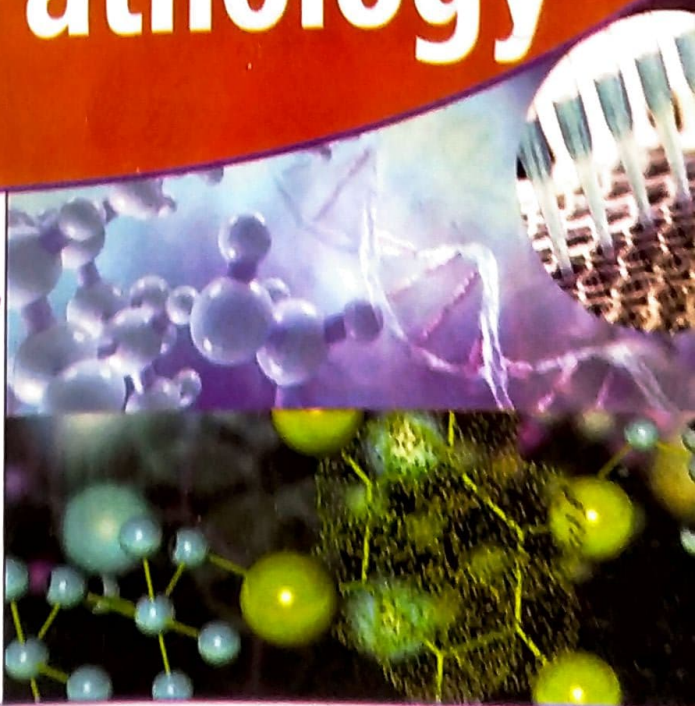
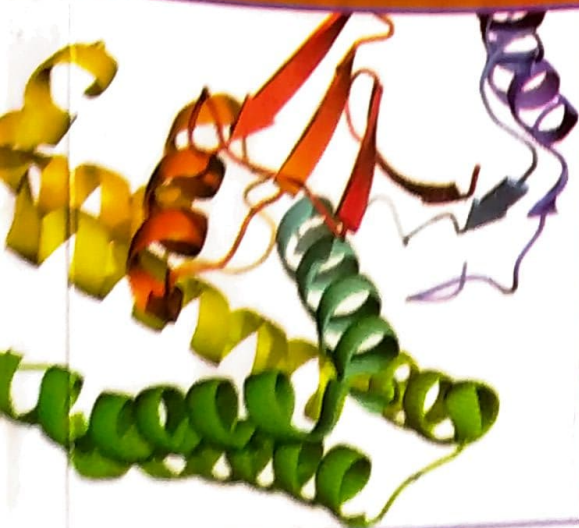




Read to Lead



Biochemistry and Clinical Pathology



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LUCKNOW

Dr. Suchandra Goswami
Dr. Pankaj Verma

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Syllabus

1.4 - BIOCHEMISTRY AND CLINICAL PATHOLOGY

Theory (50 Hours)

Introduction to Biochemistry: Brief chemistry and role of proteins, polypeptides and amino acids, classification, Qualitative tests, Biological value, Deficiency diseases.

Carbohydrates: Brief chemistry and role of carbohydrates, classification, qualitative tests, Diseases related to carbohydrate metabolism.

Lipids: Brief chemistry and role of lipids, classification and qualitative tests, Diseases related to lipids metabolism.

Vitamins: Brief chemistry and role of vitamins and coenzymes, Role of minerals and water in life processes.

Enzymes: Brief concept of enzymatic action, factors affecting it.

Therapeutics: Introduction to pathology of blood and urine, Lymphocytes and platelets, their role in health and disease, Erythrocytes-Abnormal cells and their significance, Abnormal constituents of urine and their significance in diseases.

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CHAPTER 1

Introduction to Biochemistry

1.1. BIOCHEMISTRY

1.1.1. Introduction

Biochemistry involves the study of the chemistry of life. The term **Biochemistry** was introduced by **Carl Neuberg** in **1903**. It is a hybrid branch of chemistry which specialises in the chemical processes and chemical transformations in living organisms. It ties between biology and chemistry that studies complex chemical reactions and chemical structures, giving rise to life. It is believed that life forms of today's world are descended from the same common ancestor, so they naturally have similar biochemistries.

Biochemistry can be simply defined as, "**chemistry of the living cell**". It deals with chemical basis of life in plants and animals. It relies on basic laws of chemistry, biology, and physics to explain various processes of living cells like growth, metabolism, reproduction, response towards stimuli, etc.

In general, biochemistry deals with body substances, e.g., amino acids, proteins, carbohydrates, fats, enzymes, hormones, DNA, RNA, etc. It describes their origin, formation, functions, deficiency symptoms, etc. It tries to explain life in terms of biochemical reactions. In short, it describes all the biochemical reactions at the molecular level in a living cell or living being. Other complex areas of biochemistry include the genetic code (DNA and RNA), protein synthesis, cell membrane transport, and signal transduction.

Pharmaceutical biochemistry is biochemistry related to pharmaceuticals, drug compounds, chemical entities having therapeutic value, etc., which directly influences human health and disease.

1.1.2. Proteins

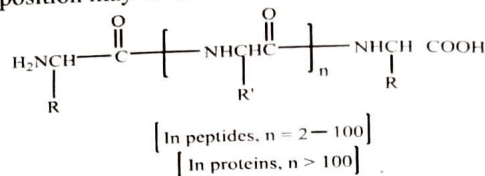
Proteins are high molecular organic compounds that consist of the long polypeptide chain of amino acid monomer. The amino acid monomers are joined together by the **peptide bonds** between the carboxyl and amino groups. Protein mainly consists of twenty different kinds of amino acid molecules that combine in different combinations or number to form different polypeptide chains.

The sequence of genes defines the amino acids sequence in a protein molecule, i.e., a specific gene encodes to a specific protein. An animal body functions with the help of thousands of different proteins.

Chemically a protein molecule consists of nitrogen, carbon, hydrogen, oxygen, sulphur, and phosphorus. However, halogens and metals may also be found in

some proteins. The composition of a protein differs with the source, but the approximate estimation of its composition may be as follows:

- 1) Carbon (50-55%),
- 2) Hydrogen (6-7.5%),
- 3) Nitrogen (15-17%),
- 4) Oxygen (21-24%),
- 5) Sulphur (0.2-2.2%), and
- 6) Phosphorous (0.1-1%).



Protein molecules form colloidal solution and difference in solubility between the two classes is related to a difference in molecular shape.

1.1.2.1. Chemistry

Most of the proteins fold up to form a unique three-dimensional structure and the shape into which a protein naturally folds is its **native conformation**. Most of the proteins fold up independently according to the chemical properties of their amino acids, while others fold up into their native states with the help of molecular chaperones.

Structure of a protein molecule can be explained as:

- 1) **Primary Structure:** The sequence of amino acids in each protein is unique. By studying the primary structure of proteins, this sequence can be determined. In 1953, **Frederick Sanger** determined the complete sequence of amino acids of **insulin protein**.

The primary structure of proteins indicates the sequence of different amino acids. The primary structures formed during the translation (the process of protein biosynthesis) are held together by covalent peptide bonds. Depending on the nature of free group on each terminal of the polypeptide chain, each extremity is referred as the **carboxyl terminus** (C-terminus) and the **amino terminus** (N-terminus). The numbering of residues always starts at the N-terminal end (NH_2 group) from where the amino group is involved in a peptide bond.

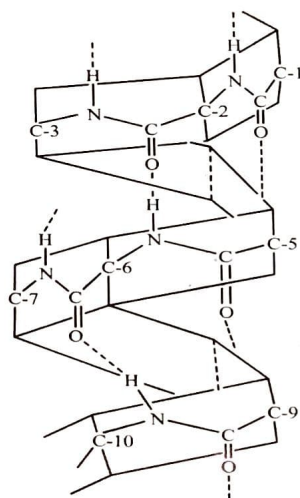


Figure 1.1: α -Helix

The primary structure of protein depends on the gene corresponding to the protein. The first step is the transcription in which mRNA is synthesised by a particular sequence of nucleotides. This mRNA is further read by the ribosome in a process known as **translation**. The sequence of amino acids is specific for a protein, which defines the function and structure of that particular protein.

Various methods like **Edman degradation** or **tandem mass spectrometry** are commonly used to determine the amino acid sequence in a protein. Post-translational modifications (like phosphorylation, glycosylation, and disulphide formation) are also included under the primary structure and cannot be read from the gene.

- 2) **Secondary Structure:** The C-atoms of protein molecule exhibit different geometric arrangements due to the presence of hydrogen bond between the amino groups and the carbonyl oxygen of peptide bonds. This arrangement of the molecule is studied under the secondary structure of protein molecule. Following two types of arrangements are included in secondary structure:

- i) **α -Helical Structure:** In the α -helix (**figure 1.1**) structure, the polypeptide chain folds around the long axis in such a way that the $>\text{C}=\text{O}$ group of each amino acid binds to the $>\text{N}-\text{H}$ group of the fourth residue by a hydrogen bond, throughout the chain. These hydrogen bonds are present parallel to the long axis with the side chains protruding outward in a manner that each turn of α -helix comprises 3.6 amino acid residues. Each amino acid residue is present at a distance of 0.15nm from one another along the axis. An **example** of α -helical protein structure is hair protein, i.e., **keratin**.

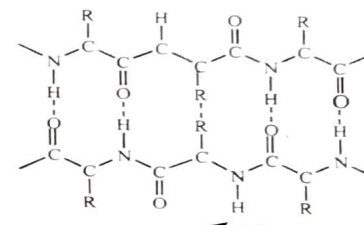


Figure 1.2: β -Pleated Structure

- ii) **β -Pleated Structure:** The β -pleated structure of polypeptide have extended polypeptide chains (**figure 1.2**) and hydrogen bond present between polypeptide strands run antiparallel to each other.

Due to the presence of hydrogen bonds between two polypeptide chains, they appear as sheet-like and are termed as **β -pleated sheet conformations** (**figure 1.3**).

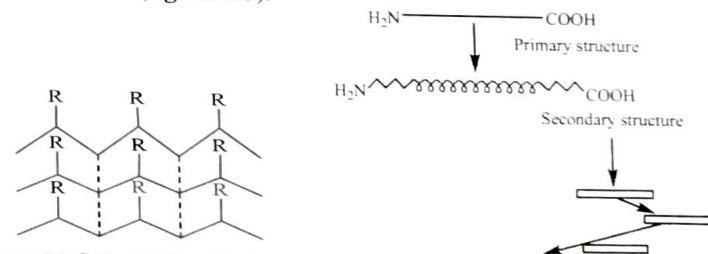


Figure 1.3: β -Pleated Sheet Conformation

Figure 1.4: Tertiary Structure

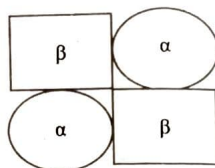
- 3) **Tertiary Structure:** This structure represents the folding of a protein that also controls the basic functioning of the protein. The stabilisation of tertiary

structure is achieved by non-local interactions (most commonly the formation of a hydrophobic core) like salt bridges, hydrogen bonds, disulphide bonds, and post-translational modifications.

Tertiary structure involves three-dimensional folding of the chain; stabilised by the bonding between the distant parts of the sequence. The long polypeptide chains are tightly folded into a compact form due to the following interactions:

- i) Hydrogen bonding,
- ii) Disulphide bonding,
- iii) Electrostatic/ionic bonding, and
- iv) Hydrophobic bonding.

- 4) **Quaternary Structure:** This structure forms when two or more polypeptide chains interact with each other and form a large assembly or complex of proteins.



Often, proteins consist of multiple polypeptide chains and are mostly referred to as protein subunits in this context. These protein subunits may have different (as in a heterodimer) or same (as in a homodimer) polypeptide chains. Thus, the quaternary structure is the interaction and arrangement of these proteins with each other to form a larger aggregate protein complex.

The proteins with two or more polypeptides linked with non-covalent interactions are **quaternary proteins**, e.g., haemoglobin ($\alpha_2\beta_2$).

1.1.2.2. Classification of Proteins

Proteins are classified into the following classes (figure 1.5) based on the composition, structure, shape, and solubility of amino acids:

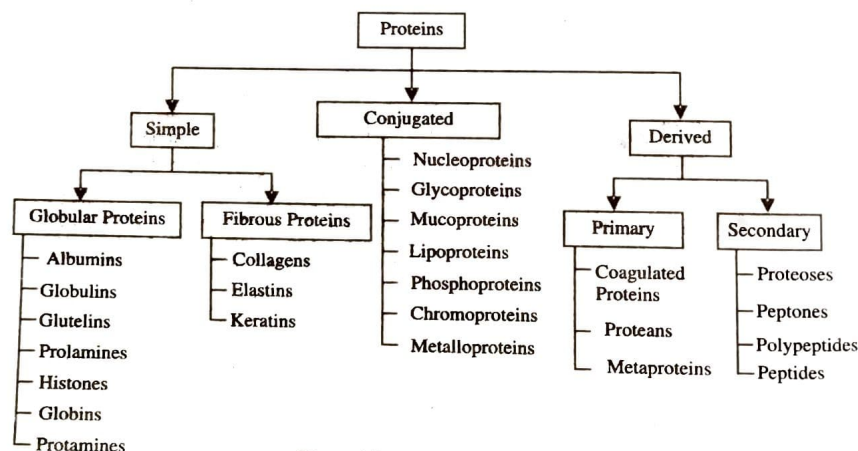


Figure 1.5: Classification of Proteins

- 1) **Simple Proteins:** These proteins only consist of amino acids and are further classified into:

- i) **Globular Proteins:** These proteins are spherical or oval-shaped, soluble in water or in other solvents, and are digestible. **For example,**

Proteins	Properties	Examples
Albumins	Soluble in water forming dilute salt solutions, but coagulates on heating.	Serum albumin, ovalbumin (egg), and lactalbumin (milk)
Globulins	Soluble in neutral and dilute salt solutions.	Serum globulins and vitelline (egg yolk)
Glutelins	Soluble in dilute acids and alkalis, and mostly found in plants.	Glutelin (wheat) and oryzenin (rice)
Prolamines	Soluble in 70% alcohol.	Gladiin (wheat) and zein (maize)
Histones	Strongly basic proteins, soluble in water and dilute acids, but insoluble in dilute ammonium hydroxide.	Thymus histones and histones of codfish sperm
Globins	Resemble histones, but are not basic in nature, and not precipitated by NH_4OH .	Thymus histones and histones of codfish sperm
Protamines	Strongly basic, resemble histones, but smaller in size, and soluble in NH_4OH .	Sperm proteins

- ii) **Fibrous Proteins:** These proteins (fibre-like in shape) are water-insoluble and cannot be digested. **For example,**

Examples	Properties
Collagens	Connective tissue proteins but do not have tryptophan. On boiling with water or dilute acids, yield gelatin which is soluble and digestible.
Elastin	Protein of elastic tissues such as tendons and arteries.
Keratins	Present in exoskeletal structures like hair, nails, and horns.

- 2) **Conjugated Proteins:** These proteins are made up of amino acids and non-protein moiety (prosthetic or conjugating group). **For example,**

Proteins	Properties	Examples
Nucleoproteins	Prosthetic group is a nucleic acid (either DNA or RNA).	Nucleohistones and nucleoprotamines
Glycoproteins	The prosthetic group is carbohydrate. If the concentration of carbohydrate is more than 4% of protein then protein is known as mucoprotein .	Mucin (saliva) and ovomucoid (egg white)
Lipoproteins	Prosthetic group is lipid.	Serum lipoproteins and membrane lipoproteins
Phosphoproteins	Prosthetic group is phosphoric acid.	Casein (milk) and vitelline (egg yolk)
Chromoproteins	Prosthetic group is coloured substances.	Haemoglobins and cytochromes
Metalloproteins	Proteins contain metal ions such as Fe, Co, Zn, Cu, Mg, etc.	Ceruloplasmin (Cu) and carbonic anhydrase (Zn)

- 3) **Derived Proteins:** These proteins are derived from simple and conjugated proteins by their denaturation or degradation. These proteins are further divided into:

- i) **Primary Derived Proteins:** These proteins are denatured or coagulated or first hydrolysed products of proteins. **For example,**

Proteins	Properties	Examples
Coagulated	Denatured proteins produced by treating with agents like heat, acids, alkalis, etc.	Cooked proteins and coagulated albumin (egg white)
Proteans	First stage products of protein hydrolysis by enzymes, dilute acids, alkalis, etc., which are insoluble in water.	Fibrin formed from fibrinogen
Metaproteins	Second stage products of protein hydrolysis obtained by treatment with slightly stronger acids and alkalis.	Acid and alkali metaproteins

- ii) **Secondary Derived Proteins:** These proteins are the degraded (due to the breakdown of peptide bonds) products of proteins or the progressive hydrolytic products of proteins undergoing hydrolysis. **For example,** proteoses, peptones, polypeptides, and peptides.

1.1.2.3. Role of Proteins (copy)

Proteins are one of the most important compounds found in living organisms as they perform the following physiological roles:

- 1) **Proteins as Enzymes:** Proteins are the macromolecules that act as enzymes (biocatalyst) and play an important role in the regulation of all the biological processes. There are about 2,000 different types of enzymes which are studied and each protein catalyses a different and specific chemical reaction, e.g., digestion of food, hydrolysis of ATP, biosynthesis of cell components (DNA, RNA), etc.
- 2) **Proteins as Hormones:** There are some proteins that function as hormones, e.g., somatotropin (growth hormone) and insulin (regulates the metabolism of glucose).
- 3) **Proteins Associated with Hereditary Characters:** Chromatin material formed by nucleoprotein condenses to form chromosomes during cell division that carries the hereditary characters (genetic information).
- 4) **Proteins and Interferons:** Interferons are major therapeutic agents used to treat viral diseases (like hepatitis, encephalitis, cancer, and common cold). These are regulatory glycoproteins with low molecular weight produced in response to viral infection, endotoxins, antigenic stimuli, rickettsia, and protozoan parasites.
- 5) **Proteins and Protection:** Immunoglobulin (or protective proteins) constitutes antibodies, which combine together to either neutralise or antagonise the effect of antigens (foreign proteins). During injury, the two blood proteins, i.e., **thrombin** and **fibrinogen**, help in the prevention of blood loss by the mechanism of blood clotting.

- 6) **Proteins and Biochemical Individuality:** Proteins show their different specific characters in all the living species and organisms. Some **examples** of biochemical individuality are:

- i) Specific proteins (antigens) are present on the surface of cells in all living beings. These surface proteins help in cell recognition and also are the basis of antigen-antibody reactions.
- ii) During blood transfusion, blood cannot be transfused from an individual of one species to another or from one organism to the other.
- iii) Cell and organ from one animal cannot be transplanted successfully into the body of another animal.

- 7) **Transport:** Few proteins bind and transport specific types of molecules through blood. **For example,**

- i) The free fatty acid molecules are bound to the serum albumin and get transported between adipose tissue and other body cells.
- ii) Blood plasma consists of lipoproteins that transport lipids between the liver, adipose tissues, and intestine.
- iii) Oxygen is transported from lungs to other tissue cells (oxygen carrier) via haemoglobin in vertebrates and via haemocyanin in certain invertebrates.
- iv) In muscles, oxygen is stored in the myoglobin.
- v) Permeases, known as transport proteins, help in the transport of molecules via cell membrane.
- vi) Organic food in plants is transported with the help of protein through the phloem.

- 8) **Toxins:** Several proteins are extremely poisonous to vertebrates, e.g., snake venom, ricin (the protein of castor bean), gossypin (the toxic protein of cottonseed), proteins of pathogenic bacteria (diphtheria toxin), etc.

- 9) **Contraction:** Proteins like actin and myosin are responsible for the contractility of all living tissues and skeletal muscles.

- 10) **Movement:** Cells containing locomotory organs, like cilia and flagella are made up of microfilaments which consist of tubulin protein.

- 11) **Receptor Proteins:** These proteins are present on the cell surface and receive stimulus from the environment.

- 12) **Protein as Structural Material**

- i) Hair, nail, scales, feathers, fur, wool, hoof, horns, etc., are made up of keratin sulphate (the derivative of keratin protein).
- ii) Protein is an integral part of all membranes and membrane-bound cell organelles, e.g., plasma membrane, nuclear membrane, ER, mitochondria, lysosome, etc.
- iii) Connective tissue and bone consist of collagen and elastin fibres made up of fibrous proteins.
- iv) Cartilage and bone matrix are formed by ossein, chondrin and proteins, respectively.

- 13) **Cell Secretions:** Cell secretions such as mucus (glycoproteins) help in protection and provide a slippery texture.

- 14) **Special Secretions:** Fibroin protein is secreted by spiders and silkworms in the form of a thick solution which is used in the weaving of web or cocoon because they have an exceptional tensile strength.
- 15) **Storage Proteins:** Proteins are stored in the form of reserve food, viz., egg albumin or in several seeds (zeins in maize, phaseolin in pea, and glutelin in rice).
- 16) **Regulatory Proteins:** The physiological and cellular activities of a cell are regulated by protein. G-protein is one of the regulatory proteins by which the hormonal signals are transmitted to the nucleus in the cell. Similar to hormones and enzymes, proteins also control DNA replication, protein synthesis, chain initiation, chain termination, etc.

1.1.3. Polypeptides and Amino Acids

Amino acids are the building blocks of all proteins. There are 20 amino acids commonly found in protein. These 20 amino acids are linked together through peptide bond, forming peptide chains and proteins. The chains containing less than 50 amino acids are called **peptides**, while those containing greater than 50 amino acids are called **proteins**.

An amino acid chain with more than 25 (10-50) amino acids is called a **polypeptide**, e.g., insulin, hormones, etc. A protein consists of a long polypeptide chain or several polypeptide subunits, e.g., interferon, clotting factors, antibodies, enzymes, etc.

1.1.3.1. Chemistry

The word **Amino Acid** itself is made of two words **amino + acid**. This means the chemical structure of amino acids contains two important functional groups:

- 1) Amine group ($-\text{NH}_2$ group), and
- 2) Acid group or carboxylic acid group ($-\text{COOH}$ group).

Any organic molecule with at least one **carboxyl** group (organic acid) and at least one **amino** group (organic base) is called an **amino acid**. The amino acids are of α -form, i.e., the amine group ($-\text{NH}_2$) is attached to the C atom next to the carboxyl group ($-\text{COOH}$).

Each amino acid has four different groups attached to α -carbon. These four groups are:

- 1) Amino group ($-\text{NH}_2$),
- 2) Carboxyl group ($-\text{COOH}$),
- 3) Hydrogen atom ($-\text{H}$), and
- 4) Side chain (R).

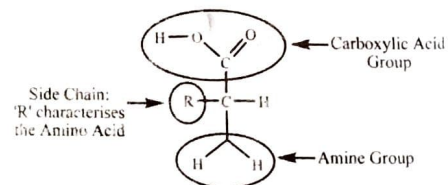
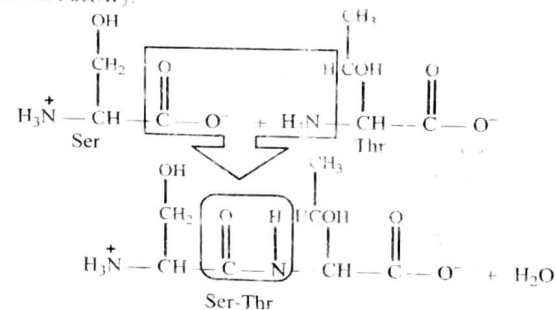


Figure 1.6: Structure of an Amino Acid

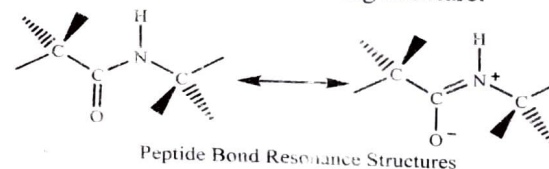
The side chains that distinguish one amino acid from another are attached to the α -carbon, so the structures are often written as shown in figure 1.6, where R stands for one of the 20 side chains. There are about 300 amino acids occurring in nature of which only 20 of them occur in proteins. All 20 amino acids in pure form are white, crystalline, and high-melting solids.

Peptide Bond

The α -amino group of amino acid is attached with α -carboxyl group of another amino acid to form a peptide bond in between two amino acids, with the removal of a water molecule. **For example**, the formation of a peptide bond by serine and threonine (shown below):



A peptide bond is a covalent bond. It is an amide ($-\text{CONH}-$) bond. A peptide bond is a link between two amino acids. It links amino acids together in a protein molecule. It shows double bond character and restricts rotation. Peptide bond undergoes resonance as shown in the following structure:



In resonance structures, the oxygen atom holds a partial negative charge and the $-\text{NH}_2$ group holds a partial positive charge. These partial negative and positive charges form a dipole. These opposite ends (charges) tend to link to form hydrogen bonds. Moreover, these hydrogen bonds are very crucial in stabilising protein structure. Due to resonance interactions, it is a rigid and planar *cis* and *trans*, both configurations are possible but only the *trans* is preferred.

1.1.3.2. Classification of Amino Acids (copy)

Amino acids can be classified as follows:

- 1) **On the Basis of the Carbon Chain:** On the basis of the carbon chain present, amino acids are of three types:
 - i) **Aliphatic Amino Acids:** These are further classified into:
 - a) **Neutral (Monoamino-Monocarboxylic Acids):** For example, glycine, alanine, serine, threonine, valine, leucine, and isoleucine.
 - b) **Acidic (Monoamino-Dicarboxylic Acids):** For example, aspartic acid, asparagine, glutamic acid, and glutamine.
 - c) **Basic:** For example, arginine, lysine, and hydroxylysine.
 - d) **Sulphur Containing Amino Acids:** For example, cysteine, cystine (dicysteine), and methionine.
 - ii) **Aromatic Amino Acids:** For example, phenylalanine, tyrosine, and thyroxine.

iii) **Heterocyclic Amino Acids:** For example, proline, hydroxyproline, tryptophan, and histidine.

2) **On the Basis of Nutritional Requirement:** On the basis of nutritional requirement, amino acids are of two types (table 1.1):

i) **Essential Amino Acids:** These amino acids are also termed as **indispensable amino acids**. They are not synthesised in the body and are obtained from dietary sources. **Examples** of such amino acids are valine, isoleucine, tryptophan, methionine, leucine, phenylalanine, threonine, and lysine.

Exceptionally, histidine and arginine are indicated as **semi-essential amino acids** as a little amount of them are synthesised in the body. Deficiency of these amino acids may give adverse effects like retarded growth, weak immunity, early ageing, etc.

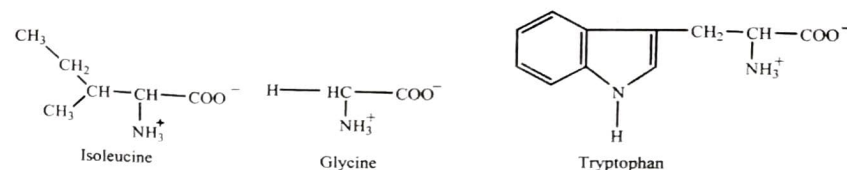
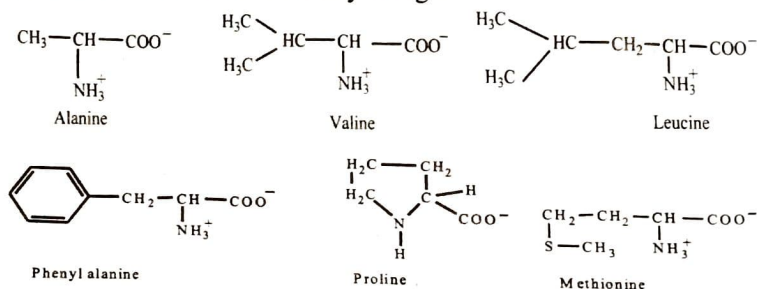
ii) **Non-Essential Amino Acids:** These amino acids are also termed as **dispensable amino acids**, and are synthesised in the body. **Examples** of these amino acids are glycine, serine, threonine, glutamate, glutamine, aspartate, asparagine, cysteine, and proline.

Table 1.1: Essential and Non-Essential Amino Acids

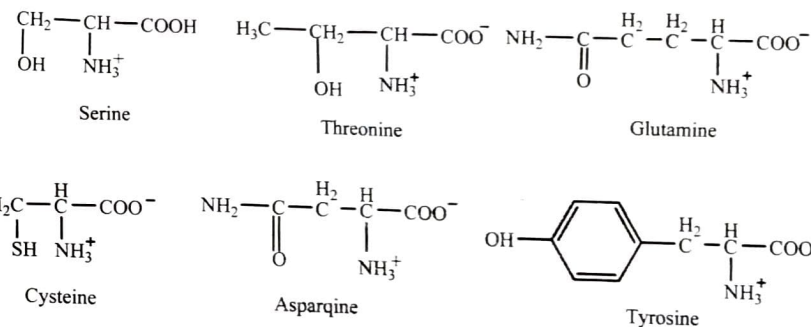
Essential	Non-Essential
Valine	Glycine
Isoleucine	Tyrosine
Tryptophan	Proline
Methionine	Cysteine
Arginine	Aspartic acid
Leucine	Alanine
Phenylalanine	Serine
Threonine	Hydroxyproline
Lysine	Cystine
Histidine	Glutamic acid

3) **On the Basis of Polarity:** On the basis of polarity, amino acids are of four types:

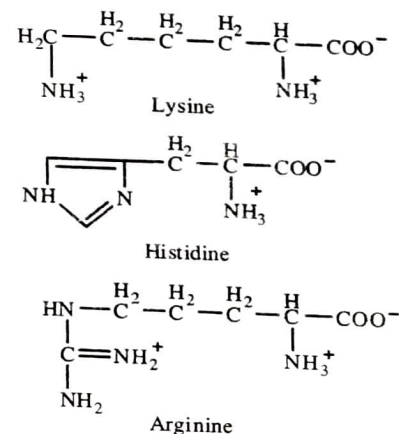
i) **Non-Polar Amino Acids:** These amino acids (e.g., alanine, valine, leucine, isoleucine, phenylalanine, glycine, tryptophan, methionine, and proline) are neutral molecules having an equal number of amino and carboxyl groups. The nature of these amino acids is hydrophobic and the 'R' side chain does not have any charges.



ii) **Polar Amino Acids with No Charge:** These amino acids do not bear any charge on the side chain 'R', (e.g., serine, threonine, tyrosine, cysteine, glutamine, and asparagine). They are involved in hydrogen bonding of protein structure.

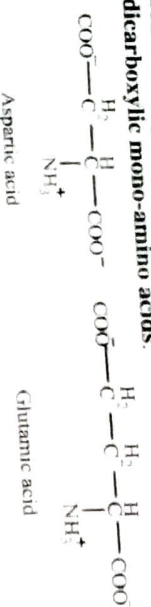


iii) **Polar Amino Acids with Positive Charge:** These amino acids (e.g., lysine, arginine, and histidine) carry more number of amino groups, thus, are basic in nature. The 'R' side chain of these amino acids bears a positive charge.



iv) **Polar Amino Acids with Negative Charge:** These amino acids (e.g., aspartic acid and glutamic acid) are acidic in nature because they carry more number of carboxyl groups. The 'R' group of these amino acids

bears a negative charge. These amino acids are also termed as **dicarboxylic mono-amino acids**.



1.1.3.3. Role of Amino Acids

Amino acids are important biomolecules which perform the following functions.

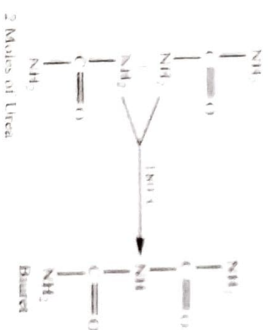
- 1) They are the building blocks of proteins.
- 2) Energy is produced from the metabolism of amino acids. During long-term starving, after the consumption of stored carbohydrate and fats, proteins are used for energy production.
- 3) Few amino acids are also used as neurotransmitters, precursor for the biosynthesis of neurotransmitters, hormones, and as many important biochemical compounds.

1.1.4. Qualitative Tests

For the identification of proteins following tests are performed:

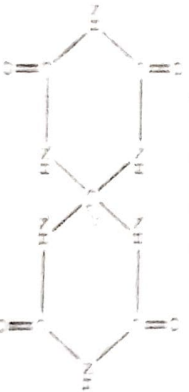
- 1) **Ninhydrin Test:** Like amino acids and peptides, proteins also give blue to violet colour with ninhydrin solution.

- 2) **Biuret Test:** Biuret is a compound formed by heating 2 moles of urea at 180°C (shown aside).



When biuret is treated with dilute copper sulphate in alkaline medium, a purple colour is obtained. This is the basis of biuret test widely used for the identification of proteins and peptides.

Biuret test is carried for the compounds containing two or more CO-NH groups, i.e., peptide bonds. All proteins and peptides possessing at least two peptide linkages, i.e., tripeptides (with 3 amino acids) give positive biuret test. The principle of biuret test is conveniently used to detect the presence of proteins in biological fluids. The mechanism of biuret test is not clearly known. It is believed that the colour develops due to the formation of a copper coordinated complex, as shown below:



The presence of magnesium and ammonium ions interferes in the biuret test. This can be overcome by using excess alkali.

- 3) **Xanthoproteic Test:** This test is given by proteins and peptides which have phenylalanine or tyrosine or tryptophan (aromatic amino acids) in their molecules. On treatment with concentrated nitric acid, these amino acids undergo nitration in the aromatic ring. These nitro compounds (particularly nitrophenols) are yellow in colour. This is the same reaction which occurs when skin comes in contact with concentrated nitric acid in the laboratories.

- 4) **Millon's Test:** When proteins or peptides are treated with Millon's reagent (a solution of mercuric and mercurous nitrate in nitric acid), a white precipitate is formed. When this is heated, a red coloured precipitate is formed. This test is given only by proteins containing tyrosine in their molecules, forming red mercury complexes with the nitrophenol formed. The test is also given by other phenolic compounds and is therefore not specific to proteins.

- 5) **Sodium Nitroprusside Test:** Sodium nitroprusside reagent gives a red colour with peptides and proteins which yield cysteine or cystine on hydrolysis. As many proteins contain cysteine or cystine, the test is given by most proteins.

- 6) **Sakaguchi Reaction:** This test is a specific reaction for the presence of arginine in proteins. Protein hydrolysates will give red colour with α -naphthol-sodium hypochlorite reagent if arginine is present. The reaction is due to the presence of guanidine group.

- 7) **Hopkins-Cole Reaction:** This test is carried out by adding glacial acetic acid followed by concentrated sulphuric acid to a solution of protein. A violet colour is produced if tryptophan is present in the protein. The reaction is due to the glyoxalic acid formed on acid hydrolysis of tryptophan.

1.1.5. Assessment of Protein Quality

The quality of a protein is evaluated by comparing with the **reference protein** (usually an egg protein). Assessment of protein quality can be carried out by the following methods:

- 1) Chemical score or amino acid score.
- 2) Net Protein Utilisation (NPU),
- 3) Protein Efficiency Ratio (PER), and
- 4) Biological Value (BV).

1.1.5.1. Chemical Score or Amino Acid Score

It is the concentration of each essential amino acid in the test protein, which is compared with the reference protein. It is calculated as follows:

$$\text{Amino acid score} = \frac{\text{Number of mg of one amino acid per gm of test protein}}{\text{Number of mg of the same amino acid per gm of egg protein}} \times 100$$

This method of chemical assessment does not consider the digestibility of dietary proteins, so, the overall protein quality is assessed by using biological methods based on growth or Nitrogen (N) retention.

1.1.5.2. Net Protein Utilisation (NPU)

It is the product of digestibility coefficient and biological value divided by 100. Both absorption and retention of protein can be expressed by biological measures of NPU. It is calculated as:

$$\text{NPU} = \frac{\text{Nitrogen retained by the body}}{\text{Nitrogen intake}} \times 100$$

The protein requirement depends on the NPU of dietary protein as lower the NPU, higher the protein requirement and *vice versa*. In India, the NPU of protein varies between 50 and 60.

1.1.5.3. Protein Efficiency Ratio (PER)

The nutritive value or the overall quality of a dietary protein can be determined in laboratory animals (like rats). The weight gained by young animals per gram of protein consumed is measured and used for determining the Protein Efficiency Ratio (PER) as follows:

$$\text{PER} = \frac{\text{Gain in body weight (in gm)}}{\text{Protein ingested (in gm)}}$$

1.1.5.4. Biological Value (BV)

The biological value of a protein is defined as nitrogen percentage retained by animals out of the nitrogen absorbed from the diet eaten. Thus, BV is the percentage of absorbed nitrogen retained by the body and is calculated as:

$$\text{Biological Value (BV)} = \frac{\text{Nitrogen retained}}{\text{Nitrogen absorbed}} \times 100$$

This test also evaluates the digestibility of protein, but not the nitrogen that loses during digestion. **Table 1.2** enlists the chemical score, BV, NPU, PER, and deficient amino acids of some important food proteins:

Table 1.2: Nutritive Values of Proteins of Some Foodstuffs

Protein Source	Protein Type	BV	Chemical Score	NPU	PER	Limiting (Deficient) Amino Acid
Animal Protein	Egg	96	100	96	3.8	Nil
	Milk	90	65	85	2.8	S-containing amino acids.
	Meat	74	70	76	3.2	S-containing amino acids.
	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 acids.
	Red gram	72	45	54	1.7	S-containing amino acids.
Oil Seeds	Groundnut	55	44	45	1.7	Lysine, Threonine
	Soya bean	62	55	55	2.1	S-containing amino acids.

S-containing = Sulphur containing.

1.1.6. Deficiency Diseases

Proteins are nitrogenous compounds, containing sulphur and phosphorus. They participate in the growth and repair of worn out tissues by providing building materials. Fish, meat, eggs, soybeans, pulses, etc., are the major sources of protein, deficiency of which results in the following two well-known disorders:

- 1) **Kwashiorkor:** This disorder is characterised by oedema (fluid retention), therefore is also named **edematous malnutrition**. It is a nutritional disorder

which mostly occurs in the geographical areas experiencing famine. It is a type of malnutrition occurring due to the deficiency of dietary protein. The affected individuals have abnormally thin or weak body parts, but have swollen ankles, feet, and belly due to fluid retention in them.

Cause: Kwashiorkor occurs due to deficiency of dietary protein. Each cell in a human body contains protein. It is an essential part of the diet as it helps in repairing worn out cells and making new cells; thus a healthy human body continually renews cells. Protein is very essential for growth during childhood and pregnancy. Protein deficiency halts the growth and normal functions of the body and gradually leads to kwashiorkor.

Symptoms: Kwashiorkor results in the following symptoms:

- i) The natural colour of skin and hair changes to a rust colour,
- ii) The texture of skin and hair also changes,
- iii) Muscle mass is lost,
- iv) Growth or weight gain is prevented,
- v) Ankles, feet, and belly swell,
- vi) Immune system is damaged, which further causes more severe infections, and
- vii) Other complications like irritability, flaky rash, and shock may also occur.

Diagnosis: The physician immediately examines for the presence of an enlarged liver (hepatomegaly) if kwashiorkor is suspected in an individual. Then blood and urine tests are conducted to measure the blood levels of protein and sugar. Other blood and urine tests may also be performed to measure signs of malnutrition and protein deficiency. These tests detect muscle breakdown and evaluate kidney function, overall health, and growth.

The diagnostic tests of kwashiorkor are:

- i) Arterial blood gas,
- ii) Blood urea nitrogen (bun),
- iii) Blood levels of creatinine,
- iv) Blood levels of potassium,
- v) Urinalysis, and
- vi) Complete Blood Count (CBC).

Treatment: Kwashiorkor can be treated by increasing the protein and calories in diet; this is more effective if early treatment is started. Firstly, carbohydrates, sugars, and fats are given to increase calories. After obtaining energy from these foods, protein diet is started. Foods should be introduced and calories should be slowly increased as the individual was deprived of proper nutrition for a long period, and need to adjust to the increased intake.

- 2) **Marasmus:** This disorder occurs in any individual having severe malnutrition; however, children mostly suffer from marasmus. It occurs in developing countries. It can be life-threatening but can be treated.

Cause: Nutrient deficiency resulting due to poverty and scarcity of food is the main cause of marasmus. It generally occurs in children not receiving proteins, calories, carbohydrates, and other essential nutrients in sufficient amounts.

Symptoms: Children affected with marasmus, experience the following symptoms:

- Underweight,
- Lose muscle mass and subcutaneous fat (the fat layer beneath the skin),
- Dry skin and brittle hair,
- Chronic diarrhoea,
- Respiratory infections,
- Intellectual disability, and
- Stunted growth.

Diagnosis: The initial stages of marasmus are often diagnosed by a physical examination by the doctor. Measurement of the height and weight of children helps in determining whether or not they are affected. For a child suffering from marasmus, these measurements are found to be much below than the measurements of a healthy child of a particular age. Lack of motion in a malnourished child also helps in diagnosing marasmus. The affected children lack energy or will power to do any task. Marasmus cannot be diagnosed by blood tests, because many affected children also suffer from other infections which can affect the results of blood tests.

Treatment: Giving the affected children dried skim milk powder mixed with boiled water is the initial treatment of marasmus. Later, a vegetable oil (e.g., sesame), casein (milk protein), and sugar can also be added in the above mixture. The added oil increases energy content and density of the mixture. Once the children start improving, they are provided with a more balanced diet to fulfil their nutritional demand. Children suffering from dehydration due to diarrhoea should be treated with rehydration by giving fluids orally.

The children having marasmus are commonly affected by other infections, thus antibiotics or other medications should also be included in the treatment. Treatment of infections and other health issues (if present) provide the best chance of recovery.

1.2. EXERCISE

1.2.1. Short Answer Type Questions

- Write a note on role of proteins.
- What is biological value?
- What is polypeptide bond?
- Give the classification of amino acids with structure.
- What are proteins? Classify them.

1.2.2. Long Answer Type Questions

- Write a detail note on chemistry of proteins.
- Write a note on deficiency diseases of proteins.
- Give qualitative tests of proteins.

CHAPTER 2

Carbohydrates

2.1. CARBOHYDRATES

2.1.1. Introduction

Carbohydrates are organic compounds with general formula $C_n(H_2O)_n$. They are composed of carbon, hydrogen, and oxygen having the ratio of oxygen and hydrogen atom 2:1. Carbohydrates are regarded as hydrates of carbon, but structurally they show resemblance with polyhydroxy aldehydes and ketones.

Carbohydrates are the primary source of energy as cells utilise carbohydrate directly for cellular respiration in the presence of oxygen. At the end of reaction, carbon dioxide, water, and energy is obtained (in the form of ATP), as shown below:



Carbohydrates contain the following **functional groups**:

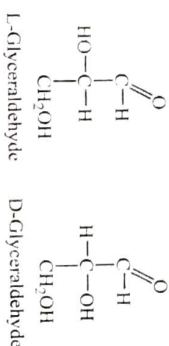
- Alcoholic hydroxyl groups ($-\text{OH}$),
- Aldehyde group ($-\text{CHO}$), and
- Ketone group ($>\text{C}=\text{O}$).

2.1.2. Chemistry

Chemistry of carbohydrate molecules can be studied on the basis of following points:

- Open-Chain Forms:** It includes:

- Asymmetric Carbons:** All monosaccharides show optical activity due to the presence of an asymmetric carbon (carbon bonded to four different atoms or groups of atoms). The nomenclature of monosaccharides involves designating them as L or D by comparing the glyceraldehyde structure.



A D-sugar matches the configuration of D-glyceraldehyde around the asymmetric carbon, farthest from the aldehyde or ketone group; similarly, an L-sugar matches the configuration of L-glyceraldehyde.

- ii) **Isomers:** These compounds have the same chemical formula. Optical isomers are similar with respect to the atoms bonded to each other, but different with respect to the orientation of atoms in space.
- iii) **Epimers:** The conformations of these isomers differ only at one carbon atom.
- iv) **Enantiomers:** These isomers are mirror images of each other. They rotate the same plane of polarised light in opposite directions to the same extent. They have similar physical properties. The enantiomers may be:
- Dextrorotatory**, if they rotate the plane polarised light to the right (i.e., in clockwise direction), and
 - Levorotatory**, if they rotate the plane polarised light to the left (i.e., in anticlockwise direction).
- 2) **Cyclic Haemiacetals and Haemiketals:** When an alcohol and an aldehyde react, hemiacetals are formed which can occur in linear or cyclic forms. The hydroxyl group on the C-5 of glucose and the carbonyl group on C-1 can react intramolecularly to yield a stable cyclic hemiacetal (**figure 2.1**). When similar reactions occur between a ketone and an alcohol, hemiketals are formed. Cyclic hemiketals are formed by ketoses, like fructose (**figure 2.1**).
- The occurrence of cyclic forms of sugar is frequent than that of open-chain sugar structures.
 - The ring structures of sugars can have either a 5-membered ring (called **furanose**) or a 6-membered ring (called **pyranose**) (**figure 2.1**).
 - Anomeric carbons are new asymmetric carbons (e.g., C-1 in glucose) created by cyclisation during formation of hemiacetal and hemiketal:
 - If the —OH group on anomeric carbon lies below the plane of the ring, the sugar is α (**figure 2.1**).
 - If the —OH group on anomeric carbon lies above the plane of the ring, the sugar is β .
 - By the process of **mutarotation**, α and β sugars in solution slowly change into an equilibrated mixture of both.

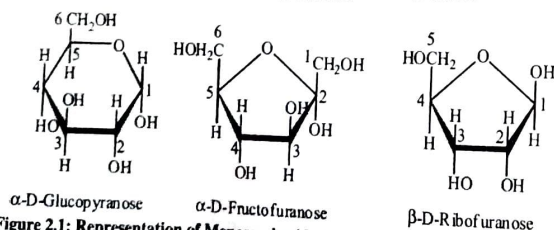
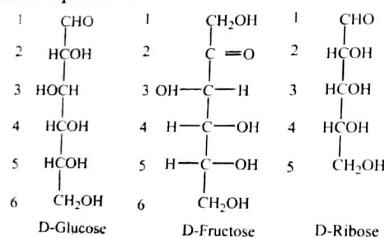


Figure 2.1: Representation of Monosaccharide Molecules. The Numbering System to identify Carbons in the Open-chain and Cyclic Structural Representations.

3) Glycosidic Linkages: It includes:

- Glycosides:** A sugar and an alcohol react to yield a glycoside. Glucoside derivative is formed if the sugar residue is glucose, galactoside derivative is formed if the sugar residue is galactose, and so on.
- Disaccharides:** A sugar and a hydroxyl (—OH) group of another sugar react to yield disaccharides. Most of the dietary sugar is in the form of disaccharides (**table 2.1**). In case disaccharides react with another sugar, the glycoside is a trisaccharide, and so on.

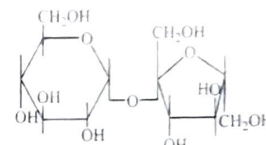
Table 2.1: Physiologically Important Disaccharides

Disaccharides	Components	Sources
Lactose	Galactose + glucose	Milk, dairy products
Maltose	Glucose + glucose	Hydrolysis of starch
Sucrose	Glucose + fructose	Cane and beet sugar

- Nomenclature:** Conventionally, glycosidic linkages are named from left to right, e.g., sucrose has an α -1,2 glycosidic linkage.

A free anomeric carbon atom, not involved in a glycosidic linkage, is present in a reducing sugar. The free aldehyde or ketone group reduces the Fehling's solution (an alkaline copper reagent).

Unlike the linkages present in simple carbohydrates, the oxygen bridge present between glucose and fructose in sucrose is between the anomeric carbons; thus, sucrose is not a reducing sugar.



Sucrose (α -D-glucopyranosyl- β -D-fructofuranoside)

iv) Polysaccharides: It includes (**table 2.2**):

- Starch:** Chemically, it occurs as a mixture of amylose and amylopectin. It is the storage form of glucose in fruits and vegetables.
- Glycogen:** It occurs as a highly branched form of amylopectin. It is the chief storage form of carbohydrates in liver and muscles of animals.
- Cellulose:** It forms cell wall of the plant cells. It does not undergo hydrolysis or digestion in the presence of human enzymes, but is an important source of dietary bulk fibre.

Table 2.2: Physiologically Important Polysaccharides Composed of Glucose

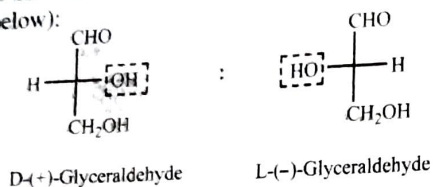
Polysaccharides	Linkages	Importance
Amylose	α -1,4 linear	Component of dietary starch.
Amylopectin	α -1,4 linear + α -1,6 branching*	Component of dietary starch.
Cellulose	β -1,4 linear	Non-digestible component of plants.
Glycogen	α -1,4 linear + α -1,6 branching*	Major storage form of carbohydrate in animals.

*In amylopectin, branching points occur every 25-30 glucose residues. In glycogen, they occur every 8-10 residues.

Isomerism in Carbohydrates

In carbohydrates, commonly found isomerism is optical isomerism, and is usually encountered when a molecule contains one or more chiral carbon atoms. With the existence of a large number of optical isomers in carbohydrates, it is necessary to have a reference compound for the nomenclature of configuration.

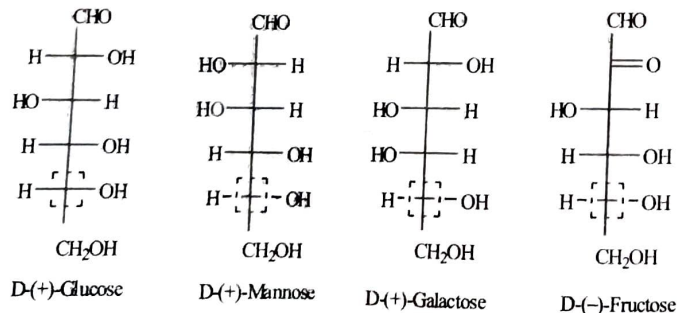
The simplest monosaccharide that possesses a chiral carbon atom is **glyceraldehyde**. It is used to relate the configuration of sugars. Since the compound has one chiral carbon atom, it can exist in two optically active forms (represented below):



It is apparent that these two forms are related to each other by a mirror image relationship, and they have the same physical properties. But they differ in the direction in which they rotate the plane polarised light. The isomer that rotates light in the clockwise direction has been assigned the symbol D-(+) as a prefix to indicate that it is a dextrorotatory compound.

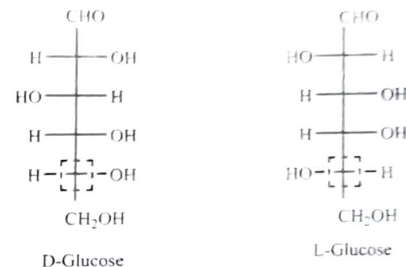
The symbol (+) describes the sign of rotation and the letter D indicates the absolute configuration shown for glyceraldehyde. In this representation, the highest numbered chiral carbon atom (counting from top to bottom) or the penultimate carbon atom has the hydroxy group on the right when the aldehyde group is written at the top of the formula. Such sugars are said to belong to the D-family. The enantiomers having the hydroxy group to the left side belong to the L-family. The D-L system of the nomenclature was proposed by **Rosanoff** in **1906**. This system specifies the configuration of only the highest numbered chiral carbon atom.

The sugars can thus be named on the basis of the absolute configuration of D- and L-glyceraldehyde. It was found that many natural sugars on degradation always gave the dextrorotatory (+) enantiomer of glyceraldehyde. On the other hand, some synthetic sugars degrade to levorotatory (-) enantiomer of glyceraldehyde. The four common hexoses with their projection formulae are given below:



All four sugars are D-sugars because they have the same configuration as D-glyceraldehyde on the penultimate carbon atom. There is necessarily no correspondence between the D- and L-designation and optical rotation. Some of the D-sugars have (+)-rotations while others have (-)-rotations.

The D-L system is used to describe the configuration, while the d, l designation indicates the sign of optical rotation or which way a sugar rotates the plane of polarised light. This must be determined experimentally by a polarimeter. The D and L configurations are determined by the bottom chiral carbon and the enantiomer of the D-sugar is always a L-sugar.



D-fructose is a structural isomer of the other three hexoses. It is a ketose rather than an aldose. The three aldoses are stereoisomers, since no one of the three is an enantiomer of either of the other two (they are diastereoisomers). If two optical isomers are not enantiomers, they are related as diastereoisomers.

There is also no simple relationship between the D-L system and R-S system. The R-S system is used to specify the configuration of each chiral carbon atom in a molecule but the D-L system is used to specify the configuration of a particular enantiomer that might contain many chiral carbons. For sugars, the D-L system is simpler.

2.1.3. Role

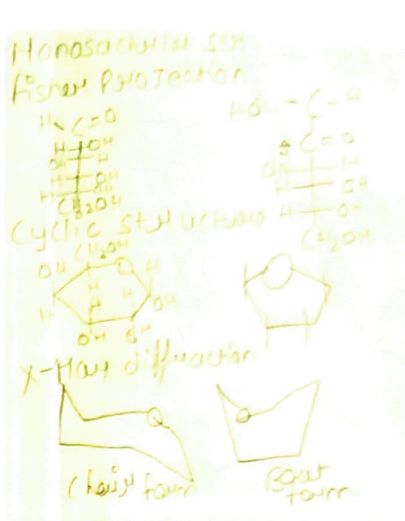
The biological significance of carbohydrates is discussed as follows:

- 1) They are an **immediate source of energy** for the living cell.
- 2) They have a **protein sparing action**.
- 3) They are involved in the breakdown of fatty acids and **preventing ketosis**.
- 4) The glycocalyx present on the cell surface is composed of carbohydrates and proteins. The glycocalyx is involved in **biological recognition** processes of immunoglobulin and MHCs.
- 5) They are major dietary fibre (cellulose) and are used as the **flavouring and sweetening agents**.
- 6) Derivatives of cellulose (such as cellulose nitrates) are used in **explosives, lacquers, celluloid, and collodion**. Cellulose acetate is used in **photographic film and packing materials**.
- 7) Carbohydrate molecules function as a key component in various central metabolic pathways and thus regulate **metabolic processes** in the body.

Carbohydrate are precursors for many organic compounds (for amino acids)

- 34 in the structure of cell membrane. D. Pharm. Part-I (Biochemistry and Clinical Pathology) B11 (U)
- functions such as cell growth, adhesion & fertilization
- 8) They also serve as **stored forms of energy** as glycogen in liver and muscles.
- 9) The bacterial cell wall is made up of polysaccharides: murein, N-Acetyl Muramic Acid (NAMA), N-Acetyl Glucosamine (NAG), along with several other amino acids linked by peptide linkage. Thus, carbohydrates provide **protection to bacterial cells**.
- 10) They are important **components of brain cells** as neuraminic acids, cerebroside (glycolipids) and gangliosides.
- 11) They form the chief **components of sulpholipids** which are present in chloroplasts and in chromatophores of photosynthetic bacteria.
- 12) **Pentose sugars** (ribose and deoxyribose) form an important component of nucleic acids.
- 13) **Heparin** (an example of the glycosaminoglycan family of carbohydrates) functions as a physiological anticoagulant.
- 14) **Hyaluronic acid** (a carbohydrate derivative) is an important component of the vitreous body (a clear gel present in the space between the lens and the retina of the eyeball) of the eye, the umbilical cord, and as a lubricant in synovial fluid of the joints.
- 15) **Trehalose** (a disaccharide) is the chief component of the haemolymph of insects, where it serves as the chief source of energy. It is also present in yeasts and other fungi.
- 16) **Pentosans** (a carbohydrate composed of pentoses) are present in plant gums and mucilage.
- 17) Many **glucosides** (carbohydrate derivative from plants) are important drugs, such as glucoside of digitalis is used in the treatment of heart diseases.
- 18) **Lactose** (a disaccharide sugar formed of galactose and glucose) is found in milk.
- 19) **Pectin** (a structural heteropolysaccharide) forms the primary cell walls of terrestrial plants. It is also used in the manufacturing of jellies and to preserve food material.
- 20) **Sialic acid** (an acetyl derivative of neuraminic acid, sugar acid) is present as a chief component in tissues especially in the mucins and blood group substances. Sialic acid with glycolyl group substituted in neuraminic acid in the place of acetyl group is found in salivary mucin and erythrocytes.
- 21) **Proteoglycans** (highly glycosylated protein molecules) are important for absorption by fibroblasts, as receptors and carriers of macromolecules, cell growth regulators, and also influence the protein synthesis and intranuclear functions.
- 22) **Inulin** (homo-polysaccharide) is used for clinical purposes in clearance tests.
- 23) **Chondroitin sulphates A and C** form the major structural components of cartilage, tendons, and bones in association with collagen. Chondroitin sulphates are sulphated glycosaminoglycan composed of a chain of alternating sugars.
- 24) **Agar-agar** (a polysaccharide of agarose) is used in the preparation of foodstuffs for a diet of invalids and is utilised in laboratories as culture media.

① Ribose is useful for nucleic acid synthesis.



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asis of complexity, carbohydrates are

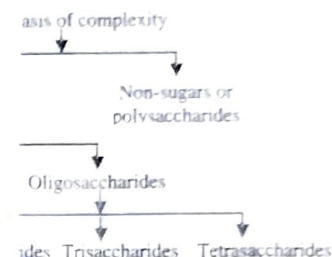
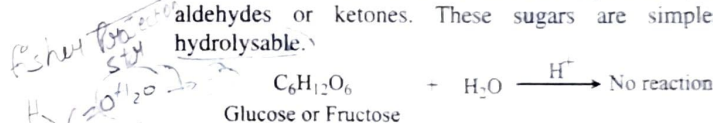


Figure 2.2: Classification of Carbohydrates on the Basis of Complexity

The above mentioned classes of carbohydrates are discussed below:

- i) **Sugars:** These are sweet, crystalline, and water-soluble carbohydrates of known molecular weights. Sugars are further **sub-divided** into:
- a) **Monosaccharides:** These are unit carbohydrates with polyhydroxy aldehydes or ketones. These sugars are simplest and non-hydrolysable.



Depending on the type of carbonyl group and the number of carbon atoms in a molecule, monosaccharides are **classified** into:

- Type of carbonyl group
 - Aldoses** (with aldehyde group)
 - Ketoses** (with ketone group)
- Number of carbon atoms, e.g., trioses, tetroses, pentoses, hexoses, heptoses, etc.
- b) **Oligosaccharides:** These are simple sugars with 2-10 units of monosaccharide. Oligosaccharides are further **classified** into:
 - **Disaccharides:** These are sugars which hydrolyse into 2 molecules of the same or different monosaccharides. **For example, sucrose hydrolysed into a molecule of each glucose (aldohexose) and fructose (ketohexose).**
 - **Trisaccharides:** These sugars are hydrolysed into 3 molecules of monosaccharides. **For example, raffinose ($C_{18}H_{32}O_{16}$) is hydrolysed into galactose, glucose, and fructose.**
 - **Tetrasaccharides:** These sugars are hydrolysed into 4 molecules of monosaccharides. **For example, stachyose ($C_{24}H_{42}O_{21}$) is hydrolysed into 2 α -D-galactose units, 1 α -D-glucose unit, and 1 β -D-fructose unit.**

2.1.4. Classification

Carbohydrates can be classified on the basis of the below mentioned three features:

- 1) **On the Basis of Complexity:** On the basis of complexity, carbohydrates are classified as shown in figure 2.2

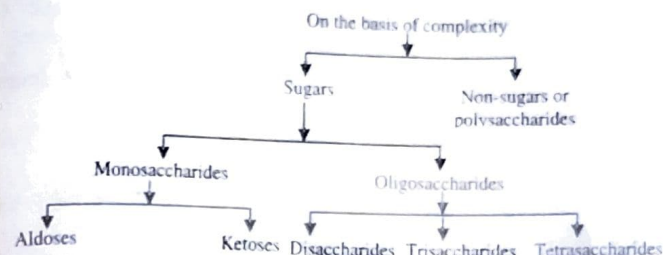
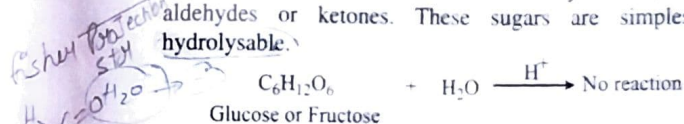


Figure 2.2: Classification of Carbohydrates on the Basis of Complexity

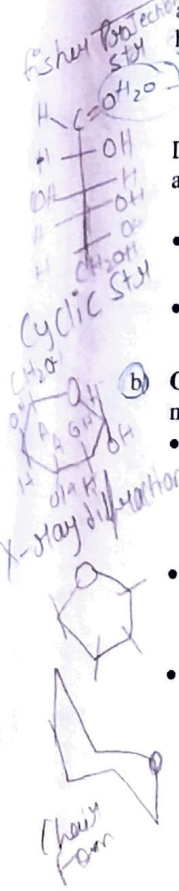
The above mentioned classes of carbohydrates are discussed below:

- Sugars:** These are sweet, crystalline, and water-soluble carbohydrates of known molecular weights. Sugars are further sub-divided into:
 - Monosaccharides:** These are unit carbohydrates with polyhydroxy aldehydes or ketones. These sugars are simplest and non-hydrolysable.



Depending on the type of carbonyl group and the number of carbon atoms in a molecule, monosaccharides are **classified** into:

- Type of carbonyl group $\begin{cases} \text{Aldoses (with aldehyde group)} \\ \text{Ketoses (with ketone group)} \end{cases}$
- Number of carbon atoms, e.g., trioses, tetroses, pentoses, hexoses, heptoses, etc.
- **Oligosaccharides:** These are simple sugars with 2-10 units of monosaccharide. Oligosaccharides are further **classified** into:
 - **Disaccharides:** These are sugars which hydrolyse into 2 molecules of the same or different monosaccharides. **For example**, sucrose hydrolysed into a molecule of each glucose (aldohexose) and fructose (ketohexose).
 - **Trisaccharides:** These sugars are hydrolysed into 3 molecules of monosaccharides. **For example**, raffinose ($C_{18}H_{32}O_{16}$) is hydrolysed into galactose, glucose, and fructose.
 - **Tetrasaccharides:** These sugars are hydrolysed into 4 molecules of monosaccharides. **For example**, stachyose ($C_{24}H_{42}O_{21}$) is hydrolysed into 2 α -D-galactose units, 1 α -D-glucose unit, and 1 β -D-fructose unit.



Useful for nucleic acid

ii) **Non-Sugars or Polysaccharides:** These are tasteless, amorphous, and water-insoluble solids which may form a colloidal suspension in water. Their properties are similar to those of high polymers, e.g., their molecular weight varies significantly. Despite being tasteless, they form sweet monosaccharide molecules when hydrolysed and when treated with dilute acids. **Cellulose** is a polysaccharide made up of glucose units β -1,4 linkages and is utilised by the plants to build their structural skeleton.

2) **On the Basis of Reactivity:** On the basis of reactivity, carbohydrates are classified into:

- Reducing Sugars:** These saccharides reduce the Fehling's and Tollen's reagents.
- Non-Reducing Sugars:** These saccharides do not reduce the Fehling's and Tollen's reagents.

Table 2.3: Differences between Reducing and Non-Reducing Sugars

Reducing Sugars	Non-Reducing Sugars
1) Give positive results to: - Tollens' test Ag^+ (antimonic complex) $\rightarrow \text{Ag} \downarrow$ Clear solution Silver mirror - Benedict's test Cu^{++} (citrate complex) $\rightarrow \text{Cu}_2\text{O} \downarrow$ Clear blue solution Yellow-red (precipitate) - Fehling's test Cu^{++} (tartrate complex) $\rightarrow \text{Cu}_2\text{O} \downarrow$ Clear blue solution Yellow-red (precipitate)	1) Give negative results to: - Tollens' test - Benedict's test - Fehling's test
2) Show mutarotation.	2) Do not show mutarotation.
3) Functional units present are: $\begin{array}{c} \text{H} \\ \\ \text{C} - \text{C} = \text{O} \\ \\ \text{OH} \end{array}$ α-Hydroxyaldehyde $\begin{array}{c} \text{O} \\ \\ \text{C} - \text{C} - \text{C} \\ \quad \quad \\ \text{OH} \quad \text{O} \end{array}$ α-Hydroxyketone $\begin{array}{c} \text{H} \\ \\ \text{O} - \text{C} - \text{OH} \end{array}$ Hemiacetal $\begin{array}{c} \text{H} \quad \text{R} \\ \quad \\ \text{O} - \text{C} - \text{OH} \end{array}$ Hemiketal	3) Functional units present are: $\begin{array}{c} \text{H} \\ \\ \text{O} - \text{C} - \text{OR} \end{array}$ Acetal $\begin{array}{c} \text{R} \\ \\ \text{O} - \text{C} - \text{OR} \end{array}$ Ketal
4) For example, D-glucose, D-fructose, Maltose, and Lactose.	4) For example, Sucrose.

3) **On the Basis of Functional Groups:** On the basis of functional groups present, carbohydrates are classified into:

- Aldoses:** These are aldehydes with a reactive aldehydic (CHO) group, e.g., glucose.
- Ketoses:** These are ketones with a reactive ketonic (C=O) group, e.g., fructose.

2.1.5. Qualitative Tests

Common tests which are carried out for identification of carbohydrates are given below:

- Fehling's Solution Test:** In this test, 1-2ml each of the Fehling's solution A and B are added to few drops of the test solution and boiled for a few minutes. A yellowish-red colour appears that confirms the presence of a reducing sugar.

$1-2\text{ml. each of A+B} + \text{F.S.} + \Delta \rightarrow \text{Yellow Red (sol.)}$

 - Fehling's Solution A:** This reagent is prepared by dissolving 34.65gm copper sulphate in distilled water and making the volume up to 500ml.
 - Fehling's Solution B:** This reagent is prepared by dissolving 125gm potassium hydroxide and 173gm Rochelle salt (potassium sodium tartrate) in distilled water and making the volume up to 500ml.
- Molisch Test:** All members of carbohydrates give a positive result for this test. In this test, 1-2 drops of Molisch's reagent (5% of 1-naphthol in alcohol) is mixed with 1-2ml of test solution. About 1ml of concentrated sulphuric acid is added along the side of the tube. A colour develops at the junction of the two liquids due to the reaction between α -naphthol and furfural and/or due to the derivative formed by dehydration of sugars with concentrated sulphuric acid.

$2-3\text{HR} + 1-2\text{T.S} + 1\text{H}_2\text{SO}_4 \rightarrow \text{Colour}$
- Osazone Formation:** A sugar on heating with phenylhydrazine hydrochloride, sodium acetate, and acetic acid forms yellow crystals of osazone.
- Resorcinol Test for Ketoses (Selivanoff's Test):** A crystal of resorcinol is added to the solution and heated with an equal volume of concentrated hydrochloric acid. The pink colour is produced in case of ketoses (e.g., fructose, honey, or hydrolysed inulin).
- Test for Pentoses:** A solution of the material is heated with an equal volume of hydrochloric acid containing a little phloroglucinol. A red colour is formed in case of pentoses.
- Furfural Test:** A carbohydrate sample is heated in a test tube with a drop of syrupy phosphoric acid to convert it into furfural. A disk of filter paper moistened with a drop of 10% solution of aniline in 10% acetic acid is placed over the mouth of the test tube. The bottom of the test tube is heated for 30-60 seconds. A pink or red stain appears on the reagent paper.
- Benedict's Test:** In this test, 1-2ml of Benedict's reagent is added to 5 drops of the test solution, boiled for 5 minutes in a water bath, and then cooled. A yellow or brownish-red precipitate appears that confirms the presence of a reducing sugar. Benedict's reagent is prepared by dissolving 173gm sodium citrate and 100gm sodium carbonate in about 500ml water. The solution is heated to dissolve the salts and filtered (if required). 17.3gm copper sulphate is dissolved separately in 100ml water and added to the above solution with stirring and volume is made up to 1000ml with water.

$1-2\text{BR} + \text{SF} + \text{sub} \rightarrow \text{Yellow}$
- Tollen's Test:** In this test, 2-3ml of an aqueous solution of carbohydrate is taken in a test tube and treated with 2-3ml of Tollen's reagent, followed by boiling in a water bath for 10 minutes. A shining silver mirror indicates the

2ml

presence of reducing carbohydrates. Tollen's reagent is prepared by adding NaOH solution to AgNO₃ solution. Then, adding NH₄OH solution drop wise until the precipitate is dissolved to give a solution of Tollen's reagent.

- 9) **Iodine Test:** Only starch gives this test, in which few drops of iodine solution are added to 1ml of test solution. A deep blue colour appears due to the presence of starch in the solution which forms a complex with iodine.

2.1.6. Diseases Related to Carbohydrate Metabolism

In the metabolism of carbohydrates (such as galactose, fructose, and glucose) different enzymatic pathways are involved. Disorders affecting these pathways produce mild, severe, or even life-threatening symptoms. The resulting clinical features are hypoglycaemia, liver enlargement, and muscle pain. With the help of dietary interventions, most of these disorders can be controlled and even treated.

2.1.6.1. Diabetes Mellitus (DM)

According to the WHO, diabetes mellitus is defined as "a metabolic disorder of multiple etiology characterised by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism, resulting from defects in insulin secretion, insulin action, or both".

On the basis of clinical stages and the etiological types, DM can be classified as follows:

- 1) **According to the Clinical Stages:** Based on the clinical stages, DM can be classified as:

- Insulin Requiring for Survival:** This corresponds to the former clinical class of **insulin-dependent DM**.
- Insulin Requiring for Control:** This class is characterised by the requirement of insulin for metabolic control rather than for survival.
- Non-Insulin Requiring:** This includes those individuals in whom diabetes can be controlled satisfactorily using non-pharmacological methods or drugs, without the use of insulin.

The latter two types correspond to the former class of **non-insulin dependent DM**.

- 2) **According to Etiology:** Based on the etiology, DM can be classified as:

- Type 1 DM:** This type is characterised by destruction of β -cells (insulin-producing cells) and thus insulin is required for survival. This type was previously referred to as **insulin-dependent diabetes** (though the term is obsolete now) and can be further classified as:
 - Type 1 Immune-Mediated DM:** In this type, autoimmune destruction of β -cells occurs. Other terms for this type of DM are **insulin dependent diabetes** or **juvenile-onset diabetes** (since it may begin during childhood).
 - Type 1 Idiopathic DM:** This form is not related to autoimmunity and the β -cell destruction is due to unknown causes.

- Type 2 DM:** This type is characterised by disorders of insulin action and insulin secretion. Patients have relative insulin deficiency (in contrast to absolute insulin deficiency in type 1 diabetes).

It is also known as **adult onset diabetes** and was earlier referred to as **non-insulin dependent diabetes** (though the term is obsolete now).

- Other Types:** This includes those in which the underlying defect or disease process can be identified in a relatively specific manner, e.g., fibrocalculous pancreatopathy.

Causes

Etiological factors for DM include:

- Genetic Factors:** DM may be caused by abnormalities in genes present on different chromosomes and include:
 - Genetic defects of β -cell function.
 - Mutation in mitochondrial DNA, and
 - Genetic defects in insulin action.
- Environmental Factors:** These include:
 - Obesity associated with modern living standards,
 - Steady urban migration, and
 - Lifestyle changes (including consumption of alcohol).
- Factors within the Individual:** These include:
 - Production of auto-antibodies that destruct β -cells,
 - Deficiency in insulin synthesis and secretion,
 - Insulin resistance (because the cells do not respond to the insulin produced),
 - Presence of diseases that may extensively damage the pancreas causing pancreatitis, trauma, infection, pancreatic carcinoma, and pancreatectomy, thus, resulting in DM,
 - Excessive secretion of hormones (e.g., growth hormone, cortisol, glucagon, epinephrine) that antagonise insulin action,
 - Impairment of insulin secretion resulting from the excessive consumption of drugs, and
 - Infections caused by viruses that cause β -cell destruction.

Symptoms

The signs and symptoms of diabetes vary widely depending upon the increase in blood sugar level. The characteristic clinical manifestations of DM include:

- Onset of Disease:** Type 1 DM usually begins during childhood and progresses rapidly. This is termed **juvenile diabetes**. If it begins in adults, it progresses relatively slowly and is termed as **Late Autoimmune Diabetes in Adults** (LADA).
- Appearance of Symptoms:** In patients with type 1 DM, symptoms appear in the initial stages and are more severe than type 2 DM. Patients with type 2 DM may experience the symptoms at a later stage and may not show any symptoms during the initial stages.

- 3) **Signs and Symptoms:** These include:
- Ketoacidosis** and presence of ketones in urine (ketones are a by-product of the breakdown of muscle and fat occurring when there is not enough available insulin).
 - Presence of glucose in urine.
 - Polydipsia** (increased thirst).
 - Polyuria** (increase in the frequency of urination).
 - Polyphagia** (extreme hunger).
 - Unexplained loss of weight.
 - Fatigue and headache.
 - Irritability.
 - Blurred vision, and
 - Frequent infections, with the retarded healing of cuts and wounds.

Diagnosis

Diagnosis of DM can be made, based on the following tests:

- Clinical Examination:** The classical symptoms of diabetes, including polydipsia, polyphagia, and polyuria help to diagnose diabetes.
- Laboratory Tests:** These tests include:
 - Urine Tests:** Urine is tested to detect the presence of glucose (glycosuria) and ketones (ketonuria).
 - Single Blood Sugar Estimation:** Fasting plasma glucose value (126 mg/dl) indicates the presence of diabetes.
 - Oral Glucose Tolerance Test (Oral GTT):** The values considered abnormal, detecting diabetes has been described in table 2.4:

Table 2.4: Criteria for Diagnosis of Diabetes by Oral GTT as per WHO

Patient Status	Plasma Glucose Value [#]	Diagnosis
1) Fasting value	Below 110mg/dl (< 6.1mmol/L)	Normal fasting value
2) Fasting value	110-126mg/dl (6.1-7.0mmol/L)	Impaired Fasting Glucose (IFG) ^{**}
3) Fasting value	126mg/dl (7.0mmol/L) or more	Diabetes mellitus
4) Two-hour after 75gm oral glucose load	140-200mg/dl (7.8-11.1mmol/L)	Impaired Glucose Tolerance (IGT) ^{**}
5) Two-hour after 75gm oral glucose load	200mg/dl (11.1mmol/L) or more	Diabetes mellitus
6) Random value	200mg/dl (11.1mmol/L) or more in a symptomatic patient	Diabetes mellitus

Note: [#] Plasma glucose values are 15% higher than whole blood glucose value.

^{**} Person with IFG and IGT is at increased risk for development of Type 2 DM later.

- Other Tests:** These include:
 - Glycosylated Haemoglobin (HbA1c):** This test detects the three-month average plasma glucose concentration. An increase in the fraction of glycated haemoglobin indicates an increase in the average amount of plasma glucose over the past 3 months.

- Extended GTT:** Oral GTT is extended for 3-4 hours for detection of symptoms of hyperglycaemia.
- Intravenous GTT:** It is used in place of oral GTT in patients with intestinal malabsorption.
- Cortisone-Primed GTT:** It is a useful aid for individuals who have a probability of diabetes.
- Insulin Assay:** It measures insulin level in plasma by radioimmunoassay and ELISA techniques.
- C-Peptide Assay:** C-peptide is a marker of insulin production.

Treatment

- Non-Pharmacological Approach:** This includes dietary treatment, along with proper physical exercise:
 - Diet:** Dietary treatment should be aimed at:
 - Controlling weight,
 - Restriction of cholesterol consumption,
 - Avoiding excessive salt intake,
 - Use of artificial sweeteners in moderation and restricting nutritive sweeteners (sorbitol and fructose), and
 - Planning of meals such that they are evenly distributed throughout the day, maintaining the consistency of food timing and energy intake, especially in patients taking insulin.
 - Physical Activity:** Regular physical activity and exercise are recommended as it promotes weight reduction and improves insulin sensitivity, thus, lowering blood glucose levels.

- Pharmacological Approach:** This includes:
 - Insulin:** Drugs used for insulin therapy can be categorised as:
 - Drugs Improving the Availability of Insulin:** The drugs included in this group are:
 - Exogenous Insulin:** Insulin is administered exogenously in the form of insulin-zinc suspension (Lente insulin), isophane, protamine zinc insulin, and insulin analogues, delivered through insulin syringes, pen devices, inhalations or pumps (that can either be inhaled or implanted).
 - Sulphonylureas:** These include tolbutamide, chlorpropamide, glimepiride, etc.
 - Meglitinide/Phenylalanine Analogues:** These include repaglinide and nateglinide.
 - Drugs Overcoming Insulin Resistance:** The drugs included in this group are:
 - Biguanides:** These include administration of metformin.
 - Thiazolidinediones:** These include rosiglitazone and pioglitazone.
 - α -Glucosidase Inhibitors:** These include acarbose and miglitol.

- ii) **Hypoglycaemic Therapy:** Hypoglycaemic drugs are administered orally and include:
- Sulfonylureas:** These include tolbutamide, chlorpropamide, glimepiride, etc.
 - Biguanides:** These include administration of metformin.
 - Meglitinide/Phenylalanine Analogues:** These include repaglinide and nateglinide.
 - Thiazolidinediones:** These include rosiglitazone and pioglitazone.
 - α -Glucosidase Inhibitors:** These include acarbose and miglitol.

2.1.6.2. Galactosaemia

Galactosaemia (galactosemia) is a genetic disorder resulting when the ability to metabolise galactose sugar is hampered. The literal meaning of term **galactosaemia** is **excess of galactose in blood**.

Cause

The absence or non-functionality of **Galactose-1-Phosphate Uridyltransferase (GALT)** enzyme leads to galactosaemia. This enzyme present in liver is responsible for the breakdown of galactose (a sugar by-product of lactose found in breast milk, cow's milk, and other dairy foods) into glucose. When this conversion does not occur, galactose and its by-products (galactose-1-phosphate) accumulate in the tissues and blood, and also affect other body parts.

Symptoms

Galactosaemia produces the following symptoms within several days or weeks after birth:

- 1) Appetite loss,
- 2) Jaundice (yellowing of skin and other body parts),
- 3) Enlargement of liver,
- 4) Liver damage,
- 5) Abdominal swelling due to fluid accumulation,
- 6) Abnormal bleeding,
- 7) Loss in weight, and
- 8) Increased risk of infection.

Diagnosis

Galactosaemia is diagnosed with the help of tests performed during a regular screening of the new-borns. A blood test is performed by withdrawing blood from the heel of the baby to detect the high levels of galactose and low levels of enzyme activity.

A urine test and genetic testing can also diagnose galactosaemia.

Treatment

The most common treatment for galactosaemia is consumption of a low-galactose diet. Thus, intake of milk and other foods containing lactose or galactose should be avoided. There is no cure for galactosaemia, nor is there any medication which could replace GALT enzymes.

Only a low-galactose diet can prevent or reduce the risk of some complications (but not all of them). In some cases, children may suffer from speech delays, learning disabilities, and reproductive issues even after taking required precautions. However, speech problems and delays can be improved by taking speech therapy, along with the treatment plan.

2.1.6.3. Fructose Disorder (Hereditary Fructose Intolerance)

Hereditary Fructose Intolerance (HFI) is a condition in which the ability of the affected individual to digest fructose is hampered. Fructose is present in fruits, sucrose (table sugar), and infant preparations containing sucrose.

Cause

HFI results due to the deficiency of **fructose-1-phosphate aldolase** enzyme, present in the liver.

Symptoms

HFI produces symptoms after fructose ingestion, thus they appear later than those of galactosemia. Failure to gain desired weight, vomiting, hypoglycaemia, liver dysfunction, and kidney defects are some symptoms of HFI.

Liver and kidney gets damaged if foods containing fructose are ingested frequently. Secondary complications like hepatomegaly (enlargement of liver), and cirrhosis (chronic liver disease) may also occur due to liver damage. If ingestion of fructose is continued, seizures and coma may occur, and the individual ultimately dies due to liver and kidney failure. Since the symptoms experienced due to fructose ingestion are severe, most of the individuals suffering from hereditary fructose intolerance start disliking fruits, juices, and other fructose-containing foods.

Treatment

The treatment of HFI involves a strictly controlled diet of the patient. All fructose-, sucrose-, and sorbitol-containing foods are strictly eliminated from their daily diet. Diet control eliminates the symptoms, which however relapses on consuming fructose, sucrose, or sorbitol. If the liver disease has reached a life-threatening stage, liver transplantation is recommended for treating HFI.

2.1.6.4. Glycogen Storage Diseases (GSD)

A constant glucose supply to the brain, RBCs, and adrenal medulla (the inner portion of adrenal gland) is essential for the proper metabolic functioning of the body. This supply begins in the small intestine, where transport proteins facilitate glucose uptake into the cells lining the gut. This glucose then enters the liver via blood circulation, and is stored in the form of glycogen. This glycogen breaks down into glucose, which is released into the blood in times of starvation, fasting, or when body suddenly demands energy. Muscle tissues also store glycogen, which degrades during exercises. Degradation of glycogen to glucose is facilitated by some enzymes, which if gets blocked results in accumulation of glycogen in the liver or muscles; thus resulting in Glycogen Storage Diseases (GSD).

Table 2.5 enlists the types of GSDs, along with the defective enzymes, affected organs, and some characteristic features:

Table 2.5: Glycogen Storage Diseases – Biochemical Lesions and Characteristic Features

Type	Name	Enzyme Defect	Organ(s) Involved	Characteristic Features
I	von Gierke's disease (type I glycogenosis)	Glucose 6-phosphatase	Liver, kidney, and intestine	Glycogen accumulates in hepatocytes and renal cells, enlarged liver and kidney, fasting hypoglycaemia, lactic acidemia, hyperlipidemia, ketosis, and gouty arthritis.
II	Pompe's disease	Lysosomal α -1,4 glucosidase (acid maltase)	All organs	Glycogen accumulates in lysosomes in almost all the tissues, the heart is mostly involved, enlarged liver and heart, the nervous system is also affected, and death occurs at an early age due to heart failure.
III	Cori's disease (limit dextrinosis, Forbe's disease)	Amylo α -1,6-glucosidase (debranching enzyme)	Liver, muscle, and heart leucocytes	Branched-chain glycogen accumulates, liver enlarged, clinical manifestations are similar but milder compared to von Gierke's disease.
IV	Anderson's disease (amylopectinosis)	Glucosyl transferase (branching enzyme)	4-6 Most tissues	A rare disease, glycogen, cirrhosis of the liver, and impairment in liver function.
V	McArdle's disease (type V glycogenosis)	Muscle glycogen phosphorylase	Skeletal muscle	Muscle glycogen stores very high, not available during exercise, the subject cannot perform strenuous exercise, suffer from muscle cramps, blood lactate and pyruvate do not increase after exercise, and muscles may get damaged due to inadequate energy supply.
VI	Her's disease	Liver glycogen phosphorylase	Liver	Liver enlarged, liver glycogen cannot form glucose (pyruvate and lactate can be precursors for glucose), mild hypoglycaemia and ketosis seen, and not a very serious disease.
VII	Tarui's disease	Phosphofructokinase	Skeletal muscle and erythrocytes	Muscle cramps due to exercise, blood lactate not elevated, and haemolysis occurs.

GSD may affect liver, muscles, or both, depending on the enzyme affected. In case of **GSD type I** (von Gierke disease), the last step in release of glucose from liver is disturbed, thereby causing hypoglycaemia. This can be treated by maintaining a continuous glucose supply to the digestive tract (e.g., by continuous drip feedings) in infants and children (in their early age). With the increasing age of children, the symptoms also improve. Glucose is supplied in sufficient amounts by repeated feedings of carbohydrates and slow-release glucose (uncooked corn starch) before the time for bed.

Liver transplantation can also cure GSD, but is performed in patients who fail to respond to the usual treatment or who develop liver cancer. If GSD has affected the muscles, strenuous exercises should be avoided.

If the earlier steps in glycogen breakdown in the liver are affected, GSD types III, IV, VI, and IX occurs which lead to milder versions of GSD type I.

2.1.6.5. Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency

Glucose-6-phosphate dehydrogenase deficiency is an X-linked genetic disorder, therefore mostly occurs in **males**. This condition **affects RBCs**, which carry oxygen from the lungs to other body tissues. In this disease, deficiency of an enzyme called **glucose-6-phosphate dehydrogenase** causes breakdown of premature RBCs. This condition of the destruction of RBCs is known as **haemolysis**.

Causes

The **bacterial** or **viral infections** or infections of certain **drugs** (such as some antibiotics and medications used to treat malaria) induce haemolytic anaemia very easily in individuals with G6PD deficiency.

Deficiency of glucose-6-phosphate dehydrogenase may also cause mild to severe jaundice in new-borns. Generally, the signs and symptoms of G6PD deficiency are not observed and the individual remains unaware of such conditions.

Deficiency of G6PD is a hereditary condition which is passed from one or both parents to their child. It is an X-linked disorder, i.e., the defective gene responsible for the deficiency of G6PD is present on the X chromosome (one of the two sex chromosomes). Women have two X chromosomes, thus mainly functions as a carrier of the disease. While men have only one X chromosome, therefore, haemolytic anaemia mostly occurs in men.

Symptoms

Haemolytic anaemia is the most common complication of G6PD deficiency. In haemolytic anaemia, the breakdown rate of RBCs is faster than their production rate. The signs and symptoms of this condition are paleness, jaundice (yellowing of skin and whites of the eyes), dark urine, fatigue, breathlessness, and tachycardia.

Diagnosis

Deficiency of G6PD can be diagnosed by a simple **blood test** to check G6PD enzyme levels. While **complete blood count**, **serum haemoglobin**, and a **reticulocyte count tests** are some other diagnostic tests used to measure the number of RBCs in the body. Thus, the doctor can diagnose the presence of haemolytic anaemia.

Treatment

Deficiency of G6PD can be treated by removing the factors responsible for haemolytic conditions. If such conditions are resulting due to any infection then the underlying infection should be treated accordingly. If haemolysis is drug-induced, then therapy must be discontinued. In such cases, most people can recover from the condition by own.

However if haemolytic anaemia has progressed, a more strict treatment is required like **oxygen therapy** to remove oxygen deficient conditions and **blood transfusion** to maintain RBC levels.

2.1.6.6. Congenital Disorders of Glycosylation

Congenital Disorders of Glycosylation (CDG; previously known as **carbohydrate-deficient glycoprotein syndrome**) are diseases affecting brain and other organs.

Cause

In CDG, the primary biochemical defects occur in the N-glycosylation pathway which takes place in the cytoplasm, endoplasmic reticulum, and in cellular organelles involved in protein and lipid synthesis. Defective phosphomannomutase-2 (a mannose-processing enzyme) results in CDG type I (the most common form). Other enzymatic defects have been identified, but the biochemical bases of some CDG sub-types are still to be determined.

Symptoms

In infants, CDG type Ia develops low muscle tone, severe delay in their development, and abnormalities in the brain.

Children with CDG type Ia have inverted nipples and unusual distribution of fat (mainly in suprapubic and buttock region). Hypoglycaemia, seizures, stroke-like episodes, damaged retina, impaired heart contractility, vomiting, liver disease, diarrhoea, and a bleeding tendency are some other symptoms.

Treatment

Any effective treatment is not available for CDG; however, CDG type Ib (occurring due to deficiency of phosphomannose isomerase enzyme) can be treated by oral administration of mannose.

2.2. EXERCISE**2.2.1. Short Answer Type Questions**

- 1) Write a note on role of carbohydrates.
- 2) Give properties of carbohydrates.
- 3) Enlist qualitative tests for carbohydrates with details of any two.

2.2.2. Long Answer Type Questions

- 1) Write a detail not on chemistry of carbohydrates and classify them.
- 2) Write a note on diseases related to carbohydrate metabolism.

**CHAPTER
3****Lipids****3.1. LIPIDS****3.1.1. Introduction**

The word **lipid** is derived from the Greek word *lipos* meaning **fat**; universally present in all plants and animal cells. They are naturally occurring waxy, greasy, or oily organic compounds and are known as **oils and fats**. Lipids are **hydrophobic** in nature, i.e., insoluble in water but soluble in non-polar solvents (chloroform, benzene, ether, etc.).

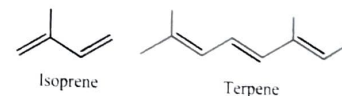
They are present in cell membrane and also found as storage molecules. They are a source of high energy value, therefore are important constituent of the diet.

3.1.2. Chemistry

Lipids do not have a single common structure. The most commonly occurring lipids are triglycerides and phospholipids. Triglycerides are fats and oils with a glycerol backbone bonded to three fatty acids.

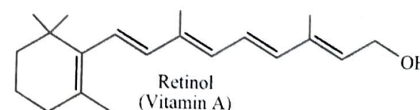
Simple Lipids

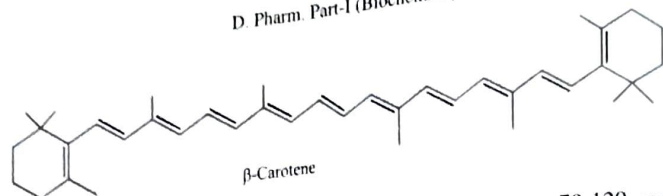
Simple lipids **comprise of a single type of lipid molecule**. A class of simple lipids are **fatty acids**. Another major class is **terpenes** (molecules formed from repeating isoprene, i.e., 2-methyl-1,3-butadiene units). A terpene molecule is made up of two isoprene molecules.



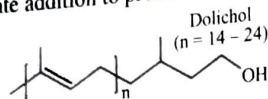
Designation	No. of Terpene Units	No. of Carbons
Monoterpene	1	10
Sesquiterpene	1.5	15
Diterpene	2	20
Triterpene	3	30

Examples of terpenes are the diterpene retinol (vitamin A) and β -carotene (a tetraterpene that acts as a precursor of retinol).

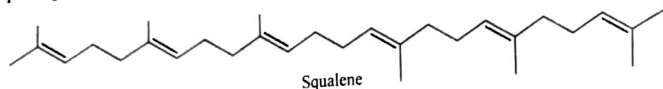




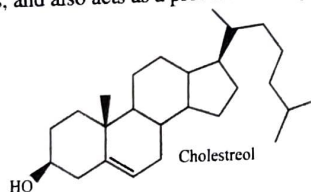
Dolichol results from 14-24 isoprene units, corresponding to 70-120 carbons. Dolichol phosphate is used as an enzyme co-factor in biochemical reaction involving carbohydrate addition to proteins and other molecules.



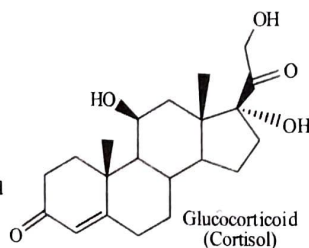
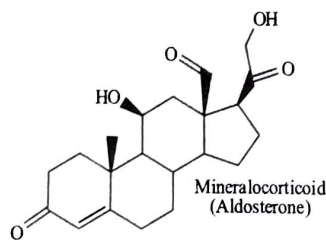
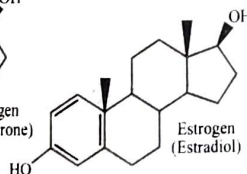
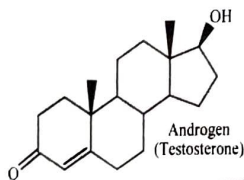
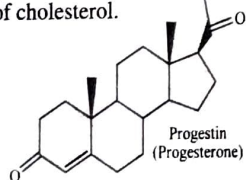
Squalene is a 30-carbon triterpene, which results from tail-to-tail linkage of two sesquiterpene molecules. It acts as a cholesterol precursor.



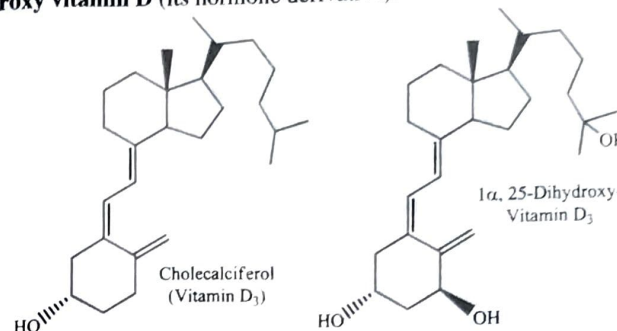
Cholesterol is a 27-carbon terpene derivative (cholesterol synthesis involves removal of 3 carbons from the original squalene, among a considerable number of other reactions). Cholesterol molecule is essentially required for proper membrane structure in animals, and also acts as a precursor for synthesis of bile acid.



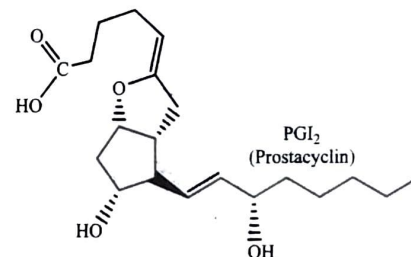
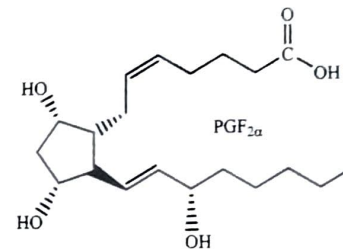
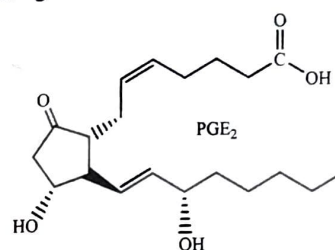
Various steroid hormones, including the **sex steroids** (androgens, oestrogens, and progestin) and the **glucocorticoids** and **mineralocorticoids** are also derivatives of cholesterol.



Cholesterol also acts as a precursor for **vitamin D** (the secosteroid) and **1 α , 25-dihydroxy vitamin D** (its hormone derivative).



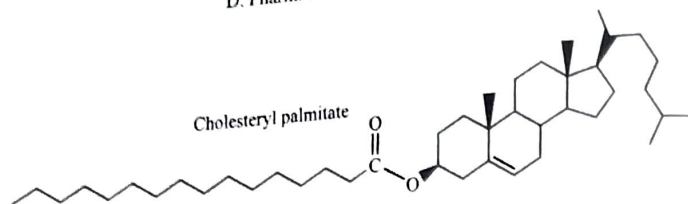
Another class of simple lipid is **prostaglandins**, which act as signalling molecules. These are derived from 20-carbon polyunsaturated fatty acids, **arachidonic acid** being the most commonly used one; however, ω^3 20:5 fatty acids can also be used. Structures of some arachidonic acid derivative prostaglandins, **PGE₂**, **PGF_{2 α}** , and **PGI₂** are given below:



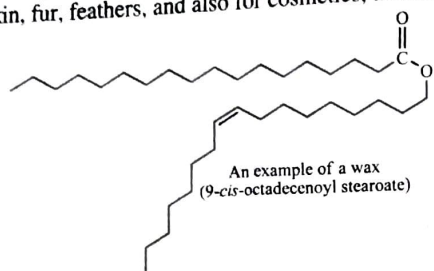
Complex Lipids

Complex lipids are **produced by covalent bond formation between one lipid molecule to another**. These lipids have various structural and functional roles like energy storage, barrier formation, and lipid transport.

An **example** of a complex lipid is **cholesteryl ester**, formed by an ester link between a fatty acid and cholesterol. Cholesteryl esters are hydrophobic, and are used for cholesterol storage and transport.

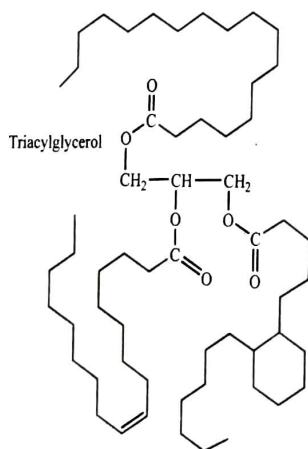
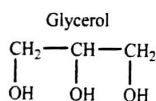


Waxes are a type of barrier molecule, made up of a long chain alcohol with an ester link to a fatty acid. They are hydrophobic, and are used for water-repellent coatings of skin, fur, feathers, and also for cosmetics, automobiles, and candles.



Triacylglycerols

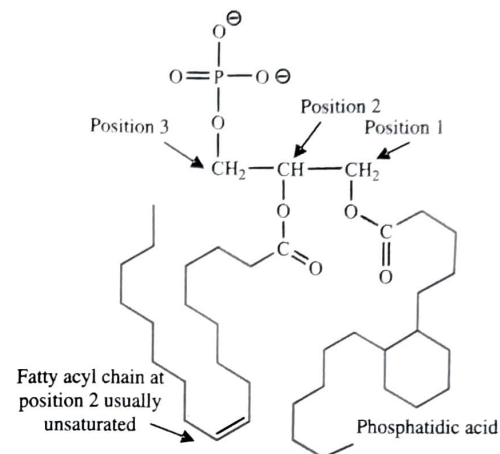
Triacylglycerols (previously named as **triglycerides**) are complex lipids. They act as energy storage molecules (mainly in adipose tissue), and are also present in lipoproteins. Due to their non-polar nature, triacylglycerols are not present in membranes. They consist of 3 fatty acid molecules attached to glycerol via ester links. A triacylglycerol molecule either comprises of different fatty acids, or 3 identical fatty acids.



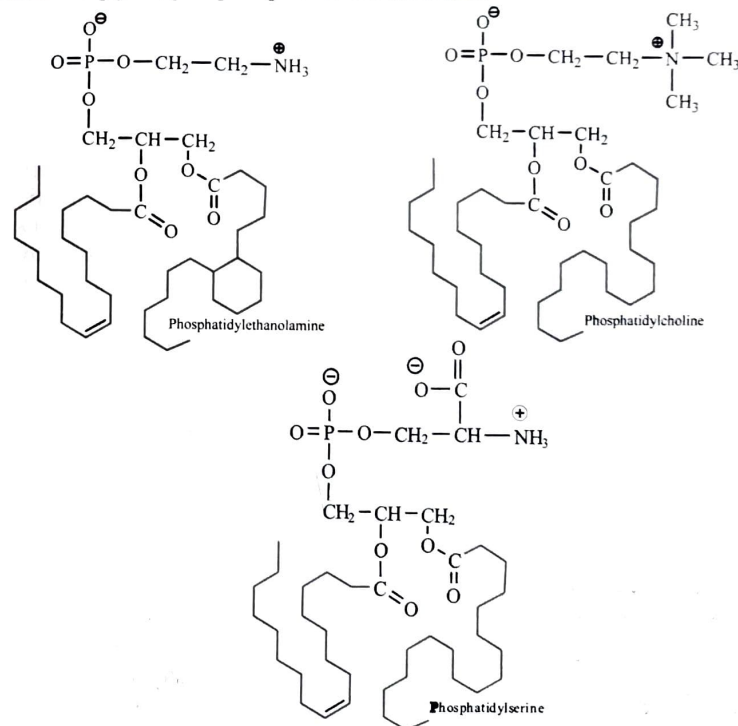
Glycerophospholipids

Glycerophospholipids form the major component of biological membranes. They are made up of a glycerol backbone attached to fatty acids via ester linkages. However, the third position is a phosphate ester (and not a fatty acyl ester). This increases the polarity of glycerophospholipid molecules, because the phosphate group becomes charged under physiological conditions.

In animal membranes, an unsaturated fatty acyl chain is attached at the 2nd position. The example shown is **1-stearoyl, 2-oleoyl phosphatidic acid**.

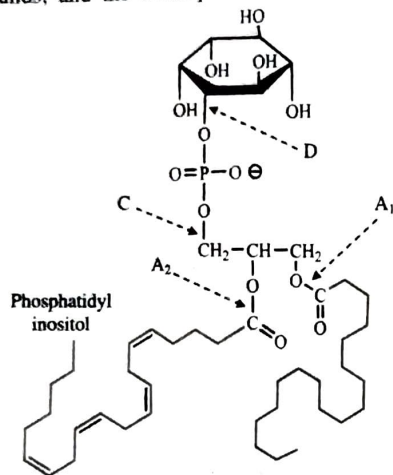


Phosphatidic acid despite of not being a major component of membrane, acts as a precursor for some glycerophospholipids. **Examples** of some common membrane glycerophospholipids are shown below:



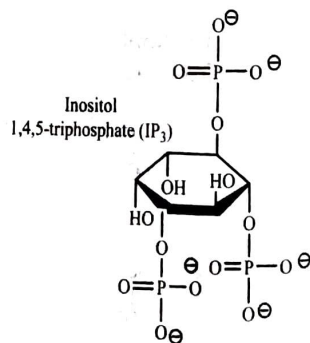
Each compound is based on the phosphatidic acid structure, with an additional compound esterified to the phosphate. The phosphate and this additional compound are together called the **head group of phospholipid**, which is a polar molecule (in each case).

Phosphatidylinositol present in animal membranes is another type of glycerophospholipid. It can be cleaved to release some important signalling molecules. The ring structure forming the part of head group is **inositol**, which is not a carbohydrate (all ring atoms are carbon). The ring hydroxyls can be repeatedly phosphorylated to produce **phosphatidylinositol bisphosphate** and related compounds; and the subsequent increased charge alters the membrane structure.



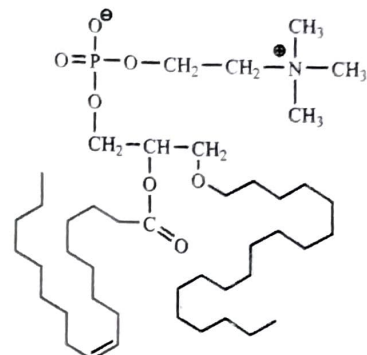
Phospholipase enzyme can hydrolyse the ester bonds in a molecule. The cleavage positions of each phospholipase type are shown in the above figure. Phospholipase A₂ removes the 2-position acyl chain (releasing the arachidonic acid which then converts into prostaglandins or other compounds). Phospholipase C removes the phosphate (and attached compounds), which leaves diacylglycerol. The polarity of diacylglycerol is much less than that of the parent glycerophospholipid, and its production can alter the membrane structure. Diacylglycerol can also activate the protein kinase C enzymes, which alter the cellular behaviour by phosphorylating the cellular enzymes.

Apart from releasing diacylglycerol, phospholipase C enzyme also releases inositol phosphates, which then alter the cellular functions. The most studied inositol phosphate is **inositol trisphosphate**, which acts to release calcium ions stored in the endoplasmic reticulum.



Ether Glycerophospholipids

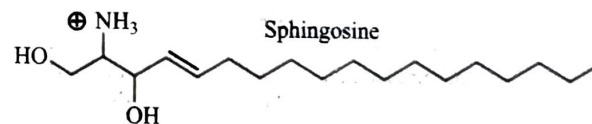
Ether glycerophospholipids (or **plasmalogens**) are another membrane component closely related to glycerophospholipids. In these compounds, the 1-position chain is formed from a long chain alcohol (and not a fatty acid). As a result, an ether linkage to the glycerol backbone is formed. Ether glycerophospholipids can have a variety of head groups (choline is shown in the **example** below). They are relatively minor membrane components, and occur less commonly than the glycerophospholipids.



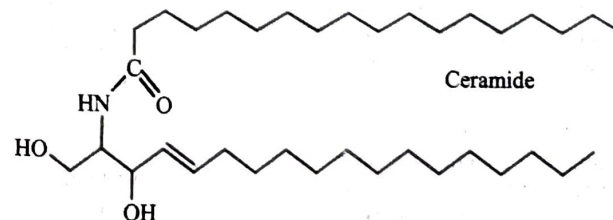
Ether Phosphatidylcholine
(1-octadecyl, 2-oleoyl Phosphatidylcholine)

Sphingolipids

Sphingolipids are another minor but important class of membrane lipids. They are not based on glycerol, but are derivatives of **sphingosine** (an 18-carbon amino alcohol containing a *trans* double bond). Sphingolipids are found in plasma membranes (of neurons mainly).

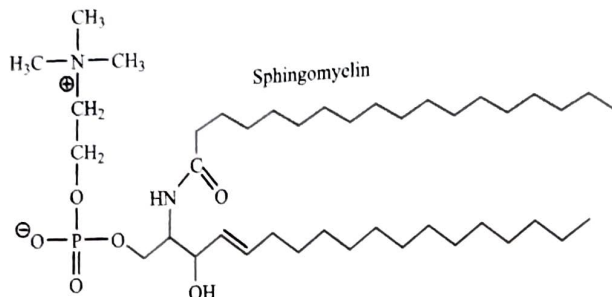


Ceramide, the simplest sphingolipid is formed from sphingosine by an amide linkage to a fatty acid.

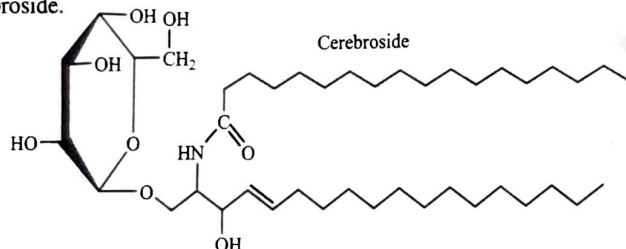


Sphingomyelins (ceramide derivatives) are more important and occur more commonly than ceramide. These are phosphate esters of ceramide. Alike other phospholipids, an additional group is found to be attached to its phosphate. The

other groups present are also similar to those found in other phospholipids, and include choline (part of the head group of sphingomyelin shown below) and ethanolamine.



Cerebrosides (ceramide derivatives) are attached to a carbohydrate via ether link to the 1-position alcohol of the sphingosine backbone. Most cerebrosides contain either β -D-glucose or β -D-galactose (a cerebroside with β -D-galactose is shown below). They do not have phosphate, and are non-ionic compounds. However, in some variants, ionisable groups are attached to the carbohydrate group of the cerebroside.



3.1.3. Role

Lipids perform several biological functions, such as:

- 1) **Storage:** They are storage compounds and in the animal body, they reserve energy in the form of triglycerides in adipocytes.
- 2) **Energy Source:** As energy source, they provide 9kcal of energy per gram. The per unit weight of fats supply two times more energy as compared to per unit weight of proteins or carbohydrates.
- 3) **Cell Membranes:** They are important components of the cell membrane. Phosphoglycerides, sphingolipids, and steroids are structural components of cell membranes.
- 4) **Membrane Permeability:** They regulate membrane permeability.
- 5) **Vitamins:** They serve as a source for fat-soluble vitamins (A, D, E, and K). Therefore, lipids are essential for the effective absorption of fat-soluble vitamins from the intestine.
- 6) **Electrical Insulation:** They act as electrical insulators to the nerve fibres, where the myelin sheath contains lipids.

- 7) **Enzyme Systems:** They are components of various enzyme systems.
- 8) **Cellular Metabolic Regulators:** Some lipids like prostaglandins and steroid hormones act as cellular metabolic regulators.
- 9) **Signalling:** Being small and insoluble in water, they act as signalling molecules.
- 10) **Thermal Insulation:** Fat deposited in the subcutaneous layer provides insulation and protection from cold.
- 11) **Fluidity and Flexibility:** Polyunsaturated phospholipids are important constituents of phospholipids. They provide fluidity and flexibility to the cell membranes.
- 12) **Transport:** Lipoproteins transport cholesterol and triglycerides from their origin to sites of use or uptake (e.g., the liver, muscle, and fat tissue). Lipoproteins that are complexes of lipids and proteins occur in blood as plasma lipoprotein.
- 13) **Essential Fatty Acids:** They also supply the essential fatty acids which are not synthesised in the human body but are required for normal growth. Essential fatty acids (like linoleic and linolenic acids) are the precursors of many different types of eicosanoids including prostaglandins and thromboxanes. These play important role in pain, fever, inflammation, and blood clotting.
- 14) **Cholesterol:** It is found in cell membranes, blood, and bile of many organisms. It maintains the fluidity of membranes by interacting with lipid complexes. It is the precursor of bile acids, Vitamin D, and steroids.
- 15) **Steroid Hormones:** They are the critical intercellular messengers.

In the table 3.1, a summary of various types of lipids and their biological role is given:

Table 3.1: Lipids and their Biological Roles

Types of Lipids	Biological Roles
Fatty acids	Principal building blocks of complex lipids.
Waxes	Esters of fatty acids (heat sensitive) and also act as moisture barrier.
Triacylglycerols	Membrane precursors and stores energy (of fats and oils).
Glycerophospholipids	Membrane components.
Sphingolipids	Form brain lipids and membrane components.
Steroids	Cholesterol, bile salts, and steroid hormones perform various functions.
Terpenes	Turpentine has many roles.
Phospholipids	Form membrane components.
Eicosanoids	Signalling molecules (prostaglandin).
Sphingomyelins	Form membrane components (important in myelin sheaths).
Glycosphingolipids	Cell recognition (ABO blood group antigen).
Steroids (sterols)	Form membrane components and hormones.
Lipid vitamins	Vitamin A, E, and K are important.
Carotenoids	Photosynthetic accessory pigments.
Chlorophyll	Major light harvesting pigment.
Plastoquinone/ubiquinone	Lipid soluble electron carriers.
Essential oils	Menthol, limonene, terpenene, myrcene, saninene, etc., play major roles.

3.1.4. Classification

Bloor in 1943 classified lipids on the basis of their chemical composition as shown in figure 3.1:

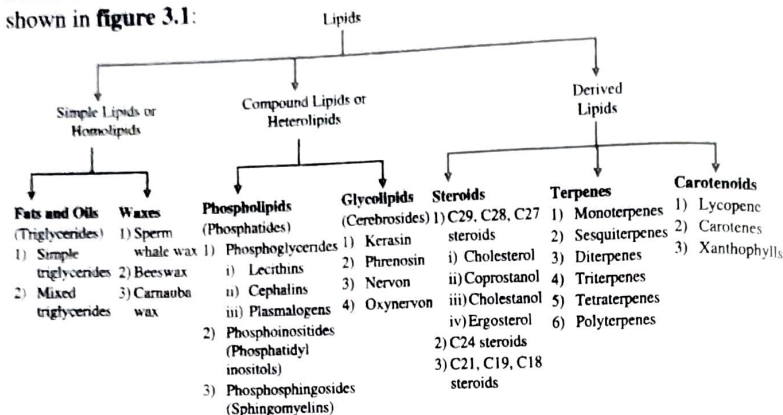


Figure 3.1: Classification of Lipids

- Simple Lipids:** These are esters of fatty acids containing various alcohols and carry no other substance.
 - Fats and Oils:** These are esters of fatty acids containing glycerol. Oils are also fats but are present in the liquid state.
 - Waxes:** These are esters of fatty acids containing high molecular weight monohydric alcohols.
- Compound Lipids:** These are esters of fatty acids, containing an alcohol, a fatty acid, and other groups.
 - Phospholipids:** These lipids consist of fatty acids, an alcohol, and a phosphoric acid residue. They frequently have nitrogen-containing bases and other substituents, e.g., in glycerophospholipids the alcohol is glycerol and in sphingophospholipids the alcohol is sphingosine.
 - Glycolipids (Glycosphingolipids):** These lipids consist of a fatty acid, sphingosine, and carbohydrate.
- Derived Lipids:** These are hydrolysed derivatives of simple or compound lipids. Examples of this class of lipids are fatty acids, glycerol, steroids, terpenes, carotenoids, fatty aldehydes, etc.

3.1.5. Qualitative Tests

The following qualitative tests are performed for identification of lipids:

- Grease Spot Test:** A small amount of oil is put on a paper. A greasy spot penetrating the paper is formed as lipids unlike water do not wet paper.
- Test for Free Fatty Acids:** A few drops of phenolphthalein solution are taken in a test tube and added with 1-2 drops of very dilute alkali solution to develop a pink colour. This solution is shaken with a few drops of oil. The pink colour disappears due to neutralisation of the alkali by the free fatty acids present in the oil.

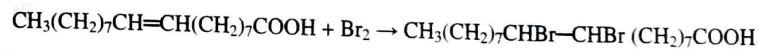
- Emulsification:** When oil or liquid fat is dispersed in water and shaken, it becomes finely divided to form an emulsion; this process is **emulsification** which is permanent and complete in the presence of emulsifying agents (e.g., bile salts, proteins, soaps, mono- and diglycerides). Emulsification aids in the processes of fat digestion in intestines. Emulsifying agents lower the liquid surface tension.

In a clean and dry test tube, 2ml water is added, and in another test tube, 2ml dilute bile salt solution is added. Each test tube is added with 2 drops of mustard oil and vigorously shaken for a minute. On leaving the tubes undisturbed for 2 minutes, the oil in the first tube breaks down in small pieces and floats on the water surface; while the oil in the second tube suspends in the bile salt liquid in the form of minute droplets (i.e., permanent emulsification).

- Saponification Test:** Hydrolysis of esters by alkali yields the parent alcohol and salt. The salt yielded by long chain fatty acids is soap; this process is **saponification**. Since oils and fats contain long chain fatty acids, they are the starting materials for soap preparation.

1ml of oil is thoroughly mixed with an equal amount of alcoholic KOH solution in a test tube. The mixture is kept during the course of warming and shaken gently with small amount of distilled water. Some oil drops appear indicating incomplete saponification; while no oil drops indicate complete saponification.

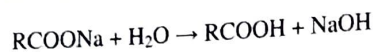
- Tests for Unsaturation of Fatty Acids:** Unsaturated fatty acids (e.g., oleic acid) react with halogens (e.g., bromine and iodine) as shown below due to the presence of double bonds.



The amount of bromine or iodine taken up indicates the amount of unsaturation of a particular acid. Approximate idea of unsaturation in different oils and fats can be obtained as follows:

- 4 clean and dry test tubes are taken and to each 5ml of CCl_4 is added.
- To the first tube, a drop of shark liver oil is added; to the second tube, a drop of coconut oil is added.
- To the third tube, a drop of vegetable ghee is added (vegetable ghee is prepared by hydrogenation of vegetable oil. Hydrogenation involves saturating unsaturated fatty acid by hydrogen).
- The fourth tube is left untouched. Thereafter, unsaturation test of the added oil is performed by adding drop wise bromine water to each tube and shaking.
- The number of drops required to obtain a permanent yellowish red colour in each tube is recorded, and the relative unsaturation in the three samples used is deduced.

- 6) **Isolation of Free Fatty Acids from Soap:** A few ml of 20% H_2SO_4 is taken in a test tube, followed by adding 5ml of soap solution. The soap hydrolyses due to which a distinct layer of fatty acids separates out.



The solution becomes hot, so it is cooled; the surface layer is skimmed off and washed several times with water to remove H_2SO_4 . Then it is dissolved in water and shaken with alkaline phenolphthalein solution. A pink colour appears due to the presence of free fatty acids.

Calcium Soap Formation: A small amount of soap solution is taken in a test tube and added with CaCl_2 solution. A white precipitate appears due to insoluble calcium salt of the fatty acid; this is the calcium soap.

Lead Soap Formation: A small amount of soap solution is taken in a test tube and added with lead acetate solution. A white precipitate appears due to the insoluble lead salt of fatty acids; this is the lead soap.

- 7) **Tests for Glycerol:** These tests are as follows:

- Acrolein Test:** Pure glycerol is taken in a dry test tube and added with a few crystals of potassium hydrogen sulphate. The mixture is gently warmed, followed by strong heating. Acrolein is formed (due to the removal of water from glycerol by potassium hydrogen sulphate) with a highly pungent odour.
- Dichromate Test:** 3 or 4ml of glycerol solution is taken in a test tube, and thoroughly mixed with a few drops of 5% potassium dichromate solution and 5ml of concentrated HNO_3 . The brown colour changes to blue. Glycerol undergoes oxidation in the presence of chromic ions, which during this process reduce to chromous ions producing the blue colour. Substances containing primary and secondary alcohol groups respond positively to this test. Reducing sugars also show positive results to this test, thus it should be assured that they are not present before confirming glycerol.

3.1.6. Diseases Related to Lipid Metabolism

Lipids (or fats) are important source of energy for the body. The fat stored within the body are constantly broken down and re-assembled to balance the energy requirement of the body with the consumed food. This whole process is catalysed by specific enzymes. But any abnormality in these enzymes leads to the formation of specific fatty substances that normally have been degraded by the enzymes. If these fatty substances accumulate within the body for the prolonged duration, the body organs may get harmed.

Lipidosis are the disorders which result due to lipid accumulation. Some enzyme abnormalities prevent the conversion of body fats into energy; this is termed **fatty acid oxidation disorders**.

In **lipid metabolism disorders**, the body lacks sufficient enzymes for lipid break down, or the enzymes fail to convert fat into energy due to improper functioning. This leads to accumulation of lipids within the body in abnormal amount, which leads to gradual damage of the cells and tissues in the brain, peripheral nervous system, liver, spleen, and bone marrow. Some of the lipid metabolism disorders are very serious, and can even lead to death.

3.1.6.1. Ketoacidosis

Ketone bodies are produced when the body burns fat for energy. If insulin secretion is decreased in the body, ketone bodies will begin to build-up in the bloodstream and will result in a life-threatening condition known as **ketoacidosis**.

Types

Along with general ketoacidosis, there are some specific types like:

- 1) **Alcoholic ketoacidosis**, caused by excessive alcohol consumption.
- 2) **Diabetic ketoacidosis** (Dka), occurring due to type 1 diabetes.
- 3) **Starvation ketoacidosis**, commonly seen in pregnant women, in their third trimester, and experiencing excessive vomiting.

These situations raise the acid level in the system and sometimes reduce the insulin production in the body. It results in the breakdown of fat cells to generate ketones.

Causes

- 1) **Alcoholic Ketoacidosis:** It occurs due to more intake of alcohol for a prolonged time. Excessive alcohol consumption causes malnourishment (fewer nutrients available in the body to function properly). Excessive drinking may also result in vomiting, thus, not eating enough nutrients, and excessive vomiting for a long period results in starvation, which further reduces insulin production in the body. A malnourished alcoholic person may develop alcoholic ketoacidosis.
- 2) **Diabetic Ketoacidosis**
 - i) Other infections and diseased conditions (like pneumonia and urinary tract infections) release higher concentration of certain hormones like adrenaline or cortisol. These hormones counteract insulin and thus trigger an episode of diabetic ketoacidosis,
 - ii) Due to missed insulin treatments or inadequate insulin therapy, the insulin level of body decreases accidentally, causing diabetic ketoacidosis,
 - iii) Trauma (either physical or emotional),
 - iv) Cardiac arrest,
 - v) Alcohol or drug abuse (like cocaine), and
 - vi) Medications like corticosteroids and some diuretics.

Symptoms

1) **Alcoholic Ketoacidosis:** Symptoms of alcoholic ketoacidosis are:

- i) Pain in the abdomen,
- ii) Confusion and agitation,
- iii) Reduced alertness or coma,
- iv) Fatigue,
- v) Slowed movement,
- vi) Irregular, deep, and fast breathing (Kussmaul's sign),
- vii) Loss of appetite,
- viii) Nausea and vomiting, and
- ix) Dehydration-like symptoms, e.g., dizziness (vertigo), lightheadedness, and thirst.

2) **Diabetic Ketoacidosis:** Ketones build-up in blood and urine, causing the following symptoms:

- i) Abdominal pain (common in children),
- ii) Confusion,
- iii) Less perspiration,
- iv) Deep and rapid breathing (a later symptom),
- v) Dry and cooler skin,
- vi) Elevated thirst and urination,
- vii) Fatigue,
- viii) Increased blood glucose level,
- ix) Nausea and vomiting,
- x) Ketones detected in the urine,
- xi) Weight loss, and
- xii) Sweet, fruity odour in breath (like nail polish remover).

Diagnosis

1) **Alcoholic Ketoacidosis:** It may be diagnosed by the following tests:

- i) **Amylase and Lipase Tests:** These tests monitor the functioning of pancreas and diagnose pancreatitis.
- ii) **Arterial Blood Gas Tests:** These tests measure levels of blood's oxygen and acid/base balance.
- iii) **Anion Gap Calculation:** This measures sodium and potassium levels.
- iv) **Blood Alcohol Tests:** These tests measure concentration of alcohol dissolved in the blood.
- v) **Blood Chemistry Panel:** This involves comprehensive study of body metabolism and functioning.
- vi) **Blood Glucose Tests:** These tests measure concentration of glucose in blood.
- vii) **Blood Urea Nitrogen and Creatinine Tests:** These tests determine kidney functioning.

viii) **Serum Lactate Tests:** These tests determine lactate levels in the blood. Lactic acidosis causes high lactate levels. This is a condition where body cells and tissues are not receiving enough oxygen.

ix) **Urine Tests:** These tests detect the presence of ketones.

2) **Diabetic Ketoacidosis:** Laboratory tests like **blood and urine tests** are performed to diagnose diabetic ketoacidosis. Home use kits for **tests for ketones** are also available.

Treatment

1) **Alcoholic Ketoacidosis:** Signs like heart rate, blood pressure, and breathing are monitored. Intravenous fluids are administered. Vitamins and following nutrients are given immediately to treat malnutrition:

- | | |
|----------------------|----------------|
| i) Thiamine, | ii) Potassium, |
| iii) Phosphorus, and | iv) Magnesium. |

2) **Diabetic Ketoacidosis:** Treatment of ketoacidosis focuses on the following:

- i) Resuscitation of fluids,
- ii) Acidosis and ketosis reversal,
- iii) Lowering plasma glucose concentration to normal,
- iv) Electrolyte replenishment along with the volume losses, and
- v) Identifying the causes.

Following medications are prescribed by the physician for management of diabetic ketoacidosis:

- i) Rapid-acting insulin (e.g., insulin aspartate),
- ii) Short-acting insulin (e.g., regular insulin),
- iii) Electrolyte supplements (e.g., potassium chloride), and
- iv) Alkalinising agents (e.g., sodium bicarbonate).

3.1.6.2. Hypercholesterolemia

Hypercholesterolemia is a condition of high cholesterol levels in the blood. Cholesterol, a wax-like, fatty substance is produced in the body as well as obtained from animal-derived foods (e.g., egg yolks, meat, poultry, fish, and dairy products). Cholesterol is an essential constituent of cell membranes, certain hormones, and also participates in fat digestion. But cholesterol in large amounts leads to disorders of the heart.

Causes

- 1) **Heredity:** Cholesterol can be inherited in large amounts as the genes may affect the LDL (bad) cholesterol metabolism.
- 2) **Foods High in Saturated Fats and Cholesterol:** Saturated fats in large amount increases the levels of LDL (bad) cholesterol.
- 3) **Other Diseases:** Diabetic patients may suffer from this disorder.
- 4) **Lifestyle Changes:** Reduced physical activity increases the levels of fat and cholesterol.

Symptoms

- 1) Angina resulting due to heart disorder.
- 2) Xanthomas (deposits of fat on elbows, buttocks, knees, and tendons).
- 3) Xanthelasma (deposits of cholesterol around the eyelids), and
- 4) Corneal arcus (deposits of cholesterol around the corneas).

Diagnosis

Lipoprotein profile is a blood test which is performed to determine the levels of total cholesterol, LDL, HDL, and triglycerides in the blood.

Treatment

The cholesterol level in plasma can be reduced by the following ways:

- 1) **Consumption of PUFA:** Consuming Polyunsaturated Fatty Acids (PUFA) in the diet lowers the plasma cholesterol level as it facilitates the transport of cholesterol. Cotton seed oil, soya bean oil, sunflower oil, corn oil, fish oils, etc., are some **examples** of PUFA-rich oils; while ghee and coconut oil are PUFA-deficient.
- 2) **Dietary Cholesterol:** Animal-derived foods (and not plant-derived) are the sources of cholesterol. Cholesterol obtained from such sources mildly affects the plasma cholesterol level. However, it is suggested to avoid cholesterol-rich diet.
- 3) **Plant Sterols:** Some plant sterols and their stanols (e.g., sitosterol esters) lower the plasma cholesterol levels by inhibiting intestinal absorption of dietary cholesterol.
- 4) **Dietary Fibre:** Vegetable fibres reduce plasma cholesterol levels by reducing its absorption through intestines.
- 5) **Avoiding High Carbohydrate Diet:** Hypercholesterolemia can be controlled by avoiding carbohydrate-rich foods (especially sucrose).
- 6) **Impact of Lifestyles:** Hypercholesterolemia is often observed in people with smoking, abdominal obesity, lack of exercise, stress, high blood pressure, consumption of soft water, etc. Thus, plasma cholesterol level can be controlled by making some adequate lifestyle changes.
- 7) **Therapeutic Agents:** Cholesterol synthesis can be decreased by using drugs which inhibit HMG CoA reductase (e.g., lovastatin). Statins currently in use are **atorvastatin**, **simvastatin**, and **pravastatin**. Some drugs like **cholestyramine** and **colestipol** bind with bile acids and thus reduce the intestinal reabsorption of cholesterol. It converts more cholesterol to bile acids and allows its excretion through faeces. **Clofibrate** decreases the plasma cholesterol and triacylglycerols levels by increasing lipoprotein lipase activity.

3.1.6.3. Atherosclerosis

Formation of plaque inside the arteries (blood vessels carrying oxygen-rich blood to the heart and other body parts) is referred to as a state of **atherosclerosis**. With time, the plaque hardens and narrows the arteries. As the arteries are narrowed, the flow of oxygen-rich blood to heart as well as to other areas of the body is reduced or stopped.

Atherosclerosis may lead to further complications like heart attack, stroke, or even death.

Causes

Atherosclerosis is a slow, progressive condition and is always present in the body up to some level. The exact mechanism of atherosclerosis is not known but it is believed that it starts with damage or injury to the inner layer of an artery. These damages can be caused by:

- 1) High blood pressure,
- 2) High cholesterol level,
- 3) High triglycerides, a type of fat (lipid) in the blood.
- 4) Smoking and other sources of tobacco,
- 5) Insulin resistance, obesity, or diabetes, and
- 6) Inflammation from diseases, such as arthritis, lupus, and infections or inflammation of unknown cause.

Symptoms

Mild atherosclerosis is usually asymptomatic. Moderate to severe atherosclerosis have symptoms depending on the arteries that are affected. Some of the common symptoms are:

- 1) Pain or pressure feeling in the chest (angina), in case atherosclerosis affects the arteries in the heart.
- 2) Numbness or weakness in arms or legs, temporary loss of vision in one eye, drooping muscles of face or difficulty in speaking or slurred speech in case atherosclerosis affects the arteries supplying the brain.
- 3) High blood pressure sometimes even results in kidney failure, in case atherosclerosis affects the arteries supplying the kidneys.

Diagnosis

In physical examination, the physician diagnoses the signs of narrowed, enlarged, or hardened arteries:

- 1) Below the narrowed area of the artery, either the pulse is absent or a weak pulse is generated.
- 2) The blood pressure in the affected limb is low, and
- 3) On hearing through stethoscope, whooshing sounds (bruits) are heard over the affected arteries.

After a physical examination, the physician may suggest one or more of the following diagnostic tests:

- 1) **Blood Tests:** The increased cholesterol and blood sugar level which may increase the risk of atherosclerosis is detected by blood tests.
- 2) **Doppler Ultrasound:** A special ultrasound device **Doppler ultrasound** is used to measure the blood pressure at various points of arm or leg. This measurement helps in detecting the degree of any blockage and the speed of arterial blood flow.
- 3) **Ankle-Brachial Index:** This test determines whether or not the arteries in legs and feet are affected. A comparison between the ankle blood pressure

and the arm blood pressure is made, which is termed as the **ankle-brachial index**. If the difference between the two is not normal, presence of peripheral vascular disease is suspected which may be the result of atherosclerosis.

- 4) **Electrocardiogram (ECG):** The electrical signals travelling through the heart are recorded by ECG. It also indicates the history of a heart attack. If the signs and symptoms of the individual appear during physical activity, he/she is asked to walk on a treadmill or ride a stationary bike while performing the ECG.
- 5) **Stress Test:** This test (also termed as **exercise stress test**), reveals the heart performance during any physical activity. In this test, the heart rhythm, blood pressure, and breathing rate of the individual are recorded while he/she walks on a treadmill or ride a stationary bike.
- 6) **Cardiac Catheterisation and Angiogram:** This test is used to detect the narrowing and blockage of coronary arteries. In this test, a catheter (a long, thin tube) filled with a liquid dye is fed through an artery (mostly of a leg) to reach the arteries in the heart. When the arteries become stained with the dye, they can be seen on X-ray, which reveals any narrow or blockage in the coronary arteries.
- 7) **Other Imaging Tests:** Other imaging techniques like ultrasound, Computerised Tomography (CT) scan, or Magnetic Resonance Angiography (MRA) are performed to determine any hardened and narrowed large arteries, any kind of swelling, and deposition of calcium on artery walls.

Treatment

The clinical management for atherosclerotic patients involves changing the current lifestyle of the patient like dietary modifications (such that the quantity of cholesterol and fat is decreased) and exercise. They improve the health of blood vessels and the heart. Administration of drugs to prevent the worsening of atherosclerosis may be needed. Such drugs include:

- 1) **Statins and fibric acid derivatives** (cholesterol-lowering drugs),
- 2) **Antiplatelet drugs and anticoagulants** (aspirin, to prevent clotting of blood and hence, clogging of the arteries),
- 3) **Beta blockers or calcium channel blockers** (to reduce blood pressure),
- 4) **Diuretics** (to lower the blood pressure), and
- 5) **Angiotensin Converting Enzyme (ACE) inhibitors** (to prevent narrowing of the arteries).

Surgery may be required in case of severe symptoms or in case of endangered skin or muscle tissue. The possible surgeries include:

- 1) **Bypass Surgery:** This is performed by using a vessel from a different site in the body or a synthetic tube to divert blood around the blocked or narrowed artery.
- 2) **Thrombolytic Therapy:** This involves injection of a drug into the affected artery to dissolve the blood clot.

- 3) **Angioplasty:** This is performed by using a catheter and a balloon for expansion of the artery, and sometimes involves insertion of a stent to avoid closure of the arteries.
- 4) **Endarterectomy:** This procedure involves surgical removal of fatty deposits from the artery.
- 5) **Atherectomy:** This procedure involves elimination of plaque from the arteries by using a catheter having a sharp blade at one end.

3.1.6.4. Fatty Liver

Fatty liver or **hepatic steatosis** defines excessive fat accumulation in the liver which accounts for more than 5-10% of liver's weight. Fatty liver condition can be controlled by making certain changes in lifestyle. Fatty liver even shows no symptoms in most of the cases, and it also does not damage the liver until it reaches the advanced stage.

Causes

Alcoholism and heavy drinking are the most common causes of fatty liver. However, some **other causes** are:

- 1) Obesity,
- 2) Hyperlipidemia (increased fat levels in the blood),
- 3) Diabetes,
- 4) Genetic inheritance,
- 5) Rapid weight loss, and
- 6) Side effects of aspirin, steroids, tamoxifen, and tetracycline.

Symptoms

Fatty liver may cause **fatigue** or **vague abdominal discomfort**. When fat accumulates in large amount, it may result in **liver inflammation**, which produces the following symptoms:

- | | |
|----------------------|-----------------|
| 1) Loss of appetite, | 2) Weight loss, |
| 3) Abdominal pain, | 4) Weakness, |
| 5) Fatigue, and | 6) Confusion. |

If not treated, fatty liver advances to liver cirrhosis and may even result in liver failure.

Diagnosis

- 1) **Physical Examination:** Inflamed liver can be diagnosed by abdominal examination.
- 2) **Blood Tests:** Presence of large amount of some enzymes indicates fatty liver.
- 3) **Ultrasound:** In this test, a picture of the liver is obtained by using sound waves. An MRI or CT-scan can also be performed to obtain additional liver images.
- 4) **Biopsy:** The affected area is anaesthetised and a needle is passed through the skin to take out a small piece of liver, which is then microscopically examined to detect fat deposits, inflammation, and damaged liver cells.

Treatment

Fatty liver cannot be treated using medicines or by any surgery. Instead, risk factors can be reduced by the following recommendations:

- 1) Avoiding alcohol.
- 2) Reducing consumption of dietary cholesterol, sugar, and saturated fatty acids, and
- 3) Losing weight.

Fatty liver due to obesity or unhealthy eating habits can be controlled by increasing physical exercise and by avoiding certain food.

Fatty liver disease can be controlled by avoiding fatty foods and foods high in sugar, and by also increasing the consumption of fresh fruits, vegetables, and whole grains. The red meats should be replaced with lean animal proteins like chicken and fish.

3.1.6.5. Obesity

Obesity is a condition of abnormal increase in body weight due to excessive fat deposition.

Obese men and women show 20% and 25% of increase in their body weight respectively due to accumulation of fat in adipose tissues. Obesity is the result of over-eating, i.e., excessive intake of calories in diet, and lack of physical activity. On consuming every 7 calories, 1gm fat deposits and 1gm body weight increases.

Body Mass Index (BMI)

Body Mass Index (BMI) is the clinical representation of obesity and is calculated by dividing weight (in kilograms) with height (in meters²).

$$\text{BMI (kg/m}^2\text{)} = \frac{\text{Weight (kg)}}{[\text{Height (m)}]^2}$$

Obesity is categorised into three grades:

- 1) Grade I obesity or overweight – BMI 25-30kg/m².
- 2) Grade II or clinical obesity – BMI > 30kg/m².
- 3) Grade III or morbid obesity – BMI > 40kg/m².

Causes

An imbalance of energy between the consumed and used calories is the major cause of obesity. In general, increased consumption of energy-dense and fat-rich foods and reduced physical activity results in obesity.

Symptoms

Increased value of BMI increases the risk of the following:

- 1) Cardiovascular disorders (heart disease and stroke),
- 2) Diabetes,
- 3) Musculoskeletal disorders (osteoarthritis, a degenerative joint disorder), and
- 4) Cancers of endometrium, breast, ovary, prostate, liver, gallbladder, kidney and colon.

Childhood obesity is concerned with higher chance of obesity, premature death, and future disability. The obese children face breathing difficulties, increased risk of fractures, hypertension, cardiovascular diseases, insulin resistance, and psychological effects.

Diagnosis

The most common method to determine obesity is the calculation of BMI. It is an indication of body fat determined by comparing the weight and height of an individual.

An **adult's BMI** can be determined using a **BMI calculator**. For adults, a BMI of:

- 1) 18.5 to 24.9 is considered normal weight,
- 2) 25.0 to 29.9 is considered overweight,
- 3) 30.0 to 39.9 is considered obese, and
- 4) 40.0 and higher is considered extremely obese.

The **BMI values for 2 year old children** are:

- 1) BMI value between 5th and the 85th percentiles indicates normal weight,
- 2) BMI value between 85th and 95th percentiles indicates overweight, and
- 3) BMI value between 95th and more percentile indicates obesity.

Apart from BMI, body fat can also be measured using other methods, e.g., by calculating waist circumference, determining waist-to-hip circumference ratios, measuring skinfold thickness (in which the thickness of a pinch of skin and fat is measured), and by ultrasound.

Treatment

Obesity can be controlled by:

- 1) Reducing the consumption of fat- and sugar-rich foods,
- 2) Increasing the consumption of fruits, vegetables, legumes, whole grains, and nuts, and
- 3) Increasing regular physical exercise (60 minutes/day for children and 150 minutes/week for adults).

3.2. EXERCISE**3.2.1. Short Answer Type Questions**

- 1) Give the classification of lipids.
- 2) Write a short note on the role of lipids in human body.
- 3) Write a brief note on chemistry of lipids.

3.2.2. Long Answer Type Questions

- 1) Give the qualitative tests of lipids.
- 2) Write note on diseases related to lipid metabolism.

CHAPTER

4

Vitamins

4.1. VITAMINS

4.1.1. Introduction

A vitamin is an essential organic compound, required in small amounts by an organism. In 1912, C. Funk coined the term **vitamine**, from the Latin words *vit* which means life and *amine*. In 1920, 'e' was dropped from **vitamine**. The name **vitamin** is obtained from "vital amines" as it was originally thought that these substances were all amines, although not all vitamins are amines. Vitamins are natural substances found in plants and animals and are known to be essential nutrients for human beings. Human body uses these substances to stay healthy and to carry many vital functions.

Vitamins are essential to keep the body healthy, and a varied diet usually gives all the vitamins required. They do not provide energy (calories) directly, but they regulate the energy-producing processes. With the exception of vitamin D and the remaining vitamins cannot be synthesised by the human body and must be obtained from the diet.

The 13 essential vitamins of the body (each having a special role to play) help in controlling metabolic processes like cell growth and repair, reproduction and digestion, etc.

Like fats, carbohydrates, proteins, minerals, salt, and water, vitamins are also essential for the proper growth and nourishment of the body. If an individual lacks sufficient amount of vitamins, diseases of nutritional deficiency will occur.

4.1.2. Classification

According to their property to be dissolved in fat or water, vitamins are classified as:

1) **Fat-Soluble Vitamins:** Vitamin A, D, E, and K are fat-soluble vitamins. These are hydrophobic in nature and are stored in the liver. These are not excreted out of the body; therefore, excessive intake can produce a toxic effect and also result in a condition known as **hypervitaminosis**.

2) **Water-Soluble Vitamins:** Vitamin B complex and vitamin C are water-soluble vitamins. These are compounds of carbon, hydrogen, oxygen, and nitrogen. These are not stored in the body, therefore, are required daily in small quantities.

Vitamins (Chapter 4)

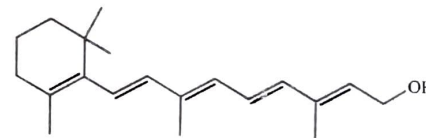
Fat-Soluble Vitamins	Water-Soluble Vitamins
Vitamin A	Vitamin C (Ascorbic acid)
Vitamin D	Vitamin B ₁₂ Complex
Vitamin E	B ₁ (Thiamine)
Vitamin K	B ₂ (Riboflavin)
	B ₃ (Niacin)
	B ₅ (Pantothenic acid)
	B ₆ (Pyridoxine)
	B ₇ (Biotin)
	B ₉ Folic Acid (Folate or Folic Acid)
	B ₁₂ (Cobalamin)

4.1.3. Chemistry

The chemical structures of many vitamins have been established along with their isolation and synthesis. Vitamins can be classified into two types, i.e., **fat-soluble vitamins** (A, D, E, and K) and **water-soluble vitamins** (B and C).

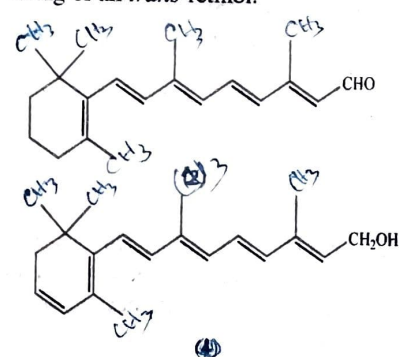
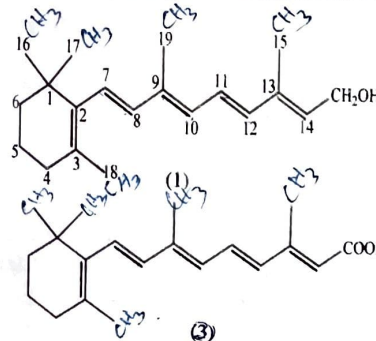
4.1.3.1. Vitamin A (Retinol)

Vitamin A, many of its analogues, and carotenoids are chemically synthesised from simple precursors which are readily available. Vitamin A and carotenoids can also undergo oxidation because of the presence of conjugated double bonds in their structure.



Vitamin A (Retinol)

Chemically, vitamin A is a sub-group of retinoids (compounds with four isoprenoid units joined head-to-tail and containing five conjugated double bonds). The term **vitamin A** is used to describe such retinoids which possess retinol's biological activity. The term **provitamin A carotenoids** is used to describe such carotenoids which possess vitamin A's biological activity. The structure (1) in figure 4.1 shows the numbering of all *trans*-retinol.



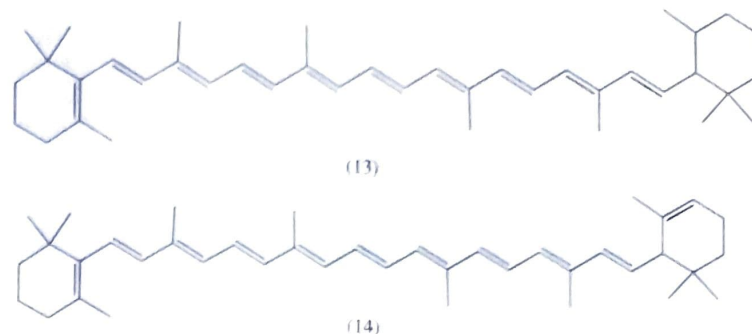
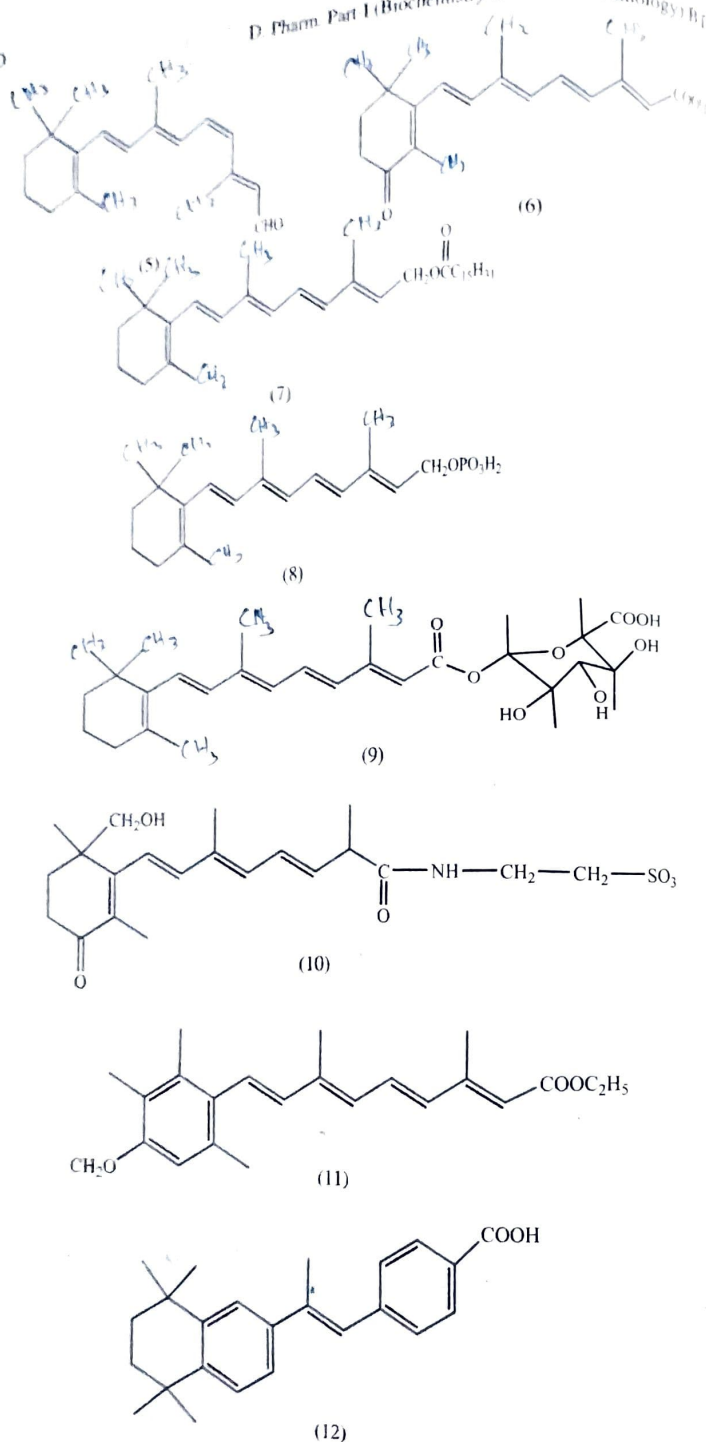


Figure 4.1: Formulas and Numbering Systems for Retinoids and Carotenoids. 1) *trans*-Retinol; 2) *trans*-Retinal; 3) *trans*-Retinoic acid; 4) 3-Dehydrorretinol (vitamin A₂); 5) 11-*cis*-Retinal; 6) 4-Oxoretinoic acid; 7) Retinyl palmitate; 8) Retinyl phosphate; 9) Retinoyl β-glucuronide; 10) Retinotaurine; 11) Trimethylmethoxyphenyl analogue of ethyl retinoate; 12) Tetrahydro, Tetramethylnaphthylisopropenylbenzoic acid; 13) all-*trans* β-Carotene; 14) α-Carotene

The retinoids (2) to (14) (**figure 4.1**) are found in nature and are biologically significant. The biological activity of retinoids (11) and (12) (synthetic compounds) is very high. The nomenclature of carotenoids is based on β-carotene or on β,β-carotene. In **figure 4.1**, the structures (13) and (14) show the formulas for β-carotene and α-carotene.

Retinol possesses 16 and β-carotene possesses 272 geometric isomers which have been already synthesised. The all-*trans* and 3 of the four mono-*cis* isomers of vitamin A (9-*cis*, 11-*cis*, and 13-*cis* forms) perform specific functions in nature. Vitamin A and carotenoids are soluble in most of the organic solvents, but are insoluble in water. When vitamin A and carotenoids are dissolved in dilute solution in the presence of light and oxygen; and at high temperatures they undergo various chemical reactions like oxidation, isomerisation, polymerisation, etc.

These compounds also undergo rapid destruction on getting adsorbed as a thin film in the presence of light and oxygen.

Stability of vitamin A can also be maintained by storing it in frozen liver tissue in the dark at a temperature below -20°C, and in frozen serum at -70°C in sealed vials under ideal conditions. Carotenoids present in stored frozen serum or tissues undergo destruction rapidly than vitamin A, but at -70°C in the dark under argon atmosphere they are stable.

Separation of vitamin A and carotenoids is done by HPLC and detection is done by UV-visible absorption spectrophotometry. However, other physical and chemical methods are also available for their detection.

Vitamin A shows a characteristic UV absorption spectrum with λ_{max} of 325nm and a molecular extinction coefficient of $53,000\text{cm}^2\text{M}^{-1}$ ($E_{1\%}^{1\text{cm}}$ of 1850) in hexane. Carotenoids also show a characteristic absorption spectrum, e.g., β-carotene has a λ_{max} of 450nm in hexane with a molecular extinction coefficient of $136,900\text{cm}^2\text{M}^{-1}$ ($E_{1\%}^{1\text{cm}}$ of 2550).

4.1.3.2. Vitamin D (Calciferol)

In the early 1920s, the term **vitamin D** was given to a group of closely related secosteroids (steroids in which one of the bonds in the steroid rings is broken) which possess anti-rachitic properties. **Cholecalciferol** or vitamin D₃ (derived from animals) and **ergocalciferol** or vitamin D₂ (derived from plants) are the two forms of vitamin D that are obtained from foods. These two dietary forms of vitamin D can also be synthesised in vertebrates and some fungi by photoirradiation from their precursors 7-dehydrocholesterol and ergosterol respectively. Vitamin D₂ and D₃ are chemically different as vitamin D₂ has a double bond and a methyl group in their side chain at C-17. **Figure 4.2** represents the structures of vitamin D and its major metabolites:

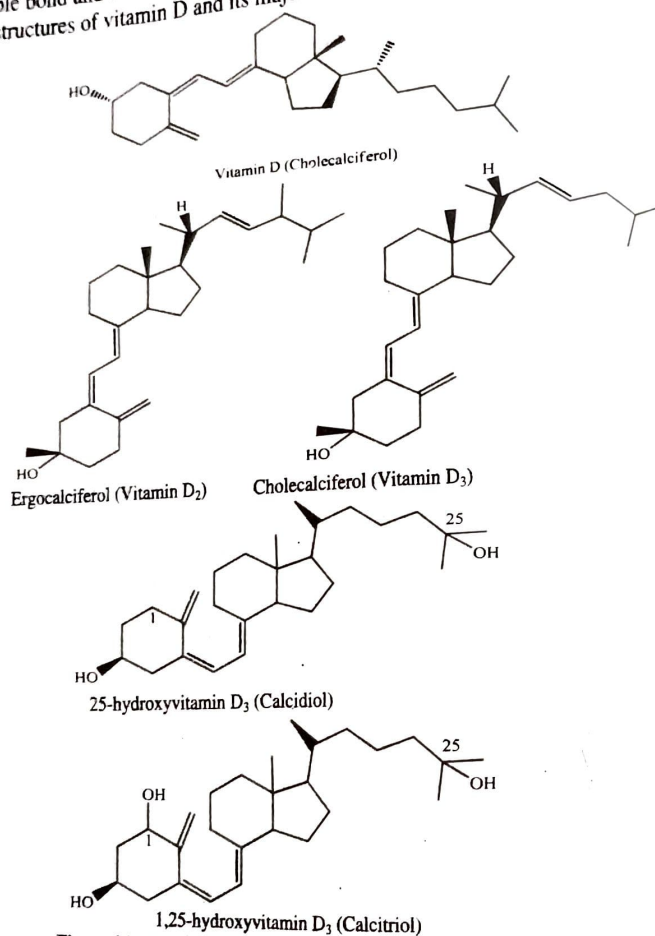


Figure 4.2: Structures of the Major Metabolites of Vitamin D

Chemically, all the forms of vitamin D are secosteroids. Ergocalciferol (vitamin D₂) is formed from ergosterol and is present in plants, while cholecalciferol (vitamin D₃) is found in animals. Both are sources for vitamin D activity and are referred to as **provitamins**.

During cholesterol biosynthesis, 7-dehydrocholesterol is formed as an intermediate which when exposed to sunlight gets converted to cholecalciferol in the skin (dermis and epidermis). Synthesis of vitamin D₃ in the skin is proportional to the exposure to sunlight.

4.1.3.3. Vitamin E (Tocopherol)

The nutritional content of vitamin E is defined in terms of the chemical activity of α -tocopherol. The molecules that contribute α -tocopherol's activity are four tocopherols and four tocotrienols, identified by the prefixes alpha- (α -), beta- (β -), gamma- (γ -), and delta- (δ -). These are derivatives of tocol. All are isoprenoid substituted 6-hydroxy-chromanes. The double ring structure in the compound is a 'chromane' ring.

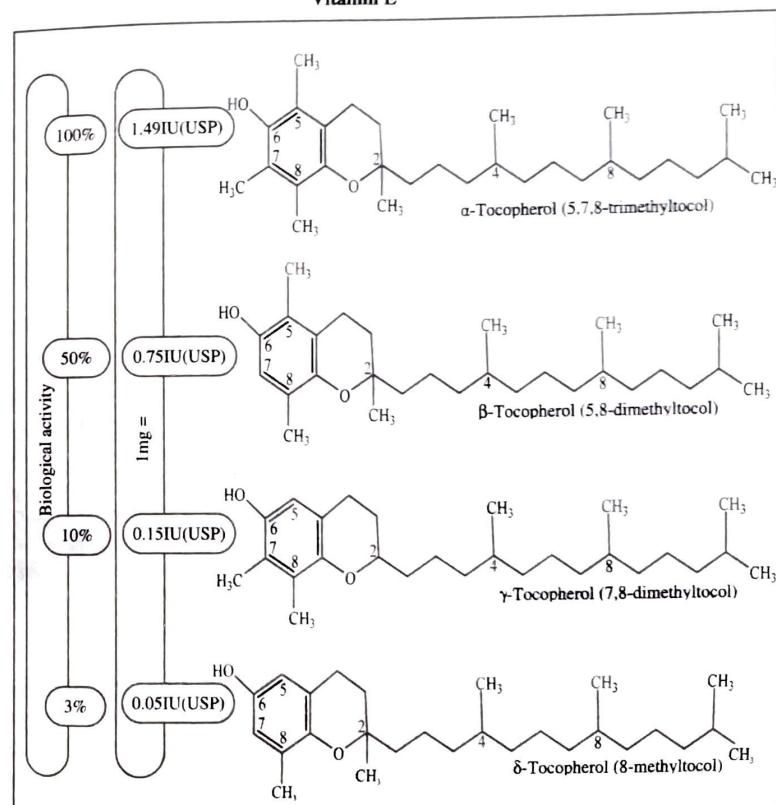
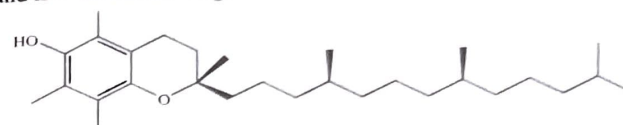


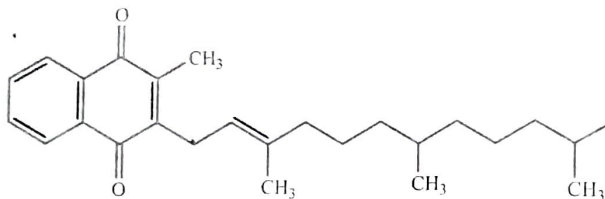
Figure 4.3: Chemistry and Biological Activity

α -Tocopherol exhibits the highest vitamin E activity. It has three chiral centres at 2', 4', 8', shown in **figure 4.3**, at which the methyl groups are in R-configuration.

When any α -tocopheryl esters undergo hydrolysis by lipases or mucosal esterases, vitamin E is absorbed in the intestine with lipids with an average absorption rate of 30%. After absorption, vitamin E is transported to the liver and only small amounts are released from the chylomicrons by LPL on the surface of endothelial cells. Vitamin E in VLDL is released into the bloodstream where a dynamic equilibrium exists between all the lipoprotein fractions due to a rapid interchange. Vitamin D uptake into the target cells is either through release by endothelium-based LPL or via receptor-mediated LDL endocytosis.

4.1.3.4. Vitamin K (Phylloquinone)

Vitamin K was discovered by DAM. in 1939. It is a fat-soluble vitamin and is essential to form prothrombin substance controlling blood coagulation in the liver. It has been proved that the phenomenon of blood clotting which is the summation of action between prothrombin, thromboplastin, and calcium-producing thrombin is related to vitamin K. If any of these factors are deficient, an abnormality in blood clotting occurs. It is notable that human blood can lack prothrombin and still possess a normal or slight subnormal clotting time. However, severe deficiencies of prothrombin extend the clotting time, and haemorrhagic diathesis in jaundice is also associated with lowered blood prothrombin.

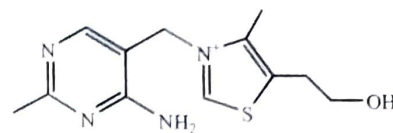


Vitamin K (Phylloquinone)

Chemically, vitamin K family comprises of 2-methyl 1, 4-naphthoquinone (3) derivatives which have poly-isoprenoid side chains. Vitamin K is a complex unsaturated hydrocarbon found in two forms, i.e., vitamin K₁ (Phylloquinone) and vitamin K₂ (Menaquinone). Phylloquinone contains phytyl side chain, whereas menaquinone contains polyisoprenoid side chain. The three synthetic forms of vitamin K (vitamin K₃, K₄, and K₅) are used in many areas. **For example**, vitamin K₃ is used in the pet food industry and vitamin K₅ is used to inhibit fungal growth.

4.1.3.5. Vitamin B₁ (Thiamine)

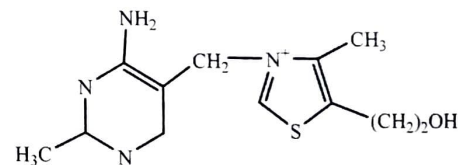
Thiamine, the first water-soluble B vitamin, is identified as an essential nutrient. Its chemical structure consists of a substituted pyrimidine ring and a thiazole connected by a methyl group. The term **vitamin B₁** includes several compounds possessing thiamine-like effects. Naturally-occurring vitamin B₁ consists of thiamine phosphates. Water-soluble thiamine derivatives (thiamine hydrochloride or nitrate) and lipophilic thiamine analogues (benfotiamine or fursultiamine) are used pharmaceutically.



Vitamin B₁ (Thiamine)

Thiamine is absorbed in the jejunum after being released into the intestinal lumen during digestion. This vitamin undergoes absorption by a saturable and rate-limiting active transport mechanism. Minor passive diffusion occurs at higher luminal concentrations. The absorption of physiological doses is nearly 100% while is of 25% pharmacological doses. Lipophilic thiamine analogues are absorbed in amounts corresponding to intakes as they cross the cell membranes more easily. Thiamine present in free form gets converted to active Thiamine Diphosphate (TDP) in intestinal mucosa. This conversion process consumes ATP. About 40% of the total body content of thiamine (~30mg) is found in the musculature. Since its storage is limited and its half-life is short, it is required from external sources daily. It is excreted via renal route either in form of sulphate ester salt of thiamine or as unidentified metabolites.

A thiamine-dependent key enzyme, **transketolase**, is used to assess thiamine status. During the pentose phosphate cycle, the reversible transfer of a C-2 fragment is catalysed by this enzyme. This transfer results in the conversion of various aldoses into ketoses and *vice versa*.



Thiamine (Vitamin B₁)

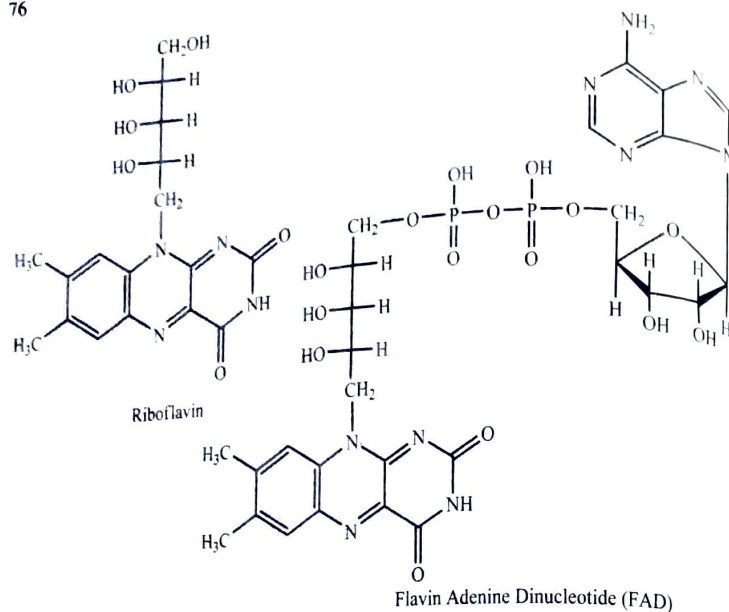
The end-product of glycolysis and breakdown product of glucogenic amino acids is pyruvate which converts into acetyl by the thiamine-dependent pyruvate dehydrogenase enzyme. After this conversion, it can enter the citrate cycle as acetyl-CoA. During the citrate cycle, succinyl-CoA is formed which requires α -ketoglutarate dehydrogenase enzyme, which also depends on a thiamine cofactor.

Thiamine is involved in reactions leading either to decarboxylation of α -keto acids, formation of α -hydroxy ketones, or transfer of an α -keto R-group. During an intermediate stage, a negatively charged C atom is formed whose charge is stabilised by TDP, thus making it available for further reactions at the enzyme complex.

4.1.3.6. Vitamin B₂ (Riboflavin)

The structure of riboflavin is a tricyclic nitrogenous ring system having a 5 carbon side chain in which the last hydroxy group can be esterified with phosphoric acid. Riboflavin is a short name suggested to vitamin B₂ by IUPAC as the old names "ovoflavin" and "lactoflavin" are not be used.

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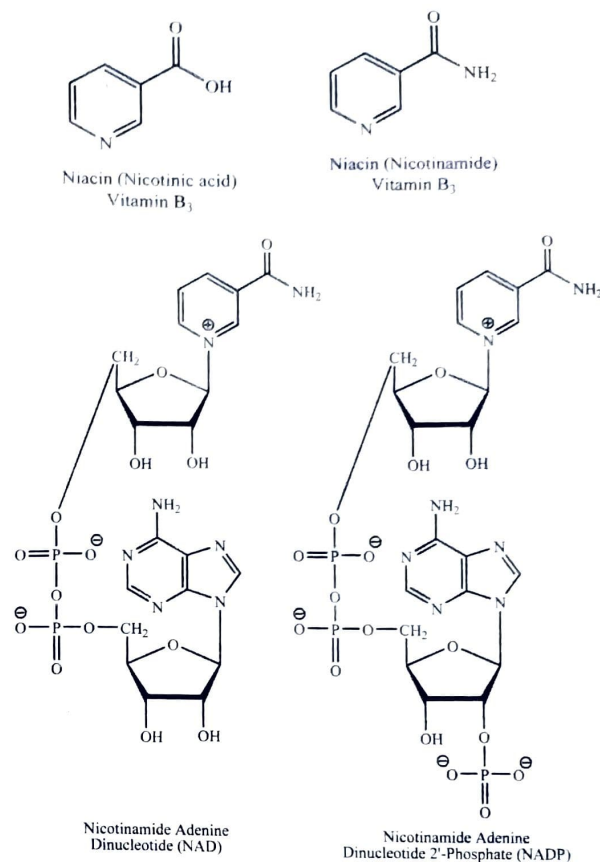


Vitamin B₂ is a component of the flavin coenzymes, FAD (Flavin Adenine Dinucleotide) and FMN (Flavin Mononucleotide). It is composed of an isoalloxazine ring system (5,7-dimethyl isoalloxazine, a heterocyclic 3 ring structure) linked to ribitol by a nitrogen atom. Ribitol is an open chain form of sugar ribose with the aldehyde group (—CHO) reduced to alcohol (CH₂OH). The ability of the ring system of riboflavin to exist as a semi-quinone allows the flavin coenzymes to accept electrons either singly or in pairs.

The free riboflavin, FMN, and FAD present in blood are either bound to albumin or to Riboflavin-Binding Proteins (RFBPs). The storage capacity of riboflavin depends on the amount of apoprotein available. Maximal reserves last for 2-6 weeks, much less in case of protein deficiency. Vitamin B₂ is excreted either as riboflavin or as metabolites like 7- α -hydroxy riboflavin into the renal tubules through active secretion. Its concentration in urine can be used as an indicator of B₂ status, e.g., riboflavin deficiency is indicated if its concentration in urine is <40 μ g riboflavin/g creatinine indicates. FMN and FAD are synthesised in liver, kidneys, and heart under hormonal control (e.g., T₃) with the help of ATP.

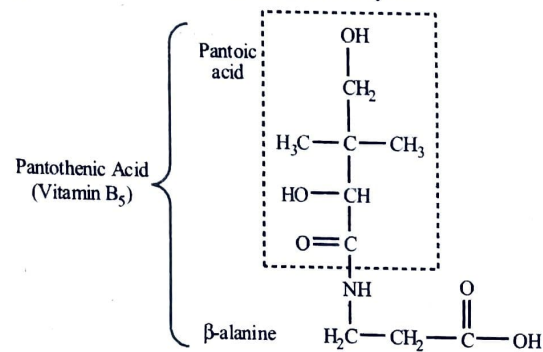
4.1.3.7. Vitamin B₃ (Niacin)

Chemically, niacin is a pyridine-3-carboxylic acid. It mainly exists as an amide. Niacin is the name for both nicotinamide and nicotinic acid, either of which can act as a precursor of nicotinamide coenzymes. Niacin is required for the synthesis of two coenzyme molecules, i.e., NAD (Nicotinamide Adenine Dinucleotide) and NADP (Nicotinamide Adenine Dinucleotide Phosphate). Both the molecules can only be differentiated by the phosphate attached to the 2'-position of the lower ribose ring in NADP.



4.1.3.8. Vitamin B₅ (Pantothenic Acid)

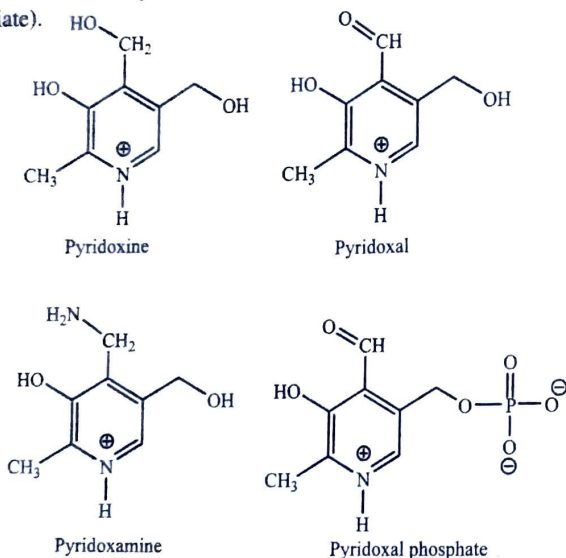
Pantothenic acid is formed from β -alanine and pantoic acid joined by a peptide bond. The active form of the cofactor is produced by the formation of a peptide bond with cysteine, followed by decarboxylation of the cysteine residue, and then by conjugation to the remainder of the coenzyme.



Coenzyme A (usually abbreviated CoA) has a free sulfhydryl group. The sulfhydryl group is used to carry the carbon compounds. The Coenzyme A (A for acyl) is derived metabolically from ATP (Adenosine triphosphate), the vitamin pantothenic acid, and β -mercapto-ethylamine.

4.1.3.9. Vitamin B₆ (Pyridoxine)

Vitamin B₆ exists in three forms, viz., pyridoxine, pyridoxal, pyridoxamine, and their respective phosphates, pyridoxal phosphate (PLP). All these forms consist of pyridine ring as a basic moiety. Pyridoxine is a 3-hydroxy-4,5-dihydroxymethyl-2-methyl pyridine. Pyridoxal contains an aldehyde group (—CHO) at the 4th position of pyridine ring in the structure of pyridoxine. The phosphate form of pyridoxal is pyridoxal phosphate and is an active form. Pyridoxamine contains a primary amide side chain (—CH₂NH₂) at the 4th position of the pyridine ring. The most important catalytic feature of the coenzyme is the electrophilic nitrogen of the pyridine ring (which acts as an electron sink, drawing electrons away from the amino acid, and stabilising a carbanion intermediate).



The three forms of vitamin B₆ namely **pyridoxal**, **pyridoxamine**, and **pyridoxine**, show equal potency in support of animal growth. The most utilised coenzyme form of vitamin B₆ is pyridoxal-5-phosphate which follows different dietary forms due to its inter-convertible nature. Pyridoxal phosphokinase is the only enzyme that can act on all the three non-phosphorylated forms and can be activated in the animal tissues by zinc ions as depicted in **figure 4.4**.

The phosphatase enzymes dephosphorylate both pyridoxal phosphate and pyridoxine phosphate. Pyridoxine oxidase converts pyridoxine and its respective phosphate into pyridoxal derivatives which further bond with the riboflavin 5'-phosphate as a prosthetic group such as B3, S3, etc.

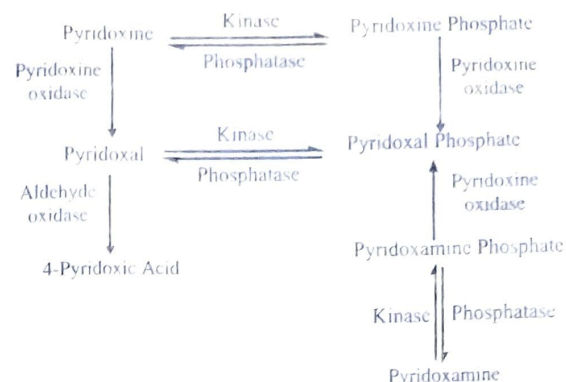


Figure 4.4: Interconversions of Vitamin B₆

In most of the systems, the coenzyme activity is shown by pyridoxal phosphate and pyridoxamine phosphate. Almost all the vitamin B₆-dependant enzymes require pyridoxal phosphate for reconstituting an active holoenzyme, whereas aminotransferases have the ability of either using pyridoxal phosphate or pyridoxamine phosphate. All enzymes (except glycogen phosphorylase) are required in the metabolism of amino acid (B3). These enzymes show two types of reactions:

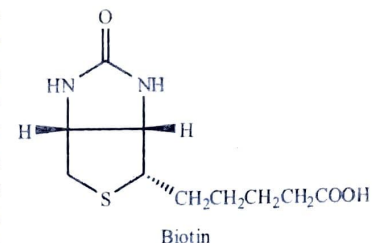
- 1) **Transaminations** (to form α -oxo-acids from α -amino acids), and
- 2) **Decarboxylations** (to form primary amines).

In bacteria, the vitamin B₆-dependent enzymes carry out racemisation of D-amino acids to L-amino acids.

Initially, these enzymes react to form Schiff base between the α -amino group and the 4'-aldehyde group of pyridoxal phosphate. Further, the reaction proceeds in a manner such that the bond on α -carbon present in the amino acid is broken down before the complex gets hydrolysed.

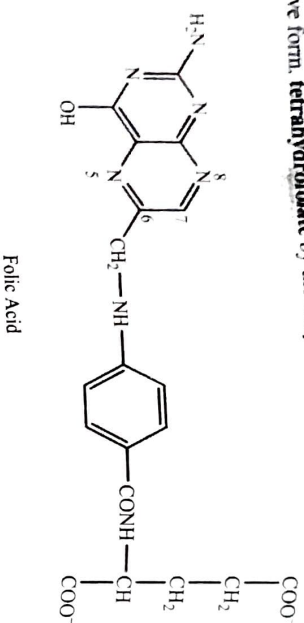
4.1.3.10. Vitamin B₇ (Vitamin H, Coenzyme R, and Biotin)

It consists of two fused 5-membered heterocyclic rings namely **ureido (tetrahydroimidazole) ring** and a **tetrahydrothiophene ring**. A valeric acid substituent is attached to one of the carbon atoms of the tetrahydrothiophene ring. Biotin acts as a coenzyme for carboxylase enzymes. **Biocytin** is the active form of biotin that is 'a lysine-biotin conjugate'. It is formed by combining the carboxyl group of biotin with the terminal nitrogen of lysine residue of enzyme protein. Biotin has two forms, α -biotin (obtained from egg yolk) and β -biotin (obtained from liver).

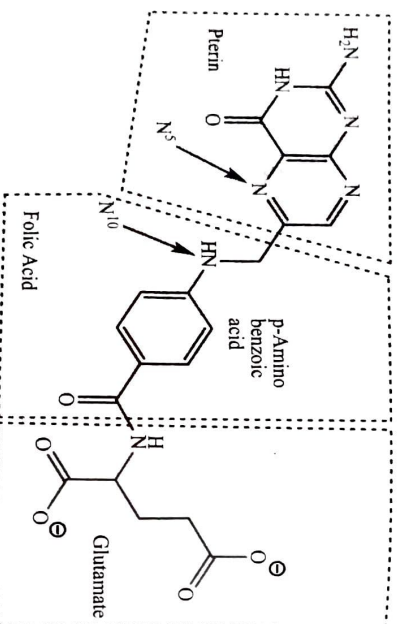


4.1.3.11. Vitamin B₉ (Folic Acid)

The folic acid molecule is composed of three separate parts called **pteridine**, **p-aminobenzoic acid**, and **glutamic acid**. A pteridine ring structure linked to PABA (p-aminobenzoic acid) forms pteroyl acid. Folic acid is then itself generated through the conjugation of glutamic acid residue to pteroyl acid. Humans require folate in the diet because they cannot synthesise PABA (the sunscreen compound) and cannot create the link with the glutamate. The physiologically active form of folate has several glutamate residues, usually 5 in humans, and 7 in plants; although the absorbed form contains a single glutamate due to the removal of the others in the intestines. Folate must be converted to the active form, **tetrahydrofolate** by the enzyme dihydrofolate reductase.

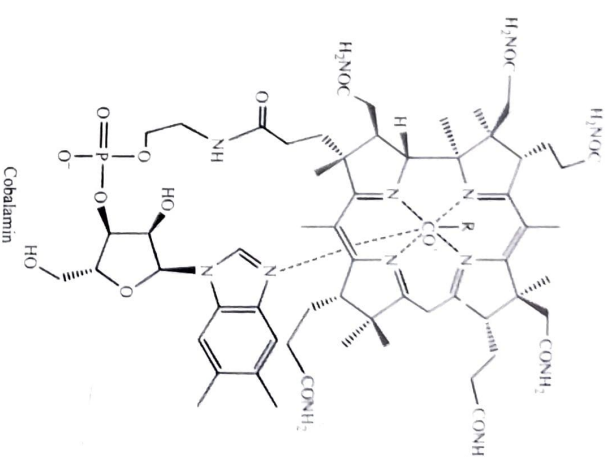


Positions 7 and 8 carry hydrogens in dihydrofolate (DHF)
Positions 5-8 carry hydrogens in tetrahydrofolate (THF)



4.1.3.12. Vitamin B₁₂ (Cyanocobalamin)

Cobalamin was not discovered until 1948; its structure became known in 1955. Chemically, cobalamin is a corrin ring system consisting of pyrrole rings holding a cobalt atom in their centre, to which variable ligands (R) are attached. The cyanide attached to the cobalt, to which variable ligands (R) are attached and is replaced by water or a hydroxyl group in cells. The presence of cobalt and amide gives vitamin B₁₂ like compounds such as **cobamides** or **cobalamines**.



The ratio between the IF/IF-receptor acts as the limiting factor for the absorptive capacity of vitamin B₁₂. There is no absorption of vitamin B₁₂ if the IF (Intrinsic Factor) is scanty or too little, whereas the excess of IF occupies the IF-receptors leading to the inability of IF-cobalamin complex to bind with the receptors. A single dose of 10µg is sufficient for maximum absorption of active cobalamin.

The active form of vitamin B₁₂ in the mitochondria is **adenosylcobalamin**, while in cytoplasm it is **methylcobalamin**. Breakdown of propionate in mitochondria leads to the conversion of methylmalonyl-CoA into succinyl-CoA, which allows the entry of C-3 body of propionate in the citrate cycle. In mitochondria, the amino group is exchanged with H⁺ ion which converts α-leucine into β-leucine.

4.1.3.13. Vitamin C (Ascorbic Acid)

In the year 1932, **Haworth** determined the exact structure of vitamin C (ascorbic acid). When compared to other vitamins, the structure of vitamin C is comparatively simple, it is **aldone-1,4-lactone of L-galacturonic acid** as shown in figure 4.5.

Ascorbic acid is a hexose (6 carbon) derivative. It exists in two forms, i.e., **L-ascorbic acid** and **D-ascorbic acid**. L-ascorbic acid is active, whereas D-ascorbic acid is inactive. The acidic property of vitamin C is due to the enolic hydroxyl groups. It is a strong reducing agent. L-ascorbic acid undergoes oxidation to form dehydroascorbic acid and this reaction is reversible. Both ascorbic acid and dehydroascorbic acid are biologically active.

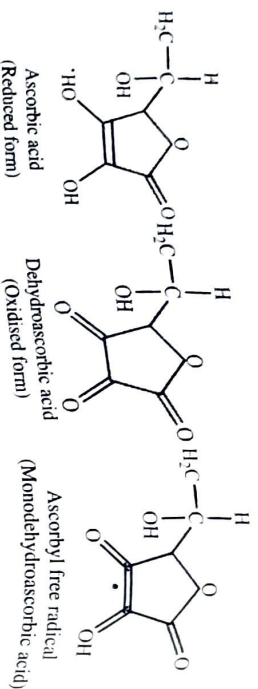


Figure 4.5: Structures of L-Ascorbic Acid, Monodehydroascorbate (MDHA) and Dehydroascorbate (DHA)

The electrons present over C-2 to C-3 region are delocalised, which gives the acidic nature to the molecule. The hydrogen present in the hydroxyl group of C-3 carbon dissociates with pK_a of 4.13. Hence, ascorbic acid exists as a monovalent anion (L-ascorbate) at physiological pH. As the ascorbate donate electrons to a variety of substrates to get converted into **Monodehydroascorbate (MDHA) radical** by oxidation. In case, the oxidation has proceeded, it results in the production of **Dehydroascorbate (DHA)**. Both MDHA and DHA can be reduced to ascorbate by the enzymatic action in plants as well as animals.

On the other hand, DHA undergoes irreversible degradation with a wide number of products ranging from oxalate to L-threonate. Vitamin C is stable in dry form but in a soluble state oxidises readily. This property of vitamin C is used along with the fact that chemical synthesis of ascorbate is relatively easy, due to which it is used as an antioxidant in food preparation to protect against browning.

4.1.4. Role

There are several roles of vitamins:

- 1) ~~They act as hormones (e.g., vitamin D).~~
- 2) ~~They act as antioxidants (e.g., vitamin E and C).~~
- 3) ~~They are mediators of cell signaling, regulators of the cell, and tissue growth and differentiation (e.g., vitamin A).~~
- 4) ~~They are precursors of enzyme cofactor or coenzymes that act as catalysts and substrates in metabolism (e.g., B complex vitamins). Biotin is part of enzymes involved in making fatty acids. Coenzymes help to carry chemical groups between enzymes, e.g., folic acid carries various forms of carbon group, methyl, formyl and methylene in the cell.~~
- 5) ~~They regulate metabolism.~~
- 6) ~~They help in the production of ATP from metabolism of fats, and carbohydrates.~~
- 7) ~~They promote growth and reproduction.~~

A multicellular organism requires vitamins for normal growth and development. The foetus requires certain vitamins and minerals in its developing stage. If one or more of these nutrients are deficient, the child may suffer from a deficiency disease.

sources, deficiency disorders, and role of vitamins can be summarised in **table 4.1:**

Table 4.1: Role of Vitamins

Vitamins	Synonyms	Sources	Deficiency Disorders	Metabolic Role
Vitamin C	Ascorbic acid Ascorbate	Citrus fruits	Scurvy	Involved in hydroxylation of proline, and acts as an antioxidant
Thiamine	B ₁	Meats, mainly pork, salmon, legumes, nuts, and grains	Beriberi, Korsakoff's syndrome, and optic neuropathy	Component of coenzymes including pyruvate dehydrogenase, α -ketoad dehydrogenase, and transketolase
Riboflavin	B ₂	Milk, cheese, green leafy vegetables, and nuts	Cheilosis and angular stomatitis, and dermatitis	Component of enzyme cofactors in many redox reactions
Pantothenic Acid	B ₅	Whole-grain cereals, legumes, eggs, meat, and yogurt	Hypertension	Component of Coenzyme A and phosphopantetheine; a prosthetic group for many enzymes
Pyridoxine	Pyridoxamine, pyridoxal, B ₆	Meat, fish, poultry, organ meats, soy products, nuts, and lentils	Depression, confusion, and convulsions	Involved in haemoglobin synthesis; numerous enzymatic reactions involving Schiff base intermediates
Biotin	B ₇	Yeast, liver and kidney. Egg yolk, soybeans, nuts, and cereals	Biotin deficiency	Coenzyme for carboxylase enzymes
Folic Acid	Folate, folic acid, folacin, vitamin B ₉	Broccoli, Brussels sprouts, liver, spinach, asparagus, peas, chickpeas, and brown rice	Anaemia, glossitis, mental symptoms, and neural tube defects	Involved in the enzymatic transfer of 1 carbon units
Cyanocobalamin	B ₁₂	Meat, salmon, cod, milk, cheese, and eggs	Pernicious anaemia and methylmalonic acidemia	Required for methylmalonyl-CoA mutase and methionine synthase reactions
Niacin	Niacinamide, B ₃	Yeast, meat, fish, milk, eggs, green vegetables, cereal, and beans	Pellagra	Component of NAD and NADP required for oxidation-reduction reactions
Vitamin A	Retinol, retinal, retinoic acid	Cheese, eggs, and yogurt	Night blindness, damage to the cornea, and GI and respiratory tracts	Required for formation of rhodopsin; hormone-like activity in growth and reproduction

Vitamin D	Cholecalciferol, vitamins D2 and D3, 1,25 dihydroxy vitamin D	Only fish, such as salmon, sardines and mackerel, eggs	Rickets and skeletal abnormalities	Regulation of levels of calcium and phosphorus and immune function
Vitamin E	α -Tocopherol and other tocopherols, α -tocotrienol and other tocotrienols	Soy, corn, and olive oil; also nuts, wheat germ, and cereals	Reduced sperm production	Antioxidant scavenger reactive species
Vitamin K	Phylloquinone	Green leafy vegetables, vegetable oils, and cereals	Subdural haemorrhaging	Required carboxylation of glutamic acid in blood clotting proteins

4.1.5. Coenzymes

Small organic molecules, transporting the chemical groups from one enzyme to another, are known as **coenzymes** (e.g., thiamine, riboflavin, and folic acid). For example, the acetyl group carried by coenzyme A, the hydride ion (H^-) carried by NAD or $NADP^+$, formyl, methenyl or methyl groups carried by folic acid, the phosphate group carried by adenosine triphosphate, and the methyl group carried by S-adenosylmethionine.

As a result of enzymatic action, the coenzymes are changed chemically and so it is beneficial to consider them as a special class of substrates or second substrates common to various enzymes. For example, coenzyme NADH is used by about 700 enzymes. There is usually continuous regeneration of the coenzymes leading to the maintenance of their concentrations (inside a cell), at a steady level.

For example, the pentose phosphate pathway regenerates NADPH, and methionine adenosyltransferase generates S-adenosylmethionine. This continuous regeneration denotes the intensive use of even a small amount of coenzyme. For example, each day a human body converts its own weight into ATP energy.

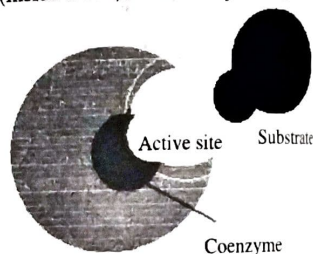


Figure 4.6: Coenzyme with Enzyme

A functional enzyme, i.e., the **holoenzyme**, is made up of a protein and a non-protein part. Protein part is the apoenzyme and non-protein part is the coenzyme.

Holoenzyme = Apoenzyme + Coenzyme
(Protein part) (Non-protein part)

Coenzyme can be defined as the organic, non-protein, dialyzable, and low molecular weight substance, related with the functions of the enzyme. These are regarded as co-substrates or second substrates because their affinity with the enzyme is comparable to that of substrates.

11.5.1. Classification

Coenzymes are classified into:

Coenzymes from B-Complex Vitamins: Important examples are given as below:

- Thiamine Pyro Phosphate (TPP) derived from thiamine (vitamin B_1).
- Flavin Mononucleotide (FMN) derived from riboflavin.
- Flavin Adenine Dinucleotide (FAD) derived from riboflavin.
- Biotin coenzyme derived from vitamin H.

Vitamins, along with their deficiency symptoms, related co-enzyme and chemical reactions catalysed are listed in table 4.2:

Table 4.2: Water-Soluble Vitamins, Effects of Deficiency, Related Coenzyme, and Chemical Reactions Catalysed

Vitamins	Deficiency Symptoms	Coenzymes	Reactions Catalysed
B_1 (Thiamine)	Beri-beri, Polyneuritis	Thiamine Pyrophosphate (TPP)	Cleavage or formation of carbon-carbon bonds adjacent to carbonyl carbon atoms
B_2 (Riboflavin)	Dermatitis, impaired growth and reproduction	Flavin Mono Nucleotide (FMN); Flavin Adenine Dinucleotide (FAD)	Oxidation - reductions including hydride anion transfer, one electron transfer
Nicotinic acid	Pellagra, dermatitis, black tongue (in dogs)	Nicotinamide Adenine Dinucleotide (NAD^+); Nicotinamide Adenine Dinucleotide Phosphate ($NADP^+$)	Oxidation-reductions involving hydride anion transfer
Pantothenic acid	Neurological problems	Co-enzyme A	Transfer of acyl groups
B_6 (Pyridoxine)	Neurological disorders, dermatitis	Pyridoxal phosphate	Transamination, decarboxylation, racemisation
Lipoic acid	None demonstrated	Lipoamide	Oxidation reduction
Biotin (B_7)	None demonstrated in absence of avidin; with avidin, dermatitis	Biotin carboxyl-carrier-protein	Carboxylation, Transcarboxylation
Folic acid (B_9)	Anaemia	Tetrahydrofolic acid	Transfer of one-carbon units
Vitamin B_{12}	Pernicious anaemia	Co-enzyme B_{12}	Rearrangements
Ascorbic acid	Scurvy	-	Hydroxylation

- 2) **Non-Vitamin Coenzymes:** Not all co-enzymes are vitamin derivatives. There are some other organic substances which have no relation with vitamins but function as co-enzymes. They may be considered as non-vitamin co-enzymes, e.g., ATP, CDP, UDP, etc. The important non-vitamin co-enzymes along with their functions are given in table 4.3:

Table 4.3: Co-Enzymes not Related to B-Complex Vitamins

Coenzymes	Abbreviations	Biochemical Functions
Adenosine Triphosphate	ATP	Donates phosphate, adenosine and Adenosine Monophosphate (AMP) moieties.
Cytidine Diphosphate	CDP	Required in phospholipid synthesis as a carrier of choline and ethanolamine.

Uridine Diphosphate	UDP	Carrier of monosaccharides (glucose, galactose, required for glycogen synthesis).
S-Adenosylmethionine (active methionine)	SAM	Donates methyl group in biosynthetic reactions.
Phosphoadenosine Phosphosulphate (active sulphate)	PAPS	Donates sulphate for the synthesis of mucopolysaccharides.

4.1.5.2. Role

Vitamins play a vital role in various metabolic processes and are also used as coenzymes. Thus, the importance of vitamins as coenzymes is similar to their biochemical function. The various vitamins are:

- 1) **Ascorbic Acid:** Also known as **vitamin C**, ascorbic acid is mainly found in citrus fruits and in many mammals, but it is absent in primates and guinea pigs. Plants and animals can synthesise ascorbic acid directly from glucose.

Occurrence: In 1932, Waugh and King first obtained ascorbic acid from fresh lemon juice. A healthy human body requires about 1mg/100ml of blood plasma of ascorbic acid. It is found in the cortex part of the adrenal gland and takes part in the synthesis of steroid hormones.

Biochemical Functions: It acts as a strong reducing agent and oxidises in the presence of ascorbic acid oxidase (an enzyme obtained from certain plants) to yield dehydroxyascorbic acid. It provides resistance against many diseases and also helps in the hydroxylation of some aromatic complexes.

- 2) **Thiamine:** It is also known as **thio-vitamine** or **vitamin B₁**. It is the sulphur-containing vitamin of B complex that is water-soluble in nature. It was firstly named as **aneurin** because it produces detrimental neurological effects due to its deficiency in the diet. Many cellular processes involve the phosphate derivatives of thiamine. TPP (Thiamine Pyro Phosphate) is the best characterised thiamine form that acts as a coenzyme in sugars and amino acids catabolism. TPP is also needed in the first step of alcoholic fermentation by the yeast.

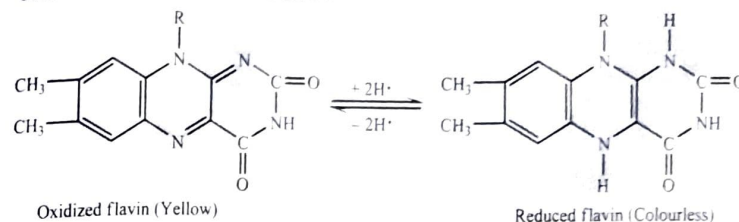
Occurrence: It occurs as a coenzyme cocarboxylase or thiamine pyrophosphate in yeast and animal tissues.

Biochemical Functions: TPP (Thiamine Pyro Phosphate) shows participation in transketolase, α -keto acid dehydrogenases, phosphoketolase, and pyruvic decarboxylase as a coenzyme. In certain bacteria, the enzyme is concerned with pentoses metabolism.

- 3) **Riboflavin:** It is also known as **vitamin B₂** or **E101 food colour**. It is a micronutrient that can be absorbed easily and plays a crucial role in the maintenance of normal metabolic processes. Riboflavin is needed by all flavoproteins because it is the central component of FMN and FAD cofactors. Therefore, Vitamin B₂ is needed for a broad range of cellular processes. It plays crucial role in the metabolism of energy, ketone bodies, fats, proteins, and carbohydrates (like other B vitamins).

Occurrence: It is found in the form of two flavin coenzymes, viz., FAD (Flavin Mono Nucleotide) and FMN (Flavin Adenine Dinucleotide).

Biochemical Functions: Due to its ability to undergo oxidation-reduction reactions, riboflavin functions as a coenzyme. The yellow colour of riboflavin disappears when it undergoes reduction since reduced flavin is colourless. But the yellow colour reappears when the reduced flavin is exposed to air, resulting in oxidation. The overall oxidation-reduction reaction consists of two hydrogen atoms in order to form a colourless reduced flavin, as shown below:



R = Remainder of FMN or FAD molecule

- 4) **Nicotinamide:** It is also known as **nicotinic acid amide** or **niacinamide**. It is the amide of vitamin B₃ or niacin or nicotinic acid. Nicotinamide is a part of the vitamin B group that is water-soluble in nature. Niacin or nicotinic acid is converted to nicotinamide *in vivo*. Both of these have identical vitamin functions but differ in toxic and pharmacologic effects.

Occurrence: The coenzyme is found in the form of vitamins, nicotinamide nucleotide coenzymes- NAD⁺ (Nicotinamide Adenine Dinucleotide) and NADP⁺ (Nicotinamide Adenine Dinucleotide Phosphate). Meat products are excellent source of vitamin B₃.

Biochemical Functions: Nicotinamide nucleotides act as coenzyme for dehydrogenases which catalyse various oxidation-reduction reactions. These coenzymes are described as co-substrates as their stoichiometric participation in dehydrogenase catalysed reaction can be easily demonstrated. For example, assume a reaction in which one mole of ethanol is reversibly oxidised by one mole of NAD⁺, to form one mole each of NADH, acetaldehyde and H⁺. This entire reaction is catalysed by an enzyme widely distributed in nature (i.e., Alcohol Dehydrogenase, ADH).

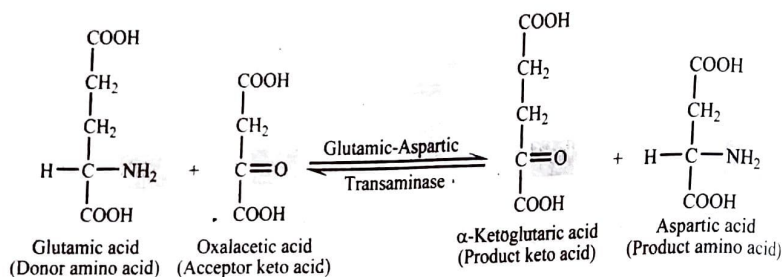


- 5) **Vitamin B₆ Group:** It is a part of vitamin B complex and is water-soluble in nature. Pyridoxal Phosphate (PLP), an active form of this vitamin among all the other forms, is a cofactor which involves in metabolism reactions of amino acids, viz., deamination, transamination, and decarboxylation. PLP is also essential for the reactions which involve the glucose release from glycogen, in the presence of enzymes.

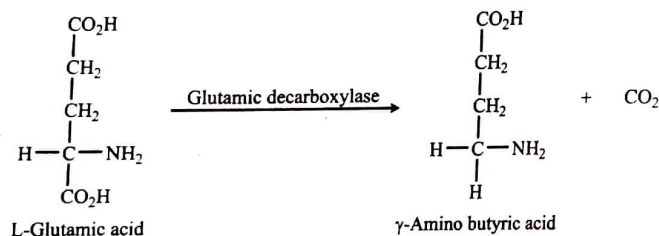
Occurrence: The phosphate pyridoxamine derivatives and pyridoxal are naturally found as coenzyme of **vitamin B₆**.

Biochemical Functions: The essential metabolism reactions of amino acid (racemisation, transamination, and decarboxylation) are catalysed by pyridoxal phosphate, thus, proving it to be a versatile coenzyme.

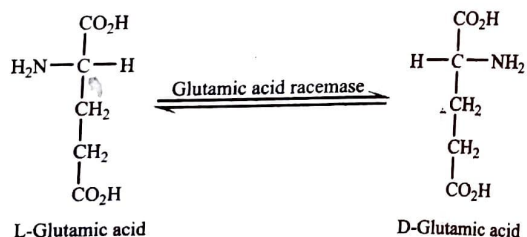
Transamination



Decarboxylation



Racemisation



- 6) **Lipoic Acid:** It is derived from the octanoic acid and itself is an organosulfur compound. The structure of LA contains 2 sulphur atoms which are attached to each other by a disulphide bond and are responsible for oxidation. The molecules of LA exist in the form of two enantiomers, i.e., S-(-)-Lipoic Acid (SLA) and R-(+)-Lipoic Acid (RLA). They also exist in the form of a racemic mixture, i.e., R/S-Lipoic Acid (R/S-LA). Out of these enantiomers, the one existing in nature, i.e., R-(+)-enantiomer is an essential cofactor for four complexes of mitochondrial enzymes.

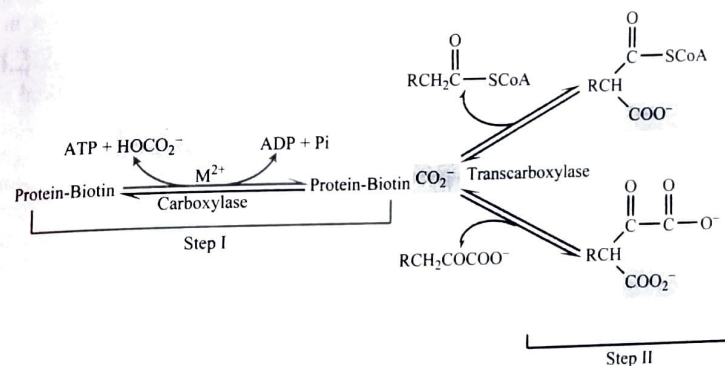
Occurrence: Liver and yeast are rich sources of lipoic acid.

Biochemical Functions LA is a cofactor of the complexes of multi-enzymes, α-ketoglutaric dehydrogenase and pyruvic dehydrogenase. The lipoyl containing enzymes present in these complexes catalyse the production and transfer of acyl groups. Also during this process, it undergoes reduction and then re-oxidation.

- 7) **Biotin:** It is a B-complex vitamin, also known as **vitamin B₇** and is water-soluble. It has a **tetrahydroimidazole (ureido) ring** which is fused with a **tetrahydrothiophene ring**. On one of the carbon atoms of the tetrahydrothiophene ring, a valeric acid substituent is attached. The biotin functions as a coenzyme in fatty acid and leucine metabolism and also plays a role in gluconeogenesis.

Occurrence: Excellent sources of biotin in nature are liver and yeast. Biotin is found in combined forms mainly where it is bound to the protein via ε-N-lysine moiety.

Biochemical Functions: Biotin, bound specifically to an enzyme protein, is associated with carboxylation reactions. Biotin-dependent carboxylases catalyse the entire reaction, occurring in two different steps as shown below:

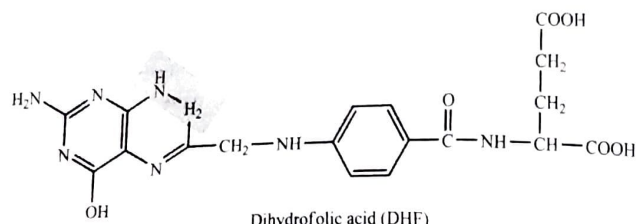


- 8) **Folic Acid:** It is also known as **folacin** or **vitamin B₉**. Other forms of water-soluble vitamin B₉ is folate (i.e., naturally occurring), and **pteroyl-L-glutamate**.

Folic acid is not a biologically active compound. After the conversion of folic acid into dihydrofolic acid in the liver, tetrahydrofolate and other derivatives are obtained.

Occurrence: Folic acid and its derivatives, tri- and hepta-glutamyl peptides, are widely spread in nature.

Biochemical Functions: Folic acid is a vitamin, and its reduced form acts as the coenzyme. Reduction of folic acid to dihydrofolic acid (DHF) occurs in the presence of enzyme L-folate reductase.

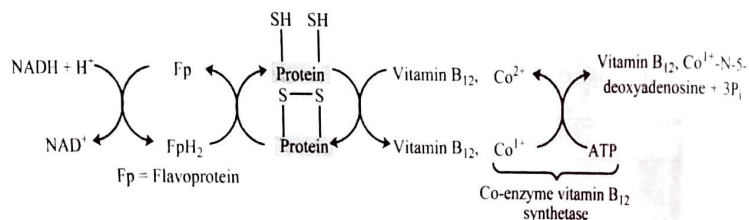


- 9) **Vitamin B₁₂:** It is also known as **cobalamin** and is a water-soluble vitamin. It plays a vital role in the proper functioning of the brain and nervous system. It is one of the eight types vitamin B and is essential for blood formation. It is involved in each and every cell metabolism in the human body.

It also affects the synthesis and regulation of DNA, the synthesis of fatty acids and production of energy. It can be industrially produced via bacterial fermentation synthesis because of its complex and large structure.

Occurrence: Vitamin B₁₂ is only found in animals and micro-organisms as a part of coenzyme B₁₂.

Biochemical Function: A specific B₁₂ coenzyme synthetase helps in the synthesis of coenzyme from vitamin B₁₂.



- 10) **Pantothenic Acid:** It is also known as a **B-vitamin, Vitamin B₅**, or **pantothenate**, and is water-soluble. It is an essential nutrient for many of the animals. Pantothenic acid is required for the synthesis of acetyl CoA, and in the synthesis and metabolism of carbohydrates, fats and proteins.

It has the ability to stimulate yeast growth. It is also used to synthesise coenzyme-A (CoA) and synthesise and metabolise carbohydrates, fats, and proteins.

Occurrence: Pantothenic acid occurs naturally, as a component of coenzyme A and Acyl-Carrier Protein (ACP).

Biochemical Functions: Coenzyme A is synthesised with the help of pantothenic acid. Pantothenic acid as a Coenzyme A is also utilised in acetylation and acylation reactions which involves in transduction of signal and activation and deactivation of enzymes, respectively.

4.2. MINERALS AND WATER

4.2.1. Introduction

Minerals mostly form complexes with insoluble fibres or phytates in the intestine; this hinders the intestinal absorption of dietary minerals, except for sodium and potassium. Few minerals are found in free and unbound form in blood circulation, while some are transported with the help of specific binding proteins- β_1 (e.g., transferrin for iron).

Specific binding proteins are required to store minerals (e.g., for storing iron, apoferritin protein is needed). Minerals are eliminated from the body by renal or hepatobiliary route. This hampers the absorption of minerals, and therefore most of the consumed minerals are eliminated through faeces.

Various specific carrier proteins are needed for the absorption of minerals; for example, the mucosal binding factor is required for absorption of zinc, and an intracellular carrier molecule is required for absorption of iron. Formation of these carrier proteins is an important factor for maintaining the level of minerals in the body.

4.2.2. Mineral Deficiency or Excess in Body

In table 4.4, total content of essential minerals in a normal adult body is given. Deficient as well as excessive conditions of these minerals can be dangerous. The circulating levels of a mineral show net result of its absorption, utilisation, storage, and excretion; therefore loss of control over these processes (besides inappropriate dietary intake) leads to mineral imbalances. For example, deficiency of iron occurs due to the following reasons:

- 1) Low consumption of iron in the diet,
- 2) Decreases in intestinal absorption,
- 3) Extreme loss due to bleeding.

Table 4.4: Total Body Content (in Moles) of Some Minerals in Adult Male of 70 kg Body Weight

Sodium	4.2	Iron	0.07
Potassium	3.0	Zinc	0.004
Chloride	0.0014	Copper	0.002
Magnesium	0.0012	Manganese	0.0002
Calcium	34	Iodine	0.0005
Phosphorous	23		

Deficiency of minerals may lead to the specific medical disorder as they are involved in various body functions, for example, microcytic hypochromic anaemia occurs due to iron deficiency. Excess concentration of these minerals in the body gives toxic effects, e.g., hemochromatosis (functional impairment of liver and pancreas) occurs due to high concentration of iron; Wilson disease (a condition of hepatic-lenticular degeneration) occurs due to high concentration of copper; Molybdenosis occurs due to high concentration of molybdenum.

4.2.3. Classification of Minerals

The following two categories of minerals essential within the body:

- 1) **Macro-Minerals or Essential Minerals:** The daily requirement of these minerals is more than 100mg per day.
- 2) **Micro-Minerals or Trace Minerals:** The daily requirement of these minerals is less than 100mg per day.

4.2.3.1. Essential Minerals

A sufficient intake of all the essential elements is vastly desired. To support the biochemical processes basically seven minerals are required, which either act as electrolytes or are required in cell structure and many other functions. In our daily diet, it has been seen that the dietary bulk "mineral elements" (RDA > 200mg/day) are arranged alphabetically in the following manner:

- 1) **Sodium:** It is the chief cation of the extra-cellular compartment. Around 50mmol/kg body weight of sodium is found in the body of a healthy adult man; of this amount around 50% is present in bones, 40% in extracellular fluids, and 10% in soft tissues. Sodium concentration in plasma is about 135-145mmol/litre, in extracellular fluids it is high, and in intracellular fluid the concentration is low, i.e., only about 35mmol/L.

Daily Requirement and Dietary Sources

Around 5-10 gram of sodium is present in a normal Indian diet in the form of table salt (sodium chloride), meat, fish, eggs, milk, cheese, cauliflower, spinach, legumes, and nuts. In a tropical country like India, the daily requirement of sodium is around 5-6 grams. Excessive consumption of sodium causes hypertension.

Biochemical Functions

Sodium performs many functions in the body like nervous tissue irritability, maintaining of osmotic pressure and pH of body fluids, and membrane transport, as mentioned below:

- i) **Neuromuscular Excitability:** Sodium together with potassium cations increase the neuromuscular excitability which is counterbalanced by divalent cations (calcium and magnesium).
- ii) **Regulation of Fluid:** Sodium cations are the major cations of extracellular fluid, therefore they are actively involved in maintaining the osmotic pressure. They also retain water in ECF.
- iii) **Acid-Base Balance:** Sodium is the element of bicarbonate buffer system in the form of sodium bicarbonate salt. In renal tubules, sodium-potassium exchange takes place to acidify urine.
- iv) **Membrane Transport:** Active absorption of various substances across the plasma membrane occurs through sodium co-transport.

Absorption

- i) Sodium is absorbed through a sodium pump which involves Na^+/K^+ -ATPase (or Na^+/K^+ -adenosine triphosphates).
- ii) Active absorption of Na^+ requires energy which is obtained by the metabolism of glucose and amino acids.

Clinical Manifestations

- i) **Hypernatraemia:** This condition is less common than hyponatraemia and occurs due to the presence of high amount of sodium. It can occur due to hyperactivity of adrenal cortex (Cushing's syndrome) or due to the continuous administration of corticosteroids. It may also be caused by excessive administration of saline. In hypernatraemia, water and sodium is retained in the body and patient suffers from puffiness (specially of the face).
- ii) **Hyponatraemia:** This is caused due to gastroenteritis with diarrhoea, vomiting, severe burns, obstructions in small intestine, Addison's disease, and by using mercurial diuretics.
- 2) **Potassium:** It is the major intracellular cation, and is generally found in tissues and body fluids.

Daily Requirement and Dietary Sources

A healthy adult human requires around 2.4gram of potassium, while a child requires around 1.3gram of potassium.

It is widely present in vegetables.

Biochemical Functions

- i) Some functions of potassium are similar to sodium.
- ii) During water retention or loss, serum potassium concentration does not change significantly.
- iii) Insulin stimulates the cellular uptake of potassium.
- iv) It maintains acid-base balance. A mutual relationship exists between the potassium and hydrogen ions. Potassium ions are disposed from cells for retaining electro neutrality during acidosis occurs. Therefore, potassium regulates acid-base balance.
- v) It helps in proper functioning of cardiac muscles and muscular system. If potassium is present in excess or low amount in the body, it may lead to life-threatening significances.
- vi) It regulates enzymatic actions, e.g., pyruvate kinase needs K^+ as a cofactor.
- vii) It helps in neurotransmission.
- viii) If glomerular filtrate is low; the kidney cannot excrete the potassium load from the body. Acidosis intensifies this condition.
- ix) An excess amount of potassium is seen in Addison's disease and in adrenalectomy.
- x) Damaged cells release potassium ions.

Absorption

It is easily absorbed in the body. It diffuses against the concentration gradient from the intracellular to the extracellular fluid. Potassium ions are transported into the cells by sodium pump; for every two potassium ions that are pumped in, three sodium ions are pumped out.

Clinical Manifestations

- i) **Hyperkalaemia:** It occurs due to:
 - a) The kidneys become unable to excrete a potassium load when GF is low,
 - b) In Addison's disease and adrenalectomy, high levels of potassium are observed, and
 - c) Potassium ions are released from damaged cells.
- ii) **Hypokalaemia:** It occurs due to:
 - a) Gastrointestinal losses, diarrhoea, vomiting, and surgical fistula,
 - b) Renal diseases (increased aldosterone production),
 - c) Administration of diuretics and corticosteroids, and
 - d) During alkalosis, potassium ions moves from the extracellular fluid to the intracellular fluid.
- 3) **Chloride:** It is the chief anion in the body. Generally, the normal serum chloride levels undergo parallel alterations with sodium levels. Around 125mmole/litre chloride is found in the Cerebrospinal Fluid (CSF) which is more than in any other body fluid; approximately 80 gram of chloride ions are present in an average adult.

Daily Requirement and Dietary Sources

An average adult requires around 5-10 gram of chloride daily. The chief sources are table salt (NaCl) and foods like meat, fish, cheese, cereals, eggs, etc. Thus, consumption of sodium and potassium occurs parallelly.

Biochemical Functions

Chloride ions regulate osmotic pressure of extracellular fluids along with sodium cations. They help in the synthesis of hydrochloric acid in the stomach and also maintains blood pH.

Absorption

Chloride ions are absorbed by passive diffusion in proximal ileum (part of small intestine); while in distal ileum and in renal tubules, absorption of chloride ions occurs in exchange for bicarbonate ions.

Serum chloride levels changes along with sodium concentration; however, it does not occur in metabolic alkalosis.

Clinical Manifestations

- i) **Hyperchloraemia** (or increased chloride concentration) occurs due to:
 - a) Loss of excess fluid (dehydration),
 - b) Adrenocortical hyperactivity of adrenal steroids which causes increased reabsorption from renal tubules,
 - c) Diarrhoea which results in loss of bicarbonate and retention of chloride ions, and
 - d) Respiratory alkalosis, metabolic acidosis, and renal tubular acidosis.

- ii) **Hypochloraemia** (or decreased chloride concentration) occurs due to:
 - a) Severe vomiting or loss of HCl, and so compensatory rise in bicarbonate occurs,
 - b) In Addison's disease, mineralocorticoid depletion occurs which in turns impairs reabsorption of chloride from renal tubules,
 - c) Prolonged gastric suction, and
 - d) Respiratory acidosis and metabolic alkalosis.

- 4) **Magnesium:** It is the fourth most abundant cation in the body after sodium, potassium, and calcium cations; while in intracellular space it is the second most abundant cation after potassium. In human, it acts as an activator for around 300 enzymes. A healthy adult contains around 25 gram of magnesium; around 70% of which is found in the skeleton, around 30% in liver, muscle (soft tissues) and body fluids, and only 1% in blood and Extracellular Fluid (ECF). Magnesium pool of skeletal and ECF can interchange easily with each other, but not with the magnesium present in intracellular spaces.

Approximately 1.6-2.3mg/dl of magnesium is found in serum; of which, around 20-25% is protein bound. Like calcium ions, the biologically active magnesium remains as ionised (unbound) part of serum magnesium. The concentration of intracellular magnesium ions (about 10mmol/L) is much higher than the magnesium present in ECF; most of the intracellular concentration of magnesium ions is found in cellular organelles. The concentration of magnesium ions in RBCs is about 20mmol/L.

Daily Requirement and Dietary Sources

Around 350mg/day of magnesium is needed for a healthy adult man; and about 300mg and 150-250mg of magnesium per day are required for adult women and children, respectively. Around 450mg/day magnesium is required to maintain positive magnesium balance during pregnancy and lactation.

Chief food sources of magnesium are nuts, cereals, beans, fish, almonds (rich source), and leafy green vegetables (high chlorophyll content).

Biochemical Functions

- i) **Enzyme Activator:** Magnesium activates many enzymes, especially those in which the substrate is a complex of ATP and magnesium complex (ATP-Mg²⁺). The enzymes requiring magnesium are involved in various metabolic reactions like trans-cellular ion transport, muscle contraction, and oxidative phosphorylation. **Examples** of some enzymes having magnesium ions are hexokinase, phosphofructokinase, pyruvate kinase, thiokinase, squalene synthase, glutamine synthetase, adenylate cyclase, etc.
- ii) **Neuromuscular Excitability:** It is decreased by high concentration of magnesium ions; while low concentration of magnesium ions increases nerve excitability.
- iii) **Glucose Tolerance:** It is improved in the presence of magnesium ions as insulin-dependent glucose uptake is increased.

- iv) **Others:** Magnesium is needed for DNA replication, transcription, and translation. It also binds to nucleoside phosphate and nucleic acids. The DNA helix is stabilised by binding of histones and magnesium to the open phosphate groups. For photosynthesis in green plants, chlorophyll (a magnesium porphyrin complex alike to haeme) is absolutely necessary.

Absorption

Magnesium is absorbed mainly in the small bowel through specific carrier mechanism. Factors increasing the absorption of magnesium ions are Vitamin D, PTH, high-protein intake, and neomycin therapy; whereas consumption of calcium and high protein, fatty acids, phylates, and phosphates decreases its absorption.

Clinical Manifestations

Hypomagnesaemia is a condition of low level of serum magnesium which contributes to a poor prognosis for the patient, especially when magnesium ion concentration is below 1.7mg/dl. Causes of hypomagnesaemia are diabetes, prolonged alcoholism, liver cirrhosis, hyperparathyroidism, aldosteronism, protein calorie malnutrition, and drug therapy. It may lead to many health problems, e.g., neuromuscular hyperirritability, tremors, increased vascular resistance, coronary vasospasm, and hypertension.

A condition of increased level of serum magnesium is known as **hypermagnesaemia** which generally occurs in conditions of renal failure.

- 5) **Calcium:** It is a chief constituent of bones and teeth; and in blood, half of the calcium is found in ionised form and rest in unionised form. A part of the unionised calcium bounds to citrate and some bounds to protein. Calcium is consumed in the diet in the form of calcium phosphate or carbonate.

Ca-to-P Ratio: The product of $\text{Ca} \times \text{P}$ should be 50mg/dl in children and 40mg/dl in adults. In rickets, it is less than 30mg/dl.

Daily Requirement and Dietary Sources

Around 0.5gm/day of calcium is required for a healthy adult man; and about 1.5gm and 1.0gm of calcium per day is required for pregnant women and children, respectively.

The chief sources of calcium are milk, eggs, fish, and vegetables. Cereals (wheat and rice) also contain a small amount of calcium.

Biochemical Functions

- Bone Calcification:** It is required for the calcification of bones and teeth.
- Blood Coagulation:** It plays a significant role in the coagulation of blood as it helps in the conversion of the prothrombin to thrombin and in the synthesis of thromboplastin.

- Action of Enzymes:** It acts as an activator for many enzymes (e.g., lipase, succinic dehydrogenase, ATPase, and some proteolytic enzymes).
- Contraction of Muscle:** Calcium ions help in the muscle contraction by neutralising the negative charge of myosin which further combines with actin. Calcium ions activate the myosin ATPase enzyme which hydrolyses the Adenosine Triphosphate (ATP), thus provides energy for muscle contraction.
- Excitability of Neuromuscular:** It is required for nerves excitation.
- Hormone Action:** It acts as an intracellular secondary messenger of different hormones.
- Hormone Release:** It facilitates the release of certain hormones such as insulin, PTH, and calcitonin.
- Membrane Permeability:** It increases the permeability of plasma membrane, and thus the effect is counterbalanced by the opposite action of sodium and potassium capillary permeability.

Absorption

Calcium is absorbed in the first and second parts of duodenum against electric and concentration gradients. Mechanisms involved in calcium absorption are simple diffusion and active transport involving energy and the Ca^{2+} pump. Calcium absorption increases in presence of vitamin D, parathyroid hormone (PTH), acidity, and amino acids (lysine and arginine). Calcium absorption decreases in the presence of phytic acid or hexaphosphate of inositol (in cereals), oxalates (in leafy vegetables), malabsorption syndrome, and foods having high-phosphate.

Clinical Manifestations

Most of the calcium deficiencies resemble vitamin D deficiencies (like rickets, osteoporosis, and hyper excitability). Recent studies show that inadequate consumption of calcium results in hypertension.

- Hypercalcaemia:** Symptoms of hypercalcaemia are nausea, lethargic (tiredness) conditions, loss of appetite, and tendency to fracture bones. Hypercalcaemia may occur in the following conditions:
 - Hyperparathyroidism,
 - Multiple myeloma,
 - Metastatic carcinoma of bone,
 - Milk-alkali syndrome,
 - Treatment with drugs such as diuretics, and
 - Hypervitaminosis D.
- Hypocalcaemia:** It may occur in the following conditions:
 - Tetany calcium levels fall below 7mg/dl,
 - Hypoparathyroidism thyroidism,
 - Fanconi's syndrome (disorder of tubular reabsorption),
 - Acute pancreatitis,
 - Vitamin D deficiency, and
 - Chronic renal failure.

- 6) **Phosphorus:** It is one of the major components of bones and helps in bone mineralisation. It is also used for the production of energy. In a healthy adult human, phosphate concentration is about 1kg 80% of which is present in bone and teeth, while 10% is in muscles.

Daily Requirement and Dietary Sources

Around 500mg/day of phosphate is required for a healthy adult man; and about 1gm and 400-600mg of phosphate per day is required by pregnant women and children, respectively.

Chief sources of phosphate are cheese, milk, nuts, eggs, etc.

Biochemical Functions

- Formation of Bones and Teeth:** After calcium, phosphorus is the major constituent of bones and teeth.
- Production of High Energy Phosphate:** It is required for the production of high-energy phosphates like Adenosine Triphosphate (ATP), Cytidine Triphosphate (CTP), Guanosine Triphosphate (GTP), etc.
- Formation of Nucleic Acid:** The backbone of DNA and RNA is formed of phosphate diester linkages.
- Activation of Enzymes:** Phosphorylation helps in the activation of some enzymes.
- Production of Biochemical Compounds:** Phosphate is also required for the formation of various biochemical compounds like phospholipids, phosphoproteins, lipoproteins, and nucleotides.

Absorption

Phosphorus absorption mostly occurs in the jejunum. Its absorption is stimulated by PTH (parathormone) and vitamin D₃.

Clinical Manifestations

- Hyperphosphatemia** is caused by:
 - Increased absorption of phosphate,
 - Increased cell lysis due to chemotherapy for cancer or due to bone secondaries,
 - Decreased excretion of phosphorus,
 - Renal impairment,
 - Massive blood transfusions, and
 - Drugs (e.g., chlorothiazide, nifedipine, and furosemide).
- Hypophosphatemia** is caused by:
 - Decreased absorption of phosphate,
 - Intracellular shift,
 - Increased urinary excretion of phosphate,
 - Hereditary hypophosphatemia,
 - Hypercalcaemia,
 - Chronic alcoholism, and
 - Drugs (e.g., antacids, diuretics, and salicylate intoxication).

4.2.3.2. Trace Minerals

Some minerals which act as cofactors for the enzymes and are needed in trace quantities are the **trace elements**. Following are the examples of some trace elements (RDA < 200mg/day):

- Cobalt:** It is significant for the biosynthesis of vitamin B₁₂ family of coenzymes. The total body content of cobalt is around 1.1mg. It is mainly absorbed from the small intestine. Around 0.26mg of cobalt is eliminated per day through urine. The main **sources** of cobalt are figs, cabbage, lettuce, spinach, and animal products like liver and kidney.

Functions

- It is a constituent of vitamin B₁₂ (around 4% of cobalt present). It is also required for the production of haemoglobin.
 - It causes polycythaemia by either increasing or preventing the destruction of erythropoietin hormone (secreted by the kidneys). It may cause the development of macrocytic anaemia.
- Copper:** It is an important component of many redox enzymes (including cytochrome-c-oxidase). In the body, copper exists in two forms, i.e., cuprous and cupric; but mainly Cu²⁺ (cupric) form is found in the body and these forms changes between each other from Cu¹⁺ to Cu²⁺. Copper helps in oxidation-reduction and in scavenging of free radicals, as it has the capacity to accept and donates the electrons.

The chief **sources** of copper are organ meats, shellfish, nuts, seeds, wheat bran cereals and whole grain products. Cupric oxide, copper gluconate, copper sulphate, and copper amino acid chelates are the **examples** of copper supplements.

Copper toxicity has many consequences like nausea, vomiting, headache, dizziness, hypertension, hepatic cirrhosis, tremors, mental deterioration, Kayser-Fleischer rings, haemolytic anaemia, renal dysfunction (Fanconi-like syndrome), and even death of an individual.

- Fluorine:** It helps in the formation of tooth enamel (highly mineralised part of the tooth that protects it from degradation), containing fluoroapatite. Around 10-20mg of fluorine is found in the blood in its ionised form. Main **sources** of fluorine are drinking water, tea, salmon and sardine. About 1-2 ppm of fluorine is required per day. As fluorine is absorbed through water, it is expressed as ppm.

It is absorbed in small intestine, and half of the ingested fluoride is eliminated through urine and remaining is deposited in bones. With ageing, this fluoride is collected in the bone.

Functions

- Tooth Development and Dental Health:** Fluorine is essential for teeth development. It prevents dental caries. In large amounts, it causes fluorosis or mottling of the enamel. In this condition, enamel is stratified and has dull white patches. Brown stains and pits are also observed on the tooth.

ii) **Bone Development:** Fluorine enhances calcium and phosphate retention, thus promotes bone development and prevents old age osteoporosis. Excessive intake causes osteoblastic activity, calcium deposition, and density of bones. Fluoride ions act as an inhibitor of enolase (enzyme inhibiting glycolysis).

4) **Iodine:** It is a non-metallic trace element and helps in the synthesis of T_3 (triiodothyronine) and T_4 (thyroxine) thyroid hormones.

The iodine content in foods depends on the iodine content of the soil in which it was grown: **for example**, seafood is rich in iodine because they can concentrate the iodine from seawater. Some seaweed (e.g., *Undaria pinnatifida* or wakame) are also very rich in iodine. Addition of iodised salt and food additives (calcium iodate and potassium iodate) to processed foods increases the iodine content.

The free iodine and inorganic iodate are firstly converted into iodide which is simply absorbed in gastrointestinal tract. It can also be absorbed from mucous membrane, lungs, and skin.

Functions

Iodine is essential for the synthesis of thyroid hormones, i.e., triiodothyronine (T_3) and tetraiodothyronine (T_4). Thyroid hormones are iodinated derivatives of the amino acid thyronine. In thyroid gland, iodine is taken up through active transport and is then oxidised to active iodine which is used to iodinate tyrosine to form iodotyrosine. The iodotyrosine combines to form T_3 and T_4 .

Iodine deficiency or hypothyroidism causes stillbirths, abortions, congenital heart anomalies, endemic cretinism, mental retardation, and neurological defects. Treatment of iodine deficiency before pregnancy prevents disorders in children.

Hyperthyroidism leads to the following complications:

- An enlarged and hyperactive thyroid is caused by exophthalmos.
- Thyroid-stimulating immunoglobulin synthesis is increased due to Grave's disease. It further activates the TSH (Thyroid Stimulating Hormone) and LATS (Long- Acting Thyroid Stimulating) factors.
- Hashimoto's disease may occur due to destruction of thyroid tissues, over production of anti-thyroid antibodies, due to overproduction of TSH, and hyperthyroidism.

5) **Iron:** It is significant for many proteins and enzymes (particularly haemoglobin). Around 3-5gm iron is found in a healthy adult human body; out of which around 75% is present in blood in the form of haemoglobin, 5% is in myoglobin, and 15% in ferritin. Around 20mg of iron is required per day for a healthy adult man; and about 40mg and 20-30mg of iron per day is required by pregnant women and children, respectively.

The chief sources of iron are leafy vegetables (20mg/100gm), pulses (10mg/100gm) cereals (5mg/100gm) liver (5mg/100gm), meat (2mg/100gm), and also from jaggery. Amount of iron in food can increase by cooking in iron utensils. Less than 0.1mg of iron is present in per 100ml of milk.

In human body, iron is stored in the form of ferritin which is found in intestinal mucosal cells, liver, spleen, and bone marrow. Partial degradation of ferritin gives haemosiderin which has more iron concentration and is found in tissues, e.g., liver, spleen, and bone marrow.

Anaemia is commonly caused due to deficiency of iron. Due to anaemia, a child loses his/her learning ability and in adults working capacity decreases.

Haemosiderosis (or iron toxicity) is the condition in which body has excess amounts of iron. Haemosiderin pigments are found in spleen and liver. They are golden brown granules. Haemosiderosis is caused due to frequent blood transfusion in an individual.

6) **Manganese:** It acts as a cofactor of antioxidant enzymes (superoxide dismutase). Around 30mg manganese is found in a healthy adult human body. The chief sources of manganese are cereals, vegetables, liver, kidney, muscle, and tea. Around 2-5mg of manganese is required per day for a healthy adult; and about 0.5-2mg of manganese per day is required by children.

It is absorbed through guts and transported in combination with β -globulin (called **transmanganin**). It is stored in the liver. Some amount of it is eliminated through urine, and the remaining through bile or faeces.

Manganese is required for the following metabolic processes:

- Role in Animal Reproduction:** Deficiency of manganese causes sterility in animals and disturbance in citric acid cycle.
- Proteoglycan Synthesis:** It promotes synthesis and deposition of proteoglycans in many tissues, including bones due to glycosyltransferase activity.
- Porphyrin Synthesis:** It is a constituent of some porphyrins of erythrocytes.
- Bone Growth:** It is also required for bone growth and cholesterol syntheses.
- Acts as Cofactor:** It acts as a cofactor for enzymes like arginase, isocitrate dehydrogenase, and leucine aminopeptidase. Enzymes containing manganese are hydrolases, kinases, decarboxylases, and transferes.

In children, **manganese toxicity** causes encephalitis; and in adults impotency, psychosis and extrapyramidal syndrome may occur.

- 7) **Molybdenum:** It is necessary for xanthine oxidase and other related oxidases. However, deficiency of molybdenum has not been yet observed in humans. The chief **sources** of molybdenum are milk, beans, breads, cereals, liver, and kidney. Around 0.15-0.5mg of molybdenum is required per day for a healthy adult. It is readily absorbed in GIT, and is excreted in urine and bile.

Functions

- It takes part in uric acid metabolism.
- It is involved in the action of various enzymes:
 - It is found in several metallo-flavoproteins containing non-haem iron (e.g., aldehyde dehydrogenase and xanthine oxidase).
 - Enzymes containing Mb participate in electron transfer.
- In trace amounts, it helps in the utilisation of copper, whereas in larger amounts it diminishes the same.

Molybdenum toxicity causes microcytic anaemia and low levels of tissue copper.

- 8) **Selenium:** It is necessary for peroxidase (antioxidant proteins). Around 5-15mg selenium is found in the total body. It protects the cell from destruction. Its chief **sources** are liver, kidney, seafoods, meats, and grains. Around 0.2mg of selenium per day is required for a healthy adult and around 0.02-0.1mg per day for children.

Functions

- It is found in selenoproteins, e.g., glutathione peroxidase, thioredoxin reductase, deiodinase, muscle selenoproteins, etc.
- It is an integral part of glutathione peroxidase, the following are the functions of it:
 - It guards vital cell components, **for example**, it protects cell membrane from hydrogen peroxide and other peroxides.
 - It increases the action of superoxide dismutase which protects the cells against superoxide (O_2^-) and other free radicals.
- Selenium releases vitamin E in the following three methods:
 - Normal pancreatic function, and digestion and absorption of lipids including vitamin E,
 - Component of glutathione peroxidase, and
 - Helps in retention of vitamin E in the blood.
- It reduces the risk of certain cancers.
- It helps in purine metabolism (thioredoxin reductase).
- It helps in sperm motility and muscle metabolism in the form of selenoproteins.

Toxicity of selenium occurs in human living in selenium-rich soil. In human, selenium toxicity leads to cardiomyopathy. In cattle, alkali disease, liver necrosis, and muscular dystrophy are caused due to excess selenium.

- 9) **Sulphur:** It acts as an enzyme cofactor and also is an essential component of cysteine and methionine (essential amino acids). Proteins contain about 1%

sulphur by weight. Sulphates are not used in the form of inorganic sulphate salts of Na^+ , K^+ and Mg^{++} , yet it is present in food.

Amino acids containing sulphur are important constituents of body proteins. The disulphide linkages keep polypeptide units together, e.g., insulin and immunoglobulin. In cartilages and bone, sulphur is present in the form of **chondroitin sulphates**. Keratin protein present in hair and nail is also a sulphur-rich protein. Many enzymes and peptides contain -SH groups at the active site, e.g., glutathione. Other compounds containing -SH group are thiamine, biotin, lipoic acid, and CoA. Sulphates also take part in detoxification mechanisms like the production of indoxyl sulphate.

- 10) **Zinc:** It is pervasive in nature and is essential for many other enzymes (carboxypeptidase, liver alcohol dehydrogenase, and carbonic anhydrase) to complete their metabolic processes. Around 2.3gm zinc is found in the body, of which 80-110mg/dl is present in the plasma. High concentrations of zinc are found in choroid of eyes, prostate, kidneys, liver, and muscles. The chief **sources** of zinc are meat, liver, seafood, eggs, vegetables, and whole grains (less available due to phytates). Around 5mg of zinc is required per day for a pregnant woman.

Absorption of zinc directly depends on the metallothionein (protein) level in intestinal mucosal cells; this protein also acts as a carrier for zinc. Its absorption is inhibited by copper, phosphate, phytate, and calcium. It is eliminated through faeces and some amount in urine and sweat.

Functions

- There are around 300 zinc-containing enzymes, e.g., lactate dehydrogenase, carbonic anhydrase, alkaline phosphatase and carboxypeptidase.
- It is also found in cytosolic superoxide dismutase which contains copper also.
- It helps in the production of DNA and proteins.

4.2.4. Role of Minerals in Life Process

Different minerals, their sources, and functions are given in table 4.5:

Table 4.5: Role of Minerals in Life Process

	Sources	Functions
Calcium	Milk, cheese, yogurt, sardines, and canned fish with bones.	Structural in bones and teeth, and signal transduction.
Phosphorus	Red meat, dairy foods, fish, poultry, bread, rice, and oats.	Structural in bones and teeth, DNA signal transduction, and enzyme regulation.
Sodium and Chloride	Found in almost all foods	Fluid and electrolyte balance, and nerve conduction.
Magnesium	Spinach and Swiss chard, bran cereals and wheat germ, brown rice, dried beans, peas, lentils (black, navy, chickpeas, nuts), and seeds (almonds, cashews, pumpkin, sunflower, and flax seeds).	Nerve and muscle function, cofactor for many enzymes, including for DNA synthesis.

Potassium	Bananas, papaya, sweet potato, dark leafy greens, avocado, prune juice, tomato juice, orange juice, milk, yogurt, dried beans such as navy, pinto and black beans, chickpeas, lentils, beef, pork, fish, nuts, and seeds (pistachio, almonds, pumpkin, flax, and sunflower seeds).	Fluid balance, nerve conduction, and blood pressure regulation.
Sulphur	Sulphur is found in nearly all foods.	Structural component in cartilage, antioxidant metabolism, and important for protein function.
Iron	Meat, fish, poultry, firm tofu, dried beans, peas, like soybeans, chickpeas, split pea, lentils, nuts and seeds, organ meats such as liver and heart, and blackstrap molasses.	Oxygen transport and signal transduction.
Manganese	Tea, bread, nuts, cereals, and green vegetables (peas and runner beans).	Enzyme cofactor.
Copper	Nuts and shellfish.	Blood cell formation and release of iron for haemoglobin function.
Iodine	Fish and shellfish.	Biosynthesis of thyroid hormones.
Cobalt	Fish, nuts, green leafy vegetables, and cereals.	Component of vitamin B ₁₂ , amino acid metabolism, and fatty acid metabolism.
Zinc	Yogurt, milk, cheese, dried beans like kidney, navy, pinto and soybeans, lentils, pumpkin seeds and sunflower seeds, liver, meat, poultry, fish, and seafood.	Cofactor for enzymes, growth and development, and immune function.
Fluoride	Seafood, tea, and gelatin.	Fluoride helps prevent tooth decay.
Boron	Green vegetables, fruits, and nuts.	Function in glucose metabolism and estrogen function.
Selenium	Fish, shellfish, red meat, grains, eggs, chicken, liver, garlic, and vegetables grown in selenium-rich soil.	Synthesis of antioxidant enzymes; immune function, cardiovascular support, bone and joint function, and brain development.

4.2.5. Water

Water is a simple natural substance which is present abundantly on earth. Chemically, it is an inorganic compound and its molecular formula is H_2O . It is present throughout the human body, forming approximately 55-60% of the body mass of average adult humans. Water constitutes most of the volume of cells and body fluids; thus, the tissues formed by such cells.

On an average 60% (range 45-70%) of total body weight consists of water. Water occupies around 74% of muscle and organ weight but only 20-30% of adipose tissue; this is because water content in a muscular body is much more than in a fatty body. Due to the high percentage of lean body mass, men in comparison to women have a higher percentage of water in their total body weight. For example, 40 years old men have 60-65% water whereas women of the same age have 50-55%. A very lean adult male has 70% of water, while in obese man 50% of water is present according to their total body weight. Total water content decreases in older individuals due to reduced muscle mass.

Table 4.6 enlists the properties and benefits of water:

Table 4.6: Properties of Water

Properties	Description	Examples of Benefit to the Body
Strong Polarity	Polar water molecules attract other polar compounds, which causes them to dissociate.	Many kinds of molecules can dissolve in cells, thereby permitting a variety of chemical reactions and allowing many substances to be transported.
High Specific Heat	Hydrogen bonds absorb heat when they break, and release heat when they form, thereby minimising temperature changes.	Body temperature stays relatively constant.
High Heat of Vaporisation	Many hydrogen bonds must be broken for water to evaporate.	Evaporation of water in perspiration cools the body.
Cohesion	Hydrogen bonds hold molecules of water together.	Waterworks as a lubricant or cushion to protect against damage from friction or trauma.

4.2.5.1. Body Water Compartments and Composition

Total body water can broadly be classified into the intracellular and extracellular water. Water found inside the cell membrane is called the **intracellular water**, while that outside the cell membrane is called the **extracellular water**, e.g., water in the plasma (intravascular water), water around the cells (interstitial fluid), the humour of eye, cerebrospinal fluid, and water present in gastrointestinal and urinary tracts. Extracellular water provides the medium for metabolic exchanges, while intracellular water provides the medium for chemical reactions of cellular metabolism. Table 4.7 shows the compartment volumes in litres for each of the major body water pools. In this table, 42 litres of water is required for maintaining the optimal fluid balance in the body of a 70kg adult. But to maintain fluid balance, the body requires other elements along with water.

Many small molecules and electrolytes (e.g., sodium, chloride, potassium, and magnesium ions) are required for maintaining the body fluid balance. Therefore, it is essential to consume electrolytes in food and beverages to maintain the fluid balance of the body. Water is easily diffusible through the cells, therefore the body water distribution in various compartments depends on the chemical activity and concentration of ions and small molecules (e.g., glucose and albumin).

If a particular compartment has high solute concentration, the osmotic pressure and solute's capacity to attract water will also be high. For example, if sodium is present in different concentrations in two neighbouring compartments of the body, water will be attracted towards the compartment of high concentration. The force required to oppose osmosis (movement) of water in a solution across a semi-permeable membrane is the **osmotic pressure**. It can also be defined as the **force that changes the movement of water from dilute to concentrated solutions**. If the membrane is permeable to the solute, it does not exert osmotic pressure across the membrane. Osmotic pressure depends on the following factors:

- 1) Total number of solute particles in solution, and
- 2) Permeability of the membrane through which the solute passes.

Osmolality of a fluid in solution is defined as the **total number of solute particles (moles) per unit volume**. Greater the solute concentration in a solution will be the osmolality of that fluid. Thus, electrolytes maintain fluid balance within the body by retaining water in a specific body compartment. In case of excess loss of electrolytes, the body fails to maintain fluid balance. For example, intake of sodium can increase fluid retention in the body as it attracts water. This effect of sodium may be beneficial for some people and may be harmful to others. A person who wants to rehydrate after vigorous exercise, intake of adequate amounts of sodium in post-exercise sports drinks or foods, may be beneficial. However, extra dietary sodium may be harmful to those who are salt sensitive. Hence, along with sufficient amount of water, adequate electrolyte balance is also necessary for maintaining fluid balance. **Osmolality** is the number of moles of particles per kilogram of water (1 litre of water weighs 1kg). It is used to measure the osmotic characteristics of beverages like a soft drink.

Table 4.7: Distribution of Body Water Volume into Various Compartments

Compartment	Percentage (%) of Body Weight	Percentage (%) of Total Body Water	Volume in 70kg Man
Total body water	60		42 L
Extracellular water	20	33	14 L
Plasma	5	8	3.5 L
Intravascular fluid	15	25	10.5 L
Intracellular water	40	67	28 L

4.2.5.2. Body and Water Electrolyte Losses

Under normal conditions, the human body loses water through urine, faeces, sweat, and respiration. The maximum loss of water (1-2litres/day) occurs through urination, however, this amount of water loss depends on fluid intake and environmental conditions. As blood weight comprises of 80% water, loss of blood also results in water loss; for example, around 378ml of body fluid is lost when an individual donates 1 pint (473ml) of blood. Table 4.8 shows various conditions under which the fluid loss occurs in the body. During exercise or in a hot environment, the amount of water loss in sweat can increase drastically (up to 1-2litres/h) as compared to non-exercise days or in a cool and moderate environment (water loss is 10ml/day water).

Table 4.8: Water Balance in Average Adults Under Various Conditions

	Normal	Hot Environment	Strenuous Work
Fluid Intake (ml/day)			
Drinking water	1200	1200	3400
Water from food	1000	1000	1150
Water of oxidation	300	300	450
Total	2500	2500	5000
Fluid Output (ml/day)			
Urine	1400	1200	500
Involuntary water			

Table 4.9: Distribution of Body Water Volume into Various Compartments

Skin	100	100	100
Lungs	100	100	100
Sweat	100	100	100
Sisal (fascia)	100	100	100
Total	2500	2500	2500

body metabolism increases by 5-10 times above resting while performing exercises. Due to this increase in metabolic rate (TR) (100%), the body becomes hot so this heat must be eliminated to normalise the body temperature. The amount of sweat produced depends on the following factors:

- Environmental conditions (like air temperature, humidity, wind speed, and radiant load),
- Insulation and moisture permeability of clothing,
- Intensity of exercise,
- Physical conditions, and
- Adjustment to the new environment

It has been noted that women, children, and aged individuals have a difference in their sweat response to exercise. When an individual runs at different speeds in different environmental conditions, the production rate of sweat (in litres per hour) varies. When an individual runs faster or the environment becomes hotter and humid, the metabolism gets increased, which in turn increases the sweat rate, and the individual may suffer from dehydration if this fluid loss is not restored. In some individuals, dehydration can adversely affect their normal physiological functioning, the capability to perform work, and the complete health; however, some individuals can better tolerate dehydration than others.

4.2.5.3. Regulation of Water and Electrolyte Balance

Regulation of water and electrolyte balance of the body is a complicated process which occurs by the stimulation of thirst centre and by the regulation of fluid loss through the kidneys (either by increasing or decreasing urine volume as required). The body constantly maintains fluid balance by adjusting both intake and output to maintain an optimum level of total body water. For example, blood volume decreases due to excessive sweating, body tries to maintain water and electrolyte balance via increasing blood pressure by the contraction of blood vessels, via reducing fluid loss through the kidneys, and via stimulating the thirst centre. All these responses help to increase the blood pressure and to restore the fluid balance of body by reducing the total vascular space, by reducing the continuous fluid losses in the urine, and by increasing fluid intake.

4.2.5.4. Role of Water In Life Processes

Water is not for just reducing the thirst, it has many other important roles within the body, as given below:

- Protection:** Water regulates the body temperature and keeps the tissues moist. It keeps the body hydrated, maintains an optimum level of moistures around the sensitive areas of body, tissues, blood, bones, and the brain. It acts as a lubricant and cushion, thus protects joints and spinal cord.

- 2) **Elimination of Waste from the Body:** The body eliminates waste through perspiration, urination, and defecation; therefore adequate water intake is essential. Excretory organs of the body (sweat glands, kidneys, liver, and intestines) utilise water to flush out waste from the body.
- 3) **Aids in Digestion:** Digestion is the process of degradation through enzymes, the medium of which is water. Water dissolves many minerals, fibres, and the medium of which is water. Water dissolves many minerals, fibres, and nutrients. It also provides medium for absorption of the breakdown products of digestion. Fibres are dissolved in water easily, thus the bowel health is improved by making well-formed, soft stool which passes smoothly.
- 4) **Prevents Dehydration:** Body loses fluids excessively during vigorous exercise, sweat in the high heat, fever, and in illness causing vomiting or diarrhoea (loss of excess fluid). If anyone is suffering from these conditions, it is necessary to restore the body's natural hydration level by increasing the fluid intake. The physician may also recommend intake of more fluid to manage many other health conditions (like heal bladder infections and urinary tract infections).
- 5) **Temperature Moderator:** Within the body various enzymatically catalysed chemical reactions occur. These enzymes show optimum activity on a specific range of temperature. Water helps in keeping the range of temperature in the human body moderate for these enzymes to act properly. This helps with the process of metabolism in the human beings. Water maintains the range of temperature in the human body for the proper working of these enzymes that help the process of metabolism.

4.3. EXERCISE

4.3.1. Short Answer Type Questions

- 1) Write the classification of vitamins.
- 2) Give a note on role of vitamins.
- 3) What are coenzymes? Give any two examples.
- 4) What are trace elements?
- 5) Give a note on role of water in life processes.

4.3.2. Long Answer Type Questions

- 1) Give classification and role of coenzymes.
- 2) Write a brief classification of minerals.
- 3) Write a detail note on chemistry of vitamins.

CHAPTER 5

Enzymes

5.1. ENZYMES

5.1.1. Introduction

In the 1850s, a French scientist Louis Pasteur said that fermentation of sugar into alcohol by the yeast is catalysed by **ferments**. After this, he proposed a hypothesis called **vitalism**, according to which the ferments cannot be separated from the structure of living yeast cells. This postulate of vitalism prevailed for decades. Later on, in the year 1897, **Eduard Buchner** found that yeast extracts could ferment sugar solution into alcohol and he proved that fermentation was facilitated by the chemicals which continued to function when removed from the cells. **Frederick W. Kühne** named such chemical molecules **enzymes**. Thus after disapproval of the idea of vitalism, the development of biochemistry progressed.

For the first time in 1926, **James Sumner** isolated and crystallised enzyme urease and provided a breakthrough in early enzyme studies. He found that crystals of urease are formed exclusively of protein, thus he postulated that chemically all enzymes are proteins. Due to the lack of other examples, this discovery remained controversial for years.

In the 1930s, this hypothesis was widely accepted, when **John Northrop** and **Moses Kunitz** crystallised pepsin, trypsin, and other digestive enzymes and also chemically found them in proteins.

Over the last decades of the 20th century, huge advances have been made in the field of biochemistry; as a result, an outline of the working of the enzyme was drawn, and isolation, purification, and structure elucidation of several enzymes have been done.

Proteins that catalyse (i.e., increase the rate of) the **chemical reactions** are known as **enzymes**.

5.1.2. Properties

Enzymes are highly specific and effective protein molecules (except RNAs) that catalyse almost all biochemical reactions. Sometimes non-protein coenzymes or cofactors are also required for the catalytic functions of enzymes.

Some properties of enzymes are briefed below:

- 1) All enzymes are proteins, except the group of catalytic RNAs.

- 2) The molecular weight of enzyme ranges from 12,000 to 1 million or more like other protein molecules.
- 3) The catalytic activity of enzymes is mainly due to the primary, secondary, tertiary, and quaternary structures of protein and their specific conformation. The catalytic activity of enzyme is destroyed in case of denaturation or dissociation into sub-units.
- 4) Most of the enzymes require only amino acid residue to show their effect, whereas in some enzymes **cofactor** or **coenzyme** is needed; while sometimes both metal ion and coenzyme are needed for enzymatic activity. Cofactors are inorganic ions (such as Fe^{2+} , Mg^{2+} , Mn^{2+} , or Zn^{2+}), whereas coenzymes are vitamin derived metallo-organic or complex organic molecules, temporarily acting as specific functional group carrier. **Prosthetic groups** are the metal ions or coenzymes which bound covalently, evenly, and tightly to the enzyme protein.
- 5) **Holoenzymes** are the catalytically active enzyme, made up of **coenzyme** or **metal ions** (non-protein part) and **apoenzyme** or **apoprotein** (protein part).
- 6) Enzyme activity can be regulated by some processes such as phosphorylation, glycosylation, etc. These processes alter the structure of a protein found in the enzyme.
- 7) Enzymes are required in very minute quantity. They are not consumed in the overall enzymatic reaction, rather are obtained as whole after completion of reaction. Enzymes do not alter the equilibrium constant (K) of a reaction but only **increase the rate of reaction**.

5.1.3. Nomenclature and IUB Classification

In early days, enzymes were named by adding the suffix “-ase” to substrate or to a word or phrase defining the activity, e.g., **DNA polymerase** enzyme catalyses polymerisation of nucleotides to form DNA; **urease** enzyme catalyses urea hydrolysis, etc.

Some enzymes were named by their discoverer on the basis of their function, e.g., **pepsin** a digestive enzyme, derived from Greek word **pepsis** means **digestion**, **lysozyme** responsible for cell wall lysis of bacteria.

Some enzyme names were derived from their source, e.g., trypsin is produced by pancreatic tissue after rubbing it with glycerine, hence named after the Greek word **tryein** means **to wear down**.

As the time passed, several new enzymes were discovered; as a result, some enzymes got multiple names or two different enzymes were named with the same name.

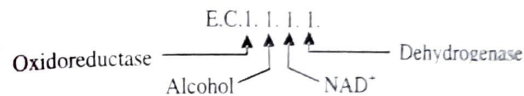
To overcome such uncertainties, enzymes were classified and named on the basis of the type of reaction they catalyse after the international agreement. As a result, all enzymes got formal **E.C. (Enzyme Commission) number** and some have their trivial names.

According to **IUB (International Union of Biochemistry)**, enzymes have been classified into six major classes, each with sub-classes, based on the type of reaction catalysed (table 5.1).

Table 5.1: International Classification of Enzymes

Classes	Types of Reaction Catalysed
Oxidoreductases	Transfer of electrons (hydride ions or H atoms).
Transferases	Group transfer reactions.
Hydrolases	Hydrolysis reaction (transfer of functional groups of water).
Lyases	Addition of groups to double bonds, formation of double bonds by removal of groups.
Isomerases	Transfer of groups within molecules to yield isomeric forms.
Ligases	Formation of C-C, C-S, C-O, and C-N bonds by condensation reactions coupled to ATP cleavage.

Each enzyme is given a systematic name and a specific four-part classification number. For example,



E.C. 1. 1. 1. 1. – Alcohol dehydrogenase (**Alcohol: NAD⁺ oxidoreductase**).

E.C. 2. 7. 1. 1. – Hexokinase (**ATP: D-hexose 6-phosphotransferase**), and

E.C. 6. 3. 1. 2. – Glutamine synthetase (**L-glutamate ammonia ligase**).

Nomenclature Committee of the International Union of Biochemistry and Molecular Biology collects and maintains the data about enzymes.

Table 5.2: Sub-Classes of Enzymes

Sub-Classes	Enzyme Names
EC 1	Oxidoreductases
EC 1.1	Acting on the CH—OH group of donors.
EC 1.2	Acting on the aldehyde or oxo group of donors.
EC 1.3	Acting on the CH—CH group of donors.
EC 1.4	Acting on the CH—NH ₂ group of donors.
EC 1.5	Acting on the CH—NH group of donors.
EC 1.6	Acting on NADH or NADPH.
EC 1.7	Acting on other nitrogenous compounds as donors.
EC 1.8	Acting on a sulphur group of donors.
EC 1.9	Acting on a haeme group of donors.
EC 1.10	Acting on diphenols and related substances as donors.
EC 1.11	Acting on peroxide as acceptor.
EC 1.12	Acting on hydrogen as donor.
EC 1.13	Acting on single donors with incorporation of molecular oxygen (oxygenases).
EC 1.14	Acting on paired donors, with incorporation or reduction of molecular oxygen.
EC 1.15	Acting on superoxide radicals as acceptor.

EC 1.16	Oxidising metal ions
EC 1.17	Acting on CH or CH ₂ groups
EC 1.18	Acting on iron-sulphur proteins as donors
EC 1.19	Acting on reduced flavodoxin as donor
EC 1.20	Acting on phosphorus or arsenic in donors
EC 1.21	Acting on X-H and Y-H to form an X-Y bond
EC 1.97	Other oxidoreductases
EC 2	Transferases
EC 2.1	Transferring one-carbon groups
EC 2.2	Transferring aldehyde or ketonic groups
EC 2.3	Acytransferases
EC 2.4	Glycosyltransferases
EC 2.5	Transferring alkyl or aryl groups, other than methyl groups
EC 2.6	Transferring nitrogenous groups
EC 2.7	Transferring phosphorus-containing groups
EC 2.8	Transferring sulphur-containing groups
EC 2.9	Transferring selenium-containing groups
EC 3	Hydrolases
EC 3.1	Acting on ester bonds
EC 3.2	Glycosylases
EC 3.3	Acting on ether bonds
EC 3.4	Acting on peptide bonds (peptidases)
EC 3.5	Acting on carbon-nitrogen bonds, other than peptide bonds
EC 3.6	Acting on acid anhydrides
EC 3.7	Acting on carbon-carbon bonds
EC 3.8	Acting on halide bonds
EC 3.9	Acting on phosphorus-nitrogen bonds
EC 3.10	Acting on sulphur-nitrogen bonds
EC 3.11	Acting on carbon-phosphorus bonds
EC 3.12	Acting on sulphur-sulphur bonds
EC 3.13	Acting on carbon-sulphur bonds
EC 4	Lyases
EC 4.1	Carbon-carbon lyases
EC 4.2	Carbon-oxygen lyases
EC 4.3	Carbon-nitrogen lyases
EC 4.4	Carbon-sulphur lyases
EC 4.5	Carbon-halide lyases
EC 4.6	Phosphorus-oxygen lyases
EC 4.99	Other lyases
EC 5	Isomerases
EC 5.1	Racemases and epimerases
EC 5.2	cis-trans-Isomerases
EC 5.3	Intramolecular isomerases
EC 5.4	Intramolecular transferases (mutases)
EC 5.5	Intramolecular lyases
EC 5.99	Other isomerases

EC 6	Ligases
EC 6.1	Forming carbon-oxygen bonds
EC 6.2	Forming carbon-sulphur bonds
EC 6.3	Forming carbon-nitrogen bonds
EC 6.4	Forming carbon-carbon bonds
EC 6.5	Forming phosphoric ester bonds
EC 6.6	Forming nitrogen-metal bonds

5.1.4. Concept of Enzymatic Action

In 1880, Charles Adolphe Wurtz gave the concept of **enzyme-substrate complex** for enzyme action in the reaction. The idea about the theory of enzyme mechanism and mathematical derivation for the kinetic mechanism of enzyme-catalysed reaction also arises after his concept.

In human body, some reactions such as muscle contraction, food digestion, nerve signal transmission, etc., essentially require enzymatic catalysis to regulate the rate of reactions. Enzymatic catalysis increases the rate of reaction with the help of an enzyme. Enzyme-catalysed reaction occurs within the **active site** (confines of a pocket on enzyme). The **substrate** molecules bind at the active site and are acted upon by the enzyme.

Active Sites

The substrate binding place of an enzyme at which catalysis occurs is known as the **active site** (figure 5.1). This site is a **cleft or big pocket-shaped structure surrounded by amino acid**. Surface of an enzyme contains residues and other side chains responsible for specificity of the substrate with respect to charge, steric hindrance, and hydrophobicity. Catalytic residues often act as acceptors or donors of protons, or even act as cofactor binders. Inhibition of enzymes is also carried out at the active site. The catalytic as well as the binding sites are contained by the active site of an enzyme. The chemical properties, as well as, the structure of the active site allow substrate binding and recognition.

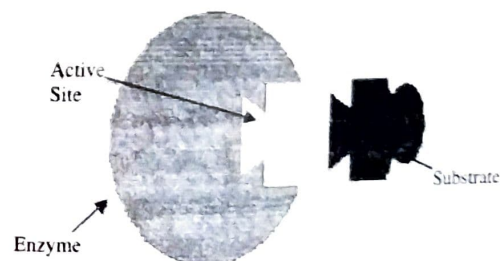


Figure 5.1: Active Site

To catalyse a reaction, an enzyme will bind to one or more reactant molecules, known as its **substrates**. In some reactions, a single substrate is broken down into multiple products. In others, two substrates may come together to create one larger molecule. Two reactants might also enter a reaction, swap pieces or change shape, and leave the reaction as two separate products.

Once substrates have entered the active site, they turn into products by following these major **strategies**:

- 1) In reactions with multiple substrates, the enzyme may hold the substrates in the correct position and orientation for formation of a new bond.
- 2) An enzyme may bend the substrate or strain its bonds, bringing it closer to the transition state and thus reducing the amount of energy needed to reach that state (the activation energy).
- 3) Enzymes may provide a chemical micro-environment that makes it easier for a reaction to proceed. This can involve making an acidic or basic "pocket" that favours a reaction, donating or receiving electrons (often, through metal ions bound in the active site), or even forming a temporary covalent bond with the substrate. All of these interactions depend on specific amino acid side chains found in the active site.

The enzyme's mechanism of action can be explained in the following two steps:

- 1) Enzyme-substrate complex formation, and
- 2) Activation energy lowering.

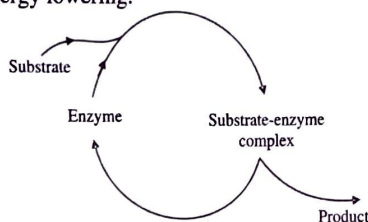
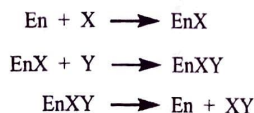


Figure 5.2: Enzyme-Substrate-Complex Cycle

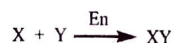
5.1.4.1. Enzyme-Substrate Complex Formation

An enzyme-substrate complex is formed when a substrate is acted upon by an enzyme (**figure 5.2**). This lowers the activation energy of the substrate. Subsequently, decomposition of substrate takes place, undergoing various chemical changes, leading to regeneration of enzyme.

For example, in the presence of enzyme (En), substrate molecules (X and Y) slowly unite together and form a larger molecule (XY). This reaction occurs in the following steps:



However, the overall reaction may be represented as:



Thus, the enzymes act as **biochemical middlemen**.

Formation of an enzyme-substrate complex can be explained by the following two models:

- 1) **Lock and Key Model:** Emil Fischer (a German Chemist) proposed the lock and key model in the 1890s. According to this model (**figure 5.3**), the enzyme allows the substrate to exactly fit in (just like a key fits into a lock), thus having the structure of both the complex components (i.e., enzyme and substrate) which are complementary to each other.

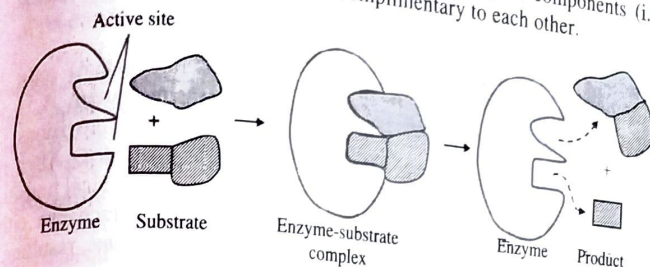


Figure 5.3: Lock and Key Model

- 2) **Induced Fit Model:** D. Koshland proposed the induced fit model in 1966. According to this model (**figure 5.4**), the active site of an enzyme is flexible. During the reaction when substrate and enzyme bind together, the appropriate shape of the enzyme molecule is made to change in such a way that it is appropriate for the substrate enzyme interaction.

The shape of the enzyme molecules so changed can put strain on the substrate molecule, which may help to break the substrate and therefore promote the reaction to form the product.

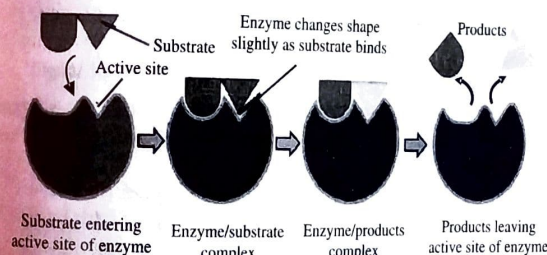


Figure 5.4: Induced Fit Model

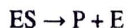
Turn Over Number

Enzymes exist in the form of a colloid due to their large-sized protein molecules. The change in substrate molecules per minute is known as the **turn over number**, which is 30 for lysozyme, 50 for flavo protein, 10,000 for sucrose, 5 million for catalase, and 36 million for carbonic anhydrase.

Various factors on which this number depends are active site numbers, reaction's rapidness, and end product's separation.

5.1.4.2. Lowering of Activation Energy

In order to convert the reactants into the products, potential energy barrier (of all the chemical reactions) needs to be overcome. To break this barrier, amount of energy needed is equivalent to activation energy. Enzyme helps in lowering down this activation energy during complex formation with substrate. Further, this enzyme is released when the product is formed.



P = Product

For example (figure 5.5), the chemical compound A (reactant) must be converted to compound C (product). But due to the energy barrier, A is not converted spontaneously to compound C. By making certain provisions for activation energy, compound A energy level must be increased. After the availability of this energy, the reactant is transformed to a hypothetical transition state B. The transition state formed is the activated state, which can either breakdown to form product C or can revert back to the reactant state, resulting in the recovery of energy E.

Figure 5.5 also clearly depicts that the energy of activation (E) in the presence of an enzyme is lowered from E to E_c . Therefore, with the lower energy requirements, A (reactant) can be converted to C (product). For example, the activation energy for the decomposition of H_2O_2 is 18kcal/mol; but in the presence of enzyme **catalase**, it is only 6.4kcal/mol (just 1/3 of the required energy in the absence of enzyme).

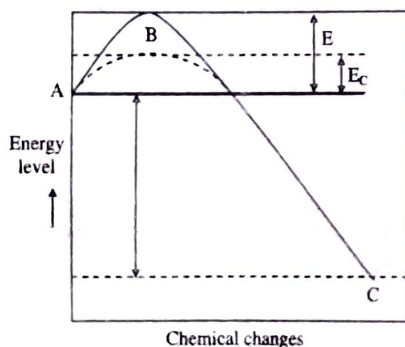


Figure 5.5: Energy of Activation of a Metastable Compound in the Presence or Absence of an Enzyme

5.1.5. Enzyme Kinetics

The study of enzyme catalysed reactions is known as **enzyme kinetics**. The rate of reaction and the effects of variation in the investigated reaction conditions are measured in enzyme kinetics. Other molecules (like enzyme's substrates) are manipulated by protein molecules. Substrate acting as target molecules bind to the active site of enzymes and get converted into products through several stages, known as the **enzymatic mechanism**.

Enzymatic mechanism can be divided into the following two types:

- 1) **Single-Substrate Mechanism:** This includes binding of one substrate on which kinetic study is conducted. For example, triosephosphate isomerase follows single substrate mechanism, wherein, the study aims to measure the affinity with which the enzyme and substrate bind to each other, and their turnover rate.
- 2) **Multiple-Substrate Mechanism:** This includes multiple substrate binding. For example, dihydrofolate reductase follows multiple substrate binding, wherein, the study shows the order of substrate binding and also the release of products.

Protease is an enzyme (with an exception) that binds to a single substrate, but releases multiple products because of the cleavage of one protein substrate into two polypeptide products. Kinetic data can be interpreted from the structure of enzymes. For example, substrates and products, which bind during catalysis, can be determined by the structure of the enzymes. Also, the changes that occur during the reaction and the role played by the particular amino acid residues in the mechanism can be determined from the enzyme structure.

5.1.5.1. Michaelis-Menten Plot

Enzyme kinetics and K_m value (i.e., Michaelis-Menten equation) depends on the effect of substrate concentration on the velocity of enzyme (figure 5.6).

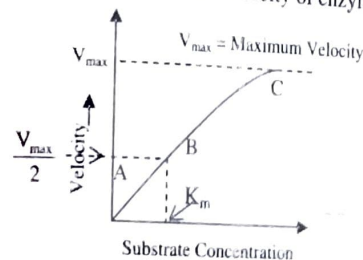
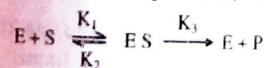


Figure 5.6: Effect of Substrate Concentration on Enzyme Velocity

The enzyme (E) and the substrate (S) react with one another to form an unstable enzyme substrate complex (ES) and produce the final product (P).



Where, K_1 , K_2 , and K_3 are the velocity constants for the respective reactions as given above.

K_m represents the **Michaelis-Menten constant** (also known as Briggs and Haldane's constant) and is given by the formula:

$$K_m = \frac{K_2 + K_3}{K_1}$$

In the above reaction, it can be assumed that the concentration of ES (Enzyme-Substrate) remains constant at steady state. So the reaction can be analysed only

at the start point, where the amount of product formed remains almost negligible. Thus, on reversing the reaction, i.e., $E + P \rightarrow ES$, the amount of reactants also turns to be negligible.

On applying the **law of mass action**:

$$\frac{d[ES]}{dt} = K_1([E] - [ES]) - K_2[ES] - K_3[ES]$$

$$K_1[E][S] - K_1[ES][S] - K_2[ES] - K_3[ES] = 0 \text{ (at steady state)}$$

$$K_1[E][S] = [ES](K_1[S] + K_2 + K_3)$$

$$[ES] = \frac{K_1[E][S]}{K_1[S] + K_2 + K_3}$$

On dividing the denominator and numerator of R.H.S with K_1 :

$$[ES] = \frac{[E][S]}{[S] + \frac{K_2 + K_3}{K_1}}$$

The initial rate (V) of formation of product:

$$V = K_3[ES]$$

And when the enzyme is saturated with substrate, maximum rate is:

$$V_{\max} = K_3[E]$$

$$\text{Therefore, } V = K_3[ES] = \frac{K_3[E][S]}{[S] + K_m}$$

$$V = \frac{V_{\max}[S]}{[S] + K_m} \text{ (Michaelis-Menten equation)} \quad \dots (1)$$

Where,

V = Measured velocity

$V_{\max}$ = Maximum velocity

$[S]$ = Substrate concentration

K_m = Michaelis-Menten constant

On assuming that the measured velocity $V = \frac{V_{\max}}{2}$. Then the **equation (1)** may be substituted as follows:

$$\frac{1}{2} V_{\max} = \frac{V_{\max}[S]}{K_m + [S]}$$

$$K_m + [S] = \frac{2V_{\max}[S]}{V_{\max}}$$

$$K_m + [S] = 2[S]$$

$$K_m = 2[S] - [S] = [S]$$

$$K_m = [S]$$

Thus, **Michaelis-Menten constant (K_m)** is the concentration of the substrate when the reaction velocity is equal to one half of the maximal velocity ($V_{\max}$) for the enzyme catalysed reaction.

Conclusions

- 1) **Characteristics of K_m :** Michaelis constant (denoted as K_m) is the main characteristic of an enzyme and its specific substrate reflects the enzyme's affinity for that particular substrate. Mathematically, K_m is equal to concentration of the substrate at which the velocity of reaction is equal to half of $V_{\max}$, i.e., $\frac{1}{2}V_{\max}$. K_m is affected by the substrate concentration and not by the enzyme concentration (**figure 5.7**).

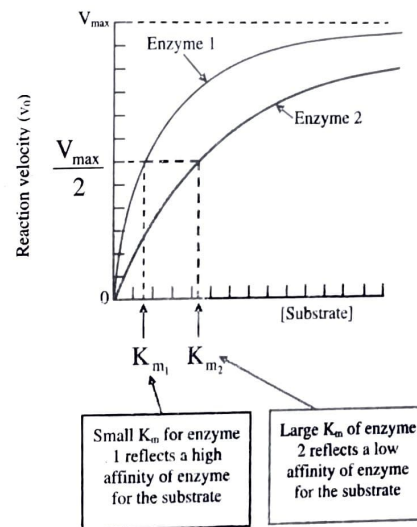


Figure 5.7: Effect of Substrate Concentration on Reaction Velocities for Two Enzymes: Enzyme 1 with a Small K_m , and Enzyme 2 with a Large K_m

Different characteristics of K_m are as follows:

- Small K_m :** An enzyme's **high affinity** for the substrate is reflected by the low value of K_m (numerically). This is because a low substrate concentration is required to saturate half of the enzyme that is basically to reach half of the velocity, i.e., $1/2V_{\max}$.
 - Large K_m :** An enzyme's **low affinity** for the substrate is reflected by the high value of K_m (numerically). This is because a high substrate concentration is required to saturate half of the enzyme.
- 2) **Relationship of Velocity to Enzyme Concentration:** There is a direct correlation between the reaction rate and the enzyme concentration. The rate of reaction at all substrate concentrations increases with the increase

in the concentration of enzyme. **For example**, the initial rate of reaction (V_0) and the maximum velocity ($V_{\max}$) becomes half of the original velocity on reducing the concentration of enzyme, to half of its original concentration.

- 3) **Order of Reaction:** When the substrate concentration is much less than the Michaelis-Menten constant, i.e., $[S] \gg K_m$, the reaction velocity is approximately proportional to the concentration of substrate and is said to follow the first order reaction with reference to substrate. When $[S] \ll K_m$, the reaction velocity remains constant, and equals the maximum velocity ($V_{\max}$). Thus, the reaction rate remains independent of substrate concentration, and is said to follow zero order reaction with respect to the concentration of the substrate (**figure 5.8**).

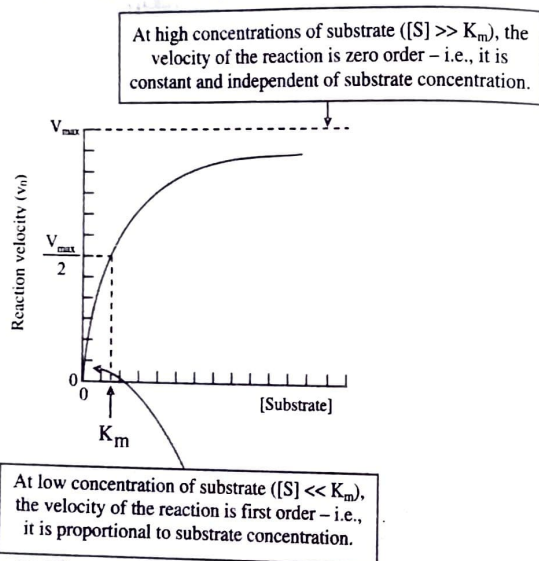


Figure 5.8: Effect of Substrate Concentration on Reaction Velocity for an Enzyme-Catalysed Reaction

5.1.5.2. Lineweaver-Burk Double Reciprocal Plot (Significance of Michaelis-Menten Equation)

Michaelis-Menten equation has the following significances:

- 1) To determine the value of K_m is the major significance of Michaelis-Menten equation. A strong enzyme-substrate affinity is indicated by the low K_m value.
- 2) Graphical representation of Lineweaver Burk double reciprocal plot can also be prepared using this equation. Further, this plot helps to understand the effect of inhibitors on the reaction.

For the determination of K_m value, the substrate saturation curve is not very accurate since $V_{\max}$ is approached asymptotically. By taking the reciprocals of the equation (1), a straight line graphic representation is obtained.

$$\frac{1}{v} = \frac{K_m + [S]}{V_{\max} [S]}$$

$$\frac{1}{v} = \frac{K_m}{V_{\max}} \times \frac{1}{[S]} + \frac{[S]}{V_{\max} [S]}$$

$$\frac{1}{v} = \frac{K_m}{V_{\max}} \times \frac{1}{[S]} + \frac{1}{V_{\max}}$$

The above equation is similar to $y = ax + b$. Therefore, a plot of the reciprocal of the velocity ($\frac{1}{v}$) versus the reciprocal of the substrate concentration ($\frac{1}{[S]}$) gives a straight line (**figure 5.9**). Here the slope is $K_m/V_{\max}$ and whose y intercept is $1/V_{\max}$.

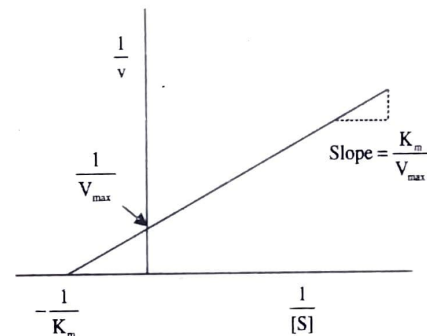


Figure 5.9: Lineweaver-Burk Double Reciprocal Plot

It is much easier to calculate the K_m value from the intercept on x-axis ($1/K_m$). Further, the double reciprocal plot is useful in understanding the effect of various inhibitions.

Enzyme Reactions with Two or More Substrates

The above discussed equations are based on the presumption of a single substrate-enzyme reaction. In fact, a majority of the enzyme-catalysed reactions involve two or more substrates. Even in case of multi-substrate enzymes, despite the complex mathematical expressions, the fundamental principles conform to Michaelis-Menten kinetics.

5.1.6. Factors Affecting Enzyme Activity

Following are the factors affecting enzyme activity:

Effect of Enzyme Concentration: The enzyme catalysed reaction rate and concentration are directly proportional to each other. Also, this enzyme property is used for determining the serum enzymes which is used for disease diagnosis. A linear straight line graph is obtained by plotting the enzyme velocity versus enzyme concentration (**figure 5.10**).



Figure 5.10: Effect of Enzyme Concentration

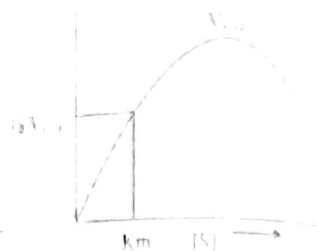


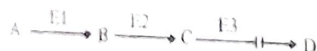
Figure 5.11: Effect of Substrate Concentration

Effect of Substrate Concentration: In an enzymatic process, reaction velocity is directly proportional to the substrate concentration, i.e., $V \propto [S]$. An increase in the concentration of substrate will ultimately result in elevated reaction velocity. The reaction velocity (V) is defined as number of micromoles of substrate converted per minute. The maximum rate of reaction obtained from an enzymatic reaction is called **maximum velocity (V_{max})**.

A flattened curve and decrease in the rate of reaction could be observed if there is a continuous increase in substrate concentration (figure 5.11).

If there is a significant rise in substrate concentration and amount of enzyme is constant, then increase in reaction velocity of an enzymatic process will be observed.

Effect of Product Concentration: The enzyme activity declines with accumulation of reaction products. This phenomena can be explained by the equation:



In the above reaction, the reason for blockage of enzymatic activity of $E2$ is accumulation of product C which is due to the absence of enzyme $E3$. This process will ultimately result in blockage of the whole pathway.

Effect of Temperature: An increase in temperature leads to increase in enzymatic activity up to a maximum and then it declines to form a bell-shaped curve (figure 5.12). An increase in activity of enzyme on increasing temperature by 10°C is known as the **temperature coefficient** or Q_{10} . **Optimum temperature** is defined as the **temperature at which enzyme is optimally active**. For most of the enzymes, it is between $40-45^\circ\text{C}$. If temperature increases beyond optimum temperature, denaturation occurs and molecular arrangement is disturbed which causes a loss of the active sites of the enzyme surfaces and efficiency loss occurs.

Effect of pH: Maximal activity of most of the enzymes is not attained throughout the pH scale (1-14). Optimal activity of several enzymes can be seen between the pH ranging from 5 to 9. Optimum pH is the pH possessed by each enzyme at which velocity of enzyme is maximum. The enzyme activity is much lower at pH below and above this, and the enzymes become totally inactive at extreme pH (figure 5.13).

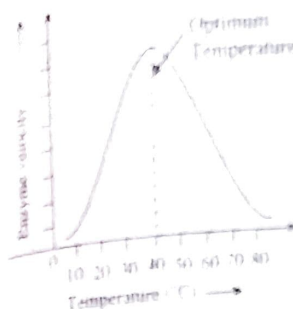


Figure 5.12: Effect of Temperature on Enzyme Velocity

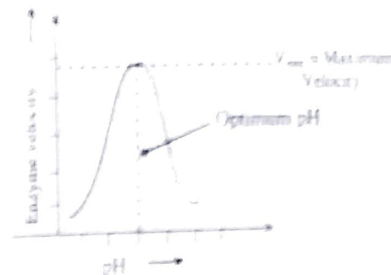


Figure 5.13: Effect of pH on Enzyme Velocity

Effect of Inhibitors and Activators: Inhibitors are the substances that decrease the catalytic activity of enzymes by preventing the formation of a normal enzyme-substrate complex. Some examples are ritonavir, disulfiram, etc. Activators are the substances that tend to increase the catalytic activity of enzymes. Some examples are chloride ions activate salivary amylase and calcium ions activate lipases.

Effect of Time: Time required for an enzymatic reaction is less provided for ideal and optimal conditions.

Effect of Light and Radiation: On exposure to X-rays, gamma rays, UV and beta rays, and certain enzymes form peroxides.

5.1.7. Role of Enzymes

Enzymes have found a significant role in therapeutic and clinical diagnosis. Some major enzymes which are used for the therapeutic purposes are listed in table 5.3:

Table 5.3: Therapeutic Enzymes and their Uses

Enzymes	Reactions	Uses
Asparaginase	$\text{L-Asparagine} + \text{H}_2\text{O} \rightarrow \text{L-aspartate} + \text{NH}_3$	Leukaemia
Collagenase	Collagen hydrolysis	Skin ulcers
Glutaminase	$\text{L-Glutamine} + \text{H}_2\text{O} \rightarrow \text{L-glutamate} + \text{NH}_3$	Leukaemia
Hyaluronidase	Hyaluronate hydrolysis	Heart attack
Lysozyme	Bacterial cell wall hydrolysis	Antibiotic
Rhodanase	$\text{S}_2\text{O}_3^{2-} + \text{CN}^- \rightarrow \text{SO}_3^{2-} + \text{SCN}^-$	Cyanide poisoning
Ribonuclease	RNA hydrolysis	Antiviral agent
β -Lactamase	Penicillin $\rightarrow$ penicilloic acid	Penicillin allergy
Streptokinase	Plasminogen $\rightarrow$ plasmin	Blood clots
Trypsin	Protein hydrolysis	Inflammation
Uricase	$\text{Urate} + \text{O}_2 \rightarrow \text{allantoin}$	Gout
Urokinase	Plasminogen $\rightarrow$ plasmin	Blood clots
Nattokinase	Plasminogen $\rightarrow$ plasmin	Blood clots

In biological fluid (especially in plasma or serum), estimation of enzyme activity is performed for clinical significance. Enzymes found in circulation are broadly classified into two categories:

- 1) **Plasma Functional or Plasma-Specific Enzymes:** These enzymes are found in the plasma and perform specific functions. Normally, these enzymes have higher activity in plasma than in the tissues. These enzymes are mainly synthesised in the liver cells and enter the blood, e.g., lipoprotein lipase, plasmin, thrombin, choline esterase, ceruloplasmin, etc.

The activity of these enzymes has decreased significantly in genetic disorders or liver damage, such as in case of Wilson's disease level of ceruloplasmin falls.

- 2) **Plasma Non-Functional or Non-Plasma Specific Enzymes:** These enzymes are either totally present or are present at a low concentration in plasma compared to their levels present in the tissues.

Non-plasma specific enzymes' activity evaluation is significant in the clinical diagnosis of certain diseases. The **secretory enzymes** of plasma are the gastrointestinal enzymes (e.g., amylase, lipase, pepsin, trypsin, etc.) which help in the digestion of food. The **constitutive enzymes** include enzymes of cellular metabolism. **Examples** of constitutive enzymes are acid and alkaline phosphatases, creatine phosphokinase, lactate dehydrogenase, and transaminases.

Estimation of the activities of non-plasma specific enzymes is significant for the diagnosis and prognosis of several diseases.

The balance between the synthesis and release of an enzyme into plasma during cell turnover reflects normal enzyme concentration in serum. The increased enzyme concentration shows cellular damage, increased rate of cell turnover, enhance enzyme synthesis, cell proliferation, etc.

There are several serum enzymes which are important in clinical diagnosis because they are excellent markers to detect cellular damage.

Some enzymes used for the diagnostic purposes are summarised in table 5.4

Table 5.4: Important Enzymes in the Diagnosis of Diseases

Serum Enzymes (Elevated)	Diseases (Most Important)
Amylase	Acute pancreatitis
Serum Glutamate Pyruvate Transaminase (SGPT)	Liver diseases (hepatitis)
Serum Glutamate Oxaloacetate Transaminase (SGOT)	Heart attacks (myocardial infarction)
Alkaline phosphatase	Rickets, obstructive jaundice
Acid phosphatase	Cancer of prostate gland
Lactate Dehydrogenase (LDH)	Heart attacks, liver diseases
Creatine Phosphokinase (CPK)	Myocardial infarction (early marker)
Aldolase	Muscular dystrophy
5'-Nucleotidase	Hepatitis
γ -Glutamyl Transpeptidase (GGT)	Alcoholism

A brief account of selected diagnostic enzymes is as follows:

- 1) **Amylase:** Serum amylase level increases in patients with acute pancreatitis (especially in urine) and chronic pancreatitis. The value of normal serum amylase is 80-180 Somogyi units/dl and in diseased condition the peak value is seen within 8-12 hours after the disease occurs which returns to normal by 3rd or 4th day. Serum amylase is used for the diagnosis of chronic pancreatitis, acute parotitis (mumps), and obstruction of pancreatic duct.
- 2) **Aspartate Transaminase (AST)/Serum Glutamic Oxaloacetic Transaminase (SGOT):** In serum, activity of SGOT is increased in myocardial infarction and also in liver diseases (normal 4-45 IU/l).
- 3) **Creatine Kinase (CK):** It has three isoenzymes. In case of muscular dystrophy (normal 10-50 IU/l) and myocardial infarction (early detection), the level of CK increases.
- 4) **Lactate Dehydrogenase (LDH):** It has five isoenzymes and is used in the diagnosis of infective hepatitis, leukaemia, muscular dystrophy, and myocardial infarction.
- 5) **Alanine Transaminase (ALT)/Serum Glutamic Pyruvic Transaminase (SGPT):** SGPT level increases above the normal range in jaundice, acute viral or toxic hepatitis, and liver cirrhosis.
- 6) **Alkaline Phosphatase (ALP):** Level of ALP increases (normal 3-13 KA units/dl) in patients with certain liver and bone disorders. It is a much specific enzyme for bone carcinoma, hyperparathyroidism, obstructive jaundice, and rickets diagnosis.
- 7) **γ -Glutamyl Transpeptidase (GGT):** It is a diagnostic marker particularly used for the detection and monitoring of alcohol abuse. It is also raised in obstructive jaundice and infective hepatitis.
- 8) **Acid Phosphatase (ACP):** Tartarate labile ACP serves as a marker for the diagnosis of prostate gland cancer in which ACP level rises above the normal. Therefore, ACP is used as a good tumour marker.

5.2. EXERCISE

5.2.1. Short Answer Type Questions

- 1) What are enzymes?
- 2) Give the properties of enzymes.
- 3) Write a note on role of enzymes.
- 4) What is lock and key hypothesis?
- 5) Define following terms:
 - i) Activation energy
 - ii) Active sites
 - iii) Turn over number

5.2.2. Long Answer Type Questions

- 1) Write a brief note on mechanism of enzymatic action.
- 2) Give a detail note on Michaelis-Menten equation.
- 3) Detail about factors affecting enzyme activity.

CHAPTER 6

Therapeutics

6.1. THERAPEUTICS

6.1.1. Introduction

The term **therapeutics** has been derived from the Greek word *therapeutikos* which means '**inclined to serve**', thus therapeutics involves treatment and care of patient for both preventing and combating disease, and relieving pain or injury.

In general, therapeutics is defined as act of helping and caring of patients, preventing disease, and solving their problems in a complete manner. Therefore, factors like exercise, diet, and psychology are important for preventing and treating diseases.

The specific methods which are used to treat specific symptoms include use of drugs to relieve pain or to treat infections, surgery to remove diseased tissues or transplantation in case of poorly functioning or non-functioning organs with fully operating ones, and counselling or psychotherapy to reduce emotional distress. The efficacy of treatment depends on the selected method and confidence in the physician.

The word **pathology** has been derived from two Greek words, i.e., *pathos* meaning **experience** or **suffering**, and *logia* meaning **study of**; thus a branch of medical science which diagnose disease on the basis of the laboratory analysis of body fluids (blood and urine) and tissues, by using the tools of chemistry, clinical microbiology, haematology, and molecular pathology is known as pathology.

6.1.2. Pathology of Blood

Blood is a connective tissue that plays a vital role in carrying out various life processes, and protects the body against diseases. Blood is a unique component for every individual as it differs in composition, number of cells of each type and its functions, from one individual to another. **Haematology** is the branch of medical science concerned with the study of blood, blood-forming tissues, and blood disorders.

Composition and Functions of Blood

Around 8% of total body weight is blood (adult female, 4-5 litres and adult male, 5-6 litres). Blood is a connective tissue, having a liquid matrix with cells and cell fragments. Two major components of blood are:

- 1) **Blood plasma**, a clear extracellular fluid, and
- 2) **Formed elements**, composed of blood cells and platelets.

Various components and functions of blood are listed in table 6.1:

Table 6.1: Components of Blood and their Functions

Components	Functions
1) Blood Cells	
i) Erythrocytes (Red Blood Cells)	Transport oxygen.
ii) Leukocytes (White Blood Cells)	Protect against infection.
iii) Platelets (Thrombocytes)	Takes part in blood clotting.
2) Plasma	
i) Water	Major part of blood, Maintains blood pressure, and Takes part in regulation of body fluid level and electrolytes.
ii) Plasma Proteins a) Albumin b) Globulin c) Fibrinogen	Maintains intravascular osmotic pressure. Takes part in immunity and inflammation. Participates in blood coagulation. Various blood clotting factors are present in it.
iii) Various Clotting Factors	Participate in blood coagulation (convert loose blood clot in stabilised form).
iv) Nutrients a) Glucose b) Amino Acids c) Lipids d) Vitamins e) Electrolytes	Source of energy. Cell growth and repair. Source of energy in absence of glucose. Participates in various biochemical actions. Allows various biochemical actions.

6.1.2.1. Formed Elements of Blood

There are major three types and several sub-types of formed elements of blood (figure 6.1):

- 1) Red blood cells (Erythrocytes)
- 2) White blood cells (Leukocytes)
 - i) Granular leucocytes or granulocytes
 - a) Neutrophils,
 - b) Eosinophils, and
 - c) Basophils.
 - ii) Agranulocytes
 - a) Lymphocytes, and
 - b) Monocytes.
- 3) Platelets (Thrombocytes)

Table 6.2 enlists the formed elements of blood, along with their characteristics and functions:

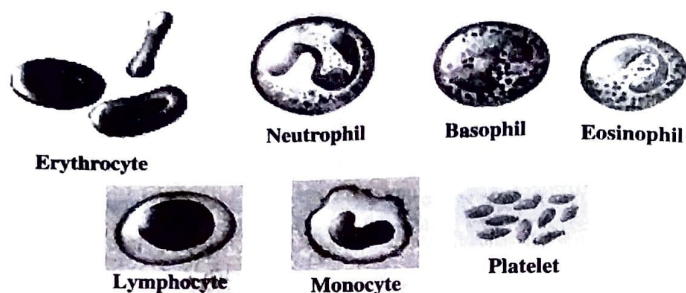


Figure 6.1: Formed Elements of Blood

Table 6.2: Formed Elements of Blood with their Characteristics and Functions

Cell Types	No. (μm^3)	% of WBCs	Description	Diameter	Functions
1) RBCs	5×10^6 (male); 4.5×10^6 (female)		Biconcave disc-like, no nucleus, have haemoglobin which gives red colour.	6.5-8.5 μm	Transports nutrients, enzymes, hormones, and respiratory gases (O_2 and CO_2).
2) WBCs					
i) Granulocytes					
a) Neutrophils	3,500-7,000	60-70%	Nucleus is 2-4 lobed and is connected with a thin filament; cytoplasmic granules are stained with neutral (light pink or reddish purple) dye.	10-12 μm	Phagocytise microbes and other substances.
b) Basophils	50-100	0.5-1%	Nucleus is 2-3 lobed and S-shaped; cytoplasmic granules are stained with basic (blue-purple) dye.	10-12 μm	Releases heparin which prevents blood clotting, and histamine which promotes inflammation.
c) Eosinophils	150-400	2-4%	2-3 lobed nucleus is connected with thin filament; cytoplasmic granules are stained with acidic (orange-red) dye.	11-14 μm	Participates in immunity, reduces inflammation.
ii) Agranulocytes					
a) Lymphocytes	3500-7000	60-70%	Rounded nucleus; cytoplasm forms a thin ring around nucleus.	6-14 μm	Produces antibodies and participates in immune system.
b) Monocytes	200-800	3-8%	Rounded, kidney-shaped (or horse-shoe shaped) nucleus; largest blood cells.	12-20 μm	Phagocytic cell in the blood; leaves the blood and modifies to macrophages, which phagocytise microbes, dead cells and other debris within tissues.
3) Platelets	250,000-400,000		Granular cell fragments, surrounded by plasma membrane.	2-4 μm	Releases blood clotting factors and forms platelet factor.

6.1.2.2. Blood Plasma and Plasma Proteins

Plasma forms the liquid or serum part of blood. Around 55% of blood (2.7-3.0 litres in an average human) constitutes blood plasma, i.e., blood's liquid medium (golden-yellow in colour). Blood plasma has 92% water, 8% blood plasma proteins, and other substances in trace amount.

Components of Plasma

1) Plasma proteins contains the following:

- Albumin:** It is formed in liver and is the most abundant plasma protein. It maintains osmotic pressure by retaining fluid in vascular compartments.
- Globulin:** It is a primary immunological agent and is of three types, i.e., α (alpha), β (beta), and γ (gamma) globulins. Gamma globulins are immunoglobulins, which help in the maintenance of osmotic pressure in blood vessels.
- Fibrinogen:** It is the key component of blood clotting and solidifies from a liquid to fibrin fibres which control bleeding.

2) Blood clotting factors, e.g., prothrombin.

3) Other substances, e.g., hormones, carbon dioxide, electrolytes, vitamins, etc.

6.1.2.3. Role of Blood Cells in Health and Diseases

Any obstruction to the haematological system results in circulatory disorder and its clinical manifestations appear in the hematologic as well as in other tissues. The symptoms of haematological disorders are oedema, thrombosis, congestion, haemorrhage, bruising, lymphedema, shock, embolism, etc. Examples of common haematological (or blood) disorders are given in table 6.3:

Table 6.3: Disorders of Blood

Disorders of Erythrocytes	Disorders of Leucocytes	Disorders of Platelets	Disorders of Clotting
1) Anaemia	1) Leucocytosis	Thrombocytopenia	1) Vitamin K deficiency
2) Polycythaemia	2) Leucopenia		2) Disseminated Intravascular Coagulation (DIC)
	3) Leukaemia		3) Haemophilia
			4) Von-Willebrand disease

Some of these disorders are described below:

- Anaemia:** In anaemia, deficiency of RBCs or haemoglobin results due to any of the reasons involving excess blood loss, increased breakdown of RBCs, of the reasons involving excess blood loss, increased breakdown of RBCs, defected RBCs production in bone marrow, low maturing factor during RBCs formation, and nutritional defects.

In anaemia, there is less haemoglobin available for oxygen transportation from lungs to the tissues. In this condition, the production rate of new RBCs formed in bone marrow is less than the haemolysis rate.

Symptoms of Anaemia

- i) Extreme fatigue,
- ii) Weakness.
- iii) Pale skin.
- iv) Chest pain, fast heartbeat, or breathlessness,
- v) Headache, dizziness, or light headedness,
- vi) Cold hands and feet,
- vii) Brittle nails.
- viii) Tongue inflammation or soreness,
- ix) Cravings for non-nutritive items (ice, dirt, or starch), and
- x) Poor appetite in infants and children.

Based on the cause, anaemia may be of the following types:

- i) **Iron Deficiency Anaemia (Microcytic Anaemia):** Throughout the world it is the most common anaemia type. Per day consumption of iron in males should be 1-2mg and for females it should be 3mg. Iron supplementation is needed during menstruation or during pregnancy for growth of foetus. Children need more iron compared to adults for fast growth. Iron deficiency anaemia occurs due to **lack of iron**. Insufficiency of iron hinders the production of haemoglobin. Hence, the iron deficiency anaemic patient shows symptoms like tiredness and breathlessness.
- ii) **Megaloblastic Anaemia (Vitamin B₁₂ and Folic Acid Deficiency):** Deficiency of **cobalamin** and/or **folate** produces **immature RBCs**, characterised as too large erythrocytes (megaloblasts) in the blood. Normally, the daughter cells formed at each stage of erythropoietic cell division are smaller than the parent cell, because the division is rapid and there is no time for cell enlargement. While in deficiency of key vitamins, DNA and RNA synthesis rate decreases which in turn hinders the RBCs maturation. So, circulating red blood cells grow unusually large, immature, and some are nucleated (MCV >94fl). The haemoglobin could be normal or elevated; the cells become fragile with decreased life span (between 40-50 days), and reduced formation with early breakdown results in anaemia.

Lack of folate in blood may occur due to:

- a) Insufficient diet in infants (with no proper mixed diet), in alcoholics, in anorexia, and in pregnancy (when more folate is required).
 - b) Coeliac disease, tropical sprue, or anticonvulsant drugs causing malabsorption of folate from the jejunum.
 - c) Cytotoxic and anticonvulsant drugs showing interaction with folic acid.
- iii) **Pernicious Anaemia:** It is the commonest form of vitamin B₁₂ deficiency anaemia in which absorption of vitamin B₁₂ is hampered due to the lack of Intrinsic Factor (IF) produced by the stomach lining. It is more prevalent in females than in males around after an age of 45 years. It is an autoimmune disease, i.e., auto-antibodies damage the Intrinsic Factor (IF) and stomach parietal cells.

- iv) **Hypoplastic and Aplastic Anaemia:** These conditions of anaemia are associated with bone marrow disease. Hypoplastic anaemia occurs due to decreased marrow function and aplastic anaemia occurs due to destruction of bone marrow.
 - v) **Haemolytic Anaemia:** At times cells are damaged or removed from circulation because the cells are abnormal or the spleen is hyperactive. Any genetic abnormality causes abnormal haemoglobin formation and increased friability of cell membrane by reducing oxygen-carrying capacity and lifespan of RBCs. Diseases under this category are **sickle cell anaemia** and **thalassemia**.
- 2) **Polycythaemia:** Unusually elevated erythrocytes count in the blood, increases blood viscosity, declines the rate of flow and increases the risk of intravascular clotting, ischemia, and infarction. Polycythaemia can be any of the two types:
 - i) **Primary Polycythaemia:** It is a consequence of the pathological condition known as polycythaemia vera. In this condition number of RBCs increases more than 7-8 millions/mm³. It is a genetic disorder in which red cells are continuously formed even if many cells are already present. There is also excess synthesis of WBCs and platelets.
 - ii) **Secondary Polycythaemia:** It occurs as a consequence of the following conditions:
 - a) **Physiological Polycythaemia:** It occurs if a person migrates from sea level to high altitude. Partial pressure of oxygen (PO₂) decreases on moving to high altitudes from sea level that causes hypoxia (decreased concentration of oxygen in body). Hypoxia stimulates erythropoietin hormone which increases red blood cell formation, resulting in physiological polycythemia.
 - b) **Pathological Polycythaemia:** It is caused by chronic hypoxia as a result of long standing lung diseases or cardiac failure.
 - 3) **Leukocytosis:** In this disorder, number of circulating WBCs increases above 11000/mm³. Leukocytosis can also be **physiological** or **pathological**. Leukocytosis may also occur under the following conditions:
 - i) Newborn infants (up to 1yr),
 - ii) Muscular exercise,
 - iii) Pregnancy,
 - iv) Exposure to very low temperature,
 - v) Mental stress,
 - vi) Parturition, and
 - vii) Emotional stress.
 - 4) **Leucopenia:** In this disorder, number of WBCs decreases below 400/mm³ of blood. Physiological leukopenia is infrequent in nature. Pathological leucopenia may occur due to:
 - i) Infection by non-pyogenic bacteria, e.g., typhoid fever,
 - ii) Irradiation by gamma rays,
 - iii) Drugs and chemicals containing benzene, and
 - iv) Replacement of bone marrow with neoplastic states.

- 5) **Leukaemia:** It is a cancerous condition characterised by multiplication of abnormal WBCs in an uncontrolled manner. These cancerous WBCs accumulate in the red bone marrow, thus, interfering with the production of blood cells (RBCs, WBCs, and platelets). This in turn reduces the oxygen carrying capacity of blood, and the patient becomes increasingly prone to infection; also abnormal blood clotting is seen in these individuals. Most of the times, leukaemia affects the lymph nodes, liver, and spleen, thus, increasing their size (resulting from the spread of WBCs).

Anaemic symptoms, i.e., fatigue, intolerance to cold, and pale skin are common in leukaemia. Weight loss, fever, night sweats, excessive bleeding, and recurrent infections may also be seen in patients of leukaemia.

Two types of leukaemia may occur:

- Acute leukaemia (symptoms develop rapidly), and
- Chronic leukaemia (symptoms develop after many years).

Adults may suffer from any of the two types, but children are generally affected by the acute type.

Causes of leukaemia are not known, however, certain risk factors, e.g., exposure to radiation or chemotherapy for other cancers, genetics (genetic disorders such as Down's syndrome), environmental factors (smoking and benzene), and microbes like the Human T cell Leukaemia-lymphoma Virus-1 (HTLV-1) and the Epstein-Barr virus have been implicated in the causation of leukaemia.

Leukaemia can be treated by chemotherapy, radiation, stem cell transplantation, interferon, antibodies, and blood transfusion.

- 6) **Thrombocytopenia:** It is characterised by blood platelet count below $150 \times 10^9/l$ ($1, 50,000/mm^3$). Capillary bleeding occurs when the count falls below $30 \times 10^9/l$ ($30,000/mm^3$) due to fall in platelet production rate or due to increased rate of destruction.

Thrombocytopenia may result due to:

- Decreased production of platelets, and
- Increased destruction of platelets.

- 7) **Vitamin K Deficiency:** Vitamin K is required for synthesis of clotting factors II, VII, IX, and X, in liver; therefore deficiency of vitamin K causes bleeding disorder in infants and in adults.

- Deficiency in New-Borns:** In infants, vitamin K deficiency is caused when vitamin K stored from mother is utilised and intestinal bacteria are not yet synthesising it. Due to this, spontaneous haemorrhage from umbilical cord and intestinal mucosa occurs in new born babies.
- Deficiency in Adults:** In adults, vitamin K deficiency occurs when bile salts are not available for its absorption. It may be in case of liver diseases, cirrhosis, hepatitis or prolonged obstruction of bile duct.

- 8) **Disseminated Intravascular Coagulation (DIC):** In this condition, the clotting mechanism becomes stimulated in general areas of blood circulation due to the following reasons:
- Large number of tissue factors is released into the blood by the large amount of traumatised or dying tissues.
 - In case of septicæmia shock, clotting mechanism is induced by bacteria and their toxins.
 - Premature separation of placenta occurs when amniotic fluid enters in the maternal blood.
 - Acute pancreatitis occurs when digestive enzymes are released into blood.
 - Malignant tumours with metastasis.

In DIC, a number of small clots are formed that plug a large share of peripheral blood vessels. Sometimes bleeding starts when clotting factors are removed by widespread clotting (some procoagulants remain to allow haemostasis of the remaining blood).

- 9) **Haemophilia:** It is an inherited bleeding disorder in which absence or low levels of clotting factors cause improper blood clotting. The affected individuals bleed easily, and the blood clot is formed after a long time. They suffer from spontaneous or internal bleeding and also experience painful, swollen joints due to bleeding joints. This condition is rare but serious which can even lead to serious complications.

Haemophilia is an inherited genetic disorder which cannot be cured, but treated so that the symptoms can be lowered and future health complications can be prevented. Acquired haemophilia occurs after birth; however it is a very rare condition, affecting individuals whose immune system develops antibodies attacking VIII or IX clotting factors.

Haemophilia is of the following three types:

- Haemophilia A (or Classic Haemophilia):** Most common form resulting due to deficiency of clotting factor VIII.
- Haemophilia B (or Christmas Disease):** Results due to deficiency of clotting factor IX.
- Haemophilia C (or Rosenthal's Haemophilia):** Mild form resulting due to deficiency of clotting factor XI. The affected individuals do not experience spontaneous bleeding. Haemorrhage may occur following trauma or surgery.

6.1.3. Pathological Blood Tests

Along with detection and diagnosis of disease, the blood pathology tests offer the following advantages:

- Treatment of disease,
- Monitoring progression of disease,
- Prevention of disease (e.g., a PAP smear or mammogram decreases the risk of some common cancers in women).

- 4) Determination of diseases in future (e.g., examining cholesterol levels or the risk of inherited conditions like familial breast cancer), and
- 5) Supporting research into new treatments, and safety of treatments and procedures.

A patient is sent for blood and pathology tests for:

- 1) **Screening of Disease:** It helps in early detection of a disease or detection of genetic or inherited disorder in an individual.
- 2) **Determination of Potential Health Risks:** Blood and pathology tests help in detection of possible health risks like diabetes, heart disease, or rheumatoid arthritis. Physician determines health risk by looking at health history of an individual which includes age, weight, lifestyle, and family history of disease.
- 3) **Detection of Causing Agents:** Blood and pathology tests help in identifying the cause which helps in determining the treatment plan for the disease.
- 4) **Determination of Prognosis:** Blood and pathology tests determine the prognosis of a disease and health outcomes. **For example**, if someone is suffering from cancer, the diagnostic tests are performed to identify other complications and stages of cancer.
- 5) **Preparation before Surgery:** Blood tests help to determine the type of blood before surgery or transfusion.
- 6) **Monitoring Illness and Medications:** Blood tests are performed to monitor whether an illness is getting better or worse or is stable. These diagnostic tests also assess the medication levels in blood and the effects of some medications on the organs.

Example of some common blood and pathology tests:

- 1) **Complete Blood Count (CBC):** It is a common test used for diagnosing a wide range of diseases, disorders, and infections. CBC provides information about the numbers and development of blood cells in body. **For example**, abnormalities in RBCs, WBCs, and platelets may cause anaemia, infection, and some blood cancer, e.g., leukaemia.
- 2) **Liver Function Tests (LFT):** It is mainly performed for measuring enzymes, proteins, and substances produced or secreted by liver. Any alteration in these secretions shows that liver is suffering from a disease or infection. These tests also help in early diagnosis and treatment of disease.
- 3) **Detection of Iron Level:** It determines whether a person have too little or too much iron, which is required for normal functioning of the body. Low level of iron in body causes anaemia which results in fatigue and lack of energy; and high level of iron in body causes hemochromatosis which increases the risk of serious conditions like liver disease, heart failure, arthritis, or diabetes.
- 4) **Thyroid Stimulating Hormone (TSH) Quantification:** This test monitors the thyroid disorders like hypothyroidism (insufficient thyroid hormone).

hyperthyroidism (excess thyroid hormone), some cancers, and autoimmune conditions. TSH stimulates thyroid gland for the synthesis of thyroid hormones which are used in various body functions like breathing, heart rate, body weight, temperature, and maintenance of energy levels.

- 5) **Urinalysis:** It is a set of tests in which urine samples are taken to detect many diseases, e.g., liver or kidney disease, diabetes and pregnancy. Urinalysis is also performed to diagnose urinary tract infections.

6.1.4. Erythrocytes – Abnormal Cells and their Significance

Normal mature erythrocytes are disc-shaped, biconcave cells which do not have nucleus. Any variations in size, shape, or colour of erythrocytes can be examined under microscope using Wright or similar Romanowsky-type stain. Appearance and morphology of normal erythrocytes may vary in various disorders or diseased conditions.

In erythrocytes, following types of variations are observed:

- 1) Variation in size,
- 2) Variation in shape,
- 3) Alteration in colour,
- 4) Inclusions in the erythrocyte, and
- 5) Alterations in the erythrocyte distribution on a peripheral blood smear.

6.1.4.1. Variations in Size

A normal erythrocyte has an average diameter of $7.2\mu\text{m}$ (ranges between $6.8-7.5\mu\text{m}$). The maximum size limit is considered to be $6.2-8.2\mu\text{m}$ and this normal size is termed as **normocytic**. A common term used for an increased variation in cell size is known as **anisocytosis**. It is a non-specific term which does not indicates types of variation, i.e., erythrocytes may be either larger than normal (**macrocytic**) or smaller than normal (**microcytic**) (figure 6.2).

Size of macrocytic erythrocytes exceeds the $8.2\mu\text{m}$ diameter limit, while size of microcytic erythrocytes remains less than the average, i.e., $6.2\mu\text{m}$ diameter.

Macrocytosis occurs due to defect in either nuclear maturation or stimulated erythropoiesis. A nuclear maturation defect resulting due to deficiency of either vitamin B_{12} or folate is known as **true macrocytes**. These cells are formed due to disrupted regular mitotic division in the bone marrow. In this defect, the cells appear as mature and enlarged erythrocytes in the circulating blood. Other type of macrocytosis occurs due to **increased erythropoietin stimulation** which increases the synthesis of haemoglobin in developing cells. In this disorder, premature reticulocytes are released into the blood circulation. These cells appear as macrocytic, basophilic, and slightly hypochromic on a periphery.

In **microcytosis**, synthesis of haemoglobin decreases due to iron deficiency, impaired globulin synthesis, and mitochondrial abnormality. Disorders leading to microcytosis are malabsorption syndrome, iron deficiency anaemia, and haemoglobinopathies (case of variant haemoglobin).

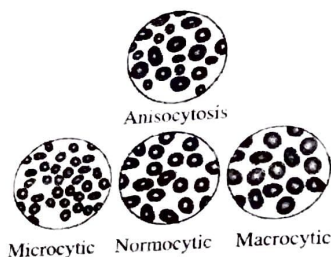


Figure 6.2: Variations in Erythrocyte Size

6.1.4.2. Variation in Shape

A term **poikilocytosis** is used to denote mature erythrocytes that have the shape other than the normal round, biconcave appearance on a stained blood smear, or variations. Poikilocytes are found in many shapes (figure 6.3) but some specific kinds of poikilocytes are acanthocytes, blister cells, burr cells, crenated erythrocytes, echinocytes, elliptocytes, keratocytes, ovalocytes, pyknocytes, schistocytes, sickle cells, speculated erythrocytes, spherocytes, stomatocytes, target cells, and teardrops:

- 1) **Acanthocytes:** They have **multiple thorny, spike-like projections** which are irregularly distributed around the cellular membrane and may vary in size (figure 6.3 B). Acanthocytes are formed mainly in two different disorders, i.e., abetalipoproteinaemia (rare hereditary disorder), and spur cell anaemia. Acanthocytes can also be observed in liver cirrhosis with associated haemolytic anaemia, following heparin administration, in hepatic haemangioma, in neonatal hepatitis, and in postsplenectomy.
- 2) **Blister Cells:** They have **one or more vacuoles** which look like a blister on the skin and have a thinned area at the periphery of the cell membrane (figure 6.3 C). The vacuoles may rupture and when it occurs, distorted cells (**keratocytes**) and cell fragments (**schistocytes**) are formed. These cell appear in case of severe burns (due to damage to the membrane), pulmonary emboli in sickle cell anaemia and microangiopathic haemolytic anaemia
- 3) **Burr Cells/Echinocytes:** They have one or more **spiny projections of cellular membrane** (figure 6.3 D). They are elongated or form irregular shapes like quarter-moon. These cells appear in a variety of anaemia, bleeding gastric ulcers, gastric carcinoma, peptic ulcers, renal insufficiency, pyruvate kinase deficiency, and uraemia.
- 4) **Poikilocytes:** They are abnormal shaped (figure 6.3 E). They result from the production of abnormal cells by the bone marrow or from damage to normal cells. They appear in primary or secondary myelofibrosis, congenital and acquired dyserythropoietic anaemia, hereditary pyropoikilocytosis, and haemoglobin H disease.
- 5) **Echinocytes (Crenated Erythrocytes):** They have **short, scalloped, or spike-like projections** which are regularly distributed around the cell membrane (figure 6.3 F). These projections vary in number and appearance. They appear in case of physical loss of intracorporeal water. Any diseased state is not related to crenation but this cellular distortion occurs due to osmotic imbalance.

- 6) **Elliptocytes:** They have **rod, cigar, or sausage-like shape**; narrower and more elongated than megalocytes (figure 6.3 G); and appear in hereditary elliptocytosis anaemia associated with malignancy, haemoglobin (Hb) C disease, haemolytic anaemia (occasionally), iron deficiency anaemia, pernicious anaemia, sickle cell trait, and thalassemia.

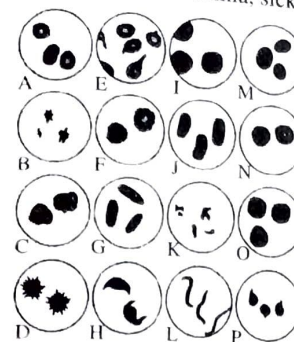


Figure 6.3: Variations in Erythrocyte Shape. A) Normal Red Blood Cells (Discocytes); B) Acanthocytes; C) Blister Cells; D) Burr Cells; E) Poikilocytes; F) Crenated RBCs (Echinocytes); G) Elliptocytes; H) Helmet cells (Schistocytes); I) Leptocytes; J) Oval Macrocytes (Megalocytes); K) Schistocytes (Schistocytes); L) Sickle Cells (Drepanocytes); M) Spherocytes; N) Stomatocytes; O) Target Cells (Codocytes); P) Teardrops (Dacryocytes).

- 7) **Leptocytes:** These are **thin or flattened RBCs**, having a central rounded area of pigmented material and a middle clear zone without any pigment, and an outer pigmented rim at the periphery of the cell (figure 6.3 I). Leptocytes occur mostly in sideropenic anaemia (an iron deficiency anaemia) occurs due to the disturbance in synthesis of haemoglobin. They can also be found in hypochromic anaemia, homozygous and heterozygous forms of C, D, and E hemoglobinopathy.
- 8) **Oval Macrocytes:** They are formed when there is a disturbance in the synthesis of DNA due to vitamin B₁₂ or folic acid deficiency (figure 6.3 J).
- 9) **Schistocytes:** These are fragments of red blood cells (figure 6.3 K) and are formed either by **fragmentation of abnormal cells** like in hereditary pyropoikilocytosis, or due to mechanical, toxin- or heat-induced damage of previously normal cells. They are seen in microangiopathic and mechanical haemolytic anaemia, collectively known as schistocytic haemolytic anaemia.
- 10) **Sickle Cells (Drepanocytes):** They are formed in sickle cell anaemia (figure 6.3 L). It is an autosomal recessive genetic disorder. Mutation (gene defect) in a single nucleotide (GAG to GTG) of β -globin gene, which causes substitution of glutamic acid by valine at position 6. This leads to formation of new haemoglobin known as **Haemoglobin S (HbS)**.
- 11) **Spherocytes:** These are **spherical or nearly spherical** in shape rather than biconcave disc shape (as normal RBCs) (figure 6.3 M). These cells loose

membrane without equivalent loss of cytosol due to inherited or acquired abnormality of the red cell cytoskeleton and membrane. **Macrospherocytes** occur in hereditary stomatocytosis (over-hydrated variant) due to the osmotic swelling. To examine the presence of spherocytes in blood film, it is necessary to examine the part of the film where the cells are just touching, because normal cells may lack central pallor near the tail of the film. Sometimes overlapping of cells give false impression of spherocytosis.

- 12) **Stomatocytes:** These cells have a **central pallor** or **stoma** on a stained blood film (figure 6.3 N). These cells are rarely seen in the blood film of a healthy person. Various abnormalities of membrane lead to stomatocytosis (due to the expansion of the inner leaflet of the lipid bilayer of RBCs). Liver diseases, hereditary spherocytosis, and autoimmune haemolytic anaemia also lead to stomatocytes formation.
- 13) **Target Cells:** They are formed as a result of excess of membrane in comparison to the volume of the cytoplasm. These cells are thinner than normal cells and have an **increased staining area** which appears in the middle of the area of central pallor (figure 6.3 O). On demonstrating through the scanning electron microscope, they appear bell-shaped. Depending on the cause of the abnormality and the mechanism of formation, target cells may be microcytic, normocytic, or macrocytic.
- 14) **Teardrop or Pear-Shaped Cells (Dacrocytes):** They are formed in case of **bone marrow fibrosis** or severe dyserythropoiesis and also in some haemolytic anaemia (figure 6.3 P). They are also characteristic of megaloblastic anaemia, thalassemia major, and myelofibrosis.

6.1.4.3. Alteration in Colour

Red blood cells have iron-containing, oxygen-transporting metalloprotein known as **haemoglobin**, which provides a characteristic red colour. Haemoglobin can be in two forms, i.e., oxyhaemoglobin (when it is oxygenated) and deoxyhaemoglobin (when it is deoxygenated). Following alterations are observed in the colour of RBCs during any disease or disorder:

- 1) **Hypochromia:** It shows a reduced staining of RBCs due to an increase in central pallor which occupies more than the normal, approximately one third of the red blood cell diameter than the normal. Severe hypochromia can be observed due to reduction in the **Mean Cell Haemoglobin Concentration (MCHC)**. In general most of the conditions causing microcytosis may also cause hypochromia (except in case of α or β thalassemia in which the blood film shows microcytosis without appreciable hypochromia). However in case of copper deficiency, hypochromia is associated with macrocytosis. It has been observed that RBCs of healthy infants are often hypochromic as compared to that of adults. Staining of RBCs depends on the thickness of the cell and on the concentration of haemoglobin, therefore hypochromia can also be observed in cells having cell membrane thinner than normal, whether or not they have a normal volume and haemoglobin concentration. Such cells are known as **leptocytes**.

- 2) **Hyperchromia:** This term is used for cells that are more intensely stained than normal or to indicate the hyperchromic cell condition. Hyperchromia is seen in spherocytes and irregularly contracted cells. It shows increase in **MCHC** which indicates that hyperchromia involves change in the shape of cells and also increase in the intracellular haemoglobin. However, hyperchromia may also be seen in macrocytes without any increase in haemoglobin concentration and these cells have outer membrane thicker than normal, or lack central pallor.
- 3) **Anisochromasia:** It indicates an increased variability in the degree of staining or haemoglobinisation of the RBCs. Practically, a spectrum of staining is seen from hypochromic to normochromic and anisochromasia indicates a changing situation, e.g., development of iron deficiency, responses to the treatment, and development or regression of anaemia.
- 4) **Dimorphism:** It shows the presence of two types of RBCs. This term is mostly used when one population of RBCs is hypochromic, microcytic cells; and other population is of normochromic cells, later which becomes normocytic or macrocytic. They vary in their size, haemoglobin content, or shape which can be seen in differential diagnosis. Dimorphism in RBCs is seen in case of iron deficiency anaemia (following administration of iron or blood transfusion), sideroblastic anaemia, the heterozygous state for hereditary sideroblastic anaemia, macrocytic anaemia post-transfusion, double deficiency of iron and either vitamin B₁₂ or folic acid, unmasking of iron deficiency following treatment of megaloblastic anaemia, and due to delayed transfusion reactions. Dimorphic cells can rarely occur in thalassemia (involving an abnormality of a constitutional chromosomal), and chimaerism post-stem cell transplantation when either the donor or the host has microcytosis with a genetic basis.
- 5) **Polychromasia/Polychromatophilia/Polychromatic Cells:** These are RBCs showing pinkish-blue colour on staining due to uptake of both eosin and basic dyes by the haemoglobin and by the residual RNA, respectively. The **reticulocyte cells** take up vital dye to form a visible reticulum due to the presence of ribosomal RNA. A correlation can be seen between reticulocytes and **polychromatic cells**. Both these cells are immature RBCs, newly released from the bone marrow. The immature reticulocytes are released from the bone marrow when there is a transient or persistent haemopoietic stress, and when erythropoietin levels are high.

Immature reticulocytes are less dense and their size is less than the mature erythrocytes due to reduced concentration of haemoglobin. They show irregular, multilobed surface on visualising under scanning electron microscope and are known as **polychromatic macrocytes**. The late reticulocytes, exclusively found in the blood of haematologically normal subjects, are cup-shaped and only slightly larger than mature erythrocytes.

The total number of reticulocytes, the proportion of early reticulocytes, and the number of polychromatic macrocytes increase when there are physiological responses such as increasing altitude or other hypoxic stimulus.

as a normal response to anaemia (when there are no factors limiting erythropoiesis).

Polychromatic erythrocytes are absent in pure red cell aplasia and in aplastic anaemia; and their concentration is low in the anaemia of chronic disease and often in renal failure when the erythropoietin response is inadequate. Absence of polychromasia in sickle cell anaemia or other haemolytic anaemia patients is significant because it may indicate complicating parvovirus B19-induced red cell aplasia.

Number of polychromatic erythrocytes increases in primary myelofibrosis and in metastatic carcinoma of the bone marrow. In these diseases, size of polychromatic cells is not increased always but morphological abnormality is seen, i.e., the cells become deeply basophilic in colour than normal cells.

6.1.4.4. Inclusions in Erythrocytes

These are elements, present in RBCs. Each type of inclusion has specific appearance, composition, and related physiology. Presence of these inclusions in RBCs indicates a disease or disorder. Following are the **examples** of common types of inclusion bodies:

- 1) **Howell-Jolly Bodies:** These are round, solid-staining, dark-blue to purple inclusions, having size of 1-2 μ m. They are not seen in normal erythrocytes, and their presence is associated with haemolytic anaemia, pernicious anaemia, particularly postsplenectomy, physiological atrophy of the spleen.

If present, only one or two bodies are found within mature RBCs that lack a nucleus; they may be seen in immature, nucleated RBCs also. Howell-Jolly bodies are developed due to the accelerated or abnormal erythropoiesis, because the spleen cannot remove these remnants from the cell.

- 2) **Pappenheimer Bodies (Siderotic Granules):** These bodies are observed as purple dots in Wright-stained smears and can be rarely seen in peripheral blood smear. **Siderotic granules** are dark-staining particles of iron (represents ferric ions, Fe^{3+}) present in erythrocyte that can be seen on staining with a special **Prussian blue** iron stain. Pappenheimer bodies and siderotic granules are almost identical structures. Pappenheimer are clinically associated with iron-loading anaemia, hyposplenism, and haemolytic anaemia. These bodies are found in mitochondria, ribosome, and iron particles.
- 3) **Heinz Body:** These inclusion bodies are 0.2-2.0 μ m in size and can be observed on staining with crystal violet or brilliant cresyl blue. They indicate precipitated and denatured haemoglobin. They are clinically associated with congenital haemolytic anaemia, G6PD deficiency, and haemolytic anaemia caused by some drugs (like phenacetin, phenylhydrazine, primaquine) or drug therapies (like haemoglobinopathies).

- 4) **Basophilic Stippling:** They appear like tiny, round, solid-staining, dark-blue granules which are evenly distributed throughout the cell. These small dots are actually accumulated RNA and ribosomes. Basophilic stippling can be of two types:
 - i) **Fine Basophilic Stippling:** It occurs when production of RBCs is increased and is commonly seen in increased polychromatophilia.
 - ii) **Coarse Basophilic Stippling:** It occurs in megaloblastic anaemia or other forms of severe anaemia, lead poisoning, and thalassemia. Coarse basophilic stippling shows impaired haemoglobin synthesis due to the instability of RNA in young cells.
- 5) **Cabot Rings:** These are loop-shaped, ring-shaped, or figure 8-shaped structures. They are present in cytoplasm of RBCs and like beads on a string. They attain red-purple colour under the Wright Stain. They are remnants of microtubules, involved in mitosis. They are associated with **lead poisoning**, **pernicious anaemia**, and **Cabot Rings Myelodysplastic Syndrome**.

6.1.4.5. Alterations in Erythrocyte Distribution

Two types of alterations, i.e., **agglutination** and **rouleaux formation**, are observed in erythrocytic distribution that may be observed on a stained peripheral smear. **Agglutination** is the clumping of erythrocytes, and in **Rouleaux formation**, erythrocytes are arranged in groups that resemble stacks of coins. **Rouleaux formation** occurs mostly in the thick portions of normal blood smears. The **rouleaux** are pathological if they are present in thin areas of the blood smear where the erythrocytes just touch each other or barely overlap. True agglutination or clumping occurs as a result of antigen-antibodies reaction on the erythrocyte. Thus, **rouleaux formation** shows presence of cryoglobulins.

6.1.5. Pathology of Urine

Urine is a sterile liquid, by-product of the body, formed by the kidneys by a process known as **urination**. It is excreted through the urethra by a process known as **micturition**. Analysis of urine is performed to diagnose various pathological conditions. Urine analysis is based on either physical or chemical characteristics of urine which help in assessment of processes occurring within the body.

Urine is an aqueous solution, having more than 95% water and 5% of other constituents mentioned below, with their decreasing concentrations:

- 1) Urea (9.3gm/l),
- 2) Chloride 1.87gm/l,
- 3) Sodium 1.17gm/l,
- 4) Potassium 0.750gm/l,
- 5) Creatinine 0.670gm/l, and
- 6) Other dissolved ions, inorganic and organic compounds (proteins, hormones, and metabolites).

In the liver, ammonia and carbon dioxide undergo ornithine cycle to synthesise urea, which is non-toxic to mammals (unlike ammonia). The processed form of ammonia is urea which is non-toxic to mammals as ammonia is highly toxic in nature. Urine is sterile until it reaches the urethra, where epithelial cells lining the urethra have facultative anaerobic, gram-negative rods, and cocci.

Physical features of urine are its colour, turbidity or transparency, odour, pH (acidity or alkalinity), and density. Many of these features can be identifiable physically while for some laboratory testing is performed:

- 1) **Colour:** Normally yellow-amber in colour, but colour may depend on recent diet and the concentration of urine. Concentration of urine reduces due to drinking of more water and it causes lighter colour of urine, whereas dark coloured urine indicates dehydration. Red urine shows the presence of RBCs in urine due to kidney damage and disease.
- 2) **Odour:** Its smell indicates various pathological conditions; for example, urine of diabetic patients has sweet or fruity smell due to the presence of ketones (organic molecules of a particular structure) or glucose. Commonly, fresh urine has a mild odour while aged urine smells stronger just like ammonia.
- 3) **pH:** The pH of normal urine ranges between 4.6-8 (average 6.0), which depends on the diet of an individual. For example, high protein diets make urine acidic while vegetarian diets result in alkaline urine.
- 4) **Density:** It is the ratio of the weight of a volume of a substance compared with the weight of the same volume of distilled water; and is also known as **specific gravity**. The density of normal urine ranges between 0.001-0.035.
- 5) **Turbidity:** The turbidity of the urine sample is evaluated from person to person and is reported as clear, slightly cloudy, cloudy, opaque, or flocculent. Generally, fresh urine is either clear or very slightly cloudy. Excess turbidity is observed in the presence of suspended particles in the urine that can be determined through the examination of microscopic urine sediment. Turbidity of the urine generally increases due to urinary tract infections or obstructions.

Any abnormalities in these physical characteristics of urine indicate the presence of a disease or metabolic imbalances. These problems may be a consequence of serious diseases like diabetes mellitus, damaged glomerulus, UTIs, etc.

6.1.5.1. Abnormal Constituents of Urine and their Significance in Diseases

Urine has the following constituents, whose abnormality indicates a pathological condition:

- 1) **Proteins:** Presence of abnormal concentration of albumin and globulin in the urine is known as **proteinuria (albuminuria)**, which may be of two types:
 - i) **Physiologic Proteinuria:** Less than 0.5% protein is observed in urine due to severe exercise, after a high protein meal, and during pregnancy.

- ii) **Pathologic Proteinuria:** Amount of proteins increases in case of glomerulonephritis and nephrotic syndrome. Proteinuria may also occur due to poisoning of renal tubules by heavy metals (like mercury, arsenic, or bismuth).
 - 2) **Glucose:** Presence of glucose in urine is known as **glycosuria** which occurs due to diabetes mellitus and endocrinal disorders such as hyperpituitarism, hyperthyroidism, Cushing's syndrome, and pheochromocytoma. Transient glycosuria may be observed rarely due to emotional stress (like in exciting athletic contest).
 - 3) **Other Sugars:** Presence of fructose sugar in urine due to disturbed fructose metabolism is known as **fructosuria**. Similarly, **gallactosuria** and **lactosuria** occur rarely in infants, pregnant women, and in lactating mother. Consumption of food rich in pentose sugars (like grapes, cherries and plums) causes **pentosuria**. The condition of pentosuria also occurs in inherited diseases in which pentoses are not metabolised.
 - 4) **Ketone Bodies:** Under normal conditions, less than 1mg of ketone bodies are excreted through urine in 24 hours. Excretion of ketone bodies increases in many conditions like starvation, diabetes mellitus, pregnancy, ether anaesthesia, and in certain types of alkalosis.
 - 5) **Bilirubin and Bile Salts:** In case of obstructive or hepatic jaundice, bilirubin is excreted in urine. A condition in which bile salts are excreted in urine is known as **bilirubinuria**. In certain stages of liver disease, bile salts are excreted without bile pigments in urine. Traces of bilirubin without bile salts are excreted in urine during excessive haemolysis.
- Bilirubin** is absent in urine of an adult healthy person. It is a waste product, produced by the liver from the haemoglobin of RBCs that are broken down and removed from circulation. It is utilised in the synthesis of bile (a fluid involved in food digestion). In some liver diseases like biliary obstruction or hepatitis, excess amount of bilirubin is formed which is eliminated through urine. Presence of bilirubin in urine indicates liver disease (like development of jaundice).
- 6) **Blood:** Blood is excreted in urine due to lesions of kidney, urinary tract infection, and in case of nephritis. Free haemoglobin molecules may also be detected after quick haemolysis, e.g., black water fever (a consequence of malaria) and severe burns.

Presence of following blood cells indicates a pathological condition:

- i) **RBCs:** Some RBCs can be found in urine sediment (0-5 RBCs per high power field, HPF) even in normal conditions. A positive chemical test for haemoglobin and increased number of RBCs seen under the microscope indicates presence of blood cells in the urine. However, this test cannot diagnose where the blood is coming from. For example, presence of RBCs in urine due to haemorrhoids or vaginal bleeding cannot be differentiated from a bleed in the urinary tract. Therefore, urine should be collected adequately.

Presence of RBCs in urine is known as **haematuria**. Sometimes haematuria has no lasting harm and can be easily controlled by treating the underlying causes. In urine, presence of blood along with WBCs and bacteria indicates UTIs which can be treated with antibiotics. However, haematuria may also occur as a consequence of some severe infections that must be monitored and treated properly.

- ii) **WBCs:** A minute concentration of WBCs (i.e., 0-5 WBCs per high power field, HPF) may be found even in normal conditions; and WBCs may also contaminate urine due to vaginal secretions. Increased number of WBCs in the urine under a microscope and/or a positive test for leukocyte esterase indicates urinary tract infection.
- iii) **Epithelial Cells:** Few epithelial cells can be normally found in the urine of both men and women. An increased number of epithelial cells are found in conditions, such as urinary tract infections, inflammation, and malignancies. Identification of the type of epithelial cells in urine helps in the detection of pathological conditions. For example, epithelial cells having large number of broken-down haemoglobin (or hemosiderin) indicate that there were RBCs or haemoglobin in the urine recently, even if they are present now.
- iv) **Urobilinogen:** During excessive haemolysis (like in **haemolytic jaundice** or **pernicious anaemia**), part of the bile pigment formed by the breakdown of haemoglobin is excreted out through urine which is known as urobilinogen. Large amounts of urobilin are found in urine during liver diseases and in constipation.

Low concentrations of urobilinogen are found in urine even under normal conditions. Urobilinogen are formed in the intestine from bilirubin and some amount of it is absorbed back into the blood. Positive test for urobilinogen indicates liver diseases like viral hepatitis, cirrhosis, liver damage due to toxic substances, and haemolytic anaemia due to increase in RBC destruction.

- v) **Porphyryns:** In a healthy adult person, around 60-280µg of coporphyrins per day is excreted in urine. Their excretion increases in some liver diseases as well as excess amount of coporphyrins in urine also indicates that patient is suffering from porphyria.

6.1.5.2. Pathological Urine Tests

A **urinalysis** is a set of physical, chemical, and microscopic tests which detect or measure the constituents excreted in the urine like the by-products of normal and abnormal metabolism, cells, cellular fragments, and bacteria.

Many disorders can be detected in early stage by diagnosing substances that are not normally present in the urine and/or by evaluating abnormal levels of some components (like **glucose**, protein, **bilirubin**, red blood cells, white blood cells, crystals, and bacteria). These substances may be present due to:

- 1) Higher level of substance in the blood and the body responds by trying to eliminate them through urine.

- 2) Presence of kidney disease, and
- 3) Urinary tract infection as in case of bacteria and white blood cells.

Whole urinalysis includes the following **three distinct testing phases**:

- i) **Visual Examination:** In this, the colour and clarity of urine sample is observed by the laboratorian to obtain an idea about the substances present in urine. These observations are used along with chemical and microscopic examination to confirm the presence of various substances.

Urine colour has a wide variety and the most common shade is yellow (ranging from very pale or colourless to very dark or amber colour). Colour of urine is affected by various conditions like disease processes, several medications (e.g., multivitamins can turn urine bright yellow), and eating of certain food (e.g., red colour of urine after eating beets containing natural pigment).

Red-colour of urine also indicates the disease or damage is limited to some part of the urinary system, whereas yellow-brown or greenish-brown colour of urine can be the symptoms of presence of bilirubin in urine.

Urine clarity means how clear the urine is. The clarity of urine can be described as clear, slightly cloudy, cloudy, or turbid. 'Normal' urine can be clear or cloudy. Cloudiness may be caused by presence of the mucus, sperm, prostatic fluid, cells from the skin, normal urine crystals, and contaminants (body lotions and powders); however presence of these substances is not considered unhealthy. While the presence of certain substances such as red blood cells, white blood cells, or bacteria, indicates an unhealthy condition and requires attention.

- 2) **Chemical Examination:** These examinations are performed chemically to determine following seven factors that give information about the concentration, and the health and diseased conditions:

- i) **Specific Gravity:** It is the measurement of urine concentration. Measurement of specific gravity compares the amount of substances dissolved in urine as compared to pure water. The specific gravity of the urine will be 1.000 (same as pure water), if any substance is not present in the urine, but it is not possible as even normal urine has certain substances. Specific gravity of urine can be very close to the water, if an individual drinks excessive amount of water in a short period of time or gets an intravenous infusion of large volumes of fluid. Urine with high specific gravity indicates concentrated urine or one with many substances in a limited amount of water.

- ii) **pH:** Generally urine is slightly acidic, having pH 6 which ranges between 4.5 to 8. The kidneys help in maintaining the acid-base balance of the body. Therefore, conditions causing acids or bases in the body (like acidosis or alkalosis, or the intake of acidic or basic foods) may directly affect the pH of urine.

iii) **Proteins:** Presence of proteins in urine can be detected by the following tests:

- Sulphosalicylic Acid Test:** In a test tube, 5ml of the protein solution is taken and added with sulphosalicylic acid drop by drop, and mixed after each addition. Formation of a white precipitate or turbidity indicates the presence of protein.
- Heller's Test:** In a test tube, 3ml of concentrated nitric acid is taken. The test tube is inclined and 3ml of the protein solution is added along the test tube wall. A white precipitate formed at the junction of the two fluids indicates the presence of protein.
- Heat Coagulation Test:** Two-third of the test tube is filled with the protein solution and 4-5 drops of chlorophenol red is added with thorough mixing. Purple red colour develops. 1% acetic acid is added drop by drop until the colour changes to a faint pink. The test tube is held at its bottom in slightly inclined position and the upper portion of the fluid is heated. Formation of a dense coagulum occurs in the upper part of the solution. Positive results are obtained for heat coagulable proteins, albumin, and globulin.

iv) **Carbohydrate: Benedict's test** (copper sulphate, sodium citrate, and sodium carbonate) is performed for the detection of carbohydrates in urine. In this test, 5ml of reagent is taken in a test tube and 8 drops of sugar solution are added in it. The test tube is placed in a boiling water bath for five minutes. Formation of a green, yellow, or orange red precipitate indicates the presence of reducing sugar.

v) **Ketone Bodies: Rothera's test** is performed for the detection of ketone bodies in urine. In this test, 5ml of urine is taken in a test tube. Solution is saturated with solid ammonium sulphate, and 2-3 drops of a freshly prepared 5% solution of sodium nitroprusside are added. 2ml of concentrated ammonium hydroxide solution is added in the solution with proper mixing. Formation of a permanganate colour indicates the presence of ketone bodies in urine. Sodium nitroprusside [$\text{Na}_2\text{Nitroferricyanide}$] decomposes to $\text{Na}_4\text{Fe}(\text{CN})_6$, NaNO_2 , $\text{Fe}(\text{OH})_3$ in an alkaline solution. These are strong oxidising agents having a rose or purple colour. The ammonium sulphate acts as a buffer maintaining the alkalinity with the range at which the complex has a purple colour.

vi) **Tests for Bile Pigments and Bile Salts:** Presence of bile pigments and bile salts can be detected by the following tests:

- Gmelin's Test for Bile Pigments:** In a test tube, 5ml of concentrated nitric acid and an equal volume of urine are added so that two layers are formed. Formation of various coloured rings (green, blue, violet, reddish yellow and red) at the junction of two liquids indicates the presence of bile pigments.
- Hay's Test for Bile Salts:** Half of a test tube is filled with water and another half is filled with urine. Some sulphur powder is sprinkled on the surface of two liquids. If the sulphur powder suddenly sinks in

the test tube containing urine, presence of bile salts is confirmed, while in the test tube containing water, sulphur powder remains on the surface.

vii) **Test for Blood:** Presence of blood in urine can be confirmed by **Benzidine Test**. In this test, a pinch of solid benzidine is taken in a test tube and 2ml of glacial acetic acid is added in it to dissolve benzidine. 2 ml of hydrogen peroxide is added and the solution is divided in two portions. In one portion, drop by drop urine is added with shaking and if blue colour appears, presence of blood in urine is confirmed, which quickly changes to brown colour. In the other portion, drop by drop water is added. No blue colour appears in the other portion.

3) **Microscopic Examination:** Microscopic examinations are not performed routinely. Generally, these tests are performed when abnormal results are obtained from physical and chemical examinations.

The microscopic tests are performed with urine sediment, i.e., the urine that has been centrifuged to concentrate the substances in it, at the bottom of a tube. The top layer of the tube is discarded and the drops of fluid remaining are analysed under a microscope. Cells, crystals, and other substances are calculated and measured either as the number observed **Per Low Power Field (LPF)** or **Per High Power Field (HPF)**. Along with this, some bodies (if present) are estimated as 'few', 'moderate', or 'many', such as epithelial cells, bacteria, and crystals.

6.2. EXERCISE

6.2.1. Short Answer Type Questions

- What is therapeutics?
- Give the normal composition of urine or blood.
- Write short notes on the following:
 - Lymphocytes
 - Platelets
 - Erythrocytes
- Give pathological tests of urine.
- What are the formed elements of blood?

6.2.2. Long Answer Type Questions

- Write a note on abnormal erythrocyte cells and their significance.
- Give a brief note on pathological test of blood.
- Write a detail note abnormal constituents of urine and their significance.

BOARD OF TECHNICAL EDUCATION UTTAR PRADESH

D. PHARM. - FIRST YEAR EXAMINATION, 2014

BIOCHEMISTRY AND CLINICAL PATHOLOGY

Time: 3 Hours

Max. Marks: 80

Note: 1) Attempt any Five questions. All questions carry equal marks.

- 2) Students are advised to specially check the Numerical Data of question paper in both versions. If there is any difference in Hindi translation of any question, the students should answer the question according to the English version.
- 3) Use of Pager and Mobile Phone by the students is not allowed.

Ques 1 a) What are essential amino acids? Give their biological importance.

(2×8=16)

Ans: Essential Amino Acids: Refer Chapter-1, Page No. 22

Biological Importance (Role of Amino Acids): Refer Chapter-1, Page No. 24

Ques 1 b) What are the abnormal constituents of urine? Explain their significance in disease.

Ans: Abnormal Constituents of Urine and their Significance in Diseases

Refer Chapter-6, Page No. 143

Ques 2 a) Give the composition of blood.

(6+5+5=16)

Ans: Composition of Blood: Refer Chapter-6, Page No. 126

Ques 2 b) Discuss the role of lymphocytes and platelets in our body.

Ans: Role of Lymphocytes

- 1) **Phagocytosis:** Leucocytes (neutrophils) engulf foreign particles and bacteria to digest them
- 2) **Antibody Formation:** Lymphocytes produce β - and γ -serum globulin.
- 3) **Fibroblasts Formation:** At the inflammation site, lymphocytes transform into fibroblasts and aid in repair process.

Role of Platelets

- 1) **Vasoconstriction:** Platelets become sticky and adhere to the damaged wall of blood vessel, secreting serotonin (5-hydroxytryptamine) that constricts the vessel, thus, lowering blood flow.
- 2) **Platelet Plug Formation:** This is a positive feedback mechanism, through which adhered platelets combine and attract passing platelets to the site of damaged vessel by secreting Adenosine-Di-Phosphate (ADP) to stick with them and secrete chemicals.
- 3) **Coagulation (Blood Clotting):** This is a positive feedback system in which blood clotting occurs at the site of injury.
- 4) **Fibrinolysis:** Breakdown of the clot and healing of damaged blood vessel is the initial stage after the clot has formed.

Ques 2 c) What are erythrocytes? Give their significance in human body.

Ans: Erythrocytes

Erythrocytes are discotic-shaped cells constituting 99% of the blood and carrying haemoglobin molecules. Haemoglobin is a red coloured protein containing iron. In humans, 5×10^{12} erythrocytes constitute 45% of blood volume. Each cell is surrounded by a colourless envelope covering a semi-liquid material having 65% water and 35% solids of which 33% constitutes haemoglobin bound to 2% stromal meshwork of protein, phospholipids, cholesterol, cholesterol esters, and neutral fat.

Significance in Human Body

Oxygen Transporter: RBCs participate in oxygen transportation

- 1) **Release of ATP and Vessel Dilation:** Constricted vessels apply their stress on RBCs, causing them to release ATP. The released ATP relaxes and dilates the vessel walls.
- 2) **Release of S-nitrosothiol:** On deoxygenation of haemoglobin, S-nitrosothiol is released by the erythrocytes which dilate vessels, providing more blood to oxygen-deficient sites.
- 3) **Immunity:** When pathogens (bacteria) break haemoglobin, it releases free radicals that rupture the bacterial cell wall and membrane, causing its death. Thereby, providing immunity to the body.

Ques 3 a) Explain the methods to analyse Glucose, Bilirubin and Cholesterol.

(2×8=16)

Ans: Methods to Analyse Glucose: Refer Chapter-6, Page No. 144

Methods to Analyse Bilirubin: Refer Chapter-6, Page No. 144

Methods to Analyse Cholesterol

Serum Cholesterol (Total): Method of Zak

Principle: Serum is treated with ferric chloride as the acid reagent to precipitate the proteins. The protein free supernatant (containing ferric chloride) is treated with conc. H_2SO_4 . A reddish purple colour is developed which is measured colorimetrically at 560nm.

Reagents

- 1) **Stock Ferric Chloride-Acetic Acid Reagent:** Dissolve 1gm $FeCl_3 \cdot 6H_2O$ in glacial acetic acid (aldehyde free).
- 2) **Working Ferric Chloride-Acetic Acid Reagent:** Dilute 5ml stock to 100ml with acetic acid.
- 3) **Stock Standard Cholesterol Solution (1mg/ml):** Dissolve 10 mg cholesterol in acetic acid and make the final volume to 100ml with acetic acid. Store in the cold.
- 4) **Working Cholesterol Standard Solution (0.04mg/ml):** Dilute 40ml stock to 100ml with working ferric chloride-acetic acid reagent. Store in the cold.
- 5) Sulphuric acid, concentrated (A.R.)

Procedure: To 0.2ml of serum in a 15ml glass stoppered centrifuge tube, add 9.8ml of working ferric chloride-acetic acid reagent. Mix and stand for 15 minutes and then centrifuge. Mark three test tubes S, T, and B for standard, test, and blank respectively. Measure 5.0ml of working ferric chloride-acetic acid reagent in 'B' and 5.0ml of protein free supernatant of serum in 'T' and add 3.0ml of concentrated H_2SO_4 to all the three tubes. Mix, stand for 30 minutes and read standard and test at 560nm against blank.

Calculation

$$\text{Serum cholesterol (mg/100ml)} = \frac{u}{s} \times \frac{100}{0.2} \times 0.4 = \frac{u}{s} \times 200$$

Interpretation: Serum cholesterol varies from 180-240mg/100ml in healthy young adults.

An increase in serum cholesterol (hypercholesterolemia) is found in diabetes mellitus, nephrotic syndrome, obstructive jaundice, hypothyroidism, xanthomatosis, and during other atherosclerotic idiopathic hypercholesterolemia of unknown etiology occurs in some families. Hypercholesterolemia of unknown etiology occurs in some families. Hypercholesterolemia, from any cause, predisposes to atherosclerosis.

A decrease in serum cholesterol (hypercholesterolemia) is found in hyperthyroidism, hepatocellular damage, anaemia (except hemorrhagic), acute infections, wasting diseases, intestinal obstruction, and terminal states of a variety of diseases.

Ques 3 b) Name any co-enzyme and explain its role in metabolic process.

Ans: Co-enzyme and their Role in Metabolic Process

Refer Chapter-4, Page No. 86

Ques 4) Write a note on the following:

a) T.C.A. Cycle

Ans: T.C.A. Cycle

Citric acid cycle is also called as **Krebs cycle** or **Tricarboxylic Acid (TCA) cycle** or **Szent-Gyorgyi-Krebs cycle**. It is named after **Albert Szent-Gyorgyi** and **Hans Krebs** who discovered the components and reactions of the citric acid cycle. It is an aerobic process, and in eukaryotes it occurs in **mitochondrial matrix**.

First time, **Hans Krebs** had given the reactions of citric acid cycle, which occurs in mitochondria when acetyl CoA enters into mitochondria. Citric acid cycle is an amphibolic pathway and its intermediates are utilised in many anabolic pathways. The significant reactions of citric acid cycle are shown in the figure 1:

1) **Formation of Citrate:** Reaction of acetyl-CoA with oxaloacetic acid and H_2O yields citrate (or citric acid), whereas coenzyme A (CoA. SH) is regenerated.

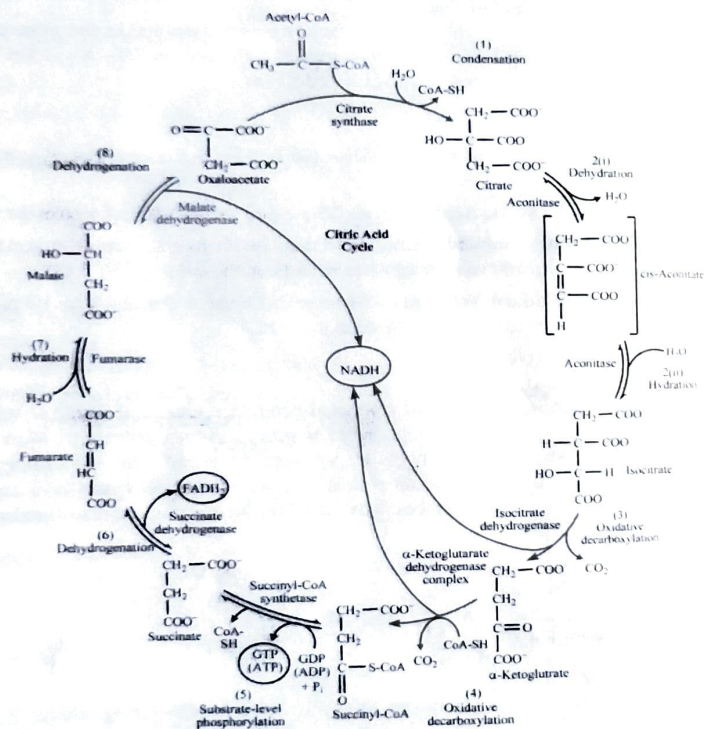


Figure 1: Reactions of the Citric Acid Cycle

2) **Isomerisation of Citrate to Isocitrate**

- Cis*-aconitic acid is produced by the dehydration of citric acid.
- Isocitric acid is formed by the hydration of *cis*-aconitic acid.

3) **Formation of α-Ketoglutarate:** Oxalosuccinic acid is produced when isocitric acid reacts with **NAD**.

α-Ketoglutaric acid is produced by decarboxylation of oxalosuccinic acid.

Solved Papers

4) **Conversion of α-Ketoglutarate to Succinyl CoA:** Succinyl-CoA is formed by oxidative decarboxylation of α-ketoglutaric acid.

5) **Formation of Succinate:** Succinic acid and coenzyme A are formed by hydrolysis of succinyl-CoA. Energy which is liberated in this reaction is utilised in the conversion of Guanine-Diphosphate to Guanine Triphosphate (GDP → GTP). This one molecule of GTP generates one equivalent molecule of ATP.

6) **Conversion of Succinate to Fumarate:** Fumaric acid and FADH₂ are formed by succinic acid reaction with FAD.

7) **Formation of Malate:** Malic acid is formed through reaction of fumaric acid with H₂O.

8) **Conversion of Malate to Oxaloacetate:** Dehydrogenation of malic acid occurs in the last step, with the generation of NAD₂H and oxaloacetic acid which further reacts with acetyl CoA in the first step of citric acid cycle. [Except succinic dehydrogenase, all other oxidative enzymes of the citric acid cycle are situated in the mitochondrial matrix. Succinic dehydrogenase (an integral protein complex, bound to the inner membrane of mitochondria) is the selected marker for inner membranes during mitochondrial fractionations].

Ques 4 b) Importance and functions of carbohydrates

Ans: Importance and Functions of Carbohydrates

Refer Chapter-2, Page No. 33

Ques 5 a) Explain the following terms:

(2×8=16)

i) Co-factor

Ans: Co-Factor

Most of the enzymes require only amino acid residue to show their effect, whereas in some enzymes cofactor or coenzyme is needed; while sometimes both metal ion and coenzyme are needed for enzymatic activity. Cofactors are inorganic ions such as Fe²⁺, Mg²⁺, Mn²⁺, or Zn²⁺.

Ques 5 a ii) Marker enzymes

Ans: Marker Enzymes

These are one of the cell biomarkers used to characterise a cell type. They are also used in the isolation of the target cellular component. For example, succinate dehydrogenase is a marker enzyme for mitochondrion whereas the acid phosphatase for lysosome.

Ques 5 a iii) Competitive inhibition

Ans: Competitive Inhibition

A competitive inhibitor is structurally much closer to any enzyme's normal substrate. It results in a competition between the inhibitor and the substrate, to bind to the active site. At one time, the enzyme may either bind to the substrate molecule or to the inhibitor molecule, but not to both of them. For example, in succinate dehydrogenase, enzyme's substrate is succinate and malonate is its competitive inhibitor.

Ques 5 a iv) Proenzyme

Ans: Proenzyme

Zymogen or proenzyme is an inactive precursor due to the presence of a supplementary polypeptide chain which blocks the active site of the enzyme by masking. Zymogen mainly protects the tissue of origin from auto-digestion.

It is also important for the rapid mobilisation of enzyme activity in response to physiological demands when needed. Trypsinogen, chymotrypsinogen, clotting factors, pepsinogen and prothrombin are few examples of zymogen.

Ques 5 b) Give an account of pharmaceutical and diagnostic importance of enzymes.

Ans: Pharmaceutical and Diagnostic Importance of Enzymes (Role of Enzymes)
Refer Chapter-5, Page No. 123

Ques 6 a) Enlist different qualitative tests carried out on proteins and give the procedure details of any two of such tests.

Ans: Qualitative Tests carried out on Proteins: Refer Chapter-1, Page No. 24

Ques 6 b) Discuss various protein-deficiency diseases in brief. (2×8=16)

Ans: Protein-Deficiency Diseases: Refer Chapter-1, Page No. 26

Ques 7 a) Write short notes on the following:

i) Saponification value

Ans: Saponification Value

It is the number of milligrams of KOH required to saponify 1gm of fat or oil.

Ques 7 a) ii) Iodine number

Ans: Iodine Number

It is the number of grams of iodine absorbed by 100gm of fat or oil. This value is constant for particular oil or fat.

Ques 7 a) iii) Ketosis

Ans: Ketosis

A metabolic state characterised by raised levels of ketone bodies in the body tissues, which is typically pathological in conditions such as diabetes, or may be the consequence of a diet that is very low in carbohydrates.

Ques 7 a) iv) Atherosclerosis

Ans: Atherosclerosis: Refer Chapter-3, Page No. 62

Ques 7 b) What are minerals and what functions do they perform in the biological system?

Ans: Minerals and their Functions in the Biological System

Refer Chapter-4, Page No. 103

Solved Papers

BOARD OF TECHNICAL EDUCATION UTTAR PRADESH

D. PHARM. - FIRST YEAR EXAMINATION, 2015

BIOCHEMISTRY AND CLINICAL PATHOLOGY

Max. Marks: 80

Time: 3 Hours

Note: 1) Attempt All questions. All questions carry equal marks.

2) Students are advised to specially check the Numerical Data of question paper in both versions. If there is any difference in Hindi translation of any question, the students should answer the question according to the English version.

3) Use of Pager and Mobile Phone by the students is not allowed.

Ques 1) Discuss the abnormal constituents of urine. Explain their significance in diseases. (16)

Ans: Abnormal Constituents of Urine and their Significance in Diseases

Refer Chapter-6, Page No. 143

(6+10=16)

Ques 2 a) Define and classify proteins.

Ans: Proteins: Refer Chapter-1, Page No. 13

Classification of Protein: Refer Chapter-1, Page No. 16

Ques 2 b) Give any four qualitative tests of proteins.

Ans: Qualitative Tests of Proteins: Refer Chapter-1, Page No. 24

Ques 3 a) What are enzymes? Discuss about the factors affecting enzymatic action. (8+8=16)

Ans: Enzymes: Refer Chapter-5, Page No. 109

Factors Affecting Enzymatic Action: Refer Chapter-5, Page No. 121

Ques 3 b) Explain the therapeutic importance of enzymes.

Ans: Therapeutic Importance of Enzymes: Refer Chapter-5, Page No. 123

Ques 4 a) Discuss the role of any two minerals in the body.

(8+8=16)

Ans: Role of any Two Minerals in the Body: Refer Chapter-4, Page No. 92, 93

Ques 4 b) Write a note on the role of co-enzymes in the body.

Ans: Role of Co-enzymes in the Body: Refer Chapter-4, Page No. 86

Ques 5 a) Define and classify carbohydrates.

(8+8=16)

Ans: Carbohydrates: Refer Chapter-2, Page No. 29

Classification of Carbohydrates: Refer Chapter-2, Page No. 35

Ques 5 b) Give any four qualitative tests of carbohydrates.

Ans: Qualitative Tests of Carbohydrates: Refer Chapter-2, Page No. 37

Or

Ques 5 a) Discuss about the diseases caused due to proteins deficiency.

(8+8=16)

Ans: Diseases Caused due to Proteins Deficiency: Refer Chapter-1, Page No. 26

Ques 5 b) Briefly discuss fat-soluble vitamins.

Ans: Fat-Soluble Vitamins: Refer Chapter-4, Page No. 69-73

BOARD OF TECHNICAL EDUCATION UTTAR PRADESH
D. PHARM. - FIRST YEAR EXAMINATION, 2016
BIOCHEMISTRY AND CLINICAL PATHOLOGY

Time: 3 Hours

- Note: 1) Attempt all questions. All questions carry equal marks.
 2) Students are advised to specially check the Numerical Data of question paper in both versions. If there is any difference in Hindi translation of any question, students should answer the question according to the English version.
 3) Use of Pager and Mobile Phone by the students is not allowed.

Ques 1) Discuss the importance of lymphocytes. Explain its role in health and disease.

Ans: Importance of Lymphocytes: Refer Chapter-6, Page No. 128

Role in Health and Disease

- 1) Phagocytosis: Leucocytes (neutrophils) engulf foreign particles and bacteria to digest them.
- 2) Antibody Formation: Lymphocytes produce β - and γ -serum globulin
- 3) Fibroblasts Formation: At inflammation site lymphocytes transform to fibroblasts for repair process.

Ques 2 a) Define and classify lipids.

Ans: Lipids: Refer Chapter-3, Page No. 47

Classification of Lipids: Refer Chapter-3, Page No. 56

Ques 2 b) Give any four qualitative tests of lipids.

Ans: Qualitative Tests of Lipids: Refer Chapter-3, Page No. 56

Ques 3) What are vitamins? Describe the importance of water soluble vitamins in our health.

Ans: Vitamins: Refer Chapter-4, Page No. 68

Importance of Water Soluble Vitamins in Health: Refer Chapter-4, Page No. 74-82

Ques 4 a) Write short note on diseases related to carbohydrate metabolism.

Ans: Diseases Related to Carbohydrate Metabolism: Refer Chapter-2, Page No. 38

Ques 4 b) Describe the importance of minerals in life processes.

Ans: Importance of Minerals in Life Processes: Refer Chapter-4, Page No. 103

Ques 5 a) What are amino acids? Discuss their importance in our life.

Ans: Amino Acids: Refer Chapter-1, Page No. 20

Importance: Refer Chapter-1, Page No. 24

Ques 5 b) Explain the therapeutic of enzymes.

Ans: Therapeutic of Enzymes: Refer Chapter-5, Page No. 123

Or

Ques 5 a) Discuss the normal constituents of urine.

Ans: Normal Constituents of Urine: Refer Chapter-6, Page No. 142

Ques 5 b) Discuss the diseases related to lipid metabolism.

Ans: Diseases Related to Lipid Metabolism: Refer Chapter-3, Page No. 58

BOARD OF TECHNICAL EDUCATION UTTAR PRADESH
D. PHARM. - FIRST YEAR EXAMINATION, 2017
BIOCHEMISTRY AND CLINICAL PATHOLOGY

Max. Marks: 80

Time: 3 Hours

- Note: 1) Attempt any Five questions. All questions carry equal marks.
 2) Students are advised to specially check the Numerical Data of question paper in both versions. If there is any difference in Hindi Translation of any question, the students should answer the question according to the English Version.
 3) Use of Pager and Mobile Phone by the students is not allowed.

Ques 1 a) What are Polypeptides? Give their biological importance.

(2×8=16)

Ans: Polypeptides: Refer Chapter-1, Page No. 20

Biological Importance: Refer Chapter-1, Page No. 18

Ques 1 b) Define carbohydrates? Give the classification of carbohydrates.

Ans: Carbohydrates: Refer Chapter-2, Page No. 29

Classification of Carbohydrates: Refer Chapter-2, Page No. 35

Ques 2 a) Mention the abnormal constituents of urine? Explain their significance in diseases.

(2×8=16)

Ans: Abnormal Constituents of Urine: Refer Chapter-6, Page No. 143

Significance in Diseases: Refer Chapter-6, Page No. 143

Ques 2 b) What are platelets and lymphocytes? Give their importance in human body.

Ans: Platelets and Lymphocytes

Refer Chapter-6, Page No. 128

Importance in Human Body: Refer Chapter-6, Page No. 128

Ques 3 a) Define amino acid? Give their classification.

(2×8=16)

Ans: Amino Acid: Refer Chapter-1, Page No. 20

Classification: Refer Chapter-1, Page No. 21

Ques 3 b) Discuss various protein deficiency diseases in brief.

Ans: Protein Deficiency Diseases: Refer Chapter-1, Page No. 26

Ques 4 a) Discuss the role of vitamins and coenzymes in our body.

(2×8= 16)

Ans: Role of Vitamins: Refer Chapter-4, Page No. 82

Role of Coenzymes: Refer Chapter-4, Page No. 86

Ques 4 b) Highlight the role of minerals and water in life process.

Ans: Role of Minerals: Refer Chapter-4, Page No. 103

Role of Water: Refer Chapter-4, Page No. 107

Ques 5 a) What are Enzymes? Given an account of their Pharmaceutical importance.

(2×8=16)

Ans: Enzymes: Refer Chapter-5, Page No. 109

Pharmaceutical Importance: Refer Chapter-5, Page No. 123

Ques 5 b) Enlist different qualitative tests for liquids.

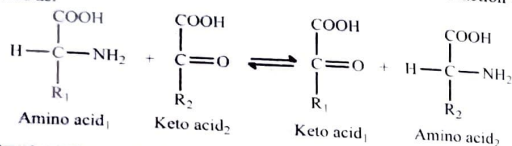
Ans: Different Qualitative Tests for Liquids: Refer Chapter-3, Page No. 56

Ques 6 a) Discuss normal and abnormal metabolism of proteins. (2x8=16)

Ans: Normal Metabolism of Proteins

In the metabolism of proteins following processes are involved:

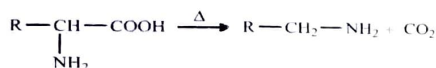
- 1) **Transamination:** Transamination reaction is defined as **transfer of an amine group from one molecule to another** in the presence of enzymes of family **transaminases**. The transamination reaction involves exchange of an amine on an acid with a ketone group on another acid, just like double replacement reaction. The basic reaction of transamination can be written as:



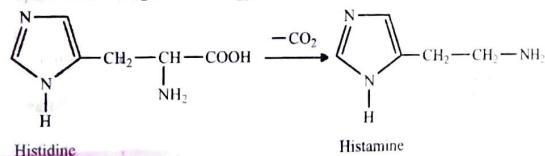
- 2) **Deamination:** Removal of amino group from amino acid as NH_3 is called deamination. The ammonia thus produced participates in urea synthesis and the remaining carbon skeleton of amino acid is catabolised into keto acid. Deamination is of two types- **oxidative** and **non-oxidative**.

Transamination and deamination are two separate processes, occurring simultaneously. In both the reactions, the glutamate is the central molecule. Hence, collectively these two reactions are termed as **transdeamination**, indicating reaction of transamination and deamination where glutamate is the key molecule.

- 3) **Decarboxylation:** Amino acids are decarboxylated by dry distillation or by heating with bases such as barium oxide. There are some specific enzymes, catalyse the decarboxylation of specific amino acids.



Decarboxylation of amino acids has biological significance. Decarboxylation of histidine to histamine has great physiological importance. Decarboxylation of histidine gives histamine which is a direct causative agent in allergy, shock, and similar states.



Abnormal Metabolism of Proteins

Abnormal metabolism of proteins results to:

- 1) **Phenylketonuria (PKU):** Phenylketonuria is a congenital or inborn error of phenylalanine metabolism, related with the inability to convert phenylalanine to tyrosine. The PKU is a genetic disorder and is inherited in an autosomal recessive manner. The rate of incidence of PKU is about 1 in 20,000 new-borns.

In PKU, phenylalanine is accumulated in tissues and blood. Thus, the new-born excretes excess of phenylalanine in urine. In PKU, the phenylalanine conversion to tyrosine through normal pathway does not occur but some minor pathway becomes active in phenylketonurics (figure 1) and various toxic phenylalanine metabolites (like phenylpyruvate, phenylacetate, phenyllactate and phenylacetyl glutamine) are accumulated in the body.

Solved Papers

The disease is termed PKU because of elevated keto acid, phenylpyruvate in urine.

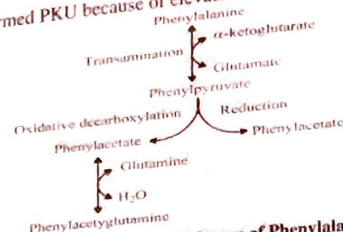


Figure 1: Alternative Pathways of Phenylalanine Catabolism in Phenylketonurics

Cause
PKU is an inherited condition and is caused by a defective PAH gene. The PAH gene is responsible for phenylalanine hydroxylase, the enzyme required for the catabolism of phenylalanine. Thus, if someone eat excess of high-protein diet (eggs, meat, etc.), phenylalanine gets accumulated to dangerous level.

Symptoms

- Mental retardation or intellectual disability.
 - Delayed development.
 - Behavioural, emotional, and social problems.
 - Psychiatric disorders.
 - Neurological problems that may include seizures.
 - Hyperactivity.
 - Poor bone strength.
 - Skin rashes (eczema).
 - A musty odour in the child's breath, skin or urine, caused by too much phenylalanine in the body.
 - Fair skin and blue eyes because phenylalanine cannot transform into melanin, (a pigment providing hair and skin colour), and
 - Abnormally small head (microcephaly).
- 2) **Albinism:** Albinism is a group of conditions, in which error occurs in tyrosine metabolism and thus defective melanin synthesis occurs. Such individuals have little or no colour (pigment) in the skin, hair, and eyes.

Cause

Albinism occurs when production or distribution of melanin is disturbed. One of several gene defects causes albinism. Melanin is a natural substance imparting colour to hair, skin, and iris of the eye.

Symptoms

- Any obstruction in melanin production causes hypo-pigmentation or no pigmentation (hair, skin, and eyes colours become white).
 - Albinos or individual with light skin colour are sensitive to sunlight and chances of skin cancer is more in such individuals.
 - Lack of melanin pigment in eyes causes photophobia (intolerance to light) and nystagmus.
- 3) **Alkaptonuria (Black Urine Disease):** Alkaptonuria is a rare genetic disorder involving less production of enzyme, Homo-Gentisic-Dioxygenase (HGD). This enzyme is utilised in the degradation of a toxic substance called homogentisic acid. Lack of HGD causes accumulation of homogentisic acid in the body.

In alkaptonuria, bones and cartilage becomes discoloured and brittle due to accumulated homogentisic acid. This leads to osteoarthritis, mainly in the spine and large joints. Alkaptonuria turns urine coloured dark brown or black upon air exposure.

Cause

A mutation on genes coding for enzyme homogentisate-1,2-dioxygenase (HGD) gene results in alkaptonuria. The enzyme homogentisate-1,2-dioxygenase (HGD) catalyses oxidation of homogentisate. Thus, the homogentisate accumulates in blood and body tissues and is excreted largely via urine.

