes.

**Fig. 12.23:** Sorbitol or polyol pathway.

### Disorders of Fructose Metabolism

#### Essential fructosuria

- Essential fructosuria is a rare and benign genetic disorders caused by a deficiency of the enzyme ***fructokinase***.
- In this disorder, fructose cannot be converted to fructose-1-phosphate.
- This is benign because no toxic metabolites of fructose accumulate in the liver and the patient remains nearly asymptomatic with excretion of fructose in urine.

#### Hereditary fructose intolerance

- It is due to deficiency of the enzyme ***Aldolase-B***.
- Fructose-1-phosphate cannot be converted to dihydroxyacetone phosphate and glyceraldehyde and therefore fructose-1-phosphate accumulates.
- This results in the inhibition of ***fructokinase*** and an impaired clearance of fructose from the blood.

### Clinical Findings

- Accumulation of fructose-1-phosphate leads to liver and kidney damage.
- Hypoglycemia due to inhibition of glycogenolysis and gluconeogenesis.

### Treatment

Elimination of fructose containing food from the diet.

## BLOOD GLUCOSE LEVEL AND ITS REGULATION

The blood glucose level must be maintained within the narrow limits of ***70–100 mg/dl***. Levels above the normal range are termed ***hyperglycemia***, those below are called ***hypoglycemia***. After the ingestion of a carbohydrate meal, it may rise to 120–140 mg/dl.

Factors involved in the ***homeostasis (regulation)*** of blood glucose are:

- Hormones
- Metabolic processes
- Renal mechanism

The liver is the organ primarily responsible for controlling the concentration of glucose in the blood. It can rapidly take up and release glucose in response to the concentration of blood glucose. The uptake and release of glucose by liver is regulated by hormones. The two major hormones controlling blood glucose levels are **(Fig. 12.24)**:

1. Insulin (hypoglycemic hormone)
2. Glucagon (hyperglycemic hormone)

### Maintenance of Glucose in Fed State (Hyperglycemic condition)

Normally, there is an increased blood glucose level shortly after each meal, a ***postprandial hyperglycemia***. Increased level of blood glucose releases ***insulin*** by β-cells of the Islets of the Langerhans. This hormone reduces the blood glucose level in a number of ways **(Fig. 12.25)** as follows:

1. By stimulating the active transport of glucose across cell membranes of muscle and adipose tissue but not

**Fig. 12.24:** Regulation of blood glucose level by insulin and glucagon.

**Fig. 12.25:** Role of insulin in regulation of blood glucose level.

the liver. ***Glucose is rapidly taken up into liver as it is freely permeable to glucose***

2. In the liver, insulin increases the use of glucose by glycolysis by inducing the synthesis of key glycolytic enzymes:
   - Glucokinase
   - Phosphofructokinase
   - Pyruvate kinase
3. In the muscle and the liver, insulin stimulates ***glycogenesis*** by stimulating ***glycogen synthase*** and thereby leading to suppression of glycogenolysis.
4. Insulin inhibits ***gluconeogenesis*** by suppressing the action of key enzymes of gluconeogenesis, e.g.
   - Pyruvate carboxylase
   - Phosphoenol pyruvate carboxykinase
   - Fructose 1,6-bisphosphatase
   - Glucose-6-phosphatase
5. In adipose tissue, glucose is converted to the glycerol-3-phosphate, needed for the formation of triacylglycerol (lipogenesis).
6. Insulin increases protein synthesis and decreases protein catabolism, thereby releasing amino acids. All these mechanisms are responsible for a drop in blood glucose level (hypoglycemia).

## *Maintenance of Blood Glucose in Fasting State (Hypoglycemic Condition)*

Decreased level of blood glucose (hypoglycemia) causes a release of hyperglycemic hormones, e.g.

- Glucagon
- Epinephrine or adrenaline
- Glucocorticoids
- Growth hormone and adrenocorticotropic hormone (ACTH)
- Thyroxine

### *Glucagon*

Glucagon is the hormone produced by the α-cells of the islets of Langerhans of the pancreas. Glucagon opposes the actions of insulin. It acts primarily in the liver as follows:

- In the liver, it stimulates glycogenolysis by activating enzyme ***glycogen phosphorylase*** and inhibits glycogen synthesis. Unlike epinephrine, glucagon does not have an effect on muscle phosphorylase due to lack of receptors.
- Glucagon enhances gluconeogenesis from amino acids and lactate by inducing the action of key enzymes of gluconeogenesis.
  - Alanine is the predominant amino acid released from muscle to liver by **glucose alanine cycle (see Fig. 12.11).**
  - Lactate formed by oxidation of glucose in skeletal muscle is transported to the liver by **lactic acid (Cori) cycle (see Fig. 12.11).**

### *Epinephrine or Adrenaline*

- It is secreted by adrenal medulla.
- It stimulates **glycogenolysis** in the liver and the muscle by stimulating **glycogen phosphorylase** activity via c-AMP.
- In muscle as a result of the **absence of glucose-6-phosphatase**, glycogenolysis results with the formation of lactate, whereas in the liver, glucose is the main product, leading to increase in blood glucose.

### *Glucocorticoids*

- These hormones are secreted by adrenal cortex, which causes increased:
  - Gluconeogenesis by increasing the activity of enzymes of gluconeogenesis.
  - Protein catabolism to provide glucogenic amino acid for gluconeogenesis.
  - Hepatic uptake of amino acids for gluconeogenesis.
- In addition, glucocorticoids inhibit the utilization of glucose in extrahepatic tissues. Thus, all the actions of glucocorticoids are antagonistic to insulin.
- Growth hormone and anterior pituitary hormones
- Growth hormone and ACTH antagonise the action of insulin by elevating the blood glucose level.
- Growth hormone decreases glucose uptake in the muscle and ACTH decreases glucose utilization by the tissue.

### *Thyroxine*

- It is secreted by thyroid gland.
- Thyroxine accelerates hepatic glycogenolysis with consequent rise in blood glucose.
- It may also increase the rate of absorption of hexoses from the intestine.

## Renal Control Mechanism

- When blood glucose rises to relatively high levels, the kidney also exerts a regulatory effect. Glucose is continuously filtered by the glomeruli but is normally reabsorbed completely in renal tubules. The capacity of the tubular system to reabsorb glucose is limited.
- If the blood glucose level is raised above ***180 mg/100 ml***, complete tubular reabsorption of glucose does not

occur and the extra amount appears in the urine causing ***glycosuria***.

- 180 mg/100 ml is the limiting level of glucose in the blood, above which tubular reabsorption does not occur which is known as ***renal threshold value for glucose***.
- Thus, by excreting extra amount of sugar in the urine during hyperglycemic state and reabsorbing sugar during the hypoglycemic state, the kidney helps in regulating the level of glucose in blood.

## GLYCOSURIA

Normally, the urine contains about 0.05 gm% of sugar. Such a small quantity cannot be detected by Benedict's test, but under certain circumstances, a considerable amount of glucose or other sugar may be excreted in the urine.

- Excretion of detectable amount of sugar in urine is known as ***glycosuria***.
- Glycosuria results from the rise of blood glucose above its renal threshold level (180 mg%)
- Glycosuria may be due to various reasons on the basis of which it is classified into the following groups:
  1. Alimentary (lag storage) glycosuria
  2. Renal glycosuria
  3. Diabetic glycosuria

### *Alimentary (Lag Storage) Glycosuria*

- The blood sugar level of some individuals after meal rises rapidly above the normal renal threshold (180 mg/dL) and results in glycosuria and known as **alimentary glycosuria.** This is due to an increased rate of absorption of glucose from the intestine. This is called alimentary glycosuria since alimentary canal (GI-tract) is involved.
- Characteristic feature of this glycosuria is that usually high blood glucose level returns to normal at 2 hours after a meal. This type of glycosuria is benign (harmless).

### *Renal Glycosuria*

- This is observed due to impaired tubular reabsorption of glucose and have lowered renal threshold (may be 130–150 mg %) for glucose.
- In such cases, the blood glucose level is below
- 80 mg%, i.e. below normal renal threshold for glucose, but glucose appears in the urine due to lowered renal threshold.
- Renal glycosuria is a benign condition, unrelated to diabetes and it may occur temporarily in pregnancy without symptoms of diabetes.
- Renal glycosuria may result from inherited defects in the kidney or it may be acquired as a result of kidney disease.

### *Diabetic Glycosuria*

- Diabetic glycosuria is a pathological condition and is due to deficiency or lack of insulin which causes diabetes mellitus.
- Although the renal threshold is normal, as blood glucose level exceeds the renal threshold, the excess glucose passes into the urine to produce glycosuria.

## DIABETES MELLITUS

### *Definition*

Diabetes mellitus is a syndrome of impaired carbohydrate, fat and protein metabolism, caused by either:

- Lack of insulin secretion or
- Decreased sensitivity of the tissues to insulin.

### *Classification of Diabetes Mellitus*

Diabetes mellitus is broadly divided into two groups namely:

1. Type I diabetes mellitus or insulin dependent diabetes mellitus (IDDM).
2. Type II diabetes mellitus or non-insulin dependent diabetes mellitus (NIDDM).

#### *Type I diabetes mellitus*

Type l diabetes is also called **insulin dependent diabetes mellitus (IDDM)** or juvenile onset diabetes.

***Cause***

It is caused by lack of insulin secretion due to destruction of pancreatic beta cells. The destructions of β-cells may be due to:

1. Viral infection
2. Autoimmune disorder (destruction of tissues by body's own antibodies)
3. There may be hereditary tendency for β-cell degeneration.

***Onset***

The usual onset of type-l diabetes occurs at about 14 years of age and for this reason it is called **juvenile** diabetes mellitus ('juvenile' means teenage in Latin).

***Symptoms***

- It develops symptoms very abruptly with:
  - Polyuria (frequent urination)
  - Polydypsia (excessive thirst)
  - Polyphagia (excessive hunger)

- These symptoms are accompanied by loss of body weight, weakness, and tiredness.
- Hyperglycemia with **glycosuria** and **ketoacidosis** are the metabolic changes.
- The patients of type-I diabetes mellitus are not obese.

***Treatment***

- Since patients of IDDM (type-I) fail to secrete insulin, administration of exogenous insulin is required.

### *Type II diabetes mellitus*

Type II is a non-insulin dependent diabetes mellitus (NIDDM) or adult onset diabetes mellitus.

***Cause***

It is caused by decreased sensitivity of target tissues to insulin. This reduced sensitivity of insulin is often referred to as ***insulin resistance***. This is perhaps due to ***inadequate insulin receptors*** on the cell surfaces of the target tissues. This syndrome is often found in an obese person.

***Onset***

Onset of the type II diabetes occurs after age 40 and the disorder develops gradually. Therefore, this syndrome is referred to as adult onset diabetes.

***Symptoms***

In type II diabetes mellitus, the symptoms are developed gradually which are similar to that of type-I **except ketoacedosis** is usually not present in type II diabetes mellitus.

***Treatment***

- NIDDM (type-II) can be treated in early stages by diet control, exercise and weight reduction and no exogenous insulin administration is required.
- Drugs that increase insulin sensitivity such as ***thiazolidinediones*** and ***metformin*** or drugs that cause additional release of insulin by the pancreas such as sulfonylureas may also be used.
- However, in the later stages insulin administration is often required to control blood glucose.

The comparison between two types of diabetes mellitus is given in **Table 12.6.**

## *Metabolic Changes in Diabetes Mellitus*

- In diabetes mellitus, the metabolic changes occur due to a deficiency of **insulin** and relative excess of **glucagon.** These changes in hormonal levels most profoundly affect metabolism in three tissues; liver, muscle and adipose tissue.
- Elevated levels of **blood glucose** and **ketone bodies** are the characteristic feature of untreated diabetes mellitus.
- The lack of insulin activity in diabetes mellitus results in failure of transfer of glucose from the blood into cells and leads to **hyperglycemia.** The body responds as it were in the fasting state (**see chapter 15**) with stimulation of:
  - Glycogenolysis
  - Gluconeogenesis
  - Lipolysis
  - Proteolysis
- An increase in glucagon favors lipolysis in adipose tissue. Increased lipolysis leads to increased mobilization of fatty acids from adipose tissue to liver, where they are converted to **ketone bodies** (β-hydroxybutyrate and acetoacetate), causing the **ketoacidosis.**

**Table 12.6:** Comparison of two types of diabetes mellitus.

| *Features* | *Type-I: Insulin dependent diabetes mellitus* | *Type-II: Non-insulin dependent diabetes mellitus* |
|---|---|---|
| Frequency | 5–10% | 90–95% |
| Age of onset | Early during childhood or puberty usually < 20 years | Later after age of 40 years |
| Onset of symptoms | Abrupt and severe | Gradual, insidious |
| Plasma insulin | Low or absent | Normal to high |
| Body weight | Low to normal | Obese |
| Blood glucose | Increased | Increased |
| Insulin sensitivity | Normal | Reduced |
| Ketosis | Common | Rare |
| Acute complications | Ketoacidosis | Hyperosmolar coma |
| Treatment with insulin | Necessary | Usually not required |

- Due to lack of insulin, the synthesis of the enzyme **lipoprotein lipase,** required for the degradation of VLDL is decreased. Decreased synthesis of lipoprotein lipase leads to elevated levels of plasma VLDL, resulting in **hypertriglycerolemia.**
- The other metabolic changes that occur in diabetes mellitus is increased mobilization of amino acids due to increased rate of proteolysis. The amino acids released from muscle are converted to glucose by gluconeogenesis.

## GLUCOSE TOLERANCE TEST (GTT)

Glucose tolerance test (GTT) is performed to assess the ability of the body to utilize glucose. GTT can be performed by two ways:

1. Oral GTT
2. Intravenous GTT

### Oral Glucose Tolerance Test

- The patient who is scheduled for oral GTT is instructed to eat a high carbohydrate diet for at least 3 days prior to the test, and come after an overnight fast on the day of the test.
- A fasting blood glucose sample is first drawn.
- Then 75 gm of glucose (or 1.75 gm per kg body weight) dissolved in 300 ml of water is given orally.
- After giving glucose, blood and urine specimens are collected at half hourly intervals for at least 2 hours.
- Blood glucose content is measured and urine is tested for glycosuria.
- A curve is plotted for time against blood glucose concentration and is called **glucose tolerance curve (Fig. 12.26).**

**Fig. 12.26:** The glucose tolerance curves.

### Intravenous Glucose Tolerance Test

- The intravenous glucose tolerance test is often used for persons with malabsorptive disorders or previous gastric or intestinal surgery.
- Glucose is administered intravenously over 30 minutes using 20% glucose solution. A glucose load of 0.5 gm/kg of body weight is used.

### *Types of Glucose Tolerance Curves*

There are three types of Glucose Tolerance Curves:

1. Normal glucose tolerance curve
2. Decreased glucose tolerance
3. Increased glucose tolerance.

#### *Normal glucose tolerance curve*

Normal response to glucose load is as follows:

- Initial fasting glucose is within the normal fasting limits (70 to 100 mg%).
- Blood glucose level rises to a peak (120 to 140 mg%) at half to 1 hour after ingestion of glucose.
- The blood glucose level then returns rapidly to the fasting normal limits in about 2 hours.
- Glucose should not be present in any of the urine specimens collected for 2 hours.

#### *Decreased glucose tolerance*

Decreased glucose tolerance means decreased ability of the body to utilize glucose. In decreased glucose tolerance:

- Fasting glucose is higher than normal limits.
- The blood glucose level rises above 180 mg/100 ml (renal threshold) after ingestion of glucose.
- The blood glucose remains high for a longer time and may not return to fasting level even after 3 hours.
- The urine samples corresponding to blood glucose level over 180 mg/100 ml may show urine Benedict's test positive (glycosuria).
- Decreased glucose tolerance occurs in **diabetes mellitus** and certain endocrine disorders like:
  - Hyperthyroidism
  - Hyperpituitarism
  - Hyperadrenalism (Cushing's syndrome)

#### *Increased glucose tolerance*

Increased glucose tolerance means increased ability of the body to utilize glucose. In increased glucose tolerance:

- Fasting blood glucose is lower than normal.

- Only small rise in blood glucose level may be observed (not more than 100 mg %) even after glucose administration.
- A flatter type of curve is obtained.
- No appearance of glucose in urine.
- This type of curve is obtained in endocrine hypoactivity like:
  - Hypothyroidism (myxoedema, cretinism)
  - Hypoadrenalism (Addison's disease)
  - Hypopituitarism

***Significance of GTT***

GTT is not necessary in symptomatic or in known cases of diabetic patients. In such cases, determination of fasting or postprandial glucose is usually sufficient for the diagnosis.

- GTT is most important in the investigation of asymptomatic hyperglycemia or glycosuria such as renal glycosuria and alimentary glycosuria.
- This test may give useful information in some cases of endocrine dysfunctions.
- It is also helpful in recognizing milder cases of diabetes.

## EXAM QUESTIONS

### Long Answer Questions (LAQs)

1. What is gluconeogenesis? Describe the reactions of gluconeogenesis. State the fate of the precursors of this pathway. How is gluconeogenesis regulated?
2. Explain glycogenolysis and its regulation.
3. Describe hexose monophosphate shunt and its biological significance.
4. What is normal blood sugar level? Why does it need to be regulated? Describe the various mechanisms of its regulation.
5. Discuss the synthesis of glucose from non-carbohydrate sources.
6. Describe the TCA cycle, its energetics and regulation.
7. Describe fructose metabolism and polyol pathway.
8. Describe glycolysis with its regulation and its biological significance.

### Short Notes

1. Metabolic changes in diabetes mellitus.
2. Glycogen storage diseases.
3. Glycosuria.
4. Galactosemia.
5. Digestion and absorption of carbohydrates.
6. Glucose tolerance test.
7. Role of TCA in transamination reactions.
8. Disorders of fructose metabolism.
9. Rapoport-Luebering cycle.
10. Regulation of citric acid cycle.
11. Regulation of gluconeogenesis.
12. Regulation of glycogen metabolism.
13. Explain high content of fructose leads to atherosclerosis.
14. Role of liver in regulation of blood sugar.
15. Explain role of TCA cycle as an amphibolic pathway.
16. Give significance of uronic acid pathway.
17. Lactic acid or Cori cycle.
18. Significance of sorbitol or polyol pathway.
19. Significance of glycolysis.
20. Fate of pyruvate and its significance.

### Solve the Following

**Case History 1**

**A 12-year-male had complained of abdominal discomfort, a feeling of being bloated, increased passage of urine and development of diarrhea after taking milk.**

**Questions**

a. Name the probable disorder.
b. Cause of disorder.
c. What will you suggest the patient to relieve the symptoms?

**Case History 2**

**The following are the findings in a patient brought to the hospital in a coma state.**

| *Findings* | *Patient* | *Normal* |
|---|---|---|
| **Blood sugar (Fasting)** | **270 mg%** | **70 to 100 mg%** |
| Urine Benedict's test | Positive | Negative |
| Urine Rothera's test | Positive | Negative |
| Plasma pH | 7.20 | 7.35 to 7.45 |

**Questions**

a. Name of the disorder.
b. Why is patient's plasma pH lower than normal?
c. What does positive Rothera's test indicate?
d. What is the renal threshold value for glucose?

**Case History 3**

**A 20-year-old male suffering from malaria was treated with chloroquine and manifested as hemolytic anemia. Provisional diagnosis of glucose-6-phosphate dehydrogenase (G-6-PD) deficiency was made.**

**Questions**

a. Which reaction is catalyzed by the enzyme G-6-PD?
b. How does deficiency of G-6-PD produce hemolytic anemia?
c. Name the pathway in which this reaction occurs.
d. Write reaction catalyzed by glucose-6-phosphate dehydrogenase

**Case History 4**

**A chronically cranky, irritable and lethargic baby girl has an extended abdomen, resulting from an enlarged liver and was diagnosed of having Von Gierke's disease.**

**Questions**

a. Which enzyme is deficient in Von Gierke's disease?
b. Name the pathways where the enzyme is required.
c. Give manifestations of the disorder.
d. Name the other disorders of the concern pathway.

**Case History 5**

**A 6-month-old infant was presented with elevated blood and urine galactose.**

**Questions**

a. Name the disease.
b. Give the biochemical steps related to the disease and point out the metabolic defect.
c. What is the clinical manifestation of the disease?

**Case History 6**

**An obese person came to the hospital with complaints of polyuria, thirst, weakness and increased appetite. On investigations, he was diagnosed having diabetes mellitus.**

**Questions**

a. What is the cause of diabetes mellitus?
b. Give names of different types of diabetes mellitus.
c. What is glycosuria? Name different types of glycosuria.
d. What is the normal blood sugar level?

**Case History 7**

**A 28-year-old man has complained of chronic leg muscle pains and cramps during exercise. This patient suffers from McArdle syndrome.**

**Questions**

a. What is McArdle syndrome? To metabolism of which biomolecule is it related?
b. What is the cause of this syndrome?
c. Name different types of disorders related to the concerned biomolecule.

**Case History 8**

**A 13-year-old diabetic boy visits a diabetic clinic for a check-up. He tells the doctor that he complies with all the dietary advice and never misses insulin. His random blood glucose level is within normal limit but his $HbA_1c$ concentration is 10% (normal 4–6%). He has no glycosuria or ketone bodies in his urine.**

**Questions**

a. What does normal blood glucose and urine glucose indicate?
b. What does elevated level of $HbA_1c$ suggest?
c. Name the type of diabetes the boy is suffering.
d. What is $HbA_1c$?

**Case History 9**

**A 3-year-old patient with mild mental retardation was found to have cataract. Biochemical investigations show high blood concentrations of a sugar alcohol and galactose.**

Questions

a. Name the probable disease.
b. Name enzyme most likely to be defective.
c. What is the cause of development of cataracts?
d. What is the treatment?

**Case History 10**

**A fasting glucose and glycosylated hemoglobin were performed for three consecutive quarters on a patient. The results are as follows:**

| *Quarter* | *Plasma glucose fasting mg/dL* | *Glycosylated hemoglobin %* |
|---|---|---|
| 1 | 280 | 7.8 |
| 2 | 85 | 15.3 |
| 3 | 91 | 8.5 |

**Questions**

a. In which quarter was the patient's glucose best controlled?
b. What is the normal fasting blood glucose?
c. What is the normal level of glycosylated Hb?
d. What is glycosylated hemoglobin?

**Case History 11**

**A 13-year-old girl was admitted to hospital. Her mother mentioned that her daughter had been losing weight and had polyuria. Doctor noticed a fruity breath. On admission following biochemical parameters of urine and blood were obtained.**

- Urine pH 5.5
- Urine glucose $4^+$
- Urine ketones moderate
- Blood glucose 480 mg/dL
- Blood ketones positive.

**Questions**

a. Identify this patient's type of diabetes.
b. What is the cause of fruity breath?
c. What is the cause of weight loss?
d. Name ketone bodies.

**Case History 12**

**A 22-year-old patient with diabetic comes to the emergency department. She gives a 3 days history of vomiting and abdominal pain. She is drowsy and her breathing is deep and rapid. There is fruity smell from her breath.**

**Questions**

a. What is the most likely diagnosis?
b. Which bedside tests could you do to confirm this diagnosis?
c. Which laboratory tests would you request?

## Multiple Choice Questions (MCQs)

1. **Which of the following enzymes produce a product used for synthesis of ATP by substrate level?**
   a. Phosphofructokinase
   b. Aldolase
   c. 1,3-bisphosphate mutase
   d. Enloase
2. **2,3-bisphosphoglycerate is:**
   a. A high energy substrate
   b. Involved in substrate level phosphorylation
   c. An intermediate in pentose phosphate pathway
   d. An allosteric effector that decreases the $O_2$ affinity of Hb
3. **Muscle glycogen is not available for maintenance of blood glucose concentration because:**
   a. Muscle lacks glucose-6-phosphatase activity
   b. There is insufficient glycogen in muscle
   c. Muscle lacks glucose transporter GLUT-4
   d. Muscle lacks glucagon receptors
4. **The primary metabolic fate of lactate released from muscle during intense exercise is:**
   a. Excretion of lactate in urine
   b. Transported to liver for replenishment of blood glucose by *gluconeogenesis*
   c. Conversion to pyruvate
   d. Gradual reuptake in muscle during the recovery phase following exercise
5. **Which of the following statement is true of TCA cycle?**
   a. It requires coenzyme biotin, FAD, NAD and coenzyme A
   b. Two NADH are produced per turn
   c. It participates in the synthesis of glucose from pyruvic acid
   d. Enzymes are located in cytosol
6. **Which of the following vitamins does not participate in the oxidative decarboxylation of pyruvate to acetyl-CoA?**
   a. Thiamine b. Biotin
   c. Niacin d. Riboflavin
7. **The following enzymes catalyze a decarboxylation reaction, *except*:**
   a. α-ketoglutarate dehydrogenase
   b. Pyruvate dehydrogenase
   c. Pyruvate carboxylase
   d. Isocitrate dehydrogenase
8. **The principal source of glucose after an overnight fast is:**
   a. Gluconeogenesis b. Glycolysis
   c. Glycogenolysis d. HMP pathway
9. **Which of the following cannot take place in the human body?**
   a. Transformation of lactate into glucose
   b. Transformation of glycerol into glucose
   c. Transformation of propionyl-CoA into glucose
   d. Transformation of acetate into glucose

**10. In a patient with galactosemia who is on a galactose free diet, the D-galactose that is required for cell membrane and other biopolymers is formed by:**
a. Isomerization of glucose-1-phosphate
b. Epimerization of UDP-glucose
c. Epimerization of D-fructose
d. Epimerization of glucose-6-phosphate

**11. Gluconeogenesis, must bypass irreversible reactions of glycolysis, *except*:**
a. Hexokinase
b. Phosphohexose isomerase
c. Pyruvate kinase
d. Phosphofructokinase

**12. In skeletal muscle, glycogen synthesis occurs during:**
a. Contraction
b. Relaxation
c. Well-fed state
d. Increased level of insulin

**13. The following hormones have hyperglycemic effect, *except*:**
a. Glucagon b. Thyroid
c. Epinephrine d. Insulin

**14. Rate controlling steps in the citric acid cycle include all of the following, *except*:**
a. Isocitrate dehydrogenase
b. Citrate synthase
c. Fumarase
d. α-ketoglutarate dehydrogenase

**15. Gluconeogenesis can proceed from all of the following, *except*:**
a. Pyruvate b. Palmitic acid
c. Propionyl-CoA d. Oxaloacetate

**16. The following are the functions of pentose phosphate pathway, *except*:**
a. Interconverts hexoses and pentoses
b. Produces NADPH
c. Supplies ribose-5-phosphate
d. Converts glucose to galactose

**17. People with diabetes mellitus are prone to develop cataracts because their elevated blood glucose concentration:**
a. Inhibit gluconeogenesis
b. Increase glycosylate hemoglobin
c. Increase glycogen synthesis within the lens
d. Allow aldose reductase to reduce glucose to sorbitol

**18. Both glycolysis and gluconeogenesis involve which of the following enzymes:**
a. Pyruvate carboxylase
b. Hexokinase
c. Aldolase
d. Phosphofructokinase

**19. Glucose-6-phosphate is involved in which of the following pathways:**
a. Glycolysis
b. Gluconeogenesis
c. Pentose phosphate pathway
d. All of the above

**20. Gluconeogenesis occurs in which of the following:**
a. Heart b. Erythrocytes
c. Liver d. Lungs

**21. Von Gierke's disease is characterized by deficiency of the enzyme:**
a. Glucokinase
b. Glucose-6-phosphatase
c. Phosphoglucomutase
d. Glycogen synthase

**22. McArdle syndrome involves a deficiency of which of the following enzymes:**
a. Hepatic phosphorylase
b. Muscle phosphorylase
c. Debranching enzyme
d. Hepatic glycogen synthase

**23. Deficiency of glucose-6-phosphate dehydrogenase causes:**
a. Cataract b. Hypoglycemia
c. Hemolytic anemia d. Galactosemia

**24. Essential pentosuria is due to metabolic defect in:**
a. Glycolysis
b. HMP-shunt
c. Uronic acid pathway
d. Glycogenolysis

**25. Rapoport-Luebering cycle in RBC produces:**
a. ATP
b. NADPH
c. 2,3-bisphosphoglycerate
d. 1,3-bisphosphoglycerate

**26. The first loss of carbon in the metabolism of glucose takes place as $CO_2$ in the formation of:**
a. Acetyl-CoA
b. Pyruvate
c. 2,3 BPG
d. Fructose 1,6-bisphos phate

**27. Anaerobic glycolysis from glycogen generates:**
a. 3 moles of ATP b. 2 moles of ATP
c. 8 moles of ATP d. 6 moles of ATP

**28. The net production of ATP when glycolysis occurs via Rapoport-Luebering route:**
a. Two b. Six
c. Eight d. Zero

**29. In erythrocytes, the end product of glycolysis is:**
a. Pyruvate b. Acetyl-CoA
c. Lactate d. 2,3-bisphosphoglycerate

**30. Which of the following step is not involved in substrate level phosphorylation?**
a. Dihydroxyacetone phosphate to glyceraldehyde-3-phosphate
b. 1,3-diphosphoglycerate to 3-phosphoglycerate
c. Succinyl CoA to succinate
d. Phosphoenol pyruvate to pyruvate

**31. How many ATP molecules are produced in the citric acid cycle itself?**
a. One b. Two
c. Ten d. Fifteen

**32. $CO_2$ is not produced in the reaction catalyzed by the enzyme.**
a. Pyruvate dehydrogenase
b. Succinate dehydrogenase
c. Isocitrate dehydrogenase
d. α-ketoglutarate dehydrogenase

**33. Which of the following statements is not true of HMP shunt pathway?**
a. $CO_2$ is not produced in it
b. NADPH is produced
c. Pentoses are produced
d. Does not produce ATP

**34. They utilize fructose but not glucose.**
a. Ovum b. Spermatozoa
c. Adipose tissue d. Mammary gland

**35. The uronic acid pathway is unique as it provides to man.**
a. Ascorbic acid b. Xylulose
c. Glucuronic acid d. All of these.

**36. The hormone does not stimulate hepatic glycogenolysis:**
a. Thyroxine b. Adrenaline
c. Glucagon d. Cortisol

**37. Suggest a test to distinguish a case of renal glycosuria from diabetic glycosuria:**
a. Bendict's test b. Blood sugar
c. Urine sugar d. GTT

**38. NADPH serves to regenerate in red cells to prevent their lysis:**
a. Cholesterol b. Glutathione
c. NADP d. Cysteine

**39. The G-6-PD deficiency causes hemolytic anemia due to lack of:**
a. NADPH b. NADP
c. Pentoses d. Cholesterol

**40. How many ATP molecules are produced on complete oxidation of acetyl CoA in the citric acid cycle?**
a. Six b. Nine
c. Ten d. Fifteen

**41. Which of the following enzyme is not involved in gluconeogenesis?**
a. Pyruvate carboxylase.
b. Phosphoenolpyruvate carboxykinase
c. Glucose 6-phosphatase
d. Hexokinase

**42. The most important initial source of blood glucose during fasting is:**
a. Muscle glycogen b. Muscle protein
c. Liver triglyceride d. Liver glycogen

**43. The major fate of glucose-6-phosphate in tissues in a well-fed state is:**
a. Hydrolysis of glucose
b. Conversion to glycogen
c. Isomerization to fructose-6-phosphate
d. Conversion to ribulose-5-phosphate

**44. The major fuel for the brain after prolonged starvation is:**
a. Glucose b. Fatty acids
c. Ketone bodies d. Glycerol

**45. The monosaccharide most rapidly absorbed from the intestine is:**
a. Glucose b. Fructose
c. Mannose d. Galactose

**46. Lactose intolerance is due to:**
a. ADH deficiency b. Deficiency of bile
c. Lactase deficiency d. Malabsorption syndrome

**47. In contrast to liver, muscle glycogen does not contribute directly to blood glucose level because:**
a. Muscles lack glucose-6-phosphatase
b. Muscles contain no glucokinase
c. Muscles lack glycogen
d. Muscles contain no glycogen phosphorylase

**48. Which of the following statement is true regarding the α-amylase?**
a. Breaks glucose from one end of the carbohydrate
b. Cleaves only α-1,4 linkages
c. Cleaves only α-1,6 linkages
d. All of the above

**49. When blood glucagon rises, which of the following hepatic enzyme activities fall?**
a. Protein kinase
b. Glycogen synthase
c. Glycogen phosphorylase
d. Adenylyl cyclase

**50. Glucose 6-phosphatse is necessary for the production of blood glucose from:**
a. Lactose
b. Amino acids
c. Liver glycogen
d. All of the above

**51. Insulin promotes hypoglycemia by a mechanism including all of the following, *except*:**
a. Repressing the synthesis of key gluconeogenic enzymes
b. Decreasing levels of C-AMP
c. Stimulating glycogen phosphorylase enzyme
d. Inducing the synthesis of key glycolytic enzymes

**52. The most severe form of galactosemia is due to:**
a. A genetic deficiency of a galactose-phosphate uridyl transferase
b. Deficiency of epimerase
c. Defect in galactokinase
d. None of the above

**53. UDP glucuronate is required for:**
a. Synthesis of proteoglycans and glycoproteins
b. Formation of Iduronate
c. Detoxification of bilirubin
d. All of the above

**54. Which of the following hormones promotes glycolysis?**
a. Growth hormone b. Glucagon
c. Insulin d. ACTH

**55. Hyperglycemic hormone secreted by pancreas:**
a. Insulin b. Glucagon
c. Epinephrine d. Growth hormone

**56. Cori's cycle involves the conversion of:**
a. Liver glucose to lactate
b. Pyruvate to lactate in muscle
c. Muscle lactate to glucose in the liver
d. Pyruvate to glucose in the liver

**57. Identity the amino acid that is the major contributor of hepatic gluconeogenesis:**
a. Alanine b. Glutamine
c. Leucine d. Lysine

**58. What is the preferred specimen for glucose analysis?**
a. EDTA plasma
b. Serum
c. Fluoride oxalate plasma
d. Heparinized plasma

**59. Glucose-6-phosphatase catalyzes the formation of:**
a. Glucose-6-phosphate
b. Glucose + Pi
c. Glucose-1-phosphate
d. Fructose-6-phosphate

**60. Enzyme hexokinase catalyzes the formation of:**
a. Glucose-6-phosphate
b. Glucose-1-phosphate
c. Acetyl CoA
d. None of the above

## Answers for MCQs

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **1.** d | **2.** d | **3.** a | **4.** b | **5.** c | **6.** b | **7.** c | **8.** a |
| **9.** d | **10.** b | **11.** b | **12.** b | **13.** d | **14.** c | **15.** b | **16.** d |
| **17.** d | **18.** c | **19.** d | **20.** c | **21.** b | **22.** b | **23.** c | **24.** c |
| **25.** c | **26.** a | **27.** a | **28.** d | **29.** c | **30.** a | **31.** c | **32.** b |
| **33.** a | **34.** b | **35.** c | **36.** b | **37.** d | **38.** b | **39.** a | **40.** c |
| **41.** d | **42.** d | **43.** b | **44.** c | **45.** d | **46.** c | **47.** a | **48.** b |
| **49.** b | **50.** d | **51.** c | **52.** a | **53.** d | **54.** c | **55.** b | **56.** c |
| **57.** a | **58.** c | **59.** b | **60.** a | | | | |

CHAPTER

# 13

# Lipid Metabolism

**Chapter outline**


- Digestion and Absorption of Lipids
- Fatty Acid Oxidation
- Metabolism of Ketone Bodies
- *De novo* Synthesis of Fatty Acid
- Synthesis of Long Chain Fatty Acids from Palmitate
- Triacylglycerol Metabolism
- Phospholipid Metabolism
- Glycolipid Metabolism
- Sphingolipidoses (Sphingolipid Storage Disease)
- Lipoprotein Metabolism
- Adipose Tissue Metabolism
- Fatty Liver
- Cholesterol Metabolism
- Atherosclerosis
- Alcohol Metabolism


## INTRODUCTION

Lipids include a wide variety of chemical substances, such as:

- Neutral fat (triacylglycerol or triglycerides)
- Fatty acids and their derivatives
- Phospholipids
- Glycolipids
- Sterols
- Fat soluble vitamins (A, D, E and K).

An adult ingests about 60–150 gm of lipids per day, of which more than 90% fat intake in the diet is as **triacylglycerols,** with the remainder consisting of cholesterol, cholesterol ester, phospholipid and free fatty acid.

## DIGESTION AND ABSORPTION OF LIPIDS

### Digestion

Little or no digestion occurs in the mouth or stomach. The major site of lipid digestion is the small intestine, where dietary lipid undergoes its major digestive processes using enzymes secreted by pancreas.

#### *Digestion in Small Intestine*

- The acidic stomach contents called ***chyme***; containing dietary fat leaves the stomach and enters the small intestine, in the duodenum. Digestion of fat occurs in the duodenum by **emulsification of fat.**
- In the duodenum, the dietary fat is **emulsified** by the action of **bile salts.**

#### *Hydrolysis of Dietary Triacylglycerols*

Emulsified triacylglycerols are hydrolyzed by ***pancreatic lipase***. Lipase hydrolyses fatty acid in the 1 and 3 positions of the triacylglycerol, producing 2 monoacylglycerols and two molecules of fatty acids **(Fig. 13.1).**

#### *Hydrolysis of Dietary Phospholipids*

Dietary glycerophospholipids are digested by pancreatic ***phospholipase-$A_2$***. This enzyme catalyzes the hydrolysis of fatty acid residues at the 2-position of the phospholipid, leaving lysophospholipids and a molecule of fatty acid **(Fig. 13.1).**

#### *Hydrolysis of Cholesterol Ester*

Cholesterol esters are hydrolyzed by pancreatic ***cholesterol ester hydrolase (cholesterol esterase)***, which produces cholesterol plus free fatty acid.

**Fig. 13.1:** Hydrolysis of dietary triacylglycerol, phospholipid and cholesterol ester in intestine.

### *Products of Lipid Digestion (Micelle Formation)*

Free fatty acids, free cholesterol, 2-monoacylglycerol, and lysophospholipid are the primary products of dietary lipid digestion. These, together with bile salts, form mixed micelles. Fat soluble vitamins A, D, E and K are also packaged in these micelles and are absorbed from the micelles along with the products of dietary lipid digestion.

## Absorption of Lipids by Intestinal Mucosal Cells

- The mixed micelles approach the brush border membrane of the intestinal mucosal cells.
- There the lipid components from mixed micelles are absorbed into the mucosal cells by diffusion.
- The net result is the transfer of monoacylglycerol, fatty acids, cholesterol, and lysophospholipid molecules into the mucosal cell.
- After absorption within the mucosal cell, the following events occur **(Fig. 13.2):**
  - 2-monoacylglycerols are reconverted to triacylglycerols. The fatty acids absorbed from the lumen are utilized for this synthesis. Fatty acids are utilized after its conversion to its active form, acyl-CoA.
  - The absorbed lysophospholipids and cholesterol are also reconverted to phospholipids and cholesterol esters.
  - The triacylglycerol resynthesized in intestinal cells combine with cholesterol, phospholipids and proteins to form **chylomicrons.**

## Transport

- Triacylglycerol, phospholipid, cholesterol esters resynthesized in the intestinal mucosa and absorbed fat soluble vitamins are transported from the mucosal cells into the lymph in the form of lipoprotein known as ***chylomicrons*** **(Fig. 13.2).** Chylomicrons are composed of:
  - Triacylglycerols (85–90%)
  - Cholesterol and cholesterol ester (5%)
  - Phospholipids (7%)
  - Protein (apolipoprotein B-48, 1–2%).
- The chylomicrons pass from the lymph into the blood through the thoracic duct. ***After a fatty meal, the plasma is milky in appearance due to the presence of chylomicrons***.

**Fig. 13.2:** Absorption and transport of lipid from intestinal lumen.
(2-MAG: 2 monoacylglycerol; FA: Fatty acid; C: Cholesterol; LysoPL: Lysophospholipid; TG: Triacylglycerol; CE: Cholesterol ester)

## FATTY ACID OXIDATION

Fatty acids are oxidized mainly by a process called β-**oxidation,** in which two carbon units sequentially removed beginning from the carboxyl end of the fatty acid in the form of **acetyl-CoA**. It is called β-oxidation because oxidation of fatty acids occurs at the β-carbon atom. β-oxidation pathway occurs in **mitochondria.** It involves following three steps:

1. Activation of fatty acid to acyl-CoA
2. Transfer of acyl CoA into mitochondria by carnitine transport system
3. Reactions of β-oxidation in mitochondria.

### Activation of Fatty Acid

- Before being catabolized, free fatty acids are converted to an active form called **acyl-CoA.** It occurs in the cytosol in the presence of ATP, coenzyme-A (CoA-SH) and the enzyme **acyl-CoA synthetase** also called **thiokinase (Fig. 13.3).** Subsequent steps of β-oxidation occur in the mitochondria of the liver and other tissue cells.

### Transport of Acyl-CoA into Mitochondria by Carnitine Transport System

Activation of fatty acids occurs in the cytosol, whereas they are oxidized in the mitochondrial matrix. The mitochondrial inner membrane is impermeable to fatty acids. So a special transport mechanism is needed.

**Fig. 13.3:** Activation of fatty acid.

Activated long chain fatty acids are carried across the inner mitochondrial membrane by ***carnitine, (β-hydroxy γ-trimethyl ammonium butyrate)***,formed from ***lysine*** and methionine in liver and kidney. This occurs in four steps **(Fig. 13.4)** as follows:

1. The acyl group of acyl-CoA is transferred to the carnitine to form acyl-carnitine. This reaction is catalyzed by ***carnitine acyltransferase-I (CAT-I)*** which is located on the cytosolic face of the inner mitochondrial membrane.
2. Acyl-carnitine is then transported across the inner mitochondrial membrane by an enzyme **translocase**.
3. The acyl group is transferred back to CoA in the mitochondrial matrix by the enzyme ***carnitine acyl transferase-ll (CAT-II)***, located on the inside of the inner mitochondrial membrane.
4. Acyl-CoA is reformed in the mitochondrial matrix with liberation of carnitine which is returned to the cytosolic side by the **translocase** in exchange for an incoming acyl-carnitine.

**Fig. 13.4:** Carnitine transport system.
(CAT I: Carnitine acyl transferase-I; CAT II: Carnitine acyl transferase-II)

## Reactions of β-oxidation of Fatty Acid

After the penetration of the acyl-CoA into mitochondria, it undergoes β-oxidation.

### *Sequence of Reactions of β-oxidation*

A saturated acyl-CoA is degraded by a repeated sequence of four reactions **(Fig. 13.5)** as follows:

1. Oxidation by FAD
2. Hydration
3. Oxidation by NAD
4. Cleavage.

1. ***Oxidation by FAD:*** The first reaction is the oxidation of acyl-CoA by ***anacyl-CoA dehydrogenase*** to give $\Delta^2$-trans enoyl-CoA (with a trans double bond between $C_2$ and $C_3$ also called α-β-unsaturated fatty acyl CoA). The coenzyme for the dehydrogenase is FAD which is converted to $FADH_2$.
2. ***Hydration:*** The next step is the hydration (addition of water) of the double bond between $C_2$ and $C_3$ by $\Delta^2$-enoyl-CoA hydratase to form L(+) β-hydroxy acyl-CoA.
3. ***Oxidation by NAD:*** The β-hydroxy derivative undergoes second oxidation reaction catalyzed by β-***hydroxyacyl-CoA dehydrogenase*** to form β-ketoacyl-CoA and generates NADH.
4. ***Cleavage:*** Finally β-ketoacyl-CoA is split at the β-carbon by *thiolase* to yield acetyl-CoA and an acyl-CoA which is shorter by two carbon atoms than the original acyl-CoA that underwent oxidation.

The new acyl-CoA, containing two carbons less than the original, re-enters the β-oxidation pathway at reaction catalyzed by acyl-CoA dehydrogenase **(Fig. 13.5).** The process continues till the fatty acid degraded completely to acetyl-CoA.

Acetyl-CoA can be oxidized to $CO_2$ and $H_2O$ via citric acid cycle in mitochondria and thus oxidation of fatty acids is completed.

### *Energy Yield from the β-Oxidation of Fatty Acids*

- Complete β-oxidation of palmitoyl CoA (16 carbon acid) occurs through 7 cycles of β-oxidation yielding finally 8 acetyl-CoA, 7 $FADH_2$ and 7 NADH **(Fig. 13.6)**.
- 2.5 ATPs are generated when each of these NADH is oxidized by respiratory chain.
- 1.5 ATPs are formed for each $FADH_2$.
- The oxidation of acetyl-CoA by the citric acid cycle yields 10 ATPs. Therefore, the number of ATPs formed in the oxidation of palmitoyl-CoA is:
  - 10.5 ATPs from the 7 $FADH_2$
  - 17.5 ATPs from the 7 NADH
  - 80 ATPs from the oxidation of 8 molecules of acetyl-CoA in TCA cycle.
  - Total of 108 ATPs.

**Fig. 13.5:** β-oxidation of fatty acids.

**Fig. 13.6:** Overall process of β-oxidation.

**Fig. 13.7:** Regulation of β-oxidation of fatty acid.

- Two high energy phosphate bonds are consumed in the activation of palmitate, in which ATP is split into AMP and PPi.
- Thus, the net yield from the complete oxidation of palmitate is 108 ATPs minus 2ATPs = 106 ATPs.

> Previously calculations were made assuming that NADH produces 3 ATPs and $FADH_2$ generates 2 ATPs. This will amount to a net generation of **129** ATPs per palmitate molecule. Recent studies show that these old values are wrong, and net generation of ATPs is only **106**.

### *Regulation of β-oxidation*

- Rate limiting step in the β-oxidation pathway is the formation of **acyl-carnitine** which is catalyzed by ***carnitine-acyl transferase-I (CAT-I)***. CAT-I is an allosteric enzyme. **Malonyl-CoA** is an inhibitor of CAT-I.
  - In well-fed state due to increased level of **insulin,** concentration of malonyl-CoA increases which inhibits CAT-I and leads to decrease in fatty acid oxidation.
  - In starvation, due to increased level of glucagon concentration of malonyl-CoA decreases and stimulates the fatty-acid oxidation **(Fig. 13.7)**.

## β-oxidation of a Fatty Acid with an Odd Number of Carbon Atoms

- Fatty acids, having an odd number of carbon atoms, are oxidized by β-oxidation in the same way as fatty acids, having an even number, except in the last and final β-oxidation cycle, of odd carbon fatty acids a three carbon fragment **propionyl-CoA** is formed rather than two carbon fragment **acetyl-CoA**.
- Propionyl-CoA is converted to succinyl-CoA **(Fig. 13.8)**, a constituent of citric acid cycle.
- Propionyl-CoA is carboxylated at the expense of an ATP to yield the D-methylmalonyl-CoA; catalyzed by ***propionyl-CoA carboxylase***, a biotin enzyme.
- The D-methylmalonyl-CoA is converted to the L-methylmalonyl-CoA by the enzyme ***methylmalonyl-CoA epimerase***.
- Succinyl-CoA is formed from L-methylmalonyl-CoA by ***methylmalonyl-CoA mutase***, which requires ***vitamin $B_{12}$*** as a coenzyme.

## Peroxisomal Fatty Acid Oxidation (Fig. 13.9)

Peroxisomes are able to conduct oxidation of long chain fatty acids. Oxidation of very long chain fatty acids, which contain 20–26 carbons begins in peroxisomes by a process very similar to β-oxidation (and is completed in the mitochondria) with significant differences.

**Fig. 13.8:** Conversion of propionyl-CoA to succinyl-CoA.

- The action of first enzyme ***acyl-CoA dehydrogenase*** differs, however, in that; it produces $H_2O_2$ rather than $FADH_2$. In peroxisomes the $FADH_2$ is not linked to ETC for generation of ATP but it transfers reducing equivalents directly to $O_2$, yielding $H_2O_2$. There is no ATP synthesized in peroxisomal β-oxidation of fatty acids.
- **Catalase** an enzyme located in peroxisomes converts $H_2O_2$ to water and molecular oxygen.

## Disorder of Peroxisomal Fatty Acid Oxidation

### *Zellweger syndrome*

- A rare inborn error of peroxisomal fatty acid oxidation is due to inherited absence of **functional peroxisomes** in all tissues.
- As a result, the long chain fatty acids are not oxidized in peroxisomes and accumulate in tissues, particularly in brain, liver, kidney and muscle and usually results in death by age six.

## α-oxidation

- Oxidation occurs at α-carbon of fatty acid in which one carbon is removed from the carboxyl end of the fatty acid chain and released as $CO_2$.
- The remaining carbons of the fatty acid can repeat the process.
- It does not require CoA intermediates and does not generate ATP.
- This process occurs in brain and other nervous tissues.

**Fig. 13.9:** Oxidation of fatty acids in peroxisomes.

## Role of α-oxidation

In α-oxidation, oxidative decarboxylation eliminates one carbon and converts the even numbered fatty acid to odd numbered chains, which are constituent of complex lipids of the brain.

## Disorders associated with Impairment of α-fatty Acid Oxidation

### *Refsum's disease (Phytanate storage disease)*

A rare neurologic inborn error of lipid metabolism results from a genetic deficiency in **phytanic acid** α-hydroxylase required for the hydroxylation of phytanic acid by α-oxidation. Persons with Refsum's disease have an inherited defect in α-oxidation that leads to accumulation of phytanic acid in the nerve tissue.

### *Symptoms*

Clinical symptoms include ***retinitis pigmentosa*** (progressive degeneration of retina), ***peripheral neuropathy*** and **ataxia.** Treatment involves elimination of dietary sources of phytol.

## ω-oxidation

Fatty acids may be, oxidized at the ω-carbon of the chain by enzymes in the ***endoplasmic reticulum***. ω-carbon is a carbon farthest from the carboxyl end.

- The ω-methyl group is first oxidized to an alcohol ($CH_2OH$) by hydroxylase that uses cytochrome $P_{450}$, molecular oxygen and NADPH in the endoplasmic reticulum.
- The alcoholic group is subsequently oxidized to—COOH by dehydrogenase and thus forming a dicarboxylic acid.

- Short chain dicarboxylic acid, such as ***pimelic acid***, a precursor of biotin formed by ω-oxidation.

## METABOLISM OF KETONE BODIES

**Acetoacetate, β-hydroxybutyrate** and **acetone,** these three substances are collectively known as ketone bodies **(Fig. 13.10)**. These are water soluble energy yielding substances. Acetone is, however, an exception, since it cannot be metabolized and is readily exhaled through lungs. Acetoacetate and acetone are true ketones, while β-hydroxybutyrate does not possess a keto group.

### Formation of Ketone Bodies (Ketogenesis)

Ketogenesis means the formation of ketone bodies. Liver is the only organ that synthesizes ketone bodies. The synthesis of ketone bodies occurs in mitochondrial matrix of hepatic cells. Ketogenesis occurs by the following reactions: **(Fig. 13.11)**.

**Fig. 13.10:** Structure of ketone bodies.

- Two molecules of acetyl-CoA condense to form **acetoacetyl-CoA.** This reaction is catalyzed by thiolase.
- Acetoacetyl-CoA then condenses with one more molecule of acetyl-CoA to give **β-hydroxy-β-methylglutaryl-CoA (HMG-CoA),** catalyzed by HMG-CoA synthase.

**Fig. 13.11:** Formation of ketone bodies.

**Fig. 13.12:** Fate or utilization of ketone bodies.

- β-HMG-CoA is then cleaved to acetyl-CoA and **acetoacetate** by the enzyme HMG-CoA lyase present in the mitochondria.
- β-**hydroxy butyrate** is formed by the reduction of acetoacetate in the mitochondrial matrix by the enzyme β-hydroxy butyrate dehydrogenase.
- Acetoacetate also undergoes a slow, nonenzymatic spontaneous decarboxylation to **acetone.**
- The concentration of total ketone bodies in the blood of well-fed condition does not normally exceed 0.2 mmol/L.

## Fate or Utilization of Ketone Bodies

The site of production of ketone bodies is the ***liver***. But, the liver cannot utilize ketone bodies because it lacks the particular enzyme ***CoA-transferase*** which is required for the activation of ketone bodies.

Acetoacetate, β-hydroxybutyrate and acetone diffuse from the liver mitochondria into the blood and are transported to peripheral tissues.

- In peripheral tissues the β-hydroxybutyrate is first reconverted to acetoacetate and the acetoacetate is then reactivated to acetoacetyl-CoA **(Fig. 13.12).**
- Activation of acetoacetate occurs by ***CoA-transferase*** in the presence of succinyl-CoA. Acetoacetate is activated by the transfer of CoA from succinyl-CoA.
- Acetoacetyl-CoA, formed by this reaction, is then cleaved by ***thiolase*** to yield two molecules of acetyl-CoA which can be oxidized in the citric acid cycle to $H_2O$ and $CO_2$.
- Further metabolism of acetone does not occur. Because acetone is volatile, it is expired by the lungs.

Schematic representation of overall metabolism (synthesis and breakdown) of ketone bodies is shown in **Figure 13.13.**

**Fig. 13.13:** Schematic representation of metabolism (formation and fate) of ketone bodies.

**Fig. 13.14:** Steps of regulation of ketogenesis.

**Fig. 13.15:** Increased formation of ketone bodies in diabetes or prolonged starvation.

**Significance of Ketogenesis**

- Ketogenesis is a mechanism that allows the liver to oxidize increasing quantities of fatty acids.
- During deprivation of carbohydrate as in starvation and diabetes mellitus, acetoacetate and β-hydroxybutyrate serve as an alternative source of energy for extrahepatic tissues such as skeletal muscle, heart muscle, renal cortex, etc.
- In prolonged starvation 75% of the energy needs of the brain are supplied by ketone bodies reducing its need for glucose.

### *Regulation of Ketogenesis*

The ketone body formation is regulated at the following three important levels **(Fig. 13.14)**.

1. **Factors regulating lipolysis:** Free fatty acids the precursors of ketone bodies arise from lipolysis of triacylglycerol in adipose tissue. Thus factors regulating lipolysis also regulate ketogenesis. The hormone **glucagon** stimulates lipolysis which in turn stimulates ketogenesis, whereas **insulin** inhibits lipolysis and ketogenesis.
2. **Factors regulating β-oxidation of fatty acids**
   - **Carnitine acyl transferase** I (CAT I) regulates the fatty acid oxidation which, in turn, regulates ketogenesis as well.
   - Its activity is high in **starvation** leading to increased fatty acid oxidation and formation of ketone bodies.
3. **Factors regulating oxidation of acetyl-CoA:** In turn, the acetyl-CoA, formed in β-oxidation in liver mitochondria, has two possible fates.
   - It may be oxidized to $CO_2$ via the citric acid cycle or it enters the pathway of ketogenesis to form ketone bodies.
   - When oxaloacetate concentration is very low, little acetyl-CoA enters the citric acid pathway, and ketone body formation is then favored.
   - Concentration of oxaloacetate is lowered if carbohydrate is unavailable or improperly utilized, e.g. in fasting or in diabetes. Under these conditions, acetyl-CoA is diverted to the formation of acetoacetate.

## Disorders of Ketone Body Metabolism

### *Ketosis*

- Normally the concentration of ketone bodies in blood is very low (less than 0.2 mmol/L) but, in fasting and in diabetes mellitus, it may reach extremely high levels.
- During fasting or in diabetes mellitus, when exogenous glucose is unavailable, insulin secretion is inhibited. The plasma insulin concentration is, therefore, low which causes increased lipolysis and therefore increased production of free fatty acids and hence ketone bodies production is increased **(Fig. 13.15).**
- When the rate of formation of the ketone bodies by liver exceeds the capacity of the peripheral tissues to use them up, their levels begin to rise in blood. An increase in concentration of ketone bodies in blood is called ***ketonemia*** and eventually leads to excretion of ketone bodies into the urine called . The overall condition (ketonemia and ketonuria) is called **ketosis.**
- In addition to β-hydroxybutyrate and acetoacetate, the blood of diabetics also contains acetone. Acetone is very volatile and is present in the breath of diabetics, to which it gives sweet fruity odor.

### *Ketoacidosis*

- Since acetoacetate and β-hydroxybutyrate are moderately strong acids, increased levels of these ketone bodies decrease the pH of the blood and cause **metabolic acidosis.** The acidosis caused by over production of ketone bodies is termed as **ketoacidosis.**
- Acetoacetate and β-hydroxybutyrate acids when present in high concentration in blood, are buffered by HCO3⁻ (alkali) fraction of bicarbonate buffer. The excessive use of $HCO_3^-$ **depletes the alkali reserve** causing **ketoacidosis.**
- Ketoacidosis is seen in type I diabetes mellitus, whereas in type II diabetes ketoacidosis is relatively rare.

## *DE NOVO* SYNTHESIS OF FATTY ACIDS (LIPOGENESIS)

In humans, fatty acid synthesis occurs mainly in the ***liver*** and ***lactating mammary glands*** and, to a lesser extent, in ***adipose tissue, kidney*** and ***brain***. **De novo** synthesis means new synthesis. Fatty acid synthesis occurs in three phases:

1. Transport of acetyl-CoA from mitochondria to cytosol.
2. Carboxylation of acetyl-CoA to malonyl-CoA.
3. Reactions of fatty acid synthase complex.

### Transport of Acetyl-CoA from Mitochondria to Cytosol

Fatty acids are synthesized in the cytosol, whereas acetyl-CoA is formed in mitochondria, hence, acetyl-CoA must be transferred from mitochondria to the cytosol. Since acetyl CoA is impermeable to inner mitochondrial membrane, it is transferred in the form of **citrate** from mitochondrial matrix to the cytosol as shown in **Figure 13.16.**

- Citrate is formed in the mitochondrial matrix by the condensation of acetyl-CoA with oxaloacetate (first reaction in the citric acid cycle).
- Then citrate is transported to the cytosol by ***translocase***, where it is cleaved by ***citrate lyase*** to oxaloacetate and acetyl-CoA.
- Oxaloacetate formed in this reaction must be returned to the mitochondria. The inner mitochondrial membrane is impermeable to oxaloacetate. Therefore, oxaloacetate is reduced to malate, catalyzed by cytosolic ***malate dehydrogenase***. Then malate is oxidatively decarboxylated to pyruvate by ***NADP-linked malic enzyme. NADPH and $CO_2$ are generated in this reaction. Both of them are utilized for fatty acid synthesis***.
- The pyruvate formed in this reaction readily diffuses into mitochondria, where it is carboxylated to oxaloacetate by ***pyruvate carboxylase***.

### Carboxylation of Acetyl-CoA to Malonyl-CoA

- Carboxylation of acetyl-CoA to malonyl-CoA is the initial and ***rate limiting reaction in fatty acid synthesis***.
- This reaction is catalyzed by an enzyme complex, ***acetyl-CoA carboxylase that*** contains **biotin** and utilizes bicarbonate (as a source of $CO_2$) in presence of ATP **(Fig. 13.17)**.

**Fig. 13.16:** Transfer of acetyl-CoA from mitochondria to the cytosol.

**Fig. 13.17:** Biosynthesis of malonyl-CoA

## Reactions of Fatty Acid Synthase Complex

- Once malonyl CoA is formed from acetyl CoA, the *de novo* synthesis of palmitic acid is carried out in cytosol by **fatty acid synthase complex**.
- Fatty acid synthase complex is a multienzyme complex possessing 7 different enzymes and one **acyl carrier protein (ACP)** molecule **(Fig. 13.18).** The seven enzymes are:

1. Malonyl/acetyl transferase (MAT)
2. Ketoacyl synthase (KS)
3. Ketoacyl reductase (KR)
4. Dehydratase (DH)
5. Enoyl reductase (ER)
6. Acyl carrier protein (ACP)
7. Thioesterase (TE)

- The fatty acid synthase complex has two active **—SH groups.**
- One —SH group is of **ACP.**
- Another —SH group is of enzyme **ketoacyl synthase (KS).**

Both —SH groups participate in fatty acid biosynthesis (**Fig. 13.18**).

- Fatty acid synthase complex is a dimer**.** Each monomer is identical consisting of all seven enzyme activities of fatty acid synthase and an ACP.
- The two subunits associate in a head-to-tail arrangement, so that the —SH group of ACP of one subunit and —SH group of KS of another subunit are closely aligned **(*see* Fig. 13.18)**.
- Though each monomer contains all the enzyme activities but only the dimer is functionally active. This is because

**Fig. 13.18:** Schematic diagram of fatty acid synthase multienzyme complex.

**Abbreviations:** 1: Malonyl/acetyl transferase (MAT); 2: Ketoacyl synthase (KS); 3: Ketoacyl reductase (KR); 4: Dehydratase (DH); 5: Enoyl reductase (ER); 6: Acyl carrier protein (ACP); 7: Thioesterase (TE); ACP: Acyl carrier protein

the functional enzyme consists of half of each subunit interacting with the complementary half of the other.

The fatty acid synthase complex catalyzes following reactions **(Fig. 13.19).**

- In the first reaction, catalyzed by ***acetyl transacylase*** the acetyl group of acetyl-CoA is transferred to SH group of the 3-ketoacyl synthase to form acetyl enzyme.
- In the second reaction, the malonyl group of malonyl-CoA is transferred to -SH group of ACP catalyzed by ***malonyl transacylase*** to form acetyl-malonyl enzyme.
- The acetyl and malonyl groups, covalently bonded to—SH groups of the fatty acid synthase, undergo a condensation reaction. This reaction is catalyzed by 3-ketoacyl synthase. The acetyl group attached to —SH of KS is transferred to malonyl group bound to —SH of ACP. Simultaneously, the malonyl moiety loses $CO_2$ forming **3-ketoacyl enzyme**.
- The 3-ketoacyl enzyme undergoes reduction at the 3-keto group, using NADPH as electron donor to form **3-Hydroxyacyl enzyme**, catalyzed by ***3-ketoacyl reductase***.
- 3-hydroxyacyl enzyme is dehydrated by ***3-hydroxyacyl hydratase*** to yield **2, 3-unsaturated acyl enzyme.**
- 2, 3- unsaturated acyl enzyme is reduced to form acyl enzyme containing 4-carbon, by the action of ***enoyl reductase*** and NADPH is the electron donor.
- This acyl group containing 4 carbons is now transferred from ACP–SH group to the KS–SH group.
- To lengthen the chain by another 2-carbon unit, the sequence of reactions is repeated, a new malonyl residue being incorporated during each sequence, until a saturated 16-carbon palmitic acid has been assembled.
- Thus, after a total of seven such cycles, palmitoyl enzyme is formed, which is liberated from the enzyme complex by the activity of a seventh enzyme in the complex ***thioesterase (deacylase)***.

### Regulation of Fatty Acid Synthesis

*De novo* fatty acid synthesis is regulated by **Acetyl-CoA Carboxylase** enzyme **(Fig. 13.20).**

- Acetyl-CoA carboxylase which catalyzes the formation of malonyl-CoA is the rate limiting enzyme in the fatty acid synthesis. It is regulated by following ways:
  1. **Allosteric regulation:** Acetyl-CoA carboxylase is allosterically activated by **citrate** and inhibited by its own product **palmitoyl-CoA.**
  2. **Hormonal regulation:** The activity of acetyl-CoA carboxylase is also controlled by hormones. **Glucagon** and **epinephrine** inactivate the enzyme. In contrast, **insulin** activates the enzyme. Thus, fatty acid synthesis is stimulated by insulin and inhibited by glucagon and epinephrine.
  3. **Nutritional regulation:** Synthesis of **acetyl-CoA carboxylase** is increased with high carbohydrate and low fat diet, which promote fatty acid synthesis. By contrast, synthesis of acetyl-CoA carboxylase decreases during starvation, diabetes mellitus or high fat diet.

## SYNTHESIS OF LONG CHAIN FATTY ACIDS FROM PALMITATE

The major product of fatty acid synthesis is palmitate. Longer fatty acids are formed by elongation reactions either in **endoplasmic reticulum (microsomes)** or in **mitochondria.**

### Microsomal Elongation of Fatty Acids

The microsomal enzyme **fatty acid elongase** elongates palmitate by the addition of 2-carbon fragments derived from malonyl-CoA and NADPH provides the reducing equivalents. The elongation reaction resembles those of fatty acid synthesis, except that the fatty acyl chain is attached to coenzyme-A rather than to phosphopantetheine group of ACP.

### Mitochondrial Elongation of Fatty Acids

Fatty acids can be elongated in mitochondria, but mitochondrial elongation of fatty acids is less active. In this case, the source of the 2-carbon units is acetyl-CoA and the substrates are usually fatty acids containing less than 16-carbons, mainly short and medium chain fatty acids.

## TRIACYLGLYCEROL METABOLISM

Triacylglycerols are esters of the alcohol glycerol and fatty acids. Fatty acids derived from endogenous synthesis or from the diet are stored in the adipose tissue in the form of triacylglycerol, called "***neutral fat***."

Triacylglycerol serves as the body's major fuel storage reserve.

### Biosynthesis of Triacylglycerols

In both liver and adipose tissue, triacylglycerols are synthesized **(Fig. 13.21).**

**Fig. 13.19:** *De novo* synthesis of fatty acid.
(KS: Ketoacyl synthase; ACP: Acyl carrier protein)

**Fig. 13.20:** Regulation of fatty acid synthesis

- First fatty acids are activated to acyl-CoA (***see* Figure 13.3).**
- Then two molecules of acyl-CoA combine with glycerol-3-phosphate to form phosphatidic acid (1,2-diacylglycerol phosphate) via formation of lysophosphatidic acid.
- Phosphatidic acid is the common precursor in the biosynthesis of the triacylglycerols and many glycerophospholipids and cardiolipin.
- Dephosphorylation of phosphatidic acid produces diacylglycerol.
- A further molecule of acyl-CoA is esterified with diacylglycerol to form triacylglycerol.
- The sources of glycerol-3-phosphate, which provides the glycerol moiety for triacylglycerol synthesis, differ in liver and adipose tissue.
- In liver glycerol-3-phosphate is produced from the phosphorylation of glycerol by ***glycerol kinase*** or from the reduction of dihydroxyacetone phosphate (DHAP), derived from glycolysis.
- Adipose tissue lacks glycerol kinase and can produce glycerol-3-phosphate only from glucose via DHAP.

### Fate of Triacylglycerol formed in Liver and Adipose Tissue

The fate of triacylglycerol in liver and adipose tissue is different.

- In the liver, little triacylglycerol is stored; instead most is exported in the form of very low density lipoprotein (VLDL). Once released into the blood stream, triacylglycerol of VLDL is hydrolyzed by lipoprotein lipase enzyme, which is located on the walls of blood capillaries. It clears the triacylglycerol in VLDL, forming free fatty acids and glycerol.
- In adipose tissue triacylglycerol is stored in the cells. It serves as "***depot fat***" ready for mobilization when the body requires it for fuel.

## PHOSPHOLIPID METABOLISM

Phospholipids are the major class of membrane lipids. There are two classes of phospholipids:

1. Those that have glycerol, a 3-carbon alcohol, as a backbone, called ***glycerophospholipids*** or ***phosphoglycerides*, e.g.**
   - Phosphatidylserine
   - Phosphatidylinositol
   - Phosphatidylcholine (Lecithin)
   - Phosphatidylethanolamine (Cephalin)
   - Cardiolipin
   - Plasmalogens
2. Those that contain **sphingosine,** a more complex amino alcohol called ***sphingophospholipid*, e.g.**
   - Sphingomyelin.

### Biosynthesis of Glycerophospholipids

The initial steps in the synthesis of glycerophospholipids are similar to those of triacylglycerol synthesis **(*see* Fig. 13.21).** Phosphatidate (diacylglycerol-3-phosphate) is a common intermediate in the synthesis of glycerophospholipids and triacylglycerols.

#### *Synthesis of Phosphatidylserine and Phosphatidylinositol (see Fig. 13.21)*

- Synthesis of phosphatidylserine and phosphatidylinositol starts with the formation of cytidine diacylglycerol (CDP-diacylglycerol) an activated phosphatidyl unit, from phosphatidate and cytidine triphosphate (CTP).
- The activated phosphatidyl unit then reacts with the hydroxyl group of alcohol.
  - If alcohol is serine, it forms **phosphatidylserine**
  - Likewise if the alcohol is inositol, the product is **phosphatidylinositol.**
- Phosphatidylserine can also be formed from phosphatidylethanolamine directly by reactions with serine.

#### *Synthesis of Phosphatidylcholine (Lecithin) and Phosphatidylethanolamine (Cephalin)*

Phosphatidylcholine is synthesized by a pathway that utilizes choline obtained from the diet.

- Choline must first be converted to active choline. This is a stage process, first is the phosphorylation with ATP to form phosphocholine, which then reacts with CTP to form CDP-choline (active form).
- The phosphorylcholine unit of CDP-choline is then transferred to a diacylglycerol to form phosphatidylcholine **(Fig. 13.21).**
- Likewise phosphatidylethanolamine can be synthesized from ethanolamine by forming a CDP-ethanolamine intermediate by analogous reactions.
- Phosphatidylserine may form phosphatidylethanolamine by decarboxylation.
- An alternative pathway in liver enables phosphatidylethanolamine to give rise directly to phosphatidylcho-

**Fig. 13.21:** Synthesis of triacylglycerol and glycerophospholipids.

line by methylation of the ethanolamine residue utilizing S-adenosyl-methionine (SAM) as the methyl donor.

### *Synthesis of Cardiolipin (Fig. 13.22)*

In the synthesis of cardiolipin first CDP-diacylglycerol combines with glycerol-3-phosphate to form phosphatidyl glycerol which, in turn, reacts with another molecule of CDP-diacylglycerol to produce cardiolipin (diphosphatidyl-glycerol).

### *Synthesis of Plasmalogens*

Plasmalogens differ from other glycerophospholipids in that the fatty acid at carbon 1 of glycerol bound by an ether linkage instead of ester linkage. The biosynthesis of plasmalogen occurs in peroxisomes and is shown in **(Fig. 13.23).**

## Biosynthesis of Sphingomyelin

- These phospholipids contain a complex amino alcohol **sphingosine** instead of glycerol.
- Palmitoyl-CoA and serine condense to form 3-ketosphinganine. The enzyme catalyzing this reaction requires **pyridoxal phosphate.**
- 3-ketosphinganine is then converted to sphingosine.
- In all sphingolipids, a long chain acyl-CoA reacts with sphingosine to form **ceramide.**
- Ceramide reacts with phosphatidylcholine to form sphingomyelin **(Fig. 13.24).**

## Degradation of Glycerophospholipids

***Phospholipases*** located in cell membranes or in lysosomes degrade glycerophospholipids. Several specific phospholipases have been isolated which are designated as $A_1$, $A_2$, B, C and D. The bonds hydrolyzed by phospholipases $A_1$, $A_2$, B, C and D are shown in **Figure. 13.25**.

- **Phospholipase-$A_1$** removes the fatty acyl group on C1 of the glycerol moiety.
- **Phospholipase-$A_2$** catalyzes the hydrolysis of the ester bond in position 2 of glycerophospholipids to form a free fatty acid and lysophospholipid, which is attacked by lysophospholipase removing the remaining 1-acyl group.
- **Phospholipase-B** hydrolyzes both acyl groups on $C_1$ and $C_2$.
- **Phospholipase**-C cleaves the bond, between phosphate and glycerol of phospholipids.

**Fig. 13.22:** Synthesis of cardiolipin

**Fig. 13.23:** Synthesis of plasmalogen and platelet activating factor.

**Fig. 13.24:** Synthesis of sphingomyelin.

- The bond between the phosphate and the nitrogen base is cleaved by ***phospholipase-D***.

## Degradation of Sphingomyelin

The sphingomyelins are hydrolyzed by lysosomal enzyme **sphingomyelinase** to ceramide and phosphorylcholine. The ceramide so formed is further hydrolyzed by another lysosomal enzyme **ceramidase** into sphingosine and free fatty acid.

# GLYCOLIPID METABOLISM

Glycolipids, as their name implies, are sugar containing lipids. Glycolipids like sphingomyelin are derived from sphingosine. Sphingosine reacts with acyl-CoA to form **ceramide.** ***Cerebrosides*** and ***gangliosides*** are the different types of glycolipids.

## Biosynthesis of Glycolipids

Cerebroside is the simplest glycosphingolipid. Incer-ebrosides, glucose or galactose is linked to the terminal hydroxyl group of ceramide to form ***glucocerebroside*** or ***galactocere-broside***.

**Fig. 13.25:** Sites of hydrolytic cleavage of glycerophospholipid by phospholipases.

Galactocerebroside is a major lipid of myelin, whereas glucocerebroside is the major glycolipid of extraneural tissues and a precursor of most of the more complex glycolipids.

- Ceramide reacts with UDP-glucose or UDP-galactose to form ***glucocerebroside*** or ***galactocerebroside*** respectively.
- **Gangliosides** are the more complex glycolipids; contain a branched chain oligosaccharide of as many as seven sugar residues.
- Gangliosides are produced from ceramide by the stepwise addition of activated sugar, e.g. UDP-glucose, UDP-galactose and sialic acid usually N-acetylneuraminic acid (NANA) **(Fig. 13.26).**

## Degradation of Glycolipids

- The glucocerebroside and galactocerebroside are hydrolyzed by lysosomal enzymes β-**glucocerebrosidase** (β-glucosidase) and β-**galactocerebrosidase** (β-glactosidase) respectively, to ceramide and hexose residues. The ceramide so formed is further cleaved by another lysosomal enzyme **ceramidase** to sphingosine and free fatty acid.
- The different gangliosides are degraded by a set of lysosomal enzymes, β-**glucosidase,** β-**hexosaminidase,** β-**galactosidase, neuraminidase,** etc.

# SPHINGOLIPIDOSES (SPHINGOLIPID STORAGE DISEASE)

- Sphingolipidoses are a group of inherited diseases that result from defective degradation and accumulation of any one of the sphingolipids (sphingomyelins or glycolipids) (Table 13.1).
- Depending on the enzyme affected several types of sphingolipidoses have been recognized. Some major types of sphingolipidoses are discussed here **(Fig. 13.27).**

**Fig. 13.26:** Biosynthesis of glycolipids.
(CMP-NANA: Cytidine monophosphate-N-acetyl neuraminic acid)

1. **Niemann-Pick disease:** The degradation of sphingomyelins is impaired in Niemann Pick disease due to inherited deficiency of **sphingomyelinase.** As a result sphingomyelins accumulate in liver, brain and spleen. The clinical findings are:
   - Enlarged liver and spleen
   - Mental retardation and death may occur in early childhood.
2. **Tay-Sachs disease:** It is due to inherited deficiency of **hexosaminidase A** required for the degradation of ganglioside GM2. Since degradation of GM2 of ganglioside is impaired, the GM2 accumulates in brain and nervous tissues.
   - The infants with Tay-Sachs disease suffer from muscular weakness, mental retardation, blindness and death occurs in early childhood.
3. **Gauchers disease:** The inherited deficiency β-**glucosidase** impairs the hydrolysis of glucocerebroside, which results in accumulation of glucocerebroside in brain, liver, spleen, and bone marrow. This disorder is associated with mental retardation and enlargement of liver and spleen.
4. **Krabbe's disease:** The inherited deficiency of the enzyme β-**galactosidase** impairs the hydrolysis of galactocerebroside, which results in accumulation of galactocerebroside in brain and other nervous tissues. There is almost complete absence of myelin in nervous tissue. The clinical features include mental retardation, blindness and deafness. Krabbe's disease is fatal in early life.
5. **Farber's disease:** The inherited deficiency of enzyme **ceramidase** impairs the hydrolysis of ceramides which results in accumulation of ceramides in the body tissue. The symptoms include skeletal deformities, and mental retardation. The disease is fatal and death occurs in early childhood.

## LIPOPROTEIN METABOLISM

- Fat absorbed from the diet and fat synthesized by the liver must be transported between the various tissues and organs for utilization and storage. Since lipids are insoluble in water, these are transported in the form of **lipoprotein.**
- Four main types of lipoproteins are (Refer Chapter 3, Lipid Chemistry for structure):
  1. **Chylomicrons:** These are principal form in which dietary lipids (exogenous lipid) are carried to the tissues.
  2. **Very low-density lipoproteins (VLDL):** These are triacylglycerol rich particles, in which endogenous triacylglycerol are carried to the tissues from the liver.
  3. **Low-density lipoproteins (LDL):** These are cholesterol rich particles, formed from VLDL by the removal of triacylglycerol from VLDL.

**Table 13.1:** Common form of sphingolipidoses.

| *Disease* | *Lipid accumulation* | *Enzyme deficiency* | *Clinical symptoms* |
|---|---|---|---|
| Niemann-Pick in infants | Sphingomyelin | Sphingomyelinase | Enlarged liver and spleen, mental retardation |
| Gaucher's | Glucocerebroside | β-glucosidase | Same as Niemann-Pick disease |
| Tay-Sachs | GM2—ganglioside | Hexosaminidase | Mental retardation, blindness, muscular weakness |
| Krabbe's | Galactocerebroside | β-galactosidase | Mental retardation, blindness and deafness, myelin almostabsent |
| Farber's | Ceramide | Ceramidase | Mental retardation, skeletal deformities |

**Fig. 13.27:** Degradation of sphingomyelin and cerebroside.

4. **High-density lipoproteins (HDL):** They transport cholesterol from peripheral cells back to the liver (reverse cholesterol transport).

Lipoprotein metabolisms include **chylomicrons, VLDL, LDL, and HDL.**

## Metabolism of Chylomicrons (Fig. 13.28)

- Cholesterol and fatty acids released from dietary fats by digestion are absorbed into intestinal mucosal cells where they are re-esterified to form cholesterol esters and triacylglycerols. Cholesterol esters and triacylglycerols, together with phospholipids and apo B-48 are converted to **chylomicrons.**
- Chylomicron shortly after entering the circulation they acquire apo C-II and apo E from circulating HDL. The apo C-II then activates **lipoprotein lipase** in the tissues and triacylglycerol is gradually removed from chylomicrons by the action of lipoprotein lipase. This enzyme is present on the walls of the blood capillaries of a number of tissues, predominantly adipose tissue and skeletal muscles.
- Lipoprotein lipase hydrolyzes triacylglycerol to fatty acid and glycerol. The fatty acids are taken up by adipose cells (for storage) and muscle cells as an energy source. The glycerol component enters the hepatic glycolytic pathway. During hydrolysis of chylomicron triacylglycerol, the apo C-II are transferred back to HDL.
- As the chylomicrons lose triacylglycerol (approximately 90% of the triacylglycerol of chylomicrons), the resulting chylomicron becomes smaller and relatively enriched in cholesterol and cholesterol esters called **chylomicron remnants**.
- These remnants are taken up by the liver. In the liver the cholesterol esters and triacylglycerols of chylomicron remnants are hydrolyzed and metabolized.

## Metabolism of VLDL

- The hepatic triacylglycerol and cholesterol are transported in the form of **nascent VLDL (Fig. 13.29)**.
- This triacylglycerol rich particle contains Apo B-100; circulating HDL donates apo C-II and apo E to convert nascent VLDL to VLDL.
- Like chylomicrons VLDL-triacylglycerol is hydrolyzed by lipoprotein lipase in the peripheral tissues with the release of free fatty acid. During the hydrolysis of VLDL triacylglycerol, the apo C-II are transferred back to HDL.
- This results in the formation of VLDL remnant an intermediate density lipoprotein (IDL), which contains cholesterol and triacylglycerol as well as apo B and apo E. Formation of IDLs are transient which are not normally present in plasma.
- VLDL remnant or IDL undergoes a further hydrolysis, in which most of the remaining triacylglycerol are removed and all apolipoproteins except B-100 are transferred to other lipoproteins and results with formation of cholesterol rich LDL.

## Metabolism of LDL (Fig. 13.29)

- LDL is formed from VLDL metabolism as discussed above. LDL is a small cholesterol rich lipoprotein containing only apo B-100.
- LDL is removed from the circulation by the binding of LDL to specific LDL receptor present on the cell of liver and other peripheral tissue. This receptor is defective in familial hypercholesterolemia.

**Fig. 13.28:** Metabolism of chylomicron.
(TG: Triacylglycerol; C: Cholesterol)

**Fig. 13.29:** Metabolism of VLDL and LDL.

- Inside the cell the cholesterol esters are hydrolyzed, thereby making unesterified cholesterol available to the cell.
  - The cells may use this cholesterol to maintain their cell membranes.
  - In specific tissue, such as adrenal cortex, or gonads, this cholesterol is utilized in steroid hormone synthesis.
  - Liver uses this cholesterol for synthesis of bile acids.
- Approximately, 30% of LDL cholesterol is degraded in extrahepatic tissues and 70% in the liver.

## Metabolism of HDL (Fig. 13.30)

- HDLs are synthesized and secreted from liver as disk-shaped nascent HDL particles that consist primarily of phospholipids (largely phosphatidyl-choline also known as lecithin), free cholesterol and apo A-1 as the main apolipoproteins together with apo C-II and Apo E. These nascent HDL particles are nearly devoid of cholesterol ester and triacylglycerol.
- After secretion of nascent HDL into the plasma, the enzyme synthesized by liver, **lecithin: Cholesterol acyltransferase** (LCAT, pronounced 'el cat) binds to the nascent HDL.
- Nascent HDL picks up cholesterol from other lipoproteins and from cell membranes of peripheral tissue. The

**Fig. 13.30:** Metabolism of high density lipoprotein (HDL) and its role in reverse cholesterol transport.
(CE: Cholesterol ester; C: Cholesterol; TG: Triacylglycerol; PL: Phospholipid; LCAT: Lecithin cholesterol acyltransferase)

cholesterol picked up by nascent HDL is converted to cholesterol esters by the action of LCAT in the presence of its activator Apo A-I within the HDL particle.

- As cholesterol in HDL becomes esterified by LCAT activity, it creates a concentration gradient and draws in free cholesterol from tissues and from other lipoproteins.
- As the nascent HDL fill with cholesterol ester, they become spherical in shape. These spherical HDL which is enriched in cholesterol ester enter the liver. In the liver the cholesterol esters are degraded to cholesterol which is utilized for synthesis of bile acids and lipoproteins or excreted into bile as cholesterol.

**Reverse cholesterol transport**

- This is the process whereby excess cholesterol contained in extrahepatic tissue is taken to the liver, through HDL metabolic cycle (Fig. 13.30) for utilization or excretion through the bile.
- The LCAT esterifies the cholesterol content of HDL to prevent it from re-entering the cells. If the cholesterol is not esterified within the HDL particle, the free cholesterol can leave the particle by the same route that it entered. Thus, esterification by LCAT serves to trap cholesterol within the lipoprotein, preventing it from deposition in the tissues.

**Significance of reverse cholesterol transport**

- By reverse cholesterol transport cellular and lipoprotein cholesterol is delivered back to the liver. This is important because the steroid nucleus of cholesterol cannot be degraded; and the liver is the only organ that can remove excess cholesterol by secreting it in the bile for excretion in the feces.
- Reverse cholesterol transport prevents deposition of cholesterol in the tissues and is thought to be anti-atherogenic. An elevated HDL cholesterol (good cholesterol) level decreases the risk of coronary heart disease.

## Disorders of Lipoprotein Metabolism

### *Hyperlipoproteinemia*

In hyperlipoproteinemia, there is elevation of circulating lipoprotein levels. This disorder may be **primary** or **secondary.** Secondary hyperlipoproteinemia is due to some other disease, e.g. diabetes mellitus, nephrotic syndrome, hypothyroidism or alcoholism. The Fredrickson or World Health Organization classification of the hyperlipoproteinemia based on electrophoretic pattern of plasma lipoprotein is the most accepted one. It is given in **Table 13.2** and briefly discussed below.

1. **Type I (Hyperchylomicronemia):** This is due to either deficiency of the enzyme, **lipoprotein lipase** or **lack of apo C** which is required as an activator of lipoprotein lipase. The enzyme defect leads to impaired hydrolysis of chylomicrons resulting in increased level of plasma chylomicron and triacylglycerol.
2. **Type IIa (Familial hypercholesterolemia):** Due to deficiency of functional **LDL receptors** in liver and extrahepatic tissues, cellular uptake of LDL is impaired resulting in high LDL and hypercholesterolemia.
3. **Type IIb (Hypercholesterolemia):** There is elevation of both **LDL** and **VLDL** resulting in elevated level of cholesterol and triacylglycerol. This is believed to be due to excessive production of apo B. Secondary type II hyperlipoproteinemia is seen in obesity, diabetes mellitus, hypothyroidism and nephrotic syndrome.
4. **Type III (Dysbetalipoproteinemia):** There is accumulation of **chylomicron remnants** and **VLDL remnants** in plasma resulting in hypercholesterolemia and hypertriglyceridemia. This is believed to be due to a genetic defect in apo E, which cannot bind to the hepatic apo E receptors.
5. **Type IV (Familial endogenous hypertriglyceridemia):** This is due to overproduction of **triacylglycerol** by liver with a concomitant elevation in plasma VLDL. It may be associated with diabetes mellitus, obesity, alcoholism and impaired glucose tolerance.
6. **Type V:** There is an increase in both **chylomicrons** and VLDL, so triacylglycerol levels are increased. This is usually secondary to other disorders like, obesity, diabetes, alcoholism and renal disease.

### *Hypolipoproteinemia*

In hypolipoproteinemia concentration of one or more lipoproteins in plasma is decreased. The commonest of these disorders are discussed below:

**Table 13.2:** Fredrickson (WHO) classification of hyperlipoproteinemia.

| *Type* | *Elevated plasma lipoprotein* | *Elevated plasma lipid* | *Metabolic defect* | *Risk* |
|---|---|---|---|---|
| I | Chylomicron | Triacylglycerol | Deficiency of lipoprotein lipase or apo C-II | Pancreatitis |
| IIa | LDL | Cholesterol | Deficiency of functional LDL-receptors | Coronary heart disease |
| IIb | LDL and VLDL | Triacylglycerol cholesterol | Overproduction of apo B | Coronary heart disease |
| III | Chylomicron remnants, VLDL remnants | Triacylglycerol cholesterol | Abnormal apo E | Vascular disease |
| IV | VLDL | Triacylglycerol cholesterol | Overproduction of triacylglycerol | Coronary heart disease |
| V | VLDL, chylomicron | Triacylglycerol | Secondary to other disease | Coronary heart disease |

### *Abetalipoproteinemia*

- Abetalipoproteinemia is an autosomal recessive defect that results from a genetic defect in the synthesis of **apo B** and the synthesis of lipoproteins that contain apo B. Both chylomicron and VLDL are affected. As a result, LDL (β-lipoprotein) which is formed from VLDL in the plasma is totally absent.
- Both apo B-48 and B-100 are affected because they are inherited from the same gene.
- Fat malabsorption occurs in abetalipoproteinemia because chylomicrons cannot be formed by the intestine due to lack of apo B-48.
- Absorption of fat soluble vitamins is severely impaired resulting in degenerative changes in retina which may lead to blindness.

### *Tangier Disease or an Alpha-lipoproteinemia*

- Alpha-lipoproteinemia also known as Tangier disease as it was first described in patients from Tangier island.
- Tangier disease is due to an inability to synthesize **apo A.** Apo A is the major apolipoprotein of HDL. This results in decreased level of HDL in plasma and impairs reverse transport of cholesterol. This leads to the accumulation of cholesterol esters in tissue.

## ADIPOSE TISSUE METABOLISM

Triacylglycerol is the major storage form of lipid in humans. Adipose tissue is the main store of triacylglycerol in the body. The triacylglycerol stored in adipose tissue is continually undergoing **lipolysis** (hydrolysis) and **resynthesis.**

### Synthesis of Triacylglycerol in Adipose Tissue

- In adipose tissue, triacylglycerol is synthesized from acyl-CoA and glycerol-3-phosphate.
- For provision of glycerol-3-phosphate, the tissue is dependent on glycolysis and a supply of glucose. The transport of glucose into adipose tissue is stimulated by insulin.
- Some fatty acids, which are incorporated into the triacylglycerol, are synthesized within the adipose tissue from glucose. The remainder is taken up from the blood, which is formed by the action of lipoprotein lipase on the triacylglycerol of chylomicrons and VLDL. Insulin stimulates this process by stimulating lipoprotein lipase.

**Figure 13.31** summarizes the process of triacylglycerol synthesis in adipose tissue.

### Degradation of Triacylglycerols in Adipose Tissue

To leave the adipose tissue, the triacylglycerol must be hydrolyzed to fatty acids and glycerol.

- Triacylglycerol undergoes hydrolysis by a ***hormone sensitive lipase*** to form free fatty acids and glycerol.
- This lipase is distinct from lipoprotein lipase that catalyzes hydrolysis of triacylglycerol before its uptake into extrahepatic tissues.
- The glycerol produced by lipolysis cannot be used by adipose tissues because they lack the enzyme ***glycerol kinase***.

**Fig. 13.31:** Adipose tissue metabolism.
(PPP: Pentose-phosphate pathway; TG: Triacylglycerol; FFA: Free fatty acid; DHAP: Dihydroxyacetone phosphate)

- Therefore, the glycerol is transported to the liver which contains ***glycerol kinase*** to metabolize glycerol to glucose by the process of gluconeogenesis.
- The free fatty acid formed by lipolysis can be reconverted in the adipose tissue to acyl-CoA by ***acyl-CoA synthetase*** and re-esterified with glycerol-3-phosphate to form triacylglycerol.

Thus, there is a continuous cycle of lipolysis and resynthesis (re-esterification) within the adipose tissue. However, when the rate of re-esterification is not sufficient to match the rate of lipolysis, free fatty acids accumulate and diffuse into the plasma and raise the concentration of plasma free fatty acids, which occurs during fasting, diabetes mellitus, anxiety or physical exertion.

**Significance of Adipose Tissue Metabolism**

Fat is stored in the adipose tissue when food is plentiful and the individual is calm and resting. Conversely, fat is made available from adipose tissue in postabsorptive state or in stressful situations, like starvation and diabetes mellitus.

## Regulation of Adipose Tissue Metabolism (Fig. 13.32)

- High levels of insulin in the blood stimulate triacylglycerol formation in adipose tissue and inhibit the lipolysis by inhibiting hormone sensitive lipase.
- Conversely, low insulin concentrations enhance the mobilization of fatty acid from adipose tissue.

**Fig. 13.32:** Regulation of lipolysis in adipose tissue.

- Other hormones like epinephrine and glucagon accelerate the rate of lipolysis of triacylglycerol stores. These hormones activate hormone sensitive lipase.

## FATTY LIVER

Fatty liver is the excessive accumulation of fat primarily ***neutral fat, triacylglycerol*** in the liver. Fat is mainly stored in adipose tissue. Liver is not a storage organ. It contains about 5% fat. In pathological conditions, this may go up to 25–30% and is known as fatty liver or fatty infiltration of liver.

When accumulation of lipid in the liver becomes chronic, fibrotic changes occur in cells which may finally lead to ***cirrhosis*** and impairment of liver function.

### Causes of Fatty Liver

Fatty liver may occur due to:

1. Overproduction of triacylglycerol in liver.
2. Impaired synthesis of VLDL.

- **Overproduction of triacylglycerol:** Fatty liver is associated with increased level of plasma-free fatty acids from the diet (high fat diet) or from the adipose tissue during starvation, diabetes mellitus and alcoholism. The increasing amounts of fatty acids are taken up by the liver and esterified to triacylglycerol. VLDL produced in liver carries this triacylglycerol from liver to the peripheral tissues. But the production of VLDL does not keep pace with the formation of triacylglycerol, allowing triacylglycerol to accumulate in liver.
- **Impaired synthesis of VLDL:** Impairment in the biosynthesis of VLDL, in turn, impairs the transport of triacylglycerol from liver, thus, allowing triacylglycerol to accumulate in the liver. The defect in the synthesis of VLDL may be due to:

1. A block in apolipoprotein synthesis
2. Defect in the synthesis of phospholipids that are found in lipoproteins.
3. A failure in the formation mechanism of lipoprotein itself from lipid and apoprotein.

### Factors that Cause Fatty Liver

1. **High fat diet:** Due to increased supply of free fatty acids from the diet, capacity of liver for lipoprotein formation is outweighed.
2. **Starvation or uncontrolled diabetes mellitus or insulin insufficiency:** Due to increased mobilization of free fatty acids from adipose tissue.
3. **Alcoholism:** Due to increased hepatic triacylglycerol synthesis and decreased fatty acid oxidation.
4. Dietary deficiency of:
   i. **Lipotropic factors:** Deficiency of lipotropic factors like choline, betaine, methionine, lecithin may cause fatty liver. Choline is required for the formation of phospholipid lecithin, which in turn, is an essential component of lipoprotein. Betain and methionine possessing methyl groups can be used to synthesize choline.
   ii. **Essential fatty acids:** Essential fatty acids are required for the formation of phospholipid. A deficiency of essential fatty acids leads to decreased formation of phospholipids.
   iii. **Essential amino acids:** Essential amino acids are required for the formation of apolipoprotein and lipotropic factor choline.
   iv. **Vitamin E and selenium:** Deficiency of vitamin E or selenium enhances the hepatic necrosis. They have protective effect against fatty liver.
   v. **Protein deficiency:** For example, ***in kwashiorkor***, deficiency of protein impairs formation of apolipoprotein.
   vi. **Vitamin deficiency:** Deficiency of pyridoxine and pantothenic acid decrease the availability of ATP, needed for protein biosynthesis.
5. **High cholesterol diet:** Excess amount of cholesterol in diet competes for essential fatty acids for esterification.
6. **Use of certain chemicals:** For example, puromycin, chloroform, carbon tetrachloride, lead and arsenic inhibit protein biosynthesis and impair formation of apolipoprotein.

## Lipotropic Factors

- The substances that prevent the accumulation of fat in the liver are known as ***lipotropic factors***. Dietary deficiency of these factors can result in fatty liver. The various lipotropic agents are:
  - Choline
  - Methionine
  - Betaine, etc.
- Choline is the principal lipotropic factor, and other lipotropic agents act by producing choline in the body, e.g. betaine and methionine possessing methyl groups are donated to ethanolamine to form choline.
- Choline is required for the formation of phospholipid, lecithin, which in turn, is an essential component of lipoprotein. And formation of lipoprotein is important in the disposal of triacylglycerol.
- Vitamin $B_{12}$ and folic acid are also able to produce lipotropic effect, as these are involved in the formation of methionine from homocysteine.
- Casein and other proteins also possess lipotropic activity.

# CHOLESTEROL METABOLISM

Cholesterol is the major sterol in human and hascyclopentanoperhydrophenanthrene ring system as a parent structure **(Fig. 13.33).**

Cholesterol is an **amphipathic** lipid which can be synthesized by most cells of the body and it is obtained from the diet in foods of ***animal origin. It is not synthesized in plants***. The major sources of dietary cholesterol are egg yolk and meat, particularly liver.

## De Novo Synthesis of Cholesterol

Cholesterol is synthesized by a pathway that occurs in most cells of the body. ***Liver*** and ***intestine*** are major sites of cholesterol synthesis.

**Fig. 13.33:** Structure of cholesterol.

All 27-carbon atoms of cholesterol are derived from the **acetyl-CoA.** The enzyme system of cholesterol synthesis is present in ***cytosol*** and ***microsomal*** (endoplasmic reticulum) fractions. The reactions of cholesterol biosynthesis occur into 5 stages **(Figs. 13.34 and 13.35).**

### *Stage 1: Synthesis of Mevalonate from Acetyl-CoA through HMG-CoA*

- First, two molecules of acetyl-CoA condense to form **acetoacetyl-CoA,** catalyzed by a cytosolic ***thiolase*** enzyme.
- Next, a third molecule of acetyl-CoA condenses with acetoacetyl-CoA catalyzed by hydroxy methylglutaryl-CoA synthase ***(HMG-CoA synthase)*** to form hydroxy methylglutaryl-CoA (**HMG-CoA).**
- This sequence of reactions in the cholesterol synthesis is similar to those for the synthesis of ketone bodies **(*see* Fig. 13.11)** except that ketone body synthesis occurs in mitochondria and cholesterol is in the cytosol.
- The next step is catalyzed by ***HMG-CoA reductase***. This enzyme uses two molecules of NADPH and converts HMG-CoA to **mevalonate.** This is the rate limiting step in the cholesterol synthesis.

### *Stage 2: Formation of Isoprenoid Unit*

Mevalonate is phosphorylated by ATP and subsequently decarboxylated to form a five carbon isoprene unit, ***isopentenyl pyrophosphate (IPP)***.

**Fig. 13.34:** Five stages of cholesterol biosynthesis.

**Fig. 13.35:** Biosynthesis of cholesterol, showing its five stages.

### *Stage 3: Formation of Squalene (Condensation of Six Isoprenoid Units)*

Six isopentenyl pyrophosphate molecules condense with loss of their pyrophosphate groups to yield the ***squalene*** (30-carbon atoms compound) through the formation of geranyl pyrophosphate and farnesyl pyrophosphate. ***Squalene was first isolated from the liver of sharks of genus Squalus.***

### *Stage 4: Cyclization of Squalene to Lanosterol*

Squalene undergoes a series of complex enzymatic reactions, in which its linear structure is folded and cyclized

to form lanosterol, which has the four condensed rings that form the steroid nucleus of cholesterol.

### Stage 5: Formation of Cholesterol from Lanosterol

The conversion of lanosterol to cholesterol is a multistep process, resulting in the:

- Shortening of the carbon chain from 30 to 27
- Removal of the three methyl groups at $C_4$
- Migration of the double bond from C8 to $C_5$
- Reduction of the one double bond between $C_{24}$ and $C_{25}$ by NADPH.

## Regulation of De Novo Synthesis of Cholesterol (Fig. 13.36)

***HMG-CoA reductase*** is the rate limiting enzyme in cholesterol biosynthesis and is subjected to different kinds of metabolic control. The following are the different kinds of metabolic control:

### Feedback Regulation

- *Cholesterol* the end product of the pathway as well as ***mevalonate***, represses the synthesis of ***HMG-CoA reductase***.
- It is also repressed by bile salts or bile acids, thus decreasing the cholesterol synthesis.

**Fig. 13.36:** Regulation of cholesterol biosynthesis.

### Hormonal Regulation

- Cholesterol synthesis is increased by **insulin** and **thyroid** hormone and is decreased by **glucagon** and **glucocorticoid** by stimulating and inhibiting HMG-CoA reductase enzyme respectively.

### Nutritional Regulation

- Dietary cholesterol suppresses the synthesis of the HMG-CoA reductase in the liver.
- Increased caloric intake stimulates cholesterol synthesis primarily by increasing the availability of acetyl-CoA and NADPH.

> Drugs like **Statin** inhibit cholesterol synthesis by acting as **competitive inhibitors** of HMG-CoA reductase. These drugs are used to decrease the serum cholesterol level in patients having elevated serum cholesterol concentration.

## Transport of Cholesterol (Fig. 13.37)

### Transport of Dietary Cholesterol from Intestine

- After digestion, dietary cholesterol is absorbed into the intestinal mucosal cells, where much of it is subsequently reconverted into cholesterol esters.

**Fig. 13.37:** Transport of cholesterol.
(C: Cholesterol; LPL: Lipoprotein lipase)

- Cholesterol esters that are synthesized in the mucosal cells, together with some unesterified cholesterol are incorporated into ***chylomicrons***, which transport cholesterol and other dietary lipids from intestine.
- When triacylglycerol of chylomicrons hydrolyzed by **lipoprotein lipase** in the peripheral tissue, only about 5% of the cholesterol ester is lost. The rest is taken up by the liver in the form of chylomicron remnants and is hydrolyzed to cholesterol.

### *Transport of Cholesterol from Liver to Peripheral Tissue*

- Cholesterol from liver is transported in the form of VLDL into the plasma.
- Like chylomicrons VLDL triacylglycerol is hydrolyzed by lipoprotein lipase in the peripheral tissues and results with formation of cholesterol rich LDL. LDL cholesterol is taken up by the liver or extrahepatic tissues by LDL receptors.

### *Transport of Cholesterol from Peripheral Tissue to Liver*

- HDL picks up cholesterol from the peripheral tissues and converts it to cholesterol esters by LCAT enzyme
- These cholesterol esters are ultimately returned to the liver and thus HDL is said to participate in ***"reverse cholesterol transport."***

## Degradation of Cholesterol

Cholesterol can undergo degradative reactions in humans with conversion of cholesterol to physiologically important products, such as:

- Bile acids which is the main catabolic pathway
- Steroid hormones
- Vitamin D

**Fig. 13.38:** Synthesis of bile acid.

### *Formation of Bile Acids*

The primary bile acids ***cholic acid*** and ***chenodeoxycholic*** acid are synthesized in the liver from cholesterol **(Fig. 13.38).**

**Importance of Bile Acids**

- The bile acids required for the emulsification of the dietary lipids and facilitate the enzymatic digestion and absorption of dietary lipids.
- Conversion of cholesterol into bile acid in the liver prevents the body from becoming overloaded with cholesterol.
- As steroidal ring of cholesterol cannot be degraded in the body, the excretion of bile salts serves as a major route for removal of the steroid ring from the body.

### *Formation of Steroid Hormones (Fig. 13.39)*

Cholesterol is the precursor of the five classes of steroid hormones:

1. Progesterone
2. Glucocorticoids
3. Mineralocorticoids
4. Sex hormones androgens
5. Estrogens

### *Formation of Vitamin D*

Cholesterol is also the precursor of vitamin D **(Fig. 13.40)** which regulates calcium and phosphorus metabolism.

## Excretion of Cholesterol

- Cholesterol is excreted in feces.
- Unlike many other metabolites, cholesterol cannot be destroyed by oxidation to $CO_2$ and $H_2O$, because of absence of enzymes capable of catabolizing the steroid ring.

**Fig. 13.39:** Formation of steroid hormones from cholesterol.

**Fig. 13.40:** The formation of vitamin $D_3$ from cholesterol.

- It is excreted in the bile either as cholesterol or after conversion to bile acids. About 1 gm of cholesterol is eliminated from the body per day. Roughly, half is excreted in the form of bile acids and half is in the form of cholesterol.
- Moreover, some dietary cholesterol is excreted in feces without being absorbed.
- Some of the cholesterol in the intestine is acted on by intestinal bacterial enzymes and converted to neutral sterols, coprostanol, and cholestanol which are excreted through feces.

## Blood Cholesterol

The normal total plasma cholesterol lies below 200 **mg per 100 ml.** Cholesterol present in blood occurs both free and as ester form. The hydroxyl group at position three of cholesterol can be esterified with fatty acids producing cholesterol esters. About 70% of the plasma cholesterol is esterified with fatty acids to form cholesterol ester; remaining 30% is free cholesterol.

**Good Cholesterol and Bad Cholesterol**

- HDL cholesterol is considered to be the good cholesterol, because it removes excess cholesterol from peripheral tissues and transport it to the liver where it is degraded or excreted in the bile. HDL thus tends to lower blood cholesterol level.
- On the other hand, LDL cholesterol is called bad cholesterol, because it transports cholesterol from liver to the peripheral tissue. For excretion, cholesterol must enter the liver (***see* Fig. 13.37).** The risk of coronary heart disease (CHD) is related to plasma levels of total cholesterol and LDL-cholesterol.

### *Hypercholesterolemia*

In a normal adult, the total plasma cholesterol ranges form **150–200 mg/100 ml.** An increase in plasma cholesterol more than 250 mg/100 ml is known as **hypercholesterolemia** and is seen in the following conditions:

1. **Diabetes mellitus:** This is due to deficiency of insulin, rate of lipolysis is increased. Increase rate of lipolysis results in more release of free fatty acids in circulation which increases acetyl-CoA. Excess of acetyl-CoA are diverted for cholesterol biosynthesis.
2. **Hypothyroidism:** This is due to decrease in the **HDL receptors** on hepatocyte in hypothyroidism and due to decreased synthesis of **7-α-hydroxylase** that requires for the conversion of cholesterol to bile acids. Thyroid hormone induces the synthesis of 7-α-hydroxylase.
3. **Obstructive jaundice:** Due to decreased excretion of cholesterol through bile.
4. **Familial hypercholesterolemia:** It is a genetic disease caused by deficiency or malfunction of the LDL receptors. In the absence of these receptors, the liver cannot take LDL and there is no release of cholesterol of LDL into the liver. Therefore, they do not cause feedback inhibition of cholesterol synthesis and lead to increased cholesterol formation.

# ATHEROSCLEROSIS

High level of serum cholesterol results in atherosclerosis. The **atherosclerosis** is characterized by hardening and narrowing of the arteries due to deposition of cholesterol and other lipids in the inner arterial wall. Deposition of cholesterol and other lipids in the inner arterial wall leads to formation of plaque (sticky deposit) and results in the endothelial damage and narrowing of the arterial lumen. The hardening and narrowing of coronary arteries result in coronary heart disease (CHD).

## Factors Responsible for Development of Atherosclerosis

### *Age*

Aging brings about changes in the blood vessel wall due to decreased metabolism of cholesterol. As age advance the elasticity of the vessel wall decreases and formation of plaques progresses. The plaques are composed of smooth muscle cells, connective tissue, lipids and debris that accumulate in the inner side of the arterial wall. Formation of plaque leads to narrowing of the lumen and invites atherosclerosis.

### *Sex*

Males are affected more than females, female incidence increases after menopause. Suggesting that male sex hormone might be atherogenic or conversely that female sex hormones might be protective.

### Genetic Factor

Hereditary genetic derangement of lipoprotein metabolism leads to high blood lipid level and familial hypercholesterolemia.

### Hyperlipidemia

Increased levels of the following components of plasma lipids are associated with increased risk of atherosclerosis:

- Total serum cholesterol and triacylglycerol.
- Low density lipoprotein (LDL) is richest in cholesterol and deposits it in tissues and has maximum association with atherosclerosis.
- **Lipoprotein (a) (LPa):** Some people have a special type of abnormal LDL called LP (a) containing an additional protein, ***apoprotein-a***. Elevated LPa levels are associated with an increased risk of coronary heart disease.

### Level of HDL

Low level of HDL is associated with atherosclerosis. HDL has protective effect against atherosclerosis. HDL participates in reverse transport of cholesterol, i.e. it transports cholesterol from cells to the liver for excretion in the bile. The higher the level of HDL, the lower is the risk of ischemic heart disease. Consequently, high ratio of HDL/LDL reduces the development of atherosclerosis. There is an inverse relationship between cardiovascular risks and HDL concentration.

### Hypertension

Hypertension is the major risk factor in patients over 45 years of age. It acts probably by mechanical injury of the arterial wall due to increased blood pressure.

### Cigarette Smoking

Ten cigarettes per day increase the risk three-fold due to reduced level of HDL and accumulating carbon monoxide that may cause endothelial cell injury. Cessation of smoking decreases risk to normal after 1 year.

### Diabetes Mellitus

The risk is due to the coexistence of other risk factors, such as obesity, hypertension, and hyperlipidemia.

### Minor or Soft Risk Factors

These include lack of exercise, stress, obesity, high caloric intake, diet containing large quantities of saturated fats, use of oral contraceptive, alcoholism and hyperuricemia, etc. The risk is due to increased LDL and decreased HDL levels.

**Prevention of Atherosclerosis**

- The most important preventive measure against the development of atherosclerosis is to eat a low fat diet that contains mainly unsaturated fat with low cholesterol content.
- Natural antioxidants, such as vitamin E, C or β-carotene may decrease the risk of cardiovascular disease by protecting LDL against oxidation.
- Moderate consumption of alcohol and exercise appears to have a slightly beneficial effect by raising the level of HDL.
- Drug therapy, e.g. Lovastatin, clofibrate, cholestyramine, which inhibit cholesterol synthesis.

## ALCOHOL METABOLISM

Alcohol, i.e. ethanol is a drug. It has high energy content, yielding about 7.1 kcal/g on oxidation. Liver is the major site of ethanol oxidation. At least three enzyme systems are capable of ethanol oxidation **(Fig. 13.41).**

1. Alcohol dehydrogenase (zinc dependent enzyme).
2. Microsomal ethanol oxidizing system (MEOS).
3. Catalase of peroxisomes.

Only 2–10% of ethanol is excreted unoxidized in the urine and lungs.

**Fig. 13.41:** The metabolism of ethanol.
(MEOS: Microsomal ethanol oxidizing system)

- ***Alcohol dehydrogenase (ADH)*** appears to be the principal pathway for ethanol oxidation. This route is operational for acute intoxication when the blood alcohol concentration is in the range 1–5 mmol/L.
- Above this, most of the ethanol is metabolized via mixed function oxidase system known as ***microsomal ethanol oxidizing system (MEOS)***, which involves ***cytochrome*** $\mathbf{P_{450}}$. MEOS appears to be a secondary enzyme system for ethanol clearance. In the chronic alcoholic, there is an increase in MEOS activity.
- The other ethanol oxidizing system is the ***catalase*** present in ***peroxisomes***. The role of catalase in biological oxidation of ethanol is controversial.
- The product of all three oxidation pathways is ***acetaldehyde***,which is rapidly oxidized to acetate by liver ***aldehyde dehydrogenase (ALDH)*** **(Fig. 13.42).** ALDH enzyme is inhibited by ***disulfiram*** and this property is used in the treatment of alcoholism.
- About 80% of the acetate produced by ALDH leaves the liver and undergoes further metabolism in tissues such as heart and skeletal muscle. Acetate is converted to acetyl-CoA, which is oxidized to $CO_2$ through citric acid cycle yielding ATP.

## Biochemical Alterations due to Alcohol

Excess intake of alcohol leads to excessive production of NADH, with a concomitant decrease in $NAD^+$, produces metabolic changes as follows:

- The increased availability of NADH favours the ***reduction of pyruvate to lactate*** and ***oxaloacetate to malate*** and decreasing its availability for gluconeogenesis, resulting in decreased rate of gluconeogenesis and decreased synthesis of glucose. This can lead to ***hypoglycemia***.
- Increased lactic acid production also can result in hyperlactacidemia. The hyperlactacidemia reduces the capacity of the kidney to excrete uric acid, leading to secondary hyperuricemia and causes aggravation of gout.
- The increased ratio of $NADH/NAD^+$ also inhibit β-oxidation of fatty acids and citric acid cycle and promotes lipogenesis, triacylglycerol synthesis and cholesterol synthesis from acetyl-CoA.
- Accumulation of lipid in most tissues results in fatty liver, fatty myocardium, fatty renal tubules and so on.

**Fig. 13.42:** Alcohol metabolism.
(ADH: Alcohol dehydrogenase; ALDH: Aldehyde dehydrogenase)

# EXAM QUESTIONS

## Long Answer Questions (LAQs)

1. Describe major stages of cholesterol synthesis. How is this regulated?
2. Explain β-oxidation of palmitic acid with energetics.
3. Describe digestion and absorption of lipids. Add a note on fatty liver.
4. Describe metabolism of lipoproteins.
5. Describe formation and breakdown of ketone bodies. Add a note on ketosis.
6. Describe *de novo* synthesis of fatty acids.
7. Describe adipose tissue metabolism.
8. Describe triacylglycerol metabolism.
9. Describe degradation and excretion of cholesterol?
10. Describe metabolic disorders of lipid metabolism.

## Short Notes

1. Fatty liver and lipotropic factors.
2. Hypercholesterolemia.
3. Ketosis.
4. Atherosclerosis.
5. Carnitine transport system of long chain fatty acids.
6. Sphingolipidoses.
7. Regulation of blood cholesterol level.
8. Metabolism of alcohol.
9. Reverse cholesterol transport.
10. Transport of cholesterol.
11. Degradation of cholesterol.

## Solve the Following

**Case History 1**

**Despite strict dietary control, a 55-year-old man had elevated serum cholesterol level. He started to take simvastatin and 3 months later his cholesterol was normal.**

**Questions**

a. Which enzyme of cholesterol biosynthesis is inhibited by simvastatin?
b. What is the role of that enzyme in cholesterol biosynthesis?
c. What is the normal serum cholesterol level?
d. Name precursors of cholesterol synthesis.

**Case History 2**

**A 32-year-old heavy smoker developed a sudden crushing chest pain. He was admitted to the casualty department. Myocardial infarction was confirmed.**

**Questions**

a. Is there any relationship between smoking and myocardial infarction? If yes, what is it?
b. What are the preventive measures against the development of heart disease?
c. Name the lipoproteins that have protective effect against development of myocardial infarction.
d. Name the enzymes likely to be elevated in myocardial infarction.

**Case History 3**

**A 50-year-old male, with history of chronic alcoholism, had fatty liver.**

**Questions**

a. What is fatty liver?
b. What are the causes of fatty liver?
c. What are lipotropic factors? Name any two.
d. What is the nutritional therapy for fatty liver?

**Case History 4**

**A 19-year-old girl was referred to a medical center because of poor exercise tolerance and muscle weakness. After biochemical investigations carnitine deficiency was confirmed.**

**Questions**

a. What is carnitine?
b. What is the role of carnitine?
c. What is cause of muscle weakness?

**Case History 5**

**A 60-year-old woman was referred to a hospital. She was noted to have hypertension. The plasma cholesterol level was 390 mg/dL. An angiogram of the right carotid artery demonstrated a narrowed lumen and the concentration of LDL was elevated.**

**Questions**

a. What is your probable diagnosis?
b. What is the normal plasma cholesterol level?

c. By which enzyme is cholesterol biosynthesis regulated?
d. Which lipoprotein has protective effect against the disorder?

**Case History 6**

**A 35-year-old female was admitted to the hospital. Analysis of her plasma lipid levels revealed an elevated amount of cholesterol and almost no measurable cholesterol esters. No lecithin-cholesterol acyltransferase (LCAT) activity was detected in the patient's plasma.**

**Questions**

a. Where does the LCAT reaction occur?
b. What is the role of LCAT?
c. What is the normal percentage of plasma cholesterol ester and free cholesterol?
d. Name the other enzyme that esterifies cholesterol in tissues.

**Case History 7**

**A 30-year-old woman was hospitalized, with an acute myocardial infarction. Her plasma cholesterol and LDL level were highly elevated. She was found to have familial hypercholesterolemia.**

**Questions**

a. How are LDL formed?
b. What apoprotein does LDL contain?
c. What is the function of LDL?
d. Why LDL is called bad cholesterol?

**Case History 8**

**A male infant was admitted to the hospital. Examination revealed the presence of Gaucher's disease.**

**Questions**

a. What is sphingolipidoses?
b. What is the cause of Gaucher's disease?
c. Name the defective enzyme.
d. What are the clinical symptoms?

## Multiple Choice Questions (MCQs)

1. **Rate controlling step of cholesterol biosynthesis is:**
   a. Lanosterol → Cholesterol.
   b. HMG-CoA → Mevalonic acid + CoA
   c. Acetoacetyl-CoA + Acetyl-CoA → HMG-CoA + CoA
   d. Squalene → Lanosterol

2. **Which one of the following transfers fatty acids from cytosol to mitochondria?**
   a. Carnitine b. Creatine
   c. Citrate d. ACP

3. **Number of β-oxidation cycles undergoes a 14-carbon saturated fatty acid:**
   a. 8 b. 7
   c. 6 d. None of the above

4. **β-oxidation of fatty acids in liver:**
   a. Requires fatty acids with an even number of carbon atoms
   b. Produces only acetyl-CoA
   c. Occurs in the mitochondria
   d. Degrades fatty acids into $CO_2$ and $H_2O$

5. **How many ATPs are produced when palmitoyl-CoA, a 16-carbon saturated fatty acid is oxidized completely to $CO_2$ and $H_2O$?**
   a. 96 b. 108
   c. 106 d. 135

6. **Which of the following intermediates in the oxidation of odd-chain fatty acids is likely to appear in the urine in vitamin $B_{12}$ deficiency?**
   a. Succinic acid b. Methylmalonic acid
   c. Propionic acid d. Butyric acid

7. **Which of the following statements is correct for fatty acid synthesis?**
   a. Occurs in mitochondria
   b. Requires NADPH as a cofactor
   c. Requires NADH as a cofactor
   d. Intermediates are linked to coenzyme A

8. **The following are the features of the fatty acid synthase complex, *except:***
   a. It is a dimer
   b. It is found within cytosol
   c. It requires pantothenic acid as a constituent
   d. It requires biotin as a cofactor

9. **Which of the following is not true for LDL?**
   a. Transport cholesterol to cells
   b. Contains apo B-100
   c. Contains apo C-II
   d. Is a marker for cardiovascular risk

10. **Free fatty acids are transported in plasma as a:**
   a. Component of VLDL
   b. Component of chylomicrons remnants
   c. Part of LDL
   d. Ligand bound to albumin

**11. The concentration of the following is inversely related to the risk of cardiovascular disease:**
a. HDL b. LDL
c. VLDL d. IDL

**12. Which of the following is an abnormal form of lipoprotein?**
a. LDL b. IDL
c. Lp(a) d. Chylomicron remnant

**13. Which of the following is true in fatty acid elongation?**
a. Occurs in mitochondria and microsomes
b. Requires provision of essential fatty acids
c. Requires biotin as a cofactor
d. Occurs only in cytosol

**14. A key intermediate in the biosynthesis of both glycerophospholipids and triacylglycerol is:**
a. Diacylglycerol
b. CDP-choline
c. Phosphatidyl choline
d. Phosphatidic acid

**15. NADPH, utilized for synthesis of fatty acid, can be generated from:**
a. Citrate lyase
b. Mitochondrial malate dehydrogenase
c. Malic enzyme
d. Citrate synthase

**16. Ethanol is converted in the liver to:**
a. Acetone b. Acetaldehyde
c. Methanol d. Lactate

**17. The risk of heart attack can be decreased by, *except:***
a. Cessation of smoking
b. Lowered level of HDL
c. Control of plasma cholesterol
d. Lowered level of LDL

**18. The form in which dietary lipids are transported from intestinal mucosal cells is:**
a. VLDL b. HDL
c. Chylomicrons d. LDL

**19. All of the following are amphipathic lipids, *except:***
a. Triacylglycerol b. Phospholipids
c. Cholesterol d. Fatty acids

**20. The triacylglycerol present in adipose tissue is hydrolyzed by:**
a. Lipoprotein lipase
b. Pancreatic lipase
c. Hormone sensitive lipase
d. Phospholipase

**21. During electrophoretic separation, the fastest moving lipoprotein is:**
a. HDL b. LDL
d. VLDL d. Chylomicrons

**22. Ketosis occurs in all of the following conditions, *except:***
a. Diabetes mellitus
b. Marasmus
c. Prolonged starvation
d. High fat diet

**23. Bile acids are derived from:**
a. Phospholipids
b. Triacylglycerol
c. Fatty acids
d. Cholesterol

**24. Which of the following pathways of oxidation of fatty acids does not require CoA intermediates?**
a. α-oxidation
b. β-oxidation
c. Peroxisomal fatty acid oxidation
d. Oxidation of unsaturated fatty acid

**25. The following are the ketone bodies, *except:***
a. Acetoacetyl-CoA
b. β-hydroxy butyrate
c. Acetone
d. Acetoacetate

**26. Satiety value of fat is due to which of the following hormone?**
a. Insulin b. Glucagon
c. Thyroxine d. Enterogastrone

**27. Which of the following ketone bodies cannot be metabolized in human body?**
a. Acetoacetate
b. Acetone
c. β-hydrdxybutyrate
d. None of the above

**28. Dietary fats after absorption appear in the circulation as**
a. HDL b. VLDL
c. LDL d. Chylomicron

**29. An important feature of Zellweger syndrome is:**
a. Hypoglycemia
b. Accumulation of phytanic acid in tissues
c. Skin eruptions
d. Accumulation of long chain fatty acid in brain tissues

**30. Gaucher's disease is due to deficiency of the enzyme:**
a. Sphingomyelinase
b. Glucocerebrosidase
c. Galactocerbrosidase
d. β-Galactosidase

**31. An enzyme required for the synthesis of ketone bodies as well as cholesterol is:**
a. Acetyl-CoA carboxylase
b. HMG-CoA synthetase
c. HMG-CoA reductase
d. All of the above

**32. Niemann-Pick disease results from deficiency of:**
a. Ceramidase
b. Sphingomyelinase
c. β-Galactosidase
d. Hexosaminidase A

**33. Chylomicron remnants are catabolized in:**
a. Intestine b. Adipose tissue
c. Liver d. Liver and intestine

**34. Free glycerol cannot be used for triglyceride synthesis in**
a. Liver b. Kidney
c. Intestine d. Adipose tissue

**35. Adipose tissue lacks:**
a. Hormone-sensitive lipase
b. Glycerol kinase
c. cAMP-dependent protein kinase
d. Glycerol-3-phosphate dehydrogenase

**36. Propionyl CoA formed by oxidation of fatty acids having an odd number of carbon atoms is converted into:**
a. Acetyl-CoA
b. Acetoacetyl-CoA
c. D-methylmalonyl-CoA
d. Butyryl-CoA

**37. α-Oxidation of fatty acids occurs mainly in:**
a. Liver b. Brain
c. Muscles d. Adipose tissue

**38. Refsum's disease results from a defect in the following pathway except:**
a. Alpha-oxidation of fatty acids
b. Beta-oxidation of fatty acids
c. Gamma-oxidation of fatty acids
d. Omega-oxidation of fatty acids

**39. Human desaturase enzyme system cannot introduce a double bond in a fattyacid beyond:**
a. Carbon 9 b. Carbon 6
c. Carbon 5 d. Carbon 3

**40. Lovastatin is a:**
a. Competitive inhibitor of acetyl-CoA carboxylase
b. Competitive inhibitor of HMG-CoA synthetase
c. Noncompetitive inhibitor of HMG-CoA reductase
d. Competitive inhibitor of HMG-CoA reductase

**41. Abetalipoproteinaemia occurs due to a block in the synthesis of:**
a. Apoprotein A b. Apoprotein B
c. Apoprotein C d. Cholesterol

**42. Mental retardation occurs in:**
a. Tay-Sachs disease
b. Gaucher's disease
c. Niemann-Pick disease
d. All of these

**43. Cholesterol is present in all of the following except:**
a. Egg b. Fish
c. Milk d. Pulses

**44. Which of the following is best described for the lipoprotein synthesized in the intestinal mucosa, containing a high concentration of triacylglycerol and mainly cleared from the circulation by adipose tissue and muscle:**
a. Chylomicron
b. Low density lipoprotein
c. Very low density lipoprotein
d. High density lipoprotein

**45. Which of the following is best described for the lipoprotein synthesized in the liver, contains high concentration of triacylglycerol and mainly cleared from the circulation by adipose tissue and muscle:**
a. Chylomicron
b. Very low density lipoprotein
c. High density lipoprotein
d. Law density lipoprotein

**46. The subcellular site of the breakdown of long chain fatty acids to acetyl CoA via β-oxidation is:**
a. Cytosol
b. Matrix of the mitochondria
c. Mitochondrial intermembrane space
d. Endoplasmic reticulum

**47. Which of the following is the major product of fatty acid synthase?**
a. Acetyl-CoA b. Acetoacetate
c. Palmitoyl-CoA d. Palmitate

**48. The class of drugs called statins reduced blood cholesterol levels by:**
a. Preventing absorption of cholesterol from the intestine
b. Inhibiting the conversion of 3-Hydroxy-3 methylglutaryl-CoA to mevalonate in the cholesterol biosynthesis
c. Increasing the excretion of cholesterol via bile acids
d. Stimulating the activity of LDL receptor

**49. Which one of following statements is correct concerning the biosynthesis of cholesterol:**
a. Synthesis occurs in the cytosol of the cell.
b. All carbon atoms in the cholesterol synthesis originates from acetyl-CoA
c. The initial precursor is mevalonate
d. Rate limiting enzyme is HMG-CoA synthase

**50. Ketone bodies formed in liver are mainly used for which of the following processes.**
a. Excretion as waste products
b. Energy generation in liver
c. Generation of energy in the tissues
d. Conversion to fatty acids

**51. Which of the following will be elevated in the bloodstream about 2 hours after eating a high fat meal?**
a. HDL
b. Ketone bodies
c. Chylomicrons
d. VLDL

**52. Which of the following will be elevated in the bloodstream about 4 hours after eating a high fat meal?**
a. Chylomicrons
b. High density lipoprotein
c. Very low density lipoprotein
d. Ketone bodies

**53. Which of the following is best described for lipoproteins formed in the circulation by removal of triacylglycerol from very low density lipoprotein and containing cholesterol taken up from high density lipoprotein, cleared by the liver.**
a. Chylomicron
b. High density lipoprotein
c. Low density lipoprotein
d. Very low density lipoprotein

## Answers for MCQs

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **1.** b | **2.** a | **3.** c | **4.** c | **5.** c | **6.** b | **7.** b | **8.** d |
| **9.** c | **10.** d | **11.** a | **12.** c | **13.** a | **14.** d | **15.** c | **16.** b |
| **17.** b | **18.** c | **19.** a | **20.** c | **21.** a | **22.** b | **23.** d | **24.** a |
| **25.** a | **26.** d | **27.** b | **28.** d | **29.** d | **30.** b | **31.** b | **32.** b |
| **33.** c | **34.** d | **35.** b | **36.** c | **37.** b | **38.** a | **39.** a | **40.** d |
| **41.** b | **42.** d | **43.** d | **44.** a | **45.** b | **46.** b | **47.** d | **48.** b |
| **49.** b | **50.** c | **51.** c | **52.** c | **53.** c | | | |

CHAPTER

# 14

# Protein Metabolism

**Chapter outline**


- Digestion and Absorption of Proteins
- Amino Acid Pool
- Protein Turnover
- Nitrogen Balance
- Catabolism of Amino Acids
- Formation of Ammonia
- Metabolic Fate of Ammonia
- Urea Cycle
- Metabolism of Glycine
- Metabolism of Aromatic Amino Acids
- Metabolism of Sulfur Containing Amino Acids
- One Carbon Metabolism
- Metabolism of Branched Chain Amino Acids
- Metabolism of Hydroxy Group Containing Amino acids
- Metabolism of Acidic Amino Acids
- Metabolism of Imino Acid
- Metabolism of Basic Amino Acids
- Biogenic Amines


## INTRODUCTION

Proteins, the primary constituents of the body, may be structural or functional. A regular and adequate supply of protein in diet is essential for cell integrity and function. Dietary proteins are the primary sources of the nitrogen that is metabolized by the body. Adult man requires ***70 to 100 gm*** protein per day. Dietary proteins serve three broad functions:

1. Their constituent amino acids are used for synthesis of the body's proteins.
2. The carbon skeletons of the amino acids can be oxidized to yield energy.
3. Their carbon and nitrogen atoms may be used to synthesize other nitrogen containing cellular constituents as well as many non-nitrogen containing metabolites.

## DIGESTION AND ABSORPTION OF PROTEINS

Proteolytic enzymes (also called proteases) break down dietary proteins into their constituent amino acids. These enzymes are produced by three different organs; the ***stomach,*** the ***pancreas*** and the ***small intestine***.

### Digestion in Mouth

There is no digestion of protein in mouth. It starts in stomach.

### Digestion in Stomach

When protein enters the stomach, it stimulates the secretion of the hormone ***gastrin,*** from gastric mucosal cells, which in turn, stimulates the release of gastric juice containing **hydrochloric acid,** proenzyme (zymogen) **pepsinogen** and **rennin** in infants.

- **Hydrochloric acid:** Denatures proteins making their internal peptide bonds more accessible to subsequent hydrolysis by proteoses and provides an acid environment for the action of pepsin.
- **Pepsin:** It is secreted as the proenzyme ***pepsinogen,*** an inactive form.
- It is converted into active pepsin in the gastric juice by the enzymatic action of pepsin itself or by high hydrogen ion concentration **(Fig. 14.1)**.
- Pepsin catalyses hydrolysis of peptide bonds adjacent to amino acids with bulky side chains like:
  - ***Aromatic amino acids***
  - ***Branched amino acids*** and ***methionine***

**Fig. 14.1:** Activation of pepsinogen and action of pepsin.

Thus, pepsin cleaves long polypeptide chains into a mixture of smaller peptides and some free amino acids.

- **Rennin** is important in the digestive processes of infants. It is absent in adults. Rennin is also called **chymosin** or **rennet**.
- Action of rennin is to clot milk. This is accomplished by the slight hydrolysis of the casein of milk to produce **paracasein,** which coagulates in the presence of calcium ions, resulting in an insoluble **calcium-paracaseinate** curd. Calcium paracaseinate is then acted on by **pepsin**.

The purpose of this reaction is to convert milk into a more solid form to prevent the rapid passage of milk from the stomach of infants.

> Rennin and renin are different. Renin is secreted by kidney and is involved in regulation of water and electrolyte balance and blood pressure.

## Digestion in Intestine by Pancreatic Enzymes

There are two types of peptidase enzymes secreted by pancreas:

1. Endopeptidase
2. Exopeptidase

### *Endopeptidase*

- Endopeptidases cleave internal peptide bonds. This results into formation of smaller peptides from large polypeptides.
- Endopeptidases secreted by pancreas, are ***trypsin, chymotrypsin*** and ***elastase***. These are secreted in proenzyme (inactive) forms, ***trypsinogen, chymotrypsinogen*** and **proelastase**.

### *Exopeptidase*

- Exopeptidase which hydrolyze the peptide bonds of terminal amino acids. Exopeptidases are of two types:

**Fig. 14.2:** Activation of pancreatic proenzymes.

  - **Carboxypeptidase** secreted by pancreas act on C-terminal amino acid.
  - **Aminopeptidases** secreted by mucosal cell act on N-terminal amino acid.

### *Activation of Pancreatic Proenzymes*

Activation of the pancreatic proenzymes occurs by the action of ***enteropeptidase (enterokinase),*** secreted by duodenal epithelial cells. Enteropeptidase activates trypsinogen to trypsin and the activated trypsin in turn activates more trypsinogen. Trypsin also activates the chymotrypsinogen, proelastase and procarboxypeptidase **(Fig. 14.2)**.The specificity of these proteolytic enzymes is given below **(Table 14.1)**.

- **Trypsin** hydrolyzes peptide bonds those contributed by ***lysine*** and ***arginine*** residues.
- **Chymotrypsin** preferentially cleaves peptide bonds involving ***aromatic*** **amino** ***acids***
- **Elastase** hydrolyzes those peptide bonds, formed by ***small neutral aliphatic amino acids***.

Trypsin, chymotrypsin and elastase, thus, hydrolyze polypeptides, resulting from the action of pepsin in the stomach into smaller peptides. Degradation of short

**Table 14.1:** Specificity of proteolytic enzymes.

| *Enzyme* | *Peptide bond specificity* |
|---|---|
| Pepsin | Peptide bonds adjacent to amino acids with bulky side chains like, Aromatic amino acids, Branched amino acids and methionine |
| Trypsin | Peptide bonds contributed by lysine and arginine |
| Chymotrypsin | Aromatic amino acids |
| Elastase | Small neutral aliphatic amino acids |
| Carboxypeptidase | Free C-terminal amino acids |
| Aminopeptidase | Free N-terminal amino acids |

peptides formed in the small intestine is continued by an **exopeptidase**.

- **Carboxypeptidase** (zinc containing enzyme), an exopeptidase removes the free carboxyl terminal amino acid residues from peptide.

### *Digestion in Intestine by Intestinal Proteoses*

The digestion of products formed by hydrolysis by pepsin, trypsin, elastase, chymotrypsin and carboxypeptidase are completed by enzymes, secreted by the mucosa of the small intestine such as **aminopeptidases** and **dipeptidases**.

- Aminopeptidase is an exopeptidase; hydrolyse free N-terminal amino acids of the short peptides.
- The dipeptidases complete digestion of dipeptides to free amino acids. These dipeptidases can then finally convert all ingested protein into free amino acids. Dipeptidases require ***cobalt*** or ***manganese*** ions for their activity.

The hydrolysis of most proteins is thus completed to their constituent amino acids which are then ready for absorption into the blood.

## Absorption of Amino Acids

- The absorption of most amino acids involves an ***active transport mechanism,*** requiring ATP and specific transport proteins in the intestinal mucosal cells.
- Many transporters have $Na^+$ dependent mechanisms, coupled with $Na^+$ $K^+$ pump, similar to those described for glucose absorption **(Fig. 14.3)**.

**Fig. 14.3:** Transport of L-amino acid across the intestinal epithelium.

- Several $Na^+$ independent transport proteins are found in the brush-border membrane that is not specific for each amino acid but rather for the groups of structurally similar amino acids.
- All are specific for only L-amino acid. D-amino acids are transported by passive diffusion.
- Thus, amino acids, released by digestion, pass from the gut through hepatic portal vein to the liver.
- Alton Meister proposed that glutathione (γ-glutamyl cysteinylglycine) participates in absorption of amino acids in **intestine, kidneys** and **brain** and the cycle is called **gamma-glutamyl cycle** or **Meister cycle**.

**Absorption of Intact Protein**

Small intestinal cells of fetus and new-born infants are able to absorb intact proteins, **e.g.** ***immunoglobulin IgA from colostrum of maternal milk are absorbed intact without loss of biologic activity, so that they provide passive immunity to the infant.***

The intact proteins are not absorbed by the adult intestine. However, in some adult individuals, small amount of intact proteins may be absorbed through the intestinal mucosa. These proteins often cause formation of antibodies against the foreign protein and are responsible for the symptoms of food allergies.

## AMINO ACID POOL

Amino acids, released by hydrolysis of dietary protein, and tissue proteins together constitute the ***amino acid pool*** **(Fig. 14.4)**.

- In contrast to carbohydrates and fat whose major function is to provide energy, the primary role of amino acids is to serve as building blocks of synthesis of tissue protein and other nitrogen containing compounds.
- Amino acids, in excess of those needed for the synthesis of proteins and other biomolecules cannot be stored in contrast with fatty acids and glucose, nor are they excreted. Surplus amino acids are oxidized for energy. Liver is the major site of amino acid oxidation.

## PROTEIN TURNOVER

Most proteins in the body are constantly being synthesized and then degraded. In healthy adults, the total amount of protein in the body remains constant because the rate of protein synthesis is just sufficient to replace the protein that is degraded. This process is called ***protein turnover,*** which leads to the hydrolysis and resynthesis of ***300 to 400 gm of body proteins each day***. The turnover is high in infancy and decreases with age advance.

**Fig. 14.4:** Amino acid pool.

## NITROGEN BALANCE

Catabolism of amino acids leads to a net loss of nitrogen from the body. This loss must be compensated by the diet in order to maintain a constant amount of body protein. Nitrogen balance studies evaluate the relationship between the nitrogen intake (in the form of protein) and nitrogen excretion.

Three situations of nitrogen balance are possible as follows:

1. Nitrogen equilibrium
2. Positive nitrogen balance
3. Negative nitrogen balance.

### Nitrogen Equilibrium

- In normal adults, nitrogen intake is equal to nitrogen excretion. The subject is said to be in nitrogen equilibrium or balance.
- In this situation, the rate of body protein synthesis is equal to the rate of degradation.

### Positive Nitrogen Balance

- In this situation, nitrogen intake > nitrogen excretion, i.e. intake of nitrogen is more than excretion.
- It shows that nitrogen is retained in the body, which means that protein is laid down.
- This occurs in growing ***infants*** and ***pregnant women***.

### Negative Nitrogen Balance

- In this situation, nitrogen intake < nitrogen excretion, i.e. nitrogen output exceeds input and this occurs during ***serious illness*** and **major *injury*** and ***trauma,*** in ***advanced cancer*** and following failure to ingest adequate or sufficient high quality protein, e.g. in ***kwashiorkor*** and ***marasmus***.
- If the situation is prolonged, it will ultimately lead to death.

## CATABOLISM OF AMINO ACIDS

The complete catabolism of amino acids includes following stages:

1. The removal of α-***amino group*** in the form of ***ammonia*** by following reactions:
   i. **Transamination** by the enzyme ***aminotransferase*** also called ***transaminases***.
   ii. **Deamination** may be oxidative or nonoxidative
      a. Oxidative deamination is by glutamate dehydrogenase or amino acid oxidase.
      b. Nonoxidative deamination is by amino acid dehydratase.
2. Disposal of **ammonia** in the form of **urea** in the liver by reactions of the **urea cycle**.
3. Disposal (catabolism) of the remaining carbon skeleton of amino acid to carbon dioxide and water by reactions of **citric acid cycle**.

## FORMATION OF AMMONIA

### Transamination

Transamination involves the transfer of α-amino group of α-amino acid to an α-keto acid to form new amino acid and a new keto acid. The enzymes that catalyze these reactions are called ***aminotransferases*** or ***transaminases*** **(Fig. 14.5)**.

**Fig. 14.5:** Transamination reaction.

**Fig. 14.6:** Reactions catalyzed by alanine transaminase and aspartate transaminase.

- Most transaminases use α-ketoglutarate (α-keto acid) as a common acceptor of amino groups.
- All transaminases require **pyridoxal phosphate (PLP)** as a coenzyme. Some of the most important transaminases are ***alanine transaminase (ALT)*** and ***aspartate transaminase (AST)*** **(Fig. 14.6)**.
- Alanine transaminase (ALT) also called ***glutamate pyruvate transaminase (GPT),*** catalyzes the transfer of amino group of alanine to α-ketoglutarate resulting in the formation of **pyruvate** and L-glutamate.
- Aspartate transaminase (AST), also called ***glutamate oxaloacetate transaminase (GOT),*** catalyzes the transfer of the amino group of aspartate to α-ketoglutarate, resulting in the formation of **oxaloacetate** and L-glutamate.

Most amino acids undergo transamination reaction except ***lysine, threonine,*** **proline** and ***hydroxyproline***.

It will be noted that there is no net loss of amino groups in transamination reactions.

### *Metabolic Significance of Transamination Reactions*

- This reaction provides a mechanism for collecting the amino groups from many different amino acids into one common product L-glutamate. This is important because glutamate is the only amino acid whose α-amino group can be directly removed at a high rate by oxidative deamination.
- Since transamination reactions are readily reversible, this permits transaminases to function both in amino acid catabolism and biosynthesis. L-glutamate, produced by transamination, can be used as an amino group donor in the synthesis of nonessential amino acids.

**Fig. 14.7:** Oxidative deamination by L-glutamate dehydrogenase.

**Clinical Significance of Transaminase Enzyme**

Serum levels of some transaminases are elevated in some disease state and measurement of these are useful in medical diagnosis, e.g. ALT (GPT) and AST (GOT) are important in the diagnosis of liver and heart damage (Refer Chapter 6: Enzyme).

## Deamination

### *Oxidative Deamination by Glutamate Dehydrogenase*

- The α-amino groups of most amino acids are ultimately transferred to α-ketoglutarate by transamination forming L-glutamate.
- Then L-glutamate undergoes oxidative deamination by the action of **L-glutamate dehydrogenase,** which requires $NAD^+$ or $NADP^+$ as an oxidizing agent **(Fig. 14.7)**.
- ***Thus, the net removal of α-amino groups to ammonia requires the combined action of glutamate transaminase and glutamate dehydrogenase.***

*Metabolic significance*

- This freely reversible reaction functions both in amino acid catabolism and biosynthesis.
- Catabolically, it channels nitrogen from glutamate to ammonia and anabolically it catalyzes amination of α-ketoglutarate by free ammonia to form glutamate.

**Fig. 14.8:** Oxidative deamination by amino acid oxidase.

**Clinical Significance of Glutamate Dehydrogenase (GLD)**

GLD is present in normal serum in trace amount only, but increased activities are observed in cases of liver disease.

### *Oxidative Deamination by Amino Acid Oxidases*

- Both L- and D-amino acid oxidases occur in the kidneys and the liver. However, their activities are low. The reaction catalyzed is given in **Figure 14.8**.
- Amino acid oxidases use auto oxidizable flavins (FMN or FAD) as coenzyme, which oxidize amino acids to an α-***imino acid.*** α-imino acid is an unstable compound which decomposes to the corresponding α-keto acid with release of ammonium ion. In this reaction, oxygen is reduced to $H_2O_2$, which is later decomposed by catalase.

*Metabolic significance*

D-amino acids present in the diet are metabolized by D-amino acid oxidase in the liver.

### *Nonoxidative Deamination by Amino Acid Dehydratase*

- The α-amino groups of ***serine*** and ***threonine*** can be directly deaminated into $NH_4^+$ ion. These amino acids contains hydroxy group attached to its β-carbon atom.
- These direct deamination are catalyzed by ***serine dehydratase*** and ***threonine dehydratase***, in which pyridoxal phosphate (PLP) is the coenzyme **(Fig. 14.9)**.

**Fig. 14.9:** Nonoxidative deamination by amino acid dehydratase.

## METABOLIC FATE OF AMMONIA

Ammonia is produced in most tissues. Since ammonia is extremely toxic, it is immediately converted to non-toxic metabolites such as, **glutamate** or **glutamine** or **alanine** and ultimately to **urea**. For the ultimate conversion of ammonia to urea, ammonia is transported to the liver.

### Transport of ammonia to the liver

Since free ammonia is highly toxic, it is never transported in free form in blood. Two mechanisms are available in humans for the transport of ammonia from the peripheral tissues to the liver for its ultimate conversion to urea.

### *Transport of Ammonia in the Form of Glutamine*

- In many tissues (liver, kidney and brain), ammonia is enzymatically combined with glutamate to yield glutamine by the action of **glutamine synthetase**.
- The glutamine, so formed is a neutral nontoxic major transport form of ammonia. The glutamine is transported by blood to the liver, where it is cleaved by **glutaminase** to yield glutamate and free ammonia **(Fig. 14.10)**
- The ammonia so formed is converted by the liver into urea.

### *Transport of Ammonia in the Form of Alanine*

- Alanine transports ammonia from muscles to the liver through **glucose alanine cycle** as shown in **(Fig. 12.11)**.
- In muscle, glutamate is formed from ammonia and α-ketoglutarate by reversal of the glutamate dehydrogenase reaction **(*see* Fig. 14.7)**.
- L-glutamate then transfers its α-amino group to pyruvate by transamination reaction to form **alanine**.
- Alanine so formed in muscle is transported to liver where it is converted to pyruvate and glutamate again by transamination reaction.
- In the liver, glutamate undergoes oxidative deamination to release free ammonia, which is converted to urea and pyruvate is converted to glucose by gluconeogenesis.

**Fig. 14.10:** Formation and breakdown of glutamine.

## Formation of Urea

- Urea is the end product of **protein metabolism**. The nitrogen of amino acids removed in the form of ammonia is detoxified by converting it to **urea**.
- Formation of urea by ***"Kreb's Henseleit urea cycle"*** is an ultimate route for the metabolic disposal of ammonia.
- Urea is produced exclusively by the liver and then is transported through blood to the kidneys for excretion in the urine.
- Urea is formed from ammonia, carbon dioxide and α-amino nitrogen of aspartate, which requires ATP.
- Enzymes catalyzing the urea cycle reactions are distributed between the ***mitochondria*** and the ***cytosol*** of the liver **(Fig. 14.11)**.

The first two reactions of urea cycle occur in the mitochondria, whereas the remaining reactions occur in the cytosol.

## UREA CYCLE

The sequence of reactions involved in the biosynthesis of urea, summarized in five steps as follows:

1. ***Formation of carbamoyl phosphate***: The biosynthesis of urea begins with the condensation of carbon dioxide, ammonia and ATP to form **carbamoyl phosphate**, a reaction catalyzed by mitochondrial ***carbamoyl phosphate synthetase-I (CPS-I)***. Formation of carbamoyl phosphate requires 2 molecules of ATP. One ATP serves as a source of phosphate and second ATP is converted to AMP and PPi.
2. ***Formation of citrulline***: Carbamoyl phosphate donates its carbamoyl group to ornithine to form **citrulline** and release phosphate, in a reaction catalyzed by ***ornithine transcarbamoylase***, a $Mg^{2+}$ requiring mitochondrial enzyme. The citrulline so formed now leaves the mitochondria and passes into the cytosol of the liver cell.
3. ***Formation of arginosuccinate***: The transfer of the second amino group (from aspartate) to citrulline occurs by a condensation reaction between the amino group of aspartate and citrulline in the presence of ATP to form **arginosuccinate**. This reaction is catalyzed by arginosuccinate synthetase of the liver cytosol, a $Mg^{2+}$ dependent enzyme.

**Fig. 14.11:** Reactions of urea cycle.

4. ***Formation of arginine and fumarate***: Arginosuccinate is cleaved by arginosuccinate lyase (arginosuccinase) to form free arginine and fumarate. The fumarate so formed returns to the pool of citric acid cycle intermediates. Though fumarate urea cycle is linked with the citric acid cycle, the two Kreb's cycles together have been referred to as the Kreb's bi cycle.
5. ***Formation of urea and ornithine***: In the last reaction of urea cycle the liver hydrolytic enzyme ***arginase***, cleaves arginine to yield urea and ornithine. Ornithine is thus regenerated and can enter mitochondria again to initiate another round of the urea cycle. The urea thus formed is excreted in the urine.

Human beings excrete about 10 kg of urea per year.

## Energy Cost of Urea Cycle

Four ATPs are consumed in the synthesis of each molecule of urea as follows:

- Two ATP are needed to make carbamoyl phosphate.
  - One ATP serves as a source of phosphate
  - Second ATP is converted to AMP + PPi.
- One ATP is required to make arginosuccinate.
- One ATP is required to restore AMP to ATP.

**Significance of Urea Cycle**

- The toxic ammonia is converted into the harmless nontoxic urea.
- It disposes off two waste products, ammonia and $CO_2$.
- It forms **semi-essential** amino acid, **arginine.**
- It participates in the regulation of blood pH, which depends upon the ratio of dissolved $CO_2$, i.e. $H_2CO_3$ to $HCO_3^-$.

## Regulation of Urea Cycle

- ***Carbamoyl phosphate synthetase-I*** is an allosteric regulatory enzyme of urea cycle, which is allosterically activated by ***N-acetylglutamate (NAG)*** **(Fig. 14.12)**. NAG is synthesized from acetyl-CoA and glutamate by NAG-synthase to activate CPS-I. It has no other function.

**Fig. 14.12:** Activation of N-acetylglutamate.

- The synthesis of NAG increases after intake of protein rich diet, by arginine and during starvation, which ultimately increases the urea formation.

## Metabolic Inborn Errors of Urea Cycle

- Five disorders associated with each of the five enzymes of urea cycle have been reported **(Table 14.2)**.
- Since urea synthesis converts toxic ammonia to non-toxic urea, all defects in urea synthesis result in ***hyperammonemia*** and ***ammonia intoxication.***
- This intoxication is more severe when the metabolic block occurs at reaction I or II, since it accumulates ammonia itself.
- Deficiency of later enzymes result in the accumulation of other intermediates of the urea cycle, which are less toxic and therefore severity of symptoms is less.

### *Symptoms*

Clinical symptoms, common to all urea cycle, disorders include:

- Ammonia intoxication
- Protein induced vomiting
- Intermittent ataxia
- Irritability
- Lethargy
- Mental retardation.

**Table 14.2:** Disorders caused by genetic defects of urea cycle enzymes.

| *Disorders* | *Defective enzyme* | *Products accumulated* |
|---|---|---|
| Hyperammonemia type-I | Carbamoyl phosphate synthetase-I | Ammonia |
| Hyperammonemia type-II | Ornithine transcarbamoylase | Ammonia |
| Citrullinemia | Arginosuccinate synthetase | Citrulline |
| Argininosuccinic aciduria | Argininosuccinate lyase | Argininosuccinate |
| Argininemia | Arginase | Arginine |

### *Treatment*

- Low protein diet, food intake should be in frequent small meals to avoid sudden increase in blood ammonia levels.

**Ammonia intoxication**

- Symptoms of ammonia intoxication include:
  - An impaired function of the brain
  - Tremor
  - Ataxia
  - Convulsions
  - Lethargy
  - Nausea
  - Vomiting
  - Slurred speech
  - Blurred vision
  - In severe cases, coma and death.
- The toxic effect of ammonia may be due to:
  - A decrease in the formation of ATP by citric acid cycle because of diversion of excessive amounts of α-ketoglutarate from citric acid cycle intermediates to form glutamate and glutamine (to detoxify ammonia) in the brain and thus lowering the rate of oxidation of glucose, the major fuel of the brain.
  - By increased formation of GABA from glutamate leads to impaired neural transmission process.
  - One more possibility is that elevated levels of glutamine, formed from $NH4^+$ and glutamate produce osmotic effect that leads directly to swelling of the brain.

## Blood Urea

- Normal range of blood urea for a healthy adult is **20 to 40 mg/dL**. High protein diet shows increase in level of blood urea concentration.
- In clinical practice, blood urea level is taken as an indicator of **renal function**.
- The term **uremia** is used to indicate increased blood urea levels. For convenience, the causes of high blood urea are subdivided into three classes **(Table 14.3)**.

**Table 14.3:** Causes of high blood urea (Uremia).

| *Types* | *Causes* |
|---|---|
| Prerenal uremia | High protein diet<br>Any cause of increased protein catabolism, e.g. trauma, surgery, starvation, diabetes mellitus.<br>Any cause of impaired renal perfusion, e.g. cardiac failure |
| Renal uremia | Any cause that leads to reduced GFR and leads to urea retention |
| Postrenal uremia | Any cause of obstruction to urine outflow, e.g. benign prostatic hypertrophy, malignant stricture or obstruction, stone |

# CATABOLISM OF CARBON SKELETON OF AMINO ACIDS

The catabolism of 20 amino acids of proteins involves the removal of α-amino groups followed by the breakdown of the resulting carbon skeletons. The carbon skeletons of 20 amino acids are converted into seven products. These are:

- Pyruvate
- Acetyl-CoA
- Acetoacetyl-CoA
- α-Ketoglutarate
- Succinyl-CoA
- Fumarate
- Oxaloacetate.

All these enter the citric acid cycle and are used for either the synthesis of glucose or lipid or in the production of energy through their oxidation to $CO_2$ and $H_2O$.

- Amino acids that are degraded to acetyl-CoA or acetoacetyl-CoA are termed ***ketogenic,*** because they give rise to ketone bodies. Among 20 amino acids, only ***leucine*** and ***lysine*** are purely ketogenic.
- Amino acids that are degraded to pyruvate, α-ketoglutarate, succinyl-CoA, fumarate or oxaloacetate are termed ***glucogenic*** because synthesis of glucose from these amino acids is possible.
- ***Isoleucine, phenylalanine, tryptophan*** **and** ***tyrosine*** are both glucogenic and ketogenic. The other fourteen amino acids are classed as purely glucogenic.

Metabolic fate of carbon skeleton of 20 amino acids is described in **Figure 14.13**.

# METABOLISM OF GLYCINE

Glycine is a nonessential or dispensable amino acid.

## Synthesis of Glycine

- It is synthesized from **serine** by removal of **hydroxymethyl** group from the side chain of serine.
- The methylene carbon group of hydroxymethyl is transferred to tetrahydrofolate and the hydroxy group is released as water **(Fig. 14.14)**.This reaction is reversible, allowing glycine and serine to be interconverted.

**Fig. 14.13:** Metabolic fate of carbon skeleton of amino acids.
Yellow shade: Glucogenic amino acids, Green shade: Glucogenic and ketogenic amino acids, Red shade: Ketogenic amino acids.

**Fig. 14.14:** Synthesis of glycine from serine (THF: Tetrahydrofolate).

## Catabolism of Glycine

Glycine is catabolized by following three ways:

1. It can be converted into serine, a 3-carbon amino acid, by addition of hydroxy methyl group carried by the coenzyme tetrahydrofolate (THF) **(Fig. 14.14)**.
2. The major pathway of glycine degradation is oxidative cleavage of glycine into $CO_2$ and $NH_4^+$ and a methylene group ($CH_2$) which is accepted by THF. This reversible reaction is catalyzed by glycine synthase **(Fig. 14.15)**. $N^5$, $N^{10}$-methylene THF is used in certain biosynthetic pathways.
3. Glycine may be oxidatively deaminated by ***glycine oxidase*** to glyoxylic acid **(Fig. 14.15)**.
   - Glyoxylic acid formed:
     - May be decarboxylated to yield formaldehyde (formate) and $CO_2$
     - May also be converted to malate and then metabolized by the citric acid cycle
     - May be oxidized to oxalate and excreted.
   - Metabolic defect of glyoxylate metabolism, associated with failure to convert glyoxylate to formate, as a result excess of glyoxylate is oxidized to oxalate.

## Metabolic Importance of Glycine (Fig. 14.16)

Glycine is necessary for the formation of following products:

1. **Synthesis of heme:** Glycine along with succinyl-CoA serves as a precursor for heme synthesis.
2. **Synthesis of glutathione:** Glycine is required for the formation of biologically important peptide, glutathione (γ-glutamyl-cysteinyl-glycine).
3. Glycine is involved in **detoxification reactions,** e.g. benzoic acid is detoxicated by conjugating with glycine to hippuric acid.

**Fig. 14.15:** Catabolic pathways of glycine.

**Fig. 14.16:** Metabolic importance of glycine.

4. **Formation of bile acids:** The bile acids cholic acid and chenodeoxy cholic acid are conjugated with glycine to form glycocholic acid and glycochenodeoxy cholic acid respectively. Conjugation lowers the pK of the bile salts, making them better detergents.
5. **Formation of purine ring:** Glycine is required for the formation of purine ring. It provides $C_4$, $C_5$ and $N_7$ of the purine ring.
6. **Synthesis of creatine:** Glycine is necessary for the formation of creatine. It is synthesized from 3 amino acids, **glycine**, **arginine** and **methionine**. Creatin is present in muscle and brain tissue as the high energy compound **creatine phosphate**. Phosphocreatine functions as a store of high energy phosphate in muscle.
7. **Collagen formation:** In collagen glycine occurs at every third position of a chain of the triple helix.
8. Glycine is **glucogenic** amino acid, thus involved in the synthesis of glucose.
9. Glycine is constituent of various tissue proteins, hormones and enzymes.
10. By way of glyoxylate, it may form formate, which by THF may be incorporated into wide variety of biologically active compounds.

## Metabolic Disorders of Glycine

### *Primary Hyperoxaluria*

- It is an inborn error, characterized by high urinary excretion of oxalate.
- The metabolic defect involves a failure to catabolize glyoxylate, which therefore gets oxidized to **oxalate**. Increased level of oxalate results in **urolithiasis** (stone in urinary tract), **nephrocalcinosis** (presence of Ca deposits in kidneys) and **recurrent infections** of urinary tract. Death occurs in childhood or early adult life from renal failure or hypertension.
- Glycinuria
- It is an inborn error characterized by increased excretion of glycine through urine, despite plasma concentration of glycine is normal.
- Since plasma levels are normal, glycinuria occurs probably due to a ***defect in renal tubular reabsorption of glycine.***
- Glycinuria is characterized by increased tendency for the formation of oxalate renal stones.

### *Non-Keto Hyperglycinemia*

Non-keto hyperglycinemia is due to defects in **glycine synthase** (also called glycine cleavage enzyme) of catabolic pathway of glycine **(Fig. 14.15)**. Non- keto hyperglycinemia is characterized by elevated serum levels of glycine leading to severe mental deficiencies and death in very early childhood. At high levels, glycine inhibits neurotransmitters leading to neurological defects.

## METABOLISM OF AROMATIC AMINO ACIDS

- **Phenylalanine, tyrosine** and **tryptophan** are the aromatic amino acids.
- Phenylalanine and tryptophan are nutritionally essential amino acids but tyrosine is not as it can be synthesized from phenylalanine.

### Metabolism of Phenylalanine and Tyrosine

#### *Catabolism of Phenylalanine and Tyrosine*

Phenylalanine metabolism is initiated by its oxidation to tyrosine which then undergoes oxidative degradation. Thus, catabolic pathway for phenylalanine and tyrosine is same as follows **(Fig. 14.17)**:

1. The first step is the hydroxylation of phenylalanine to tyrosine, a reaction catalyzed by ***phenylalanine hydroxylase.*** This enzyme requires tetrahydrobiopterin ($H_4$-biopterin) as a cofactor. The cofactor is oxidized to dihydrobiopterin ($H_2$-biopterin) during this reaction. The dihydrobiopterin produced is reduced back to $H_4$-biopterin by ***dihydrobiopterin reductase***.
2. The next step is transamination of tyrosine with α-ketoglutarate to P-hydroxyphenyl pyruvate, catalyzed by ***tyrosine transaminase***.
3. P-Hydroxyphenyl pyruvate then reacts with $O_2$ to form **homogentisate.** This reaction is catalyzed by ***P-hydroxy-phenyl-pyruvate*** **hydroxylase,** is called a ***dioxygenase*** because both atoms of $O_2$ become incorporated into product.
4. Homogentisate is then cleaved by $O_2$ to yield 4-maleylacetoacetate. This reaction is catalyzed by ***homogentisate oxidase.***
5. 4-Maleylcetoacetate is then isomerized to 4-fumarylacetoacetate, by an enzyme maleylacetoacetate isomerase that uses ***glutathione*** as a cofactor.

**Fig. 14.17:** Catabolic pathway for phenylalanine and tyrosine.

**Fig. 14.18:** Alternative pathways of phenylalanine catabolism in phenylketonuria.

6. Finally, 4-fumarylacetoacetate is hydrolyzed by ***fumaryl acetoacetate hydrolase*** to fumarate, a glucogenic intermediate and acetoacetate, a ketogenic intermediate. Phenylalanine and tyrosine are therefore, both glucogenic and ketogenic.

## Metabolic Disorders of Phenylalanine and Tyrosine

### *Phenylketonuria (PKU)*

- It is an inborn error of phenylalanine metabolism, associated with the inability to convert **phenylalanine to tyrosine**.
- The phenylketonuria is inherited in an autosomal recessive manner. The incidence of phenylketonuria is about 1 in 20,000 newborns.
- In phenylketonuria, there is an accumulation of phenylalanine in tissues and blood and results in its increased excretion in urine.
- Since phenylketonuric patients cannot convert phenylalanine to tyrosine, by normal pathway, some minor pathway of phenylalanine becomes prominent in phenylketonurics **(Fig. 14.18)** and accumulation of toxic metabolites of phenylalanine such as, ***phenylpyruvate, phenylacetate, phenyl-lactate*** and ***phenylacetyl glutamine*** occurs.
- The disease acquired its name (PKU) from the high levels of the keto acid, phenylpyruvate in urine.
- Almost all untreated phenylketonurics are severely **mentally retarded**.
- Untreated phenylketonuria is life threatening, half are dead by age 20 and three quarters by age 30.

**Table 14.4:** Types of phenylketonuria with their defects.

| *Type of PKU* | *Defect* |
|---|---|
| Classic phenylketonuria or Hyperphenylalaninemia type-I | Defect in phenylalanine hydroxylase |
| Atypical phenylketonuria or Hyperphenylalaninemia type-II and III | Defect in dihydrobiopterin reductase |
| Hyperphenylalaninemia type-IV and V | Defect in dihydrobiopterin synthesis |

### *Classification of PKU*

Phenylketonuria may be classified into three broad groups **(Table 14.4)**. PKU caused by deficiency of ***phenylalanine hydroxylase,*** is the most commonly encountered error.

**Characteristics of PKU**

- **Increased level of:** Phenylalanine, phenylacetate, phenyllactate, phenylpyruvate and phenylacetylglutamine, in tissues, plasma and urine. Phenylacetate gives the urine a **mousy odor**.
- **Neurological symptoms:** Mental retardation, failure to walk, to talk, seizures, psychoses, tremor and failure to grow.
- **Hypopigmentation:** Phenylketonurics have a lighter skin color, fair hair and blue eyes due to deficiency of pigment melanin. The hydroxylation of tyrosine by ***tyrosinase*** is the first step in the formation of the pigment melanin is competitively inhibited by the high levels of phenylalanine in PKU **(*see* Fig. 14.22)**.

### *Treatment of PKU*

The therapy for PKU is a low phenylalanine diet. The aim is to provide just enough phenylalanine to meet the needs for growth and replacement.

- Proteins that have a low content of phenylalanine such as casein from milk are hydrolyzed and phenylalanine is removed by adsorption.
- A low phenylalanine diet must be started very soon after birth to prevent irreversible brain damage.
- Diagnostic tests for PKU
- In the past years, the urine of newborns was assayed by the addition of ***$FeCl_3$*** which gives an ***olive color*** in the presence of phenylpyruvate.
- The phenylalanine level in blood is detected by screening by using ***Guthrie test***.
- The gene for human ***phenylalanine hydroxylase*** has been ***cloned,*** so that prenatal diagnosis of PKU is now possible with DNA probes.

### *Tyrosinemia*

There are three types of tyrosinemia:

1. Tyrosinemia type-I (Tyrosinosis/Hepatorenal tyrosinemia)

2. Tyrosinemia type-II (Richner-Hanhart syndrome)
3. Neonatal tyrosinemia.

### *Tyrosinemia type-I*

- Tyrosinemia type-I, also called **tyrosinosis** or **hepato-renal tyrosinemia**. It is caused by a genetic deficiency of **fumarylacetoacetate hydroxylase**.
- This defect results in accumulation and excretion of tyrosine and its metabolites such as P-hydroxyphenylpyruvate, P-hydroxyphenyllactate, P-hydroxyphenylacetate, N-acetyltyrosin and tyramine.
- The deficiency of enzyme fumaryl acetoacetate hydrolase causes liver failure, kidney dysfunction, polyneuropathy and vitamin D resistant rickets.

#### ***Classification of tyrosinemia type-I***

1. Acute tyrosinosis
2. Chronic tyrosinosis.

**Clinical Features**

- In acute tyrosinosis, the infant exhibits diarrhea, vomiting and cabbage like odor. Death may occur in infancy due to acute liver failure (within first year of life).
- Whereas in chronic tyrosinosis in later life it develops liver cirrhosis and death occurs by age 10.

### *Treatment*

The patient should be kept on diet low in phenylalanine and tyrosine.

### *Tyrosinemia type-II (richner-hanhart syndrome)*

#### ***Cause***

Tyrosinemia type-II is caused by genetic deficiency of hepatic enzyme **tyrosine aminotransferase** (tyrosine transaminase).

**Clinical Features**

- Due to deficiency of hepatic enzyme **tyrosine aminotransferase,** the tyrosine cannot be metabolized by its routine pathway. As a result, the tyrosine and its toxic metabolites accumulate in blood and tissues and appear in urine.
- The accumulation of tyrosine produces lesions in eye and skin and causes mild to moderate mental retardation.

#### ***Treatment***

Diet low in tyrosine and phenylalanine is recommended.

### *Neonatal tyrosinemia*

It is caused by absence of the enzyme **P-hydroxyphenylpyruvate hydroxylase** (dioxygenase).

In neonatal tyrosinemia, serum tyrosine levels are high in premature infants resulting from an immature liver and its limited ability to synthesize the enzyme, **P-hydroxyphenylpyruvate hydroxylase**.

As the liver matures, the accumulated tyrosine is metabolized and serum levels decrease to adult levels within 4 to 8 weeks of age. It is benign condition and responds well to ascorbic acid.

## *Alkaptonuria*

### *Cause*

This inherited metabolic disorder is due to defect in the enzyme ***homogentisate* oxidase,** that catalyzes oxidation of homogentisate **(Fig. 14.17)**. As a result, the homogentisate accumulates in blood and body tissues and is excreted in large amounts in urine.

**Clinical Features**

- The urine of alkaptonuric patients becomes dark after being exposed to air. In presence of oxygen, the colorless homogentisate present in urine undergoes spontaneous oxidation to yield **benzoquinone acetate,** which polymerizes to form black brown pigment **alkapton (Fig. 14.19)**. The alkapton imparts a characteristic black-brown color to urine.
- Alkaptonuria is a harmless condition. Later in life patients may suffer from deposition of dark colored alkapton pigments in connective tissues and bones. This results in black pigmentation of the sclera, ear, nose and cheeks and the clinical condition is known as **ochronosis** (because ochre color of the deposit). Ochronosis leads to tissue damage and may develop joint pain, arthritis and backache.

### *Treatment*

Since alkaptonuria is not considered life threatening, this condition is not treated. Later in life, the symptoms of arthritis may be treated but the condition itself is not.

**Fig. 14.19:** Formation of alkapton bodies.

*Diagnosis*

The urine sample of patients of alkaptonuria turns dark on standing in air. The urine gives positive test with **ferric chloride** and **silver nitrate** due to reducing activity of homogentisate.

### Albinism

*Cause*

It is an inborn error of tyrosine catabolism. It is due to inherited deficiency of enzyme **tyrosinase**. The inherited deficiency of tyrosinase impairs the synthesis of melanin from tyrosine **(Fig. 14.22)**. Melanin is dark pigment of skin, hair, iris, and retinal epithelial cells. Melanin protects the body from harmful radiation of sunlight.

**Clinical Features**

- Impaired synthesis of melanin results in either hypo-pigmentation or no pigmentation of hair, skin and eyes and leads to white hair and skin.
- Albinos are highly sensitive to sunlight and can lead to skin cancer.
- The lack of melanin pigment in eyes is responsible for photophobia (intolerance to light) and nystagmus.

## Biologically Important Compounds Derived from Tyrosine

Tyrosine serves as a precursor for following several biologically important compounds **(Fig. 14.20)**.

1. Catecholamines
   - Dopamine
   - Norepinephrine
   - Epinephrine
2. Melanin pigment
3. Thyroxine.

**Fig. 14.20:** Biosynthesis of biologically important compounds from tyrosine.

### Biosynthesis of Catecholamines

- Epinephrine (adrenaline), norepinephrine (noradrenaline) and dopamine are collectively called **catecholamines**. They are synthesized from tyrosine **(Fig. 14.21)**
- Epinephrine and norepinephrine are produced by adrenal medulla and serve as **hormones,** whereas dopamine and norepinephrine produced in the CNS and postganglionic sympathetic nerves act as **neurotransmitter**.

In **Parkinson's disease,** dopamine levels in the CNS are decreased because of a deficiency of cells that produce dopamine.

### Biosynthesis of Melanin Pigment

Melanin is a pigment. The synthesis of melanin occurs only in pigment producing cells called ***melanocytes***.

- The first step is the conversion of tyrosine to DOPA **(Fig. 14.22)**
- In melanocyte, a different enzyme ***tyrosinase*** catalyzes this reaction.
- Tyrosinase also catalyzes the subsequent oxidation of dopa to dopaquinone.
- Dopaquinone is then converted to melanin. Tyrosinase does not require tetrahydrobiopterin but it utilizes copper as a cofactor.

**Fig. 14.21:** Synthesis of catecholamines.

**Fig. 14.22:** Biosynthesis of melanin.

### Biosynthesis of Thyroxine and Tri-iodothyroxine

The hormones ***thyroxine*** **($T_4$)** and ***tri-iodothyroxine*** ***($T_3$)*** are formed in the follicle cells of the thyroid gland by iodination of tyrosine residues of protein **thyroglobulin**. Monoiodo and diiodotyrosine residues are first formed and these then react to form $T_3$ and $T_4$ **(Fig. 14.23)**.

**Fig. 14.23:** Biosynthesis of $T_3$ and $T_4$.

## Metabolism of Tryptophan

- Tryptophan is an essential amino acid, containing ***indol ring.*** Tryptophan is oxidized to produce alanine, which is glucogenic and acetyl-CoA, which is ketogenic. ***Thus, tryptophan is both glucogenic and ketogenic.***
- Tryptophan is a precursor for the synthesis of:
  - Vitamin niacin (vitamin $B_3$)
  - Neurotransmitter serotonin
  - Hormone melatonin.
- Tryptophan is metabolized by two pathways:
  1. Kynurenine pathway
  2. Serotonin pathway.

### Kynurenine Pathway

In this pathway tryptophan is oxidized to kynurenine and alanine. Kynurenine is then converted to either **vitamin niacin** or **acetyl-CoA (Fig. 14.24)**.

- The initial reaction is an oxidation of tryptophan to formylkynurenine, catalyzed by the enzyme ***tryptophan oxygenase*** also called ***tryptophan pyrrolase***, which is feedback inhibited by nicotinic acid derivatives, e.g. NADH or NADPH.
- Formylkynurenine is converted to kynurenine by removal of formyl group with the enzyme ***kynurenine formylase***.
- Kynurenine is then metabolized to 3-hydroxy kynurenine by ***kynurenine hydroxylase***.
- Next 3-hydroxykynurenine is converted to 3-hydroxyanthranilate and alanine by ***kynureninase***, a PLP-dependent enzyme. A deficiency of ***vitamin $B_6$ (pyridoxin)*** results in failure to catabolize the hydroxykynurenine, forming ***xanthurenate*** **(*see* Fig. 14.22)** and excreted in urine in vitamin B6 deficiency.
- Next 3-hydroxyanthranilate undergoes decarboxylation forming vitamin ***niacin*** which can be converted to ***NAD***$^+$ and **NADP**$^+$ or 3-hydroxyanthranilate can also be converted through a number of steps to acetyl-CoA. ***For every 60 mg of tryptophan, 1 mg equivalent of niacin can be generated.***

### Serotonin Pathway

- Tryptophan is first oxidized to 5-hydroxytryptophan by ***tryptophan hydroxylase***, which requires, tetrahydro-biopterin as a cofactor **(Fig. 14.25)**.

**Fig. 14.24:** Kynurenine metabolic pathway of tryptophan and biosynthesis of vitamin niacin.

**Fig. 14.25:** Serotonin metabolic pathway of tryptophan and formation of melatonin.

- 5-hydroxytryptophan undergoes decarboxylation to yield ***serotonin*** (5-hydroxytryptamine)
- Acetylation of serotonin followed by methylation in the pineal gland forms a hormone ***melatonin***.

## Serotonin

- Serotonin is synthesized from tryptophan by neurons, pineal glands and intestinal argentaffin cells. In normal adult, about 1% of tryptophan is converted to serotonin.

### *Functions of serotonin*

- Serotonin is a **neurotransmitter** and stimulates cerebral activity. Therefore, serotonin deficiency causes a decrease in cerebral (brain) activity, which leads to depression.
- In humans, serotonin is involved in a variety of behavioral patterns, including sleep, body temperature and blood pressure.
- Serotonin produced in intestinal cells stimulates the release of gastrointestinal peptide hormones.
- Serotonin serves as precursor of **melatonin** in the pineal gland.
- Serotonin is also a powerful **vasoconstrictor** and stimulator of smooth muscle contraction.

**Argentaffinomas or Carcinoid Syndrome**

Argentaffinomas or carcinoid syndrome is the cancer of argentaffin cells of the gastrointestinal tract. Normally, about 1% of tryptophan is converted to **serotonin;** however, in carcinoid syndrome about 60% of tryptophan is diverted to serotonin formation resulting in decreased synthesis of **vitamin niacin.** Therefore, these patients develop the symptoms of **pellagra**.

## Melatonin

- Melatonin is a hormone produced from serotonin by the pineal gland **(Fig. 14.25)**.
- Synthesis of melatonin is regulated by **light dark cycle**. It is synthesized mostly at night.
- It is an inhibitor of **melanocyte stimulating hormone (MSH)** and **adrenocorticotropic hormone (ACTH)**.
- Melatonin is a sleep inducing substance and is involved in regulation of **circadian rhythm** of body. It may also be involved in regulating **reproductive functions**.

# Metabolic Disorder of Tryptophan

## Hartnup's Disease

- Hartnup's disease is an inherited disorder of tryptophan metabolism. This disorder was first of all reported in the family of **Hartnup,** therefore, named Hartnup's disease.
- It is due to defect in the intestinal and renal **transport of tryptophan** and other neutral amino acids and leads to tryptophan deficiency. Tryptophan deficiency ultimately leads to decreased synthesis of **vitamin niacin** and **serotonin**.
- Decreased synthesis of niacin leads to **pellagra** like symptoms and decreased serotonin synthesis is responsible for **neurological symptoms** observed in Hartnup's disease.
- In addition to the neurological and pellagra like symptoms, there is **amino aciduria** due to failure of transport of amino acids from kidney.
- As well, any unabsorbed tryptophan remaining in the intestine is metabolized by intestinal bacteria to **indolacetic acid** and **indolpyruvic acid** which are subsequently excreted in urine.

### *Therapy*

- Oral nicotinic acid supplementation will permit adequate synthesis of $NAD^+$ in patients with Hartnup disease. This corrects pellagra like symptoms of the disorder. The aminoaciduria remains unaltered.

## METABOLISM OF SULFUR CONTAINING AMINO ACIDS

The three sulfur containing amino acids are ***cystine, cysteine***, and ***methionine***. Among these methionine is an essential amino acid whereas cysteine and cystine are non-essential amino acids. Cysteine and cystine are synthesized from two amino acids, **methionine,** which is nutritionally essential and **serine** is non-essential. Cystine and cysteine are readily interconvertible in the body.

### Metabolism of Methionine

Methionine is metabolized by:

1. Transfer of methyl group of methionine by transmethylation reactions.
2. Conversion of demethylated portion of the methionine to cysteine and cystine.

#### *Transfer of Methyl Group of Methionine (Transmethylation Reactions)*

Transfer of methyl group ($-CH_3$) from methionine to an acceptor molecule is termed as **transmethylation**. The methyl group of methionine becomes available for transmethylation only in an active form of methionine, **S-adenosylmethionine (SAM)**.

- **ATP** and an enzyme **methionine adenosyltransferase** are required for the activation of methionine to S-adenosylmethionine **(Fig. 14.26)**.
- The methyl group of active methionine (SAM) is very reactive and can be enzymatically transferred in the synthesis of many compounds. Some important transmethylation reactions are given below:
  - Norepinephrine to epinephrine
  - Phosphatidylethanolamine to phosphatidylcholine
  - Gunaidoacetoacetate to creatine
  - Ethanolamine to choline
  - Acetyl serotonin to melatonin
  - Nucleotides to methylated nucleotides

#### *Conversion of Demethylated Portion of the Methionine to Cysteine and Cystine (Fig. 14.27)*

- Removal of the methyl group from SAM forms **S-adenosyl homocysteine**.
- Hydrolytic cleavage of S-adenosylhomocysteine occurs to **homocysteine** and **adenosine**.
- Homocysteine then condenses with serine forming **cystathionine** by the enzyme **cystathionine synthase**.
- Hydrolytic cleavage of cystathionine forms **homoserine** plus **cysteine** by the enzyme **cystathionine lyase,** which requires cofactor pyridoxal phosphate (PLP).
- The cysteine then may be oxidized to **cystine**.
- Next homoserine is converted to α-ketobutyrate by **homoserine deaminase**.
- Then α-ketobutyrate undergoes oxidative decarboxylation to form propionyl-CoA which is catabolized by way of methylmalonyl-CoA and succinyl-CoA.

> It should be noted that cysteine is synthesized from two amino acids, **methionine** and **serine.** Methionine furnishes the sulfur atom and serine furnishes the carbon skeleton.

#### *Synthesis of Methionine from Homocysteine (Fig. 14.27)*

- Methionine can be regenerated by remethylation of homocysteine, which requires both **tetrahydrofolate (THF)** and **vitamin $B_{12}$**.
- In this reaction methyl group of **$N^5$-methyl THF** is transferred to vitamin $B_{12}$ forming **methyl $B_{12}$**. Then methyl $B_{12}$ transfers the methyl group to homocysteine which is converted to methionine.

> It should be noted that there is no net synthesis of methionine because homocysteine which serves as precursor for methionine has to be derived from methionine.

**Fig. 14.26:** Formation of active methionine.

**Fig. 14.27:** Catabolic pathway of methionine or biosynthetic pathway of cysteine. (THF: Tetrahydrofolate)

## Metabolism of Cysteine and Cystine

### *Synthesis of Cysteine and Cystine*

- Cysteine is a nutritionally non-essential, glucogenic amino acid.
- As discussed above, it is synthesized from two amino acids, ***methionine,*** which is nutritionally essential and ***serine,*** which is not. Methionine furnishes sulfur atom and serine furnishes the carbon skeleton **(Fig. 14.27)**.
- Cystine is formed by oxidation of cysteine.

### *Catabolism of Cystine and Cysteine*

- The major catabolic fate of cystine is conversion of cystine to cysteine **(Fig. 14.28)** a reaction catalyzed by ***cystine reductase***.
- Catabolism of cystine then occurs simultaneously with that of cysteine.
- Cysteine can be catabolized by two routes as follows:
  1. The transamination pathway
  2. Direct oxidative pathway

### *Transamination Pathway*

- One principal pathway is its transamination in the presence of α-ketoglutarate by the enzyme ***cysteine transaminase*** to form β-mercaptopyruvate and glutamate **(Fig. 14.29)**.
- β-Mercaptopyruvate then undergoes desulfuration with ***sulfur transferase*** to form pyruvate and $H_2S$, which is converted to sulfide by reduced glutathione.
- The sulfide formed in the reaction is converted to sulfite and then to sulfate ($SO^{4-}$).

**Fig. 14.28:** Conversion of cystine to cysteine.

**Fig. 14.29:** Transamination pathway for the catabolism of cysteine. (PAPS: Phosphoadenosyl phosphosulfate)

- The sulfate thus formed is either excreted in the urine or converted to active sulfate, ***phosphoadenosyl phosphosulfate (PAPS)*** by using ATP. PAPS is used as a source of sulfur in the synthesis of ***polysaccharides, glycolipids*** and ***sulpholipids*** or used for ***detoxification*** reactions.

*Direct oxidative pathway (Fig. 14.30)*

- Cysteine is oxidized to cysteine sulfinate by ***cysteine dioxygenase*** which is then decarboxylated to **hypotaurine**. Hypotaurine may be oxidized to taurine (an inhibitory neurotransmitter), that conjugates with bile acid ***cholic acid*** to form taurocholic acid.
- Cysteine sulfinate may undergo transamination and give rise to sulfate.

## Importance of Cysteine

- The conversion of cysteine to pyruvate accounts for glucogenic nature and involved in formation of glucose.
- Cysteine is most important dietary source of sulfur.
- Besides taurine, other physiologically important sulfur containing compounds such as ***insulin, coenzyme-A, glutathione*** and ***vasopressin*** are derived from cysteine.
- It is also involved in detoxification mechanisms.

## Metabolic Disorders of Sulfur Containing Amino Acids

### *Cystinuria (Cystin-lysinuria)*

- Cystinuria is the most common inborn error of amino acid transport.
- Cystinuria is an inherited disorder in which kidney tubules fail to reabsorb the amino acids cystine, ornithine, arginine and lysine (the mnemonic is COAL).
- This is characterized by massive urinary excretion of cystine, ornithine, arginine and lysine.

**Fig. 14.30:** Direct oxidative pathway for catabolism of cysteine.

*Cause*

- Normally, these amino acids are filtered by the glomerulus and reabsorbed in the proximal renal tubule by specific carrier proteins.
- In cystinuria defect in this carrier system leads to the excretion of all these four amino acids.

*Clinical features*

Since cystine is the least soluble it's over excretion often leads to precipitation and formation of **cystine calculi** (stones) in the renal tubules and leads to obstruction, infection and renal insufficiency in cystinuric patient.

*Treatment*

Treatment involves ingestion of large amounts of water, which increases cystine solubility through maintenance of alkaline urine.

### Cystinosis (Cystine Storage Disease)

Cystinosis is a rare but serious lysosomal disorder.

*Cause*

It is caused by a defective carrier that transports cystine across the lysosomal membrane from lysosomal vesicles to the cytosol.

**Clinical Features**

- Cystine accumulates in the lysosomes in many tissues and forms crystals impairing their function.
- Cystinosis is usually accompanied by a generalized aminoaciduria.
- Patients usually die within 10 years due to acute renal failure.

### Homocystinuria

Homocystinurias are a group of disorder of methionine metabolism. It is characterized by high blood and urinary levels of **homocysteine** and **methionine**. Four metabolic defects cause four types of homocystinuria **(Table 14.5)**.

*Homocystinuria type-I*

- It is due to defect in the enzyme **cystathionine synthase,** which converts homocysteine to cystathionine **(Fig. 14.27)**. As a result, homocysteine accumulates in blood and appears in urine.

**Table 14.5:** Different types of homocystinuria with their defects.

| *Type* | *Defect* |
|---|---|
| Homocystinuria-I | Cystathionine-β-synthase |
| Homocystinuria-II | Synthesis of $N^5$ methyl THF |
| Homocystinuria-III | Deficiency of methyl $B_{12}$ |
| Homocystinuria-IV | Defective intestinal absorption of vitamin $B_{12}$ |

- The accumulation of homocysteine causes skeletal abnormalities, ectopia lentis (dislocation of the lenses in the eyes), osteoporosis, mental retardation and thrombosis.
- Thrombosis may result in myocardial infarction, pulmonary embolism or stroke.
- A deficiency of cystathionine synthase is the most common cause of homocystinuria. Other types of homocystinuria are due to defects in the remethylation of homocysteine to form methionine.

*Homocystinuria type-II and III*

- In type-II and III, there is deficiency in synthesis of **$N^5$-methyltetrahydrofolate** and **methyl $B_{12}$** respectively. Both $N^5$-methyl THF and methyl $B_{12}$ are required for the remethylation of homocysteine to form methionine.

*Homocystinuria type-IV*

It is due to defective intestinal absorption of vitamin $B_{12}$.

***Treatment***

- The biochemical defect in cystathionine synthase can be corrected in some cases by providing pyridoxine (vitamin $B_6$). Pyridoxine is needed to activate the enzyme cystathionine synthase.
- Those with complete enzyme deficiency should be treated with a diet low in methionine and supplemented with cysteine.
- Vitamin $B_{12}$ may be given in instances of vitamin $B_{12}$ deficiency.

## ONE CARBON METABOLISM

- Groups, containing single carbon atoms are called one carbon groups. One carbon groups are formed from several amino acids during their metabolism. These include **serine, glycine, histidine** and **tryptophan**. One carbon groups formed during metabolism are:
  - Methyl ($CH_3$)
  - Methylene ($CH_2$)
  - Methenyl (CH)
  - Formyl (CHO)
  - Formimino (CH=NH)
- These one carbon groups are transferred by way of **tetrahydrofolate (THF)**. Tetrahydrofolate is formed from vitamin folic acid **(Fig. 14.31)**. It should be noted that $CO_2$ is also a one carbon group, is carried by vitamin **biotin**. However, biotin is not considered as a member of one carbon pool.
- One carbon groups carried by THF are attached either to nitrogen $N^5$ or $N^{10}$ or to both $N^5$ and $N^{10}$ **(Fig. 14.32)**.

**Fig. 14.31:** Formation of tetrahydrofolate from folic acid.

- The different one carbon derivatives of THF are as follows:
  - $N^5$-methyl THF
  - $N^5$, $N^{10}$-methylene THF
  - $N^5$, $N^{10}$-methenyl THF
  - $N^5$-formyl THF
  - $N^5$-formimino THF.

  These different derivatives of THF are interconvertible.

**Figure 14.33** shows sources of one carbon groups and their utilization.

## Sources of One Carbon Groups (Fig. 14.33)

1. Formate produced in the degradation of glycine and tryptophan reacts with THF to form **$N^{10}$-formyl THF**.
2. Histidine during its degradation produces **formimino glutamate (FIGLU),** which reacts with THF forming **$N^5$-formimino THF** which on releasing ammonia generates **$N^5$, $N^{10}$- methenyl THF**.
3. Serine is the major one carbon source. When serine is converted to glycine **$N^5$, $N^{10}$-methylene THF** is formed.
4. Methyl groups of choline and betaine reacts with THF to form **$N^5$-methyl THF**.

**Fig. 14.32:** Structure of tetrahydrofolate.

5. Active form of methionine, **S-adenosylmethionine (SAM)** carries methyl groups and acts as a methyl group donor.

## Utilization of one Carbon Groups (Fig. 14.33)

One carbon groups carried by THF are used for the synthesis of other biologically important compounds, e.g.

1. Synthesis of serine from glycine.
2. Synthesis of pyrimidine nucleotide thymidylate (TMP).
3. Synthesis of purine bases.
4. Synthesis of methionine from homocysteine.
5. Methyl group carried by SAM is used for the synthesis of choline, creatine, epinephrine, carnitine, t-RNA, DNA, etc.

**Importance of One Carbon Group Metabolism**

One carbon groups at different levels of oxidation are transferred and made available by way of the tetrahydrofolate and vitamin $B_{12}$ coenzymes for use in a wide variety of vital anabolic processes.

**Fig. 14.33:** One carbon metabolism showing sources of one carbon groups and utilization of one carbon groups.

## METABOLISM OF BRANCHED CHAIN AMINO ACIDS (FIG. 14.34)

Valine, isoleucine and leucine are branched essential amino acids.

### Catabolism of Branched Chain Amino Acids

The first three metabolic reactions for these branched chain amino acids are similar and are catalyzed by common enzymes. Therefore, the metabolisms of three branched chain amino acids are considered together.

1. **Transamination: The** initial step in the degradation of the branched chain amino acids is a transamination reaction to yield corresponding α-keto acids.
2. **Oxidative decarboxylation: In** the second step, the α-keto acids are oxidatively decarboxylated to the corresponding acyl-CoA thioesters by branched-chain α-keto acid dehydrogenase complex. TPP, FAD, Lipoic acid, $NAD^+$ and CoA are required as coenzymes in this reaction.
3. **Dehydrogenation: The** third reaction is a FAD dependent dehydrogenation, a reaction that resembles the first step of β-oxidation.
4. The subsequent reactions of all the three branched chain amino acids differ and are catabolized as follows:
   - Valine is converted to succinyl-CoA accounting for the glucogenic nature of the valine.
   - Isoleucine is converted to succinyl-CoA and acetyl-CoA, accounting for the ketogenic and glucogenic nature of the isoleucine.
   - Leucine forms acetoacetate and acetyl-CoA but does not produce succinyl-CoA, which accounts for exclusively the ***ketogenic nature of the leucine***. Leucine produces hydroxymethyl glutaryl-CoA (HMG-CoA) intermediate product which is a precursor of cholesterol biosynthesis and ketone body formation.

**Fig. 14.34:** Degradation of the branched chain amino acids. (HMG-CoA: Hydroxymethylglutaryl-CoA)

## Metabolic Disorder of Branched Chain Amino Acids

### *Maple Syrup Urine Disease (MSUD) or Branched Chain Keto Aciduria*

- It is an inborn error or branched chain amino acids namely leucine, isoleucine and valine catabolism **(Fig. 14.34)**.

*Biochemical cause*

- Maple syrup urine disease is due to inherited defect in the **branched chain α-keto acid dehydrogenase**. Due to this defect α-keto acids of leucine, isoleucine and valine cannot be further metabolized. As a result, the branched chain amino acids, leucine, isoleucine and valine, and their α-keto acids accumulate in blood, urine and CSF.
- α-keto acids impart a characteristic sweet odor to the urine of the affected individuals which resembles with maple syrup or burnt sugar hence the name.

*Symptoms*

- Maple syrup urine disease is characterized by vomiting, dehydration severe metabolic acidosis and a characteristic **maple syrup** odor to the urine.
- If untreated, it leads to mental retardation, coma and even death within one year after birth.

*Diagnosis*

- Maple syrup disease is diagnosed by estimating increased levels of branched chain amino acids and their keto acids in plasma and urine.

*Treatment*

- Treatment involves replacing dietary protein by mixture of amino acids that contain low or no leucine, isoleucine and valine.
- To monitor the effectiveness of the dietary treatment, plasma and urinary levels of branched chain amino acids should be measured constantly.

# METABOLISM OF HYDROXY GROUP CONTAINING AMINO ACIDS

Serine and threonine are the hydroxy group containing amino acids.

## Metabolism of Serine

Serine is a non-essential amino acid.

### *Synthesis of Serine*

- Serine is synthesized from glycine by transfer of hydroxymethyl group from $N^5N^{10}$-methylene THF **(*see* Fig. 14.14)**.

### *Catsabolism of Serine*

- In humans, serine is catabolized via glycine and $N^5$, $N^{10}$-methylene tetrahydrofolate. The reaction is catalyzed by ***serine hydroxy methyl transferase*** **(*see* Fig. 14.14)**. Further catabolism of serine merges with that of glycine.
- In many mammals, serine is degraded by ***serine dehydratase,*** an enzyme that requires pyridoxal phosphate (PLP) and produces $NH_4^+$ and pyruvate **(Fig. 14.35)**.This pathway appears to be a minor one in humans.

## Importance of Serine

- Serine is a constituent of phospholipid, ***phosphstidylserine,*** found in brain.
- After decarboxylation serine gives rise to **ethanolamine** which is the constituent of another phospholipid, ***phosphatidyl ethanolamine*** (cephalin).
- Serine also takes part in the synthesis of cysteine **(Fig. 14.27)**.

## Metabolism of Threonine

Threonine is an essential and glucogenic amino acid.

### *Catabolism of Threonine*

Threonine is degraded by two pathways:

1. Threonine is cleaved to acetaldehyde and glycine by ***threonine aldolase.*** Acetaldehyde is then oxidized to acetate, which then is converted to acetyl-CoA **(Figs. 14.36A and B)**.
2. ***Threonine dehydratase*** produces α-ketobutyrate. Subsequently, α-ketobutyrate is oxidatively decarboxylated to yield propionyl-CoA, which is then carboxylated to methylmalonyl-CoA, which, in turn, is isomerized to succinyl-CoA. Succinyl-CoA enters the Kreb's cycle, **(Figs. 14.36A and B)** and gives rise to pyruvate. Threonine is thus glucogenic amino acid.

## Metabolism of Alanine

Alanine is a nonessential amino acid.

**Fig. 14.35:** Degradation of serine.

**Figs. 14.36A and B:** Catabolic pathways of threonine.

**Fig. 14.37:** Synthesis and catabolism of alanine.

### *Synthesis and Catabolism of Alanine*

It is produced from pyruvate by a transamination reaction catalyzed by alanine transaminase (ALT) and may be metabolized back to pyruvate by a reversal of the same reaction **(Fig. 14.37)**. Alanine is a major glucogenic amino acid.

**Role of Alanine in Regulation of Blood Glucose**

- In fasting, alanine plays a key role in hepatic gluconeogenesis.
- Alanine is released from muscle (for the transport of ammonia to the liver) during fasting and undergoes transamination to form pyruvate.
- The pyruvate is then converted to glucose by the gluconeogenesis.
- Thus, alanine has an important role in maintaining the fasting blood glucose level by way of gluconeogenesis.

## METABOLISM OF ACIDIC AMINO ACIDS

### Metabolism of Glutamic Acid

Glutamic acid is a nonessential, glucogenic amino acid.

### *Synthesis of Glutamic Acid*

It is synthesized from α-ketoglutarate an intermediate of citric acid cycle by:

- Transamination **(Fig. 14.38A)**
- By reductive amination of a ketoglutarate by $NH_4^+$, catalyzed by ***glutamate dehydrogenase*** **(Fig. 14.38 B)**.

### *Catabolism of Glutamic Acid*

When glutamate is degraded, it is converted back to α-ketoglutarate either by transamination or by glutamate dehydrogenase reaction **(Figs. 14.38A and B)**.

### Importance of Glutamic Acid

- A number of other amino acids like glutamine, proline and arginine are derived from glutamate.

**Figs. 14.38A and B:** Synthesis and degradation of L-glutamate.

- Glutamate involved in the synthesis of glutathione, (γ-glutamyl-cysteinyl glycine) which is involved in the reduction of $H_2O_2$ to $H_2O$ and transport of amino acids into cells of kidney and intestine.
- Glutamate is decarboxylated at C-1 to form amine ***gamma aminobutyric acid (GABA),*** which serves as a neurotransmitter.

## Metabolism of Glutamine

Glutamine is an amide of glutamate.

### *Synthesis of Glutamine*

It is produced from glutamate by ***glutamine synthetase***, which adds $NH_4^+$ to the carboxyl group of the side chain, forming an amide **(Fig. 14.39)**.

### *Degradation of Glutamine*

Glutamine is reconverted to glutamate by a different enzyme, ***glutaminase***, which is particularly important in the kidney.

## Importance of Glutamine

- **Glutamine is the major transport form of ammonia**.
- Glutamine is the principal source of ammonia in the kidney, the ammonia it produces enters the urine and decreases its acidity ($NH_3 + H^+ \rightarrow NH_4^+$) and in this way it plays an important role in the ***maintenance of acid-base balance.***
- Glutamine participates in a number of biosynthetic reactions, usually by supplying amino or ammonia nitrogen, e.g. in the formation of arginine, carbamoyl phosphate, purines, etc.

## Metabolism of Aspartic Acid

Aspartic acid is a nonessential, glucogenic amino acid.

**Fig. 14.39:** Synthesis and degradation of glutamine.

### *Synthesis of Aspartic Acid*

Aspartate is synthesized from Kreb's citric acid cycle intermediate, oxaloacetate by transamination reaction **(Fig. 14.38A)**.

### *Degradation of Aspartic Acid*

Because the transamination reaction is readily reversible, aspartate can be converted to oxaloacetate, an intermediate of citric acid cycle **(Fig. 14.38A)**.

## Functions of Aspartic Acid

- In the urea cycle, aspartate reacts with citrulline to form arginosuccinate, which is cleaved, forming an essential amino acid *arginine* and fumarate.
- Aspartate reacts with inosine monophosphate (IMP) to form ***AMP***.

## Metabolism of Aspargine

Aspargine is an amide of aspartate.

### *Synthesis of Aspargine*

- Aspargine is formed from aspartate by a reaction in which glutamine provides the nitrogen for formation of the amide group **(Fig. 14.40)**

### *Degradation of Aspargine*

- Hydrolytic release of the amide nitrogen of aspargine as ammonia and aspartate is catalyzed by **asparginase (Fig. 14.40)**.

**Clinical Importance of Asparginase**

- Certain types of tumor cells, particularly leukemic cells, require aspargine. Therefore, asparginase has been used as an antitumor agent.
- It acts by converting aspargine to aspartate, decreasing the amount of aspargine available for tumor cell growth.

**Fig. 14.40:** Synthesis and degradation of aspartate and aspargine.

**Fig. 14.41:** Synthesis of proline from glutamate.

# METABOLISM OF IMINO ACID

## Metabolism of Proline

Proline is a non-essential, glucogenic amino acid.

### *Synthesis of Proline*

- Proline is formed from glutamate by reversal of the reactions of proline catabolism **(Fig. 14.41)**.
- Glutamate is first phosphorylated and then converted to glutamate γ-semialdehyde by reduction.
- This semialdehyde spontaneously cyclizes and reduction of this cyclic compound yields proline.

### *Degradation of Proline*

- Proline rather than undergoing direct transamination is oxidized to dehydroproline, which adds water, forming glutamate γ-semialdehyde.
- This is further oxidized to glutamate and transaminated to α-ketoglutarate **(Fig. 14.42)**.

## Importance of Proline

- Proline serves as a precursor of hydroxyproline. Hydroxyproline is an important constituent of collagen which stabilizes the collagen triple helix. Collagen contains about one-third glycine and one- third proline plus hydroxyproline.
- Proline and ornithine are readily interconverted in the body. Thus, proline may yield ornithine for urea synthesis or ornithine may be broken down via proline **(Fig. 14.41)**.

## Metabolic Disorders of Proline

- Two autosomal recessive hyperprolinemias have been reported. Mental retardation occurs in half of the known cases but neither type is life-threatening. Two types of

**Fig. 14.42:** Degradation of L-proline and L-arginine to α-ketoglutarate.

hyperprolinemia, called ***type-I*** and ***type-II,*** resulting in increase in blood and urine levels of proline are as follows:

### Hyperprolinemia Type-I

- The metabolic block in type-I is at ***proline dehydrogenase*** **(Fig. 14.42)**.
- There is no associated impairment of hydroxyproline catabolism.

### Hyperprolinemia Type-II

- The metabolic block occurs at ***glutamate γ-semialdehyde dehydrogenase*** **(Fig. 14.42)**.
- Since the same dehydrogenase functions in hydroxyproline catabolism, both proline and hydroxyproline catabolism are affected.
- The urine contains the hydroxyproline catabolites.

## METABOLISM OF BASIC AMINO ACIDS

### Metabolism of Arginine

- Arginine is considered to be a semi-essential amino acid. It can be synthesized in the body but not in quantities sufficient to permit normal growth. It is thus an amino acid, which is essential for growth but not for maintenance.

### Synthesis of Arginine

- Arginine is synthesized from glutamate **(Fig. 14.43)**.
- Glutamate is reduced to glutamate-γ-semialdehyde, which is then transaminated to yield ornithine, an intermediate of urea cycle.

**Fig. 14.43:** Synthesis and degradation of arginine.

- The reactions of the urea cycle convert ornithine to arginine.

### Degradation of Arginine

- Arginine is cleaved by **arginase** to form urea and ornithine.
- If ornithine is present in amounts in excess of those required for the urea cycle, it is transaminated to glutamate semialdehyde which is reduced to glutamate **(Fig. 14.43)**.

### Importance of Arginine

- Arginine takes part in the formation of urea.
- Arginine is involved in the synthesis of creatine, an important constituent of muscle. In the formation of creatine, the guanidinium group of arginine is transferred to glycine.
- Nitric oxide is synthesized from arginine **(Fig. 14.44)**. Nitric oxide is a biological messenger in a variety of physiological responses including vasodilation, neurotransmission and the ability to kill tumor cells and parasites.

### Metabolic Disorders of Arginine

The five hereditary defects in the biosynthesis of urea, mentioned earlier **(*see* Table 14.2),** may be considered inherited disorders in the metabolism of arginine as they share common biosynthetic and catabolic pathways.

### Metabolism of Histidine

Histidine like arginine is a nutritionally semi-essential amino acid and is glucogenic.

### Degradation of Histidine

Degradation of histidine occurs mainly in the liver. The major pathway and principal intermediates are shown in **Figure 14.45**.

- In a series of steps, histidine is converted to formiminoglutamate (FIGLU).

**Fig. 14.44:** Synthesis of nitric oxide.
(NOS: Nitric oxide synthase, NO: Nitric Oxide)

**Fig. 14.45:** Degradation of histidine.

- The subsequent reactions transfer one carbon group, i.e. formimino group of FIGLU to the tetrahydrofolate (THF) to form $N_5$-formimino THF leaving L-glutamate.
- $N_5$-formimino THF may be used in one carbon metabolism **(Fig. 14.33)**.

**Clinical Significance of FIGLU**

- In folic acid deficiency transfer of formimino group of FIGLU to THF reaction is partially or totally blocked, and FIGLU is excreted in the urine.
- Excretion of FIGLU, therefore, provides a diagnostic test for folic acid deficiency.

## Importance of Histidine

Histidine has several other important functions in addition to the general role of amino acids in tissue protein formation as follows:

- Upon decarboxylation, it forms ***histamine,*** which reduces blood pressure, is a vasodilator and increases the secretion of gastric juice. Allergic reactions stimulate an excessive liberation of histamine.
- Histidine is involved in the formation of biologically important peptides like ***carnosine*** and ***anserine*** present in muscle, ***ergothioneine*** present in erythrocytes, liver and brain.

## Metabolic Disorders of Histidine

Two benign hereditary disorders of histidine catabolism are:

- **Histidinemia:** Histidinemia is characterized by elevated blood and urine histidine. The defective enzyme is ***histidase,*** resulting in impaired conversion of histidine to urocanate.
- **Urocanic aciduria:** It is characterized by elevated excretion of urocanate; it results from a defective ***urocanase*** enzyme.

## Metabolism of Lysine

- Lysine is a nutritionally essential amino acid.
- Lysine is both glucogenic and ketogenic amino acid.
- It contains two amino groups, neither of which can undergo direct transamination.

### *Catabolism of Lysine*

Lysine is degraded by a complex pathway in which ***saccharopine, α-ketoadipate*** and ***crotonyl-CoA*** are intermediates **(Fig. 14.46)**. Ultimately, lysine generates acetyl-CoA.

## Importance of Lysine

Lysine is involved in the synthesis of ***carnitine that*** serves to shuttle fatty acyl groups across mitochondrial membrane.

## Metabolic Disorder of Lysine

Two inherited disorders of lysine metabolism have been reported:

- Periodic hyperlysinemia
- Persistent hyperlysinemia.

**Fig. 14.46:** Degradation of L-lysine.

### *Periodic Hyperlysinemia*

- It is characterized by hyperammonemia and elevated plasma lysine.
- Elevated liver lysine levels competitively inhibit liver arginase, causing hyperammonemia and show the clinical symptoms of ammonia intoxication.
- The biochemical cause of hyperlysinemia is uncertain.

### *Persistent Hyperlysinemia*

- Persistent hyperlysinemia is believed to be inherited as an autosomal recessive trait.
- In addition to impaired conversion of lysine and α-ketoglutarate to saccharopine, some patients cannot cleave saccharopine.
- Persistent hyperlysinemia is not associated with hyperammonemia.
- Some patients are mentally retarded.

## BIOGENIC AMINES

Decarboxylation of amino acids results in the formation of amines. These amines are called **biogenic amines**. They have diverse biological function. Decarboxylation reactions are catalyzed by **PLP dependent decarboxylases**. The important biogenic amines formed by decarboxylation of amino acids are given below **(Fig. 14.47)**.

- **γ-amino butyric acid (GABA):** It is an inhibitory neurotransmitter derived from glutamate on decarboxylation. In vitamin B6 deficiency, underproduction of GABA leads to convulsions (epileptic seizures) in infants and children.
- **Serotonin and melatonin:** Serotonin and melatonin are produced from tryptophan. Serotonin is a neurotransmitter and stimulates the cerebral activity. Melatonin is a sleep inducing substance and is involved in regulation of **circadian rhythm** of body.

**Fig. 14.47:** Synthesis of biogenic amines by PLP dependent decarboxylation of amino acids.

- **Histamine:** Histamine is produced by decarboxylation of histidine. It is a vasodilator and lowers blood pressure. It is involved in allergic reactions.
- **Catecholamines** (dopamine, norepinephrine and epinephrine). Synthesis of catecholamines from tyrosine requires PLP-dependent DOPA decarboxylase. Catecholamines are neurotransmitters and involved in metabolic and nervous regulation. Some of the most important biogenic amines and their functions are given in **Table 14.6**.

## Polyamines

- Biological amines made up of multiple amino acids called polyamines, e.g.
  - Putrescine
  - Spermidine
  - Spermine.

**Table 14.6:** Some important biogenic amines and their function.

| *Amine* | *Amino acid precursor* | *Function* |
|---|---|---|
| Dopamine | Tyrosine | Neurotransmitter |
| Norepinephrine | Tyrosine | Neurotransmitter |
| Epinephrine | Tyrosine | Hormone |
| Tyramine | Tyrosine | Vasoconstrictor |
| Serotonin | Tryptophan | Vasoconstrictor |
| Melatonin | Tryptophan | Vasoconstrictor |
| GABA | Glutamate | Neurotransmitter |
| Histamine | Histidine | Vasodilator |
| Taurine | Cysteine | Neurotransmitter |
| Spermine | Ornithine and methionine | Growth factor, regulator of transcription and translation |

- Polyamines are positively charged at physiological pH and associate with negatively charged nuclear DNA. These are present in high concentration in semen. The concentration of polyamines in brain is about 2 mm.

### *Functions of polyamines*

- Polyamines are involved in regulation of transcription and translation.
- They act as a growth factor and function in cell proliferation and growth.
- Polyamines are involved in stabilization of intact cells, subcellular organelles and membranes.

### *Biosynthesis of polyamines*

- **Putrescine, spermidine** and **spermine** are derived from ornithine and methionine.
- Ornithine is derived from arginine. Arginine undergoes a decarboxylation to form **putrescine** and carbon dioxide **(Fig. 14.48)** by an enzyme **ornithine decarboxylase**.
- S-Adenosylmethionine undergoes a decarboxylation to form 5-adenosylmethiopropylamine, by an enzyme S-adenosylmethionine decarboxylase.
- S-Adenosylmethiopropylamine donates aminopropyl group to putrescine and then to spermidine to form spermine.
- It is presumed that the 15% of methionine which cannot be used for cysteine synthesis in minimal diets is used for polyamine synthesis.

### *Catabolism and excretion of polyamines*

- The enzyme **polyamine oxidase** present in liver peroxisomes oxidizes spermine to spremidine and spermidine to putrescine.
- Putrescine is then oxidized by a copper containing diamine oxidase to $CO_2$ and $NH_3$.
- Major portions of putrescine and spermidine are excreted in urine after conjugation with acetyl-CoA as acetylated derivatives.

**Fig. 14.48:** Biosynthesis of polyamines.
(SAM: S-Adenosylmethionine)

*Clinical significance of polyamines*

- Polyamines and their derivatives have application in diagnosis and treatment of cancer.
- Their levels have been shown to increase in response to cell growth and differentiation.
- Their concentration is elevated in body fluids of cancer patients.
- Assays of urinary and blood polyamines have been used to detect cancer and to determine the success of therapy (diagnostic indicator).

## EXAM QUESTIONS

### Long Answer Questions (LAQs)

1. Describe the metabolism and inherited disorders of phenylalanine and tyrosine.
2. Explain the routes of ammonia disposal via: (i) Glutamate (ii) Glutamine (iii) Urea. Mention the disorders associated with the urea cycle.
3. Describe the digestion and absorption of proteins. Briefly mention the fate of amino acids.
4. What is the fate of ammonia and describe urea cycle?
5. Describe the metabolism of sulfur containing amino acids and associated inherited disorders.
6. Describe one carbon metabolism.
7. Describe the metabolism of glycine and associated disorders.
8. Describe the metabolism of amino acid tryptophan and associated disorders.
9. Describe metabolic disorders of aromatic and sulfur containing amino acids.
10. Describe formation and fate of ammonia.

### Short Notes

1. Transamination.
2. Nitrogen balance.
3. Deamination.
4. Metabolic disorders of urea cycle.
5. Phenylketonuria.
6. Alkaptonuria

7. Disorders of sulfur containing amino acids.
8. Hartnup disease.
9. Absorption of amino acids.
10. Glycine.
11. Tyrosinemia.
12. Albinism.
13. Serotonin.
14. Transmethylation.
15. Importance of histidine.
16. Formation of ammonia and its toxicity in brain.
17. Proteolytic enzymes and their specificity.
18. Give significance of urea cycle.
19. Biologically important compound derived from tyrosine and tryptophan.
20. Maple-syrup urine disease.

## Solve the Following

**Case History 1**

**A 3-year-old boy was admitted to hospital with the symptoms of pellagra, accompanied by mental retardation and excessive excretion of neutral amino acids. He was diagnosed as having Hartnup disease.**

**Questions**

a. What is the cause of Hartnup disease?
b. Why does it show mental retardation and pellagra like symptoms?
c. How will you treat pellagra like symptoms?
d. Can aminoaciduria be treated?

**Case History 2**

**A 5-month-old female infant was admitted to hospital with a complaint of vomiting and a failure to gain weight. The mother also reported that the child would oscillate between periods of irritability and lethargy, biochemical investigations of the patient indicated markedly increased concentration of plasma ammonia (550 μg/dL).**

**Questions**

a. Name the probable disorder.
b. What is the cause of the disorder?
c. Name the transport form of ammonia.
d. What will be the nutritional therapy for the patient?

**Case History 3**

**A full term infant was observed to have a lack of pigmentation, blue eyes, white hair and confirmed as a case of albinism.**

**Questions**

a. Name the deficient pigment.
b. Name the enzyme responsible for the defect.
c. Write biochemical reaction catalyzed by the enzyme.
d. Name the amino acid, from which the pigment is synthesized.

**Case History 4**

**A 20-year-old man came to the emergency room with severe pain in his right side and back. Subsequent examination and evaluation indicated a kidney stone and increased excretion of cystine, arginine and lysine in the urine and diagnosed as a case of cystinuria.**

**Questions**

a. What is the cause of cystinuria?
b. Why is there formation of kidney stone?
c. How is the condition to be treated?
d. Name the biosynthetic precursor of cysteine.

**Case History 5**

**A 5-month-old female infant was hospitalized. A diagnosis of classic phenylketonuria (PKU) was made.**

**Questions**

a. Name the defective enzyme of classic phenylketonuria.
b. Name the other types of PKU with their defective enzymes.
c. What are the characteristics of PKU?
d. Name diagnostic test for PKU.

**Case History 6**

**A patient was diagnosed as having alkaptonuria.**

**Questions**

a. Outline the biochemical pathway and point out metabolic defect which leads to this condition.
b. State the changes in urine, on standing, in such patients. Why?
c. What is ochronosis?
d. What is the treatment?

## Multiple Choice Questions (MCQs)

1. **Histidine is converted to histamine by:**
   a. Transamination
   b. Decarboxylation
   c. Hydroxylation
   d. Reduction

**2. In Maple-syrup urine disease, which of the following compounds is accumulated?**
a. Homogentisate
b. Methylmalonyl-CoA
c. Branched chain α-keto acids
d. Homocysteine

**3. The methylene group, transferred to glycine in converting it to serine, comes from:**
a. S-Adenosylmethionine
b. Methylene-$B_{12}$
c. Carboxybiotin
d. $N^5$,$N^{10}$-methylene-THF

**4. Which of the following does not take part in the human urea cycle?**
a. Arginine
b. Aspartate
c. Arginosuccinate
d. Urease

**5. Which type of reaction is conversion of norepinephrine to epinephrine?**
a. Transamination
b. Decarboxylation
c. Transmethylation
d. Phosphorylation

**6. Phenylketonuria results due to the absence of the enzyme:**
a. Phenylalanine oxidase
b. Phenylalanine hydroxylase
c. Phenylalanine transaminase
d. Phenylalanine oxygenase

**7. Which of the following amino acid cannot undergo transamination?**
a. Lysine b. Alanine
c. Aspartic acid d. Glutamic acid

**8. The amino acid required for synthesis of heme is:**
a. Glutamine b. Glutamic acid
c. Glycine d. Lysine

**9. All of the following are synthesized from tyrosine, except:**
a. Melanin b. Serotonin
c. Dopamine d. Epinephrine

**10. The fate of ammonia in brain is:**
a. Conversion to urea
b. Conversion to glutamate
c. Conversion to aspartate
d. Remains as such

**11. Which of the following amino acids can undergo deamination by dehydration?**
a. Threonine b. Alanine
c. Tryptophan d. Glycine

**12. Transamination of oxaloacetate results in the formation of:**
a. Aspartic acid b. Valine
c. Alanine d. Serine

**13. All of the following are intermediates formed by amino acid degradation, *except*:**
a. α-Ketoglutarate b. Oxaloacetate
c. Fumarate d. Citrate

**14. In alkaptonuria, which of the following accumulates abnormally in the urine?**
a. Phenylalanine b.Acetoacetate
c. Homogentisated.Fumarate

**15. The reactions of urea cycle occur in:**
a. The cytosol
b. The mitochondria
c. The mitochondrial matrix and the cytosol
d. Lysosomes

**16. All of the following statements are true in the transamination of amino acids, *except*:**
a. Ammonia is neither consumed nor produced
b. Requires pyridoxal phosphate
c. The amino group acceptor is α-keto acid
d. All amino acids can undergo transamination

**17. Tetrahydrobiopterine is used as a coenzyme in the metabolism of:**
a. Folic acid b. Phenylalanine
c. Glycine d. Aspargine

**18. Which of the following pathways occurs in part in the mitochondria and in part in the cytoplasm?**
a. Urea cycle
b. TCA cycle
c. Glycolysis
d. Oxidative phosphorylation

**19. Which of the following amino acid is involved in the synthesis of carnitine?**
a. Lysine b. Phenylalanine
c. Tryptophan d. Threonine

**20. The following enzymes are involved in digestion and absorption of protein, *except*:**
a. Trypsinogen b. Amylase
c. Pepsin d. Chymotrypsin

**21. Norepinephrine is converted to epinephrine by:**
a. Transamination
b. Transmethylation
c. Transcarboxylation
d. Decarboxylation

**22. Following enzymes are endopeptidase, *except*:**
a. Pepsin b. Trypsin
c. Chymotrypsin d. Aminopeptidase

**23. Which of the following enzyme is secreted by mucosal cell?**
a. Trypsin b. Proelastase
c. Aminopeptidase d. Carboxypeptidase

**24. Carboxypeptidase contains which of the following mineral?**
a. Fe b. Cu
c. Ca d. Zn

**25. For metabolic disorder of urea cycle which statement is not correct?**
a. Ammonia intoxication is most severe when the metabolic block occurs prior to reactions 3 of the urea cycle.
b. Clinical symptoms include mental retardation
c. Clinical signs include hyperammonemia
d. Glutamate provides the second nitrogen of arginosuccinate

**26. Select incorrect statement of the following:**
a. Threonine contribute to biosynthesis of coenzyme A
b. Histamine arises by decarboxylation of histidine
c. Serotonin and melatonin are metabolites of tryptophan
d. Glycine, arginine and methionine each contribute atoms for biosynthesis of creatine

**27. In the formation of urea from ammonia all of the following are true, *except*:**
a. Ornithine transcarbamoylase catalyzes the rate limiting step
b. Aspartate supplies one of the nitrogen found in urea.
c. This is an energy required process
d. Fumarate is produced

**28. Following components are required for the urea formation, *except*:**
a. Ammonia
b. α-amino group of aspartate
c. $CO_2$
d. NADPH

**29. Which of the following is true for aminotransferase?**
a. Catalyze irreversible reaction
b. Require pyridoxal phosphate as a cofactor
c. Catalyze reaction that result in net loss of amino group in the form of ammonia
d. Usually require glutamine as one of the reacting pair

**30. Which of the following statements are true for the reaction catalyzed by glutamate dehydrogenase? *Except*:**
a. It catalyzes oxidative deamination
b. Requires NADH or NADPH
c. Net removal of α-amino group of amino acid to ammonia
d. Is an irreversible reaction

**31. The number of ATP required for urea synthesis is:**
a. 0 b. 1
c. 2 d. 4

**32. A compound that link citric acid cycle and urea cycle is:**
a. Malate b. Citrate
c. Succinate d. Fumarate

**33. The two nitrogen atoms in urea are contributed by:**
a. Ammonia and glutamate
b. Glutamine and glutamate
c. Ammonia and aspartate
d. Ammonia and alanine

**34. A coenzyme required for the synthesis of glycine from serine is:**
a. ATP
b. Pyridoxal phosphate
c. Tetrahydrofolate
d. NAD

**35. Cysteine is synthesized from methionine and which of the following amino acid**
a. Serine b. Homoserine
c. Homocysteine d. Threonine

**36. Methionine is synthesized in human body from:**
a. Cysteine and homoserine
b. Homocysteine and serine
c. Cysteine and serine
d. None of these

**37. Free ammonia is released during:**
a. Oxidative deamination of glutamate
b. Catabolism of purines
c. Catabolism of pyrimidines
d. All of these

**38. Ammonia is transported from muscles to liver mainly in the form of:**
a. Free ammonia b. Glutamine
c. Asparagine d. Alanine

**39. Maple syrup urine disease is an inborn error of metabolism of:**
a. Sulfur-containing amino acids
b. Aromatic amino acids
c. Branched chain amino acids
d. Dicarboxylic amino acids

**40. Pancreatic juice contains of all of the following, *except*:**
a. Trypsin b. Chymotrypsin
c. Carboxypeptidase d. Aminopeptidase

**41. Normal range of serum urea is:**
a. 0.6–1.5 mg/dL b. 9–11 mg/dL
c. 20–40 mg/dL d. 60–100 mg/dL

**42. The symptom of ammonia intoxication includes:**
a. Blurring of vision b. Constipation
c. Skin lesion d. Diarrhea

**43. The milk protein in the stomach of the infants is digested by:**
a. Pepsin b. Trypsin
c. Chymotrypsin d. Rennin

**44. Rennin acts on casein of milk in infants in presence of:**
a. $Mg^{++}$ b. $Zn^{++}$
c. $Co^{++}$ d. $Ca^{++}$

**45. Neonatal tyrosinemia improves on administration of:**
a. Thiamin b. Riboflavin
c. Pyridoxine d. Ascorbic acid

**46. Absence of phenylalanine hydroxylase causes:**
a. Neonatal tyrosinemia
b. Classic phenylketonuria
c. Primary hyperoxaluria
d. Albinism

**47. Richner-Hanhart syndrome is due to defect in:**
a. Tyrosinase
b. Phenylalanine hydroxylase
c. Hepatic tyrosine transaminase
d. Fumarylacetoacetate hydrolase

**48. Tyrosinosis is due to defect in the enzyme:**
a. Fumarylacetoacetate hydrolase
b. P-hydroxyphenylpyruvate hydroxylase
c. Tyrosine transaminase
d. Tyrosine hydroxylase

**49. An important finding in glycinuria is:**
a. Excess excretion of oxalate in the urine
b. Deficiency of enzyme glycinase
c. Significantly increased serum glycine level
d. Defect in renal tubular reabsorption of glycine

**50. Increased urinary indole acetic acid is diagnostic of:**
a. Maple syrup urine disease
b. Hartnup disease
c. Homocystinuria
d. Phenylketonuria

**51. An inborn error, maple syrup urine disease is due to deficiency of the enzyme:**
a. Glycinase
b. Phenylalanine hydroxylase
c. Fumarylacetoacetate hydrolase
d. α-Ketoacid dehydrogenase

**52. Alkaptonuria occurs due to deficiency of the enzyme:**
a. Maleylacetoacetate isomerase
b. Homogentisate oxidase
c. P-hydroxyphenylpyruvate hydroxylase
d. Fumarylacetoacetate hydrolase

**53. Ochronosis occurs in:**
a. Tyrosinemia
b. Tyrosinosis
c. Alkaptonuria
d. Richner Hanhart syndrome

## Answers for MCQs

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **1.** b | **2.** c | **3.** d | **4.** d | **5.** c | **6.** b | **7.** a | **8.** c |
| **9.** b | **10.** b | **11.** a | **12.** a | **13.** d | **14.** c | **15.** c | **16.** d |
| **17.** b | **18.** a | **19.** a | **20.** b | **21.** b | **22.** d | **23.** c | **24.** d |
| **25.** d | **26.** a | **27.** a | **28.** d | **29.** b | **30.** d | **31.** d | **32.** d |
| **33.** c | **34.** b | **35.** a | **36.** d | **37.** a | **38.** d | **39.** c | **40.** d |
| **41.** c | **42.** a | **43.** d | **44.** d | **45.** d | **46.** b | **47.** c | **48.** a |
| **49.** d | **50.** b | **51.** d | **52.** b | **53.** c | | | |

CHAPTER

# 15 Integration of Metabolism and Metabolism in Starvation

**Chapter outline**


- Integration of Metabolism
- Integration of Metabolism at Cellular Level
- Integration of Metabolism at Tissue or Organ Level
- Metabolism in Starvation
- Metabolic Changes occur during Short-term Starvation
- Metabolic Changes occur during Prolonged Starvation


## INTRODUCTION

Carbohydrates, lipids and proteins are the principal foods providing necessary nutrients to the body. The metabolism of all of them is inter-related, and occurs simultaneously **(Fig. 15.1)**. The deficiency of one is made up by another to some extent.

## INTEGRATION OF METABOLISM

### Definition

The various ***anabolic*** and ***catabolic*** pathways by which carbohydrates, lipids and proteins are processed for energy supply or for the biosynthesis of compounds required by the cell (for maintenance or growth) are closely co-ordinated.

**Fig. 15.1:** Inter-relationship between **metabolism** of three principal nutrients. The amino acids, glucose and fatty acids catabolized to a common degradative product, acetyl-CoA, which in turn is degraded to $CO_2$ and $H_2O$ with formation of ATP.

***This coordination between three metabolites is called integration of metabolism.***

Integration of metabolism is considered at two levels:

1. Cellular level
2. Tissue or organ level.

## INTEGRATION OF METABOLISM AT CELLULAR LEVEL

Integration of metabolism at cellular level includes the different metabolic pathways of glucose, fatty acids, glycerol and amino acids, at the cellular level which results in: **(Fig. 15.2)**.

1. Conversion of carbohydrates into fats and fats into carbohydrates.
2. Conversion of carbohydrates into proteins and proteins into carbohydrates.
3. Conversion of proteins into fats and fats into proteins.

### Conversion of Carbohydrates into Fats and Fat to Carbohydrate

- Carbohydrates (glucose) are metabolized via glycolytic pathway to pyruvate and then pyruvate to acetyl-CoA.
- Acetyl- CoA is the starting material for synthesis of ***fatty acids.*** Fatty acids, that are produced, combine with glycerol to form ***triacylglycerol***.
- Glycerol may also be supplied from the glucose by glycolysis as glycerol-3-phosphate. Thus, carbohydrates can easily form fat.
- There cannot be a net conversion of fatty acids having an **even number** of carbon atoms (which form acetyl-CoA) to glucose or glycogen.
- Only a fatty acid having an **odd number** of carbon atoms is glucogenic (i.e. can form glucose) as it forms a molecule of propionyl-CoA upon β-oxidation.
- **Propionyl-CoA** can be converted to succinyl-CoA, an intermediate of citric acid cycle, which can be converted to glucose by gluconeogenesis.
- The glycerol moiety of triacylglycerol is converted to glucose by gluconeogenesis after activation to glycerol-3-phosphate and this is an important source of glucose in starvation.

### Conversion of Carbohydrates into Proteins and Proteins into Carbohydrates

- Many carbon skeletons of the nonessential amino acids can be produced from carbohydrate via the intermediates of citric acid cycle and transamination.
- By reversal of these processes, glucogenic amino acids yield intermediates of the citric acid cycle. They are therefore readily converted by gluconeogenesis to glucose and glycogen.

### Conversion of Proteins to Fats and Fats into Proteins

- Conversion of carbon skeletons of glucogenic amino acids to fatty acids is possible either by formation of pyruvate and acetyl-CoA.
- Generally, however, the net conversion of amino acids to fat is not a significant process.
- It is not possible for a net conversion of fatty acids to amino acids to take place.

## INTEGRATION OF METABOLISM AT TISSUE OR ORGAN LEVEL

Integration of metabolism at tissue or organ level includes the inter-relationship of different tissues and organs to meet metabolic demands for the whole body **(Fig. 15.3)**. The major organs along with their most important metabolic functions are discussed here.

### Role of Liver

Major roles of the liver include the following:

- Maintenance of blood glucose levels.
- During the fed state, the liver takes up excess glucose and stores it as glycogen or converts it to fatty acids.
- During the fasting state, liver provides glucose for the body by the **glycogenolysis** and **gluconeogenesis**.
- The liver serves as the major site of fatty acid synthesis.
- The liver synthesizes ketone bodies during starvation and supplies to the peripheral tissues as a source of energy.

### Role of Skeletal Muscle

- Skeletal muscle maintains large stores of glycogen, which provide energy during exertion.
- During starvation, free fatty acids and ketone bodies supplied by liver are oxidized in preference to glucose in muscle.
- The protein present in muscle may be used as a fuel source, if no other fuel is available.
- Pyruvate, the product of glycolysis in the skeletal muscle, may be converted to either lactate or alanine and transported to the liver, where it is used to regenerate glucose via gluconeogenesis ***(See Fig. 12.11)***.

**Fig. 15.2:** Integration of three metabolisms at cellular level.

## Role of Adipose Tissue

- The primary function of the adipose tissue is the storage of metabolic fuel in the form of triacylglycerols.
- During the fed state, the adipose tissue synthesizes triacylglycerols from glucose and free fatty acids.
- During the fasting state, triacylglycerols are converted to glycerol and fatty acids, which are exported to the liver and other tissues.

## Role of Heart Muscle

Heart muscle contains essentially no fuel reserves and must be continuously supplied with fuel from liver and adipose tissue.

## Role of Brain

- Brain tissue normally uses glucose as an exclusive fuel, except during starvation, when it can adapt to use ketone bodies as an energy source.

**Fig. 15.3:** Integration of metabolism among major tissues of the body.

- The brain contains essentially no fuel reserves and must be continuously supplied with fuel from the liver.

**Significance of Integration of Metabolism**

- Integration of metabolism ensures a supply of suitable fuel for all tissues, at all times from the fully fed state to the totally starved state.
- Under positive caloric balance, i.e. in well fed state, a significant proportion of the food energy intake is stored as either glycogen or fat.
- Under negative caloric balance, i.e. in starvation, fatty acids are oxidized in preference to glucose, to spare glucose for those tissues, (e.g. brain and erythrocytes) that require it under all conditions.

## METABOLISM IN STARVATION

### Definition

- Starvation is the deprivation of the food and thereby deprivation of exogenous supply of calories to meet the energy demands of the body for basal metabolism and other activities.
- Starvation is not always the result of unavailability or scarcity of food.
- Any medical condition, which prevents consumption or utilization of available food will lead to starvation, e.g. trauma, surgery, cancer cachexia, infections, malabsorption, etc.

### Fuel Reserve of a Normal Healthy Person

- In starvation, energy has to be derived from the body's own stores. A typical well-nourished 70 kg man has fuel reserve of:
  - 1600 kcal in glycogen
  - 2400 kcal in mobilizable protein
  - 135000 kcal in triacylglycerols.
- The energy, needed for 24-hr period, ranges from about ***1600 kcal in the basal state to 6000 kcal*** depending on the extent of activity.
- Thus, stored fuels are enough to meet caloric needs in starvation for one to three months and in the case of some obese individuals, much longer.

## Phases of Starvation

Starvation can be divided into two phases characterized by distinct metabolic changes. In starvation, the changes in metabolism are not abrupt but gradual **(Fig. 15.4)**.

1. ***Short-term starvation,*** which covers the 12 hour overnight fast and can extend to 24 hour.
2. ***Prolonged starvation,*** which lasts longer than 24 hour and can extend to several days or weeks.

# METABOLIC CHANGES OCCUR DURING SHORT-TERM STARVATION

## Changes in Carbohydrate Metabolism and Role of Liver

- The first phase of starvation begins four to five hours after a meal. Within about 1 hr after a meal, blood glucose levels begin to fall. Consequently, **insulin levels decline** and **glucagon, epinephrine levels rise**.
- The brain, the erythrocytes, the bone marrow, the renal medulla and peripheral nerves have to be supplied with glucose, for their energy needs. However, the tissues such as muscle can readily use free fatty acids, released from adipose tissue.

The **first priority** of metabolism in starvation is to provide sufficient glucose to the brain and other tissues that are absolutely dependent on glucose.

- In this phase, the main source of blood glucose is ***liver glycogen***. The liver first uses **glycogenolysis** (glycogen degradation) then **gluconeogenesis** to maintain blood glucose levels and provide sufficient glucose to the brain and other glucose requiring tissues.
- The fall of **blood glucose** and **insulin concentration** and rise of **glucagon** stimulates glycogenolysis. Glucagon stimulates c-AMP formation, essential for degradation of glycogen.

The liver glycogen is capable of maintaining the blood glucose concentration at normal values for 8 to 12 hours.

- As the liver glycogen store begins to be depleted, gluconeogenesis becomes active, which ensures a continuous supply of glucose to the brain and other tissues. The sources of substrates for gluconeogenesis are:

**Fig. 15.4:** Metabolic changes occurring during starvation.
The circled number indicates the approximate order in which processes begin to occur.
(TG: Triacylglycerol; FA: Fatty acid; KB: Ketone bodies)

- Pyruvate
- Lactate which is a product of glycolysis in red blood cell and exercising muscle
- Glucogenic amino acids released from muscle
- Glycerol released by degradation of triacylglycerols.

Gluconeogenesis plays an essential role in maintaining blood glucose during both short-term and prolonged starvation.

### Changes in Protein Metabolism and Role of Muscles

- The decrease in plasma insulin and increase in epinephrine inhibits glycolysis and the uptake of glucose by muscle, whereas fatty acids enter freely in muscle.
- Increased proteolytic activity occurs due to decreased concentration of insulin. The main precursor for gluconeogenesis is, however, tissue protein, in the form of amino acids, released after proteolytic degradation.
- During short-term starvation there is a rapid breakdown of muscle protein, providing amino acids that are used by the liver for synthesis of glucose by gluconeogenesis. The major gluconeogenic amino acids, coming from muscle, are ***alanine*** and ***glutamine.***

### Changes in Fat Metabolism and Role of Adipose Tissue

- Increased levels of **epinephrine** stimulates breakdown of triacylglycerol of adipose tissue to free fatty acids and glycerol. Free fatty acids can be used by some tissues such as skeletal muscle, as an alternative source of fuel, and glucose is spared for the brain and other glucose requiring tissues.
- The glycerol derived from the cleavage of triacylglycerol is a source for the synthesis of glucose by gluconeogenesis by the liver.

## METABOLIC CHANGES OCCUR DURING PROLONGED STARVATION

- The processes which take place in short-term starvation cannot go on indefinitely, because, although gluconeogenesis provides glucose efficiently for the body's energy requirements, it will soon deplete the substantial proportion of body protein and it is known that death results when 30 to 50% of the body protein is lost.
- Adjustments to metabolism are made after 24 to 48 hr, which conserve body protein.

***Thus, the second priority of metabolism in starvation is to preserve protein.*** This is accomplished by using fatty acids and ketone bodies in place of glucose as a fuel.

### Changes in Carbohydrate Metabolism in Prolonged Starvation

- The blood glucose levels drop from about 100 mg/dL to about 70 mg/dL after 24 h but, are thereafter maintained **(Fig. 15.5)**.
- Conservation of body proteins is accomplished by a reduction in glucose production by gluconeogenesis.

### Changes in Fat Metabolism in Prolonged Starvation

- During prolonged fasting, adipose tissue continues to breakdown its triacylglycerol store to fatty acids and glycerol. These fatty acids serve as the major source of fuel for the body. The glycerol is converted to glucose while fatty acids are oxidized to $CO_2$ and $H_2O$ by muscle and in the liver where they are converted into ketone bodies.
- The synthesis of ketone bodies from acetyl-CoA increases markedly producing the state of **ketosis (Fig. 15.5)**. As the citric acid cycle is unable to oxidize all of the acetyl-CoA generated by the degradation of fatty acids, these are converted to ketone bodies. Gluconeogenesis depletes the supply of oxaloacetate, which is essential for the entry of acetyl-CoA into the citric acid cycle. Consequently, liver produces large quantities of ketone bodies.

***Ketosis is a metabolic adaptation to starvation, arises as a result of deficiency in available carbohydrate.***

**Fig. 15.5:** Changes in the concentration of fuels in the blood during prolonged fasting (1 mm glucose = 18 mg/dL glucose).

- As the supply of ketone bodies increases and the supply of glucose diminish, the brain reduces its utilization of glucose and begins to consume appreciable amounts of ketone bodies in place of glucose. Although the brain still has a residual glucose requirement, not only for provision of energy, but also for synthesis of ***neurotransmitters***.
- After several weeks of starvation, ketone bodies become the major fuel of the brain which diminishes the need for glucose. Therefore, in prolonged starvation, less muscle is degraded than in the first days of starvation. This sequence of events leads to at least partial preservation of the protein stores of the body.

## Changes in Protein Metabolism in Prolonged Starvation

- During the first few days of starvation, there is a rapid breakdown of muscle protein, providing amino acids, alanine and glutamine for gluconeogenesis.
- After several weeks of starvation, the rate of muscle breakdown decreases due to decreased need of glucose as a fuel for brain which has begun using ketone bodies as a source of energy.
- Because of these adaptive mechanisms the duration of starvation of the adult human is determined by the size of the triacylglycerol depot. When the stored triacylglycerol are completely exhausted, muscle proteins the largest single source of energy are heavily drained.
- At that time, the protein stores once again enter a stage of rapid depletion. As proteins are also essential for the maintenance of cellular function, death ordinarily results when the proteins of the body have been depleted to about half their normal level.

**Fig. 15.6:** Changes in urea excretion during starvation.

- During the conversion of amino acid to glucose by gluconeogenesis, the nitrogen of the amino acid is converted to urea and so production of urea decreases during prolonged starvation, as compared to its production in short-term starvation **(Fig. 15.6)**.

## Effect on BMR

- Apart from increase in the ratio of glucagon to insulin concentrations thyroxine ($T_3$), production is reduced and that leads to the decreased:
  - BMR
  - Body temperature
  - Pulse rate
  - BP.
- The body is more susceptible to infections, all these lead death in prolonged starvation.

# EXAM QUESTIONS

## Long Answer Questions (LAQs)

1. Define integration metabolism. Discuss integration of metabolism at various levels and its significance.
2. Define starvation. Describe metabolic changes occur during starvation.

## Short Notes

1. Biochemical changes in starvation.
2. Integration metabolism at cellular level.
3. Integration metabolism at tissue or organ level.
4. Short-term starvation.
5. Prolonged starvation.
6. Role of liver in integration metabolism.
7. Significance of integration metabolism.
8. What is short-term and prolonged starvation?

## Solve the Following

**Case History 1**

**A patient attending an obesity clinic is found to have ketonuria. The patient is on a low carbohydrate diet in order to lose weight. There is no glycosuria. Blood glucose level was, found 80 mg/100 mL:**

a. Explain the cause of ketonuria
b. What is ketoacidosis?
c. Name ketone bodies
d. What is ketonemia?
e. What is ketosis?

## Multiple Choice Questions (MCQs)

1. **The first priority of metabolism in starvation is to provide which of the following substances to the brain and other tissue?**
   a. Fatty acids b. Ketone bodies
   c. Glucose d. Cholesterol
2. **During starvation, blood or tissue levels of all of the following are elevated, *except:***
   a. Free fatty acids b. Ketone bodies
   c. Glucagon d. Insulin
3. **Which of the following processes plays an essential role in maintaining blood glucose during early and prolonged starvation?**
   a. Glycolysis
   b. Gluconeogenesis
   c. Ketogenesis
   d. Glycogenolysis
4. **In starvation, muscle protein is spared by:**
   a. Using fatty acids and ketone bodies
   b. By increasing rate of gluconeogenesis
   c. By inhibiting glucose utilization
   d. By stimulating protein synthesis
5. **In prolonged starvation, the main energy source of brain is:**
   a. Glucose b. Ketone bodies
   c. Fructose d. Fatty acids
6. **The major glucogenic amino acid coming from muscle to liver for gluconeogenesis during starvation is:**
   a. Threonine b. Methionine
   c. Alanine d. Glycine
7. **During early fasting glucose is derived from:**
   a. Glycogen b. Amino acids
   c. Fatty acids d. Ketone bodies
8. **Fatty acid or ketone bodies are used as energy source in:**
   a. Intraprandial phase
   b. Post absorptive phase
   c. Prolonged starvation
   d. Fully fed state
9. **During starvation free fatty acids and ketone bodies are oxidized in preference to glucose in muscle by impairing, *except:***
   a. Uptake of glucose by the cell
   b. Phosphorylation by hexokinase and phosphofructokinase
   c. Pyruvate dehydrogenase activity
   d. Lactate dehydrogenase activity
10. **During starvation, the first reserve nutrient to be depleted is:**
    a. Glycogen b. Proteins
    c. Triglycerides d. Cholesterol
11. **Synthesis of the following enzymes is increased during starvation:**
    a. Digestive enzymes
    b. Gluconeogenic enzymes
    c. Glycolysis enzymes
    d. Glycogenesis enzymes
12. **In fasting which of the following metabolites will be elevated in blood after 24 hours?**
    a. Triacylglycerol
    b. Glucose
    c. Free fatty acid
    d. Glycogen
13. **On prolonged fasting which of the following metabolites will be elevated in blood after 3 days?**
    a. Ketone bodies b. Glycogen
    c. Triacylglycerol d. Glucose
14. **After overnight fast one would expect increased activity of what?**
    a. Hepatic glycogen synthase
    b. Pyruvate dehydrogenase
    c. Hormone sensitive lipase
    d. Pancreatic lipase
15. **After overnight fast one would expect glucose transporter activity to be what?**
    a. Decreased in brain cell
    b. Enhanced in adipocytes
    c. Decreased in red blood cells
    d. Decreased in muscle cells

**16. Which one of the following statements is correct for metabolism during prolonged starvation compared to early or short term starvation?**
a. The degradation of proteins is increased
b. The rate of gluconeogenesis in liver is decreased
c. The formation of ketone bodies is deceased
d. Heart generates more of its energy from glucose.

**17. Which of the following statements are correct for amino acid metabolism during the fasting state compared with the fed state? *Except:***
a. Glutamine and alanine are released in increased amounts
b. Glucose is preserved for the brain
c. Alanine is utilized for glucose synthesis
d. Alanine is utilized for protein synthesis

**18. Which of the following occurs in cardiac muscle but not in the brain, even in the fasted state?**
a. Fatty acid oxidation
b. Glycolysis
c. TCA cycle
d. Glucose uptake via a glucose transporter

**19. During starvation, ketone bodies are used as a fuel by:**
a. Erythrocytes
b. Brain
c. Liver
d. All of these

## Answers for MCQs

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

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CHAPTER

# 16

# Water Metabolism

**Chapter outline**

- Importance of Water
- Total Body Water (TBW) and its Distribution
- Normal Water Balance
- Electrolytes
- Regulation of Water and Electrolyte Balance
- Disorders of Water and Electrolyte Balances

## INTRODUCTION

Water is the most abundant constituent of the human body accounting approximately 60 to 70% of the body mass in a normal adult. Water content of the body changes with age. It is about 75% in the newborn and decreases to less than 50% in older individuals. Water content is greatest in brain tissue and least in adipose tissue.

## IMPORTANCE OF WATER

- It is a medium in which body solutes, both organic and inorganic, are dissolved and metabolic reactions take place.
- It acts as a vehicle for transport of solutes.
- Water itself participates as a substrate and a product in many chemical reactions, e.g. in glycolysis, citric acid cycle and respiratory chain.
- The stability of subcellular structures and activities of numerous enzymes are dependent on adequate cell hydration.
- Water is involved in the regulation of body temperature because of its highest latent heat of evaporation.
- Water also acts as a lubricant in the body so as to prevent friction in joints, pleura, peritoneum and conjunctiva.
- Both a relative deficiency and an excess of water impair the function of tissues and organs.

## TOTAL BODY WATER (TBW) AND ITS DISTRIBUTION

Total body water, includes water both inside and outside of cells and water normally present in the gastrointestinal and genitourinary systems.

**Fig. 16.1:** Body water compartments

Total body water can be theoretically divided into two main compartments **(Fig. 16.1):**

1. Extracellular water (ECW) and
2. Intracellular water (ICW).

- The ECW includes all water external to cell membranes. The ECW can be further subdivided into:
  - Intravascular water, i.e. plasma
  - Extravascular water, i.e. interstitial fluid.
- The ICW includes all water within cell membranes and constitutes the medium in which chemical reactions of cell metabolism occur.

### Distribution of Water

- In a 70 kg adult the total body water is about **42 L.**
- **28 L** is of intracellular water (ICW) and **14 L** of extracellular water (ECW).
- The ECW is distributed as **3.5 L** plasma water (intravascular water) and **10.5 L** interstitial water (extravascular) **(Table 16.1)**

**Table 16.1:** Distribution of water.

| *Compartment* | *Percentage of TBW* | *Volume in normal adult* |
|---|---|---|
| Total body water (TBW) | – | 42 L |
| Extracellular water (ECW) | 33% | 14 L |
| a. Plasma | 8% | 3.5 L |
| b. Interstitial water | 25% | 10.5 L |
| Intracellular water (ICW) | 67% | 28.0 L |

### *Factors Affecting Distribution of Water*

- Two important factors influence the distribution of water between intracellular and extracellular compartments are:
  - Osmolality or osmolarity
  - Colloidal osmotic pressure.
- Osmolarity or osmolality is a measure of solute particles present in fluid medium.
- Osmolarity is the number of moles per liter of solution and osmolality is the number of moles per kg of solvent.
- All molecules dissolved in the body water contribute to the **osmotic pressure.** Thus, osmolarity or osmolality determines the osmotic pressure exerted by a solution across a membrane. However, for biological fluids, the osmolality is more commonly used.
- The osmotic pressure of a solution is directly proportional to the concentration of osmotically active particles in that solution.
- In a normal person, the osmotic pressure of ECF (mainly due to $Na^+$ ions) is equal to the osmotic pressure of ICF (which is mainly due to $K^+$ ions). Due to this osmotic equilibrium there is no net movement of water in or out of the cells.
- A change in the concentration of osmotically active ions in either of the water compartments creates a difference of osmotic pressure and consequently movement of water between compartments occur.
- Water diffuses from a compartment of low osmolality to one of high osmolality until the osmotic pressures are identical in both of them.

## NORMAL WATER BALANCE

- The body water is maintained within the fairly constant limits by a regulation between the **intake** and **output** of water as shown in **Table 16.2**.
- Average daily water turnover in the adult is approximately 2500 mL. However, the range of water turnover depends on **intake**, **environment** and **activity**.

### Water Intake

Under normal conditions:

- Approximately, one-half to two-thirds of water intake is in the form of **oral fluid intake,** and
- Approximately, one-half to one-third is in the form of oral **intake of water in food**.
- In addition, a small amount of water (150 to 350 ml/day) is produced during metabolism of food called **metabolic *water***.
- Oral water intake is regulated by a **thirst center** located in hypothalamus. Increase in the osmolality of plasma causes increased water intake by stimulating thirst center.

### Water Output

Water is lost from the body by following routes.

- Urinary water loss via kidney
- Insensible water loss via skin and lungs
- Sensible perspiration (sweating)
- Gastrointestinal water loss through stool.

**Table 16.2:** Average water balance in normal adult.

| *Daily intake of water* | | *Daily output of water* | |
|---|---|---|---|
| *Source* | *mL* | *Source* | *mL* |
| Drinking water | 1200 | Urine | 1400 |
| Water from food | 1000 | Insensible water loss<br>Through skin<br>Through lungs | <br>400<br>400 |
| Water derived during metabolism of food (metabolic water) | 300 | Sensible perspiration water loss | 100 |
| | | Gastrointestinal water loss through stool | 200 |
| Total | 2500 | | 2500 |

### Urinary Water Loss

In a normal individual, 1200 to 1500 mL of water is lost in urine per day. Urine volume varies in response to changes in ECW volume and osmolality.

### Insensible Water Loss

Loss of water by diffusion through skin and through the lungs is known as insensible water because it is not apparent. It is the only route by which water is lost without solute. Normally, half of the insensible water loss occurs through the skin (about 400 mL) and half through the lungs (about 400 mL). Insensible water loss increases with increase in surrounding temperature, body temperature and physical activity.

### Sensible Perspiration

Sensible perspiration via skin is negligible in cool environment but increases with surrounding temperature, body temperature or physical activity. An increase in plasma osmolality causes a decrease in the rate of sensible perspiration.

### Gastrointestinal Water Loss

Water loss from the gastrointestinal tract through stool is approximately 200 mL/day.

## ELECTROLYTES

Electrolytes are the inorganic substances which are readily dissociated into **positively charged (cations)** and **negatively charged (anions)** ions.

Normal cellular functions and survival requires electrolytes which are maintained within narrow limits. The concentration of electrolytes is expressed as **milliequivalent per liter (mEq/L) rather than milligrams.**

### Distribution of Electrolytes

- The electrolytes are well distributed in body fluids and play an important role in distribution and retention of body water by regulating the osmotic equilibrium.
- Total concentration of cations and anions in each compartment (ECF and ICF) is equal to maintain electrical neutrality. The concentration of electrolytes in extracellular and intracellular fluid is shown in **Table 16.3**. There are striking differences in composition between the two fluids.

**Table 16.3:** Electrolyte content of ECF and ICF.

| *Ions* | *Extracellular fluid mEq/L* | *Intracellular fluid mEq/L* |
|---|---|---|
| Cations | | |
| $Na^+$ | 142 | 10 |
| $K^+$ | 5 | 150 |
| $Ca^{++}$ | 5 | 2 |
| $Mg^{++}$ | 3 | 40 |
| Total | 155 | 202 |
| Anions | | |
| $Cl^-$ | 103 | 2 |
| $HCO_3^-$ | 27 | 10 |
| $HPO_4^-$ | 2 | 140 |
| $SO_4^-$ | 1 | 5 |
| Organic acids | 6 | 5 |
| Protein | 16 | 40 |
| **Total** | **155** | **202** |

- **Sodium** is the principal cation of the **extracellular fluid** and comprises over 90% of the total cations, but has a low concentration in intracellular fluid and constitutes only 8% of the total cations
- **Potassium** by contrast, is the principal cation of **intracellular fluid** and has a low concentration in extracellular fluid.
- Similar differences exist with the anions. **Chloride ($Cl^-$)** and **bicarbonate ($HCO_3^-$)** predominate in the **extracellular fluid,** while **phosphate** is the principal anion within the cells.

The term electrolytes applied in medicine to the four ions in plasma, ($Na^+$, $K^+$, $Cl^-$ and $HCO_3^-$) that exert the greatest influence on water balance and acid-base balance.

## REGULATION OF WATER AND ELECTROLYTE BALANCE

Water and electrolyte balance are regulated together. It is regulated through following hormones:

- Antidiuretic hormone (ADH) or vasopressin
- The renin-angiotensin-aldosterone system (RAAS)
- Atrial natriuretic factor (ANF).

### Antidiuretic Hormone (ADH)

- Water **intake** is normally controlled by the sensation of **thirst** and its **output** by the action of hormone **vasopressin,** also known as antidiuretic hormone

**Fig. 16.2:** Regulation of water balance.

(ADH). The major role of ADH is to increase the reabsorption of water from the kidney.

- An increase in plasma osmolality (due to deficiency of water) causes sensation of thirst and stimulates hypothalamic thirst center, which results in an increase in water intake. An increase in plasma osmolality also stimulates hypothalamus to release ADH. ADH then increases water reabsorption by the kidney. All these events ultimately help to restore the plasma osmolality **(Fig. 16.2)**.
- Conversely, a large intake of water causes fall in osmolality suppresses thirst and reduces ADH secretion, leading to a diuresis, producing large volume of dilute urine.

## Renin-Angiotensin-Aldosterone System (RAAS)

- Renin is secreted in response to a decreased level of $Na^+$ in the fluid of the distal tubule. Renin converts angiotensinogen in plasma to angiotensin I, which in turn is converted to **angiotensin II** by angiotensin converting enzyme (ACE). Angiotensin II stimulates aldosterone secretion, thirsting behavior and ADH secretion.

Aldosterone stimulates $Na^+$ reabsorption in the renal tubules in the exchange of $H^+$ and $K^+$. As a consequence of $Na^+$ reabsorption, water is retained by the body **(Fig. 16.3)**

**Fig. 16.3:** Renin-angiotensin-aldosterone system (RAAS) in regulation of water and electrolyte balance.

## Atrial Natriuretic Factor (ANF)

ANF is a polypeptide **hormone** secreted by the right atrium of the heart. It **increases $Na^+$** and **water excretion** by the kidney. Thus, kidney plays an important role in maintenance of electrolyte and water balance.

# DISORDERS OF WATER AND ELECTROLYTE BALANCES

**Dehydration** and **overhydration** are the disorders of water balance, which are due to an imbalance of water intake and output or sodium intake and output.

## Dehydration

Dehydration may be defined as a state in which loss of water exceeds that of intake, as a result of which body's water content gets reduced and the body is in negative water balance. Dehydration may be of two types:

### *Dehydration due to Pure Water Deficiency, without Loss of Electrolytes, Called Simple Dehydration*

- Simple dehydration or pure water deficiency is due to deprivation of water without corresponding loss of electrolytes. Simple dehydration is associated with **hypernatremia,** i.e. increased level of sodium and **increase in ECW osmolality** due to loss of water from the body.

### *Dehydration due to Combined Deficiency of Water and Electrolyte, Sodium*

- Dehydration due to combined water and electrolyte sodium deficiency is more common than simple dehydration.

*Causes of dehydration*

- Simple dehydration results from deprivation of water either due to no or inadequate intake of water or due to excessive loss of water from body, e.g. in diabetes insipidus.
- Dehydration due to combined deficiency of water and electrolyte occur as a result of vomiting, diarrhea, excessive sweating, salt wasting renal disease, and adrenocortical insufficiency (Addison's disease).

*Symptoms of dehydration*

- Symptoms of simple dehydration are intense thirst, mental confusion, fever and oliguria (decreased urine output).
- Symptoms of dehydration due to combined deficiency of water and electrolytes are wrinkled skin, dry mucous membranes, muscle cramps, sunken eyeballs and increased blood urea nitrogen. With increasing severity, weakness, hypotension and shock may occur.

*Treatment*

- **Treatment of simple dehydration:** The patient is asked to drink plenty of water. If oral administration is not possible, an isotonic solution of 5% dextrose is given intravenously.
- **Treatment of dehydration due to combined deficiency of water and electrolyte:** An isotonic solution of sodium chloride (normal saline) is given intravenously.

## Overhydration or Water Intoxication

*Overhydration is a state of pure* **water excess** *or* **water intoxication.** More often, water intoxication results due to the retention of excess water in the body, which can occur due to:

- Renal failure
- Excessive administration of fluids parenteral
- Hypersecretion of ADH (syndrome of inappropriate ADH secretion, SIADH).

This results in reduced plasma electrolytes with decreased osmolality.

*Symptoms of overhydration*

Nausea, vomiting, headache, muscular weakness confusion and in severe cases convulsions, coma and even death occurs.

## EXAM QUESTIONS

### Short Notes

1. Water balance and its regulation in the body.
2. Composition of extracellular fluid.
3. Water distribution and its balance in the body.
4. Body water compartments and their composition.
5. Factors affecting distribution of water.
6. Distribution of electrolytes.
7. Dehydration.
8. Overhydration.
9. Regulation of water and electrolyte balance.

### Solve the Following

**Case History**

**A 40-year-old female was brought to the hospital with complaints of persistent vomiting, loose motions, cramps and extreme weakness, sunken eyes and dry tongue.**

**Questions**

a. Name the condition arising due to the above symptoms.
b. What are the causes for the condition?
c. Which are the different types of the condition?
d. Suggest the treatment.

## Multiple Choice Questions (MCQs)

**1. Chief anion of ECF is:**
a. $Cl^-$ b. $HCO_3^-$
c. $HPO_4^-$ d. Protein

**2. In ICF, main cation is:**
a. $Na^+$ b. $K^+$
c. $Ca^{++}$ d. $Mg^{++}$

**3. Which of the following is correct about intracellular water (ICW)?**
a. Amount less than ECW
b. Amount more than ECW
c. Amount equal to ECW
d. None of the above

**4. Which of the following hormones affects fluid and electrolyte balance?**
a. Epinephrine b. Glucagon
c. Thyroxine d. Aldosterone

**5. Distribution of water between intracellular and extracellular compartments depends on all of the following, *except*:**
a. Osmolality
b. Osmolarity
c. Colloidal osmotic pressure
d. Surface tension

**6. Source of daily output of water is:**
a. Urine
b. Insensible water (skin and lungs)
c. Sensible water (sweats and stool)
d. All of the above

**7. Metabolic water is:**
a. Water from food
b. Drinking water
c. Water derived from metabolism
d. Total body water

**8. Water and electrolyte balance is regulated by, *except*:**
a. ADH
b. Renin-angiotensin-aldosterone system (RAAS)
c. Atrial natriuretic factor (ANF)
d. Insulin

**9. Main anions of ICF is:**
a. $Cl^-$ b. $HPO_4^-$
c. $HCO_3^-$ d. $SO_4^{--}$

**10. Main cation of ECF is:**
a. $Na^+$ b. $K^+$
c. $Ca^{++}$ d. $Mg^{++}$

**11. Which of the following has greatest water content?**
a. Liver b. Adipose tissue
c. Brain d. Kidney

**12. Which of the following has least water content?**
a. Pancreas b. Brain
c. Liver d. Adipose tissue

**13. In a 70 kg adult, the total body water content is:**
a. 42 L b. 28 L
c. 14 L d. 3.5 L

**14. The largest portion of total body water is found in which of the tissue?**
a. Intracellular fluid b. Extracellular fluid
c. Interstitial fluid d. Plasma

**15. The daily water allowance for normal adult (60 kg) is about:**
a. 200–600 mL b. 500–800 mL
c. 800–1500 mL d. 1800–2500 mL

**16. Insensible loss of body water of normal adult is about:**
a. 50–100 mL b. 100–200 mL
c. 300–500 mL d. 600–1000 mL

**17. Vasopressin (ADH):**
a. Enhance reabsorption of water from kidney
b. Decreases reabsorption of water
c. Increases excretion of calcium
d. Decreases excretion of calcium

**18. Osmotically active substances in plasma are:**
a. Sodium b. Chloride
c. Proteins d. All of these

**19 The water produced during metabolic reactions in an adult is about:**
a. 100 mL/day b. 300 mL/day
c. 500 mL/day d. 700 mL/day

**20. The daily water loss through gastrointestinal tract in an adult is about:**
a. 500 mL/day b. 200 mL/day
c. 300 mL/day d. 400 mL/day

**21. Body water is regulated by the hormone:**
a. Oxytocin b. ACTH
c. FSH d. Epinephrine

### Answers for MCQs

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

CHAPTER

# 17

# Mineral Metabolism

**Chapter outline**

- Metabolism of Sodium, Potassium and Chloride
- Metabolism of Calcium, Phosphorus and Magnesium
- Metabolism of Sulfur
- Metabolism of Trace Elements (Microminerals)

## INTRODUCTION

Minerals are inorganic elements, required for a variety of functions. The minerals required in human nutrition can be grouped into ***macrominerals*** *and* ***microminerals (trace elements)*** **(Table 17.1)**.

- The macrominerals are required in excess of 100 mg/day.
- The microminerals or trace elements are required in amounts less than 100 mg/day.
- The principal functions and deficiency manifestations of each of the macro- and microminerals are summarized in **Table 17.2**.

## METABOLISM OF SODIUM, POTASSIUM AND CHLORIDE

### Sodium

Sodium is the major cation of extracellular fluids.

**Table 17.1:** Minerals required in human nutrition.

| *Macrominerals* | *Microminerals or Trace elements* |
|---|---|
| Sodium | Chromium |
| Potassium | Cobalt |
| Chlorine | Copper |
| Calcium | Fluoride |
| Phosphorus | Iodine |
| Magnesium | Iron |
| Sulfur | Manganese |
| | Molybdenum |
| | Selenium |
| | Zinc |

*Dietary food sources*

Table salt (NaCl), salty foods, animal foods, milk and some vegetables.

*Recommended dietary allowance per day*

- 1–5 gm
- 5 gm NaCl per day is recommended for adults without history of hypertension and 1 gm NaCl per day with history of hypertension.

*Absorption and excretion*

Sodium readily absorbed from the gut and is excreted from the body via urine. There is normally little loss of sodium occur through skin (sweat) and in the feces. Urinary excretion of sodium is regulated by aldosterone, which increases sodium reabsorption in kidney.

*Metabolic functions*

- It maintains the ***osmotic pressure*** and ***water balance***.
- It is a constituent of ***buffer*** and involved in the maintenance of ***acid-base balance***.
- It maintains ***muscle*** and nerve ***irritability*** at the proper level.
- Sodium is involved in ***cell membrane permeability***.
- Sodium is required for intestinal absorption of glucose, galactose and amino acids.

### Plasma Sodium

The plasma concentration of sodium is **135-145 mEq/L,** whereas blood cells (intracellular) contain **35 mEq/L**.

**Table 17.2:** Principal functions and deficiency manifestations of macrominerals and microminerals

| *Element* | *Metabolic function* | *Deficiency manifestation* |
|---|---|---|
| **Macrominerals** | | |
| Sodium | Principal extracellular cation, buffer constituent, water and acid base balance, cell membrane permeability | Dehydration, acidosis, excess leads to edema and hypertension |
| Potassium | Principal intracellular cation, buffer constituent, water and acid base balance, neuromuscular irritability | Muscle weakness, paralysis and mental confusion, acidosis |
| Chloride | Principal extracellular anion, electrolyte balance, osmotic balance, and acid base balance, gastric HCl formation | Deficiency secondary to vomiting and diarrhea |
| Calcium | Constituent of bone and teeth, blood clotting, regulation of nerve, muscle and hormone function | Tetany, muscle cramps, convulsions, osteoporosis, rickets |
| Phosphorus | Constituent of bone and teeth, nucleic acids, and NAD, FAD, ATP, etc. Required for energy metabolism | Growth retardation, skeletal deformities, muscle weakness, cardiac arrhythmia |
| Magnesium | Cofactor for phosphate transferring enzymes, constituent of bones and teeth, muscle contraction, nerve transmission | Muscle spasms, tetany, confusions, seizures |
| Sulfur | Constituent of proteins, bile acid, glycosoaminoglycans, vitamins like thiamine, lipoic acid, involved in detoxication reactions | Unknown |
| **Microminerals or trace elements** | | |
| Chromium | Potentiate the effect of insulin | Impaired glucose metabolism |
| Cobalt | Constituent of vitamin $B_{12}$ | Macrocytic anemia |
| Copper | Constituent of oxidase enzymes, e.g. tyrosinase, cytochrome oxidase, ferroxidase and ceruloplasmin, involved in iron absorption and mobilization | Microcytic hyporchromic anemia, depigmentation of skin, hair. Excessive deposition in liver in Wilson's disease |
| Fluoride | Constituent of bone and teeth, strengthens bone and teeth | Dental caries |
| Iodine | Constituent of thyroid hormones ($T_3$ and $T_4$) | Cretinism in children and goiter in adults |
| Iron | Constituent of heme and non-heme compounds and transport, storage of $O_2$ | Microcytic anemia |
| Manganese | Cofactor for number of enzymes, e.g. arginase, carboxylase, kinases, etc. | Not well-defined |
| Molybdenum | Constituent of xanthine oxidase, sulfite oxidase and aldehyde oxidase | Xanthinuria |
| Selenium | Antioxidant, cofactor for glutathione peroxidiase, protects cell against membrane lipid peroxidation | Cardiomyopathy |
| Zinc | Cofactor for enzymes in DNA, RNA and protein synthesis, constituent of insulin, carbonic anhydrase, carboxypeptidase, LDH, alcohol dehydrogenase, alkaline phosphatase, etc. | Growth failure, impaired wound healing, defects in taste and smell, loss of apetite |

## Clinical Conditions Related to Plasma Sodium Level Alterations

### *Hypernatremia*

Hypernatremia is an increase in serum sodium concentration above the normal range of 135–145 mEq/L.

***Causes of hypernatremia***

- ***Water depletion,*** may arise from a decreased intake or excessive loss with normal sodium content, e.g. diabetes insipidus.
- ***Water and sodium depletion,*** if more water than sodium is lost, e.g. diabetes mellitus (osmotic diuresis), excessive sweating or diarrhea in children
- ***Excessive sodium intake or retention*** in the ECF due to excessive aldosterone secretion, e.g. **Cohn's syndrome** and in **Cushing's syndrome**.

***Symptoms of hypernatremia***

It is due to loss of water and the symptom is therefore those of dehydration and if it is due to excess salt gain, leads to hypertension and edema.

*Hyponatremia*

It is a significant fall in serum sodium concentration below the normal range 135 to 145 mEq/L.

***Causes of hyponatremia***

- **Retention of water:** Retention of water dilutes the constituents of the extracellular space causing hyponatremia, e.g. in heart failure, liver disease, nephrotic syndrome, renal failure, syndrome of inappropriate ADH secretion (SIADH).
- **Loss of sodium:** Such losses may be from gastrointestinal tract, e.g. vomiting, diarrhea, or in urine. Urinary loss may be due to aldosterone deficiency (Addison's disease).

***Symptoms of hyponatremia*** are constant thirst, muscle cramps, nausea, vomiting, abdominal cramps, weakness and lethargy.

## Potassium

Potassium is the main intracellular ***cation.*** About 98% of total body potassium is in cells (150–160 mEq/L), only 2% in the ECF (3.5–5 mEq/L).

*Dietary food sources*

Vegetables, fruits, whole grain, meat, milk, legumes and tender coconut water.

*Recommended dietary allowance per day*

2–5 gm.

*Absorption*

Potassium is absorbed readily by passive diffusion from gastrointestinal tract.

*Excretion*

- Potassium excretion occurs through three primary routes, the ***gastrointestinal tract***, the ***skin*** and the ***urine***. Under normal conditions, loss of potassium through gastrointestinal tract and skin is very small. The major means of $K^+$ excretion is by the kidney.
- When sodium is reabsorbed by distal tubule cations (e.g. $K^+$ or $H^+$) in the cell move into the lumen to balance the charge. ***Thus during the sodium reabsorption there is an obligatory loss of potassium.***

*Serum potassium*

The concentration of potassium in serum is around ***3.5–5 mEq/L.*** Serum potassium concentration does not vary appreciably in response to water loss or retention.

*Metabolic functions*

- Potassium maintains the intracellular ***osmotic pressure, water balance*** and ***acid-base balance***.
- It influences activity of cardiac and skeletal muscle.
- Several *glycolytic enzymes* need potassium for their formation.
- Potassium is required for ***transmission of nerve impulses***.
- ***Nuclear activity*** and ***protein synthesis*** are dependent on potassium.

### Clinical Conditions Related to Plasma Potassium Level Alterations

*Hyperkalemia*

Hyperkalemia is a clinical condition associated with elevated plasma potassium above the normal range (3.5–5 mEq/L).

***Causes of hyperkalemia***

- **Renal failure:** The kidney may not be able to excrete a potassium load when GFR is very low.
- **Mineralocorticoid deficiency:** For example, in Addison's disease.
- **Cell damage:** For example, in trauma and malignancy.

***Symptoms of hyperkalemia***

First manifestation is cardiac arrest, changes in electrocardiogram, cardiac arrhythmia, muscle weakness which may be preceded by paraesthesia (abnormal tingling sensation).

*Hypokalemia (low plasma concentration)*

***Causes of hypokalemia***

- **Gastrointestinal losses:** Potassium may be lost from the intestine due to vomiting, diarrhea.
- **Renal losses:** Due to renal disease, administration of diuretics.

***Symptoms of hypokalemia***

Muscular weakness, tachycardia, electrocardiographic (ECG) changes (flattering of ECG waves), lethargy, and confusion.

## Chloride

Chloride is the major **anion** in the **extracellular fluid** space.

*Dietary food sources*

Table salt, leafy vegetables, eggs and milk.

*Recommended dietary allowance (RDA) per day*

2–5 gm.

*Absorption*

Rapidly and almost totally absorbed in the gastrointestinal tract.

*Excretion*

Under normal conditions chloride excretion occurs by way of three routes; the **gastrointestinal tract,** the skin and **urinary tract.** Chloride is excreted, mostly as sodium chloride and chiefly by way of the kidney.

*Plasma chloride*

The concentration of chloride in plasma is 95–**105 mEq/L**.

Functions

- As a part of sodium chloride, chloride is essential for water balance, regulation of osmotic pressure, and acid-base balance.
- Chloride is necessary for the formation of HCl by the gastric mucosa and for activation of enzyme amylase.
- It is involved in ***chloride shift.***

### Clinical Conditions Related to Plasma Chloride Level Alterations

*Hyperchloremia*

- An increased chloride concentration occurs in dehydration, metabolic acidosis and Cushing's syndrome.

*Hypochloremia*

- A decreased chloride concentration is seen in severe vomiting, metabolic alkalosis, excessive sweating and Addison's disease.

## METABOLISM OF CALCIUM, PHOSPHORUS AND MAGNESIUM

### Calcium

Calcium is the most abundant mineral in the body. The adult human body contains about 1 kg of calcium. About 99% the body's calcium is present in bone together with phosphate as the mineral ***hydroxyapatite*** **[$Ca_{10}$ $(PO_4)_6$ $(OH)_2$]**, with small amounts in soft tissue and extracellular fluid.

*Functions*

1. ***Formation of bone and teeth***: 99% of the body's calcium is located in bone in the form of **hydroxyapatite crystal [$3Ca_3$ $(PO_4)_2$ Ca $(OH)_2$]**. The hardness and rigidity of bone and teeth are due to hydroxyapatite.
2. ***Blood coagulations***: Calcium present in platelets involved in blood coagulation, the conversion of an inactive protein prothrombin into an active **thrombin** requires calcium ions.
3. **Muscle contraction:** Muscle contraction is initiated by the binding of calcium to troponin**.**
4. **Release of hormones:** The release of certain hormones like parathyroid hormone, calcitonin, etc. requires calcium ions.
5. **Release of neurotransmitter:** Influx of $Ca^{2+}$ from extracellular space into neurons causes release of neurotransmitter.
6. **Regulation of enzyme activity:** Activation of number of enzymes requires $Ca^{2+}$ as a specific cofactor. For example:
   - Activation of enzyme **glycogen phosphorylase kinase** which then triggers glycogenolysis.
   - Activation of salivary and pancreatic α-amylase.
7. **Second messenger:** Calcium acts as a second messenger for hormone action. For example, it acts as a **second messenger** for epinephrine or glucagon. Ca also functions as a **third messenger** for some hormones such as antidiuretic hormone (ADH).
8. ***Membrane excitability***: **Calcium ions** activate the sodium channels. Deficiency of calcium ions lead to decreased activity of Na-channels, which ultimately leads to decrease in membrane potential so that the nerve fiber becomes highly excitable causing muscle tetany.
9. ***Cardiac activity***: Cardiac muscle depends on extra-cellular $Ca^{2+}$ for contraction. Myocardial contractility increases with increased $Ca^{2+}$ concentration and decreases with decreased calcium concentration.
10. ***Membrane integrity and permeability:*** Calcium is required for maintenance of integrity and permeability of the membrane.
11. ***Hydrolysis of casein of milk***: Calcium is required for the formation of Ca-paracaseinate (insoluble curd).

The significance of this reaction is to convert milk into a more solid form to increase its retention in the stomach for a longer period of time and facilitate its gastric digestion in infants.

*Dietary sources*

The main dietary sources of calcium are milk and dairy products, (half a liter of milk contains approximately 1,000 mg of calcium) cheese, cereal grains, legumes, nuts and vegetables.

*Recommended dietary allowance (RDA) per day*

- Adults: 800 mg/day
- Women during pregnancy: 1200 mg/day and lactation and for teenagers.
- Infants: 300–500 mg/day.

*Absorption*

***Factors affecting absorption***: The absorption of calcium from the intestine depends on several factors. Some of these are discussed below:

***Factors that stimulate calcium absorption***

1. **Vitamin D** stimulates absorption of calcium from intestine by inducing the synthesis of calcium binding protein, necessary for the absorption of calcium from intestine.
2. **Parathyroid hormone** (PTH) stimulates calcium absorption indirectly via activating vitamin D.
3. **Acidic pH:** Since, calcium salts are more soluble in acidic pH, the acidic foods and organic acids (citric acid, lactic acid, pyruvic acid, etc.) favour the absorption of calcium from intestine.
4. **High protein diet** favours the absorption of calcium. Basic amino acids, lysine and arginine derived from hydrolysis of the dietary proteins increase calcium absorption.
5. **Lactose** is known to increase the absorption of calcium, by forming soluble complexes with the calcium ion.

***Factors that inhibit calcium absorption***

1. **Phytates** and **Oxalates** bind dietary calcium forming insoluble salts which cannot be absorbed from the intestine. Phytates present in many cereals and oxalates present in green leafy vegetables.
2. **High fat diet** decreases the absorption of calcium. High amounts of fatty acids derived from hydrolysis of dietary fats react with calcium to form insoluble calcium soaps which cannot be absorbed.
3. **High phosphate content** in diet causes precipitation of calcium as calcium phosphate and thereby lowers the ratio of Ca: P in the intestine. The Ca: P ratio should be 1:2–2:1 for optimum absorption of calcium. Absorption of calcium is maximum when food contains almost equal amounts of calcium and phosphorus.
4. **High fiber diet** decreases the absorption of calcium from intestine.

*Excretion*

The excretion of calcium is partly through the kidneys but mostly by way of the small intestine through feces. Small amount of calcium may also be lost in sweat.

## Plasma Calcium

The plasma calcium concentration in normal individual is **9–11 mg%.**

### *Regulation of Plasma Calcium Level (Fig. 17.1)*

Homeostasis of plasma calcium is dependent on the:

- Function of three main organs:
  1. Bone
  2. Kidney
  3. Intestine.
- Function of three main hormones:
  1. Parathyroid hormone (PTH)
  2. Vitamin D or cholecalciferol or calcitriol
  3. Calcitonin.
- The four major processes are **(Fig. 17.1):**
  1. Absorption of calcium from the intestine, mainly through the action of vitamin D.
  2. Reabsorption of calcium from the kidney, mainly through the action of parathyroid hormone and vitamin D.
  3. Demineralization of bone mainly through action of parathyroid hormone, but facilitated by vitamin D.
  4. Mineralization (calcification) of bone through the action of calcitonin.

***Role of parathyroid hormone (PTH):*** It is secreted by the parathyroid in response to drop in the blood calcium level. It acts on two main target organs, bone and kidney and indirectly via the activation of vitamin D on the intestine to increase the plasma calcium concentration.

***Action on bone:*** PTH stimulates mobilization of calcium and phosphate from bones by stimulating osteoclast activity. Osteoclast activity results in demineralization of the bone.

- Uptake of calcium and phosphate by bone is also decreased by PTH resulting in an increase in blood calcium and phosphate level.

***Action on kidney:*** In kidney PTH increases the tubular reabsorption of calcium and decreases renal excretion of calcium. PTH increases excretion of phosphate by inhibiting its renal reabsorption.

***Action on intestine:*** Action of PTH on intestine is indirect via the formation of calcitriol; active form of vitamin D. PTH stimulates the production of calcitriol. Calcitriol then increases absorption of calcium from intestine.

***Role of vitamin D (calcitriol):*** It is the active form of vitamin D, which causes the increase in plasma calcium

**Fig. 17.1:** Regulation of plasma calcium.
(25-HCC: 25-Hydroxycholecalciferol)

and phosphate concentration by stimulating the following processes:

- Absorption of calcium and phosphorus from intestine by inducing synthesis of calcium binding protein necessary for the absorption of calcium from intestine.
- Reabsorption of calcium and phosphorus from the kidney.
- Mobilization of calcium and phosphorus from the bone.
- Thus, overall effects of PTH and calcitriol elevate plasma calcium and phosphate level.

***Role of Calcitonin:*** The secretion of calcitonin is stimulated by increase in blood calcium level.

- Action of calcitonin on the bones is opposite to that of the PTH. It inhibits calcium mobilization from bone and increases bone calcification (mineralization) by increasing the osteoblasts activity.
- In the kidney it stimulates the excretion of calcium and phosphorus, thereby decreasing the blood calcium level.

## Clinical Conditions Related to Plasma Calcium Level Alterations

### Hypocalcemia

Hypocalcemia is characterized by lowered levels of plasma calcium. The causes of hypocalcemia include:

- **Hypoparathyroidism:** The commonest cause of hypoparathyroidism is neck surgery, or due to magnesium deficiency (See functions of magnesium).
- **Vitamin D deficiency:** This may be due to dietary deficiency, malabsorption or little exposure to sunlight. It may lead to bone disorders, **osteomalacia** in adults and **rickets** in children ***(See Chapter 7).***
- **Renal disease:** The diseased kidneys fail to synthesize calcitriol due to impaired hydroxylation.

***Clinical features of hypocalcemia***

The clinical features of hypocalcemia include:

- Neuromuscular irritability
- Neurologic features such as tingling, tetany, numbness (fingers and toes).
- Muscle cramps
- Cardiovascular signs such as an abnormal ECG.
- Cataracts.

### Hypercalcemia

Hypercalcemia is characterized by increased plasma calcium level. The commonest causes of hypercalcemia are:

- Hyperparathyroidism
- Malignant disease.

***Clinical features of hypercalcemia***

- Neurological symptoms such as depression, confusion, inability to concentrate.
- Muscle weakness.

- Gastrointestinal problems such as anorexia, abdominal pain, nausea and vomiting and constipation.
- Renal features such as polyuria and polydypsia.
- Cardiac arrhythmias.

## Phosphorus

Adults contain about **400–700 gm** of phosphorus, about 80% of which is combined with calcium in bones and teeth.

*Functions*

- **Constituent of bone and teeth:** Inorganic phosphate is a major constituent of **hydroxyapatite** in bone, thereby playing an important role in structural support of the body.
- **Acid-base regulation:** Mixture of $HPO_4^{--}$ and $H_2PO_4^-$ constitutes the phosphate buffer which plays a role in maintaining the pH of body fluid.
- **Energy storage and transfer reactions:** High energy compounds, e.g. ATP, ADP, creatin phosphate, etc. which play a role of storage and transport of energy, contain phosphorus.
- **Essential constituent:** Phosphate is an essential element in phospholipid of cell membrane, nucleic acids (RNA and DNA), nucleotides (NAD, NADP, c-AMP, c-GMP, etc.)
- **Regulation of enzyme activity:** Phosphorylation and dephosphorylation of enzymes modify the activity of many enzymes.

*Dietary sources*

The foods rich in calcium are also rich in phosphorus, i.e. milk, cheese, beans, eggs, cereals, fish and meat.

*Recommended dietary allowance per day*

- The recommended dietary allowance for both men and women is **800 mg/day**.
- The amount during pregnancy and lactation is **1200 mg/day**.

*Absorption*

- Like calcium, phosphorus is absorbed from small intestine and the degree of absorption is similarly affected by different factors as that of calcium.
- Vitamin D stimulates the absorption of phosphate along with calcium.
- Acidic pH favours the absorption of phosphorus.
- Phytates and oxalates decrease absorption of phosphate from intestine.
- Optimum absorption of calcium and phosphate occurs when dietary Ca: P ratio is 1:2–2:1.

*Excretion*

Phosphates are mainly excreted by the kidneys as $NaH_2PO_4$ through urine (unlike calcium). PTH decreases the reabsorption of phosphorus from the tubules and cause increased excretion of phosphorus in urine.

*Plasma phosphorus*

Plasma contains **2.5–4.5 mg/dL** of inorganic phosphate. Plasma phosphate concentration is controlled by the kidney, where tubular reabsorption is reduced by PTH. ***The phosphate which is not reabsorbed in the renal tubule acts as an important urinary buffer***.

### Clinical Conditions Related to Plasma Phosphorus Concentration Alterations

*Hypophosphatemia*

In hypophosphatemia serum inorganic phosphate concentration is less than 2.5 mg/dL.

***Causes of hypophosphatemia***

- **Hyperparathyroidism:** High PTH increases phosphate excretion by the kidneys and this leads to low serum concentration of phosphate.
- **Congenital defects** of tubular phosphate reabsorption, e.g. **Fanconi's syndrome,** in which phosphate is lost from body.

***Clinical symptoms of hypophosphatemia***

- As phosphate is an important component of ATP, cellular function is impaired with hypophosphatemia and leads to muscle pain and weakness and decreased myocardial output.
- If hypophosphatemia is chronic; rickets in children or osteomalacia in adults may develop.

*Hyperphosphatemia*
*(High serum phosphate concentration)*

***Causes of hyperphosphatemia***

- **Renal failure:** This is the commonest cause in which phosphate excretion is impaired.
- **Hypoparathyroidism:** Low PTH decreases phosphate excretion by the kidney and leads to high serum concentration.

***Clinical symptoms of hyperphosphatemia***

Elevated serum phosphate may cause a decrease in serum calcium concentration; therefore ***tetany*** and ***seizures*** may be the presenting symptoms.

## Magnesium

The body contains about **25 gm** of magnesium, most of which (55%) is present in the bones in association with calcium and phosphorus, a small proportion of the body's content is in the ECF.

*Functions*

- Magnesium is essential for the activity of many enzymes. Magnesium is a cofactor for more than 300 enzymes in the body; in addition, magnesium is allosteric activators of many enzyme systems. It plays an important role in oxidative phosphorylation, glycolysis, cell replication, nucleotide metabolism, protein synthesis and many ATP dependent reactions.
- Magnesium influences the secretion of PTH by the parathyroid glands.
- Hypomagnesemia may cause hypoparathyroidism.
- Magnesium along with sodium, potassium and calcium controls the neuromuscular irritability.
- It is an important constituent of bone and teeth.

*Dietary sources*

Cereals, pulses, nuts, green leafy vegetables, meat, eggs and milk.

*Recommended dietary allowance per day*

- RDA of the adult man is 350 mg/day and for women 300 mg/day.
- More magnesium is required during pregnancy and lactation (450 mg/day).

*Absorption*

- About 30–40% of the dietary magnesium is absorbed from the small intestine.
- Vitamin D and PTH increase the absorption of magnesium from intestine.
- Large amounts of calcium and phosphate in diet reduce the absorption of magnesium from intestine.

*Excretion*

Magnesium is excreted mainly by way of intestine. All unabsorbed magnesium as well as that in biliary excretion and intestinal secretion is excreted through feces. A fraction of absorbed magnesium is excreted by the kidneys through urine.

### Serum Magnesium

Human blood serum magnesium concentration is **1–3.5 mg/dL**.

The mechanism of control is poorly understood

- Renal conservation of magnesium is partly controlled by PTH and aldosterone.
- PTH increases tubular reabsorption of magnesium similar to that of calcium.
- Aldosterone increases its renal excretion as it does for potassium.

### Clinical Conditions Related to Plasma Magnesium Concentration Alterations

*Hypomagnesemia*

Hypomagnesemia is an abnormally low serum magnesium level.

- It is usually associated with magnesium deficiency.
- Since magnesium is present in most common food stuffs, low dietary intakes of magnesium are associated with general nutritional insufficiency, accompanied by intestinal malabsorption, severe vomiting, diarrhea or other causes of intestinal loss.
- The symptoms of hypomagnesemia are very similar to those of hypocalcemia, impaired neuromuscular function such as tetany, hyperirritability, tremor, convulsions and muscle weakness.

*Hypermagnesemia*

Hypermagnesemia is uncommon but is occasionally seen in renal failure.

Depression of the neuromuscular system is the most common manifestation of hypermagnesemia.

## METABOLISM OF SULFUR

The body receives sulfur through the proteins, as sulfur containing amino acids, e.g. methionine and cysteine.

*Food sources*

Plant and animal proteins, legume, eggs, cereals and cauliflower.

*Functions*

- Sulfur is a constituent of:
  - Protein
  - Glycosaminoglycans, e.g. heparin and chondroitin sulfate.
  - Vitamins, e.g. thiamine, biotin, lipoic acid, CoA of pantothenic acid.
  - Bile acids, e.g. taurocholic acid.
  - Active form of sulfate, phosphoadenosine phosphosulfate (PAPS) is involved in detoxication

reactions, e.g. some phenolic compounds are detoxified by conjugating with PAPS and eliminated from the body in the form of etheral sulfate.
- Non-heme iron enzyme such as mitochondrial NADH dehydrogenase and Fe-S protein.
- Compounds like glutathione and insulin.

*Excretion*

Sulfur is excreted by the kidneys in urine in the form of inorganic, organic and etheral sulfate.

*Deficiency manifestation*

Not well defined.

## METABOLISM OF TRACE ELEMENTS (MICROMINERALS)

Microminerals or trace elements are present in the body in very small amount (micrograms to miligrams) that is essential for certain biochemical processes. Trace elements required by humans are:

- Chromium
- Cobalt
- Copper
- Fluoride
- Iodine
- Iron
- Manganese
- Molybdenum
- Selenium
- Zinc.

### Chromium (Cr)

The adult human body contains only **6 mg** of chromium.

*Dietary food sources*

Yeast, molasses, meat products, cheese, whole grains.

*Recommended dietary allowance per day*

For healthy adults it is **0.05–20 mg**.

*Functions*

- Chromium functions in the control of glucose and lipid metabolism.
- It acts as a ***cofactor for insulin*** in increasing glucose utilization and transport of amino acids into cells.
- ***Chromium is also reported to lower the cholesterol levels.***

*Absorption and excretion*

The biologically active form ($Cr^{3+}$) is absorbed poorly from the diet. The majority of orally absorbed chromium is excreted through urine.

*Deficiency manifestation*

Deficiency of chromium can develop symptoms of glucose intolerance and weight loss, that are reversed with complementary chromium.

*Toxicity*

Chromium has toxic properties. The hexavalent ($Cr^{6+}$) form of chromium has strong oxidizing properties and is much more toxic than the trivalent ($Cr^{3+}$) form. Chromium toxicity is known to result in:

- Inflammation and necrosis of the skin and nasal passages.
- Allergic contact dermatitis and lung cancer.
- Oral ingestion can result in damage to the gastrointestinal tract and renal failure.

### Cobalt (Co)

Cobalt is necessary for biological activity of vitamin $B_{12}$. Cobalt fits into the corrin ring of vitamin $B_{12}$ **(*see* Fig. 7.14)**.

*Dietary food sources*

Liver, pancreas and vitamin $B_{12}$.

*Recommended dietary allowance per day*

Not established.

*Functions*

The only known function of cobalt is that it is an integral part of vitamin $B_{12}$.

*Absorption and excretion*

Dietary cobalt is poorly absorbed and is stored in the liver, probably as vitamin $B_{12}$. Cobalt is excreted in bile.

*Deficiency manifestation*

A cobalt deficiency is accompanied by all the signs and symptoms of a vitamin $B_{12}$ deficiency. The most important is anemia.

*Toxicity*

*An excess of cobalt can lead to* **polycythemia.**

### Copper (Cu)

A 70 kg human adult body contains approximately 80 mg of copper. It is present in all tissues. The highest concentrations are found in liver and kidney, with significant amount in cardiac and skeletal muscle and in bone.

*Dietary food sources*

Shellfish, liver, kidneys, egg yolk and some legumes are rich in copper.

*Recommended dietary allowance per day*

2–3 mg.

*Functions*

- Copper is an essential constituent of many enzymes including:
  - Ceruloplasmin (ferroxidase)
  - Cytochrome oxidase
  - Superoxide dismutase
  - Dopamine β-hydroxylase
  - Tyrosinase
  - Tryptophan dioxygenase
  - Lysyl oxidase.
- Copper plays an important role in iron absorption. Ceruloplasmin, the major copper containing protein in plasma has ferroxidase activity that oxidizes ferrous ion to ferric state before its binding to transferrin (transport form of iron).
- Copper is required for the synthesis of hemoglobin. Copper is a constituent of **ALA synthase** enzyme required for heme synthesis.
- Being a constituent of enzyme **tyrosinase,** copper is required for synthesis of **melanin pigment**.
- Copper is required for the synthesis of **collagen** and **elastin. Lysyl oxidase,** a copper containing enzyme converts certain lysine residues to allysine needed in the formation of collagen and elastin.

*Absorption and Excretion*

About 10% of the average daily dietary copper is absorbed mainly from the duodenum. Absorbed copper is transported to the liver bound to albumin and exported to peripheral tissues mainly (about 90%) bound to ceruloplasmin and to a lesser extent (10%) to albumin. The main route of excretion of copper is in the bile into the gut.

## Plasma Copper

Normal plasma concentrations are usually between **100** to **200 mg/dl** of which 90% is bound to ceruloplasmin.

*Deficiency Manifestation*

Signs of copper deficiency include:

- ***Neutropenia*** (decreased number of neutrophils) and ***hypochromic anemia*** in the early stages.
- ***Osteoporosis*** and various bone and joint abnormalities, due to impairment in copper-dependent cross-linking of bone collagen and connective tissue.
- ***Decreased pigmentation of skin*** due to depressed copper dependent **tyrosinase** activity, which is required in the biosynthesis of skin pigment melanin.
- In the later stages ***neurological abnormalities*** probably caused by depressed **cytochrome oxidase** activity.

## Inborn Errors of Copper Metabolism

There are two inborn errors of copper metabolism:

1. Menkes syndrome
2. Wilson's disease.

*Menkes Syndrome Or Kinky-Hair disease*

It is very rare, fatal, X-linked recessive disorder. The genetic defect is in absorption of copper from intestine. Both serum copper and ceruloplasmin and liver copper content are low. Clinical manifestations occur early in life and include:

- Kinky or twisted brittle hair (steely) due to loss of copper catalyzed disulfide bond formation.
- Depigmentation of the skin and hair.
- Mental retardation.

*Wilson's Disease*

- Wilson's disease is an inborn error of copper metabolism. It is an autosomal recessive disorder in which excessive accumulation of copper occurs in tissues.

***The possible causes are:***

- Impairment in binding capacity of copper to ceruloplasmin or inability of liver to synthesize ceruloplasmin or both.
- Impairment in excretion of copper in bile.

***Symptoms***

- Accumulation of copper in liver, brain, kidney and eyes leading to copper toxicosis.
- Excessive deposition of copper in brain and liver leads to neurological symptoms and liver damage leading to cirrhosis.
- Copper deposition in kidney leads to renal tubular damage and those in cornea form yellow or brown ring around the cornea, known as **Kayser-Fleisher (KF)** rings.
- The disease is also characterized by low levels of copper and ceruloplasmin in plasma with increased excretion of copper in urine.

***Treatment***

The excess copper is removed from the body by treatment with penicillamine.

## Fluorine (F)

In the form of fluoride, fluorine is incorporated into the structure of teeth and bone.

*Dietary food sources*

The body receives fluorine mainly from drinking water. Some sea fish and tea also contain small amount of fluoride.

*Recommended Dietary Allowance Per Day*

**1.5–4 mg** per day or **1–2 ppm** (since it is present in water it is expressed as ppm).

*Absorption and excretion*

Inorganic fluoride is absorbed readily in the stomach and small intestine and distributed almost entirely to bone and teeth. About 50% of the daily intake is excreted through urine.

*Functions*

Fluoride is required for the proper formation of **bone** and **teeth.** Fluoride becomes incorporated into hydroxyapatite, the crystalline mineral of bones and teeth to form **fluoroapatite.** Fluoroapatite increases hardness of bone and teeth and provides protection against dental caries and attack by acids.

### Deficiency Symptoms

Deficiency of fluoride leads to ***dental caries*** and ***osteoporosis.***

*Toxicity*

- Excessive amounts of fluoride can result in ***dental fluorosis.*** This condition results in teeth with a patch, dull white, even chalk looking appearance. A brown mottled appearance can also occur.
- It is known to ***inhibit*** several enzymes especially ***enolase*** of glycolysis.

## Iodine (I2)

The adult human body contains about **50 mg** of iodine. The blood plasma contains 4–8 mg of protein bound iodine (PBI) per 100 ml.

*Dietary Food Sources*

Seafood, drinking water, iodized table salt, onions, vegetables, etc.

*Recommended Dietary Allowance Per Day*

100–150 mg for adults

*Functions*

The most important role of iodine in the body is in the synthesis of thyroid hormones, **triiodothyronine ($T_3$)** and **tetraiodothyronine ($T_4$),** which influence a large number of metabolic functions.

*Absorption and excretion*

Iodine in the diet absorbed rapidly in the form of iodide from small intestine. Normally, about 1/3rd of dietary iodide is taken up by the thyroid gland, a little by the mammary and salivary glands. The rest is excreted by the kidneys.

Nearly 70–80% of iodine is excreted by the kidneys; small amounts are excreted through bile, skin and saliva. Milk of lactating women also contains some iodine.

### Deficiency Manifestation

Deficiency of iodine occurs in several regions of the world, where the iodine content of soil and therefore of plants is low. A deficiency of iodine in children leads to ***cretinism*** and in adult endemic ***goiter.***

- ***Cretinism:*** Severe iodine deficiency in mothers leads to intrauterine or neonatal hypothyroidism results in cretinism in their children. Cretinism is characterized by mental retardation, slow body development, dwarfism and characteristic facial structure.
- ***Goiter:*** A goiter is an enlarged thyroid with decreased thyroid hormone production. An iodine deficiency in adults stimulates the proliferation of thyroid epithelial cells, resulting in enlargement of the thyroid gland. The thyroid gland collects iodine from the blood and uses it to make thyroid hormones. In iodine deficiency, the thyroid gland undergoes compensatory enlargement in order to extract iodine from blood more efficiently.

## Iron (Fe)

A normal adult possesses **3–5 gm** of iron. This small amount is used again and again in the body. Iron is called a ***one way substance,*** because very little of it is excreted. Iron is not like vitamins or most other organic or even inorganic substances which are either inactivated or excreted in course of their physiological function.

*Dietary Food Sources*

The best sources of food iron include liver, meat, egg yolk, green leafy vegetables, whole grains and cereals. There are two types of food iron:

- **Heme iron:** Iron associated with porphyrin is found in green leafy vegetables.
- **Non-heme iron:** Iron without porphyrin, and is found in meat, poultry and fish.

*Recommended Dietary Allowance Per Day*

- Adult men and post-menopausal women: 10 mg
- Premenopausal women: 15–20 mg
- Pregnant women: 30–60 mg.

Women require greater amount than men due to the physiological loss during menstruation.

*Functions*

Iron is required for:

- Synthesis of heme compound like hemoglobin, myoglobin, cytochromes, catalase and peroxidase. Thus iron helps mainly in the ***transport, storage*** and ***utilization of oxygen.***
- Synthesis of non-heme iron (NHI) compounds, e.g. iron-sulfur proteins of flavoprotein, succinate dehydrogenase and NADH dehydrogenase.

*Absorption (Fig. 17.2)*

The normal intake of iron is about 10–20 mg/day. Normally, about 5–10% of dietary iron is absorbed. Most absorption occurs in the duodenum.

- Non-heme iron bound to organic acids or proteins is absorbed in the ferrous ($Fe^{2+}$) state into the mucosal cell as follows:
  - The gastric acid, HCl and organic acids in the diet convert bound non-heme compound of the diet into **free ferric ($Fe^{3+}$)** ions.
  - These free ferric ions are reduced with ascorbic acid and glutathione of food to more soluble ferrous ($Fe^{2+}$) form which is more readily absorbed.
  - After absorption $Fe^{2+}$ is oxidized in mucosal cells to $Fe^{3+}$ by the enzyme **Ferroxidase,** which then combines with **aproferritin** to form **ferritin.** Ferritin is a temporary storage form of iron.

**Fig. 17.2:** Absorption, storage and utilization of iron.

- Heme of food is absorbed as such by the intestinal mucosal cells. It is subsequently broken down and iron is released with the cells.

*Transport*

- The transfer of iron from the storage ferritin ($Fe^{3+}$ form) to plasma involves reduction of $Fe^{3+}$ to $Fe^{2+}$ in the mucosal cell with the help of ***ferroreductase***.
- $Fe^{2+}$ then enters the plasma where it is reoxidized to $Fe^{3+}$ by a copper protein, ***ceruloplasmin*** (ferroxidase).
- $Fe^{3+}$ is then incorporated into **transferrin** by combining with **apotransferrin**.
- Apotransferrin is a specific iron binding protein. Each apotransferrin can bind with two $Fe^{3+}$ ions.

*Storage*

Iron in plasma is taken up by cells and either incorporated into heme or stored as ***ferritin*** or ***hemosiderin.*** Storage of iron occurs in most cells but predominantly in cells of liver, spleen and bone marrow.

- Ferritin is the major iron storage compound and readily available source of iron. Each apoferritin molecule can take up about 4500 **iron atoms.**
- In addition to storage as ferritin, iron can also be found in a form of ***hemosiderin***. The precise nature of hemosiderin is unclear. Normally very little hemosiderin is to be found in the liver, but the quantity increases during iron overload.

*Excretion*

- Iron is not excreted in the urine, but is lost from the body via the ***bile, feces*** and in ***menstrual blood***.
- Iron excreted in the feces is exogenous, i.e. dietary iron that has not been absorbed by the mucosal cells is excreted in the feces.
- In male, there is an average loss of endogenous iron of about 1 mg/day through desquamated cells of the skin and the intestinal mucosa.
- Females may have additional losses due to menstruation or pregnancy.

*Factors Affecting Iron Absorption*

- **State of iron stores in the body:** Absorption is increased in iron deficiency and decreased when there is iron overload.
- **Rate of erythropoiesis** (the process of red blood cell production). When rate of erythropoiesis is increased, absorption may be increased even though the iron stores are adequate or overloaded.
- **The contents of the diet:** Substances that form soluble complexes with iron, e.g. ascorbic acid (vitamin C) facilitates absorption. Substances that form insoluble complexes, e.g. phosphate, phytates and oxalates inhibit absorption.
- **Nature of gastrointestinal secretions and the chemical state of the iron:** Iron in the diet does not usually become available for absorption unless released in free form during digestion. This depends partly on gastric acid (HCl) production. Ferrous ($Fe^{2+}$) is more readily absorbed than ferric form ($Fe^{3+}$) and the presence of HCl, helps to keep iron in the $Fe^{2+}$ form.

## Disorders of Iron Metabolism

**Iron deficiency** and **iron overload** are *the major disorders of iron metabolism.*

*Iron deficiency*

A deficiency of iron causes a reduction in the rate of hemoglobin synthesis and erythropoiesis, and can result in **iron deficiency anemia**.

Iron deficiency anemia is the commonest of all single nutrient deficiencies. The main causes are:

- **Deficient intake:** Including reduced bioavailability of iron due to dietary fiber, phytates, oxalates, etc.
- **Impaired absorption:** For example, intestinal malabsorptive disease and abdominal surgery.
- **Excessive loss:** For example, menstrual blood loss in women and in men from gastrointestinal bleeding (in peptic ulcer, diverticulosis or malignancy).

Iron deficiency causes low hemoglobin resulting in ***hypochromic microcytic anemia*** in which the size of the red blood cells are much smaller than normal and have much reduced hemoglobin content.

***Clinical features of anemia:*** Weakness, fatigue, dizziness and palpitation. Nonspecific symptoms are nausea, anorexia, constipation, and menstrual irregularities. Some individuals develop pica, a craving for unnatural articles of food such as clay or chalk.

*Iron Overload*

Hemosiderosis and hemochromatosis are the conditions associated with iron overload.

- ***Hemosiderosis:*** Hemosiderosis is a term that has been used to imply an increase in iron stores as hemosiderin without associated tissue injury. Hemosiderosis is an initial stage of iron overload.

- ***Hemochromatosis:*** Hemochromatosis is a clinical condition in which excessive deposits of iron in the form of hemosiderin are present in the tissues, **with injury** to involved organs as follows:
  - **Liver:** Leading to cirrhosis
  - **Pancreas:** Leading to fibrotic damage to pancreas with diabetes mellitus
  - **Skin:** Skin pigmentation, bronzed diabetes
  - **Endocrine organ:** leading to hypothyroidism, testicular atrophy
  - **Joints:** Leading to arthritis
  - **Heart:** Leading to arrhythmia and heart failure.

## Manganese (Mn)

The adult human body contains about **15–20 mg** of manganese. The liver and kidney are rich in Mn. Mn mainly found in the nuclei, where it gives stability to the nucleic acid structure.

*Dietary Food Sources*

Meat (liver and kidney), wheat germs, legumes and nuts

*Recommended Dietary Allowance Per Day*

2.5–5.0 mg.

*Functions*

- Manganese acts as a cofactor or activator of many enzymes such as arginase, pyruvate carboxylase, glucosyl transferase, mitochondria superoxide dismutase, decarboxylase, etc.
- Manganese is required for synthesis of glycoproteins, proteoglycans, Hb, and cholesterol.
- Manganese is required for the physical growth and reproductive functions.
- It plays important role in formation of connective and bony tissue.
- Manganese also functions with vitamin K in the formation of prothrombin.

*Absorption and Excretion*

Dietary manganese is absorbed poorly from the small intestine. Most of the manganese is excreted rapidly in the bile and pancreatic secretion in the feces.

### *Deficiency Manifestation*

Because of wide distribution of manganese in plant and animal foods, the deficiency of manganese is not known in humans. However, in animals manganese deficiency leads to sterility and bone deformities.

## Molybdenum (Mo)

*Dietary Food Sources*

Liver and kidney are good meat sources, whole grains, legumes and leafy vegetables serve as vegetable sources.

*Recommended Dietary Allowance Per Day*

0.15–0.5 mg

*Functions*

Molybdenum is a constituent of the following enzymes:

- Xanthine oxidase
- Aldehyde oxidase
- Sulphite oxidase.

*Absorption and Excretion*

Dietary molybdenum is readily absorbed by the intestine and is excreted in urine and bile.

*Deficiency Manifestation*

Deficiency of molybdenum has been reported to cause ***xanthinuria*** with low plasma and urinary uric acid concentration.

## Selenium (Se)

*Dietary Food Sources*

Liver, kidney, seafood and meat are good sources of selenium. Grains have a variable content depending on the region where they are grown.

*Recommended Dietary Allowance Per Day*

50–200 mg for normal adults

*Functions*

- Selenium functions as an ***antioxidant*** along with vitamin E.
- Selenium is a constituent of ***glutathione peroxidase***. Glutathione peroxidase has a cellular ***antioxidant*** function, which protects cell membrane, against oxidative damage by H2O2 and a variety of hydroperoxides.
- Selenium, as a constituent of glutathione peroxidase is important in preventing lipid peroxidation and protecting cells against superoxide ($O2^-$) and some other free radicals.
- Selenium also is a constituent of ***iodothyronine deiodinase,*** the enzyme that converts thyroxine to triiodothyronine.

*Absorption and Excretion*

The principal dietary forms of selenium ***selenocysteine*** and **selenomethionine** are absorbed from gastrointestinal tract. Selenium homeostasis is achieved by regulation of its excretion via urine.

### Deficiency Manifestations

Selenium deficiency has been associated in some areas of China with ***Keshan disease***, a cardiomyopathy that primarily affects children and women of child bearing age. Its most common symptoms include dizziness, loss of appetite, nausea, abnormal electrocardiograms, and congestive heart failure.

#### Selenium Toxicity (Selenosis)

Excessive selenium intake results in ***alkali disease***, characterized by loss of hair and nails, skin lesions, liver and neuromuscular disorders that is usually fatal.

## Zinc (Zn)

Total zinc content of the adult body is about 2 gm. In blood, RBCs contain very high concentration of zinc as compared to plasma.

#### Dietary Food Sources

Meat, liver, seafood, and eggs are good sources. Milk including breast milk also is a good source of zinc. ***The colostrum is an especially rich source.***

#### Recommended Dietary Allowance Per Day

**15 mg per day** for adults with an additional 5 mg during pregnancy and lactation

#### Functions

- Zinc is a constituent of a number of enzymes. For example,
  - Carbonic anhydrase
  - Alkaline phosphatase
  - DNA and RNA-polymerases
  - Porphobilinogen (PBG) synthase of heme synthesis.
- Because it is required by many of the enzymes needed for DNA and RNA synthesis, ***zinc is necessary for the growth and division of cells.***
- Zinc is an important element in wound healing as it is a necessary factor in the biosynthesis and integrity of connective tissue.
- Zinc stabilizes structure of protein and nucleic acids.
- Zinc is required for the secretion and storage of insulin from the β-cells of pancreas.
- Gustin, a Zn containing protein present in saliva is required for the development and functioning of taste buds. Therefore, zinc deficiency leads to loss of taste acuity.

#### Absorption And Excretion

Approximately 20–30% of ingested dietary zinc is absorbed in small intestine. It is transported in blood plasma mostly by albumin and a2-macroglobulin. Zinc is excreted in urine, bile, in pancreatic fluid and in milk in lactating mothers.

### Deficiency Manifestation

Zinc deficiency has many causes, but malnutrition and malabsorption are the most common. Clinical symptoms of zinc deficiency include:

- Growth failure
- Hair loss
- Anemia
- Loss of taste sensation
- Impaired spermatogenesis
- Neuropsychiatric symptoms.

***Acrodermatitis enteropathica:*** A rare inherited disorder of zinc metabolism is due to an inherited defect in zinc absorption that causes low plasma zinc concentration and reduced total body content of zinc; it is manifested in infancy as skin rash.

## EXAM QUESTIONS

### Long Answer Questions (LAQs)

1. Describe the metabolism of calcium and phosphorus in the body.
2. Describe the metabolism of iron in the body.
3. Describe the metabolism of sodium and potassium in the body.
4. Describe functions of trace elements in the body.
5. Describe deficiency manifestation of copper, iodine, zinc and fluoride.

### Short Notes

1. Absorption, transport and storage of iron.
2. Factors affecting calcium absorption.
3. Regulation of serum calcium level.

4. Disorders of iron overload.
5. Metabolic functions of sodium and potassium.
6. Clinical conditions related to potassium, sodium, calcium or phosphorus level.
7. Wilson's disease.
8. Menke's syndrome.
9. Hemosiderin.
10. Functions of selenium.

## Solve

**Case History 1**

**A 15 years old girl presented with abdominal pain. She became jaundiced and she subsequently died of liver failure. At postmortem of her liver copper concentration was found to be grossly increased.**

**Questions**

a. What is the diagnosis?
b. What is the cause of liver failure?
c. What is the cause of increased copper concentration in liver?
d. What is the normal plasma concentration of copper?

**Case History 2**

**A patient in the hospital had seizures and usually appeared weak and tired. Physical finding was deposition of copper in the eyes as brown pigment (the Kayser-Fleischer ring) and hepatomegaly. A diagnosis of Wilson's disease was made.**

**Questions**

a. What is the biochemical problem in Wilson's disease?
b. Name two copper containing enzymes.
c. Give functions and sources of copper.

**Case History 3**

**A 35-year-old man, who required total intravenous feeding (with no assessment of his trace metal status), for four months, developed a skin rash, with accompanying hair loss, reduced taste acuity and delayed wound healing. He was clearly diagnosed zinc deficient.**

**Questions**

a. Give food sources of zinc.
b. RDA for zinc.
c. Functions of zinc.
d. Name two enzymes having zinc as a constituent.

**Case History 4**

**A 40-year-old woman complains of tiredness and appears pale. She is experiencing a heavy and prolonged monthly menstrual flow and her hemoglobin concentration is 90 g/L (normal range 120–160 g/L).**

**Questions**

a. What is your probable diagnosis?
b. How can the complaints be relieved?
c. Give RDA and factors affecting absorption of the deficient biochemical substance.

**Case History 5**

**A 18-year-old female comes to her physician complaining about feeling tired and her physician performs a physical examination and diagnosis of anemia was made. Her blood investigations showed decreased level of ceruloplasmin.**

**Questions**

a. What is the cause of anemia.
b. What type of anemia does this patient exhibit?
c. What is ceruloplasmin.
d. What are the functions of ceruloplasmin?

**Case History 6**

**A 57-year-old man was admitted to clinic who exhibits a brown pigment ring (KF ring) around his cornea and also some signs of neurological impairment.**

**Questions**

a. What is the probable diagnosis?
b. Cause of disorder.
c. Suggest treatment for the disorder.
d. Name supportive investigations.

**Case History 7**

**A 50-year-old woman presented at clinic, which is pale and tired she has iron deficiency anemia.**

**Questions**

a. What is the normal reference plasma level of iron?
b. Write RDA for iron.
c. What is the transport form of iron?
d. Write storage form of iron.

## Multiple Choice Questions (MCQs)

1. **Normal serum sodium level is:**
   a. 135–145 mEq/L b. 150–160 mEq/L
   c. 120–130 mEq/L d. 170–180 mEq/L

**2. In wound healing the following trace element is involved:**
a. Iron b. Copper
c. Zinc d. Selenium

**3. The mineral having sparing action of vitamin E:**
a. Chromium b. Iron
c. Iodine d. Selenium

**4. Wilson's disease is characterized by impaired:**
a. Copper excretion into bile
b. Reabsorption of copper in the kidney
c. Hepatic incorporation of copper into ceruloplasmin
d. All of the above

**5. Glutathione peroxidase contains:**
a. Calcium b. Iron
c. Selenium d. Chromium

**6. Hemochromatosis is due to excessive deposition of:**
a. Iron in the form of hemosiderin
b. Copper
c. Zinc
d. Iodine

**7. Transferrin is involved in:**
a. Hormone metabolism
b. Diagnosis of Wilson's disease
c. Transport of iron
d. Transport of bilirubin

**8. The major storage form of iron is:**
a. Transferrin b. Ceruloplasmin
c. Ferritin d. Hemosiderin

**9. The element that prevents the development of dental caries:**
a. Fluorine b. Calcium
c. Phosphorus d. Selenium

**10. Carbonic anhydrase contains mineral:**
a. Copper b. Iodine
c. Zinc d. Iron

**11. Molybdenum is a constituent of all of the following, except:**
a. Xanthine oxidase
b. Aldehyde oxidase
c. Sulfite oxidase
d. Cytochrome oxidase

**12. Transport form of iron is:**
a. Transferrin b. Ferritin
c. Hemosiderin d. Ceruloplasmin

**13. Iodine is required for the formation of:**
a. Vitamin $B_{12}$ b. Thyroxine
c. Insulin d. Calcitonin

**14. Which of the following minerals is known as glucose tolerance factor (GTF)?**
a. Chromium b. Cobalt
c. Calcium d. Copper

**15. Intestinal absorption of iron is enhanced by:**
a. Phytic acid b. Ascorbic acid
c. Oxalic acid d. Alkaline pH

**16. Element called "one way substance" is:**
a. Iodine b. Iron
c. Copper d. Calcium

**17. Which of the following minerals stimulates secretion of PTH?**
a. Iodine b. Magnesium
c. Copper d. Sodium

**18. Iron is a component of:**
a. Hemoglobin b. Ceruloplasmin
c. Transferase d. Transaminase

**19. Daily requirement of iron for normal adult male is about:**
a. 5 mg b. 10 mg
c. 15 mg d. 20 mg

**20. The total iron content of the human body is:**
a. 400–500 mg b. 1–2 g
c. 2–3 g d. 4–5 g

**21. A good source of iron is:**
a. Spinach b. Milk
c. Tomato d. Potato

**22. The deficiency of copper decreases the activity of the enzyme:**
a. Lysine oxidase b. Lysine hydroxylase
c. Tyrosine oxidase d. Proline hydroxylase

**23. Wilson's disease is a condition of toxicosis of:**
a. Iron b. Copper
c. Chromium d. Molybdenum

**24. In Wilson's disease:**
a. Copper fails to be excreted in the bile
b. Copper level in plasma is decreased
c. Ceruloplasmin level is increased
d. Intestinal absorption of copper is decreased

**25. Menke's disease is due to an abnormality in the metabolism of:**
a. Iron b. Manganese
c. Magnesium d. Copper

**26. Menke's disease (Kinky or steel hair disease) is a X-linked disease characterized by:**
a. High levels of plasma copper
b. High levels of ceruloplasmin
c. Low levels of plasma copper and of ceruloplasmin
d. High level of hepatic copper

**27. Mitochondrial superoxide dismutase contains:**
a. Zinc b. Copper
c. Magnesium d. Manganese

**28. Mitochondrial pyruvate carboxylase contains:**
a. Zinc b. Copper
c. Manganese d. Magnesium

**29. Molybdenum is a constituent of:**
a. Hydroxylases b. Oxidases
c. Transaminases d. Transferases

**30. Metallic constituent of "Glucose tolerance factor" is:**
a. Sulfur b. Cobalt
c. Chromium d. Selenium

**31. Selenium is a constituent of the enzyme:**
a. Glutathione peroxidase
b. Homogentisate oxidase
c. Tyrosine hydroxylase
d. Phenylalanine hydroxylase

**32. Excess intake of cobalt for longer periods leads to:**
a. Polycythemia
b. Megaloblastic anemia
c. Pernicious anemia
d. Microcytic anemia

**33. Fluorosis occurs due to:**
a. Drinking water containing less fluorine
b. Drinking water containing high calcium
c. Drinking water containing high fluorine
d. Drinking water containing heavy metals

**34. An important zinc containing enzyme is:**
a. Carbonic anhydrase
b. Isocitrate dehydrogenase
c. Cholinesterase
d. Lipoprotein lipase

**35. Acrodermatitis enteropathica is due to defective absorption of:**
a. Manganese b. Molybdenum
c. Cobalt d. Zinc

**36. Intestinal absorption of calcium is hampered by:**
a. Phosphate b. Phytates
c. Proteins d. Lactose

**37. What are the functions of potassium?**
a. In muscle contraction
b. Cell membrane function
c. Enzyme action
d. All of these

**38. Hypocalcemia can occur in all the following *except*:**
a. Rickets
b. Osteomalacia
c. Hyperparathyroidism
d. Intestinal malabsorption

**39. Normal range of serum potassium is:**
a. 2.1–3.4 mEq/L b. 3.5–5.3 mEq/L
c. 5.4–7.4 mEq/L d. 7.5–9.5 mEq/L

**40. Iron is stored in the form of:**
a. Ferritin and transferrin
b. Transferrin and hemosiderin
c. Hemoglobin and myoglobin
d. Ferritin and hemosiderin

**41. Iron is transported in blood in the form of:**
a. Ferritin b. Hemosiderin
c. Transferrin d. Hemoglobin

**42. Zinc is involved in storage and release of:**
a. Histamine b. Acetylcholine
c. Epinephrine d. Insulin

**43. Molybdenum is a cofactor for:**
a. Xanthine oxidase b. Aldehyde oxidase
c. Sulfite oxidase d. All of these

**44. A trace element having antioxidant function is:**
a. Selenium b. Tocopherol
c. Chromium d. Molybdenum

**45. Selenium is a constituent of:**
a. Glutathione reductase
b. Glutathione peroxidase
c. Catalase
d. Superoxide dismutase

**46. Selenium decreases the requirement of:**
a. Copper
b. Zinc
c. Vitamin D
d. Vitamin E

**47. The general functions of minerals are:**
a. The structural components of body tissues
b. In the regulation of body fluids
c. In acid-base balance
d. All of these

**48. Hyponatremia caused by each of the following, *except*:**
a. Prolonged vomiting or diarrhea
b. Aldosterone deficiency
c. Renal failure
d. Excessive aldosterone secretion

**49. A hemolytic sample will cause falsely increased levels of each of the following, *except*:**
a. Potassium
b. Sodium
c. Phosphate
d. Magnesium

## Answers for MCQs

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **1.** a | **2.** c | **3.** d | **4.** d | **5.** c | **6.** a | **7.** c | **8.** c |
| **9.** a | **10.** c | **11.** d | **12.** a | **13.** b | **14.** a | **15.** b | **16.** b |
| **17.** b | **18.** a | **19.** b | **20.** d | **21.** a | **22.** a | **23.** b | **24.** a |
| **25.** d | **26.** c | **27.** c | **28.** c | **29.** b | **30.** c | **31.** a | **32.** a |
| **33.** c | **34.** a | **35.** d | **36.** b | **37.** d | **38.** c | **39.** b | **40.** d |
| **41.** c | **42.** d | **43.** d | **44.** a | **45.** b | **46.** d | **47.** d | **48.** d |
| **49.** b | | | | | | | |

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CHAPTER

# 18

# Hemoglobin Metabolism

**Chapter outline**

- Synthesis of Heme
- Disorder of Heme Biosynthesis
- Porphyrias
- Breakdown of Hemoglobin
- Fate of Bilirubin
- Jaundice
- Inherited Hyperbilirubinemias

## INTRODUCTION

Heme is the prosthetic group of several proteins and enzymes including hemoglobin, myoglobin, cytochrome, cytochrome $P_{450}$, enzymes like catalase, certain peroxidase and tryptophan pyrrolase. Heme is synthesized from **porphyrin** and **iron.** Porphyrin ring is coordinated with an atom of **iron** to form heme **(Fig. 18.1)**.

- Porphyrins are cyclic molecule formed by the linkage of 4-pyrrole rings through methen bridges.
- Eight side chains serve as substituents on the porphyrin ring, two on each pyrrole.
- These side chains may be acetyl (A), propionyl (P), methyl (M) or vinyl (V) groups.
- The side chains of the porphyrin can be arranged in four different ways. Designated by Roman numerals, I to IV. Only type III isomer is physiologically important in humans.

**Fig. 18.1:** Structure of heme molecule.

## SYNTHESIS OF HEME

Heme synthesis takes place in all cells, but occurs to the greatest extent in the ***bone marrow*** and **liver**.

### Stages of Heme Synthesis

Biosynthesis of heme may be divided into three stages **(Fig. 18.2)**:

1. Biosynthesis of δ-**aminolevulinic acid (ALA)** from the precursor glycine and succinyl-CoA
2. Formation of **porphobilinogen (PBG)** from δ-aminolevulinic acid.
3. Formation of porphyrins and heme from porphobilinogen.

#### *Biosynthesis of δ-Aminolevulinic Acid (δ-ALA)*

- The first step in the biosynthesis of porphyrins is the condensation of glycine and succinyl-CoA to form δ-**aminolevulinic acid,** which occurs in mitochondria. This reaction is catalyzed by δ-**aminolevulinic acid synthase (δ-ALAS)** and PLP (pyridoxal phosphate) is also necessary in this reaction. **This is the rate controlling step in heme synthesis.**

#### *Formation of Porphobilinogen (PBG)*

- In the cytosol, two molecules of δ-ALA condense to form **porphobilinogen.** This reaction is catalyzed

**Fig. 18.2:** Biosynthetic pathway of heme.

by δ-***aminolevulinate dehydratase,*** also called ***porphobilinogen synthase (PBGS),*** which is a ***zinc*** containing ***enzyme.***

- δ-aminolevulinate dehydratase enzyme is inhibited by relatively low concentrations of lead (Pb). This accounts for the large excretion of ALA in the urine of a person who is affected with lead poisoning.

Indeed, ***the quantitative determination of ALA in urine is one of the better analytical means of monitoring the severity and control of lead poisoning in human subjects.***

### *Formation of Porphyrins and Heme*

- Four porphobilinogens condense head-to-tail to form a linear tetrapyrrole, hydroxy-methylbilane. The reaction is catalyzed by ***uroporphyrinogen-I synthase,*** also known as ***PBG deaminase.***
- Hydroxymethylbilane cyclizes to form uroporphyrinogen-III by the action of ***uroporphyrinogen-III synthase.*** *At this point basic ring structure (porphyrin skeleton) is formed.* Under normal conditions, the uroporphyrinogen formed is almost ***exclusively the III isomer*** but in certain of the porphyrias (discussed later), the type I isomers of porphyrinogens are formed in excess.
- Uroporphyrinogen-III is converted to coproporphyrinogen III by decarboxylation. The reaction is catalyzed by **uroporphyrinogen decarboxylase.**
- Coproporphyrinogen-III then enters the mitochondria, where it is converted to **protoporphyrinogen-IX** by the mitochondrial enzyme **coproporphyrinogen oxidase.**
- ***This enzyme is able to act only on type-III coproporphyrinogen; that is why type-I protoporphyrins do not generally occur in nature.***
- The oxidation of protoporphyrinogen-IX to protoporphyrin-IX is catalyzed by another mitochondrial enzyme, **protoporphyrinogen oxidase.**
- The final step in heme synthesis involves the incorporation of **ferrous iron** into protoporphyrin-IX in a reaction catalyzed by mitochondrial ***heme synthase*** or ***ferrochelatase***.

### Regulation of Heme Synthesis

- ***δ-aminolevulinic acid synthase (ALAS),*** a ***itochondrial allosteric enzyme*** that catalyzes first step of heme biosynthetic pathway, is a regulatory enzyme. It is feedback inhibited by ***heme***.
- Regulation also occurs at the level of enzyme synthesis. Increased level of heme represses the synthesis of δ-aminolevulinic acid synthase.

## DISORDER OF HEME BIOSYNTHESIS

### Porphyrias

Porphyrias are rare inherited (or occasionally acquired) disorder due to deficiencies of enzymes in heme synthesis. This leads to accumulation and increased excretion of ***porphyrins*** or ***porphyrin precursors*** (ALA and PBG).

### Classification of Porphyria

The hereditary porphyrias are classified into two categories on the basis of the organs or cell that are mostly affected. Thus, porphyria is classified into:

1. **Erythropoietic porphyria:** Enzyme deficiency occurs in erythropoietic cells of the bone marrow.
2. **Hepatic porphyria:** Enzyme deficiency occurs in hepatic cell.

Different types of hereditary porphyrias that fall into these two classes are given in **Table 18.1.**

### Clinical Symptoms of Porphyrias

- Acute abdominal pain
- Neuropsychiatric symptoms
- Photosensitivity and skin lesions (in some porphyrias only).

Where the enzyme deficiency occurs early in the pathway prior to the formation of porphyrinogen, ALA and PBG will accumulate in the body tissues and fluids. These compounds can impair the function of abdominal nerve and central nervous system, resulting in **abdominal pain** and **neuropsychiatric** symptoms. The "madness" of George III, king of England during the American Revolution, is believed to have been due to this porphyria.

On the other hand, enzyme deficiency occurs later in the pathway, results in the accumulation of the **porphyrinogens** which on exposure to light auto-oxidized to corresponding porphyrin derivatives, causes ***photosensitivity*** and ***skin lesions.***

### Acquired Porphyria

- The most common acquired form of porphyria is due to lead poisoning. δ-**ALA dehydratase** and **ferrochelatase** are inactivated by lead.
- Thus, in lead poisoning, δ-ALA and protoporphyrin accumulate, and the production of heme is decreased.
- Anemia results from lack of hemoglobin and energy production decreases due to lack of cytochromes required for the electron transport chain.

## BREAKDOWN OF HEMOGLOBIN

After approximately 120 days, red blood cells are degraded by reticuloendothelial (RE) system, particularly in the liver and spleen.

- First hemoglobin is dissociated into heme and globin.
- Globin is degraded to its constituent amino acids, which are reused.
- The catabolism of heme is carried out in the microsomal fractions of cells by a complex enzyme system called ***heme oxygenase,*** in the presence of NADPH and $O_2$. Ferric ion and carbon monoxide (CO) are released with production of the green pigment **biliverdin**.

**Fig. 18.3:** Catabolic pathway of hemoglobin.

**Table 18.1:** Types of porphyrias.

| *Classes* | *Types* | *Enzyme defect* | *Signs and symptoms* |
|---|---|---|---|
| Hepatic | Acute intermittent porphyria | Uroporphyrinogen-I synthase | Abdominal pain, neuropsychiatric symptoms |
| Erythropoietic | Congenital erythropoietic | Uroporphyrinogen-III synthase | Photosensitivity |
| Hepatic | Porphyria Cutanea Tarda | Uroporphyrinogen decarboxylase | Photosensitivity |
| Hepatic | Hereditary coproporphyria | Coproporphyrinogen oxidase | Photosensitivity, abdominal pain neuropsychiatric symptoms |
| Hepatic | Variegate porphyria | Protoporphyrinogen oxidase | Photosensitivity, abdominal pain neuropsychiatric symptoms |
| Erythropoietic | Protoporphyria | Ferrochelatase | Photosensitivity |

- Biliverdin is reduced to red orange colored **bilirubin**. This reaction is catalyzed by an NADPH dependent **biliverdin reductase (Fig. 18.3).** Approximately, 250 to 350 mg of bilirubin is produced per day in human adults. Bilirubin and its derivatives are collectively termed ***bile pigments***.

## FATE OF BILIRUBIN

The further metabolism and excretion of bilirubin occurs in the **liver** and **intestine.** It can be divided into four processes:
1. Uptake of bilirubin by liver
2. Conjugation of bilirubin in the liver
3. Secretion of conjugated bilirubin into the bile
4. Excretion of bilirubin.

### Uptake of Bilirubin by Liver

- Since bilirubin is hydrophobic and insoluble in aqueous plasma, it is transported to the liver by binding noncovalently to **plasma albumin**.
- In the liver, the bilirubin is removed from albumin and taken up by hepatocytes.

### Conjugation of Bilirubin

- Hepatocytes convert insoluble bilirubin to a soluble form by conjugation with two molecules of glucuronate supplied by UDP-glucuronate. This reaction is catalyzed by **bilirubin glucuronyl transferase**.
- Bilirubin monoglucuronide is an intermediate and is subsequently converted to the bilirubin diglucuronide **(Fig. 18.4)**.

### Secretion of Bilirubin into Bile

Bilirubin diglucuronide (conjugated bilirubin) formed in the liver is secreted in the bile and is a rate limiting step for the entire process of hepatic bilirubin metabolism. Unconjugated bilirubin is not secreted into bile.

**Fig. 18.4:** Conjugation of bilirubin with glucuronic acid in the liver.

### *Excretion of Bilirubin*

- Following secretion into bile conjugated bilirubin passes through the hepatic and common bile ducts into the intestinal lumen.
- Bilirubin diglucuronide is hydrolyzed in the intestine by bacterial enzymes β-***glucuronidase*** to liberate free bilirubin. The free bilirubin so formed is further reduced to a colorless **urobilinogen.** A part of which is absorbed from the gut into the portal circulation **(Fig. 18.5)**.
- The urobilinogen which is absorbed into portal circulation can take two alternative routes.
  1. A part of it enters the systemic circulation and transported to the kidneys, where it is oxidized to **urobilin** (orange yellow pigment) and excreted in urine. The normal umber yellow color of urine is due to urobilin.
  2. A part of the urobilinogen is returned to the liver and re-excreted through liver to the intestine, known as **enterohepatic urobilinogen cycle.**
- The major portion of urobilinogen which remains within intestinal lumen is reduced further in the intestine to **stercobilinogen,** which is excreted as an oxidized brown pigment **stercobilin** in the feces. The characteristic brown color of stool is due to stercobilin. **Figure 18.5** shows the four major processes involved in the metabolism of bilirubin.

**Serum Bilirubin**

The normal concentration of serum bilirubin is:
1. Total bilirubin = 0.1 to 1.0 mg/dL
2. Conjugated (Direct) bilirubin = 0.1 to 0.4 mg/dL
3. Unconjugated (Indirect) bilirubin = 0.2 to 0.7 mg/dL.

## JAUNDICE

When bilirubin in blood exceeds 1 mg/dL is called **hyperbilirubinemia** and when it reaches a certain concentration approximately 2.2 to 5 mg/dL it diffuses into the tissues. The skin and sclera appear yellowish due to deposition of bilirubin in the tissues. This clinical condition is called **jaundice** (French: jaune = yellow) or **icterus.**

### Classification of Jaundice

Jaundice can be classified into three types:
1. Hemolytic or prehepatic
2. Hepatocellular or hepatic
3. Obstructive or post hepatic.

**Fig. 18.5:** Schematic representation of normal bilirubin metabolism.

## *Prehepatic or Hemolytic Jaundice*

- In prehepatic or hemolytic jaundice, there is increased breakdown of hemoglobin to bilirubin at a rate in excess of the ability of the liver cell to conjugate and excrete it. Excess hemolysis may be due to:
  - Sickle hemoglobin
  - Deficiency of enzyme glucose-6-phosphate dehydrogenase
  - Incompatible blood transfusion.
- In hemolytic jaundice, more than normal amounts of bilirubin are excreted into the intestine, resulting in an increased amount of **urobilinogen** in feces and urine.

- The main biochemical features of the hemolytic jaundice are:
  - Increased plasma concentration of unconjugated bilirubin
  - Increased amount of urobilinogen in urine and feces
  - Absence of bilirubin in the urine. Since the excess bilirubin is unconjugated, it is not excretable in the urine.

### *Hepatocellular or Hepatic Jaundice*

- In this kind of jaundice, there is some disorder of the liver cells. Hepatic parenchymal cell damage impairs uptake and conjugation of bilirubin and results in ***unconjugated hyperbilirubinemia.*** Liver damage is usually caused by:
  - Infections (viral hepatitis)
  - Toxic chemicals (such as alcohol, chloroform, carbon tetrachloride, etc.)
  - Drugs
  - Cirrhosis.
- Patients with jaundice due to hepatocellular damage commonly have obstruction of the liver biliary tree that leads to increased plasma level of conjugated bilirubin also.
- The main biochemical features of hepatocellular jaundice are:
  - Increased plasma concentration of conjugated and unconjugated bilirubin
  - Decreased amount of urobilinogen in urine and feces
  - Presence of bilirubin in the urine
  - Raised level of alanine transaminase (ALT) and aspartate transaminase (AST) enzymes.

### *Post Hepatic or Obstructive Jaundice*

- This occurs when there is an obstruction in the common bile duct that prevents the passage of conjugated bilirubin from the liver cells to the intestine. The obstruction may be due to:
  - Blockage of the common bile duct by gallstones
  - Carcinoma of the head of the pancreas
  - Carcinoma of the duct itself.
- The main biochemical manifestations of obstructive jaundice are:
  - Increased plasma concentration of conjugated bilirubin
  - Absence of urobilinogen in feces and urine
  - The presence of bilirubin and bile salts in the urine
  - Raised level of plasma alkaline phosphatase (ALP). ALP is normally excreted through bile. Obstruction to the flow of bile causes regurgitation of enzyme into the blood resulting in increased serum concentration.

**Table 18.2** summarizes laboratory findings in the differential diagnosis of jaundice.

### Neonatal or Physiologic Jaundice

- Mild jaundice in the first few days after birth is common and physiological.
- It results from an **increased hemolysis** and **immature liver enzyme system** for conjugation of bilirubin in the newborn.
- The liver of newborn is deficient in enzyme ***UDP-glucuronyl transferase,*** necessary for conjugation.
- The enzyme deficiency is more in premature infants.
- Since the increased bilirubin is unconjugated, it is capable of penetrating the blood-brain barrier when its concentration in plasma exceeds 20 to 25 mg/dL. This results in a ***hyperbilirubinemic toxic encephalopathy*** or ***kernicterus,*** which can cause mental retardation.

## INHERITED HYPERBILIRUBINEMIAS

### Gilbert's Syndrome

Gilbert's syndrome is an inherited disease characterized by mild benign (harmless) unconjugated hyperbilirubinemia due to:

**Table 18.2:** Laboratory findings in the differential diagnosis of jaundice.

| Condition | Serum bilirubin | | Urine urobilinogen | Urine bilirubin | Fecal urobilinogen |
|---|---|---|---|---|---|
| | Conjugated (Direct) | Unconjugated (Indirect) | | | |
| Normal | 0.1–0.4 mg/dL | 0.2–0.7 mg/dL | 0.4 mg/24 hours | Absent | 40-280 mg/24 hours |
| Hemolytic | Normal | Increased | Increased | Absent | Increased |
| Hepatocellular | Increased | Increased | Decreased | Present | Decreased |
| Obstructive | Increased | Normal | Absent | Present | Trace to absent |

- Impaired hepatic uptake of bilirubin
- Partial conjugation defect due to reduced activity of **UDP-glucuronyl transferase**.

## Crigler-Najjar Syndrome

This is a rare autosomal recessive disorder due to deficiency of **hepatic glucuronyl transferase** enzyme. There are two forms of this condition:

- **Type-I** is characterized by complete absence of the conjugating enzyme **glucuronyl transferase** and therefore no conjugated bilirubin is formed. It causes severe jaundice with kernicterus and early death.
- **Type-II** is a less severe form in which the enzyme deficiency is partial and is compatible with more prolonged survival.

## Dubin-Johnson Syndrome

This is harmless autosomal recessive disorder and is due to defective hepatic secretion of **conjugated bilirubin** into the bile and is characterized by slightly raised plasma conjugated bilirubin level. It is characterized by abnormal black pigment in the hepatocytes, imparting a dark brown to black color to the liver.

# EXAM QUESTIONS

## Long Answer Questions (LAQs)

1. Describe in brief heme biosynthesis and its regulation. Add a note on porphyria.
2. Describe in brief degradation heme and fate of its degradative product.
3. Describe in brief formation and fate of bilirubin. Add a note on jaundice.

## Short Notes

1. Formation and fate of bilirubin.
2. Porphyria.
3. Unconjugated hyperbilirubinemia.
4. Differential diagnosis of jaundice
5. Congenital hyperbilirubinemia

## Solve

**Case History 1**

**A 53-year-old woman developed a hyperpigmentation and rash on her neck and photosensitive nature, a diagnosis of porphyria Cutanea Tarda was considered.**

**Questions**

a. What is porphyria?
b. What are the types of porphyria?
c. Which enzyme is defective in porphyria Cutanea Tarda?

**Case History 2**

**A 45-year-old woman complains of acute abdominal pain and vomiting following fatty food. The biochemical investigations are:**

a. Raised serum conjugated bilirubin.
b. Significantly raised serum alkaline phosphatase.
c. Excretion of dark yellow colored urine.
d. Fouchet's test on fresh urine shows green color.

**Questions**

a. Name the disease.
b. Give differential diagnosis of the condition.

**Case History 3**

**A 50-year-old woman visited hospital with history of anorexia, nausea and flu like symptoms. She had noticed that her urine had been dark in color over the past 2 days. Her LFTs were as follows:**

i. Total bilirubin (direct and indirect): Increased
ii. AST and ALT: Marked increased activity
iii. Alkaline phosphatase: Increased

**Questions**

a. Comment on these results
b. What is the differential diagnosis

**Case History 4**

**A 30-year-old male presented at clinical with history of intermittent abdominal pain and episodes of confusion and psychiatric problems. Laboratory tests revealed increase of urinary δ-aminolevulinate and porphobilinogen. Mutational analysis revealed a mutation in the gene for uroporphyrinogen synthase (porphobilinogen deaminase)**

**Questions**

a. What is the probable diagnosis?
b. What is the cause of abdominal pain and psychiatric problem?
c. The disorder concern with which metabolic pathway
d. Name some other disorders related to same pathway.

**Case History 5**

**A 62-year-old female presented at clinic with intense jaundice she had noted that her stools had been very pale. Lab test revealed a very high level of direct bilirubin. The level of alkaline phosphates was markedly elevated.**

a. What is the probable diagnosis?
b. Why color of stool is very place?
c. What is the status of urine bilirubin?
d. What is direct and indirect bilirubin?

**Case History 6**

**A 58-year-old man complained of increased skin lesion formation on his hands, forehead, neck and ears on exposure to the sun. Laboratory findings showed an increase in urinary uroporphyrin with normal levels of ALA and porphobilinogen. Mutational analysis showed mutation in the gene for uroporphyrinogen decarboxylase.**

a. What is the probable type of porphyria?
b. Which are the other types of porphyria?
c. What is the cause of skin lesion?
d. What is porphyria?

## Multiple Choice Questions (MCQs)

1. **Acute intermittent porphyria is accompanied by increased urinary excretion of:**
   a. Porphobilinogen b. Heme
   c. Bilirubin d. Biliverdin
2. **The normal brown red color of feces results from the presence of:**
   a. Stercobilin b. Bilirubin
   c. Biliverdin d. Bilirubin diglucuronide
3. **The end product of catabolism of heme is:**
   a. Bile acids b. Bile salts
   c. Bile pigment d. Uric acid
4. **Unconjugated bilirubin is raised mostly in:**
   a. Hemolytic jaundice
   b. Obstructive jaundice
   c. Carcinoma of pancreas
   d. Stone in gallbladder
5. **The rate limiting reaction in heme synthesis is catalyzed by:**
   a. δ-ALA dehydratase
   b. δ-ALA synthase
   c. Ferrochelatase
   d. Uroporphyrinogen decarboxylase
6. **Which of the following statements is not applicable to bilirubin?**
   a. Bilirubin is carried in the plasma to the liver bound to albumin
   b. High levels of bilirubin can cause damage to brain of newborn infants.
   c. Bilirubin is water soluble
   d. Bilirubin does not contain iron
7. **The biosynthesis of heme requires all of the following, *except*:**
   a. Succinyl CoA b. Glycine
   c. Ferrous ion d. Glutamine
8. **Lead poisoning leads to increased level of:**
   a. Bilirubin
   b. δ-aminolevulinic acid
   c. Porphobilinogen
   d. Coproporphyrin
9. **Increased level of conjugated bilirubin in blood occurs in the following condition, *except:***
   a. Hemolytic jaundice
   b. Hepatocellular jaundice
   c. Obstructive jaundice
   d. Dubin Johnson syndrome
10. **Which of the following substance is deposited in skin and sclera in jaundice?**
    a. Only unconjugated bilirubin
    b. Only conjugated bilirubin
    c. Both unconjugated and conjugated bilirubin
    d. Biliverdin
11. **High levels of δ-aminolevulinic acid and porphobilinogen in the urine occurs in:**
    a. Acute intermittent porphyria
    b. Erythropoietic porphyria
    c. Hereditary coproporphyria
    d. Porphyria Cutanea Tarda
12. **The porphyria which does not show photosensitivity is:**
    a. Acute intermittent porphyria
    b. Erythropoietic porphyria
    c. Hereditary coproporphyria
    d. Porphyria cutanea tarda

**13. Hepatic glucuronyl transferase deficiency occurs in which of the following condition:**
a. Dubin Johnson syndrome
b. Crigler Najjar syndrome
c. Lesch Nyhan syndrome
d. None of the above

**14. Protoporphyria is due to deficiency of:**
a. Protoporphyrinogen oxidase
b. Coproporphyrinogen oxidase
c. Ferrochelatase
d. Uroporphyrinogen decarboxylase

**15. Porphyrias is an inherited disorder of which of the following synthetic pathway:**
a. Glycogen
b. Heme
c. Purine nucleoids
d. Cholesterol

## Answers for MCQs

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **1.** a | **2.** a | **3.** c | **4.** a | **5.** b | **6.** c | **7.** d | **8.** b |
| **9.** a | **10.** c | **11.** a | **12.** a | **13.** b | **14.** c | **15.** b | |

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CHAPTER

# 19 Purine and Pyrimidine Nucleotide Metabolism

**Chapter outline**


- *De Novo* Biosynthesis of Purine Nucleotides
- Salvage Pathway
- Catabolism of Purine Nucleotides
- Disorders of Purine Catabolism
- Immunodeficiency Disorders of Purine Metabolism
- *De Novo* Biosynthesis of Pyrimidine Nucleotides
- Catabolism of Pyrimidine Nucleotides
- Disorders of Pyrimidine Catabolism


## INTRODUCTION

Purines and pyrimidines are **dietary nonessential** components. Dietary nucleic acids and nucleotides do not provide essential constituents for the biosynthesis of endogenous nucleic acids. Humans can synthesize purine and pyrimidine nucleotides *de novo,* i.e. from amphibolic intermediates.

## BIOSYNTHESIS OF PURINE NUCLEOTIDES

- The two purine nucleotides of nucleic acids are:
  1. Adenosine monophosphate, AMP
  2. Guanosine monophosphate, GMP
- Purine nucleotides can be synthesized by two pathways:
  1. ***De novo pathway*** (New synthesis from amphibolic intermediates).
  2. **Salvage pathway**.

## *DE NOVO* BIOSYNTHESIS OF PURINE NUCLEOTIDES

In de novo pathway, the purine ring formed from variety, of precursors **(Fig. 19.1)** is assembled on ribose-5-phosphate.

### Precursors for the De Novo Synthesis of Purine

- Glycine provides $C_4$, $C_5$ and $N_7$
- Aspartate provides $N_1$
- Glutamine provides $N_3$ and $N_9$
- Tetrahydrofolate derivatives furnish $C_2$ and $C_8$
- Carbon dioxide provides $C_6$.

### Major Steps of *De Novo* Synthesis of Purine Nucleotides

1. The biosynthesis of purine begins with ribose-5-phosphate, derived from **pentose phosphate pathway**, which is converted to **phosphoribosyl pyrophosphate,**

**Fig. 19.1:** Source of carbon and nitrogen atoms in the purine ring.

**PRPP** by the transfer of pyrophosphate group from ATP to C-1 of the ribose-5-phosphate. This reaction is catalyzed by the enzyme ***PRPP synthetase.*** PRPP is required for both pyrimidine and purine *de novo* synthesis. It is an intermediate in the purine salvage pathway.

2. PRPP is aminated by the addition of the amide group from **glutamine** to form amino sugar **5 phosphoribosylamine**. The enzyme that catalyzes the transfer of the amide nitrogen is called **glutamine PRPP amidotransferase.** *The synthesis of phosphoribosylamine from PRPP is the* ***first committed (rate limiting) step*** *in the formation of inosine monophosphate (IMP).* The amino nitrogen of phosphoribosylamine provides $\mathbf{N_9}$ of the purine ring **(Fig. 19.2)**.
3. $\mathbf{C_4}$, $\mathbf{C_5}$, and $\mathbf{N_7}$ are next provided by the addition of amino acid **glycine** to form **glycinamide ribonucleotide (GAR).** ATP is consumed in this reaction and the enzyme ***glycinamide ribonucleotide synthetase*** is required.
4. A **one carbon unit** is next transferred to the free amino group of GAR by an *enzyme* ***GAR formyltransferase*** to form **formylglycinamide ribonucleotide (FGAR)**. **$N^5$, $N^{10}$-methenyl tetrahydrofolate** serves as the carrier of one carbon unit. This reaction adds a carbon that will become $\mathbf{C_8}$ of the purine ring.
5. The $\mathbf{N_3}$ of the purine structure is introduced by another amination using **glutamine** and ATP to form **formyl glycinamidine *ribonucleotide* (FGAM)** catalyzed by ***FGAR amidotransferase***.
6. In a reaction catalyzed by ***FGAM cyclase (AIR cyclase)*** loss of water accompanied by ring closure forms ***amino-imidazole ribonucleotide (AIR)***.
7. Addition of $\mathbf{CO_2}$ to AIR adds the atom that will become $\mathbf{C_6}$ of the purine structure. The reaction, catalyzed by ***amino imidazole ribonucleotide carboxylase (AIR carboxylase),*** requires neither ATP nor biotin and forms **carboxy amino imidazole ribonucleotide (CAIR)**.
8. Condensation of **aspartate** with (CAIR), catalyzed by ***succinyl amino imidazole carboxamide ribonucleotide (SAICAR) synthetase*** forms N-succinyl-5-amino-imidazole-4-carboxamide nucleotide *(SAICAR).*
9. Liberation of the succinyl group of *(SAICAR) as* fumarate, catalyzed by ***SAICAR lyase*** forms **amino imidazole carboxamide ribonucleotide (AICAR)**. Reactions 8 and 9, add the atom that becomes **nitrogen-1** of the purine structure.
10. Carbon-2 of the purine is added by **$N^{10}$ formyl tetrahydrofolate** by **AIRCAR transformylase** to form **formyl amino imidazole carboxamide ribonucleotide (FAICAR)**.
11. Ring closure of FAICAR catalyzed by **IMP synthase** forms the first purine nucleotide, **inosine monophosphate, IMP**.

**Fig. 19.2:** Contd...

*Contd...*

**Fig. 19.2:** *De novo* pathway for synthesis of purine nucleotides.

12. Both **adenosine** and **guanosine monophosphate** are produced from IMP, using nitrogen of **aspartate** and **glutamine** respectively. Addition of aspartate to IMP forms adenylosuccinate in the presence of ***adenylosuccinate synthetase*** and GTP.
13. ***Adenylosuccinase*** in turn catalyzes the removal of fumarate to yield AMP.
14. To produce GMP, the IMP must first be oxidized to **xanthosine monophosphate (XMP)** by $NAD^+$ linked enzyme ***IMP dehydrogenase***.
15. XMP then accepts an amino group from glutamine in the presence of ATP and the enzyme ***XMP-glutamine amidotransferase.***

## Regulation of De Novo Synthesis of Purine Nucleotide (Fig. 19.3)

The synthesis of purine nucleotides is controlled by:

- Concentration of PRPP
- Feedback regulation at several sites.

### *Concentration of PRPP*

Increased concentration of PRPP stimulates the purine nucleotides synthesis. Concentration of PRPP depends on:

- Availability of ribose-5-phosphate
- On the activity of PRPP synthetase.

### *Feedback Regulation*

Three major feesdback mechanisms cooperate in regulating the overall rate of de novo purine nucleotide synthesis.

1. The first of these control mechanisms is exerted on the first reaction, i.e. the conversion of PRPP into phosphoribosylamine by glutamine PRPP amidotransferase which is feedback inhibited by the end products IMP, AMP, and GMP **(Fig. 19.3)**.
2. In the second control mechanism, exerted at a later stage, in which AMP and GMP regulate their formation from IMP. An excess GMP in the cell inhibits formation of XMP from IMP without affecting the formation of AMP. Conversely an accumulation of AMP inhibits formation of adenylosuccinate without affecting the biosynthesis of GMP.
3. The third and final control mechanism is the inhibition of formation of PRPP. This reaction is catalyzed by an allosteric enzyme ***PRPP* synthetase,** which is feedback inhibited by ADP and GDP **(Fig. 19.3)**.

**Fig. 19.3:** Regulation of *de novo* synthesis of purine nucleotides.

## SYNTHESIS OF PURINE NUCLEOTIDES BY SALVAGE PATHWAY

- The pathway involved in the conversion of free purines to nucleotides is called **salvage pathway** (Salvage means property saved from loss).
- Free purine bases (adenine, guanine and hypoxanthine) are formed in cells during the metabolic degradation of nucleic acids and nucleotides. However, free purines are salvaged and used over again to remake purine nucleotides. This occurs by a pathway that is quite different from the *de novo* biosynthesis of purine nucleotides described earlier, in which the purine ring system is assembled step by step on ribose-5-phosphate in a long series of reactions.
- The salvage pathway is much simpler and requires far less energy than does *de novo* synthesis. It consists of a single reaction.

**Significance of Salvage Pathway**

Salvage pathway provides purine nucleotides for tissues, which are incapable to synthesize purine nucleotides by *de novo* pathway, e.g. human brain, erythrocytes and polymorphonuclear leukocytes.

## Salvage Reaction (Fig. 19.4)

In salvage reaction ribose phosphate moiety of PRPP is transferred to the purine to form the corresponding nucleotide. There are two salvage enzymes with different specificities as follows:

- Adenine phosphoribosyl transferase (APRTase) catalyzes the formation of adenine nucleotide (AMP) from adenine.
- Whereas, **Hypoxanthine Guanine phosphoribosyl transferase (HGPRTase),** catalyzes the formation of ionosine (IMP) from hypoxanthine and guanine nucleotide (GMP) from guanine **(Fig. 19.4)**.

**Fig. 19.4:** Salvage pathway of purine nucleotide synthesis.

## CATABOLISM OF PURINE NUCLEOTIDES

The end product of purines (adenine and guanine) in humans is sparingly soluble uric acid **(Fig. 19.5)**.

- Purine nucleotides (AMP and GMP) are degraded by a pathway, in which the phosphate group is removed by the action of **nucleotidase;** to yield the nucleoside, adenosine or guanosine.
- Adenosine is then deaminated to **inosine** by **adenosine deaminase**.
- Inosine is then hydrolyzed by ***purine nucleoside phosphorylase*** to yield its purine base **hypoxanthine** and ribose-1-phosphate.
- Hypoxanthine is oxidized successively to **xanthine** and then **uric acid,** by ***xanthine oxidase,*** *a* molybdenum and iron containing flavoprotein. In this reaction, molecular oxygen is reduced to $H_2O_2$, which is decomposed to $H_2O$ and $O_2$, by catalase.
- Guanosine is cleaved to **guanine** and ribose-1-phosphate by **phosphorylase** enzyme.
- Guanine undergoes hydrolytic removal of its amino group by **guanase** to yield **xanthine**, which is converted to **uric acid** by **xanthine oxidase**.

## DISORDERS OF PURINE CATABOLISM

The catabolism of the purines, adenine and guanine produces **uric acid.** At physiological pH, uric acid is mostly ionized and present in plasma as ***sodium urate.***

An elevated serum urate concentration is known as ***hyperuricemia.*** Uric acid and urate are relatively insoluble

**Fig. 19.5:** Catabolism of purine nucleotides.

molecules which readily precipitate out of aqueous solutions such as urine or synovial fluid. The consequence of this is the condition, ***gout.***

The average normal blood serum level of uric acid is **4 to 7 mg per 100 ml.**

## Gout

Gout is a metabolic disorder associated with an elevated level of **uric acid** in the serum. The increased serum uric acid is due to either increased formation of uric acid or its decreased renal excretion. *Whatever is the cause,* **gout is associated with hyperuricemia but hyperuricemia is not always associated with gout**.

### *Classification of Gout*

Gout is classified into two broad types:

1. Primary gout
2. Secondary gout

#### *Primary gout*

- Primary gout is an inborn error of metabolism due to **overproduction of uric acid.** In primary gout, increased level of uric acid is associated with increased synthesis of purine nucleotides. Increased synthesis of purine nucleotides is caused by defective enzymes of purine nucleotide biosynthesis, such as:
  - PRPP synthetase
  - PRPP glutamyl amidotransferase
  - HGPRTase
- In normal course **PRPP synthetase** and **PRPP glutamyl amidotransferase** are allosterically feedback regulated by its own product AMP and GMP. But due to loss of allosteric feedback regulation, abnormally high level of PRPP synthetase and PRPP glutamyl amidotransferase results in excessive production of PRPP, which in turn accelerates the rate of *de novo* synthesis of purine nucleotides. Increased synthesis is associated with increased break down to uric acid.
- **HGPRTase deficiency:** The enzyme HGPRTase catalyzes the synthesis of GMP and IMP by salvage pathway **(*see* Fig. 19.4).** Deficiency of HGPRTase leads to reduced synthesis of IMP and GMP by salvage pathway and increases the level of PRPP. Increased level of PRPP accelerates the purine nucleotide biosynthesis by *de novo* pathway.

***Symptoms of primary gout***

- Patients with primary gout often show deposition of urate as tophi (clusters of urate crystals) in soft tissues **(*see* Fig. 19.6)** that affects the joints and leads to painful arthritis.
- The kidneys are also affected, since excess urate is also deposited in the kidney tubules **(Fig. 19.7)** and leads to renal failure.

#### *Secondary gout*

Secondary gout results from a variety of diseases that cause an ***elevated destruction of cells*** or ***decreased elimination of uric acid*** as follows:

- Elevated destruction of cells is accompanied by increased degradation of nucleic acids to uric acid, which occurs in cancers (leukemia, polycythemia), psoriasis and hyper catabolic states (starvation, trauma, etc.).

**Fig. 19.6:** Deposition of urate as tophi in soft tissues.

**Fig. 19.7:** Urate crystals deposited in kidney tubules.

- Decreased elimination of uric acid occurs in chronic renal disease due to reduced glomerular filtrate rate.
- **Glucose-6-phosphatase deficiency:** Glucose-6-phosphatase enzyme is not directly involved in purine synthesis. It is involved indirectly with biosynthesis of purine nucleotide.

In type-I glycogen storage disease, **Von Gierkes disease** due to deficiency of **glucose-6-phosphatase**, glucose-6-phosphate cannot be converted to glucose. Accumulated glucose-6-phosphate is then metabolized via HMP shunt, which in turn generates large amounts of ribose-5-phosphate, a precursor of PRPP. The increased synthesis of PRPP then enhances de novo synthesis of purine nucleotides.

### *Treatment of Gout*

Gout can be treated by a combination of nutritional therapy and drug therapy.

- Foods especially rich in nucleotides and nucleic acids such as liver or coffee and tea, which contain the purines **caffeine** and **theobromin,** are withheld from the diet. Restriction in intake of alcohol is also advised.
- Major improvement follows the use of the drug ***allopurinol,*** an analog of hypoxanthine which inhibits **xanthine oxidase competitively**, the enzyme responsible for converting purines into uric acid. This leads to reduced formation of uric acid and accumulation of xanthine and hypoxanthine, which are more soluble and thus easily excreted.

## Lesch-Nyhan Syndrome

Lesch-Nyhan syndrome is an inherited X-linked disorder that affects only males. It was first described by Michael Lesch and William Nyhan. It is caused by a ***complete deficiency of HGPRTase,*** an enzyme which is involved in purine salvage pathway **(*see* Fig. 19.4)**.

- In the absence of HGPRTase, the salvage pathway is inhibited and purines cannot be reconverted to nucleotides; instead they are degraded to uric acid.
- The lack of HGPRTase also causes an overproduction of PRPP, which stimulates purine biosynthesis. Because of increased purine synthesis, the degradation product uric acid also increases. This results in increased concentration of uric acid in plasma and urine.

#### *Symptoms*

The symptoms include:

- Hyperuricemia
- Gout
- Urinary tract stones
- The neurological symptoms or mental retardation
- Spasticity (resistance to the passive movement of a limb)
- Self-mutilation (self-painful, destructive behavior of biting of fingers and lips).

The basis for neurologic symptoms is unknown. However, brain cells normally have much higher levels of purine salvage enzyme (HGPRTase) than other cells. Brain normally use salvage pathway for the synthesis of IMP and GMP, because brain is incapable to synthesize purine nucleotides by *de novo* pathway. In Lesch-Nyhan syndrome

due to lack of HGPRTase, synthesis of purine nucleotides (which are precursors of DNA) by brain is decreased.

### *Treatment*

Allopurinol reduces uric acid formation but does not alleviate the neurologic symptoms.

### Xanthinuria

Deficiency of the enzyme, ***xanthine oxidase,*** due to either genetic defect or severe liver damage results in **hypouricemia** and increased urinary excretion of xanthine and hypoxanthine.

Xanthine lithiasis (formation of stones) and secondary renal damage may occur in severe xanthine oxidase deficiency.

## IMMUNODEFICIENCY DISORDERS OF PURINE METABOLISM

### Adenosine Deaminase (ADA) Deficiency

- ADA deficiency causes severe combined immunodeficiency (SCID).
- In ADA deficiency both thymus-derived lymphocytes (T-cells) and bone marrow derived lymphocytes (B-cells) are dysfunctional.
- A deficiency of ADA results in an accumulation of adenosine which in turn results in accumulation of deoxyadenosine and d ATP.
- As d ATP levels rise, ribonucleotide reductase is inhibited which is required for the formation of deoxyribonucleotides. Consequently, cells cannot make DNA and divide.
- Elevated levels of d ATP are toxic to lymphocytes. Thus, lymphocytes are killed resulting in impairment of both cell and humoral immunity.
- The disease is fatal and victims usually die in early childhood (within 2 years of age).

## *DE NOVO* BIOSYNTHESIS OF PYRIMIDINE NUCLEOTIDES

The pyrimidine nucleotides are:

1. Cytidine monophosphate CMP
2. Uridine monophosphate UMP
3. Thymidine monophosphate TMP.

Unlike the synthesis of purine nucleotide, six membered pyrimidine ring is made first and then attached to ribose phosphate, which is donated by PRPP.

**Fig. 19.8:** Sources of the carbon and nitrogen atoms in the pyrimidine ring.

### Precursors for the De Novo Synthesis of Pyrimidine (Fig. 19.8)

- Glutamine provides N3
- Aspartic acid furnishes C4, C5, C6 and N1
- Carbon dioxide provides C2.

### Major Steps for De Novo Synthesis of Pyrimidine Nucleotide (Fig. 19.9)

1. Pyrimidine biosynthesis starts with the formation of carbamoyl phosphate from glutamine, ATP and $CO_2$. This reaction is catalyzed by **cytosolic carbamoyl phosphate synthase-l (CPS-II)** an enzyme different from mitochondrial carbamoyl phosphate synthase-l (CPS-I) required in the synthesis of urea.
2. Condensation of carbamoyl phosphate with aspartate forms carbamoyl aspartate in a reaction catalyzed by ***aspartate transcarbamoylase and is the committed step in the biosynthesis of pyrimidine.***
3. By removal of water from carbamoyl aspartate, catalyzed by ***dihydroorotase,*** the pyrimidine ring is closed with formation of dihydro-orotic acid.
4. Dihydro-orotic acid is now oxidized to yield pyrimidine derivative orotic acid, a reaction catalyzed by mitochondrial $NAD^+$ dependent **dihydro-orotate dehydrogenase.** All other enzymes of pyrimidine biosynthesis are cytosolic.
5. The next step is the acquisition of a ribose phosphate group. Transfer of ribose phosphate moiety from PRPP to orotate, forming orotidine monophosphate, OMP (orotidylate). This reaction is catalyzed by **orotate phosphoribosyl transferase.**
6. Orotidylate is then decarboxylated to yield uridylate (UMP).
7. UMP is phosphorylated to UDP by kinase using ATP as a phosphate donor. UDP serves as a precursor for the synthesis of UTP, CTP, dUMP and dTMP.
8. UDP undergoes an ATP dependent kinase reaction to produce UTP.

9. UTP is aminated by accepting amino group from glutamine to form cytidine triphosphate (CTP).
10. Ribonucleotide reductase converts UDP to dUDP.
11. Deoxyuridine diphosphate (dUDP) is dephosphorylated to dUMP which acts as a substrate for thymidine monophosphate (TMP).
12. Methylation of dUMP by $N^5N^{10}$-methylene tetrahydrofolate, catalyzed by ***thymidylate synthase,*** forms deoxythymidine monophosphate (dTMP).

**Fig. 19.9:** *De novo* pathway for the synthesis of pyrimidine nucleotide.

## Regulation of De Novo Synthesis of Pyrimidine Nucleotides

The first two enzymes **carbamoyl phosphate synthase-II** and **aspartate transcarbamoylase** are allosteric enzymes and are regulated allosterically **(Fig. 19.10)**.

- Carbamoyl phosphate synthase-II, reaction 1 is feedback inhibited by UTP and activated by PRPP.
- Aspartate transcarbamoylase, reaction 2, is feedback inhibited by CTP and activated by ATP.

## CATABOLISM OF PYRIMIDINE NUCLEOTIDES (FIG. 19.11)

Unlike the purines which degraded to sparingly soluble product, uric acid, the end products of pyrimidine catabolism are highly water soluble:

- $CO_2$

**Fig. 19.10:** Regulation of *de novo* pyrimidine nucleotide synthesis. (CP: Carbamoyl phosphate; CA: Carbamoyl aspartate).

**Fig. 19.11:** Catabolism of pyrimidines.

- $NH_3$
- β-alanine
- β-aminoisobutyrate

Human probably transaminate β-aminoisobutyrate into methylmalonate semialdehyde. This is then converted to succinyl-CoA via methylmalonyl-CoA. β-alanine can serve as precursor of acetyl-CoA.

## DISORDERS OF PYRIMIDINE CATABOLISM

Since the end products of pyrimidine catabolism are highly water soluble, overproduction of pyrimidine catabolites is rarely associated with clinically significant abnormalities.

### Disorder of Pyrimidine Synthesis

#### *Orotic Aciduria*

- Orotic aciduria is a hereditary disorder which can result from a defective enzyme in pyrimidine synthesis.
- A defect in the multifunctional enzyme ***UMP synthase*** that converts orotic acid to UMP results in the excretion of orotic acid in the urine.
- UMP synthase is a multifunctional enzyme containing both orotate phosphoribosyl transferase and orotidylate decarboxylase activity **(Fig. 19.9)**.
- There are two types of orotic acidemia:
  i. **In type-I,** there is deficiency of both enzymes, orotate phosphoribosyl transferase and orotidylate decarboxylase.
  ii. **In type-II,** only orotidylate decarboxylase is deficient.

The deficiency in UMP and other pyrimidine nucleotides results in inhibition of DNA and RNA synthesis, which causes ***megaloblastic anemia*** and ***failure to thrive*** (growth retardation).

#### *Reye's Syndrome*

Reye's syndrome is a secondary orotic aciduria, which may be due to inability of severely damaged mitochondria to utilize carbamoylphosphate in the formation of urea which then may be diverted for cytosolic overproduction of orotic acid.

## EXAM QUESTIONS

### Short Notes

1. Purine salvage pathway.
2. Catabolism of pyrimidines and related disorders.
3. Gout.
4. Catabolism of purines and related disorder.
5. Lesch-Nyhan syndrome.
6. Regulation of purine nucleotide *de novo* biosynthesis.
7. Regulation of pyrimidine nucleotide *de novo* biosynthesis.
8. Immunodeficiency disorders of purine metabolism.
9. Explain how patients with glycogen storage disease (glucose-6-phosphatase deficiency) develop gout in early adult life.
10. Enumerate the sources for purine ring formation, show diagrammatic representation of, which atoms in purine ring is contributed by them?

### Solve

**Case History 1**

**A 50-year-old female patient complains of pain and swelling in joints and shows hyperuricemia.**

**Questions**

a. Name the probable disease.
b. Name the enzyme defect.
c. What is the normal value of serum uric acid?
d. Suggest nutritional therapy.

**Case History 2**

**A 4-year-old boy suffered from pain in joints, showed signs of mental retardation and had self-mutilation (self-destructive behavior of biting fingers and lips). His serum uric acid level was 10 mg/dL.**

**Questions**

a. What is the probable diagnosis?
b. Which enzyme is defective in this disease?
c. What is the normal level of serum uric acid in human?
d. What is the mode of inheritance of this disease?

**Case History 3**

**A 50-year-old man was awakened by a severe pain in his left toe and the pain became so intense that he could not bear the weight of the bedclothes. On investigation, it is found that his serum uric acid level was high.**

a. Name the probable disorder.
b. What is uric acid?
c. Write pathway for the formation uric acid.
d. Give normal level of serum uric acid.

## Multiple Choice Questions (MCQs)

1. **Rate controlling step of pyrimidine biosynthesis is catalyzed by:**
   a. Orotidylate decarboxylase
   b. Aspartate transcarbamoylase
   c. Carbamoyl phosphate synthetase I
   d. Orotate phosphoribosyl transferase
2. **Which statement for purine biosynthesis is incorrect?**
   a. Requires vitamin $B_{12}$
   b. Assembled on ribose phosphate
   c. Requires PRPP
   d. Requires glycine
3. **Purines and pyrimidines are:**
   a. Dietary essential
   b. Dietary nonessential
   c. Derived from essential fatty acids
   d. Derivatives of essential amino acid
4. **The two nitrogen of the pyrimidine ring are contributed by:**
   a. Glutamate and ammonia
   b. Glutamine and aspartate
   c. Glutamate and glutamine
   d. Aspartate and carbamoyl phosphate
5. **Nitrogen or carbon atoms are contributed to the structure of the purine ring by amino acids, *except*:**
   a. Glycine
   b. Glutamine
   c. Aspartate
   d. Glutamate
6. **Lesch-Nyhan syndrome may lead to:**
   a. Self-destructive behavior
   b. Gout
   c. Elevated levels of PRPP
   d. All of the above
7. **Hyperuricemia can result from defect in enzymes, *except*:**
   a. Carbamoyl phosphate synthase II
   b. HGPRTase
   c. PRPP synthase
   d. Glucose-6-phosphatase
8. **The end product of purine metabolism in human is:**
   a. Creatinine
   b. Uric acid
   c. Urea
   d. Ammonia
9. **The salvage pathway for purines involves enzyme:**
   a. PRPP amidotransferase
   b. PRPP synthase
   c. HGPRTase
   d. Xanthine oxidase
10. **Enzyme involved with immunodeficiency disease of purine metabolism is:**
    a. Adenosine deaminase
    b. Xanthine oxidase
    c. PRPP synthetase
    d. HGPRTase
11. **Purine salvage occurs in the tissues, *except*:**
    a. RBC
    b. Brain
    c. Liver
    d. Polymorphonuclear leukocytes
12. **Allopurinol is used in the treatment of:**
    a. Rickets b. Cancer
    c. Gout d. Pellagra
13. **Lesch-Nyhan syndrome is due to the lack of:**
    a. HGPRTase
    b. APRTase
    c. Adenosine deaminase
    d. PRPP amidotransferase
14. **Hereditary orotic aciduria type II is due to deficiency of the following enzyme:**
    a. Dihydro-orotate dehydrogenase
    b. Orotate phosphoribosyl transferase
    c. Aspartate transcarbamoylase
    d. Orotidylate decarboxylase
15. **The *de novo* biosynthesis of purine nucleotides differs from the *de novo* biosynthesis of pyrimidine nucleotides in which one of the following features?**
    a. Utilizes glutamine
    b. Utilizes aspartate
    c. PRPP stimulates both
    d. Utilizes glycine
16. **The source of sugar phosphate incorporate in the *de novo* synthesis of purine nucleotides is:**
    a. Ribulose-5-phosphate
    b. 5-phosphoribosyl pyrophosphate (PRPP)
    c. Glucose-6-phosphate
    d. Deoxyribose-5 phosphate
17. **Which of the following statements describe pyrimidine biosynthesis in mammals?**
    a. Carbamoyl phosphate synthase II (CPSII) is feedback inhibited by UTP

b. CPS II activated by PRPP
c. CPS II utilizes glutamine as a source of amino group
d. a, b, and c are all correct

**18. The enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRTase) is involved in:**
a. Salvage of pyrimidine bases
b. Salvage of purine bases
c. *De novo* synthesis of purine nucleotides
d. *De novo* synthesis of pyrimidine nucleotides

**19. In purine biosynthesis carbon atoms at 4 and 5 position and N at 7 positions are contributed by:**
a. Glycine b. Glutamine
c. Alanine d. Threonine

**20. $N^{10}$-formyl and $N^5N^{10}$-methenyl tetrahydrofolate contributes purine carbon atoms at position:**
a. 4 and 6 b. 4 and 5
c. 5 and 6 d. 2 and 8

**21. In purine nucleus nitrogen atom at 1 position is derived from:**
a. Aspartate b. Glutamate
c. Glycine d. Alanine

**22. In purine biosynthesis ring closure in the molecule formyl glycinamide ribosyl-5-phosphate requires the cofactors:**
a. ADP b. NAD
c. FAD d. ATP and $Mg^{++}$

**23. Conversion of inosine monophosphate to xanthine monophosphate is catalyzed by:**
a. IMP dehydrogenase
b. Formyl transferase
c. Xanthine-guanine phosphoribosyl transferase
d. Adenine phosphoribosyl transferase

**24. An enzyme which acts as allosteric regulator of *de novo* biosynthesis of purine nucleotides is:**
a. PRPP synthetase
b. PRPP glutamyl amidotransferase
c. HGPRTase
d. Formyl transferase

**25. In the biosynthesis of purine nucleotides the AMP feedback regulates:**
a. Adenylosuccinase
b. Adenylosuccinate synthetase
c. IMP dehydrogenase
d. HGPRTase

**26 Pyrimidine and purine nucleoside biosynthesis share a common precursor:**
a. PRPP
b. Glycine
c. Fumarate
d. Alanine

**27. Pyrimidine biosynthesis begins with the formation using glutamine, ATP and $CO_2$, of:**
a. Carbamoyl aspartate
b. Orotate
c. Carbamoyl phosphate
d. Dihydroorotate

**28. The two nitrogen of the pyrimidine ring are contributed by:**
a. Ammonia and glycine
b. Aspartate and glutamine
c. Glutamine and ammonia
d. Aspartate and ammonia

**29. The first true pyrimidine ribonucleotide synthesized is:**
a. UMP b. UDP
c. TMP d. CTP

**30. UDP and UTP are formed by phosphorylation from:**
a. AMP b. ADP
c. ATP d. GTP

**31. Conversion of deoxyuridine monophosphate to thymidine monophosphate is catalyzed by the enzyme:**
a. Ribonucleotide reductase
b. Thymidylate synthase
c. CTP synthetase
d. Orotidylic acid decarboxylase

**32. d-UMP is converted to TMP by:**
a. Methylation b. Decarboxylation
c. Reduction d. Deamination

**33. UTP is converted to CTP by:**
a. Methylation b. Isomerization
c. Amination d. Reduction

**34. The enzyme aspartate transcarbamoylase of pyrimidine biosynthesis is inhibited by:**
a. ATP b. ADP
c. AMP d. CTP

**35. Gout is a metabolic disorder of catabolism of:**
a. Pyrimidine b. Purine
c. Alanine d. Phenylalanine

**36. Gout is characterized by increased plasma levels of:**
a. Urea
b. Uric acid
c. Creatine
d. Creatinine

**37. Lesch-Nyhan syndrome, the sex linked, recessive absence of HGPRTase, may lead to:**
a. Compulsive self-destructive behavior with elevated levels of urate in serum
b. Hypouricemia due to liver damage
c. Failure to thrive and megaloblastic anemia
d. Protein intolerance and hepatic encephalopathy

**38. The major catabolic product of pyrimidines in human is:**
a. β-alanine
b. Urea
c. Uric acid
d. Guanine

**39. Orotic aciduria type I reflects the deficiency of enzymes:**
a. Orotate phosphoribosyl transferase and orotidylate decarboxylase
b. Dihydroorotate dehydrogenase
c. Dihydroorotase
d. Carbamoyl phosphate synthetase

**40. An autosomal recessive disorder, xanthinuria is due to deficiency of the enzymes:**
a. Adenosine deaminase
b. Xanthine oxidase
c. HGPRTase
d. Transaminase

**41. Adenosine Deaminase (ADA) deficiency leads to:**
a. Severe combined immunodeficiency (SCID)
b. Orotic aciduria
c. Gout
d. Lesch-Nyhan syndrome

**42. The key substance in the synthesis of purine nucleotide phosphoribosyl pyrophosphate is formed by:**
a. α-D-ribose 5-phosphate
b. 5-phospho β-D-ribosylamine
c. D-ribose
d. Deoxyribose

## Answers for MCQs

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **1.** b | **2.** a | **3.** b | **4.** b | **5.** d | **6.** d | **7.** a | **8.** b |
| **9.** c | **10.** a | **11.** c | **12.** c | **13.** a | **14.** d | **15.** d | **16.** b |
| **17.** d | **18.** b | **19.** a | **20.** d | **21.** a | **22.** d | **23.** a | **24.** a |
| **25.** b | **26.** a | **27.** c | **28.** b | **29.** a | **30.** c | **31.** b | **32.** a |
| **33.** c | **34.** d | **35.** b | **36.** b | **37.** a | **38.** a | **39.** a | **40.** b |
| **41.** a | **42.** a | | | | | | |

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CHAPTER

# 20 Replication, Transcription and Translation

**Chapter outline**

- Replication (DNA Synthesis)
- Transcription (RNA Synthesis)
- Genetic Code
- Wobble Hypothesis for Codon-Anticodon Interactions
- Translation (Protein Biosynthesis)
- Folding and Processing (Post-translational Modification)
- Inhibitors of Protein Synthesis
- Protein Targeting and Degradation

## INTRODUCTION

The important role of DNA in transfer of information in living cells, formulated by Francis Crick, is called **the central dogma of molecular biology (Fig. 20.1)**, which defines three major steps in the processing of genetic information.

1. The first is **replication,** the copying of parent DNA to form daughter DNA molecules having nucleotide sequences identical to those of the parent DNA.
2. The second step is **transcription,** the process in which the genetic messages in DNA are rewritten in the form of ribonucleic acid (RNA).
3. The third step is **translation** in which the genetic message coded by RNA is translated by the ribosomes into the protein structure.

This flow of genetic information from **DNA** to **RNA** to **protein** is called **the central dogma** of molecular biology.

In this chapter, we shall study the successive steps in this sequence.

**Fig. 20.1:** The central dogma of molecular biology.

## REPLICATION (DNA SYNTHESIS)

DNA is a major store of genetic information. To transfer this genetic information from a parent cell to a daughter cell during cellular reproduction, the DNA must be duplicated. ***The duplication or synthesis of DNA is called replication.***

This method of DNA replication results in ***semi-conservative mechanism,*** in which each replicated duplex daughter DNA molecule contains one parent strand and one newly synthesized strand **(Fig. 20.2)**.

**Fig. 20.2:** Semiconservative DNA molecules.

**Table 20.1:** A comparison of prokaryotic and eukaryotic DNA polymerase.

| *Prokaryotic polymerase* | *Eukaryotic polymerase* | *Function* |
|---|---|---|
| I | α | Gap-filling and synthesis between Okazaki fragments of lagging strand |
| II | ε<br>β<br>γ | DNA proofreading and DNA repair<br>DNA repair<br>Mitochondrial DNA synthesis |
| III | δ | Leading and lagging strand synthesis |

## Prokaryotic Replication

Replication in prokaryotes is much better understood than replication in eukaryotes. The basic requirements and components of replication are the same for prokaryotes as for eukaryotes. Therefore, an understanding of how prokaryotes replicate provides the understanding of how eukaryotes replicate.

- The DNA synthesis is catalyzed by enzyme called ***DNA-dependent DNA polymerase.*** They are called ***DNA-dependent*** as they require DNA template. They are more commonly called ***DNA polymerases.*** These polymerases are required for:
  - DNA chain elongation
  - DNA repair (5'–3' exonuclease activity)
  - Proofreading (3'–5' exonuclease activity).
- There are three types of polymerases in prokaryotes:
  1. **DNA polymerase I (Pol I):** DNA polymerase I completes chain synthesis between Okazaki fragments on the lagging strand.
  2. **DNA polymerase II (Pol II):** Pol II is mostly concerned with proofreading and DNA repair.
  3. **DNA polymerase III (Pol III):** Pol III catalyzes leading and lagging strand synthesis.
- At least five DNA-polymerases exist in eukaryotic cells, α, β, γ, δ and ε **(Table 20.1).**

**Fig. 20.3:** Formation of replicating fork.

## Stages of Replication

The process of replication can be divided into three stages.

1. Initiation
2. Elongation
3. Termination.

### *Initiation*

- Initiation of DNA replication involves unwinding (separation) of two complementary DNA strands and formation of replicating fork.
- Unwinding occurs at a single, specific site at a particular DNA sequence on circular DNA of prokaryotes. The site is called the **origin of replication, 'Ori'** where active synthesis occurs. This region is called **replicating fork (Fig. 20.3).**
- Replication of double-stranded DNA is bidirectional.
- In eukaryotes, replication begins at multiple sites composed almost exclusively of A-T base pairs along the DNA helix and is referred to as a ***consensus sequence***.

#### *Steps Involved in Initiation*

1. First **DNA A protein** recognizes and binds to the **'ori'** of the DNA and successively denatures the DNA.
2. **DNA B protein (helicase)** then binds to this region and unwinds the parental DNA, and form a 'V' where active synthesis occurs. This region is called the **replicating fork (Fig. 20.4).**
3. The stress produced due to unwinding by helicase is released by **topoisomerases** by cutting either one or both DNA strands.
4. The **single-stranded binding (SSB)** protein stabilizes the separated strands and prevents their reassociation.

**Fig. 20.4:** Replicating fork.

5. To initiate the DNA synthesis by DNA polymerase III, it requires **RNA** primer. The **RNA primers** are short pieces of RNA (some 5–50 nucleotides in length) formed by the enzyme **primase** (RNA polymerase) using DNA as a template.

*Elongation*

- Once RNA primer has been synthesized at each of the replicating forks, a **DNA polymerase III** initiates the synthesis of new DNA strand by adding deoxyribonucleotide to the 3' end of the RNA primer. Thus, DNA polymerase III can synthesize a new chain only in the 5' to 3' direction. Both the DNA strands are synthesized simultaneously but in opposite direction, one is in direction towards the replication fork, the other in a direction away from the replication fork.
- The DNA chain which runs in the 3' → 5' direction is copied by polymerase III as a continuous strand, requiring one primer. This new strand is known as the ***leading strand.***
- The DNA chain which runs in the 5' → 3' direction is copied by polymerase III as a discontinuous manner because synthesis can only proceed in the 5' to 3' direction. This new strand is known as the ***lagging strand.*** This requires numerous RNA primers. As the replication fork moves, RNA primers are synthesized at specified intervals. These RNA primers are extended by DNA polymerase III into short pieces of DNA called **Okazaki fragments**.
- Upon completion of lagging strand synthesis, the RNA primers are removed from fragments by **DNA polymerase I.** DNA Polymerase I also fills the gaps that are produced by removal of the primer leaving only a nick. It cannot join two polynucleotide chains together; an additional enzyme **DNA ligase** is required to perform this function. This enzyme catalyzes the formation of a phosphodiester bond to seal the Okazaki fragments.

*Termination*

Termination sequences, e.g. ***'ter'***, direct termination of replication. A specific protein, ***ter binding protein,*** binds these sequences and prevents the helicase (DNA B protein) from further unwinding of DNA and facilitates the termination of replication.

### *Proofreading*

- DNA is copied by DNA polymerase with high fidelity (accuracy). Incorrect nucleotides are incorporated with a frequency of one in $10^8$–$10^{12}$ bases, which could lead to **mutation**. But the error ratio during replication is kept at a very low level by specific process. This process is known as ***proofreading***.
- Mismatches occur more frequently but do not lead to stable incorporations because all the three **DNA polymerases** have **3' to 5' exonuclease** activity (proofreading activity).
- DNA polymerase I and II are known to excise mismatched nucleotides before the introduction of the next nucleotide.

## Eukaryotic Replication

DNA replication in eukaryotic organisms resembles that in prokaryotic cells and proceeds by a mechanism similar to that of prokaryotic replication but is not identical. Some differences are given in **Table 20.2**.

**Inhibitors of DNA replication**

Some **antibacterial, antiviral** and many **chemotherapeutic** drugs inhibit replication.

- Many antibacterial drugs, like **Novobiocin**, **Nalidixic acid** and **Ciprofloxacin** inhibit the prokaryotic enzyme, **topoisomerase** (DNA gyrase).
- These topoisomerase inhibitors are widely used for the treatment of urinary tract and other infections.
- **Camptothecin,** an antitumor drug, inhibits human topoisomerase.
- Certain anticancer and antiviral drugs inhibit elongation of DNA chain by incorporating certain nucleotide analogs, e.g. **2, 3 deoxyinosine.**

**Table 20.2:** Some differences between prokaryotic and eukaryotic DNA replication.

| *Features* | *Prokaryotes* | *Eukaryotes* |
|---|---|---|
| RNA primer length | ~50 nucleotides | 9 nucleotides |
| DNA polymerase | Three types and designated by Roman numerals: I, II, III | Five types and designated by Greek numerals α, β, γ, δ and ε |
| Number of origins | Single | Multiple |
| Nucleotide length of Okazaki fragments of lagging strand | 1000–2000 nucleotides | ~200 nucleotides |
| Rate of replication | ~500 nucleotides per sec | 50 nucleotides per sec (10 times slower than prokaryotes) |

## TRANSCRIPTION (RNA SYNTHESIS)

Transcription is defined as the synthesis of RNA from DNA that results in the transfer of the information stored in double stranded DNA into a single stranded RNA, which is used to direct the synthesis of its proteins.

Transcription process is best understood in prokaryotes, the description of RNA synthesis in prokaryotes is applicable to eukaryotes even though the enzyme involved and regulatory signals are different.

### Basic Requirements for RNA Synthesis

#### *Template*

A single-strand of DNA acts as a template to direct the formation of complementary RNA transcript. The strand that is transcribed to RNA molecule is referred to as the **template strand** of the DNA. The other DNA strand is referred to as the **coding strand** of the gene.

#### *Substrate*

The substrates for RNA synthesis are the four ribonucleoside triphosphates:

1. ATP
2. GTP
3. CTP
4. UTP

#### *Enzyme*

***DNA-dependent RNA polymerase,*** called ***RNA polymerase (RNAP),*** is responsible for the synthesis of RNA, 5′ to 3′ direction, using DNA template. Prokaryotes have single RNA polymerase ***(RNAP)*** that transcribes all three RNAs, i.e. mRNA, rRNA and tRNA.

RNAP contains five subunits (2α, β′, β, ω) which form the core enzyme. The active enzyme, the holoenzyme contains core enzyme and a sixth subunit called **sigma (σ) factor (Fig. 20.5)**. The σ subunit binds transiently to the core enzyme and directs the enzyme to specific binding sites on the DNA.

**RNA polymerases lack a separate proofreading 3′ to 5′ exonuclease activity** as that of DNA polymerases and the error rates for transcription are higher than for chromosomal DNA replication. RNA polymerase requires $Mg^{2+}$ as well as $Zn^{2+}$ for its activity.

### Transcription in Prokaryotes

- DNA-dependent RNA polymerase, called **RNA polymerase (RNAP)** responsible for the synthesis of RNA using DNA template.
- Prokaryotes have single RNA polymerase that transcribes all three RNAs, i.e. mRNA, tRNA and rRNA.
- RNAP contains five subunits (2α, β′ β, ω) which form the core enzyme. The active enzyme, the holoenzyme contains core enzyme and a sixth subunit called **sigma (σ) factor (Fig. 20.5)**. The sigma subunit binds transiently to the core enzyme is required for binding of the RNA-polymerase to specific regions (promoter region) of DNA template.

**RNA polymerase lack a separate proofreading 3′ to 5′ exonuclease activity** as that of DNA polymerases and the error rates for transcription is higher than for chromosomal DNA replication. RNA polymerase requires $Mg^{2+}$ as well as $Zn^{2+}$ for its activity.

#### *Stages of Transcription*

RNA synthesis involves **(Fig. 20.6)**

1. Initiation
2. Elongation
3. Termination

##### *Initiation*

- Initiation of transcription involves the binding of RNA polymerase (core enzyme + σ factor) to the DNA template at the promoter site. The sigma factor enables the RNA polymerase (holoenzyme) to recognize and bind to promoter sequences. Promoters are characteristic sequences of DNA which are different in prokaryotes and eukaryotes **(Fig. 20.7)**.

**Fig. 20.5:** Prokaryotic RNA polymerase.

**Fig. 20.6:** Process of transcription in prokaryotes
**A:** Recognition of promoter by sigma factor
**B:** Binding of core enzyme and starts the synthesis of RNA
**C:** Elongation continues until termination region is reached
**D:** Termination of transcription by Rho factor
**E:** Newly synthesized RNA (primary transcript)

- Promoter sequences are responsible for directing RNA polymerase to initiate transcription at a particular point known as ***start point*** or ***initiation site***.
- Unlike the initiation of replication, transcriptional initiation does not require a primer.

**Prokaryotic promoters**

Prokaryotic genes have two promoter sequences **(Fig. 20.7)**.

1. **Pribnow box** (–10 region) has the nucleotide sequence TATAAT and is usually found 10 base pairs away from (upstream) the start point.
2. **The –35 region** has the nucleotide sequence TTGACA. It is named –35 sequence because it is found 35 base pairs away from (upstream) the start point.

### *Elongation*

- Once the promoter region has been recognized and bound by the RNA polymerase holoenzyme, local unwinding of the DNA helix continues.
- RNA polymerase then begins to synthesize a transcript of DNA sequence and short piece of RNA is made. As with replication, transcription is always in the 5′ to 3′ direction. The first base is usually a purine nucleotide.
- By the time 10 nucleotides have been added, the σ factor dissociates. The elongation phase is said to begin when the transcript exceeds ten nucleotides in length.
- Sigma is then released and the RNAP (core enzyme) is able to move along the template stand and continues the elongation of the transcript. The process of elongation of the RNA chain continues until a termination signal is reached.

**Fig. 20.7:** Promoter sites for transcription in prokaryotes and eukaryotes.

### *Termination*

In prokaryotes, termination of transcription occurs by one of the two well-characterized mechanisms:

1. Rho-dependent
2. Rho-independent.

***Rho-dependent termination***

Rho-dependent termination requires a protein factor called **rho (ρ)** which recognizes the termination signal and has an ATP-dependent helicase activity that displaces the RNA polymerase from template, resulting in termination of RNA synthesis.

***Rho-independent termination***

Rho-independent termination brought about by the formation of a secondary structure (hair-pin loop) in the newly synthesized RNA **(Fig. 20.8)**, which removes the RNA polymerase from DNA template, resulting in the release of the transcript.

This hairpin loop structure is followed by a sequence of four or more uracil residues, which also are essential for termination. The RNA transcript ends within or just after then.

In eukaryotic cells, termination is less well-defined. It is believed that it is similar to that described for rho-independent prokaryotic termination.

## Transcription in Eukaryotes

The description of RNA synthesis in prokaryotes is applicable to eukaryotes even though the enzyme involved and regulatory signals are different.

### *Eukaryotic RNA Polymerase*

In contrast to prokaryotes eukaryotic cells have three RNA polymerases I, II and III found in nucleus. Each of these RNA polymerase is responsible for the transcription of different sets of genes.

1. **RNA Polymerase I:** It catalyzes the synthesis of ribosomal RNA.

**Fig. 20.8:** A hair-pin loop structure of newly synthesized RNA.

2. **RNA Polymerase II:** It catalyzes the synthesis of m-RNA and small nuclear RNAs (sn-RNA).
3. **RNA Polymerase III:** It catalyzes the synthesis of tRNA.

Besides the three nuclear RNA polymerases, in eukaryotic cell, a fourth type of RNA polymerase is found in mitochondrial matrix known as **mitochondrial RNA polymerase (mtRNAP).** Similar to prokaryotic RNA polymerase, mtRNA polymerase catalyzes the synthesis of all the three types of RNA, i.e. mRNA, tRNA and rRNA.

### *Eukaryotic Promoter Sites (Fig. 20.7)*

Each type of eukaryotic RNA polymerase uses different promoters. These are:

1. **Hogness box or TATA box:** It is a stretch of six nucleotides and located 25 nucleotides upstream of the transcription-starting point.
2. **CAAT box:** It is stretch of eight nucleotides and located about 75 nucleotides upstream of the transcription starting point.
3. **GC box:** It is a stretch of six nucleotides and is located about 90 nucleotides upstream of the transcription starting point.

## Post-transcriptional Processing

- The RNAs formed during transcription are called **primary transcript**. The primary transcript normally undergoes further enzymatic alteration, called **post-transcriptional processing**. Post-transcriptional processing is required to convert the primary RNAs into functional or active forms. Processing may involve either of these:
  - Cleavage of large precursor of RNA to a smaller molecule by the action of endonuclease or exonuclease.
  - Splicing
  - Terminal addition of nucleotide
  - Nucleoside modifications.
- Post-transcriptional processing is more extensive in eukaryotes than in prokaryotes.

### *m-RNA processing*

- In prokaryotes, mRNA is not post-transcriptionally processed. Prokaryotic mRNA is functional immediately upon synthesis.
- In eukaryotes, the primary transcript of mRNA is the hnRNA (heterogenous nuclear RNA). After transcription, hnRNA is extensively modified to form functional mRNA. These modifications are as follows:

1. **The 5'-capping:** The 5' end of eukaryotic mRNA is capped with **7-methylguanylate**. This cap functions in protein biosynthesis and to stabilize the structure of mRNA.
2. **Addition of poly A tail:** The 3' end of most eukaryotic mRNAs possess a chain of 200–300 adenine nucleotides and called **Poly A tail.** Poly A tail is not transcribed by DNA but rather is added after transcription. Poly A tail may enhance translation efficiency and involved in stabilization of mRNA.
3. **Removal of introns:** Introns are the nucleotide sequences on mRNA that do not code for proteins. These are the intervening sequences between exons. Exons are the coding sequences that code for proteins. The process by which introns are excised and exons are linked to form functional mRNA is called **splicing.**

### *t-RNA Processing*

t-RNA precursors are converted to mature tRNAs by following alterations:

1. Cleavage of a 5' leader sequence.
2. Splicing to remove introns.
3. Replacement of the 3' terminal UU by CCA.
4. Modification of several bases, e.g. Uridylate is modified after transcription to form ribothymidylate and pseudouridylate.

### *r-RNA Processing*

The rRNA precursors called **preribosomal** RNAs are cleaved and trimmed to produce the mature functional r-RNAs.

**Inhibitors of Transcription**

A number of compounds inhibit RNA synthesis. Those that inhibit RNA synthesis in prokaryotes but not in eukaryotes serve as therapeutic drugs, antibiotics. For example:

- **Rifampin:** It is an antituberculosis drug, which inhibits the initiation of transcription by binding β-subunit of prokaryotic RNA polymerase. Rifampin has no effect on eukaryotic nuclear RNA polymerases.
- **Dactinomycin (actinomycin D):** Dactinomycin is a therapeutic agent in the treatment of some cancer. It binds tightly and specifically to double helical DNA and thereby prevents the movement of the RNA polymerase.

**Amphibolic Nature of Citric Acid Cycle**

Viral DNA polymerase is called ***reverse transcriptase*** because it uses RNA as a template to synthesize DNA. It is therefore, an ***RNA-dependent DNA polymerase***.

Some viruses, referred to as ***'retroviruses'***, carry RNA as their genetic material and can synthesize double-stranded DNA from their genomic RNA by a process known as **reverse transcription**. An example of retrovirus is the ***human immunodeficiency virus (HIV)*** which causes ***AIDS***.

## GENETIC CODE

The information needed to direct the synthesis of protein is contained in the mRNA in the form of a **genetic code**. Codons are a group of three adjacent bases that specify the amino acids of protein.

**The genetic code is the system of nucleotide sequences of mRNA that determines the sequence of amino acids in protein.**

### Characteristics of Genetic Code

- **Number of codons:** There are 64 possible codon sequences. Because four nucleotide bases A, G, C and U are used to produce the three base codons, there are, therefore, the 43 or 64 possible codon sequences **(Fig. 20.9)**.
- **Stop or termination or nonsense codons:** Three of the 64 possible nucleotide triplets, UAA, UAG and UGA do not code for any amino acids. They are called **nonsense codons** that normally signal termination of polypeptide chains. These nonsense codons are arbitrarily named **amber**, **ochre** and **opal**.
- **The code is degenerate but unambiguous:** As there are 61 codons for 20 amino acids, one amino acid has more than one codon and the code is referred to as degenerate, indicating that there are redundancies. Although an amino acid may have more than one codon, each codon specifies only one amino acid. Thus, the genetic code is unambiguous. Degeneracy minimizes the deleterious effects of mutations.
- Codons that designate the same amino acid are called **synonyms**. Two amino acids **methionine (AUG)** and **tryptophan (UGC)** each have only one codon. The remaining amino acids have multiple codons, e.g. arginine is specified by six different codons **(Fig. 20.9)**.
- **The code is almost universal:** That is, the meaning of each codon is the same in almost all known organisms. Exceptions to the universality of the genetic code are found in human mitochondria, where the code:
  - UGA codes for tryptophan instead of serving as a stop codon

| First letter | Second letter: U | C | A | G | Third letter |
|---|---|---|---|---|---|
| U | UUU, UUC – Phe<br>UUA, UUG – Leu | UCU, UCC, UCA, UCG – Ser | UAU, UAC – Tyr<br>UAA*<br>UAC* | UGU, UGC – Cys<br>UGA*<br>UGG – Trp | U<br>C<br>A<br>G |
| C | CUU, CUC, CUA, CUG – Leu | CCU, CCC, CCA, CCG – Pro | CAU, CAC – His<br>CAA, CAG – Gln | CGU, CGC, CGA, CGG – Arg | U<br>C<br>A<br>G |
| A | AUU, AUC, AUA – Ile<br>AUG – Met | ACU, ACC, ACA, ACG – Thr | AAU, AAC – Asn<br>AAA, AAG – Lys | AGU, AGC – Ser<br>AGA, AGG – Arg | U<br>C<br>A<br>G |
| G | GUU, GUC, GUA, GUG – Val | GCU, GCC, GCA, GCG – Ala | GAU, GAC – Asp<br>GAA, GAG – Glu | GGU, GGC, GGA, GGG – Gly | U<br>C<br>A<br>G |

* Termination code

**Fig. 20.9:** The genetic code.

– AUA codes for methionine instead of isoleucine
– CUA codes for threonine instead of leucine.

- **The code is nonoverlapping and without punctuation:** During translation, the code is read sequentially, without spacer bases, from a fixed starting point, as a continuous sequence of bases, taken 3 at a time, e.g. **A U G C U A G A C U U U** is read as: **AUG/CUA/GAC/UUU** without punctuation between the codons.

## WOBBLE HYPOTHESIS FOR CODON-ANTICODON INTERACTIONS

- When several different codons specify one amino acid, the difference between them usually lies at the third base position (at the 3′ end), e.g. alanine is coded by the triplets GCU, GCC, GCA and GCG. For most amino acids, the first two bases of each codon are the primary determinants of specificity.
- The tRNA base pair with mRNA codons at a three base sequence on the tRNA called the **anticodon**. The first base of the codon in mRNA (read in the 5′ to 3′ direction) pairs with the third base of the anticodon **(Fig. 20.10)**. If the anticodon triplet of a tRNA recognized only one codon triplet through Watson Crick base pairing at all three positions, cells would have a different tRNA for each amino acid codon. This is not the case, however because the anticodons in some tRNAs include the nucleotide inosinate (designated 'I') which contains the uncommon base hypoxanthine.
- Inosine can form hydrogen bonds with three different nucleotides, U, C, and A although these pairings are much weaker than the hydrogen bonds of Watson Crick base pairs G ≡ C and A = U. Crick proposed a hypothesis to explain these data on the basis of set of four relationships called the **wobble hypothesis**.
  1. The first two bases of an mRNA codon always form strong Watson-Crick base pairs with the corresponding bases of the tRNA anticodon and confer most of the coding specificity.
  2. The first base of the anticodon (reading in the 5' to 3' direction; this pairs with the third base of the codon) determines the number of codons recognized by the tRNA.
     – When the first base of the anticodon is C or A, base pairing is specific and only one codon is recognized by the tRNA.

**Fig. 20.10:** Pairing relationship of codon and anticodon.

- When the first base is U or G, binding is less specific and two different codons may be read.
- When inosine (I) is the first (wobble) nucleotide of an anticodon, three different codons can be recognized.

3. When an amino acid is specified by several different codons, the codons that differ in either of the first two bases require different tRNAs.
4. A minimum of 32 tRNAs are required to translate all 61 codons (31 to encode the amino acids and 1 for initiation).

## TRANSLATION (PROTEIN BIOSYNTHESIS)

The pathway of protein Biosynthesis is called ***translation***.

- Translation is the process by which ribosomes convert the information carried by mRNA in the form of genetic code to the synthesis of new protein.
- Translation occurs in cytosol on ribosomes and is guided by mRNA.

The basic plan of protein synthesis in eukaryotes is similar to that in prokaryotes. However, eukaryotic protein synthesis involves more protein components and some steps are more intricate.

Eukaryotic mRNA is referred to as ***monocistronic***, meaning that there is only one coding sequence on each mRNA molecule producing only one polypeptide chain. Prokaryotic mRNA is ***polycistronic***, often encoding more than one polypeptide on the same mRNA. Some differences are listed in **Table 20.3.**

### Basic Requirements for the Translation

- mRNA
- tRNAs
- Ribosomes
- Energy in the form of ATP and GTP
- Enzymes and specific protein factors, e.g. initiation factors, elongation factors, etc.

### Stages of Eukaryotic Translation

There are four major stages in protein synthesis, each requiring a number of components:

1. Activation of amino acids
2. Initiation
3. Elongation
4. Termination.

#### *Activation of Amino Acid*

Activation of amino acid takes place in the cytosol. In activation of amino acid each of the 20 amino acids, is covalently attached to their respective tRNA, at the expense of ATP, by **aminoacyl tRNA synthases (AAS)** enzyme **(Fig. 20.11)**.

**Table 20.3:** Differences between eukaryotic and prokaryotic protein synthesis.

| *Characters* | *Eukaryotes* | *Prokaryotes* |
|---|---|---|
| Ribosome | Larger, 80S, consists of 60S and 40S subunits | Smaller, 70S, consists of 50S and 30S subunits |
| Initiator tRNA | Met-tRNA$^{Met}$ | f-met-tRNA$^{f\ met}$ |
| Start signal | Initiating AUG of mRNA is preceded by a cap, methylguanosyl triphosphate | Initiating AUG of mRNA is preceded by a purine rich sequence |
| Initiation factors | Contains more initiation factors than do prokaryotes Nine are known and several consists of multiple subunits The prefix eIF denotes an eukaryotic initiation factor | Contains three initiation factors, $IF_1$, $IF_2$ and $IF_3$ |
| Termination factors | Termination is carried out by a single release factor eRF | Termination is carried by three releasing factors $RF_1$, $RF_2$ and $RF_3$ |
| mRNA | Monocistronic | Polycistronic |

**Fig. 20.11:** Activation of amino acid. (AAS: Aminoacyl tRNA synthase)

**Fig. 20.12:** Eukaryotic 80S ribosome.

When an amino acid is attached to the tRNA to form aminoacyl tRNA, the latter can recognize a codon on mRNA by virtue of an anticodon in the tRNA structure.

## *Initiation*

Initiation can be divided into four steps:

1. Ribosomal dissociation
2. Formation of 43S pre-initiation complex.
3. Formation of 48S initiation complex
4. Formation of 80S initiation complex.

### *Ribosomal dissociation*

Eukaryotic ribosomes are 80S particles composed of two subunits of 40S and 60S **(Fig. 20.12)**. (S = sedimentation coefficient depends on the size and shape of a particle as well as molecular mass, so that the values given for the two subunits cannot simply be added up to give the value for the whole ribosome). Ribosomes have three sites that bind tRNAs:

1. Aminoacyl site (A site)
2. Peptidyl (P) site
3. Exit (E) site.

The A and P sites bind to aminoacyl–tRNAs, whereas E site binds only to uncharged tRNAs that have completed their task on the ribosome. 40S subunit of ribosome binds two initiation factors elF3 and elF-1A and dissociates 80S into two subunits 40S and 60S **(Fig. 20.13)**.

### *Formation of 43S pre-initiation complex*

- First it involves the binding of GTP with initiation factors elF2. This complex then binds to Met-tRNA$^{Met}$ (a tRNA specifically involved in binding to the initiation codon AUG on mRNA).

> - In prokaryotes, the first amino acid methionine is modified by formylation of its amino group to N-formyl-methionine (f Met) and initiator tRNA is fmet-tRNA$^{fMet}$.

- This ternary (GTP-elF2-tRNA) complex binds to the 40S ribosomal subunit to form 43S pre-initiation complex, which is stabilized by association with elF3 and elF-1A **(Fig. 20.13)**.

### *Formation of 48S initiation complex*

- Binding of mRNA to the 43S pre-initiation complex forms 48S initiation complex.
- The 5' terminals of most mRNA molecules in eukaryotic cells are capped by methylguanosyl triphosphate, which facilitates the binding of mRNA to the 43S pre-initiation complex to form 48S initiation complex.
- The association of mRNA with 43S initiation complex requires cap binding protein (CBP) or elF4 and ATP **(Fig. 20.13)**.

> In prokaryotes, a sequence of nucleotide bases on mRNA known as ***Shine-Dalgarno sequence*** facilitates the binding of mRNA to the preinitiation complex.

### *Formation of 80S initiation complex*

- Combination of the 48S initiation complex with 60S ribosomal subunit forms 80S initiation complex.
- The binding of the 60S ribosomal subunit to the 48S initiation complex involves the hydrolysis of the GTP bound to elF2, by elF5 with the release of the initiation factors bound to the 48S initiation complex. These factors are then recycled.
- At this stage, the Met-tRNA$^{Met}$, is on the P site of the ribosome so that its anticodon pairs with the initiating AUG codon on the mRNA and is now ready for the elongation process **(Fig. 20.13)**.

**Fig. 20.13:** Initiation of protein synthesis: *Abbreviation:* CBP = Cap-binding protein (eIF4)

Ribosomal complex with elongated polypeptide and appearance of termination codon of mRNA in A site

**Fig. 20.14:** Elongation of protein biosynthesis.

### Elongation

Elongation can be divided into four steps:

1. Binding of aminoacyl tRNA specified by the next codon in the mRNA to the A site
2. Formation of peptide bond between amino acids present in P and A site of ribosome
3. Translocation of the ribosome along the mRNA
4. Removal of the deacylated tRNA from the P and E sites.

#### *Binding of aminoacyl tRNA to the A site*

- The next aminoacyl tRNA specified by the next codon in the mRNA is first bound to a complex of elongation factor eEf-1A-containing a molecule of bound GTP
- The resulting aminoacyl tRNA-eEF-1A-GTP complex then allows aminoacyl tRNA to enter the A site on the ribosome with the release of eEF-1A-GTP and phosphate **(Fig. 20.14)**.

#### *Formation of peptide bond*

- In the second step of the elongation cycle, a new peptide bond is formed between the amino acids whose tRNAs are located on the A and P sites of the ribosome.
- This step occurs by the transfer of the initiating methionine from its tRNA to the amino group of the new amino acid that has just entered the A site.
- This step is catalyzed by peptidyl transferase (ribozyme).
- As a result of this reaction, a dipeptide tRNA is formed on the A site and the 'empty' initiating $tRNA^{Met}$ remains bound to P site **(Fig. 20.14)**.

#### *Translocation*

- In the third step of the elongation cycle, the ribosome moves along the mRNA toward its 3′ end. The deacylated tRNA is attached by its anticodon to the P site at one end and by the free CCA tail to an exit (E) site of large ribosomal subunit.
- The elongation factor EF2 binds and displaces the peptidyl tRNA form the A site to the P site. In turn, the deacylated tRNA is in E site from which it leaves the ribosome.
- The hydrolysis of GTP to GDP provides energy to move mRNA forward toward its 3′ end, by a distance of one codon (three bases) and leaving the A site open for occupancy by another aminoacyl tRNA–EF-IA-GTP complex and another cycle of elongation which proceeds in precisely the same way. Thus, the process of peptide synthesis occurs until a termination codon is reached **(Fig. 20.14)**.

### Termination

- The elongation steps are repeated until one of the three (UAA, UAG, UGA) termination or nonsense codon of mRNA appears in the A site.
- Once the ribosome reaches a termination codon, releasing factors are capable of recognizing the termination signal present in the A site.
- Prokaryotes have three release factors, RF-1, RF-2, and RF-3 but eukaryotes have only single release factor, eRF.
- Releasing factors in conjunction with GTP and peptidyl transferase hydrolyze the peptidyl tRNA bond (the bond between the peptide and the tRNA), when a nonsense codon occupies the A site.
- This hydrolysis releases the polypeptide and tRNA from P site.
- Upon hydrolysis and release of polypeptide, the mRNA is then released from the ribosome.
- Ribosome then dissociates into 60S and 40S subunits, which are then recycled **(Fig. 20.15)**.

  An overview of the five stages of protein synthesis is given in **Figure 20.16**.

## FOLDING AND PROCESSING (POST-TRANSLATIONAL MODIFICATION)

In order to achieve native biologically active form of the polypeptide, it must undergo folding into its proper three-dimensional conformation. The **chaperones** (a group of specialized proteins) ensure the folding of a protein into

**Fig. 20.15:** Termination of protein synthesis.

**Fig. 20.16:** An overview of the five stages of protein synthesis.

**Stage 1: Activation of amino acids:** Amino acids are activated by attaching the amino acid to a tRNA in the first stage of protein synthesis. Each of the 20 amino acids is covalently attached to a specific tRNA at the expense of ATP energy using $Mg^{2+}$ dependent, aminoacyl tRNA synthetases.

**State 2: Initiation:** The mRNA containing codons for the protein to be synthesized binds to the smaller ribosomal subunits and to the initiating aminoacyl tRNA. The large ribosomal subunit then binds to form an initiation complex. The initiating aminoacyl tRNA base pairs with the mRNA codon AUG and signals the beginning of the polypeptide synthesis. This process which requires GTP is promoted by cytosolic proteins called **initiation factors**.

**Stage 3: Elongation:** The nascent polypeptide is lengthened by covalent (peptide bond) attachment of successive amino acid units, each carried to the ribosome and correctly positioned by its tRNA, which base pairs to its corresponding codon in the mRNA. Elongation requires elongation factors. The biding of each incoming aminoacyl tRNA and the movement of the ribosome along the mRNA are facilitated by GTP as each residue is added to the growing polypeptide.

**Stage 4: Termination and ribosome recycling:** Completion of the polypeptide chain is signaled by a termination codon in the mRNA. The new polypeptide is released from the ribosome aided by release factors, and the ribosome is recycled for another round of synthesis.

**Stage 5: Folding and post-translational processing:** In order to achieve its biologically active form, the new polypeptide must fold into its proper three-dimensional conformations. Before or after folding, the new polypeptide may undergo enzymatic processing, including removal of one or more amino acids (usually form the amino terminus), addition of acetyl, phosphoryl, methyl, carboxyl, or other groups to certain amino acid residues, proteolytic cleavage; and/or attachment of oligosaccharides or prosthetic groups.

its native form. Before or after folding, the polypeptide may undergo processing by enzymatic action. Collectively these alterations are known as ***post-translational modifications.*** These modifications may include removal of part of the translated sequence or the covalent addition of one or more chemical groups required for protein activity. Some types of post-translational modifications are listed below.

### Amino Terminal Modifications

- Initially, all polypeptides begin with a residue of N-formyl methionine (in prokaryotes) or methionine (in eukaryotes).
- The formyl group and the amino terminal methionine residue are removed enzymatically.

### Loss of Signal Sequence

- 15–30 residues of amino terminal end of some proteins required to direct the proteins to its ultimate destination in the cell. Such signal sequences are ultimately removed by specific peptidase.

### Covalent Modification of Proteins

- **Glycosylation: Glycoproteins** and **proteoglycans** are attached covalently during or after the synthesis of polypeptide chain.
- **Phosphorylation:** Covalent addition of phosphate group to hydroxyl group of serine, threonine and tyrosine.
- **Carboxylation:** It involves carboxylation of amino acids like glutamic acid residues of prothrombin and other blood-clotting factors.
- **Hydroxylation:** During formation of collagen, the proline and lysine residues are hydroxylated to hydroxyproline and hydroxylysine respectively.
- **Methylation:** In some proteins lysine residues are methylated enzymatically, e.g. methylated lysine residues present in muscle proteins and cytochrome C.
- **Addition of prosthetic group:** It includes covalent attachment of non-protein substance to protein, e.g. biotin is covalently bound to acetyl CoA carboxylase and heme group of cytochrome.

### Proteolytic Processing

Insulin and proteases such as trypsin and chymotrypsin are initially synthesized as larger, inactive precursor proteins. These precursors are proteolytically trimmed to produce their final, active forms.

## INHIBITORS OF PROTEIN SYNTHESIS

A number of commonly used variety of antibiotics act by inhibiting selectively the process of prokaryotic protein biosynthesis. The most useful antibiotics do not interact with eukaryotic protein synthesis and thus are not toxic to eukaryotes. Such antibiotics can be used as therapeutic drugs **(Table 20.4).** Puromycin and cycloheximide are not clinically useful because they inhibit protein biosynthesis in eukaryotes also but are used for research purpose.

## PROTEIN TARGETING AND DEGRADATION

The eukaryotic cell is made up of many compartments and organelles each with specific functions that require distinct sets of proteins and enzymes. These proteins are synthesized on ribosomes in the cytosol. From cytosol, they are directed to their final cellular destinations. The pathways by which proteins are sorted and transported to their proper cellular location are referred to as **protein-targeting pathways**.

Protein targeting system is depending on the short sequence of amino acids called **signal sequence** at amino terminus of a newly synthesized polypeptide chain. The signal sequence directs a protein to its appropriate location in the cell and is removed during transport or when the protein reaches its final destination. Different targeting mechanisms are discussed here.

**Table 20.4:** Inhibitors of protein synthesis.

| *Antibiotic* | *Action* |
|---|---|
| Streptomycin | It binds to the 30S subunit of prokaryotes at the A site, thereby inhibiting chain elongation by preventing the binding of additional aminoacyl tRNA |
| Tetracycline | Binds to the 30S subunit and inhibits binding of aminoacyl tRNA to mRNA in prokaryotes |
| Chloramphenicol | Binds to the 50S ribosomal subunit and blocks the peptidyl transferase reaction in prokaryotes |
| Erythromycin | Binds to the 50S ribosomal subunit that inhibits the translocation reaction in prokaryotes |
| Lincomycin and Clindamycin | Binds to the 50S subunit and inhibits peptidyl transferase, thereby preventing peptide bond Formation in prokaryotes |
| Puromycin | Causes premature chain termination in both prokaryotes and eukaryotes |

## Secretory Membrane Protein Targeting

Most lysosomal, membrane and secreted proteins have an amino terminal signal sequence that marks them translocation into the lumen of the ER. The carboxyl terminus of the signal sequence is defined by a cleavage site, where protease action removes the sequence after the protein is imported into the ER. Signal sequences vary in length from 13 to 36 amino acid residues. The signal sequence itself helps to direct the ribosome to the ER as given here:

The targeting pathway begins with initiation of protein synthesis on ribosomes.

- The signal sequence appears early in the synthesis process, because it is at the amino terminus, which, as we have seen, is synthesized first.
- The signal sequence and the ribosome itself are bound by the large **signal recognition particle (SRP)**. SRP then binds GTP and halts elongation of the polypeptide when it is 70 amino acids long and the signal sequence has completely emerged from the ribosome.
- The GTP bound SRP now directs ribosome and the incomplete polypeptide to SRP receptors of the ER. The nascent polypeptide is then delivered to a **peptide translocation complex** in the ER, which may interact directly with the ribosome.
- SRP dissociates from the ribosomes, accompanied by hydrolysis of GTP in both SRP and the SRP receptor.
- Elongation of the polypeptide now resumes, into the ER lumen until the complete protein has been synthesized.
- The signal sequence is removed by a signal peptidase within the ER lumens; the ribosome dissociates and recycled.

In the ER lumen, newly synthesized proteins are further modified in several ways. Following the removal of signal sequences, polypeptides are folded, disulfide bonds formed and many proteins glycosylated to form glycoproteins. Suitable modified proteins can now be moved to a variety of intracellular destinations. Proteins travel from ER to the Golgi complex in transport vesicles. Golgi complex also sorts proteins and sends them to their final destinations (lysosomes, the plasma membrane or secretory vesicles).

## Nuclear and Mitochondrial Protein Targeting

Like membrane and secretory protein, both mitochondrial and nuclear proteins have signal sequences that mark them to be transported from the ER to their respective organelles. In contrast to secretory and membrane proteins, mitochondrial and nuclear proteins are transported after their translation is complete.

In the case of proteins destined for mitochondria may have two signal sequences on their N-terminal ends, depending on whether they are destined for the matrix or for the intramembrane space? Mitochondrial proteins must be unfolded before their transport.

A variety of nuclear proteins like RNA and DNA polymerases, histones, topoisomerases that regulate gene expressions are synthesized in the cytosol and imported into the nucleus. The proteins targeted to the nucleus have an internal signal sequence called **Nuclear-localization sequence (NLS)**. Unlike other signal sequences it is not cleaved after the protein arrives at its destination. An NLS unlike other signal sequences are located almost anywhere the length of the proteins and mark them to pass through nuclear pores. NLSs can vary considerably, but many consist of four to eight amino acid residues and include several consecutive basic (Arg or Lys) residues. Some eukaryotic cells import proteins by receptor-mediated endocytosis, e.g. LDL, transferring, peptide hormones, etc.

## Protein Degradation/Protein Turnover

Protein degradation is mediated by specialized systems in all cells. Protein degradation prevents the buildup of abnormal or unwanted proteins and permits the recycling of amino acids.

- Defective proteins and those with characteristically short half-lives are generally degraded in both bacterial and eukaryotic cells by ATP dependent pathway. The ATP dependent pathway in eukaryotic cells involves the protein **ubiquitin**, which as its name suggests, occurs throughout the eukaryotic kingdoms. Ubiquitin is covalently linked to proteins scheduled for destruction via ATP-dependent pathway involving three separate types of enzymes, called:
  E1—activating enzymes
  E2—conjugating enzymes
  E3—ligases

  Different $E_1$, $E_2$ and $E_3$ enzymes exhibit different specificities for target proteins.
- Ubiquitinated proteins are degraded by a large complex known as the **26S proteasome**. Ubiquitin-dependent proteolysis is important for the regulation of the elimination of defective proteins. Many proteins required at only one stage of eukaryotic cell cycle are rapidly degraded by the ubiquitin-dependent pathway after completing their function.

## EXAM QUESTIONS

### Long Answer Questions (LAQs)

1. Describe various steps of replication (DNA synthesis) and discuss its proofreading.
2. Describe various steps of transcription.
3. Describe various types of post-transcriptional processing.
4. Describe various steps of protein biosynthesis.

### Short Notes

1. Replication of DNA.
2. Okazaki fragments.
3. Inhibitors of DNA synthesis.
4. Reverse transcription.
5. Different types of prokaryotic DNA polymerase.
6. Enzymes or proteins involved in replication.
7. Differences between prokaryotic and eukaryotic replication.
8. What is central dogma of molecular biology?
9. Draw and label diagram of DNA replication.
10. Inhibitors of RNA synthesis in prokaryotes.
11. Inhibitors of protein biosynthesis.
12. Genetic code.
13. Protein targeting and degradation.
14. Wobble hypothesis for codon-anticodon interactions.

### Multiple Choice Questions (MCQs)

1. **Formation of Okazaki fragments occur in the process of:**
   a. Transcription b. Translation
   c. Replication d. Reverse transcription
2. **Which of the following enzymes joins Okazaki fragments?**
   a. DNA polymerase b. DNA ligase
   c. RNA polymerase d. Peptidyl transferase
3. **Which the following enzymes fills the gap between Okazaki fragments?**
   a. DNA polymerase b. RNA polymerase
   c. Translocase d. Helicase
4. **Which of the following eukaryotic DNA polymerases is required for mitochondrial DNA synthesis?**
   a. DNA polymerase α
   b. DNA polymerase β
   c. DNA polymerase γ
   d. DNA polymerase δ
5. **Which of the following inhibits eukaryotic protein synthesis?**
   a. Tetracycline b. Erythromycin
   c. Streptomycin d. Puromycin
6. **All of the following enzymes are required for the synthesis of DNA except:**
   a. DNA-dependent DNA polymerase
   b. Helicase
   c. DNA ligase
   d. DNA-dependent RNA polymerase
7. **Primer (a short piece of RNA) is required for:**
   a. DNA synthesis b. M-RNA synthesis
   c. t-RNA synthesis d. r-RNA synthesis
8. **Synthesis of DNA is also known as:**
   a. Duplication b. Replication
   c. Transcription d. Translation
9. **Replication of DNA is:**
   a. Conservative b. Semiconservative
   c. Nonconservative d. None of these
10. **Direction of new DNA strand synthesis is:**
    a. $5' \rightarrow 3'$ b. $3' \rightarrow 5'$
    c. Both (a) and (b) d. None of these
11. **Formation of RNA primer:**
    a. Leads replication b. Leads translation
    c. Leads transcription d. All of the above
12. **In replication, after formation of replication fork:**
    a. Both the new strands are synthesized discontinuously
    b. One strand is synthesized continuously and the other discontinuously
    c. Both the new strands are synthesized continuously
    d. RNA primer is required only for the synthesis of one new strand
13. **An Okazaki fragment contains about:**
    a. 10 Nucleotides
    b. 100 Nucleotides
    c. 1,000 Nucleotides
    d. 10,000 Nucleotides
14. **In replication, RNA primer is formed by the enzyme:**
    a. Ribonuclease b. Primase
    c. DNA polymerase I d. DNA polymerase III

**15. In eukaryotes, during replication, the template DNA is unwound:**
a. At one of the ends b. At both the ends
c. At multiple sites d. Nowhere

**16. During replication, unwinding of double helix is initiated by:**
a. DNA A protein
b. DNA B protein
c. Topoisomerase
d. Single strand binding protein

**17. In replication, the unwound strands of DNA are held apart by:**
a. DNA A protein
b. DNA B protein
c. Single strand binding protein
d. Topoisomerase

**18. In replication, deoxyribonucleotides are added to RNA primer by:**
a. DNA polymerase I
b. DNA polymerase II
c. DNA polymerase III
d. All of these

**19. In replication, ribonucleotides of RNA primer are replaced by deoxyribonucleotides by the enzyme:**
a. DNA polymerase I b. DNA polymerase II
c. DNA polymerase III d. All of these

**20. In replication, Okazaki fragments are sealed by:**
a. DNA polymerase II
b. DNA ligase
c. DNA gyrase
d. DNA topoisomerase II

**21. DNA polymerase III holoenzyme possesses:**
a. Polymerase activity
b. 3′ → 5′ Exonuclease activity
c. 5′ → 3′ Exonuclease and polymerase activities
d. 3′ → 5′ Exonuclease and polymerase activities

**22. Reverse transcriptase catalyses:**
a. Synthesis of RNA from DNA
b. Breakdown of RNA
c. Synthesis of DNA from RNA
d. Breakdown of DNA

**23. Consensus sequence is:**
a. Initiation site of replication in eukaryotes
b. Initiation site of replication in prokaryotes
c. Initiation site of transcription in eukaryotes
d. Initiation site of transcription in prokaryotes

**24. Eukaryotic DNA polymerase γ is located in:**
a. Nucleus b. Nucleolus
c. Mitochondria d. Cytosol

**25. The mammalian DNA polymerase involved in error correction is:**
a. DNA polymerase α
b. DNA polymerase β
c. DNA polymerase γ
d. DNA polymerase δ

**26. A promoter site on DNA:**
a. Transcribes repressor
b. Codes for RNA polymerase
c. Initiates transcription
d. Regulates termination

**27. Which of the following is true for the process of RNA synthesis?**
a. Occurs in the 3′ to 5′ direction
b. Requires primer
c. Does not require primer
d. Is initiated at special DNA sequences known as start condons

**28. What sequence feature of mature mRNAs listed below is thought to protect mRNAs from degradation?**
a. Special post-translational modifications
b. 3′ poly (A)n tail
c. 5′ methyl guanosine triphosphate cap
d. Introns

**29. Synthesis of RNA from DNA template is known as:**
a. Replication
b. Translation
c. Transcription
d. Mutation

**30. Direction of RNA synthesis is:**
a. 5′→ 3′ b. 3′ → 5′
c. Both (a) and (b) d. None of these

**31. The termination site for transcription is recognized by:**
a. α–Subunit of DNA-dependent RNA polymerase
b. β–Subunit of DNA-dependent RNA polymerase
c. Sigma factor
d. Rho factor

**32. Post-transcriptional modification of hnRNA involves all of the following except:**
a. Addition of 7-methylguanosine triphosphate cap
b. Addition of polyadenylate tail

c. Insertion of nucleotides
d. Deletion of introns

**33. Newly synthesized tRNA undergoes post-transcriptional modifications which include all the following *except*:**
a. Reduction in size
b. Methylation of some bases
c. Formation of pseudouridine
d. Addition of C-C-A terminus at 5′ end

**34. Post-transcriptional modification does not occur in:**
a. Eukaryotic tRNA b. Prokaryotic tRNA
c. Eukaryotic hnRNA d. Prokaryotic mRNA

**35. A consensus sequence on DNA, called TATA box, is the site for attachment of:**
a. RNA-dependent DNA polymerase
b. DNA-dependent RNA polymerase
c. DNA-dependent DNA polymerase
d. DNA topoisomerase II

**36. Introns in genes:**
a. Encode the amino acids which are removed during post-translational modification
b. Encode signal sequences which are removed before secretion of the proteins
c. Are the noncoding sequences which are not translated?
d. Are the sequences that occur between two genes?

**37. All of the following statements about post-transcriptional processing of tRNA are true *except*:**
a. Introns of some tRNA precursors are removed
b. CCA is added at 3′ end
c. 7-Methylguanosine triphosphate cap is added at 5′ end
d. Some bases are methylated

**38. Noncoding sequences in a gene are known as:**
a. Cistron b. Nonsense codons
c. Introns d. Exons

**39. Pribnow box is present in:**
a. Prokaryotic promoters
b. Eukaryotic promoters
c. Both (a) and (b)
d. None of these

**40. Hogness box is present in:**
a. Prokaryotic promoters
b. Eukaryotic promoters
c. Both (a) and (b)
d. None of these

**41. CAAT box is present in:**
a. Prokaryotic promoters 10 bp upstream of transcription start site
b. Prokaryotic promoters 35 bp upstream of transcription start site
c. Eukaryotic promoters 25 bp upstream of transcription start site
d. Eukaryotic promoters 70 to 80 bp upstream of transcription start site

**42. Eukaryotic promoters contain:**
a. TATA box 25 bp upstream of transcription start site
b. CAAT box 70 to 80 bp upstream of transcription start site
c. Both (a) and (b)
d. None of these

**43. Transcription is the formation of:**
a. DNA from a parent DNA
b. RNA from a parent RNA
c. RNA from DNA
d. Protein from mRNA

**44. Sigma and Rho factors are required for:**
a. Replication b. Transcription
c. Translation d. Polymerization

**45. RNA synthesis requires:**
a. RNA primer b. RNA template
c. DNA template d. DNA primer

**46. The site to which RNA polymerase binds on the DNA template prior to the initiation of transcription is:**
a. Intron/exon junction
b. Promoter
c. Terminator
d. Initiator methionine codon

**47. The DNA segment from which the primary transcript is copied or transcribed is called:**
a. Coding region b. Initial codon
c. Translation unit d. Transcriptome

**48. The formation of an initiation complex for polypeptide synthesis in eukaryotes requires:**
a. A promoter
b. Formylmethionyl-tRNA Met
c. The 60S subunit but not the 40S subunit
d. Both 60S and 40S subunits

**49. A codon AUG is a:**
a. Chain-initiating codon
b. Chain-terminating codon
c. Releasing factor for peptide chains
d. Recognition site on the tRNA

**50. Release of a polypeptide chain from a ribosome is catalyzed by:**
a. Translocase
b. Dissociation of ribosomes
c. Peptidyl transferase
d. Stop codons

**51. In contrast to eukaryotic mRNA, prokaryotic mRNA:**
a. has a poly A tail
b. has 7-methylguanosine at the 5′ end
c. can be polycistronic
d. none of the above

**52. Erythromycin prevents synthesis of polypeptide:**
a. By inhibiting binding of aminoacyl tRNA to mRNA
b. By inhibiting translocation reaction
c. By inhibiting binding of tRNA to mRNA
d. By blocking mRNA formation from DNA

**53. The stop codons are present on:**
a. The coding strand of DNA
b. The template strand of DNA
c. The mRNA
d. The tRNA

**54. Termination of protein biosynthesis requires presence of:**
a. Termination sequences 'ter'
b. Rho factor
c. Nonsense codons
d. Sigma factor

**55. Which one of the following causes frameshifts mutation?**
a. Transition
b. Transversion
c. Deletion
d. Substitution of purine to pyrimidine

**56. Translocase is an enzyme required in the process of:**
a. DNA replication
b. RNA synthesis
c. Initiation of protein synthesis
d. Elongation of peptides in protein synthesis

**57. Which of the following nucleotide bases is not present in codons?**
a. Adenine b. Guanine
c. Thymine d. Cytosine

**58. Total number of codons is:**
a. 64 b. 61
c. 62 d. 63

**59. A gene is:**
a. A single protein molecule
b. A group of chromosomes
c. An instruction for making a protein molecule
d. A bit of DNA molecule

**60. Degeneracy of the genetic code means that:**
a. Codons are not ambiguous
b. A given amino acid can be coded for by more than one base triplet
c. A given codon can code for more than one amino acid
d. There is no punctuation in the code sequence

**61. The first amino acyl tRNA which initiates translation in prokaryotes is:**
a. Methionyl tRNA
b. Formylmethionyl tRNA
c. Tyrosinyl tRNA
d. Alanyl tRNA

**62. The first codon to be translated on mRNA is:**
a. AUG b. GGU
c. GGA d. AAA

**63. AUG, the codon for methionine, is important as:**
a. A releasing factor for peptide chains
b. A chain-terminating codon
c. Recognition site on tRNA
d. A chain-initiating codon

**64. In biosynthesis of proteins, the chain-terminating codons are:**
a. UAA, UAC and UGA
b. UGG, UGU and AGU
c. AAU, AAG and GAU
d. GCG, GCA and GCU

**65. The first amino acyl tRNA which initiates translation in eukaryotes is:**
a. Methionyl tRNA
b. Formylmethionyl tRNA
c. Tyrosinyl tRNA
d. Alanyl tRNA

**66. Formation of initiation complex in protein biosynthesis involves:**
a. Dissociation of ribosome into its 40S and 60S subunits
b. Formation of 48S initiation complex
c. Formation of 80S initiation complex
d. All of the above

**67. Initiation of protein synthesis requires:**
a. ATP b. AMP
c. GDP d. GTP

**68. The enzyme amino acyl tRNA synthetase is involved in:**
a. Dissociation of discharged tRNA from 80S ribosome
b. Charging of tRNA with specific amino acids
c. Termination of protein synthesis
d. Formation of peptide bond

**69. Translation results in a product known as:**
a. Protein b. tRNA
c. mRNA d. rRNA

**70. Tetracycline prevents synthesis of polypeptide by:**
a. Blocking mRNA formation from DNA
b. Releasing peptides from mRNA-tRNA complex
c. Competing with mRNA for ribosomal binding sites
d. Preventing binding of aminoacyl tRNA

**71. A polycistronic mRNA can be seen in:**
a. Prokaryotes
b. Eukaryotes
c. Mitochondria
d. All of these

**72. Synthesis of DNA is also known as:**
a. Duplication b. Replication
c. Transcription d. Translation

**73. Anticodons are present on:**
a. Coding strand of DNA
b. mRNA
c. tRNA
d. rRNA

**74. Codons are present on:**
a. Noncoding strand of DNA
b. mRNA
c. tRNA
d. None of these

**75. Nonsense codons are present on:**
a. mRNA
b. tRNA
c. rRNA
d. None of these

**76. All of the following statements about nonsense codons are true *except*:**
a. They do not code for amino acids
b. They act as chain termination signals
c. They are identical in nuclear and mitochondrial DNA
d. They have no complementary anticodons

**77. All the following statements about genetic code are correct *except*:**
a. It is degenerate
b. It is unambiguous
c. It is nearly universal
d. It is overlapping

## Answers for MCQs

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **1.** c | **2.** b | **3.** a | **4.** c | **5.** d | **6.** d | **7.** a | **8.** b |
| **9.** b | **10.** a | **11.** a | **12.** b | **13.** c | **14.** b | **15.** c | **16.** b |
| **17.** c | **18.** c | **19.** c | **20.** b | **21.** d | **22.** c | **23.** a | **24.** c |
| **25.** b | **26.** c | **27.** c | **28.** c | **29.** c | **30.** a | **31.** d | **32.** c |
| **33.** d | **34.** d | **35.** b | **36.** c | **37.** c | **38.** c | **39.** a | **40.** b |
| **41.** d | **42.** c | **43.** c | **44.** b | **45.** c | **46.** b | **47.** a | **48.** d |
| **49.** a | **50.** c | **51.** c | **52.** b | **53.** c | **54.** c | **55.** c | **56.** d |
| **57.** c | **58.** a | **59.** d | **60.** b | **61.** b | **62.** a | **63.** d | **64.** a |
| **65.** a | **66.** d | **67.** d | **68.** b | **69.** a | **70.** d | **71.** a | **72.** b |
| **73.** c | **74.** b | **75.** a | **76.** c | **77.** d | | | |

CHAPTER

# 21 Regulation of Gene Expression and Mutation

**Chapter outline**

- Gene Expression
- Regulation of Gene Expression in Prokaryotes
- Lactose Operon or Lac Operon
- Mutations

## INTRODUCTION

Gene expression is the combined process of the transcription of a gene into mRNA and its translation into protein. Genetic mutation alters the regulation or expression of gene and results in dysfunctional or non-functional protein synthesis.

The aim of this chapter is to introduce the basic concepts involved in the regulation of genes and how these processes may involve in the causation of mutation.

## GENE EXPRESSION

*Information, encoded in DNA, is transcribed into RNA and then translated into protein. It is called the* ***gene expression****. Thus, gene is expressed in terms of synthesis of protein*. The regulation of gene expression is essential for metabolic functions, growth and development and differentiation of tissues.

The rate of expression of prokaryotic genes is controlled mainly at the level of transcription, mRNA synthesis.

Eukaryotes however have a much larger and more complex genome than prokaryotes. Gene expression in eukaryotes is controlled by many ways.

### Types of Gene Regulation

There are two types of gene regulation:

- Positive regulation
- Negative regulation

#### *Positive Regulation*

When the expression of genetic information is increased by the presence of a specific regulatory element, regulation is said to be ***positive.*** The element or molecule mediating the positive regulation is said to be an ***activator*** or ***inducer.***

#### *Negative Regulation*

When the expression of genetic information is diminished by the presence of a specific regulatory element, regulation is said to be **negative** and element mediating negative regulation is said to be a **repressor.**

### Types of Genes

There are two types of genes:

- Inducible gene
- Constitutive gene

#### *Inducible Gene*

Inducible genes are expressed only when a specific positive regulatory substance, i.e. an inducer or activator is present, e.g. the production of the enzyme β-galactosidase is induced by the presence of lactose in the prokaryotes or insulin is an inducer of the gene glucokinase of glycolysis in human beings.

#### *Constitutive Gene*

Constitutive genes refer to genes whose expression is not regulated. They are expressed at a constant rate. These are often referred to as ***housekeeping genes.***

## REGULATION OF GENE EXPRESSION IN PROKARYOTES

In prokaryotes, the genes involved in a metabolic pathway are often present in a linear fashion, called an **operon.**

Operons occur in prokaryotes but not in eukaryotes. For example:

- Lactose operon (Lac operon for regulation of lactose metabolism)
- Arabinose operon (Ara operon for regulation of arabinose metabolism)
- Galactose operon (Gal operon for regulation of galactose metabolism).

## LACTOSE OPERON OR LAC OPERON

Jacob and Monod, in 1961, described their operon model for the regulation of lactose metabolism (lac operon) by E. coli. Lac operon is a coordinated unit of gene expression to make the enzymes necessary to metabolize lactose.

### Structure of the Lac Operon (Fig. 21.1)

The lac operon is a region of DNA in the genome of E.coli that contains the following genetic elements:

1. **Regulatory gene (lac i)** produces a repressor protein
2. **A promoter site (P)** for the binding of RNA polymerase. Promoter site contains two specific regions:
   i. Catabolite activator protein binding site (CAP site)
   ii. RNA polymerase entry site, to which RNA polymerase first becomes bound.
3. **An operator site (O)**, a regulatory protein called the **lac repressor protein** binds to this site and blocks initiation of transcription.
4. **Three structural genes, Z, Y and A,** that code for β-galactosidase, galactoside permease and transacetylase respectively, required for lactose metabolism.

This genetic arrangement of the structural genes and their regulatory genes allows for the coordinated expression of the three enzymes concerned with lactose metabolism.

### Regulation of Lac Operon

Lac operon is regulated by following mechanism:

1. Negative regulation by **lac repressor** in absence of lactose and presence of glucose.
2. Positive regulation by:
   i. **Induction of expression**, in presence of lactose and absence of glucose.
   ii. **Catabolite repression** in presence of both glucose and lactose.

#### *Negative Regulation by Lac Repressor in Absence of Lactose and Presence of Glucose*

- *E. coli* bacteria usually depend on glucose as their source of energy. However, in absence of glucose, E. coli can use lactose as their energy source.
- In the absence of lactose, the cell has no need for the production of β-**galactosidase, galactoside permease** and **transacetylase** which are required for metabolism of lactose. Hence regulatory molecule, the **lac repressor protein,** prevents expression of the lac operon in the absence of lactose.
- In the absence of lactose, repressor protein (a product of regulatory gene i) can bind to the operator, thus preventing the binding of RNA polymerase and subsequent transcription of the structural genes Z, Y and A. Under these conditions, β-galactosidase and other two enzymes are not made by the cells **(Fig. 21.2A)**.

#### *Positive Regulation by Induction of Expression, in Presence of Lactose and Absence of Glucose*

- In presence of lactose and absence of glucose in the medium, lactose acts as an inducer. It binds to repressor protein and inactivates it.

**Fig. 21.1:** Schematic diagram of lac operon.
(P: Promoter, O: Operator, i: Lac i gene, Z: Lac Z gene, Y: Lac Y gene, A: Lac A gene)

**Figs. 21.2A and B:** Regulation of lac operon.
(A) In presence of glucose and absence of lactose; (B) In presence of lactose and absence of glucose.

- The inactive repressor protein no longer binds to the operator.
- RNA polymerase now can bind to the promoter and transcribe the three structural genes of the operon.
- The result is the continued production of the enzymes needed for the metabolism of lactose **(Fig. 21.2B)**.

## *Positive Regulation by Catabolite Repression in Presence of Both Glucose and Lactose*

When *E. coli* is exposed to both lactose and glucose, they first metabolize the glucose, although lactose is present. The cell does not induce those enzymes necessary for catabolism of lactose until the glucose has been exhausted. This phenomenon was thought to be due to repression of the lac operon by some catabolite of glucose; therefore, it was termed ***catabolite repression.*** It is now known that ***"catabolite repression",*** is in fact mediated by a **catabolite activator protein (CAP)** in conjunction with **cAMP**. This protein is also referred to as the ***cAMP regulatory protein (CRP).***

- The levels of cAMP are inversely linked to the levels of glucose. As glucose levels decrease, cAMP levels increase. In the presence of cAMP, a complex of **cAMP-CAP** forms, which binds to the CAP binding site of promoter, stimulating the binding of the RNA polymerase to the promoter and transcription occurs **(Fig. 21.2 B)**.
- As glucose levels increase, cAMP levels decrease and in the absence of cAMP, CAP does not bind to CAP binding

**Fig. 21.3:** Types of mutation.

**Fig. 21.4:** Diagrammatic representation of transition and transversion mutations.

site of promoter and transcription does not occur. Thus, the enzymes for metabolism of lactose are not produced if cells have an adequate supply of glucose even if the alternative energy source, lactose, is present at very high levels.

## MUTATIONS

The term mutation refers to the permanent changes in the DNA sequence.

- Mutations in germ cells are transmitted to the next progeny and may give rise to inherited diseases.
- Mutations in somatic cells are not transmitted to the progeny but are important in the causation of cancers and some congenital malfunctions.

### Causes of Mutations

Mutation arises by a number of different means:

- **Errors in replication**, if a mismatch base pair is not corrected during proofreading and post replication repair system.
- **Error due to recombination events**.
- **Spontaneous change in DNA**, e.g. deamination of cytosine to uracil or spontaneous depurination.
- **Environmental factors** like chemical mutagens and irradiations, e.g. UV light or ionizing radiation can alter the structure of DNA.

### Types of Mutations (Fig. 21.3)

#### *Base Substitution or Point Mutation*

- Point mutation occurs when only one base in DNA is altered, which may be transcribed into mRNA and therefore, may result in the translation of a protein with an abnormal amino acid sequence. Single base change can be of **transition type** or **transversion type**.
- **Transition** in which one purine is replaced by another purine or one pyrimidine is replaced by another pyrimidine **(Fig. 21.4)**.
- **Transversion** in which a purine is replaced by a pyrimidine or a pyrimidine is replaced by a purine **(Fig. 21.4)**.
- The point mutation can lead to:
  1. Silent mutation
  2. Missense mutation
  3. Nonsense mutation

*Silent mutation*

Point mutations are said to be silent when there is no detectable effect. Silent mutation leads to the formation of a codon synonym and no change in the amino acid sequence of the protein occurs due to degeneracy of the codon, e.g. a codon change from CGA to CGG does not affect the proteins because both of these codons specify arginine.

*Missense mutation*

Missense mutation will occur when a different amino acid is incorporated at the corresponding site in the protein molecule. Depending upon the location of the mistaken amino acid (missense) in the specific protein, missense mutation might be **acceptable, partially acceptable** or **unacceptable** with respect to the function of that protein.

***Acceptable Missense Mutations***

Example of Acceptable missense mutation is Hb-Hikari. This Hb has asparagine substituent for lysine at the 61 position in the β-globin chain. Hb-Hikari is a type of transversion mutation, in which either AAA or AAG changed to either AAU or AAC. The replacement of the specific lysine with asparagine does not alter the normal function of the β-chain in these individuals and is therefore called acceptable missense mutation.

***Partially Acceptable Missense Mutation***

For example, HbS, sickle hemoglobin, in which the normal amino acid in position 6 of the β-chain, glutamic acid has been replaced by valine. The corresponding transversion might be either GGA or GAG of ***glutamic acid*** to GUA or GUG of ***valine***.

***Unacceptable Missense Mutation***

For example, Hb M (Methemoglobin) in which normal amino acid in position 58 of α-chain, ***histidine*** has been replaced by ***tyrosine*** and non-functional Hb molecule is generated which cannot transport oxygen.

*Nonsense mutations*

Nonsense mutation leads to the conversion of an amino acid codon to a stop or nonsense codon. Nonsense mutation causes the premature termination of a polypeptide chain, which is usually non-functional, e.g. one type of ***thalassemia***, in which codon 17 of the β-chain is changed from UGG to UGA and results in the conversion of a codon tryptophan to a nonsense codon.

## Frame Shift Mutations (Fig. 21.5)

Frame shift mutation occurs when there is insertion or deletion of one or two nucleotides in DNA that generates altered mRNAs.

**Fig. 21.5:** Diagrammatic representation of frame shift mutation.

*Deletion frame shift mutation*

- The deletion of a single nucleotide from the coding strand of a gene results in an altered reading frame in the mRNA.
- Since there is no punctuation in the reading of codons, the translating machinery does not recognize that a base was missing.
- Such frame shifts would result in the production of an entirely different protein after transcription and translation or indeed, no protein, at all, if a stop codon is encountered, as in cystic fibrosis of pancreas.

*Insertion frame shift mutation*

Insertion may be of one or two nucleotides. As with deletions, insertions of nucleotides into genes can lead to severe frame shift mutations, e.g. thalassemia.

If the number of nucleotides involved in deletion or insertion is three or multiples of three, frame shift does not occur. Instead, an abnormal protein, missing one or more amino acids, is synthesized. Such a mutation is likely to be less severe than a frame shift mutation.

## EXAM QUESTIONS

### Long Answer Question (LAQs)

1. Describe lac operon concept and its regulation.
2. Describe various types of mutation with examples.

### Short Notes

1. Negative regulation by lac repressor in absence of lactose and presence of glucose.
2. Positive regulation by induction of expression, in presence of lactose and absence of glucose.
3. Positive regulation by catabolite repression in presence of both glucose and lactose.
4. Structure of the lac operon.

### Multiple Choice Questions (MCQs)

**1. The lac operon includes:**
a. i gene
b. Control site
c. Z, Y and A genes
d. i, Z, Y, A genes and control site

**2. An operon is best described by:**
a. A constitutively expressed gene system
b. An unregulated gene system
c. A co-ordinately regulated gene system
d. A gene that produces a monocistronic mRNA

**3. All of the following statements about the repressor of lac operon of *E. coli* are true, *except*:**
a. It is the gene product of regulatory gene
b. It prevents the transcription of structural genes
c. It binds to the operator region of the lac operon
d. It requires corepressor

**4. cAMP regulates the lac operon *except*:**
a. By forming cAMP-CAP (catabolite activator protein) complex
b. By binding to the promoter site
c. By stimulating binding of RNA polymerase to promoter and by activating transcription
d. By binding to the lac repressor to prevent transcription

**5. The portion of an operon to which a repressor binds is:**
a. A regulatory gene b. A promoter
c. An initiation site d. An operator

**6. Lac operon is a cluster of genes present in:**
a. Human beings b. *E. coli*
c. Lambda phage d. All of these

**7. Lac operon is a cluster of:**
a. Three structural genes
b. Three structural genes and their promoter
c. A regulatory gene, an operator and a promoter
d. A regulatory gene, an operator, a promoter and three structural genes

**8. The regulatory *i* gene of lac operon:**
a. Is inhibited by lactose
b. Is inhibited by its own product, the repressor protein
c. Forms a repressor protein which regulates the expression of structural genes
d. None of the above

**9. RNA polymerase holoenzyme binds to lac operon at the following site:**
a. i gene b. z gene
c. Operator locus d. Promoter region

**10. Transcription of z, y and a genes of lac operon is prevented by:**
a. Lactose b. Allolactose
c. Repressor d. cAMP

**11. Transcription of structural genes of lac operon is prevented by binding of the repressor protein to:**
a. i gene b. Operator locus
c. Promoter d. *z* gene

**12. Binding of RNA polymerase holoenzyme to the promoter region of lac operon is facilitated by:**
a. Catabolite gene activator protein (CAP)
b. cAMP
c. CAP-cAMP complex
d. None of these

**13. Lactose or its analog act as positive regulators of lac operon by:**
a. Attaching to i gene and preventing its expression
b. Increasing the synthesis of catabolite gene activator protein
c. Attaching to promoter region and facilitating the binding of RNA polymerase holoenzyme
d. Binding to repressor subunits so that the repressor cannot attach to the operator locus

**14. Expression of structural genes of lac operon is affected by all the following:**
a. Lactose or its analog
b. Repressor protein
c. CAP-cAMP complex
d. All of the above

**15. Mutations can be caused by:**
a. Ultraviolet radiation
b. Ionizing radiation
c. Alkylating agents
d. All of these

**16. Substitution of an adenine base by guanine in DNA is known as:**
a. Transposition
b. Transition
c. Transversion
d. Frame shift mutation

**17. Substitution of a thymine base by adenine in DNA is known as:**
a. Transposition b. Transition
c. Transversion d. Frame shift mutation

**18. A point mutation results from:**
a. Substitution of a base
b. Insertion of a base
c. Deletion of a base
d. All of these

**19. Substitution of a base can result in a:**
a. Silent mutation b. Missense mutation
c. Nonsense mutation d. All of these

**20. Amino acid sequence of the encoded protein is not changed in:**
a. Silent mutation
b. Acceptable missense mutation
c. Both (A) and (B)
d. None of these

**21. Hemoglobin S is an example of:**
a. Silent mutation
b. Acceptable missense mutation
c. Unacceptable missense mutation
d. Partially acceptable missense mutation

**22. If the codon UAC on mRNA changes into UAG as a result of a base substitution in DNA, it will result in:**
a. Silent mutation
b. Acceptable missense mutation
c. Nonsense mutation
d. Frame shift mutation

**23. Insertion of a base in a gene can cause:**
a. Change in reading frame
b. Garbled amino acid sequence in the encoded protein
c. Premature termination of translation
d. All of these

**24. A frameshift mutation changes the reading frame because the genetic code:**
a. Is degenerate
b. Is overlapping
c. Has no punctuations
d. Is universal

## Answers for MCQs

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **1.** d | **2.** c | **3.** d | **4.** d | **5.** d | **6.** b | **7.** d | **8.** c |
| **9.** d | **10.** c | **11.** b | **12.** c | **13.** d | **14.** d | **15.** d | **16.** b |
| **17.** c | **18.** a | **19.** d | **20.** a | **21.** d | **22.** c | **23.** d | **24.** c |

CHAPTER

# 22

# Genetic Engineering

**Chapter outline**

- Recombinant DNA
- Cloning of the DNA
- Applications of Recombinant DNA Technology
- DNA Library
- Technique for Identifying DNA Sequence
- Restriction Fragment Length Polymorphism (RFLP)
- Polymerase Chain Reaction (PCR)

## INTRODUCTION

Engineering is the designing, construction and repair of structures of non-living things. Genetic engineering is the construction and repair of the genes of living things. Now it is possible to synthesize a gene in a laboratory and then introduce it into a living cell which will synthesize protein corresponding to that gene. Rapid progress in biotechnology is a result of following few key techniques:

- **Restriction enzyme analysis:** Restriction enzymes allow an investigator to manipulate DNA segment.
- **Blotting techniques:** Southern and Northern blots are used to separate and characterized DNA and RNA respectively. The western blot uses antibodies to characterize proteins.
- **DNA sequencing:** The precise nucleotide sequence of the molecule of DNA can be determined. Sequencing provides information concerning gene architecture, the control of gene expression and protein structure.
- **Solid-phase synthesis of nucleic acids:** Precise sequences of nucleic acids can be synthesized *de novo* and used to identify other nucleic acids.
- **Polymerase chain reaction (PCR):** The polymerase chain reaction leads to several fold amplification of a segment of DNA. This technique can be used to detect pathogens and genetic diseases, determine the source of a hair left at the scene of a crime and regenerate genes from the fossils of extinct organisms.
- We begin by outlining the principles of recombinant DNA, DNA cloning, and then illustrate the range of applications and potentials of many newer technologies.

## RECOMBINANT DNA

Recombinant DNA is a DNA formed by the hybrid combination of two DNA fragments, derived from different sources. *In vivo* genes often undergo recombination. The normal biological exchange or addition of genes from different sources to form an altered chromosome, which can be replicated, transcribed, and translated, is called ***genetic recombination***, it occurs in a number of different biological situations.

In eukaryotic organisms genetic recombination occurs by the sexual union of egg and sperm cell, in which both parental chromosomes contribute certain genes to the new daughter chromosomes appearing in the progeny cell. In this process, the chromosomes of both the sperm and egg cells undergo cleavage at homologous point, and pieces of the chromosomes from the two parent cells are then exchanged and spliced together to yield new combinations of genes. Such naturally occurring cutting, reassembly and splicing of genes during sexual conjugation of eukaryotes occur without disturbing the reading frame of the DNA sequence.

### In Vitro Recombinant DNA

Genes or sets of gene can also be recombined *in vitro* (in the test tube) to produce new combinations that do not occur biologically. ***Recombinant DNA technology involves isolation and manipulation of DNA to make chimeric or hybrid DNA molecule***.

## Chimeric DNA

Chimeric DNA is a recombinant DNA containing genes from two different species, e.g. molecules containing both human and bacterial DNA sequences (Chimera was a mythological creature with the head of the lion, the body of goat and the tail of the snake). A number of techniques have been used to construct recombinant DNA.

## Construction of Recombinant DNA

Following steps are involved in the construction of recombinant DNA:

- Fragmentation of DNA (restriction fragments) by restriction endonuclease enzyme.
- Isolation of specific human DNA.
- Insertion of isolated human DNA into vector to form chimeric or hybrid DNA molecule.
- Joining of two different cut DNA fragments by DNA ligase.

### *Fragmentation of DNA (Restriction Fragments) by Restriction Endonuclease Enzyme*

Restriction endonuclease also called as ***restriction enzyme***. These are found in wide variety of prokaryotes. These enzymes were originally called restriction enzymes because their presence in a given bacterium restricted the growth of foreign infective bacterial viruses (bacteriophages). The cell's own DNA is not degraded because the sequences recognized by its own restriction enzymes are methylated, thereby protected from the action of restricted endonuclease enzyme.

Restricted enzymes cut DNA of any source in a sequence specific manner in contrast to action of most other endonuclease which breaks DNA randomly. The restricted enzymes are named according to the bacteria from which they are isolated. Their name consists of a three letter abbreviation for the host organism.

- The first letter of the name is from genus of the bacteria
- The next two letters are from the name of the species
- These followed by a strain and Roman numeral indicate the order in which the enzyme was discovered in the particular organism, e.g.
  - **EcoRI** is from *Escherichia coli*
  - ***BamHI*** is from *Bacillus amyloliquefaciens*
  - ***Hae III*** is for *Haemophilus aegyptius*

Restriction endonuclease recognizes specific base sequences of double helical DNA, usually 4 to 6 base-pairs in length and cleaves both strands within this sequence. Most of the DNA sequences recognized by restriction endonucleases are ***palindromic*** (in Greek palindromic = running back again), i.e. both strands of DNA have the same sequence when read in a 5′ to 3′ direction (**Fig. 22.1**).

**Fig. 22.1:** Palindromic sequence when read in 5' to 3' direction.

*Action of restriction endonucleases*

- some restriction endonucleases cleave both strands of DNA so as to leave no unpaired bases on either end. These ends are often called ***blunt ends,*** e.g. restriction enzyme from Hae III (**Haemophilus aegyptius**).
- Others (restriction enzyme from EcoRI) make staggered cuts on the two strands, leaving two to four nucleotides of one strand unpaired at each resulting end. These are referred to as **cohesive ends** or ***sticky ends*** **(Fig. 22.2)**.

Restriction enzymes are used to cleave DNA molecules into specific fragments that are more readily analyzed and manipulated than the entire parent molecule. Different restriction fragments of DNA can base-pair with each other if they have sticky ends which are complementary. Therefore, two unrelated DNA fragments can base-pair with each other, if they were cleaved by the same restriction endonuclease enzyme.

### *Isolation of Specific Human DNA*

Once DNA has been cleaved into fragments by restriction endonuclease, a particular fragment of interest can be separated by others, by **electrophoresis** or **HPLC**.

### *Insertion of Isolated Human DNA into Vector to form Chimeric or Hybrid DNA Molecule*

A specific selected fragment of DNA of human genome is inserted into vector (carrier). *A vector is a carrier DNA molecule*, to which the fragment of DNA of interest is, attached **(Fig. 22.3)**. Normally, foreign DNA fragments cannot self-replicate in a cell. Therefore, they are joined together to a vector that can replicate within the host cell.

**Most commonly used cloning vectors are:**

- **Bacterial plasmids:** Small circular duplex DNA molecule whose natural function is to confer antibiotic resistance to the host cell and replicate independently from the bacterial chromosomal DNA.
- **Bacteriophages:** Viral DNA capable of replicating in bacterial cell.
- **Cosmids:** Plasmids that contain DNA sequences from the lambda phage.

**Fig. 22.2:** Action of restriction endonuclease EcoRI produces sticky ends and Hae III produces blunt ends.

A segment of the foreign DNA and the DNA vector are usually cleaved with the same restriction enzyme to produce complementary sticky ends in both the foreign DNA and the DNA vector. These complementary sticky ends can base pairs.

### *Joining of Two Different Cut DNA Fragments by DNA Ligase*

After the fragments of DNA (one from human genome and another from vector DNA) have base paired, the ends are covalently joined by the action of ***DNA ligase***. Restriction enzymes in conjunction with DNA ligase, therefore, can produce vector containing recombinant or hybrid or chimeric DNA.

## CLONING OF THE DNA

Cloning is a technique developed for amplifying the quantity of DNA, *in vivo*. A clone is a large population of

**Fig. 22.3:** Construction of recombinant DNA molecules.

identical molecules, bacteria, or cells that arise from a common ancestor. Cloning allows for the production of a large number of identical DNA molecules, which can be produced as follows:

- **Introduction of recombinant DNA into appropriate host cell:** The recombinant DNA inserted back into bacterial cell (host cell) by the process called transformation. Host cells that contain recombinant DNA are called transformed cells.
- **Amplification of recombinant DNA:** The host cells containing the recombinant DNA are incubated under conditions in which they replicate rapidly. As the host cell divide in addition to replicating their own DNA, they also replicate the DNA of the vector which includes the human DNA. Subsequently, relatively large quantities of human DNA can be isolated from cells.
- If the host cells are grown under conditions permitting expression of the human DNA, the human protein produced from this DNA can be isolated **(Fig. 22.4)**.

## APPLICATIONS OF RECOMBINANT DNA TECHNOLOGY

Let us consider how the recombinant DNA technology has been used in medicine **(Table 22.1)**.

- **Molecular basis of disease:** Recombinant DNA technology is used to understand the molecular basis of a number of diseases like:
  - Familial hypercholesterolemia
  - Sickle cell disease
  - Thalassemia
  - Cystic fibrosis
  - Muscular dystrophy, etc.
- **Diagnosis of disease:** Recombinant DNA technology is used to diagnose existing diseases.
- **Production of proteins:** Using recombinant technology, human proteins can be produced in abundance for therapy, research and diagnosis, e.g. it can supply large amounts of material that could not be obtained by conventional purification methods, e.g. interferon, tissue plasminogen activating factor, etc.

**Table 22.1:** Applications of recombinant DNA technology.

| | |
|---|---|
| 1. | It offers a rational approach to understanding the molecular basis of number of diseases, e.g. familial hypercholesterolemia, sickle cell disease, thalassemia, cystic fibrosis, muscular dystrophy, vascular and heart disease, cancer and diabetes |
| 2. | Human proteins can be produced in abundance for therapy (e.g. insulin, growth hormone, tissue plasminogen activator, etc.) |
| 3. | Proteins for vaccine, e.g. hepatitis B and for diagnostic testing, e.g. Ebola and AIDS tests) can be obtained |
| 4. | This technology is used both to diagnose existing diseases as well as to predict the risk of developing a given disease and individual response to pharmacological therapeutics |
| 5. | Special techniques (**DNA fingerprinting/DNA genotyping** or **DNA profiling**) are used in forensic medicine |
| 6. | Gene therapy for curing diseases caused by a single gene deficiency such as sickle cell disease, thalassemia, adenosine deaminase deficiency and others may be planned |
| 7. | Genetic counseling prenatal diagnosis transgenic animal formation is possible |
| 8. | *Commercial applications:* Agricultural application and Industrial applications |

**Table 22.2:** Human proteins and their role produced by recombinant technology.

| *Protein produced* | *Function* |
|---|---|
| Anticoagulant | Tissue plasminogen activator (TPA). It activates plasmin, an enzyme involved in dissolving clots. Used in treating heart attack victims |
| Human growth hormone | It is used to treat children with growth hormone deficiencies (dwarfism) |
| Growth factors | Stimulate differentiation and growth of various cell types |
| Colony stimulating factors | Immune system growth factors. It stimulates leukocyte production used to treat immune deficiencies |
| Erythropoietin | Stimulates erythrocyte production used to treat anemia |
| Blood factors | Factor VIII promotes clotting and is used to treat hemophilic patients |
| Insulin | A hormone used to treat diabetes |
| Interferons | Used to treat cancer |
| Interleukins | Activates and stimulates leukocytes used in wound healing, HIV infections, cancer, immune deficiencies |
| Monoclonal antibodies | Used in diagnostic tests, also to transport drugs, toxins, or radioactive compounds to tumors or a cancer therapy |
| Superoxide dismutase | Prevent tissue damage from reactive oxygen species (ROS) when tissue is deprived of $O_2$ for short periods during surgery |
| Proteins for diagnosis | For example, for AIDS test of human and other animal diseases |
| Vaccines | Prevent disease |

It can provide human material, e.g. insulin and growth hormone **(Table 22.2)**. The primary aim of recombinant technology is to supply proteins for treatment (insulin) and diagnosis (AIDS, Ebola testing) of human and other animal diseases and for prevention of disease (hepatitis B vaccine).

Before the introduction of recombinant DNA technology, vaccines were made from infectious agents, which were either killed or attenuated (altered so that they can no longer multiply in an inoculated individual). Both types of vaccines were potentially dangerous because they could get contaminated with the live infectious agents. By recombinant DNA techniques, the proteins could be produced, completely free of the infectious agent, and used in the vaccines. Thus, the risk of infection could be eliminated. The first successful recombinant DNA vaccine to be produced was for the **hepatitis B virus**.

- **Prenatal diagnosis:** If the genetic lesion is understood and a specific probe (probe is a single stranded polynucleotide of DNA or RNA that is used to identify the sequence on a target DNA) is available, prenatal diagnosis is possible. DNA from cells collected from amniotic fluid can be analyzed by **Southern blot transfer**.
- **Genetic counselling:** One means of preventing disease is avoid passing defective genes to offspring. If members of families are tested for genetic diseases, which are known to carry a defective gene, genetic counsellors can inform the individuals of their risk and options. With this information people can decide in advance whether to have children.

**Fig. 22.4:** Cloning of human DNA in bacteria using recombinant DNA technology.

- **Screening tests:** Tests based on the recombinant DNA techniques have been developed for many inherited diseases. Screening can be performed on the prospective parents before conception. If they decide to conceive, the fetus can be tested for the genetic defect. In some cases if the fetus has the defect, treatment can be instituted at an early stage, even *in utero*. For certain diseases, early therapy leads to a more positive outcome.
- **Gene therapy:** The ultimate cure for genetic diseases is to introduce normal genes into individuals who have

defective genes. The strategy is to clone a normal copy of the relevant gene into a suitable vector and incorporate into genome of a host cell. Bone marrow precursor cells are being investigated for this purpose. The introduced gene would begin to direct the expression of its protein product and this would correct the deficiency in the host cell. It is not possible at present to replace a defective gene with a normal gene at its usual location in the genome of the appropriate cells.

Gene therapy for **sickle cell disease**, **thalassemia, adenosine deaminase** deficiency and other diseases may be devised. Currently gene therapy is at experimental level.

- **Transgenic animals:** Transgenic means containing genetic material into which DNA from a different organism has been artificially introduced. The introduction of normal genes into somatic cells with defective genes corrects the defect only in the treated individuals, not in their offspring. To dominate the defect for future generations the normal genes must be introduced into the germ cell line (the cells that produce sperm in males or eggs in females). Experiments with animals indicate that gene therapy in germ cells is feasible. Genes can be introduced into fertilized eggs from which transgenic animals develop, and these transgenic animals can produce apparently normal offspring. Transgenic approach has been used to correct a genetic deficiency in mice.
- **In forensic medicine:** Special techniques, e.g. **DNA fingerprinting** also called **DNA genotyping** or **DNA profiling** (analysis of DNA sequence differences) can be used to determine family relationships or to help identify the criminals of a crime.
- **Commercial applications**
  - **Agricultural application:** In agriculture, plants that are resistant to disease, insects, herbicides, and drought and temperature extremes or more efficient at fixing nitrogen are being produced using recombinant DNA technology.
  - **Industrial applications:** Industrial applications include the production of enzymes used in detergents, sugar and cheese.

## DNA LIBRARY

**DNA** library sometimes called ***shotgun collection***. DNA library is a collection of cloned restriction fragments of DNA that represents the entire genome. The total genetic information content of an organism is represented by all the fragments in the library in the same way as human knowledge is represented by all the volumes in a book library. DNA libraries may be either:

1. Genomic DNA library, in which both introns (non-coding sequences) and exons (coding sequences) are represented.
2. Complementary DNA (cDNA) library in which only exons are represented.

### Genomic DNA Library

In genomic DNA library both introns and exons are represented and thus, contain every sequence from the genome of a specific organism.

The entire genomic DNA of an organism is cut into small pieces by restriction endonucleases. These cut pieces are then introduced into vectors. A collection of these different recombinant clones is called ***genomic DNA library***. To produce the complete gene library about 1500 fragments are required in the case of *E. coli*, but about 10 lakh fragments for human DNA.

### Complementary DNA (cDNA) Library

In cDNA library only exons are represented. It is constructed so as to include only those genes that are expressed in a given organism or even in certain cells or tissues. ***cDNA library is a more specialized and exclusive DNA library***.

To construct cDNAs library, the mRNAs from an organism is extracted and complementary double stranded DNAs (cDNAs) are produced from this mRNAs by reverse transcriptase. The resulting DNA fragments are then inserted into a suitable vector and cloned, creating a population of clones called a cDNA library. Since there will be no introns in the mRNA, the expression of the genes is easier.

## TECHNIQUE FOR IDENTIFYING DNA SEQUENCE

DNA libraries contain thousands or even millions of irrelevant DNA fragments. It is difficult to pick out a specific gene (a DNA sequence of interest) from these fragments. ***Probes are used to search specific gene from the DNA libraries***. Probe is a molecule used to identify a specific fragment of DNA or RNA in a genetic library or during analysis by blot transfer (Southern, Northern or Western) techniques.

A probe is a single stranded polynucleotide of DNA or RNA that is used to identify a complementary sequence on a larger single stranded DNA or RNA molecule. Formation of base pairs with a complementary strand is called

**Fig. 22.5:** Identification of DNA sequences by using labelled probes.

**Fig. 22.6:** The blot transfer procedures. (Asterisk signify radio labelling)

annealing or hybridization. Probes can be composed of cDNA produced from mRNA (by reverse transcriptase), fragments of genomic DNA (cleaved by restriction enzymes from the genome), chemically synthesized oligonucleotides or occasionally RNA. To identity the target sequence, the probe must carry a label. If the probe has radioactive label, it can be detected by autoradiography **(Fig. 22.5)**.

## Gel Electrophoresis

Gel electrophoresis is a technique that uses an electrical field to separate molecules on the basis of size. Because DNA contains negatively charged phosphate groups it will migrate in an electrical field toward the positive electrode. Shorter molecules migrate more rapidly through the pores of a gel than do longer molecules.

Polyacrylamide gel that can separate DNA molecules that differ in length by only one nucleotide are used to determine the base sequence of DNA. Agarose gels are used to separate longer DNA fragment that have larger size differences.

The bonds of DNA in the gel can be visualized by various techniques. Staining with dyes such as **ethidium bromide** allows direct visualization of DNA bands under ultraviolet light. Specific sequences are generally detected by means of a labelled probe.

## Blots Transfer Techniques

Blot transfer is a technique used for visualization of specific DNA, RNA or proteins among the thousands of molecules. These are **(Fig. 22.6)**:

Southern blot (for DNA)
Northern blot (for RNA)
Western blot (for protein)

EM Southern developed a technique which bears his name for identifying DNA sequences on gels and the other two names Northern and Western began as laboratory jargons.

### *Southern or DNA Blots Transfer*

Southern blot transfer procedure is useful in determining the number of copies of a gene present in a given tissue and mutations in the gene. In Southern blots transfer:

- First DNA is extracted from cells, which is digested into many fragments with restriction enzyme. The resulting fragments are separated by agarose or polyacrylamide gel electrophoresis. The smaller fragments move more rapidly than larger fragments.
- After a suitable time the DNA is denatured by mild alkali and transferred to nitrocellulose paper in an exact replica of the pattern on the gel by blotting technique, hence the name blot. The paper is then

exposed to the labelled cDNA probe, which hybridizes to complementary fragments on the paper. The paper is exposed to X-ray film, which shows specific bands corresponding to the DNA fragment that recognized the sequence in the cDNA probe.

### Northern or RNA Blots Transfer

The Northern blot transfer technique is used to size and quantitate specific RNA molecules. In Northern blot procedures RNA is subjected to electrophoresis before blot transfer and treated similarly except that alkali is not used (alkali hydrolyzes RNA). The hybrids formed between RNA and cDNA can be identified by radio autography.

### Western or Protein Blots Transfer

- Western blots technique is used to size and quantitate specific protein molecules. Western blot are currently being used as one of the tests for the **AIDS** and **Ebola** virus. In this case, the presence of viral proteins in the blood is detected by antibodies.
- Western blot procedures proteins are electrophoresed and transferred to nitrocellulose paper and then probed with labelled antibodies. The probes are labelled to visualize the bands with which they hybridize.

## RESTRICTION FRAGMENT LENGTH POLYMORPHISM

The human genome is heterogeneous. There are normal variations of DNA sequence. ***Variations of DNA sequence is called polymorphism***, which occurs approximately once in every 500 nucleotides or about $10^7$ times per genome. In normal healthy individual, these alterations (deletion, insertion of DNA or single base substitution) occur in non-coding region of DNA or at sites that cause no change in function of encoded protein.

The differences in DNA sequences can result in variations of restriction sites and thus, in the length of restriction fragments. ***An inherited difference in the pattern of restriction sites is known as restriction fragment length polymorphism or RFLP***.

**Application of RFLP**

- Restriction fragment length polymorphism can be used to detect human genetic defects in prospective parents or in fetal tissue, for example sickle cell anemia and thalassemia.
- In forensic medicine RFLPs is used to establish a unique pattern of DNA fragments (fingerprinting) for an individual.

## POLYMERASE CHAIN REACTION

Polymerase chain reaction (PCR) is an *in vitro* method that can be used for rapid production (amplification) of very large amounts of specific segments of DNA **(Fig. 22.7)**. It is particularly used for amplifying DNA for clinical or forensic testing procedures. By PCR DNA can be amplified from a single strand of hair or single drop of blood or semen.

DNA can be amplified *in vivo* by cloning by recombinant DNA (discussed earlier). Amplification of DNA by PCR is faster and technically less difficult than laborious cloning methods using recombinant DNA techniques.

### Events of the PCR

Millions of copies of the target sequences can be readily obtained by PCR if the **flanking sequences** of the target are known. A PCR is carried out by adding the following components to a solution contacting DNA sequences of interest (Fig. 22.7):

- A pair of ***"primers"*** (synthetic oligonucleotide of 20 to 35 sequences) that bind with the **flanking sequences** of the target. One primer is complementary to flanking sequences in one strand of the DNA to be amplified and the other is complementary to flanking sequences in other DNA strand).
- All four deoxyribonucleoside triphosphates (dNTPs)
- A heat stable DNA polymerase (this polymerase is isolated from *thermus aquaticus,* a bacterium that grows in hot springs)

### A PCR Cycle Consists of Three Steps:

1. **Strand separation:** The two strands of the parent DNA are separated by heating the solution to 95°C for 15s.
2. **Binding of primers:** The solution is then cooled to 54°C to allow each primer to bind to a separated DNA strand. One primer binds to the 3′ end of the target on one strand, and other primer binds to the 3′ end on the complementary target strand.
3. **DNA synthesis:** The solution is then heated to 72°C (the optimal temperature for heat stable polymerases). Deoxyribonucleotide triphosphates and heat stable DNA polymerase are added to the mixture to initiate the synthesis. One such DNA polymerase enzyme is ***Taq polymerase*** is obtained from thermophilic bacterium, ***Thermus aquatics***. The polymerase elongates both primers in 5′ to 3′ direction.

**Fig. 22.7:** The polymerase chain reaction (PCR).
The two double stranded products of the first PCR cycle are recycled.

These three steps, **strand separation**, **binding of primers**, and **DNA synthesis** constitute one cycle of the PCR amplification and can carried out repetitively just by changing the temperature of the reaction mixture. The thermostability of polymerase makes it possible to carry out PCR in a closed container. No reagents are added after the first cycle. At the completion of the second cycle, four duplexes containing the targeting sequence have been generated. Subsequent cycles will amplify the target sequence exponentially. Ideally, after *n* cycles, the desired sequence is amplified 2*n*-fold. The amplification is million fold after 20 cycles and billion fold after 30 cycles, which can be carried out in less than one hour.

## Applications of PCR

Polymerase chain reaction is a very powerful technique in **medical diagnostics**, **forensics**, and studies of **molecular evolution**.

### *Diagnostic Importance*

PCR can provide valuable diagnostic information in medicine. **Bacteria** and **viruses** can be readily detected with the use of specific primers. For example,

- PCR can reveal the presence of small amounts of DNA from the human immunodeficiency virus (HIV) in

persons who have not yet involved an immune response to this pathogen. In these patients, conventional assays designed to detect antibodies against the virus would yield a false negative test result.

- Finding *Mycobacterium tuberculosis* bacilli in tissue specimens is slow and laborious. With PCR, as few as 10 tubercle bacilli per million human cells can be readily detected.
- PCR is promising method for early detection of cancers. PCR can identify mutations of certain growth control genes, such as the ras genes.
- The capacity to greatly amplify selected regions of DNA can also be highly informative in monitoring cancer chemotherapy.
- Tests using PCR can detect when cancerous cells have been eliminated and treatment can be stopped; they can also detect a relapse and the need to immediately resume treatment.
- PCR is ideal for detecting leukemias caused by chromosomal rearrangements.
- In clinical biochemistry and molecular biology it is used in the antenatal diagnosis of single gene mutation and in studying structural gene polymorphism.

### Forensics and Legal Medicine

PCR is also having an effect in Forensics and legal medicine. The root of a single shed hair found at a crime scene contains enough DNA for capturing by PCR.

- PCR amplification of multiple genes is being used to establish biological parentage in disputed paternity and immigration cases.
- Analysis of blood stains and semen samples by PCR have implicated blame or innocence in numerous assault and rape cases.

### Studies of Molecular Evolution

PCR provides an ideal method for amplifying ancient DNA molecules so that they can be detected and characterized.

PCR can also be used to amplify DNA from micro-organisms that have not yet been isolated and cultured. The sequences from these PCR products can be sources of significant awareness into evolutionary relationships between organisms.

## EXAM QUESTIONS

### Short Notes

1. Restriction endonuclease.
2. DNA library.
3. What is PCR, and give applications of PCR?
4. What is recombinant DNA? Give applications of recombinant DNA technology.
5. Process of recombinant DNA technology.
6. Techniques used for visualization of specific DNA, RNA and proteins.
7. Blot transfer technique.
8. What is restriction fragment length polymorphism (RFLP)? Give its application.
9. Cloning.

### Multiple Choice Questions (MCQs)

1. **Restriction endonuclease has the following characteristics, *except:***
   a. Cut DNA in a sequence specific manner
   b. Named according to the bacteria from which they are isolated
   c. Most of the DNA sequences recognized by it are palindromic
   d. Cut DNA randomly
2. **Most commonly used cloning vectors are:**
   a. Bacterial plasmids b. Bacteriophages
   c. Cosmids d. All of the above
3. **Cloning is a technique developed for:**
   a. Amplifying the quantity of DNA *in vivo*
   b. Amplifying the quantity of DNA *in vitro*
   c. To search specific gene from DNA library
   d. To visualize specific DNA, RNA or proteins
4. **Southern blot is a technique used for visualization of specific:**
   a. RNA b. DNA
   c. Protein d. Carbohydrate
5. **Polymerase chain reaction (PCR) is a technique for:**
   a. Amplification of DNA *in vitro*
   b. Amplification of DNA *in vivo*
   c. Amplification of protein
   d. Amplification of RNA

**6. Which one of the following enzymes is required for PCR?**
a. DNA ligase b. Thymidine kinase
c. Taq polymerase d. Restriction endonuclease

**7. Restriction endonucleases are enzymes:**
a. Used to joining DNA to cloning vector
b. Which cleaves DNA at specific sequence
c. Which cleaves DNA randomly
d. Which digest DNA molecule from ends

**8. Construction of recombinant DNA requires which of the following steps?**
a. Fragmentation of DNA by restriction endonuclease enzyme
b. Isolation of specific human DNA
c. Insertion of isolated human DNA into vector to form hybrid DNA
d. All of the above

**9. Which of the following is correct for complementary DNA (cDNA) library?**
a. Is reverse transcribed from functional eukaryotic mRNA
b. Remains as a single stranded molecule
c. Exons as well as introns are presented
d. All of the above

**10. Restriction endonucleases:**
a. Cut RNA chains at specific locations
b. Excise introns from mRNA
c. Remove Okazaki fragments
d. Act as defensive enzymes to protect the host bacterial DNA from DNA of foreign organisms

**11. Restriction endonucleases recognize and cut a certain sequence of:**
a. Single stranded DNA
b. Double stranded DNA
c. RNA
d. Protein

**12. Restriction endonucleases are present in:**
a. Viruses
b. Bacteria
c. Eukaryotes
d. All of these

**13. Restriction endonucleases can recognize:**
a. Palindromic sequences
b. Chimeric DNA
c. DNA-RNA hybrids
d. Homopolymeric sequences

**14. All of the following statements about restriction endonucleases are true *except*:**
a. They are present in bacteria
b. They act on double stranded DNA
c. They recognize palindromic sequences
d. They always produce sticky ends

**15. Fragments of DNA can be identified by the technique of:**
a. Western blotting b. Eastern blotting
c. Northern blotting d. Southern blotting

**16. A particular RNA in a mixture can be identified by:**
a. Western blotting b. Eastern blotting
c. Northern blotting d. Southern blotting

**17. The first protein synthesized by recombinant DNA technology was:**
a. Streptokinase
b. Human growth hormone
c. Tissue plasminogen activator
d. Human insulin

**18. Trials for gene therapy in human beings were carried out in which following genetic disease?**
a. Sickle cell disease
b. Thalassemia
c. Adenosine deaminase deficiency
d. All of the above

**19. Chimeric DNA:**
a. Is found in bacteriophages
b. Contain unrelated genes
c. Has no restriction sites
d. Is palindromic

**20. In addition to Taq polymerase, polymerase chain reaction requires all of the following *except*:**
a. A template DNA
b. Deoxyribonucleoside triphosphates
c. Primers
d. Primase

## Answers for MCQs

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

CHAPTER

# 23 Mechanism of Hormone Action

**Chapter outline**

- Classification of Hormones
- Mechanism of Hormone Action at Cytosolic or Nuclear Level
- Cell Membrane Receptor Mechanism of Hormone Action

## INTRODUCTION

The word hormone is derived from a Greek verb meaning ***to excite***. Hormones are produced by special cells or glands such as adrenals, ovaries, parathyroids, pituitary, testes and thyroid. These glands secrete their hormones directly into the bloodstream and are known as ***endocrine glands***. Endocrinology is the study of hormones and their interactions.

A hormone is a chemical messenger, secreted in trace amounts by one type of tissue and carried by the blood to a target tissue elsewhere in the body to stimulate a specific biochemical or physiological activity.

**Figure 23.1** shows a schematic master plan of the regulatory relationship between the endocrine glands and their target tissue.

- Nerve impulses received by the hypothalamus cause it to send specific hormones to the pituitary gland, stimulating (or inhibiting) the release of different tropic hormones.
- The anterior pituitary hormones in turn can stimulate other endocrine glands to secrete their characteristic hormones, which in turn, stimulate specific target tissues.

## CLASSIFICATION OF HORMONES

Hormones can be classified according to:

- Chemical structure
- Mechanism of hormone action.

### Classification Based on Chemical Structure

Hormones are usually classified into three main groups on the basis of their chemical structure as follows:

1. Peptide or protein hormones.
2. Amine hormones or amino acid derivatives.
3. Steroid hormones.

#### *Peptide or Protein Hormones*

Most hormones fall into this class. These are water soluble and may have 3 to over 200 amino acid residues, e.g. hormones of the hypothalamus, pituitary and insulin and glucagon of the pancreas.

#### *Amine Hormones or Amino Acid Derivatives*

These are small, water soluble compounds containing amino groups. For example:

- Adrenaline of the adrenal medulla
- Thyroid hormones.

#### *Steroid Hormones*

These are fat soluble (lipophilic) and all are derivatives of cholesterol. For example:

- Adrenal cortical hormones
- Androgen (male sex hormones)
- Estrogens (female sex hormones).

**Fig. 23.1:** Schematic master plan of the regulatory relationship between the endocrine glands and their ultimate target tissue. 1: First target tissue; 2: Second target tissue; 3: Third target tissue.

## Classification Based on Mechanism of Hormone Action

Hormones can be classified according to mechanism of hormone action to:

1. Group I hormones
2. Group II hormones.

This classification is based on location of the hormone receptors.

- The hormones of **Group I** are **lipophilic** which readily pass through the lipophilic plasma membrane of the target cells and interacts with receptors which are located intracellularly in either the **cytosol** or the **nucleus.** The hormones that have intracellular receptors are given in **Table 23.1**.
- The receptors for the different **steroid hormones** are found mainly in the **cytoplasm** and the receptors for the **thyroid hormones** are found in the **nucleus.**
- The hormones of **group II** are **water-soluble peptides** and **amine (except $T_3$ and $T_4$)** hormones **(Table 23.1)** which do not penetrate lipophilic cell membrane readily. The receptors for such hormones are located on the outer surface of the target cell (cell surface receptors).

The first step of hormone action is binding of hormone to specific receptors of the target cell. Hormone receptor complex activates the receptor itself and the activated receptor initiates the hormonal effects. Hormonal receptors are large proteins, which are highly specific for a single hormone. Due to this specificity a particular hormone will act on a particular tissue.

**Table 23.1:** Classification for hormone based on mechanism of action.

| *Class* | *Second messenger or mediator* | *Examples* |
|---|---|---|
| **Group I**<br>Cytosolic or nuclear<br>Receptor | Hormone-receptor complex | Androgens<br>Estrogens<br>Glucocorticoids<br>Mineralocorticoids<br>Progesterol<br>Thyroid hormones ($T_3$ and $T_4$) |
| **Group II**<br>Cell membrane receptor | c-AMP<br><br><br>Calcium or Phosphatidylinositol or both | Epinephrine<br>Norepinephrine<br>Glucagon<br>Parathyroid hormone<br>Vasopressin<br>Oxytocin |

## MECHANISM OF HORMONE ACTION AT CYTOSOLIC OR NUCLEAR LEVEL

- Several hormones, e.g. steroid hormones (adrenal and gonadal) and thyroid hormones bind with receptors inside the cell rather than in the cell membrane. Because these hormones are lipid soluble, they readily cross the cell membrane and interact with receptors in the cytoplasm or nucleus.
- The receptors for the steroid hormones are found mainly in the cytoplasm and the receptors for the thyroid hormones are found in the nucleus **(Fig. 23.2)**.
- The **hormone receptor complex** is assumed to be the **intracellular messenger** for these (Group I) hormones.
- The activated hormone receptor complex undergoes conformational change which then binds with a specific regulatory (promoter) sequence of DNA called the

**Fig. 23.2:** Schematic representation of mechanism of hormone action at cytosolic or nuclear level. (H: Hormone; R: Receptor; R-H: Hormone receptor complex; HRE: Hormone response element)

**hormone response element (HRE)** which activates transcription of specific genes and formation of mRNA.

- These mRNA get translate into proteins. Newly formed proteins in the cell control the cellular metabolic functions.

## CELL MEMBRANE RECEPTOR MECHANISM OF HORMONE ACTION

- The receptors for group II hormones are located on the **outer surface of the target cell** because these hormones are water soluble which do not penetrate lipophilic cell membrane.
- Hormones that bind to surface receptors of the cells communicate their action through intermediary molecules called **second messenger** (the hormone itself is the first messenger).
- On binding of the hormone to the receptor, a **conformational change** occurs in the receptor that causes activation of the G-protein (GTP-binding protein). This results from the exchange of GDP to GTP on the α-subunit. G-protein is a group of three subunits α, β, γ. In absence of the hormone the G-protein is in an inactive form. Inactive G-protein is bound to GDP (**Fig. 23.3**).
- Activation of G-protein by hormone leads to dissociation of α-subunit (to which GTP is bound) from β, γ subunits of the G-protein.
- The dissociated α-subunit with bound GTP interacts with other intracellular signaling enzymes such as **adenylate cyclase** or **phospholipase-C** which generates second messenger. Interaction of α-subunit with **adenylate cyclase** generates **c-AMP** as a second messenger and interaction with **phospholipase C** generates **inositol triphosphate ($IP_3$)** and **diacylglycerol (DAG)** as a second messenger from **phosphatidylinositol**. These second messengers serve as mediators of the hormone action, as discussed below.

### c-AMP Second Messenger

c-AMP derived from ATP through the action of **adenylate cyclase** is the second messenger for many hormones, e.g. epinephrine, glucagon, calcitonin, PTH, etc.

- c-AMP then activates c-AMP dependent **protein kinase,** which phosphorylates specific proteins (enzymes) in the cell. Phosphorylation alters activities of these enzymes, some are activated (e.g. glycogen phosphorylase) while some are inactivated (e.g. glycogen synthase).

### Phosphatidylinositol/Calcium Second Messenger

- Certain hormone-receptor interaction result in the activation of the enzyme **phospholipase C** through a specific G-protein **(Fig. 23.4)**.

**Fig. 23.3:** Cell membrane receptor mechanism of hormone action through c-AMP as a second messenger.

**Fig. 23.4:** Cell membrane receptor mechanism of hormone action through phosphatidylinositol or calcium or calcium calmodulin as a second messenger.
(H: Hormone, R: Receptor, $PIP_2$: Phosphatidylinositol; DAG: Diacylglycerol; $IP_3$: Inositol triphosphate)

- Phospholipase C enzyme catalyzes the breakdown of phospholipids in cell membrane especially phosphatidylinositol bisphosphate ($PIP_2$) into two different second messenger products:
  1. Inositol triphosphate ($IP_3$)
  2. Diacylglycerol (DAG).
- The $IP_3$ liberates stored intracellular calcium ions from mitochondria and endoplasmic reticulum.
- The calcium in turn acts as a third messenger which influences a variety of biochemical processes through the mediation of calmodulin.
- **DAG,** the other second messenger, activates the enzyme **protein kinase C** (PKC), which then alter physiological processes. The hormones thyrotropin releasing hormone (TRH) gastrin, cholecystokinin acts through this second messenger.

## EXAM QUESTIONS

### Long Answer Questions (LAQs)

1. Describe the mechanism of hormone action for group I hormones.
2. Describe the mechanism of hormone action for group II hormones.

### Short Notes

1. cAMP as a second messenger for hormone action.
2. Cell membrane receptor mechanism of hormone action.
3. Cytosolic or nuclear receptor mechanism of hormone action.
4. Phosphatidylinositol as a second messenger for hormone action.
5. Mechanism of steroid hormone action.

### Multiple Choice Questions (MCQs)

1. **Binding of the following hormones to receptor activate adenylate cyclase, *except*:**
   a. Vasopressin
   b. Glucagon
   c. Thyroxine
   d. Epinephrine

**2. The following are the intracellular second messenger for hormone action, *except*:**
a. cAMP
b. cGMP
c. Phosphatidylinositol
d. NADPH

**3. Water soluble hormones have cell surface receptors, *except*:**
a. Glucagon
b. Epinephrine
c. Thyroxine
d. Vasopressin

**4. Hormone that have intracellular receptor:**
a. Glucocorticoids
b. ACTH
c. TSH
d. Glucagon

**5. Second messenger that mobilizes intracellular calcium is:**
a. Phosphatidic acid
b. Diacylglycerol
c. Inositol triphosphate
d. cGMP

## Answers for MCQs

**1.** c **2.** d **3.** c **4.** a **5.** c

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CHAPTER

# 24

# Acid-Base Balance

**Chapter outline**


- Acids, Bases and Buffers
- Normal pH of the Body Fluids
- Regulation of Blood pH
- Buffer Systems and their Role in Acid-Base Balance
- Respiratory Mechanism in Acid-Base Balance
- Renal Mechanism in Acid-Base Balance
- Acidosis and Alkalosis
- Anion Gap


## INTRODUCTION

To achieve acid-base balance, there must be a balance between the intake or production of hydrogen ions and net removal of hydrogen ions from the body. The various mechanisms that contribute to the regulation of hydrogen ion concentration are discussed in this chapter.

## ACIDS, BASES AND BUFFERS

- An **acid** is defined as a substance that releases protons or hydrogen ions ($H^+$), e.g. hydrochloric acid (HCl), carbonic acid ($H_2CO_3$).

$$HCl \longrightarrow H^+ + Cl^-$$

$$H_2CO_3 \longrightarrow H^+ + HCO_3^-$$

- A **base** is a substance that accepts protons or hydrogen ions, e.g. bicarbonate ion ($HCO_3^-$), and $HPO4^{--}$

$$HCO_3^- + H^+ \longrightarrow H_2CO_3$$

$$HPO_4^{--} + H^+ \longrightarrow H_2PO_4^-$$

  Proteins in the body also function as bases, because some of the amino acids accept hydrogen ions, e.g. **hemoglobin** in red blood cells and plasma protein, especially **albumin** is the most important of the body's bases.
- **Buffer** is a solution of weak acid and its corresponding salt, which resists a change in pH when a small amount of acid or base is added to it. By buffering mechanism, a strong acid (or base) is replaced by a weaker one.

## NORMAL pH OF THE BODY FLUIDS

- **The normal pH of arterial blood is 7.4**, whereas the pH of venous blood and interstitial fluids is about 7.35 because of the extra amounts of carbon dioxide ($CO_2$), released from the tissues to form $H_2CO_3$ in these fluids. ***Thus, the pH of blood is maintained within a remarkable constant level of 7.35-7.45.***
- Normal pH of body fluids are shown in **Table 24.1**.
- The maintenance of a constant pH is important because, the activities of almost all enzyme systems in the body are influenced by hydrogen ion concentration. Therefore, changes in hydrogen ion concentration alter virtually all cell and body functions, the conformation of biological structural components and uptake and release of oxygen.

### Metabolic Sources of Acids and Bases which Tend to Alter pH of the Body Fluids

#### *Metabolic Sources of Acids*

During metabolic processes, two types of acids are produced:

**Table 24.1:** Normal pH of body fluids

| *Body fluid* | *pH* |
|---|---|
| Extracellular fluid | |
| • arterial blood | 7.40 |
| • venous blood, and interstitial fluid | 7.35 |
| Intracellular fluid | 6.0–7.4 |
| Urine | 4.5–8.0 |
| Gastric HCl | 0.8 |

### *Fixed Acids or Nonvolatile Acids*

Fixed acids are nongaseous acids, such as:

- Phosphoric and sulfuric acids, produced from the sulfur and phosphorus of proteins and lipoproteins.
- Organic acids, such as pyruvic acid, lactic acid, ketoacids (acetoacetic and β-hydroxybutyric acid), and uric acid.

### *Volatile Acids*

The physiologically important volatile acid is **carbonic acid ($H_2CO_3$)**. It is equivalent to 36 liters of 1.0 N acid.

### *Metabolic Sources of Bases*

Catabolism of few food materials produces bases. For example:

- Citrate salts of fruit juices may produce bicarbonate salt.
- Deamination of amino acids produces ammonia.
- Formation of biphosphate and acetate also contributes to alkalinizing effect.

## REGULATION OF BLOOD pH

To maintain the blood pH at **7.35–7.45**, there are three primary systems that regulate the hydrogen ion concentration in the body fluids. These are:

1. **Buffer mechanism:** First line of defense.
2. **Respiratory mechanism:** Second line of defense.
3. **Renal mechanism:** Third line of defense.

The first two lines of defense keep the hydrogen ion concentration from changing too much until the more slowly responding third line of defense, the kidneys, can eliminate the excess acid or base from the body.

## BUFFER SYSTEMS AND THEIR ROLE IN ACID-BASE BALANCE

- The buffer systems of the blood, tissue fluids and cells; immediately combine with acid or base to prevent excessive changes in hydrogen ion concentration.
- Buffer systems do not eliminate hydrogen ions from the body or add them to the body but only keep them tied up until balance can be re-established.

### Blood Buffers

Various buffer systems present in human body are given in **Table 24.2**.

1. Buffers of **extracellular fluid** present in plasma.
   i. Bicarbonate buffer ($NaHCO_3/H_2CO_3$).
   ii. Phosphate buffer ($Na_2HPO_4/NaH_2PO_4$).
   iii. Protein buffer (Na protein/H protein).
2. Buffers of **intracellular fluid** present in RBCs
   i. Bicarbonate buffer ($KHCO_3/H_2CO_3$).
   ii. Phosphate buffer ($K_2HPO_4/KH_2PO_4$).
   iii. Hemoglobin buffer (KHb/HHb), ($KHbO_2/HHbO_2$).

### *Bicarbonate Buffer System ($HCO_3^-/H_2CO_3$)*

The bicarbonate buffer system is the most important extracellular buffer.

> Under physiological conditions, with a plasma pH 7.4, the ratio of bicarbonate to carbonic acid ($HCO_3^-/H_2CO_3$) is 20:1.

#### *Mechanism of action of bicarbonate buffer*

- When a strong acid, such as HCl, is added to the bicarbonate buffer solution, the increased hydrogen ions are buffered by $HCO_3^-$.

$$HCO_3^- + H^+ \longrightarrow H_2CO_3$$

- Thus, hydrogen ions from strong acid HCl react with $HCO_3$ to form very weak acid $H_2CO_3$.
- The opposite reactions take place when a strong base, such as sodium hydroxide (NaOH), is added to the bicarbonate buffer solution.

$$NaOH + H_2CO_3 \longrightarrow NaHCO_3 + H_2CO_3$$

- In this case, the hydroxyl ion ($OH^-$) from NaOH combines with $H_2CO_3$ to form weak base $HCO_3^-$. Thus, strong base NaOH is replaced by a weak base $NaHCO_3$.

> Plasma bicarbonate is a measure of the base that remains after all acids, stronger than carbonic, have been neutralized. It represents the reserve of alkali available for the neutralization of such strong acids and it has been termed as the ***alkali reserve***.

**Table 24.2:** Principal buffers of the blood

| *Buffer system* | *Plasma (extracellular) buffer* | *Erythrocyte (intracellular) buffer* |
|---|---|---|
| Bicarbonate | $NaHCO_3/H_2CO_3$ | $KHCO_3/H_2CO_3$ |
| Phosphate | $Na_2HPO_4/NaH_2PO_4$ | $K_2HPO_4/KH_2PO_4$ |
| Protein | Na Protein/H Protein | KHb/HHb $KHbO_2/HHbO_2$ |

## Phosphate Buffer System ($HPO_4^{--}/H_2PO_4^-$)

- The phosphate buffer system is not important as a blood buffer, it plays a major role in buffering renal tubular fluid and intracellular fluids.
- Its concentration in both plasma and erythrocytes is low, i.e. only 8% of the concentration of the bicarbonate buffer. Therefore, the total buffering power of the phosphate system in the blood is much less than that of the bicarbonate buffering system.

### *Mechanism of action of phosphate buffer*

- The main elements of the phosphate buffer system are $HPO_4^{--}$ and $H_2PO_4^-$. When a strong acid such as HCl is added to a phosphate buffer system, the $H^+$ is accepted by the base $HPO_4^{--}$ and converted to $H_2PO_4^-$ and strong acid HCl is replaced by a weak acid $H_2PO_4$ and decrease in pH is minimized.

$$HCl + Na_2HPO_4 \longrightarrow NaH_2PO_4 + NaCl$$

- When strong base, such as NaOH, is added to the buffer system, the $OH^-$ is buffered by the $H_2PO_4^-$ to form $HPO_4^{--}$ and water. Thus, strong base NaOH is replaced by weak base $HPO_4^{--}$, causing slight increase in the pH.

$$NaOH + NaH_2PO_4 \longrightarrow Na_2HPO_4 + H_2O$$

At a plasma pH of 7.4, the ratio $HPO_4^{--} : H_2PO_4^-$ is 4:1.t

## Protein Buffer

### *Plasma protein buffer (Na protein/H protein)*

- In the blood, plasma proteins especially **albumin** acts as buffer because:
  - Proteins contain a large number of dissociable *acidic (COOH)* and *basic ($NH_2$)* groups in their structure.
  - In acid solution they act as a buffer in that, the basic amino group ($NH_2$) takes up excess $H^+$ ions forming ($NH_3^+$).
  - Whereas in basic solutions, the acidic COOH groups give up hydrogen ion forming $OH^-$ of alkali to water.
  - Other important buffer groups of proteins in the physiological pH range are the **imidazole groups of histidine**. Each albumin molecule contains **16 histidine** residues.

### *Hemoglobin buffer (KHb/HHb and $KHbO_2/HHbO_2$)*

Hemoglobin is the major **intracellular buffer** of the blood which is present in erythrocytes. Hemoglobin has high concentration of histidine residues. Each Hb molecule contains **38 molecules of histidine**. The imidazole group of histidine has a pKa of approximately 7.3, fairly close to 7.4. High concentration and appropriate pKa makes hemoglobin the dominant buffering agent of blood at physiological pH. It buffers **carbonic acid ($H_2CO_3$)** and its **anhydride $CO_2$** from the tissues.

**Fig. 24.1:** Action of hemoglobin buffer in tissue. (CA: Carbonic anhydrase).

**Action of Hemoglobin Buffer:** Hemoglobin works effectively in cooperation with the bicarbonate system. The several reactions occurring in regulation of body pH by hemoglobin are given below.

#### *In The Tissues*

- The oxyhemoglobin arriving at the tissues cells, dissociates to release deoxyhemoglobin (KHb) and oxygen. **In the tissues**, the $CO_2$ formed by metabolic processes diffuses into red blood cell and is converted to carbonic acid ($H_2CO_3$) by **carbonic anhydrase (CA)**. The $H_2CO_3$ thus formed ionizes to form $H^+$ and $HCO_3^-$ and results in decrease in blood pH. Deoxyhemoglobin (KHb) acts as a buffer and accepts these $H^+$ ions to form HHb (weak acid) and $KHCO_3$. Thus, $H^+$ ions produced from $H_2CO_3$ do not cause any change in pH **(Fig. 24.1)**.
- Now, the increase in bicarbonate concentration in the erythrocyte leads to diffusion of these ions from the erythrocytes into the plasma, where its concentration is low. To preserve electroneutrality, some negative ion must enter the erythrocyte for each bicarbonate ion coming out. Since the cell is readily **permeable to chloride** ions, chloride ion enters the red blood cell for each bicarbonate molecule that leaves it. This change is called the **chloride shift**.

#### *In The Lungs*

- The bicarbonate diffused from erythrocyte to plasma is carried back by the venous blood to the lungs. In the lungs, deoxyhemoglobin (HHb) carried from tissue

**Fig. 24.2:** Action of hemoglobin buffer in lungs. (CA: Carbonic anhydrase).

is oxygenated to oxyhemoglobin ($HHbO_2$). Since, **oxyhemoglobin ($HHbO_2$)** is a **stronger acid**, it results in the release of $H^+$, which is buffered by $KHCO_3^-$ to give $H_2CO_3$ and $KHbO_2$. This buffering effect reduces the pH change as a result of the oxygenation of HHb **(Fig. 24.2)**.

- As the concentration of $HCO_3^-$ in the erythrocytes is reduced, as these are used to buffer $HHbO_2$, $HCO_3^-$ from the plasma, where its concentration is higher, enters into the erythrocyte. Again a shift of chloride ions in exchange for the bicarbonate ions occurs in the lungs but, this time, chloride ions must leave erythrocyte for each bicarbonate molecule that enters it. The carbonic acid formed is converted quickly in the presence of the carbonic anhydrase (CA) to carbon dioxide and water which is eliminated by ventilation.

## RESPIRATORY MECHANISM IN ACID-BASE BALANCE

- The second line of defense against acid-bases disturbances is by regulating the concentration of carbonic acid ($H_2CO_3$) in the blood and other body fluids by the lungs.
- The respiratory center regulates the removal or retention of $CO_2$ and, thereby, $H_2CO_3$ from the extracellular fluid by the lungs. Thus, lungs function by maintaining one component ($H_2CO_3$) of the bicarbonate buffer as follows:
  - An increase in ($H^+$) or ($H_2CO_3$) stimulates the respiratory center to increase the rate of respiratory ventilation. When the ventilation rate increases, more $CO_2$ is released from the blood and pH increases.
  - Similarly, an increase in ($OH^-$) or ($HCO_3^-$) depresses respiratory ventilation. A decrease in ventilation rate will cause a decrease in release of $CO_2$ from the blood. The increased blood $CO_2$ will result in the formation of more $H_2CO_3$. Thus, there will be decrease in pH.
- Thus, when the rate of ventilation is increased, excess acid ($H_2CO_3$) in the form of $CO_2$ is quickly removed. Similarly, when the rate of ventilation is decreased, acid ($H_2CO_3$) in the form of $CO_2$ is added to neutralize excess alkali ($HCO_3^-$).

Respiratory system acts as a **feedback controller** of $H^+$ concentration **(Fig. 24.3).** Increased $H^+$ concentration stimulates respiratory center and alveolar ventilation. This decreases the concentration of $CO_2$ in extracellular fluid and reduces $H^+$ concentration back to normal. Conversely, decreased $H^+$ concentration below normal depresses respiratory center and alveolar ventilation and $H^+$ concentration increases back to normal.

## RENAL MECHANISM IN ACID-BASE BALANCE

Renal mechanism is the third line of defense in acid-base balance. Long-term acid-base control is exerted by renal mechanisms. Kidney participates in the regulation of acid-base balance primarily by **conservation of $HCO_3^-$ (alkali reserve)** and excretion of acid, as the case may be.

- The pH of the initial glomerular filtrate is approximately 7.4, same as that of plasma, whereas the average urinary pH is approximately 6.0 due to the renal excretion of nonvolatile acids produced by metabolic processes.
- The pH of the urine may vary from 4.5 to 8.0 corresponding to the case of acidosis or alkalosis. In acidosis, excretion of acids is increased and base is conserved, in alkalosis, the opposite occurs. This ability to excrete

**Fig. 24.3:** Feedback control of $H^+$ concentration by respiratory system

variable amounts of acid or base makes the kidney the final defense mechanism against change in body pH.

- Renal conservation of $HCO_3^-$ and excretion of acid occur through four key mechanisms **(Fig. 24.4)**.
  1. Exchange of $H^+$ for $Na^+$ of tubular fluid.
  2. Reabsorption (reclamation) of bicarbonate from tubular fluid.
  3. Formation of ammonia and excretion of ammonium ion ($NH_4^+$) in the urine.
  4. Excretion of $H^+$ as $H_2PO_4^-$ in urine.

## Exchange of $H^+$ for $Na^+$ of Tubular Fluid

- In renal tubular cells, the carbonic anhydrase catalyzes the formation of carbonic acid ($H_2CO_3$) from $CO_2$ and water. The carbonic acid thus formed dissociates to yield $H^+$ and $HCO_3^-$.
- The $H^+$ ions formed in tubular cells are secreted into the tubular fluid in exchange for $Na^+$ present in tubular fluid **(Fig. 24.4)**.
- The bicarbonate anion formed by the dissociation of $H_2CO_3$ in the tubular cell diffuses into the blood as the accompanying ion to $Na^+$ and $HCO_3^-$ is thus conserved and increases the **'alkali reserve'** of the body.

## Reabsorption of Bicarbonate from Tubular Fluid

- Some $H^+$ that are secreted into the tubular fluid in exchange of $Na^+$ react with $HCO_3^-$ in the tubular fluid to form $H_2CO_3$, which is dehydrated to $CO_2$ and $H_2O$ by an enzyme carbonic anhydrase.
- The increase in $CO_2$ in tubular fluid causes carbon dioxide to diffuse into the tubular cell, where it reacts with $H_2O$ to form $H_2CO_3$ and subsequently, $H^+$ and $HCO_3^-$. Thus, reabsorption of bicarbonate is in terms of diffusion of $CO_2$ into tubular cells and its subsequent conversion to $HCO_3^-$ **(Fig. 24.4)**.
- The process of bicarbonate reabsorption is enhanced in states of acidosis and decreased in alkalosis.
- The kidney reabsorbs almost all filtered bicarbonates at plasma bicarbonate concentration below 25 mEq/L. Only when bicarbonate levels become elevated above 25 mEq/L, bicarbonate will be excreted into the urine.

## Formation of Ammonia and Excretion of Ammonium Ions ($NH_4^+$) in the Urine

- Ammonia (the urinary buffer) is produced by deamination of glutamine in renal tubular cell. **Glutaminase** present in tubular cells catalyzes this reaction.

**Fig. 24.4:** Renal mechanism in acid-base balance

- Ammonia is a gas and diffuses readily across the cell membrane into the tubular lumen, where it buffers hydrogen ions to form ammonium ($NH_4^+$) ions **(Fig. 24.4)**.
- The $NH_4^+$ ions formed in the tubular lumen cannot diffuse back into tubular cells and thus, is trapped in the tubular urine and excreted with anions, such as phosphate, chloride or sulfate.
- The rate of glutamine uptake from the blood and its utilization by the kidney depends on the amount of acid which must be excreted to maintain a normal blood pH. As the level of $H^+$ ions of the blood increases (pH decreases), glutamine uptake increases. During a metabolic acidosis, the excretion of $NH_4^+$ by the kidney increases several fold.
- The removal of hydrogen ions as $NH_4^+$ decreases the requirement of bicarbonate to buffer the urine.

### Excretion of $H^+$ Ions as $H_2PO_4^-$ in Urine

- The hydrogen ions secreted into the tubular fluid in exchange of $Na^+$ are buffered by $HPO_4^{--}$ of phosphate buffer. $HPO_4^{--}$ combines with the secreted $H^+$ and is converted to $H_2PO_4^-$ and is excreted in the urine as $NaH_2PO_4$.
- This process depends on the amount of phosphate filtered by the glomeruli and the pH of urine. Acidemia increases phosphate excretion and, thus, provides additional buffer for reaction with $H^+$.
- A decrease in the glomerular filtration rate (GFR) with renal disease may result in a decrease of $H_2PO_4^-$ excretion.

## ACIDOSIS AND ALKALOSIS

Acid-base balance depends on the ***ratio $HCO_3^-$/ $H_2CO_3$*** which is constant at ***20:1*** at physiological pH. Any alteration produced in the ratio between carbonic acid and bicarbonate results in an acid-base imbalance and leads to **acidosis** or **alkalosis**.

- ***Acidosis*** may be defined as an abnormal condition caused by the accumulation of excess acid in the body or by the loss of alkali from the body.
- ***Alkalosis*** is an abnormal condition caused by the accumulation of excess alkali in the body or by the loss of acid from the body.

Acidosis and alkalosis are classified, in terms of their immediate cause, as follows:

1. **Metabolic acidosis:** Decrease in bicarbonate ($HCO_3^-$) concentration.
2. **Respiratory acidosis:** Increase in $H_2CO_3$ concentration.
3. **Metabolic alkalosis:** Increase in bicarbonate ($HCO_3^-$) concentration.
4. **Respiratory alkalosis:** Decrease in $H_2CO_3$ concentration.

### Metabolic Acidosis

A fall in blood pH due to a decrease in bicarbonate levels of plasma is called ***metabolic acidosis.***

- Decrease in bicarbonate levels may be due to:
  - Increased production of acids. In uncontrolled diabetes mellitus and starvation, there is an excessive production of acetoacetic acid and β-hydroxybutyric acid. These acids are buffered by utilizing base component (i.e. $HCO_3^-$) of the bicarbonate buffer. Consequently, the concentration of bicarbonate ions falls giving rise to bicarbonate deficit and results in metabolic acidosis (ketoacidosis).
  - Excessive loss of bicarbonate occurs in the urine in renal tubular dysfunction and from GI tract in severe diarrhea.

#### *Compensatory Mechanisms*

- Metabolic acidosis is compensated by:
  1. Increasing rate of respiration to wash out $CO_2$ (hence $H_2CO_3$) faster. Consequently, the ratio $HCO_3^-$ : $H_2CO_3$ is elevated.
  2. Increasing excretion of $H^+$ ions as $NH_4^+$ ions.
  3. Increasing elimination of acid ($H_2PO_4^-$) in the urine.

All these compensatory mechanisms tend to reduce carbonic acid to keep the pH in the normal range and a compensated acidosis results.

### Respiratory Acidosis

It results from an increase in concentration of carbonic acid ($H_2CO_3$) in plasma. An increase in concentration of $H_2CO_3$ is due to decrease in alveolar ventilation, and that leads to retention of $CO_2$. Decreased alveolar ventilation may occur in the following circumstances:

- **Obstruction to respiration:** This may occur in pneumonia, emphysema, asthma, etc.
- **Depression of respiration:** Administration of respiratory depressant toxic drugs, e.g. morphine depresses the respiratory center.

### *Compensatory Mechanisms*

1. Increase in renal reabsorption of bicarbonate.
2. Rise in urinary acid ($H_2PO_4^-$) and ammonia.

## Metabolic Alkalosis

A rise in blood pH due to rise in the bicarbonate levels of plasma is called **metabolic alkalosis**. This is seen in the following conditions:

- Loss of gastric juice along with $H^+$ ions in prolonged and severe vomiting.
- Therapeutic administration of large dose of alkali (as in peptic ulcer) or chronic intake of excess antacids.

### *Compensatory Mechanisms*

1. Increased excretion of alkali ($HCO_3^-$) by the kidney
2. Diminished formation of ammonia
3. Respiration is depressed to conserve $CO_2$.

## Respiratory Alkalosis

A rise in blood pH due to lowered concentration of $CO_2$ or $H_2CO_3$, due to hyperventilation. This occurs in the following conditions:

- Anxiety or hysteria
- Fever
- Hot baths
- At high altitude
- Working at high temperature, etc.

### *Compensatory Mechanisms*

1. Reduction of urinary ammonia formation
2. Increased excretion of bicarbonate.

## Mixed Acid-Base Disturbances

- Respiratory and metabolic disorders of acid-base balance can occur together and called **mixed acid-base disturbances**.
- For example, some patients with chronic renal failure (which causes a primary metabolic acidosis) may also have chronic obstructive airways disease, which causes a primary respiratory acidosis.
- Plasma ($H^+$) will be increased in these patients, but the results for plasma $CO_2$ and concentration of ($HCO_3^-$) cannot be predicted. The history and clinical findings must be taken into account.

# ANION GAP

- The concept of anion gap originally was devised as a quality control rule when it was found that if the sum of the $Cl^-$ and $HCO_3^-$ values was subtracted from the $Na^+$ and $K^+$ values the difference or 'gap' averaged 16 mmol/L in healthy individuals.
- The concentration of anions and cations in plasma must be equal to maintain electrical neutrality. Therefore, there is no real anion gap in the plasma. Anion gap is not a physiological reality.
- The anion gap is the difference between **unmeasured anions** and **unmeasured cations** and is estimated as:

$$\begin{aligned}\text{Anion gap} &= ([Na^+] + [K^+]) - ([Cl^-] + [HCO_3^-]) \\ &= (142 + 4) - (103 + 27) \\ &= 146 - 130 \\ &= 16 \text{ mEq/L}\end{aligned}$$

- The most important unmeasured cations include **calcium, magnesium,** and the major unmeasured anions are **albumin, phosphate, sulfate** and other organic anions. The anion gap ranges between 8–16 mEq/L.
- Acid-base disorders are often associated with alterations in the anion gap.
- In metabolic acidosis, the anion gap can increase or remain normal depending on the cause of acidosis.

**Clinical significance of anion gap**

The anion gap is a biochemical tool which sometimes helps in assessing acid-base problems. It is used for the diagnosis of different causes of metabolic acidosis.

# EXAM QUESTIONS

## Long Answer Questions (LAQs)

1. Discuss the various mechanisms for regulation of acid-base balance.
2. Discuss disorders of acid-base balance.

## Short Notes

1. Explain the role of various blood buffers in the maintenance of blood pH.
2. Respiratory acidosis.
3. Causes of metabolic acidosis and their compensatory mechanisms.
4. Role of kidney in regulation of blood pH.
5. Hemoglobin as a buffering agent.
6. Metabolic acidosis.
7. Anion gap.

## Solve

**Case History 1**

**A 38-year-old man reported in the emergency ward of a hospital emergency with complaints of persistent vomiting for one week. He had generalized muscular cramps. On examination, he appeared dehydrated and had shallow respiration. Blood sample was analyzed with the following results:**

| | | |
|---|---|---|
| pH | = | 7.8 |
| Bicarbonates | = | elevated |
| $pCO_2$ | = | elevated |
| $Na^+$ | = | 145 mEq/L |
| $K^+$ | = | 2.9 mEq/L |

**Questions**

1. Identify the nature of acid-base disorder.
2. What could be the cause of this acid-base disorder?
3. What is the cause of shallow respiration?
4. Give reason for development of muscle cramps.

**Case History 2**

**A 50-year-old male was admitted with a history of chronic obstructive airways disease for many years. On examination, he was found cyanosed, and breathless. Blood sample was analyzed with the following results:**

| | | |
|---|---|---|
| Blood pH | = | below normal |
| $pCO_2$ | = | markedly elevated |
| $(HCO_3^-)$ | = | markedly elevated |

**Questions**

1. Identify the nature of acid-base disorder.
2. What could be the cause of elevated $pCO_2$?
3. What could be the cause of elevated $(HCO_3^-)$?
4. What are the compensated mechanisms for this acid-base disorder?

**Case History 3**

**A person presents himself with untreated diabetes mellitus. He is treated for acidosis.**

**Questions**

1. What is the type of acidosis?
2. What is the normal bicarbonate/carbonic acid ratio? What will happen to the ratio in this patient?
3. How will compensation occur?
4. What is the role of kidney in correcting acidosis?

**Case History 4**

**A 28-year-old man was admitted to hospital with a crushed chest. The biochemical results on arterial blood gases were found with high $[H^+]$ and $pCO_2$.**

**Questions**

a. What is the acid-base disorder?
b. Write compensatory mechanism for the disorder.
c. What is the normal blood pH?
d. What is the normal blood $pCO_2$?

**Case History 5**

**A 56-year-old woman was admitted seriously ill and confused. The patient had systemic edema. Diagnosis was that the patient had mixed acid-base disorder. On admission, the following biochemical results were obtained:**

i. Low $[H^+]$ concentration (Increased blood pH)
ii. Increased bicarbonate concentration
iii. Increase in $pCO_2$ is too high

**Questions**

a. What is the evidence for the disorder?
b. Which are the other acid-base disorders?
c. What is the normal blood pH?
d. What is the normal blood $pCO_2$?

## Multiple Choice Questions (MCQs)

1. **Metabolic acidosis is primarily due to:**
   a. Increase in carbonic acid
   b. Decrease in carbonic acid
   c. Decrease in bicarbonate
   d. Increase in bicarbonate

2. **In compensated metabolic acidosis plasma:**
   a. $HCO_3^-/H_2CO_3$ ratio is increased
   b. Total $CO_2$ content is decreased
   c. $HCO_3^-/H_2CO_3$ ratio is decreased
   d. None of the above

3. **Important buffer in extracellular fluid is:**
   a. Hemoglobin
   b. Bicarbonate
   c. Protein
   d. Phosphate

4. **All of the following are associated with metabolic alkalosis *except:***
   a. Rise in blood pH
   b. Rise in bicarbonate plasma levels
   c. Loss of $H^+$ ions
   d. Decrease in bicarbonate plasma levels

5. **Metabolic acidosis is associated with all of the following *except:***
   a. Increased elimination of acid in urine
   b. Increased formation of ammonia
   c. Increased respiration
   d. Diminished formation of ammonia

6. **The normal pH of plasma is maintained by all of the following *except:***
   a. Plasma buffer
   b. Lung's mechanism
   c. Heat mechanism
   d. Renal mechanism

7. **Normal pH of blood is:**
   a. 7.0 b. 7.2
   c. 7.4 d. 7.6

8. **At blood pH 7.4, the ratio of $NaHCO_3/H_2CO_3$ will be:**
   a. 5:1 b. 10:1
   c. 20:1 d. 4:1

9. **At blood pH 7.4, the ratio of $HPO_4^-/H_2PO_4^-$ will be:**
   a. 4:1 b. 5:1
   c. 20:1 d. 1:20

10. **Respiratory acidosis results from:**
    a. Obstruction to respiration
    b. Diabetes mellitus
    c. Starvation
    d. Hyperventilation

11. **Which of the following is volatile acid?**
    a. Phosphoric acid
    b. Carbonic acid
    c. Sulfuric acid
    d. Lactic acid

12. **Physiologically important carbonic acid ($H_2CO_3$) present in the body is equivalent to:**
    a. 36 liters of 0.1 N acid
    b. 56 liters of 1 N acid
    c. 36 liters of 1 N acid
    d. 36 liters of 0.01 N acid

13. **Carbonic acid ($H_2CO_3$) is buffered by:**
    a. Bicarbonate buffer
    b. Phosphate buffer
    c. Hemoglobin buffer
    d. None of the above

14. **Plasma bicarbonate is decreased in:**
    a. Respiratory alkalosis
    b. Respiratory acidosis
    c. Metabolic alkalosis
    d. Metabolic acidosis

15. **Plasma bicarbonate is increased in:**
    a. Respiratory alkalosis
    b. Metabolic alkalosis
    c. Respiratory acidosis
    d. Metabolic acidosis

16. **Total $CO_2$ is increased in:**
    a. Respiratory acidosis
    b. Metabolic acidosis
    c. Respiratory alkalosis
    d. All of the above

17. **Metabolic acidosis is caused in:**
    a. Pneumonia
    b. Prolonged starvation
    c. Depression of respiratory center
    d. Hysterical hyperventilation

18. **Respiratory alkalosis occurs in:**
    a. Hysterical hyperventilation
    b. Depression of respiratory center
    c. Renal diseases
    d. Loss of intestinal fluids

19. **Morphine poisoning causes:**
    a. Metabolic acidosis
    b. Respiratory acidosis

c. Metabolic alkalosis
d. Respiratory alkalosis

**20. Respiratory acidosis results from:**
a. Retention of carbon dioxide
b. Excessive elimination of carbon dioxide
c. Retention of bicarbonate
d. Excessive elimination of bicarbonate

**21. Respiratory acidosis can occur in all of the following *except*:**
a. Administration of respiratory depressant toxic drugs
b. Hysterical hyperventilation
c. Pneumonia
d. Emphysema

**22. Respiratory alkalosis can occur in:**
a. Bronchial asthma
b. Collapse of lungs
c. Hysterical hyperventilation
d. Bronchial obstruction

**23. Anion gap is the difference in the plasma concentrations of:**
a. (Chloride) – (Bicarbonate)
b. (Sodium) – (Chloride)
c. (Sodium + Potassium) – (Chloride + Bicarbonate)
d. (Sum of cations) – (Sum of anions)

**24. Normal anion gap in plasma is about:**
a. 5 meq/L
b. 16 meq/L
c. 25 meq/L
d. 40 meq/L

**25. Anion gap is normal in:**
a. Hyperchloremic metabolic acidosis
b. Diabetic ketoacidosis
c. Lactic acidosis
d. None of the above

**26. Metabolic alkalosis can occur in:**
a. Severe diarrhea
b. Renal failure
c. Recurrent vomiting
d. All of the above

**27. Which of the following is true for anion gap in plasma?**
a. There is no real anion gap in the plasma.
b. Anion gap is not a physiological reality.
c. The anion gap is the difference between unmeasured anions and unmeasured cations
d. All of the above

## Answers for MCQs

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **1.** c | **2.** c | **3.** b | **4.** d | **5.** d | **6.** c | **7.** c | **8.** c |
| **9.** a | **10.** a | **11.** b | **12.** c | **13.** c | **14.** c | **15.** b | **16.** a |
| **17.** b | **18.** a | **19.** b | **20.** a | **21.** b | **22.** c | **23.** c | **24.** b |
| **25.** a | **26.** c | **27.** d | | | | | |

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CHAPTER

# 25

# Organ Function Tests

**Chapter outline**

- Liver Function Tests
- Renal Function Tests
- Thyroid Function Tests
- Lipid Profile Tests
- Cardiac Markers

## INTRODUCTION

A large number of biochemical tests are carried out in the investigation of diseases. Many of them are well associated with the impairment of the function of particular organ and called ***organ function tests.*** Thus, organ function tests are the tests carried out to assess whether a particular organ is functioning normally or not. The following organ function tests are most common:

- Liver function tests
- Renal function tests
- Thyroid function tests
- Lipid profile tests

## LIVER FUNCTION TESTS

### Classification of Liver Function Tests

The liver performs four major functions:

1. Excretion/Secretion
2. Metabolism
3. Detoxification
4. Storage

As liver performs a multiple of functions, a number of tests are required to assess hepatic functions: All the tests need not be performed in every case. The tests should be selected according to the clinical symptoms and signs. Several tests used earlier, e.g. **icterus index, thymol turbidity, serum cholesterol: cholesteryl ester ratio**, etc. have now become outdated due to lack of specificity and or sensitivity. The following are the commonly used liver function tests. Liver function tests can be classified into five classes according to the function of the liver as given below:

**Tests based on excretory function** (related to excretion of toxic endogenous bilirubin and exogenous substances). It includes measurement of:

- Serum bilirubin
- Urine bilirubin
- Urine bile salts
- Bromosulphophthalein (BSP) dye tests

**Tests based on detoxification function**

It includes determination of:

- Blood ammonia and bilirubin
- Hippuric acid test

**Tests based on synthetic function**

It includes determination of:

- Plasma proteins, albumins and globulins
- Prothrombin time

**Tests based on metabolic function**

It includes:

- Test related to carbohydrate metabolism
- Galactose tolerance test
- Test related to lipid metabolism

  Determination of serum cholesterol and ratio of free to esterified cholesterol
- Test related to protein metabolism

  Serum protein estimation

  Serum ammonia estimation

**Tests related to enzymes in diagnosis of liver disease**

- Serum alanine transaminase (ALT)
- Serum aspartate transaminase (AST)
- Serum alkaline phosphatase (ALP)

## Liver Function Tests Based on Excretory Function

An important physiologic role of the liver is the removal of toxic endogenous and exogenous substances from the blood. The tests based on excretory function of liver are related to bilirubin metabolism.

### *Tests Based on Bilirubin Metabolism*

Bilirubin is the excretory end product of heme. It is conjugated in the liver to form bilirubin diglucuronide. Bilirubin is insoluble in water but bilirubin diglucuronide is soluble in water. The bilirubin glucuronide is excreted in the bile and through the bile goes to the intestine. There, it is reduced by to bacterial enzymes to urobilinogen.

- Bilirubin exists in the serum in two forms.
  - Conjugated or direct bilirubin which is water soluble.
  - Unconjugated or indirect bilirubin which is water insoluble.
- The normal concentration of total serum bilirubin is 0.1 to 1 mg/dL of which direct serum bilirubin ranges from 0.1 to 0.4mg/dL and indirect serum bilirubin is 0.2 to 0.7 mg/dL.

### *Serum Bilirubin Estimation by Van Den Bergh Reaction*

Estimation of serum bilirubin is based on Van Den Bergh reaction. In this reaction, when serum bilirubin is allowed to react with **Van den Bergh's diazo** reagent (sulfanilic acid and sodium nitrite in HCL) a purple colored **azobilirubin** is formed.

- Conjugated bilirubin being water soluble can react directly with aqueous solution of diazo reagent and so called ***direct bilirubin.***
- Whereas the unconjugated bilirubin is water insoluble and does not react in aqueous solution. It requires addition of methyl alcohol to react with diazo reagent in the determination method and called **indirect bilirubin**.
- If both conjugated and unconjugated bilirubin are present in increased amounts, a purple color is produced immediately and the color is intensified on addition of alcohol. Then, the reaction is called biphasic.

#### *Clinical interpretation*

Estimation of **direct** and **indirect bilirubin** is useful for the **differential diagnosis of jaundice.** Bilirubin metabolism is deranged in three important diseases. They are:

- Hemolytic jaundice
- Hepatic jaundice
- Obstructive jaundice

- In hemolytic jaundice, unconjugated bilirubin is increased. Hence, Van den Bergh test is indirect positive.
- In obstructive jaundice, conjugated bilirubin is elevated and Van den Bergh test is direct positive.
- In hepatic jaundice both conjugated and unconjugated bilirubin are increased hence a biphasic reaction is observed.
- Laboratory results in normal persons and patients with three different types of jaundice are shown in **Table 25.1**.

### *Urine Bilirubin*

- In normal individuals, bilirubin is not excreted in the urine. When it is present in the urine, it indicates some disease of the liver.
- Only conjugated bilirubin is soluble in water and is excreted in urine but not the unconjugated which is water insoluble.
- In urine, conjugated bilirubin can be detected by Fouchet's test.

#### *Clinical interpretation*

- Conjugated bilirubin appears in urine of patients in obstructive and hepatic jaundice.

**Table 25.1:** Laboratory results in normal and patients with three types of jaundice

| *Condition* | *Serum bilirubin* | | *Urine urobilinogen* | *Urine bilirubin* |
|---|---|---|---|---|
| | *Conjugated (Direct)* | *Unconjugated (Indirect)* | | |
| Normal | 0.1–0.4 mg/dL | 0.2–0.7 mg/dL | 0.4 mg/24 h | Absent |
| Hemolylic or prehepatic jaundice | Normal | Increased | Increased | Absent |
| Hepatic jaundice | Increased | Increased | Normal or Decreased | Present |
| Obstructive or posthepatic jaundice | Increased | Normal | Absent | Present |

- In hemolytic jaundice, unconjugated bilirubin is increased in blood, it does not appear in urine.

### Urobilinogen in Urine

The amount of urobilinogen present in urine depends on the amount of bilirubin entering the intestine.

Urine urobilinogen is estimated semi-quantitatively, by Ehrlich's aldehyde reagent.

*Clinical interpretation*

- Normally, trace amounts of urobilinogen are present in urine.
- An increase in urobilinogen in urine, is found in hemolytic jaundice due to excess production of bilirubin.
- In hepatitis, the urobilinogen in urine may be normal or decreased.
- In posthepatic obstructive jaundice, due to the complete or almost complete biliary obstruction, no urobilinogen is found in urine because bilirubin is unable to enter the intestine.

## Dye Excretion Test for Excretory Function

In addition to excreting bilirubin, the liver is capable of eliminating various dyes or drugs by the same excretory pathway as bilirubin.

### Bromosulfophthalein Excretion Test

A 5% solution of BSP is injected intravenously (the dose is 5 mg/kg body weight) and a sample of blood is tested 45 minutes later for percentage of injected dye remaining in the blood.

*Clinical interpretation*

- In normal, the retention of BSP at 45 minutes is less than 5%. Impairment of liver cell function causes an increase in BSP retention.

## Tests Based on the Detoxification Function

The liver is involved in the detoxification and removal of potentially hazardous substances from the body. These may be endogenous, e.g. **ammonia**, liver detoxicates ammonia to form urea and **bilirubin** which detoxicated to **bilirubin diglucuronide** (discussed earlier) or exogenous chemicals and drugs.

### Hippuric Acid Test

Hippuric acid test is based on detoxicating function of the liver. The liver removes **benzoic acid** by conjugating it with **glycine** to hippuric acid which is excreted in the urine.

In hippuric acid test, a dose of sodium benzoate is given either orally or intravenously and the amount of hippuric acid excreted in a fixed time is determined.

For the oral test, the patient ingests 6 gm sodium benzoate dissolved in about 250 mL water. Urine collections are made for the next 4 hours and the amount of hippuric acid excreted is estimated.

*Clinical interpretation*

- In normal subjects, at least 3 to 3.5 gm hippuric acid should be excreted in the bile during the 4 hours period.
- It is decreased in hepatitis, tumors, cirrhosis and obstructive jaundice.
- Excretion is normal in hemolytic jaundice.

### Determination of Blood Ammonia

Liver detoxicates ammonia to form urea. In a liver disease, the ability to remove ammonia may be impaired. The normal level of blood ammonia is 40–70 mg/100 mL of blood.

*Clinical interpretation*

High blood levels of ammonia are found in acute hepatitis and cirrhosis.

### Determination of Serum Bilirubin

Determination of serum bilirubin (discussed earlier).

## Tests Based on Synthetic Function

Liver is the main source of synthesis of plasma proteins, e.g. albumin, globulin (except γ-globulins which are synthesized in the reticuloendothelial system), blood clotting factors, e.g. fibrinogen, prothrombin and factors V, VII, IX, X. Impaired function of liver results in decreased protein synthesis.

### Determination of Serum Albumin and Globulin

The normal concentrations of serum proteins are given below:

- Total serum protein = 6 to 8 gm/dL
- Serum albumin = 3.5 to 5.5 gm/dL
- Serum globulin = 2 to 3.5 gm/dL
- Albumin/globulin ratio = 1.2:1 to 2.5:1

*Clinical interpretation*

- Hypoalbuminemia may occur in hepatocellular disease, e.g. cirrhosis.
- Hyperglobulinemia may be present in chronic inflammatory disorders such as cirrhosis and in infectious hepatitis.

- In advanced stages of liver disease, albumin is decreased and globulins are increased, so that the A/G ratio may be reversed. The concentration of total globulins increases due to a rise in the γ-globulins (synthesized by reticuloendothelial system and not by the liver) to compensate for a possible fall in the α-globulins by liver.

### Determination of Prothrombin Time

Hepatic synthetic function of several clotting factors can be assessed by a simple coagulation test, e.g. prothrombin time.

Various proteins that participate in blood coagulation are synthesized in the liver, e.g. fibrinogen, prothrombin (factor II) and factors V, VII, IX and X. If any one of these factors is deficient, the deficiency causes prolonged prothrombin time.

#### *Clinical interpretation*

- An increased prothrombin time indicates the failure of hepatic synthesis of one or more of the above mentioned clotting factors.
- As vitamin K is required for the synthesis of blood clotting factors, deficiency of vitamin K can also cause prolonged prothrombin time, which must be ruled out by estimating the prothrombin time, before and after administration of vitamin K. In case of liver disease, the prothrombin remains prolonged even after administration of vitamin K.

## Tests Based on Metabolic Function of Liver

### Tests Related to Carbohydrate Metabolism

#### *Galactose tolerance test*

Galactose is converted to glucose exclusively in liver, but this metabolic process is impaired in hepatocellular damage. In this test, the person is given 40 gm of galactose dissolved in water and if he has a normal liver, he should not excrete more than 3 gm of galactose in a 5 hour collection of urine after administration of galactose. However, in hepatocellular damage 4 to 5 gm or more of galactose is excreted in urine within 5 hours.

The normal liver is able to convert galactose to glucose. In patients with hepatic disease, this ability is defective.

### Tests related to Lipid Metabolism

Determination of **serum total cholesterol** and ratio of free to esterified cholesterol: Liver is involved in the synthesis, esterification and excretion of cholesterol. In a normal individual, the serum cholesterol level ranges between ***150 to 200 mg/dL,*** approximately 70% of which is esterified. Esterification occurs in liver and blood. Changes in the ratio of free to esterified cholesterol are often found in liver diseases, as the liver is also a site of esterification of cholesterol. Normally, the ester fraction predominates in plasma but in liver damage, it goes down.

#### *Clinical interpretation*

- Increase in total blood cholesterol is found in obstructive jaundice due to retention of cholesterol, which is normally excreted in the bile.
- Serum cholesterol is normal or depressed in hepatic jaundice. In severe acute liver necrosis, the total serum cholesterol value is usually low with a marked reduction in the percentage of the esterified cholesterol.

### Tests Related to Protein Metabolism

#### *Serum estimation of proteins*

The liver is the principal site of metabolism and synthesis of plasma proteins and amino acids. Amino acids are metabolized in the liver to ammonia and urea. Based on these metabolic functions of the liver, serum estimation of proteins, and ammonia (discussed earlier) are employed to assess the liver cell damage.

## Enzymes in Diagnosis of Liver Disease

Liver cells contain several enzymes. In liver damage, these enzymes are released into blood and levels of these enzymes increase in blood.

- A large number of different enzymes have been used in the diagnosis of liver disease. But most commonly and routinely employed in laboratory are **(Table 25.2):**
  - Serum aspartate transaminase (AST)
  - Serum alanine transaminase (ALT)
  - Serum alkaline phosphatase (ALP).
- Other enzymes which have been found to be useful but not routinely done in the laboratory are:
  - Serum 5,-nucleotidase
  - Lactate dehydrogenase
  - Isocitrate dehydrogenase
  - γ-Glutamyl transferase.

**Table 25.2:** Enzyme assays in differential diagnosis of jaundice.

| *Enzyme assays* | *Hemolytic or prehepatic jaundice* | *Hepatic jaundice* | *Obstructive or Posthepatic jaundice* |
|---|---|---|---|
| ALT or AST | Usually normal | Marked increase | Increased |
| ALP | Normal | Increased slightly | Marked increase |

### Serum Transaminases

- Liver is the richest source of:
  - **Aspartate transaminase (AST)** which is previously called serum glutamate oxaloacetate transaminase (SGOT).
  - **Alanine transaminase (ALT)** which is previously called serum glutamate pyruvate transaminase (SGPT).
- The normal range for these enzymes is as follows:
  - AST or SGOT = 4–17 IU/L
  - ALT or SGPT = 3–15 IU/L.
- Although, both AST and ALT are commonly thought of as liver enzymes because of their high concentrations in liver, ***only ALT is markedly specific for liver since AST is widely present in myocardium, skeletal muscle, brain and kidney and may rise in acute necrosis of these organs besides liver cell injury.***

#### Clinical interpretation

- Alanine transaminase (ALT) estimations are useful in early diagnosis to evaluate severity and prognosis of liver disease.
- In hepatitis, the levels of both these enzymes (ALT and AST) are increased, which go in thousand units, usually 500 to 1500 IU/L.
- In obstructive jaundice also an increase occurs but usually does not exceed 200 to 300 IU/L.
- In hemolytic jaundice, the level of these enzymes is normal.

### Alkaline Phosphatase (ALP)

ALP is produced by many tissues, especially bone, liver, intestine and placenta and is excreted in the bile. Elevation in activity of the enzyme can thus be found in diseases of bone, liver and in pregnancy. In the absence of bone disease and pregnancy, there are elevated ALP levels generally due to hepatobiliary disease. The normal level of ALP in the plasma is 3–13 KA units/100 mL (King Armstrong units).

#### Clinical interpretation

- The greatest elevation (3 – 10 times normal) occurs in obstructive jaundice. ***The enzyme ALP is normally excreted through bile. Obstruction to the flow of bile causes regurgitation of enzyme into the blood resulting in increased serum concentration.***
- Slight to moderate increase is seen in hepatitis and cirrhosis.
- Normal serum ALP values are found in hemolytic jaundice.

## RENAL FUNCTION TESTS

Kidney performs following important functions. The functional unit in the kidney is the **nephron**. The component parts of the nephron are given in **Figure 25.1**.

- The chief function of kidney is excretion of water and metabolic wastes in urine.
- The glomerular filtration and renal tubular reabsorption are the two major functions of kidney that are involved in the formation of urine.
- In order to assess kidney function several kidney function tests (renal function tests) are performed. The various renal function tests have been divided into following groups **(Table 25.3).**

1. **Urine analysis**
   Physical examination
   Chemical examination
   Microscopic examination
2. **Serum and urine markers of renal function**
   Serum creatinine
   Serum urea (or blood urea nitrogen, BUN)
   Protein in urine (Proteinuria)
   Red blood cells in urine (Hematuria)
3. **Estimation of glomerular filtration rate (GFR)**
   Creatinine clearance test
   Urea clearance test
   Inulin clearance test
4. **Tests of renal tubular function**
   Urine concentration test (Water deprivation test)
   Urine dilution test (Excess water intake test)
   Acid load test (Urine acidification test)
   Phenolsulfonphthalein (PSP) test or phenol red test

**Fig. 25.1:** Components of nephron.

**Table 25.3:** Major renal function tests.

- **Urine analysis**
  - *Physical characteristics: Assessment of volume, color,* odor, appearance, specific gravity, and pH.
  - *Chemical characteristics: Checking for the presence of* protein, reducing sugar, ketone bodies, blood, bile salts, and bile pigments.
  - Microscopy: Checking for the presence of WBCs, RBCs, and casts.
- **Serum markers of renal function**
  - *Serum creatinine*
  - *Serum urea or blood urea nitrogen* **(Bun)**
- **Estimation of glomerular filtration rate (GFR)**
  - *Creatinine clearance*
  - *Inulin clearance*
- **Tests of renal tubular function**
  - *Water deprivation test*
  - *Urine dilution test*
  - *Urine acidification test*

## Urine Analysis

Routine urine examination is usually the first test undertaken to assess the renal function and very often it gives some important information like **proteinuria, hematuria** to do further renal investigation. Its analysis, therefore, is important in evaluating kidney function. The standard urine analysis includes:

1. Physical examination
2. Chemical examination
3. Microscopic examination of urine

### Physical Examination

A. Physical examination includes:
   a. The 24 hours urinary output (volume)
   b. Appearance (color)
   c. Specific gravity and osmolality
   d. pH
   e. Odor

### Volume

The daily output of urine in adult is 800 to 2,500 mL with an average of **1,500 mL/day.** The quantity normally depends on the water intake, the external temperature, the diet and the mental and physical state, cardiovascular and renal function.

***Polyuria:*** Volume more than 2,500 mL/day occur in

- Diabetes mellitus, upto 5–6 L/day
- Diabetes insipidus, 10–20 L/day
- Later stages of chronic glomerulonephritis, 2–3 L/day.

***Oliguria:*** Volume 500 mL/day due to: Fever, diarrhea, acute nephritis, early stages of glomerulonephritis, cardiac failure.

***Anuria:*** Complete cessation of urine occurs in: Acute tubular necrosis, bilateral renal stones, surgical shock.

#### *Appearance (color)*

Normal urine is transparent pale yellow or amber color. Variation in color may be physiological or pathological.

- Darkening from the normal pale yellow color indicating more concentrated urine or presence of another pigment.
  - Hemoglobin and myoglobin in urine produce a brownish coloration.
  - Turbidity in a fresh sample may indicate infection but also may be due to fat particles in an individual with nephrotic syndrome.
- Reddish coloration in hematuria is due to renal stones, cancer, some injury or disease of kidneys or urinary tract.

#### *Specific gravity and osmolality*

- The specific gravity indicates the concentrating ability of the kidney. It normally varies from 1.016 to 1.025 with an average 1.020. It can vary widely depending on diet, fluid intake and renal function. If renal function is impaired, the quantity of eliminated urine will be very less. In this condition increased specific gravity may be seen.
- The urine osmolality of normal individuals varies widely depending on the state of hydration. After excessive intake of fluids, the osmotic concentration may fall as low as 50 mOsm/kg, whereas with restricted fluid intake it is up to 1,200 mOsm/kg have been observed. On average, fluid intakes 300 to 900 mOsm/kg are found.

#### *pH*

The urine is normally acidic in reaction with a pH of about 6.0 (range 5.5 to 7.5). Alkaline urine is found in urinary tract infection.

#### *Odor*

Fresh urine is normally aromatic. Foul smell indicates bacterial infection.

### Chemical Examination

Chemical examination includes detection of the abnormal constituents of urine like: **Protein**, **blood**, **glucose**, **ketone bodies**, **bile salts** and **bile pigment**. The abnormal constituents appear in different disease conditions.

### *Glucose*

Normal urine contains small amounts of glucose which cannot be detected by routine test. Excretion of detectable amounts of reducing sugar in urine is called **glycosuria.** It may be benign or pathological (Refer glycosuria).

### *Protein*

Increased amount of protein in urine, i.e. proteinuria can be caused by:

- Increased glomerular permeability
- Reduced tubular reabsorption

  Most common type of proteinuria is due to albumin.

### *Blood*

Presence of blood in urine is called **hematuria** and is commonly seen due to some injury or disease of kidneys or urinary tract. It may be found in renal stones, cancer, tuberculosis, trauma of kidney or acute glomerulonephritis.

### *Ketone bodies*

Detectable levels in urine (ketonuria) are seen in conditions characterized by increased ketogenesis e.g. diabetic ketoacidosis and starvation ketoacidosis.

### *Bile salts and bile pigments*

Presence of these in urine is associated with obstruction of the biliary tract Gallstone or carcinoma of the head of pancreas obstructing the common bile duct.

## *Microscopic Examination*

- Microscopic examination of the centrifuged urinary sediment is done to detect:
- Cells, e.g. RBC, WBC, pus cells
- Crystals, e.g. calcium phosphate, calcium oxalate, amorphous phosphates, etc.
- Casts, e.g. hyaline casts, granular casts, red blood casts, etc.
- Presence of crystals in the urine may be a clue to the diagnosis of a specific type of renal calculus. Various components are observed on microscopic examination of urine in renal disease.

## Serum and Urine Markers of Renal Function

### *Serum Creatinine and urea*

- Serum **urea** and **creatinine** are markers of renal function. Both these substances are primarily excreted in the urine. Deterioration of renal function is, therefore associated with increases in the serum levels of these substances.
- Creatinine is considered a better marker of renal function than urea because urea is affected by dietary protein intake and liver function.
- An impaired glomerular filtration results in retention of urea and creatinine, which causes in elevation of **blood urea** (normal range 20–40 mg/dL) and **creatinine** (normal range 0.5–1.5 mg/dL). An increase of these end products in the blood is called **azotemia.**

## *Test for Proteins in Urine*

- Protein in urine is an indicator of **leaky glomeruli** and is the first sign of **glomerular injury** before a decrease in GFR.
- The glomeruli of kidney are not permeable to plasma proteins and therefore plasma proteins are absent in normal urine.
- When glomeruli are damaged in diseased conditions, they become more permeable and plasma proteins may appear in urine and the condition is known as **proteinuria**.
- Excretion of albumin more than 300 mg/day is indicative of significant damage to the glomerular membrane.
- Excretion of albumin in the range 30–300 mg/day is termed **microalbuminuria.**

Microalbuminuria is the earliest sign of renal damage due to **diabetes mellitus** and **hypertension.**

**Red Blood Cells in Urine (Hematuria)**

Intact glomerulus does not allow the passage of RBC. But with severe glomerular damage, RBC leakage occurs. Thus, detection of microscopic hematuria or RBC casts confirms glomerular damage and is an earliest sign before the decrease in GFR.

## Estimation of Glomerular Filtration Rate

These tests are performed to assess the glomerular filtration rate (GFR). GFR provides a useful index of the status of functioning glomeruli. Renal clearance tests are performed to determine GFR.

## *Clearance Test*

Clearance test is performed to assess the glomerular filtration rate (GFR). Clearance is defined as the volume of plasma (in mL) that could be completely cleared off a

substance per minute and is expressed as milliliter per minute. It may also be defined as that volume of plasma (in mL) which contains the amount of the substance which is excreted by kidney in urine in 1 minute. Renal clearance (C) is calculated by using following formula.

$$C = \frac{U \times V}{P}$$

Where,

C: Renal clearance = GFR of a substance in mL/minute
U: Concentration of substance in urine (mg/100 mL)
V: Volume of urine in mL excreted per minute
P: Concentration of substance in plasma (mg/100 mL).

## Creatinine Clearance Tests

Creatinine clearance test is the renal function test based on the rate of excretion of creatinine by the kidneys. Creatinine is an excretory product derived from creatin phosphate. The excretion of creatinine is not influenced by metabolism or dietary factors.

Creatinine is freely filtered at the glomerulus and is not reabsorbed by the tubules. A small amount of creatinine is secreted by tubules. Because of these properties, the creatinine clearance can be used to estimate the GFR.

Creatinine clearance is defined as the volume of plasma (in mL) that would be completely cleared off creatinine per minute.

The creatinine clearance is determined by collecting urine over a 24 hour period and a sample of blood is drawn during the urine collection period. The clearance of creatinine from plasma is directly related to the GFR, which is calculated as follows:

$$\text{Creatinine clearance} = \text{GFR} = \frac{U \times V}{P}$$

Where,

U is urinary creatinine (mg/dL)
P is plasma creatinine (mg/dL)
V is volume of urine excreted (mL/minute).

### *Clinical interpretation*

- The normal range for creatinine clearance is **90 to 120 mL/minute.**
- A decreased creatinine clearance is a very sensitive indicator of a decreased glomerular filtration rate.
- The reduced filtration rate may be caused by acute or chronic damage to the glomerulus or any of its components.
- Reduced blood flow to the glomeruli may also produce a decreased creatinine clearance.

## Urea Clearance Test

Urea is the end product of protein metabolism. Urea clearance may also be employed as a measure of the GFR. But urea clearance is not as sensitive as creatinine clearance because:

- Unlike creatinine, 40–60% of urea is reabsorbed by the renal tubules after being filtered at glomeruli. Hence, its clearance is less than GFR.
- Moreover, urea clearance is influenced by number of factors, e.g. dietary protein, fluid intake, infection, surgery, etc.

Urea clearance is defined as the volume of plasma (in mL) that would be completely cleared off urea per minute. It is calculated by the formula.

$$\text{Urea clearance} = \frac{U \times V}{P}$$

Where,

U is urinary urea (mg/dL)
P is plasma urea (mg/dL)
V is volume of urine in mL excreted per minute.

### *Clinical interpretation*

- The normal value of urea clearance is 75 mL/minute.
- Urea clearance between 40–70 mL/min indicates mild impairment, between 20–40 mL/min indicates moderate impairment and below 20 mL/min indicates severe impairment of renal function.

## Inulin Clearance Test

- Inulin clearance is the method of choice when accurate determination of GFR is required.
- Inulin is a polysaccharide of fructose, which is filtered by the glomerulus but not reabsorbed, secreted or metabolically altered by the renal tubule.
- The normal value of inulin clearance is **120 mL/min**.

Inulin clearance is calculated by the following formula:

$$\text{Inulin clearance} = \frac{U \times V}{P}$$

Where,

U is urinary inulin (mg/dL)
P is plasma inulin (mg/dL)
V is volume of urine in mL excreted per minute.

- The main disadvantages in the measurement of inulin clearance is that inulin is required to be injected intravenously.
- From a practical point of view, creatinine clearance (inspite of secretion of small fraction of creatinine) is used in clinical medicine as an assessment of the GFR because:
  - In creatinine clearance test, there is no need of intravenous administration of creatinine, since creatinine is produced endogenously. It fulfills all the requirements of an ideal substance.
  - Creatinine is freely filtered through glomerulus and is not reabsorbed by the tubules.

## Tests of Renal Tubular Function

Assessment of the **concentrating** and **diluting** ability of the kidney can provide the most sensitive means of detecting early impairment in renal function.

- The ability to concentrate or dilute urine is dependent upon renal **tubular reabsorption** function and presence of **antidiuretic hormone (ADH)**.
- The kidneys fail to concentrate urine either due to renal tubular damage or due to ADH deficiency (endocrine disorder).

The urinary ***specific gravity*** and ***osmolality*** are used to measure the concentrating and diluting ability of the tubules.

### *Urine Concentration Test (Fluid Deprivation Test)*

- In the fluid deprivation test, fluid intake is withheld for 15 hours.
- The first urine sample in the morning is collected and osmolality or specific gravity is measured.
- If it exceeds osmolality 850 mOsmol/kg or specific gravity of 1.025, the renal concentrating ability is considered normal.
- Dehydration maximally stimulates ADH secretion. If kidney is normal, water is selectively reabsorbed resulting in excretion of urine of high solute concentration and urine osmolality should be at least three times that of plasma (286 mOsmol/kg).

*Clinical interpretation*

In case, the urine does not have specific gravity 1.025 or osmolality 850 mOsmol/kg, it is sure that renal concentrating ability is impaired due to tubular defect.

### *Urine Dilution Test*

In dilution test, after emptying the bladder, 1,000 to 1,200 mL of water is given to the patient. Urine specimens are then collected every hour for the next 4 hours.

*Clinical interpretation*

- Under these circumstances, if the functioning of renal tubule is normal, the urinary specific gravity should fall to 1.005 or less or an osmolality of less than 100 mOsm/kg.
- If the renal tubules are diseased, the concentration of solutes in the urine will remain constant irrespective of excess water intake.
- Renal or cardiac failure may be cause of decreased output. If concentrating ability is normal, the cause may be extra renal.

### *Acid Load Test or Ammonium Chloride Loading Test*

- The acid load test is occasionally used for the diagnosis of **renal tubular acidosis** in which metabolic acidosis arises due to diminished tubular secretion of $H^+$ ions.
- Ammonium chloride is administered orally in gelatin capsule (100 mg/kg body weight) to cause metabolic acidosis and the capacity of kidneys is assessed for the production of acidic urine. Urine samples are collected hourly for the following 8 hours.

*clinical interpretation*

- In normal subjects, the urine pH falls below 5.5 in at least one sample. Normal urine pH is 5.5 to 7.5.
- In an individual with renal tubular acidosis this decrease does not occur, which remains between 5.7 and 7.0.

### *Phenolsulfonphthalein (PSP) Test or Phenol Red Test*

Dyes are widely used for excretion tests. PSP dye is nontoxic and exclusively excreted by kidney and hence, is the dye of choice for excretory function of the kidney.

- The test is conducted by measuring the rate of excretion of the dye following intravenous administration.
- After intravenous injection of 6 mg of PSP in 1 mL of saline. Urine specimen may be collected at 15, 30, 60 and 120 minutes.

*Clinical interpretation*

- If the 15 minute urine contains 25% or more of the injected PSP, the test is normal.
- Forty to Sixty percent of the dye is normally excreted in the first hour and 20 to 25% in the second.
- Excretion of less than 23% of the dye during the 15 minute urine indicates impaired renal excretory function. Two hour excretion may be normal.

## THYROID FUNCTION TESTS

The thyroid gland secrets the hormones: thyroxine ($T_4$) and triiodothyronine ($T_3$). Clinical conditions associated with increased or decreased synthesis of thyroid hormones (***hyperthyroidism*** *or* ***hypothyroidism*** *respectively*) occur commonly. Laboratory determinations of thyroid functions are useful in distinguishing patients with euthyroidism (having a normally functioning thyroid gland) from those with hyperthyroidism or hypothyroidism. The main thyroid function tests commonly done in clinical practice are shown in **Table 25.4**. To understand the thyroid function tests, it is necessary to understand the following basic concepts:

- The function of the thyroid gland is to take iodine from the circulating blood, combine it with the amino acid tyrosine of thyroglobulin and convert it to the thyroid hormones thyroxine ($T_4$) and triiodothyronine ($T_3$).
- Hormone production by the thyroid gland is tightly regulated through ***hypothalamic pituitary thyroid axis (HPTA)*** **(Fig. 25.2).** Thyroid hormones are released in response to stimulation of the thyroid gland by the pituitary hormone called ***thyroid stimulating hormone (TSH)***. TSH in turn secreted in response to stimulation of the pituitary gland by the hypothalamic, ***thyrotropin releasing hormone (TRH)***.
- $T_3$ and $T_4$ (70–80%) are transported in plasma by a ***thyroid binding globulin (TBG)***, a plasma protein. The remaining 20 to 30% of $T_3$ and $T_4$ is transported by ***thyroxine binding prealbumin (TBPA)*** and ***albumin***.
- Only a small amount of the hormone is free which is not bound to protein. However, it is the free portion of the thyroid hormones that is the true determinant of the thyroid status of the patient.

  The evaluation of the thyroid status is not a simple procedure because it does not depend solely on the measurement of circulating thyroid hormone. One or more of the following factors may be abnormal and these have to be sorted out and evaluated.
- The concentration of TBG and its degree of saturation with $T_3$ and $T_4$.
- Concentration of free $T_3$ and $T_4$.
- The status of hypothalamus and anterior pituitary with their respective outputs of TRH and TSH.
- The response of the pituitary to TRH and response of the thyroid to TSH.

**Table 25.4:** Major thyroid function tests.

1. Serum thyroid stimulating hormone (TSH)
2. Serum free thyroxin ($T_4$) and triiodothyronine ($T_3$)
3. Tests for autoimmune thyroid diseases include tests for
   - *Anti-TSH receptor antibodies*
   - *Antithyroglobulin antibodies*
   - *Antimicrosomal antibodies*
   - *Antithyroperoxidase antibodies.*

### Major Thyroid Function Tests

#### *Serum Thyroid Stimulating Hormone*

The measurement of plasma thyroid stimulating hormone (TSH) in a basal blood sample provides the single most sensitive, specific and reliable test of thyroid status. Stimulation of the thyroid gland by the TSH, which is produced by the anterior pituitary gland, will cause the release of stored thyroid hormones.

- When $T_4$ and $T_3$ are too high, TSH secretion decreases.
- When $T_4$ and $T_3$ are too low, TSH secretion increases.

This measurement is used in the diagnosis of **primary hypothyroidism** (thyroid gland failure).

**Normal values of serum TSH**

2 to 6 μU/mL

*Clinical interpretation*

- Increased levels are seen in primary hypothyroidism due to absence of negative feedback control on the pituitary **(Fig. 25.2).**
- Decreased levels are associated with:
  - Primary hyperthyroidism
  - Secondary (anterior pituitary failure) hypothyroidism
  - Tertiary (hypothalamic failure) hypothyroidism

**Fig. 25.2:** Hypothalamic pituitary thyroid axis (HPTA).

### Serum Free $T_3$ and $T_4$

This is a measure of that fraction of circulatory $T_4$ and $T_3$ that exists in free state in the blood unbound to protein. Free thyroid hormone concentrations provide more reliable means of diagnosing thyroid dysfunction than measurement of total serum $T_4$ and $T_3$.

**Normal Values of free $T_4$ and $T_3$**

- Free $T_4$ = 10 to 27 pmol/L
- Free $T_3$ = 3 to 9 pmol/L

**Clinical Interpretation**

- Increased values are associated with hyperthyroidism and thyrotoxicosis.
- Decreased values are associated with hypothyroidism.

### Serum Total $T_4$ and $T_3$

Changes in total thyroid hormone concentration are far less sensitive indices of thyroid function than is TSH; for this reason, they should not be measured alone. This test is commonly done to rule out **hyperthyroidism** and **hypothyroidism.** This test is a good index of thyroid function when TBG is normal.

**The normal values of total $T_4$ and $T_3$**

- $T_4$ = 5 to 12.5 μg/dL
- $T_3$ = 70 to 200 ng/dL

**Clinical Interpretation**

Values can be increased in:

- Hyperthyroidism
- Increased concentration of TBG (as in pregnancy and with estrogen therapy)

Values can be decreased in:

- Hypothyroidism
- Decreased concentration of TBG (as in nephrosis due to loss of TBG in urine and in liver disease in which there is a decreased synthesis of TBG).

### Thyroid Autoantibodies

Several types of antibody against thyroid tissue have been detected in serum of patients with thyroid disease. Measuring these antibodies helps to demonstrate the presence of the autoimmune disorders. For example:

- Graves' disease is commonly associated with the presence of **anti TSH receptor antibodies**.
- Hashimoto's thyroiditis is associated with the presence of **anti-thyroid peroxidase antibodies** (Anti-TPO antibodies, previously called **antimicrosomal antibodies**).
- ***Thyroglobulin (Tg)***: It is made uniquely by the thyroid. Measurement of Tg is of particular use in assessing the presence of any remnant thyroid tissue after thyroidectomy performed for malignancy. Measurement of Tg is an important tool in assessment of patients with differentiated thyroid malignancies. After successful thyroidectomy for thyroid malignancy Tg concentration in blood will fall to undetectable levels. The reappearance of Tg in blood is strongly suggestive of tumor recurrence.

**Clinical Interpretation**

- **Thyroid peroxidase** (microsomal antigen) and **thyroglobulin** antibodies are present in the serum of patients with immunological mediated thyroid disease, e.g. Hashimoto's thyroiditis and Graves' disease.
- They may also be found in a small proportion of healthy individuals, the incidence being higher in relations of patients with hyperthyroidism.

## LIPID PROFILE TESTS

Lipid profile tests are used to estimate increased risk of cardiovascular disease which includes measurement of:

1. Total serum cholesterol
2. Serum triglycerides
3. HDL cholesterol
4. LDL cholesterol

### Total Serum Cholesterol

*Enzymatic method for estimation of cholesterol*

- Commercially available cholesterol reagents commonly combine all enzymes and other required components into a single reagent.
- The reagent usually is mixed with 3 μL to 10 μL aliquot of serum or plasma, incubated under controlled conditions for color development and absorbance is measured at about 500 nm.
- The reagents typically use a bacterial **cholesterol ester hydrolase** to hydrolyze cholesterol esters to cholesterol and fatty acids **(Fig. 25.3)**.
- The 3-OH group of cholesterol is then oxidized to a ketone derivative and $H_2O_2$ by cholesterol oxidase.
- $H_2O_2$ is then measured in a peroxidase catalyzed reaction that forms dye.

### Normal Values and Interpretation

- The normal range for healthy young adults is less than 200 mg/dL.
- It may be lower in children.
- The concentration increases with age.

**Fig. 25.3:** Reaction occurs in enzymatic method of total serum cholesterol estimation.

- The concentration in the women is generally somewhat lower than in men up to the time of menopause but then increase and may exceed that in men of the same age.

*Increased concentration*

- The total concentration is increased in:
  - Hypothyroidism
  - Uncontrolled diabetes mellitus
  - Nephrotic syndrome
  - Extrahepatic obstruction of the bile ducts
  - Various hyperlipidemias
- Long time elevated cholesterol concentration (more than 240 mg/dL) is a serious **risk factor for the development of coronary artery disease**.
- Lowering of plasma cholesterol concentration reduces the incidence of coronary heart diseases.
- National Cholesterol Education Program (NCEP) defined the levels of serum cholesterol believed to be **desirable, tolerable** or a **serious risk factor** for development of coronary artery disease. The report classifies total cholesterol concentration **(Table 25.5)** which is applicable to all individuals over 20 years age and sex.

*Decreased concentration*

Hypocholesterolemia is usually present in:

- Hyperthyroidism
- Hepatocellular disease
- Certain genetic defects, e.g. abetalipoproteinemia.

## Serum Triglycerides (TG)

*Enzymatic method for estimation of TG* ***(Fig. 25.4)***

- Single reagents that consist of all the required enzymes, cofactors and buffers generally is used.
- The first step is the hydrolysis of triglycerides to glycerol and fatty acid by lipase.
- Glycerol is then oxidized to dihydroxyacetone and $H_2O_2$ by glycerophosphate oxidase enzyme.

**Fig. 25.4:** Reaction of enzymatic estimation of triglycerides.

- The $H_2O_2$ formed in the reaction subsequently is measured as described in enzymatic method for total serum cholesterol.

### *Normal Values and Clinical Interpretation*

- The normal range of serum triglycerides is **40–145 mg/dL**. Mean values rise slowly with age after the third decade.
- Values below the normal range are of little clinical significance.
- Elevated concentration is often found in disturbances of **lipid metabolism** and in **atherosclerosis** and **coronary artery disease.** The classification of triglyceride concentration according to the NCEP is listed in **Table 25.5**.
- The serum triglyceride concentration is greatly elevated in hyperlipoproteinemia type I and V and moderately increased in type II b and III.
- The cause of hyperlipoproteinemia is a genetic origin but hypertriglyceridemia occur commonly secondary to the following pathologic conditions:
  - Hypothyroidism
  - Nephrotic syndrome
  - Alcoholism
  - Obstructive liver diseases
  - Acute pancreatitis
  - Uncontrolled diabetes mellitus
  - Glycogen storage disease (type I)

**Table 25.5:** Classification of serum cholesterol and serum triglycerides concentration according to the NCEP.

| *Category* | *Serum cholesterol mg/dL* | *Serum triglycerides mg/dL* | *Serum LDL-cholesterol mg/dL* |
|---|---|---|---|
| Normal (desirable, safer side) | Below 200 | Below 200 | Below 130 |
| Borderline high risk | 200 to 240 | 200 to 400 | 130 to 160 |
| High risk | Above 240 | 400 to 1000 | Above 160 |

NCEP = National Cholesterol Education Program. All values are in mg/dL; to convert to mmL/L, multiply by 0.259

*Decreased concentration*

The plasma triglyceride concentration is low in the rare disease, abetalipoproteinemia (absence of low density lipoproteins).

## HDL Cholesterol

Method for HDL-cholesterol estimation

Commercial kits are available for the HDL cholesterol determination.

### *Principle*

LDL, VLDL and chylomicrons are precipitated by polyanions in the presence of magnesium ions to leave HDL in solution. The cholesterol content of the supernatant fluid is then determined by an enzymatic method.

### *Normal Values and Clinical Significance of HDL Cholesterol*

- Serum level of HDL cholesterol for:
  - Men is 30–60 mg/dL
  - For women 40–80 mg/dL which is 20 to 30% higher than men.
- Studies have indicated that when the HDL cholesterol value is lower than 45 mg/dL in men and lower than 55 mg/dL in women there is an increased risk for heart disease and the relative risk increases with lower HDL cholesterol concentrations.
- Higher HDL cholesterol concentrations may be associated with decreased risk of coronary disease.
- Thus, HDL cholesterol levels are inversely related to the risk of cardiovascular disease.
- HDL cholesterol level above 60 mg/dL indicates very low risk for coronary artery disease (CAD).
- HDL below 35 mg/dL increases the risk of CAD.
- Decreased levels are associated with stress, obesity, androgens, cigarette smoking and diseases like diabetes mellitus, augments the risk of coronary artery disease. HDL cholesterol is very low in genetic disorder, **Tangier disease.**
- The ratio of total cholesterol to HDL cholesterol gives a more accurate and definite assessment of heart disease risk **(Table 25.6)**.

## LDL Cholesterol

- The value of LDL cholesterol may be calculated, if the concentrations of total and HDL cholesterol and triglycerides are measured.

**Table 25.6:** Ratio of total cholesterol/HDL cholesterol and LDL cholesterol/HDL cholesterol for the assessment of risk of coronary artery disease.

| | *Total cholesterol/HDL cholesterol* | | *LDL cholesterol/HDL cholesterol* | |
|---|---|---|---|---|
| Category | Men | Women | Men | Women |
| Safer side | 3.40 | 3.25 | 1.00 | 1.45 |
| Borderline high risk | 4.95 | 4.45 | 3.50 | 3.20 |
| High risk | 9.50 | 7.00 | 6.25 | 5.00 |

- In practice, LDL can be measured indirectly by use of **Friedewald equation** assuming that total cholesterol is composed primarily.
  Total cholesterol = Cholesterol in (VLDL + LDL + HDL).
  LDL cholesterol = Total cholesterol – (HDL cholesterol + 1/5 × Triglyceride (TG)).

**The concentrations of all constituents should be expressed in the same units mg/dL or mg/L. 1/2.22 × TG is used when LDL cholesterol is expressed in mmol/L.**

The factor 1/5 × TG is an estimate of the VLDL cholesterol concentration.

### *Normal Values and Clinical Interpretation*

- The LDL cholesterol in women is somewhat lower than in men but increase after menopause.
- Low levels of LDL cholesterol lower the risk.
- Values above 160 mg/dL indicate high risk.
- Values between 130–160 mg/dL are in borderline risk.
- Values below 130 mg/dL are safer side **(Table 25.5)**.

Thus, the risk of cardiovascular disease is correlated directly with a high concentration of LDL cholesterol.

- The highest correlations have been obtained as a risk factor by the ratio of LDL cholesterol to HDL cholesterol **(Table 25.6)**.

# CARDIAC MARKERS

After myocardial infarction, a number of intracellular **enzymes** and **proteins** are released from the damaged cells. They have diagnostic importance and are called **cardiac markers.** Cardiac markers are useful in the detection of acute myocardial infarction (AMI) or minor myocardial injury.

- The cardiac markers of major diagnostic interest includes, enzymes such as:
  - Creatine kinase (CK)
  - Lactate dehydrogenase (LD)

- Serum aspartate aminotransferase (AST) or serum glutamate transaminase (SGOT).
- Non-enzyme proteins such as:
  - Myoglobin (Mb)
  - Cardiac troponin T and I (cTnT and cTnI).

## Creatine Kinase (CK)

Creatine kinase has three isoenzymes ***(Refer Chapter 6)*** CK-2 or CK-MB isoenzyme is specific for the heart.

### Reference Values

- Normal values for total CK ranges from 10 to 100 U/L
- The upper limit for CK-MB activity = 6 U/L.

### Clinical Interpretation

#### *Increased activity*

- There is a rise in total CK activity following a myocardial infarction. The degree of increase varies with the extent of the tissue damage. CK is the first enzyme to appear in serum in higher concentration after myocardial infarction and is probably the first to return to normal levels if there is no further coronary damage.
- The serum total CK activity may be increased in some cases of coronary insufficiency without myocardial infarction. So, the simultaneous determination of CK-MB isoenzyme and LDH1 isoenzyme help to make the diagnosis.
- The CK-MB isoenzyme starts to increase within 4 hours after an acute myocardial infarction (AMI) and reaches a maximum within 24 hours.
- CK-MB is a more sensitive and specific test for AMI than total CK.

## Lactate Dehydrogenase (LDH)

LDH is distributed widely in liver, cardiac muscle, kidney, skeletal muscle, erythrocytes and other tissues. Because LDH is not tissue specific enzyme, serum total LDH is increased in a wide variety of disease including heart disease. LDH has five isoenzymes ***(Refer chapter 6)***. LDH1 is specific for the heart.

### Reference values

Total LDH = 125 to 290 U/L
LDH1 = 100 U/L
LDH2 = 115 U/L
LDH3 = 65 U/L
LDH4 = 40 U/L
LDH5 = 35 U/L

The use of LDH and LDH isoenzymes for detection of AMI is declining rapidly. The troponin tests are much more useful, as will be explained later in the chapter.

### Clinical Interpretation

- For patients having an AMI, serum total LDH values become elevated at 12 to 18 hours after onset of symptoms, peak at 48 to 72 hours and returns to normal after 6 to 10 days **(Fig. 25.5)**.
- The LDH1 increase over LDH2 in serum after AMI (the so called flipped pattern, in which the LDH1/LDH2 ratio becomes greater than 1).
- The combination of an elevated CK-MB and a flipped LDH isoenzyme ratio in a patient suspected of having a myocardial infarct makes the diagnosis certain. The combination never occurs in coronary insufficiency without a myocardial infarction.

## Serum Aspartate Aminotransferase (AST)/ Serum Glutamate Oxaloacetate Transaminase (SGOT)

AST is found practically in every tissue of the body, including red blood cells. It is in particularly high concentration in cardiac muscle and liver, intermediate in skeletal muscle and kidney.

### Reference Values

The normal concentration of serum AST is 6 to 25 U/L

### Clinical interpretation

- The serum activity of AST begins to rise about 6 to 12 hours after myocardial infarction and usually reaches its maximum value in about 24 to 48 hours.

**Fig. 25.5:** Enzyme activity after myocardial infarction (troponin which is not an enzyme is also shown).

- It usually returns to normal 4 to 6 days after the infarct.
- The increase in activity is not as great as for CK, nor does it rises as early after the infarct.
- It is much less specific indication of myocardial infarction than the rise in CK, because so many other conditions, e.g. liver, muscle or hemolytic disease, can cause a rise in serum AST. Prolonged myocardial ischemia, congestive heart failure is also associated an increased AST level.

## Myoglobin (Mb)

- Myoglobin is an oxygen binding protein of cardiac and skeletal muscle.
- Its low molecular weight probably account for its early appearance in the circulation after muscle injury.
- Increase in serum myoglobin occurs after AMI.
- The major advantage offered by myoglobin as a serum marker for myocardial injury is that it is released early from damaged cells.

### *Clinical Interpretation*

- As shown in **Figure 25.6**, serum concentrations of myoglobin rise above the normal values as early as 1 hour after the occurrence of an AMI with peak activity in the range of 4 to 12 hours.
- Myoglobin is cleared rapidly and thus has no clinical significance after 12 hours.
- The role of myoglobin in the detection of AMI is within the first 0 to 4 hours, the time period in which CK-2 and cardiac troponin are still within their normal values.

**Disadvantages of Myoglobin as a Cardiac Marker**

- The measurement of serum myoglobin has not been used extensively in clinical laboratories for the routine analysis of AMI because it is non-specific, since it is raised following any form of muscle damage.
  Even minor injury to skeletal muscle may result in an elevated concentration of serum myoglobin which may lead to the misdiagnosis of AMI.

**Fig. 25.6:** Temporal pattern of serum myoglobin and creatine kinase-2 in patients with myocardial infarction.

## Cardiac Troponin

- The contractile proteins include the regulatory protein troponin ***(See Chapter 33)***.
- Troponin is a complex of three protein subunits:
  - Troponin C (TnC, the calcium binding component)
  - Troponin I (TnI, the inhibitory component)
  - Troponin T (TnT, tropomyosin binding component).
- The troponin subunits exist in a number of isoforms. However, cardiac specific **troponin T (cTnT)** and **troponin I (cTnI)** forms are the most useful cardiac markers of acute myocardial infarction.

### *Clinical Interpretation*

The initial rise in cardiac troponins (cTnI and cTnT) after myocardial infarction occurs at about the same time as CK and CK–MB but this rise continues for longer than for most of the enzyme.

- For patients having AMI serum cTnT and cTnI values become elevated above the normal level at 4 to 8 hours after the onset of the symptoms.
- Secondly, cTnT and cTnI also can remain elevated up to 5 to 10 days respectively, after an AMI occurs.

# EXAM QUESTIONS

## Long Answer Questions (LAQs)

1. Classify liver function tests (LFT). Describe any two tests in detail.
2. Thyroid function tests (TFT). Describe any two tests in detail.
3. Classify kidney function tests (KFT). Describe any two tests in detail.
4. Discuss tests based on differential diagnosis of jaundice.
5. Describe tests used to estimate increased risk of cardiovascular disease.
6. Describe tests for diagnosis of AMI.

## Short Notes

1. Explain the role of various enzymes in liver function tests.
2. Clearance tests.
3. Tests based on metabolic and excretory functions of liver.
4. Liver function tests based on bile pigment metabolism.
5. What is renal clearance? How creatinine clearance is superior to urea clearance?
6. Kidney function tests based on glomerular function.
7. Clinical interpretation of alkaline phosphatase and alanine transaminase with respect to liver function.
8. Lipid profile tests.
9. Cardiac markers.
10. Enzyme assay in AMI.
11. Role of HDL-cholesterol in lowering the risk of CHD.

## Solve the Following

**Case History-1**

**A man aged 38 years presenting with a serum creatinine of 1.7 mg/dL. 24 hours urine of 2160 mL is collected and found to have creatinine concentration in urine of 0.85 g/dL.**

**Questions**

a. Calculate the creatinine clearance and comment on the results.
b. Write normal creatinine clearance value.
c. Define creatinine clearance.
d. Which are the other clearance tests?

**Case History-2**

**A 50-year-old woman presented at the accident and emergency department with severe chest pain which had been present for the past hour.**

**Questions**

1. What specific test would you request from the biochemistry laboratory?
2. Explain their clinical interpretation with normal rage.

## Multiple Choice Questions (MCQs)

1. **Conjugated hyperbilirubinemia with raised alkaline phosphatase levels are found in:**
   a. Viral hepatitis
   b. Obstructive jaundice
   c. Hemolytic jaundice
   d. Neonatal physiological jaundice
2. **Inulin clearance is used to assess:**
   a. Renal threshold
   b. Concentrating ability of tubules
   c. GFR
   d. Diluting ability of tubules
3. **In hemolytic jaundice:**
   a. Urinary urobilinogen excretion is increased
   b. Serum conjugated bilirubin is increased
   c. Serum alkaline phosphatase is increased
   d. Bilirubin is excreted in urine
4. **Prothrombin time in obstructive jaundice:**
   a. Decreases
   b. Increases after parenteral injection of vitamin K
   c. Normal
   d. Normalizes after parenteral injection of vitamin K
5. **Positive direct van den Bergh's reaction indicates presence of:**
   a. Conjugated bilirubin
   b. Unconjugated bilirubin
   c. Urobilinogen
   d. Bile salts
6. **Failure of concentrating capacity of urine is assessed by measurement of:**
   a. Inulin clearance test
   b. Fluid deprivation test
   c. Acid load test
   d. Creatinine clearance test

**7. Which of the following tests measures GFR accurately?**
a. Creatinine clearance
b. Urea clearance
c. Inulin clearance
d. None of the above

**8. Which of the following is not a feature of obstructive jaundice?**
a. Increased level of serum unconjugated bilirubin
b. Increased level of serum conjugated bilirubin
c. Absence of urobilinogen in stool
d. Increased level of alkaline phosphatase

**9. Absence of urobilinogen in urine occurs in:**
a. Obstructive jaundice
b. Hemolytic jaundice
c. Hepatic jaundice
d. All of the above

**10. In obstructive jaundice which of the following enzymes is diagnostically important?**
a. Alkaline phosphatase
b. Acid phosphatase
c. Lactate dehydrogenase
d. Creatine phosphokinase

**11. The normal value of urea clearance is:**
a. 54 mL/min b. 75 mL/min
c. 110 mL/min d. 130 mL/min

**12. Average creatinine clearance in an adult man is about:**
a. 54 mL/min b. 75 mL/min
c. 110 mL/min d. 130 mL/min

**13. Inulin clearance in an average adult man is about:**
a. 54 mL/min b. 75 mL/min
c. 110 mL/min d. 120 mL/min

**14. A simple way to assess tubular function is to withhold food and water for 12 hours and, then, measure:**
a. Serum urea
b. Serum creatinine
c. Urine output in one hour
d. Specific gravity of urine

**15. Among the following, the most sensitive indicator of glomerular function is:**
a. Serum urea
b. Serum creatinine
c. Urea clearance
d. Creatinine clearance

**16. All the following statements about inulin are correct, *except*:**
a. It is completely nontoxic
b. It is completely filtered by glomeruli
c. It is not reabsorbed by tubular cells
d. It is secreted by tubular cells

**17. Excretion of albumin in microalbuminuria occurs in the range of:**
a. 30–300 mg/day
b. 30–800 mg/day
c. 0.3–3 gm/day
d. None of the above

**18. Azotemia is:**
a. An increase of blood urea and creatinine
b. An increase of blood glucose and ketone bodies
c. An increase of blood $T_3$ and $T_4$
d. An increase of urine urea and creatinine

**19. Serum urea and creatinine are markers of:**
a. Liver function
b. Renal function
c. Pancreatic function
d. Gastric function

**20. Tests of renal tubular function is:**
a. Urine concentration test (Water deprivation test)
b. Urine dilution test (Excess water intake test)
c. Acid load test (Urine acidification test)
d. All of the above

**21. Following are the cardiac markers, *except*:**
a. Troponin b. Myoglobin
c. CK-MB d. Alkaline phosphatase

**22. Increased plasma level of which of the following is a risk factor of CHD, *except*:**
a. LDL-cholesterol
b. HDL-cholesterol
c. Triglyceride
d. Homocysteine

**23. Which of the following cardiac marker release first following myocardial infarction?**
a. Myoglobin
b. Troponin
c. LDH (heart specific)
d. AST

**24. Which of the following has protective effect against myocardial infarction?**
a. LDL b. VLDL
c. HDL d. Chylomicrons

**25. Which of the following protein is considered as a high-risk protein for AMI?**

a. Albumin
b. α-fetoprotein
c. Ceruloplasmin
d. C-reactive protein (CRP)

**26. Which of the following amino acid has a high-risk for CHD?**

a. Homocysteine
b. Methionine
c. Glycine
d. None of the above

## Answers for MCQs

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **1.** b | **2.** c | **3.** a | **4.** d | **5.** a | **6.** b | **7.** c | **8.** a |
| **9.** a | **10.** a | **11.** b | **12.** c | **13.** d | **14.** d | **15.** d | **16.** d |
| **17.** a | **18.** a | **19.** b | **20.** d | **21.** d | **22.** b | **23.** a | **24.** c |
| **25.** d | **26.** a | | | | | | |

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CHAPTER

# 26 Radioisotopes in Medicine

**Chapter outline**

- Radioactivity
- Use of Radioisotopes in Medicine
- Radiation Hazards
- Radiation Health Safety and Protection

## INTRODUCTION

A radioisotope of an element may be defined as a special kind of atom which has the physical property of giving out radiations. Isotope means **'same *place*'** and implies that these have the same place in ***Mendeleev periodic table*** of the chemical elements.

***Isotope is defined as an element with same atomic number but different atomic weight.*** The radioactive isotope or radioisotope, therefore, would behave in the same way both chemically and metabolically as the stable element with the additional advantage of giving out radiations which can be detected or can be used to destroy the tissue.

## RADIOACTIVITY

An atom is made up of a central core called the nucleus, which consists of closely packed ***protons*** (positively charged particle) and ***neutrons*** (uncharged particle), surrounded by a cloud of ***electrons*** moving around the nucleus in well-defined orbits **(Fig. 26.1)**. The nucleus has a positive charge, while the electrons have an equivalent negative charge, so that the complete atom is electrically neutral.

The phenomenon of radioactivity is associated with changes in the nucleus which consists of protons and neutrons. The ratio of neutrons to protons in a nucleus of a given element may vary due to variations in the number of neutrons. Since, an atom is electrically neutral; the number of electrons does not change, while the number of protons remains the same. In different isotopes of an element, therefore, the number of protons (Z) remains the same but the number of neutrons (N) and mass number (A) differ.

**Fig. 26.1:** Structure of an atom of helium showing two protons, two neutrons and two electrons.

- Some of these ratios of protons to neutrons give stability to the nucleus and the isotope is then referred to as a ***'stable' isotope;*** while other ratios lead to instability
- An unstable nucleus has excess of energy and it undergoes spontaneous transformation of the number of protons or neutrons or of their internal arrangement, in order to achieve stability. During this process, the excess of energy is given out in the form of radioactivity. Such isotopes are called ***radioactive isotopes*** *or* ***radioisotopes.***

### Nature of Radioactivity

There are different types of transformations which can take place within the nucleus, resulting into various types of radioactivity. These are **alpha, beta** and **gamma** rays.

### Properties of Radioisotopes

#### *Penetrating Ability*

Radiations given out can travel through or penetrate the matter.

- Alpha particles have poor penetrability.

**Fig. 26.2:** Penetrating ability of alpha, beta and gamma radiations.

- Beta particles can penetrate only a few, 4–5 millimeters in the soft tissue.
- The gamma rays, however, can penetrate deeper into the tissues and have greater penetrating power **(Fig. 26.2)**.

**Alpha radiations cannot penetrate the skin and are not of medical interest. The beta and gamma emissions, however, are useful both for *diagnostic* and *therapeutic* purposes.**

### *Radioactive Decay*

Unlike the ordinary therapeutic drugs, radioisotopes do not have an expiry date, but they are continuously losing their activity.

- The time taken for reduction of the activity to half of its zero hour value is known as **physical half-life** of that isotope
- The time taken by the element to reduce its body concentration to half of that administered is known as the **biological half-life**.

Thus, labeled compounds using carbon-14 with half-life of 5,760 years may be metabolized and eliminated by the body within less than one day.

### *Ionization*

Ionization is mainly responsible for the biological effects of radiation and it forms the basis for the measurement of radioactivity with detectors like Geiger Muller (GM) counters.

### Unit of Radioactivity

The unit of radioactivity is based, not on the mass of the element, but on the rate of disintegrations per second.

- The unit is the Becquerel (Bq). It is the quantity of radioactive material in which one nuclear disintegration occurs in one second.
- The curie (Ci) was formerly used as the unit of activity; $1 Ci = 3.7 \times 10^{10}$ Bq.
- In order to express the radiation effect on the tissues, units like ***'roentgen',*** or more commonly 'rad' is used. The rad (r) expresses the dosage to the tissue in terms of energy dissipated and one rad is equivalent to 100 ergs of energy absorbed per 1 gm of tissue.

## USE OF RADIOISOTOPES IN MEDICINE

Radioisotopes are now extensively used in various branches of medicine for ***diagnostic, research*** and **therapeutic** purposes. The radioisotope may be used as such or by attaching it to an organic compound; the latter is then called a ***labeled*** compound.

### Therapeutic Applications of Radioisotopes

When radioisotopes are focused carefully on the malignant tissue, death of the tumor cell is caused mainly due to the direct **ionization effect on its DNA**. The radiation also acts indirectly by creating free radicals that attack the DNA of the tumor cell but the process is slow. The radioisotopes used therapeutically are given below:

- The γ-rays of radio cobalt ($^{60}Co$) are used to treat cancer of the cervix and many other types of internal cancer.
- Radio phosphorus, $^{32}P$ is used for the treatment of polycythemia and chronic myeloid leukemias.
- Pleural and intra-abdominal effusions arising as secondary complication of neoplasm of thoracic and abdominal viscera is often treated with radioactive chromic sulfate $^{32}CrSO_4$.
- Superficially involved cancer, as in the cornea of the eye may be treated with $^{32}P$ or $^{90}Sr$.
- Radio cesium $^{137}Cs$ is used in the treatment of chronic leukemia.

- Radio tantalum $^{182}Ta$ is used in the treatment of bladder cancer.
- Radio gold $^{198}Au$ is introduced to treat malignant pleural/peritoneal effusions.
- Radio iodine $^{131}I$ is used for the treatment of thyroid cancer and thyrotoxicosis.
- Radio yttrium $^{90}Y$ is used to treat arthritis of hemophilic patients.
- $^{32}P$ is used in dermatology for the treatment of squamous cell carcinoma.

### Diagnostic Applications of Radioisotopes

Radioisotopes can be used in the diagnosis of many diseases. For example:

- Thyroid disorder can be diagnosed with radioiodine $^{131}I$.
- $^{24}Na$ and $^{51}Cr$ have been used in the determination of blood volume and circulation time.
- $^{32}P$ is used for the diagnosis of cancer of liver, bone, lymph nodes and spleen.
- Radioactive iron $^{59}Fe$ has been used to diagnose obscure anemias and some other blood diseases.
- $^{131}I$, $^{90}Sr$ and $^{131}I$-hippuran are used in the scanning of organs, thyroid, bone and kidney respectively.
- $^{131}I$ diodrast (iodopyracet) is used to detect internal hemorrhages.
- $^{24}Na$, $^{59}Fe$, $^{131}I$-labels are used to calculate ECF, circulating blood volume and cardiac output respectively.

## RADIATION HAZARDS

Biological effects of radiation may be divided into two groups:

1. Somatic effect
   i. Immediate effect
   ii. Delayed effect.
2. Genetic effect.

Somatic effects are recognizable within the lifespan of the irradiated person, while genetic effects would be manifest in the offspring.

### Somatic Radiation Hazards

#### *Immediate Effects*

An immediate effect of radiation leads to ***acute radiation syndrome.*** The acute radiation syndrome is characterized by:

- Severe nausea, vomiting and prostration. It is due to necrosis and ulceration of the gastrointestinal tract and may be accompanied by bleeding.
- Large dose causes high fever which is a bad prognostic sign.
- Various blood elements such as red cells, granulocytes, lymphocytes and platelets are markedly decreased and this may cause anemia, bleeding from various sites.
- Radiation decreases antibody formation and lowers the body resistance.

There is general deterioration of health, and death ensues within 2–3 weeks.

#### *Delayed Effects*

Chronic exposure to low level radiation can give rise to certain delayed effects. These are:

- **Aging effect:** Radiation can accelerate the physical processes of aging and shorten the lifespan. There is an early
  - Greying of the hair
  - Epilation (removal of hair by its roots)
  - Early development of vascular and degenerative changes
  - Lenticular cataracts.
- **Induction of neoplasms (cancer),** e.g.
  - Carcinoma of the skin following radiation.
  - Osteogenic sarcoma following radium deposition in bones.
  - Lung cancer following the inhalation of radon in uranium mine workers.
  - The incidence of leukemia in children exposed to radiation *in utero*, during the diagnostic radiological procedures in pregnancy.

### Genetic Radiation Hazards

Genetic effects result from injury to chromosomes, which lead to mutations.

## RADIATION HEALTH SAFETY AND PROTECTION

Basic knowledge of radiation safety is vital to every laboratory worker who has frequent contact with radioactive substances as these are hazardous to health. The protection techniques are designed to prevent radioactive material from entering the body. The protection techniques include:

- Prevention of external exposure
- Prevention of internal exposure
- Proper storage of radioactive substance
- Proper disposal of radioactive substance.

## Prevention of External Exposure

Three principal methods that help to reduce exposure from external sources are:

1. **Reduce time:** Minimize the time spent in the vicinity of a source of radiation. Work efficiently, but do not rush.
2. **Increase distance:** The radiation intensity from a source diminishes rapidly as the distance from the source is increased. Maintain as large a distance from the source as practical.
3. **Use shielding:** In case of X-rays, shielding should be used to reduce the exposure.
   Pregnant women should not be allowed to work in places where there is continuous exposure.

## Prevention of Internal Exposure

Prevent the entry of radioactive materials into the body through ***inhalation, ingestion*** or ***absorption*** by the ***skin.***

- **Limiting inhalation:** This is accomplished through proper laboratory design, including attention to adequate air exchange and air flow patterns, use of fume hoods to minimize inhalation.
- **Limiting ingestion:** This is accomplished through good laboratory hygiene, such as wearing of protective gloves and gowns, knowledge of proper glove removal, hand washing before eating, and prohibition of pipetting by mouth, as well as eating, drinking, smoking, cosmetics applications in the laboratory.
- **Limiting absorption through skin:** Individuals should exercise extra care to properly cover the hands, forearms and other parts that could become contaminated.
- **Proper storage:** All radioactive material should be stored in special refrigerators or cabinets conspicuously labeled with appropriate signs indicating the presence of radioactive elements.
- **Proper disposal:** Proper disposal of radioactive waste is a must. All waste containers should be clearly marked as containing radioactive material, so that waste will not be incinerated and handed over to the atomic energy authorities.

# EXAM QUESTIONS

## Short Notes

1. Name isotopes and mention their applications in medicine.
2. Give radiation monitoring precautions.
3. Give therapeutic applications of radioisotopes.
4. Give diagnostic applications of radioisotopes.
5. Write radiation hazards.
6. Give radiation health safety and protection.

## Multiple Choice Questions (MCQs)

**1. Becquerel (Bq) is a:**
a. Unit of light energy
b. Unit of radioactivity
c. Type of radiation
d. Measure of half-life of radio isotope

**2. The most penetrating rays are:**
a. α-rays b. γ-rays
c. β-rays d. X-rays

**3. Radio isotope used for treatment of polycythemia is:**
a. $^{32}P$ b. $^{131}I$
c. $^{60}Co$ d. $^{90}Ya$

**4. Which of the following emitters is more suitable for the diagnostic purpose?**
a. α-rays b. β-rays
c. γ-rays d. UV-rays.

**5. Unit of activity is based on:**
a. Mass of the element
b. Disintegration per sec
c. Number of protons in the element
d. Mass number of the element

**6. Unit of radioactivity is:**
a. Becquerel
b. Curie
c. Rad (r)
d. All of the above

### Answers for MCQs

**1.** b **2.** b **3.** a **4.** c **5.** b **6.** d

CHAPTER

# 27

# Free Radicals and Antioxidants

**Chapter outline**

- Formation of Reactive Oxygen Species
- Exogenous Causes of Formation of Free Radical
- Antioxidant

## INTRODUCTION

Free radicals are atoms or molecules containing one or more unpaired electrons in an outer orbit. They are a highly reactive species and have a tendency either to lose an electron, thereby acting as reducing agents or gain an electron, acting as oxidizing agents. Thus, they can initiate chain reactions by extracting an electron from a neighbouring molecule to complete its own orbit.

The most important radicals are derived from molecular oxygen and certain oxides of nitrogen especially nitric oxide. These free radicals may act as signaling molecules in physiological and biochemical activities or may provide defense against invading micro-organisms. Failure of these protective mechanisms may lead to pathological conditions. Oxygen derived free radicals and related non-radical compound ($H_2O_2$) is referred to as reactive oxygen species (ROS). Not all reactive oxygen species are free radicals, e.g. singlet oxygen and hydrogen peroxide.

## FORMATION OF REACTIVE OXYGEN SPECIES

When the oxygen molecule ($O_2$) is introduced into a reducing environment, it may undergo a series of reactions leading to the formation of reactive oxygen species (ROS) **(Fig. 27.1)**. The reactive intermediates which are formed usually remain tightly bound in the active sites of the enzymes, until the reaction is finished. These free radicals usually have only a transient existence in the catalytic process. But occasionally, they may escape from the active site of the enzyme and lead to a destructive effect.

**Fig. 27.1:** Formation of reactive intermediates from molecular oxygen.

### Enzyme Reactions in which Free Radicals are By-products

Various reactions occurring in cells depend on a supply of oxygen. In such type of reactions free radicals are generated as by-products. The enzyme catalyzing oxygen requiring reactions can be classified into:

- Oxidases
- Mono-oxygenases
- Dioxygenases.

**Oxidases:** Oxidase catalyzes the reduction of dioxygen to yield water or hydrogen peroxide, e.g. **cytochrome oxidase** of mitochondria and **xanthine oxidase** which generate superoxide during reduction of dioxygen.

**Mono-oxygenases:** Enzymes that incorporate one atom of oxygen into the substrate and other is reduced to water, e.g. **cytochrome $P_{450}$ enzyme complex** found in endoplasmic reticulum and responsible for the hydroxylation of a wide range of endogenous and exogenous (xenobiotics) molecules, generates superoxide as an intermediate.

**Dioxygenases:** Enzymes that incorporate both atoms of oxygen into the substrate to form derivatives of hydroxyl and peroxy radicals, e.g. the reactions catalyzed by **cyclo-oxygenase** and **lipo-oxygenase** (in the synthesis of

prostaglandins from arachidonic acid) both lead to the formation of hydroxyl and peroxyl radicals.

## Enzyme Reactions in Which Free Radicals are Functional Products

Functional free radicals may be involved in:

- Cellular signalling in various physiological or biochemical reactions
- Cellular defense mechanism

### *Intracellular Signaling Free Radical*

The free radical nitric oxide is produced in various cells in mammalian system by a reaction catalyzed by **nitric oxide synthase (NOS)**. NOS is a very complex enzyme, requiring five redox cofactors: NADPH, FAD, FMN, heme and tetrahydrobiopterin. There are three forms (isoenzyme) of NOS, which differ in their cellular distribution as follows:

- NOS isoenzyme found in endothelial cells
- NOS isoenzyme found in brain
- NOS isoenzyme found in macrophages.

Nitrous oxide (NO) produced, acts as an intracellular signalling molecule and performs many biological functions:

- Relaxes smooth muscle and blood vessels
- Acts as a neurotransmitter
- Kills bacterial and tumor cells
- Prevents platelet aggregation by increasing synthesis of cGMP.

$NO^{\bullet}$ has a very short half-life (approximately 3–4 seconds) in a tissue because it reacts with oxygen and superoxide and forms peroxynitrite ($ONOO^{--}$), which decomposes to form the highly reactive $OH^{\bullet}$ radical.

### *Cellular Defense Mechanism*

- Phagocytic cells, such as monocytes, macrophages, neutrophils and eosinophils form large quantities of free radical **superoxide** for the destruction and removal of foreign cells.
- During phagocytosis of foreign particles such as bacteria, the phagocytic cells are activated and they exhibit a rapid increase in oxygen consumption known as **respiratory burst (Fig. 27.2)**. This phenomenon reflects the rapid utilization of oxygen and production of large amounts of reactive derivatives such as $O_2^{-\bullet}$, $H_2O_2$, $OH^{-\bullet}$ and $OCl^-$ (hypochlorite ion). Some of these products are potent microbicidal agents.

**Fig. 27.2:** The respiratory burst during phagocytosis by neutrophil.

- **NADPH oxidase** located in plasma membrane is responsible for the respiratory burst. NADPH oxidase is inactive in resting phagocytic cells, and is activated upon contact with various ligands with receptors in the plasma membrane. During phagocytosis NADPH oxidase, converts molecular oxygen from the surrounding tissue into superoxide.
- Next superoxide is converted into hydrogen peroxide by superoxide dismutase (SOD). In the presence of myeloperoxidase (MPO), a lysosomal enzyme present within the phagolysosome, peroxide plus chloride ions are converted into hypochlorous acid (HOCl) that kills the bacteria. Excess $H_2O_2$ is either neutralized by catalase or by glutathione peroxidase.

Genetic deficiencies of NADPH oxidase system cause **chronic granulomatosis**, a disease characterized by persistent and multiple infections of the skin, lungs, bone, liver and lymphocytes.

## EXOGENOUS CAUSES OF FORMATION OF FREE RADICAL

- Environmental factors responsible for the generation of harmful free radicals are:
  - Exposure to ionizing radiation (X-rays and UV rays)
  - Exposure to toxic chemicals (including pollutants).

- These free radicals are highly reactive and are capable of damaging biological compounds. The main biological compounds that are damaged by free radicals are:
  - Polyunsaturated fatty acids (PUFAs)
  - Proteins
  - Nucleic acids especially DNA

Damage to biological compounds lead to many pathological conditions.

### Polyunsaturated Fatty Acids

The hydroxy radical can remove a hydrogen atom from the polyunsaturated fatty acid (PUFA) to produce a lipid free radical ($L^{\bullet}$).

$$LH^{\bullet} + OH \longrightarrow L^{\bullet} + H_2O$$

The interaction of molecular oxygen with these lipid free radicals gives rise to a **peroxyl radical ($LOO^{\bullet}$)**.

$$L^{\bullet} + O_2 \longrightarrow LOO^{\bullet}$$

The peroxyl radical can react with another PUFA to produce a lipid hydroperoxide (LOOH) and another lipid free radical ($L^{\bullet}$).

$$LOO^{\bullet} + LH \longrightarrow LOOH + L^{\bullet}$$

This new lipid radical can, in turn, be converted into another lipid peroxyl radical and lipid peroxidation proceeds as a chain reaction until the available PUFA gets oxidized with the generation of harmful products.

### Protein

Cellular proteins are susceptible to attack by free radical and lead to cellular malfunction, impaired membrane and transport function.

### DNA

Damage to DNA results in mutation and thereby leads to cancer.

## ANTIOXIDANT

Antioxidants are the compounds that protect the body against toxic effect of free radicals. The protective mechanisms of antioxidants serve to scavenge (remove) the free radicals.

Chemical compounds and reactions capable of generating potential toxic oxygen species can be referred to as **pro-oxidants**. On the other hand, compounds and reactions disposing of these species, scavenging them, suppressing their formation or opposing their actions are **antioxidants**.

### Oxidative Stress

In a normal cell, there is an appropriate **pro-oxidant: antioxidant balance**. Pro-oxidants are the compounds capable of generating toxic ROS. However, this balance can be shifted towards the pro-oxidants when production of ROS is increased greatly (e.g. following ingestion of certain chemicals or drugs, exposure to ionizing radiation) or when levels of antioxidants are diminished. This state is called **"oxidative stress"** and can result in serious cell damage if the stress is massive or prolonged.

Free radicals are responsible for the causation and progress of several diseases. These include:
- Atherosclerosis
- Some forms of cancer
- Cataract formation
- Rheumatoid arthritis
- Ulcerative colitis
- Crohn's disease.

### Action of Antioxidant

Different antioxidants act at different levels:
- They may prevent the initiation of chain reactions by removing free radicals
- They may scavenge free radicals generated in chain reactions, thereby interrupting the chain sequence
- They may remove peroxides, thereby preventing further generation of ROS.

### Types of Antioxidant System

There are two main lines of defense against ROS:
1. Enzymatic antioxidant systems also called scavenger enzymes
2. Nonenzymatic (nutrients) antioxidant systems.

#### *Enzymatic Antioxidant System or Scavenger Enzymes*

Such systems include enzymes:
- Superoxide dismutase (SOD)
- Catalase
- Glutathione peroxidase.

**Superoxide Dismutase (SOD):** It has two isomers:
- SOD-1 is a cytosolic copper and zinc containing enzymes, and
- SOD-2 is a mitochondrial manganese containing enzyme.

These enzymes catalyze the following reactions.

**Catalase:** Catalase is present in peroxisomes. The $H_2O_2$ which is generated by SOD can be scavenged by catalase.

**Fig. 27.3:** Role of glutathione as an antioxidant.

***Glutathione peroxidase:*** Glutathione peroxidase is a selenium containing enzyme and can act on lipid hydroperoxides as well as $H_2O_2$ using glutathione (GSH) as the reducing agents **(Fig. 27.3)**.

## *Nonenzymatic (Nutrients) Antioxidant System*

Such systems include:

**Vitamins:** Vitamin E and C, and carotenoids

**Minerals:** Manganese, copper, zinc or selenium.

### *Vitamins antioxidant system*

Few vitamins have antioxidant activity and help in detoxification of free radical. These vitamins are:

- Tocopherol (vitamin E)
- β Carotenes (vitamin A)
- Ascorbic acid (vitamin C).

Reduced form of vitamin E (EH) can break the chain process by reacting with lipid peroxide radical and itself forming a free radical tocopheroxy radical ($E^{\bullet}$).

The resulting vitamin $E^{\bullet}$ radical is stable, as it is able to delocalize the unpaired electron within its structure and does not propagate the chain reaction. Vitamin C and carotenoids are able to generate vitamin E from $E^{\bullet}$, permitting the vitamin E once more to act as an antioxidant **(Fig. 27.4)**. Vitamin C, β-carotene, are also able to reduce and detoxify oxygen intermediates in cells.

**Fig. 27.4:** Inter-relationship between antioxidant systems.

*Minerals antioxidant system*

The activity of the antioxidant enzymes depends on supply of minerals:

- Manganese
- Copper
- Zinc
- Selenium
  - Manganese, copper and zinc are required for the activity of **superoxide dismutase**
  - Selenium is required for the activity of **glutathione peroxidase**.

**Figure 27.4** shows the inter-relationship between antioxidant systems and **Table 27.1** shows ROS and their antioxidants.

**Table 27.1:** ROS and their antioxidants.

| *Reactive species* | *Antioxidant* |
|---|---|
| Superoxide free radical ($O_2^{-\bullet}$) and Hydroxyl free radical ($OH^\bullet$) | Superoxide dismutase, Vitamin E, β-Carotene |
| Hydrogen peroxide ($H_2O_2$) | Catalase, Glutathione peroxidase |
| Lipid peroxides ($LOO^\bullet$) | Glutathione peroxidase |
| Peroxy free radical ($ROO^\bullet$) | Vitamin E and C |

## EXAM QUESTIONS

### Short Notes

1. Antioxidant vitamins.
2. Antioxidant enzymes and minerals.
3. Free radicals.
4. What is reactive oxygen species (ROS)? How are they formed?
5. Name enzyme reactions in which free radicals are functional products.
6. Which are the biological targets for attack by free radicals?
7. Oxidative stress?
8. Inter-relationship between antioxidant systems.
9. Give different ROS and their antioxidants.
10. Action of antioxidant.

### Multiple Choice Questions (MCQs)

**1. The following enzymes act as antioxidants, *except*:**
a. Superoxide dismutase
b. Lactate dehydrogenase
c. Catalase
d. Glutathione peroxidase

**2. All of the following are antioxidant nutrients, *except*:**
a. Vitamin E
b. Vitamin C
c. Carotenoid and flavonoids
d. Phylloquinone

**3. Free radicals are:**
a. Chemical species with unpaired electron
b. Ion having both positive and negative charge
c. Positively charged ion
d. Negatively charged ion

**4. The minerals function as antioxidants, *except*:**
a. Manganese
b. Copper
c. Zinc
d. Iron

**5. α-Tocopherol prevents rancidity by virtue of this property:**
a. Antioxidant
b. Oxidant
c. Hydrogenation
d. Phosphorylation

**6. During respiratory burst, all the following ROS are formed, *except*:**
a. Superoxide
b. Hydrogen peroxide
c. Hydroxy radical
d. Nitric oxide

**7. The main biological target for attack by free radicals are, *except:***
a. PUFA
b. Protein
c. DNA
d. Glycosaminoglycans

**8. Enzyme responsible for respiratory burst is:**
a. NADPH-oxidase
b. Nitric oxide synthase
c. Glutathione peroxidase
d. Catalase

**9. Which of the following is not generating oxygen radicals?**
a. Action of xanthine oxidase
b. Action of superoxide dismutase
c. Reactions catalyzed by cyclooxygenase
d. Ultraviolet radiation

**10. Which of the following is not the result of oxygen radical action?**
a. Peroxidation of unsaturated fatty acids in membranes
b. Damage to DNA
c. Oxidations of amino acids in proteins which leads to impaired transport function
d. Activation of macrophages

**11. Regarding respiratory burst, which the following is correct?**
a. During phagocytosis NADPH oxidase leads to the production of superoxide
b. Genetic deficiency of NADPH oxidase cause chronic granulomatosis disease
c. Rapid consumption of the oxygen
d. All of the above

## Answers for MCQs

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **1.** b | **2.** d | **3.** a | **4.** d | **5.** a | **6.** d | **7.** d | **8.** a |
| **9.** b | **10.** d | **11.** d | | | | | |

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CHAPTER

# 28 Detoxification (Metabolism of Xenobiotics)

**Chapter outline**

- Detoxification and Biotransformation
- Mechanism of Detoxification of Xenobiotics
- Phase I Reactions
- Phase II Reactions

## INTRODUCTION

Certain unwanted and harmful chemical agents may enter the human body through various routes such as inhalation, ingestion, skin contact, etc. These foreign compounds are known as **xenobiotics** (Greek, Xenos = stranger).

- These foreign compounds may be in the form of food, drugs, chemical carcinogens, additives, preservatives or pollutants.
- Besides, foreign molecules are also produced in intestine through bacterial enzymatic actions upon normal digestion products. Xenobiotics can produce a variety of biological effects, including pharmacologic responses, toxicity, immunologic reactions, and cancer.

## DETOXIFICATION AND BIOTRANSFORMATION

Most of these foreign compounds are subjected to metabolism (chemical alteration) in the human body. In the past the metabolic processes that lead to the disposition of foreign compounds have been referred to as **detoxification** mechanisms. However, the term is not always appropriate, because the detoxified products are sometimes more toxic than the original substance. **Biotransformation** has been suggested as a preferable term.

The primary purpose of the biotransformation process is to convert the lipophilic, nonpolar, toxic compound to more polar, water soluble forms to decrease the permeability of the toxic compound through lipid cell membrane, thus, protecting cell interior and to facilitate their excretion from the body through urine or bile.

## MECHANISM OF DETOXIFICATION OF XENOBIOTICS

The liver is the principal organ responsible for detoxification of xenobiotics. The pathways for hepatic detoxification include either:

1. Oxidation
2. Reduction
3. Hydrolysis
4. Conjugation
5. Some combinations of all these metabolic reactions.

For convenience, the detoxification reactions of xenobiotics are grouped into two phases **(Fig. 28.1):**

1. Phase I reactions
2. Phase II reactions.

## PHASE I REACTIONS

Phase I reactions involve **oxidation, reduction** and **hydrolysis** and metabolites may be excreted without further reactions or is subsequently conjugated with conjugating agent (phase II reaction) for excretion.

### Oxidation

A large number of foreign substances which include alcohols, aldehydes, amines, aromatic hydrocarbons and certain drugs are destroyed in the body by oxidation. Reactions of this type include.

**Fig. 28.1:** Phase I and Phase II reactions of detoxification.

**Fig. 28.2:** Mode of action of cytochrome $P_{450}$.

## Hydroxylation

**Hydroxylation** is the chief reaction involved in oxidative metabolism of foreign compounds, catalyzed by **monooxygenase** or **cytochrome $P_{450}$** (so named because the enzyme absorbs light at 450 nm, P stands for pigment). Monooxygenase enzyme, is also called **mixed function oxidases (MFOs).**

- Microsomal cytochrome $P_{450}$ enzymes hydroxylated many xenobiotics such as drugs, carcinogens and environmental agents (pollutants). Their hydroxylated products are more water soluble facilitating their excretion.
- The reaction catalyzed by cytochrome $P_{450}$ can be represented as shown in **Figure 28.2**. Approximately, 50% of the drugs that are ingested in humans are metabolized by microsomal cytochrome $P_{450}$ enzyme.

## Oxidation of Alcohols and Aldehydes to Acids

- Alcohols and aldehydes are converted to corresponding acids by oxidation. For example:

Ethanol → Acetic acid

Methanol → Formic acid

Benzaldehyde → Benzoic acid } Conjugated later on

Benzylalcohol → Benzoic acid

- Oxidation of methanol results in the production of formic acid, a more toxic compound than methanol which may be removed by phase II conjugation reaction.

## Oxidation of Aromatic Hydrocarbons

Aromatic hydrocarbons oxidized to phenols. Phenols may be removed by conjugation reaction. For example:

Benzene → Phenol

Acetanilide → Aminophenol } Conjugated later on

## Oxidative Deamination of Amines

Aliphatic amines undergo oxidative deamination to the corresponding acids and nitrogen is converted to urea. For example:

Butylamine → Acetoacetic acid

Benzylamine → Benzcic acid

## Oxidative Dealkylation of Hydrocarbons

Aromatic hydrocarbons containing alkyl group is oxidized to acids. For example:

Toluene → Benzoic acid

## Reduction

Reduction is less common than oxidation. The major group of compounds which are reduced and detoxified by the liver are **azo compounds** and **nitro compounds.** Some important reactions are:

- Aldehydes may be reduced to alcohol, e.g.

Chloral → Trichloroethanol

- Aromatic nitro compounds are reduced to amines, e.g.

Picric acid ⟶ Picramic acid

- Compounds with S-S bonds reduced to -SH, e.g.

Disulfiram (used in the treatment of alcoholism) ⟶ Dithiocarbamic acid

### Hydrolysis

Foreign compounds that are esters, amides or glycosides are subject to hydrolysis by esterases, amidases and glycosidases, e.g.

Procaine (Anesthetic) ⟶ P-Aminobenzoic acid + Diethylaminoethanol

Aspirin ⟶ Salicylic acid + Acetic acid

Atropine ⟶ Tropin acid + Tropin

Cyclohexane ⟶ Cyclohexane diol

## PHASE II REACTIONS

In phase II, the hydroxylated or other compounds produced in phase I are converted by specific enzymes to various polar (water soluble) metabolites by **conjugation reactions**.

### Conjugation Reaction

- Conjugation means chemical combination of one compound with some other.
- Xenobiotics may undergo conjugation reactions without prior metabolism (phase I reaction) but conjugation occurs more frequently as a phase II reaction, after preliminary modification of the molecule by phase I reactions (oxidation, reduction and hydrolysis).
- The effect of conjugation is to make the compound more polar (water soluble) and therefore, more easily excreted in the urine or bile. Many conjugating agents are known. These are as follows:
  - Glucuronic acid
  - Glycine
  - Glutamine
  - Cysteine
  - Glutathione
  - Acetyl-CoA
  - Phosphoadenosyl phosphosulfate (PAPS)
  - S-Adenosyl methionine (SAM)
  - Thiosulfate
  - British antilewisite (BAL).

### Conjugation with Glucuronic Acid

- Glucuronic acid is formed from oxidation of glucose by glucuronic acid pathway. Glucuronic acid participates in detoxification reactions as its UDP-derivative, UDP-glucuronic acid. **UDP-glucuronic** acid combines with a number of xenobiotics.
- A general conjugation reaction with UDP-glucuronic acid is shown in **Figures 28.3A and B**. **UDP-glucuronyl transferase** enzyme catalyzes the formation of glucuronide conjugates, in which glucuronic acid being **transferred** to various compounds.
- Various drugs that form glucuronides are:
  - Morphine
  - Chloramphenicol
  - Salicylic acid
  - Indomethacin
  - Dapsone
  - Sulfathiazole, etc.

**Formation of bilirubin diglucuronide is a normal metabolic reaction for detoxification of bilirubin by phase reactions (Figs. 28.3A and B).**

### Conjugation with Glycine

- Many aromatic carboxylic acids are conjugated with glycine. The most common example is conjugation of benzoic acid with glycine to form hippuric acid. Some more examples are given below:

Glycine + Benzoic acid ⟶ Hippuric acid

Glycine + Salicylic acid ⟶ Salicyluric acid

Glycine + Nicotinic acid ⟶ Nicotinuric acid

Glycine + Cholic acid ⟶ Glycocholic acid

Glycine + Deoxycholic acid ⟶ Deoxyglycocholic acid

**Figs. 28.3A and B:** (A) Representation of the general reaction for conjugation with glucuronic acid; (B) Conjugation reaction with bilirubin.

Cholic acids and deoxycholic acid are conjugated with glycine to form glycocholic acid and deoxyglycocholic acid respectively are the examples of normal metabolic detoxification reactions.

## Conjugation with Glutamine

In normal metabolic pathway, phenylacetate conjugates with glutamine.

## Conjugation with Cysteine

Cysteine is acetylated first with acetic acid whereby acetyl-cysteine is formed which gets conjugated with toxic substances like bromobenzene, chlorobenzene, iodobenzene, naphthalene, anthracene, benzyl chloride and number of other substances which are converted to nontoxic **mercapturic acids**. For example:

## Conjugation with Glutathione (GSH)

- Glutathione (γ-glutamyl-cysteinyl-glycine) is a tripeptide of glutamic acid, cysteine and glycine. A wide range of xenobiotics are known to be excreted following conjugation with glutathione. These include:
  - Aromatic nitro compounds
  - Halogenated compounds
  - Aliphatic halides
  - The epoxide formed in phase I oxidation of hydrocarbons.
- The general reaction can be represented as follows:

Where,

X = xenobiotic
GSH = glutathione
X-S-G = Glutathione conjugate.

- Glutathione conjugates are subjected to further metabolism and excreted in the form of mercapturic acid (sulfur containing compound).

## Conjugation with Acetic Acid (Acetylation)

- It has just been stated that acetate is used together with cysteine in the formation of mercapturic acids. However, a range of compounds with an amino group undergo conjugation with acetic acid alone as acetyl-CoA in a reaction catalyzed by a **acetyltransferase.**
- The drugs that undergo acetylation prior to their excretion are:
  - Sulphanilamide (sulpha drug)
  - Isoniazid (antituberculosis drug)
  - Para-aminobenzoic acid (PABA).
- Acetylation is represented by:

## Conjugation with Sulfate (Sulfation)

- The sulfur donor for detoxification reactions is ***phosphoadenosyl phosphosulfate (PAPS)***. This compound is called **active sulfate**.
- Sulphotransferase transfers sulfate from PAPS to the alcoholic OH of the phenol, cresol, indole, skatole, steroids or to the $NH_2$ of aliphatic and aromatic amines to form various **etheral sulfates.**
- The general reaction can be represented as follows:

## Conjugation by Methylation

A few xenobiotics are subjected to methylation for their excretion. The methyl donor is **S-adenosylmethionine (SAM),** active form of methionine. The reaction is catalyzed by methyltransferase. For example:

- Pyridine, nicotinic acid, nicotinamide, thyroxine
- Estrogen
- Epinephrine and norepinephrine are methylated before their excretion.

## Conjugation with Thiosulfate

- Cyanides, highly toxic compounds are conjugated with thiosulfate and converted to relatively nontoxic **thiocyanate (-SCN)**.
- Small quantities of cyanide formed during the course of normal metabolism is converted to thiocyanate by an enzyme called **rhodanese (thiosulfate-sulfur transferase)**.

## Conjugation with British Antilewisite (BAL)

- Certain heavy metals like arsenic, cadmium, gold and mercury are detoxified by British antilewisite (BAL) which is 2, 3 **mercaptopropanol**.
- Heavy metals impair the functioning of the enzymes by binding with -SH group of the enzymes, BAL acts as an antidote, it has an high affinity for these metal ions and removes these ions from the enzyme.

# EXAM QUESTIONS

## Short Notes

1. Detoxification by conjugation.
2. Xenobiotics phase II reactions.
3. Oxidation of xenobiotics by cytochrome ($P_{450}$).
4. What is biotransformation? Name different pathways for biotransformation.
5. Detoxification by oxidation and reduction.

## Multiple Choice Questions (MCQs)

**1. Toxic cyanides are conjugated with:**
a. Cysteine b. Active sulfate
c. Glucuronic acid d. Thiosulfate

**2. All of the following are detoxifying agents, *except*:**
a. Glycine b. Glutathione
c. Glucuronic acid d. Glycogen

**3. All of the following are true for cytochrome $P_{450}$, *except*:**
a. Is also called mixed function oxidase
b. Uses NADPH
c. Associated with smooth endoplasmic reticulum
d. Is an allosteric enzyme

**4. Biotransformation by oxidation is catalyzed by:**
a. Cytochrome c b. Cytochrome $aa_3$
c. Cytochrome b d. Cytochrome $P_{450}$

**5. All of the following compounds are detoxified by sulphation, *except*:**
a. Indole b. Phenol
c. Cresol d. Benzoic acid

**6. Phase II reactions include all the following, *except*:**
a. Glucuronidation b. Sulfation
c. Hydroxylation d. Methylation

**7. Which of the following statement is correct for glutathione, *except*?**
a. Glutathione (GSH) can be conjugated with toxic molecules and detoxify them.
b. Glutathione is a dipeptide derived from glutamic Acid and cysteine
c. Glutathione helps to maintain the SH groups of certain proteins in reduced state.
d. Glutathione involved in the transport of certain amino acids

**8. Phase I reactions include:**
a. Oxidation b. Reduction
c. Hydrolysis d. All of the above

### Answers for MCQs

**1.** d **2.** d **3.** d **4.** d **5.** d **6.** c **7.** b **8.** d

CHAPTER

# 29

# Cancer

Chapter outline


- Characteristics of Cancer Cells
- Types of Cancer
- Carcinogenesis
- Carcinogens
- Proto-oncogenes and Oncogenes
- Tumor Suppressor Genes
- Apoptosis
- Tumor Markers
- Cancer and Diet


## INTRODUCTION

The term cancer applies to a group of diseases in which cells grow abnormally. It may be defined as "***malignant neoplasm***." Neoplasm means new growth. Neoplasia is a general term given to diseases that cause abnormal growth of cells.

A mass of tissue formed as a result of abnormal excessive, uncoordinated, autonomous and purposeless proliferation of cells is called ***tumor***. The branch of science dealing with the study of neoplasm or tumor is called oncology (oncos = tumor, logos = study). Tumors may be "benign" (that is, it does not invade or spread to distant sites in the body and does not destroy the tissue in which it originates, i.e. a non-cancerous tumor) or "malignant" (that is, it invades and destroys the tissue in which it originates and can spread to other sites in the body via the blood stream and lymphatic systems). The term used for all malignant tumors is ***cancer*.**

## CHARACTERISTICS OF CANCER CELL

Cancer cells are characterized by following properties:

1. Differentiation and anaplasia
2. Rate of growth
3. Invasion and metastasis (distant spread) to other parts of the body (meta = transformation, stasis = residence).
4. Biochemical changes

### Differentiation and Anaplasia

Differentiation means the extent to which a neoplasm resembles both histologically and functionally the normal cells. In general, benign tumor cell very closely resemble the both histologically and functionally to the normal cells (well differentiated). On the other hand, a malignant neoplasm varies greatly in its resemblance with the normal tissue**.** Thus based on its differentiation it is often classified into three types.

1. **Well differentiated:** This maximally resembles normal cells.
2. **Undifferentiated or poorly differentiated:** These neoplasms show hardly any resemblance to normal.

Malignant neoplasms that are composed of poorly differentiated cells are said to be **anaplastic**. Lack of differentiation (anaplasia) is considered as a hallmark of malignancy. Lack of differentiation is often associated with many other morphologic changes as follows:

- **Pleomorphism:** Pleomorphism is the variation in **size** and **shape** of the cells or nucleus. Thus, the cells within the same tumor are not uniform but range from large cells to extremely small and primitive appearing.
- **Abnormal nuclear morphology:** Characteristically the nuclei contain abundant chromatin and are dark staining (hyperchromatic). The nuclei disproportionately large for the cell and, nuclear to cytoplasm ratio may approach 1:1 instead of the normal 1:4 or 1:6. The nuclear shape is

variable and often irregular and the chromatin is often coarsely clumped.

- **Mitosis:** The rate of mitosis increases in malignant neoplasia. Some of the tumors are classified on the rate of mitosis.
- **Loss of polarity:** This means loss of normal arrangement of the tumor cells. For example in a normal squamous epithelium the cells are arranged in a pavement pattern. When this becomes malignant the cells are arranged in disorganized, haphazard and disordered fashion.
- **Formation of tumor giant cell:** Some anaplastic cell possesses only a single huge polymorphic nucleus and others having two or more large hyperchromatic nuclei.

### Rate of Growth

Malignant tumors show faster rate of growth due to loss of contact inhibition of growth transformed cells and form multilayers instead of a monolayer usually formed by normal cells, e.g. leukemia, lymphomas, lung cancers. In comparison, some malignant tumors have slower growth rate, e.g. breast cancer, colon cancer. Rate of growth is important from the point of view of response to chemotherapy. Faster growing tumors are more responsive to chemotherapy compared to slower growing tumor.

### Invasion and Metastasis

Benign tumors do not invade (direct spread) or metastasize (distant spread). Invasion and metastasize is the hallmark of malignant tumors.

### Biochemical Changes which Occur in Cancer Cell

Biochemical changes which occur in cancer cell are:

- Increased synthesis of RNA and DNA.
- Increased activity of **ribonucleotide reductase** required for the formation of deoxyribonucleotides from ribonucleotides.
- Increased rates of aerobic and anaerobic **glycolysis.** Thus, more pyruvate is produced than can be metabolized. This in turn results in excessive production of lactate and **lactic acidosis** results.
- Synthesis of certain fetal proteins, e.g. Carcino embryonic antigen.
- Inappropriate synthesis of certain growth factors and hormones.
- Alterations of the cell surface due to changes in the composition of glycoproteins or glycosphingolipids.

## TYPES OF CANCER

Cancers are classified according to the tissue and cell types from which they arise, e.g.

- Carcinomas: Cancer arises from epithelial tissues
- Sarcomas: Cancer arises from mesenchymal tissues
- Leukemia: Cancer of blood forming organs
- Lymphoma: Cancer of lymph node
- Hepatoma: Carcinoma of hepatocytes.

## CARCINOGENESIS (MOLECULAR BASIS OF CANCER)

**Carcinogenesis** means mechanism of induction of tumors (cancer). Agents which can induce cancer are called ***carcinogens.*** Carcinogenesis is a gradual multi step process involving many generations of cells.

- Cell growth of normal as well as abnormal types is under genetic control. In cancer there are either genetic abnormalities in the cell, or there are normal genes with abnormal expression.
- The abnormalities in genetic composition may be from inherited or induced mutations (induced by carcinogenic agents, namely chemicals, viruses, and radiation). The mutated cells transmit their characters to the next progeny of cells and result in cancer.
- In normal cell growth, there are four regulatory genes.
  1. Proto-oncogenes are growth promoting genes.
  2. Antioncogenes are growth inhibiting or growth suppressor genes.
  3. Apoptosis regulatory genes control the programed cell death.
  4. DNA repair genes are those normal genes which regulate the repair of DNA damage that has occurred during mitosis.
- In cancer the transformed cells are produced by abnormal cell growth due to genetic damage to these normal controlling genes. The corresponding abnormalities in these four genes are as under.
  1. Activation of **growth promoting oncogenes** causing transformation of cell (mutant form of normal proto-oncogene in cancer is termed oncogene). Many of these cancers associated genes (oncogenes), were first discovered by viruses, and hence named as **V-onc**. Gene products of oncogenes are called **oncoproteins**.
  2. Inactivation of cancer **suppressor genes** (i.e. inactivation of antioncogenes) permitting the cellular proliferation of transported cells.

**Table 29.1:** Important chemical carcinogens.

| *Class* | *Examples* |
|---|---|
| Anticancer drugs | Cyclophosphamide, Nitrosourea, and Acetyl imidazole |
| Aromatic hydrocarbons | Benzo[a]pyrene, tobacco, smoke, smoked animal foods, industrial and atmospheric pollutants |
| Aromatic amines | β-Naphthylamine, Benzidine, Azo dyes used in rubber industries |
| Naturally occurring compounds | Aflatoxin $B_1$ |
| Inorganic compounds | Vinyl chloride, Asbestos, metals like Nickel, Lead, Chromium, Cobalt, |
| Nitrosamine compounds | Dimethylnitrosamine, diethylnitrosamine |
| Miscellaneous compounds | Phenols, Hormones (estrogen), Phenobarbital, and Artificial sweeteners like saccharine and cyclamates |

3. Abnormal apoptosis regulatory genes which may act as oncogenes or antioncogene.
4. Failures of DNA repair genes and thus inability to repair the DNA damage resulting in mutation.

## CARCINOGENS

Agents which can induce cancer are called ***carcinogens***. Carcinogens are a variety of external agents which are divided into three groups:
1. Chemical carcinogens
2. Physical carcinogens
3. Biologic carcinogens.

### Chemical Carcinogens (Table 29.1)

Depending upon the mode of action of carcinogenic chemicals, they are divided into two groups:
1. ***Initiators*** of carcinogenesis
2. ***Promoters*** of carcinogenesis.

#### *Initiators of Carcinogenesis*

These are the chemical carcinogens which can initiate the process of abnormal new growth of cells. These are further classified into two subgroups:
a. ***Indirect*** acting carcinogens
b. ***Direct*** acting carcinogens.

*Indirect acting carcinogens (procarcinogens)*
Indirect acting carcinogens require prior metabolism to become carcinogenic. One or more enzyme catalyzed reactions convert procarcinogens to active carcinogens. This is called metabolic activation of procarcinogens. Examples of procarcinogens are: **(Table 29.1).**
- **Aromatic hydrocarbons,** e.g. Benzo[a]pyrene, Tobacco smoke, industrial and atmospheric pollutants.
- **Aromatic amines,** e.g. Benzidine, β-naphthylamine, azo dyes used in rubber industries.
- **Naturally occurring products,** e.g. Aflatoxin $B_1$.
- **Inorganic compounds,** e.g. Vinyl chloride, Asbestos, metals like nickel, lead, chromium, etc.
- **Nitrosamine compounds,** e.g. Dimethylnitrosamine, diethylnitrosamine found in whisky, new car interiors, tobacco smoke.

*Direct acting carcinogens*
These do not require metabolic activation. These include mainly various anticancer drugs, e.g. cyclophosphamide, nitrosourea, acetyl imidazole, etc.

#### *Promoters of Carcinogenesis*

Certain chemical substances are not carcinogenic but they help the initiated cell to proliferate further are called promoters of carcinogenesis **(Table 29.1).** For example, phenols, phenobarbital, artificial sweeteners like saccharine and cyclamates.

#### *Action of Chemical Carcinogens (Fig. 29.1)*

Direct or indirect acting carcinogens are usually **electrophiles,** i.e. they are deficient in electrons (free radicals). These free radical carcinogens can covalently bind to purines, pyrimidines and phosphodiester bonds of DNA causing unrepairable damage. This unrepaired damage generates mutations in DNA and mutation in DNA may lead to cancer.

### Physical Carcinogens

- Physical carcinogenic agent is radiant energy both **ultraviolet light** and **ionizing radiation,** i.e. **X-rays,** α, β and γ-**rays.**
- These rays damage DNA which is the basic mechanism of carcinogenicity with radiant energy.
- The main source of UV radiation is the sunlight, others are UV lamps, Welder's arcs, etc. In humans, excessive

**Fig. 29.1:** Action of chemical carcinogens.

exposure of UV rays can cause various forms of skin cancers.

- Ionizing radiation of all kinds like X-rays, α, β and γ-rays, radioactive isotopes, protons, and neutrons can cause cancer.

### Mode of Action of Radiation

- Ultraviolet light and ionizing radiation differ in their mode of action.
- UV rays damage the DNA by formation of **pyrimidine dimmers** in DNA or by formation of **apurinic** or **pyrimidine** sites in DNA.
- While ionizing radiations cause the formation of highly reactive **free radicals that** can interact with DNA leading to molecular damage.

## Biologic Carcinogens

- Biologic carcinogens are chiefly ***viruses***, ***parasites*** and ***bacteria***. The role of viruses in the causation of cancer is more significant.
- Oncogenic (carcinogenic) viruses contain either DNA or RNA as their genome. The two types of carcinogenic viruses are:
  1. DNA oncogenic viruses
  2. RNA oncogenic viruses.

### DNA Oncogenic Viruses

DNA oncogenic viruses are classified into five subgroups. These are:

a. Papovavirus
b. Herpes viruses
c. Adenoviruses
d. Pox viruses
e. Hepadnaviruses.

### RNA Oncogenic Viruses

The RNA viruses use RNA as the genome. RNA oncogenic viruses are **retroviruses** they contain the enzyme ***reverse transcriptase***. All retroviruses are not oncogenic. The examples of RNA oncogenic viruses are:

- Rous sarcoma virus
- Leukemia sarcoma virus
- Mouse mammary tumor virus, etc.

### Mechanism of Viral Carcinogenesis

DNA and RNA oncogenic viruses differ in their mode of action.

#### *Mode of action of DNA oncogenic virus*

The DNA virus infects the host cell. Then, DNA virus binds tightly to host cell DNA and causes alterations in gene expression of host cell DNA and thus causes cancer by altering the types of protein made in cell. Viral oncoproteins bind to tumor suppressors and inactivate them.

#### *Mode of action of RNA oncogenic virus*

The RNA viruses use RNA as the genome. The RNA gets copied by **reverse transcriptase** to produce single strand of viral DNA. Single strand of viral DNA is then copied to form another strand of complementary DNA, resulting in double stranded ***viral DNA or provirus***. The provirus is then integrated into the DNA of the host cell genome and may transform the cell into cancer cell.

## PROTO-ONCOGENES AND ONCOGENES

- Proto-oncogenes are normal genes which stimulate cell division.
- Their normal activity during somatic growth and in regeneration and repair are strictly controlled.
- Cellular proto-oncogenes code for a number of proteins, e.g. growth factors, receptors, transcription factors and other proteins involved in cell proliferation.
- When proto-oncogenes get mutated they become **oncogenes.**
- Oncogenes are genes capable of causing cancer. In the cancer cell, their normal proto-oncogenes are permanently changed to oncogenes and the balance

between factors stimulating and the factors inhibiting cell growth is permanently lost resulting in increased proliferation.

- When oncogenes are expressed, they produce mutated proteins, e.g. growth factors, receptors, transcription factors and other proteins involved in cell proliferation.

Thus, various factors that cause cancer may all act through their effects on proto-oncogene. Radiation, chemical carcinogens and viruses may cause mutations in the proto-oncogene.

## TUMOR SUPPRESSOR GENES

Tumor suppressor genes are normal genes, which **inhibit cell proliferation** and which normally suppresses tumor formation. These tumor suppressor genes, sometimes called **recessive oncogenes** or **anti-oncogenes**. Inactivation by mutation of tumor suppressor gene can cause some types of tumor.

Two of the most widely studied tumor suppressor genes are **retinoblastoma (RB) gene** and $P^{53}$ gene.

- The retinoblastoma (RB) gene was the first tumor suppressor gene discovered. Retinoblastoma is a rare tumor in children. The RB gene codes for nuclear phosphoprotein.
- $p^{53}$ gene codes for a nuclear protein. Mutations of $p^{53}$ gene are found in at least 30% of the all cancers.

## APOPTOSIS

Apoptosis (Greek "***dropping off***" **or** ***falling off***) is the normal and programmed destruction of cells during embryogenesis, development and adult life. Disruption of apoptosis can promote inappropriate cell survival and the development of cancer.

## TUMOR MARKERS

Tumor marker is a biological substance synthesized and released by cancer cells and found in an increased amount in the blood, other body fluids or tissues which may suggest the presence of a type of cancer. Different types of tumor marker are **(Table 29.2)**:

### Oncofetal Oncogenes

- Oncofetal antigens are proteins produced during fetal life. These proteins are present in high concentrations in the sera of fetuses and decreases to low level or disappear after birth.
- These proteins reappear in cancer patients because certain genes are reactivated as the result of the malignant transformation of cells, e.g. α-**fetoprotein (AFP)** and **carcinoembryonic antigen (CEA)**.

### Hormones

- Several hormones that are used as tumor markers are:
  - Calcitonin
  - Growth hormone
  - Parathyroid hormone
  - Prolactin
  - Human chorionic gonadotropin (hCG).

**Table 29.2:** Tumor markers commonly used in clinical practice.

| *Tumor markers* | *Associated cancer or tumor* |
|---|---|
| **Oncofetal antigens** | |
| α-Fetoprotein (AFP) | Hepatocellular, germ cell |
| Carcinoembryonic antigens (CEA) | Colorectal, gastrointestinal, pancreatic, lung |
| **Hormones** | |
| Calcitonin | Thyroid |
| Growth hormone | Pituitary adenoma, renal, lung |
| Parathyroid hormone | Liver, renal, breast, lung |
| Prolactin | Pituitary adenoma, renal, lung |
| Human chorionic gonadotropin (hCG) | Teratoma of testes, choriocarcinoma |
| **Carbohydrate markers** | |
| CA 125 | Ovarian, endometrial |
| CA 549 | Breast, ovarian |
| **Proteins** | |
| β-microglobulin | Multiple myeloma, B-Cell lymphoma, |
| C-peptide | Insulinoma |
| Ferritin | Liver, lung, breast, leukemia |
| Immunoglobulin (Bence Jones protein) | Multiple myeloma |
| Prostatic specific antigen (PSA) | Prostate |
| **Enzymes** | |
| Amylase | Pancreatic |
| Alkaline phosphatase | Bone, liver, leukemia, sarcoma |
| Prostatic acid phosphatase | Prostate |
| Lactate dehydrogenase | Liver, lymphomas, leukemia |

### Carbohydrate Markers

- Carbohydrate markers are high molecular weight glycoproteins. They usually are abbreviated CA for carbohydrate antigen. These are:
  - CA 125
  - CA 549

### Proteins

Some of the protein markers are:
- β-microglobulin
- C-peptide
- Ferritin
- Immunoglobulin (Bence Jones protein)
- Prostate specific antigen (PSA).

### Enzymes

Several enzymes that are used as tumor markers are:
- Amylase
- Alkaline phosphatase
- Prostatic acid phosphatase
- Lactate dehydrogenase.

**Clinical Applications of Tumor Markers**

Tumor markers can be used in a number of ways including:
- Differential diagnosis in symptomatic individuals
- Clinical staging of cancer
- Prognosis of disease
- Evaluation of success of treatment
- Detection of recurrence of cancer
- Monitoring of response to therapy.

## CANCER AND DIET

- Increased fat and animal protein intake in the diet may increase the risk of cancer.
- This diet may favor bacterial flora capable of increasing the conversion of acid and neutral sterols to carcinogens.
- The feces produced from high beef diets also contain carcinogens such as **nitrosamide**.
- A **high fiber diet** is associated with reduced incidence of cancer of colon.
- Intake of food high in **antioxidant** (e.g. β-carotene, vitamin E, vitamin C, trace element selenium) eicosapentaenoic acid and also certain compounds found in the cruciferous vegetables, broccoli, cabbage, and cauliflower have protective effect against some forms of cancer.

## EXAM QUESTIONS

### Short Notes

1. Tumor suppressor genes.
2. Tumor marker.
3. Chemical carcinogen.
4. Biological carcinogen.
5. Physical carcinogen.
6. Proto-oncogene and oncogene.
7. Mechanism of carcinogenesis.
8. Characteristic features of cancer cells.

### Multiple Choice Questions (MCQs)

1. **Activation of proto-oncogenes to oncogenes occurs by following mechanisms, *except*:**
   a. Promoter insertion
   b. Chromosomal translocation
   c. Gene amplification
   d. Post-translational modification
2. **All of the following statements regarding proto-oncogene are true, *except*:**
   a. They are present in normal cells
   b. They are present only in cancer cells
   c. They code for growth factors, receptors and other proteins involved in cell proliferation
   d. When they get mutated, they become oncogenes.
3. **Which of the following statements regarding tumor suppressor genes are true?**
   a. Normal genes
   b. Antioncogenes
   c. Mutation of this gene causes tumor formation
   d. All of the above
4. **Regarding oncogenes which of the following is true:**
   a. When proto-oncogene get mutated they become oncogene
   b. Oncogenes are genes capable of causing cancer

c. When oncogenes are exposed they produce mutated version of growth factors
d. All a, b, and c are true

**5. Retinoblastoma (RB) gene is a:**
a. Proto-oncogene b. Oncogene
c. Carcinogen d. Antioncogene

**6. Genes capable of causing cancer are, *except*:**
a. Mutagens b. Oncogenes
c. Carcinogens d. Antioncogenes

**7. Which one of the following is not a tumor marker?**
a. Alpha-fetoprotein
b. Carcino embryonic antigen
c. Prostatic acid phosphatase
d. Parathormone

**8. Tumor suppressor genes are sometimes called:**
a. Antioncogenes b. Proto-oncogenes
c. Oncogenes d. Proximate carcinogens

**9. A normal cell can be transformed into a cancer cell by which of the following:**
a. Ionizing radiation b. Mutagenic chemicals
c. Oncogenic bacteria d. All of the above

**10. Proto-oncogenes are present in:**
a. Oncoviruses
b. Cancer cells
c. Healthy human cells
d. Prokaryotes

**11. Retinoblastoma can result from a mutation in:**
a. ras proto-oncogene b. erbB proto-oncogene
c. $p^{53}$ gene d. RB1 gene

**12. RB1 gene is:**
a. A tumor suppressor gene
b. Oncogene
c. Proto-oncogene
d. Activated proto-oncogene

## Answers for MCQs

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **1.** d | **2.** b | **3.** d | **4.** d | **5.** d | **6.** d | **7.** d | **8.** a |
| **9.** d | **10.** c | **11.** d | **12.** a | | | | |

CHAPTER

# 30

# Environment and Health

**Chapter outline**

- Classification of Environment
- Environmental Pollution and their Effects on Human Health
- Metabolic Responses or Adaptations to an Altered Environmental Temperature
- Heat Stress
- Cold Stress

## INTRODUCTION

The term environment implies all the external factors, living and non-living, material and nonmaterial with which man is in constant interaction. This includes air, water, food, soil, etc. The modern concept of environment includes not only the water, air, food and soil, but also social and economic conditions under which we live.

Changes in environment are tolerated within normal limits and the organism tends to adapt itself to the altered situation. Environmental biochemistry involves environmental pollution, as well as the response of the body to the various factors like temperature, cold, etc.

The metabolic response to environmental factors and the limits of metabolic changes leading to adaptation constitute environmental biochemistry.

## CLASSIFICATION OF ENVIRONMENT

For convenience the environment has been divided into four groups, which are closely related to each other **(Fig. 30.1).** These are:

1. Physical environment
2. Chemical environment
3. Biological environment
4. Psychosocial environment.

***Physical environment:*** The term physical environment is applied to non-living things and physical factors, with which man is in constant contact. For example, water, air, soil, heat, noise, climate, radiation, etc.

**Fig. 30.1:** Different groups of environment related to man.

***Chemical environment:*** It includes chemical factors affecting health for example, food pollutants, natural toxins, xenobiotics, etc.

***Biological environment:*** The biological environment is of living things which surrounds man. For example, viruses, bacteria and other microbial agents, insects, rodents, animals and plants, etc.

***Psychosocial environment:*** It includes psychosocial factors affecting personal health, healthcare and community well-being. For example, culture, customs, habits, occupation, income, religion, etc.

## ENVIRONMENTAL POLLUTION AND THEIR EFFECTS ON HUMAN HEALTH

Pollution is defined as the addition of certain substances into the environment (air, water, food and soil) resulting

in undesirable and unwanted changes in the environment that are harmful to human beings. The pollutants may be organic, inorganic, biological or physical factors. We shall discuss water and air pollution and metabolic responses or adaptation to an altered environmental temperature.

## Water Pollution

Water intended for human consumption should be free from:

- Harmful chemical substances
- Pathogenic organisms
- Color and odor

Water is said to be polluted or contaminated when it does not fulfil the above criteria. Pure uncontaminated water does not occur in nature. It contains impurities of various kinds, natural and man-made which are discussed below.

### *Natural Impurities*

Natural impurities are not essentially dangerous. The natural impurities picked up during rainfall include:

- Dissolved gases, e.g. nitrogen, carbon dioxide, hydrogen, sulfide
- Dissolved minerals, e.g. salts of calcium, magnesium, sodium, etc.

### *Man-made Impurities or Pollutants*

Man-made impurities which cause water pollution are due to:

- Human activity
- Urbanization
- Industrialization
- Agricultural and domestic wastes.

Water pollutants resulting from these factors could be ***organic, inorganic*** or ***physical substances.***

#### *Organic water pollutants*

- Sewage contains decomposable organic matter. As a result, microbial population increases and oxygen gets depleted. Normal ***dissolved oxygen (DO)*** content of water is **4 to 6 ppm.** Decrease in DO content of water indicates pollution due to organic matter.
- Organic wastes are measured by **biochemical oxygen demand (BOD)** or **chemical oxygen demand (COD).**
- Agricultural pollutants include fertilizers and pesticides run off from agricultural lands. Pesticides that are added to water are:
  - Chlorinated hydrocarbons and their derivatives
  - Herbicides
  - Soil insecticides, etc.

#### *Inorganic water pollutants*

Industrial wastes contain toxic agents and include:

- Detergent solvents
- Cyanides
- Heavy metals (arsenic, cadmium, lead and mercury)
- Minerals (chromium, fluoride, selenium)
- Organic acids
- Nitrogenous substances
- Bleaching agents
- Dyes
- Pigments
- Sulphides
- Ammonia, etc.

#### *Physical water pollutants*

Physical water pollutants include radioactive substances generated in:

- Nuclear power plant
- Medical, industrial and research field
- Nuclear weapon.

## Effect of Water Pollutant on Human Health

Human health may be affected adversely by the ingestion of polluted water and lead to certain diseases or metabolic impairment. This water related disorders may be classified as follows:

- Biological water borne diseases
- Chemical water borne disorders.

### *Biological Water Borne Diseases*

Biological water borne diseases caused by presence of infective pathogenic organisms and due to presence of aquatic hosts is listed in **Table 30.1**.

### *Chemical Water Borne Disorders*

***Nitrates:*** This is a rare occurrence; occur when surface of the water from farmland treated with fertilizer, gain access to the water supply. In infants high nitrate content water is associated with **methemoglobinemia that** leads to ***cyanosis.***

***Fluoride:*** Mottling of the dental enamel (dental fluorosis). High levels of fluoride cause mottling of the dental enamel. The presence of fluoride at about 1 mg/liter in drinking water has protective effect against dental caries. Excess fluoride causes inhibition of an enzyme enolase in glycolysis.

**Table 30.1:** Biological water borne diseases caused by infective pathogens and aquatic hosts.

| | *Organism* | *Disease* |
|---|---|---|
| By infective pathogens | Virus | Viral hepatitis A, poliomyelitis, rotavirus diarrhea in infants |
| | Bacteria | Typhoid, paratyphoid fever, Bacillary dysentery, *E. coli* diarrhea, cholera |
| | Protozoa | Amebiasis, giardiasis |
| | Helminthic | Roundworm, threadworm |
| | Leptospira | Weil's disease |
| By an aquatic hosts | Snail | Schistosomiasis |
| | Cyclops | Guinea worm, fish tapeworm |

***Cyanide:*** It causes anoxia by chelating ferric ions in the cytochrome-$aa_3$ complex within mitochondria, thereby inhibits mitochondrial respiratory chain. Chronic toxicity of cyanide affects functioning of thyroid and nervous system.

***Lead:*** Lead is toxic to both central and peripheral nervous system. Lead inhibits δ-aminolevulinic acid (ALA) dehydratase, δ-ALA synthase, and ferrochelatase enzymes involved in synthesis of porphyrin and heme and leads to anemia.

***Arsenic:*** Arsenic inhibits the sulfhydryl groups of enzymes and interferes with cellular metabolism.

It competes with phosphate for reaction with ADP, resulting in the formation of low energy products.

It may also cause intravascular hemolysis leading to hemoglobinemia and hemoglobinuria.

The toxic effects of arsenic lead to dizziness, cramps and paralysis leading to death.

***Mercury:*** Hg being lipid soluble, once it is absorbed it crosses the blood-brain barrier and placenta, which is retained by the kidney and brain for many years and causes neurological disorders or death.

***Cadmium:*** Cadmium accumulates primarily in the kidneys. It acts through displacing zinc from the enzymes requiring zinc as a catalytic or structural component. It is a potent uncoupler of oxidative phosphorylation.

## Air Pollution

Air pollution is the presence, in the surrounding atmosphere, of excess concentration of certain substances, generated mostly by the activities of man and animal, such as, gases, mixture of gases and particulated matter, which interfere with human health, safety or comfort or which are injurious to vegetation and animals.

Air is a mechanical mixture of gases. The normal composition of air by volume is approximately as follows:

- *Nitrogen:* 78.1%
- *Oxygen:* 20.93%
- *Carbon dioxide:* 0.03%
- *Argon, neon:* Traces of krypton, xenon, and helium.
- Air also contains water vapors, traces of ammonia and suspended matter such as dust, bacteria, spores, and vegetable debris.

Under ordinary conditions, the composition of outdoor air is remarkably kept constant by certain natural self-cleaning mechanisms such as wind, sunlight, rain and plants.

### Air Pollutants

More than 100 substances are known which pollute air. Air pollutants may be in the form of solids, liquids (vapors) or gases. ***The combination of smoke and fog is called "smog".***

Air pollution is one of the present day health problems throughout the world. About 1.3 billion urban residents worldwide are exposed to air pollution level above recommended limits, which can affect health and social and economic state. Some important pollutants are given below:

- Carbon monoxide
- Carbon dioxide
- Sulfur dioxide
- Photochemical oxidants
- Lead
- Hydrogen sulfide
- Polynuclear aromatic hydrocarbons (PAH)
- Cadmium
- Chlorofluorocarbons (CFCs) and halons (ozone depleting substances)
- Particulate matter.

### Causes of Air Pollution

Air is polluted by:

- Respiration of men and animals

- Combustion of coal, gas, oil, etc.
- Decomposition of organic matter
- Trade, traffic and manufacturing process which give off dust, fumes, vapours and gases.

### Sources of Air Pollution

The main sources of air pollution are:
- Industries
- Automobiles
- Domestic sources
- Miscellaneous sources.

***Industries:*** Industries discharge into the atmosphere their wastes from chimneys at high temperature and high speed. Combustion of fuel to generate heat and power produces smoke, sulfur dioxide, nitrogen oxides, and fly ash into the atmosphere.

Petrochemical industries generate hydrogen fluoride, hydrochloric acid and organic halides.

Many industries discharge carbon dioxide, carbon monoxide, hydrogen sulfide, sulfur dioxide and ozone.

***Automobiles:*** Motor vehicles emit hydrocarbons, lead, carbon monoxide, nitrogen oxides and particulate matter. In the presence of strong sunlight, certain hydrocarbons and oxides of nitrogen are converted in the atmosphere into photochemical oxidants, which are oxidizing agents.

***Domestic sources:*** Domestic combustion of coal, wood or oil is a source of smoke, sulfur dioxide and nitrogen oxides.

***Miscellaneous sources:*** These include tobacco smoke, burning refuse, incinerators, pesticide spraying, natural sources, e.g. wind-borne dust, fungi, molds, and bacteria.

## Effect of Air Pollution on Human Health

Air pollution mainly affects respiratory system. The effects may be ***immediate*** or ***delayed.***
- Immediate effects result in acute bronchitis. If the air pollution is intense it may result even in death by suffocation
- The delayed effects result in chronic bronchitis, lung cancer, bronchial asthma, emphysema and respiratory allergies.

### Effect of Air Pollution on Social and Economic State

These include destruction of plant and animal life, corrosion of metals, damage of building, cost of cleaning and maintenance and repairs. It also reduces visibility in towns and damages soil and clothing.

**Acid Rain**

Acid rain results from the reaction of $\mathbf{SO_2}$ and $\mathbf{NO_2}$ with water vapors in the air to form nitric acid ($HNO_3$) and sulphuric acid ($H_2SO_4$) respectively, which are soluble in water and pollute rivers, streams and soils. They also decrease the fertility of the soils.

### Adverse Effect of Some Air Pollutants

#### *Carbon monoxide*

Carbon monoxide has high affinity for hemoglobin than oxygen. Carbon monoxide combines with hemoglobin to form carboxy hemoglobin and impairs the functioning of hemoglobin and leads to hypoxia with decreased mental performance.

#### *Carbon dioxide*

Carbon dioxide is a natural constituent of the air. This is not commonly regarded as an air pollutant; Excess $CO_2$ traps heat, **("Greenhouse" effect)** and causes increase in earth's temperature, leading to ***"global warming".*** This in turn leads to melting of polar icecaps, with increased levels of seas all over the world and low lying towns and cities at the risk of flooding.

#### *Sulfur dioxide*

It damages the lung tissue by destroying lung surfactant, dipalmitoyl lecithin, leading to respiratory disorders. Prolonged exposure results in bronchitis and lung cancer. It also causes spotting and burning of leaves, destruction of crops and retarded growth of plants.

#### *Photochemical oxidants (nitrogen dioxide and hydrocarbons)*

Nitrogen dioxide and hydrocarbons, in the presence of sunlight form complex secondary products, which are oxidizing agents. They lead to eye and lung irritation. Prolonged exposure causes asthma. They also damage vegetation.

#### *Lead*

Discussed earlier in this chapter.

#### *Hydrogen sulfide*

Hydrogen sulfide has an unpleasant odor, and can cause conjunctival irritation and show neurological and mental symptoms.

*Polynuclear aromatic hydrocarbons*

Polynuclear aromatic hydrocarbons (PAHs) are a large group of organic compounds. These are carcinogenic substances. They are formed by incomplete combustion of organic materials. There are about 500 PAH in the air. The best known is ***Benzo[a]pyrine (BaP)*** present in the smoke of cigarette. It can cause skin and lung cancer.

*Cadmium*

It is produced in the steel industries, waste incineration, volcanic action and zinc production. Tobacco contain cadmium and smoking contributes to the uptake of cadmium (Hazards discussed earlier in this chapter).

*Chlorofluorocarbons and halons (ozone depleting substances)*

Chlorofluorocarbons (CFCs) are used in refrigerators, air conditioners, perfumes, room fresheners, foam and cushion industries. Halons are used as fire extinguishers. Both CFCs and halons, on leaking into the air, get photo-dissociated when they reach the upper atmosphere (stratosphere) and the chlorine atoms are released. These chlorine atoms destroy the ozone layer and hence CFCs and halons are known as **"ozone depleting substances".** Ozone is a protective gas which shields the earth from the harmful ultraviolet (UV) rays of the sun, which cause skin disorders.

*Particulate matter*

It is an air borne mixture of organic and inorganic substances, emitted from a number of sources, may be natural, e.g. dust storms or man-made, e.g. power plants and industrial processes, vehicular traffic, domestic coal burning and industrial incinerators.

Various pulmonary abnormalities occur due to inhalation of:

- Dust particles (pneumoconiosis)
- Silica dust (silicosis)
- Asbestos (asbestosis)
- Cotton dust (byssinosis)
- Sugarcane dust (bagassosis).

## METABOLIC RESPONSES OR ADAPTATIONS TO AN ALTERED ENVIRONMENTAL TEMPERATURE

The environmental temperature may be –20°C or + 50°C. In both cases living tissues can function optimally only within a very narrow range of temperature. Therefore, accurate regulation is a great boon. The organism tends to adapt itself to an altered temperature.

## HEAT STRESS

Heat stress is the burden or load of heat that must be dissipated if the body is to remain in thermal equilibrium. The factors which influence heat stress are:

- Metabolic rate
- Air temperature
- Humidity
- Air movement
- Radiant temperature.

The amount of heat gained by the body must be equalled by the amount of heat lost from it.

### Thermoregulatory Responses

The temperature control system uses three important mechanisms to reduce body heat when body temperature becomes too great.

#### *Vasodilation*

The skin blood vessels become intensely dilated and increase the blood flow. Increased blood flow rises the temperature of skin. Thus, full vasodilatation can increase the rate of heat transfer to the skin as much as eight fold.

#### *Sweating*

If the environmental temperature exceeds the body temperature, sweating is the major thermoregulatory response.

#### *Decrease in Heat Production*

Mechanisms that cause excess heat production, such as shivering and chemical thermogenesis are inhibited.

### Effects of Heat Stress

#### *Heat Stroke*

This is a condition of failure of the temperature regulatory mechanism when the body temperature rises beyond a critical temperature 105° to 108°F.

#### *Symptoms*

- The symptoms include dizziness, abdominal distress, sometimes with vomiting, sometimes delirium and eventually unconsciousness if the body temperature is not decreased

- Because of high temperature, there is heat denaturation of the proteins of the brain, cellular damage of vital organs
- In fact, even a few minutes of very high body temperature can sometimes be fatal.

### *Treatment*

The treatment consists of reducing the body temperature by immersing the patient in a tub of ice cold water. If that is not possible, the body should be covered with wet ice cold towels and sheets. Cooling should be continued till the body temperature falls below 102°F.

## Heat Exhaustion

Unlike heat stroke, heat exhaustion is not because of failure of thermoregulation. It is caused by imbalance or inadequate replacement of water and salts lost in perspiration due to thermal stress. Heat exhaustion occurs after several days of exposure to a high temperature. Body temperature may be normal or moderately elevated. The symptoms are dizziness, weakness and fatigue due to circulatory distress.

## Heat Cramps

This is a painful condition of limb muscles due to spasmodic contractions of the skeletal muscles. Heat cramps are primarily due to water and salt depletion and can therefore be treated with water and sodium chloride.

## Heat Syncope

The person standing in the sun becomes pale, his blood pressure falls and he collapses suddenly. Due to dilatation of blood vessels, blood is pooled in lower limbs, with the result that the amount of blood returning to the heart is reduced, which in turn is responsible for lowering of blood pressure and lack of blood supply to the brain.

The treatment is the patient should be made lie in the shade with the head slightly down.

# COLD STRESS

The ability to survive cold stress lies in the capacity of the body to produce heat. When the body is too cold, the temperature control system operates exactly opposite procedures that occur in heat stress.

## Thermoregulatory Responses

**Skin vasoconstriction:** Vasoconstriction occurs throughout the body.

**Piloerection:** Piloerection means hairs "standing on end". This upright projection of the hairs allows them to entrap a thick layer of insulatory air, next to the skin, so that transfer of heat of the surroundings is greatly depressed. This is not important in human being, but in lower animals.

**Increase in heat production:** Heat production by the metabolic systems is increased by promoting; shivering thermogenesis and non-shivering or chemical thermogenesis.

### *Shivering Thermogenesis*

This occurs during short periods of exposure of cold. Skeletal muscles play a key role in the production of heat by a process of shivering.

- The shivering center located in the hypothalamus becomes activated when the body temperature falls.
- It then transmits signals that cause shivering. These signals increase the tone of the skeletal muscles throughout the body with production of heat.
- During maximum shivering body heat production can rise to four-to-five times the normal heat production.
- During this phase, the muscle stores of glycogen are used and heat is produced by hydrolysis of ATP.

### *Nonshivering or Chemical Thermogenesis*

This occurs during prolonged exposure to cold. Non-shivering mechanisms involve chemical thermogenesis. Cold stimulates the release of norepinephrine, epinephrine and thyroxine

- Increased levels of these hormones increase the rate of cellular metabolism throughout the body with the increased heat output
- Norepinephrine and epinephrine can cause an immediate increase in rate of cellular metabolism; but thyroxine requires several weeks of exposure of the body to cold.

The effect of chemical thermogenesis is partially due to the ability of these hormones to uncouple oxidative phosphorylation, and excess food stuffs are oxidized to release energy in the form of heat. This ***type of chemical thermogenesis is directly proportional to the amount of brown fat.*** In adult human beings, who have almost no brown fat, heat production by this chemical thermogenesis is not more than 10 to 15%. However, in infants who do have a small amount of brown fat, heat production can increase 100%.

## Metabolic Adaptations Occur during Chemical Thermogenesis

Various metabolic adaptations, which occur during chemical thermogenesis, are:

- Increased food intake
- The body tries to use all components of the diet for combustion, because thermoregulation is more important than energy balance during cold exposure
- In addition to carbohydrates and fat, proteins are also utilized as a source of calories
- The flow of carbohydrate through glycolysis is enhanced by way of activation of enzymes
- Gluconeogenesis is increased with increased utilization of non-carbohydrate sources of energy
- Increased activity of citric acid cycle enzymes and electron transport chain components occurs
- A lipase is activated which releases fatty acids for oxidation. The total free fatty acids in serum come back to normal on acclimatization due to increased oxidation for heat production
- As a response of cold stress transamination reactions are activated, which stimulate the catabolism of amino acids and carbon skeleton can be used for oxidation
- Increased arginase facilitates formation and excretion of urea.

## Effect of Cold Stress

### *Hypothermia*

It is due to universal lowering of the body temperature as a result of acute exposure to severe cold.

***Symptoms:*** Numbness, loss of sensation, muscular weakness, sleepiness, coma and death.

If a person is exposed to cold, there is peripheral vasoconstriction resulting in decreased blood supply to the tissues. This causes a decrease in oxygen supply to the tissue and accumulation of metabolites, resulting in tissue necrosis. Tissue damage occurs because of freezing of intracellular protoplasm and extracellular fluid.

**Local cold injury,** when it occurs below freezing temperature, is known as **"frostbite",** *i.e. tissues freeze with formation of ice crystals between cells.* **"Trench foot"** *or* **immersion** *is an example of local cold injury above freezing temperatures.*

# EXAM QUESTIONS

## Long Answer Questions (LAQs)

1. What is environmental biochemistry? Enumerate metabolic responses to cold and heat stress.
2. Give causes, sources and health hazards of air pollution or water pollution.

## Short Notes

1. Nonshivering thermogenesis and effect of cold stress.
2. Heat stress.
3. Cold stress.
4. Classification of environment with examples.
5. Natural impurities of water.
6. Man-made impurities (pollutants) of water.
7. Effect of water pollutants on human health.
8. Health hazards due to air pollution.
9. Causes and sources of air pollution.
10. Heat stroke or effect of heat stress.
11. Hypothermia or effect of cold stress.
12. Metabolic adaptations occur during chemical thermogenesis.
13. What is frostbite?

## Multiple Choice Questions (MCQs)

**1. All of the following are air pollutants, *except:***
a. $CO_2$ b. CO
c. $SO_2$ d. $H_2S$

**2. All of the following are effects of heat stress, *except:***
a. Heat stroke b. Heat syncope
c. Heat exhaustion d. Heat coagulation

**3. Greenhouse effect is due to:**
a. $CO_2$ b. CO
c. $SO_2$ d. $O_2$

**4. One of the following air pollutants is responsible for acid rain:**
a. $H_2S$ b. CO
c. $CO_2$ d. $SO_2$

**5. Normal dissolved oxygen (DO) content of water is:**
a. 10 to 14 ppm
b. 4 to 6 ppm
c. 8 to 10 ppm
d. 20 to 25 ppm

**6. Organic wastes of water are measured by:**
a. Biochemical or chemical oxygen demand
b. Centrifugation
c. Chromatography
d. Electrophoresis

**7. Ozone depleting substances are:**
a. Photochemical oxidants
b. Polynuclear aromatic hydrocarbons (PAH)
c. Chlorofluorocarbons (CFCs) and halons
d. Cadmium and lead

**8. Failure of temperature regulatory mechanism causes:**
a. Heat stroke b. Heat cramps
c. Heat exhaustion d. Heat syncope

**9. Which of the following for acid rain is not true?**
a. It results from air pollutants $SO_2$ and $NO_2$
b. It forms nitric acid and sulphuric acid
c. It decreases fertility of the soil
d. It results from water pollutants, nitrogen and hydrogen sulfide gas

## Answers for MCQs

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

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CHAPTER

# 31 Biomedical Waste Management

**Chapter outline**

- Classification of Hazardous Waste
- Different Locations of Biomedicals Waste Generation
- Types of Hazards
- Biomedical Waste Management Process

## INTRODUCTION

Biomedical waste is the waste that is generated during the diagnosis, treatment or immunization of human beings or animals, or in research activities pertaining thereto, or in the production or testing of biologicals.

Healthcare activities like immunization, diagnostic test, medical treatments and laboratory examinations protect and restore health and save lives. At the same time, however, health services may generate large quantity of wastes and by-products that need to be handled safely and disposed of properly.

The hospital waste, in addition to the risk for patients and personnel who handle these wastes poses a threat to public health and environment. Proper management of biomedical waste is essential to maintain hygienic, esthetics, cleanliness and control of environmental pollution.

Public concern about medical waste dates back to early 1980s when large quantities of syringes and needles were found on the beaches of the East coast and in Florida, USA. The public hue and cry due to scare regarding the spread of infectious diseases from this waste led to the first legislation on biomedical waste management in USA. Later, other countries adopted similar legislation to manage their biomedical waste effectively.

The government of India notified the National Biomedical Waste (Management and Handling) Rules in July, 1998. All the health care facilities in the country are covered under these rules, making it mandatory for such health facilities to manage their waste.

## CLASSIFICATION OF HAZARDOUS WASTE

Hospital waste/healthcare waste includes all the waste generated by health care establishments, research facilities and laboratories including minor or scattered source, such as care taken at home (insulin injection).

About 75 to 90% of the waste produced by healthcare providers is non-hazardous **general waste** comparable to domestic waste. About 20% of the waste is considered hazardous and/or infectious. If segregation does not take place, all the waste produced should be considered as infectious as it is mixed. WHO has classified hazardous waste into following categories:

- **Infectious waste:** (Suspected to contain pathogens), e.g. laboratory culture, waste from isolation
- **Pathological waste:** (Containing human tissue or fluids), e.g. body parts, blood and other body fluids, fetuses
- **Sharps:** (Sharp material), e.g. needles, infusion sets, scalpels, knives, blades, broken glass
- **Pharmaceutical waste:** (Containing pharmaceuticals), e.g. expired drugs, contaminated bottles, boxes
- **Genotoxic waste:** Waste containing cytostatic drugs (drugs used for the treatment of cancer) genotoxic chemicals
- **Chemical waste:** (Substance containing chemical substance), e.g. laboratory reagents, film developer, expired disinfectants, solvents
- **Waste with heavy metals:** Batteries, broken thermometer

- **Pressurized containers:** Gas cylinders, gas cartridges, aerosol cans
- **Radioactive material:** (Substances containing radioactive substances), e.g. unused liquid from radiotherapy, contaminated glassware, urine, excreta from patient treated with unsealed radio nucleotides.

## DIFFERENT LOCATIONS OF BIOMEDICALS WASTE GENERATION

Different locations or points of biomedical waste generation are:

- Hospitals: Government/private
- Nursing homes
- Dental clinics
- Dispensaries
- Primary healthcare centers
- Vaccinating centers
- *Laboratories*: Clinical/research
- Medical research and training centers
- Blood banks and collection centers
- Mortuaries
- Animal house
- Slaughter houses
- Biotechnology institutions/production unit.

## TYPES OF HAZARDS

Exposure to hazardous healthcare waste can result into:

- Infection
- Genotoxicity and cytotoxicity
- Chemical toxicity
- Radioactivity hazards
- Physical injuries
- Public sensitivity.

### Infection

The infectious agents can enter in the body through a puncture, abrasion, or cut in the skin; through mucous membranes; by inhalation and ingestion. The most common infections, which can result from mishandling of hospital/health care waste, are gastroenteric through feces and/or vomit (*Salmonella*, *Shigella* spp, *Vibrio cholerae*, Helminths; Hepatitis A), respiratory through inhaled secretions; saliva (*Mycobacterium tuberculosis*; measles virus; *Streptococcus pneumonia*), ocular infections through eye secretions (Herpes virus), genital infections (*Neisseria gonorrhoeae*; herpes virus), skin infection through pus (*Streptococcus* spp), meningitis through cerebrospinal fluid (*Neisseria meningitides*), AIDS through blood and sexual secretions (HIV), hemorrhagic fevers through body fluids (Junin, Lassa, Ebola and Marburg viruses), Septicemia and bacteremia through blood (*Staphylococcus aureus*, *Enterococcus*, *Enterobacter*, *Klebsiella* and *Streptococcus*) and Viral Hepatitis B and C through blood and body fluids (hepatitis B and C viruses).

### Genotoxicity and Cytotoxicity

Many cytotoxic drugs are extremely irritable and have harmful local effects after direct contact with skin and eyes (alkylating agents; Intercalating agents; vinca alkaloids and derivatives and epipodophyllotoxins). Many neoplastic drugs are carcinogenic and mutagenic; secondary neoplasia is known to be associated with chemotherapy.

### Chemical Toxicity

Many of chemicals and pharmaceutical drugs used in healthcare establishments are hazardous (e.g. toxic, genotoxic, corrosive, flammable, reactive, explosive and shock-sensitive). They may cause intoxication by acute or chronic exposure, injuries including burns, poisoning.

### Radioactivity Hazards

The radioactive waste exposure may cause headache, dizziness, vomiting, genotoxicity and tissue damage.

### Physical Injuries

It may result from sharps, chemicals and explosive agents.

### Public Sensitivity

The general public is very sensitive about visual impact of the anatomical waste, recognizable body parts including foetuses, if handled improperly.

## BIOMEDICAL WASTE MANAGEMENT PROCESS

- To control the indiscriminate disposal of hospital waste/biomedical waste, the ministry of environment and forest, Government of India has issued a notification on biomedical waste management under the Environment Protection Act
- In accordance with these rules, (Rule-4) it is the duty of every "occupier" (a person who has the control over the

institution and or its premises) generating biomedical waste, to take all steps to ensure that waste generated is handled without any adverse effect to the human health and the environment

- Every occupier generating the biomedicals waste need to install an appropriate facility in the premises or set up a common facility to ensure requisite treatment of waste in accordance with Schedule-I **(Table 31.1)**
- There are various types of biomedical waste. These are categories as mentioned in Schedule I **(Table 31.1).** Each type has to be disposed according to its characteristics.

## Various Steps in Biomedical Waste Management

Various vital steps for safe and scientific management of biomedicals waste are:

- Segregation

**Table 31.1:** Categories of biomedical waste.

| *Option* | *Waste category* | *Disposal* |
|---|---|---|
| Category No. 1 | **Human Anatomical Waste** (Human tissues, organs, body parts) | Incineration [@]/deep burial* |
| Category No. 2 | **Animal Waste** (Animal tissues, organs, body parts carcasses, bleeding parts, fluid, blood and experimental animals used in research, waste generated by veterinary hospitals, colleges, discharge from hospitals, animal houses) | Incineration [@]/deep burial* |
| Category No. 3 | **Microbiology and Biotechnology Waste** (Wastes from laboratory cultures, stocks or microorganisms live or vaccines, human and animal cell culture used in research and infectious agents from research and industrial laboratories, wastes from production of biologicals, toxins, dishes and devices used for transfer of cultures) | Local autoclaving/microwaving/incineration[@] |
| Category No. 4 | **Waste Sharps** (Needles, syringes, scalpels, blade, glass, etc. that may cause puncture and cuts. This includes both used and unused sharps) | Disinfection (chemical treatment [@@@]/ autoclaving/microwaving and mutilation/ shredding [# #] |
| Category No. 5 | **Discarded Medicines and Cytotoxic Drugs** (Waste comprising of outdated, contaminated discarded medicines) | Incineration[@]/destruction and drugs disposal in secured landfills |
| Category No. 6 | **Soiled Waste** (Items contaminated with blood, and body fluids including cotton, dressings, soiled plaster casts, lines, bedding, other material contaminated with blood) | Incineration[@] autoclaving/microwaving |
| Category No. 7 | **Solid Waste** (Waste generated from disposal items other than the sharps such tubing, catheters, intravenous sets, etc.) | Disinfection by chemical treatment [@@] autoclaving/microwaving and mutilation/ shredding [# #] |
| Category No. 8 | **Liquid Waste** (Waste generated from laboratory and washing, cleaning, housekeeping and disinfecting activities) | Disinfection by chemical treatment [@@] and discharge into drains |
| Category No. 9 | **Incineration Ash** (Ash from incineration of any biomedical waste) | Disposal in municipal landfill |
| Category No. 10 | **Chemical Waste** (Chemicals used in production of biologicals, chemicals used in production of biologicals, chemicals used in disinfection, as insecticides, etc.) | Chemical treatment[@@] and discharge into drains for liquids and secured landfill for solids |

Note:

[@] There will be no chemical pre-treatment before incineration. Chlorinated plastics shall not be incinerated.

* Deep burial shall be an option available only in towns with population less than five lakhs and in rural areas.

[@@] Chemicals treatment using at least 1% hypochlorite solution or any other equivalent chemical reagent. It must be ensured that chemical treatment ensures disinfection.

[# #] Multilation/shredding must be such so as to prevent unauthorized reuse.

- Storage
- Transportation
- Treatment
- Disposal.

## Importance of Segregation

As mention earlier only about 20% of the biomedical waste is infectious. If segregation does not take place all the waste produced should be considered as infectious as it is mixed.

- Segregation of the biomedical waste is the first step in waste management
- Segregation at source of different types of biomedical wastes and their appropriate storage and/or disinfections, sterilization, etc. would ensure that infectious wastes do not get mixed with noninfectious wastes as this would infect the entire waste.
- Segregation at source makes:
  - It easier to prevent spread of infection
  - Makes it easier to choose among the options of disposal
  - Can reduce the load on the incineration and prevent injuries.

## How are Wastes to be Segregated?

- Infected waste can be segregated into:
  - Dressings
  - Organs and organ parts, placenta, associated body fluids (blood, pus, plural, or peritoneal fluids, etc.)
  - Broken glassware
  - Intact glassware
  - Needles, razor, blades, scalpel blades, nails, pins, bones, etc.
  - Microbiology waste, non-reusable specimen containers
  - General waste
- The rules have laid down certain directions regarding segregation and storage to ensure safe and hygienic handling of infectious and non-infectious waste. Among these:
  - No biomedical waste shall be mixed with other wastes
  - Biomedical waste be segregated into container/bags at the point of generation
  - These containers/bags are to be made of different materials and have different **color coding** signifying the different kinds of wastes **(Table 31.2)**

Segregation is mandatory prior to storage, transportation, treatment and disposal.

## Storage of Biomedical Waste after Segregation

- Clinical and general wastes should be segregated at source and placed in **color coded plastic bags** and

**Table 31.2:** Categories, color-coding and types of container recommended for segregation of biomedical waste by regulations of the Ministry of Environment and Forest, Government of India.

| *Color-coding* | *Types of container* | *Waste category* | *Treatment as per Schedule–I* |
|---|---|---|---|
| Yellow | Plastic bag | Cat–1 Human anatomical waste<br>Cat–2 Animal waste<br>Cat–3 Microbiology waste<br>Cat–6 Soiled waste | Incineration/deep burial |
| Red | Disinfected container/plastic bags | Cat–3 Microbiological waste<br>Cat–6 Soiled waste<br>Cat–7 Solid waste | Autoclaving/microwaving/ chemical treatment |
| Blue/white/translucent | Plastic bag/puncture proof containers | Cat–4 Waste sharps<br>Cat–7 Plastic disposable tubings, etc. | Autoclaving/microwaving Chemical treatment and Distraction/shredding |
| Black | Plastic bag | Cat–5 Discarded medicines<br>Cat–9 Incineration ash<br>Cat–10 (Solid) Chemical waste | Disposal in secured landfill |

*Note:*
1. Color-coding of waste categories with multiple treatment options as defined in Schedule I, shall be selected depending on the treatment option chosen, which shall be as specified in Schedule I.
2. Waste collection bags for waste types needing incineration shall not be made of chlorinated plastics.
3. Categories 8 and 10 (liquid) do not require containers/bags.
4. Category 3 if disinfected locally need not be in containers/bags.

**containers** of definite specifications prior to collection and disposal **(Table 31.2)**.

- The biomedical rules have recommended different color codes for waste containers in which different types of wastes needs to be stored **(Table 31.2)**.
- The container should comprise of an inner plastic bag of varied color depending on the type of waste.
  - It should be of a minimum gauge of 55 micron (if of low density) or 25 micron (if of high density)
  - Leak proof and puncture proof
  - Should match the chosen outer container
- The outer container is a plastic bin with handles, and of a size which will depend on the amount of waste-generated
- The inner polythene bag should fit into the container with one-fourth of the polythene bag turned over the rim
- Labelling has been recommended to indicate:
  - The type of waste
  - Site of generation
  - Name of generating hospital or facility.

This will allow the waste to be traced from the point of generation to the disposal area.

- Bags and containers should be clearly labelled with words **Biohazard** and the **biohazard symbol (Fig. 31.1)**.
- The containers are then to be transported in closed trolleys or wheeled containers that should be designed for easy cleaning and draining.

## Transport

- Biomedical waste should be transported within the hospital by means of wheeled trolleys, containers or carts that are not used for any other purpose

**Fig. 31.1:** The biohazard symbol.

- The trolleys have to be cleaned daily
- Offsite transportation vehicle should be marked with the name and address of carrier
- Biohazard symbol should be painted
- Suitable system for securing the load during transport should be ensured
- Such a vehicle should be easily cleaned with rounded corners.

### *Care to be taken in storage and transportation*

- Untreated biomedical waste shall be transported only in specially designed vehicles
- No untreated biomedical waste shall be stored beyond a period of 48 hours
- If for any reasons it becomes necessary to store the waste beyond such period, permission from the prescribed authority (established by the government of every state and union territory) must be taken and it must be ensured that it does not adversely affected human health and the environment.

## Treatment of Biomedical Waste

- If biomedical waste treated in accordance with the following procedures, the waste shall no longer be considered biomedical waste and may be combined and handled with regular solid waste
- Biomedical waste shall be treated by one of the following method prior to disposal at a permitted solid waste disposal facility.

### *Incineration*

Incineration is a thermal treatment (high temperature dry oxidation) technology facility that provides complete combustion of waste to render it non-pathogenic.

- Waste types not to be incinerated are:
  - Large amount of reactive chemical wastes
  - Pressurized gas containers
  - Halogenated plastics such as PVC
  - Silver salts and photographic or radiographic wastes
  - Wastes with high mercury or cadmium content such as broken thermometers
  - Used batteries
  - Lead-lined wooden panels
  - Sealed ampules or ampules containing heavy metals.

### *Wet and dry thermal treatment*

Wet and dry thermal treatment process is decontamination by heating with steam under pressure (autoclave) so as to render the biochemical waste non-infectious. The process

is inappropriate for the treatment of anatomical waste and animal carcasses, and will not efficiently treat chemical and pharmaceutical waste.

*Screw-feed technology*

Screw-feed technology is the basis of a nonburn, dry thermal disinfection process in which waste is shredded and heated in a rotating auger. This process is suitable for treating infectious waste and sharps, but it should not be used to process pathological, cytotoxic or radioactive waste.

*Chemical disinfection*

Chemicals such as sodium hypochlorite or chlorine dioxide are added to waste to kill or inactivate the pathogens it contents, this treatment usually results in disinfection rather than sterilization. Chemical disinfection is most suitable for treating liquid waste such as blood, urine stools or hospital sewage.

*Microwave irradiation*

Most organisms are destroyed by heating the waste at frequency of about 2450 MHz and a wavelength of 12.24 cm by means of microwaves. The water contained within the waste is rapidly heated by the microwaves and the infectious components are destroyed by heat.

*Land disposal*

There are two types of land disposal:

- Open dumps landfilling and
- Sanitary landfills.

- Biomedical waste should not be deposited on or around open dumps, because people or animals may come into contact with infectious pathogens
- **Sanitary landfills:** Sanitary landfills have advantages over open dumps.

*Inertization*

- Inertization involves mixing biomedical waste with cement and other substances before disposal, in order to minimize the risk of toxic substances contained in the wastes migrating into the surface water or ground water
- Biomedical wastes is mixed in a proportion of 65% pharmaceutical waste 15% lime +15% cement and 5% water
- A homogenous mass is formed and cubes are produced on site and then transported to suitable storage site.

## Disposal of Biomedical Waste

Biomedical wastes treated in accordance with the regulations shall be properly disposed of at a properly permitted facility.

## EXAM QUESTIONS

### Long Answer Question (LAQ)

1. Define biomedical waste write management and handling.

### Short Notes

1. Segregation of biomedical waste.
2. Different methods of biomedical treatment.
3. Storage of biomedical waste.
4. Types of hazards of biomedical waste.
5. Categories of biomedical wastes.

CHAPTER

# 32

# Connective Tissue

**Chapter outline**


- Basic Components of Connective Tissue
- Structure and Function of Collagen
- Structure and Function of Elastin
- Structure and Function of Proteoglycan
- Structure and Function of Glycoproteins
- Disorders of Connective Tissue


## INTRODUCTION

As the name implies, connective tissue serves a connecting function. It supports and binds other tissues. Unlike epithelial tissue, connective tissue typically has cells scattered throughout an extracellular matrix. The chief function of connective tissue is to give strength, support and shape to the tissues.

Connective tissue is a system of insoluble protein fibers embedded in a matrix called the ground substance. Connective tissue is widely distributed in the body, the tendons, ligaments, cartilage and matrix of bone.

## BASIC COMPONENTS OF CONNECTIVE TISSUE

Connective tissue consists of four major components:

1. Collagen
2. Elastin
3. Proteoglycans
4. Glycoproteins

## STRUCTURE AND FUNCTION OF COLLAGEN

### Functions of Collagen

- Collagen is the main protein of connective tissue. It has great tensile strength and serves to hold cells together.
- Collagen is involved in proper alignment of cells. This, in turn, helps in proliferation and differentiation of cells.

### Structure of Collagen

- The basic structural unit of collagen is **tropocollagen,** which consists of three polypeptide chains called **α-chains.** These three polypeptide chains twisted around each other in a **triple helix** forming a rope like structure, which has great tensile strength **(Fig. 32.1).**
- The three helically interwind polypeptides are of equal length, each having about 1000 amino acids residues. The three polypeptide chains are held together by **hydrogen bonds** between chains.
- Multiple types of collagen in human tissues arise from different triple helical combinations of polypeptides. In

**Fig. 32.1:** Right-handed collagen triple helix formed from three α-chains.

**Table 32.1:** Most abundant types of collagen found in human tissues and their distribution.

| *Type of Collagen* | *Distribution* |
|---|---|
| I | Skin, tendon, bone, cornea |
| II | Articular cartilage, intervertebral disk, vitreous body |
| III | Fetal skin, cardiovascular system, reticular fibers |
| IV | Basement membrane |
| V | Placenta, skin |

human tissues, 19 distinct types of collagen have been identified. **Table 32.1** summarizes the most abundant types of collagen found in human tissues.

## *Structure of α-chain of Collagen*

- Collagen has an unusual amino acid composition with 33% of the total residues being **glycine (Gly),** 10% **proline (Pro),** 10% **hydroxyproline (Hyp)** and 1% **hydroxylysine (Hyl).**
- Two amino acids that are found in collagen, **hydroxyproline** and **hydroxylysine** are not present in most proteins.
- The primary structure of collagen is unusual in that, collagen has regular arrangement of amino acids in each of the α-chains of the tropocollagen.
- The sequence generally follows the pattern **(Gly-X-Y),** where Gly for glycine and X and Y, for any amino acid residues. Most of the time X is for proline and Y is for hydroxyproline **(Fig. 32.2)**.
- Thus, glycine, the smallest amino acid is found in every third position of the polypeptide chain. This is necessary because **glycine is the only amino acid small enough to be accommodated in the limited space available in the central core of the helix (Fig. 32.2)**.

**Fig. 32.2:** Representation of primary structure of α-chain of collagen and cross-section of triple helical structure of collagen, where, G = Glycine, X and Y = Any other amino acid mostly proline and hydroxyproline.

Mutation of a single glycine residue in collagen leads to connective tissue disorder which can be lethal, e.g. **osteogenesis imperfecta** (see disorders of connective tissue).

- Collagen is unique in its high content of helix destabilizing amino acids, **proline, hydroxyproline** and **glycine.** These prevent the formation of the usual α-helical and β-pleated structure. Instead, it forms a **triple helical** secondary structure.
- The three α-chains are wound around each other to form a tight, **right handed triple helix**.
- Interchain hydrogen bonding stabilizes the triple helical structure.

## *Formation of Collagen Fibrils*

- Individual tropocollagen molecules spontaneously laterally aggregate to form fibril. They arrange themselves under physiological conditions into staggered, parallel and overlapping array structures.
- Each tropocollagen molecule overlaps its neighbor by a length approximately three quarters of a molecule **(Figs. 32.3A to C)**.
- The regularity of gaps and overlaps is responsible for the banded appearance of these fibers in connective tissues.

**Figs. 32.3A to C:** (A) The quarter staggered, parallel and overlapping array structural arrangement of tropocollagen to form collagen fiber; (B) The regularity of gaps and overlaps generates the banded appearance in the collagen fiber; (C) Electron micrographic banded appearance of fibriller collagen.

**Fig. 32.4:** Types of cross-links in collagen.

- These fibriller array of tropocollagen molecules become connected and subsequently stabilized by intra and intercovalent cross-links through action of **copper requiring enzyme lysyl oxidase.**
- Three types of inter or intramolecular cross-links that stabilize the collagen fibril are **(Fig. 32.4)**:

1. Aldol condensation
2. Schiff base
3. Lysinonorleucine

These cross-links are important for the tensile strength of the fibers. Because of the tightness of coiling of triple helix of tropocollagen and its cross-linkages, it has no capacity to stretch.

## STRUCTURE AND FUNCTIONS OF ELASTIN

### Functions of Elastin

- Elastin occurs with collagen in connective tissues. Elastin is a **rubber-like protein,** which can stretch to several times their length and then rapidly return to their original size and shape when the tension is released.
- Elastin is present in large amounts, particularly in tissues that require these physical properties, e.g. lung, blood vessels and ligaments. Smaller quantities of elastin are also found in skin, ear cartilage and several other tissues.
- Elastin differs from collagen in several properties. **Table 32.2** summarizes the main differences between collagen and elastin.
- In contrast to collagen, there is only one genetic type of elastin.

### Structure of Elastin

- The basic subunit of elastin fibrils is **tropoelastin** which contains about 800 amino acid residues.
- Unlike collagen, elastin does not contain repeat (Gly-X-Y) sequences. Although elastin and collagen contain similarly high amounts of glycine and proline, elastin contains less hydroxyproline and no hydroxylysine.
- Elastin has very high content of **alanine** and other nonpolar aliphatic residue, i.e. **valine, leucine** and **isoleucine.**

#### *Cross-Links of Elastin*

- The cross-links in elastin are more complex than those in collagen.
- The major cross-links formed in elastin are the **desmosines,** which is derived from the condensation of three allysine (oxidized form of lysine) residues with lysine.
- These cross-links permit the elastin to stretch in two dimensions and subsequently recoil during the performance of its physiologic functions.

Destruction of elastin by **elastase** is normally inhibited by **α-trypsin,** the genetic deficiency of which can result in **emphysema.**

**Table 32.2:** Difference between collagen and elastin.

| *Collagen* | *Elastin* |
|---|---|
| Many different genetic type | One genetic type |
| It has no capacity to stretch | It has capacity to stretch and subsequently to recoil |
| Primary structure has repeating (Gly-X-Y) sequences | Primary structure has no repeating (Gly-X-Y) sequences |
| Formation of triple helical secondary structure | No formation of triple helix |
| Presence of hydroxylysine | No hydroxylysine present |
| Glycosylated hydroxylysine is present | No glycosylated hydroxylysine present |
| Formation of intramolecular aldol cross-links | Formation of intramolecular desmosine cross-links |

## STRUCTURE AND FUNCTION OF PROTEOGLYCANS

- Intracellular ground substance of connective tissue contains **proteoglycans** and **glycoproteins** in which fibrous elements of connective tissues are embedded. They connect cell and other connective elements in the extracellular matrix.
- Proteoglycan is a complex formed by polysaccharide called glycosaminoglycans (about 95%) and protein (about 5%).
- Hyaluronic acid, chondroitin sulfate, keratan sulfate, heparan sulfate and heparin are the major glycosaminoglycans, ***(For details please Refer Chapter 2)***.

## STRUCTURE AND FUNCTION OF GLYCOPROTEINS

- Glycoproteins are proteins to which oligosaccharides are covalently attached.
- The main function of glycoprotein is to facilitate adhesion between various elements of connective tissue, ***(For details please Refer Chapter 2)***.
- Some glycoproteins present in connective tissue are:
  - Fibrillin
  - Fibronectin
  - Lamin
  - Tenascin

## DISORDERS OF CONNECTIVE TISSUE

Mutations in genes that are responsible for production of collagen can lead to number of disorders. Some of them are discussed below.

### Osteogenesis Imperfecta

- Osteogenesis imperfecta, also called **brittle bone syndrome.** This is a congenital disorder caused by multiple genetic defects in the synthesis of **type-I collagen.**
- The disorder is characterized by fragile bones, thin skin, abnormal teeth and weak tendons. The sclera are often abnormally thin and translucent and may appear blue owing to a deficiency of connective tissue.
- Many of these mutations are single base, substitutions that replace glycine in the (Gly-X-Y) repeat sequence by another bulkier amino acid **cysteine,** preventing the correct folding of the collagen chains into a triple helix and their assembly to form collagen fibrils.

### Ehlers-Danlos Syndrome

- Ehlers-Danlos syndrome is a group of inherited disorder of connective tissue.
- This is characterized by hyperextensibility of the skin, hypermobility of the joints, fragile skin and laxity in the musculoskeleton.

### Epidermolysis Bullosa

Epidermolysis bullosa is a rare heritable disorder due to mutation in gene encoding collagen. It is characterized by skin breaks and blistering of the skin and epithelial tissue.

### Marfan's Syndrome

- Marfan's syndrome is an inherited disease of connective tissue. Abraham Lincoln may have had this condition. Marfan's syndrome is relatively rare genetic disorder caused by mutations in genes coding for **fibrillin,** a glycoprotein, which gives stability to the elastic fibers.
- Mutations in fibrillin affect the eyes, the skeletal system and cardiovascular system.
- Marfan's syndrome is characterized by:
  - Ectopia lentis, i.e. dislocation of the lens (due to weakness of the suspensory ligaments).
  - Tall structure, long arms and legs, hyperextensibility of the joints.

### Scurvy

Scurvy is a nutritional disorder, not a genetic disease caused by a deficiency in ascorbic acid (vitamin C).

#### *Symptoms seen in Scurvy*

- Abnormal bone development in infants and children
- Osteoporosis
- Loosening of teeth and swollen bleeding gums
- Poor wound healing
- Easy bruising and bleeding due to fragile capillaries.

Vitamin C plays a role in collagen biosynthesis by acting as a cofactor in the hydroxylation reactions of proline and lysine. Without hydroxylation of lysine and proline, the procollagen that is formed, is unstable and degradable.

### Lathyrism

- Lathyrism is a dietary disease due to inhibition of **lysyl oxidase,** an enzyme required for the cross- linking of collagen chains.
- Lathyrism is characterized by deformation of spine, demineralization of bone, dislocation of joints and aortic aneurysm.

## EXAM QUESTIONS

### Long Answer Question (LAQ)

1. Describe structure, functions and disorders of collagen.

### Short Notes

1. Elastin.
2. Proteoglycans and glycoproteins of connective tissue.
3. Lathyrism.
4. Marfan's syndrome.
5. Osteogenesis imperfecta.
6. Ehlers-Danlos syndrome.
7. Cross-links of collagen.
8. Cross-links of elastin.
9. Effect of aging on collagen.
10. Triple helical structure of collagen.

### Multiple Choice Questions (MCQs)

**1. Basic components of connective tissue are, *except*:**
a. Collagen b. Elastin
c. Proteoglycans d. Cholesterol

**2. Hydroxylation of proline requires which of the following?**
a. Vitamin C b. $Fe^{2+}$
c. α-ketoglutarate d. All of the above

**3. Collagen contents of helix destabilizing amino acid is:**
a. Glycine b. Serine
c. Alanine d. Threonine

**4. Cross-links present in collagen are, except:**
a. Aldol condensation b. Schiff base
c. Lysinonorleucine d. Desmosine

**5. The basic subunit of elastic fibrils is:**
a. Troponin b. Tropoelastin
c. Tensin d. Laminin

**6. Which of the following is not a genetic disorder of collagen?**
a. Marfan's syndrome b. Osteogenesis imperfecta
c. Scurvy d. Epidermolysis bullosa

**7. A deficiency of copper affects the formation of normal collagen by reducing the activity of:**
a. Propyl hydroxylase b. Lysyl oxidase
c. Lysyl hydroxylase d. Glucosyl transferase.

### Answers for MCQs

**1.** d **2.** d **3.** a **4.** d **5.** b **6.** c **7.** b

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CHAPTER

# 33

# Muscle

Chapter outline


- Classification of Muscle
- Structure of Skeletal Muscle
- Mechanism of Muscle Contraction
- Muscle Disorders


## INTRODUCTION

Muscle is the major biochemical machine that converts chemical (potential) energy into mechanical (kinetic) energy. Muscle tissue is responsible for movement of the body and its parts and for changes in the size and shape of internal organs.

Muscle tissue is characterized by aggregates of specialized, elongated cells arranged in parallel array, whose primary role is contraction.

## CLASSIFICATION OF MUSCLE

Muscle is classified on the basis of the appearance of the contractile cells. Two principal types of muscle are:

### Striated Muscle

Striated muscles are those in which cells appear striated upon microscopic observation. Striated muscle tissue is further subclassified on the basis of its location as follows:

- Skeletal muscle which is attached to bone.
- Cardiac muscle which is found in the wall of the heart.

### Smooth Muscle

Smooth muscle cells are non-striated.

## STRUCTURE OF SKELETAL MUSCLE (FIGS. 33.1A TO D)

- The cells of skeletal muscle are cylindrical in shape. Each cell is commonly called **muscle fiber.**
- Each muscle fiber is **multinucleated (syncytial)** cell. Each cell is surrounded by an electrically excitable plasma membrane, the **sarcolemma.**
- Each of the muscle fibers are made of many myofibrils, held together by connective tissue.
- Myofibrils are composed of bundles of myofilaments.
- Myofilaments consist of **thick filament** and **thin filament.**
  - The thick filament consists of protein **myosin.**
  - The thin filament consists of protein **actin, tropomyosin** and **troponin.**
- Myofilaments are the actual contractile elements of striated muscle.
- When myofibril is examined by electron microscope alternating dark A (anisotropic) bands and light I (isotropic) bands can be observed.
- The central region of the A-band termed the **H-band** is less dense than the rest of the band (in German, Hell means light).
- The I (light) band is bisected by a very dense, narrow **Z-line** or **Z-disks.** The Z-line is made of the protein **α-actinin** and **desmin.**
- The portion of the myofibril that lies between two successive Z-lines is called **sarcomere,** i.e. sarcomere, is a Z-line to Z-line repeat **(Figs. 33.1A to D)** which repeats every 2.3 mm (2300 Å) along the fibril axis.
- **Sarcomere represents the smallest functional unit of myofibril.** It is the basic contractile unit of striated muscle.

**Figs. 33.1A to D:** Structure of muscle fiber.
(A) Skeletal muscle; (B) Muscle fiber composed of myofibrils; (C) Myofibril composed of myofilaments; (D) Arrangement of myofilaments in striated muscle.

- The thick and thin filaments of sarcomere do not change length or width during muscle contraction.
- During muscle contraction, the thick and thin filaments overlap each other. Consequently, the H bands and I bands shorten **(Figs. 33.2A and B)**.
- The length of the sarcomere which is 2300 nm in an extended form of myofibril is reduced to 1500 nm in a contracted form.

## Protein Composition of Muscle Fibers

Muscle contains following types of proteins:

1. Contractile proteins
   i. Myosin
   ii. Actin
2. Regulatory proteins
   i. Tropomyosin
   ii. Troponin
3. Minor or accessory proteins.

### *Contractile Protein*

#### *Myosin*

- Each myosin molecule is composed of six polypeptide chains, two identical **heavy chains** and four **light chains.** Light chains are of two types **(Figs. 33.3A and B)**.

**Figs. 33.2A and B:** Arrangement of muscle filaments.
(A) Relaxed or extended position; (B) Contracted position.

**Figs. 33.3A and B:** (A) The thick filament consists of bundles of myosin molecules; (B) Schematic structure of myosin molecule.

- Two identical heavy chains are wound around each other to form a double helix, which is called the **tail of the myosin** molecule.
- The amino terminal end of each of these chains is folded into a globular structure called the **myosin head.** Thus, there are two free heads lying side-by-side at one end of the double helix myosin molecule.
- One each type of light chain is associated with each of the myosin heavy chain heads. These light chains help to control the function of the head during muscle contraction.
- Globular head of myosin has two specific binding sites, one for ATP and one for actin. lt also exhibits ATPase activity which can hydrolyze ATP to ADP and Pi.
- Tails of the myosin molecules bundled together to form the thick filament, while many heads of the myosin molecule hang outward to the sides of the thick filament except H-region, where there are no myosin heads.

***Functions of Myosin***

Myosin has three important functions as follows:

- Constituent of thick filament of muscle fiber.
- The amino terminal globular ends of myosin exhibit **adenosine triphosphatase (ATPase)** activity. It hydrolyzes ATP to ADP + Pi and provides free energy for muscle contraction.
- Myosin interacts with actin and generates the force (power stroke) that moves the thick and thin filament past each other.

*Actin*

- Actin is the major constituent of thin filament of muscle fiber.
- Actin is a polymer of globular-shaped subunit called **G-actin**.
- In presence of $Mg^{2+}$ ions, G-actin polymerizes spontaneously into a fibrous or filamentous form called F-actin **(Fig. 33.4)**.
- Each G-actin molecule of the thin filament has a binding site for myosin.
- In association with myosin, actin plays a major role in muscle contraction (discussed later).

## Regulatory Proteins

*Tropomyosin*

- Tropomyosin is a constituent of thin filaments of the muscle.
- Tropomyosin is made up of two polypeptide chains, which is wrapped spirally around the sides of the F-actin helix **(Fig. 33.4)**.
- In the resting state, the tropomyosin molecules lie on top of the active sites of the actin strands, so that attraction cannot occur between actin and myosin filaments to cause contraction.
- Tropomyosin is involved in the contraction process by regulating the attachment of actin and myosin.

**Fig. 33.4:** Assembly of proteins of thin filament.

*Troponin*

- Troponin is also a constituent of thin filaments of the muscle.
- Troponin is a complex of three polypeptide chain, **TnC, TnI** and **TnT,** which is attached to tropomyosin **(Fig. 33.4)** and each of which plays a specific role in controlling muscle contraction as follows:

**TnC (Troponin C):** It binds calcium ions, the essential step in the initiation of muscle contraction.

**TnI (Troponin I)**: It binds to actin and inhibits actin-myosin attachment.

**TnT (Troponin T)**: It binds to tropomyosin, anchoring the troponin complex.

### *Minor or Accessory Proteins of Myofibril*

- There are a large number of other proteins that are involved in stabilizing the structure and functions of muscle are called minor or accessory proteins. These include
  - α-actinin
  - Cap-Z
  - Titin
  - Nebulin
  - Desmin
  - Dystrophin.

## MECHANISM OF MUSCLE CONTRACTION

**"Sliding filament model",** is the most accepted model of muscle contraction proposed in 1954. According to this mechanism, the thin filaments slide past the thick filaments during contraction so that the total length of the fiber is shortened. Although the length of the sarcomere decreased during muscular contraction, the lengths of the individual thick and thin filaments did not change but the H-zones and I-bands shortened **(*see* Fig. 33.2).**

### Biochemical Events Occurring during Muscle Contraction

Each contraction cycle consists of five stages as follows **(Fig. 33.5):**

1. **Binding of ATP to head of myosin** of thick filaments and detachment of myosin from actin (thin filament).
2. **Conformational changes** in the myosin head as a result of hydrolysis of ATP to ADP+Pi.
3. **Binding of myosin-ADP-Pi** complex to actin.

**Fig. 33.5:** Schematic representation of myosin-actin interaction during contraction.

4. **Force generation** in which myosin head releases inorganic phosphate and power stroke occurs.
5. **Reattachment of myosin head** to the new actin molecule of the thin filament and formation of rigor complex **(Fig. 33.5)**.

## *Stage 1*

- In the first stage, ATP binds to the myosin head of the thick filament which is tightly bound to the actin molecule of the thin filament.
- Binding of ATP dissociates actomyosin into actin and myosin.
- Binding of ATP induces conformational changes in the myosin head. This change reduces the affinity of the myosin head for the actin molecule of the thin filament causing the myosin head to detach from the thin filament.

## *Stage 2*

- Adenosine triphosphatase (ATPase) of the myosin hydrolyzes ATP to ADP and Pi and remain bound to myosin head.
- The energy released by the splitting of ATP is stored in the myosin molecule.

- Myosin in the form of **myosin ADP-Pi complex** is now in a high energy state. This is the predominant state at **rest, i.e. relaxed state**.

### *Stage 3*

- Upon muscle stimulation via calcium, the inhibition of actin-myosin attachment imposed by the regulatory proteins (tropomyosin and troponin) is removed and consequently the myosin with bound ADP and Pi attaches to actin.
- It is believed that the angle of myosin head attachment is 90°.

### *Stage 4*

- The actin-myosin reassociation stimulates the release of inorganic phosphate (Pi) from myosin head; which results in increasing the strength of the myosin-actin attachment.
- This tight binding of myosin to actin results in a conformational change in myosin head, tilting the angle of head from 90° to 45°.
- This change in myosin head, causes, the generation of force called the **'Power Stroke'** of muscle contraction which pulls the thin filament a distance about 70°A (10 nm) towards the center the sarcomere.

### *Stage 5*

- The myosin head is again tightly bound with release of ADP to a new actin molecule of the thin filament which is known as **rigor configuration**.
- The actin-myosin complex remains intact until another molecule of ATP binds to the head of myosin molecule.
- With a new ATP, a new cycle may begin and the cycling may continue as long as regulatory calcium and ATP are present.

**Rigor Mortis**

- At the beginning of the contraction cycle, the myosin head is tightly bound to the actin molecule of the thin filament and ATP is absent. This arrangement is known as **rigor configuration**.
- ATP is needed for the detachment of myosin from the actin during muscle contraction. In case of ATP depletion, the cycle is arrested and actin does not dissociate and relaxation does not occur.
- When actin and myosin are permanently bound in the absence of ATP, the muscle becomes rigid and stiff. The muscular rigidity and stiffening that begins at the moment of death due to lack of ATP is known as **rigor mortis**.

## Source of Energy in Muscle Contraction

- The immediate source of energy in muscle for contraction is ATP. ATP can be generated from the following ways:
  - By substrate level phosphorylation of glycolysis using glucose or muscle glycogen
  - By oxidative phosphorylation
  - From creatine phosphate.

# MUSCLE DISORDERS

## Muscular Dystrophy

- Muscular dystrophies are a group of genetic conditions characterized by progressive muscle weakness and wasting without involvement of nervous system.
- This is an X-linked disorder and caused by mutations in the gene coding for the protein dystrophin.
- The different types of muscular dystrophy affect different sets of muscles and result in different degrees of muscle weakness.
- The commonest and best characterized types are:
  - Duchenne muscular dystrophy (DMD)
  - Becker muscular dystrophy (BMD).
- Duchenne and Becker types of muscular dystrophy primarily affect the skeletal muscles, which are used for movement, and the muscles of the heart.

# EXAM QUESTIONS

## Long Answer Question (LAQ)

1. Describe biochemical events occur during muscle contraction with its regulation.

## Short Notes

1. Rigor mortis
2. Role of calcium in muscle contractions

3. Source of energy in muscle contraction
4. Types of skeletal muscle fibers
5. Sarcomere
6. Protein composition of muscle fibers
7. Muscular dystrophy.

## Multiple Choice Questions (MCQs)

**1. The primary components of thin filaments include which of the following:**
   a. Myosin
   b. Tropomyosin and myosin
   c. Actin, tropomyosin and troponin
   d. None of the above

**2. Which of the following does not change length during muscle contraction?**
   a. Sarcomere
   b. I band
   c. A band
   d. H-zone

**3. Which of the following proteins involved in muscle contraction, *except:***
   a. Myosin
   b. Myoglobin
   c. Actin
   d. α-Actinin

**4. Which of the following acts as source of energy in muscle contraction?**
   a. Blood glucose
   b. Oxidative phosphorylation
   c. Fatty acids
   d. All of the above

**5. Which of the following is true for sarcomere?**
   a. It is the functional unit of muscle
   b. It consists of thick filament myosin
   c. It consists of thin filament actin
   d. All of the above

### Answers for MCQs

**1.** c **2.** c **3.** b **4.** d **5.** d

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CHAPTER

# 34

# Neurotransmitters

**Chapter outline**


- Overview of the Nerve Cell
- Classification of Neurotransmitters
- Mechanism of Release of Neurotransmitters
- Regulation of Action of Neurotransmitters
- Different Common Neurotransmitters


## INTRODUCTION

Neurotransmitter is a chemical substance or chemical messenger (within brain) that is secreted by neurons and that allow communication between nerve cells to produce physiological response such as **muscle contraction**.

## OVERVIEW OF THE NERVE CELL

- **The neuron:** The neuron is the structural and functional unit of the nervous system **(Fig. 34.1).**
- Neurons do not divide; they must last for a lifetime.
- Neurons function by the production, propagation and transfer of nerve impulses.
- The functional components of a neuron consist of:
  - The cell body (perikaryon) having the nucleus (karyon) and those organelles that maintain the cell
  - The processes extending from the cell body which consists of **axon** and **dendrites**.
- Axon, usually the longest process, which transmits impulses away from the cell body to other neurons or effector (target) cells such as muscle cell or gland (secretory cells).
- Dendrites, usually the shorter processes that transmit impulses from the periphery (i.e. from other neuron) towards the cell body.

### Synapses

- A synapse is a site of functional contact between neurons that facilitate transmission of impulses from one (presynaptic) neuron to another (postsynaptic) neuron or effector (target) cells such as muscle and gland cells. Thus, a synapse is a site of neuron-to-neuron or neuron-to-effector cell communication.
- A typical synapse contains:
  - Presynaptic bouton or knob
  - Synaptic cleft
  - Postsynaptic membrane.

### *Presynaptic Bouton or Knob*

- The end of a neuro process is terminated in a bulb like end called a **bouton** or **presynaptic knob** from which neurotransmitters are released.

**Fig. 34.1:** Structure of typical neuron.

**Fig. 34.2:** Structure of typical synapse.

- It consists of **synaptic vesicles** in which neurotransmitters are stored **(Fig. 34.2)**.

### *Synaptic Cleft*

- There is no actual continuity between one neuron and another, a minute gap exists between them.
- The space that separates one neuron from another neuron or effector (target) cell, which the transmitter must cross is called **synaptic cleft (Fig. 34.2)**.

### *Postsynaptic Membrane*

It is a portion of the plasma membrane of the postsynaptic neuron which contains receptor sites with which the neurotransmitter interacts **(Fig. 34.2)**.

## CLASSIFICATION OF NEUROTRANSMITTERS

A classification of neurotransmitters based on chemical composition is given in **Table 34.1**. All are synthesized at the nerve ending and packaged into vesicles there.

## MECHANISM OF RELEASE OF NEUROTRANSMITTERS

- Various stimuli (physical, chemical or electrical) activate neurons from their resting state and cause them to produce nerve impulses.
- At rest, a neuron maintains a difference in potential (i.e. voltage) of about –50 to –80 mV between the inside and outside of its surface membrane. Thus, the cell surface is polarized in respect to the cell interior.
- When a nerve impulse reaches the bouton, the voltage reversal across the membrane produced by the impulse (called depolarization) causes $Ca^{2+}$ channels to open in the plasma membrane of the bouton.
- The influx of $Ca^{2+}$ from the extracellular space causes the synaptic vesicles to migrate to, and fuse with the presynaptic membrane, thereby releasing the neurotransmitter into the synaptic cleft by exocytosis.
- The neurotransmitter then diffuses across the synaptic cleft and binds to specific receptors on the postsynaptic membrane causing $Na^+$ channels in that membrane to open and allowing $Na^+$ to enter the neuron.
- Influx of $Na^+$ causes local depolarization in the postsynaptic membrane and thereby generating a new action potential or nerve impulse.
- The release of neurotransmitter by the presynaptic bouton can cause either **excitation** or **inhibition** at postsynaptic membrane.

### Excitatory Neurotransmitters

Excitatory neurotransmitters like acetylcholine and serotonin open **cation** channels, prompting an influx of $Na^+$ that causes local depolarization in the postsynaptic membrane. This leads to initiation of an action potential and generation of a nerve impulse.

**Table 34.1:** Classification of most common neurotransmitters and their site of synthesis.

| *Class* | *Example* | *Derived from* | *Site of synthesis* |
|---|---|---|---|
| Amine | Epinephrine<br>Norepinephrine<br>Dopamine<br>Serotonin<br>(5-Hydroxytryptamine) | Tyrosine<br>Tyrosine<br>Tyrosine<br>Tryptophan | Adrenal medulla, some CNS cells<br>CNS, sympathetic nerves<br>CNS<br>CNS, Chromaffin cells |
| Amino acid derivatives | Histamine<br>GABA | Histidine<br>Glutamate | Hypothalamus<br>CNS |
| Miscellaneous | Acetylcholine | Choline | Parasympathetic nerves, CNS |

### Inhibitory Neurotransmitters

- Inhibitory neurotransmitters like GABA open anion channels causing $Cl^-$ to enter the cell and hyperpolarize the postsynaptic membrane, making it even more negative so that generation of an action potential (i.e. making membrane depolarized) becomes more difficult and inhibit the production of new impulse.
- Each CNS neuron, in general, receives thousands of synapse, some excitatory and some inhibitory. Whether a given neuron will generate an impulse or not depends on the summation of the excitatory and inhibitory transmitters acting upon its surface at any particular time.

## REGULATION OF ACTION OF NEUROTRANSMITTERS

- When neurotransmitters serve their function, they must be removed from the synaptic space.
- The binding of neurotransmitter to receptor appears to be reversible, so that bound transmitter dissociates from its receptors when the local transmitter concentration falls.
- The most common process of removal of the neurotransmitter after its release into the synaptic cleft is by **high affinity reuptake mechanism**.
- In high affinity reuptake mechanism neurotransmitters are re-incorporated into the presynaptic vesicles by endocytosis and are available for recycling.
- About 80% of the released neurotransmitters are removed by this mechanism. Remaining 20% of the neurotransmitters are degraded by the enzymes associated with the postsynaptic membrane. For example, acetylcholinesterase (AChE) degrades acetylcholine into acetate and choline, catechol-o-methyltransferase (COMT) and monoamine oxidase (MAO) degrade norepinephrine.
- The degradation or reuptake of neurotransmitters is necessary to limit the duration of stimulation or inhibition of the postsynaptic membrane.

## DIFFERENT COMMON NEUROTRANSMITTERS

### Acetylcholine

- The first chemical neurotransmitter identified was acetylcholine. It is a neurotransmitter between axons and striated muscle at the neuromuscular junction (contact made by the terminal branches of the axon with muscle).

**Fig. 34.3:** Synthesis and hydrolysis of acetylcholine.

- Neurons that synthesize and release acetylcholine are termed **cholinergic neurons**.
- Acetylcholine is synthesized in neuronal cytoplasm from choline and acetyl-CoA through the action of choline acetyltransferase **(Fig. 34.3)**.
- Acetylcholine is then incorporated into synaptic vesicles and stored therein.

#### *Release and Action*

- Release of acetylcholine in response to an action potential is $Ca^{2+}$ dependent, as explained above in the mechanism of release of neurotransmitter.
- The released acetylcholine diffuses rapidly across the synaptic cleft to its receptors on the postsynaptic membrane (muscle membrane) causing opening of the $Na^+$ channels in the receptor that permits a flux of cations across the membrane.
- The consequent entry of $Na^+$ results in depolarization of the muscle membrane and action potential generated and transmitted along the fiber, resulting in contraction of the muscle.

### Catecholamines

- The principal catecholamines are:
  - Norepinephrine (noradrenaline)
  - Epinephrine (adrenaline)
  - Dopamine.

#### *Synthesis*

- These neurotransmitters are synthesized from phenylalanine and tyrosine **(*see* Fig. 14.21)**.

- Tyrosine is produced in the liver from phenylalanine through the action of phenylalanine hydroxylase.
- Tyrosine is then transported to catecholamine secreting neurons where a series of reactions convert it to dopamine, to norepinephrine and finally to epinephrine.

Dopamine is a major transmitter in nerves that control voluntary movement. Damage to these nerves causes **Parkinson's disease** which is characterized by tremor and difficulties in initiating and controlling movement.

### *Storage and Release*

- After synthesis, catecholamines are stored in synaptic vesicles.
- Catecholamines undergo $Ca^{2+}$ dependent release from synaptic vesicles in response to action potentials.
- After action, the catecholamine dissociates from its receptor quickly, causing the duration of the biological response to be brief.

### *Functions of Catecholamines*

- Catecholamines exhibit peripheral nervous system excitatory and inhibitory effects as well as respiratory stimulation in the CNS.
- The excitatory effects are exerted upon:
  - Smooth muscle cells of the vessels that supply blood to the skin and mucous membranes.
  - Heart, which lead to an increase in heart rate and force of contraction.
- Inhibitory effects, by contrast, are exerted upon smooth muscle cells in the wall of the gut, the bronchial tree of the lungs, and vessels that supply blood to skeletal muscle.
- In addition to their effects as neurotransmitters, norepinephrine and epinephrine can influence the rate of metabolism by increasing the rate of glycogenolysis and fatty acid mobilization.
- In the periphery, dopamine causes vasodilatation and it is therefore used clinically to stimulate renal blood flow, and is important in the treatment of renal failure.
- Dopamine found in limbic systems of the brain are involved in emotional responses and memory.

## Serotonin or 5-Hydroxytryptamine

- Serotonin also called **5-Hydroxytryptamine (5-HT),** is derived from **tryptophan (*see* Fig. 14.25)**.
- Neurons that secrete 5-HT are termed serotonergic neurons.

### *Storage and Release*

- Serotonin is stored in vesicles in the axon terminals of these neurons.
- Serotonin undergoes $Ca^{2+}$ dependent release from synaptic vesicles.

- The degradation of serotonin results in the formation of 5-hydroxyindoleacetic acid (5-HIAA). This is a useful marker of excess production of serotonin in diseases such as **carcinoid syndrome.**

### *Functions*

- Serotonin is involved with a wide range of functions such as platelet aggregation, and smooth muscle contraction, appetite, mood, hormonal balance, sleep/wake cycles, alertness, sexual behavior and temperature control.

## GABA (Gamma Aminobutyric Acid)

GABA is an amino acid derivative and is the most abundant inhibitor in the brain. It balances the brain by inhibiting over excitation.

### *Synthesis*

- GABA is synthesized from glutamate **(Fig. 34.4)** and stored in vesicles in axon terminals.
- Glutamate decarboxylase is present in many nerve endings of the brain as well as in the β-cells of the pancreas.

**Fig. 34.4:** Synthesis of GABA.

### Receptors and Action

- Neurons that secrete GABA are called **GABAergic neurons.** GABA exerts its effects by binding to receptors.
- Binding of GABA to receptors increases the $Cl^-$ conductance from outside to inside the neuron creating hyperpolarization and decreases nerve excitability.

### Functions of GABA

- GABA contributes to motor control, vision and many other cortical functions. Anxiety is also regulated by GABA. **Some drugs that increase the level of GABA in the brain are used to treat epilepsy** and **Huntington's** disease.
- GABA also stimulates the anterior pituitary, leading to higher levels of human growth hormone (HGH) which contributes significantly to muscle growth and also **prevents the formation of fat cells.**

## Histamine

- Histamine is found mainly in the hypothalamus. It is synthesized from histidine by the reaction catalyzed by **histidine decarboxylase,** a pyridoxal phosphate containing enzyme **(Fig. 34.5)**.
- Histamine is released from synaptic vesicles by exocytosis and interacts with its receptors.
- There are three classes of histamine receptors denoted $H_1$, $H_2$ and $H_3$.

**Fig. 34.5:** Histidine metabolism.

### Functions

- Histamine has been shown to control the release of pituitary hormones and play a role in sleep-wake cycles and food intake.

### Degradation

- After its action, it is inactivated metabolically. In contrast to other neurotransmitters, a high affinity $Na^+$ dependent transport system does not exist in brain. In brain, histamine undergoes methylation followed by oxidation to methylimidazole acetate **(Fig. 34.5)**.

## EXAM QUESTIONS

### Long Answer Question (LAQ)

1. Describe synthesis, storage, release, receptor action, re-uptake, and degradation of amine neurotransmitters.

### Short Notes

1. Acetylcholine as a neurotransmitter.
2. Classification of neurotransmitter.
3. Regulation of neurotransmitters.
4. Natural cholinergic agonist and antagonists.
5. Therapeutic applications of agonists and antagonists of catecholamine receptor.
6. Therapeutic applications of GABA agonist and antagonists.
7. Therapeutic applications of histamine receptor agonist and antagonist.
8. Inhibitory neurotransmitters.

### Multiple Choice Questions (MCQs)

1. **Which of the following is an inhibitory neurotransmitter?**
   a. GABA
   b. Dopamine
   c. Epinephrine
   d. Histamine

**2. Following are the amine neurotransmitters, *except*:**
a. Histamine b. Norepinephrine
c. Dopamine d. Acetylcholine

**3. Neurotransmitter serotonin is derived from:**
a. Tryptophan b. Tyrosine
c. Histidine d. Glutamine

**4. Which of the following neurotransmitters is a catecholamine?**
a. Serotonin b. GABA
c. Histamine d. Dopamine

**5. Which of the following is not a neurotransmitter?**
a. GABA b. Serotonin
c. Histamine d. Serine

## Answers for MCQs

**1.** a **2.** d **3.** a **4.** d **5.** d

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CHAPTER

# 35 Laboratory Investigation Techniques

**Chapter outline**

- Chromatography
- Electrophoresis
- Colorimetry
- Flame Photometry
- Immunochemical Techniques (RIA and ELISA)

## INTRODUCTION

The clinical biochemistry laboratory measures chemical changes in the body for **diagnosis, therapy** and **prognosis of disease.** The primary work of its technologists is the assay of various chemical constituents in blood, urine and other fluids or tissues.

The test accuracy is a prerequisite for the proper interpretation of a laboratory test for which knowledge of basic principles of laboratory instruments is essential. Accordingly, this chapter summarizes some of the basic principles common to the most biochemistry laboratory methods and essential to good laboratory practice and technique.

## CHROMATOGRAPHY

### Definition

Chromatography is the process for separation of components in a solution by differences in migration rate as the solution mixture (mobile phase) is passed over or through a stationary phase.

The separation may make use of one or more of the following physicochemical principles, depending upon the particular chromatographic system **(Table 35.1).**

### Technique

- The chromatographic technique is used for the separation of amino acids, proteins and carbohydrates.

**Table 35.1:** Physicochemical principle used for chromatographic separations.

| *Type of chromatography* | *Physicochemical principle* |
|---|---|
| Gel filtration | Size and shape |
| Adsorption chromatography | Adsorption |
| Partition chromatography | Solubility |
| Ion exchange chromatography | Ionization |

**Applications of Chromatography Technique**

- Purification of proteins, enzymes, nucleic acids, immunoglobulins and membrane receptors.
- Determination of molecular weight of proteins.
- Analysis of drugs, hormones vitamins and brain amines.
- Qualitative and quantitative analysis of complex mixtures.
- In the clinical laboratory for the identification of sugars, amino acids and drugs in serum or urine.

### Classification of Chromatography

- Chromatographic methods are generally classified according to the physical state of the mobile phase **(Fig. 35.1)**. This is further subclassified according to how the stationary phase is contained for a particular chromatographic method.

#### *Paper Chromatography*

*Principle of paper chromatography*

- Paper chromatography is a partition type of chromatography.

**Fig. 35.1:** Classification of chromatography according to the mobile phase.

- Partition chromatography is a process whereby the solutes of a sample are separated by differences in their relative solubilities between two liquid phases.
- The separation depends on the relative tendencies of the molecule in a mixture to associate more strongly with one or the other phases.
- In paper chromatography, Whatman filter paper No. 3 serves as a solid support to hold the stationary phase.
- Solvent system provides both stationary phase and mobile phase.
- For amino acid chromatography, butanol: acetic acid: water in the proportion of 4:1:5 v/v is used as solvent system.
- **Butanol with a little acetic acid** acts as the mobile phase and **water and acetic** acid forms the stationary phase, which will be held on the paper.

*Technique for paper chromatography*

- A Whatman filter paper No. 3 of appropriate size is soaked in solvent mixture in a tray, which absorbs aqueous part of solvent mixture.
- The sample which is to be separated is then spotted near the lower edge of filter paper along with known standards with the help of fine capillary.
- Keep the paper in the chamber dipping the end of the paper close to spots inside the solvent as shown in **Figure 35.2**.
- Solvent mixture is then allowed to run almost to the upper edge of paper.
- The paper is then removed, dried and sprayed with appropriate staining solution **(Figure 35.3)**.
- The colored spots seen are identified on the basis of $R_f$ values, which are specific for different substances.
- The ratio of fronts termed $R_f$, which is given by:

$$R_f \text{ (ratio of fronts)} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

- For quantitation, the spots may be cut out and eluted, i.e. dissolves in suitable solvent and intensity of color is measured on photoelectric colorimeter.

*Application of paper chromatography*

Paper chromatography is used for detection of **amino acids, sugars, pigments,** etc.

***Clinical Application of Paper Chromatography***

Identification of type of amino aciduria like cystinuria, phenylketonuria, etc.

## High Performance Liquid Chromatography (HPLC)

- HPLC is a highly sensitive and popular technique which can be applied to resolution of different types of compounds and drugs available in very small quantities and can be performed in a short time.
- In HPLC, a high pressure pump is used to force the solvent and sample through a relatively short and narrow-bore, tightly packed column of an adsorbent to give constant flow rate. Pressure of about 1000 to 3000 psi is frequently used in routine separations.
- As the separated components elute from the column, they pass through the detector (UV detector) where they are detected.

*Applications*

The method is applicable for the separation of carbohydrates, proteins, peptides, amino acids, vitamins, steroids, amines like biogenic amines and the polyamines, neuropeptides, hormones and drugs.

**Fig. 35.2:** Ascending paper chromatography.

**Fig. 35.3:** Paper chromatogram of amino acids. (G: Glycine, L: Leucine)

## ELECTROPHORESIS

A charged particle placed in an electric field migrates towards the **anode** or **cathode,** depending on the net charge carried by the particle **(Fig. 35.4)**. Application of electric field to solution of ions makes ions to move.

### Definition

Electrophoresis is the process of separating the **charged constituents** of a solution by means of an electric current.

### Electrophoresis Apparatus (Fig. 35.5)

- The electrophoresis apparatus is designed so that the circuit between the two poles is bridged by the support medium holding the sample, and the current flow is partially carried by the components of the sample.
- An electrophoresis chamber consists of two compartments separated from each other by a dividing wall; one side contains the **anode** and the other, the **cathode**.
- Each side is filled to the same level with a buffer (barbital buffer, pH 8.6) is often used for serum protein separation.
- A "bridge", across the top of the dividing wall holds a membrane or other support material so that each end of it is in contact with the buffer in one of the compartments.
- First membrane is immersed in buffer, blotted and placed in the chamber, and then sample is applied.
- When a voltage is applied to the cell, the current is carried across the porous membrane by the buffer ions.
- At pH 8.6, all the serum proteins carry a net negative charge and tend to migrate towards the anode.
  - **Albumin** carries the largest charge and therefore, moves the **fastest**.
  - The γ-**globulins** have the smallest net charge and move the least distance **(*see* Fig. 5.1)**.

### Classification of Electrophoresis

Depending upon the mode of operation and separation, electrophoresis is classified into various types as shown in **Figure 35.6**.

**Fig. 35.4:** Application of electric field to solution of ions makes ions to move.

**Fig. 35.5:** Electrophoresis apparatus.

**Fig. 35.6:** Classification of electrophoresis.

**Clinical Applications of Electrophoresis**

- The electrophoresis procedure is used in clinical laboratory for separation of serum proteins, lipoproteins, isoenzymes, hemoglobin and other classes of macromolecules.
- Immuno-electrophoresis is used to determine specific classes of immunoglobulins.
- Western blot technique to identify a specific protein used to confirm the presence of antibodies to human immunodeficiency virus (HIV) is based on electrophoretic principle.
- Southern blot techniques to identify specific nucleic acid sequences (DNA or RNA) used for prenatal diagnosis of inborn errors, diagnosis of viral infections and identification of risk factors for cancer is also based on electrophoretic principle.

## COLORIMETRY

- The most widely used method for determining the concentration of biochemical compounds is **colorimetry,** which makes use of the following principle.

### Principle

- When white light passes through a colored solution, some specific wavelengths of light are absorbed which is related to color intensity. The color intensity will be proportional to the concentration of the chemical responsible for producing the color.

### Components of the Colorimeter (Fig. 35.7)

- **A lamp (light source):** A lamp provides light in visible region of the spectrum. Usually, tungsten lamp is the source of light.
- **Adjustable slit:** The light emerging from tungsten lamp is allowed to pass through a narrow adjustable slit.
- **Condensing lens:** Provides parallel beam of light.
- **Filter:** Filter provides the desired monochromatic light (of single wavelength) by filtering other wavelengths. The color of the filter is complementary to the color of the solution **(Table 35.2)**. This allows only appropriate wavelength of light to pass through the colored solution.

**Fig. 35.7:** Basic components of colorimeter.

- **Cuvette (sample holder):** A special glass tube, which holds the solution to be analyzed in a colorimeter, is called cuvette. Cuvette should have uniform thickness, inner diameter and refractive index. Cuvettes usually have 1 cm light path.
- **Photodetector:** This produces a current in response to the light impinging upon it.
- **Galvanometer readout device**: This measures electric current generated by the photocell to optical density or % transmittance.
- The measurement of color intensity of a colored solution by colorimetry is governed by two laws:
  - Beer's law
  - Lambert's law.

### Beer's Law

- When a ray of monochromatic light passes through an absorbing medium, its intensity decreases exponentially as concentration of the light absorbing material increases.
- If **A** is the light absorbed (absorbance) and **C** is the concentration of the solution, then

$$A \propto C$$

### Lambert's Law

When a ray of monochromatic light passes through an absorbing medium its intensity decreases exponentially as the length of the light path through light absorbing material increases. If L be the length through which light passes, then

$$A \propto L$$

**Table 35.2:** Complementary colors for selection of filters.

| *Filter* | *Color of solution* |
|---|---|
| Blue | Red |
| Purple | Green |
| Yellow | Violet |
| Orange | Blue green |

### Calculations

Percent concentration of the test

$$= \frac{\text{OD of Test} - \text{OD of Blank}}{\text{OD of Standard} - \text{OD of Blank}} \times \frac{\text{Concentration of std.}}{\text{Volume of test sample}} \times 100$$

**Applications of Colorimeter**

Colorimetric procedures are widely used in hospital and laboratory for the estimation of various biochemical compounds in various biological samples like blood, plasma, serum, cerebrospinal fluid (CSF), urine and other body fluids.

- The biochemical compounds such as glucose, urea, creatinine, uric acid, bilirubin, lipids, total proteins, and enzymes like AST, ALT, ATP, minerals like calcium, phosphorus, etc. are routinely estimated by colori meter.

## FLAME PHOTOMETRY

Flame photometry is a device for measuring the concentration of **alkali metals like Na, K** and **Li** by measuring the intensities of light emitted by the same metals when their solutions are sprayed into a gas flame.

### Principle

When sufficient heat energy is supplied by a gas flame, atoms of alkali metals like Na, K and Li become excited, i.e. the electrons at the ground state of such atoms become excited and attain a higher energy state. These electrons while coming back to their original ground state emit energy in the form of photons of light of characteristic wavelength unique to each alkali metal. This emitted light passes through the suitable filter to isolate it from unwanted light. The emitted light then falls on a photocell, generating current which is measured by galvanometer.

### Components of the Flame Photometer (Fig. 35.8)

The basic components of the flame photometer are:

#### An Aspirator/Nebulizer/Automizer

The solution under analysis is sucked in by an aspirator system, consisting of a fine capillary tube, which dips into it. The nebulizer produces a fine spray of droplets of uniform size necessary for constant emission of light. The droplets are then driven to the flame of the gas burner.

**Fig. 35.8:** Basic components of flame photometer.

#### Burner and Flame

Flame provides heat energy. The heat of the flame evaporates the water and vaporizes the substance which is converted into atomic state. The heat energy also excites the electron in the ground state and moves it into higher energy state.

#### Monochromator/Filter

Monochromatic light is obtained by means of filter. The light of particular wavelength emitted by electrons passes through the suitable filter, e.g.

- For sodium 580 nm (orange), filter is used
- For potassium 766 nm (red), filter is used.

#### Phototube/Detector

A detector is a phototube which converts light energy into electrical energy.

#### Readout/Galvanometer

The electrical energy generated by detector is measured by galvanometer.

### Technique

- The solution which is to be analyzed is sprayed as a fine mist of droplets on the flame, which decomposes the compound into atoms.
- The electrons of free atoms absorb heat energy and get excited.
- When they return to ground state they emit characteristic light. This emitted light passes through the suitable filter to isolate it from unwanted light.
- The emitted light then falls on a photocell, generating current which is measured by galvanometer.

**Application of Flame Photometer**

- This special technique of emission of flame photometry is widely used in the clinical laboratory to determine the concentrations of sodium and potassium in biological fluids like serum, urine and sweat.
- The serum lithium levels can also be measured in connection with the therapeutic use of lithium salts in the treatment of psychiatric disorders.

## IMMUNOCHEMICAL TECHNIQUES (RIA AND ELISA)

- The immunochemical techniques are available to detect or quantitate the **antigen** or **antibody.** They are:
  1. Radioimmunoassay (RIA)
  2. Enzyme-linked immunosorbent assay (ELISA).
- RIA and ELISA are used to measure hormones, drugs, tumor markers, proteins and antigens in biological sample.
- Any immunoassay technique involves the reactions between an antigen and its specific antibody.

### Radioimmunoassay (RIA)

Yalow and Berson devised a radioimmunoassay (RIA) for the measurement of the insulin concentration in plasma that was far more sensitive and specific than any method in existence at that time. Now, it is possible to determine by RIA methods the concentration of nearly all of the hormones as well as many drugs, proteins and other compounds.

This immunoassay technique utilizes radioactive isotopes to label antigen. The most commonly used label is $^{125}I$, $^{3}H$ and $^{14}C$.

#### *Disadvantages of Radioimmunoassay*

The use of radioisotopes as label is associated with the following problems:

- Health hazard due to exposure to radiation.
- Waste disposal is costly and inconvenient.
- Short shelf-life of labeled reagents because of radioactive decay.
- High cost of equipment and reagents.
- Long duration of assay.

**Applications of Radioimmunoassay**

Variety of compounds of serum or other biological fluids present in very small amount can be estimated, e.g.

- Almost all hormones
- Tumor markers like prostate specific antigen (PSA), Alpha-fetoprotein (AFP), etc.
- Vitamins
- Drugs.

### ELISA (Enzyme-Linked Immunosorbent Assay or Enzyme Immunoassay)

ELISA is a **non-isotopic** immunoassay to overcome the disadvantages of using radioactivity; enzymes can be attached to the analyses and used in place of the radioactive antigen.

#### *Technique*

The technique of enzyme immunoassay is similar in principle to that of radioimmunoassay except that the antigen is labeled with a stable enzyme **(Table 35.3)** instead of a radioisotope compound.

**Applications of ELISA**

- ELISA is used in clinical laboratory to measure:
  - All hormones in the serum
  - Tumor markers in the serum, e.g. PSA, AFP, HCG, CEA, etc.
  - Antibodies in the serum in infectious diseases, e.g. antiviral, antibodies and antibacterial antibodies.
  - Autoantibodies, e.g. anti-DNA, ANA (antinuclear antibody).
- ELISA is used in the study of infectious diseases like defection of bacterial toxins, viruses, hepatitis B surface antigens, etc.

#### *Difference between RIA and ELISA*

- ELISA is non-isotopic technique
- Lacks radiological hazards of RIA
- Reagents have more shelf-life compared to RIA
- ELISA is cheaper than RIA
- Suitable for use even in small laboratories.

**Table 35.3:** Enzyme labels for immunoassays.

| *Enzyme* | *Source* | *Specific activity (Units/mg)** |
|---|---|---|
| Alkaline phosphatase | Calf intestine | 400 |
| β-Galactosidase | *E. coli* | 400 |
| Glucose oxidase | *Aspergillus Niger* | 200 |
| Glucose-6-phosphate dehydrogenase | Leuconostoc mesenteroides | 250 |
| Peroxidase | Horseradish | 900 |

* A unit of enzyme activity represents the conversion of 1 μmol of enzyme substrate to product per minute.

## EXAM QUESTIONS

### Long Answer Questions (LAQs)

1. What is chromatography? Describe different types of chromatography and its applications?
2. Define electrophoresis. Give classification, principle and applications of electrophoresis.

### Short Notes

1. RIA.
2. ELISA.
3. Flame photometer.
4. Partition chromatography.
5. HPLC.
6. Applications of chromatography.
7. Applications of electrophoresis.
8. Principle of flame photometer.
9. Disadvantages of RIA.
10. Difference between RIA and ELISA.
11. Name enzyme used in ELISA.
12. Lambert and Beer's law.

### Multiple Choice Questions (MCQs)

**1. Which of the following chromatographic techniques is based on molecular size?**
a. Gel filtration chromatography
b. Ion exchange chromatography
c. Paper chromatography
d. Affinity chromatography

**2. Which of the following is used as a label in ELISA?**
a. Antibody b. Antigen
c. Enzyme d. Radioisotope

**3. The pH of the buffer used for separation of serum proteins by electrophoresis on agar gel is:**
a. 10.01 b. 9.6
c. 5.6 d. 8.6

**4. Which of the following physicochemical principles is used for chromatographic separation?**
a. Adsorption
b. Partition
c. Difference in molecular size
d. All of the above

### Answers for MCQs

**1.** a **2.** c **3.** d **4.** d

# Glossary

**Abetalipoproteinemia:** An inherited inability to form chylomicrons and very-low-density lipoprotein (VLDL).
**Acetyl-CoA carboxylase:** The rate-limiting enzyme of fatty acid biosynthesis.
**Acetylcholinesterase:** An extracellular acetylcholine degrading enzyme in cholinergic synapses.
**Achondroplasia:** A dominantly inherited form of dwarfism caused by constitutive activation of a growth factor receptor.
**Acid:** A proton donor.
**Acid phosphatase:** A marker for prostatic cancer.
**Acidosis:** Abnormally low blood pH.
**Acrodermatitis enteropathica:** A disease caused by an inherited defect of intestinal zinc absorption.
**Actin:** A globular protein that polymerizes into microfilaments.
**Actinomycin D:** An inhibitor of transcription that binds to double-stranded DNA.
**Active site:** The place on the enzyme protein to which the substrate binds and where catalysis takes place.
**Acute intermittent porphyria:** A hepatic porphyria with abdominal pain and neurological symptoms.
**Acute-phase reactants:** Plasma proteins whose levels are elevated or reduced within 1 to 2 days of an acute stress.
**Acyl-CoA:** The activated form of a fatty acid.
**Adenomatous polyposis coli (APC):** An inherited cancer susceptibility syndrome.
**Adenosine deaminase deficiency:** A cause of severe combined immunodeficiency.
**Adenosine triphosphate (ATP):** The "energetic currency" of the cell.
**S-Adenosylmethionine (SAM):** A co-substrate that supplies an activated methyl group.
**Adenylate cyclase:** The cyclic adenosine monophosphate (cAMP) synthesizing enzyme, located in the plasma membrane.
**Adrenergic receptors:** Receptors for epinephrine and norepinephrine.
**Adrenodoxin:** A mitochondrial iron-sulfur protein that participates in hydroxylation reactions of steroids.
**Adrenogenital syndrome:** Inherited defects of corticosteroid synthesis that lead to over production of adrenal androgens.
**Aggrecan:** A large, aggregating proteoglycan in cartilage.
**Agonist:** A stimulatory ligand for a receptor.
**Alanine cycle:** Cycling of alanine and glucose between muscle and liver during fasting.
**Alanine transaminase:** A liver enzyme: its serum level is elevated in liver diseases.
**Albumin:** A plasma protein that accounts for approximately 60% of the total plasma protein.
**Alcohol dehydrogenase:** A cytosolic liver enzyme that oxidizes ethanol to acetaldehyde.
**Aldose:** Monosaccharide with an aldehyde group.
**Alkaline phosphatase:** A diagnostically useful enzyme in bones and biliary system.
**Alkalosis:** Abnormally high blood pH.
**Alkaptonuria:** A rare inborn error of tyrosine metabolism, with accumulation of homogentisate.
**Allele-specific oligonucleotides probes:** Probes that can distinguish between different alleles (variants) of a gene.
**Allelic heterogeneity:** Different disease-causing mutations in the same gene.
**Allopurinol:** An inhibitor of xanthine oxidase, used to treat gout.
**Allosteric effector:** A ligand that affects the equilibrium between the alternative conformations of an allosteric protein.
**Allosteric protein:** A protein that can exist in alternative conformations.
**Alport syndrome:** Genetic disorder of type IV collagen leading to kidney failure.
**Alu sequences:** A large family of short interspersed elements.
**Alzheimer disease:** The most common type of age-related dementia.

**α-Amanitine:** A mushroom poison that inhibits RNA polymerase II.
**Aminoacyl-tRNA:** A transfer RNA (tRNA) with an amino acid covalently bound to its 3′ terminus
**Aminoacyl-tRNA synthetase:** Cytoplasmic enzymes that attach an amino acid to a tRNA.
**γ-Aminobutyric acid (GABA):** An inhibitory neurotransmitter in the brain, synthesizes from glutamate.
**Aminolevulinic acid (ALA) synthase:** The regulated enzyme of heme biosynthesis.
**Amphipathic:** Containing hydrophilic and hydrophobic portions in the same molecule.
**α-Amylase:** A starch-degrading endoglycosidase in saliva and pancreatic juice.
**Amylin:** A hormone that can form amyloid deposits in the endocrine pancreas.
**Amyloid:** Abnormally folded, insoluble proteins with β-pleated sheet structure.
**Amyloidogenic:** Amyloid-forming.
**Anabolic pathway:** Biosynthetic pathway.
**Anaerobic:** Oxygen deficient.
**Anaplerotic reactions:** Reactions that result in the net production of a tricarboxylic acid (TCA) cycle intermediate.
**Anchorage dependence:** The inability of cultured cells to grow in the absence of a solid support.
**Androgen insensitivity syndrome (testicular feminization):** Sex reversal caused by the absence of functional androgen receptor in a genotypic male.
**Androgens:** Male sex steroids derived from progestins by a side chain cleavage reaction.
**Anemia:** Abnormal decrease of the blood hemoglobin concentration.
**Aneuploidy:** Deficiency or excess of a chromosome.
**Angiotensin II:** The active form of the vasoconstrictor angiotensin.
**Angiotensin-converting enzyme (ACE):** An enzyme of angiotensin synthesis; important drug target.
**Anion:** Negatively charged ion.
**Annealing:** Formation of a double strand from two complementary nucleic and strands.
**Anomers:** Monosaccharide that differ only in the orientation of substituent around their carbonyl carbons.
**Antagonist:** An inhibitory ligand for a receptor.
**Anticodon:** The base triplet of the tRNA that base pairs with the codon during protein synthesis.
**Antigen-antibody complex:** A noncovalent aggregate between antigen and antibody.
**Antimycin A:** An inhibitor of electron flow through the $QH_2$–cytochrome *c* reductase complex.
**Antiport:** The coupled membrane transport of two substrates in opposite directions.
**$\alpha_1$-Antiprotease:** A circulating protease inhibitor, deficiency of which causes lung emphysema.
**Antithrombin III:** A circulating inhibitor of thrombin and some other activated clotting factors.
**AP endonuclease:** An endonuclease that cleaves a phosphodiester bond formed by a baseless (apurinic) nucleotide in DNA.
**Aplastic anemia:** Anemia caused by bone marrow failure.
**ApoA-I, apoA-II:** The major apolipoproteins of high density lipoprotein (HDL).
**ApoB-100:** The major apolipoprotein of very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL).
**ApoB-48:** The major apolipoprotein of chylomicrons.
**ApoC-II:** An apolipoprotein that activates lipoprotein lipase.
**ApoC-III:** An apolipoprotein that inhibits lipoprotein lipase.
**ApoE:** An apolipoprotein that mediates the endocytosis of remnant particles by binding to hepatic apo-E-receptors.
**Apolipoprotein:** Protein component of a lipoprotein.
**Apoprotein:** The polypeptide component of a conjugated protein.
**Apoptosis:** Programmed cell death.
**Apoptosome:** A cytoplasmic protein complex that activates caspases, causing apoptosis.
**Arachidonic acid:** A 20-carbon polyunsaturated fatty acid, precursor of prostaglandins and leukotrienes.
**Arginase:** The urea-forming enzyme of the urea cycle.
**Aromatase:** The enzyme that converts androgens to estrogens.
**Arrestin:** A cytoplasmic protein that binds to activated hormone receptors, in activating them and marking them for endocytosis.
**Arsenate:** A poison that competes with phosphate in many phosphate-dependent reactions.
**Arsenite:** An inhibitor of pyruvate dehydrogenase that binds to dihydrolipoic acid.
**Ascorbic acid:** Vitamin C, a water-soluble antioxidant.
**Asialoglycoprotein:** Glycoprotein that has lost the terminal sialic acid residues from its oligosaccharides; undergoes endocytosis by the liver.
**Ataxia-telangiectasia:** A syndrome that is caused by impaired repair of DNA double-strand breaks.
**Atheromatous plaque:** The defining lesion of atherosclerosis.
**Atrial natriuretic factor:** A hormone from the heart that stimulates a membrane-bound guanylate cyclase.
**Autophagy:** A process leading to the lysosomal destruction of defective organelles and intracellular bacteria.
**Autosome:** Nonsex chromosome.

**Avidin:** A biotin-binding protein in egg white.
**B lymphocyte:** A types of lymphocyte that produces membrane-bound immunoglobulin.
**Bacteriophage:** "Phage"; bacteria-infecting virus.
**Basal metabolic rate:** The energy consumed in the absence of physical activity.
**Basal mutation rate:** Mutation rate in the absence of mutagens.
**Base:** A proton acceptor.
**Base excision repair:** Removal of an abnormal base by a DNA glycosylase.
**Base pairing:** The specific interaction between two bases in opposite strands of a double-stranded nucleic acid.
**Base stacking:** The noncovalent interaction between successive bases within a nucleic acid strand.
**Basement membrane:** An extracellular matrix structure beneath single-layered epithelia.
**Bence Jones protein:** Immunoglobulin light chains overproduced by some patients with multiple myeloma (a malignant plasma cell dyscrasia).
**Beriberi:** Thiamine deficiency, with severe neuromuscular weakness.
**Bile acids:** Emulsifiers in bile; required for lipid absorption.
**Bilirubin:** A yellow pigment formed from biliverdin.
**Biliverdin:** A green pigment formed from heme.
**Biotin:** The prosthetic group of carboxylase enzymes.
**2,3-Bisphosphoglycerate (BPG):** An allosteric effector that reduces the oxygen affinity of hemoglobin.
**Blood group substances:** Polymorphic constituents of the erythrocyte membrane.
**Blood urea nitrogen:** A measure for the accumulation of urea in uremia.
**Body mass index:** Weight/height$^2$.
**Bohr Effect:** Decreased inhibitor that is effective against some cancers.
***BRCA1, BRCA2*:** Two tumor suppressor genes associated with inherited susceptibility to breast and ovarian cancers.
**Bromouracil:** An analog of thymine that causes mutations after being incorporated in DNA.
**Brush border enzymes:** Enzymes on the luminal surface of intestinal mucosal cells.
**Buffer:** A solution whose pH value is stabilized by the presence of ionizable groups.
**Burkitt lymphoma:** A B-cell malignancy in which the *myc* proto-oncogene has been translocated to an immunoglobulin locus.
**C-peptide:** A biologically inactive fragment of pro-insulin that is released together with insulin.
**C-reactive protein:** The most sensitive acute-phase reactant.
**Cadherins:** Membrane-spanning cell adhesion proteins in the zonula adherens.
**Calcitriol:** 1,2-5-Dihydroxycholecalciferol, the active form of vitamin D.
**Calcium channel blockers:** Drug that block voltage gated calcium channels in excitable cells.
**Calmodulin:** A calcium-dependent enzyme activator.
**cAMP response element-binding protein (CREB):** Protein that mediates effects of cyclic AMP on gene transcription.
**Cap:** A methylguanosine-containing structure at the 5′ end of eukaryotic messenger RNA.
**Capsid:** The protein coat of the virus particle.
**Carbamino hemoglobin:** Hemoglobin with carbon dioxide covalently bound to its terminal amino groups.
**Carbamoyl phosphate:** An intermediate in the synthesis of urea and pyrimidines.
**Carbohydrate loading:** A procedure aimed at the buildup of large muscle glycogen stores in endurance athletes.
**α-Carbon:** The carbon next to the carboxyl carbon.
**Carbon monoxide:** A competitive inhibitor of oxygen blinding to hemoglobin and myoglobin.
**Carbonic anhydrase:** An enzyme that establishes equilibrium among carbon dioxide, water, and carbonic acid.
**γ-Carboxyglutamate:** A post-translationally formed amino acid in prothrombin and factors VII, IX, and X.
**Carboxypeptidase A and B:** Two pancreatic carboxypeptidases.
**Carcinoma:** Cancer of epithelial tissues.
**Cardiolipin:** A phosphoglyceride in the inner mitochondrial membrane.
**Cardiotonic steroids:** A class of drugs that inhibit the sodium-potassium ATPase.
**Carnitine:** A coenzyme that carries long-chain fatty acids into the mitochondrion.
**Carnosine:** The dipeptide β- alanyl-histidine**:** used as a pH buffer in muscle tissue.
**Carotenes:** Dietary precursors of vitamin A in vegetables.
**Caspases:** Proteases whose activation triggers apoptosis.
**Catabolic pathway:** Degradative pathway.
**Catabolite repression:** Repression of catabolic operons in the presence of glucose.
**Catalase:** A hydrogen peroxide-degrading enzyme.
**Catalytic rate constant:** The rate constant K cat that describes the rate of product formation from the enzyme-substrate complex.

**Catecholamines:** Tyrosine-derived: biogenic amines dopamine, norepinephrine, and epinephrine.
**Catechol-O-methyltransferase (COMT):** A catecholamine-inactivating enzyme.
**Cation:** Positively charged ion.
**cDNA library:** A collection of complementary DNA (cDNA)–containing bacterial clones.
**Centromere:** The region of the chromosome that interacts with microtubules during mitosis.
**Ceramide:** A lipid consisting of sphingosine and a fatty acid.
**Cerebrosides:** Sphingolipid containing monosaccharide.
**Ceruloplasmin:** A copper-containing plasma protein.
**Chaperone:** A protein that assists in the folding of other proteins.
**Chelators:** Small molecules that bind a metal with high affinity.
**Chirality:** Optical isomerism, created by alternative configurations of substituent around an asymmetrical carbon.
**Cholecalciferol:** The form of vitamin D that is made photochemically in the skin.
**Cholera toxin:** The enterotoxin of *Vibrio cholerae*. It causes cAMP accumulation in the intestinal mucosa by covalent modification of the $G_s$ protein.
**Cholestasis:** Lack of bile flow.
**Cholesterol:** The only important membrane steroid in humans.
**Cholesterol ester transfer protein:** A protein that transfer cholesterol esters from high-density lipoprotein (HDL) to other lipoproteins.
**Cholesterol esters:** Esters of cholesterol with a fatty acid.
**Cholestyramine:** A bile acid-binding resin that is used as a cholesterol-lowing agent.
**Choline-acetyltransferase:** The acetylcholine synthesizing enzyme in nerve terminals.
**Cholinesterase:** A serum enzyme that participates in the metabolism of some drugs.
**Choluric jaundice:** Jaundice accompanied by the urinary excretion of bilirubin diglucuronide.
**Chondrodysplasias:** A group of skeletal deformity syndromes, often caused by abnormal cartilage collagens.
**Chondroitin sulfate:** The most abundant glycosaminoglycan (GAG) in many connective tissues.
**Chromatin:** DNA complexed with histones.
**Chylomicrons:** Lipoproteins that carry dietary lipids from the intestine to other tissues.
**Chymotrypsin:** A serine protease from the pancreas.
**Ciprofloxacin:** An antibiotic that inhibits bacterial topoisomerases.
**Cirrhosis:** Fibrous degeneration of the liver.
**Clathrin:** The protein that forms the coat of coated vesicles.
**Clonal selection:** Selective growth stimulation by an antigen of a B-cell clone carrying a matching surface antibody.
**Clone:** A population of genetically identical cells that are descended from the same ancestral cell.
**Cloning vector:** A plasmid, bacteriophage or artificial chromosome contains foreign DNA.
**Cobalamin:** Vitamin $B_{12}$.
**Cockayne syndrome:** Neurological degeneration and early senility caused by inherited defects of transcription-coupled nucleotide excision repair.
**Coding strand (sense strand):** The strand of a gene whose base sequence corresponds to the base sequence of the RNA transcript.
**Codon:** A base triplet on messenger RNA that specifies an amino acid.
**Coenzyme A (CoA):** A co-substrate that forms energy-rich thioester bonds with many organic acids.
**Colchicine:** An alkaloid that inhibits the formation of microtubules.
**Collagen:** A family fibrous proteins in the extracellular matrix, with a characteristic triple-helical structure.
**Colloid-osmotic pressure:** The osmotic pressure of macromolecules.
**Committed step:** The first irreversible reaction unique to a metabolic pathway.
**Comparative genomic hybridization:** A method for the detection of copy number variations (deletions, duplications) in DNA.
**Competitive inhibition:** Inhibition by an agent that binds non-covalently to the active site of the enzyme.
**Complementary DNA (cDNA):** The double-stranded DNA copy of a single-stranded RNA.
**Compound heterozygote:** Person who carries two different mutations in different copies of the same gene.
**Condensation reaction:** Reactions in which a hydrophilic molecule becomes covalently linked to a chemical.
**Conformation:** The noncovalent higher order of protein.
**Congenital:** Present at birth. (The opposite of congenital is acquired).
**Conjugation reaction:** Reaction in which the hydrophilic molecule becomes covalently linked to a chemical.
**Connexin:** A channel-forming transmembrane protein in gap junctions.
**Consensus sequence:** The sequence of the most commonly encountered bases in a functionally defined nucleic acid sequence.

**Constitutive proteins:** Proteins that are synthesized at all times.
**Contact inhibition:** Inhibition of cell proliferation by contact with neighboring cells.
**Contact-phase activation:** The very first reactions in the intrinsic pathway of blood clotting.
**Cooperativity:** Interactions between multiple binding sites for the same ligand in an allosteric protein.
**Copper:** A trace mineral; present in many enzymes that use molecular oxygen as a substrate.
**Cori cycle:** The shuttling of glucose and lactate between muscle and liver during physical exercise.
**Corticosteroids:** Glucocorticoids and mineralocorticoids; C-21 steroids that are derived from progestins by hydroxylation reactions.
**Cortisol:** The most important glucocorticoid; a stress hormone.
**Coumarin-type anticoagulants:** Vitamin K antagonists; inhibit the post-translational formation of γ-carboxyglutamate in some clotting factors.
**Covalent bond:** Strong chemical bond formed by a binding electron pair.
**Covalent catalysis:** A catalytic mechanism that involves the formation of a covalent bond between enzyme and substrate.
**Cer recombinase:** An integrase from a bacteriophage, used for highly selective splicing reactions in recombinant DNA technology.
**Creatine:** A metabolite in muscle that forms the energy-rich compound creatine phosphate.
**Creatine kinase:** An enzyme whose level is elevated in the serum of patients with muscle diseases and acute myocardial infarction
**Cretinism:** The result of untreated congenital hypothyroidism; characterized by mental deficiency and growth retardation.
**Creutzfeldt-Jakob disease:** A sporadic or inherited prion disease.
**Crigler-Najjar syndrome:** Inherited defect of bilirubin conjugation.
**Crohn disease:** A type of inflammatory bowel disease.
**Crossing-over:** The exchange of DNA between homologous chromosomes during meiosis and (rarely) mitosis.
**Cryoprecipitate:** A plasma protein preparation enriched in some clotting factors.
**Curare:** An arrow poison that blocks acetylcholine receptors in the neuromuscular junction.
**Cyanide:** A poison that prevent the reduction of molecular oxygen by cytochrome oxidase.
**Cyclic adenosine monophosphate (cAMP):** A second messenger of many hormones.
**Cyclic guanosine monophosphate (cGMP):** Another second messenger in many cells.
**Cyclin-dependent kinases (CDKs):** Positive regulators of cell cycle progression.
**Cyclins:** Activators of cylin dependent protein kinases.
**Cyclooxygenase:** The key enzyme for the synthesis of prostaglandins, prostacyclin, and thromboxane.
**Cystic fibrosis:** A genetic disease cause by malfunction of a chloride channel in secretary epithelia.
**Cystinuria:** An inherited defect in the transport of dibasic amino acids in kidney and intestine; causes kidney stones.
**Cytochrome oxidase:** Complex IV in the respiratory chain, transfers electrons to $O_2$.
**Cytochromes:** Heme proteins that function as electron carries.
**Cytokines:** Biologically active protein released by activated lymphocytes and monocytes/macrophages.
**Death receptors:** Receptors for extracellular ligands; their activation triggers apoptosis.
**7-Dehydrocholesterol:** A steroid that is photochemically cleaved to cholecalciferol (vitamin $D_3$) in the skin.
**Dehydrogenase reactions:** Hydrogen transfer reactions.
**Dementia:** Loss of mental capacities.
**Denaturation:** Destruction of a protein's or nucleic acids higher-order structure.
**Desaturase:** Enzyme that introduce double bonds into fatty acids.
**Desmolase:** The rate-limiting enzyme for the synthesis of steroid hormones.
**Desmosine:** A covalent cross-link in elastin.
**Desmosomes:** Spot welds that hold neighboring cells together.
**1,2-Diacylglycerol:** A second messenger formed by phospholipase C.
**Dialysis:** Removal of small molecules and inorganic ions through a semipermeable membrane.
**Dihydrofolate reductase:** An enzyme that reduces dihydrofolate to tetrahydrofolate.
**Dihydrotestosterone:** A potent androgen formed from testosterone by 5a-reductase in androgen target tissues.
**Dioxygenases:** Enzymes that incorporate both oxygen atoms of $O_2$ in their substrate.
**Dipalmitoyl phosphatidylcholine:** The main constituent of lung surfactant.
**Diphtheria toxin:** A bacterial toxin that inactivates an elongation factor of eukaryotic protein synthesis.

**Dipole:** A structure with asymmetrical distribution of electric charges.
**Dipole-dipole interaction:** Attraction force between the components of two polarized bonds.
**Disulfide bond:** A covalent bond formed by an oxidative reaction between two sulfhydryl groups.
**Disulfiram:** Antabuse; an aldehyde dehydrogenase inhibitor used for treatment of alcoholism.
**DNA fingerprinting:** DNA-based methods for the identification of persons in criminal cases.
**DNA glycosylase:** Enzymes that remove abnormal bases from DNA.
**DNA ligase:** An enzyme links two DNA strands.
**DNA Methylation:** A covalent modification of DNA that suppresses transcription.
**DNA microarray:** "DNA chip", used for studies of gene expression or genetic variation.
**DNA polymerases:** DNA-synthesizing enzymes.
**Dolichol phosphate:** A lipid that participates in the synthesis of N-linked oligosaccharides in glycoproteins.
**Dominant:** Determining the phenotype in the heterozygous (as well as the homozygous) state.
**Dopamine neurons:** Neurons that use dopamine as a neurotransmitter.
**Dot blotting:** A rapid screening method for mutations and DNA polymorphism.
**Doxorubicin:** An anticancer drug that inhibits human topoisomerases.
**Duchenne muscular dystrophy:** A severe inherited muscle disease caused by defects of the structural protein dystrophin.
**Dynein:** The ATPase that moves cilia and flagella.
**E2F:** A transcription factor that is negatively regulated by the retinoblastoma protein.
**E6, E7:** The oncogenes of the human papilloma virus; they bind and inactivate p53 and p RB, respectively.
**Edema:** Abnormal fluid accumulation in the interstitial tissue spaces.
**Ehlers-Danlos syndrome:** A group of genetic diseases characterized by stretchy skin and loose joints.
**Elastase:** An endopeptidase from pancreas and other sources.
**Elastin:** The major component of elastic fibers.
**Electrogenic transport:** Net transport of electrical charges across a membrane.
**Electronegativity:** The tendency of an atom to attract electrons.
**Electrophoresis:** Separation of molecules in an electrical field.
**Electrostatic interaction:** "Salt bond"; the attraction force between oppositely charges ions.
**Emphysema:** A lung disease, characterized by degeneration of the alveolar walls.
**Enantiomers:** Isomers that are mirror images.
**Endergonic reaction:** Reaction with positive ΔG.
**Endocytosis:** Cellular uptake of soluble macromolecules or particles through an endocytic vesicle.
**Endorphins:** Peptides that activate opiate receptors.
**Endosome:** An organelle derived from endocytic vesicles.
**Endosymbiont hypothesis:** The hypothesis that mitochondria (and chloroplasts) are derived from symbiotic prokaryotes.
**Energy charge:** A measure for the energy status of a cell.
**Energy-rich bonds:** Bonds whose hydrolysis releases an unusually large amount of energy.
**Enhancers:** DNA sequences that increase the rate of transcription by binding regulatory proteins.
**Enterohepatic circulation:** The cycling of bile acids (and other compounds) through liver and intestine.
**Enteropeptidase:** The energy content of a molecule.
**Entropy:** The randomness of a thermodynamic system.
**Envelope:** A membrane, acquired from the host cell that surrounds many animal viruses.
**Enzyme-substrate complex:** Enzyme with a noncovalent bound substrate.
**Epidermal growth factor (EGF):** A growth factor that is mitogenic for epithelial cells.
**Epidermolysis bullosa:** Inherited skin-blistering diseases caused by abnormalities of proteins in the dermal-epidermal junction.
**Epimers:** Monosaccharides differ in the configuration of substituents around one of their asymmetrical carbons.
**Epinephrine:** Synonym for adrenaline, a stress hormone from the adrenal medulla.
**Equilibrium constant:** A thermodynamic constant that is defined as product concentration(s) divided by substrate concentration (s) dividend by substrate concentration (s) at equilibrium.
**Erythropoietin:** A kidney-derived growth factor that stimulated erythropoiesis in the bone marrow.
***Escherichia coli (E. coli)*:** An intestinal bacterium.
**Estrogens:** 18-Carbon steroids, synthesized from androgens by the aromatization of ring A.
**Euchromatin:** A dispersed transcriptionally active form of chromatin.
**Eukaryotes:** Cells with a membrane-bounded nucleus.
**Exergonic reaction:** Reaction with negative ΔG.

**Exocytosis:** Secretion of water-soluble substances by fusion of an exocytic vesicle with the plasma membrane.
**Exons:** The parts of the gene that are represented in the mature RNA.
**Expression vector:** A cloning vector from which the cloned cDNA can be transcribed.
**Extracellular:** Outside the cells.
**Extracellular signal-regulated kinases (ERKs):** Serine/threonine kinases of the mitogen-activated protein (MAP) kinase family.
**F factor:** A self-transmissible plasmid in *Escherichia coli.*
**Fab:** The antigen-binding fragment of immunoglobulins.
**Facilitated diffusion:** A passive type of carrier mediated transport.
**Familial combined hyperlipoproteinemia:** A genetic predisposition to type II AND TYPE IV hyperlipoproteinemia.
**Familial hypercholesterolemia:** Inherited deficiency of low-density lipoprotein (LDL) receptors.
**Fatty liver:** A reversible lesion caused by increased hepatic triglyceride synthesis or impaired very low density lipoprotein (VLDL) formation.
**Fatty streak:** Accumulation of cholesterol esters in arterial walls.
**Fc:** The crystallizable fragment of immunoglobulins, formed from the carboxyl-terminal halves of the heavy chains.
**Feedback inhibition:** Inhibition of a metabolic pathway by its end product.
**Feedforward stimulation:** Stimulation of a metabolic pathway by its substrate.
**Ferritin:** The principal intracellular iron storage protein; trace amounts are present in the plasma.
**α-fetoprotein:** A fetal plasma protein, levels of which and in amniotic fluid if the fetus has an open neural tube defect.
**Fibrate drugs:** Lipid-lowering drugs that activate peroxisome proliferator-activated receptor-α (PPAR-α).
**Fibrillin:** Protein in microfibrils that is defective in Marfan syndrome.
**Fibrillinogen:** The circulating precursor of fibrin.
**Fibronectin:** A glycoprotein that binds to cell surfaces and extracellular matrix constituents.
**First-order reaction:** Reaction whose velocity is proportional to the concentration of a substrate.
**Flavin adenine dinucleotide (FAD):** Hydrogen transferring prosthetic group of flavoprotein.
**Flavoprotein:** Proteins contain a flavin coenzyme (either flavin mononucleotide (FMN) prosthetic group; participate in hydrogen transfer reactions.
**Fluid-mosaic model:** A model of membrane structure that assumes globular proteins embedded in a lipid bilayer.
**Fluorescent *in situ* hybridization (FISH):** Method for the detection of DNA sequences in a chromosome spread.
**Fluorouracil:** A base analog used in cancer chemotherapy.
**Foam cell:** A macrophage filled with droplets of cholesterol esters.
**Follicular hyperkeratosis:** Gooseflesh; occurs in deficiency of vitamin C and vitamin A.
**Frame shift mutation:** Insertion or deletion that changes the reading frame of the messenger RNA.
**Free energy:** The "useful" energy in chemical reactions.
**Fructokinase:** The major fructose-metabolizing enzyme.
**Freuctose-1,6-bisphosphatase:** An important regulated enzyme of gluconeogenesis.
**Fructose-2,6-bisphosphate:** A regulatory metabolite that mediates hormonal effects on phosphofructokinase and fructose-1, 6-bisphosphatase.
**Fructose intolerance:** Hereditary disorder caused by deficiency of a fructose-metabolizing enzyme.
**Furanose ring:** Five-member ring in monosaccharides.
**Futile cycle:** Simultaneous activity of two opposing metabolic reactions, leading to ATP hydrolysis.
**G proteins:** GTP-binding signal transducing proteins that mediate most hormone effects.
**$G_0$ phase:** The nondividing state of a cell.
**$G_1$ phase:** The time between mitosis and S phase.
**$G_2$ phase:** The time between S phase and mitosis.
**Galactokinase:** The enzyme that phosphorylates galactose to galactose-1-phosphorylates.
**Galactosemia:** A hereditary disease caused by the deficiency of a galactose-metabolizing enzyme.
**β-Galactosidase:** A lactose-hydrolyzing enzyme.
**Gallstones:** Calculi formed from cholesterol or other poorly soluble substances in the biliary system.
**Ganglioside:** Sphingolipid containing an acidic oligosaccharide.
**Gap junction:** A small aqueous channel connecting the cytoplasm of neighboring cells.
**Gas gangrene:** A severe type of wound infection caused by collagenase-producing anaerobic bacteria.
**Gaucher disease:** A lipid storage disease in which glucocerebroside accumulates.
**Gelatin:** Denatured collagen.
**Gene:** A length of DNA directing the synthesis of a polypeptide or a functional RNA.
**Gene families:** Structurally related genes with a common evolutionary origin.

**Gene therapy:** The introduction of a functional gene into the patient's cells.
**Genome-wide association study:** A study that relates a large number of DNA variants to a disease or other phenotype.
**Genomic library:** A collection of bacterial clones containing fragments of genomic DNA.
**Germline mutation:** Mutation arising in the cell lineage that gives rise to gametes.
**Gilbert syndrome:** Benign hyperbilirubinemia caused by a promoter mutation in the gene foe bilirubin UDP-glucuronyl transferase.
**Glitazone drugs:** Pharmacological activators of peroxisome proliferator-activated receptor-γ (PPAR-γ) that sensitize cells to insulin.
**Glucagon:** A pancreatic hormone that stimulates the glucose-producing pathways of the liver.
**Glucogenic:** Glucose forming compounds.
**Glucokinase:** The liver isoenzyme of hexokinase.
**Gluconeogenesis:** Synthesis of glucose from noncarbohydrates.
**Glucose oxidase test:** An enzymatic method for the selective determination of glucose in the clinical laboratory.
**Glucose-6-phosphatase:** The glucose-producing enzyme in gluconeogenic tissues and glucose-transporting epithelia.
**Glucose-6-phosphate dehydrogenase:** The first enzyme in the oxidative branch of the pentose phosphate pathway.
**Glucose-6-phosphate dehydrogenase deficiency:** A common enzyme deficiency that leads to hemolysis after exposure to certain drugs.
**Glucose tolerance test:** A laboratory test that determines the effect of glucose ingestion on the blood glucose level.
**Glucose transporter 4 (GLUT-4):** An insulin-dependent glucose carrier in muscle and adipose tissue.
**Glutamate dehydrogenase:** An enzyme that catalyzes the oxidative deamination of glutamate and the reductive amination of α-ketoglutarate.
**Glutaminase:** A hydrolytic enzyme that releases ammonia from glutamine.
**Glutathione:** A tripeptide with reducing properties.
**Glutathione reductase:** An NADPH-dependent enzyme that keeps glutathione in the reduced state.
**Glycemic index:** A measure for the extent to which a food raises the blood glucose level.
**Glycerol phosphate shuttle:** The transfer of electrons from cytoplasmic NADH to the respiratory chain.
**Glycogen phosphorylase:** The enzyme that cleaves glycogen to glucose-1-phosphate.
**Glycogen synthase:** The enzyme that synthesizes glycogen from UPD-glucose.
**Glycolipids:** Carbohydrate-containing lipid.
**Glycoprotein:** A protein contains covalently bound carbohydrate.
**Glycosaminoglycans (GAGs):** Polysaccharides containing an amino sugar in other position.
**Glycosidases:** Enzymes that cleave glycosidic bonds.
**Glycosidic bond:** Bond formed by the anomeric carbon of a monosaccharide.
**Glycosyltransferases:** Biosynthetic enzymes that use activated monosaccharides.
**Goiter:** Enlargement of the thyroid gland; seen in some forms of hypothyroidism and hyperthyroidism.
**Gouty arthritis:** Arthritis caused by sodium urate deposits in the joints.
**Graves's disease:** An autoimmune disease leading to hyperthyroidism.
**Growth factors:** Soluble extracellular protein that stimulates the proliferation or differentiation of cultured cells.
**Guanylate cyclase:** Cyclic GMP-synthesizing enzymes.
**Half-life:** The time that it takes for half of the substrate molecules to react in a first-order reaction.
**Haptoglobin:** A hemoglobin-binding protein in serum.
**Hartnup disease:** An inherited defect in the renal and intestinal transport of large neutral amino acids, with pellagra-like symptoms.
**Hashimoto disease:** Autoimmune thyroiditis, the most common cause of hypothyroidism.
**Heat shock proteins:** Chaperones that are induced by heat exposure.
**Heinz bodies:** Abnormal protein aggregates in erythrocytes.
**Helicases:** Enzymes separate the strands of double-stranded DNA.
**α-helix:** A compact secondary structure in proteins that is stabilized by intrachain hydrogen bonds between peptide bonds.
**Hematin:** An oxidized derivative of heme containing a ferric iron.
**Hematocrit:** The percentage of the blood volume that is occupied by blood cells.
**Hemochromatosis:** An iron overload syndrome.
**Hemodialysis:** The major procedure for treatment of renal failure.
**Hemoglobin $A_{1C}$:** Hemoglobin A modified by a reaction between terminal amino groups and glucose.
**Hemoglobin Bart:** A $\gamma_4$ tetramer, in patients with α-thalassemia.
**Hemoglobin S:** Sickle cell hemoglobin.
**Hemoglobinopathy:** Genetic diseases caused by abnormalities of hemoglobin structure or synthesis.

**Hemolysis:** Destruction of erythrocytes.
**Hemopexin:** A heme-binding protein in serum.
**Hemophilia:** A group of inherited clothing disorders; the most common is factor VIII deficiency.
**Hemorrhagic disease of the newborn:** A neonatal bleeding disorder caused by vitamin K deficiency.
**Hemosiderin:** A partially denatured form of ferritin.
**Hemosiderosis:** Abnormal accumulation of hemosiderin.
**Henderson-Hasselbalch equation:** The equation that relates protonation states to pK and pH.
**Heparan sulfate:** A glycosaminoglycans (GAG) in many cell surface and connective tissue proteoglycans.
**Heparin:** A sulfated glycosaminoglycan (GAG) made by made cells and basophils.
**Hepatic encephalopathy:** Brain dysfunction caused by hyperammonemia and other aberrations in patients with liver cirrhosis.
**Hepatic lipase:** An extracellular enzyme in the liver that hydrolyzes triglycerides and phospholipids in remnant particles and high-density lipoprotein (HDL).
**Hereditary fructose intolerance:** Inherited defect in hepatic fructose metabolism.
**Hereditary nonpolyposis colon cancer (HNPCC):** An inherited cancer susceptibility syndrome caused by defects of post-replication mismatch repair.
**Hereditary persistence of fetal hemoglobin:** $\gamma$-chain production in an adult.
**Heterochromatin:** A condensed, transcriptionally inactive form of chromatin.
**Heteroplasmy:** Presence of both normal and pathogenic mitochondrial DNA.
**Heterozygote advantage:** Improved survival and/or reproduction of heterozygous mutation carries.
**Heterozygous:** Carrying two different variants of a gene.
**Hexokinase:** The enzyme that phosphorylates glucose to glucose-6-phosphate.
**Hexose:** Six-carbon sugar.
**High-density lipoprotein:** A protein-rich lipoprotein that transports cholesterol to the liver.
**Histidinemia:** A relatively benign inborn error of histidine metabolism.
**Histones:** A Small, basic proteins that are tightly associated with DNA in chromatin.
**Homocysteine:** An amino acid derived from methionine; possible risk factor for atherosclerosis.
**Homocystinuria:** An inborn error in the metabolism of the sulfur amino acids, causing skeletal deformities and mental deficiency.
**Homologous recombination:** Reciprocal exchange of DNA between two DAN molecules of related sequence.
**Homozygous:** Carrying two identical variants of gene.
**Hormone-sensitive lipase:** An enzyme in adipose cells that hydrolyzes stored triglycerides.
**Human artificial chromosomes:** Vectors for germline genetic engineering.
**Huntington disease:** An inherited neurodegenerative disease caused by expansion of a trinucleotide repeat.
**Hutchinson-Gilford progeria:** Premature aging caused by inherited defects in nuclear lamins.
**Hyaluronic acid:** A large, unsulfated glycosaminoglycans (GAG), not bound to a core protein.
**Hybridization:** Annealing of nucleic acids from different sources.
**Hydrogen bond:** Dipole-dipole interaction involving a hydrogen atom.
**Hydrogen peroxide:** A toxic product of some oxidative reactions.
**Hydrolase:** Enzyme catalyzing hydrolytic cleavage of water.
**Hydrophobic interactions:** Interactions between hydrophobic groups, resulting from deduction of the aqueous-nonpolar interface.
**3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase:** The regulated enzyme of cholesterol synthesis.
**Hydroxyl radical:** An extremely reactive and highly toxic byproduct of oxidative metabolism.
**Hydroxylase:** Enzyme that introduces a hydroxyl group in its substrate.
**7 $\alpha$-Hydroxylase:** The regulated enzyme of bile acid synthesis.
**Hyperammonemia:** Too much ammonia in the blood.
**Hyperbaric oxygen:** Oxygen applied under increased pressure.
**Hyperbilirubinemia:** Too much uric acid in the blood.
**Hypervariable regions:** The most variable parts of the variable domains in the immunoglobulins; sites of contact with the antigen.
**Hypochromia:** Reduced hemoglobin content of erythrocytes.
**Hypoglycemia:** Too little blood glucose, resulting in brain dysfunction.
**Hypoxanthine:** A deamination product of adenine.
**Hypoxanthine-guanine phosphoribosyl transferase (HGPRT):** The most important salvage enzyme for purine bases.
**Hypoxia:** Oxygen deficiency.
**I-cell disease:** A lysosomal storage disease caused by misrouting of lysosomal enzymes.

**Imprinting:** Silencing of specific genes in the germline, usually by DNA methylation.
**Indels:** Insertions and deletions.
**Induced-fit model:** A model that assumes a flexible substrate-binding site in the enzyme.
**Inducible proteins:** Proteins whose synthesis is regulated.
**Inosine:** A nucleoside containing hypoxanthine and ribose.
**Inositol 1,4,5-trisphosphate ($IP_3$):** A second messenger formed by phospholipase c; releases calcium from the endoplasmic reticulum.
**Insertion sequence:** A mobile element in prokaryotes that contains a gene for transposase.
**Insulinoma:** An insulin-secreting tumor of pancreatic β-cells.
**Integral membrane proteins:** Proteins that are embedded in the lipid bilayer.
**Integrases:** Enzymes that catalyze site-specific recombination.
**Integrins:** Integral membrane proteins that function as receptors for components of the extracellular matrix.
**Intermediate filament:** A type of cytoskeletal fiber formed from proteins in a coiled coil conformation.
**International unit (IU):** The enzyme activity that converts 1 μmol of substrate to product per minute.
**Intracellular:** A glycoprotein from parietal cells in the stomach, required for efficient vitamin $B_{12}$ absorption.
**Introns:** The parts of a gene that do not appear in the mature, functional RNA product.
**Ion channel:** A pore in the membrane that is selectively permeable for specific ions.
**Ionizing radiation:** Energy-rich radiation that forms free radicals in the body.
**Iron-sulfur proteins:** Nonheme iron proteins that participate in electron transfer reactions.
**Irreversible inhibition:** Inhibition by the formation of a covalent bond with the enzyme.
**Irreversible reaction:** A reaction that proceeds in only one direction under physiological conditions.
**Ischemia:** Interruption of the blood supply.
**Isoelectric point:** The pH value at which the number of positive charges on the molecule equals the number of negative charges.
**Isoenzymes:** Enzymes catalyzing the same reaction but composed of different polypeptides.
**Isomerase:** Enzyme that interconverts isomers.
**Isomers:** Alternative molecular forms with identical composition.
**Isoniazid:** A tuberculostatic that can induce vitamin $B_6$ deficiency.
**Isoprenoids:** Lipids synthesized from branched-chain five-carbon units.
**J chain:** A polypeptide in polymeric immunoglobulins.
**J gene:** A small gene that participated in the assembly of an immunoglobulin gene in developing B lymphocytes.
**Janus kinase (JAK):** A type of tyrosine kinase that associates with activated receptors for cytokines, growth hormone, prolactin, and erythropoietin.
**Jaundice:** Yellow discoloration of skin and sclera in patients with hyperbilirubinemia.
**Junk DNA:** Noncoding DNA of unknown function.
**Kartagener syndrome:** A recessively inherited condition with immotile cilia, male infertility, and situs inversus.
**Keratin:** A type of intermediate filament protein in epithelial cells.
**Kernicterus:** Brain damage caused by deposition of bilirubin in the basal ganglia.
**Ketoacidosis:** A metabolic emergency in insulin dependent diabetics, with hyperglycemia, acidosis, dehydration, and electrolyte imbalances.
**Ketogenesis:** Synthesis of ketone bodies.
**Ketogenic:** Ketone body forming.
**Ketone bodies:** Acetoacetate, β-hydroxybutyrate, and acetone.
**Ketose:** Monosaccharide with a keto group.
**Kinase:** An enzyme that transfers a phosphate group from a nucleotide.
**Kinetics:** The description of reaction rates.
**Kinetochore:** A proteinaceous structure on the centromere to which spindle fibers attach.
**Knockout mouse:** A mouse in which a gene has been disrupted by genetic manipulations.
**β-Lactamase:** Penicillinase; an enzyme that inactivates penicillin and related antibiotics.
**Lactate dehydrogenase:** The enzyme that interconverts pyruvate and lactate.
**Lactic acidosis:** Decrease of the blood pH resulting from lactic acid accumulation.
**Lactose intolerance:** Digestive disturbances after the ingestion of milk or milk products, caused by low activity of intestinal lactase.
**Lactose operon:** An operon in *Escherichia coli* that codes for enzymes of lactose metabolism.
**Lagging strand:** The strand that is synthesized in fragments during DNA replication.

**Lamin:** A type of intermediate filament protein in the nucleus.
**Laminin:** A glycoprotein in basement membranes.
**LDL receptor:** A lipoprotein receptor that mediates the endocytosis of low-density lipoprotein (LDL).
**Leading strand:** The strand that is synthesized continuously during DNA replication.
**Leber hereditary optic neuropathy:** Adult-onset blindness caused by mutations in mitochondrial DNA.
**Lecithin:** Synonym for phosphatidylcholine, a phosphoglyceride.
**Lecithin-cholesterol acyltransferase (LCAT):** An enzyme in high-density lipoprotein (HDL) that converts free cholesterol esters.
**Leprechaunism:** A serious disorder caused by congenital absence of functional insulin receptors.
**Leptin:** A polypeptide hormone from overfed adipose cells that reduces appetite.
**Lesch-Nyhan syndrome:** A neurological disorder caused by complete deficiency of hypoxanthine-guanine phosphoribosyl transferase (HGPRT).
**Leucine zipper:** A structural feature of some DNA-binding proteins, required for dimerization.
**Leukotrienes:** Biologically active products formed in asthma and other allergic diseases.
**Lewy bodies:** Intracellular inclusions of insoluble proteins in neurons, found in some neurodegenerative diseases.
**Li-Fraumeni syndrome:** A cancer susceptibility syndrome caused by germline mutations of the p53 gene.
**Ligand:** A small molecule that binds noncovalent to a protein.
**Ligand-gated ion channels:** Ion channels that are regulated by the blinding of a neurotransmitter.
**Ligase:** A type of enzyme that forms a bond while hydrolyzing a high-energy phosphate.
**Lineweaver-Burk plot:** A double-reciprocal plot that describes the relationship between substrate concentration and reaction rate.
**Linoleic acid:** An $\omega_6$ polyunsaturated fatty acid.
**Linolenic acid:** An $w_3$ polyunsaturated fatty acid.
**Lipase:** Triglyceride degrading enzyme.
**Lipofuscin:** "Age pigment," formed from partially oxidized lipids and partially denatured proteins.
**Lipoic acid:** A prosthetic group of pyruvate dehydrogenase.
**Lipolysis:** Triglyceride hydrolysis.
**Lipoprotein:** High-density lipoprotein (HDL).
**β-Lipoprotein:** Low-density lipoprotein (LDL).
**Lipoprotein (a):** A form of low-density lipoprotein (LDL) that promotes atherosclerosis.
**Lipoprotein lipase:** An endothelial enzyme that hydrolyzes triglycerides in chylomicrons and very-low-density lipoprotein (VLDL).
**Lipoxygenases:** Enzymes that produce leukotrienes and other biologically active products from polyunsaturated fatty acids.
**Lock-and-key model:** A model that assumes a rigid substrate-binding site in the enzyme.
**Locus heterogeneity:** A situation where mutations in more than one gene can lead to the same disease.
**Long terminal repeats:** The terminal repeat sequences of retroviral cDNAs.
**Low-density lipoprotein (LDL):** The lipoprotein that delivers cholesterol to the cells.
**Lung surfactant:** A lipid secretion that reduces the surface tension in the lung alveoli.
**Lyase:** An enzyme that removes a group nonhydrolytically from its substrate.
**Lysogenic pathway:** A reproductive strategy of some bacteriophages that involves the integration of the viral DNA into the host-cell chromosome.
**Lysophosphoglyceride:** A phosphoglycerate with one fatty acid missing.
**Lysozyme:** An enzyme that cleaves the peptidoglycan in bacterial cell walls.
**Lysyl oxidase:** An enzyme that produces allysyl residues in collagen; required for cross linking.
**Lytic pathway:** The reproductive strategy of bacteriophages that destroy their host cell.
**$\alpha_2$-Macroglobulin:** A circulating protease inhibitor, with a very high molecular weight (725,000 D).
**Malate-aspartate shuttle:** The reversible shuttling of hydrogen, in the form of malate, across the inner mitochondrial membrane.
**Malondialdehyde:** A chemically reactive product formed during lipid peroxidation.
**Maple syrup urine disease:** An inborn error of branched-chain amino acid metabolism, causing mental and neurological deficits.
**Marfa syndrome:** Dominantly inherited disorder caused by abnormalities of the connective tissue protein fibrillin.
**Mc Ardle disease:** Deficiency of glycogen phosphorylase in muscle, causing muscle weakness.
**Mdm2:** A protein that inactivate the p53 protein.
**Medium-chain acyl-CoA dehydrogenase deficiency:** An inherited defect of β-oxidation that causes fasting hypoglycemia.

**Megaloblastic anemia:** A type of anemia characterized by oversized red blood cells, caused by impaired DNA synthesis.
**Melanin:** The dark pigment of skin and hair.
**Melatonin:** A pineal hormone derived from serotonin.
**Menkes disease:** An inherited defect of copper absorption.
**Messenger RNA (mRNA):** Specifies the amino acid sequence during protein synthesis.
**Metabolic syndrome:** The abnormalities seen in people who eat more than they can metabolize.
**Metallothionein:** A protein that binds heavy metals.
**Metastable:** Stable kinetically but not thermodynamically.
**Metastatic calcification:** Abnormal calcification of soft tissues.
**Metformin:** Nonfunctional hemoglobin in which the heme irons is oxidized to the ferric state.
**Methemoglobin:** Nonfunctional hemoglobin in which the heme iron is oxidized to the ferric state.
**Methemoglobinemia:** Methemoglobin in the blood (suffix-emia means "in the blood"; suffix-uria means "in the urine").
**Methotrexate:** An anticancer drug that inhibits dihydrofolate reductase.
**Methylation reaction:** Transfer of a methyl group from one molecule (often S-adenosyl methionine (SAM) to another.
**Methylmalonic aciduria:** Excretion of methylmalonic acid in the urine, caused by an inherited enzyme deficiency or by vitamin $B_{12}$ deficiency.
**Methylxanthines:** Caffeine and related purines inhibit many phosphodiesterase.
**Micelle:** Small globule or sheet formed from amphipathic lipids.
**Michaelis constant ($k_m$):** The substrate concentration at which the rate of an enzymatic reaction is half maximal.
**Microcytosis:** Presence of abnormally small erythrocytes.
**Micro-RNA (miRNA):** A type of small RNA that inhibits mRNA translation.
**Microfilaments:** Cytoskeletal fibers formed by the polymerization of actin.
**Microsatellites:** Very small tandem repeats.
**Microsomes:** Fragments of the endoplasmic reticulum obtained by cell fractionation.
**Microtubules:** Cytoskeletal fibers formed by the polymerization of tubulin.
**Minisatellites:** Small tandem repeats.
**Mismatch repair:** A repair system that corrects replication errors.
**Missense mutation:** Mutation leading to a single amino acid substitution.
**Mitogen:** Mitosis-inducing agent.
**Mitogen-activated protein (MAP) kinases:** A family of protein kinases that are activated in response to growth factors or stress.
**Monoamine oxidase (MAO):** An enzyme that inactivates catecholamines and serotonin.
**Monoclonal gammopathy:** Overproduction of a single immunoglobulin by a plasma cell clone.
**Monooxygenases:** Enzymes that incorporate a single oxygen atom from $O_2$ in their substrate.
**Monosaccharide:** Polyalcohol containing a carbonyl group.
**Monounsaturated fatty acid:** Fatty acid with one carbon-carbon double bond.
**Mucopolysaccharidose**s**:** Lysosomal storage diseases caused by deficiencies of glycosaminoglycans (GAN) degrading enzymes.
**Muscarinic receptors:** G protein-linked receptors for acetylcholine.
**Mutagen:** Mutation-inducing agent.
**Mutarotation:** Spontaneous interconversion of anomeric forms in a monosaccharide.
**Mutation:** Heritable change in DNA structure.
**Mutational load:** "Genetic garbage" that accumulates from generation to generation.
**myc:** An oncogene or proto-oncogene coding for a nuclear transcription factor, amplified in many spontaneous cancers.
**Myoglobin:** An oxygen-binding protein in muscle tissue.
**Myosin:** The protein of thick filaments in muscle.
**Myosin light-chain kinase:** A calcium-calmodulin activated protein kinase that induces contraction in smooth muscle.
**Myxedema:** Hypothyroidism in adults.
**N-Acetylglutamate:** An activator of mitochondrial carbamoyl phosphate synthetase.
**N-Linked oligosaccharides:** Oligosaccharides bound to asparagine side chains in glycoproteins.
**Natural selection:** The process of differential survival and reproduction that changes allele frequencies from generation to generation.
**Neoplasia:** Abnormal growth of either a benign or a malignant nature.
**Nephrotic syndrome:** A type of kidney disease with massive proteinuria.
**Niacin:** Nicotinic acid; also used as a generic name for nicotinamide.

**Nicotinamide adenine dinucleotide (NAD), nicotinamide adenine dinucleotide phosphate (NADP):** Two cosubstrates that accept or donate electrons ($H^+$ protein) in many dehydrogenase reactions.
**Nicotinic receptors:** Acetylcholine-operated cation channels.
**Nitric oxide (NO):** An unstable, diffusible messenger molecule produced in endothelial cells and some other tissues.
**Nitrogen balance:** The differences between ingested nitrogen and excreted nitrogen.
**Nitroglycerin:** A vasodilator drug that is metabolized to nitric oxide.
**Nitrous acid:** The acid form of the nitrites, cause mutations by deaminating DNA bases.
**Noncompetitive inhibition:** Inhibition by an agent that binds the enzyme noncovalently outside its active site.
**Nonketotic hyperglycinemia:** An inform error of glycine metabolism causing brain damage and early death.
**Nonketotic hyperosmolar coma:** A metabolic emergency in patients with type II diabetes, with hyperglycemia and dehydration but no acidosis.
**Nonsense mutation:** A mutation that creates a stop codon.
**Nonsteroidal anti-inflammatory drugs (NSAIDs):** Drugs that inhibit cyclooxygenase.
**Nonviral retroposons:** DNA sequences produced by the reverse transcription of a cellular RNA.
**Northern blotting:** A method for the identification of RNA after gel electrophoresis, using a probe.
**Nucleases:** Enzymes cleaving phosphodiester bonds in a nucleic acid.
**Nucleocapsid:** The particle formed from viral nucleic acid and protein coat.
**Nucleoside:** A structure formed from a pentose sugar and a base.
**Nucleosome:** The structural unit chromatin, formed from DNA and histones.
**Nucleotide:** A structure formed from pentose sugar, base, and a variable number of phosphate groups.
**Nucleotide excision repair:** A DNA repair system for bulky lesions.
**O-Linked oligosaccharides:** Oligosaccharides bound to hydroxyl group I proteins.
**Okazaki fragments:** Pieces of DNA synthesized in the lagging strand during DNA replication.
**Oleic acid:** A C-18 monounsaturated fatty acid.
**Oligomycin:** An inhibitor of the mitochondrial ATP synthase.
**Oncogene:** A growth-promoting gene in cancer cells.
**Operator:** A repressor-binding regulatory DNA sequence in bacterial operons.
**Operon:** The unit promoter, operator, and structural genes in bacteria.
**Opsonization:** Stimulation of phagocytosis by an antibody or other protein bound to the surface of a particle.
**Organophosphates:** Irreversible inhibitors of acetylcholinesterase, used as pesticides and as nerve gases.
**Oriental flush:** Hypersensitivity to alcohol, caused by deficiency of a mitochondrial aldehyde dehydrogenase in many Asians.
**Orotic acid:** An intermediate of pyrimidine biosynthesis.
**Orphan receptors:** Proteins that resemble known receptors but whose natural ligands are unknown.
**Osteogenesis imperfecta:** Disorder characterized by brittle bones, caused by inherited defects of type I collagen.
**Osteomalacia:** Rickets in adults.
**Osteoporosis:** Brittle, fragile bones in elderly people.
**Oxalic acid:** A component of kidney stones that can be formed from glycine.
**α-Oxidation:** A minor catabolic pathway that shortens fatty acids by one carbon.
**β-Oxidation:** The major pathway of fatty acid oxidation.
**ω:** Oxidation of the last carbon in a medium-chain fatty acid.
**Oxidoreductase:** Enzyme catalyzing oxidation reduction reactions.
**Oxygenation:** Reversible binding of oxygen.
**Palindrome:** A type of symmetrical DNA sequence.
**Palmitic acid:** A saturated C-16 fatty acid.
**Pancreatic lipase:** The major enzyme of fat digestion.
**Pantothenic acid:** A nutritionally essential constituent of coenzyme A.
**Papillomavirus:** A DNA virus associated with cervical cancer.
**Para-aminobenzoic acid (PABA):** A constituent of folic acid.
**Paracrine signaling:** The action of an extracellular messenger on neighboring cells within its tissue or origin.
**Parkinson disease:** Age-related degeneration of dopamine neurons, causing a motor disorder.
**Passive diffusion:** Nonsaturable diffusion across a membrane.
**Pellagra:** Niacin deficiency; symptoms include dermatitis, diarrhea, and dementia.
**Penicillamine:** A metal chelator used to treat Wilson disease and rheumatoid arthritis.
**Pentachlorophenol:** A wood preservative that uncouples oxidative phosphorylation.

**Pentose:** Five carbon sugar.
**Pepsin:** An endopeptidase in gastric juice.
**Peptidases:** Enzymes that cleave peptide bonds in polypeptides.
**Peptide bond:** Amide bond between two amino acids.
**Peptidyl transferase:** The enzymatic activity of the large ribosomal subunit that forms the peptide bond.
**Peripheral membrane proteins:** Proteins that are attached to the surface of the membrane.
**Peroxidases:** Enzymes that consume hydrogen peroxide or organic peroxides.
**Peroxidation:** The nonenzymatic, free radical mediated oxidation of polyunsaturated fatty acids.
**Peroxisome proliferator-activated receptors (PPARs):** Nuclear receptors that regulate lipid and carbohydrate metabolism
**Peroxisomes:** Catalase-rich organelles that contain oxidative enzymes.
**Pertussis toxin:** A toxin produced by *Bordetella pertussis*; cause cyclic AMP accumulation by inactivation of the $G_i$ protein.
**PH value:** The negative logarithm of the hydrogen ion concentration.
λ **Phage:** A temperate bacteriophage of *Escherichia coli.*
**Phagocytosis:** "Cell eating"; the cellular uptake of a solid particle.
**Phenylketonuria (PKU):** Inherited deficiency of phenylalanine hydroxylase, causing mental retardation.
**Phenylpyruvate:** A phenylalanine-derived metabolite in phenylketonuria (PKU).
**Pheochromocytoma:** Endocrine tumor secreting catecholamines.
**Philadelphia chromosome:** A chromosomal translocation in patients with chronic myelogenous leukemia.
**Phosphatase-1:** The enzyme that dephosphorylates glycogen synthase, glycogen phosphorylase, and phosphorylase kinase.
**Phosphatidic acid:** Glycerol + two fatty acids + phosphorylase kinase.
**Phosphatidic acid:** Glycerol + two fatty acids + phosphate.
**Phosphoadenosine phosphosulfate (PAPS):** The "activated sulfate" used for sulfation reactions.
**Phosphodiester bond:** Bond between phosphate and two hydroxyl groups.
**Phosphodiesterase:** Enzymes that hydrolyze cyclic AMP, cyclic GMP, or both.
**Phosphoenolpyruvate (PEP):** An energy-rich intermediate of glycolysis and gluconeogenesis.
**Phosphoenolpyruvate (PEP) carboxykinase:** A gluconeogenic enzyme that is induced by glucagon and glucocorticoids.
**Phosphofructokinase:** The enzyme that catalyzes the committed step of glycolysis.
**Phosphoglycerides:** Lipids structurally related to phosphatidic acid.
**Phosphoinositide 3-kinase:** A lipid kinase that mediates effects of growth factors and insulin.
**Phospholipase C:** A type of enzyme that cleaves phosphoglycerides between glycerol and phosphate.
**Phospholipases:** Phosphoglyceride-hydrolyzing enzymes.
**Phospholipids:** Lipids with phosphate in their hydrophilic head group.
**Phosphopantetheine:** A prosthetic group of fatty acid synthase.
**Phosphoprotein:** A protein containing covalently bound phosphate.
**Phosphoribosyl pyrophosphate (PRPP):** The precursor of the ribose in purine and pyrimidine nucleotides.
**Phosphorylase:** A type of enzyme that cleaves a bond by the addition of phosphate.
**Phosphorylase kinase:** A protein kinase that phosphorylates glycogen phosphorylase.
**Phosphorylation reactions:** Reactions in which a phosphate group becomes covalently attached to an acceptor molecule.
**Phototherapy:** Treatment by exposure to light, used for hyperbilirubinemia in newborns.
**Physiological jaundice:** The common jaundice of newborns.
**Phytosterols:** Steroids from plants.
**Pinocytosis:** Nonselective uptake of fluid droplets into the cell.
**pK value:** The negative logarithm of the dissociation constant for an acid.
**Plasma cells:** Immunoglobulin-producing cells in blood and lymphatic tissues.
**Plasmalogens:** Phosphoglycerides containing α, β unsaturated fatty alcohol.
**Plasmids:** Circular, double-stranded DNAs that function as "accessory chromosomes" in bacteria.
**Platelet activation:** A change in shape and membrane structure of platelets, accompanied by the release of various mediators.
**Platelet-activation factor (PAF):** A soluble, biologically active phosphoglyceride released from white blood cells.
**Platelet-derived growth factor (PDGF):** Released from activated platelets during blood clotting.

**β-pleated sheet:** An extended secondary structure in proteins stabilized by hydrogen bonds between polypeptides.
**Point mutation:** Change in a single base pair.
**Poly-A tail:** A structure at the 3′ end of eukaryotic messenger RNA.
**Polyadenylation signal:** A conserved sequence (AAUAAA) at the 3′ end of eukaryotic genes.
**Polycistronic mRNA:** A messenger RNA (mRNA) that has been transcribed from more than one gene.
**Polyclonal gammopathy:** Nonspecific overproduction of immunoglobulins.
**Polygenic diseases:** Diseases caused by interactions between multiple genes and the environment.
**Polymerase chain reaction (PCR):** A method for amplifying selected DNA sequences.
**Polymorphism:** Sequence variation in DNA.
**Polyol pathway:** A pathway that synthesizes fructose from glucose.
**Polyp:** A benign tumor of mucous membranes.
**Polypeptide:** Polymer of amino acids.
**Polysaccharide:** Polymer of monosaccharides.
**Polyunsaturated fatty acids:** Fatty acids containing more than one C=C double bond.
**Pompe's disease:** A systemic glycogen storage disease causing death in childhood.
**Porphyria Cutanea Tarda:** A Porphyria characterized by cutaneous photosensitivity.
**Porphyrias:** Diseases caused by impairment of heme biosynthesis, with accumulation of biosynthetic intermediates.
**Porphyrin:** A type of pigment contains four pyrrole rings.
**Postprandial thermogenesis:** Metabolic heat production in response to food intake.
**Post-transcriptional processing:** Chemical modification of RNA after synthesis.
**Post-translational processing:** Chemical modification of protein after synthesis.
**Preimplantation genetic diagnosis:** Diagnosis of genetic diseases in the early embryo after *in vitro* fertilization.
**Prenatal diagnosis:** The diagnosis of diseases in the fetus during early pregnancy.
**Preprocollagen:** The earliest precursor of collagen.
**Primary bile acids:** Bile acids that are synthesized by the liver.
**Primary structure:** The covalent structure of a protein.
**Primase:** A special RNA polymerase that synthesizes a primer during DNA replication.
**Probe:** A labeled oligonucleotides or polynucleotide that is used to identify a specific base sequence in DNA.
**Processed pseudogenes:** Nonviral retroposons derived by the reverse transcription of a messenger RNA.
**Processivity:** The ability of a DNA or RNA polymerase to synthesize long strands without interruption.
**Progestins:** A class of steroid hormones that are precursors for the other steroid hormones.
**Prohormone:** The inactive or less active biosynthetic precursor of an active hormone.
**Prokaryote:** A cellular organism without a nucleus.
**Proliferating cell nuclear antigen (PCNA):** A clamp protein that holds the DNA template during eukaryotic DNA replication.
**Promoter:** A regulatory DNA sequence at the upstream end of a gene or an operon.
**Proopiomelanocortin:** A prohormone in the anterior pituitary gland.
**Propeptides:** The N- and C-terminal extensions in procollagen.
**Prophase:** Bacteriophage DNA that has been integrated into the host-cell chromosome.
**Prostate-specific antigen (PSA):** A marker for prostatic cancer.
**Prosthetic group:** A nonpolypeptide component in a protein.
**Proteasome:** A particle that destroys worn-out cellular proteins.
**Protein kinase A:** The cyclic AMP activated protein kinase.
**Protein kinase B:** A protein kinase that mediates effects of growth factors and insulin.
**Protein kinase C:** The diacylglycerol activated protein kinase.
**Protein kinase G:** The cyclic GMP activated protein kinase.
**Proteoglycans:** Products consisting of core protein and covalently bound sulfated glycosaminoglycans (GAGs).
**Proteome:** The totality of proteins made by a particular cell at a particular time.
**Prothrombin time:** A clotting test that detects deficiencies in the extrinsic and final common pathway.
**Protonation/deprotonation:** Reversible binding and release of a proton by an ionizable (acidic or basic) group.
**Protoporphyrin IX:** The organic portion of the heme group.
**Proximal histidine:** A histidine residue in hemoglobin and myoglobin that is bound to the heme iron.
**Pseudogene:** Degenerate, nonfunctional gene that is related to a functional gene.

**Pseudohypoparathyroidism:** Reduced responsiveness to parathyroid hormone.
**PTEN:** A lipid phosphatase that hydrolyzes 3-phosphorylated phosphoinositide; the product of an important tumor suppressor gene.
**Pyranose ring:** Six-member ring in monosaccharides.
**Pyridoxal phosphate:** The coenzyme form of vitamin $B_6$, used as prosthetic group in many enzymes of amino acid metabolism.
**Pyrimidine dimer:** A DNA lesion caused by ultraviolet radiation.
**Pyruvate carboxylase:** The mitochondrial enzyme that turns pyruvate into oxaloacetate.
**Pyruvate dehydrogenase:** The mitochondrial enzyme complex that turns pyruvate into acetyl-CoA.
**Pyruvate kinase:** The glycolytic enzyme that turns phosphoenolpyruvate (PEP) into pyruvate.
**$Q_{10}$ value:** The factor by which the reaction rate increases in response to a 10°C rise in the temperature.
**Quaternary structure:** The submit interactions of an oligomeric protein.
**R factor:** A plasmid that carries genes for antibiotic resistance.
**Radioimmunoassay (RIA):** A highly sensitive analytical method used for the determination of hormone levels.
**RAS:** A (proto) oncogene coding for the Ras protein, a membrane associated, mitogen activated G protein.
**Rate constant:** A measure for the rate of a reaction.
**Reading frame:** The frame in which the codons on the messenger RNA are translated.
**Receptor:** A cellular protein that causes physiological effects after binding an extracellular agent.
**Recessive:** Determining the phenotype in the homozygous but not the heterozygous state.
**Recombinational repair:** Repair of DNA double strand breaks guided by the homologous DNA sequence.
**Recommended daily allowance:** Dietary reference intake, the dietary intake considered optimal under ordinary conditions.
**Redox reaction:** Electron transfer reaction.
**Reduction potential:** A measure for the tendency of a redox couple to donate electrons in a redox reaction.
**Refsum's disease:** Inherited defect in α-oxidation, causing neurological degeneration.
**Remnant particles:** Lipoproteins that are produced by the action of lipoprotein lipase on very low-density lipoprotein (VLDL) or chylomicrons.
**Renin:** The rate-limiting enzyme of angiotensin synthesis.
**Repressor:** A DNA-binding protein that prevents transcription.
**Respiratory chain:** A system of electron carriers in the inner mitochondrial membrane.
**Respiratory distress syndrome:** Dyspnea with cyanosis, resulting from insufficient lung surfactant in newborns.
**Respiratory quotient:** The ratio of respiratory $CO_2$ produced to $O_2$ consumed.
**Response element:** A regulatory DNA sequence that mediates the effects of a hormone, second messenger, nutrient, or metabolite on gene transcription.
**Restriction endonucleases:** Bacterial enzymes that cleave specific palindromic sequences in double stranded DNA.
**Restriction fragment:** DNA fragment produced by a restriction endonuclease.
**Restriction site polymorphism:** DNA sequence variation that changes the cleavage site of a restriction endonuclease.
**Retinoblastoma:** A rare tumor of immature retinal calls in children: can be either sporadic or inherited.
**Retinoblastoma protein (pRB):** The "guardian of the $G_1$ checkpoint," encoded by the retinoblastoma (*RB1*) tumor suppressor gene.
**Retinol binding protein:** A plasma protein that carries retinol from the liver to other tissues.
**Retrovirus:** A type of virus that inserts a double stranded DNA copy of its RNA genome into the host cell genome.
**Rett syndrome:** A neurodevelopmental disorder caused by mutations in a methyl-cytosine binding protein.
**Reverse cholesterol transport:** The transport of cholesterol from extrahepatic tissues to the liver.
**Reverse transcriptases:** Enzymes that transcribe RNA into a double stranded DNA.
**Rhodopsin:** The light-sensing protein in retinal rod cells.
**Riboflavin:** Vitamin $B_2$, a constituent of flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN).
**Ribonucleotide reductase:** The enzyme that reduces ribose to 2-deoxyribose in the nucleoside diphosphates.
**Ribozymes:** Catalytic RNA.
**Rickets:** Bone demineralization caused by vitamin D deficiency in children.
**Rifampicin:** An inhibitor of bacterial RNA polymerase.
**Rigor:** Stiffness of skeletal muscles.
**RNA editing:** Enzymatic modification of a base in messenger RNA.
**RNA polymerases:** RNA synthesizing enzymes.
**RNA replicase:** A viral enzyme that synthesizes RNA on an RNA template.
**RNase H:** An enzyme that degrades the RNA strand in a DNA-RNA hybrid.

**Rotenone:** A fish poison that inhibits electron flow through NADH-Q reductase.
**Rous sarcoma virus:** A retrovirus that causes sarcomas in chickens.
**S phase:** The phase of DNA replication.
**Salvage reactions:** Reactions that convert free bases to nucleotides.
**Sarcomere:** A functional compartment of the muscle fiber.
**Sarcoplasmic reticulum:** The endoplasmic reticulum of muscle cells, a specialized calcium storage organelle.
**Saturated fatty acids:** Fatty acids without C=C double bond.
**Scavenger receptors:** Lipoprotein receptors with broad substrate specificity that mediate the uptake of low-density lipoprotein (LDL) by macrophages.
**Schizophrenia:** A group of severe psychiatric diseases in which new mutations are frequently observed.
**Scurvy:** Disease caused by vitamin C deficiency, with impaired collagen synthesis and connective tissue abnormalities.
**Second messengers:** Small, diffusible molecules that mediate many effects of hormones.
**Secondary active transport:** Transport that dissipates an ATP-dependent ion gradient.
**Secondary bile acids:** Bile acids that are synthesized from the primary bile acids by intestinal bacteria.
**Secondary structure:** The repetitive folding pattern of a polypeptide.
**Secretory pathway:** The organelles through which secreted proteins are processed; endoplasmic reticulum, Golgi, apparatus, and secretory vesicles.
**Selectable marker:** A gene that permits the selective survival of genetically modified cells.
**Semiconservative replication:** The mechanism of DNA replication that leads to a daughter molecule with one old strand and one new strand.
**Serotonin:** 5-hydroxytryptamine (5-HT), the major indolamine.
**Serum:** Blood plasma from which clothing factors have been removed.
**Severe combined immunodeficiency:** Inherited diseases with combined B-cell and T-cell defects.
**Shine-Dalgarno sequence:** A ribosome binding sequence in the 5′ untranslated region of bacterial mRNAs.
**Sickle cell trait:** Heterozygosity for hemoglobin S.
**Sideroblastic anemia:** A microcytic anemia in the presence of high iron stores; seen in vitamin $B_6$ deficiency.
**Signal peptidase:** An enzyme in the endoplasmic reticulum that cleaves off the signal sequence.
**Signal recognition particle:** A cytoplasmic ribonucleoprotein that binds the signal sequence.
**Signal sequence:** An amino acid sequence at the amino end of secreted protein that directs them to the endoplasmic reticulum.
**Sildenafil (Viagra):** A phosphodiesterase inhibitor that prevent the degradation of cyclic GMP in the corpora cavernosa.
**Silencers:** DNA sequences that reduce the rate of transcription by binding regulatory proteins.
**Single nucleotide polymorphism (SNP):** The most common type of polymorphism in the human genome.
**Site directed mutagenesis:** The production of specific mutations in the test tube.
**7 SL RNA:** A component of the signal recognition particle and the grandfather of the Alu sequences.
**Small interfering RNA (si RNA):** Small double stranded RNAs that inhibit translation and/or cause degradation of the messenger RNA.
**Small nuclear ribonucleoproteins (Sn RNPs, pronounced "snurps"):** Components of the spliceosome.
**Sodium co-transport:** A symport system that brings a substrate into the cell together with a sodium-ion.
**Sodium-potassium ATPase:** The sodium-potassium pump in the plasma membrane of all cells.
**Sodium urate:** The poorly soluble uric acid salts that deposit in the joints of patients with gout.
**Somatic mutation:** Mutation in a somatic (nongermline) cell.
**Sorbitol:** A sugar alcohol formed by aldose reductase.
**Southern blotting:** A method for the identification of DNA fragments after gel electrophoresis, using a probe.
**Spectrin:** A membrane-associated cytoskeletal protein in erythrocytes.
**Spectrin repeat:** A three-stranded coiled-coil module in spectrin and dystrophin.
**Spherocytosis:** Inherited defects in the membrane skeleton of erythrocytes, leading to spherical shape of the cells.
**Sphingolipidoses:** Lipid storage disease, a type of disease caused by the deficiency of a sphingolipid-degrading lysosomal enzyme.
**Sphingomyelin:** A phosphosphingolipid.
**Sphingosine:** A long-chain, hydrophobic amino alcohol.
**Splice site mutation:** Mutation causing aberrant introns-exon splicing.
**Spliceosomes:** Nuclear ribonucleoproteins that remove introns from primary transcripts.

**Spongiform encephalopathies:** Rapidly progressive neurodegenerative diseases caused by misfolded prion protein.
**SRC:** A (proto) oncogene coding for a nonreceptor tyrosine protein kinase.
**Standard conditions:** Conditions with 1 mol/L concentrations of all reactants at a pH of 7.
**Statins:** Cholesterol lowering drugs that inhibit 3-hydroxy-3-methyglutary-coenzyme A (HMG-CoA) reductase.
**Steady state:** A state in which the rate of synthesis equals the rate of degradation.
**Stearic acid:** A saturated C-18 fatty acid.
**Steatorrhea:** Fatty stools.
**Steroids:** Lipids containing a cyclopentane-perhydro-phenanthrene ring system.
**Sterol response element binding protein:** A cholesterol regulated transcription factor.
**Storage disease:** A type of inborn error of metabolism in which a non-metabolizable macromolecule accumulates.
**Streptokinase:** A plasmin-activating bacterial protein.
**Streptomycin:** An antibiotic that binds to the small (30S) subunit of bacterial ribosomes.
**Stress fiber:** Actin microfilaments that underlying the plasma membrane.
**Structural gene:** Gene coding for a functional, nonregulatory protein product.
**Substrate:** The starting material for an enzymatic reaction.
**Substrate-level-phosphorylation:** The use of an energy rich metabolic intermediate for the synthesis of ATP OR GTP.
**α subunit:** A subunit of bacterial RNA polymerase required for promoter recognition.
**Sulfonamides:** Bacteriostatic agents that inhibit bacterial folate synthesis.
**Superoxide dismutase:** An enzyme that turns superoxide into oxygen and hydrogen peroxide.
**Superoxide radical:** A free radical formed by the transfer of a single electron to molecular oxygen.
**Super twisting:** Over winding or under winding of double-helical DNA.
**Symport:** Coupled membrane transport of two substrates in the same direction.
**T-cell receptor:** The antigen-binding receptor on the surface of T lymphocytes.
**T lymphocytes:** Lymphocytes possessing no surface antibody but an antigen-recognizing T-cell receptor.
**Tandem repeats:** Head-to-tail repeat sequences in eukaryotic genomes.
**Tangier disease:** An inherited disease with near absence of high-density lipoprotein (HDL).
**Taq polymerase:** A heat-stable DNA polymerase used for polymerase chain reaction (PCR).
**TATA box:** A sequence motif in some eukaryotic promoters.
**Tau protein:** An axonal protein that is misfolded in many neurodegenerative diseases.
**Tay-Sachs disease:** A lipid storage disease caused by deficiency of a ganglioside degrading lysosomal enzyme.
**Telomerase:** RNA containing enzyme that extends the telomeres.
**Telomere:** The end piece of the eukaryotic chromosome.
**Template strand:** The DNA strand complementary to a newly synthesized DNA or RNA.
**Tenase complex:** The complex of clotting factors VIII and IX that activates factor X.
**Tertiary structure:** The overall folding of a polypeptide.
**Tetrahydrobiopterin:** A coenzyme for the hydroxylation of aromatic amino acids.
**Tetrahydrofolate:** The coenzyme form of folic acid; acts as a carrier of one-carbon units.
**Thalassemia:** Under production of hemoglobin α-chains (α-thalassemia) or β-chains (β-thalassemia).
**Thermodynamics:** The description of reaction equilibria and free energy changes.
**Thermogenin:** A mitochondrial uncoupling protein in brown adipose tissue.
**Thiamin:** The dietary precursor of thiamin pyrophosphate.
**Thiamin pyrophosphate:** A prosthetic group that transfers carbonyl compounds.
**Thioester bond:** An energy-rich bond between a sulfhydryl group and a carboxyl group.
**Thrombin:** An activated serine protease in the blood clotting system that acts on fibrinogen and on factors V, VII, VIII, XI, and XIII.
**Thrombomodulin:** An anticoagulant protein in endothelial cells.
**Thromboxane:** A prostaglandin-related product made by platelets.
**Thrombus:** A clot formed in an intact blood vessel.
**Thymidylate synthase:** The folate-dependent enzyme that methylates uracil to thymine in **deoxyuridine monophosphate.**
**Thyroglobulin:** A glycoprotein secreted into the thyroid follicle; precursor of the thyroid hormone synthesis.
**Thyroxine-binding globulin:** The main binding protein for thyroid hormones in the blood.
**Tight junction:** Belt like cell-cell adhesion in epithelial tissues that impairs the diffusion of extracellular solutes and of membrane constituents.

**Tissue-type plasminogen activator:** A fibrin-binding protease that activates plasminogen to plasmin.
**Titration:** Treatment of a weak acid or base with a strong base or acid respectively.
**α-Tocopherol:** The most active form of vitamin E; an important lipid-soluble antioxidant.
**Tophi:** Subcutaneous deposits of sodium urate in gouty patients.
**Topoisomerases:** Enzymes that regulate the supertwisting of double helical DNA.
**TP53:** A tumor suppressor gene encoding the p53 protein; mutated in at least half of all spontaneous cancers.
**Transaminases:** Enzymes catalyzing the reversible, vitamin $B_6$ dependent transfer of amino group from an amino acid to an α-keto acid.
**Transcortin:** A cortisol-binding protein in plasma.
**Transcription:** Synthesis of RNA on DNA template.
**Transcription factors:** DNA-binding proteins that are required for transcription or that increase the rate of transcription.
**Transcriptome:** The totality of RNAs transcribed in particular cell at a particular time.
**Transcytosis:** Vesicular transport across a single layered epithelium.
**Transducin:** A G-protein in retinal rod cells that mediates the effects of light exposure on a cyclic GMP specific phosphodiesterase.
**Transduction:** The cell-to-cell transfer of DNA by a bacteriophage.
**Transfection:** Virus mediated gene transfer for gene therapy.
**Transferase:** Enzyme that transfer a group between substrates.
**Transferrin:** The iron transport protein in the blood.
**Transformation:** Nonselective uptake of foreign DNA by a cell.
**Transgenic mouse:** Mouse with an artificially inserted gene.
**Transition state:** The most unstable intermediate in a chemical reaction.
**Translation:** Ribosomal protein synthesis.
**Translocation:** (1) Movement of the ribosome along the messenger RNA (2) Transfer of DNA from one chromosome to another.
**Transmembrane helix:** A membrane spanning, hydrophobic α-helix in integral membrane proteins.
**Transposase:** An enzyme that catalyzes the movement of an insertion sequence or transposon.
**Transposon:** A mobile element in prokaryotes that contains a gene for transposase and other genes.
**Transthyretin ("Prealbumin"):** A plasma protein that binds thyroxine and retinol-binding protein.
**Tremor:** Trembling.
**Triose:** Three-carbon sugar.
**Tropocollagen:** A long, thin, fibrous protein associated with actin microfilaments.
**Troponin:** A calcium-sensing regulatory protein on the thin filaments of striated muscle.
**Trypsin:** A protease secreted from the pancreas.
**Tryptophan operon:** An operon in *Escherichia coli* that codes for enzymes of tryptophan biosynthesis.
**Tubulin:** A globular protein that polymerizes into microtubules.
**Tumor necrosis factor:** An apoptosis inducing cytokine.
**Tumor suppressor gene:** A growth inhibiting gene in normal cells, whose inactivation contributes to neoplasia.
**Turnover number:** The number of substrate molecules converted to product by one enzyme molecule per second.
**Tyrosine hydroxylase:** The enzyme catalyzing the committed step of catecholamine synthesis.
**Ubiquinone:** Coenzyme Q, a lipid that carries hydrogen in the inner mitochondrial membrane.
**Ubiquitin:** A ubiquitous protein that marks worn-out cellular protein for destruction by the proteasome.
**Ubiquitin ligases:** Enzymes that transfer ubiquitin to cellular proteins.
**Urea:** The major nitrogen containing waste product in urine.
**Urease:** An enzyme in some bacteria and plants that cleaves urea to carbonic acid and ammonia.
**Uremia:** Retention of nitrogenous wastes in patients with kidney failure.
**Uric acid:** The end product of purine degradation.
**Uric–acid nephropathy:** Kidney damage caused by urate deposits.
**Urobilinogens:** Uncolored products formed from bilirubin by intestinal bacteria.
**Urobilins:** Colored products formed from bilirubin by intestinal bacteria.
**Uronic-acid pathway:** The pathway of glucuronic acid metabolism.
**Valinomycin:** A potassium ionophore.
**van der Waals forces:** Nonspecific attractive and repulsive forces between molecules.
**Very low-density lipoprotein (VLDL):** The lipoprotein that carries triglycerides from the liver to other tissues.
**Virion:** The virus particle.
**Vitamin K:** A fat-soluble vitamin that is required for the synthesis of blood clotting factors.

**Voltage-gated ion channels:** Ion channels that open in response to membrane depolarization.
**Von Gierke's disease:** Deficiency of glucose-6-phosphatase, causing hepatomegaly and severe fasting hypoglycemia.
**Von Willebrand factor:** A plasma protein that mediates the binding of platelets to collagen.
**Watson-Crick double helix:** The principal higher-order structure of double-stranded DNA.
**Wernicke-Korsakoff syndrome:** Encephalopathy and amnesia caused by thiamin deficiency in alcoholic persons.
**Western blotting:** A method for the identification of separated proteins, with the use of antibodies.
**Wild-type allele:** The inherited copper transport defect, with abnormal copper accumulation in liver and brain.
**Wobble:** Freedom of base-pairing between the third codon base and the first anticodon base.
**Xanthine oxidase:** A flavoprotein enzyme that produces uric acid.
**Xanthoma:** Visible subcutaneous lipid deposit.
**Xenobiotics:** Foreign substances without nutritive value.
**Xeroderma pigmentosum:** An inherited defect of nucleotide excision repair, characterized by cutaneous photosensitivity.
**Xerophthalmia:** Dry eyes, in vitamin A deficiency.
**Zero-order reaction:** Reaction whose velocity is independent of the substrate concentration.
**Zeta-associated protein 70 (ZAP-70):** A protein kinase in T lymphocytes, activated by antigen binding to the T-cell receptor.
**Zinc:** A trace mineral in the body; constituent of many enzymes.
**Zinc finger protein:** A type of DNA binding protein.
**Zwitterions:** A molecule that contains at least one positive and at least one negative charge.
**Zymogen:** The catalytically inactive precursor of an enzyme.

# Reference Values for Selected Biochemical Laboratory Tests

| Blood analyst | System of international units (SI units) | Conventional units |
|---|---|---|
| 1. Anion gap | 7–16 mmol/L | 7–16 meq/L |
| 2. Arterial blood gas (ABG) analysis | | |
| • pH | 7.35–7.45 | 7.35–7.45 |
| • Bicarbonate | 22–30 mmol/L | 22–30 meq/L |
| • $pCO_2$ | 4.3–6.0 k Pa | 32–45 mm Hg |
| • $pO_2$ | 9.6–13.8 kPa | 72–104 mm Hg |
| 3. Electrolytes and other ions | | |
| • Sodium | 136–146 mmol/L | 136–146 meq/L |
| • Potassium | 3.5–5.0 mmol/L | 3.5–5.0 meq/L |
| • Chloride | 102–109 mmol/L | 102–109 meq/L |
| • Calcium (Total) | 2.2–2.6 mmol/L | 8.7–10.2 mg/dL |
| • Phosphorus (inorganic) | 0.81–1.4 mmol/L | 2.5–4.3 mg/dL |
| • Magnesium | 0.62–0.95 mmol/L | 1.5–2.3 mg/dL |
| 4. Glucose | | |
| • Fasting | | |
| – Normal | 4.2–6.1 mmol/L | 75–100 mg/dL |
| – Impaired glucose tolerance | 6.2–6.9 mmol/L | 111–125 mg/dL |
| – Diabetes mellitus | > 7.0 mmol/L | > 125 mg/dL |
| • 2-hour postprandial | 3.9–6.7 mmol/L | > 70–120 mg/dL |
| 5. Glycated hemoglobin ($HbA_{1c}$) | 0.04–0.06 Hb fraction | 4.0–6.0% |
| 6. Iron homeostasis parameters | | |
| • Ferritin | | |
| – Male | 29–248 μg/L | 29–248 ng/mL |
| – Female | 10–150 μg/L | 10–150 ng/mL |
| • Iron | 7.25 μmol/L | 41–141 μg/dL |
| • Iron binding capacity | 45–73 μmol/L | 251–406 μg/dL |
| • Transferrin saturation | 0.16–0.35 | 16–35% |
| • Transferrin | 2.0–4.0 g/L | 200–400 mg/dL |
| 7. Kidney function tests | | |
| • Creatinine | | |
| – Male | 53–106 μmol/L | 0.6–1.2 mg/dL |
| – Female | 44–80 μmol/L | 0.5–0.9 mg/dL |
| • Urea | 5.4–14.3 mmol/L | 15–40 mg/dL |
| • Blood urea nitrogen (BUN) | 2.5–7.1 mmol/L | 7–20 mg/dL |
| • Creatinine clearance | 1.5–2.2 mL/s | 91–130 mL/min |

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| Blood analyst | System of international units (SI units) | Conventional units |
|---|---|---|
| 8. Lipid profile | | |
| • Total cholesterol | | |
| – Desirable | < 5.17 mmol/L | < 200 mg/dL |
| – Borderline high | 5.17–6.18 mmol/L | 200–239 mg/dL |
| – High | > 6.21 mmol/L | > 240 mg/dL |
| • LDL cholesterol | | |
| – Optimal | < 2.59 mmol/L | <100 mg/dL |
| – Above optimal/near optimal | 2.59–3.34 mmol/L | 100–129 mg/dL |
| – Borderline high | 3.36–4.11 mmol/L | 130–159 mg/dL |
| – High | 4.14–4.89 mmol/L | 160–189 mg/dL |
| – Very high | > 4.91 mmol/L | > 190 mg/dL |
| • HDL cholesterol | | |
| – Low | <1.03 mmol/L | < 40 mg/dL |
| – High | >1.55 mmol/L | 60 mg/dL |
| • Triglycerides (fasting) | 0.34–2.26 mmol/L | 30–200 mg/dL |
| 9. Liver function tests | | |
| • Total protein | 67–86 g/L | 6.7–8.6 g/dL |
| • Albumin | 40–50 g/L | 4.0–5.0 g/dL |
| • Bilirubin | | |
| – Total | 5.1–22 μmol/L | 0.3–1.3 mg/dL |
| – Direct | 1.7–6.8 μmol/L | 0.1–0.4 mg/dL |
| – Indirect | 3.4–15.2 μmol/L | 0.2–0.9 mg/dL |
| • Alanine transaminase (ALT/SGPT) | 0.12–0.70 μkat/L | 7–41 U/L |
| • Aspartate transaminase (AST/SGPT) | 0.2–0.65 μkat/L | 12–38 U/L |
| • Alkaline phosphatase (ALP) | 0.56–1.63 μkat/L | 33–96 U/L |
| • Gamma glutamyl transferase (γGT/GGT) | 0.15–0.99 μkat/L | 9–58 U/L |
| 10. Osmolality | 275–295 mOsmol/kg | 275–295 mOsmol/kg |
| 11. Thyroid profile | | |
| • Thyroid stimulating hormone (TSH) | 0.34–4.25 mIU/L | 0.34–4.25 μIU/mL |
| • Thyroxin | | |
| – Free | 10.3–21.9 pmol/L | 0.8–1.7 ng/dL |
| – Total | 70–151 nmol/L | 5.4–11 ng/mL |
| 12. Troponin T | 0–0.1 μg/L | 0–0.1 ng/mL |
| 13. Uric acid | | |
| • Male | 0.18–0.41 μmol/L | 3.1–7.0 mg/dL |
| • Female | 0.15–0.33 μmol/L | 2.5–5.6 mg/dL |
| *Cerebrospinal fluid (CSF)* | | |
| 1. Glucose | 2.22–3.89 mmol/L | 40–70 mg/dL |
| 2. Protein (lumbar) | 0.15–0.5 g/L | 15–50 mg/dL |
| 3. Red cells | 0 | 0 |
| 4. Leukocytes (WBCs) | 0–5 mononuclear cells/μL | 0–5 mononuclear cells/$mm^3$ |
| *Urine* | | |
| 1. Acidity, titratable | 20–40 mmol/day | 20–40 mEq/day |
| 2. Creatinine | 8.8–14 mmol/day | 1.0–1.6 g/day |
| 3. Albumin | | |
| • Normal | 0.0–0.03 g/day | 0–30 mg/day |
| • Microalbuminuria | 0.03–0.30 g/day | 30–300 mg/day |
| • Clinical albuminuria | > 0.3 g/day | >300 mg/day |
| 4. Total protein | < 0.15 g/day | <150 mg/day |
| 5. pH | 5.0–9.0 | 5.0–9.0 |

A variety of factors such as the population studied, the duration and means of transport of specimen, laboratory methods and instrumentation, etc. can affect the normal values given in this table.

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