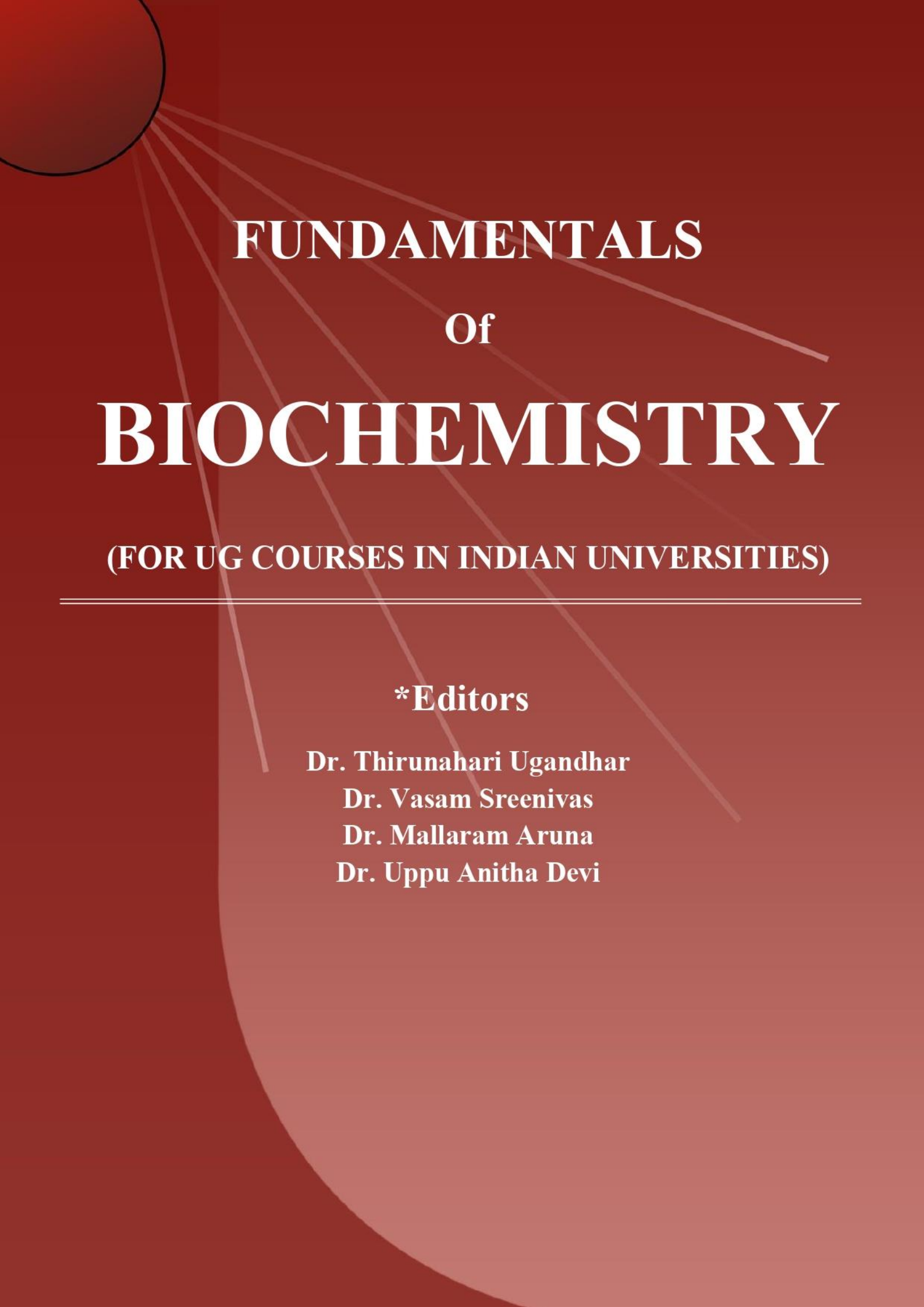




FUNDAMENTALS Of BIOCHEMISTRY

(FOR UG COURSES IN INDIAN UNIVERSITIES)

***Editors**

Dr. Thirunahari Ugandhar

Dr. Vasam Sreenivas

Dr. Mallaram Aruna

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PREFACE

The chemistry of life in all its forms whether it be human, animal, plant, or microorganism is the focus of biochemistry, which is possibly the most singular of all academic disciplines. As a result of this, biochemistry is now a fundamental subject that is studied in a variety of fields, including agriculture, biotechnology, biomedical engineering, home science, and other areas of the life sciences (botany, zoology, microbiology, and genetics). Students in these disciplines should have a fundamental understanding of the concepts and principles of biochemistry, as well as their applications, even though biochemistry is a very expansive field of study.

This book, titled "**Fundamentals of Biochemistry**," is a redistillation of our knowledge, condensing the core of the topic in a condensed form. It will fulfil the criteria of the curriculum for beginning students learning biochemistry in a variety of fields. The complexity of the structures and reactions taught in biochemistry might be intimidating to a lot of pupils. This book is going to be a godsend for students who are like that because most of the structures have been removed from it without sacrificing any of the topic knowledge, including the most recent advancements. This book, which is written in straightforward English and features color drawings, is intended to pique the readers' curiosity and inspire them to study as much as they can about the topic in as little time as possible. Developing clear notions in biochemistry will be made easier with the help of the summary and the biological and clinical concepts. The pupils will be prepared to confidently tackle the tests thanks to the self-assessment assignments (Essays, MCQs and Fill in the blanks).

Dr. T. Ugandhar

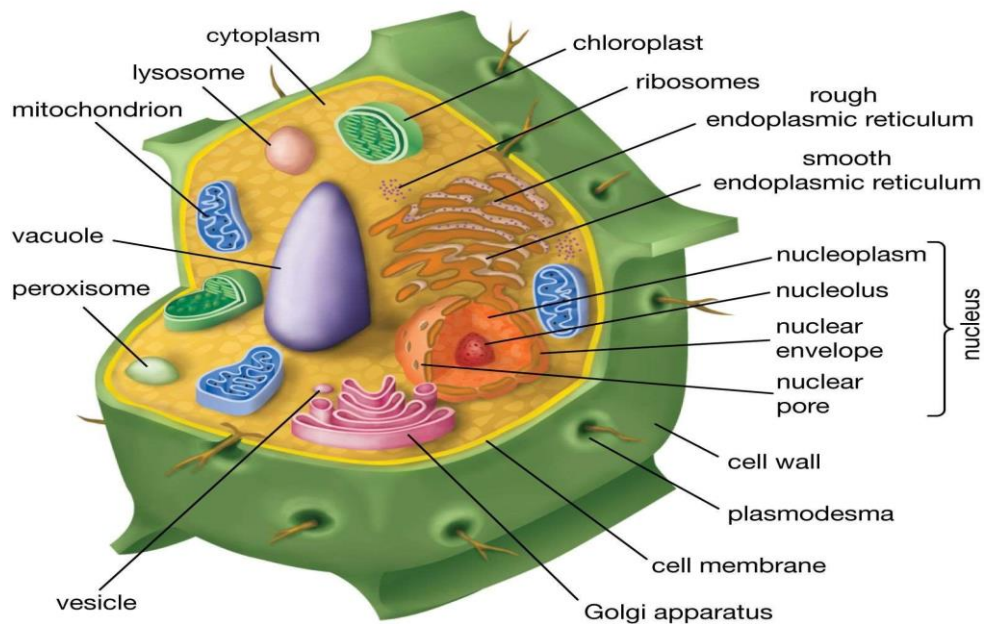
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CHAPTER-I**Plant Cell****Plant cell**

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CHAPTER-I**The Cell and their Molecules**

It is usually easy to decide whether something is alive. This is because living things share many common attributes, such as the capacity to extract energy from nutrients to drive their various functions, the power to actively respond to changes in their environment, and the ability to grow, differentiate, and perhaps most telling of all—to reproduce. Of course, a given organism may not have all these traits. For example, mules, which are obviously alive, rarely reproduce. Conversely, the inanimate matter may exhibit some lifelike properties. For instance, crystals may grow larger when immersed in a supersaturated solution of the crystalline material. Therefore, life, as are many other complex phenomena, is perhaps impossible to define in a precise fashion. Norman Horowitz, however, proposed a useful set of criteria for living systems: Life possesses the properties of replication, catalysis, and mutability. Much of this text is concerned with the way living organisms exhibit these properties.

Biochemistry is the study of life on the molecular level. The significance of such studies is greatly enhanced if they are related to the biology of the corresponding

organisms or even communities of such organisms. This introductory chapter, therefore, begins with a synopsis of the biological realm. This is followed by an outline of biochemistry, a review of genetics, a discussion of the origin of life, and finally, an introduction to the biochemical literature.

1 PROKARYOTES

It has long been recognized that life is based on morphological units known as **cells**. The formulation of this concept is generally attributed to an 1838 paper by Matthias Schleiden and Theodor Schwann, but its origins may be traced to the seventeenth-century observations of early microscopists such as Robert Hooke. There are two major classifications of cells: the **eukaryotes** (Greek: eu, good or true _ karyon, kernel or nut), which have a membrane-enclosed **nucleus** encapsulating their **DNA (deoxyribonucleic acid)**; and the **prokaryotes** (Greek: pro, before), which lack this organelle. Prokaryotes, which comprise the various types of bacteria, have relatively simple structures and are invariably unicellular (although they may form filaments or colonies of independent cells). They are estimated to represent about half of Earth's biomass. Eukaryotes, which may be multicellular as well as unicellular, is vastly more complex than prokaryotes. (**Viruses**, which are much simpler entities than cells, are not classified as living because they lack the metabolic apparatus to reproduce outside their host cells. They are essentially large molecular aggregates.) This section is a discussion of prokaryotes. Eukaryotes are considered in the following section.

Form and Function

Prokaryotes are the most numerous and widespread organisms on Earth. This is because their varied and often highly adaptable metabolisms suit them to an enormous variety of habitats. Besides inhabiting our familiar temperate and aerobic environment, certain types of bacteria may thrive in or even require conditions that are hostile to eukaryotes such as unusual chemical environments, high temperatures (as high as 130⁰C), and lack of oxygen. Moreover, the rapid reproductive rate of prokaryotes (optimally ≤ 20 min per cell division for many species) permits them to take advantage of transiently favourable conditions, and conversely, the ability of many bacteria to form resistant **spores** allows them to survive adverse conditions.

a. Prokaryotes Have Relatively Simple Anatomies

Prokaryotes, which were first observed in 1683 by the inventor of the microscope, Antonie van Leeuwenhoek, have sizes that are mostly in the range of 1 to 10 μm . They have one of three basic shapes (Fig. 1-1): spheroidal (**cocci**), rodlike (**bacilli**), and helically coiled (**spirilla**), but all have the same general design (Fig. 1-2). They are bounded, as are all cells, by a 70-Å-thick **cell membrane (plasma membrane)**, which consists of a lipid bilayer containing embedded proteins that control the passage of molecules in and out of the cell and catalyse a variety of reactions. The cells of most prokaryotic species are surrounded by a rigid, 30- to 250-Å-thick polysaccharide **cell wall** that mainly functions to protect the cell from mechanical injury and to prevent it from bursting in media more osmotically dilute than its contents. Some bacteria further encase themselves in a gelatinous polysaccharide **capsule** that protects them from the defences of higher organisms. Although prokaryotes lack the membranous subcellular organelles characteristic of eukaryotes (Section 1-2), their plasma membranes may be infolded to form multi-layered structures known as **mesosomes**. The mesosomes are thought to serve as the site of DNA replication and other specialized enzymatic reactions.

The prokaryotic **cytoplasm** (cell contents) is by no means a homogeneous soup. Its single **chromosome** (DNA molecule, several copies of which may be present in a rapidly dividing cell) is condensed to form a body known as a **nucleoid**. The cytoplasm also contains numerous species of **RNA (Ribo Nucleic Acid)**, a variety of soluble **enzymes** (proteins that catalyse specific reactions), and many thousands of 250-Å-diameter particles known as **ribosomes**, which are the sites of protein synthesis. Many bacterial cells bear one or more whiplike appendages known as **flagella**, which are used for locomotion (Section 35-3I). Certain bacteria also have filamentous projections named **pili**, some types of which function as conduits for DNA during sexual conjugation (a process in which DNA is transferred from one cell to another; prokaryotes usually reproduce by binary fission) or aid in the attachment of the bacterium to a host organism's cells. The bacterium **Escherichia coli** (abbreviated **E. coli** and named after its discoverer, Theodor Escherich) is the biologically most well-characterized organism because of its intensive biochemical and genetic study over the past 70 years. Indeed, much of the subject matter of this text deals with the biochemistry of *E. coli*. Cells of this normal inhabitant of the higher mammalian

colon are typically $2 \mu\text{m m}^{-1}$ long rods that are $1 \mu\text{m}$ in diameter. Altogether an *E. coli* cell contains 3 to 6 thousand different types of molecules, including proteins, nucleic acids, polysaccharides, lipids, and various small molecules and ions (Table 1-1).

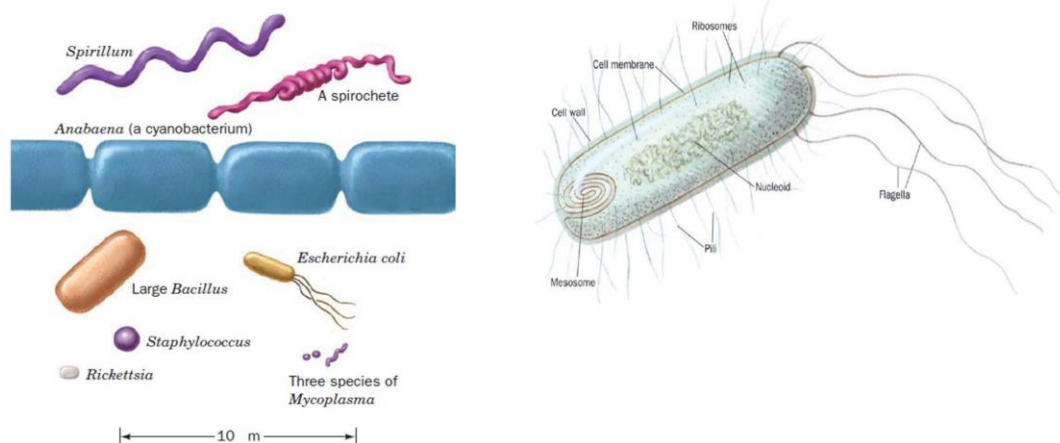
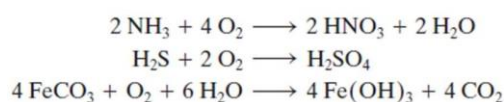


Figure 1-1: Scale drawings of some prokaryotic cells. Figure 1-2 Schematic diagram of a prokaryotic cell.

b. Prokaryotes Employ a Wide Variety of Metabolic Energy Sources

The nutritional requirements of prokaryotes are enormously varied. **Autotrophs** (Greek: autos, self-trophies, to feed) can synthesize all their cellular constituents. from simple molecules such as H_2O , CO_2 , NH_3 , and H_2S . Of course, they need an energy source to do so as well as to power their other functions. **Chemolithotrophs** (Greek: lithos, stone) obtain their energy through the oxidation of inorganic compounds such as NH_3 , H_2S , or even Fe^{2+} . Indeed, studies have revealed the existence of extensive al.

Table 1-1 Molecular Composition of *E. coli*.



Component	Percentage by Weight
H_2O	70
Protein	15
Nucleic acids:	
DNA	1
RNA	6
Polysaccharides and precursors	3
Lipids and precursors	2
Other small organic molecules	1
Inorganic ions	1

best extremely slow-growing colonies of chemolithotrophs that live as far as 5 kilometres underground and whose aggregate biomass appears to rival that of surface-dwelling organisms.

Photoautotrophs are autotrophs that obtain energy via **photosynthesis**, a process in which light energy powers the transfer of electrons from inorganic donors to CO₂-yielding **carbohydrates** [(CH₂O)_n]. In the most widespread form of photosynthesis, the electron donor in the light-driven reaction sequence is H₂O.

This process is carried out by **cyanobacteria** (e.g., the green slimy organisms that grow on the walls of aquariums; cyanobacteria were formerly known as **blue-green algae**), as well as by plants. This form of photosynthesis is thought to have generated the O₂ in Earth's atmosphere. Some species of cyanobacteria can convert N₂ from the atmosphere to organic nitrogen compounds. This **nitrogen fixation** capacity gives them the simplest nutritional requirements of all organisms: Except for their need for small amounts of minerals, they can literally live on sunlight and air. In a more primitive form of photosynthesis, substances such as H₂, H₂S, thiosulfate, or organic compounds are the electron donors in light-driven reactions such as.

The **purple** and the **green photosynthetic bacteria** that carry out these processes occupy such oxygen-free habitats as shallow muddy ponds in which H₂S is generated by rotting organic matter.



Heterotrophs (Greek: hetero, other) obtain energy through the oxidation of organic compounds and hence are ultimately dependent on autotrophs for these substances. **Obligate aerobes** (which include animals) must utilize O₂, whereas **anaerobes** employ oxidizing agents such as sulfate (**sulfate-reducing bacteria**) or nitrate (**denitrifying bacteria**). Many organisms can partially metabolize various organic compounds in intramolecular oxidation–reduction processes known as **fermentation**. **Facultative anaerobes** such as E. coli can grow in either the presence or the absence of O₂. **Obligate anaerobes**, in contrast, are poisoned by the presence of O₂. Their metabolisms are thought to resemble those of the earliest life forms (which arose over 3.8 billion years ago when Earth's atmosphere lacked O₂; Section 1-5B). At any rate,

there are few organic compounds that cannot be metabolized by some prokaryotic organisms.

EUKARYOTES

Eukaryotic cells are generally 10 to 100 μm in diameter and thus have a thousand to a million times the volume of typical prokaryotes. It is not size, however, but a profusion of membrane-enclosed organelles, each with a specialized function, that best characterizes eukaryotic cells (Fig. 1-5). In fact, eukaryotic structure and function are more complex than those of prokaryotes at all levels of the organization, from the molecular level up. Eukaryotes and prokaryotes have developed according to fundamentally different evolutionary strategies. Prokaryotes have exploited the advantages of simplicity and miniaturization: Their rapid growth rates permit them to occupy ecological niches in which there may be drastic fluctuations of the available nutrients. In contrast, the complexity of eukaryotes, which renders them larger and more slowly growing than prokaryotes, gives them the competitive cell specifies around 800 megabytes of information about. 200 times the information content of this text]. Within the nucleus, the genetic information encoded by the DNA is transcribed into molecules of RNA, which, after extensive processing, are transported to the cytoplasm (eukaryotes, the cell contents exclusive of the nucleus), where they direct the ribosomal synthesis of proteins. The nuclear envelope consists of a double membrane that is perforated by numerous 90-Å-wide pores that regulate the flow of proteins and RNA between the nucleus and the cytoplasm. The nucleus of most eukaryotic cells contains at least one dark-staining body known as the **nucleolus**, which is the site of ribosomal assembly. It contains chromosomal segments bearing multiple copies of genes specifying ribosomal RNA. These genes are transcribed in the nucleolus, and the resulting RNA is combined with ribosomal proteins that have been imported from their site of synthesis in the **cytosol** (the cytoplasm exclusive of its membrane-bound organelles).

The resulting immature ribosomes are then exported to the cytosol, where their assembly is completed. Thus, protein synthesis occurs almost entirely in the cytosol.

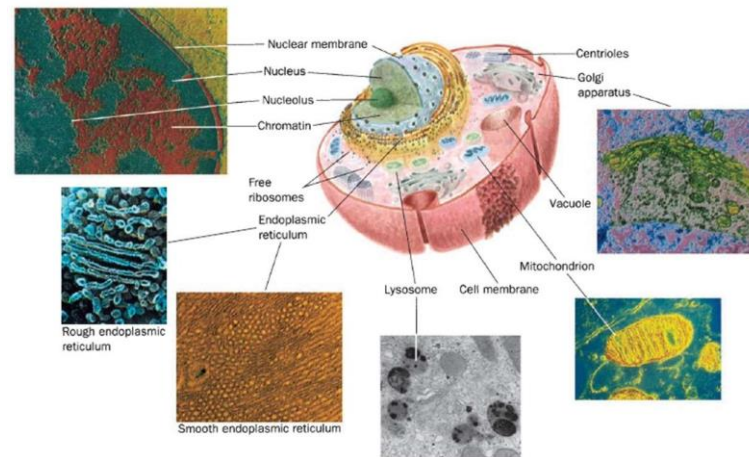


Figure 1-5 Schematic diagram of an animal cell accompanied by electron micrographs of its organelles.

[Nucleus: Tektoff-RM, CNRI/Photo Researchers; rough endoplasmic reticulum: Pietro M. Motta & Tomonori Naguro/Photo Researchers, Inc. and Golgi apparatus: Secchi-Lecaque/Roussel-UCLAF/CNRI/Photo Researchers, Inc.; smooth endoplasmic reticulum: David M. Phillips/ Visuals Unlimited; mitochondrion: CNRI/Photo Researchers; lysosome: Bio photo Associates/Photo Researchers.

Nucleus

The nucleus is the largest cellular organelle, surrounded by a double membrane nuclear envelope. The outer membrane is continuous with the membranes of the endoplasmic reticulum. At certain intervals, the two nuclear membranes have nuclear pores with a diameter of about 90 nm. These pores permit the free passage of the products synthesized in the nucleus into the surrounding cytoplasm.

The nucleus contains DNA, the repository of genetic information. Eukaryotic DNA is associated with basic proteins (histones) in the ratio of 1:1, to form nucleosomes. An assembly of nucleosomes constitutes chromatin fibers of chromosomes (Greek: chroma- color; soma-body). Thus, a single human chromosome is composed of about a million nucleosomes. The number of chromosomes is a characteristic feature of the species. Humans have 46 chromosomes, compactly packed in the nucleus.

The nucleus of the eukaryotic cell contains a dense body known as a nucleolus. It is rich in RNA, particularly the ribosomal RNA which enters the cytosol through

nuclear pores. The ground material of the nucleus is often referred to as nucleoplasm. It is rich in enzymes such as DNA polymerases and RNA polymerases.

Mitochondria

The mitochondria (Greek: mitos - thread; chondros-granule) are the centers for cellular respiration and energy metabolism. They are regarded as the powerhouses of the cell with variable sizes and shapes. Mitochondria are rod-like or filamentous bodies, usually with dimensions of $1.0 \times 3 \mu\text{m}$. About 2,000 mitochondria, occupying about 1/4th of the total cell volume, are present in a typical cell.

The mitochondria are composed of a double membrane system. The outer membrane is smooth and completely envelops the organelle. The inner membrane is folded to form cristae (Latin - crests) which occupy a larger surface area. The internal of mitochondria is referred to as matrix or mitosol.

The components of the electron transport chain and oxidative phosphorylation (flavoprotein, cytochromes b, c₁, c, a, and a₃, and coupling factors) are buried in the inner mitochondrial membrane. The matrix contains several enzymes concerned with the energy metabolism of carbohydrates, lipids, and amino acids (e.g., citric acid cycle, β -oxidation). ATP, the energy currency, generated in mitochondria is exported to all parts of the cell to provide energy for cellular work. The mitochondrial matrix contains circular double-stranded DNA (mt DNA), RNA, and ribosomes. It is estimated that about 10% of the mitochondrial proteins are produced in the mitochondria. The structure and functions of mitochondria closely resemble prokaryotic cells. It is hypothesized that mitochondria have evolved from aerobic bacteria. Further, it is believed that during evolution, the aerobic bacteria developed a symbiotic relationship with primordial anaerobic eukaryotic cells that ultimately led to the arrival of aerobic eukaryotes.

Endoplasmic reticulum

The network of membrane-enclosed spaces that extend throughout the cytoplasm constitutes the endoplasmic reticulum (ER). Some of these thread-like structures extend from the nuclear pores to the plasma membrane. A large portion of the ER is studded with ribosomes to give a granular appearance which is referred to as rough endoplasmic reticulum. Ribosomes are the factories of protein biosynthesis.

The smooth endoplasmic reticulum does not contain ribosomes. It is involved in the synthesis of lipids (triacylglycerols, phospholipids, sterols) and the metabolism of drugs, besides supplying Ca^{2+} for cellular functions.

Golgi apparatus

Eukaryotic cells contain a unique cluster of membrane vesicles known as dictyosomes which, in turn, constitute the Golgi apparatus (or Golgi complex). The newly synthesized proteins are handed over to the Golgi apparatus which catalyses the addition of carbohydrates, lipids, or sulphate moieties to the proteins. These chemical modifications are necessary for the transport of proteins across the plasma membrane.

Lysosomes

Lysosomes are spherical vesicles enveloped by a single membrane. Lysosomes are regarded as the digestive tract of the cell since they are actively involved in the digestion of cellular substances—namely proteins, lipids, carbohydrates, and nucleic acids. Lysosomal enzymes are categorized as hydrolases. These include the following enzymes (with the substrate in brackets).

a-Glucosidase (glycogen)

Cathepsins (proteins) Lipases (lipids)

Ribonucleases (RNA).

The pH of the lysosomal matrix is more acidic ($\text{pH} < 5$) than the cytosol ($\text{pH} \sim 7$) and this facilitates the degradation of different compounds. The lysosomal enzymes are responsible for maintaining the cellular compounds in a dynamic state, by their degradation and recycling. The degraded products leave the lysosomes, usually by diffusion, for reutilization by the cell. Sometimes, however, certain residual products, rich in lipids and proteins, collectively known as lipofuscin accumulate in the cell. Lipofuscin is the age pigment or wear and tear pigment which has been implicated in the aging process.

The digestive enzymes of cellular compounds are confined to the lysosomes in the best interest of the cell. The escape of these enzymes into cytosol will destroy the functional macromolecules of the cell and result in many complications. The occurrence of several diseases (e.g. arthritis, muscle diseases, allergic disorders) has been partly attributed to the release of lysosomal enzymes.

Peroxisomes

Peroxisomes, also known as microbodies, are single-membrane cellular organelles. They are spherical or oval and contain the enzyme catalase. Catalase protects the cell from the toxic effects of H_2O_2 by converting it to H_2O and O_2 . Peroxisomes are also involved in the oxidation of long-chain fatty acids ($> C_{18}$) and synthesis of plasmalogens and glycolipids. Plants contain glyoxisomes, a specialized type of peroxisomes, which are involved in the glyoxylate pathway.

Cytosol and cytoskeleton

The cellular matrix is collectively referred to as cytosol. Cytosol is basically a compartment containing several enzymes, metabolites, and salts in an aqueous gel-like medium. More recent studies however indicate that the cytoplasm actually contains a complex network of protein filaments, spread throughout, that constitutes the cytoskeleton. The cytoplasmic filaments are of three types-microtubules, actin filaments, and intermediate filaments. The filaments which are polymers of proteins are responsible for the structure, shape, and organization of the cell.

SUMMARY: Prokaryotes

Prokaryotes are single-celled organisms that lack a membrane-enclosed nucleus. Most prokaryotes have similar anatomies: a rigid cell wall surrounding a cell membrane that encloses the cytoplasm. The cell's single chromosome is condensed to form a nucleoid. *Escherichia coli*, the biochemically most well-characterized organism, is a typical prokaryote. Prokaryotes have quite varied nutritional requirements. The chemolithotrophs metabolize inorganic substances. Photolithotrophs, such as cyanobacteria, carry out photosynthesis. Heterotrophs, which live by oxidizing organic substances, are classified as aerobes if they use oxygen in this process and as anaerobes if some other oxidizing agent serves as their terminal electron acceptor. Traditional prokaryotic classification schemes are rather arbitrary because of the poor correlation between bacterial form and metabolism. Sequence comparisons of nucleic acids and proteins, however, have established that all life forms can be classified into three domains of evolutionary descent: the Archaea (archaebacteria), the Bacteria (eubacteria), and the Eukarya (eukaryotes).

2 Eukaryotes

Eukaryotic cells, which are far more complex than those of prokaryotes, are characterized by having numerous membrane-enclosed organelles. The most conspicuous of these is the nucleus, which contains the cell's chromosomes, and the nucleolus, where ribosomes are assembled. The endoplasmic reticulum is the site of the synthesis of lipids and of proteins that are destined for secretion. Further processing of these products occurs in the Golgi apparatus. The mitochondria, wherein oxidative metabolism occurs, are thought to have evolved from a symbiotic relationship between an aerobic bacterium and a primitive eukaryote. The chloroplast, the site of photosynthesis in plants similarly evolved from a cyanobacterium. Other eukaryotic organelles include the lysosome, which functions as an intracellular digestive chamber, and the peroxisome, which contains a variety of oxidative enzymes including some that generate H_2O_2 . The eukaryotic cytoplasm is pervaded by a cytoskeleton whose components include microtubules, which consist of tubulin; microfilaments, which are composed of actin; and intermediate filaments, which are made of different proteins in different types of cells. Eukaryotes have enormous morphological diversity on the cellular as well as on the organismal level. They have been classified into four kingdoms: Protista, Plantae, Fungi, and Animalia. The pattern of embryonic development in multicellular organisms partially mirrors their evolutionary history.

3 Biochemistry: A Prologue Organisms have a hierarchical structure that extends down to the sub-molecular level. They contain but three basic types of macromolecules: proteins, nucleic acids, and polysaccharides, as well as lipids, each of which are constructed from only a few different species of monomeric units. Macromolecules and supramolecular assemblies form their native biological structures through a process of self-assembly. The assembly mechanisms of higher biological structures are largely unknown. Metabolic processes are organized into a series of tightly regulated pathways. These are classified as catabolic or anabolic depending on whether they participate in degradative or biosynthetic processes. The common energy “currency” in all these processes is ATP, whose synthesis is the product of many catabolic pathways and whose hydrolysis drives most anabolic pathways. DNA, the cell's hereditary molecule, encodes genetic information in its sequence of bases. The complementary base sequences of its two strands permit them to act as templates for their own replication and for the synthesis of complementary strands of RNA. Ribosomes

synthesize proteins by linking amino acids together in the order specified by the base sequences of RNAs.

EXERCISE

- (1) Prokaryotic Cell
- (2) Eucaryotic Cell
- (3) Mitochondria
- (4) Chloroplast
- (5) Endoplasmic reticulum.
- (6) Ribosomes
- (7) Lysosomes

II Multiple choice question

1. What is the name of the region where double-stranded single circular DNA is found in the prokaryotic cell? ()

- (a) Protonucleus (b) Nucleus (c) Nucleoid (d) Nucleoplasm.

2. In prokaryotic cells, ribosomes are ()

- (a) 70 S (b) 80 S (c) 60S + 40S (d) 50S + 40S.

3. The two domains to which prokaryotes are classified into are: ()

- (a) Bacteria and Protista (b) Bacteria and Archaea (c) Archaea and Eukarya
(d) Eukarya and Monera

4. In prokaryotes, the hair-like outgrowths which attach to the surface of other bacterial cells are. ()

- (a) Flagella (b) Pili (c) Capsule (d) Plasmids

5. A component of prokaryotic cells: ()

- (a) Plasma membrane (b) DNA (c) Cytoplasm (d) All of these

6. The process of recombination in prokaryotes takes place in this way ()

- (a) Transformation (b) Conjugation (c) Transduction (d) All of the above

7. Which of these are not eukaryotic? ()

- a) Plants b) Fungi c) Protists d) Monerans.

8. Which of these structures are absent in eukaryotes? ()

- a) Organised nucleus b) Membrane-bound organelles c) Mesosome
d) Nuclear envelope

9. Which of these are absent in plant cells? ()

- a) Large central vacuole b) Cell wall c) Centriole d) Plastid

10. Which of these structures of the phospholipid bilayer is correctly matched with its property? ()

- a) Phosphate head – hydrophobic b) Phosphate head – hydrophilic c) Lipid tail – hydrophilic
d) Lipid tail – Polar.

III Fill in the Blanks.

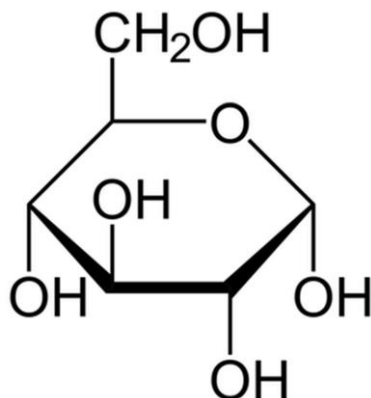
1. _____ is the study of the cell, its types, structure, functions, and its organelles.
2. _____ is a jellylike substance found floating inside the plasma membrane.
3. _____ is involved in the synthesis of phospholipids.
4. The cell is not applied for _____
5. The term cell was given by _____
6. Lysosomes are known as “suicidal bags” because _____
7. _____ cell organelle is involved in apoptosis
8. A cell without a cell wall is termed as _____
9. A plant cell wall is mainly composed of _____
10. The membrane around the vacuole is known as _____

II Answers: 1) c 2) a 3) b 4) b 5) d 6) d 7) d 8) c 9) c 10) b.

III Answers: 1) Cell Biology. 2) Cytoplasm. 3) Smooth Endoplasmic Reticulum. 4) Virus 5) Robert Hooke 6) Hydrolytic activity 7) Mitochondria 8) Protoplast 9) Cellulose 10) Tonoplast.

CHAPTER-II

CARBOHYDRATES



2.1 INTRODUCTION

Carbohydrates are an enormous variety of chemical compounds that are most often known as starches or sugars. The name "carbohydrate" refers to this group of chemicals. Carbohydrates are a type of macronutrient that may be found in both plant and animal foods. Most of all organic substances discovered in the natural world are found in the form of carbohydrates. They have carbon, hydrogen, and oxygen as the primary basic components that make up most of their makeup. The phrase "hydrates of carbon" is where the word "carbohydrate" originates from.

Definition

Carbohydrates are chemicals that, when hydrolyzed, form polyhydroxy-aldehydes, ketones, or other compounds that may be characterized as carbohydrates. In other words, carbohydrates are compounds that generate other carbs when they are hydrolyzed. Sugar is a collective term for a class of carbohydrates that, when taken, result in a taste sensation that is best described as cloyingly sweet.

Functions of carbohydrates

Carbohydrates are involved in an extensive variety of processes and activities.

1. They are the most readily available nutritional energy source for every kind of creature since each gram contains four calories.
2. Carbohydrates are essential components used to construct a wide range of organic compounds. (Fats, amino acids).
3. Carbohydrates, in the form of glycoproteins and glycolipids, are involved in the production of the structure of cell membranes as well as the performance of numerous

cellular tasks. These processes include cell development, adhesion, and fertilization.

4. Carbohydrates, in addition to their role as a source of immediate energy for the body, also play a role in the body's production of glycogen, which is a sort of energy storage.

Biological Significance

1. The metabolization of this substance results in the production of carbon dioxide gas, water, and energy. This is the principal function that this substance serves in the body. They supply most of the vitality that is required for live cells to operate correctly and are the primary source of this vitality.
2. The activity of certain byproducts of the metabolism of carbohydrates, which are known as catalysts, accelerates the combustion of food and drink. These byproducts are known collectively as "catalysts."
3. It is a beginning ingredient for the biological synthesis of compounds such as fatty acids and amino acids, and it plays an essential part in the process of the biological production of these compounds.

Occurrence

The spread of these organisms across the natural world is helped along in part by the animals and plants that live there. Starch is the component in plants that is the most fundamental. They are most prevalent in the plant's leaves, vegetables, tubers, roots, and grains, all of which have considerable amounts. The form that glucose adopts when it is discovered in animals is known as glycogen. The liver and the skeletal muscles are the principal organs in which this sort of glucose storage may be found. It is a natural process that occurs in all mammals. Sugarcane is the principal source of sucrose, which is an ingredient that has a wide range of applications in the culinary world. In addition to this, it is present in the nectar of flowers as well as in the fruit that they produce. There are several types of plant life that include glucosides, which are compounds that are generated from carbs but have their own special qualities. Certain glucosides have an important role in the treatment of several different cardiac diseases. Both glucose and fructose, two types of simple sugars, may be found in large levels in plant matter. Glucose and fructose are examples of simple sugars. Lactose is naturally present in milk in its unprocessed form.

2.2 : CHEMICAL AND PHYSICAL CHARACTERISTICS OF CARBOHYDRATES.

In terms of their composition, they are composed of Carbon, Hydrogen, and Oxygen. These components are represented by the proposition $C_nH_{2n}O_n$, which can also be written as $(CH_2O)_n$ times or $C_n(H_2O)_n$. Every type of simple sugar has an aldehyde or ketone molecule lurking within its structure. Because of their association with reducing properties, aldehyde and ketone groups are very essential in organic chemistry.

2.3 : CLASSIFICATION OF CARBOHYDRATES

Carbohydrates are classified into 3 major groups. They are.

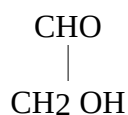
1. Monosaccharides
2. Oligosaccharides
3. Polysaccharides

I. Monosaccharides

They have only one molecule of sugar, and the hydrolysis process cannot be used to break them down into molecules of lower complexity. As can be seen in the table below, they are further classified based on the total amount of carbon atoms that are present in the structure.

Dioses

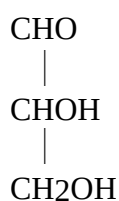
Dioses has two carbon atoms.(e.g.) Glycolaldehyde

**2) Triose**

Triose has three carbon atoms.

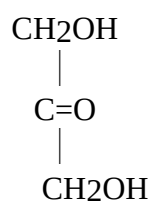
(e.g.) Aldehyde form

Glyceraldehyde



Ketone form

Dihydroxy acetone

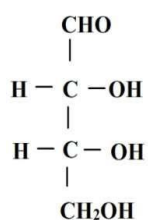


3) Tetrose

Tetrose has four carbon atoms (e.g.)

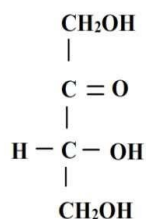
Aldehyde form

D - Erythrose



Ketone form

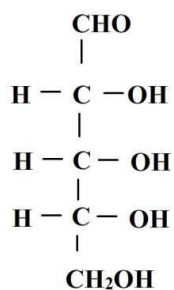
D - Erythrulose

**4) Pentose**

Pentose has five carbon atoms.

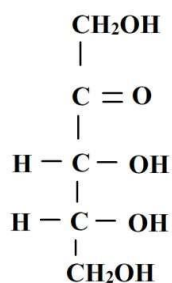
Aldehyde form

D - Ribose



Ketone form

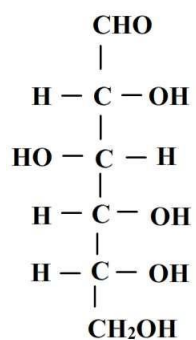
D - Ribulose

**5) Hexose**

Hexose has six carbon atoms

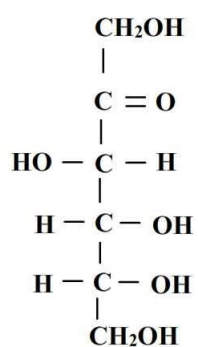
Aldehyde form

D - Glucose



Ketone form

D - Fructose

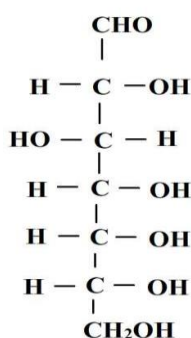


6) Heptose

Heptose has seven carbon atom

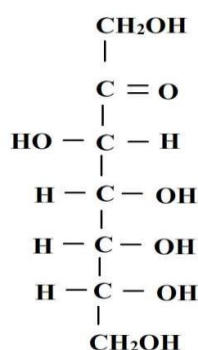
Aldehyde form

Glucoheptose



Ketone form

Sedoheptulose



II. Oligosaccharides

On hydrolysis, these compounds give 2 to 9 monosaccharide unit on hydrolysis. It includes.

a) Disaccharides

The molecular formula is $\text{C}_{12}\text{H}_{22}\text{O}_{11}$. They yield 2 molecules of monosaccharides on hydrolysis (e.g.) Sucrose, Lactose, Maltose.

b) Trisaccharides

This yield 3 molecules of monosaccharides on hydrolysis. (e.g.) Raffinose, Maltotriose.

III. Polysaccharides

This yield more than 10 molecules of monosaccharides on hydrolysis. The molecular formula of this is $(\text{C}_6\text{H}_{10}\text{O}_5)_n$. It can be classified as Homo and Hetero polysaccharides.

i) Homo Polysaccharides

It contains some monosaccharides. (eg) Starch, Cellulose, Glycogen, Dextrins, Dextrins, and Inulin.

ii) Hetero Polysaccharides

It contains more than one simple sugar in an alternating repeating sequence. (e.g.) Heparin, Chondroitin Sulphate, Hyaluronic acid, Dermatin sulphate, Heparin,

Keratansulphate, and Heparan sulfate.

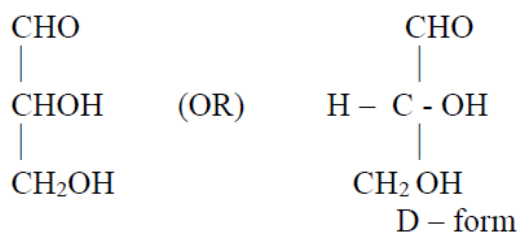
ISOMERISM

Isomerism can take place when asymmetric carbon is present since it paves the way for its creation. Stereoisomers, also known as geometric isomers, are a type of structural isomer that refers to a group of substances that have the same structural formula but change solely in their spatial configuration.

Asymmetric carbon atom

A carbon atom is said to be asymmetric when it bears 4 different atoms or groups of atoms at four valency bonds.

(e.g.) Glyceraldehyde

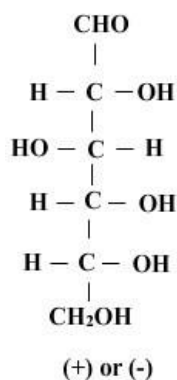


The second carbon atom above is an asymmetric carbon atom.

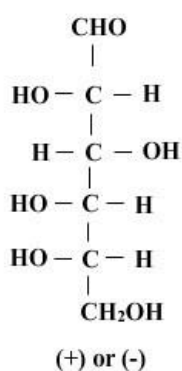
The number of isomers of any compound depends upon the number of asymmetric in the molecule. It is given by the formula $I = 2^n$. Where I represent the number of isomers & represents several an asymmetric carbon atom. For example, Glucose has 4 asymmetric carbon atom & a number of possible isomers is $I = 2^4 = 16$.

Out of this one 8 represent the series & other 8 represent L series. Both D & L are non – super imposable and are mirror images of each other.

D - Glucose



L - Glucose



D – form

In D – form the carbon atom just adjacent to the terminal primary alcohol hydroxyl group (i.e.) 5th carbon atom in D Glucose has a secondary alcoholic hydroxyl group on the right side.

L – form

In L – form the carbon atom just adjacent to the terminal primary alcoholic hydroxy group (i.e.) the 5th carbon atom in L Glucose has a secondary alcoholic hydroxy group on the leftside.

D and L have no significance to the direction of optical rotation. They simply designate the spatial arrangement of the secondary alcoholic group to the right or left respectively whereas the sign (+) and (-) represent optical isomerism.

Optical isomerism

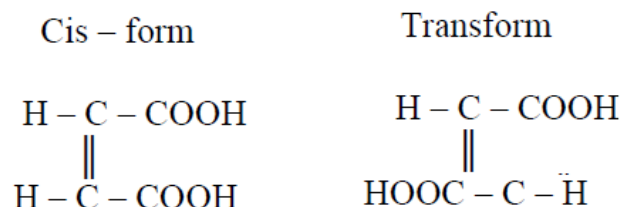
When a beam of plane-polarized light is passed through a solution of an optical isomer & if the plane-polarized light is found to rotate to the left it is called Levo rotation & is expressed as (-) sign or l. If it is rotated to the right, it is called Dextro rotation & is expressed as (+) sign or d.

When it is necessary to represent both the spatial configuration and the optical activity of your isomer simultaneously, they are expressed as D (-) or D (+) and L (-) or L (+).

When an equal amount of dextro rotatory and levo rotatory isomers are present, the resulting mixture has no optical activity since the activities of each isomer cancel the other such a mixture is called a Racemic mixture or DL mixture. The separation of optically active isomers from the racemic mixture is called Resolution.

Cis – trans isomerism: (Geometric isomerism)

This occurs in compounds with double bonds. As the double bond is rigid, the atoms attached to it are not free to rotate like those attached to a single bond. The structures are.



Maleic acid Fumaric acid

These structures have different chemical & physiological properties.

The two D and L forms of a compound constitute a pair of enantiomers. Thus, D Glucose and L Glucose are enantiomers. The enantiomers are chemically identical in their real sense. They agree in the melting point, solubility, etc., But differ in their ability to rotate the plane of polarized light in a polarimeter (i.e.) a solution of 1 of the 2 enantiomers rotates the plane to right (d isomer) other to left (l isomer).

Diastereomer isomers

These are isomers where they are not mirror images of each other. Thus, if two optical isomers are not enantiomers, they are referred to as diastereo isomerism. They differ in solubility, Melting point, and chemical properties in general. (e.g.) Galactose and Mannose are diastereomer – isomers. D – Erythrose & D Threose are diastereo isomers.

Epimers

When sugars are different from one another only in the configuration about a single carbon, atom, they are called Epimers. (e.g.) Glucose & Mannose are epimeric pair which differ only with respect to carbon atom two. Similarly, Galactose is the 4th epimer of glucose.

2.4 : STRUCTURE OF GLUCOSE

Linear form

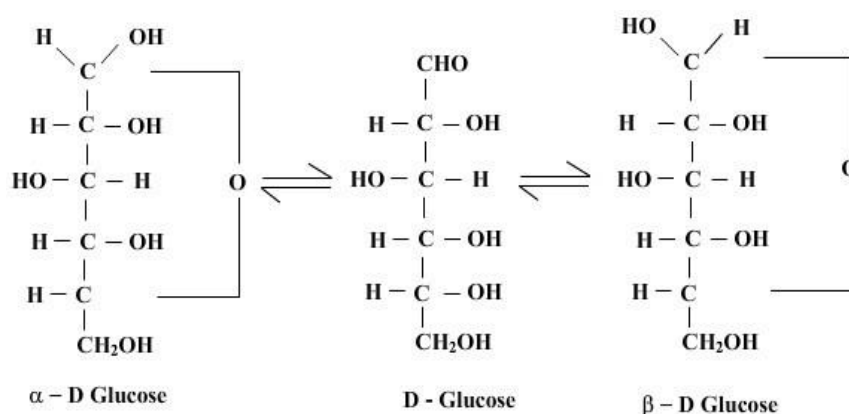
Glucose is represented as a straight-chain compound having six carbon atoms. The aldehyde group represents the first attachment & the rest of the carbon has H & - OH group attached to each carbon atom. Fischer who worked out the structural configuration of the monosaccharide formulated D & L Glucose.

The ring structure of Glucose

Aldehydes and ketones groups of monosaccharides containing five or more carbon atoms exist in a condensed form, as a hemiacetal or hemiketal by combination with one of the -OH hydroxyl groups in the same molecule. This was again the work of Fischer.

Mechanism of ring formation

When the -CHO group at C, the atom condensed with -OH group at C5, ring formation occurs. This ring formation has made C1 atom asymmetric because it has 4 different groups attached to it and C1 is referred as an Anomeric carbon atom. Because of anomeric carbon Glucose can exist as isomeric for α & β anomer.



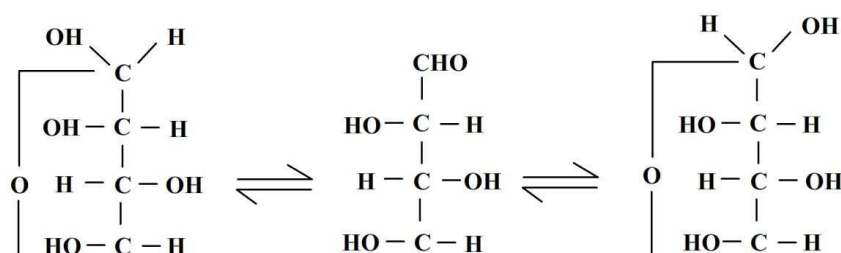
α - Anomers:

If the hydroxyl group in the 1st C atom is in the same side of the ring formation it is referred to as α anomer.

β - Anomer

If the hydroxyl group in 1st C atom is in the opposite side of the ring formation it is referred to as β - anomer.

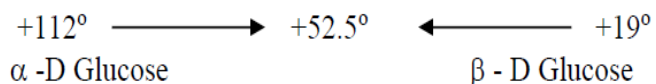
Thus 2 anomers for L glucose are α L Glucose & β L glucose.



changing of optical rotation. When a solution of freshly prepared crystalline Glucose

of α - D form was observed in a polarimeter. Its initial specific rotation which is 112° gradually decreases & finally becomes steady at $+52.5^\circ$. If however, the solution of pure crystalline β - D Glucose is observed the initial specific rotation is $+19^\circ$ for β - D Glucose gradually increase becomes steady at 52.5° .

The change in rotation is illustrated as.



This change in optical rotation undergone by Glucose is called Muta rotation. Thus both α & β D Glucose in solution attains a stage of equilibrium at $+52.5^\circ$. It has been established that all monosaccharides exist in three forms in nature. They are.

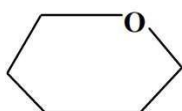
1. Aldehyde form
2. α - ring form
3. β - ring form

Of the 3 forms aldehyde form is said to be unstable and exists as an intermediate. When Glucose solutions it is assumed that approx. $2/3^{\text{rd}}$ of the sugar exists as β - form at equilibrium.

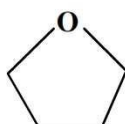
Pyranoses & Furanoses

Haworth proposed a scheme by which all sugars forming six-membered rings are to be called pyranoses which are composed of five Carbon atoms & one oxygen atom. The sugars having five-membered rings are called Furanoses which are composed of four carbon atoms & one oxygen atom.

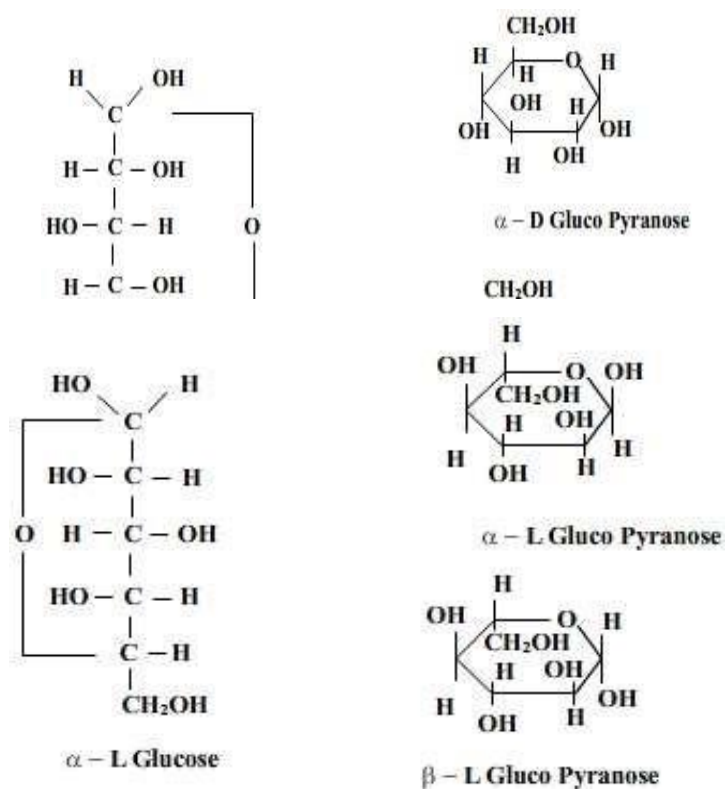
Pyranose ring



Furanose ring

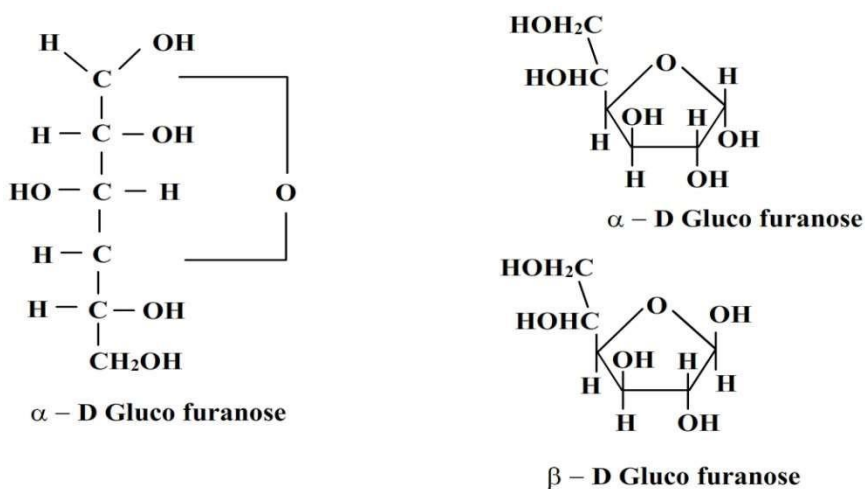


According to Haworth's projection formula groups of atoms projecting to the right in the straight line formula are shown projecting downwards whereas those of atoms projecting to the left is projected upwards except 5th carbon atom (i.e.) 5th carbon atom does not obey Haworth's formula.



Sugars in Furanose structure

The structures of α & β forms Glucose D series existing as five membered rings. Here ring formation takes place by condensation of aldehyde at C1 with -OH at C4. (Hydroxyl group).

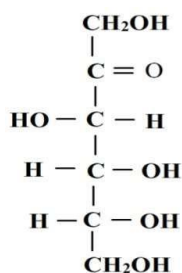


Structure of Fructose

Fructose is called levulose or fruit sugar. It is called, so because it is laevorotatory and it is found abundantly in sweet fruits. It is also present in honey, which is sweeter than sucrose, D-Fructose is in keto form. Its structure can be given in three forms. They

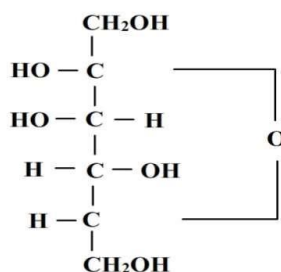
are,

1. Keto form
2. α & β form
3. Haworth formula as Furanose & Pyranose.

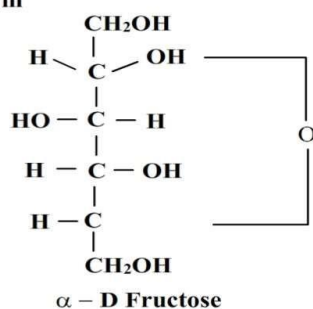


D Fructose

Keto form

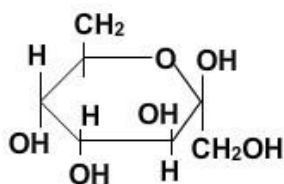


β - D Fructose

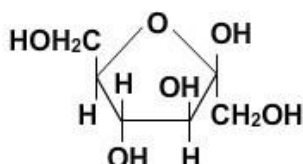


α - D Fructose

Because of Hemiketal linkage the 2nd carbon atom in Fructose is becoming anomeric carbon atom. So, in α D Fructose the -OH group at C2 is on the same side as the ring, in β - D Fructose, the -OH at C2 is on the opposite side of the ring. Fructose exists in Furanose form when combined with natural products but in free state it occurs in Pyranose form.



b- D Fructo Pyranose



b - D Fructo Furanose

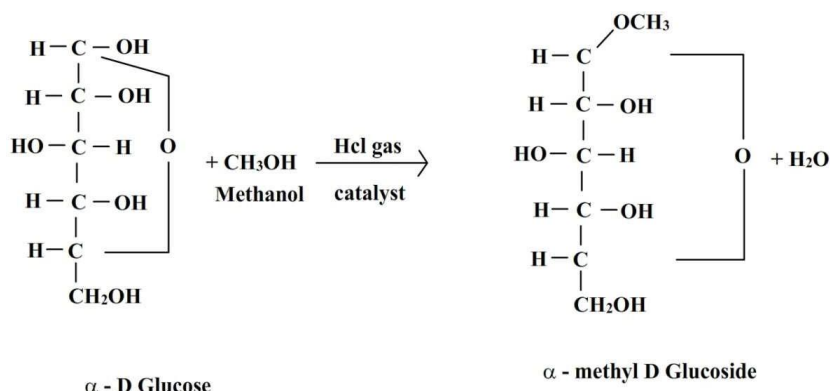
Properties of Monosaccharides

Carbohydrates possess active groups which are responsible for their chemical behaviors.

These groups are glycosidic -OH and -CHO or (-CO).

I Action of glycosidic - OH group

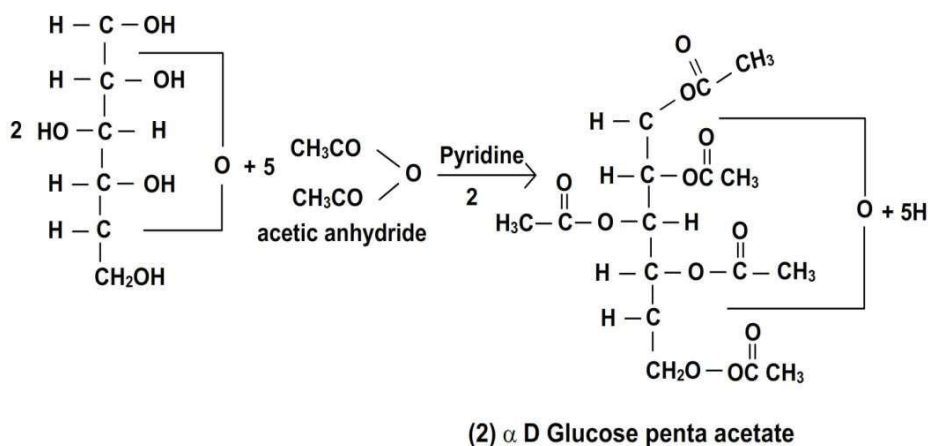
1. Reaction to alcohol



A glycosidic -OH group react with alcohols to form α or β Glycosides.

2. Reaction involving both glycosidic and alcoholic -OH group.

Reaction with acetic anhydride.



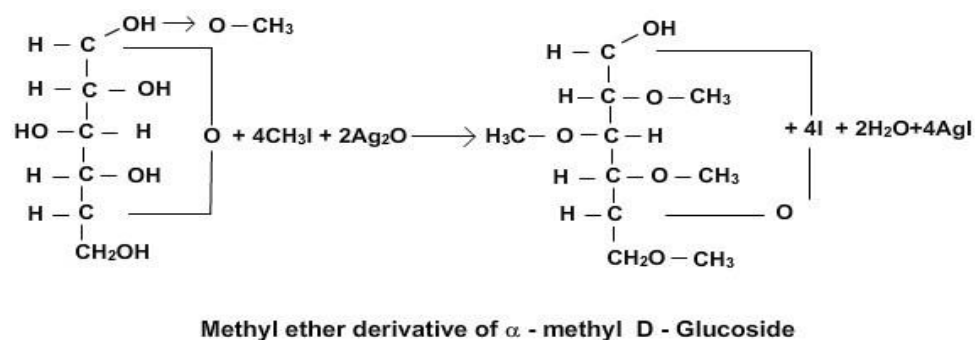
Glycosidic & alcoholic -OH groups of monosaccharides & disaccharides react with an acetylating agent like acetic anhydride in pyridine to form acetate derivatives called esters.

Significance

The ability of sugars to form esters indicates the presence of -OH groups in their molecule, glucose upon acetylation yield penta acetate derivative. It obviously contains 5 -OH groups.

II. Reaction of alcoholic –OH groups

Reaction with Methyl iodide.



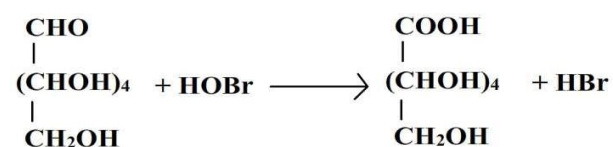
The alcoholic – OH groups of monosaccharides & disaccharides are converted into ether groups upon treatment with a methylating agent. The glycosidic – OH group of sugar is 1st protected with an R group which is then allowed to react with a methylating agent.

Significance

This reaction has been extensively used to ascertain which – OH groups in the sugar are free and available for reaction. Thus α -methyl D – Glucoside is methylated at C 2,3,4 and C6 but not at C5 and C1 which is involved in the hemiacetal link.

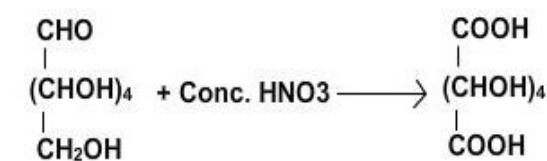
4. Reaction involved in both alcoholic –OH and CHO or CO groups.

a. Oxidation: a) With weak oxidizing agent

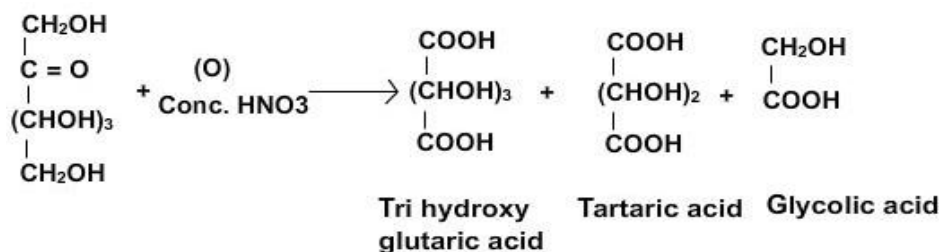


Mono carboxylic acid or Gluconic acid

b. With strong oxidizing agent



Di carboxylic acid or Glucaric acid (saccharic acid)

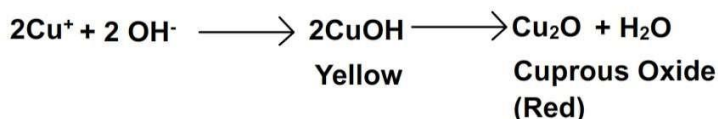
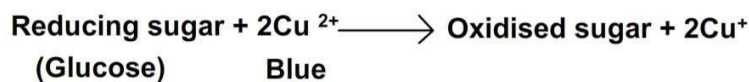
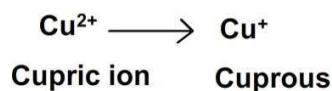


With strong oxidants like Conc. HNO₃ both aldehyde & 1° alcohol groups are oxidized to yield dicarboxylic acids. With aldoses gives acids with the same number of carbons is obtained whereas ketose react to produce acids with fewer carbon atoms

III. Reactions of -CHO or -CO group.

Oxidation.

1. Oxidation with metal hydroxides.

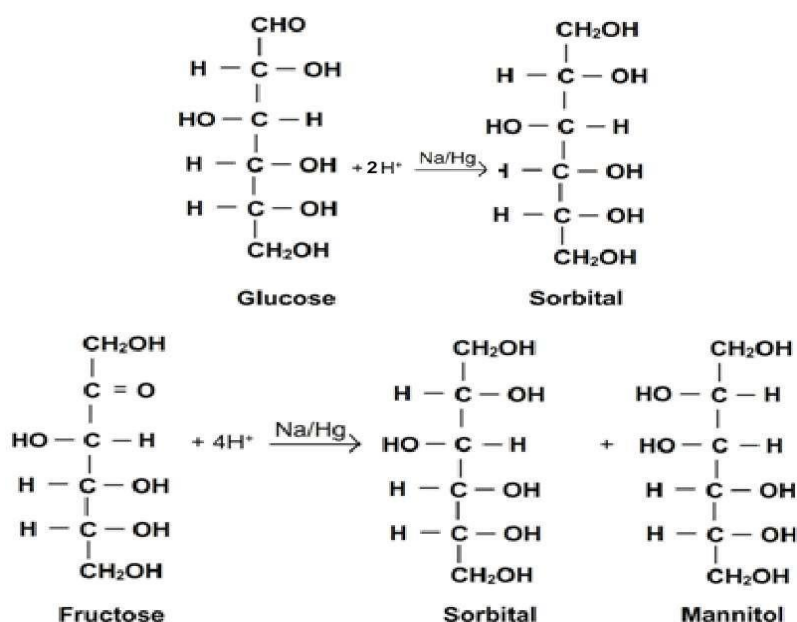


Metal hydroxides like Cu (OH)₂, AgOH, and Bi (OH)₃ oxidize the free -CHO or -CO group of mutarotating mono & disaccharides, and the metal oxides are reduced to the lower oxide or to the free metals. Thus Cu (OH)₂ is reduced to Cu₂O and AgOH & Bi (OH)₃ to metals Ag or Bi. Cupric ion (Cu²⁺) is the most common oxidizing agent. It is the active ingredient in Fehling's, Benedict's and Barfoed's reagents.

2. Sugars may be reduced in many ways depending upon the type of reducing agent used.

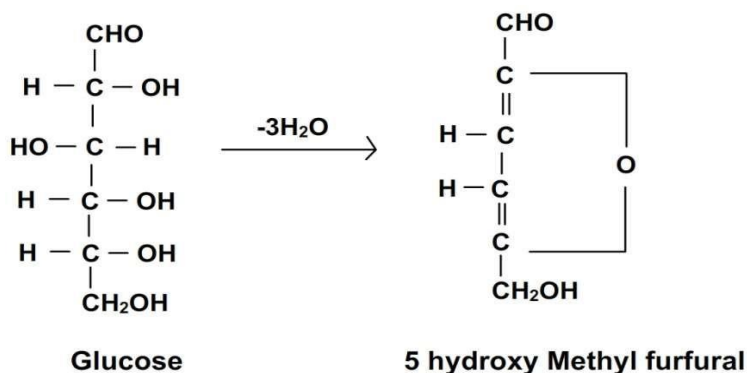
a. With Na/Hg

The Monosaccharides are reduced to their corresponding alcohols by treating them with reducing agents like Na/Hg. Thus, glucose yield Sorbitol, Mannose yield Mannitol, Galactose Dulcitol & Fructose yield a mixture of sorbitol & Mannitol.

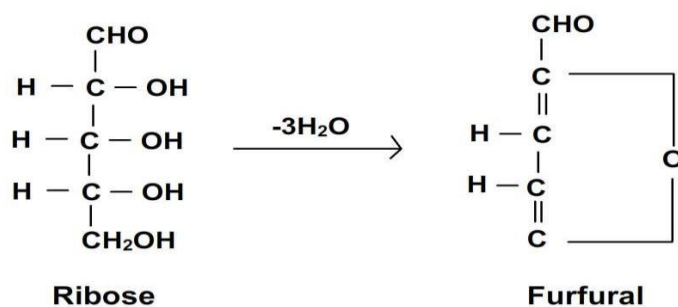


b. With Strong mineral acids

With hexoses, reduction leads to the formation of 5 hydroxy methyl Furfura



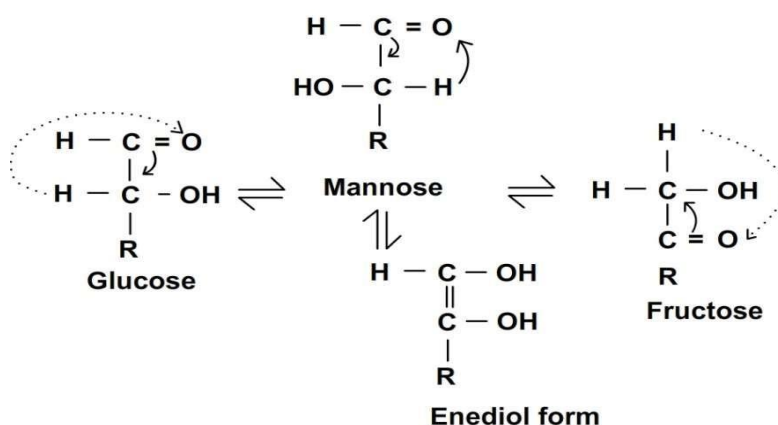
This reaction forms the bases for some colored test (e.g.) Molisch's test & Bials original test. For sugars as –CHO products of this reaction condense with certain Organic phenols to give characteristic-colored products. With pentoses, the product furfural is obtained on heating.



Tetrodes & trioses do not undergo this reaction.

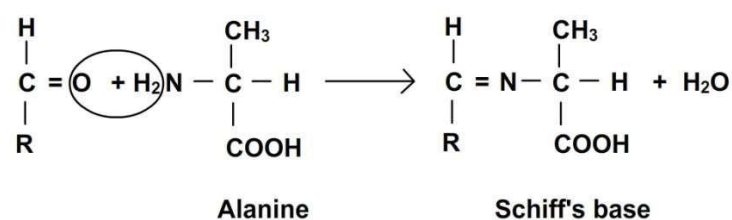
c. With dilute alkalis

Glucose, Fructose & Mannose are interconvertible in a weak alkaline solution such as $\text{Ca}(\text{OH})_2$ & $\text{Ba}(\text{OH})$ at low temperatures. This reaction is known as Lobry de Bruyn Alberda Van Ekenstein's transformation. The mechanism of this reaction involves enolization (i.e.) the migration of proton from the carbon atom or to the Oxygen of an adjacent – CO group resulting in the formation of unsaturated alcohol called enol. The interconversion between glucose, Fructose & Mannose may be occurring through a common enediol form.

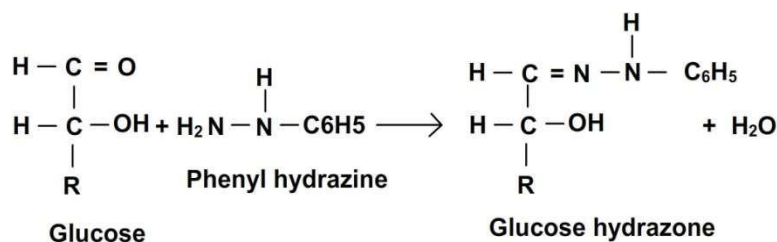


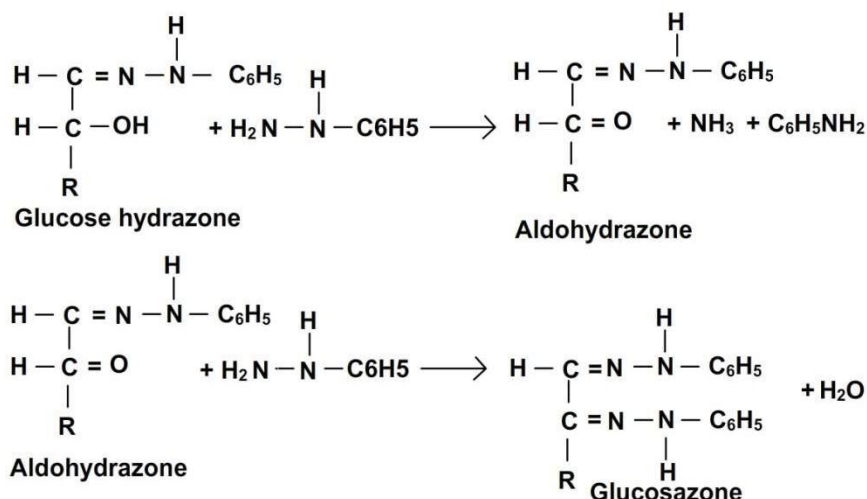
3. Reaction with alanine

The aldehyde group of carbohydrates condenses with – NH_2 group of alanine to form Schiff's base.



4. Reaction with Phenyl hydrazine.





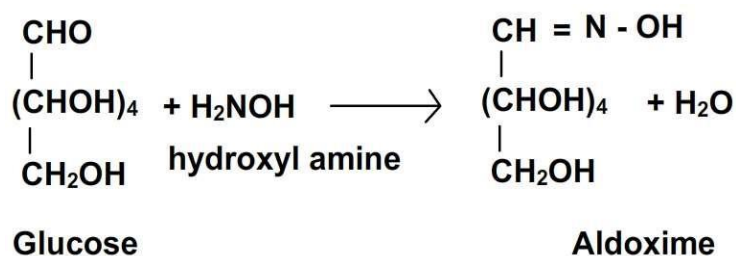
The reaction with Phenyl hydrazine involves only 2 carbon atoms (i.e.) Carbonyl (-CHO or -CO) and the adjacent one 1 Mole of phenyl hydrazine reacts with 1 mole of aldose or ketose to form a mole of hydrazone with 2nd mole of phenyl hydrazine the hydrazone is oxidized to C₆H₅NH₂ & NH₃. Finally, the 3rd mole of phenyl hydrazine reacts with aldohydrazone to produce Osazone.

The Osazone of reducing sugar is crystalline yellow & usually insoluble Compounds Glucose, Fructose & Mannose would yield the same Osazone owing to the similar structure in unaffected part of the molecule (C3 to C6).

Osazone formation is also found in disaccharides except for sucrose.

5. Reaction with hydroxyl amine

Simple sugars react with NH₂OH to yield Oximes.

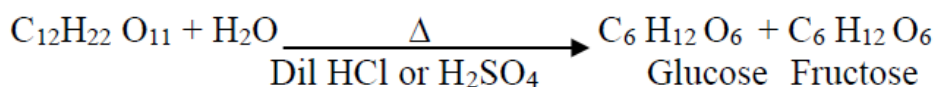


Preparation of Glucose

1. From Sucrose

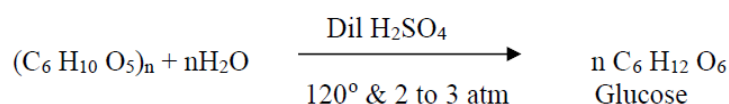
In the laboratory, Glucose is made by the hydrolysis of sucrose by boiling with Dil HCl or H₂SO₄ in an alcoholic solution. Glucose and Fructose are obtained in equal

amounts. On cooling resulting solution contains Glucose that is being much less soluble than Fructose separates out.



2. From starch

Commercially Glucose is obtained by the hydrolysis of starch by heating with very dilute H_2O at 120°C under a pressure of 2 to 3 atm.



The resulting solution is neutralized with CaCO_3 , and precipitated calcium salts are filtered off. Filtrate is decolorized by boiling with animal charcoal & then concentrated under reduced pressure crystallized.

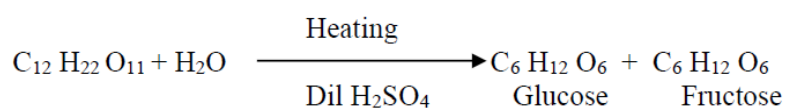
3. From Cellulose

Glucose may also be obtained by the hydrolysis of cellulose by heating with dil H_2SO_4 the procedure is the same as the manufacture of Glucose from starch.

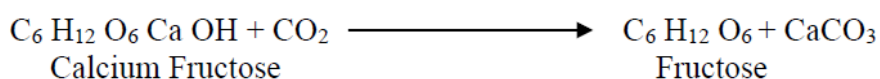
Preparation of Fructose

1. From sucrose

In the laboratory, Fructose is prepared by hydrolysis of sucrose by boiling with dil H_2SO_4 Heating.

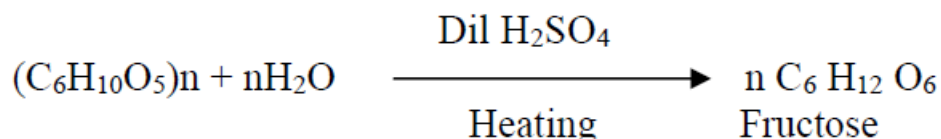


On completion of hydrolysis, the excess of H_2SO_4 is neutralized with BaCO_3 & filtrate concentrated. It is cooled in ice & then Ca(OH) is added to precipitate Calcium Fructosate, Calcium Glucosate which is also formed remains in solution. The calcium Fructosate is removed by filtrations & is converted into Fructose by passing CO_2 through its suspension in water.



After the removal of CaCO_3 by filtration, the solution is evaporated to a syrup. The addition of a crystal of fructose causes the syrup to solidify & crystals obtained may be recrystallized from alcohols.

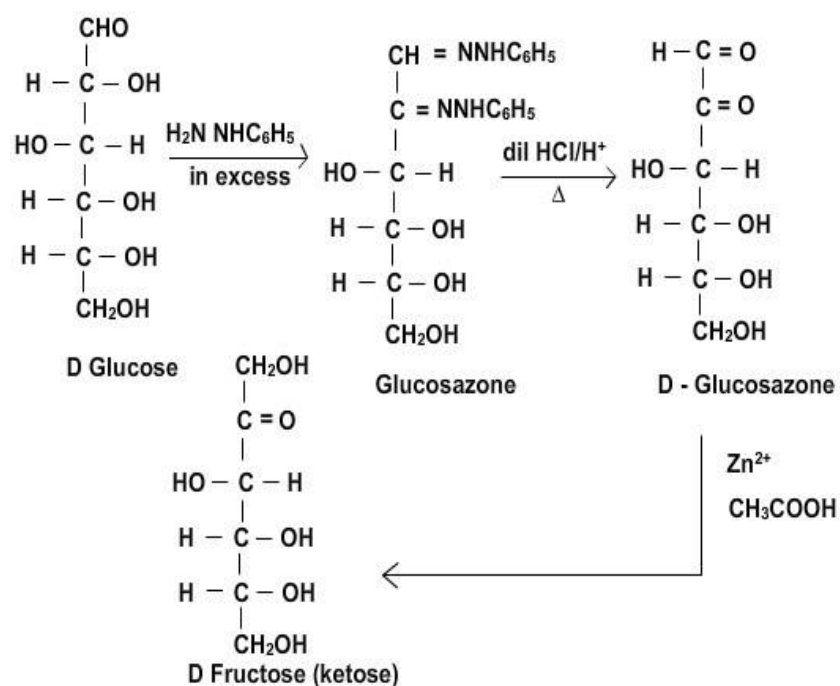
From Inulin: Fructose is obtained commercially by the hydrolysis of Inulin by heating with Dil H_2SO_4 .



The resulting solution is neutralized BaOH and the precipitate BaSO_4 is removed filtrations. The solution thus obtained is concentrated under reduced pressure to yield crystals of fructose.

2.5 : INTER CONVERSION

Conversion of aldose into corresponding ketose (From glucose to fructose).

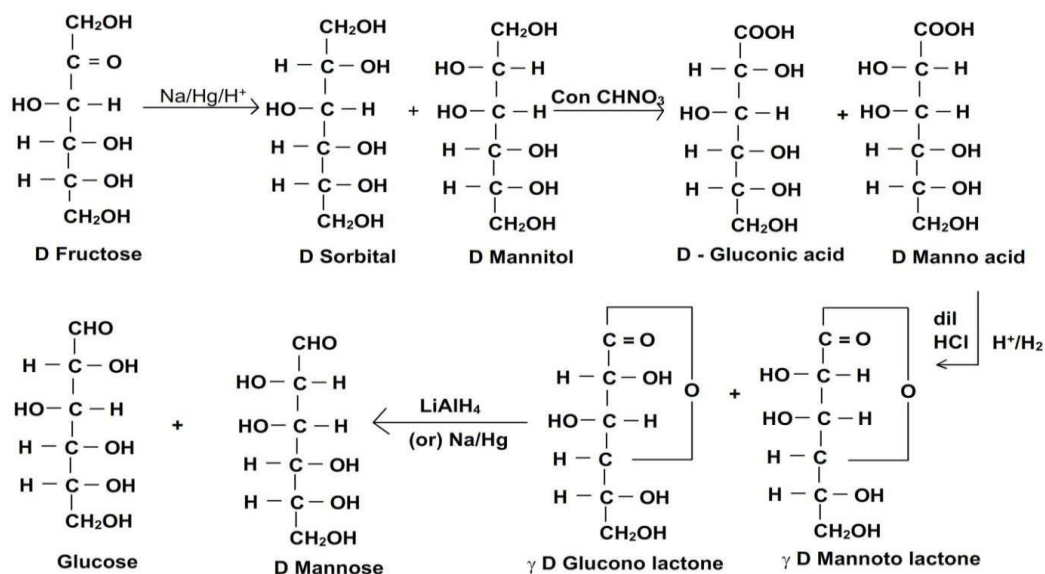


(Ketohehexose)

Glucose reacts with $\text{NH}_2\text{NHC}_6\text{H}_5$ giving corresponding Osazone. It is then hydrolysis with Dil HCl to give Ozone. It is then reduced with Zn & glacial acetic acid to give ketose. It should be noted that in glacial acetic acid, Zinc reduces the aldehyde group in preference to the ketone group.

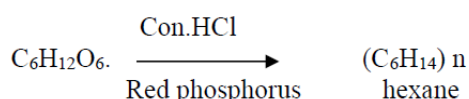
Conversion of ketoses into the corresponding aldoses

The ketose is first reduced with Na/Hg in the presence of a trace amount of acid. This process introduces a new asymmetric center & results in the formation in two different polyhydric alcohols. The alcohols are next oxidized with HNO₃ to give mono basic aldonic acid. The aldonic acids on treatment with dil HCl give γ lactones. These lactones are solids and are separated by fractional crystallization. The individual lactones are then reduced with LiAlH₄ (or) Na/Hg in a weakly acidic solution to yield aldoses that are isomeric with original ketose. Thus D- Fructose may be converted to D Glucose & Mannose as shown below.



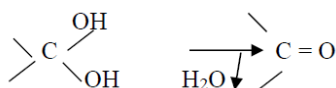
2.5.1: Structural Elucidation of Glucose

1. Elemental analysis & molecular weight determination show that the molecular formula of Glucose is C₆ H₁₂ O₆.
2. Complete reduction of glucose with conc hydrochloric acid in the presence of red phosphorus reduces n-Hexane (C₆ H₁₄) as a major product. This indicates that the 6- carbon atom in Glucose forms an unbranched chain.



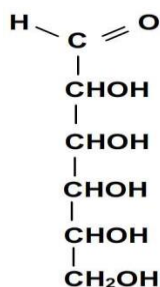
3. Glucose readily dissolves in H₂O to give a neutral solution. This indicates that it does not contain - COOH group.
4. It reacts with NH₂OH to form a mono oxime or adds only one mole of HCN to give acyanohydrin. This reaction indicates the presence of either an -CHO or -CO group but not both.

5. Mild oxidation of it with bromine water given Gluconic acid, a monocarboxylic acid. This indicates the presence of an -CHO group that must occupy one end of the chain.
6. Further oxidation of Gluconic acid with HNO_3 gives glucaric acid, a dicarboxylic acid with molecular formula $\text{C}_6\text{H}_{10}\text{O}_8$. This indicates the presence of 1° alcohol group.
7. Glucose reduces an ammoniacal solution of Silver Oxide to metallic Ag (or) a basic solution of Cu^{+2} (Fehlings) to red cuprous oxide. These reactions further confirm the presence of a terminal -CHO group.
8. Glucose reacts with $(\text{CH}_3\text{CO})_2\text{O}$ in the presence of pyridine, to form a penta acetate. This reaction indicates that 5 -OH groups are present in it.
9. Organic compounds with 2 -OH groups attached to a single carbon atom are rare & those which are known usually lose H_2O to produce a carbonyl group.



This suggests that each one of the -OH group is attached to a different carbon atom in Glucose.

From the above evidence, we conclude that glucose is Penta hydroxyl hexanal & it can be represented by the following structure.



Uses of Glucose

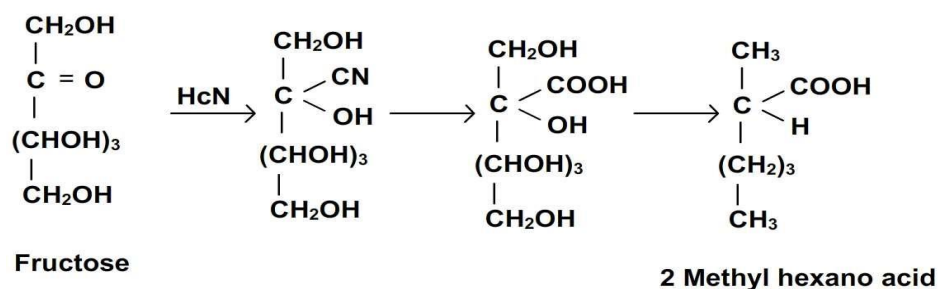
1. Normal level of glucose is 60-120 mg/100ml.
2. Glucose is used in the making of candy.
3. Glucose is used as a food.
4. As a raw material in the preparation of Vinegar in the flavoring syrups, in Jellies & in preserves.
5. It is also used as a reducing agent in silvering of mirrors & in the reduction of indigo blue to white.

Limitation

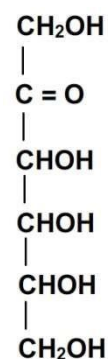
Increased glucose level leads to hyperglycemia.

2.5.2: Structural elucidation of Fructose

1. Elementary analysis & Molecular weight determination show that the Molecular formula for Fructose is $C_6H_{12}O_6$
2. 2nd, 3rd, 4th & 8th points are same as that of Glucose.
3. Oxidation of fructose with conc. HNO_3 gives a mixture of glycolic acid & Tartaric acid. Since this oxidation occurs with the rupture of the carbon chain, the carbonyl group must be present as a keto group.
4. Partial reduction of fructose with Na/Hg & H_2O reduces a mixture of two epimeric alcohols sorbitol & Mannitol because a new asymmetric center has been created at C_2 . This confirms the presence of the ketonic group.
5. When fructose is treated with HCN it forms an additional product which upon hydrolysis & subsequent reduction of hydroiodic acid & red phosphorus gives two methyl hexanoic acid. This indicates that the ketone group is adjacent to 1 of the terminal carbon atoms.



From the above evidence we conclude that fructose is a penta hydroxyl hexanone & can be represented by the following structure.



Fructose

Uses of Fructose

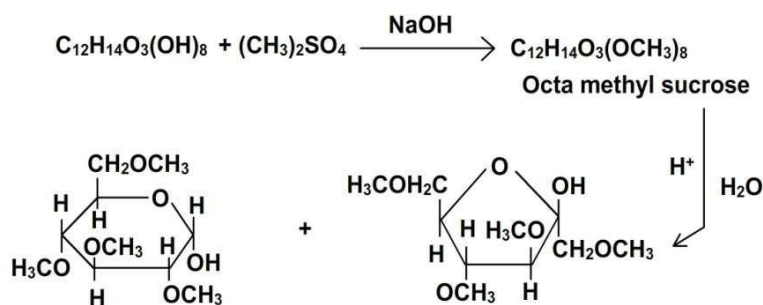
1. It is used as a sweetening agent in the confectionery.
2. It acts as a substitute of cane sugar for persons suffering from diabetes.

Sucrose

It is ordinary table sugar that we eat every day. It occurs in Sugarcane, Sugar beet, Almonds, and Pineapples. Maple sap, Apricot, Banana, Mango & Honey.

2.5.3: Structural elucidation of sucrose

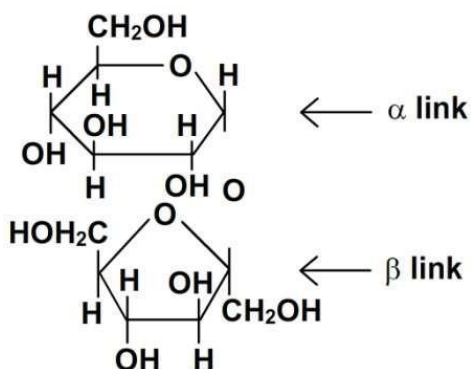
1. Elemental analysis & Molecular weight determination show that molecular formula of sucrose is $C_{12}H_{22}O_{11}$.
2. It reacts with $(CH_3CO)_2O$ in the presence of Na acetate to form sucrose octa acetate. This indicates the presence of 8 hydroxyl groups in the sucrose molecule. Since it is a stable compound, the 8 -OH group must be present on separate carbon atoms.
3. Hydrolysis of sucrose with dilute acids yields an equimolecular mixture of D Glucose & D Fructose. This indicates that the sucrose molecule is made up of one unit of each of these monosaccharides.
4. Sucrose does not reduce Tollen's reagent or Fehling's solution, does not form an Osazone, does not form Methyl glycosides & does not undergo Mutarotation. All these observations indicate that the cyclic forms of Glucose & Fructose joined together through glycosidic linkage at points where the carbonyl groups present (i.e) C1 in glucose & C2 in fructose.
5. Sucrose reacts with dimethyl sulfate in an alkaline solution to form Octa methyl sucrose which on hydrolysis yields a mixture of 2,3,4,6 tetramethyl.
6. D Gluco pyranose & 1,3,4,6 tetramethyl D Fructo Furanose. The formation of these compounds indicates that the Glucose unit in sucrose has a pyranose form & Fructose unit has a furanose form.



2,3,4,6 tetra methyl D Gluco pyranose 1,3,4,6 tetra methyl D Fructo Furanose

- Sucrose is hydrolyzed by Maltase, an enzyme that hydrolysis only α Glycosides. It is also hydrolyzed by invertase, an enzyme that hydrolysis only β but not α fructo furanosides. These observations indicate that it is both α glucoside & β fructoside.

The above evidence clearly indicate that sucrose has the following structure. It should be noted that fructose unit in sucrose has furanose form but when it is hydrolyzed it is pyranose form fructose which is obtained.



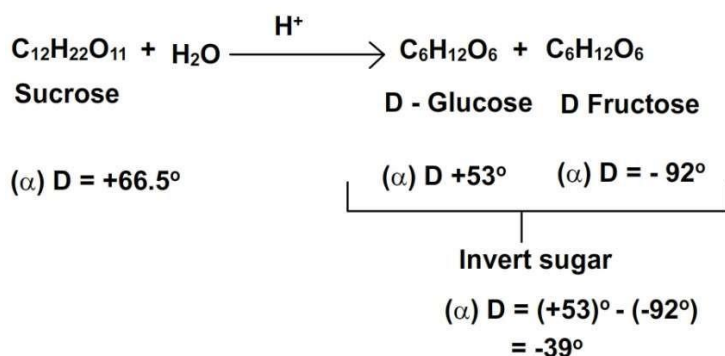
Properties

Physical properties

- It is a colorless, Odorless, and Crystalline solid.
- It is melting point is 184° to 185° CC.
- It is very soluble in H_2O .

Chemical Properties

- It is a non-reducing sugar. It does not react with Tollen's reagent, Fehling's solution, HCN , hydroxyl amine, or phenylhydrazine.
- Hydrolysis of sucrose with hot dilute acid yield D Glucose & D Fructose.



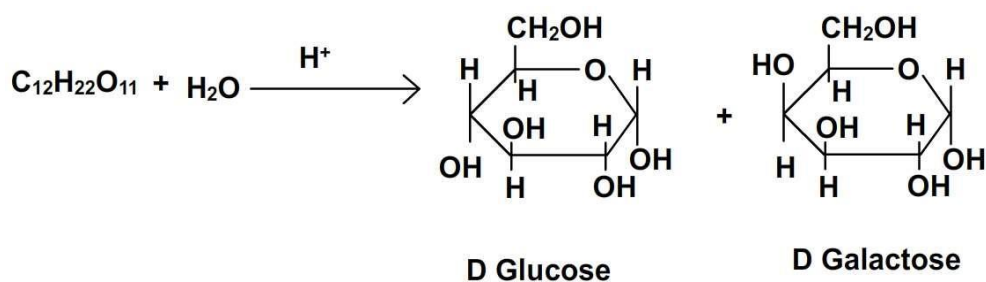
It is dextro rotatory, its specific rotation $+66.5^\circ$, D Glucose is also D – rotatory $(\alpha) \text{D} = +53^\circ$ but D Fructose has large –ve rotation & $(\alpha) \text{D} = -92^\circ$. Since D Fructose has greater specific rotation than D Glucose so, the resulting mixture is levo rotatory. Due to this sucrose hydrolysis is known as Inversion of Sucrose & equimolecular mixture of Glucose & Fructose is known as inverted sugar or Invertase. It is also brought about by enzyme invertase which is found in yeast.

Uses of Sucrose.

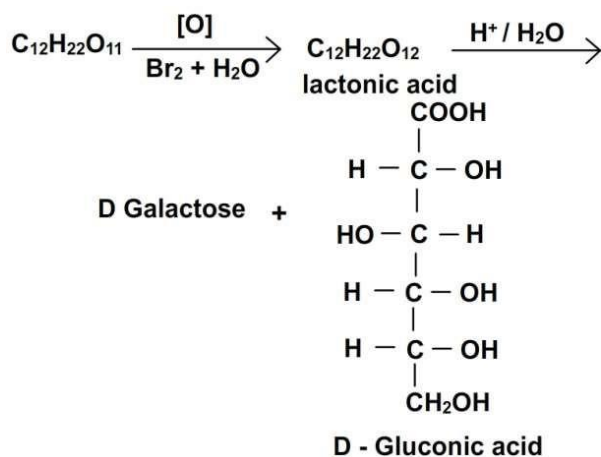
1. It is used as a food.
2. It is an ingredient of jellies, jams, canned fruits, preserves, confections, condensed milk & other foods.

2.5.4: Structural elucidation of Lactose: (Milk sugar)

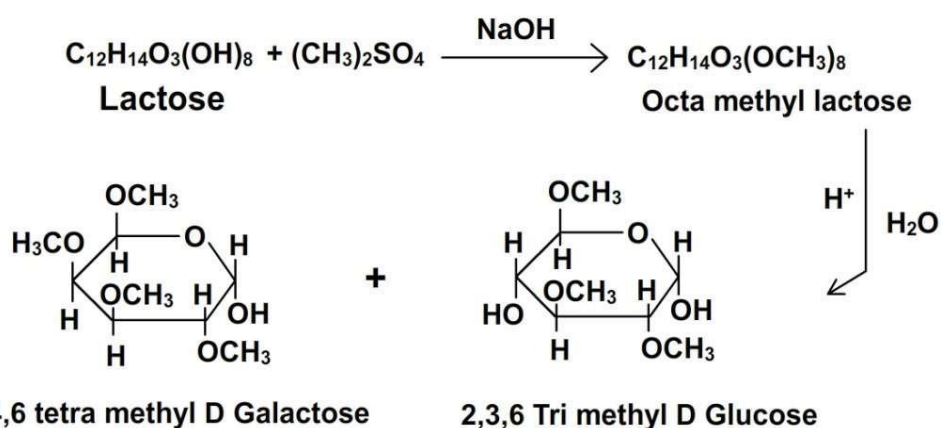
1. Elemental analysis & Molecular weight determination show that Molecular formula of Lactose is $\text{C}_{12}\text{H}_{22}\text{O}_{11}$.
2. Lactose reacts with $(\text{CH}_3\text{CO})_2\text{O}$ in the presence of Na acetate to form lactose octa acetate. This indicates the presence of 8 – OH groups in Lactose molecule. Since it is a stable compound, 8 – OH groups must be present on separate carbon atoms.
3. Hydrolysis of lactose with dilute acid yields an equimolecular mixture of D – Glucose & D – Galactose. This indicates that it contains 1 unit of D – Glucose & D – Galactose.



- It reduces Tollen's reagent & Fehling's solution, reacts with HCN and forms an Osazone. All these reactions indicate that 1 free hemiacetal group must be present & this is in equilibrium with some of the free -CHO form.
- Oxidation of Lactose with $\text{Br}_2 + \text{H}_2\text{O}$ give lactonic acid which on hydrolysis yield a mixture of D Galactose & D Gluconic acid. It indicates that it is a Glucose unit that contains free hemi acetal-CHO group.



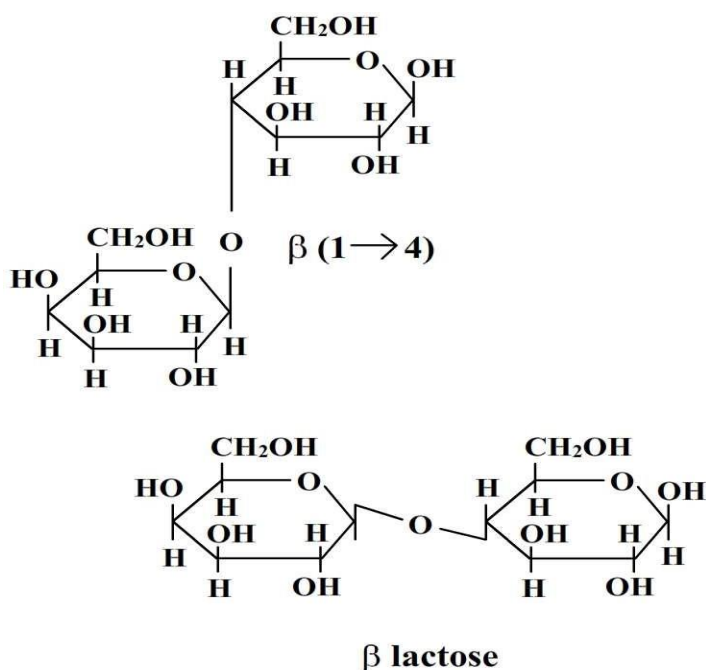
- It reacts with di methyl sulphate in an alkaline medium form Octa methyl lactose which on hydrolysis yield a mixture of 2,3,4,6 tetra methyl D -Gal & 2,3,6 tri methyl D - Glucose. Formation of these compounds indicates that both unit will exist in 6 membered Pyranase form & Glycosidic linkage involves. OH group at C4 in Glu



It is also hydrolyzed by emulsion an enzyme that hydrolysis β - Glycosides rather than α -Glycosides. This indicates that lactose is a β - Galactoside.

The above evidence clearly indicates the lactose has the following structure.

β – Lactose



Properties

1. It is dextro rotatory. The α and β forms have a specific rotation of $+90^\circ$ and $+35^\circ$ respectively.
2. Lactose is less soluble in water.
3. It is a reducing sugar reduces Cu^{2+} ions to Cu^+ ions & also forms Osazone & Oxime.
4. On hydrolysis it yields an equimolar mixture of Glucose & Galactose.

Uses

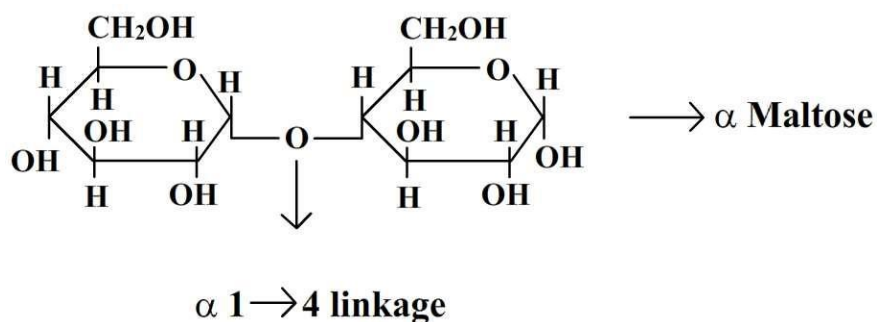
It is used in baby food and in pharmacy as a base for compressed tablets.

2.5.5: MALTOSE

Malt prepared from sprouting barley is an excellent source of Maltose.

Structure

Hydrolysis of Maltose with dilute acids yield only D – Glucose. This indicates that maltose is made up of two units. Other structural studies similar for lactose indicate that 2 Glucose units are joined by an α glycosidic linkage of C1 of one unit & C4 of other.



Properties

1. It is a reducing sugar, forms Osazone with phenyl hydrazine.
2. It is easily hydrolyzed into 2 glucose units by the enzyme Maltase found in intestine.

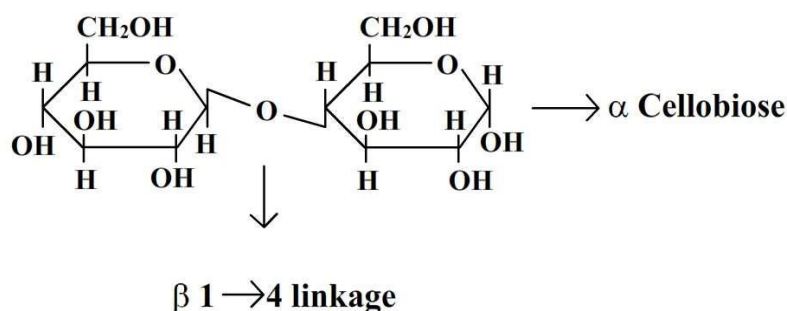
Uses

It is used in infant foods and in Malted milk.

2.5.6: CELLOBIOSE

Structure

Hydrolysis with dilute acids or by the enzyme emulsion yield only D – Glucose. This indicates that it is made up of 2 Glucose units. Further experiments show that 2.



glucose units are joined by β – Glycosidic linkage between C1 of one unit and C4 of other. It should be noted that it has the same structure as Maltose except for the type of glycosidic linkage.

Properties

1. It is a colorless crystalline solid.
2. It is soluble in H₂O
3. It is d – rotatory
4. It is also a reducing sugar.

2.6 : POLYSACCHARIDES

Polysaccharides are more complex substances. Some are polymers of single monosaccharides and are termed as homo polysaccharides. (e.g.) Starch, Cellulose, Glycogen.

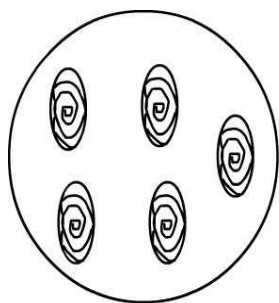
Homopolysaccharides: (Homo glycans) Starch

It is the polymer of Glucose and occurs in many plant as storage food. It may be found in the leaves and stem as well as in roots, fruits, and seeds where it is usually present in greater concentration.

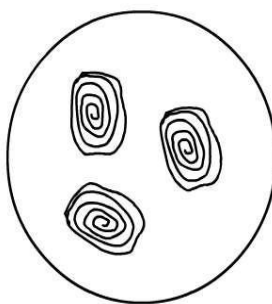
Starch granules

They appear under microscope as particles made up of concentric layers of materials.

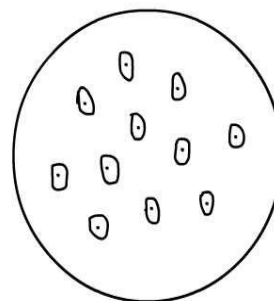
They differ in shape, size, and markings according to the source.



Potato



Wheat



Rice

A large amount of starch is present in cereals. Such as wheat, rice, corns and barley, potatoes, legumes and in nuts.

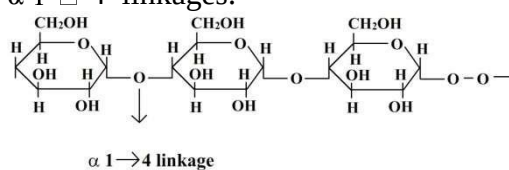
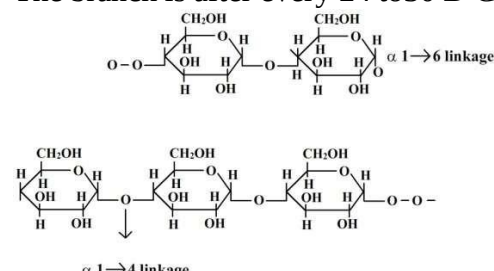
Composition

Starch granules consist of 2 polymeric units of glucose called Amylose and Amylopectin.

But they differ in molecular structure and in certain properties.

Starch granules are insoluble in cold water but when their suspension is heated, water is taken up and swelling occurs at first to a slight degree but later to several hundred times to their original volume. Viscosity increases and starch gel or paste are formed.

Difference between Amylose and Amylopectin

S. No	Amylose	Amylopectin
1	Occurs to the extent of 15 to 20%	Occurs to the extent of 80 to 85%
2	Have low molecular weight. approximately 60,000D	Have high molecular weight. approximately 5,00,000D
3	Soluble in water	Insoluble in water but can absorb water and swells up
4	Gives blue color with dilute iodine solution	Gives reddish violet color with iodine solution.
5	Structure	Structure
	Straight chain	Branched (highly)
	Unbranched	More D- Glucose units joined together by α 1 $\rightarrow$ 4 linkage and at branch point by α 1 $\rightarrow$ 6 linkages
	2500 to 3000 D Glucose units linked by α 1 $\rightarrow$ 4 linkages.	The branch is after every 24 to 30 D Glucose units.
		

Properties

1. Reaction with iodine

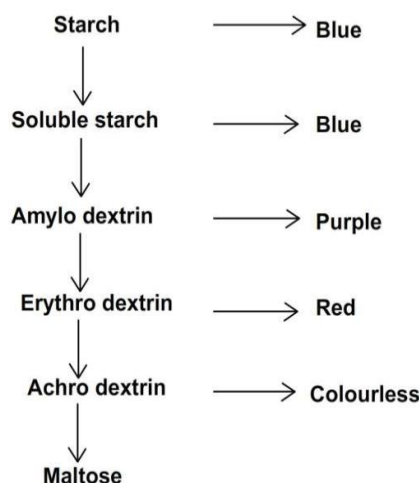
Amylose + iodine $\longrightarrow$ Blue color

Amylopectin + iodine $\longrightarrow$ Blue violet or purple colour.

2. Hydrolysis of starch

This yields succession of polysaccharides of gradually decreasing molecular size.

Course of hydrolysis $\longrightarrow$ Reaction with iodine



Enzyme hydrolysis (Amylase) ends at Maltose but traces of dextrin's also formed.

If the hydrolysis is accomplished by acids much of the starch will be converted into glucose.

Types of Amylases

1. **α – Amylase:** Present in saliva and pancreatic juice
2. **β – Amylase:** Present in sprouted grains & Malts

Both of them hydrolyze only α - Glycosidic linkage. α –Amylases produces a random cleavage of glycosidic bonds well inside the starch molecule yielding a mixture of Maltose & Dextrins. β – Amylase splits off maltose moieties liberating maltose units commencing at non – reducing end of the starch molecule & ends in limit dextrins.

Enzyme hydrolysis



Acid hydrolysis

Acid

Starch $\xrightarrow{\quad}$ Glucose

2. Glycogen: (Animal starch)

It is also called Animal starch. It is stored in the liver and muscles are readily available as an immediate source of energy.

The formation of glycogen from glucose is called Glycogenesis and breakdown of glycogen to form glucose is called as Glycogenolysis. The molecular weight of glycogen varies from 10 lakhs to 40 lakhs.

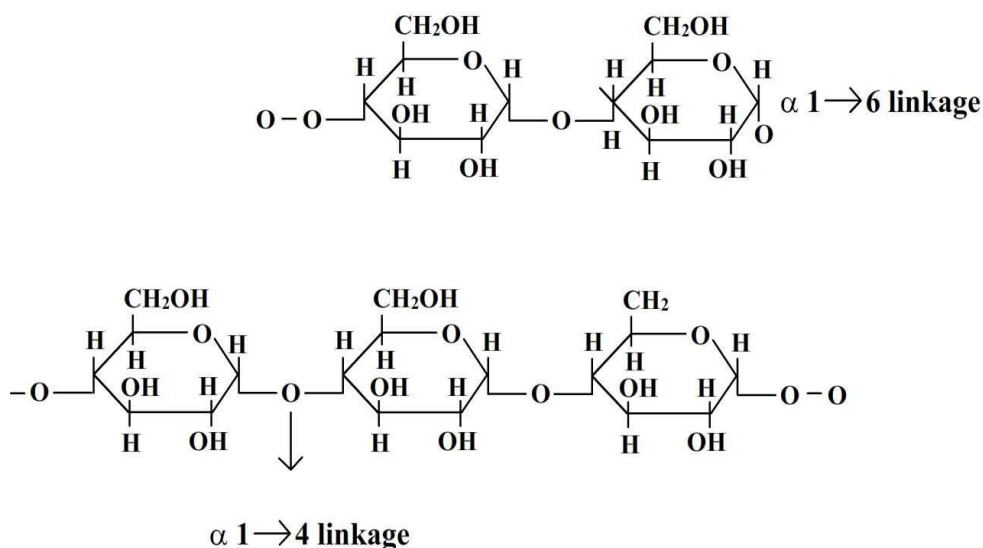
Properties

1. It is a dextro rotatory
2. Glycogen is not readily soluble in water.
3. Reaction with iodine gives red color (a deep)

Structure

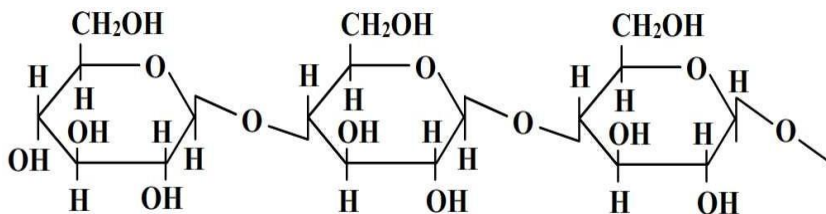
Glycogens have the complex structure of highly branched chains. It is the polymers of

D – Glucose and resembles Amylopectin, but the difference is branching point occurs for every 12 to 18 glucose units in glycogen.



3. Cellulose

It is a polymer of glucose. It is hydrolysed to cellobiose and D – Glucose when it is heated with conc. acid. Here the glucose molecules are joined together by $\beta 1 \rightarrow 4$ linkages.



Importance

It is a fibrous tough white solid insoluble in water. Because of the lack of chemical reactivity it is of no nutritive value. But these characteristics have made it so useful as fibres for the manufacture of paper and cloth. In man cellulose is not digested since it does not possess an enzyme cellulase which is capable of catalyzing the cleavage of β – Glucoside bonds. Hence it serves as an important source of bulk in the diet and it is essential for the treatment of constipation. However ruminants and certain wood-eating insects are able to digest it because the microorganisms present in their digestive tract present digestive enzymes like cellulase.

2.7 : HETERO POLYSACCHARIDES

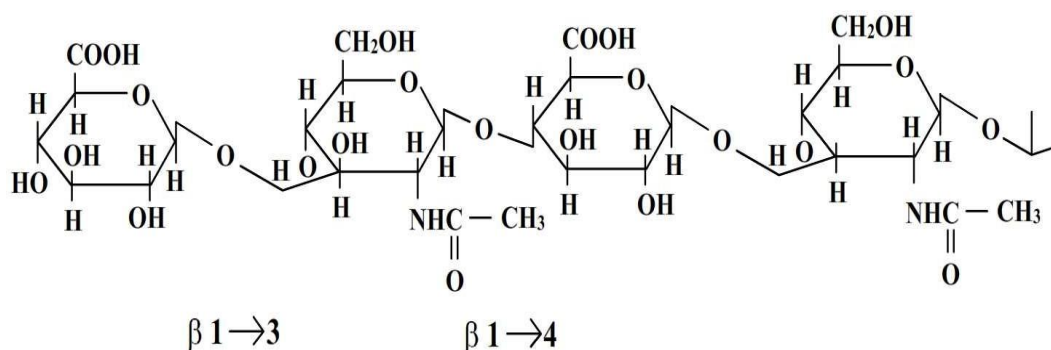
(Muco polysaccharides or Glycosamino glycans)

They situated in the extracellular matrix is called glycosamino glycans such as Hyaluronic acid, chondroitin sulphate, Heparin, Keratin sulphate etc.

Hyaluronic acid

1. It has a molecular weight of 1 to 4 million.
2. It forms viscous solutions in water and is important in the body as lubricant.
3. It has an enormous capacity to hold water.
4. It occurs in synovial fluid, in skin, and vitreous humor of eye.
5. Its presence affects the rate of diffusion in the skin. The enzyme hyaluronidase reduces its viscosity and helps diffusion in tissue space.

Structure



It consists of alternate units of D – Glucuronic acid and N acetyl D Glucosamine links to give a thread like structure.

Functions

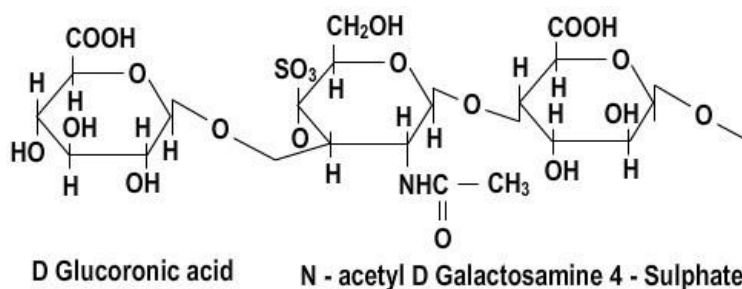
1. The presence of enzyme hyaluronidase increases the rate of diffusion of substance through tissues containing hyaluronic acid. Richest source of the enzyme in mammals is the testis and its presence in the sperm facilitates fertilization.
2. It is secreted by pathogenic substance like bacteria which can therefore invade the tissues of the host animal more easily.
3. Hyaluronic acid in tissues act as a cementing substance.

2.8 : CHONDROITIN SULPHATES:

1. Chondroitin sulphate A :(Chondroitin 4 sulphate)

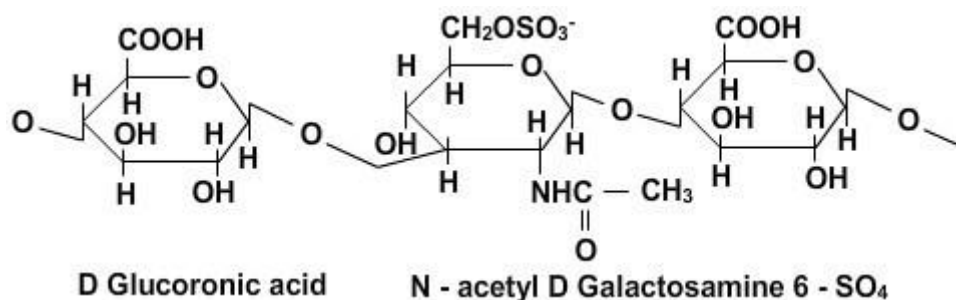
It is major component of cartilage and other connective tissue and has alternate units

of N – acetyl D Galactosamine 4 – sulphate and D Glucuronic acid.



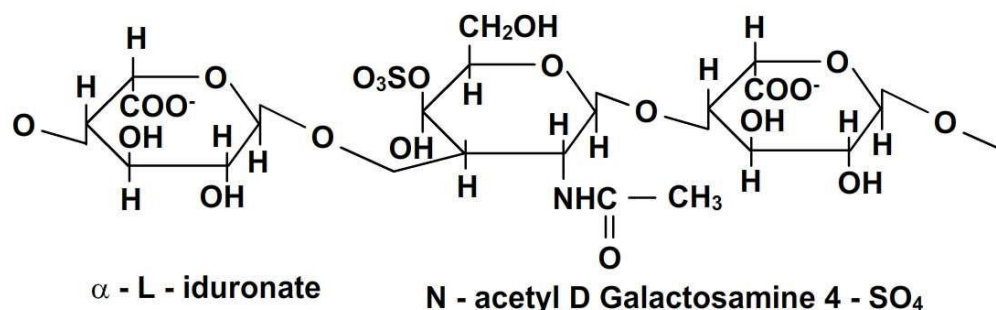
2. Chondroitin sulphate C: (Chondroitin 6 sulphate)

It is sulphated at 6th position of its N-acetyl D Galactosamine residues. The 2 chondroitinsulphate A and C occurs separately or in mixtures depending on the tissue.



3. Dermatan sulphate: (Chondroitin sulphate B)

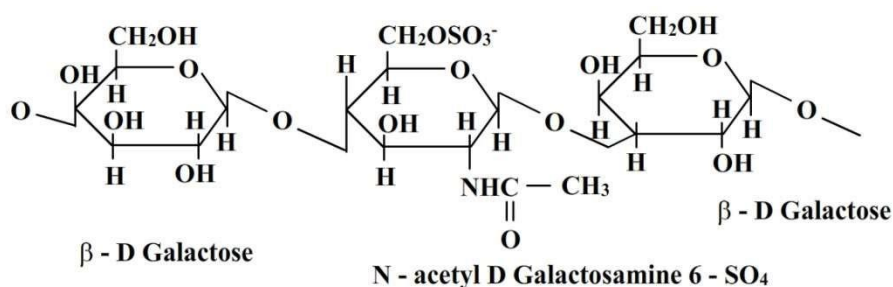
This is named as because of its prevalence in skin, differs from chondroitin 4 sulphate only by an inversion of configuration about C5 of β D – Gluconate residue to form α – L iduronic acid. This results from the enzymatic epimerization of these residues after the formation of chondroitin. The epimerization is usefully incomplete, so dermatan sulphate also contains Gluconate residues.



4. Keratan sulphate

It contains alternating β 1 → 4 linked D – Galactose and N acetyl D Glucosamine 6 sulphate residues. It is the most heterogenous of the major glycosamino glycans in that its sulphate content is variable and it contains small amount of Fructose,

Mannose, N – acetyl glucosamine and sialic acid.

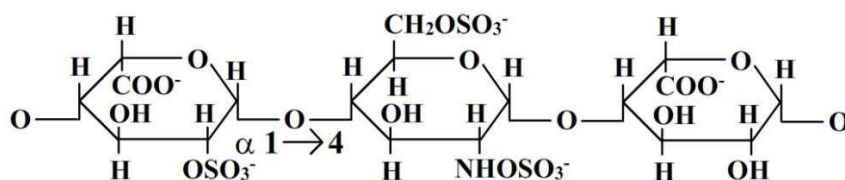


Functions

They have a marked capacity to bind water and contribute to resistance to compression of connective tissue.

They are polyvalent anions and they may also regulate the flow and concentration of cations round the cells.

5. Heparin



It is a variably sulphated glycosaminoglycon that consists of alternating α 1 $\rightarrow$ 4 linked residues of L iduronate 2 sulphate and N sulpho D Glucosamine 6 SO₄. They occur in intracellular granules of mast cells that line arterial walls, especially in liver, lungs and skin.

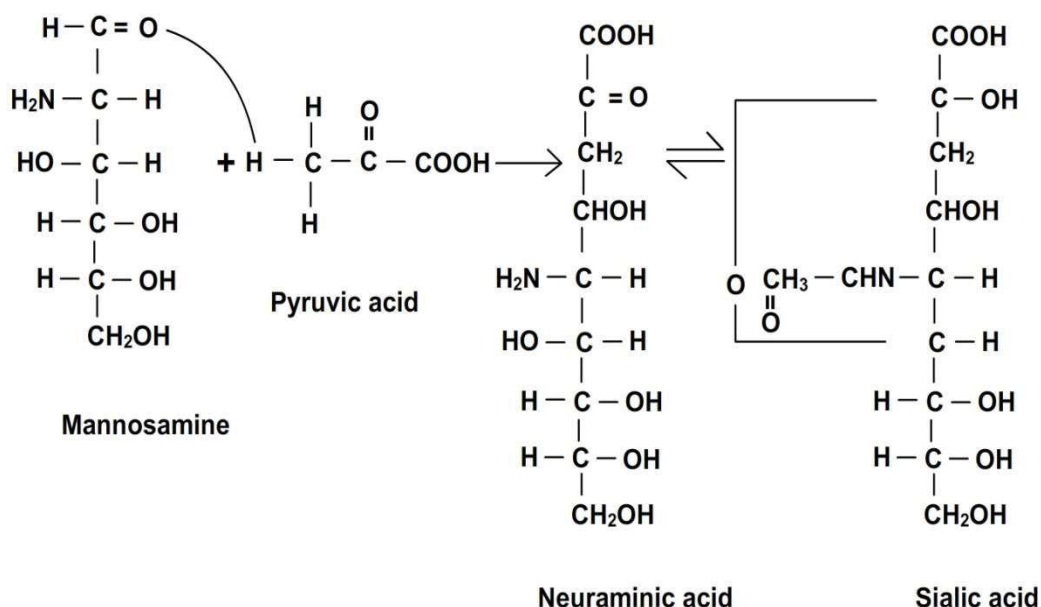
It inhibits clotting of blood and therefore has wide clinical use to inhibit blood clotting. For example, in post-surgical patients.

Heparan sulfate is a cell surface component as well as an extracellular substance in blood vessel walls and brain. Structurally it resembles heparin with fewer N and O sulphate groups and more N – acetyl groups.

Sialic acid

The sialic acids represent naturally occurring substances widely distributed in tissues, particularly in Mucins and blood group substances.

Sialic acids are acetyl derivatives of neuraminic acid. Neuraminic acid is a 9 C, 3 deoxy, 5 amino sugar acid. When the $-NH_2$ group is acetylated in neuraminic acid (i.e) is called as sialic acid. And in some cases the $-OH$ group is also acetylated. Neuraminic acids may be considered to be derived from an amino sugar (D – Mannose amine) and pyruvic acid by an aldol condensation. Certain bacterial enzymes hydrolyze neuraminic acid to an amino sugar and pyruvic acid.



The sialic acids present in Mucopolysaccharides linked with sugar by a glycosidic bond at the sialic acid. They contain glycolyl (CH_2OH) substituted in the place of the acetyl group in neuraminic acid has been found in salivary mucin and Erythrocytes.

2.9 : SUMMARY

Carbohydrates include a large group of compounds commonly known as starches or sugars, widely distributed in plants and animals.

It may be defined as polyhydroxy alcohols with aldehydes or ketones and their derivatives. In general, carbohydrates are white solids, freely soluble in water. They are widely distributed in nature in plants and animals. The most important of them in plants is starch. They occur abundantly in leaves, vegetables, tubers, roots & grains. They present in animals is Glycogen. It forms a storage form of carbohydrates in animals and it is found largely in the liver and muscles.

Carbohydrates of lower molecular weight compounds of sweet taste. The chief function of this in body is that when they are oxidized in the body, they liberate CO_2 , H_2O , and energy. They supply the major portion of the energy required by living cells. Certain products of carbohydrate metabolism act as catalysts to promote the oxidation of foodstuffs. Certain of this is used as starting material for the biological synthesis of compounds such as fatty acids and amino acids.

2.10 : KEYWORDS

Carbohydrates

It may be defined as polyhydroxy alcohols with aldehydes or ketones and their derivatives.

Monosaccharides

They contain only one molecule of sugar and they cannot be broken into simpler substances by hydrolysis.

Disaccharides

The molecular formula for the above is $\text{C}_{12}\text{H}_{22}\text{O}_{11}$. They yield two molecules of monosaccharides on hydrolysis. (eg) Sucrose, Lactose, Maltose

Lactose

Elemental analysis & Molecular weight determination show that Molecular formula of Lactose is $\text{C}_{12}\text{H}_{22}\text{O}_{11}$.

Lactose reacts with $(\text{CH}_3\text{CO})_2\text{O}$ in the presence of Na acetate to form lactose octa acetate. This indicates the presence of 8 – OH groups in Lactose molecule. Since it a stable compound, 8 – OH groups must be present on separate carbon atoms.

Maltose

Malt prepared from sprouting barley is an excellent source of Maltose.

1. It is a reducing sugar, forms Osazone with phenylhydrazine.
2. It is easily hydrolyzed into two glucose units by the enzyme Maltase found in intestine.
3. It is used in infant foods and in Malted milk.

Sucrose

1. Elemental analysis & Molecular weight determination show that molecular formula of sucrose is $C_{12}H_{22}O_{11}$.
2. It reacts with $(CH_3CO)_2O$ in the presence of Na acetate to form sucrose octa acetate. This indicates the presence of 8 hydroxyl groups in the sucrose molecule. Since it is a stable compound, the 8 – OH group must be present on separate carbon atom.
3. Hydrolysis of sucrose with dilute acids yield an equimolecular mixture of D Glucose & D Fructose. This indicates that the sucrose molecule is made up of 1 unit of each of These monosaccharides.

Polysaccharides

Polysaccharides are more complex substances. Some are polymers of single monosaccharides and are termed as homo polysaccharides. (eg) Starch, Cellulose, Glycogen. Homopolysaccharides (Homo glycans).

Starch

It is the polymer of Glucose and occurs in many plant as storage food. It may be found in the leaves and stem as well as in roots, fruits and seeds where it is usually present in greater concentration.

Cellulose

It is a polymer of glucose. It is hydrolysed to cellobiose and D – Glucose when it is heated with conc. acid. Here the glucose molecules are joined together by β 1 $\rightarrow$ 4 linkages.

EXERCISE

1. What are monosaccharides? Explain its classification with examples?
2. What are disaccharides? Explain its classification with examples.
3. Explain the structural elucidation of lactose and maltose?
4. Give an account on the physical and chemical properties of monosaccharides and disaccharides?
5. What are polysaccharides and mention its classification with examples?
6. Explain the structural elucidation of starch in detail?

7. Write a note on the structural and characteristic features of cellulose?
8. Write a note on sucrose in detail?

II Multiple choice question

1. Starch is an example of ()
a) Monosaccharide b) oligosaccharide.
c) Polysaccharide d) trisaccharide.
2. Which of the following compounds reduces tollens reagent. ()
a) Glucose b) sucrose c) Methanol d) Acetic acid
3. The reagent that can be used to differentiate an aldose and a ketose is ()
a) Bromine water b) Fehling's solution c) Tollen's reagent d) None
4. Which of the following is a non-reducing Sugar ()
a) Glucose b) fructose c) mannose d) Sucrose
5. α -D Glucose and β -D Glucose are ()
a) Anomers b) epimers c) both a & b d) None.
6. Glucose and mannose are. ()
a) Anomers b) epimers c) both a & b d) None.
7. Ktilliani-fischer Synthesis is used to convert ()
a) Aldo pentose to aldohexose b) Aldo hexose to Aldo pentose
c) Glucose to Mannose d) Glucose to fructose
8. D-Glucose is converted to D-Arabinose by ()
a) tilliani-fischer Synthesis, b) Hoffmann method.
c) Labry de bruyn gan ekenstein rearrangment d) Ruffs degradation
9. Fructose on reduction with NaBH_4 forms. ()
a) Sorbital b) n-hexane c) mixture of sorbital and mannitol
d) mannitol.
- 10) According to Haworth representation, the ring size of glucose is. ()
a) five-membered b) Six membered. c) Four-membered. d) none.

III Fill in the Blanks

1. Milk sugar is _____
2. The compounds which on acidic hydrolysis give polyhydroxy aldehydes and ketones are _____
3. Carbohydrates which give +ve Tollens and Fehling tests are called _____
4. Glucose is having asymmetric carbon atoms.

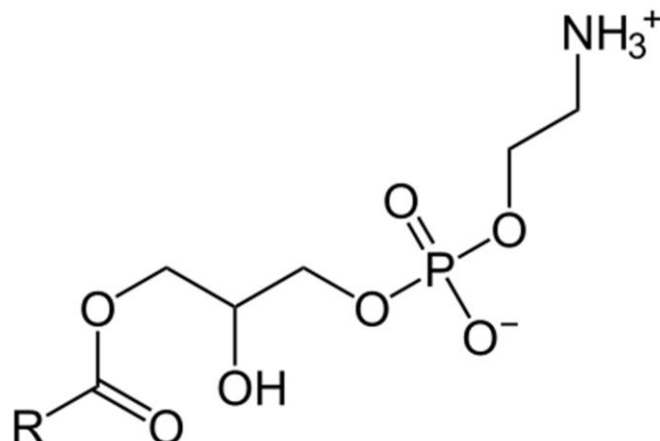
5. An aldohexose is having optically active isomers.
6. The change in optical rotation of a solution of either form of glucose until constant values are obtained is called _____
7. The pair of diastereomers which differ in Configuration of C1, only are called _____
8. In an aqueous solution α -D (+) Glucose exists in _____ % and β -D (+) exists _____ % in an equilibrium mixture
9. Glucose on reaction with phenylhydrazine and forms _____
10. The process of Conversion of glucose to Mannose is called _____

III Answers: 1) c 2) a 3) a 4) d 5) a 6) b 7) a 8) d 9) c 10) b

III Answers: 1, Lactose 2) Carbohydrate 3) Reducing sugars 4) Four 5) Sixteen 6) Mutarotation 7) Anomers 8) 37% & 63% 9) Glucosazone 10) Epimerisation.

CHAPTER-III

Lipids



3.0 INTRODUCTION

“Lipids are organic compounds that contain hydrogen, carbon, and oxygen atoms, which form the framework for the structure and function of living cells.”

These organic compounds are nonpolar molecules, which are soluble only in nonpolar solvents and insoluble in water because water is a polar molecule. In the human body, these molecules can be synthesized in the liver and are found in oil, butter, whole milk, cheese, fried foods, and in some red meats.

3.1 OCCURRENCE

Utilizing a fat solvent, it is possible to extract fats, which are also known as lipids, from a wide variety of plant and animal tissues. Typically, they may be found in plant components such as nuts, seeds, fruits, and others. The principal storage sites in animals are in the adipose tissue, the nerve tissue, and the bone marrow. Cholesterol, phospholipids, and glycolipids are only a few examples of the many different types of lipids that are present in high quantities in the brain and spinal cord. There is evidence that the blood contains lipoproteins. There are lipids present in a cell's cytoplasm as well as in its plasma membrane.

Food sources: Rich in fats are. **Animal source:** Milk, Meat, Liver etc. **Plant source:** Seed, nut, oil.

3.2 BIOLOGICAL IMPORTANCE OF LIPIDS:

1. They are essential components of a healthy diet and serve as a source of fuel for the body. In terms of heat and energy production, they are superior to carbs. (e.g.)

When 1 gramme of fat is oxidized, 9.5 kilocalories of heat are produced, yet there is no change in the amount of Carbohydrates only provide 4.5 kilocalories per gramme when burned.

2. Water does not dissolve them at all. Because of this, the body can conveniently store them as a food reserve (for example, triglycerides).
3. They are an essential component of the cell membrane and play a role in the transport of cations across the membrane. For example, lipoproteins, glycolipids, and phospholipids are all found in the membrane of a cell, and each of these types of lipids plays a specific role.
4. They provide the function of an insulator and shield the body from the adverse effects of extreme heat or cold.
5. They perform the function of an electric insulating substance within the Myelin sheath or within the neuron.
6. Certain molecules that are produced from lipids are essential building blocks for many biologically active components. For example: the manufacture of cholesterol, steroid hormones, and other related compounds requires acetyl CoA, which is produced when fatty acids are oxidized.
7. Their presence near key organs like the kidney helps to protect those organs from being injured by movement.
8. They serve as vehicles for the transport of fat-soluble vitamins, including A, D, E, and K.
9. The provision of the necessary fatty acids is the function of the lipids in the diet. They could be needed at relatively modest levels for the group to function at its best. Deficiency symptoms can appear when these are lacking in the body.

Examples of Essential fatty acids are linoleic acid, linolenic acid, and Arachidonic acid.

By lowering surface tensions, some lipids can behave like surfactants.

3.3 CLASSIFICATION OF LIPIDS

The lipids are classified into four main types.

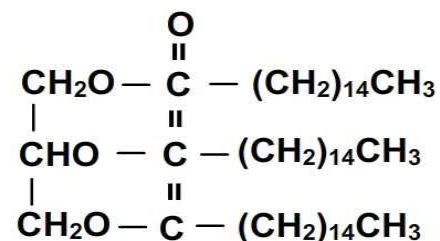
1. Simple lipids
2. Compound lipids
3. Derived lipids and
4. Miscellaneous lipids.

I. Simple lipids

These are esters of fatty acids with alcohol.

1. Neutral fat (or) Triglycerides

(e.g.) Tripalmitin, Oledistearin



Tripalmitin

2. Waxes

These are ester of fatty acids with monohydroxy aliphatic alcohols other than glycerol.

a. True waxes

These are esters of Palmitic, steric, Oleic, or other fatty acids with ethyl alcohol [$\text{CH}_3(\text{CH}_2)_{14}\text{CH}_2\text{OH}$] or other straight chain alcohols.

b. Cholesterol esters

These are esters of fatty acids with cholesterol.

c. Vitamin A esters

d. Vitamin B esters

II. Compound lipids

These are esters of fatty acids with alcohol and containing other groups in alcohols and acids.

1. Phospho lipids

These are esters containing H_3PO_4 and nitrogenous bases or amino acid or some other moiety like inositol. Phospholipids are compound lipids, consisting of phosphoric acids, nitrogenbases, alcohol, and fatty acids. These compound lipids are major components of the cell membrane and provide a fluid character to the membranes. In cell membranes, these phospholipids have a hydrophilic head and a hydrophobic tail, which forms the inside of the bilayer.

Properties Of Phospholipids

1. They are signal mediators.
2. They are amphipathic molecules.
3. They anchor proteins within the cell membranes.
4. They are the major constituents of cell membranes.
5. They are the components of bile and lipoproteins.

Functions Of Phospholipids

1. It regulates the permeability of the membrane.
2. It is also involved in the absorption of fat from the intestine.
3. It helps in ETC- Electron Transport Chain in the mitochondria.
4. Phospholipids help by preventing the accumulation of fats in the liver.
5. It plays a major role in the transportation and removal of cholesterol from the cells.
6. It forms the structural components of the cell membrane with the association of proteins.
7. They act as surfactants in the respiratory system and are also involved in the coagulation of blood cells.
8. It helps in the synthesis of different lipoproteins, prostacyclins, prostaglandins and thromboxanes.

(e.g.) (i) Lecithin (Phosphatidyl choline)

(ii) Cephalin [Phosphatidyl ethanol amine]

(iii) Phosphatidyl serine

(iv) Plasmalogen

(v) Phosphatidyl Inositol

(vi) Sphingo myelin

2. Phosphatidic acid

These are phospho lipids without Organic base. (eg) cardiolipin.

3. Glycolipids

These are esters containing a carbohydrate and a nitrogenous base but no H_3PO_4 and glycerol. (eg) Kerotroside, cerebroside, ganglia side, sulphatides.

4. Sulpho lipids

These are esters containing H_2SO_4 .

5. Lipo proteins

These are lipids attached to proteins. (eg) HDL (High Density Lipoproteins), LDL (Low Density Lipoproteins), VLDL (Very Low-Density Lipoproteins) and Chylomicrons.

III. Derived lipids

These are derivatives obtained by the hydrolysis of simple and compound lipids and still possess the physical characteristic of lipids.

1. Saturated and unsaturated fatty acids
2. Monoglyceride and Diglycerides
3. Alcohols including sterol

IV. Miscellaneous lipids

These includes.

1. Carotenoids
2. Vitamin D, E, and K
3. Steroid hormones
4. Bile acids

Compound lipids

These are the lipids containing some chemical groups in addition to fatty acids.

(FA) and alcohols. Based on the nature of additional chemical groups, compound lipids are subdivided into the following.

Phospho lipids

They are also known as phosphatides or derivatives of phosphatidic acid. Phospho lipids are abundantly present in brain, heart, kidney, egg yolk, etc.

Based on the nature of nitrogenous bases – they are again classified into Lecithin, Cephalin, Phosphatidyl serine, phosphatidyl inositol, plasmalogens, sphingomyelin.

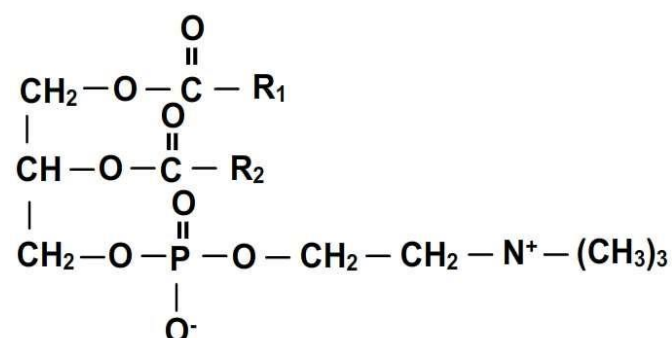
3.3.1 Phosphotidyl choline (or) Lecithin

1. Lecithin

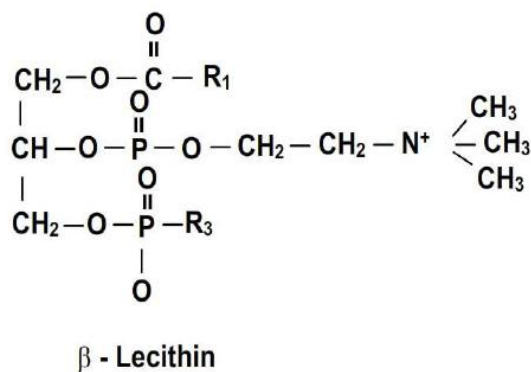
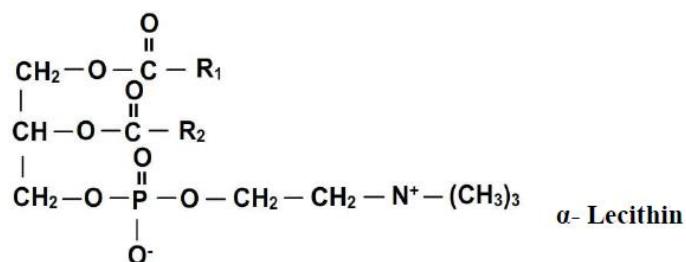
It occurs in all plants and animals. They are mainly present in brain, egg yolk etc. While in plants they are particularly abundant in seeds.

Structure

They consist of 1 glycerol molecule esterified to 2 molecules of FA. One of this FA may be saturated and other unsaturated. They also contain H₃PO₄ and a nitrogenous base called choline.



Depending on the position of H₃PO₄ and choline complex there are 2 types of Lecithin, α and β Lecithin.



Physical properties

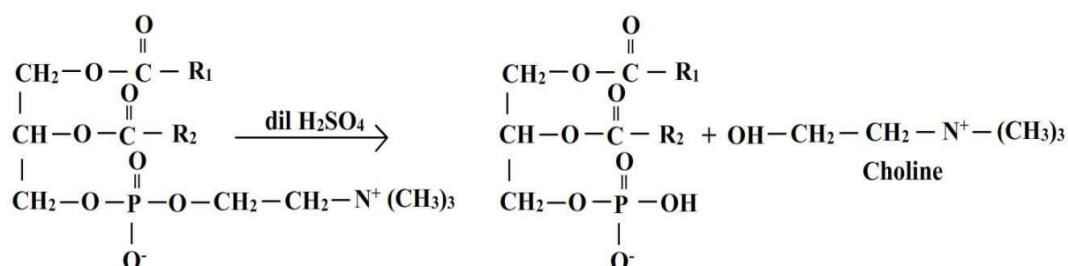
1. They are white waxy substances that become brown when exposed to air and light

due to auto oxidation and decomposition. This is due to presence of unsaturated fatty acids (UFA) in Lecithin.

2. They are soluble in all fat solvents except acetone
3. It is hygroscopic nature. They can mix well with H₂O and form colloidal solution.
4. Pure lecithin is mild emulsifying agent and when combined with carbohydrates and protein, they become more effective emulsifying agent.

Chemical properties

1. Acid hydrolysis



When lecithin is treated with dilute H₂SO₄ it is hydrolysed to choline and phosphatidic acid.

2. Alkali hydrolysis

When it is boiled with alkalies it 1st gives choline and phosphatidic acid. Phosphatidic acid is again split to give 2 molecules of FA and glycerol phosphatidic acid.

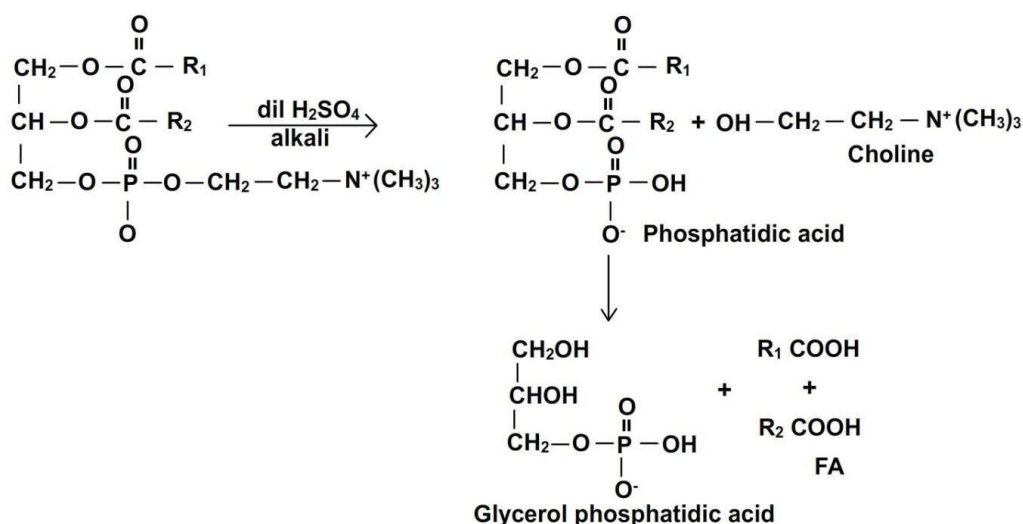
Enzymatic hydrolysis

They are hydrolyzed by enzymes called phospholipases or lecithinases to various components. There are 4 types of lecithinase occurring in nature. They are lecithinase A, B, C & D.

Lecithinase A

This is again classified into 2 types, Lecithinase A₁ & A₂. Lecithinase A₁ occurs in human mammalian tissue. It is also isolated from cobra venom and spider venom.

Lecithinase A₁ attacks ester bond in position 1 of phospholipids and forms lysolecithin.



3. Enzymatic hydrolysis

They are hydrolysed by enzymes called phospho lipases or lecithinases to various components. There are 4 types of lecithinases occurring in nature. They are lecithinase A,B,C & D.

Lecithinase A.

This is again classified into 2 types, Lecithinase A1 & A2.

Lecithinase A1 occurs in human mammalian tissue. It is also isolated from cobra venom and spider venom.

Lecithinase A1 attacks ester bond in position 1 of phospho lipids and form lysolecithin and 1 molecule of saturated fatty acids (SFA). Lysolecithin is a powerful hemolytic poison which cause hemolysis of Erythrocyte.

Lecithinase A2 attacks ester bond in β position or 2nd position and form lyso lecithin and 1 molecule of unsaturated fatty acids (UFA).

Lecithinase B

This is found in pancreas and also in certain fungi like *Penicillium notatum*. It acts on lysolecithin and forms 1 molecule of glycerol phosphonyl choline and 1 molecule of SFA or UFA also known as lysolecithinase.

Lecithinase C

This is found in plant kingdom and it is recently isolated from mammalian brain.

Lecithinase C hydrolyses lecithin at the position between glycerol and H₃PO₄. With

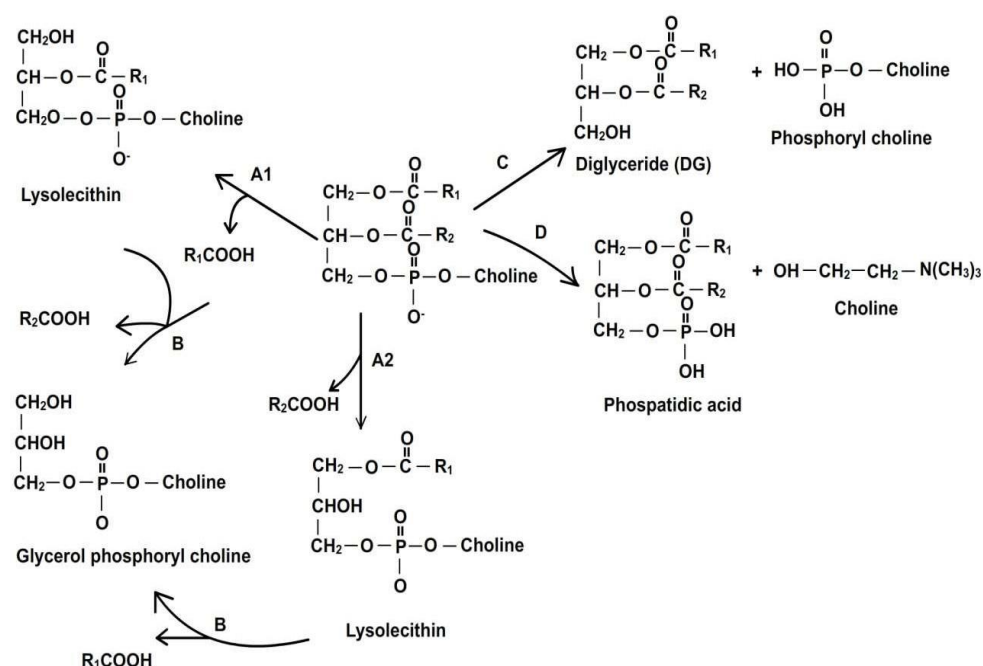
liberation of diacyl glycerol and phosphoryl choline.

Lecithinase D

This is found in plants like cabbage, carrot etc. This hydrolysis lecithin by splitting only choline and leave behind phosphatidic acid.

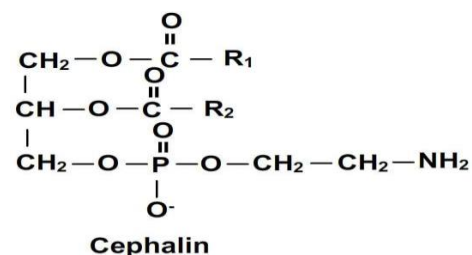
Biological importance of Lecithin

1. Lecithins are needed for transport and utilization of other lipids especially in the liver. This lecithins are important in the prevention of fatty liver.
2. They are important constituents of cell membrane.



2. Cephalin: (Phosphatidyl ethanolamine)

They occur along with lecithin in all animal and plant cells. They contain ethanol amine instead of choline. It is also called as phosphatidyl ethanol amine.



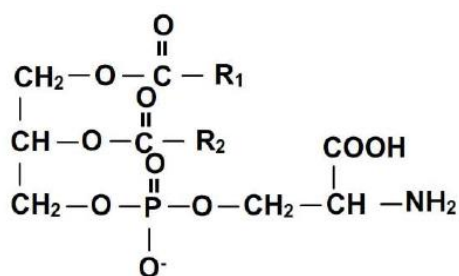
Properties

1. They behave like lecithin. They are soluble in most of fat solvents except acetone, Ethyl, and methyl alcohols.

2. They are unstable in air and turn dark when oxidized.
3. They are hydrolyzed in the same manner as Lecithin
4. They play an important role in the mechanism of coagulation of blood.
Thromboplastin which is an important factor in the coagulation of blood is a phospho lipid.

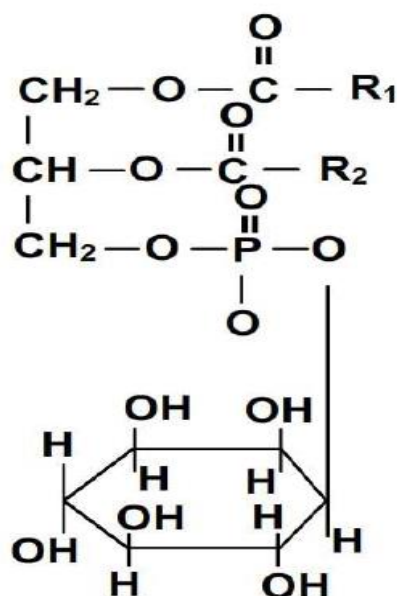
Phosphatidyl serine

It is found in brain tissues. Traces are also detected in blood.



Phosphatidyl Inositol

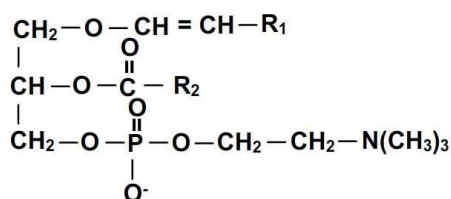
This is present in plasma membrane. Their derivative such as inositol tri phosphate and diglyceride (DG) have a role as a 2nd messenger in regulating Ca²⁺ fluxes. That will be mediating some cellular response.



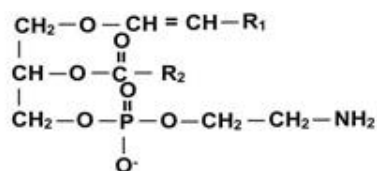
3.4.2: Plasmalogens

This constitute about 10% of phospho lipids of brain and muscle. It is also found in seeds of higher plants. Structurally these resemble lecithin and cephalin but have

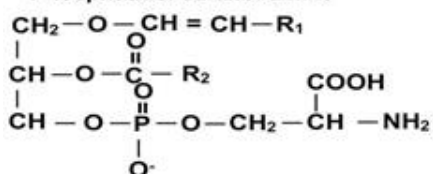
one of the FA replaced by an unsaturated alcohol and it is linked by ether linkage with – OH group of glycerol. It is soluble in all fat solvent. There are 3 types of plasmalogens depending upon base present.



Phosphatidol choline



Phosphatidol ethanol amine

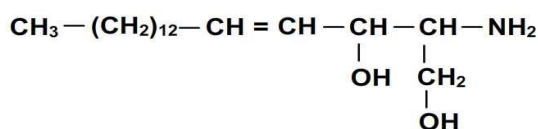


Phosphatidol serine

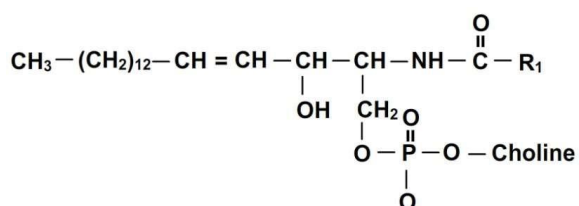
On hydrolysis they yield 1 molecule of long chain aliphatic aldehyde, a FA, glycerol phosphate and Nitrogenous base.

3.4.3 Sphingomyelin

It is found in large quantities in brain and nervous tissue and very small amount in other tissues. In place of glycerol, it contains an 18 carbon unsaturated amino alcohol called sphingosine or sphingol



Sphingosine



Sphingomyelin

Sphingomyelin is composed of sphingosine with a FA in amide linkage on the – NH₂ group and a phosphoryl choline group attached to terminal - OH group.

Combination of sphingosine with FA without H₃PO₄ and choline is called ceramide or acyl sphingosine. FA present in sphingomyelin may be stearic, lignoceric or nervonic.

Sphingomyelinase is an enzyme which hydrolyses sphingomyelin to ceramide and phosphoryl choline. Sphingomyelins are white crystalline substances and insoluble in fats solvent like ether and acetone.

Glyco lipids

They are compound lipids containing lipids and carbohydrates. They are classified into 2 types.

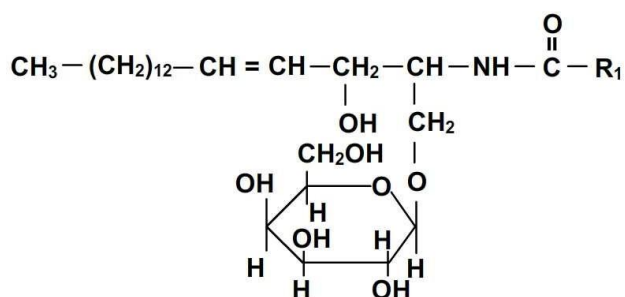
1. Cerebrosides
2. Gangliosides

3.4.5 : Cerebrosides

These occur in large amount in white matter of brain and myelin sheath of nerve fibre.

Structure

It contains high molecular weight FA sphingosine and either galactose or Glucose. There is no choline and H₃PO₄.



Galacto Cerebroside

Different types of cerebroside

There are different types of cerebroside according to the type of FA. They are

1. **Keresin:** It contains FA lignoceric acid.
2. **Cerebron:** It contains FA cerebronic acid
3. **Nervon:** It contains UFA nervonic acid

4. Oxynerve: It contains FA – OH nervonic acid

By prolong hydrolysis of any cerebroside with Ba(OH)₂, FA is removed and psychosine is formed. Psychosine is further hydrolysed to yield sphingosine and galactose or Glucose.

3.4.6 : Gangliosides

These are large complex lipids and then molecular weight varies from 130,000 to 250,000.

Structure

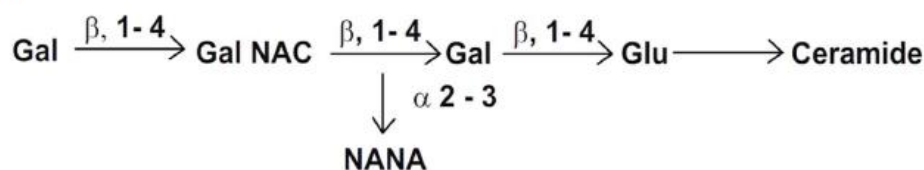
On hydrolysis they yield the following

1. Long chain FA
2. Sphingosine
3. A carbohydrate moiety which usually contains Glucose or galactose, 1 molecule of N –actyl galactosamine and atleast 1- 5 molecule of N- acetyl neuraminic acid (NANA)

Types of Ganglioside

Over 3 types of gangliosides have been isolated from brain. 3 important types are GM₁, GM₂, GM₃.

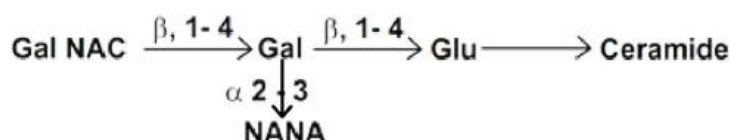
GM₁



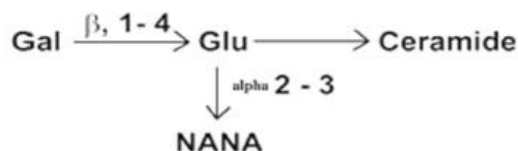
Gal NAC - N acetyl Galactosamine

NANA - N acetyl neuraminic acid

GM₂



GM₃



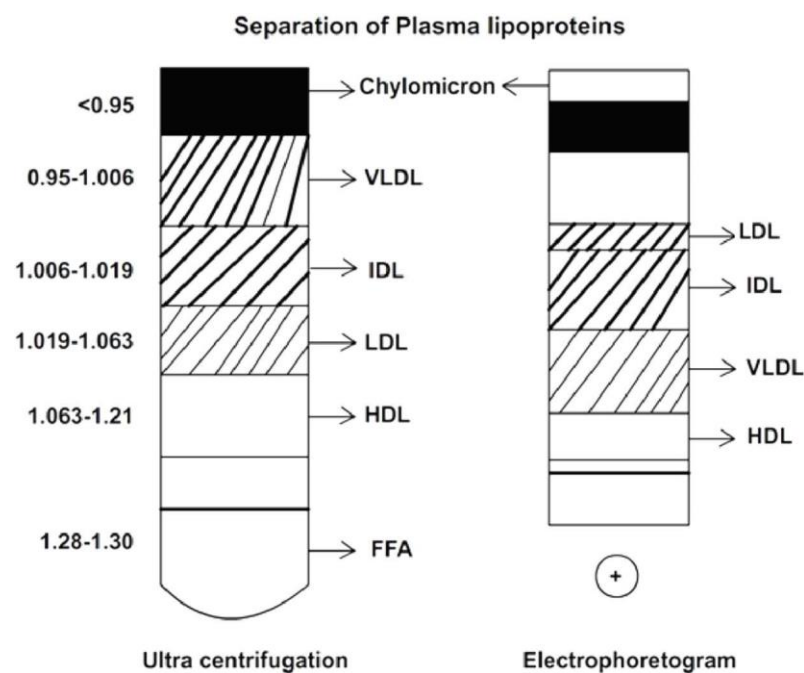
Sulpho lipids

Glyco lipids containing – SO₄ group is known as sulpho lipids. They are present in liver, kidney, brain, salivary gland and certain tumor cells.

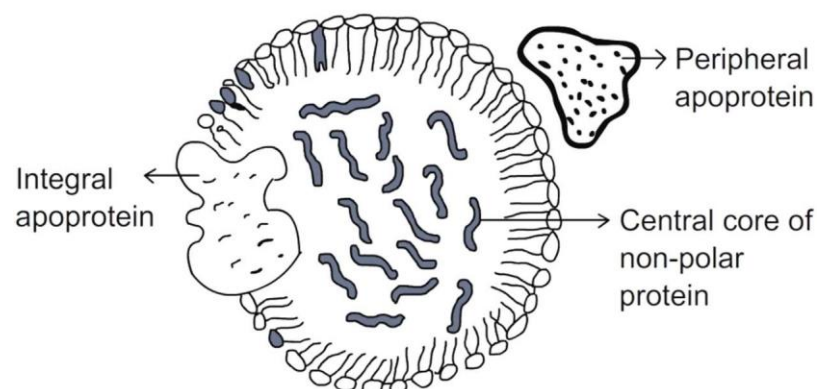
They are abundant in white matter of brain. When their SO₄ group is esterified with –OH group of hexose moiety of molecule.

LIPOPROTEINS

Since lipids are insoluble in H₂O they cannot be transported as such in plasma. Therefore they are complexed with proteins to form lipo proteins. The major lipids in plasma are organized into different types of lipo proteins by complexing with called apo lipo protein.



Structure of lipo protein



Depending on the density or on the electrophoretic mobility the lipo proteins in plasma are classified into 5 major types.

1. Chylomicrons.
2. VLDL (β lipo proteins)
3. IDL (Broad β lipo proteins)
4. LDL (β – lipo proteins)
5. HDL (α – lipo proteins)

The protein components of plasma lipo proteins are called apo proteins and classified into 3 major groups A,B and C. The A group are mainly present in HDL, the B are present in LDL mainly and also in VLDL and chylomicrons. Apo protein C are small polypeptides found in HDL, VLDL and chylomicrons. Apo lipo protein or apo proteins are usually abbreviated as apo A, apo 'D' and 'E', apo B and apo C.

Apo proteins other than A, B and C are also present in small quantities. Apo D is present in HDL, Apo E is origin rich and is present in chylo microns, VLDL and HDL.

Chylomicrons

These are mainly of intestinal origin and their quantity increases in plasma following a fat meal. The plasma becomes opalescent. The protein component has mainly Apo B. They are rapidly taken up from blood by adipose tissue, heart and skeletal muscles lipoprotein lipases or clearing factor hydrolyses lipids of chylomicrons (and VLDL) and help the passage into tissue cells. The enzyme is loosely attached to capillary endothelium.

Lipolysis occurs after the chylomicrons or VLDL combine with enzyme capillary endothelium. The lipid liberated from chylomicron and VLDL are used for energy production.

After removal of triglycerides (TG) from chylomicrons and VLDL, the molecular size and TG content of remnants decreases. These remnants are called IDL. They are finally converted to LDL in the liver. The chylomicrons still contain cholesterol and cholesterol esters and are now taken up by the liver.

VLDL

The bulk of VLDL is secreted by the liver in a manner similar to chylomicrons by the intestines. The protein component in them is also mostly apo B. The clearing factor acts on VLDL in a manner similar to its action on chylomicrons and helps in removing FA from them.

LDL

These seem to be a product of degradation of VLDL and chylomicrons. These fraction is rich in cholesterol and is taken up by all cells including liver cells and metabolized.

HDL

These are synthesized and secreted by both liver and intestinal Mucosa. The HDL secreted by intestines contain only apo A whereas those from liver contain also apo C. The lipids are mainly cholesterol and phospho lipid. The enzyme LCAT (Lecithin Cholesterol acetyl transferases) transfers FA from lecithin to cholesterol and form cholesterol ester and lyso lecithin. The cholesterol then get transferred from HDL to VLDL and LDL.

This transfer is facilitated by a protein called cholesterol ester transfer protein.

Composition of plasma lipo protein.

Name of fraction	Diameternm	Mg/100ml plasma	Protein	PL	% Composition cholesterol free	Ester	TG	FA
Chylomicrons	300 – 5000	100 – 250	2	7	2	6	83	-
VLDL	300 – 750	130 – 200	9	18	7	15	50	1
LDL	200 – 250	210 – 400	21	22	8	38	10	1
HDL	100 – 150	50 – 130	33	29	7	23	8	-

3.5 : DERIVED LIPIDS**STEROL**

Sterols are the steroids containing - OH groups. These are crystalline compounds containing 2 alcoholic group and they differ from common alcohol being solid and due to this reason they are called as sterols.

They include cholesterol, 7 dehydro cholesterol, Ergosterol, Cytosterol and Calciferol.

CHOLESTEROL

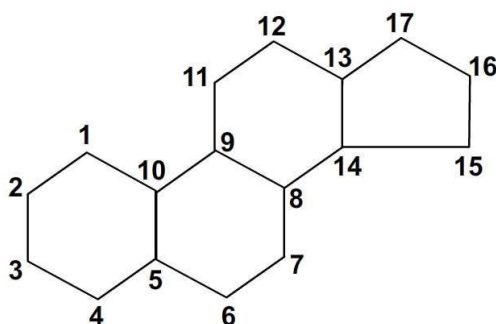
Cholesterol are the solid bile alcohol. It derives its name from Greek word “Cholesterine” which means bile solid. (Chole = bile and Sterine = Solid) As it was 1st

isolated from gall stone.

Cholesterol is widely distributed in all cells of body. Its concentration in human plasma is 150 -250 mg/100 ml of blood.

Structure

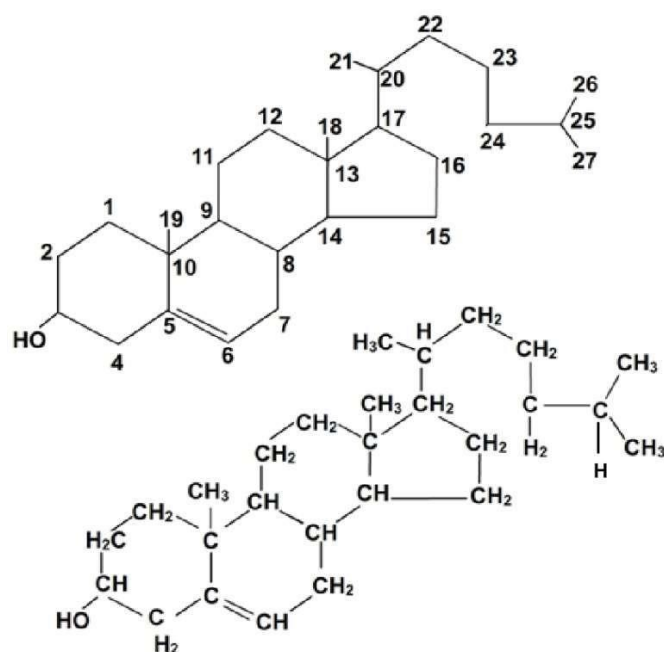
It is composed of a characteristic ring called cyclopentanoperhydrophenanthrene (Steroid nucleus)



This ring system has a total of 17 C atoms. In addition to the ring system, it has a long aliphatic chain and an alcoholic group.

The structure of cholesterol has the following characteristic features.

1. Cyclopentanoperhydrophenanthrene ring system
2. An aliphatic side chain is attached to C¹⁷
3. A –OH group attached to C³
4. A double bond is present between C⁵ and C⁶
5. 2 – CH₃ groups are present, one attached to C¹⁰ and the other at C¹³. Cholesterol is an alcohol because of the presence of a double bond.



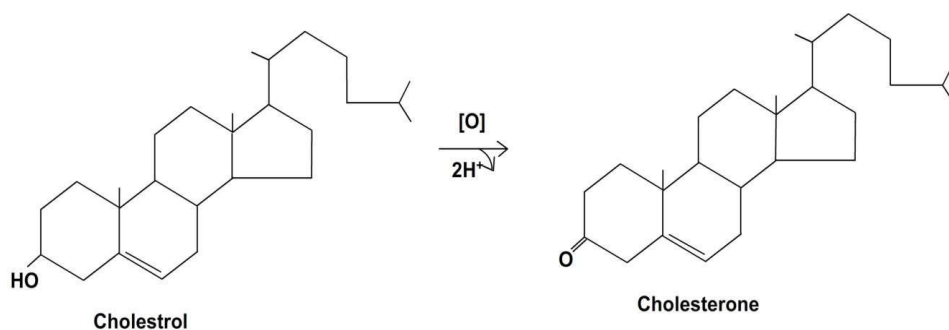
Properties

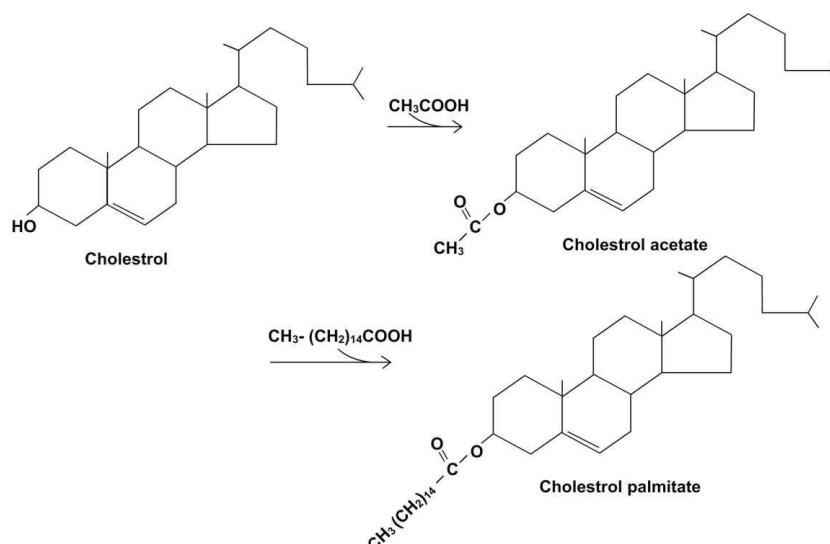
Physical properties

1. They are white waxy substance
2. They are tasteless and odorless
3. It has high melting point
4. It is insoluble in H₂O but soluble in organic solvent

Chemical Properties

1. When it is oxidized under suitable condition undergo rapid oxidation to form the corresponding ketone called cholesterol

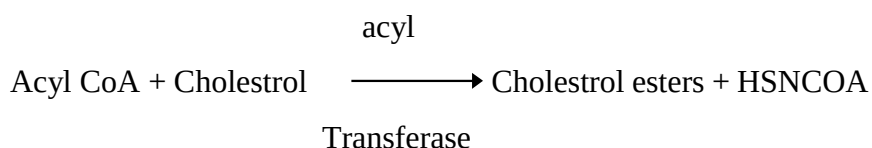




2. Cholesterol esterification

The –OH group of cholesterol readily forms esters with acids. The FA esters namely cholesterol acetate, cholesterol palmitate, etc are found in blood and tissues.

The cholesterol esters are formed in the tissues by the transfer of acetyl group from acetyl CoA to cholesterol by the enzyme acetyl transferase.

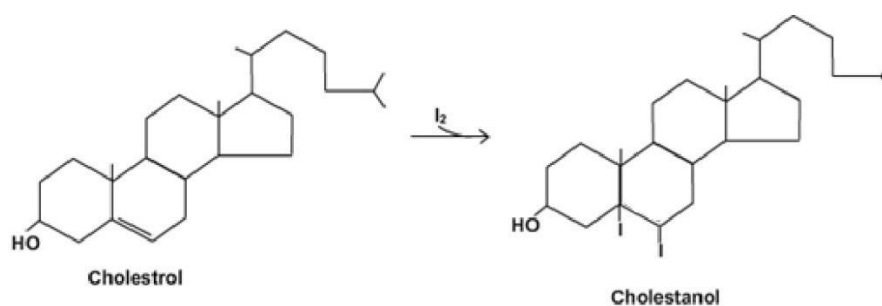


Most of plasma cholesterol esters are produced in the plasma excel by the transfer of acetyl group from β – position of lecithin to cholesterol by an enzyme LCAT

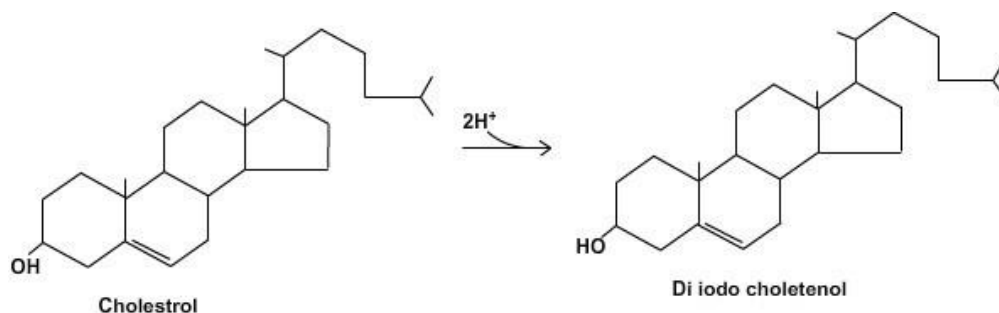


3. Because of the double bond it undergoes additional reactions including halogenation and hydrogenation. By hydrogenation, it is converted into cholesterol.

Hydrogenation



Halogenation



4. Colour reactions of cholesterol:

a. Liebermann's Burchard reaction:

A CHCl_3 solution of cholesterol when treated with $\text{CH}_3(\text{CO})_2\text{O}$ and Conc. H_2SO_4 it gives green color solution.

b. Sal Kowski reaction:

When CHCl_3 solution of sterol is treated with equal volume of Conc. H_2SO_4 develops a red to purple colour.

c. Formaldehyde – H₂SO₄ test:

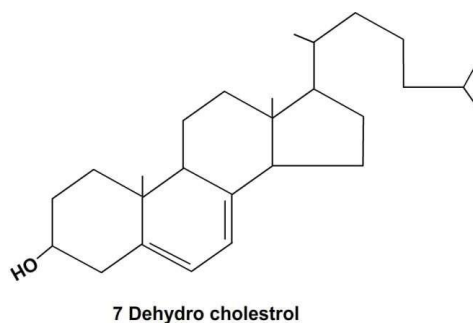
On adding a 1:50 mixture of HCHO and H₂SO₄ into a CHCl₃ solution of cholesterol the CHCl₃ layer turns cherry red. The addition of CH₃(CO)₂O to this CHCl₃ layer changes the cherry red to blue color.

d. Zak's method:

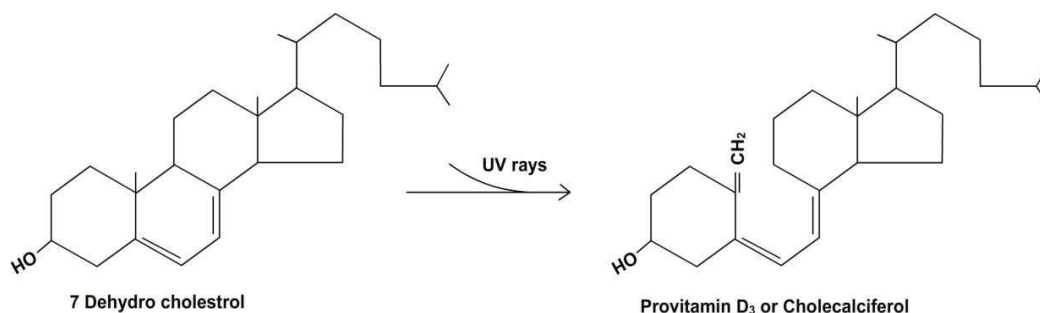
When glacial CH_3COOH aldehyde-free solution of cholesterol is treated with FeCl_3 and Concentrated H_2SO_4 produces a red colour. This reaction forms a basis or the colorimetric estimation of cholesterol.

5. 7 Dehydro cholesterol:

This is an important sterol present in the skin. It has 1 more double bond at C7 and C8.



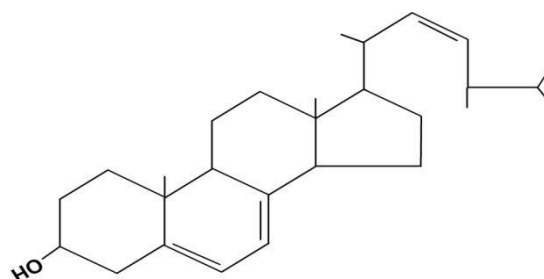
In man 7 dehydro cholesterol may be obtained from cholesterol and it occurs in skin or intestinal wall. In epidermis of skin, UV rays of sunlight converts 7 dehydro cholesterol to cholecalciferol. Hence 7 dehydro cholesterol is also called as Provitamin D₃.



Ergosterol: (provitamin D₃)

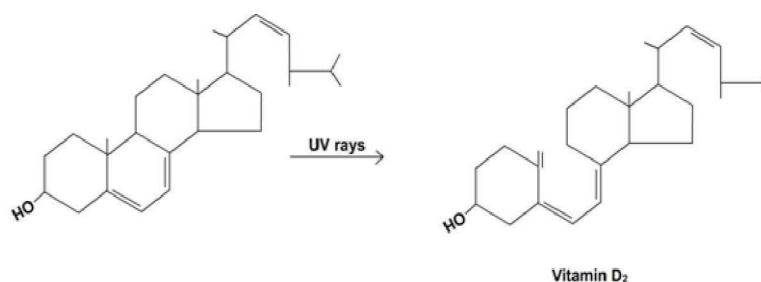
It is a plant sterol 1st isolated from Ergot a fungus of ripe plant and latter from yeast.

Structure



Structurally this sterol has the same links as in 7 dehydro cholesterol but differ slightly in its sidechain. It has a double bond at the side chain and also –CH₃ group in side chain. It has 3 double bond at C5, C7 and C22.

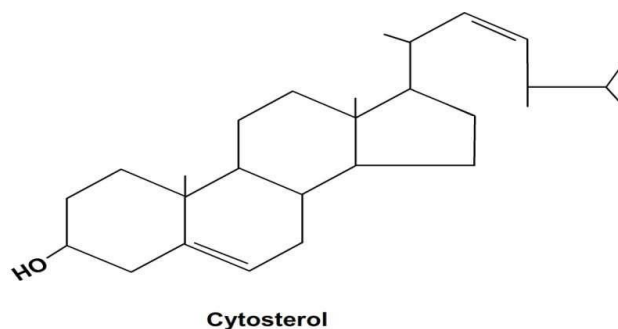
When irradiated with uv rays it is converted into vitamin D₂



Cytosterol

It is a plant and also sterol occurring in higher plants and also isolated from soya bean and wheat germ oil. It has no nutritional value for human beings. It decreases

intestinal absorption of cholesterol and thus lowers the blood cholesterol level.



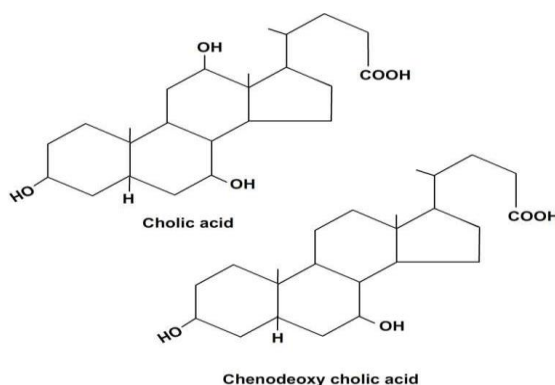
Bile acids and bile salts

Bile acids are important end products of metabolism of cholesterol in liver. Their important factors are the digestion and absorption of lipids in intestine.

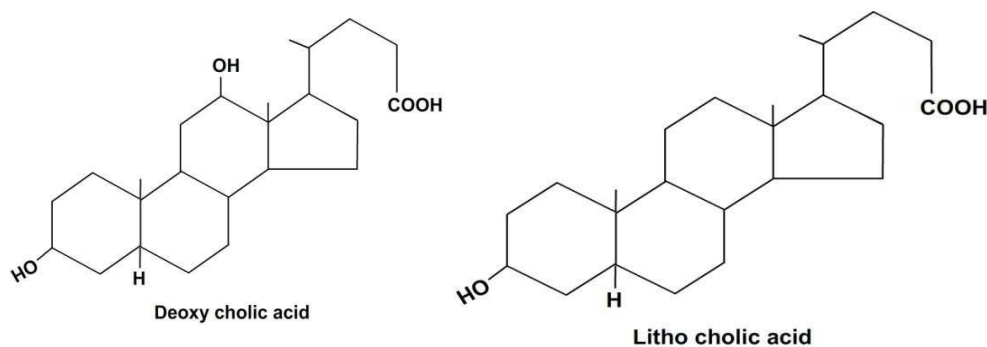
Bile acids contain addition –OH group attached to steroid nucleus. The side has –COOH group 4 bile acids have been isolated from human bile.

Cholic and chenodeoxy cholic acid are Primary bile acids and are formed in the liver from cholesterol. Deoxy cholic and lithocholic acid are Secondary bile acids formed from cholic and chenodeoxy cholic acid respectively.

Primary bile acids



Secondary bile acids



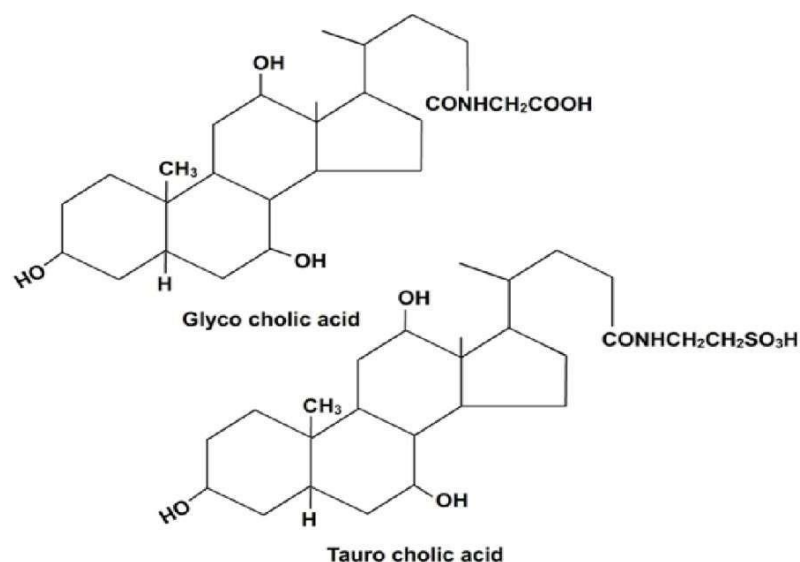
Cholic acid is the saturated sterol having 3-OH groups in C3, C7, and C12. Chenodeoxy cholic acid has 2-OH groups in C3 and C12. Lithocholic acid has only 1-OH group at C3.

Bile contains significant amount of Na^+ and K^+ and pH is alkaline. The bile acids that conjugate with glycine and taurine and then combined with Na^+ and K^+ are called Bile salt.

Before excretion in the bile cholic acid and chenodeoxy cholic acid are conjugated in the liver either with glycine or taurine through peptide linkages forming glycocholic acid and Taurocholic acid respectively. They then combined with Na^+ and K^+ and form water soluble alkaline salt namely sodium glycocholate and sodium taurocholate and then excreted in the bile.

Bile salts are powerful emulsifying agents secreted by liver into the bile which then enter into upper portion of the small intestine. In the small intestine these bile salts convert all fats into fat droplets and monoglycerides. These compounds are absorbed in the lower small intestine along with the bile salt, so they return to the liver for further use. The bile salt thus constantly cycle is known as enterohepatic circulation of bile salts.

A small amount of bile salt escape reabsorption during each enterohepatic circulation cycle.



They pass into large intestine where bacteria in the choline remove gly or taurine and form secondary (2^0) bile acids and their excreted in the feces.

Functions of bile acids and bile salts

(i) The bile salts enhance the digestion of fat in the intestine.

In intestine bile acids combined with monoglyceride (MG) and diglyceride (DG) to form tiny particles called micelles and these particles facilitate absorption of fat in intestine.

(ii) They help in the absorption of cholesterol, fat soluble vitamins A, D, E and K and form water soluble compounds.

(iii) Bile salts stimulate the secretion of hepatic bile.

(iv) Bile salts activate pancreatic lipase and cholesterol esterase.

MICELLES

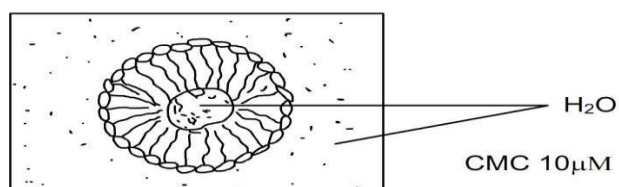
Molecules like phospholipids carrying both polar and non-polar group are called amphipathic molecules. When amphipathic substances occur in an aqueous medium in excess of specific concentration is called its critical micelle concentration (CMC). Its molecules aggregate into micelles. These are small stable droplets each containing 50 – 100 of amphipathic molecules. The polar head of all the molecules are oriented towards surroundings the aqueous phase lies on the outer surface of the micelles and remain associated with adjoining H₂O molecules by H bond or ion dipole interactions. But the non-polar tails of molecules lie in the non-aqueous central core of the micelles and are held close together by Vander Waals force and hydrophobic interaction.

Amphipathic molecules having single non-polar tail (eg) Soap, bile salt possess the tapered effective shape due to a tapering Vander Waals envelope around the wider polar head and the much narrower non-polar tail. This enables them to be closely packed into a spheroidal micelle at or above an average CMC of about 10 μ m.

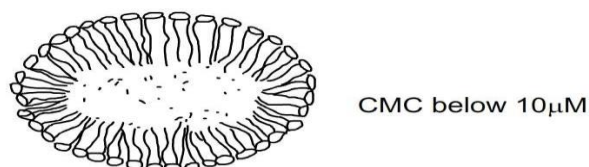
On the contrary phospholipid molecules have 2 non-polar tails and consequently possess a rectangular effective shape with Vander Waals envelope covering the polar head and both the tails. It enables these molecules to form stable ellipsoidal disordered micelles at or above an average CMC lower than 10 μ m.

The micelle formation is important for the absorption of fats in the intestine.

Spheroidal micelles



Ellipsoidal micelles



FATTY ACIDS

They are aliphatic - carboxylic acids and have the general structural formula $R - COOH$ where $-COOH$ represents functional group. Depending upon the R groups fatty acids may vary.

Classification of Fatty acids

I. Depending upon total number of C atoms, they are classified into 2 types.

1. Even chain having C atoms 2,4,6,8 etc. Most of the naturally occurring lipids contain even chain fatty acids.
2. Odd chain having C atoms 3,5,7 etc. They are seen in microbial cell walls.

II. Depending upon the length of hydrocarbon chain, they are classified into 3 types:

1. Short chain fatty acids with 2-6 C atoms.
2. Medium chain fatty acids with 8 – 14 C atoms.
3. Long chain fatty acids with 16 and above usually upto 24 C atoms.

III. Depending upon the nature of hydrocarbo chain, they are classified into 5 types:

1. Saturated fatty acids.
2. Unsaturated fatty acids.
3. Branched chain fatty acids.
4. Hydroxy fatty acids
5. Cyclic fatty acids.

1. Saturated fatty acids

These do not have double bond.

List of fatty acids

S. No	Commonname	Systemic name	No. of catom	Structure	Commonsource
1	Formic acid	Mehtanoic acid	1	HCOOH	-
2	Acetic acid	Ethanoic acid	2	CH ₃ COOH	Vineger
3	Propionicacid	Propanoic acid	3	CH ₃ CH ₂ COOH	-
4	Butyric acid	Butanoic acid	4	CH ₃ CH ₂ CH ₂ COOH	Butter
5	Valeric acid	Pentanoic acid	5	CH ₃ (CH ₂) ₃ COOH	-
6	Caproic acid	Hexanoic acid	6	CH ₃ (CH ₂) ₄ COOH	Butter
7	Heptoicacid	Heptanoic acid	7	CH ₃ (CH ₂) ₅ COOH	-
8	Caprylicacid	Octanoic acid	8	CH ₃ (CH ₂) ₆ COOH	Butter
9	Pelargonicacid	Nanonic acid	9	CH ₃ (CH ₂) ₇ COOH	-
10	Capric acid	Decarnoic acid	10	CH ₃ (CH ₂) ₈ COOH	Butter andCoconut oil
11	-	Hendecanoic	11	CH ₃ (CH ₂) ₉ COOH	-
12	Lauric acid	Didecanoic	12	CH ₃ (CH ₂) ₁₀ COOH	Coconut oil
13	-	Tri decanoicacid	13	CH ₃ (CH ₂) ₁₁ COOH	-
14	Myristicacid	Tetra Decanoicacid	14	CH ₃ (CH ₂) ₁₂ COOH	Coconut oil
15	-	Penta Decanoicacid	15	CH ₃ (CH ₂) ₁₃ COOH	-
16	Palmiticacid	Hexa Decanoicacid	16	CH ₃ (CH ₂) ₁₄ COOH	Body fat(adipose tissue)
17	-	Hepta Decanoicacid	17	CH ₃ (CH ₂) ₁₅ COOH	-
18	Stearic acid	Octa Decanoicacid	18	CH ₃ (CH ₂) ₁₆ COOH	Bodyfat
19	-	Naro Decanoicacid	19	CH ₃ (CH ₂) ₁₇ COOH	-
20	Arachidicacid	Eicosanoic acid	20	CH ₃ (CH ₂) ₁₈ COOH	Aracis oil

(2) Unsaturated fatty acids

They have double bond in its structure. They are further classified into Monounsaturated having single double bond called Monoenoic acid and Poly unsaturated having 2 or more double bonds (2 – Dienoic acid; 3 – Trienoic acid; 4 – Tetraenoic acid)

Monoenoic acid	Palmitoleic acid	Δ ⁹ Hexa Decenoic acid	16	CH ₃ – (CH ₂) ₅ – CH = (CH ₂) ₇ COOH	Body fat
	Oleic acid	Δ ⁹ Octa Decenoic acid	18	CH ₃ – (CH ₂) ₇ – CH = (CH ₂) ₇ COOH	Body fat
Dienoic acid	Linoleic acid	Δ ^{9, 12} Octa Decadienoic acid	18	CH ₃ – (CH ₂) ₄ – CH = CH – CH ₂ – CH = CH – (CH ₂) ₇ – COOH	Vegetable oil
Trienoic acid	Linolenic acid	Δ ^{9, 12, 15} Octa Decatrienoic acid	18	CH ₃ – CH ₂ – CH = CH – CH ₂ – CH = CH – (CH ₂) ₇ – COOH	Vegetable oil
Tetraenoic acid	Arachidonic acid	Δ ^{5, 8, 11, 14} Eiqosa tetraenoic acid	20	CH ₃ – (CH ₂) ₄ – CH = CH – CH ₂ – CH = CH – CH ₂ – CH = CH – (CH ₂) ₃ COOH	Phospho lipids

(3) Branched chain fatty acids

They have branched structure. (eg) Isovaleric acid, Iso caproic acid.

Isovalericacid	3methyacid butanoic	5	$\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_3 - \text{CH} - \text{CH}_2 - \text{COOH} \end{array}$	Metabolic intermediate
Isocaproic acid	4methyl pentonoic acid	6	$\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_3 - \text{CH} - \text{CH}_2 - \text{CH}_2 \\ \\ \text{COOH} \end{array}$	Metabolic intermediate

(4) Hydroxy fatty acids

They have hydroxyl group in their structure (eg) Cerebronic acid, Ricinoleic acid.

Ricinoleic acid	12 – OH Δ^9 Octa decenoic acid	18	$ \begin{array}{c} \text{OH} \\ \\ \text{CH}_3 - (\text{CH}_2)_5 - \text{CH} - \text{CH}_2 - \\ \text{CH} = \text{CH} - (\text{CH}_2)_7 - \text{COOH} \end{array} $	Castor oil
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5 Cyclic fatty acids

They have cyclic ring structure (e.g.) Chaulmoogric acid.

Chaulmoogric acid	$ \begin{array}{c} \text{HC} \\ \\ \text{H}_2\text{C} \quad \text{CH}_2 \\ \quad \quad \quad \diagup \\ \text{COOH} \quad \quad \quad \text{C} = \text{CH} - (\text{CH}_2)_2 - \end{array} $	Chaulmogra
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Essential fatty acids: (EFA)

Certain FA which have specific nutritional importance are called EFA. They are long chain poly unsaturated FA (PUFA) that cannot be synthesized by the body. They resemble vitamins, proteins in that they are required in small quantity but are essential constituents of (eg) Linoleic acid, Linolenic acid and Arachidonic acid.

Deficiency of EFA produce symptoms like skin lesions like Eczema cessation of growth and abnormalities in pregnancy, lactation. They are reversed to normal condition by the administration of EFA.

It has been found that animal fat contain more saturated FA where as vegetable fats contain more of unsaturated FA and later are rich in EFA. The unsaturated FA and EFA tend to lower the serum cholesterol.

This property of EFA is important in management in hypercholesterolemia which is associated with Atherosclerosis or thickening of arteries. The other functions of EFA are.

1. They are important for growth of cells
2. They are important reproductive function
3. They prolong clotting time and increase the fibrinolytic activities.

Diet rich in EFA are.

1. Vegetable oils rich in EFA (eg) Safflower oil contains 75% EFA, corn oil contains 50% EFA, cotton seed oil contains 45% EFA.
2. Animal fats are poor sources of EFA. (eg) Egg contains 9% EFA, Lard contains

9%EFA.

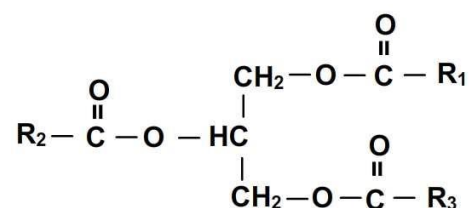
Fats and Oils

They are esters of EFA with glycerol. Fats are solid and oils are liquid at room temperature. Fats are classified into 3 types according to no. of FA present in them.

1. Triglycerides (Triceyl glyceros) (TG)
2. Diglycerides (DG)
3. Monoglycerides (MG)

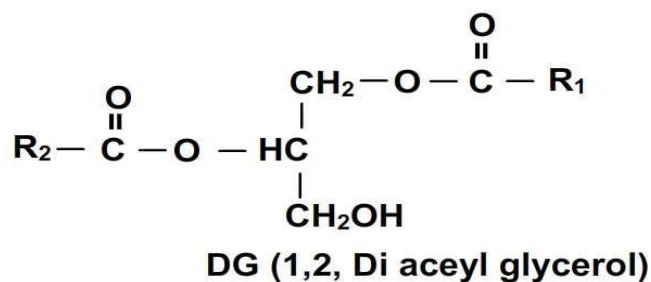
Triglycerides: (TG)

They contain 3 FA esterified into 1 glycerol molecule



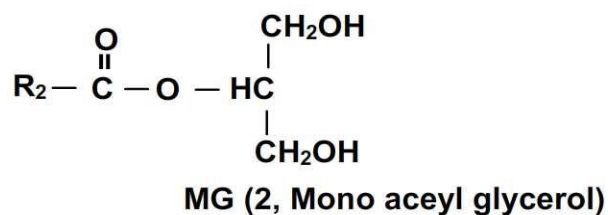
Diglycerides: (DG)

They contain 2 FA residues esterified to 1 glycerol molecule.



MONOGLYCERIDES: (MG)

They contains 1 FA residue esterified into 1 glycerol molecule.

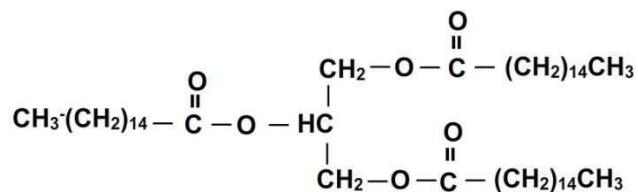


According to the type of FA, Triglycerides are classified into 2 types. They are,

1. Simple triglycerides
2. Mixed triglycerides

Simple tryglycerides

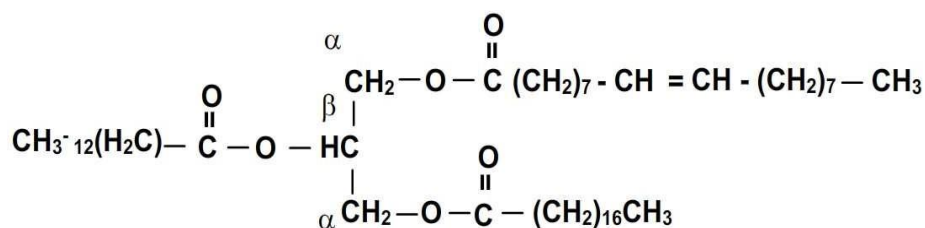
This contains 3 identical FA residues and a glycerol. (eg) Tripalmitin.



Tripalmitin

Mixed triglycerides

This contains more than 1 type of FA and glycerol. (eg)

 α Oleo, β Palmito, α Stearin

Properties of Fat

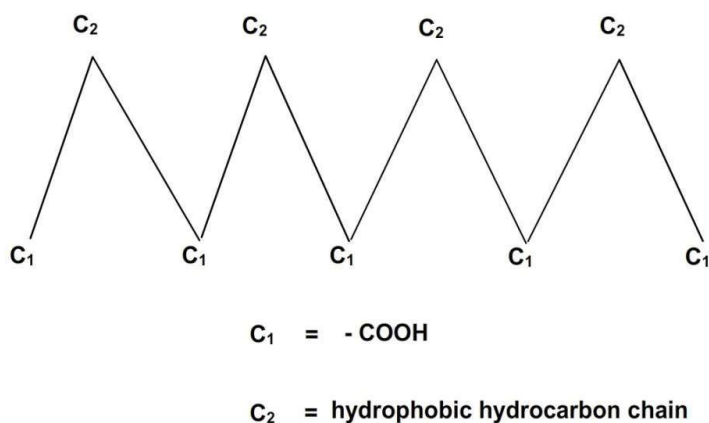
Physical properties

1. They are greasy to touch and leave an oily impression on paper.
2. They are insoluble in H₂O but readily soluble in fat solvent like ether, alcohol, chloroform etc.
3. Pure glycerides are tasteless, Odorless, Colorless and neutral in reaction but after exposure to air, for sometimes they become acidic and develop yellow colour due to partial hydrolysis and oxidation of unsaturated FA
4. Fats used in foods have specific flavour and odour. Flavour is attributed by presence of certain foreign substances. (e.g.) The flavour of butter is due to bacterial Flora.
5. Hardness or consistency of fats depends upon the amount of SFA and UFA present in fats. Fat containing SFA are solids at room temp and fats containing UFA are liquid at room temperature and these are called oils.
6. They have definite melting point. Presence of SFA in fats increases melting point and UFA lowers the melting point.
7. Fats have lesser specific gravity than H₂O. Therefore, they float in H₂O.

8. Spreading of fat

When liquid fat is placed on water, it spreads uniformly over the surface of H₂O and form a layer of molecule thick. The effect of this property is to lower the surface tension and help to transport of fat. Hydrophobic hydrocarbon chain (C₂) present in FA forms a layer on surface and anchored to a H₂O soluble – COOH group (C₁). The layer formed so as thickness of a mole therefore it forms a mono molecular layer.

9. Though fats are insoluble in H₂O they can be broken down into minute droplets and dispersed in H₂O. This is an emulsion (eg) of naturally occurring emulsions are Milk, Egg yolk. Emulsions greatly increase the surface area of fat and this is essential for The digestion of fat in the Intestine.



It is brought about by mechanical and chemical action.

1. Mechanical

This can be brought about by mixing mechanically or passing a super-heated stream.

2. Chemical

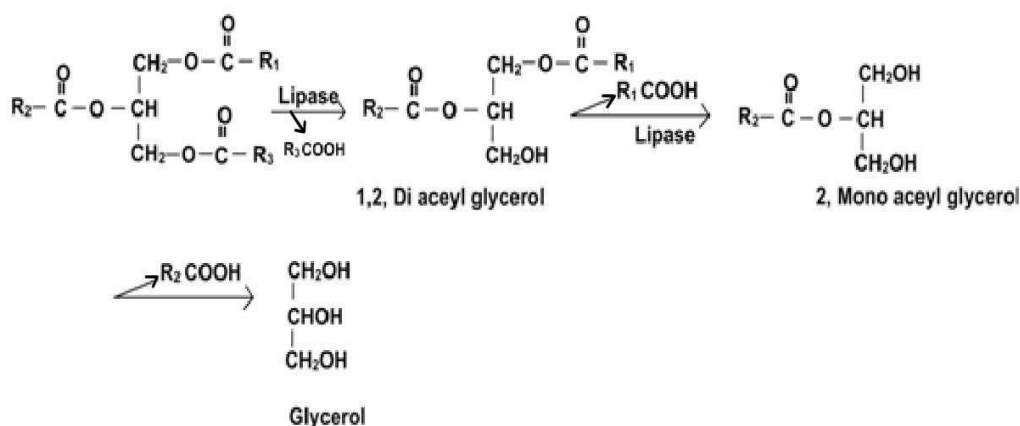
This is accomplished by bile salt which lowers the surface tension.

Chemical properties

I. Reaction involving – COOH group

1. Hydrolysis

Fats are readily hydrolyzed to glycerol and FA by acids, alkalis, super-heated stream and enzymes known as lipases. 2 kinds of hydrolysis are known.



Enzymehydrolysis.

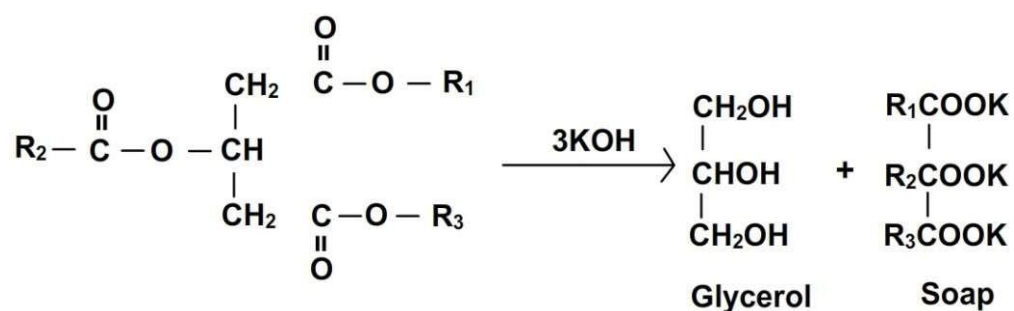
It is effective by lipase or pancreatic juice. This is involved in the digestion of fats.

Alkali hydrolyses.

It refers to the hydrolysis of fats by boiling them with alkalis. The process of alkali hydrolysis is called saponification which means soap-making.

2. Alkali hydrolysis

It refers to the hydrolysis of fats by boiling them with alkalies. The process of alkali hydrolysis is called saponification which means soap-making.



II. Auto oxidation

UFA in fat reacts with Ozone and Oxygen in presence of moisture, light, warmth and catalytic substances to form Ozonides, per-oxides, aldehydes and ketones.

Rancidity

Auto oxidation occurring in natural edible fat is called Rancidity. The fat which has become Rancid has a disagreeable odour and taste and is unfit for consumption. Rancidity can be prevented by proper storage of fat in air-tight, nonmetallic

receptacles, keeping in cool, place away from light, warmth, and moisture.

III. Reactions involving double bonds:

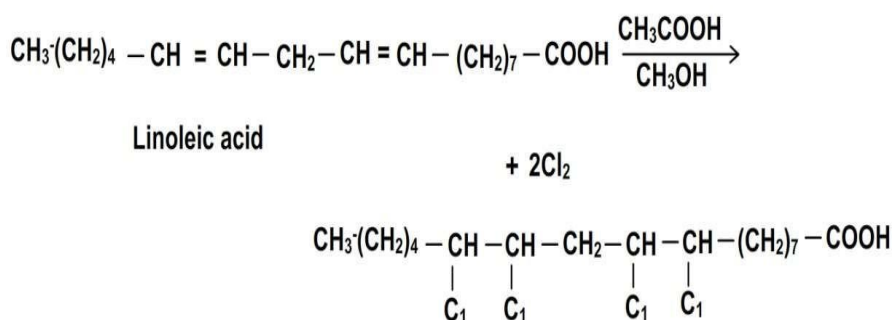
1. Hydrogenation:

UFA can combine with H₂ under the influence of suitable catalysts like finely divided Ni, Platinum and Cu and a high temperature become more saturated.



2. Halogenation

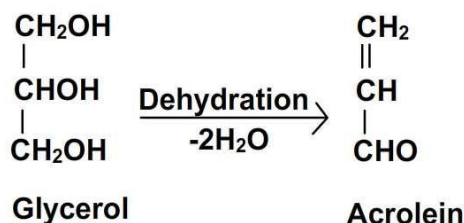
UFA and their ester can take up halogen like a Br, and Iodine at their double bond at room temperature in the presence of CH₃COOH or CH₃OH.



IV. Reactions involving - OH group

Dehydration: (Acrolein test)

Fat when heat in presence of the dehydrating agent like (NaHSO₄) sodium, hydrogen, sulphate produce an UFA called Acrolein from the glycerol moiety of fat. It is easily recognized by pungent odour and forms a basis of a test for the presence of glycerol in fat molecules.



CHARACTERISATION OF FATS

Composition, Quality and purity of a given fat or oil is determined by means of no. of physical and chemical test. Usual physical test includes determination of MP, specific gravity, viscosity etc., While chemical tests includes determination of Iodine no,

saponification no, Acid no, Acetyl no, Reichert Meissl no and Polanski no.

1. Saponification number

It refers to (number of mg of KOH required to neutralize or saponify 1g of fat). This no gives information about average chain length of FA in the fat and also Molecular weight. This value is high for fats containing low molecular weight or short chain FA. The lower saponification no indicates presence of long chain FA or high Molecular weight.

2. Iodine number

It is the (number of gms of I₂ absorbed by 100g of fat) I₂ is absorbed only at double bonds or UFA residue of fat. Thus this no is a measure of degree of unsaturation of FA present in particular fat. Higher I₂ value indicates presence of higher amount of UFA. Oils like soya bean, corn, cotton seed oil have higher I₂ values than the solid fat like beef fat.

3. Acid number

It is (number of milligrams of KOH required to neutralize the free FA present in 1g of fat or oil). The acid no indicates the amount of free FA present in fat. Free FA liberated in neutral fat due to hydrolyses by lipases and it leads to Rancidity of fat. So, the high acid no indicates higher tendency for Rancidity.

4. Acetyl number

It is (number of ml of KOH required to neutralize CH₃COOH obtained by saponification of 1g of acetylated fat). The acetyl group is accepted only by –OH group or – OH FA residucin fat. Thus, it is a measure of the amount of –OH FA in fat molecule (eg) Acetyl no: of castor oil is 146 -160 which contains higher amount of - OH FA, than olive oil whose acetylation no is 10 – 12.

5. Reichert Meissl number (or) Volatile FA number

It is (number of ml of 0.1N KOH required to neutralize the soluble volatile FA derived from 5g of fat. This no thus measures the quantity of short chain FA or soluble volatile FA in fat molecule. (eg) Butter has high R.M value 26 -33, lard has less R.M value 0.6.

6. Polanski number

It is the (number of ml of 0.1N KOH required to neutralize the insoluble non volatile FA derived from 5g of Fat). This is used to measure the amount of long chain insoluble non-volatile FA. The value is low in pure ghee.

3.6 : SUMMARY

Lipids are a heterogenous group of water-insoluble oily, greasy organic compounds soluble in Organic solvents like C₆H₆, ether, acetone, alcohol, chloroform. Fats or lipids are widely distributed in plants and animal tissues from which they can be extracted by fat solvent. In plants, they are particularly present in nuts, seeds, fruits, etc. In animals they are stored in adipose tissue; nerve tissues and bone marrow.

3.7 : KEYWORDS**Lipids**

Lipids are a heterogenous group of water-insoluble oily, greasy organic compounds soluble in Organic solvents like C₆H₆, ether, acetone, alcohol, Chloroform.

Compound lipids

These are the lipids containing some chemical groups in addition to FA and alcohols. Based on the nature of additional chemical groups, compound lipids are subdivided into the following.

Rancidity

Auto oxidation occurring in natural edible fat is called Rancidity. The fat which has become Rancid has a disagreeable odour and taste and is unfit for consumption. Rancidity can be prevented by proper storage of fat in air tight, nonmetallic receptacles, keep in cool, place away from light, warmth, and moisture.

Polanski number

It is the (number of ml of 0.1N KOH required to neutralize the insoluble nonvolatile FA derived from 5g of Fat). This is used to measure the amount of long-chain insoluble non-volatile FA. The value is low in pure ghee.

Reichert Meissl number (or) Volatile FA number

It is (the number of ml of 0.1N KOH required to neutralize the soluble volatile FA derived from 5g of fat. This no thus measures the quantity of short-chain FA or soluble volatile FA in fat molecules. (eg) Butter has a high R.M value 26 -33, lard has less R.M value 0.6.

Saponification number

It refers to (the number of mg of KOH required to neutralize or saponify 1g of fat). This no gives information about the average chain length of FA in the fat and also Molecular weight. This value is high for fats containing low molecular weight or short-chain FA. The lower saponification no indicates the presence of long-chain FA or high molecular weight.

Iodine number

It is the (number of gms of I₂ absorbed by 100g of fat) I₂ is absorbed only at double bonds or UFA residue of fat. Thus this no is a measure of degree of unsaturation of FA present in particular fat. A higher I₂ value indicates the presence of higher amount of UFA. Oils like soya bean, corn, and cotton seed oil have higher I₂ values than solid fat like beef fat.

Cholesterol

Cholesterol are the solid bile alcohol. It derives its name from Greek word "Cholesterine" which means bile solid. (Chole = bile and Sterine = Solid) As it was 1st isolated from gall stone.

Cholesterol is widely distributed in all cells of body. Its concentration in human plasma is 150 -250 mg/100 ml of blood.

3.8 : ANSWERS TO CHECK YOUR PROGRESS:

- (1) Pre-Beta- lipoproteins
- (2) Lipids
- (3) The deposition of yellow-orange colour lipids in liver, spleen and flat bones of the skull is known as xanthomatosis (Greek: xanthos – yellow). This is usually associated with severe hyperlipidemia and hypercholesterolemia.
- (4) 15

(5) Essential Fatty Acids

The Fatty acid that cannot be synthesized by the body and have to be supplied in the diet are known as essential fatty acids. They are polyunsaturated Fatty Acids.

The Essential Fatty acids are

1. Linoleic acid and
2. Linolenic acid

Linoleic acid: $\omega 6$ (18; 2 - 9, 12)

Linolenic acid: $\omega 3$ (18; 3 - 9, 12, 15)

They are essential because, our body lacks the enzyme that can introduce double bonds between carbons 9 and 10.

Biochemical Function of EFA

1. Required for the membrane structure and functions.
2. Transport of cholesterol
3. Formation of Lipoproteins
4. Prevention of Fatty Liver
5. Required for the synthesis of Eicosanoids.

(6) Deficiency manifestations are

Phrynodema or toad skin with horny eruptions on posterior and lateral part of limbs, back
Loss of hair
Poor wound healing.

Most of the vegetable oils: e.g.: groundnut oil, sesame oil, mustard oil, cotton seed oil, soybean
Oil is a good source of essential fatty acids

EXERCISE

I; Essay Questions

1. What are lipids and mention the classification of lipids?
2. Explain in detail about the physical and chemical properties of lipids?
3. What are fatty acids and mention their classification with examples?
4. Write a note on Bile acids?
5. Write a note on the structure and characteristic features of cholesterol as a steroid?

II Multiple choice question

1. This molecule acts as molecular chaperones to assist the folding of proteins. ()

- (a) Vitamins (b) Carbohydrates (c) Amides (d) Lipids
2. Which of these is not a lipid? ()
- (a) Fats (b) Oils (c) Proteins (d) Waxes
3. Beta-oxidation of fatty acids occurs in ()
- (a) Peroxisome (b) Peroxisome and Mitochondria (c) Mitochondria (d) Peroxisome, Mitochondria, and ER
4. The abundantly distributed enzyme in germinating seeds and adipocytes is ()
- (a) Lipase (b) Proteases (c) Cellulase (d) Nuclease
5. An example of is Carnauba wax ()
- (a) Soft wax (b) Liquid wax (c) Hard wax (d) Archaeobacterial wax
6. In fats, the number of OH groups can be expressed as ()
- (a) Reichert-Meissil number (b) Polenske number (c) Iodine number (d) Acetyl numbe
7. Rancidity of lipids of lipid-rich foodstuff is because of ()
- (a) Reduction of fatty acids (b) Hydrogenation of unsaturated fatty acids (c) Dehydrogenationof saturated fatty acids (d) Oxidation of fatty acids
8. This is an example of derived lipids ()
- (a) Terpenes (b) Steroids (c) Carotenoids(d) All of the above
9. The degree of unsaturation of lipids can be measured as ()
- (a) Iodine number (b) Saponification number (c) Reichert Meissel number (d) Polenskenumber
10. The specific gravity of lipid is ()
- (a) 1.5 (b) 1.0 (c) 0.8 (d) 0.2

III Fill in the Blanks

- _____ is the solubility of lipids in water.
- _____ the two essential fatty acids
- The melting point of fatty acids depends upon chain length and_____
- _____ the reagent which is used in Saponification?
- _____ would move faster in thin-layer chromatography
- A mixture of fatty acyl methyl esters are separated based on_____
- The dye used in TLC for detecting separated lipids by spraying the plate is_____
- Phosphatidylinositol, phosphatidylglycerol and phosphatidylserine are easily separated by_____

9. For the determination of fatty acid composition, transesterification is done in a warm aqueous solution of _____
10. _____ technique is preferred in the separation of fatty acyl methyl esters from a mixture.

II Answers: 1) d 2) c 3) c 4) a 5) c 6) d 7) d 8) d 9) a 10) c

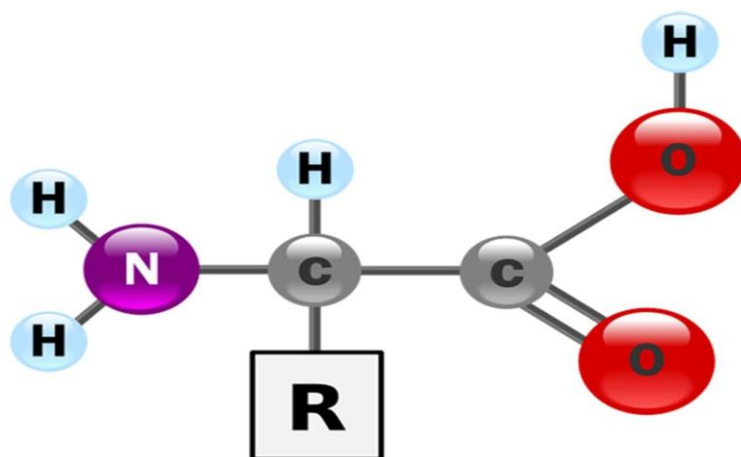
III Answers: 1) Insoluble 2) Linoleate and linoleate 3) Degree of unsaturation

4) NaOH/KOH 5) Beeswax 6) Chain length and degree of saturation

7) Rhodamine 8) TLC 9) NaOH+methanol 10) Gas-liquid chromatography

CHAPTER-IV

Amino Acids



4.0 INTRODUCTION

Chapter IV

AMINOACIDS

Amino acids make up the proteins. They are organic compounds of nitrogen, carbon, hydrogen, and oxygen with a variable side chain group. Human beings need 20 essential amino acids for the proper development and functioning of various organs in the body. Out of the 20, 9 are deemed as the most important amino acids.

An amino acid is an organic compound that consists of a basic amino group (NH₂), an acidic carboxyl group (COOH), and a unique organic R group (or side chain). Each molecule has a core carbon (C) atom, referred to as the α -carbon, to which amino and a carboxyl group are linked. A hydrogen (H) atom and the R group usually satisfy the remaining two bonds of the α -carbon atom. Proteins are made up of amino acids, which are the building blocks of the molecule. They offer many of a cell's structural constituents and aid in the formation of tissues by binding cells together. Animals and plants contain about 200 proteins. But the human body contains only 60 amino acids of these 20 amino acids are used as building blocks in the synthesis of proteins. These 20 amino acids are repeatedly used to synthesize millions of proteins.

Properties of Amino acid

- The melting and boiling points of these are extremely high.
- Amino acids are crystalline white solids with a high molecular weight.
- Few amino acids have a pleasant, flavorless, or bitter flavor.

- Inorganic solvents are insoluble in most amino acids, which are soluble in water.

How Important are Amino Acids

Amino acids are the compounds that form the building blocks of proteins. Proteins are essential components that makeup 75% of the human body. Proteins are responsible for proper overall development.

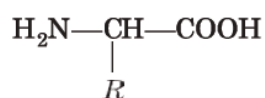
Functions of Essential and Non-Essential Amino Acids

- Essential amino acids like phenylalanine, valine threonine helps maintain the nervoussystem, promote muscle growth, and strengthen the immune system.
- Essential amino acid Tryptophan is involved in the production of vitamin B3 and serotoninhormones that play a vital role in regulating sleep, boosting mood, and maintaining appetite.
- Isoleucine plays a vital role in the formation of hemoglobin along with stimulating the pancreas to synthesize insulin and helps the transportation of oxygen in the body.
- Nonessential amino acids like alanine, cysteine, and glutamine help in the production ofglucose and other amino acids, provide resistance, and help promote healthy brain function.

4.1 COMPOSITION OF AMINO ACIDS

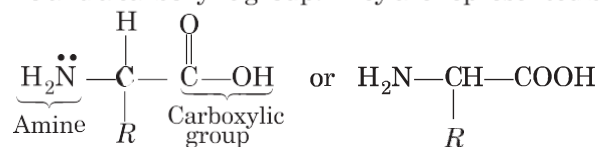
1. An amino acid is made up of four components namely
2. A carbon atom- C
3. An amino group- NH₂
4. A carboxyl group- COOH
5. A-side chain or Residue ®

The general formula of an amino acid is.



In the structure of amino acid 'R' represents the groups other than amin

As the term suggests, amino acids are bifunctional compounds containing both an amino and a carboxylic group. They are represented by general formula

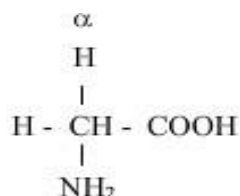


o and carboxyl groups. It may be aliphatic or aromatic. It may also be a hydrogen atom (H) or a methyl group (CH₃) or a more complex structure. In glycine, the simplest amino acid 'R' represents the H atom.

α , β , γ amino acids

In amino acids, the numbering of carbon atoms is made from the carbon atom situated next to the –COOH group. The carbon atom situated next to the –COOH group is C atom no:1 and is called α carbon atom. The second carbon atom is called β carbon atom and the third carbon atom is called γ carbon atom.

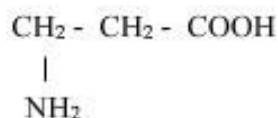
The amino acid is named α , β & γ amino acids according to the position of the –NH₂ group. When the –NH₂ group is attached to the α carbon atom, the amino acid is called α amino acid. (e.g.) α amino acid. (glycine).



Glycine

When the –NH₂ group is attached to the β carbon atom, the amino acid is called β amino acid.

(eg) β Alanine.



When the –NH₂ group is attached to the γ carbon atom, the amino acid is called γ -amino acid. (e.g.) GABA Almost all the amino acids especially in animals are α -amino acids.

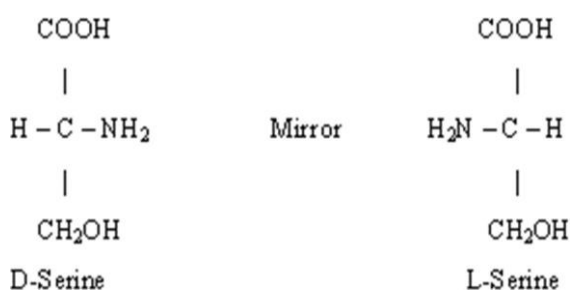
Asymmetry in amino acids

In all α -amino acids (α -NH₂) the α carbon atom is asymmetric because it is attached to four different groups. They exhibit optical activity, and they rotate plane-polarized light to the right or left. As a result, they exist in two optical isomers.

One isomeric α -amino acid rotates the plane polarized light to the right and is called dextrose rotatory form and this α -amino acid is represented by the plane polarized light to the left and it is called levo rotatory form and this α -amino acid is represented by (-) or (L).

4.2 D AND L α -AMINO ACIDS

Based on the configuration. Two types of α -amino acids exist. They are D and L α -amino acids. The amino acids having the α -amino group on the right side are called D amino acids. The amino acids having α -amino group on the left side is called L amino acids. These isomers are mirror images of each other.



In nature, L- amino acids are more common. Therefore L- amino acids are called natural amino acids.

4.3 CLASSIFICATION OF AMINO ACIDS

The human body contains about 60 amino acids. But on hydrolysis, proteins yield only 25 amino acids. Out of the 25 amino acids, 23 are α amino acids and the remaining 2 are proline and γ -hydroxy proline.

Amino acids are classified in various ways

1. Classification based on their incorporation in proteins.
2. Classification based on the structure of the side chain.
3. Classification based on the position of the α -amino group.
4. Classification based on the reaction in solution.
5. Classification based on polarity.
6. Classification based on biological importance.

I. CLASSIFICATION OF AMINO ACIDS BASED ON THEIR INCORPORATION IN PROTEINS

Based on this the amino acids are classified into 2 groups. They are proteogenic and non- proteogenic amino acids.

1. Proteo-genic amino acids

The amino acids which are used for the synthesis of proteins are called proteo-genic (or) proteinogenic amino acids. They are usually components of proteins. The proteo-genic amino acids are of two types of namely major amino acids and rare amino acids. The major amino acids are widely involved in the synthesis of a vast number of proteins irrespective of the species concerned. The 20 amino acids from alanine to valine are major amino acids.

- a) Alanine- Ala
- b) Arginine- Arg
- c) Asparagine-Asn
- d) Aspartic acid- Asp
- e) Cysteine- Cys
- f) Glutamine-Gln
- g) Glutamic acid- Glu
- h) Histidine- His
- i) Isoleucine- Ile
- j) Leucine-Ieu
- k) Lysine- lys
- l) Methionine-Met
- m) Phenylalanine- Phe
- n) Proline- Pro
- o) Serine- Ser
- p) Threonine- Thr
- q) Tryptophan- Trp
- r) Tyrosine-Tyr
- s) Valine- Val

The rare amino acids are derivatives of the 20 major amino acids.

(eg) Hydroxy proline, Hydroxy glycine.

2. non-proteogenic amino acids

Some of the amino acids are not incorporated with proteins. These amino acids are called non-proteogenic amino acids. They do not participate in protein synthesis. They occur in the cells either in the free state as metabolites or as part of non-protein compounds.

(eg) Ornithine, Citrulline, amino butyric acid, beta-alanine, pantothenic acid, Thyroxine, and many others.

Certain non-proteogenic amino acids such as canavanine, and beta-cyano alanine isolated from plants are toxic for man.

II. CLASSIFICATION BASED ON THE STRUCTURE OF THE SIDE CHAIN.

On the basis of the structure of the side chain (R group), amino acids are classified into the following 3 types.

1. Aliphatic amino acids.
2. Aromatic amino acids.
3. Heterocyclic amino acids.

ALIPHATIC AMINO ACIDS

The amino acids containing straight chains in the R group are called Aliphatic amino acids.

They are further classified into four types based on the number of amino and –COOH groups in the side chain.

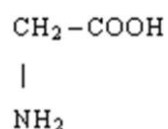
1. Mono amino mono carboxylic amino acids.
2. Mono amino dicarboxylic amino acids.
3. Di amino mono carboxylic amino acids.
4. Di amino dicarboxylic amino acids.

I. MONO AMINO MONO CARBOXYLIC AMINO ACIDS

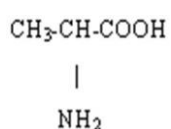
These amino acids have one –NH₂ group and one –COOH group in the structure.

These are neutral amino acids. (e.g.) Glycine, alanine, serine, Threonine, methionine, Cysteine, leucine, Isoleucine, and Valine.

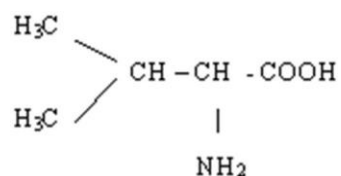
1. Glycine:



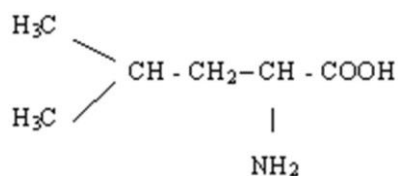
2. Alanine



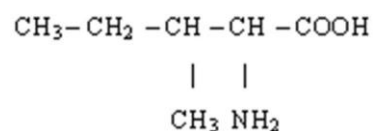
3. Valine:



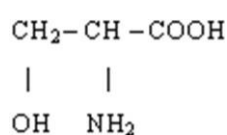
4. Leucine:



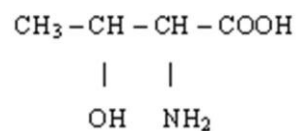
5. Isoleucine:



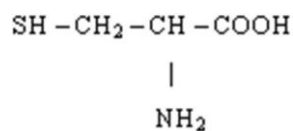
6. Serine:



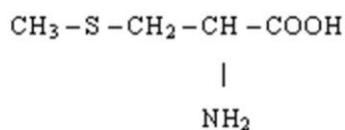
7. Threonine:



8. Cysteine:



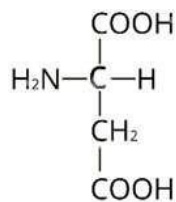
9. Methionine



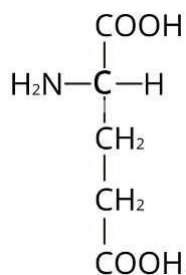
II. MONO AMINO DI CARBOXYLIC AMINO ACIDS

These amino acids contain 1- NH_2 group and 2- COOH groups. These are acidic amino acids. **(eg)** Aspartic acid, Glutamic acid and their derivatives are Asparagine and Glutamine.

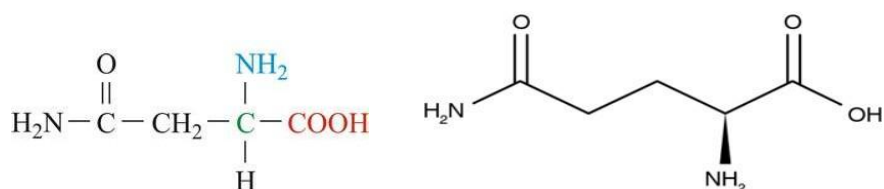
Aspartic acid



Glutamic acid:



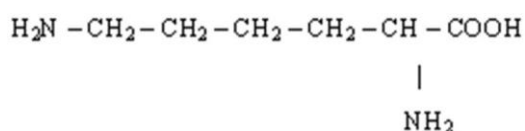
Asparagine: Glutamine



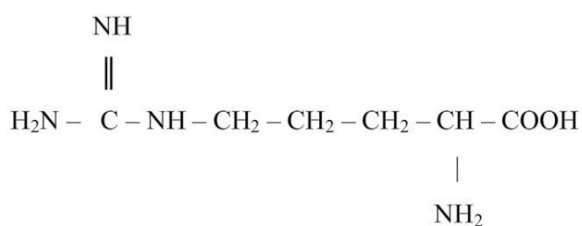
III. DI AMINO MONO CARBOXYLIC AMINO ACIDS:

These amino acids contain two -NH_2 group and one -COOH groups. These are basicamino acids. (eg) Lysine and Arginine.

Lysine



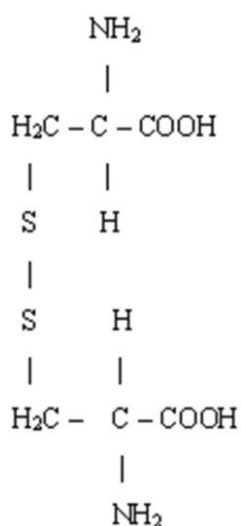
Arginine



IV. DIAMINO DI CARBOXYLIC AMINO ACIDS

These amino acids contain two -NH_2 group and two -COOH groups. (eg) cystine.

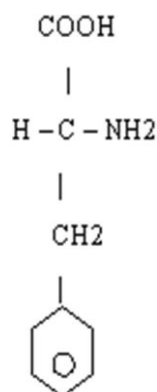
Cystine or Di cysteine



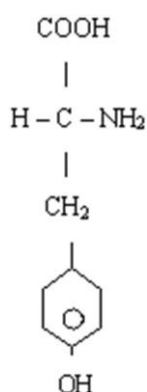
AROMATIC AMINO ACIDS

They contain aromatic rings. They are mono amino mono carboxylic acids.(eg) Phenylalanine and Tyrosine and Tryptophan.

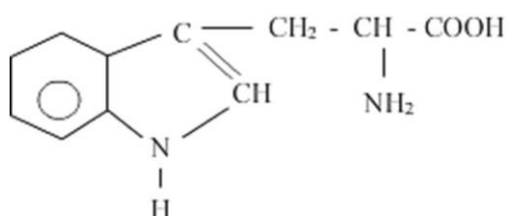
Phenyl alanine:



Tyrosine



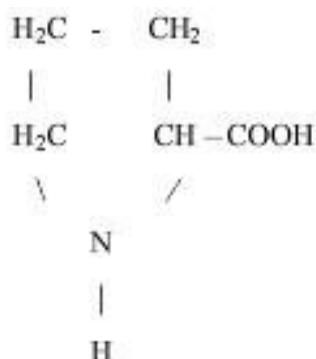
Tryptophan



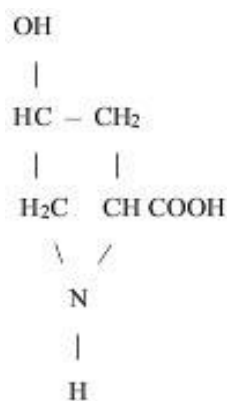
HETEROCYCLIC AMINO ACIDS

These amino acids contain a heterocyclic ring in the structure. (e.g.) Proline, Histidine, and Tryptophan.

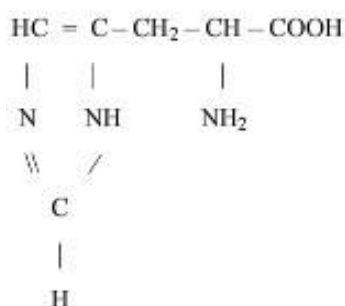
Proline:



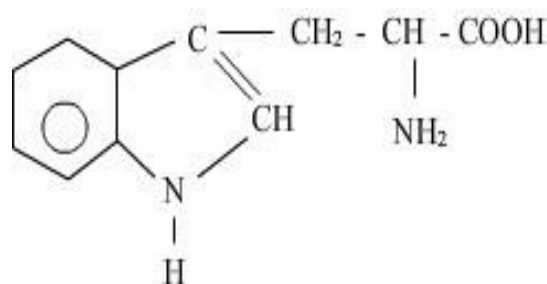
Hydroxy proline



Histidine:



Tryptophan:



III. CLASSIFICATION BASED ON THE POSITION OF THE AMINO GROUP.

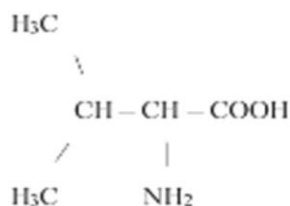
According to the position of the $-\text{NH}_2$ group amino acids are classified into 3 groups.

1. Alpha amino acids.
2. Beta amino acids.
3. Gamma amino acids.

Alpha amino acids

The carbon atom next to the $-\text{COOH}$ group is called the alpha carbon group. If the $-\text{NH}_2$ group is attached to an alpha-carbon atom, the amino acid is called Alpha-amino acid.

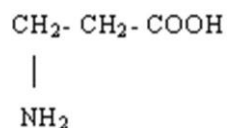
(eg) Valine.



Beta amino acids

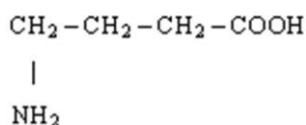
The second atom from the acid group is called beta carbon atom. If -NH_2 is attached to beta carbon atom, the amino acid is called beta amino acid.

(eg) beta alanine.

**Gamma amino acid**

The third carbon atom from the acid group is called gamma carbon atom. If -NH_2 group is attached to gamma carbon atom, the amino acid is called Gamma amino acid.

(eg) Gamma butyric acid (GABA)

**IV. CLASSIFICATION BASED ON THE REACTION IN SOLUTION**

Based on a chemical reaction in solution amino acids are classified into 3 groups, namely.

1. Neutral amino acid
2. Acidic amino acid
3. Basic amino acids

1. Neutral amino acids

The amino acids which do not contain any amino or carboxyl group in the side chain are called neutral amino acids.

They contain one -NH_2 and one -COOH group and hence they are neutral in character. (e.g.) Glycine, alanine, serine, etc.

2. Acidic amino acids

The amino acids which contain the attached -COOH group in the side chain are called acidic amino acids. As they contain an additional -COOH group, they impart acidic properties. (eg) Aspartic acid and Glutamic acid.

3. Basic amino acids

The amino acid which contains an additional -NH_2 group in the side chain is called

basic amino acid. They impart basic properties.

(eg) Lysine and Arginine.

V. CLASSIFICATION BASED ON POLARITY OF SIDE CHAIN OR R GROUP:

The tendency to interact with water at biological Ph.

The R groups of amino acids vary widely with respect to their polarity from totally non-polar (or) hydrophobic (water-hating) R groups to highly polar (or) hydrophilic (water-loving) R groups.

1. Amino acids with non-polar R groups:

The R groups in this category of amino acids are hydrophobic. This group includes.

- a. Alanine, Valine, leucine, and proline- Aliphatic R group
- b. Phenylalanine, tyrosine- Aromatic R groups
- c. Methionine which contains sulphur groups

2. Amino acids with polar but uncharged R groups:

The R groups of these are more soluble in H₂O (more hydrophilic). They contain this category includes 7 amino acids, like Gly, Ser, Thr, Tyr, Cys, Asn, Gln. The polarity of these amino acids may be due to either the $-OH$ group, Sulphydryl group or an amide group.

3. Amino acids with negatively charged R groups:

These are mono-amino dicarboxylic acids. In other words, their side chain contains an extra $-COOH$ group with a dissociable proton. The 2 amino acids which belong to this category are Asp and Glu.

4. Amino acid with positively charged R groups:

These are di-amino monocarboxylic acids. In other words, their R group contains an extra $-NH_2$ group which imparts basic properties to them (eg) Lys, Arg.

VI CLASSIFICATION BASED ON BIOLOGICAL IMPORTANCE:

Based on this classification amino acids are classified into essential and non-essential amino acids.

Essential amino acids

Certain amino acids cannot be synthesized by organisms. They must be compulsorily included in the diet for normal health. Hence these amino acids are

called essential amino acids. Essential amino acids are Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Tyr, Val and Try.

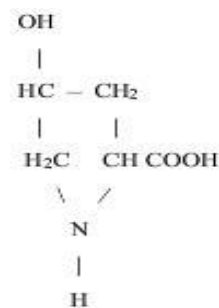
Non-essential amino acids

Certain amino acids can be synthesized in the body. These amino acids need not be included in the diet. These amino acids are thus called as non-essential amino acids. (e.g.) Ala, Asp, Asn, Glu, Gln, Gly, Pro, Cys and Ser.

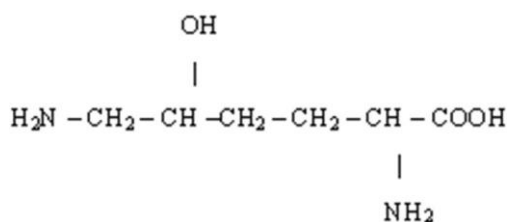
Less common amino acids

In Addition to the above-said 20 amino acids, which are building blocks of proteins and have a wide range of distribution in proteins several other amino acids also exist. These have a limited distribution but may be present in high amounts in a few proteins. (e.g.) Hydroxy proline has a limited distribution in nature but constitutes as much as 12% of the composition of collagen, an important structural protein of animals. Similarly, hydroxy lysine is also a component of collagen, where it accounts for about 1% of the total amino acids.

Hydroxy praline:



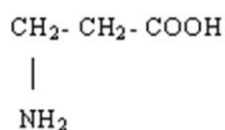
Hydroxy lysine:



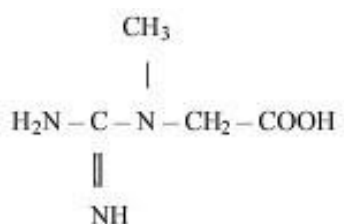
L-Ornithine and L citrulline occur in free state in the animal tissues and are metabolic intermediates in the urea cycle.

Beta-alanine an isomer of alanine, occurs free in nature and as a constituent of an important vitamin pantothenic acid, and of co-enzyme A. It is also found in naturally occurring peptides, creatine, a derivative of glycine plays an important role in the energy storage process in vertebrates. Lastly gamma amino butyric acid (GABA) is found in free form in the brain.

Beta alanine:



Creatine



Creatine

Gamma amino butyric acid (GABA) (or) Gamma amino butyrate.

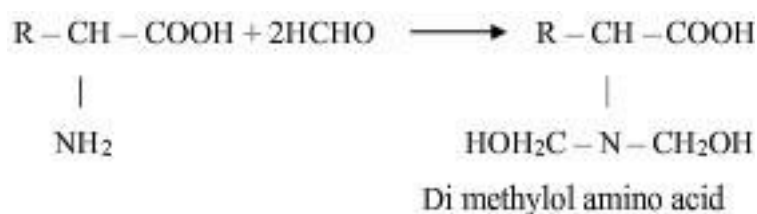
3.4 CHEMICAL PROPERTIES

Amino acids are functional compounds. So chemical properties of amino acids mainly depend on the presence of -NH_2 groups and -COOH groups.

I. REACTIONS OF AMINO ACIDS DUE TO THE PRESENCE OF -NH_2 GROUP

1. Reactions with HCHO:

Amino acids react with HCHO and form hydroxy methyl derivation.



These derivatives are insoluble in water and exhibit resistance to attack by microorganisms. Because of this action, HCHO is the principal reagent in Embalming fluids and it is used to harden and preserve certain fibers obtained from globular proteins.

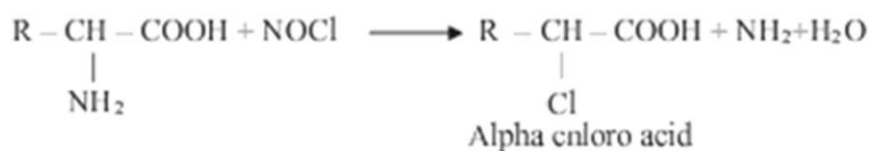
This reaction is the basis of Sorenson's formal titration for determining the purity of the individual amino acids.

2. Reaction with HONO: (Vanslyke's reaction)

The amino acids react with HONO to liberate N_2 gas and produce the corresponding alpha hydroxy acids.

This method is the basis for Vanslyke's nitrous acid method for the estimation of amino acids by measuring the value of nitrogen gas liberated.

3. Reaction with nitrosyl halides

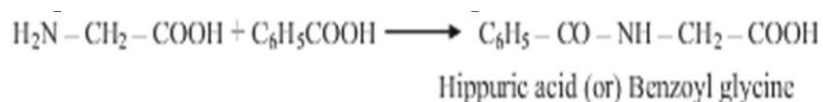


Amino acid react with nitrosyl halides to form corresponding Alpha-halo acids.

4. Reaction of Glycine with Benzoic acid

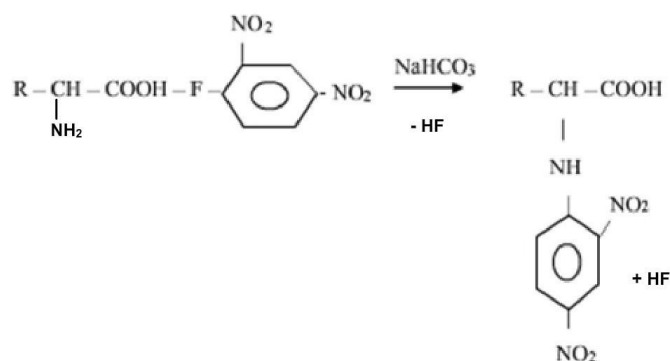
The $-\text{NH}_2$ group glycine combines with $\text{C}_6\text{H}_5\text{COOH}$ to form hippuric acid.

This phenomenon occurs in man as a process for detoxification of benzoic acid.



5. Reaction with Sanger's reagent

1 fluoro 2,4 di nitro benzene (FGNB) this reagent combines with free $-\text{NH}_2$ group in presence of mild alkaline solution like NaHCO_3 to give Dinitrophenyl amino acid.

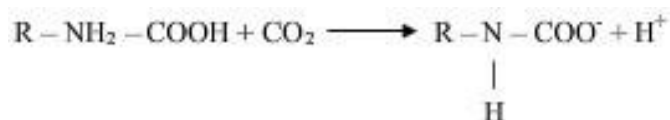


Dinitro phenyl amino acid (DNP)

The DNP amino acid is brought yellow in color and are identified by the chromatography method. This reaction is a very important reaction. Sanger used this reagent to identify and to separate the N-terminal in a peptide chain of insulin. By using this technique Sanger succeeded in determining the sequence of amino acid in the molecule of insulin which contains 51 amino acids (2 peptide chains) 1 polypeptide chain contains 20 amino acids and the other contains 31 amino acids.

6. Reaction Siegfreds carbamino reaction

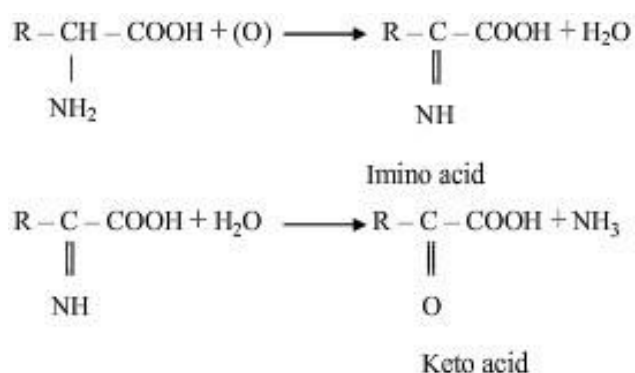
This reaction is called Siegfried's carbamino reaction.



Carbamino compounds are formed in the blood, when the $-NH_2$ group in hemoglobin reacts with CO_2 . Carbamino compounds of hemoglobin are important in the transport of CO_2 .

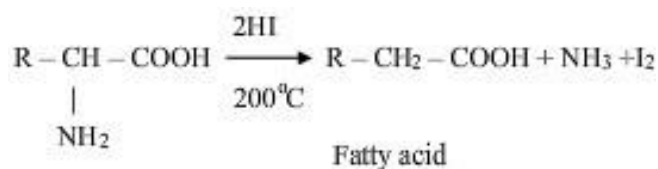
7. Reaction with ninhydrin

(Oxidative deamination): The -NH_2 group of an amino acid may be easily removed by oxidation. This process is called oxidative deamination. When an amino acid is heated with ninhydrin, the amino acid is deaminated and NH_3 and keto acid are released by oxidative deamination. The reaction occurs in two stages.



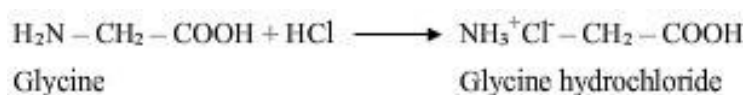
8. Reaction with HI

When amino acids are heated with hydroiodic acid at 200°C, the -NH_2 group is eliminated from the amino acid and they form fatty acid.



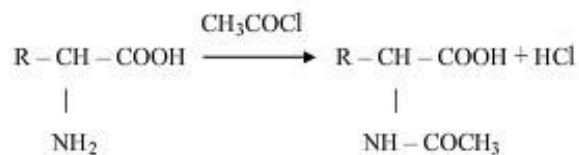
9. Reaction with strong acids

Glycine reacts with HCl to form glycine hydrochloride.



10. Acylation

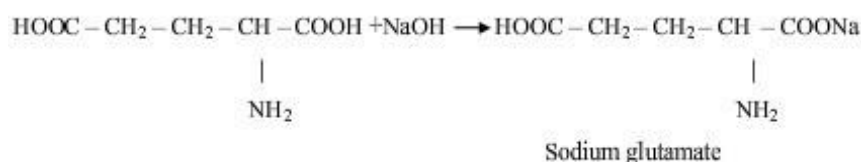
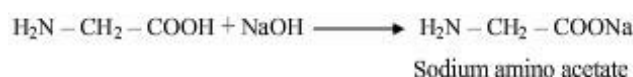
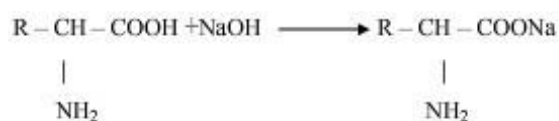
Amino acid reacts with acetyl chloride to form N acetyl amino acid.



II. Reaction due to the presence of –the COOH group

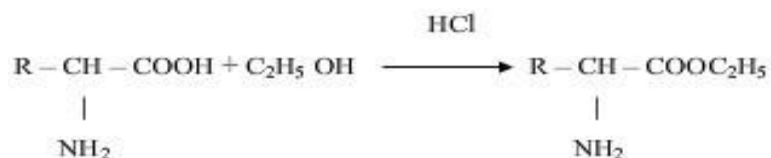
1. Reactions with alkalis: (Salt formation)

-COOH group of amino acids can release H^+ ions to the formation of COO^- . This can be neutralized by cations like Na^+ and to Ca^{2+} form salts. Thus, amino acid reacts with alkalis to form salts.



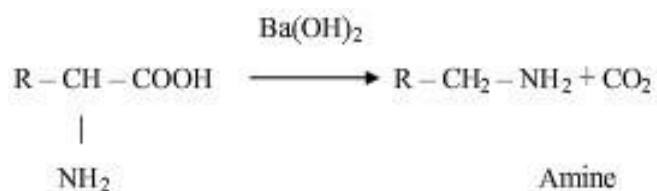
2. Formation of esters

Amino acids react with alcohol in the presence of HCl to form esters.

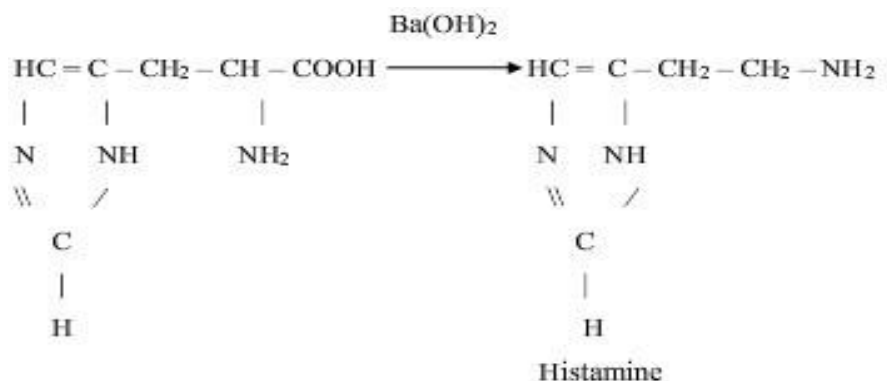


3. Decarboxylation Reaction and Formation Of Amines.

When amino acids are heated in the presence of $\text{Ba}(\text{OH})_2$, CO_2 is lost, and the corresponding amine is formed.

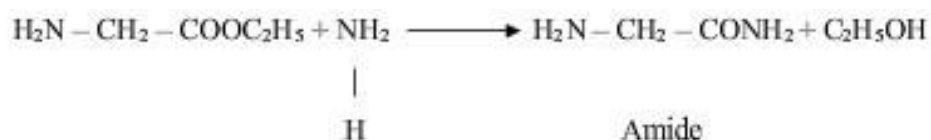


Decarboxylation of histidine to histamine takes place by enzymatic action of bacteria in the large intestine.



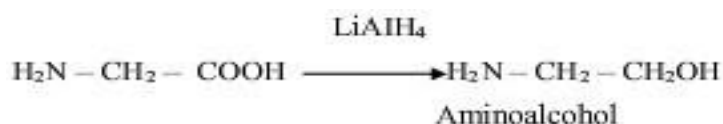
4. Amide formation

Amino acid esters when treated with alcoholic NH_3 to form corresponding amides.



5. Reaction with LiAlH_4

When amino acids are treated with LiAlH_4 amino alcohol is formed on reduction of $-\text{COOH}$ group to CH_2OH .

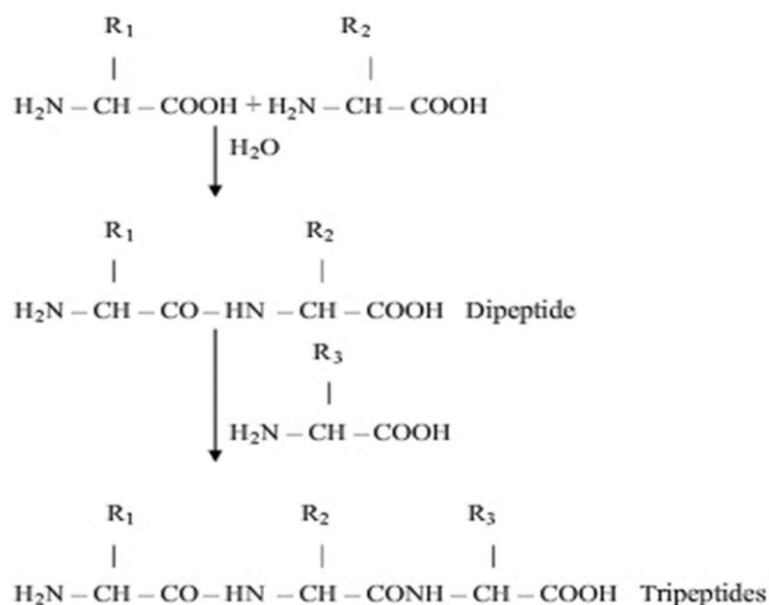


III. Reaction due to the presence of both $-\text{NH}_2$ and $-\text{COOH}$ group

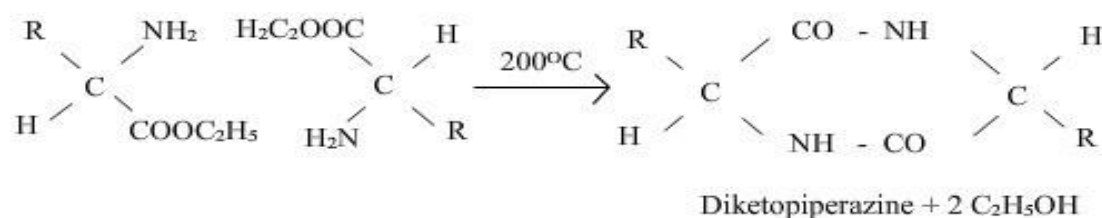
1. Peptide formation

$-\text{NH}_2$ group of 1 amino acid and $-\text{COOH}$ group of another condense to form peptide.

If 2 amino acids are involved in the peptide formation, the resulting product will be dipeptide. Similarly, when 3 amino acids condense it gives tripeptides.

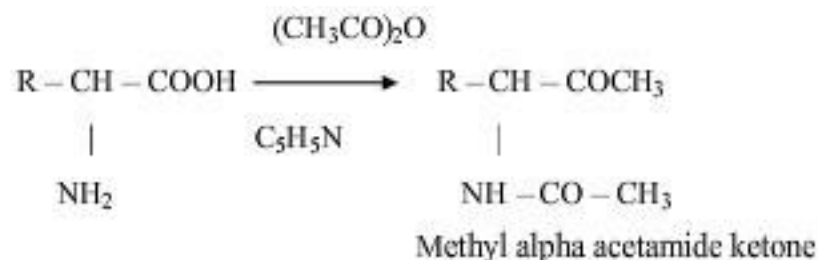


2. The esters of amino acids condense to form ring structure containing amino acid groups. These substances or compounds are called diketopiperazine.



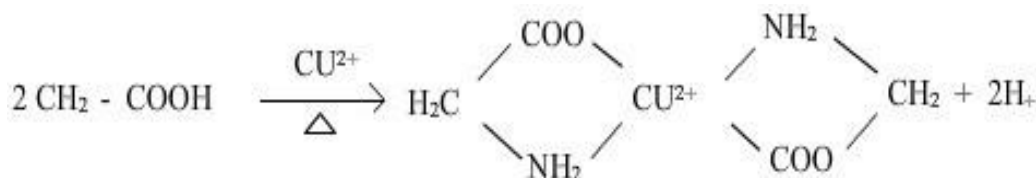
3. Darkin- West reaction

When amino acids are treated with acetic anhydride in pyridine solution, they are converted to methyl alpha acetamide ketones. This reaction is often referred as Darkin – West reaction.



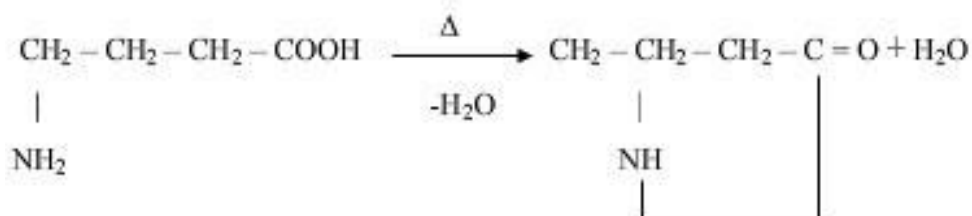
4. Chelation of amino acids with metal ions

Amino acids form complexes with several metal ions like Mn²⁺, Cu²⁺ and Fe²⁺ etc and metals are chelated to the amino acids and form complexes. For example, gly react with Cu²⁺ to form cupric glycinate.



5. Lactum ring formation

Aliphatic amino acids on heating eliminate H₂O molecule and form a cyclic structure known as lactum ring.



4.5 PHYSICAL PROPERTIES OF AMINO ACIDS

1. Most of the naturally occurring amino acids are solids. They are in the form of crystals. The crystals forms vary from slender needles to hexagonal plates.
2. **Colour:** All the amino acids are colorless and they are somewhat white in color.
3. **Taste:** Amino acids may be tasteless or sweet in taste or bitter in taste. Sodium salt of Glu-Na glutamate is valuable as a flavoring agent in the preparation of various food substances and sauce.
4. **Solubility:** In general, the amino acids are readily soluble in water(cold) but more soluble in hot water. Pro hydroxy pro are soluble in alcohol and ether.
5. **Melting point:** The amino acids in general have their melting points between 200 to 300°C. Most of them undergo decomposition at or near their melting point.
6. **Optical activity:** All amino acids except gly are optically active. Optical activity is the property of the molecules by virtue of which they can rotate the plane polarized light most of them rotate the plane polarized light to the left and they are called levo rotatory amino acid and are designated by (l) or (-) symbols. Some of them rotate the plane polarized light to right and they are called dextro rotatory amino acids and are designated by (d) or (+) symbols. This property is due to the presence of asymmetric carbon atom in amino acid molecules.
7. **Amphoteric (or) Ampholytes nature:** As amino acids contain 1-COOH and 1-NH₂ group they are called ampholytes or amphoteric compounds. They are capable of both donating and accepting protons. Due to the presence of an acidic

and basic group in molecules amino acids largely exist as dipolar or Zwitter ion or hybride ion in which a proton from the -COOH group has been transformed to the -NH_2 group and thus the dipolar ion contains both positive and negative charge.

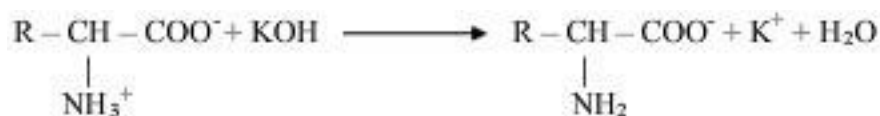
8. **Isoelectric point:** The isoelectric PH is the PH midway between PK values on either side of the isoionic species. Amino acids can exist as ampholytes or zwitter ion in solution, depending on the pH of the medium. The pH at which the molecule carries no net charges is known as isoelectric point or Iso electric pH (pI). In acidic solution, they are cationic in form and in alkaline solution, they behave as anions. At pI, of an amino acid solubility and buffering capacity will be minimum. The amino acids forming proteins contain both acidic and basic groups and react with both alkalies and acids to form salts. These amino acids are amphoteric and are called ampholytes or Zwitterion.



As amino acids are amphoteric compounds and as they contain both acidic and basic group they can react with both alkalis and acid to form salts. In acid solution, amino acid carry a ositive charge and move towards cathode in an electric field.



When an alkali is added to Zwitter ion, the amino acids becomes negatively charged and it move towards an anode.



At certain reactions around neutrality, the amino acids are electrically neutral. This is because the amino acid at this stage carries an equal number of positive and negative charges. The pH at which the amino acid has no tendency to move towards either a positive or negative electrode is known as isoelectric pH.

4.6 SUMMARY

Amino acids are compounds containing amino groups ($-\text{NH}_2$) and Carboxyl groups ($-\text{COOH}$). So amino acids are also called Amino Carboxylic acids.

Amino acids are essential components of all living cells. They are the building blocks of proteins. They are monomers of protein polymers.

Animals and plants contain about 200 proteins. But the human body contains only 20 amino acids of these 20 amino acids are used as building blocks in the synthesis of proteins. These 20 amino acids are repeatedly used to synthesize millions of proteins.

4.7 KEYWORDS**Amino acids**

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Composition of amino acids

1. An amino acid is made up of four components namely
2. A carbon atom- C
3. An amino group- NH_2
4. A carboxyl group- COOH
5. A side chain or Residue R

Classification of amino acids

1. Classification based on their incorporation in proteins.
2. Classification based on the structure of the side chain.
3. Classification based on the position of $-\text{NH}_2$ group.
4. Classification based on the reaction in solution.

5. Classification based on polarity.
6. Classification based on biological importance.

Amphoteric

Amphoteric (or) Ampholytes nature: As amino acids contain one COOH and one -NH_2 group they are called ampholytes or amphoteric compounds. They are capable of both donating and accepting protons.

D and L form of amino acids

Based on the configuration. Two types of -NH_2 exist. They are D and L -NH_2 . The amino acids having the -NH_2 group on the right side is called D amino acids. The amino acids having -NH_2 group on the left side are called L amino acids. These isomers are mirror images of each other.

Ninhydrin

The -NH_2 group of an amino acid may be easily removed by oxidation. This process is called oxidative deamination. When an amino acid is heated with ninhydrin, the amino acid is deaminated and NH_3 and keto acid are released by oxidative deamination. The reaction occurs in two stages.

EXERCISE

I; Essay Questions

1. What are amino acids?
2. Mention the composition of amino acids?
3. Write a note on the D and L form of amino acids?
4. Explain the physical and chemical properties of amino acids?
5. Give a detailed account on the classification of amino acids?
6. Write a note on the structural and characteristic function of insulin and glutathione
7. Write the structures of (i) a neutral amino acid, (ii) an acidic amino acid (iii) a basic amino acid
8. How is phenylalanine prepared? 3. What is α -amino acid? How are they related to proteins?
9. How are α -amino acids prepared? 5. Describe the important reactions of amino acids.

10. Write a note on isoelectric points of amino acids.
11. Write a note on Zwitter ion. (C) Multiple Choice Type Questions.

II Multiple Choice Questions

1. All natural amino acids: ()
(a) are d-rotatory (b) are l-rotatory
(c) have L-configuration (d) none
2. Amino acids are building blocks of: ()
(a) Carbohydrates (b) Fats
(c) Proteins (d) Lipids
3. Which of the following essential amino acids is not synthesized by the body? ()
a) Arginine b) Glutamine c) Histidine d) Proline
4. From which of the following components amino acids are generally synthesized?
()
a) Fatty acids b) α -ketoglutaric acid c) Mineral Salts d) Volatile acids
5. **Amino acids with the non-polar aliphatic 'R' group are ()**
a) Glycine, alanine, leucine b) Serine, threonine, cysteine c) Lysine, arginine, histidine d) Phenylalanine, tyrosine, and tryptophan
6. **Amino acids with aromatic side chains are ()**
a) tryptophan, asparagine, tyrosine b) tryptophan, threonine, tyrosine c) phenylalanine, tryptophan, serine d) phenylalanine, tryptophan, tyrosine.
7. **Which of the following amino acids yield Acetyl CoA during catabolism? ()**
a) Ketogenic b) Glucogenic c) Essential d) None of the above
8. **Out of these, which one is the non-essential amino acid? ()**
a) Lysine b) Threonine c) Serine d) Histidine
9. **Which of the following is an essential amino acid?**
a) Cysteine b) Asparagine c) Glutamine d) Phenylalanine
10. **The difference between amino acid and imino acid is ()**
a) Bonding of carbonyl group with the amide group b) Bonding of nitrogen in the amide group c) Bonding of nitrogen in the carbonyl group d) Presence of covalent bond between amide and carbonyl group

III Fill in the Blanks.

- 1) _____ is the amino acid that is both glucogenic and ketogenic in nature
- 2) _____ is the first amino acid in a polypeptide chain

- 3) The 21st amino acid is_____
- 4) One example of a non-essential amino acid_____
- 5) One example of an essential amino acid_____
- 6) _____ example of both glucogenic and ketogenic
- 7) The two amino acids having R groups with a negative net charge at pH 7.0 are_____
- 8) If $pK_1 = 2.34$ and $pK_2 = 9.60$, then the isoelectric point pI is_____
- 9) _____ is one example of an imino acid
- 10) Number of chiral centers in isoleucine is_____

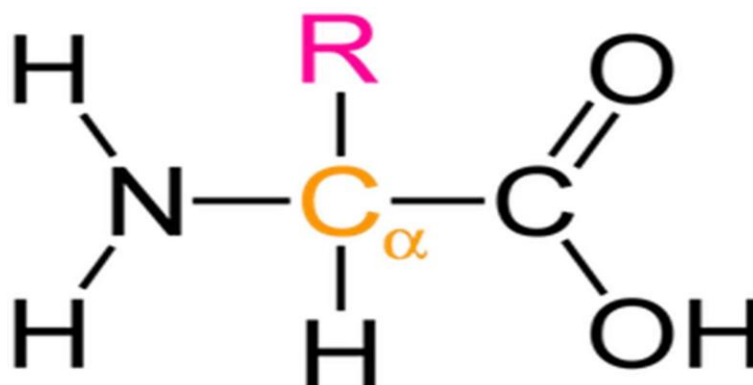
II Answers: 1) a 2) c 3) c 4) b 5) a 6) d 7) a 8) c 9) d 10) b

III Answers 1) Isoleucine 2) Methionine 3) Selenocysteine 4) Serine

5) Phenylalanine 6) soleucine 7) Aspartate and glutamate 8) 5.97 9) Proline 10) 2

CHAPTER-V

Proteins



5.0 INTRODUCTION

The word "protein" comes from the Greek word "protos," which translates to "of first importance." Proteins are essential for life. It is representative of a very large set of nitrogenous compounds that are of a complex nature and have a high molecular weight. Chains of alpha-amino acids are linked to one another in protein molecules via peptide connections. Protein molecules make up their parts. Like how carbohydrates are composed of monosaccharides, amino acids may be broken down into their constituent parts.

More than fifty percent of the cellular dry weight is made up of proteins, making them one of the most plentiful and varied classes of macromolecules. The crucial role that proteins play in practically every aspect of cellular structure and function is reflected in both their variety and abundance in cells. Only because of the intrinsic adaptability of proteins, each of which is precisely adapted to its biological job, is it possible for cells to carry out such an astonishing variety of different types of activities. DNA contains information that is encoded in a specific sequence of nucleotide bases. This sequence determines the pattern that is used to personalize each component of the organism. A gene is defined as a particular section of encoded information, and the expression of a gene results in the production of the protein that is encoded by that gene. This provides the cell with the capabilities that are special to that protein. Proteins are the primary determinants of cellular activity; in addition, they are the vehicles through which genetic information is expressed.

OCCURRENCE

Proteins are present in every cell that is alive. They mix with lipids and nucleic acids to generate complex proteins, which are key components of protoplasm, the cell membrane, and other cellular structures. Nucleoproteins make up genes, which are responsible for passing on inherited features. They are the fundamental components of cellular and organic structures, which means they are extremely important to the functioning of living organisms. Proteins are the building blocks of enzymes, which are the catalytic molecules that are necessary for all the metabolic reactions in the body.

5.1 CLASSIFICATION OF PROTEINS

Based on the shape of protein molecules, the proteins can be grouped under two categories.

1. Globular (or) Corpuscular proteins

These have an axial ratio <10 , usually not more than 3 or 4, and hence, possess a relatively spherical or ovoid shape.

These are usually soluble in H_2O or in an aqueous medium containing acids, bases, salts, or alcohol and diffuse readily. They are more complex in conformation than fibrous proteins and have a greater variety of biological functions. Nearly all enzymes are globular proteins. Protein hormones, antibodies, and nutrient storage proteins are also examples of globular proteins. A simple functional classification of these is not possible because.

- a. These proteins perform a variety of different functions.
- b. Many widely differing globular proteins perform almost similar functions.

2. Fibrous or Fibrillar proteins

These have an axial ratio > 10 and hence resemble long ribbons or fibers in shape. These are mainly of animal origin and insoluble in all common solvents such as H_2O , dilute acids, alkalis, and inorganic solvents.

Most of them are extremely strong and play a structural and protective role. It includes the proteins of connective tissues, bones, blood vessels, skins, hairs, nails, horns, hoofs, wool, and silk the important examples are.

a. Collagens

These form the major proteins of connective tissues and bones. More than half the total protein mammalian body is collagen; acted upon by boiling H₂O, dilute acids or alkalis, collagens produce soluble gelatins. They have high content –OH proline (12%) poor in sulfur- containing amino acids.

b. Elastin

These **form** the major constituents of yellow elastic tissues (ligaments, blood vessels) that differ from collagens in not being converted into soluble gelatin.

c. Keratins

These form major constituents of epithelial tissues (skin, hair, feather, hoofs, nails, horns) and usually contain large amounts of sulfur-containing amino acids. Human hair has about 14% of Di cysteine.

d. Fibroin

It is the principal constituent of fibers of silk, composed mainly of gly, ala, and ser units.

5.2 CLASSIFICATION BASED ON COMPOSITION AND SOLUBILITY

This system of classification divides the proteins into three major groups. They are.

1. Simple
2. Conjugated
3. Derived proteins

I. SIMPLE PROTEINS

These are of globular type except for sclera proteins which are fibrous in nature. This group includes proteins containing only amino acids as structural compounds. On decomposition with acids, these liberate the constituent amino acids. These are further classified mainly their solubility bases and are as follows.

1. Protamine and Histones

These are basic proteins and occur almost entirely in animals; soluble in H₂O not coagulated by heat, strongly basic containing lys and arg. Forms salts with mineral acids. Histones are somewhat weaker bases, and are therefore insoluble in NH₄OH

solution, whereas the protamines are soluble. (eg) Protamines – saline Histones – Nucleo histones of nuclei.

2. Albumins

These are widely distributed in seeds; soluble in H₂O and dilute solutions of acids, bases and salts; precipitated with saturated solution NH₄SO₄ or NaSO₄ coagulated by heat. **(eg)** Serum albumin from blood plasma Lactalbumin of milk.

3. Globulins

These are of two types, Pseudo globulins and Euglobulins. euglobulin are more widely distributed in nature than pseudo globulins, either soluble (Pseudo globulin) or insoluble (euglobulin) in H₂O, Precipitate with half saturated solution of NH₄SO₄ coagulated by heat. **(eg)** Pseudo globulin- milk whey Euglobulin- Serum globulin, Legumin from peas.

4. Glutelin

Isolated from plant seeds; insoluble in H₂O and dilute salt solutions in dilute acids and alkalis; coagulated by heat. **(e.g.)** Glutelin from corn.

5. Prolamines

Isolated from plant seeds, insoluble in H₂O and dilute salt solution but soluble in dilute acids and alkalis; not coagulated by heat **(e.g.)** Zein from corn.

6. Sclero protein (or) Albuminoids

These occur almost entirely in animals and are therefore commonly known as the animal skeleton proteins; insoluble in H₂O, dilute solutions acid bases, and salts; not attacked by enzymes. **(e.g.)** Collagen of bones. Elastin in ligaments Keratin in hair Fibroin in silk.

II. 5.2.1: CONJUGATED (OR) COMPLEX PROTEINS

These are also globular types. These are the proteins linked with a separable non-protein part called a prosthetic group. The prosthetic group is either a metal or a compound. On decomposition with acids, these liberate constituent amino acid as well as a prosthetic group. Their further classification is based on the nature the of prosthetic group present in the various divisions are.

1. Metallo proteins
2. Chromo proteins
3. Glyco proteins
4. Phospho proteins
5. Lipo proteins
6. Nucleo proteins

1. Metallo proteins

These are the proteins linked with various metals. Based on their reactivity with metal ions, the metalloproteins can be classified into two groups.

1. Metals strongly bound by proteins: Some heavy metals like Cu, Zn become strongly bound with proteins like collagen, albumin, kaesin etc., through the –SH radicals of the side chain. Some other proteins have a strong affinity for Fe (Siderophilin) and Cu (ceruloplasmin).
2. Metals weakly bound by proteins: Ca belongs to this category. Here the binding takes place with the help of radicals possessing e^- charge.

2. Chromo proteins

These are the proteins that couple with colored pigments. Such pigments have also been found among enzyme catalase and flavor enzymes. Similarly, chlorophyll is present in leaf cells in the form of alpha proteins chloroplasts. Other examples include hemoglobin and cytochrome.

3. Glycoproteins and mucoproteins

These are the proteins that contain carbohydrates as a prosthetic group. Glycoproteins contain small amounts of carbohydrates (<4%) whereas mucoproteins contain comparatively higher amounts (>4%) (**e.g.**) Glycoproteins – Egg albumin, serum albumin, and globulins.

(**e.g.**) Mucoproteins – mucin from saliva

4. Phospho proteins

These are proteins linked with H_3PO_4 ; mainly acidic. (**e.g.**) casein from milk, vitellin from egg yolk.

5. Lipoproteins

These are proteins that form complexes with lipid soluble in H₂O but insoluble in organicsolvents. (e.g.) lipo vitellin form egg yolk.

6. Nucleo proteins

These are compounds containing nucleic acid and proteins. (e.g.) Protamines and Histones. These are present in nuclear substances as well as in cytoplasm and are considered as the sites for the synthesis of proteins and enzymes and are attached to each other by means of electrostatic forces.

III. 5.2.2: DERIVED PROTEINS

These are derivatives of proteins resulting from the action of heat, acid, alkali, etc.

1. Primary derived proteins

These are derivatives of proteins in which the size of a protein molecule is not altered.

a. Proteans

Insoluble in H₂O appear as the first product produced by the action of acids, enzymes or H₂O (Eg)Myosan from myosin.

b. Meta proteins

Insoluble in H₂O. But soluble in dilute acids or alkalis, produced by acids or alkalis on proteins at 30-60°C (e.g.) Acid and Alkali- infra proteins.

c. Coagulated proteins

Insoluble in H₂O produced by the action of heat or alcohol on proteins (e.g.) egg white.

2. Secondary-derived proteins

These are derivatives of proteins in which hydrolysis has occurred and are smaller than the original proteins.

a. Proteoses

Soluble in H₂O coagulated by heat; produced when hydrolyses proceed beyond the level of meta proteins. Primary proteoses are salted out by half-saturated NH₄SO₄ and precipitated by HNO₃ and picric acid. Secondary proteoses are salted out by

completely saturated NH_4SO_4 but not precipitated by HNO_3 or picric acid (e.g.) Albuminoses from albumin.

b. Peptones

Soluble in H_2O not coagulated by heat produced by the action of dilute acids or enzymes when hydrolysis proceeds beyond proteoses; neither salted out by NH_4SO_4 or precipitated by HNO_3 or picric acid.

c. Poly peptides

These are combinations of two or more amino acid units, in fact, proteins are essentially long-chain polypeptides.

PHYSICAL PROPERTIES

1. Color and taste

These are colorless and usually tasteless.

2. Shape and size

They range in shape from simple crystalline spherical structures to long fibrillar structures. The two distinct patterns of the shape of proteins include. Globular-Pepsin, RNase. Fibrillar proteins- Fibrinogen, Myosin.

3. Molecular weight

The extraordinary size and high reactivity of proteins has rendered the determination of molecular weight of proteins as a difficult task. However, the proteins generally have a large molecular weight range between 5×10^3 and 1.1×10^6 .

4. Colloidal nature

Because of their greater size they exhibit many colloidal properties like Tyndall effect.

5. Denaturation

It refers to the changes in properties of proteins, in other words it is a loss of biological activity.

6. Amphoteric nature

Iso electric point is the pH value at which the number of cations is equal to a number of anions. Thus proteins will have net electric charge of zero at this point. At pH

values lower than IEP, the protein will have a net positive charge as cation and migrate towards negative pole (cathode). Similarly at pH value higher than I.E.P., the protein will have a net negative charge as an anion and move towards positive pole (anode).

7. Solubility

The solubility of proteins is influenced by pH. The solubility is lowest at I.E.P. This is because when the protein molecule exists as either cations or anions, repulsive forces between the ions are high. Thus they will be more soluble than in I.E.P.

a. Salting-in effect

Globulins were sparingly soluble in H₂O. But their solubility is greatly increased by salts like NaCl. This phenomenon is described as salting-in effect.

b. Salting out effect

Proteins are precipitated by high concentrations of salts like NH₄SO₄, Na₂SO₄ etc.

c. I.E. Precipitation

Some proteins like casein of milk are precipitated at or near their I.E. point, and is referred to as I.E. precipitation.

8. Optical activity

All most all the proteins rotate the plane polarized light to the left and thus they are levo rotatory.

5.3 : CHEMICAL PROPERTIES

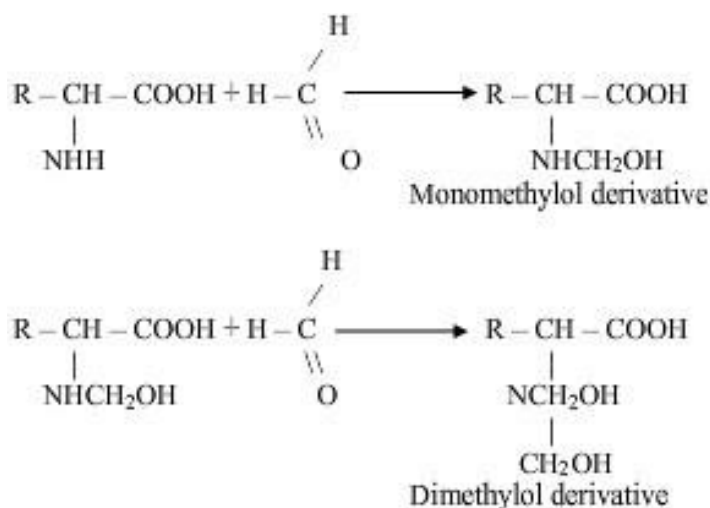
I. Reactions involving –NH₂ group

1. Reaction with HCl

When proteins are treated with mineral acids like HCl, the acid salts are formed.



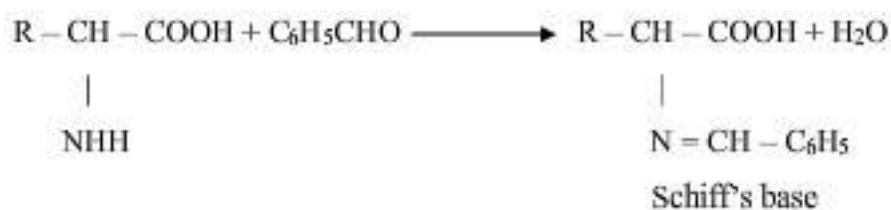
2. Reaction with HCHO: With HCHO, the hydroxy methyl derivatives are formed. These derivatives are insoluble in H₂O and resistant to attack by microorganisms. Because of this property it is used as preservative.



This reaction is the basis for Sorenson formal titration method for determining the purity of amino acid.

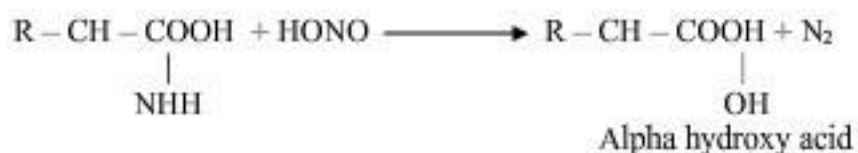
3. Reaction with C₆H₅CHO

Schiff's bases are formed on its reaction C₆H₅CHO



4. Reaction with HONO (Vanslyke's reaction)

When amino acid reacts with HONO to liberate N₂ gas and produce corresponding alpha-hydroxy acids.



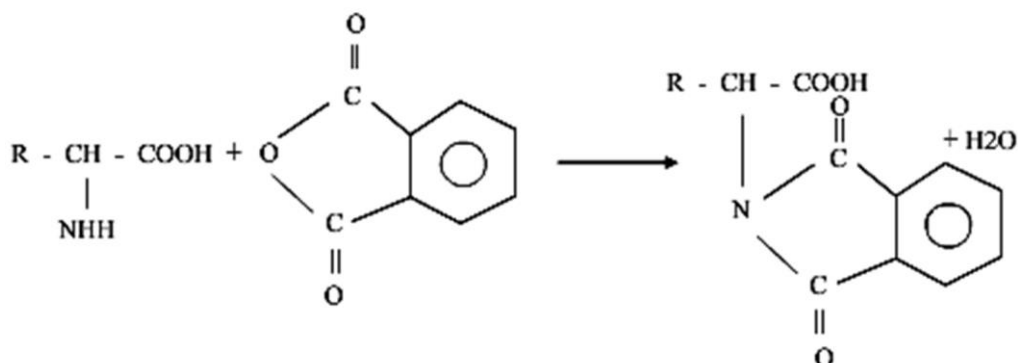
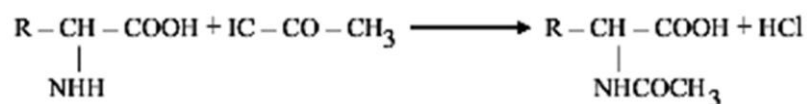
Proline and hydroxyproline do not respond to this reaction.

5. Reaction with acylating agent

This reaction is brought about by CH₃COCl, C₆H₅COCl, and phthalic anhydride and also by acetic anhydride.

Reaction with Sanger's reagent

Amino acid reacts with FDNB to produce a yellow colour derivative –DNP amino acid.



This reaction is valuable in the elucidation of protein structure.

II. Reaction Based On – COOH group

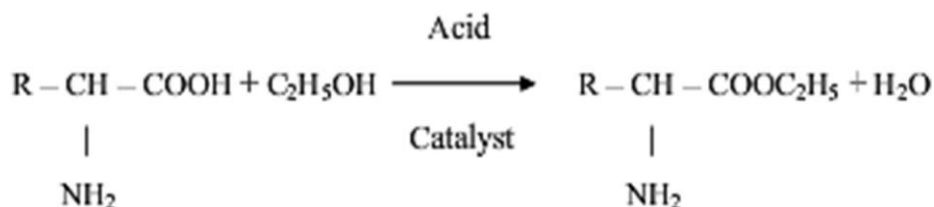
1. The – COOH group of amino acid can release H^+ ion with the formation of COO^- ion, which may be neutralized by cations like Na^+ and Ca^{2+} to form salts. Thus amino acid with alkalis to form salts.



Na salt of glu used commercially as flavouring agent.

2. Reaction with alcohol (Esterification)

With alcohols corresponding esters are produced.



3. Reaction to amine

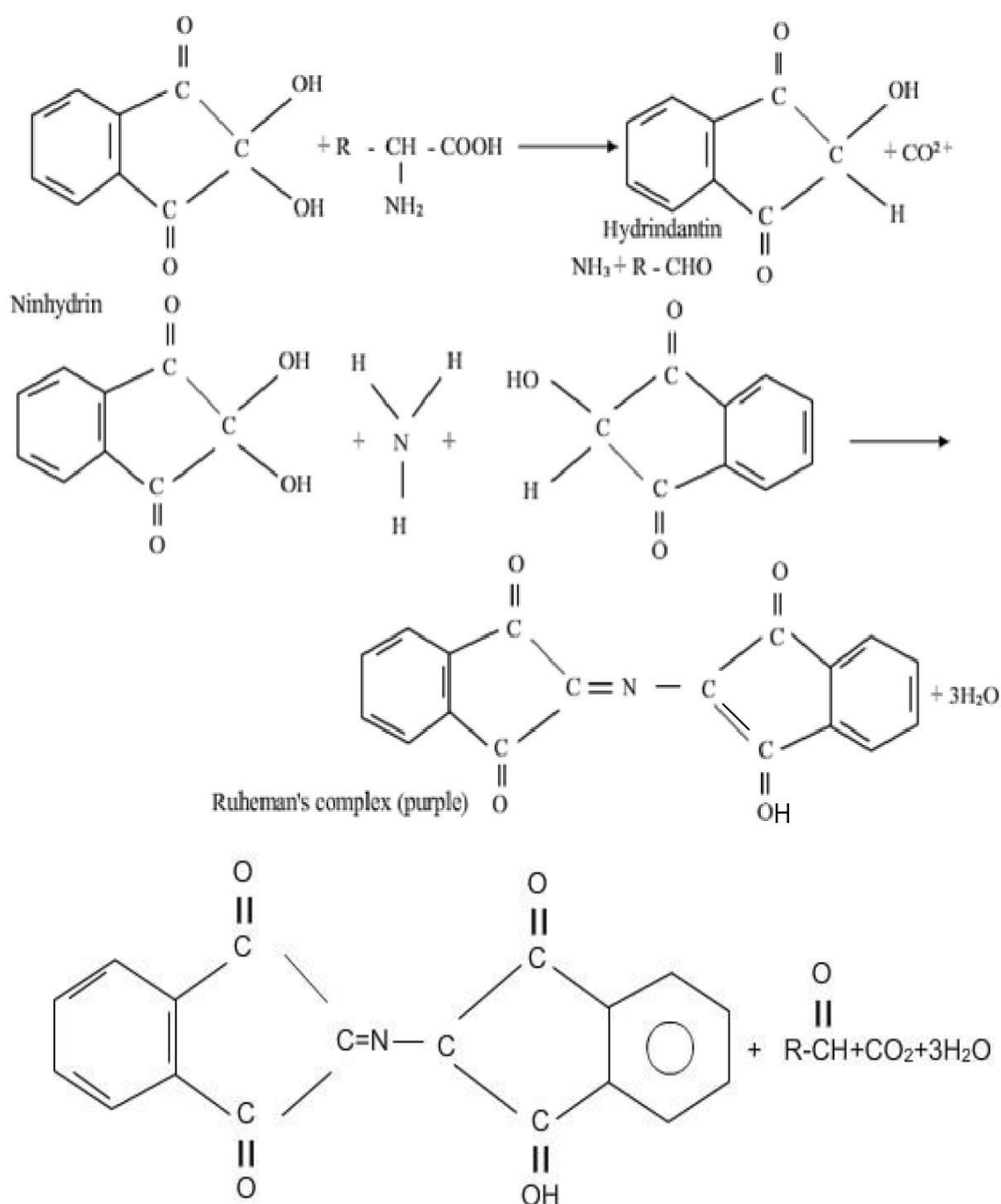
Amino acid reacts with amines and form amide.



REACTIONS INVOLVING BOTH NH₂ AND –COOH GROUPS

1. Reaction with ninhydrin

Ninhydrin (Indane – 1,2,3, trione hydrate) is a powerful oxidizing agent and causes oxidative-decarboxylation of alpha amino acid, producing CO₂, NH₃, and an-RCHO. The reduced ninhydrin (Hydrindantin) reacts with liberated NH₃ and a mole of ninhydrin forms Ruheman's complex.

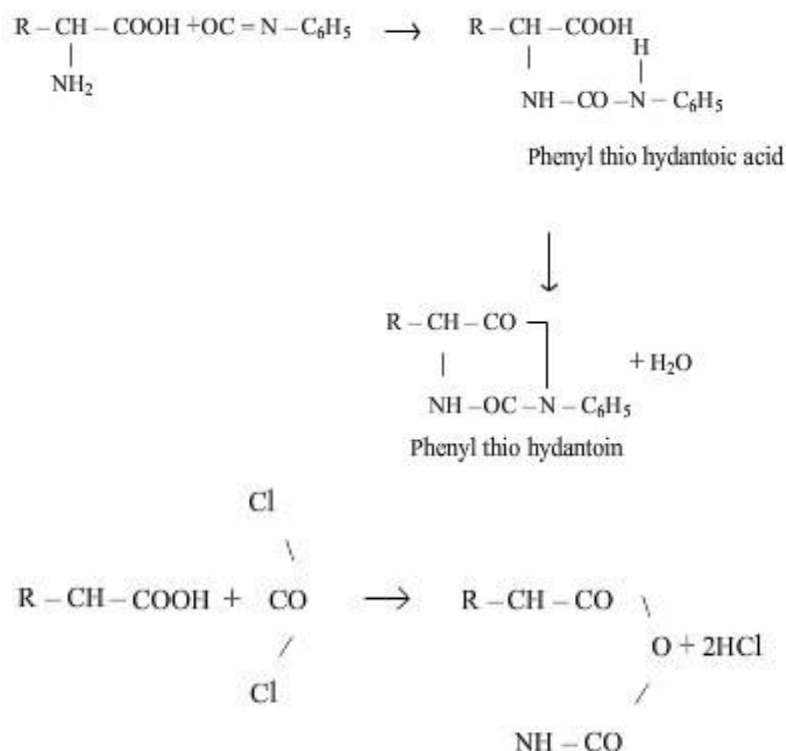


This reaction is extremely sensitive amines other than alpha amino acid react with ninhydrin forming a blue complex but without evolving CO₂. The evolution of CO₂ is thus an indication of the presence of alpha amino acid. Proline and –OH proline

however produce yellow complexes with ninhydrin.

2. Reaction with phenyl isocyanate

With phenyl isothiocyanate, phenyl thiohydantoic acid is formed which in turn can be converted into thiohydantoin.

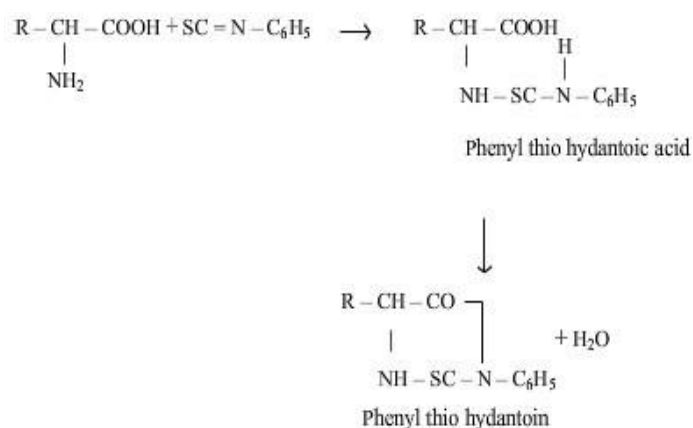


Reaction with phenyl isothiocyanate

(Edmann's reagent): Phenyl isothiocyanate also reacts with amino acid and produces thiohydantoic acid which then cyclises to form thiohydantoin.

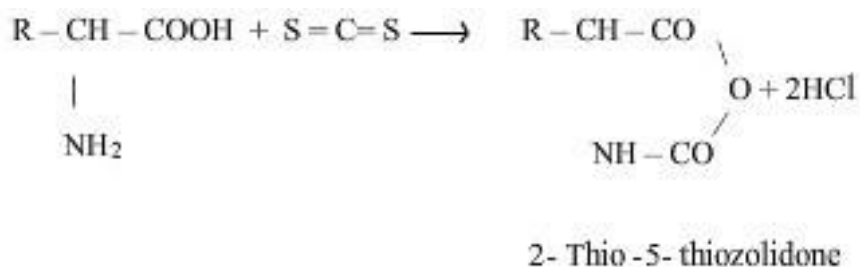
Reaction with phosgene

With phosgene amino acid produces carboxy anhydride.



4. Reaction with carbon di sulphide

With CS₂ amino acid produces two thio thiolidone.



BIOLOGICAL FUNCTIONS OF PROTEINS

Proteins play a number of important biological functions and they are.

1. Enzyme catalyst

All most all chemical reactions in biological system are catalyst by enzymes. These enzymes exhibit enormous catalytic power. They increase the reaction rate atleast million- fold.

2. Transport

Proteins transport irons and small molecules.

- a.** Haemoglobin: A conjugated protein of blood transport O₂
- b.** Transferrin :Carries iron in plasma of blood .
- c.** The membrane proteins transport mucous, amino acid and other nutrients across the membrane in to cell.
- d.** The lipoproteins or plasma carry lipids from liver to other organs.

3. Storage

Certain proteins functions as storage molecule. Ferritin a protein stores iron in the liver.

4. Nutrients

Certain proteins function as nutrients. (eg) ovaalbumin of egg, casein of milk.

5. Contraction and movement

The contraction of muscle is brought about by actin, myosin. The micro tubules of flagella, cilia are built on tubulin which are proteins.

6. Mechanical support

Many proteins serve as supporting structure and also give strength and protection.

- a. **Collagen:** Many components of tendons
- b. **Elastin :** Structural protein of ligament
- c. **Keratin :** Main component of hair, nail etc

Proteins

7. Immune protection

Many proteins protect our body against invasion of many micro organisms like bacteria,virus etc. Antibodies of our body neutralize the foreign microbes are proteins.

8. Blood clotting

Blood leakage is stopped by formation of clot. Clotting is brought about by bloodclotting factors (proteins) such as fibrinogen and thrombin.

9. Hormonal action

Many hormones are proteins (eg) TSH: It is essential for growth and to reproduction.

10. Thermo regulation

The blood plasma of antartic fishes contain antifreeze proteins which protect their blood from clotting/freezing.

11. Transmission of nerve impulse

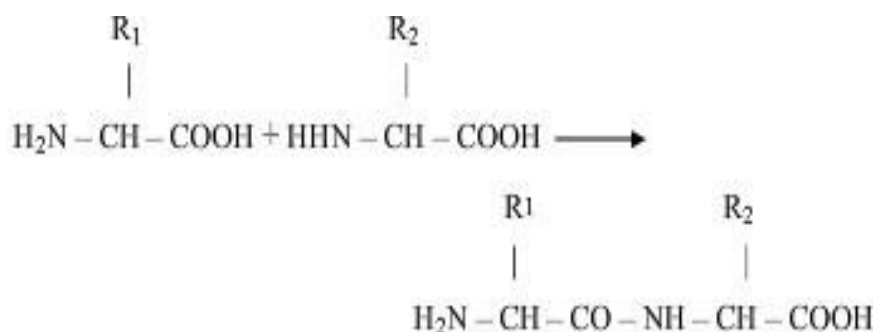
The nerve impulse is transmitted through synapse with the help of receptor proteins.

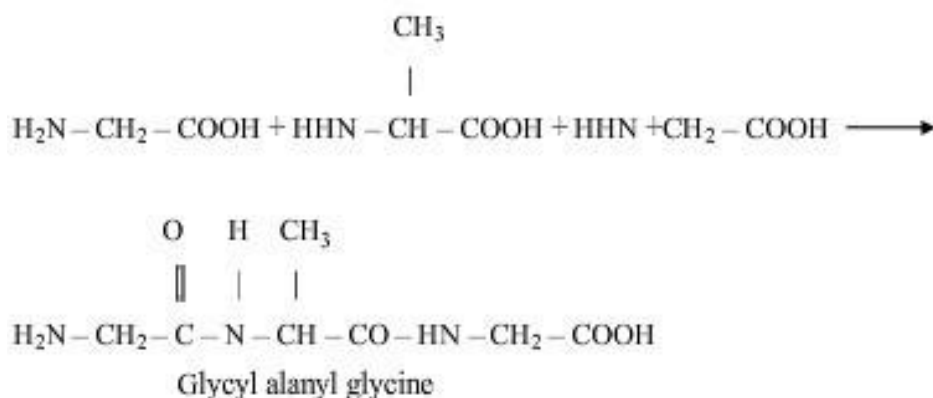
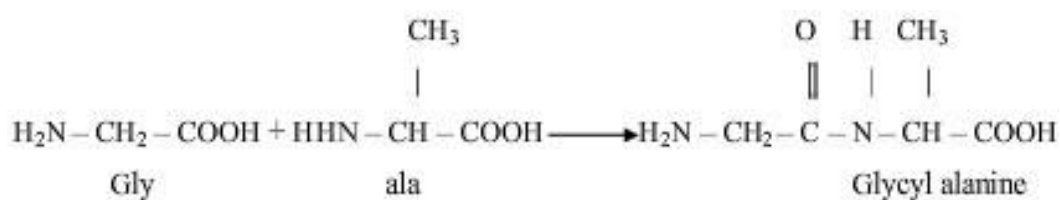
12. Gene expression

In a cell all the genes are not activated at a time, and they remain inactive . The inactivation of gene is brought about by repressor proteins.

PEPTIDES

Peptides consists of two or more amino acids linked by peptide bond which is formed between -COOH group of one amino acid and -NH_2 group of another amino acid with removal of one molecule of H_2O . When two amino acids combine dipeptide is formed.

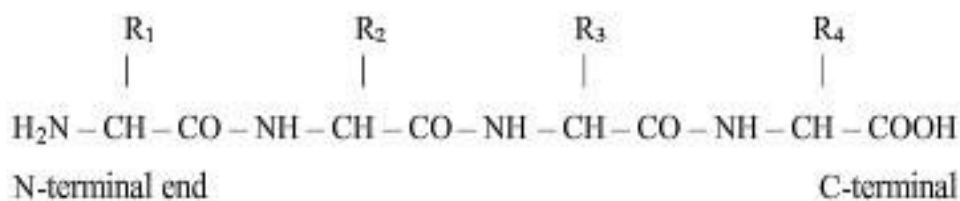




Similarly, tripeptides are formed by linkage of three amino acid, tetrapeptides by linkage of four amino acid and so on. When many amino acid are joined together, compound is called poly peptide.

PRIMARY STRUCTURE OF PEPTIDES

The sequence of amino acid in a polypeptide is referred as primary structure of peptides. The N- terminal amino acid (which contains free $-\text{NH}_2$ group) is always written on left side and C-Terminal amino acid (which contains $-\text{COOH}$ group) in the right side of polypeptide chain.



Even small changes in primary structure may produce remarkable effects. (eg) sickle cell anemia. In this condition glutamic acids of beta- chain at sixth position is replaced by Valine.

Nomenclature of polypeptides

When peptides are named amino or N-Terminal end denoted the end with free $-\text{NH}_2$ group and C-terminal with free $-\text{COOH}$ group. The names of amino acid in a

polypeptide are written from left to right replacing the terminal 'ine' with 'yl' whose –COOH has been involved in linkage.

NATURALLY OCCURRING PEPTIDES

There are many naturally occurring peptides that are of biologically important compounds. These peptides include mammalian peptide hormones, growth factors, and bacterial products.

S.No	Peptide	Biological importance	No.of amino acid
1	ACTH	Stimulates cortex of adrenal glands	39
2	Glutathione	Reducing agents	3
3	Gramicidin -A	Antibiotic	10

5.4 CLASSIFICATION OF PEPTIDES

Peptides have been classified in different ways. Those peptides which on hydrolysis gives only amino acid are called Homeomeric peptides. While those peptides which give other products in addition to amino acid are called Heteromeric peptides.

5.5 DETERMINATION OF AMINO ACID SEQUENCE OF A POLYPEPTIDE

To understand the primary structure of peptide we have to determine.

1. An at N-terminal end of polypeptide chain
2. An at C-terminal end of polypeptide chain
3. Those amino acid that occurs between N and C-terminal amino acid and their sequential arrangement.

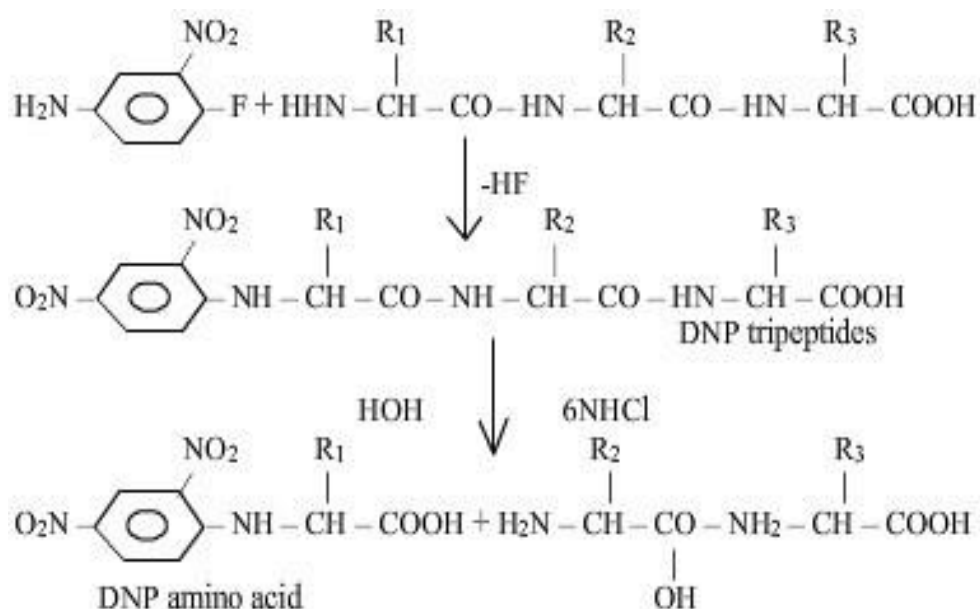
I. Determination N-Terminal Amino Acid:

There are several methods for determination of N terminal amino acid.

1. Sanger's method

The first useful method for N terminal residue of polypeptide was described by Sanger.

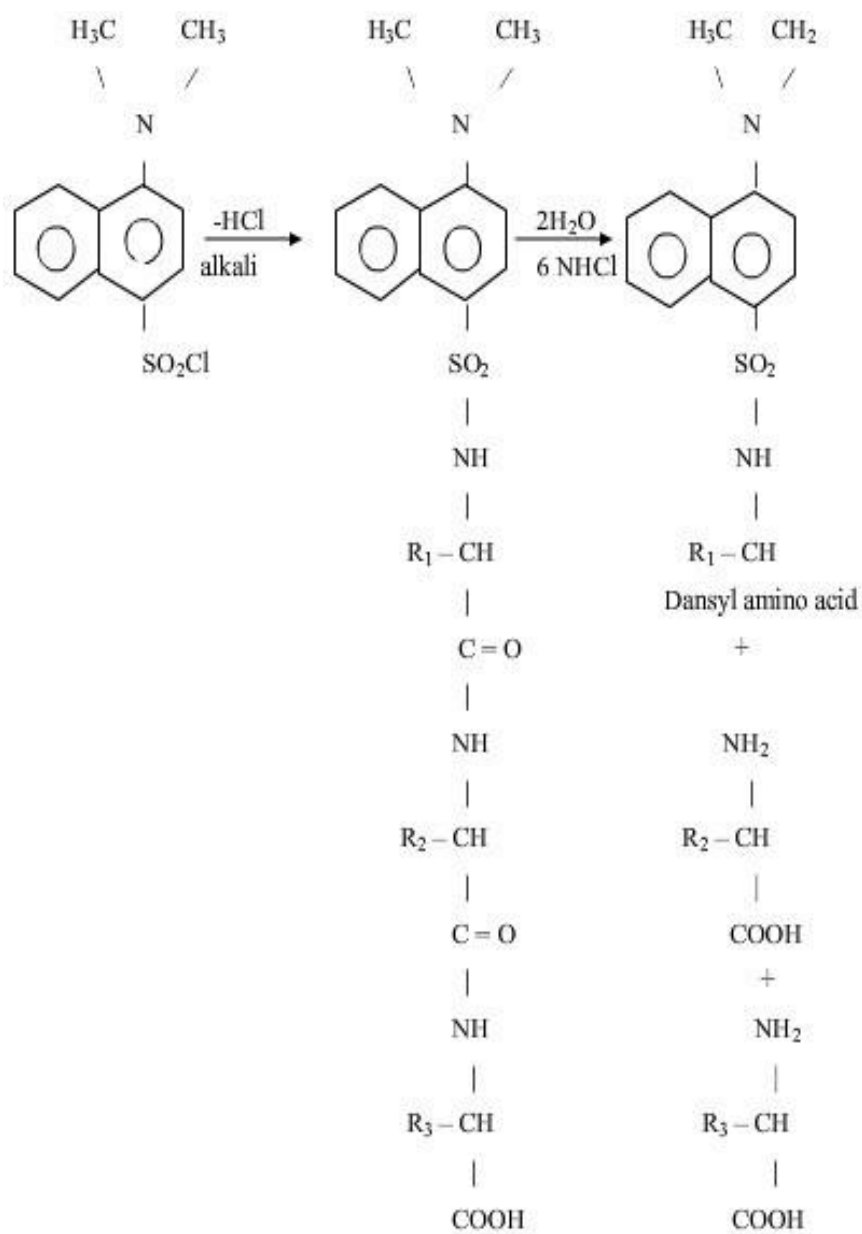
In this method Sanger's reagent- FDNB was used.



Free $-\text{NH}_2$ group reacts with FDNB to form yellow colour 2,4 di nitrophenyl derivative, which is then subjected to hydrolysis with 6NHCl. All the peptide bonds are hydrolysed but the bond between DNP group and free $-\text{NH}_2$ group of N-terminal amino acid is relatively stable to acid hydrolysis. This DNP labeled amino acid can easily be separated from free amino acid and identified by paper chromatographic technique.

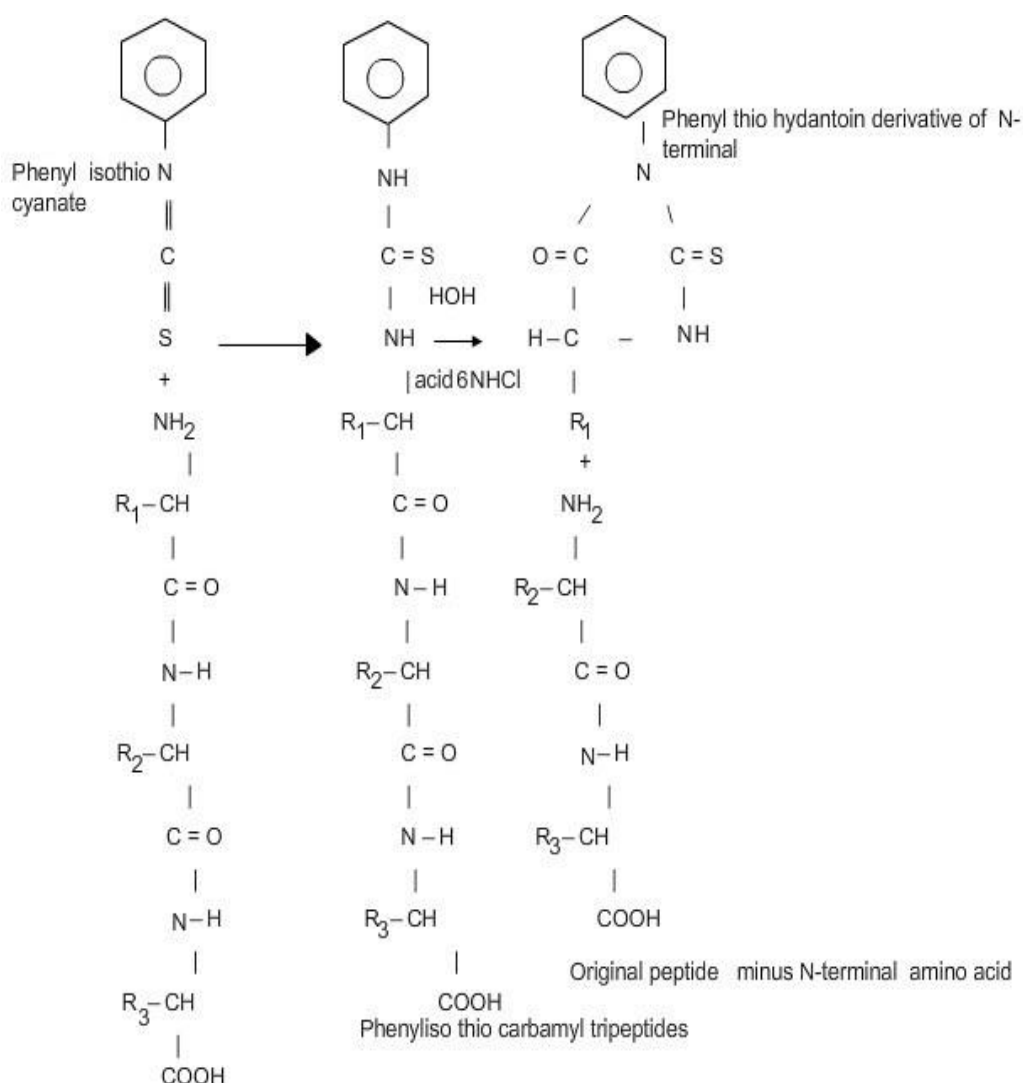
2. Dansyl method

The highly sensitive method called Dansyl procedure has been used for the determination of N-terminal amino acid. In this method the reagent used is Dimethyl amino naphthalene sulphonyl chloride. The reaction of Dansyl chloride and peptide produce an acidstable Dansyl derivative of N-terminal residue along with other amino acid. This is represented as Dansyl peptide. The hydrolyses of Dansyl peptide with 6 NHCl produces free amino acid and Dansyl derivative of N-terminal amino acid which can be identified by paper electrophoresis.



3. Edmann's method

In this method the polypeptide is treated with Edmann's reagent (phenyl iso thio cyanate) in the presence of dilute acid. The various step are as follows



Thus the terminal amino acid residue having free amino acid have been picked up by the reagent as phenyl iso thio hydantoin while the remainder of the polypeptide left as such at the end of the reaction. The phenyl iso thio hydantoin derivative may be separated and identified by paper chromatography. The reagent can be used again on the newly formed polypeptide (remainder of the polypeptide) to give the second end amino acid and so on. This method is now used to determine the amino acid sequence in polypeptide.

3. Enzymatic method

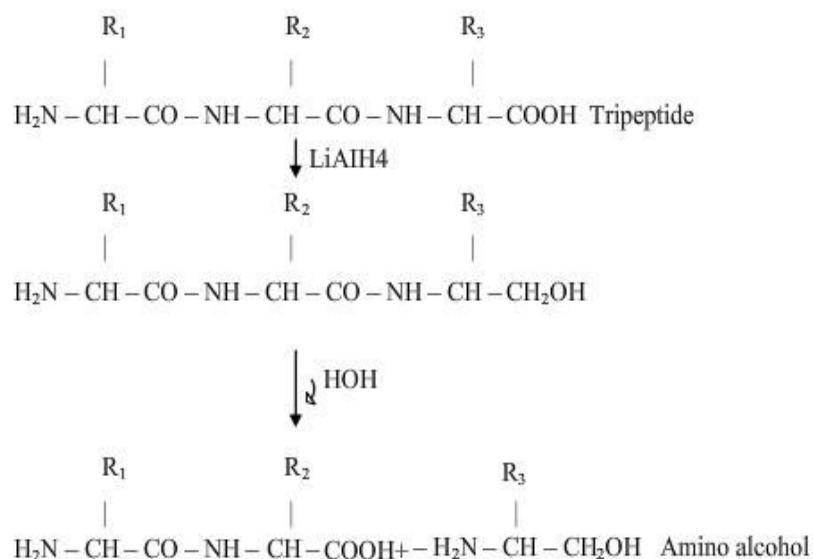
The enzyme amino peptidase attacks peptides only at the end which contains free. -NH_2 group.

II. DETERMINATION OF C- TERMINAL AMINO ACID

There are three methods in the determination of C-terminal amino acid.

1. Reductive method

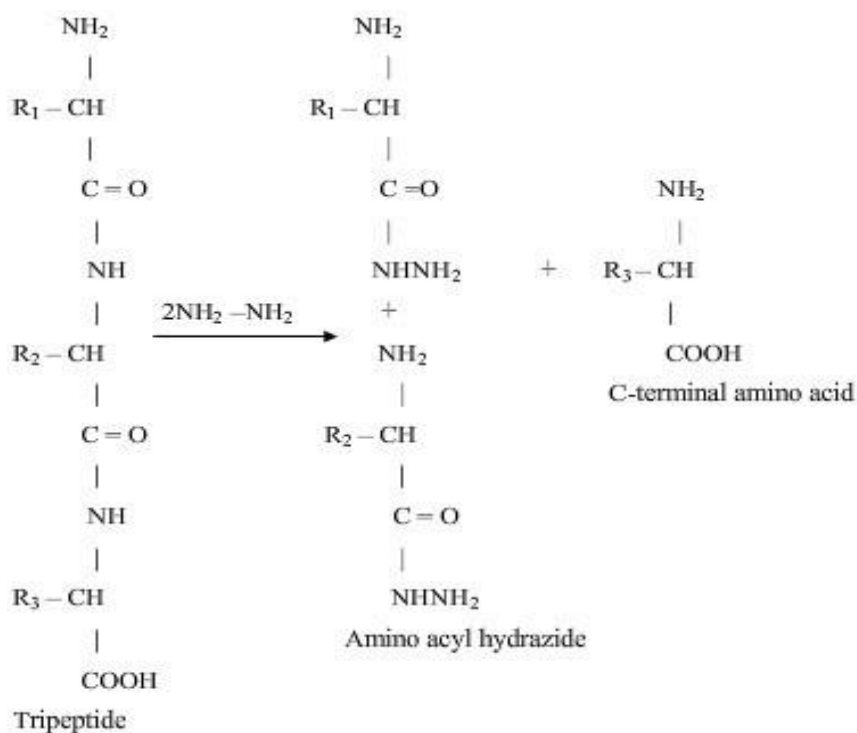
This method is because LiAlH_4 reduces the free terminal $-\text{COOH}$ group of the peptide to a primary alcoholic group and the product on hydrolysis gives amino acid along with remaining peptide.



The amino alcohol is identified by paper chromatography and the process is repeated on the remaining peptide.

4. Hydrazinolysis

The peptide is heated with anhydrous hydrazine 100°C which converts all the amino acid except the $-\text{COOH}$ end of the chain into amino acyl hydrazides.



Here the C- terminal amino acid appears as a free amino acid which can be readily identified chromatographically.

5. Enzymatic method

Carboxyl peptidase is an enzyme which attacks peptides only at the end which contains free -COOH group.

III. DETERMINATION OF SEQUENCE OF AMINO ACID

Once the N-terminal and C- terminal amino acid residue of a polypeptide chain have been identified, the next step is the determination of sequence of amino acid. This is accomplished by the partial or selective hydrolysis of the polypeptide chain. Sometimes this can be achieved by partial hydrolysis with dilute acid.

The enzyme proteases which hydrolyze peptide bonds are also used for the partial hydrolysis of peptides. Several highly purified proteases have been used for this purpose. The most specific enzyme is trypsin, a digestive enzyme secreted from pancreas in the form of the inactive precursor trypsinogen. Trypsin catalyzes the hydrolysis of only those peptide bonds in a polypeptide chain whose -COOH group is donated by either a lysine or an arg residue. On the other hand, chymotrypsin is able to split peptide bonds in which the -COOH group forms a part of Phe and Tyr or Try residue. By using these two enzymes separately the peptide or protein may be splitted in different ways to yield large fragments of peptide. Some other enzymes like carboxy peptidase, amino peptidase, pepsin, papain is also known. These also have specific amino acid residues. The best chemical method is the action of cyanogen bromide on the peptide in aqueous HCOOH at to room temperature. Cyanogen bromide breaks peptide bonds whose -COOH group is contributed by a Methionine residue.

After the hydrolysis the resulting products obtained are identified by paper chromatography, paper electrophoresis etc.

III ORDERING PEPTIDE FRAGMENTS BY ESTABLISHING OVERLAPS.

After the identification of N terminal and C- terminal amino acid residues and the amino acid sequences in the fragments of peptides by applying Trypsin and Cyanogen bromide. The final step is the ordering the peptide fragments and to check out for

possible errors in determining the amino acid sequences of each fragment.

To determine the position ordering of fragments in polypeptide chain, it is necessary to prepare overlapping peptides. These are formed by treating separate samples of the original protein with reagents that cleave the polypeptide chain at different sites. Overlapping peptides act as bridges in ordering the peptide fragments because a peptide produced by one reagent overlaps sequences corresponding to N and C terminal sequence of fragments produced by a different reagent.

For example, the cleavage of a polypeptide by cyanogen bromide provides a peptide, that overlaps the end of the peptide produced by the action of Trypsin.

1. Cleavage with cyanogen bromide:Original peptide.

Gly- His- Phe- Leu- Arg –Ala- met – Lys-gly- Val –Leu



Cleavage with cyanogen bromide at met

Gly- His- phe –leu- Arg- Ala- met -lys- gly-val- Leu = peptide X

2. Use cyanogen bromide peptides to determine the order of trypsin peptides

Peptide A Peptide B Peptide C Gly- His- phe- Leu- Arg / Ala- gly- met – lys / gly – val- Leu Gly His- phe –leu –Arg- ala – gly – met / lys – gly –Val – Leu

Peptide X Peptide Y

Original sequence of peptides

Gly- His – Phe – Leu – Arg – Ala –Gly- Met – Lys – Gly – Val- Leu

5.6 DENATURATION OF PROTEINS

This organization of the native (naturally occurring) protein molecule by which the specific configuration of a regular arrangement of a protein molecule is altered to an irregular diffused arrangement. So, this leads to a change in the physical and chemical properties of affected proteins. The changes of properties in proteins is called Denaturation. In other words, it is the loss of biological activity. In many incidents, this process is followed by coagulation. A process where denatured protein molecules tend to form large aggregates.

Denaturation involves a mere unfolding of peptide chains or splitting of proteins into smaller units.

Denaturation can be brought about by a variety of agents.

1. Physical agents

Heat, UV rays, X-rays, high pressure, violent shaking, Cooling, freezing, and rubbing operations.

2. Chemical agents

Acids, alkalies, organic solvents, alcohols, urea, high concentration of salts or heavy metals, detergents, and ionizing radiations.

The following changes takes place in

1. Physical changes:

- a. Solubility decreases.
- b. Surface tension is altered.
- c. Viscosity increases
- d. Denatured proteins cannot be crystallized because crystallization depends on high degree of organization of molecules (eg) Albumin of egg white.

2. Chemical changes

The structure of peptide chains of proteins have in coiled form by H, disulphide, salt (ionic) linkage and hydrophobic bonds.

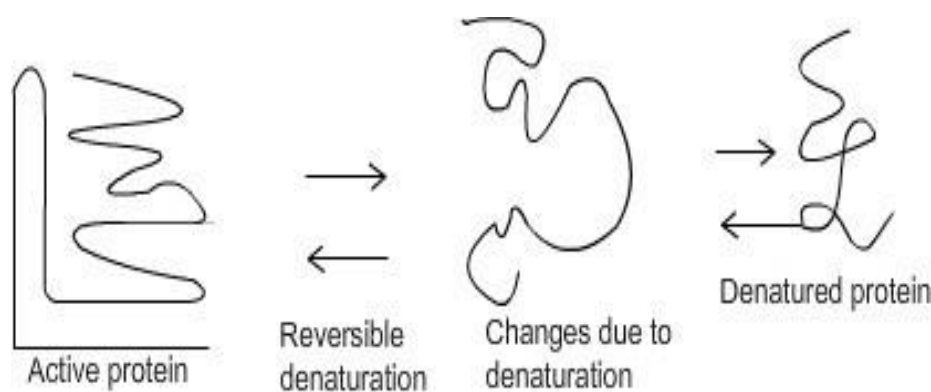
Denaturation results in splitting of 1 or more of these linkages, uncoiling or unfolding of peptide chain which inturn produce certain chemical changes as a result of unfolding process chemical groups that are seen in active site which are essential for the activity of protein molecules lose its characteristic activity. (eg)

- a. Sulph –hydrl group of cysteine.
- b. Di—sulphide group of cysteine.
- c. Phenolic group of Tryosine.

3. Biological changes

The denaturated proteins are digested more easily by proteolytic enzymes. The protein undergo Denaturation, no covalent bonds of polypeptide chains are broken. The specific manner in which the chain is folded gives each protein a characteristic

biological activity. When a protein is denatured, the characteristic 3 D arrangement is affected and it unfolds in random without any change in backbone covalent bonds. The active proteins become fragile and readily disturbed by heat and other reagents.



CLASSIFICATION

Some proteins, when denatured can be brought back to their original state and this type is called Reversible Denaturation or refolding or Renaturation.

Some proteins cannot be brought back to their original state and the cases are known as irreversible Denaturation.

Example

1. Ovalbumin irreversible type

When denatured by urea because of two cystine bridges, which hold the structure of protein molecules are broken and it is difficult to restore the structure on account of few linkages.

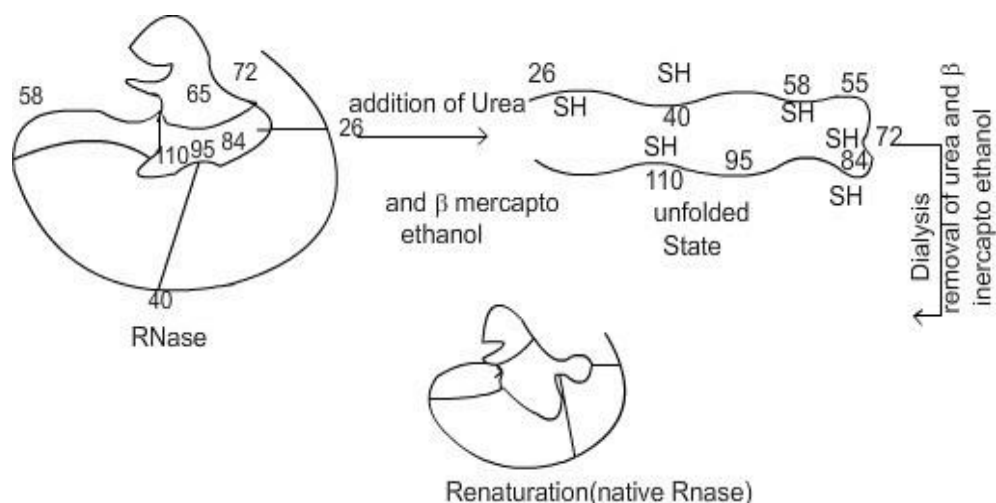
2. Egg albumin – irreversible type

Denaturation occurs due to boiling tertiary structure is destroyed and forms a disorganized mass of polypeptide chain.

3. Trypsin (or) RNase: Reversible type

Trypsin when exposed to temperature to 80-90°C, it denatures and when this temperature is reduced to 37°C, the solubility and activity of this is regained. The process of regaining normal protein after Denaturation is called Renaturation or Refolding.

RNase can be denatured by exposure to concentration urea solution in presence of reducing agent which cleaves the disulphide bonds of 4 cys residues to yield 8 cysteine residues, and thereby the entire polypeptide chain is changed from its folded conformation. Under this condition, the enzyme loses its catalytic activity and undergoes complete unfolding. Now place denatured RNase into a dialysis bag and suspend it in dialyzing solution. Small molecular weight urea reducing agent will diffuse in the bag into the solution. As this agent are removed gradually randomly coiled denatured RNase slowly, and spontaneously into its correct 3D conformation with full restoration of its catalytic activity.



Detailed study of Anfinsen showed that all the original enzymatic activity was regained if SH groups are oxidized under suitable condition. All of the measured physical and chemical properties of renatured enzymes was identical with that of native enzyme.

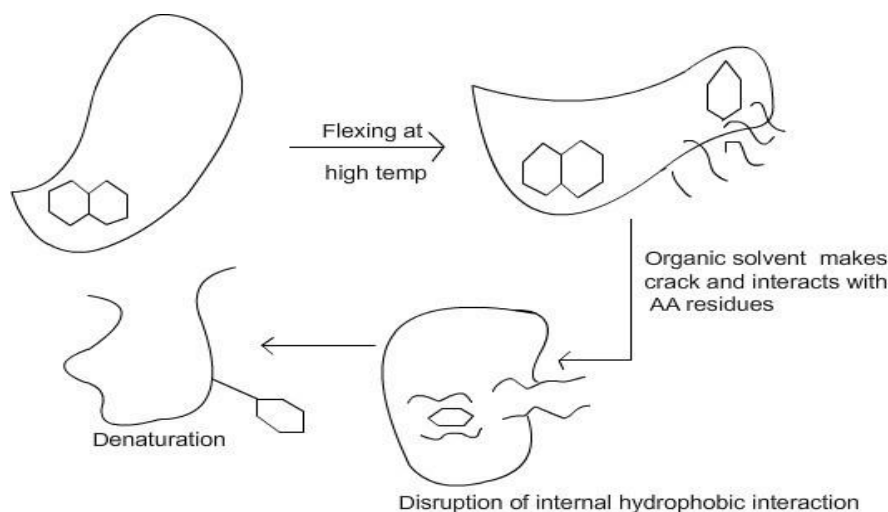
Quite different result was obtained when reduced RNase was reoxidised when it is still in 8M urea.

RNase oxidized in this way had only few of the enzyme activities of native proteins. The reason is that wrong disulphide pairing performed when random, coiled form of reduced molecules was re-oxidised. This RNase is termed as 'Scrambled RNase'.

Denaturation of protein by organic solvents and high temperature

The protein structure is maintained by intra molecular hydrophobic interactions. As a result of Denaturation protein loses its characteristic 4^o structure due to disturbance in hydrophobic interactions.

During Denaturation at high temperature by organic solvents make crack on the surface which of internal residues. At high temperature by internal hydrophobic forces between amino acid of proteins, which are relatively more important in maintaining molecular form of protein, lose its interaction and quickly results in Denaturation.



1.7 : CHEMICAL AND SOLID STATE PEPTIDE SYNTHESIS

The ability to manufacture polypeptides not available in nature has enormous biomedical importance.

1. To investigate the properties of polypeptides by systematically varying in their sidechains.
2. To obtain polypeptides with unique properties
3. To manufacture pharmacologically, active poly peptides that are biologically scarce or not existence.
4. One of the most promising applications of poly peptide synthesis is the production of synthetic vaccine.

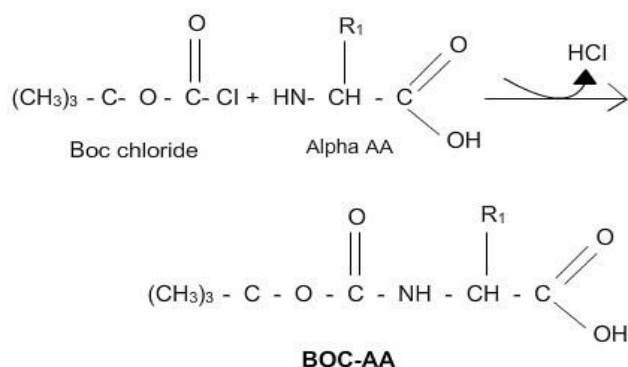
Synthetic Procedure

Polypeptides are chemically synthesized by covalently linking amino acid at a time to the terminus of growing poly peptide chain. Imagine that a polypeptide is being synthesized from its C-terminus towards its N-terminus (i.e) the growing chain ends with free $-NH_2$ group. Each amino acid is added to chain must already have its own alpha- NH_2 group chemically protected (block) or it would react with other like molecules as well as with N-terminal $-NH_2$ group of chain. Once a new amino acid is couple, its N-terminal $-NH_2$ group must be de-protected (deblocked) so that the next peptide bond can be formed. Every cycle of amino acid addition therefore requires a

coupling step and deblocking step. Further more reactive side chains must also be blocked.

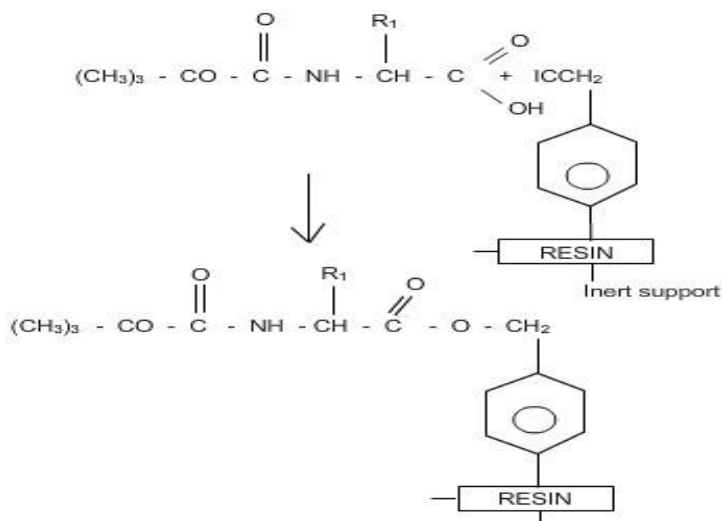
In 1962 Bruce Merrifield introduced solid phase synthesis. In this method the growing poly peptide is covalently anchored, usually by its C-terminus to an insoluble solid support such as beads of poly- styrene resin and the appropriately blocked amino acid and reagents are added in proper sequence. This permits when poly peptide chains are synthesized by amino acid addition to their N-terminus, the alpha -NH₂ group of each sequentially added amino acid must be chemically protected during the coupling reaction. The tertiary butyloxy carbonyl (t- BOC) group is frequently used for this purpose.

Protein synthesis then proceeds in a step- wise fashion.

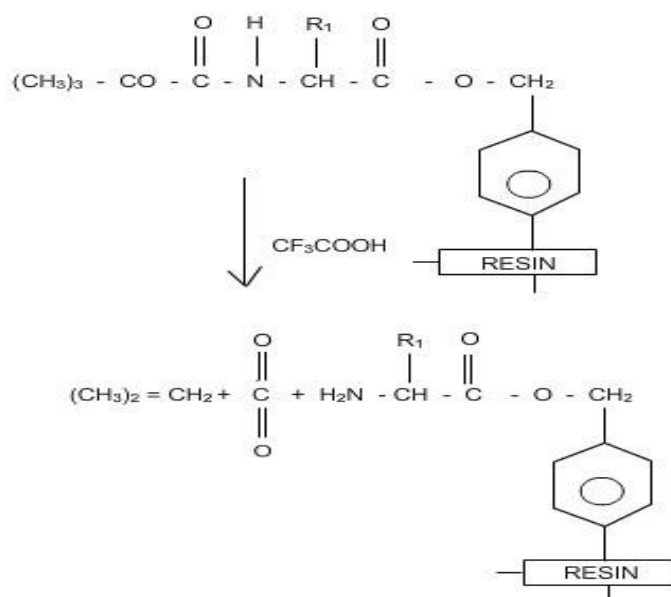


ANCHORING THE CHAIN TO THE INERT SUPPORT

The first step of solid phase Polypeptide synthesis is coupling the C-terminal amino acid to a solid support. The most used support is a cross link polystyrene resin with chloro -CH₂ groups.

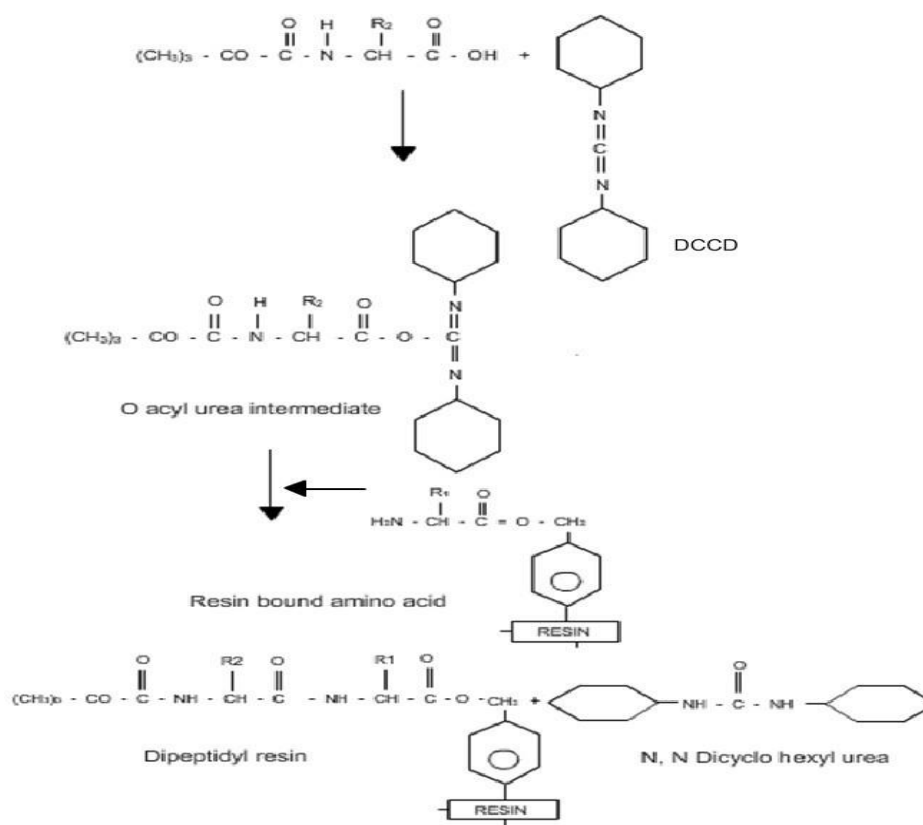


The resulting alpha amino acid derivatized resin is splitted and washed. The $-NH_2$ group is then deblocked by treatment with anhydrous acid such as tri fluoro CH_3COOH .



COUPLING THE AMINO ACID

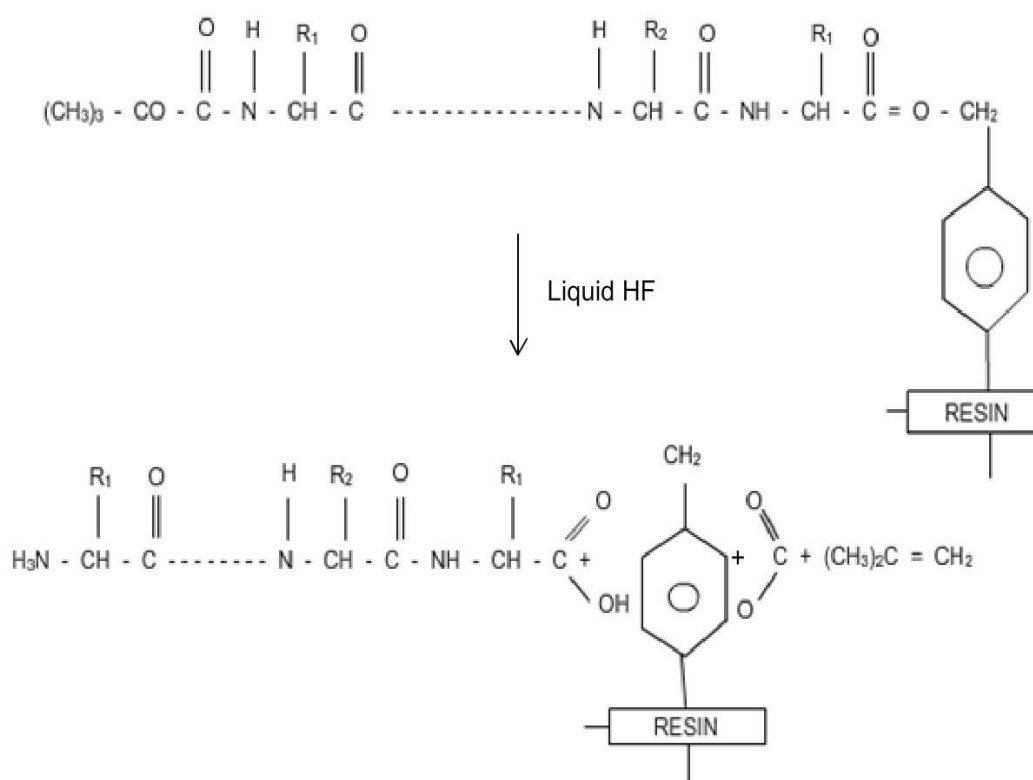
The reaction coupling two amino acids through a peptide bond is endergonic and therefore must be activated to obtain significant yields. The activating agent most often used is Dicyclohexyl carbodiimide (DCCD).



The O-acyl urea intermediate that results from the reaction of DCCD with $-\text{COOH}$ group of BOC-protected alpha amino acid readily reacts with resin-bound alpha amino acid to form the desired peptide bond in high yield. By subsequently alternative deblocking coupling reaction a polypeptide with the desired amino acid sequence can be synthesized. The repetitive nature of these operations allows the solid phase synthesis to be easily automated.

RELEASING THE POLY PEPTIDE FROM THE RESIN

The final step in solid phase polypeptide synthesis is cleavage of polypeptide from the solid support by treatment with liquid HF.



The BOC group linked to the polypeptide N-terminus, as well as the benzyl group protecting its side chains are also removed by this treatment.

5.8 ESTIMATION OF PROTEINS

There are three methods for it. Each one of them varying in sensitivity and applicability.

The processing of samples for protein estimation must also be considered.

1. It is generally advised to use acid precipitable material for protein estimation. For fluid samples this is achieved by adding 5% cold TCA which precipitates out almost

all proteins and interfering materials like carbohydrates, pigments remain in solution. For solid samples the sample can be extracted with 5% TCA and supernatant is discarded.

2. When sample is rich in lipid like substance, it becomes necessary to detect before protein estimation. This is easily carried out by extracting sample with ethanol ether mixture (2:1). The sediment thus obtained is once washed with ether and then dried in air. The dried solid can be now extracted with 5% TCA solution to precipitate out the proteins. Plant tissue extract contains considerable number of polyphenols which get precipitated with TCA and cause interference to protein estimation. It is a general practice to include 1% solution of PVP in the extracting medium. When plant tissues are used PVP complexes with polyphenols which get precipitated and can be removed by slow speed centrifugation.

ESTIMATION OF PROTEIN BY LOWRY'S METHOD

This is more sensitive. The aromatic amino acids Tyr, try present in protein react with Folin's Ciocalteu reagent and produce a dark blue color.

1. Lowry's reagent

Solution A: 2% Na_2CO_3 in 0.1 N NaOH Solution B: 0.5% CuSO_4 solution in 1% NaK tartarate (SPT) Mix solution A and B in the ratio of 50% before use.

2. Folin's ciocaltea reagent: It is commercially available and diluted with equal volume of H_2O before use.

3. Stock standard protein solution: 40mg of Bovine serum albumin is dissolved in 100ml of distilled H_2O

4. Working standard solution: 10 ml of stock standard solution is diluted to 100ml with distilled H_2O . Various concentration of working standard are pipetted out into various test tube and made up to 1 ml with of H_2O . The blank containing 1 ml of diluted H_2O is also taken. 0.1 ml of serum is taken as test sample. To all the above tubes add 4.5 ml of Lowry's reagent and allow to stand for 10 min, and then add 0.5 ml of Folin's reagent and allow to stand for 15 min and colour developed is read at 620 nm standard graph is drawn with concentration on X-axis and OD and Y-axis. And then amount of protein present in serum sample is calculated.

Normal serum protein level – 6 to 8 g/ 100 ml. M

ESTIMATION OF PROTEIN BY BIURET METHOD

Sub which contains CO –NH group form purple complex or violet color complex with Cuions in alkaline medium. The reaction takes this name from the complex form (i.e) Biuret.

1. Stock Biuret reagent: It is prepared by dissolving 45g SPT in 400 ml of 0.2N NaOH and 15g of CuSO₄ and 5g of KI and make up to a litre with 0.2N NaOH.
2. Dilute Biuret reagent: Dilute 200ml of stock Biuret reagent to a litre with 0.2 N NaOH.
3. Stock standard protein solution: 2g of Bovine serum albumin is dissolved in 100ml of diluted H₂O.
4. Working standard solution: 10 ml of the stock is dilute to 100ml with dilute H₂O. Pipetted out various concentration of working standard solution into a series of test tubes and make up volume to 2.5ml with dilute H₂O. For the test 0.4ml of serum is diluted to 10ml. From this 2ml is taken. The blank is also maintained by taking 2.5ml of dilute H₂O. To all the tubes add 3ml of diluted Biuret reagent and allow to stand for 30 min. The colour developed is read at 500nm. The standard graph is drawn by taking concentration of protein on X-axis and OD on Y-axis, and then the amount of protein serum sample is calculated from the graph.

Normal total serum protein is 6 to 8g/100ml.

5.9 PAPER CHROMATOGRAPHY

The term chromatography is introduced in 1906 the procedure is where the mixture of different color pigments was separated from each other.

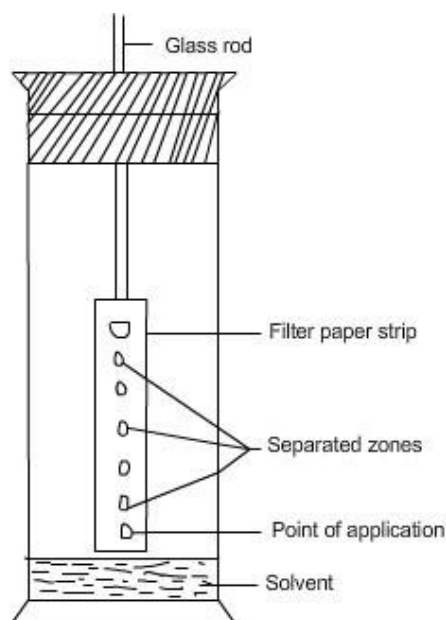
It can be defined as the techniques of separation of sub according to their partition coefficient. This method, the separation of components of mixture is the function of their different affinities for fixed stationary phase and the differential solubility in a moving mobile phase.

Technique

This method is especially useful for the detection and separation of amino acids. Filter paper strips are used to support the stationary H₂O phase while a mobile organic phase moved down the suspended paper strip in a cylinder.

A drop of solution containing a mixture of amino acids to be separated is applied at the marked point about 3 cm from 1 end of filter paper (Whatman number 1 paper is widely used for this purpose). The filter paper is then dried by putting it into an air tight cylindrical jar and also equilibrated by an aqueous solution of solvent – mixture of N-Butanol acetic acid: H₂O [4:1:5]. The end of filter paper nearest the applied drop is inserted into the solvent mixture at the bottom of the jar. The paper is suspended in such a manner so that it hangs freely without touching the sides of the container. Thus, the solvent will ascend into the paper and process is called ascending chromatography.

Alternatively, the same end of the filter paper may be put into the solvent mixture contained in a narrow trough mounted near the top of the container. In this case, the solvent will descend into the paper and this process is known as Descending chromatography.



LOCATING THE COMPOUNDS

Strip is removed when the solvent has migrated over most of the available space. The distance to which the solvent has run is marked. In most cases, the completed chromatogram is colorless and has no indication of the presence of any compounds. Such a chromatogram is known as undeveloped. For locating various compounds, the filter paper is 1st dried and then sprayed with 0.5% ninhydrin in acetone and at least increased for a few min at 80-100°C. The reaction occurs and colored spots appear at the sites of amino acid. Such a chromatogram is now called developed.

IDENTIFYING THE COMPONENT

In chromatography, the distance traveled by a particular component of a mixture is used to identify it. The ratio of distance traveled by a component (i.e) amino acid to that traveled by solvent front, both measured from the marked point of application of mixture, is called Resolution front (RF). RF value for that component.

Thus

$$RF = \frac{\text{Distance from origin run by Compound}}{\text{Distance from origin run by Solvent}}$$

The RF value for the given comp varies with the type of solvent used. It is preferable to chromatogram known amino acid standards along with the unknown mixture so that Rf values by easily be compared and amino acid can be identified.

PAPER ELECTROPHORESIS

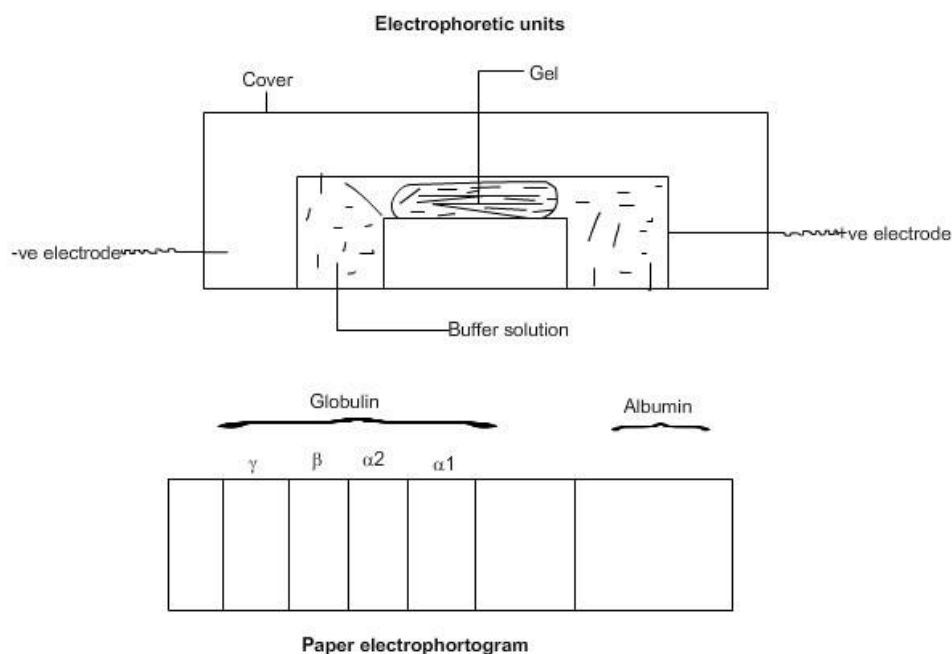
Electrophoresis is the migration of charged particles towards the electrodes bearing opposite charge when electric charge is created in solution between two electrodes. The rate of migration of charged particles depends on

1. The amount of net charge of the solution
2. The size and shape of solute particles
3. pH of solution
4. Electrical gradient established in solution

Particles of different protein migrate at different rates towards the respective electrodes, depending on their charges, sizes and shapes.

In paper electrophoresis, the sample is applied transversely across the central path of long strip of filter paper moistened with a buffer solution of desired pH. The ends of paper are fixed to dip in buffer solution in two troughs fitted with electrodes. On establishing an electric field. The charged particles of sample migrate along with strips towards the respective opposite electrodes, according to their net charge and size. Each group of particles migrate as separate band. Final locations of bands are identified on electro photogram. The sample component is estimated in each band as a.

1. By spray staining the bands with suitable reagent and measuring the density of stain by densitometer.
2. By scintillation spectrometer in terms of radio isotopes components.



5.10 SUMMARY

The term Protein is derived from the Greek words 'Protos' which means 'of first importance'. It represents an enormous group of complex nitrogenous compounds, having high molecular weight. Protein molecules are composed of chains of alpha – amino acids, united to one another by peptide linkages. The individual amino acids may be considered analogous to the monosaccharides of carbohydrates.

5.11 KEYWORDS

Proteins

The term Protein is derived from the Greek words 'Protos' which means 'of first importance'. It represents an enormous group of complex nitrogenous compounds, having high molecular weight. Protein molecules are composed of chains of alpha – amino acids, united to one another by peptide linkages. The individual amino acids may be considered analogous to the monosaccharides of carbohydrates.

Peptides

Peptides consist of two or more amino acids linked by a peptide bond which is formed between the $-\text{COOH}$ group of 1 amino acid and the $-\text{NH}_2$ group of another amino acid with the removal of one molecule of H_2O . When two amino acids combine dipeptide is formed.

Scelero protein (or) Albuminoids

These occur almost entirely in animals and are therefore commonly known as the animalskeleton proteins; insoluble in H₂O, dilute solutions acid and bases and salts; not attacked by enzymes. (eg) Collagen of bones, Elastin in ligaments, Keratin in hair, and Fibroin in silk.

Collagens

These form the major proteins of connective tissues and bones. More than half the total protein the mammalian body is collagen; acted upon by boiling H₂O, dilute acids or alkalis, collagens produce soluble gelatins. They have high content –OH proline (12%) poor in sulfur- containing amino acids.

Nucleo proteins

These are compounds containing nucleic acid and proteins. (eg) Protamines and Histones. These are present in nuclear substance as well as in cytoplasm and are consider as the sites for the synthesis of proteins and enzymes and are attached to each other by means of electrostatic forces.

Denaturation proteins

This organization of the native (naturally occurring) protein molecule by which the specific configuration of a regular arrangement of a protein molecule is altered to an irregular diffused arrangement. So, this leads to a change in the physical and chemical properties of affected proteins. The changes in properties in proteins is called Denaturation. In other words, it is the loss of biological activity. In many incidents, this process is followed by coagulation. A process where denatured protein molecules tend to form large aggregates.

EXERCISE

I; Essay Questions

1. What are proteins?
2. Explain the classification of proteins in detail?
3. Give an account on the chemical properties of proteins and peptides?
4. Mention the denaturation process of proteins?
5. How to estimate the proteins. Mention the techniques to estimate the proteins?

6. How to determine peptides?
7. List out the bonds involved in the peptide synthesis?
8. Explain in detail about the structure levels and organization of proteins?

II Multiple Choice Questions

- 1) Which of the following is a 39-residue hormone of the anterior pituitary gland? ()
a) Corticotropin b) Glucagon c) Insulin d) Bradykinin
- 2) Which of the following is not the classified form of conjugated proteins?
()
a) Lipoproteins b) Glycoproteins c) Metalloproteins d) Complete proteins
- 3) Which part of the amino acid gives it uniqueness ()
a) Amino group b) Carboxyl group c) Sidechain d) None of the mentioned
- 4) Which of the following information is responsible to specify the three-dimensional shape of a protein? ()
a) The protein's peptide bond b) The protein's amino acids sequence c) The protein's interaction with other polypeptides d) The protein's interaction with molecular chaperons
- 5) What of the following is not a factor responsible for the denaturation of proteins ()
a) pH change b) Organic solvents c) Heat d) Charge
- 6) The nucleoproteins of conjugated protein contain the following except? ()
a) Nucleic acid b) Nitrogen c) Phosphorous d) Hydrochloric acid
- 7) The protein that is combined with amino sugars, Sugar acids and present in ()
a) DNA b) Saliva c) Legume d) Bones
- 8) The of milk is a phosphoprotein.
a) Casein b) Ovovitellin c) Tendamucoid d) Mucin
- 9) The amino acids of protein are linked with one another through Bonds. ()
a) Cystic b) Elastin c) Melittin d) Peptide
- 10) The protein which are simple and conjugated proteins is ()
a) Ornamental b) Acidic c) Derived d) Basic.

III Fill in the Blanks.

1. Peptide bond is a _____
2. A tripeptide has _____
3. The factor which does not affect pK_a value of an amino acid is _____
4. The average molecular weight of an amino acid residue in a protein is about _____

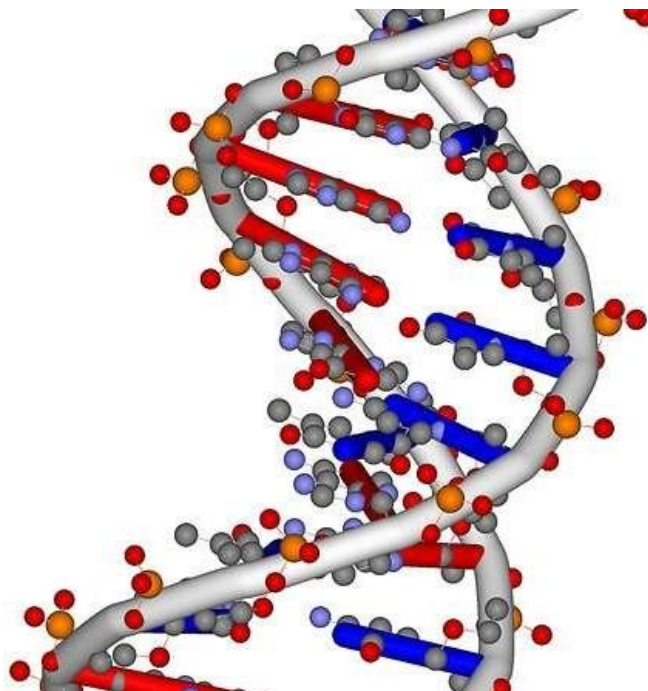
5. Unfolding of a protein can be termed as _____
6. The _____ of a protein molecule may range from two to several thousands.
7. Protein molecule contains _____
8. The proteins are the basis for the major structural components of _____
9. The proteins which give single amino acid only upon hydrolysis is called as _____
10. The simple protein which is in Blood is _____

II Answers: 1) a 2) d 3) c 4) b 5) d 6) d 7) b 8) a 9) d 10) c

III Answers: 1) Covalent bond 2) 3 amino acids and 2 peptide bonds 3) Molecular weight 4) 110 5) Denaturation 6) Amino acids 7) Oxygen and Nitrogen 8) Animal Tissues 9) Simple proteins 10) Serumbumin.

CHAPTER-VI

Nucleic Acids

**6.0 INTRODUCTION**

Since 1871, when a 22-year-old Swiss physician and scientist called Friedrich Miescher isolated the nucleic acid from the nucleus of pus cells and gave it the term nuclein, it has been the topic of study into the biochemical properties of the nucleic acid. This molecule was very unlike the proteins and lipids that were found in carbohydrates. In later years, it came to be known as nucleic acids.

Information about a person's genes is stored in their nucleic acids. DNA, or deoxyribonucleic acid, is a molecule that is generated by the repeating of nucleotides, which results in DNA having the properties of a polymer. This is how hereditary information is stored in all creatures that are alive. DNA is comprised of four distinct nucleotides, each of which contributes to the development of a universal code for transmitting genetic information. DNA is not the only type of nucleic acid; there is also a molecule known as ribonucleic acid (RNA), which is linked to DNA. Additionally, it is a polymer made up of four nucleotides, three of which are identical to those found in DNA and the fourth of which is quite different. It has a variety of purposes in the cell, the most important of which is to act as a link between DNA and proteins. There are even viruses that will store their genome in the form of an RNA

molecule as opposed to DNA if they can. Although their name indicates that they are found in the nucleus of cells, some nucleic acids may also be found in the cytoplasm of those cells. Hereditary information is stored in nucleic acids, which are found in all living things. They are the micromolecules that can be found in most cells either in their free state or coupled to proteins as nucleo-proteins. They may be found in either condition. The mononucleotide serves as the repeating unit for the biopolymers that make up nucleic acids, which are high-molecular-weight biopolymers. In terms of their chemical makeup, nucleic acids consist of carbon, hydrogen, oxygen, nitrogen, and phosphorus. Phosphorus is also present. The remaining two components account for around 15 and 10 percent of the total, respectively.

6.1 COMPOSITION OF NUCLEIC ACIDS:

There are two kinds of nucleic acids Deoxy Ribo nucleic acid (DNA) and ribonucleic acid(RNA). On hydrolysis, the two nucleic acids yield 3 components.

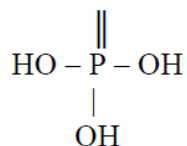
1. Phosphoric acid.
2. Pentose sugar.
3. Nitrogenous bases.

HYDROLYTIC COMPONENTS OF NUCLEIC ACID

S. No	Components	RNA	DNA
1	Acid	Phosphoric acid	Phosphoric acid
2	Pentose sugar	D-ribose	D-deoxy ribose
3	Nitrogenous bases		
	Purines	AdenineGuanine	Adenine Guanine
	Pyrimidine	CytosineUracil	CytosineThymine

Phosphoric acid

The molecular formula of phosphoric acid is H_3PO_4 . It contains 3 mono valent hydroxyl groups and a divalent oxygen atom, all linked to the penta valent phosphorus atom.

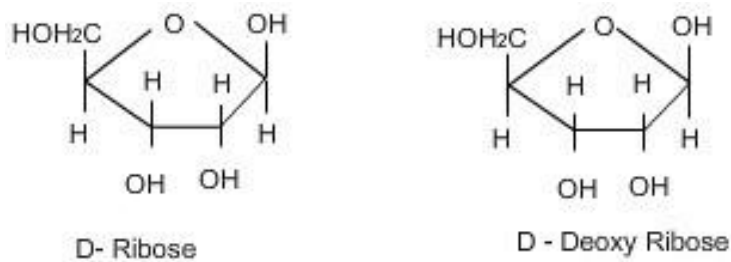


Phosphoric acid (H_3PO_4)

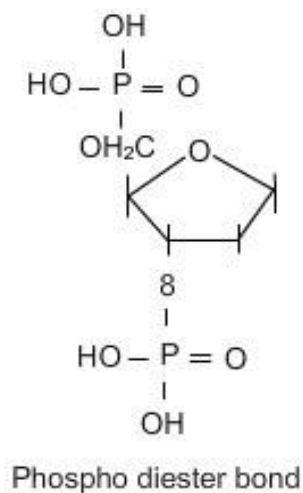
Pentose Sugar

The two types of nucleic acids are distinguished primarily based on pentose which they possess. One possesses deoxyribose, hence the name deoxy ribo nucleic acid,

while the other contains ribose, hence the name ribonucleic acid. Both these sugars in nucleic acids are present in furanose form and are of Beta configuration.



An important property of the pentose is its capacity to form esters with H₃PO₄. In this reaction, the -OH groups of the pentose, especially those at C3 & C5 are involved in forming a phosphor diester bond between adjacent pentose residues.

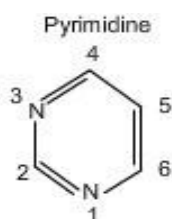


Nitrogenous bases

Two types of nitrogenous bases are found in all nucleic acids. These are derivatives of pyrimidine and purine.

Pyrimidine derivatives

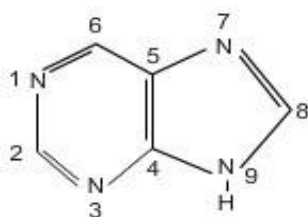
These are all derived from their parent heterocyclic compound Pyrimidine.



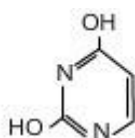
The common Pyrimidine derivatives found in nucleic acids are uracil, thymine, and cytosine.

Purine derivatives

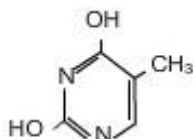
These are all derived from their parent compound purine which contains 6 membered pyrimidine rings fused to five membered imidazole ring.



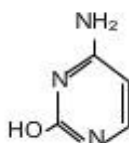
Purine



Uracil- (2,4 dioxy pyrimidine)

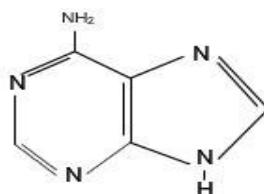


Thymine- (2, 4 dioxy 5 methyl pyrimidine)

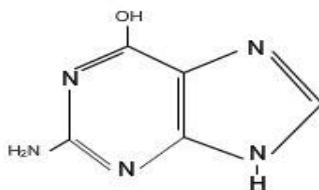


Cytosine - (2 oxy 4 amino pyrimidine)

The pre-valent purine derivatives found in nucleic acids are adenine and guanine.

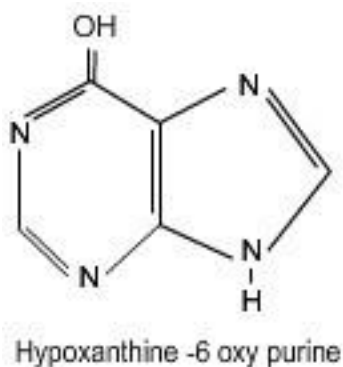


Adenine - 6 amino purine



Guanine - 2 amino 6 oxy purine

In recent years, several unusual purine derivatives have been found in transfer RNA's (tRNA). The important ones are hypoxanthine, xanthine.



The other purine derivatives also are found in plants. For example : caffeine (1,3,7 trimethyl xanthine) is present in coffee and tea and theobromine (3,7 dimethyl xanthine) is found in tea and cocoa.

6.2 NUCLEOSIDES

The nucleosides are compounds in which nitrogenous bases (purines and Pyrimidine) are conjugated to the pentose sugars (ribose and deoxyribose) by a beta glycosidic linkage. The glycosidic linkage involves the C1 atom of sugar and the H atom of N9 (in the case of purines) or N1 (in the case of pyrimidines), thus eliminating a molecule of H₂O. The nucleosides are generally named for the purine or Pyrimidine present nucleosides containing ribose are ribonucleosides while those possessing deoxy ribose are called deoxy ribonucleosides.

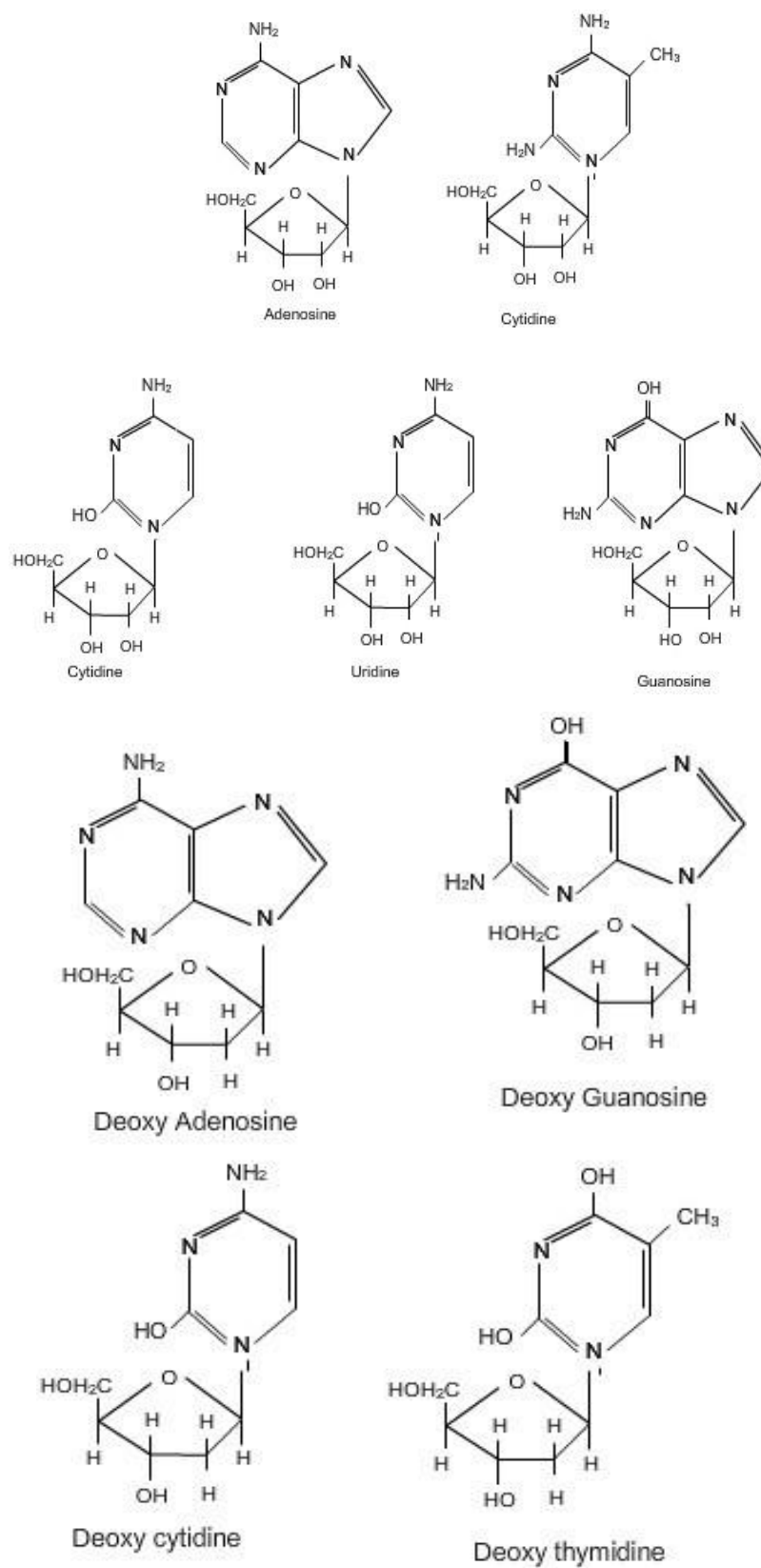
Ribo Nucleosides

S.No	Pentose sugar	Nitrogenous base	Name of nucleosides
1	Ribose	Adenine	Adenosine (adenine ribo nucleoside)
2	Ribose	Guanine	Guanosine(guanine ribo nucleoside)
3	Ribose	Cytosine	Cytidine(cytosine ribo nucleosides)
4	Ribose	Uracil	Uridine (Uracil ribo nucleoside)
5	Ribose	Thymine	Thymidine (Thymine ribo nucleoside)

Deoxy ribo nucleosides

S.No	Pentose sugar	Nitrogenous base	Name of nucleosides
1	Deoxy Ribose	Adenine	Deoxy Adenosine (adenine deoxy ribo nucleoside)
2	Deoxy Ribose	Guanine	Deoxy Guanosine (guanine deoxy ribo nucleoside)
3	Deoxy Ribose	Cytosine	Deoxy Cytidine (cytosine deoxy ribo nucleosides)
4	Deoxy Ribose	Uracil	Deoxy Uridine (Uracil deoxy ribo nucleoside)
5	Deoxy Ribose	Thymine	Deoxy Thymidine (Thymine deoxy ribo nucleoside)

RIBO-NUCLEOSIDES: DEOXY RIBO NUCLEOSIDES



Nucleotides: Nucleotides = nucleosides + H_3PO_4

RIBO NUCLEOTIDES

S.No	Ribo nucleotides	Name	Abbreviation
1	Adenosine mono phosphate	Adenylic acid	AMP
2	Guanosine mono phosphate	Guanylic acid	GMP
3	Cytidine mono phosphate	Cytidylic acid	CMP
4	Uridine mono phosphate	Uridylic acid	UMP

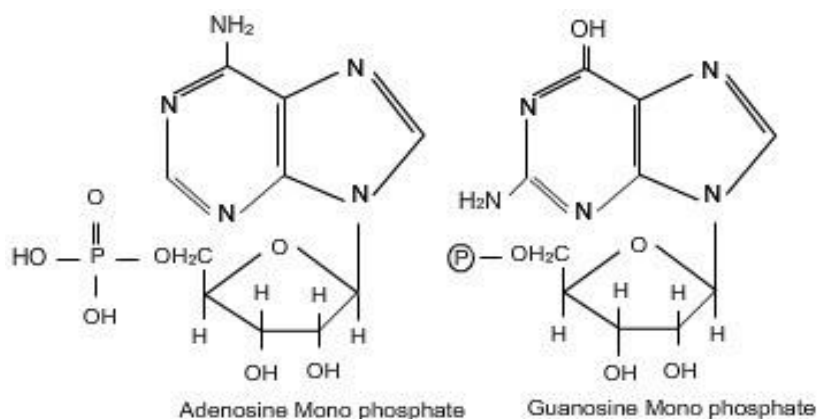
DEOXY RIBO NUCLEOTIDES

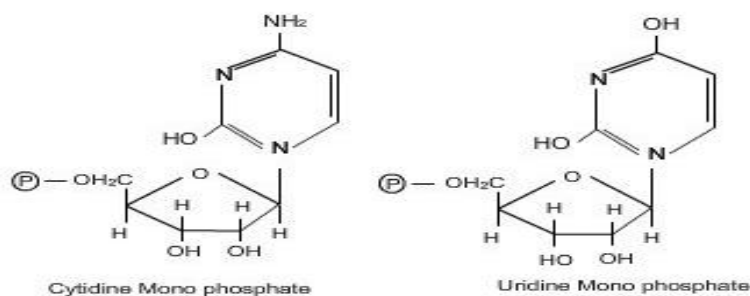
S.No	Deoxy Ribo nucleotides	Name	Abbreviation
1	Deoxy Adenosine mono phosphate	Deoxy Adenylic acid	dAMP
2	Deoxy Guanosine mono phosphate	Deoxy Guanylic acid	dGMP
3	Deoxy Cytidine mono phosphate	Deoxy Cytidylic acid	dCMP
4	Deoxy thymidine mono phosphate	Deoxy thymidylic acid	dTMP

Nucleotides are the H_3PO_4 esters of nucleosides. These occur either in the free form or as subunits in nucleic acids. The phosphate is always esterified to the sugar moiety (group).

In the ribose moiety of a ribonucleosides phosphor relation is possible only at 3 positions. (C2, C3 & C5). Since C1 and C4 are involved in the furanose ring formation. In other words, the phosphate group could be esterified only at these 3 places.

In the deoxy ribose component of deoxy ribonucleosides only two positions (C3 & C5) are available for phosphor relation. In this sugar C1 & C4 are involved in furanose ring and C2 does not bear a – OH group.

Ribo nucleotides

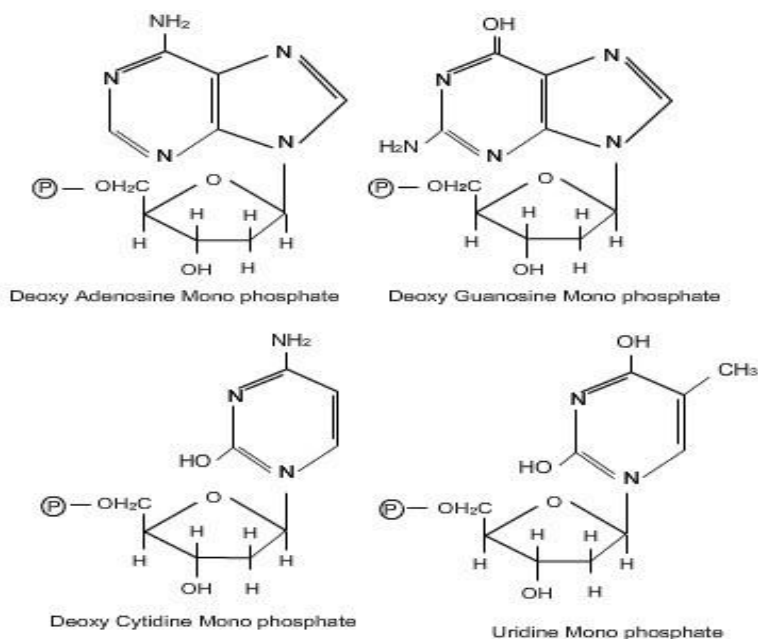


Representation of nucleic acids

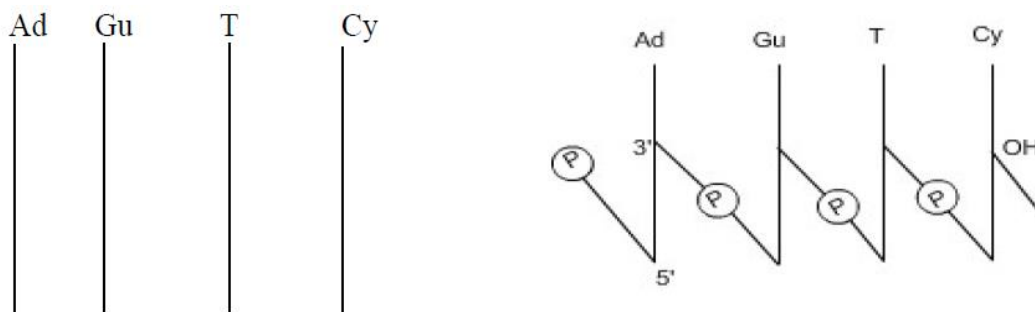
In nucleic acids, it may be visualized as a polymer of nucleotides. In other words, it maybe considered as a poly nucleotide. (Base- sugar- phosphate).

The constituent units are coupled by means of phospho di ester bonds. The nature properties and function of 2 nucleic acids (DNA, RNA) depend on the exact order of the purine and pyrimidine bases in the molecule. This sequence of specific bases is termed primary structure. The purine and pyrimidine base of DNA carry genetic information whereas sugar and phosphate groups perform a structural role. The backbone of DNA which is constant throughout the molecule consist of de oxy riboses link by phospho di ester linkage or bridges. The 3¹ hydroxyl of adjacent sugar moiety of 1 deoxy ribo nucleotide is joined to 5¹ hydroxyls of adjacent sugar by a phospho diester bond. The variable part of DNA is its sequence of bases.

Deoxy ribo nucleotides

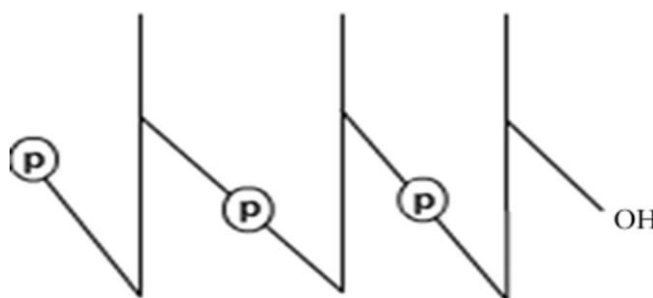


The writing of the structure of DNA chain is tedious and time taking. Hence a system of schematic representation has been evolved according to this system.



The bold line refers to the sugar and the letters, Ad, Gu, T and Cy represent the bases.

The encircled phosphorus group represents a phosphor di ester bond. In this example 3' hydroxyl of de oxy adenylic acid is linked to 5' hydroxyl of de oxy guanylic acid by a phosphate group. In the above nucleotide, suppose de oxy thymidylic is added the resulting in nucleotide can be represented.



6.3 : ISOLATION AND SEPARATION OF NUCLEIC ACIDS:

The 3 major steps involved in the process of nucleic acid isolation are.

1. Disruption
2. Separation
3. Purification

Disruption

The first step in isolation of nucleic acid is its disassociation from the other cellular constituents and its separation from associate proteins.

The methods employed in disruption of cells include.

1. Mechanical method
2. Chemical method
3. Enzymatic method

Mechanical method may be employed such as grinding with the mortar and Pistle, with sand, Alumina or fine glass beads. It is a useful method for small amounts of nucleic acid isolation.

In chemical methods the cells are treated with sodium dodecyl sulphate or sodium lauryl sulphate which causes disruption of the cells.

For bacterial cells, they are treated with the enzyme, lysozyme, lysis (breaking) the bacterial cell wall and cause the release of the nucleic acids from the cells.

Other common methods employed for the disruption of cells include freezing and thawing, busting by osmotic shock and exposure of the cells to ultrasonic vibrations. Thus the process of disruption is carried out.

Separation

After cell disruption the nucleic acid must be separated from associated proteins. It may be achieved in 2 ways.

1. Chemical method
2. Enzymatic method

In chemical method, the separation of nucleic acids from associated proteins is achieved using phenol or by repeated treatment of chloroform and isoamyl alcohol.

Proteins may also be removed by digestion with enzymes such as pronase. Pronase is a broad-spectrum protease (proteolytic enzyme) which hydrolyses almost any proteins to amino acids.

In the isolation of RNA care must be taken to avoid degradation of RNA by ribonuclease either from experimental materials or from the operators' fingers. Suitable ribonuclease inhibitors may have to be employed to avoid the action of ribonuclease. In the preparation of DNA, degradation of deoxy ribonucleases must be prevented. Special cases where even the slightest trace of nuclease must be avoided. Sterilized glassware must be used, and operator should wear gloves.

Conversely purified ribonucleases (RNase) may be used to remove contaminating RNA from preparation of DNA. Similarly purified deoxy ribonuclease (DNase) is

used to remove contamination DNA from the preparations of RNA.

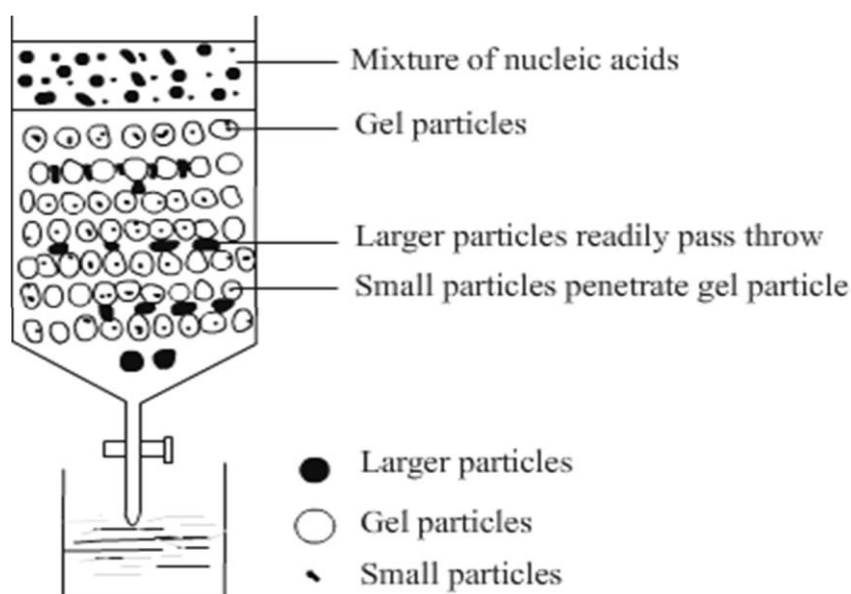
Purification

a. Purification of DNA

The purification of nucleic acids is based on molecular size, secondary structures and base composition. Purification of nucleic acids molecules according to size may be achieved by gel filtration. Sephadex is the most widely used gel. This technique is also known as molecular exclusion chromatography.

During the gel filtration, smaller molecules penetrate the gel particles to a varying extent.

Whereas large molecules are not retained and pass through readily.



The molecules are therefore separated from a column in the order of decreasing molecular size. Poly acrylamide gels can also be used instead of sephadex .

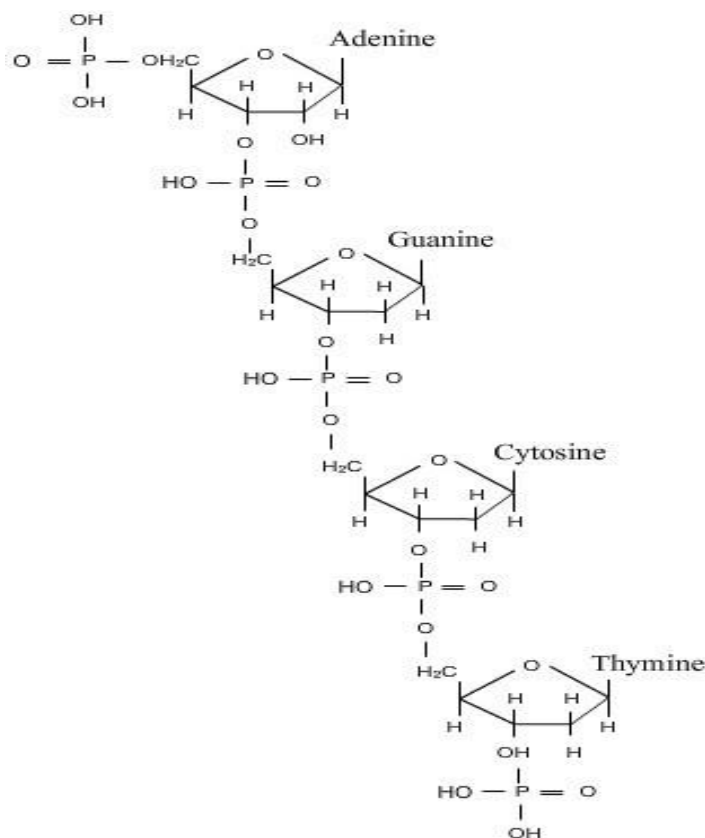
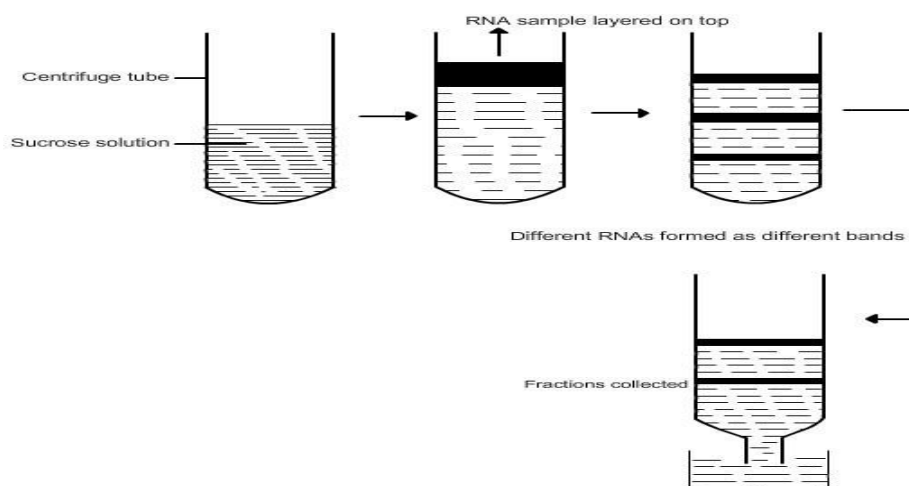
b. Purification of RNA

After the completion of isolation, RNA tends to be a complex mixture of nucleotide of various chain lengths. The separation procedure may be carried out in many ways. One such method employed is Gradient centrifugation. The functions of RNA can readily be separated by the process of centrifugation through sucrose gradients. In this process, the centrifuge tube is filled with sucrose solution. The solution of mixture of RNA carefully layered on the top and the tube is centrifuged at high speed for several hours. The different types of RNA's are separated as different bands. The bottom of

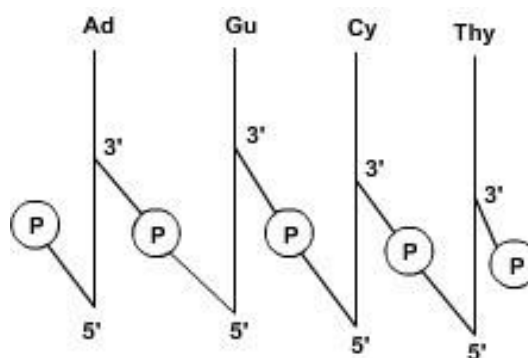
the tube is then punched with a needle and a series of samples were collected. The nucleic acid content of each sample is estimated by various analytical methods.

6.4 PRIMARY STRUCTURE OF NUCLEIC ACIDS

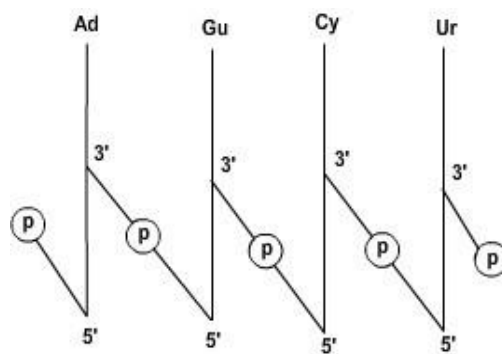
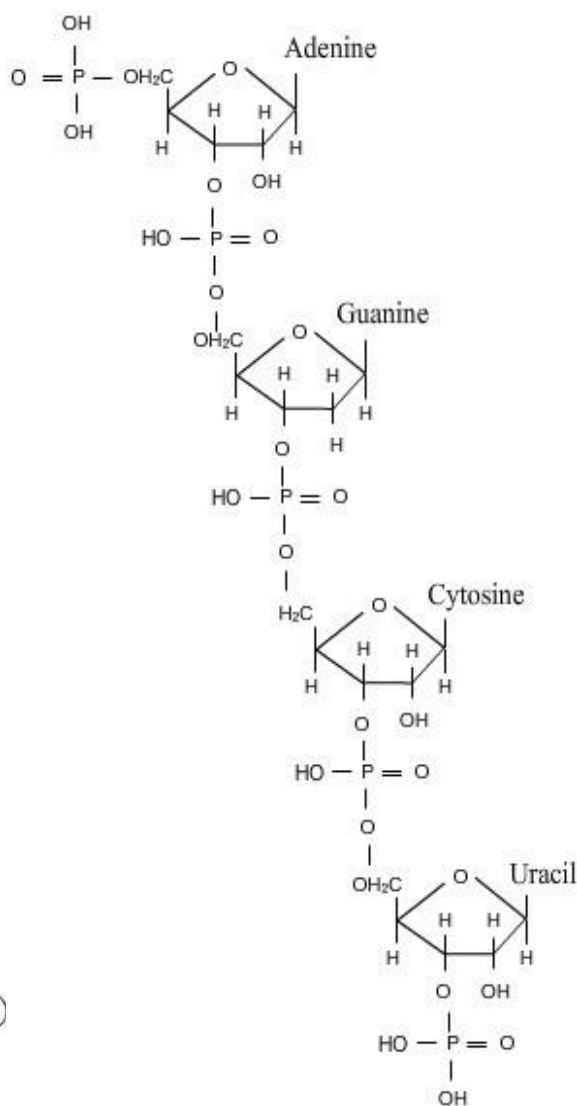
Nucleic acids are polymers made up of thousands or millions of nucleotides coupled together by phosphor diester linkages. In the case of DNA C4 in the sugar is occupied in ring formation and C2 doesn't contain a hydroxyl group. Only the hydroxyl groups at C3 & C5 are available for inter-nucleotide linkages. The primary structure of the poly nucleotide chain in DNA is shown in the following figure.



In short form it may be represented as follows



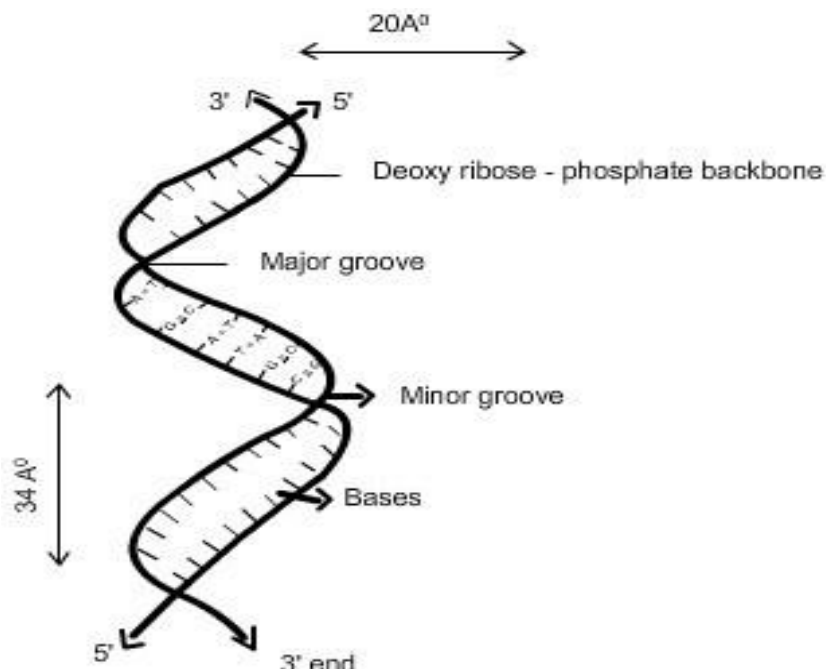
In **the** case of RNA a hydroxyl group is present at C2. So it is possible to form a 2, 5 linkage rather than 3,5 linkage which occurs in DNA. However, hydrolysis of RNA with phospho diesterase from snake venom yields nucleosides 3 mono phosphates . This indicates that inter nucleotide linkage in most RNA is identical to the DNA(3, 5).



Short representation of RNA

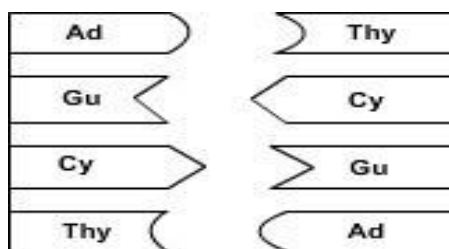
6.5 SECONDARY STRUCTURE OF DNA

According to Watson & Crick (1953), DNA molecules consist of 2 very long single poly nucleotide chains wrapped around each other in the form of a double helix. The two chains are joined together by specific hydrogen bonds.



The width of the double stranded helix is 20Å . The distance between each turn of the helix is 34Å . In the double helix, the two chains are coiled around a common axis. The chains are paired in an anti-parallel manner. In the DNA helix, the hydrophilic deoxy ribose phosphate backbone of each chain is on the outside of the molecule, whereas the hydrophobic bases are stacked inside, perpendicular to the axis of the helix. The overall structure resembles a twisted ladder.

The twisting of the strands results in the formation of major and minor grooves. Approximately there are 8 to 10 nucleotide units in 1 lot of each strand. In the molecule, the pentose phosphate group in the 2-poly nucleotide chain run in opposite direction. The 2 chains constituting DNA double helix are said to be 5' anti parallel.

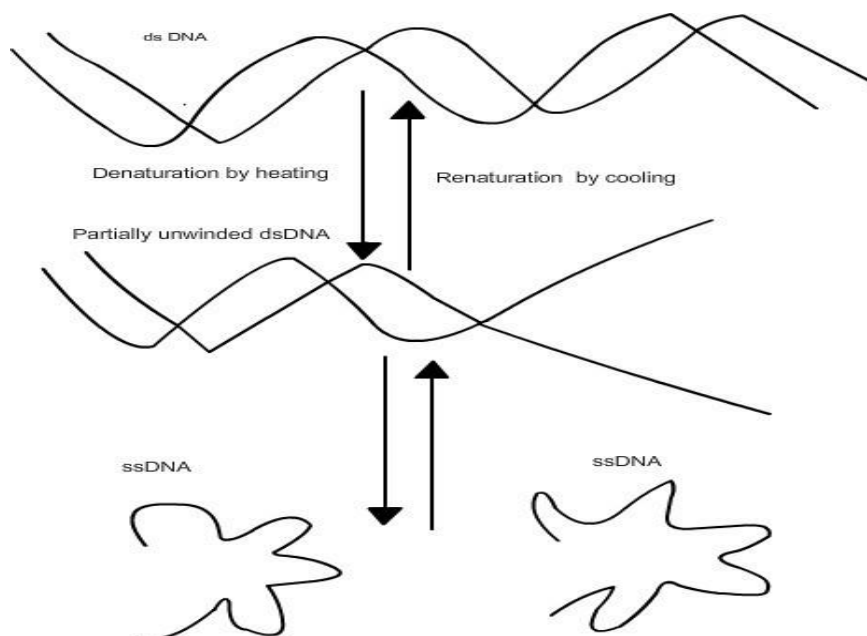


In the DNA molecule each base on one chain is forming bonds with the opposite base on the 2nd chain. The nature of the bonds between the bases is decided by the size and chemical structure of the bases. Every base pair consists of a purine from one strand and pyrimidine from the other strand.

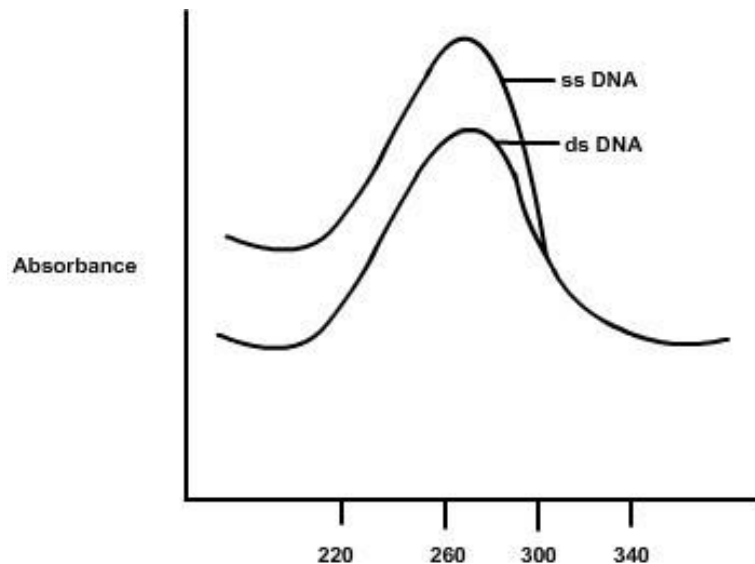
The chemical structure of the bases decide that only possible combination are between adenine and thymine (A-T- OR T-A) and between guanine and cytosine (G-C- OR C-G). adenine and thymine bases are connected by 2 hydrogen bonds while Gu and Cy are connected by 3 hydrogen bonds. According to Watson & Crick model the number of Ad bases is equal to number of Thy bases . Similarly, number of Gu bases is equal to number of Cy bases.

6.6 DENATURATION

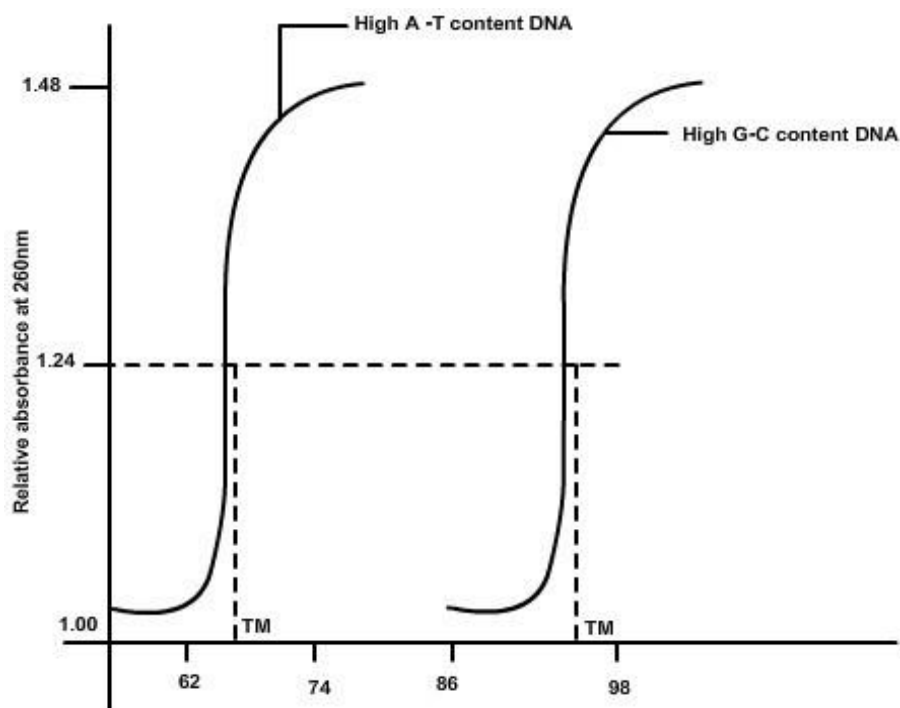
The 2 strands of a DNA helix readily separate when the hydrogen bonds between its paired bases are disrupted. This can be achieved by heating as a solution of DNA or by adding acid or alkali to ionize its bases. The unwinding of the double helix is called melting because it occurs abruptly at a certain temperature. This process is known as Denaturation.



The melting temperature (T_m) is defined as the temperature at which half of the helix structure is lost. The melting of DNA is readily monitored by measuring its absorbance at 260nm. The unstacking of bases pairs, result in increased absorbance, an effect called hyperchromism(hyperchromic effect).



The T_m of DNA molecule depends markedly on its base composition. DNA molecules rich in G- C base pair have higher T_m . But DNA molecules rich in A-T base pairs have comparatively lower T_m G- C base pair are more stable than A-T base pairs because their bases held together by 3' hydrogen bonds. Hence A-T rich regions of DNA which are held by 2 hydrogen bonds are the first to melt.



Separated complementary strands of DNA spontaneously reassociate , to form a double helix when the temperature is lowered below T_m (i.e) by cooling. This process is called renaturation and is sometimes also known as Annealing . The following with which double helixs can be melted and then reassociated is crucial for the biological

functions of DNA.

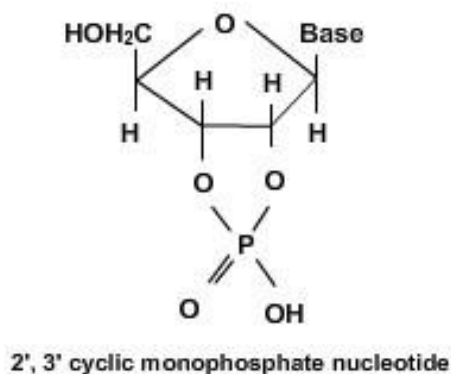
6.7 STRUCTURE OF RNA

RNA like DNA is a long unbranched macro molecule consisting of nucleotides joined by 3' to 5' phosphor diester bonds. The number of ribo nucleotides in RNA ranges from as few as 75 to many thousands.

Differences with DNA

Although sharing many features with DNA, the RNA molecules possess several specific differences.

1. The sugar moiety in RNA to which the phosphate and nitrogen bases are attached is ribose rather than deoxyribose of DNA. Ribose contains a 2-OH group that is not present in deoxyribose.
2. RNA contains the pyrimidine base uracil in place of thymine which is present in DNA. Uracil, like thymine can form a base pair with adenine by 2 hydrogen bonds.
3. The native RNA is single stranded rather than a double stranded helical structure which is characteristic of DNA, however, the single strand of RNA may fold back on itself like a hair-pin and thus acquire the double stranded pattern. In the region of hair-pin loops 'S' pairs with 'U' & 'G' pairs with 'C'. The base pairing in RNA hair pins is frequently imperfect. Some of the opposing bases may not be complementary and 1 or more bases along a single strand may be looped out to facilitate the pairing of others.
4. Since the RNA molecule is single stranded and it of need not have complementary base ratios. In other words, its adenine content does not necessarily equal its uracil content similarly; its guanine content doesn't equal its cytosine content.
5. RNA can be hydrolyzed by weak alkali to 2, 3 cyclic diesters of mono nucleotides. This intermediate, however cannot be formed in alkali treated DNA because of the absence of 2-OH group. Thus, RNA is alkali labile whereas DNA is alkali stable.



Types of RNA

There are mainly 3 types of RNA found in human beings. They are.

1. m-RNA (Messenger RNA)
2. t- RNA (Transfer RNA or soluble RNA)
3. r- RNA (Ribosomal RNA)

The main function of each of these RNA is protein bio-synthesis. In human cells there are small nuclear RNA or sn-RNA which are not involved in protein bio-synthesis directly. A large precursor of m-RNA called as heterogeneous nuclear RNA or hn-RNA, is also found in the nucleus.

1. MESSENGER RNA

1. This is the most heterogeneous class of RNA with respect to its size and stability.
2. The molecular weight varies from 310^4 TO 210^6 . They consist of 10^3 to 10^4 ribonucleotides.
3. It carries mainly adenine, guanine, cytosine and uracil as the major bases. Methyl purines and methyl pyrimidines are the minor bases present in m-RNA.
4. The m- RNA molecules are formed with the help of DNA template strands during the process called transcription.
5. The m- RNA carries a specific sequence of nucleotides in triplets called codons, which are responsible for the synthesis of a specific protein molecule.
6. The 3' end of most m-RNA molecules carries a polymer of adenylate ribonucleotides consisting of 20-250 residues in length. This is called as poly A tail. Its function is preventing the action of nucleases. (Exo nucleases).
7. On the other hand, the 5' ends of the m-RNA carry a cap structure consisting of 7-methyl GTP (Guanosine tri phosphate). The cap is involved in preventing the attack of exonucleases.

8. The protein synthesis begins at 5' end of the capped structure of m-RNA.

m- RNA

In mammalian cells the m-RNA that comes to cytoplasm is the product of processing of a precursor called heterogeneous nuclear RNA. These molecules are very large and may have a molecular weight of 10^7 .

2. TRANSFER –RNA- (t-RNA)

These t- RNA molecules are also called as soluble RNA. They remain largely in cytoplasm. The t- RNAs are relatively small, single stranded, globular molecules with molecular weight 2×10^4 to 30,000. There are at least 20 different t- RNA molecules.

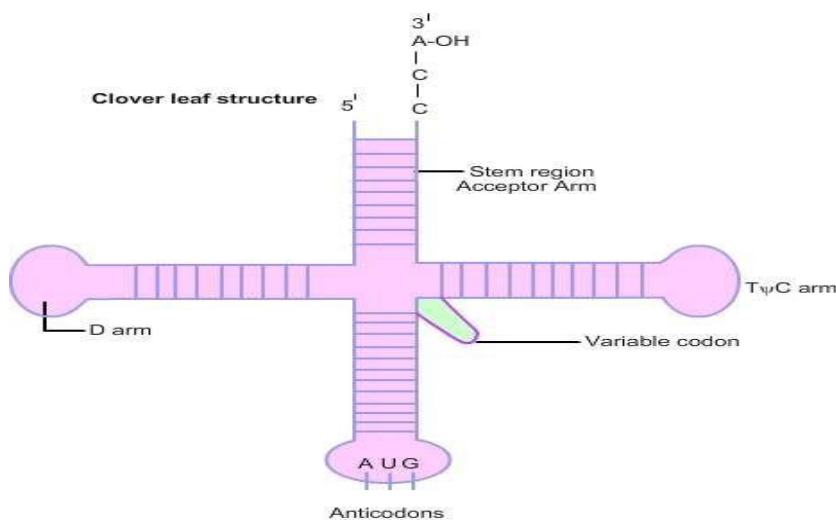
PRIMARY STRUCTURE OF t- RNA

t- RNA molecules consist of approximately 75 nucleotides. Their bases include Ad, Gu, Ur and Cy and Pseudo uridine (Ψ), dihydro uridine (DHU) and Thymine are present in the loops.

SECONDARY STRUCTURE OF t- RNA

Each single stranded t- RNA molecule remains folded to form a cloverleaf secondary structure. These folds of secondary structure are stabilized by hydrogen bonds between complementary bases in different portions of the same strand. These double stranded helical structures are called as stems.

Clover Leaf Structure



All t- RNA molecules contain 4 main arms or loops

1. Acceptor arm
2. Anticodon arm
3. D-arm
4. T ΨC arm

Acceptor arm

These consists of unpaired sequences of Cy-cy- Ad at the 3¹ end also called as Acceptor end. The 3¹-OH terminal of adenine may bind with the alpha –COOH of a specific amino acid and carries it. Now, it is called amino acyl t- RNA which is involved in the process of protein biosynthesis.

Anticodon arm

This is another unpaired and non-bonded loop carrying specific sequences of 3 bases constituting the anti-codon. The bases of anti- codon are hydrogen bonded with 3complementary bases of codon of m-RNA, during protein synthesis (eg). The AUG codon present in the m-RNA codes for methionine. In the corresponding amino acyl t- RNA carrying Methionine (methionyl-t-RNA), the Anticodon has the complementary UAC base sequence in it.

D-arm: The 3rd is the D-arm because it contains the base dihydro uridine.

T ΨC arm: This 4th arm contains thymine, pseudo uridine and cytosine.

Variable arm

Variables are is the most variable structure of t- RNA and it forms the basis of its classification.

1. Class -1 t- RNA: About 45% of all t- RNA belong to this class and have 3 to 5 base pairs in its extra arm. (e.g.) alanine –t-RNA
2. Class-2 t- RNA: This forms about 25% of total t- RNA and has 13 to 21 base pairs in theextra arm (e.g.) Phenyl alanine –t-RNA.

TERTIARY STRUCTURE OF t- RNA:

The L- shaped tertiary structure is formed by further folding of the clover leaf structure due to hydrogen bonds between D and T ΨC arm.

3. Ribosomal RNA (r-RNA)

The ribosome is present in the cytoplasm and is a nucleo-protein . In the ribosome, the m- RNA and t- RNA interact during protein biosynthesis. Ribosomes contain the 3rd type of RNA known as r-RNA. The r- RNA forms 80% of the total cellular RNA.

The r- RNA molecules are necessary for the assembly of ribosomal subunits . They also seen to play key roles in the binding of m-RNA to ribosomes and its translation. Ribosomal RNA is found in association with a few different proteins in the ribosomes. The ribosome serve as the site for protein synthesis.

In prokaryotic cells there are 3 distinct size species of r- RNA namely 5S, 16S, 23S, r- RNA, 16S r-RNA and 5s r-RNA. In eukaryotic cells there are 4 r-RNA species namely 5S, 18S, 28S, 5.8S r-RNA.

‘S’ is the swed berg unit which is related to the molecular weight of the compound.

Comparison of RNA with DNA

Similarities

1. Both have Ad, Gu and Cy
2. The nucleotides are linked together by phosphor diester bonds.
3. The bonding is in 3¹ to 5¹ directions.
4. Main function involves protein biosynthesis.

S.No	DNA	RNA
1	Thymine is present and uracil is absent	Uracil is present and thymine is absent
2	Pentose sugar is de oxy ribose	Pentose sugar is ribose
3	Present in nucleus	In addition to nucleus RNA is also found incytoplasm
4	Generally, they are ds	Generally, they are single strands
5	There are A,B, & Z forms ofDNA	There are m-RNA, t-RNA and r-RNA, hn-RNA, sn-RNA
6	Purine and pyrimidine contentsare equal	Purine and pyrimidine contents are unequal
7	Alkali stable doesnot give cyclic esters.	Alkali hydrolysis gives 2 ¹ , 3 ¹ cyclic di estermono nucleotides

6.8 NUCLEASES

Enzymes that hydrolyze nucleic acids are called nucleases. Virtually all cells contain various nucleases that have important roles in the normal nucleic acid metabolism. Organs that provide digestive fluids such as pancreas, are rich in nucleases. Pancreases secrete more amounts of nuclease to hydrolyze ingested nucleic acids. Fungi are also often good sources of nucleases.

Nucleases are phosphor diesterases because they catalyze the cleavage of phosphor di ester bonds by water. Because each internal phosphate in a polynucleotide backbone is involved in two phospho di ester linkages, cleavage can occur on either side of the phosphorus.

Cleavage on 'a' site leaves the phosphate attached to 5' at adjacent nucleotide. The 'b' site hydrolysis yields 3' phosphate products.

NUCLEASE SPECIFICITY

Like most enzymes nucleases exhibit specificity for the nature of the substance on which they act. That is some nuclease act only on DNA and are called DNase (De oxy ribo nuclease), white the others specific for RNA are ribonucleases (RNase), still same nucleases are not specific and are referred simply as nucleases.

Another classification denotes whether nucleic acid chain was cleaved at some internal location (Endo) or at terminal portions (Exo). Exo nucleases degrade NA sequential removing nucleotides from their ends. Endo nucleases cleaves NA chains at some internal locations.

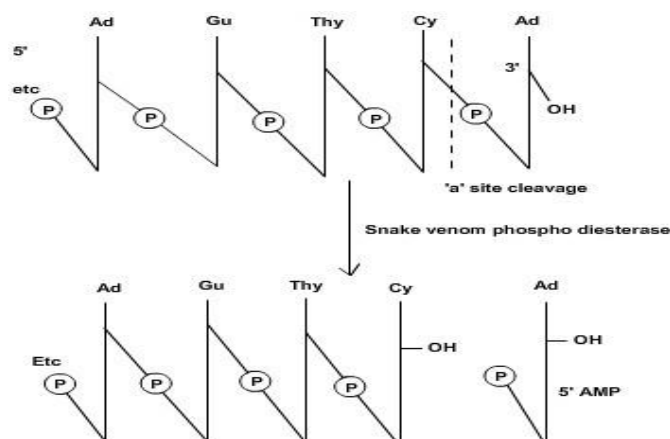
EXO NUCLEASES

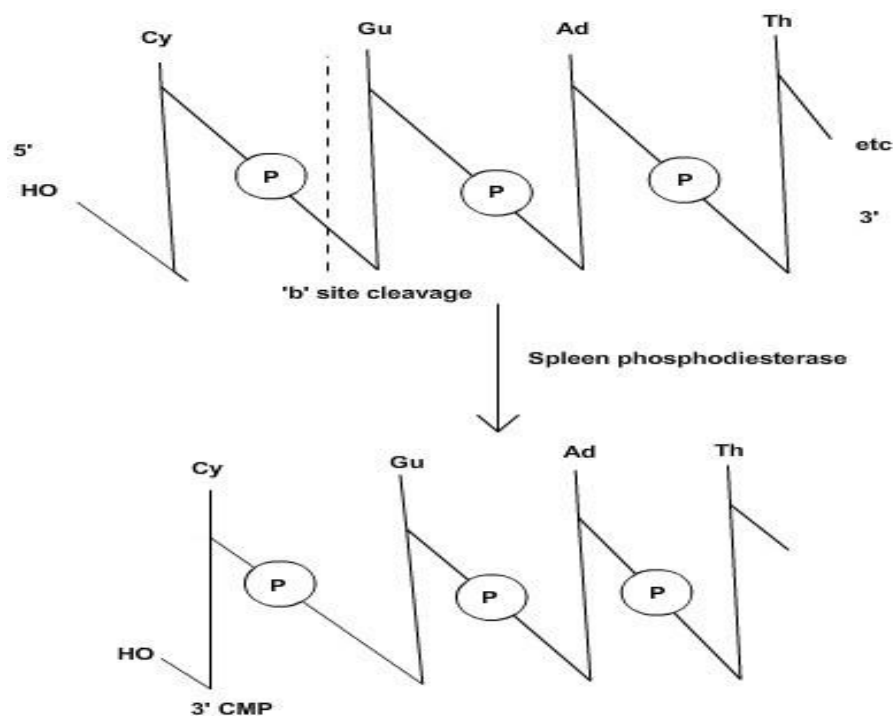
The two common exo nucleases are.

1. Snake venom phospho di esterase
2. Spleen phospho diesterase

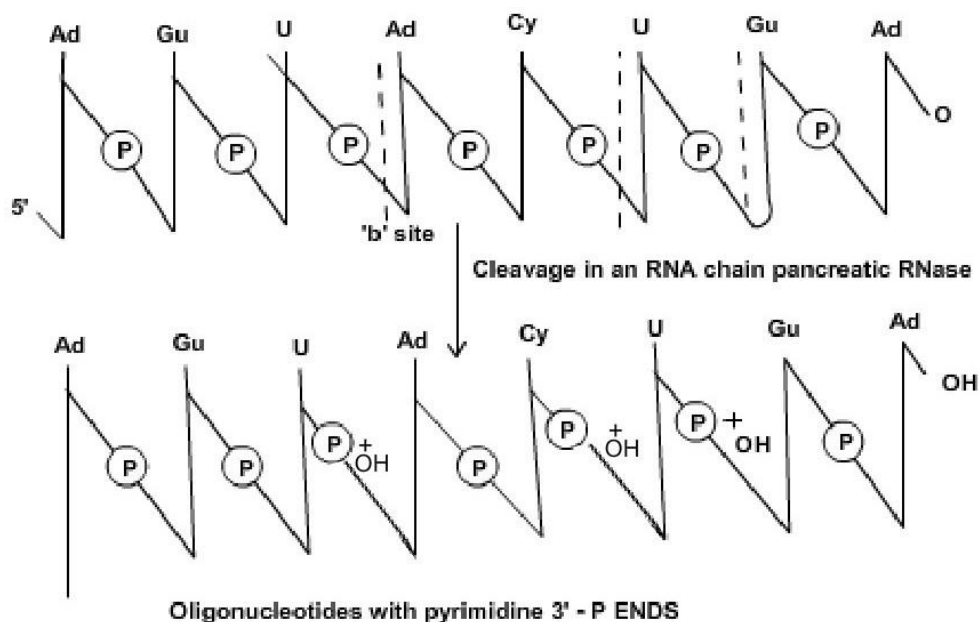
Since they can act on either DNA or RNA they are generally referred as phospho di esterase.

Snake venom phospho di esterase acts by a cleavage and starts at the free 3' OH end of apoly nucleotide chain liberating nucleoside 5' mono phosphates.





In contrast spleen phospho diesterase starts at the 5¹ end of the nucleic acid, clearing 'b' and releasing 3¹ nucleotide monophosphate.

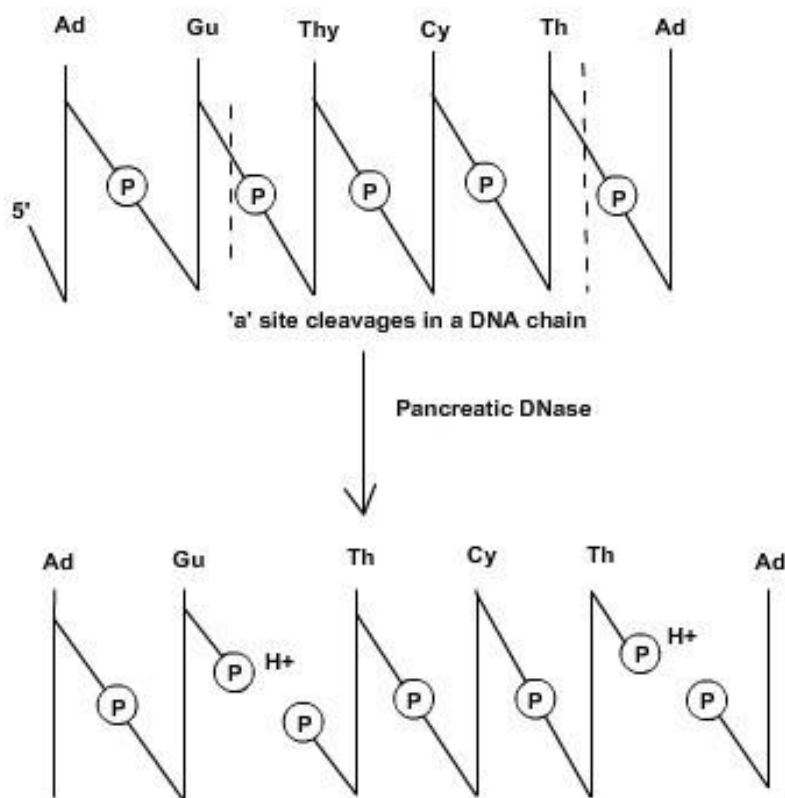


ENDONLEASE

The best studied and most widely used ribonuclease (RNase) is an Endonuclease. This pancreatic ribonuclease is an enzyme specific for 'b' cleavage where a pyrimidine base lies to the 3¹ side of the phosphodiester bond. The products are oligo nucleotides with pyrimidine 3¹ phosphate ends.

Deoxy ribonucleases or DNase

They hydrolyse either single stranded or double stranded DNA chain. DNase obtained from pancreas is an 'a' acting Endonuclease that shows from preference for cleavage between pyrimidine and purines . It creates 3¹ hydroxyl ends at random internal positions.



S.No		Enzyme	DNA, RNA	Site of cleavage	Specificity
1	Exonucleases	Snake venom phospho diesterase	Both	"a"	Starts at 3 ¹ end and yields 5 ¹ nucleotide mono phosphate products (5 ¹ NMP)
		Spleen phospho diesterase	Both	"b"	Starts at 5 ¹ end and yields 3 ¹ NMP products
2	Endonucleases	Pancreatic RNase	RNA	"b"	Catalyses 'b' cleavage where 3 ¹ phosphate bond is to pyrimidine
		Pancreatic DNase	DNA	"a"	Nicks double stranded DNA preferably between pyrimidine and purine creating 3 ¹ hydroxyl ends.

6.9 ESTIMATION OF RNA

Acid hydrolyses of RNA release ribose. This ribose releases in presence of strong acids like concentration HCl, dehydrates to yield furfural. Orcinol reacts with furfural in the presence of FeCl_3 to give a green colour. This forms the principle behind the estimation of RNA.

Pure yeast RNA is available commercially and is used (100 micro gram/ml) concentration for standardization set up different tubes with increasing concentration of RNA and make up the volume to 2ml with distilled water. Prepare the orcinol reagent . 300mg of orcinol dissolved in 5ml of ethanol. From this 3.5 ml is added 0.1% solution of FeCl_3 in concentration HCl. Add 3 ml of orcinol reagent to each test tube and mix well. Heat the tubes in a boiling water bath for 15 min. After which cool them to room temperature.

Measure the optical density at 665nm and draw a standard graph. Carryout the same procedure with test samples also. Thus the amount of RNA in any isolated sample may be estimated from the standard graph.

ESTIMATION OF DNA

The de-oxy ribose in DNA in presence of acid (concentration H_2SO_4) forms hydroxy leuvallic aldehyde. This hydroxy leuvallic aldehyde reacts with diphenyl amine to give a blue color.

Calf thymus DNA is available commercially and solution (100 microgram/ml concentration) is prepared and used for standard graph. Pipette out different volumes of DNA solution into test tubes and make up the volume to 3 ml with H_2O .

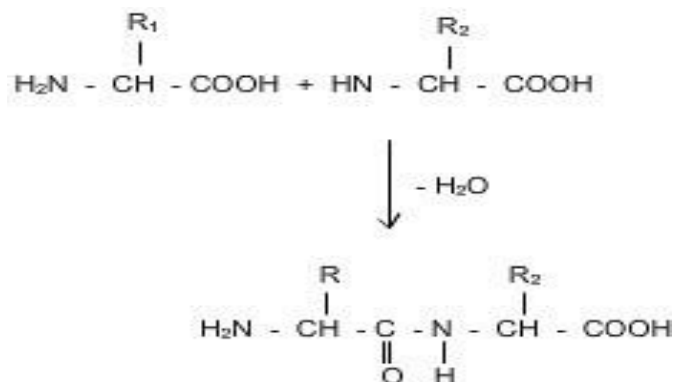
Prepare the fresh reagent by dissolving 1g of diphenyl amine in 100ml glacial acetic acid and by adding 2.5ml of concentration H_2SO_4 . Add 5ml of the reagent to all test tubes, mix well and heat in a boiling water bath for 10 min. After cooling measure, the optical density at 595 nm using colorimeter and then draw a standard graph. Carryout the same procedure for test sample and estimate the amount of DNA present in isolated sample.

6.10 PROTEINS

Forces or bonds involved in proteins Bonds involved in protein structure are of different types. They are.

1. Peptide bond

The -COOH group of 1 amino acid can be joined to the -NH_2 group of another amino acid by a covalent bond called as peptide bonds. In the process of formation of a peptide bond a molecule of H_2O is eliminated.



Peptide linkage

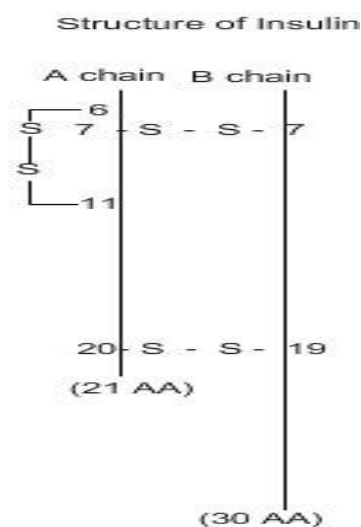
When two amino acids are joined together by one peptide bond, such a structure is called as Dipeptide. The 3rd amino acid can form a 2nd peptide bond and is called Tripeptide.

Thus the peptide linkage holds various amino acids together in a specific sequence and number. Peptides varying from the simplest dipeptide to very long poly peptides are present in human body and serve specific functions.

2. Disulphide bond (bridges linkages)

It is an important bond which interconnects two parallel peptide chains and is formed between two cysteine residues of the adjacent chains. Sometimes it is formed between two cysteine residues within a same chain. A good example is insulin which contains three disulphide linkages. Of these two are inter chain disulphide linkages and one is intra chain disulphide linkage.

Structure of insulin



3. Hydrogen bonds

These are weak low energy non-covalent bonds sharing a single hydrogen by 2 electro negative atoms such as oxygen and nitrogen. These are formed in secondary structure by sharing hydrogen atoms between oxygen of -CO and nitrogen of -NH (amide group) of different peptide bonds. This bond is symbolized by dotted lines.



Silk fibroin is an example for the presence of hydrogen bonds involving -NH and CO groups of the peptide bonds.

4. Hydrophobic bonds

Many amino acids Ala, Val, Leu, Isl, Met, have the side chain or R groups which are essentially hydrophobic such R groups can unite among themselves with elimination of H_2O to form linkages between various segments of the chain or between different chains.

These hydrophobic bonds constitute the major stabilizing for 3° structure forming a compact 3-dimensional structure.

5. Electrostatic bonds: (Ionic bond)

Ions possessing similar charge repel each other whereas ions having dissimilar charge attract each other.

The ionic bonding may be the interaction between acidic and basic groups of constituent amino acid in proteins. Thus ionic bond may occur between positively charged group (side chains of Lys, Arg) and negatively charged groups

(side chain of glu, Asp). These ionic bonds although weaker than hydrogen bonds are regarded as responsible for maintaining the tertiary of globular proteins.

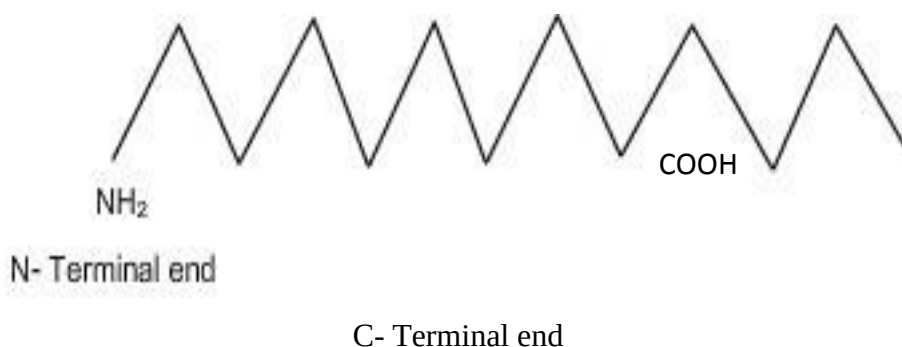
Structural organization of proteins

Protein structure is normally described at 4 levels of organization.

1. Primary level of organization (or) 1^o structure
2. Secondary level of organization (or) 2^o structure
3. Tertiary level of organization (or) 3^o structure
4. Quaternary level of organization (or) quaternary structure

Primary structure: (1^o structure)

Primary structure is the linear sequence of amino acid held together by peptide bond in protein. The peptide bond from the back bone and side chains of amino acid residues project outside the peptide back bone. The free –NH₂ group of the terminal amino acid is called as N- terminal end and the free –COOH group end is called as C-terminal end. It is a tradition to number the amino acid from N-terminal end as number one towards the C-terminal end.



Presence of specific amino acid at a specific number is very significant for a particular function of a protein. Any change in the sequence is abnormal and may affect the function and properties of proteins. For example hemoglobin contains four polypeptides (2 alpha and beta chains) In the beta chain the 6th amino acid is glutamic acid. In the sickle cell anemia the glu is replaced by Valine.

SECONDARY STRUCTURE OF PROTEINS

Secondary structure of proteins is known as conformation of peptide chain in the protein molecule. The folding of the polypeptide chain into a specific coiled structure held together by disulphide bonds and hydrogen bonds is referred to as the secondary

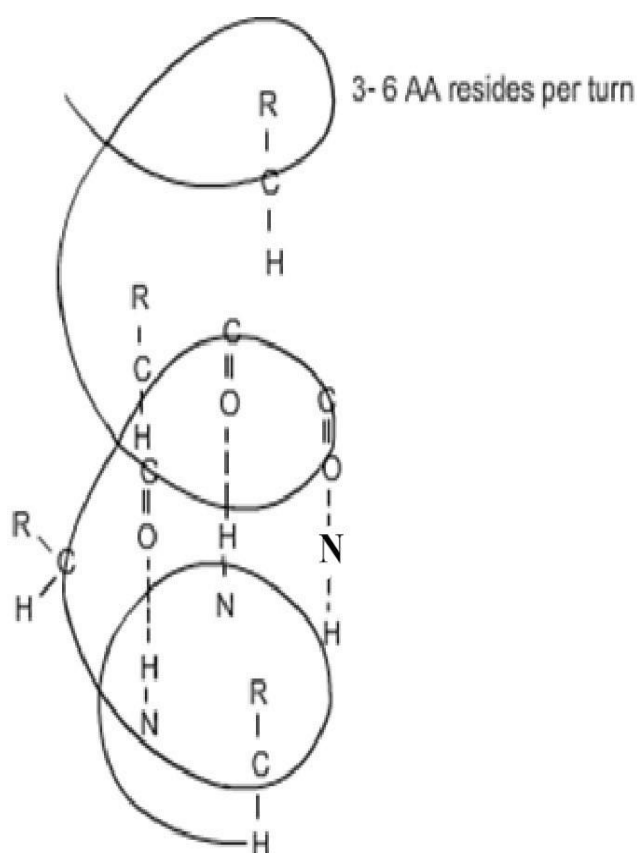
structure of proteins.

Two basic types of secondary structures of proteins have been recognized.

1. Alpha helix structure
2. Beta pleated sheet structure.

Alpha helix

This model was proposed by Pauling and Corey. A peptide chain forms regular helical coils called alpha helix. These coils are stabilized by hydrogen bonds between carbonyl oxygen of first amino acid and amide nitrogen of the 4th amino acid residues/ Thus in alpha helix intra chain hydrogen bonding is present.



The alpha helix can be either right handed or left handed. Right handed alpha helix is more stable than left handed alpha helix. Only the right handed alpha helix has been found in protein structure. This model permits maximum number of hydrogen bonds. There are 3.6 amino acid residues per turn of the helix. The distance between two equivalent point on turn is 0.54 nm (5.4 Å) and is called a pitch.

Small or uncharged amino acid residues such as ala, leu, phenyl alanine are often found in alpha helix. The main exemption is proline which do not fit in alpha helix

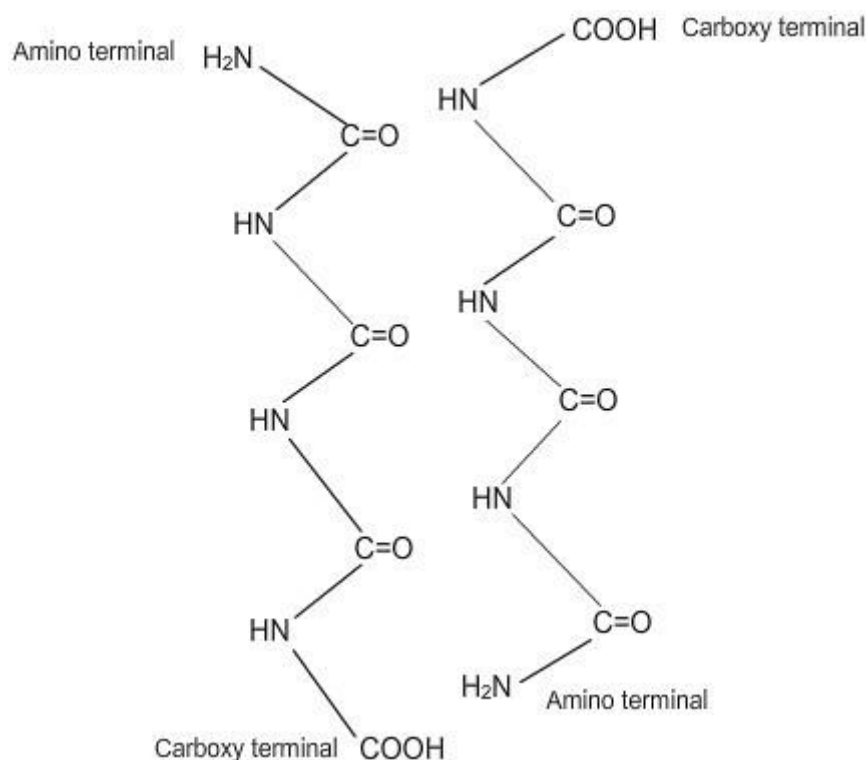
due to its ring structure. Other exceptions are val, and Ile. These are the amino acid with bulky side chain which break the helical structure. Amino acid which doesn't fit into helix are termed as helix breakers.

The proteins of hair, nail and skin contain a group of proteins called keratin rich in alpha helical structure.

Beta pleated structure or (Beta conformation)

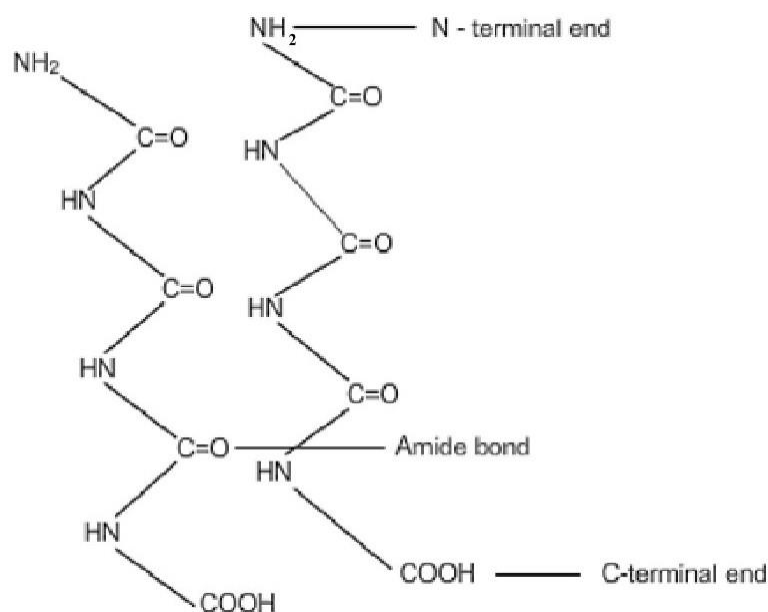
This model was also proposed Pauling. In this model the poly peptide chain are arranged side by side in close contact and these chain one are stabilized by inter molecular H₂ bonding. Two types of pleated sheets are possible.

1. Parallel pleated sheet structure: In this type of structure, the poly peptide chains lie side by side in the same direction so that their N-terminal residues are on the same end. This pleated sheet conformation is stabilized by H₂ bonding. Here the H₂ bonds are formed between an amide hydrogen of a peptide in one chain and carbonyl oxygen of a neighboring chain.



2. Anti-parallel pleated sheet structure: In this type of structure the poly peptide chain lie in opposite direction so that N-terminal end of one chain and C-terminal end of other chain face each other. It is also stabilized by inter chain.

H₂ bonding.



TERTIARY STRUCTURE OF PROTEINS

The poly peptide chain with secondary structure may be further folded, super folded, twisted about itself forming many sizes. Such a structural conformation is called tertiary structure. It is only one such conformation which is biologically active. The protein in this conformation is called a native protein.

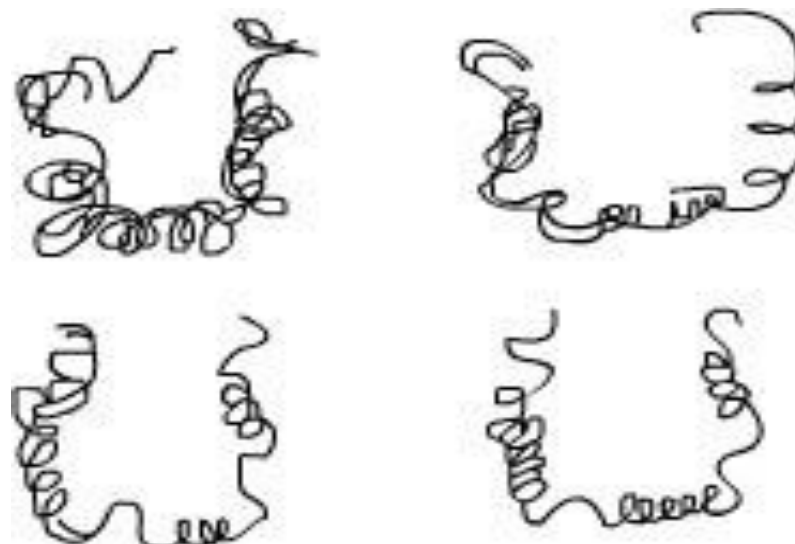
The tertiary structure of proteins describes the overall special arrangement of poly peptide chain thus gives an exact account of molecular shape in most of the small and medium sized proteins. The tertiary structure is constituted by steric relationship between amino acid located far apart but brought closer by folding.

The bonds responsible for interaction between groups of amino acid hydrophobic interaction, H₂ bonds, ionic bond and Van der Waals force.

QUATERNARY STRUCTURE OF PROTEINS

Quaternary structure of proteins refers to the number, and arrangement of poly peptide chain in naturally occurring proteins. Most of the large and even some medium sized proteins exist as an association of several chains. In such cases, the protein is known as Oligomer and the individual chain is known as Monomers or sub units.

Each subunit has its own primary, secondary and tertiary structures. The subunits may or may not be identical. (e.g.) Hemoglobin is a tetramer made up of four subunits or monomers. It consists of two identical alpha chain and two identical beta chains. The chains fit together in an approximate tetrahedral arrangement in the quaternary structure of hemoglobin.

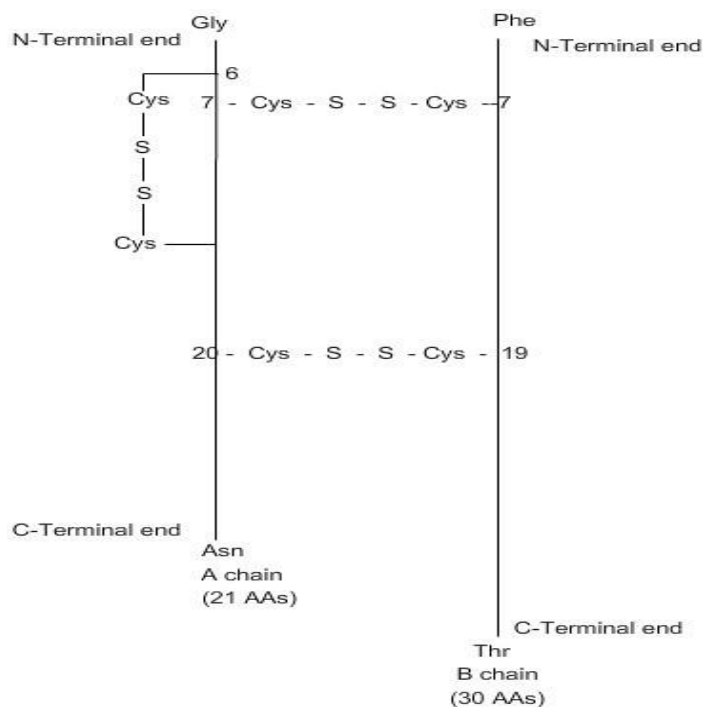


6.11 : INSULIN – HORMONE

Insulin is a peptide hormone secreted by the beta cells of islets of Langerhans of the pancreas. It plays an important role in the body by regulating carbohydrate metabolism. Its deficiency causes a disease called diabetes. Which is characterized by increased blood sugar and the excretion of glucose in urine. This disease is hereditary and can be treated by injecting insulin which is now prepared from the pancreas of cattle or other animals.

STRUCTURE OF INSULIN

Insulin was the first protein whose amino acid sequence was worked out by Sanger *et.al.*, (1951-1955). The structure of insulin was established in 1955 by scientist. Sanger for which he was awarded Nobel Prize. Insulin on complete hydrolysis gives 51 amino acid molecules.



Insulin is a hetero diametric protein which has been isolated from pancreas and is prepared in crystalline form. Insulin molecule is composed of 2 polypeptide chain called A and B chain containing total of 51 amino acid. 'A' chain contains 21 amino acid 'B' chain contain 30 amino acid. In A chain N-terminal amino acid is glycine and C-terminal is Asn. In B chain N-terminal amino acid is phenyl alanine and C-terminal is Threonine.

Both the chains are held together by d di-sulphide linkages. The Cys-7 of A chain is joined to Cys-7 of B chain. The cys-20 of A chain is joined to Cys-19 of B chain. In addition the a chain carries an intra chain disulphide linkage between Cys-6 and Cys-11 of A chain. Breaking of the disulphide bonds with alkali or reducing agents inactivate insulin. Digestion of insulin protein with proteolytic enzymes also inactivates the hormone. Hence insulin cannot be given orally.

FUNCTIONS OF INSULIN

Insulin is an anabolic hormone which acts on variety of tissues. Major target tissues of insulin are the muscles, liver and adipose tissues. The various function of insulin are.

1. Insulin lowers the blood sugar level by accelerating glycogenesis (glycogen synthesis in liver).
2. It increases the rate of oxidation of glucose (i.e) glycolysis.
3. The net effect is lowering of blood glucose level and increase glycogen storage.

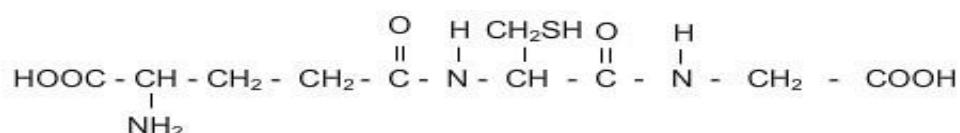
4. The effects of insulin on lipid metabolism are lowering of free fatty acid level and increase in triglyceride storage.
5. Insulin promotes protein synthesis.

BIOMEDICAL IMPORTANCE

1. Insulin was the first protein proved to have hormonal action.
2. It was the first protein crystallized (Abel 1926) and 1st protein sequenced. (Sanger et al, 1955)
3. It was the first protein synthesized by chemical techniques and prepared for commercial use by recombinant DNA technology.

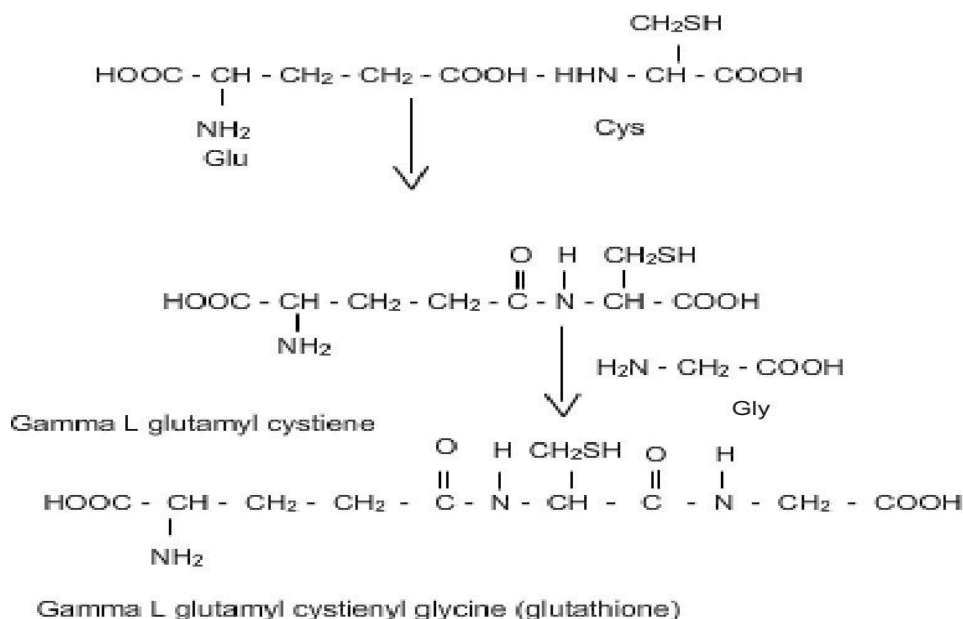
6.12: GLUTATHIONE

Glutathione is a tripeptide of three amino acids Gly, Cys and Glu. Chemically it is γ -L-glutamyl cysteinyl glycine.



SYNTHESIS OF GLUTATHIONE

Glutathione is synthesized in the cytosol outside the ribosomes from three amino acids Glu, Cys and Gly. N-glutamate 1st condenses with N-cysteine to form γ -L-glutamyl cysteine which then combines with Gly to form glutathione.



Functions

It functions as co-enzymes of various enzymes such as malyl aceto acetate isomerase and glyoxylase, in the variety of metabolic processes.

Besides this function glutathione plays a protective role in various tissues including erythrocytes, liver and pancreas. A few of protective roles are as follows.

1. Erythrocytes are protected by glutathione against oxidative damage.
2. Sulphydryl group of reduced glutathione helps to maintain structure of RBC and the globin part of HB molecule.
3. Glutathione prevents the effects of alloxan which is a toxic substance that causes diabetes mellitus. In other words, it protects the pancreas against the diabetogenic action of alloxan.
4. Glutathione protects the liver by detoxification of foreign substances by converting them into mercapturic acid
5. Glutathione reduces ascorbic acid into more stable reduced form and hence it protects the body storage of ascorbic acid.

OXYTOCIN

Oxytocin is a hormone secreted by pituitary gland. It is cyclic poly peptide containing amino acid and a disulphide linkage. Its molecular weight is about 1000.

Functions

1. It causes contraction of stomach muscles of lactating mammary gland resulting in ejection of milk.
2. It plays a major role during parturition and causes the rhythmic contraction of uterus.
3. It stimulates contraction of gallbladder, intestine, and urinary bladder.

VASOPRESSIN (ADH)

Vasopressin is another hormone secreted by posterior pituitary gland. It is a cyclic poly peptide. Its structure is quite like that of oxytocin except Ile which is replaced by phenyl alanine and leu which is replaced by lys.

FUNCTIONS

1. It has a marked effect on kidney tubules accelerating the rate of H₂O reabsorption from the distal tubules and thus produces a marked antidiuretic effect. Hence it is

termed as antidiuretic hormone.

2. Diabetes insipidus

occurs in the absence of ADH which is characterized by large volume of excretion of urine up to 30 liters of urine per day.

6.13: SUMMARY

The nucleic acid has been subject of bio-chemical investigations since 1871 when a 22 year old Swiss physician and chemist Friedrich Miescher isolated from nucleus of pus cells and named it as nuclein. This substance was quite different from the carbohydrates proteins and fats. Later it was renamed as nucleic acids.

6.14: KEYWORDS

Nucleic acids

The nucleic acid has been subject of bio-chemical investigations since 1871 when a 22 year old Swiss physician and chemist Friedrich Miescher isolated from nucleus of pus cells and named it as nuclein. This substance was quite different from the carbohydrates proteins and fats. Later it was renamed as nucleic acids.

Composition

There are two kinds of nucleic acids Deoxy ribo nucleic acid (DNA) and Ribo nucleic acid(RNA). On hydrolysis the two nucleic acids yield 3 components.

1. Phosphoric acid
2. Pentose sugar
3. Nitrogenous bases

Nucleosides

The nucleosides are compounds in which nitrogenous bases (purines and Pyrimidine) are conjugated to the pentose sugars (ribose and deoxy ribose) by a beta glycosidic linkage. The glycosidic linkage involves the C1 atom of sugar and H atom of N9 (in the case of purines) or N1 (in the case of pyrimidines), thus eliminating a molecule of H₂O. The nucleosides are generally named for the particular purine or Pyrimidine present nucleosides containing ribose are ribonucleosides while those possessing deoxy ribose are called deoxy ribonucleosides.

Nucleotides

Nucleotides: Nucleotides = nucleosides + H_3PO_4

Nucleotides are the H_3PO_4 esters of nucleosides. These occur either in the free form or as subunits in nucleic acids. The phosphate is always esterified to the sugar moiety (group).

Denaturation

The 2 strands of a DNA helix readily separate when the hydrogen bonds between its paired bases are disrupted. This can be achieved by heating as a solution of DNA or by adding acid or alkali to ionize its bases. The unwinding of the double helix is called melting, because it occurs abruptly at a certain temperature. This process is known as Denaturation.

Insulin

Insulin was the first protein whose amino acid sequence was worked out by Sanger et al (1951-1955). The structure of insulin was established in 1955 by scientist . Sanger for which he was awarded Nobel prize. Insulin on complete hydrolysis gives 51 amino acid molecules.

Glutathione

Glutathione is a tripeptide of 3 amino acid Gly, Cys and gly. Chemically it is, Lglutamyl cysteinyl glycine.

Oxytocin

Oxytocin is a hormone secreted by pituitary gland. It is cyclic poly peptide containing amino acid and a disulphide linkage. Its molecular weight is about 1000.

Messenger RNA

EXERCISE

I; Essay Questions

1. What are nucleic acids?
2. Write a note on the composition of nucleosides and nucleotides?
3. How to isolate nucleic acids and mention the separation technique of nucleic acids?
4. Explain in detail the primary and secondary structure of nucleic acids?

5. Write a detailed note on the structure and types of RNA?
 6. What are nucleases?
 7. Give an account of the denaturation and renaturation process?
 8. Mention the melting temperature with its G: C content of DNA?
 9. Explain in detail the structure of proteins?
 10. How to estimate DNA and RNA?
- II Multiple Choice Questions:**

1. Nucleoside is a pyrimidine or purine base. ()
a) covalently bonded to a sugar b) ionically bonded to a sugar.
c) hydrogen bonded to a sugar d) none of the above
2. in gel electrophoresis, what fragments will move most quickly through a gel ()
a) Large fragments b) Small fragments c) Large genome d) None of these.
3. Which pyrimidine base contains an amino group at carbon 4 ()
a) Cytosine b) Thymine c) Uracil d) Adenine
4. Nucleotide bases and aromatic amino acids absorb light respectively at () a) 280 and 260 nm b) 260 and 280 nm c) 270 and 280 nm d) 260 and 270 nm
5. The glycosidic bonds in DNA and RNA ()
a) Connect the sugar to the base b) Can be hydrolyzed by OH⁻ ion c) Stabilize Watson-Crick H-bonds d) are free to rotate over about 180°
6. Nucleic acids can be analyzed experimentally by their ()
a) molecular weight b) absorption of visible light c) absorption of uv light
d) none of these
7. Thymidine ()
a) can participate in hydrophobic interactions due to its methyl group.
b) is replaced by uracil in RNA c) normally forms two hydrogen bonds with adenosine
d) all the above
8. A nucleotide consists of ()
a) a sugar, a base, and a phosphate b) a sugar and a phosphate c) paired bases d) a sugar, a base, and three phosphates.
9. A five-carbon sugar lacking a hydrogen at the number 2 carbon is found in ()
a) DNA b) RNA c) mRNA d) rDNA
10. The most stabilizing force for nucleic acids is ()
a) hydrogen bonds b) electrostatic bond c) Van der Waals d) conformational entropy

III Fill in the Blanks.

1. The sugar in RNA is_____, the sugar in DNA is_____
2. A purine with an amine (NH₂) group on the 6th carbon is _____
3. _____ is found on RNA but not DNA
4. _____ is an example of purine?
5. Nucleic acids _____
6. A nucleotide is composed of _____
7. Oligonucleotides are formed from 2 to 10 _____
8. The variable portion of DNA is the sequence of _____
9. Gene expression is the activation of a gene to produce a specific_____
10. _____ is the process of removing the _____ from pre-mRNA and joining the remaining _____ together to form an mRNA molecule.

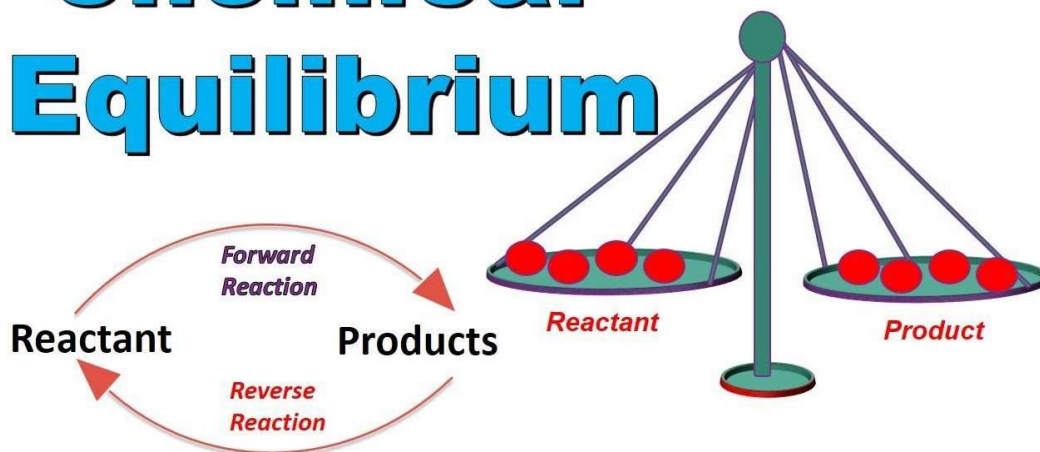
II Answers: 1) a 2) b 3) a 4) b 5) a 6) c 7) d 8) a 9) a 10) c

III Answers: 1) ribose, deoxyribose **2)** adenine **3)** Uracil **4)** adenine/Thymine **5)** are polymers. **6)** a base, a sugar, and a phosphate **7)** nucleotides **8)** bases **9)** protein **10)** splicing, introns, exons

CHAPTER-VII

Chemical Equilibrium

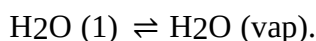
Chemical Equilibrium



7.0 INTRODUCTION

Chemical reactions

Chemical equilibria are important in numerous biological and environmental processes. For example, equilibria involving O₂ molecules, and the protein hemoglobin play a crucial role in the transport and delivery of O₂ from our lungs to our muscles. Similar equilibria involving CO molecules and hemoglobin account for the toxicity of CO. When a liquid evaporates in a closed container, molecules with relatively higher kinetic energy escape the liquid surface into the vapour phase, and a number of liquid molecules from the vapour phase strike the liquid surface and are retained in the liquid phase. It gives rise to a constant vapour pressure because of an equilibrium in which the number of molecules leaving the liquid equals the number returning to liquid from the vapour. We say that the system has reached equilibrium state at this stage. However, this is not static equilibrium and there is a lot of activity at the boundary between the liquid and the vapour. Thus, at equilibrium, the rate of evaporation is equal to the rate of condensation. It may be represented by.



The double half arrows indicate that the processes in both directions are going on simultaneously. The mixture of reactants and products in the equilibrium state is called an equilibrium mixture.

Equilibrium can be established for both physical processes and chemical reactions. The reaction may be fast or slow depending on the experimental conditions and the nature of the reactants. When the reactants in a closed vessel at a particular temperature react to give products, the concentrations of the reactants keep on decreasing, while those of products keep on increasing for some time after which there is no change in the concentrations of either of the reactants or products. This stage of the system is the dynamic equilibrium and the rates of the forward and reverse reactions become equal. It is due to this dynamic equilibrium stage that there is no change in the concentrations of various species in the reaction mixture. Based on the extent to which the reactions proceed to reach the state of chemical equilibrium, these may be classified in three groups.

- (i) The reactions that proceed nearly to completion and only negligible concentrations of the reactants are left. In some cases, it may not be even possible to detect these experimentally.
- (ii) The reactions in which only small amounts of products are formed and most of the reactants remain unchanged at the equilibrium stage.
- (iii) The reactions in which the concentrations of the reactants and products are comparable when the system is in equilibrium.

The extent of a reaction in equilibrium varies with the experimental conditions such as concentrations of reactants, temperature, etc. Optimization of the operational conditions is very important in industry and laboratory so that equilibrium is favorable in the direction of the desired product. Some important aspects of equilibrium involving physical and chemical processes are dealt in this unit along with the equilibrium involving ions in aqueous solutions which is called as ionic equilibrium. Chemical equilibrium is called Dynamic equilibrium because it gives products and reactants simultaneously.

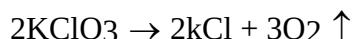
Reversible Reactions

This is also called a balanced reaction. It is one which proceeds in both directions and gives the products and reactants simultaneously.



Irreversible reactions

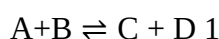
If the reactions proceed only in one direction, it is said to be irreversible reactions.



7.1 LAW OF MASS ACTION AND EQUILIBRIUM CONSTANT

The law of mass action provides an explanation for the phenomenon of chemical equilibrium. The rate of a chemical reaction is directly proportional to the product of the active mass of the reactants, according to this law, which asserts that this relationship holds true. Both the molecular concentration and the active mass are equivalent to one another. It is the number of grammes of a substance's molecules that are contained in one liter. Putting the formula for the sub in square brackets provides a visual representation of this concept. Both gases and solids have active masses that are equal to their respective partial pressures. There is no change in the active mass.

Let us apply this law to the following reversible reactions.



Let [A],[B], [C] and [D] represents the active masses of A,B,C and D respectively at equilibrium.

Then the rate of combination of A and B will be proportional to [A] and [B].

$$\therefore r_f = k_f [\text{A}] [\text{B}] \quad 2$$

When r_f and k_f are forward rate and forward rate constant respectively.

Similarly, the rate of formation of A and B (backward reaction) will be $\propto$ to [C] and [D]

$$\therefore r_b = k_b [\text{C}] [\text{D}] \quad 3$$

Since at equilibrium $r_f = r_b$, it follows that $k_f [\text{A}] [\text{B}] = k_b [\text{C}] [\text{D}]$

$$\boxed{K_c = \frac{k_f}{k_b} = \frac{[\text{C}][\text{D}]}{[\text{A}][\text{B}]}} \quad \text{-----} \quad 4 \quad K_c = \frac{[\text{C}][\text{D}]}{[\text{A}][\text{B}]}$$

Where K_c is known as equilibrium constant and is defined as the ratio of the product of active masses of products and product of active masses of reactants.

Many reactions involve more than one mole of reactants and products and such reactions may be represented generally as



When a, and b are number of moles of reactants and c and d are number of moles of products.

$$K_c = \frac{[C]^c [D]^d}{[A]^a [B]^b} \text{----- 6}$$

So, we have

If the sub is involved in reversible reaction α gases, it is more convenient to use partial pressure, since the partial pressure of the sub is proportional to its concentration in a gaseous phase at the given temperature.

Then the equilibrium constant K_p is obtained as,

$$\boxed{K_p = \frac{P_C^c P_D^d}{P_A^a P_B^b}} \text{----- 7}$$

When K_p is the equilibrium constant expressed in terms of partial pressure P_A , P_B , P_C & P_D .

Relation between K_c and K_p

Consider the reversible reaction involving gaseous substances behaving ideally.



$$K_c = \frac{[C]^c [D]^d}{[A]^a [B]^b}$$

$$K_p = \frac{P_C^c P_D^d}{P_A^a P_B^b}$$

We have

According to ideal gas equation $PV = nRT$, bringing this V to the right side we get.

$$P = \frac{n}{v} RT$$

Where $\frac{n}{v}$ = active mass

$$P = \left(\frac{n}{v} \right) RT$$

By using this relation, the partial pressure of A, B, C, D molecules,

$$P_A^a = [A]^a RT^a$$

$$P_B^b = [B]^b RT^b$$

$$P_C^c = [C]^c RT^c$$

$$P_D^d = [D]^d RT^d$$

Substitute these 4 values in K_p equation, we get

$$K_p = \frac{[C]^c [D]^d RT^{c+d}}{[A]^a [B]^b RT^{a+b}}$$

$$\text{Since } \frac{[C]^c [D]^d}{[A]^a [B]^b} = K_c,$$

$$\text{Now, } K_p = K_c RT^{(c+d)-(a+b)}$$

Since $\Delta^n = (c+d) - (a+b)$, where Δ^n is difference in total number of moles of the products and that of number of moles of the reactants.

$$\therefore K_p = K_c RT^{\Delta^n}$$

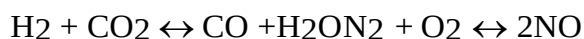
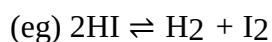
On the basis of Δ^n values, the reactions are classified into 2 categories.

a) $\Delta^n = 0$

b) $\Delta^n \neq 0$

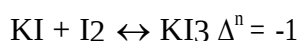
a) $\Delta^n = 0$

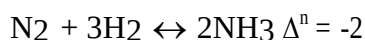
Gaseous reactions in which the number of molecules remained are the same



b) $\Delta^n \neq 0$

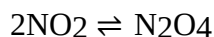
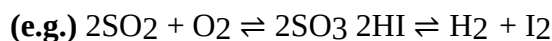
Gaseous reactions in which there is a change in the no; of molecules. (eg) PCl_5



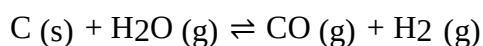
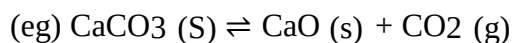
**7.2 TYPES OF CHEMICAL EQUILIBRIA**

They are two types. They are **1. Homogenous equilibria:**

In this, the subs that is present are in single pH

**2. Heterogenous Equilibria**

If the subs participating in chemical equilibrium are in more than one phase, it is said to be heterogenous.

**7.3 LE CHATLIERS PRINCIPLE**

If states as “when a stress is applied on a system in equilibrium, the system tends to adjust itself so as to reduce the stress”. There are three ways in which stress can be caused on it.

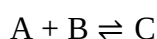
- i) Changing the concentration of the Reactant or product.
- ii) Changing the volume or pressure of the system
- iii) Changing the temperature.

When applied to the chemical reaction in equilibrium this principle can be stated as, if change in Conc, Temp or pres is caused to a chemical reaction in equilibrium, the equili shifts to the right or left to minimize the change.

7.4 EFFECT OF CHANGE IN CONCENTRATION

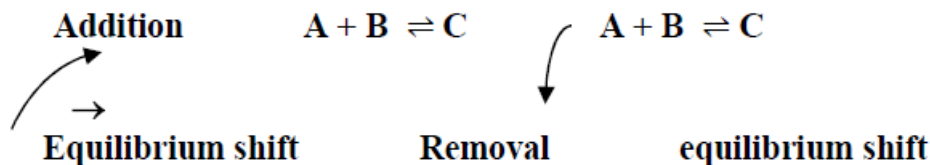
When Conc of any reactant or product is changed, the equili shifts in the direction to reduce the change in Conc it was made.

Let us Consider, general reaction.



When a reactant say A is added at equilibrium, its conc has increased. Forward reaction alone occurs momentarily. According to this principle, a new equilibrium

will be established to reduce the conc of A. Thus, addition of A causes equilibrium to shift to right. This increases the conc of product C,



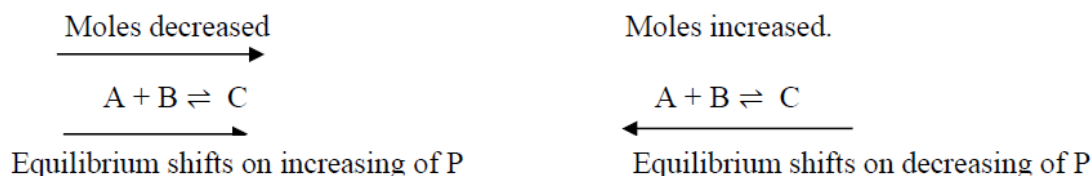
A decrease in the Concentration of A by its removal from the equilibrium mixture will lead to the shift of the equilibrium position to the left. This reduces the Concentration of product C.

7.4.1: Effect of change in Pressure

When pressure is increased on a gaseous equilibrium reaction, the equilibrium will shift in a direction that tends to decrease the pressure.

The pressure of the gaseous reaction at equilibrium is determined by the total no ; of molecules. While the decomposition of C into A results in the increase of molecules. $\therefore$ by the increases of pressure on equilibrium, it shifts to the right and gives more C.

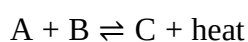
The decrease in pressure will cause the opposite effect. The equilibrium shifts to the left when C will decompose to form more of A and B.



By increase of pressure on chemical equilibrium shift in that direction in which the number of molecules decreases and vice versa.

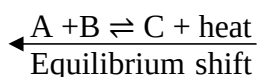
1.4.2: Effect of temperature

When a temp of the reaction is increased, the equilibrium shift in a direction in which heat is absorbed. Let us consider an exothermic reaction.



When the temp of the system is increased heat is supplied to it from outside. According to Le chatliers principles, the equilibrium will shift to the left which involves absorption of heat. This would result in an increase of the concentration of the reactants A and B.

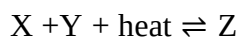
Temperature increase heat added.



In an endothermic reaction, $X + Y + \text{heat} \leftrightarrow Z$ when increase the temperature, it shifts the equilibrium to the right as it involves the adsorption of heat. This increases now concentration of product Z.

The increases in temperature, favor the reverse change in an exothermic reaction and forward change in endothermic reaction.

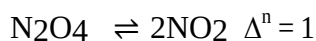
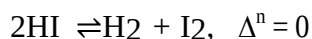
Temperature increases, heat added.



$\xrightarrow{\hspace{1.5cm}}$
 Equilibrium shifts

Application of law of mass action in chemical equilibrium:

Amongst homogenous equilibrium, gaseous systems are of 2 types ($\Delta^n = 0$ and $\Delta^n \neq 0$) will be discussed.



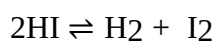
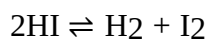
Under heterogenous equilibrium, we will discuss the decomposition of limestone.



7.5 HYDROGEN IODIDE EQUILIBRIUM: The equilibrium may be represented as, $2\text{HI} \rightleftharpoons \text{H}_2 + \text{I}_2$

Let 'a' mole of HI be increased in a sealed bulb having a volume 'v' liter kept in a thermostat at 721 K till an equilibrium is established. Let, x/2 moles of H_2 and x/2 moles of I_2 are formed from x moles of HI.

The equilibrium concentration of various species per litre are.



Initial Conc	$\frac{a}{v}$	a	0
equilibrium Conc	$\frac{a-x}{v}$	$\frac{x}{2v}$	$\frac{x}{2v}$

Applying the law of mass action,

$$K_c = \frac{[\text{H}_2][\text{I}_2]}{[\text{HI}]^2} = \frac{\left(\frac{x}{2v}\right)\left(\frac{x}{2v}\right)}{\left(\frac{a-x}{v}\right)^2} = \frac{x^2}{4v^2} \times \frac{v^2}{(a-x)^2}$$

$$\therefore K_c = \frac{x^2}{4(a-x)^2}$$

Alternatively, we can use partial pressure of the species instead of Molar concentrations. Since the no; of moles of the species H₂, I₂ and HI at equilibrium x/2, x/2 and (a – x) respectively, the total no ; of moles present at equilibrium is x/2 + x/2 + a – x = a. If the total pressure is P, the partial pressure at equilibrium is.

$$P_{H_2} = \frac{x}{2a} \cdot P$$

$$P_{I_2} = \frac{x}{2a} \cdot P$$

$$P_{HI} = \left(\frac{a-x}{a} \right) \cdot P$$

Applying the law of mass action

$$K_p = \frac{P_{H_2} \cdot P_{I_2}}{(P_{HI})^2} = \frac{\left(\frac{x}{2a} \cdot P \right) \left(\frac{x}{2a} \cdot P \right)}{\left(\frac{a-x}{a} \cdot P \right)^2}$$

$$= \frac{x^2 P^2 a^2}{4a^2 (a-x)^2 P^2} = \frac{x^2}{4(a-x)^2}$$

$$\therefore K_p = \frac{x^2}{4(a-x)^2}$$

Since, in this case $\Delta^n = 0$, we have $K_p = K_c$

$$K_p = K_c = \frac{x^2}{4(a-x)^2}$$

Effect of temperature

There is no temp term in K_p and K_c equation. Hence the increase or decrease in the temp will not affect the equilibrium position.

Effect of pressure

There is no pressure term in K_p and K_c equation. Hence, the increase or decrease in the pressure will not affect the equilibrium position.

Effect of Catalyst

Catalyst will increase the rate of both forward and backward reaction. There is no change in equilibrium position.

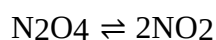
Effect of concentration

If we increase the Concentration of H_2 or I_2 we will get more amount of HI . If we increase the Concentration of HI , we will get more amount of H_2 and I_2 are formed.

Check your progress

7.6 DISSOCIATION OF N_2O_4

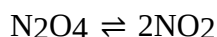
Let us consider the decomposition of N_2O_4 into NO_2 which is a reversible reaction.



Applying the law of mass action to equilibrium we get,

$$K_c = \frac{[NO_2]^2}{[N_2O_4]}$$

Taking 'a' moles N_2O_4 initially and assuming 'x' moles are dissociated on heating to a constant α / v temp in a closed container of 'v' litre capacity the equilibrium constant K_c can be calculated in the following way.

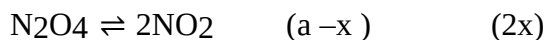


Initial concentration	a	0
	$\frac{a-x}{v}$	$\frac{2x}{v}$
equilibrium concentration		

$$K_c = \frac{[NO_2]^2}{[N_2O_4]} = \frac{\left(\frac{2x}{v}\right)^2}{\frac{a-x}{v}} = \frac{4x^2}{v^2} \times \frac{v}{a-x}$$

$$\therefore K_c = \frac{4x^2}{(a-x)v}$$

Alternatively, the equilibrium constant K_p can be calculated in following manner. As seen above, number of moles of each species at equilibrium are.



The total number of moles at equilibrium is $(a-x) + 2x = a + x$ since pressure is proportional to number of moles, total pressure P is proportional to $a + x$. Similarly, pressure of N_2O_4 is proportional to $a - x$ and that of NO_2 is proportional to $2x$. Hence

$$P_{\text{N}_2\text{O}_4} = \frac{(a-x)}{(a+x)} P$$

$$P_{\text{NO}_2} = \frac{2x}{a+x} P$$

By definition,

$$K_p = \frac{[P_{\text{NO}_2}]^2}{P_{\text{N}_2\text{O}_4}} = \frac{\left(\frac{2x}{a+x} P\right)^2}{\frac{a-x}{a+x} P} = \frac{4x^2 P^2 \cdot a+x}{(a+x)^2 (a-x) P}$$

$$K_p = \frac{4x^2 P}{(a+x)(a-x)} = \frac{4x^2 P}{a^2 - x^2} = L$$

$$\therefore K_p = \frac{4x^2 P}{a^2 - x^2}$$

If the dissociation is very small then x^2 may be neglected when compared with a^2 and then equation becomes,

$$K_p = \frac{4x^2 P}{a^2}$$

Effect of pressure

There is pressure term in K_p equation in the numerator. So, the increase in pressure increases the K_p value, but K_p is constant. The increase in numerator value is compensated by increase in denominator value (i.e) more amount of N_2O_4 is formed.

Effect of temperature

Both K_p and K_c equation are independent of temp. So increase or decrease in temperature will not affect the equilibrium position.

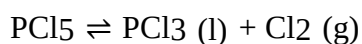
Effect of concentration

If we increase the concentration of N_2O_4 , the excess amount of N_2O_4 are decomposed to give more amount of NO_2 . If we increase the concentration of NO_2 , more amount of N_2O_4 are formed.

Effect of catalyst

It may increase the rate of both forward and backward reaction. So, there is no change in equilibrium position.

7.7 APPLICATION OF LAW OF MASS ACTION TO PCl_5 SYSTEM:



1 mole of PCl_5 is taken in 'v' volume of solution.

No : of moles at 1 0 0

Initial

At equilibrium $(1 - \alpha)$ α α

' α ' moles of PCl_5 from 1 mole is decomposed to give α moles of PCl_3 and α moles of Cl_2 .

Number of moles

$$\text{Active mass} = \frac{n}{v} = \quad v$$

Active masses of PCl_3 , Cl_2 and PCl_5 are α / v , α / v , $\frac{1 - \alpha}{v}$

Substitute these values in K_c equation.

$$K_c = \frac{[PCl_3][Cl_2]}{[PCl_5]} = \frac{\left(\frac{\alpha}{v}\right)\left(\frac{\alpha}{v}\right)}{\left(\frac{1 - \alpha}{v}\right)} = \frac{\alpha^2 X v}{v^2 X (1 - \alpha)}$$

$$K_c = \frac{\alpha^2 v}{v^2 (1 - \alpha)} = \frac{\alpha^2}{v(1 - \alpha)}$$

$$\therefore K_c = \frac{\alpha^2}{v(1 - \alpha)}$$

Equilibrium constant at partial pressure

$$K_p = \frac{P_{PCl_3} \cdot P_{Cl_2}}{P_{PCl_5}} = \frac{\text{Partial pressure of products}}{\text{Partial pressure of reactant}}$$

Partial pressure = Total pressure X Mole fraction of that

Subtotal no. of moles = $1 - \alpha + \alpha + \alpha = 1 + \alpha$

$$P_{PCl_3} = p \cdot \frac{\alpha}{1 + \alpha}, P_{Cl_2} = \frac{\alpha}{1 + \alpha}, P_{PCl_5} = p \cdot \frac{1 - \alpha}{1 + \alpha}$$

Substituting these values in K_p equation

$$\begin{aligned} K_p &= \frac{p \cdot \frac{\alpha}{1 + \alpha} \times p \cdot \frac{\alpha}{1 + \alpha}}{p \cdot \frac{1 - \alpha}{1 + \alpha}} \\ &= \frac{p^2 \cdot \alpha^2 \cdot 1 + \alpha}{(1 + \alpha)^2 (1 - \alpha) \cdot p} = \frac{p \alpha^2}{(1 + \alpha)(1 - \alpha)} \end{aligned}$$

$$K_p = \frac{p \alpha^2}{1 - \alpha^2}$$

Effect of temperature

There is no temp term in K_p and K_c equation. Therefore, increase or decrease in temp do not affect the equilibrium.

Effect of Pressure

The pressure term is present in K_p equation. Pressure term at K_p are directly proportional. If we increase the pressure of the system, the value of K_p is also increased. But K_p is a constant. So, the increase in numerator value is compensated by increase in denominator value. (i.e) More amount of PCl_5 is formed.

Effect of catalyst

Catalyst will increase the rate of both directions (i.e) forward and backward reaction.

There is no change in the equilibrium.

Effect of concentration

If we increase the Concentration of PCl_5 , the excess of PCl_5 is decomposed to give more amount of PCl_3 and Cl_2 . If we increased the concentration of either PCl_3 or Cl_2 , we get more amount of PCl_5 .

7.8 DIPOLES

A dipole is constituted by a collection of equal number of +ve and – ve charges separated by an extremely small distance. It carries no net charge but possesses local charges of opposite signs and equal magnitudes, centered a short distance apart. A dipole is therefore endowed with a +ve pole and a –ve pole as well as potential resulting from the electrical asymmetry or polarity.

The potential of a dipole is directly proportional to its dipole moment (μ) The latter is the turning force on the molecule due to an external electrical field and amounts to the product of the distance between the electrical centers of opposite charges and the magnitude (q) of the electronic charge at either pole.

$$\mu = q/r$$

Dipole moment is given in esu-cm units. When q is expressed in esv and r in cm. μ is usually expressed in Debye (D). $1\text{D} = 10^{-18} \text{ esu-cm}$ ($3.33 \times 10^{-28} \text{ (m)}$)

When 2 non identical atoms remain covalently bonded, the electrons are usually displaced towards the more electronegative atom having a stronger tendency to acquire e^- from the other. So, the –ve and + ve electrical centers of the combination fail to coincide and an electrical polarity result. Unless a molecule is perfectly symmetrical. Such a phenomenon at each of its covalent bonds causes a net resultant displacement of the e^- s to one end of the molecule.

The +ve charges of the atomic nuclei have their electrical center near the other end. The molecule thus gains an electrical polarity and is called Polar molecules.

Groups such as OH, SH, NH_2 , COOH and NO_2 are called polar groups because their presence displaces the 3 electrical centers away from one another and endows the molecule with polarity.

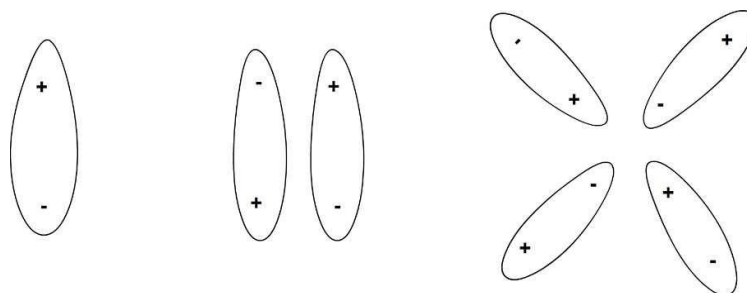
Each polar molecule possesses a dipole moment which is the vector sum of the dipole moments associated with all of its covalent bonds and is usually of the order of 10^{-18} esu-cm.

Vanderwalls force: Types of dipole interactions

Vanderwalls force are very short lived inter-molecular attractive forces which are believed to exist between all kinds of atoms, molecules, and ions, when they are sufficiently close to each other. Vanderwalls force which operates in liquid / solid is almost absent in gases.

Types: Dipole – Dipole interaction

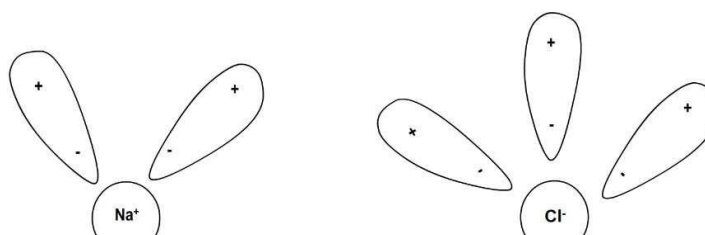
Found in polar molecules having permanent dipole. It is also called Keesom forces and are the strongest of all other types of V.W. forces. The intensity of these forces is generally hampered by increase / rise in temperature.



Ion Dipole

Polar molecules are attracted towards ions. The -ve end of dipole is attracted towards the cation while the +ve end towards the anion.

(eg) Dissociation of NaCl in H₂O



Dipole – induced dipole

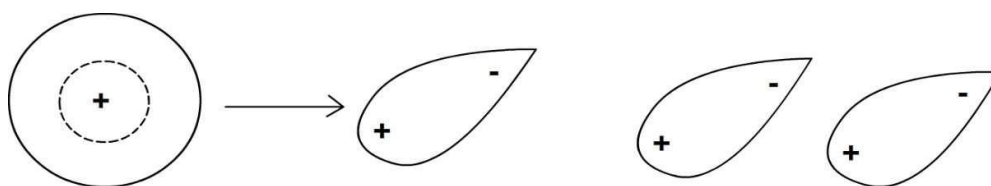
Found in mixture containing polar and non – polar molecules. When a non – polar molecule is brought near a polar molecule, the +ve end of the polar molecule.

Attracts the mobile e^- of non – polar molecules and thus polarity is induced in non – polar molecules. Now both the molecules become dipole and hence attracts. These types of force are called Debye forces.

Instantaneous dipole – induced dipole interactions:

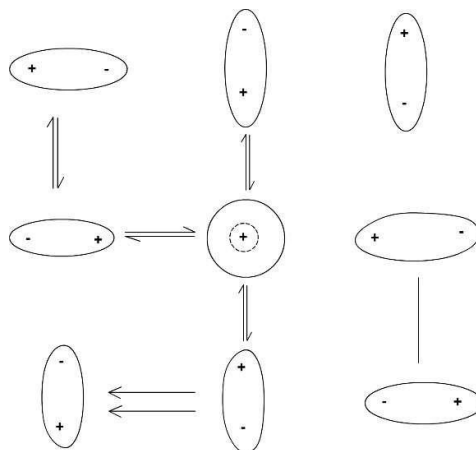
These forces are found in non – polar molecules such a di atomic gases like H_2 , O_2 , Cl_2 , N_2 etc., a well as mono atomic noble gases like He, Ne, Ar etc.

A non – polar molecule/ atom may be visualized as a +ve centre surrounded by asymmetrical –ve election cloud, both in equilibrium. But the e^- cloud ascillates, the e^- cloud becomes move dense on one side of the molecule then on the other sides. Thus, the equilibrium gets disturbed for a moment. The displacement of e^- cloud creates and instantaneous dipole, temporarily. Thus, the non – polar molecule is temporarily, self-polarized and becomes, polar. The self-polarized molecule when brought near to non – polar molecules, it polarizes the neighboring molecule by disturbing its electronic distribution and indeed dipole is created init.



London forces

(Responsible for bringing about condensation of gases, even of noble gases)



Fluctuations

London forces become stronger with increase in no; of e^- s in molecules / atoms and also with increase in B.Pof molecules.

Factors affecting V.W. forces

Large number of e^- in molecules.

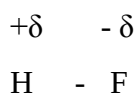
Large molecular sizes.

Low temperature (Randomness decreases and molecules come closer)

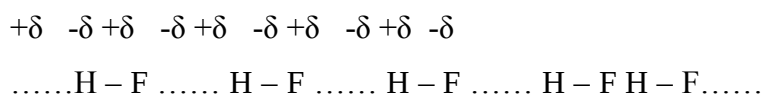
High pressure

7.9 HYDROGEN BOND:

In compounds of hydrogen with strongly electronegative elements, such as fluorine oxygen and Nitrogen, the electron pair shared between 2 atoms lies far away from hydrogen atom because of which the hydrogen atom highly becomes electro positive with respect to the other atom which becomes electronegative. This phenomenon of charge separation in the case of HF is represented as



Such a molecule is said to be polar. The molecule behaves as a dipole because 1 end carries a +ve charge and the other end a -ve charge. The electrostatic force of attraction between such molecules should be very strong. This is because the +ve end of 1 molecule attracts and is attracted by the -ve end of the other molecule. Thus 2 or more molecules may associate together to form large clusters of molecules. This is illustrated below for the association of several molecules of HF.

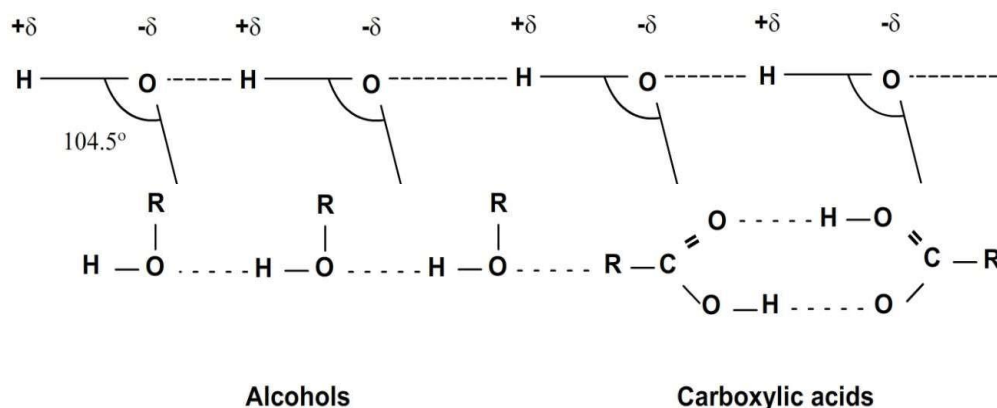


In this case, while the length of the covalent bond between F and H atoms is found to be $1.00\text{\AA}$, the length of the hydrogen bond between F and H atoms of neighbouring molecules has been found to be $1.55\text{\AA}$.

The cluster of HF molecule may be described as $(\text{HF})_n$ "The attractive force which binds hydrogen atom of 1 molecule with electronegative atom (such as fluorine) of another molecule, generally of the same substance, is known as Hydrogen bond".

Water molecule, because of its bent structure, is also a dipole, the oxygen end carrying a -ve charge and the hydrogen end carrying a +ve charge. Hydrogen bonding takes place in this case as well, as represented below.

The cluster of H_2O molecules may be described as $(\text{H}_2\text{O})_n$. Alcohols and carboxylic acids also form associated molecules for the same reason, as illustrated below.

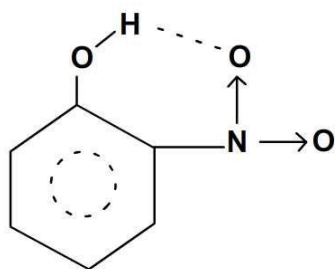


The strength of a hydrogen bond has been found to vary between 10-40 KJmol^{-1} while that of a covalent bond found to be of the order of 400 KJmol^{-1} . Thus, a hydrogen bond is very much weaker than a covalent bond.

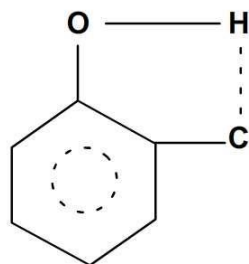
Intra molecular hydrogen bonding

In addition to intermolecular hydrogen bonding resulting from electrostatic forces of attraction between the +ve and -ve ends of different molecules of some substance, cases of intramolecular hydrogen bonding are also known. These involve the electrostatic forces of attraction between hydrogen and an electronegative element both present in the same molecule.

Interesting examples are provided by O-nitrophenol and O-chlorophenol.

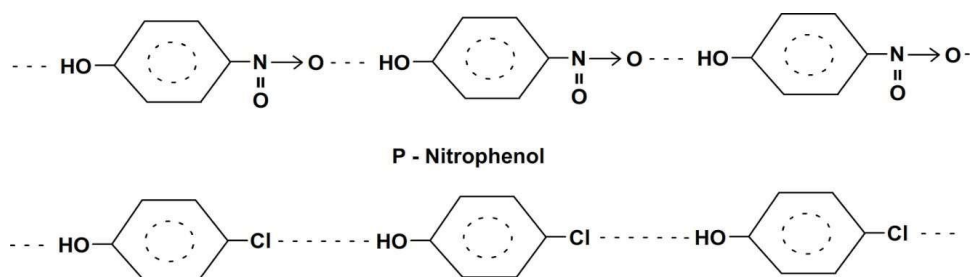


O - nitrophenol showing intramolecular H bonding



O - Chlorophenol showing intramolecular H bonding

Para - nitrophenol and para - chlorophenol, on account of larger distances between the 2 groups, do not show any intramolecular H bonding. On the other hand, they show the usual intermolecular H bonding as illustrated below.



As a result of inter molecular H bonding, the para derivatives undergo association, as shown above, resulting in an increase in Mol. wt and here an increase in boiling point. In Ortho derivatives on account of intramolecular hydrogen bonding, no such association is possible. Consequently, the ortho derivatives are more volatile than the para derivatives.

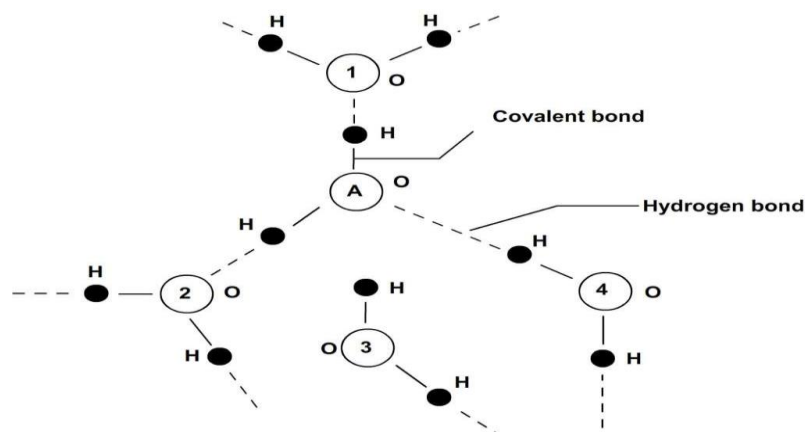
7.10 SOME UNIQUE PROPERTIES OF WATER:

Water has some unique properties. Two of these are discussed below.

Density of the solid state (ice) is less than that in the liquid state. This is somewhat unusual because in most substances density in the solid state is more than that in the liquid state.

Water contracts when ΔT is between 0° to 4°C . This is again unusual because most substance expand when heated in all temperature ranges. Both these peculiar features can be explained as due to hydrogen, bonding, as discussed below.

1. The hydrogen bonding between H_2O molecules is more extensive in ice than in liquid H_2O . A substance in solid state has a definite. Structure and the molecules are more rigidly fixed relative to one another than in liquid state. In Ice, the H_2O , molecules are tetrahedrally oriented with respect to one another. At the same time, each oxygen atom is surrounded tetrahedrally by 4 hydrogen atoms, 2 of these are bonded covalently and the other 2 are by hydrogen bonds.



The hydrogen bonds are weaker and therefore, longer, than the covalent bonds. This arrangement gives rise to an open Cage-like structure with a no. of 'holes' open spaces. These 'holes' are formed because the H bonds holding the H₂O such H bonds are fewer in number. Therefore, as ice melts, many H bond are broken. The molecules, therefore, move into the 'holes or open spaces and come closer to one another than they were in the solid state. This results in a sharp increase in density than that of ice.

2. As liquid H₂O is cooled from 0° to 4°C, H bonds continue to be broken and the molecules come closer and closer together. This leads to contraction. However, there is also some expansion of H₂O due to rise in temperature as in other liquids. It appears that the net contraction in volume. Above 4°C, the normal expansion effect, due to rise in temperature, predominates and hence the volume increases as the temperature rises.

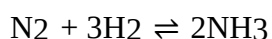
Importance of hydrogen bonding in Sustaining life

It can be easily realized that without H bonding H₂O. In that case no life would have been possible on this globe.

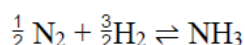
H bonding also exists in all living organisms whether of animal or of vegetable kingdom. Thus, it exists in various tissues, organs, blood, skin, and bones in animal life. It plays an important role in determining structure of proteins which are so essential for life.

H bonding plays an important role in making wood fibres more rigid and thus makes it an article of great utility to meet requirements of housing, furniture's etc., The cotton silk or synthetic fibres owe their rigidity and tensile strength to H bonding. Thus, H bonding is of our food materials also consist of hydrogen bonded molecules. Sugars and carbohydrates, for example, have many –OH groups. The Oxygen of another molecule through hydrogen bonded. The stickiness of glue (a protein) or honey (which consists mainly of water and other such groups of different molecules with one another. H bonding is thus a phenomenon of great importance in everyday life.

7.11 FORMATION OF AMMONIA



The equation for formation of 1 mole of NH₃ is



Initial conc 1:3

Partial pressure 1:3 at equilibrium

At equilibrium, total pressure $P = P_{\text{N}_2} + P_{\text{H}_2} + P_{\text{NH}_3}$

$$= 1 + 3 + P_{\text{NH}_3}$$

$$\therefore P - P_{\text{NH}_3} = 1 + 3 = 4$$

$$P_{\text{N}_2} = \frac{1}{4} (P - P_{\text{NH}_3})$$

$$P_{\text{H}_2} = \frac{3}{4} (P - P_{\text{NH}_3})$$

$$\therefore P_{\text{NH}_3} = P_{\text{NH}_3}$$

Substitute these values in K_p equation.

$$\begin{aligned} \therefore K_p &= \frac{P_{\text{NH}_3}}{P_{\text{N}_2}^{\frac{1}{2}} \cdot P_{\text{H}_2}^{\frac{3}{2}}} \\ &= \frac{P_{\text{NH}_3}}{\left(\frac{1}{4}\right)^{\frac{1}{2}} (P - P_{\text{NH}_3}) \left(\frac{3}{4}\right)^{\frac{3}{2}} (P - P_{\text{NH}_3})} \\ K_p &= \frac{P_{\text{NH}_3}}{(P - P_{\text{NH}_3})^2 \left(\frac{1}{4}\right)^{\frac{1}{2}} \left(\frac{3}{4}\right)^{\frac{3}{2}}} \\ \therefore \left(\frac{1}{4}\right)^{\frac{1}{2}} \left(\frac{3}{4}\right)^{\frac{3}{2}} K_p &= \frac{P_{\text{NH}_3}}{(P - P_{\text{NH}_3})^2} \end{aligned}$$

$$0.325 K_p = \frac{P_{\text{NH}_3}}{(P - P_{\text{NH}_3})^2}$$

Equilibrium constant K_c for the formation of NH₃ is

$$K_c = \frac{[\text{NH}_3]^2}{[\text{N}_2][\text{H}_2]^3}$$

Effect of temperature

Formation of NH₃ is an exothermic reaction (i.e) heat is liberated in the product side.

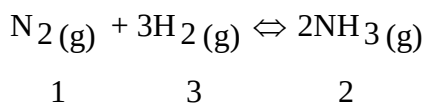
If we increased the temp of the equilibrium, system the equilibrium shifted to the direction of backward reaction. If we apply high temp to this system Δ is absorbed

by the NH₃ molecules, and it gives N₂ and H₂. At low temp, reaction is favorable to the formation of NH₃. The low temp and high-pressure condition is favorable for good yield of NH₃.

Effect of catalyst

Catalyst will increase the speed of both $\rightarrow$ and $\leftarrow$ reaction. There is no effect of catalyst on equilibrium system.

Effect of Change of pressure:



In the equation the forward reaction is accompanied by a decrease in the no: of moles. If the pressure increased, volume would decrease, and therefore the no: of moles / unit volume will increase. According to Le Chatelier's principle in which there is decrease in the no. of moles. (i.e) in favour of formation of NH₃. It is no account of this consideration, that pressure of above 200 atm is maintained in Haber's process in the manufacture of NH₃.

7.12 HETEROGENEOUS EQUILIBRIA

In this equilibrium reaction the reacting species are in different phases.



The concentration of the solid fixed by the density of solid and cannot be changed by the progress of reaction of chemical methods.

The concentration and partial pressure of solid is taken as constant / unity. The equilibrium constant only depends upon the gaseous species, K_c for this system.

$$K_c = \frac{[\text{CaO}][\text{CO}_2]}{[\text{CaCO}_3]}$$

The concentration of CaO and CaCO₃ are constant. So the K_c is only depends upon the concentration of CO₂

$$K_c = [\text{CO}_2]$$

In this system, $K_p = \frac{P_{\text{CaO}} \cdot P_{\text{CO}_2}}{P_{\text{CaCO}_3}}$

Cao and Caco₃ are solid species [Cao] and [Caco₃] = 1

$$K_p = P_{CO_2}$$

7.13 BIOLOGICAL APPLICATIONS OF VAPOUR PRESSURE

The atmospheric air inhaled at external temperature lower than human body temperature contains relatively small amounts of H₂O vapour depending on temperature and relative humidity. The vapour pressure of atm air even when saturated with H₂O at 20°C amounts to 17.5 mm mercury only. The temp of inspired air is raised in lungs to 37°C at which saturation vapour pressure is about 47mm Hg. So, more H₂O vapour can be added to the alveolar air from the alveolar fluid until the air is saturated with moisture and its vapour pressure reaches 47 mm HG. Thus the expired air has a far higher moisture content and a vapour pressure of about 47mm Hg in contrast to low moisture content and vapour pressure of inspired air.

The alveolar air raised to body temp has vp of about 47 mm Hg vapour than the colder and drive inspired air with a much lower Vp. But during expiration contact with the cooled nasal surfaces cooles the air again to about 23 -28°C at which its Vp cannot exceed 30 mm Hg. This re condense about 50-85% of the vapour in it and minimizes the respiratory loss of H₂O.

7.14 SUMMARY

It means, some of the matter will combine with another to form products, that each change are represented by chemical equations that is called chemical reactions. Some of the chemical reactions are proceeded in both forward and backward directions. This reactions are never completed. The reaction mixture contains half of the reactant and half of the products. At one stage the rate of the forward reaction is equal to the rate of the backward reaction. This stage is called chemical equilibrium.

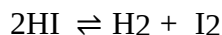
7.15 KEY WORDS

Law of mass action

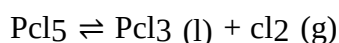
Chemical equilibrium is explained by law of mass action. This law states that the rate of chemical reaction is directly proportional to the product of the active mass of the reactants.

Hydrogen iodide equilibrium

The equilibrium concentration of various species per litre are $2\text{HI} \rightleftharpoons \text{H}_2 + \text{I}_2$



Let 'a' mole of HI be increased in a sealed bulb having a volume 'v' litres kept in a thermostat at 721 K till an equilibrium is established. Let, x/2 moles of H_2 and x/2 moles of I_2 are formed from x moles of HI.

Law of mass action of PCl_5 

1 mole of PCl_5 is taken in 'v' volume of solution.

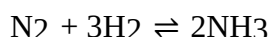
No : of moles at 1 0 0

Initial

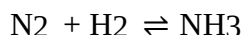
At equilibrium $(1 - \alpha)$ α α

Dipoles

A dipole is constituted by a collection of equal number of +ve and – ve charges separated by an extremely small distance. It carries no net charge but possesses local charges of opposite signs and equal magnitudes, centered a short distance apart. A dipole is therefore endowed with a +ve pole and a –ve pole as well as potential resulting from the electrical asymmetry or polarity.

Formation of ammonia

The equation for formation of 1 mole of NH_3 is

**Biological application of vapour pressure**

The atmospheric air inhaled at external temperature lower than human body temperature contains relatively small amounts of H_2O vapour depending on temperature and relative humidity. The vapour pressure of atm air even when saturated with H_2O at 20°C amounts to 17.5 mm mercury only. The temp of inspired air is raised in lungs to 37°C at which saturation vapour pressure is about 47mm Hg. So, more H_2O vapour can be added to the alveolar air from the alveolar fluid until the air is saturated with moisture and its vapour pressure reaches 47 mm HG. Thus

the expired air has a far higher moisture content and a vapour pressure of about 47mm Hg in contrast to low moisture content and vapour pressure of inspired air.

EXERCISE

I; Essay Questions

1. Define chemical equilibrium?
2. Explain in detail about the law of mass action and the chemical equilibrium constant?
3. Write a note on the relationship between the K_p and K_c ?
4. Mention the types of dipole?
5. Explain in detail about the Le chatlier Braun Principle?
6. Write a note on the effects of changes in concentration, pressure, and temperature?
7. Write a note on the formation of ammonia?
8. Write a note on the heterogeneous equilibria?
9. Mention the application of law of mass action on PCl_5 system?
10. Write a note on hydrogen iodide equilibrium?

II Multiple Choice Questions:

1. Find the pH of a solution when 0.01 M HCl and 0.1 M NaOH are mixed in equal volumes (a) 12.65 (b) 1.04 (c) 7.0 (d) 2.0
2. Which of the following aqueous solution will be the best conductor of electricity ()
(a) NH_3 (b) CH_3COOH (c) HCl (d) $C_6H_{12}O_6$
3. Highest pH will be recorded for which of the following solutions if they are equimolar
(a) $AlCl_3$ (b) $BaCl_2$ (c) $BeCl_2$ (d) LiCl ()
4. On increasing the concentration of reactants in a reversible reaction, the equilibrium constant will ()
(a) depend on the concentration (b) increase (c) unchanged (d) decrease
5. Find the conjugate acid of NH_2^- ()
(a) NH_3 (b) NH_4OH (c) NH_4^+ (d) NH_2^-
6. According to Lewis concept, an acid is ()
a) proton donor (b) electron pair donor (c) electron pair acceptor (d) proton acceptor
7. Ostwald's dilution law is applicable to: ()

- (a) Strong electrolytes only (b) Weak electrolyte only (c) Non-electrolytes (d) Strong and weak electrolytes
8. The molarity of the solution is () (a) 4.0 (b) 0.4 (c) 0.0001 (d) 0.04
9. The strong conjugate base is ()
- (a) NO^{2-} , (b) Cl^- (c) SO^{2-} (d) CH_3COO^-
10. Which of the following pairs ()
- (a) NaOH and HCl (b) HNO_3 and NH_2NO (c) HCl and KCl (d) HNO_2 and NaNO_2

III Fill in the Blanks

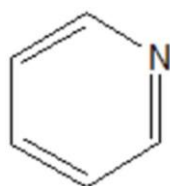
- When KOH is dissolved in water, heat is evolved. If the temperature is raised, the solubility of KOH. _____
- For the liquefaction of gas, the favourable conditions are _____
- A reaction takes place in two steps with equilibrium constants 10^{-2} for slow step and 10^2 for fast step. The equilibrium constant of the overall reaction will be ____
- According to Le Chatelier's principle adding heat to a solid and liquid in equilibrium with endothermic nature will cause the _____
- _____ would happen to a reversible reaction at equilibrium when temperature is raised, given that its ΔH is positive
- _____ of the equilibrium, the value of K_P is less than K_C ?
- K for the synthesis of HI (g) is 50. The degree of dissociation of HI is _____
- One mole of $\text{N}_2\text{O}_4(\text{g})$ at 300 K is kept in a closed container under one atmosphere. It is heated to 600 K when $\text{N}_2\text{O}_4(\text{g})$ decomposes to $\text{NO}_2(\text{g})$. If the resultant pressure is 2.4 atm, the percentage dissociation by mass of $\text{N}_2\text{O}_4(\text{g})$ is _____
- 40% of a mixture of 0.2 mol of N_2 and 0.6 mol of H_2 react to give NH_3 according to the equation: $\text{N}_2(\text{g}) + 3\text{H}_2(\text{g}) \rightleftharpoons 2\text{NH}_3(\text{g})$ at constant temperature and pressure. Then the ratio of the final volume to the initial volume of gases is _____
- K_P for a reaction at 25°C is 10 atm. The activation energy for forward and reverse reactions are 12 and 20 kJ / mol respectively. The K_C for the reaction at 40°C will be _____

II Answers: 1) a 2) c 3) b 4) c 5) a 6) c 7) b 8) c 9) d 10) d

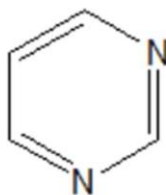
III Answers: 1) Decreases 2) Low T and high P 3) 1 4) Amount of solid to decrease 5) More of the products are formed 6) $\text{N}_2 + 3\text{H}_2 \rightleftharpoons 2\text{NH}_3$ 7) 0.22 8) 20% 9) 4:5 10) $3.33 \times 10^{-1} \text{ M}$

CHAPTER-VIII

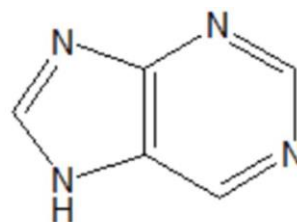
Hetero Cyclic Compounds



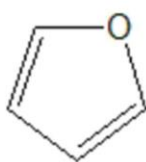
pyridine



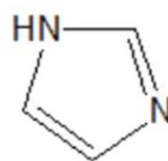
pyrimidine



purine



furan



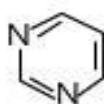
imidazole

8.0 INTRODUCTION

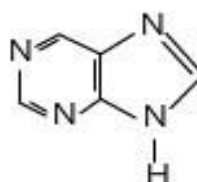
Those cyclic compounds which in addition to carbon contain at least one atom of another element in the ring are called heterocyclic compounds or simply heterocyclic. The common hetero compound atoms present in the carbon rings are O, N, and S. When the hetero atom is part of an aromatic ring, the compound is referred to as an aromatic heterocyclic compound.

EXAMPLE

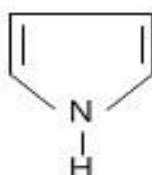
Pyrimidine



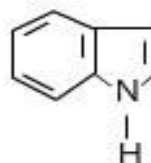
Purine



Pyrrole



Indole



8.1 : CAROTENOIDS

Carotenoids are fat-soluble yellow or red pigments that are composed of isoprene units. Carotenoids occur in both plants and animals. In plants, they occur as colloidal

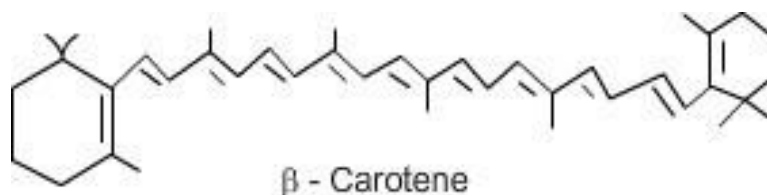
suspensions of their esters. While in animals they occur in solution in fats or in combination with proteins.

The Carotenoids may be classified according to their source of isolation. They are.

Phyto Carotenoids: These are Carotenoids that occur in plants.

Zoo Carotenoids: These are Carotenoids that occur in animals. Examples of carotenoids are α -carotene, β -carotene, γ -carotene, and xanthophylls.

STRUCTURE



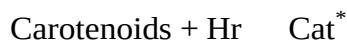
Characteristics

They are yellow or red pigments.

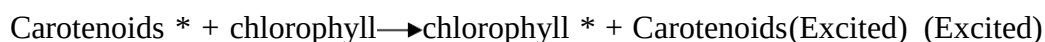
They are insoluble in water, acids and alkalis. But are soluble in fat, CHCl_3 and ethers. In plants chlorophyll occurs in combination with Carotenoids. When chlorophyll is absent in fungi then Carotenoids are responsible for color.

FUNCTIONS

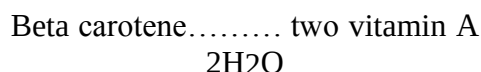
The exact function of Carotenoids in plants is not known certainly. However different views given by various workers are as follows. Carotenoids act as light filters in plants which protect chlorophyll from destruction with strong light. The main function of Carotenoids is to protect sensitive enzymes in the cell. It helps in assimilation. It plays a role in some redox reactions that are taking place in plants. They act as H^+ acceptors. When a plant is exposed to light, they take energy from light photons and get excited, and they transfer excitation energy to chlorophyll.



light photon Excited



In animals, they play the following role. In the intestinal track or in the liver most of them act as a provitamin. Beta carotene on absorbing two molecular H_2O is converted to two molecules of vitamin A.



8.2 TERPENES

Terpenes are a class of compounds that give plants their odor, flavor, and in some cases color. They occur widely in the leaves and fruits of higher plants such as conifers, citrus and Eucalyptus.

The term Terpenoids may be defined as follow “ It includes hydrocarbons of plant origin of the general formula $(C_5H_8)_n$ as well as their oxygenates hydrogenated and dehydrogenated derivatives”.

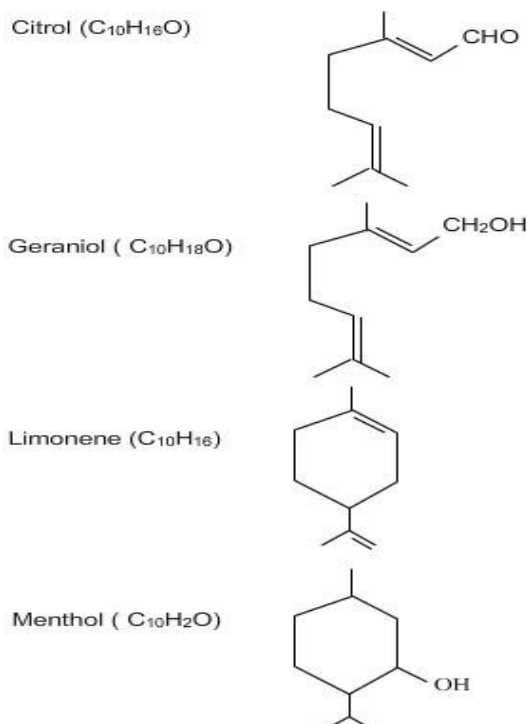
They generally occur in Terpentine oils and many other essential oils. The molecules of Terpenes are constructed of two or more isoprene units, usually joined in a head-tail fashion.

CLASSIFICATION

Terpenes are classified according to their number of isoprene (C_5) units in the molecules.

S.No	Name	Number of isoprene units	Number of Cn
1	Monoterpenes	2 isoprene units	C ₁₀
2	Sesquiterpenes	3 isoprene units	C ₁₅
3	Di terpenes	4 isoprene units	C ₂₀
4	Tri terpenes	6 isoprene units	C ₃₀
5	Tetra terpenes	8 isoprene units	C ₄₀

The important terpenes are



Applications

Citrol is used extensively in the perfume and flavor industry to stimulate lemon-like odor and the manufacture of vitamin A. Recently it has become important as a drug for reducing BP.

Limonene is used as a flavoring agent in foods, beverages, and shaving creams. Menthol is used in the pharmaceutical as a local anesthetic and for the relief of headaches.

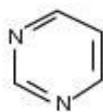
Menthol is used in mouth washers, nasal sprays and inhalers, cough drops, chewing, and mentholated cigarettes.

Menthol is used in cosmetics like toothpaste, shaving creams and lotions, face creams, and powders.

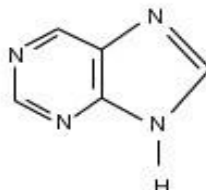
Some terpenoids exhibit biological activity like insecticidal, and antiseptic and are used in pharmacy.

8.3 PURINES AND PYRIMIDINES

Pyrimidine



Purine



The heterocyclic bases purines and pyrimidine are the parent molecules of nucleotides and nucleosides. Nucleotides are present in living cells where they perform numerous key functions.

BIOLOGICAL IMPORTANCE OF PURINE AND PYRIMIDINES

They are incorporated as their Ribose mono phosphate (RNA) or as deoxy ribose mono phosphate (DNA). Purine ribonucleotides serve as high energy sources (e.g.) ATP. Some are components of co-enzymes (e.g.) NAD^+ , NADP^+ , FMN, FAD. S-Adenosyl methionine (SAM) is a form of active met and serves as a methyl donor in methylation reactions. Synthetic analogues of naturally occurring nucleotides find application in cancer chemotherapy. The such compound includes 5-fluoro uracil, 6-thio guanine, 6-aza uridine. Allo purinol, a purine analog is widely used in the treatment of GOUT.

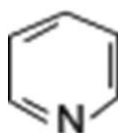
Methylated purines with pharmacologic properties include Caffeine or 1,3,7 trimethyl xanthine is present in coffee.

Theophylline or 1,3 di methyl xanthine present in tea Theobromine or 3,7 di methyl xanthine is present in cocoa.

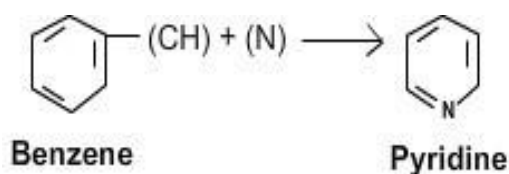
Some purine nucleotides acts as a second messenger in hormone action (eg) Cyclic AMP (cAMP), and cyclic GMP (cGMP).

The pyrimidine nucleotide serve as a high energy intermediates such as UDP glu and UDP Gal in carbohydrate metabolism and CDP acyl glycerol in lipid metabolism.

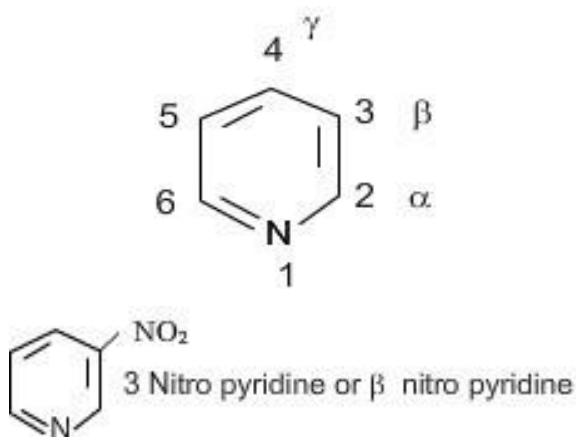
8.4 PYRIDINE



Pyridine is a six membered aromatic hetero cyclic with 1 N atom in the ring. Its systematic name is Aza benzene since it could be derived from C₆H₆ by replacing a – CH by N. The prefix aza stands for N.



The positions of pyridine ring are indicated by numbers alpha, beta, gamma as shown below



The molecular formula of pyridine is (C₅H₅N)

USES

It is used as a basic solvent in organic reaction. It is used to denature alcohol.

It is used to destroy the insects.

It is used as a catalyst in many reactions.

It is used in manufacture of drug intermediate like vitamin B6 and sulpha pyridine.

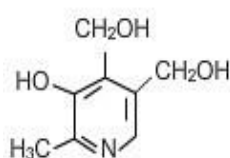
Sulpha pyridine has been used in the treatment pneumonia and other infectious diseases.

BIOLOGICAL IMPORTANCE

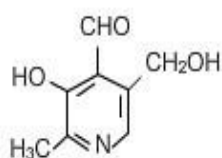
Pyridine is present in biologically important compounds like vitamin B6, B5 and some co-enzymes.

1. Vitamin B6

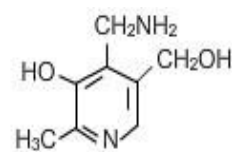
Vitamin B6 refers to a group of three compounds namely pyridoxine, pyridoxal and pyridoxamine.



Pyridoxin



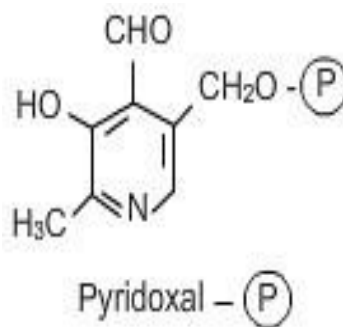
Pyridoxal



Pyridoxamine

2. Pyridoxal phosphate (PLP)

In phosphate related derivatives of pyridoxal and pyridoxal phosphate is an important co-enzyme in several metabolic reactions.



Pyridoxal - (P)

3. Niacin (vitamin B5 or Nicotinic acid)

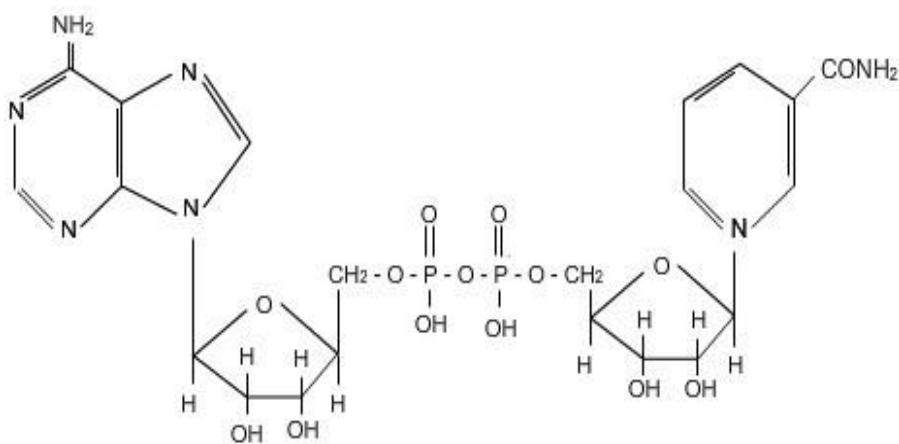
It is chemically pyridine three carboxylic acid



Nicotinamide forms two phosphorylated related compound which act as co-enzyme to dehydrogenases in metabolic reaction. They are.

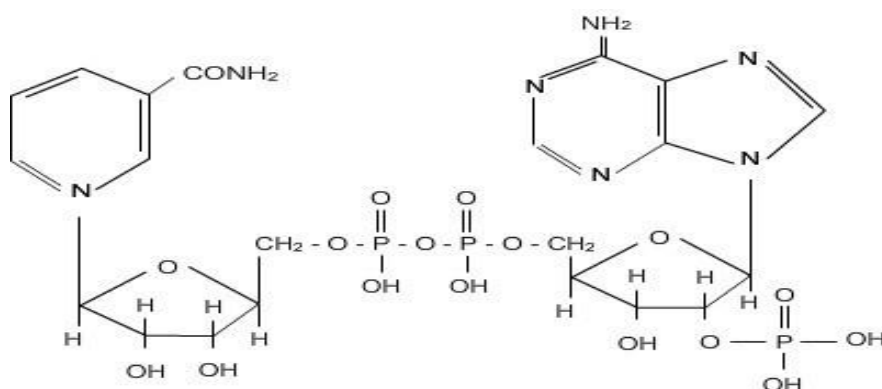
Nicotinamide adenine dinucleotide (NAD⁺)

It contains one molecule of nicotinamide and two molecule of D-ribose and two molecule of phosphate and one molecule of Adenine.



4. Nicotinamide adenine dinucleotide phosphate (NADP⁺)

It contains one nicotinamide, two molecule of D-ribose, three molecule of H₃PO₄ and one Adenine molecule



8.5 HEME

Heme is a complex of porphyrin and iron. The iron is held at the centre of the heme molecule by bonds to the four nitrogen of the porphyrin ring. The Fe²⁺ of

heme can form two additional bonds one on each side of the planar porphyrin ring. For example: In myoglobin and Hb one of these positions is co-ordinated to the side chain of a Histidine residue of the globin molecule, where as the other position is available to bind oxygen.

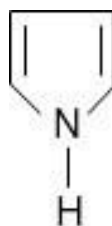
Hemoproteins are a group of specialized proteins that contain heme as a tightly bound prosthetic group of compounds containing heme are Hb , myoglobin, cytochromes, catalase etc.

BIOLOGICAL FUNCTIONS

1. The role of heme group is dictated by the environment created by the three D structure of the proteins for example.
2. The heme group of the cytochrome functions as an electron carrier that is alternatively oxidized and reduced.
3. In contrast, the heme group of the enzyme catalase is part of the active site of the enzyme that catalyzes the breakdown of H_2O_2
4. In Hb and myoglobin, the two most abundant hemoproteins in humans, the heme group serves to bind oxygen reversibly.

STRUCTURE

1. Heme is an iron porphyrin compound. The porphyrins are complex compounds with a tetra pyrrole structure. Each pyrrole ring has the following structure.

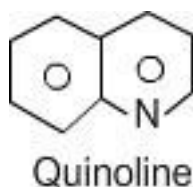


2. such pyrroles are combined through $-CH=$ bridges called as methyne or methylene bridges to form a porphyrin nucleus
3. The outer carbons of the 4 pyrrole rings which are linked with the methylene bridges are numbered 1,2,3.....8.
4. The methylene bridges are referred to as α , β , γ , δ respectively.
5. Fe^{2+} in the center besides its linkages to 4 N of pyrrole ring, is also linked internally to the imidazole ring of His of the polypeptide chain.
6. In addition to the hydrogens at positions 1 to 8 are substituted by different

groups like methyl, vinyl, propionyl groups.

8.6 QUINOLINE

It is a bicyclic heterocyclic having a C_6H_6 ring fused with pyridine ring. Other name is 1 az naphthalene.



USES

It is used as a solvent.

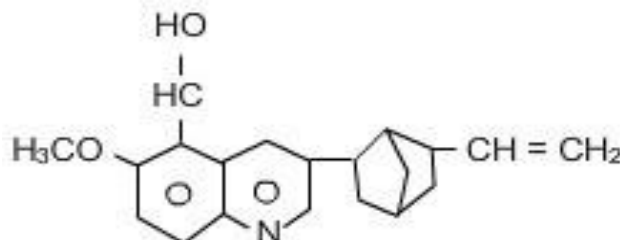
It is used in the manufacture of pharmaceuticals, dyes and insecticides.

It is also used in the precipitation of certain metals.

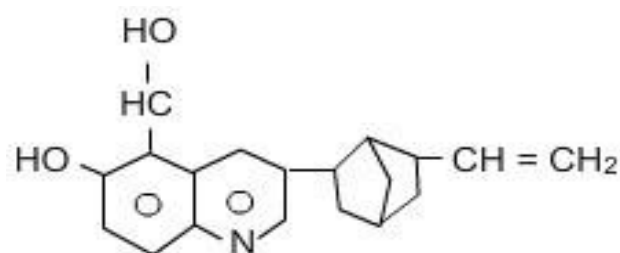
BIOLOGICAL IMPORTANCE

It is present in plant alkaloids like quinine, chinconine.

1. Quinine: It is one of the important alkaloid isolated from chincona tree

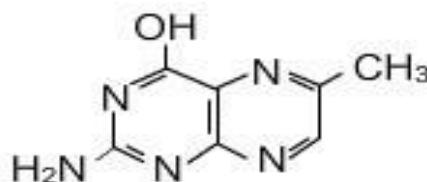


2. Quinine destroys the micro organisms which cause malaria and it also lowers the body temperature so it is used as anti-malarial and anti-pyretic drug Chinconine. It is also isolated from chincona tree along with quinine. It is used as a strong local anesthetic and also used as anti pyretic and anti malarial drug

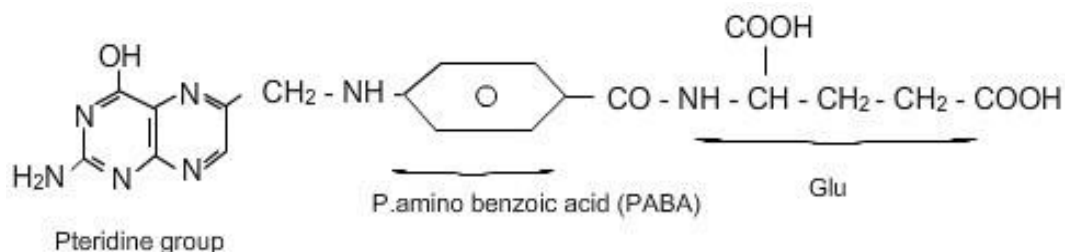


8.7 PTERIDINE

It is a combination of pyrimidine and pteridine ring.

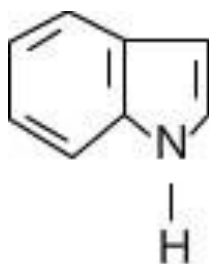


Biological importance: Pteridine ring is present in B complex vitamin folic acid.



1. Folic acid is first reduced to tetra hydro folate by the enzyme folic acid reductase. This tetra hydro folate serves as a co-enzyme and acts as a hydrogen donor for reduction reactions.
2. Folic acid participates in the reactions concerned with synthesis of purine, pyrimidine and Nucleic acid, which are required for normal growth and reproduction of cells.
3. It is required for normal growth and maturation of RBCs. Deficiency of folate results in the production of immature RBC and consequently Anemia.

8.8 INDOLE

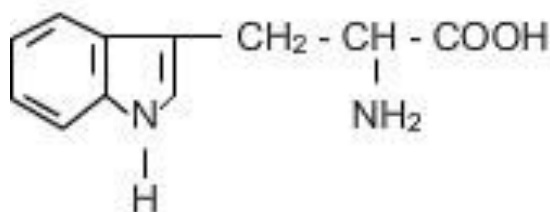


Its another name is benzo pyrrole. It is made up of C₆ H₆ ring fused with pyrrole ring.

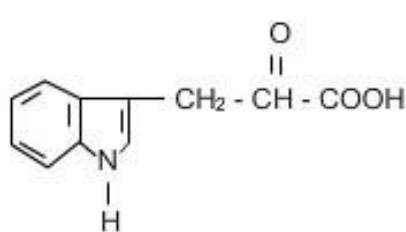
BIOLOGICAL IMPORTANCE

Indole ring is present in the essential amino acid Tryptophan (alpha-amino beta indolepropionic acid). It is essential for many biological functions.

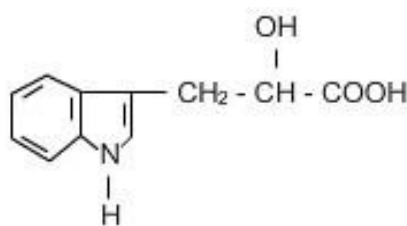
Tryptophan



Indole pyruvic acid and indole lactic acid are the derivative of indole which are also used as essential nutrients.



Indole pyruvic acid



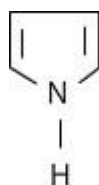
Indole lactic acid

Indole acetic acid (Auxins) is a derivative of indole which acts as plant growth hormone.

Indole formed in the gut by the action of intestinal bacteria are absorbed and transported to liver where they are conjugated to the less toxic form indoxyl and excreted in urine.

8.9 : PYRROLE

Pyrrole is an important five membered heterocyclic cyclic compound because many naturally occurring such contains pyrrole ring. (eg) Chlorophyll, Hb , Myoglobin, cytochrome, catalase.



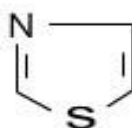
BIOLOGICAL IMPORTANCE

1. Hb consists of a tetra pyrrole structure which are combined through methylene bridges. Hb is involved in the transport of oxygen in our body.
2. Pyrrole is an important constituent of myoglobin which is seen in muscle tissue.

3. Catalase is an enzyme which is involved in the break down of H_2O_2 .
4. Chlorophyll, is the important plant pigment involved in the process of photosynthesis also consists of pyrrole ring in its structure .
5. The cytochrome which are involved in electron transport chain (ETC) are also made up of pyrrole nucleus.

8.10: THIOZOLE

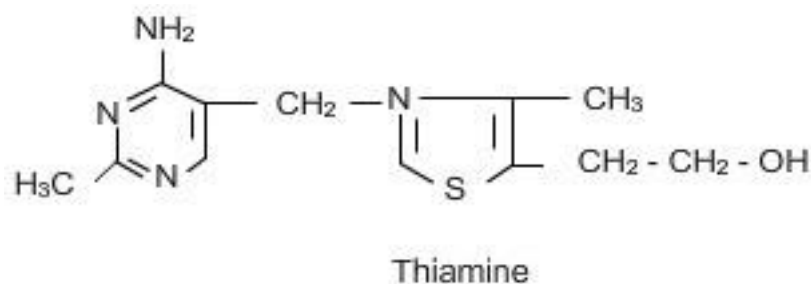
Thiazole is a five membered ring with two hetero atoms. It contains one nitrogen atom and one sulphur atom and three carbon atoms.

**BIOLOGICAL IMPORTANCE**

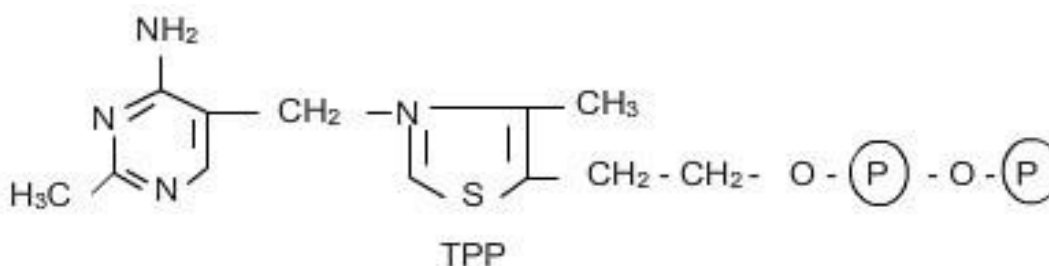
Thiazole ring is present in vitamin B1 (Thiamine), Thiamine pyrophosphate (TPP) and the antibiotic penicillin.

1. Thiamine

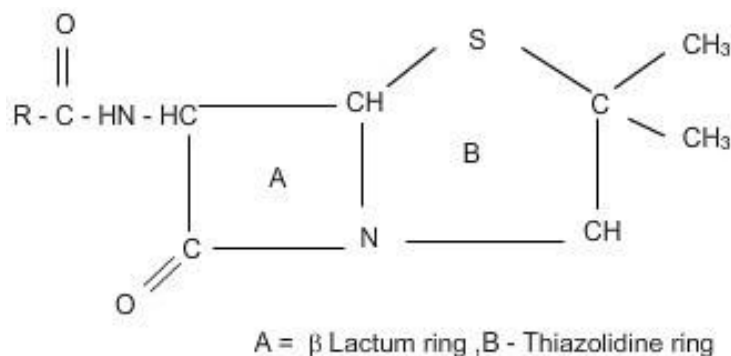
Chemically vitamin contains a substituted pyrimidine ring and Thiazole ring. Thiamine is a H_2O soluble B complex vitamin whose deficiency causes beriberi.

**2. Thiamine pyro phosphate**

TPP is a derivative of thiamine which acts as a co-enzyme in decarboxylation reaction in the metabolism of macromolecules.

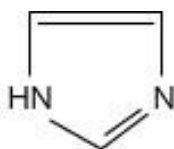


4. Penicillin is a broad spectrum antibiotic used in the treatment of many bacterial infections.



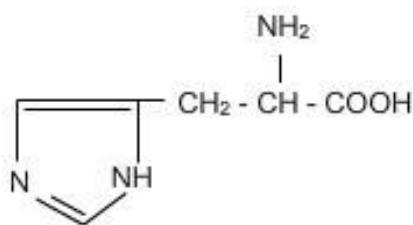
8.11IMIDAZOLE

STRUCTURE



Biological importance

Imidazole is an important heterocyclic compound present in the histidine and histamine.1.The amino acid histidine contains the Imidazole ring in it



2. Histamines contain the Imidazole ring in them. They are the derivatives of the amino acid Histidine which are produced in large quantities during allergic conditions. Histamines acts as neurotransmitters .

8.12: SUMMARY

Those cyclic compounds which in addition to carbon contain at least 1 atom of another element in the ring are called heterocyclic compounds or simply hetero cyclic. The common hetero compound atoms present in the carbon rings are O, N, and S. When the hetero atom is part of an aromatic ring, the compound is referred to as aromatic heterocyclic compound..

8.13: KEYWORDS**Carotenoids**

Carotenoids are fat soluble yellow or red pigments which are composed of isoprene units. Carotenoids occur in both plants and animals. In plants they occur as colloidal suspension of their esters. While in animals they occur in solution in fats or in combination with proteins.

Heme

Heme is a complex of porphyrin and iron. The iron is held at the centre of the heme molecule by bonds to the four nitrogen of the porphyrin ring. The Fe^{2+} of heme can form two additional bonds one on each side of the planar porphyrin ring. For example: In myoglobin and Hb one of these positions is co-ordinated to the side chain of a Histidine residue of the globin molecule, where as the other position is available to bind oxygen.

Hemoproteins are a group of specialized proteins that contain heme as a tightly bond prosthetic group. (eg) of compounds containing heme are Hb , myoglobin, cytochromes, catalase etc.

Purines and Pyrimidines

The hetero cyclic bases purines and pyrimidine are the parent molecules of nucleotides and nucleosides. Nucleotides are present in living cells where they perform numerous key function.

Pyrrole

Pyrrole is an important five membered heterocyclic cyclic compound because many naturally occurring such contains pyrrole rine. (eg) Cholorophyll, Hb, Myoglobin, cytochrome, catalase.

Thiazole

Thiazole is a five membered ring with two hetero atoms. It contains one nitrogen atomand one sulphur atom and three carbon atoms.

8.14: ANSWERS TO CHECK YOUR PROGRESS

- (1) Those cyclic compounds which in addition to carbon contain atleast 1 atom of another element in the ring are called hetero cyclic compounds or simply hetero

cyclics.

- (2) They are incorporated as their Ribose mono phosphate (RNA) or as deoxy ribose mono phosphate (DNA).

Purine ribo nucleotides serve as high energy source (eg) ATP.

Some are components of co-enzymes (eg) NAD^+ , NADP^+ , FMN, FAD

S- adenosyl methionine (SAM) is a form of active met serves as a methyl donor in methylation reactions.

Allo purinol, a purine analogue is widely used in the treatment of GOUT.

Some purine nucleotides acts as a second messenger in hormone action (eg) cyclic Amp (cAMP), cyclic GMP (cGMP).

The pyrimidine nucleotide serve as a high energy intermediate such as UDP glu and UDP Gal in carbohydrate metabolism and CDP acyl glycerol in lipid metabolism.

- (3) It is used as a basic solvent in organic reaction.

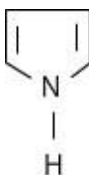
It is used to denature alcohol It is used to destroy the insects

It is used as a catalyst in many reactions.

It is used in manufacture of drug intermediate like vitamin B6 and sulpha pyridine.

Sulpha pyridine has been used in the treatment pneumonia and other infectious diseases.

- (4) Pyrrole is an important five membered hetero cyclic compound because many naturally occurring such contains pyrrole ring. (eg) Chlorophyll, Hb, Myoglobin, cytochrome, catalase.



- (5) (i) Histamines contain the Imidazole ring in them. They are the derivatives of the amino acid Histidine which are produced in large quantities during allergic conditions. Histamines acts as neurotransmitters.

- (ii) Imidazole is an importance heterocyclic compound present in the histidine and histamine.

EXERCISE

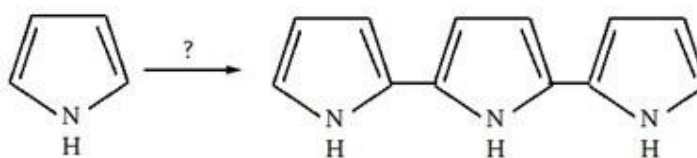
I; Essay Questions

1. Define heterocyclic compounds?

2. Write a note on terpenes?
3. Write a note on carotenoids?
4. Write a note on purines and pyrimidines?
5. Give an account of the structural elucidation of niacin, pyridoxal phosphate, pyridine, quinoline, cinchonine, thiazole, and thiamine?
6. Write a note on the heme structure?

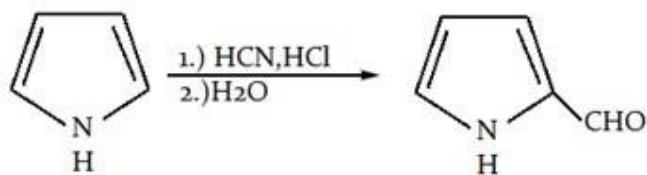
II Multiple Choice Questions

1. Which of the following is not true about the five-membered rings? ()
 - a) Five-membered rings are more stable than 4 membered rings
 - b) Five-membered rings are more stable than 6 membered rings
 - c) Five-membered rings are more stable than 7 membered rings
 - d) Five-membered rings are more stable than 8 membered rings
2. Which of the following is not a five-membered ring? ()
 - a) Pyridine
 - b) Pyrrole
 - c) Furan
 - d) Thiophene
3. Which of the following five-membered rings is most resonance stabilized? ()
 - a) Furan
 - b) Thiophene
 - c) Pyrrole
 - d) Pyridine
4. Five membered rings come under which category of heterocycle classification based on chemical behavior? ()
 - a) -excessive heterocycle
 - b) -deficient heterocycle
 - c) -equivalent heterocycle
 - d) Can't say about the five-membered rings
5. What is the reactivity order in the following five-membered heterocyclic compounds?
 - a) Pyrrole
 - b) Furan
 - c) Thiophene
 - d) Pyridine
6. What is the product when thiophene reacts with Br₂ in benzene? ()
 - a) 2-bromothiophene
 - b) 3-bromothiophene
 - c) 2,5-dibromothiophene
 - d) 3,4-dibromothiophene
7. What is the product when pyrrole reacts with Br₂ in ethanol? ()
 - a) 2,3-dibromopyrrole
 - b) 2,3,4,5-tetrabromopyrrole
 - c) 2,5-dibromopyrrole
 - d) 3,4-dibromopyrrole
8. What will be the reagent used for the completion of the following reaction ()



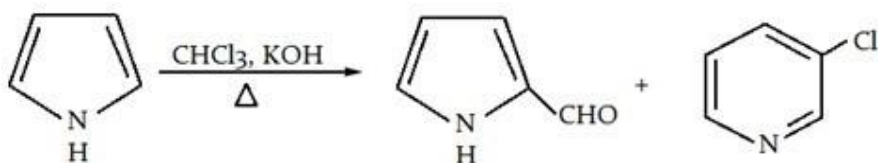
a) Concentrated acid b) Dilute acid c) Concentrated base d) Dilute base

9. What is the name of the following reaction ()



a) Gattermann reaction b) Riemer tiemann reaction c) Friedal craft reaction
d) Blanc's chloromethylation

10. What is the name of the following reaction?



a) Gattermann reaction b) Riemer tiemann reaction c) Friedal craft reaction
d) Blanc's chloromethylation

III Fill in the Blanks.

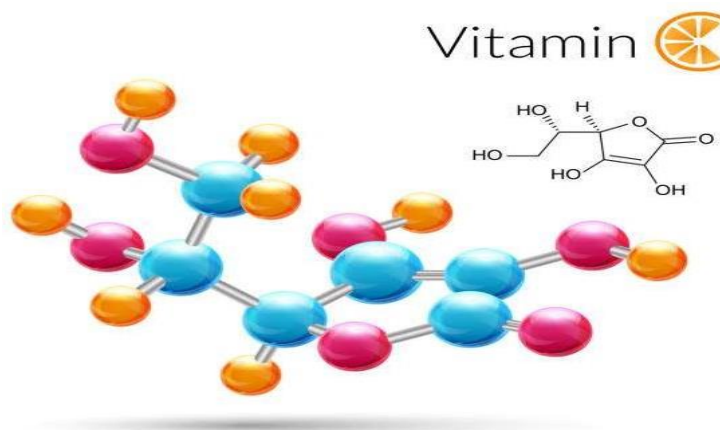
- _____ type of ring system is found in the compound pyridine?
- _____ compounds contain a nitrogen atom in its ring structure
- _____ of the compound with the chemical formula C₆H₇N
- _____ type of heterocyclic compound contains a nitrogen atom and a sulfur atom in its ring structure?
- _____ type of heterocyclic compound is found in the compound quinolone.
- _____ type of compounds contains a nitrogen atom in its ring structure and is a base
- _____ type of heterocyclic compound is found in the compound purine?
- _____ of the compounds contains a nitrogen atom in its ring structure and is a building block of DNA?
- _____ type of heterocyclic compound is found in the compound caffeine
- _____ of the compounds contains a nitrogen atom in its ring structure and is a common component of flavoring agents.

II Answers: 1) b 2) a 3) b 4) a 5) a 6) c 7) b 8) b 9) a 10) a

III Answers: 1) Heterocyclic 2) Pyridine 3) Aniline 4) Thiophene 5) Indole 6) Pyrrole 7) Pyrimidine 8) Guanine 9) Xanthine 10) Pyridine N-oxide

CHAPTER-XI

Vitamins

**9.0 INTRODUCTION**

Vitamins are organic compounds required in the diet in small amounts to perform specific biological functions for the normal maintenance of optimum growth and health of the organism.

The term vitamin was coined from the word vital amine since the earlier identified ones had amino groups; later works showed that most of them did not contain groups. Most of the vitamins are supplied by the diet. Very few vitamins are synthesized in the intestine.

It is difficult to define vitamins precisely. Vitamins may be regarded as organic compounds required in the diet in small amounts to perform specific biological functions for normal maintenance of optimum growth and health of the organism.

Synthesis of vitamins by intestinal bacteria

Vitamins, as per the definition, are not synthesized in the body. However, the bacteria of the gut can produce some of the vitamins, required by man and animals.

As far as humans are concerned, it is believed that the normal bacterial synthesis and absorption of vitamin K and biotin may be sufficient to meet the body's requirements. For other B-complex vitamins, the synthesis and absorption are relatively less. Administration of antibiotics often kills the vitamin-synthesizing bacteria present in the gut, hence additional consumption of vitamins is recommended.

The bacterium *E.coli* does not require any vitamins, as it can synthesize all of them. It is believed that during evolution, the ability to synthesize vitamins was lost. Hence,

the higher organisms must obtain them from the diet. The vitamins are required in small amounts since their degradation is relatively slow.

1. They are distinguished from other organic substances by the fact that, unlike proteins, they do not enter the tissue structures of the body.
2. In contrast to hormones, they are not created by the organism themselves, and the majority of them must be obtained from the consumption of food.

Vitamins, which are synthesized by intestinal flora are Vitamin K, Thiamine (Vitamin - B1), Riboflavin (B2), Pyridoxine, Folic acid, Niacin, and Biotin.

9.1 CLASSIFICATION OF VITAMINS

Classification

All Vitamins are broadly divided into two groups according to solubility. Many water-soluble Vitamins of different structures and having coenzyme function were discovered and they were grouped as “B. Complex” group. Vitamins are classified as fat-soluble and water-soluble. The fat-soluble vitamins are A, D, E, & K.

The water-soluble vitamins are. B complex vitamins & vitamins ‘C’. The B complex vitamin are further subdivided into energy-releasing (B1, B2, B6, Biotin, etc.) and hematopoietic (folic acid and B12).

9.2 Fat-Soluble Vitamins

The four vitamins namely vitamins A, D, E, & K are known as fat or Lipid soluble. Their availability in the diet, absorption, and transport are associated with fat. They are soluble in fats and oils and fat solvents like alcohol, acetone, etc.

Fat-soluble vitamins can be stored in the liver and adipose tissue. They are not readily excreted via urine.

Excess consumption of these vitamins (Particularly A and D) leads to their accumulation and toxic effects.

9.2.1

VITAMIN – A

(Retinol, Growth promoting vitamin, anti-infective vitamin, Anti Xerophthalmia)

Chemistry

Vitamin - A as such present only in foods of animal origin. The pro-vitamin β carotene is present in plants.

Vitamin A occurs in nature in different forms. The usual form of vitamin A₁. Described above predominates except in freshwater fish, in them another form namely vitamin A₂ is present. Differences between vitamin A₁ and A₂ are shown below in a tabular form.

Vitamin A₁	Vitamin A₂
1. Found predominantly in major species of animal.	1. Found in freshwater fish liver and other tissues.
2. show absorption maximum at 639 mμ when treated with SbCl ₃ .	2. Shows absorption maximum at 620 mμ on treatment with SbCl ₃ .
3. Only one double bond is present in β-ionone ring.	3. Two double bonds in β-ionone ring (additional double bond between (C ₃ -C ₄).
4. More potent in its activity than vitamin A ₂ .	4. Less potent, biological activity is approximately 40% that of A ₁ .

Retinol (Vitamin - A alcohol)

It is a primary alcohol-containing β- ionone ring. The side chain has two isoprenoid units.

Four double bonds and one hydroxyl group.

It is not destroyed by heat due to the presence of double bonds.

Retinal (Vitamin - A – aldehyde)

This is an aldehyde form of vitamin A previously it is called retinine.

Retinoic acid

This is produced by the oxidation of retinol.

β- **Carotene (Provitamin – A)**: This is found in plants. It is cleaved in the intestine to produce two moles of retinol.

Sources: Animal sources: Liver, Kidney, Egg yolk, Milk, Cheese, Butter, Fish liver oil.

Vegetable Sources

Yellow and dark green vegetables and fruits such as carrots, spinach, amaranths, pumpkins, mango, papaya, etc are good sources of β- Carotene.

Recommended Daily Allowances

Adult men – 5000 IU (IU = 0.3 μg retinol), Adult women – 4000 IU.

The requirement increases in growing children, pregnant women, and lactating

mothers.

Absorption, Storage, and Transport

1. Vitamin A and its carotene precursors are absorbed in the small intestine.
2. It is believed that the presence of tocopherols (vitamin E) and other antioxidants protect them against oxidation and destruction in the intestinal lumen.
3. Dietary vitamin A is chiefly in ester form, which is hydrolyzed by cholesterol esterase into fatty acid and free vitamin A. Free retinol is absorbed and undergoes re-esterification in the intestinal epithelial cells. It is stored in the liver as retinyl ester normally as retinyl palmitate.
4. Conversion of carotenes to retinol occurs in intestinal epithelial cells in few animals, although the liver may also participate in conversion. However, in man liver is the only organ where carotenes are converted to vitamin A.
5. Retinol is transported in the blood in association with a specific retinol-binding protein (RBP). There is another Retinoic acid binding protein (RABP) specific for retinoic acid. Plasma RBP and RABP are different from that are found inside the cell in cytoplasm and nucleoplasm. Authors have found out that the K_d for plasma RBP is much less than that for cytosolic RBP and nucleosolic RBP indicating that the transport is maintained in uni-directional manner until the target cell chromatin is involved in specific gene expression as a result of vitamin A function.
6. About 95% of vitamin A is stored as its ester mainly as palmitate in the liver. It is released in the plasma as and when required. About 10-20 mg of vitamin A is present per 100 g of the liver.
7. Normal blood level of vitamin A is found to be 18-60 $\mu\text{g/dl}$ and that of carotenoids 100 - 300 $\mu\text{g/dl}$.

Functions of vitamin A

The important functions of vitamin A are.

1. Vitamin A and vision

Vitamin A plays a critical role in vision in dim light. Perhaps the only function of vitamin A, which is clearly understood in its molecular details is its role in vision. The overall mechanism through which vitamins function in the visual system is known as “Wald’s visual cycle” or “Rhodopsin cycle” discovered by George Wald for which he

was awarded Nobel Prize.

Retina contains two types of receptor cells.

- (i) **Cones:** which are specialized for color and detail vision in bright light.
- (ii) **Rods:** which are specialized for visual activity in dim light (night vision).
- (iii) Light waves striking these receptors produce chemical changes which in turn give rise to nerve impulses. Vitamin A Plays a significant role in the photochemical phase of this process. The visual activity of rod cells is dependent on their content of photosensitive pigment called “rhodopsin” or “visual purple” which is a conjugated protein with a molecular weight of 40,000. It contains Opsin as its apoprotein and retinene as its prosthetic group. Retinene or retinal or retinaldehyde present in rhodopsin is 11-cis retinal. The aldehyde group of 11-cis-retinal is bound to amino group of lysine of opsin. Rhodopsin has a light-absorbing property due to polyene group of 11-cis retinal. Even dim light can break rhodopsin as shown below.

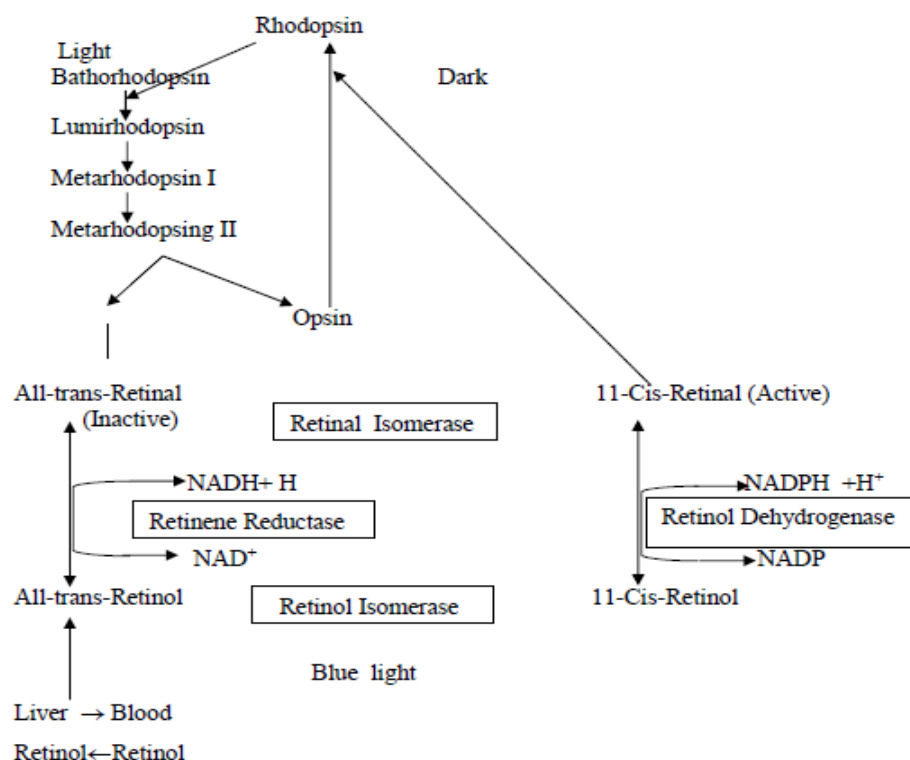
When the light falls on rhodopsin it is split into opsin and all-trans retinal in a series of events. It first forms bathorhodopsin, then lumirhodopsin, then metarhodopsin I and II. Finally, metarhodopsin gets split into opsin and all-trans-retinal.

At this stage the eye becomes less sensitive to light. All-trans-retinal is inactive in synthesis of rhodopsin it must be converted to 11-cis-retinal. It can take place in the following ways.

1. All-trans-retinal may be isomerized to its 11-cis-isomer in presence of blue light but in the eye, this isomerization is not significant.
2. The all-trans-retinol from blood can be converted to all-trans-retinal by retinene reductase by making use of NAD^+ and all-trans-retinal then can be isomerized to its cis isomer.
3. All-trans-retinol from blood can be first isomerized to 11-cis-retinol. All 11-cis-retinol then can be converted to 11-cis-retinal by retinol dehydrogenase.

Now 11-cis-retinene which is active, can combine with opsin to form back rhodopsin in the dark. Thus, the visual process involves the continual removal of the active retinol from the blood into retina. Photolysis of rhodopsin loses sodium channels in rod membrane through either a release of Ca^+ into cytosol or a hydrolytic fall in the cytosolic cyclic GMP level. This results in lowering the membrane permeability to

Na^+ causing hyperpolarization of the rod membrane and changes its conformation through 50 and initiates the visual signal from the rod. Briefly, the basic factors stimulating the rod cells are the (i) cis-trans-isomerization and (ii) conformational changes in the rhodopsin due to c-GMP.



2. Vitamin A and epithelial tissues

Vitamin A is essential for the integrity of the mucous-secreting cells of epithelial tissues.

In vitamin A deficiency, the epithelial tissues are keratinized.

3. **Vitamin A** is essential for the normal formation of epithelial tissues. Vitamin A is essential for glycoprotein synthesis.

4. **Retinoids and cancer prevention:** The term 'retinoids' includes both naturally occurring forms of vitamin A and its synthetic analogues with or without vitamin A activity. Vitamin A helps in cancer prevention.

5. **Vitamin A** is essential for nerve function and normal bone formation and

6. **Vitamin A** is essential for the synthesis of mucoproteins and glycoproteins

Colour Vision

The color vision is governed by colour-sensitive pigments like Porphyropsin - red

Iodopsin - green

Cyanopsin - blue

When bright light strikes the retina, one or more of these pigments are bleached and then the nerve impulse goes to the brain, now brain can read that colour.

Dark adaptation time

When a person shifts from a bright light to dim light, Rhodopsin stores are depleted and vision is impaired. However, within few minutes Rhodopsin is synthesized and vision will be normal it is called dark adaptation time (entering into a cinema theatre)

Visual cascade and c GMP

When light strikes the retina, a number of biochemical changes leading to membrane hyperpolarization occurs resulting in the genesis of nerve impulse. This brought – about by cGMP.

When a photon (from light) is absorbed by Rhodopsin, Metarhodopsin II is produced. The protein, transducin activated by Metarhodopsin II, it required GTP.

The activated transducin activates cyclic GMP in the rod cells.

The rapid decrease in cGMP closes the Na^+ channels in the membrane of the rod cells. This results in hyperpolarization, which is an excitatory response, transmitted through the neuron network to the visual cortex of the brain.

Effects of vitamin A deficiency

The deficiency states due to lack of vitamin A in the diet are

Night blindness

Xerosis of conjunctiva

Xerosis of cornea

Bitot's spots and

Keratomalacia

The stages in development of vitamin A deficiency are

Night Blindness

In early stages of vitamin A deficiency, the individual cannot see well in dim light. Difficulty in reading or driving the car in dim light is experienced. In advanced deficiency, the subject cannot see objects in dim light.

Xerophthalmia

This condition refers to xerosis of the conjunctiva and cornea.

Xerosis of conjunctiva

The conjunctiva is dry, thickened, wrinkled and pigmented. This is due to the keratinisation of the epithelial cells. The pigmentation gives conjunctiva a smoky appearance.

Xerosis of cornea

When dryness spreads to cornea, it enables on a dull, lacy, beustreless appearance. This is due to the keratinisation of the epithelial tissue over the cornea.

Bitot's spots

Bitot described this condition in 1863. Grayish or glistening white plaques formed of desquamated thickened conjunctival epithelium usually triangular in shape and firmly adhering to the conjunctiva.

Keratomalacia

When xerosis of the conjunctiva and cornea is not treated it may develop into the condition known as "keratomalacia". Metaplasia and degeneration of the corneal epithelium occur, producing capillaries and the cornea becomes vascularised, oedematous, and infiltrated with leucocytes. Necrosis, leading to ulceration and bacterial invasion brings about the destruction of the cornea. Blindness often results from corneal scarring or perforation.

Hypervitaminosis A (vitamin A toxicity)

A toxicity was well reviewed by Moore(1957) and more recently by Multer et al (1971). The later authors reviewed 17 cases, ranging from those patients who took 41,000 I.U/ day for eight years to those who ingested 200,000 I.U. vitamin A/ day for two years. Signs and symptoms were the following dryness, maculopapular rash, fissures, desquamation and purities of the skin ;hair loss; weakness and fatigue bone and joint fracture and pain , muscle stiffness, tenderness of the bones to palpation, anorexia, headache, hepatomegaly, absent, or decreased menses in females and papilledema. The liver should have signs of damage sinusoidal filurosis, portal hypertension areas of congestion, cell atrophy. There was an increase in CSF pressure.

These are all symptoms of chronic Hypervitaminosis A. Acute symptoms are nausea, violet headache, dizziness, and skin desquamation. In all cases signs and symptoms disappear completely within a few weeks of stopping the excessive intake.

Recommended intakes of vitamin A or carotene (µg/day)

S.no	Age group	I.C.M.R expert group	
		Vitamin A retinol(µg)	Carotene(µg)
1	Up to 1 year	300-400	1,200-1,600
2	1-3 years	250	1,000
3	4-6 years	300	1,200
4	7-9 years	400	1,600
5	10-12 years	600	2,400
6	13-15 years	750	3,000
7	Adolescents	750	3,000
8	Adults (men and women)	750	3,000
9	Pregnancy	750	3,000
10	Lactation	1150	4,600

The beneficial effect of βCarotene

Increased consumption of β Carotene is associated with decreased incidence of heart attacks, skin and lung cancers. This is because of its antioxidant property. Ingestion of large doses of βCarotene is not toxic like vitamin A.

VITAMIN – D

(Calcitriol)

Ergocalciferol (Vit - D₂) is formed from Ergosterol present in plants. Cholecalciferol (Vit - D₃) is found in animals.

Ergocalciferol and Cholecalciferol are the sources for vitamin – D.

Sources of vitamin – D

1. Fatty fish.
2. Fish liver oil.
3. Egg yolk.

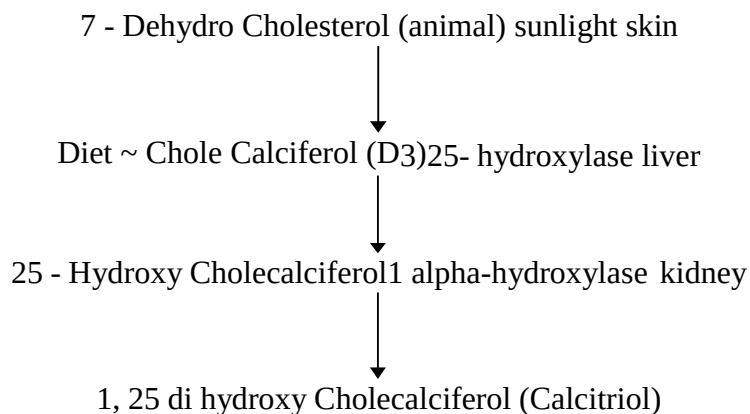
Absorption, storage and transport

It is absorbed from small intestine for which bile is required through Lymph, vitamin - D enters the circulation bound to plasma alpha 2 globulin and is distributed throughout the body.

Metabolism

1. Vitamins D₂ and D₃ are not biologically active.
2. They are metabolized identically in our body and converted to active form. The active form is called calcitriol.

Synthesis



Regulation of synthesis of calcitriol (1.25, DHCC)

1. Plasma levels of calcium and phosphorous.
2. Parathyroid hormone.

Vitamin- D can be provided to the body by three ways:

1. Consumption of natural foods.
2. By irradiating foods like yeast.
3. Exposure of skin to sunlight for the synthesis of Vitamin - D

Recommended dietary allowances

Adult - 10 micro gms of Vitamin - D₃ (400 IU.), during pregnancy and lactation period requirement will be more.

Biochemical functions

1. It regulates the plasma level of calcium and phosphate. Calcitriol acts at 3 different levels intestine, kidney, and bone.
2. Action of calcitriol on the intestine.
Calcitriol increases the intestinal absorption of calcium and Phosphate.
3. Action on bone.

In the osteoblasts of bone, calcitriol stimulates calcium uptake for deposition as

Calcium phosphate.

4. Action in the kidney

Calcitriol involves in minimizing the excretion of calcium and phosphate through kidney.

Functions

1. Vitamin - D₃ is synthesized in the skin by ultraviolet rays of sunlight
2. The biologically active form of Vitamin - D (calcitriol) produced in the Kidney.
3. It has target organs - intestine, bone, and kidney.
4. Calcitriol action is similar to steroid hormone.

Deficiency of vitamin D

1. Vitamin - D deficiency occurs in strict vegetarians, chronic alcoholics, and individuals with liver and kidney diseases.
2. Deficiency of Vitamin - D leads to demineralization of bone. This results in Rickets in children and Osteomalacia in adults.

Rickets

1. Bone deformities due to incomplete mineralization, resulting in soft and reliable bones and delay in teeth formation.
2. In rickets plasma level of calcitriol is decreased and alkaline phosphatase increased.

Osteomalacia (in adults)

1. Demineralization of bones.
2. Increasing the susceptibility to fractures.

Renal rickets (renal osteodystrophy)

This is seen in patients with chronic renal failure. This is due to decreased synthesis of calcitriol in the kidney

Hyper vitaminosis of vitamin D.

Symptoms

1. Demineralization of bone and calcium absorption from intestine will be increased, so calcium level will be increased in blood. Prolonged hypercalcemia is associated with deposition of calcium in many soft tissues such as kidney and arteries (renal

calculi)

2. Loss of appetite.
3. Nausea.
4. Increased thirst.
5. Loss of weight etc.

9.2.2 : VITAMIN - E

(TOCOPHEROL)

(Anti- sterility vitamin)

Chemistry

1. Vitamin - E is the name given to a group of tocopherols, possessing Vitamin - E activity.
2. Four tocopherols are namely alpha, beta, gamma, delta among these alpha – tocopherol is more active.
3. The tocopherols are methyl derivatives of the parent compound (tocol).

Sources

Many vegetable oils like.

1. Wheat germ oil.
2. Cotton seed oil.
3. Peanut oil.
4. Corn oil.
5. Sunflower oil.
6. It is also present in meat, milk, butter and eggs.

Recommended Daily Allowances

Man - 20 mg/day Women - 8 mg/day.

Biochemical functions

1. Major functions of Vitamin - E is related to its antioxidant property. It prevents the non- enzymatic oxidations of various cell components. (e.g) unsaturated fatty acids) by molecular oxygen and free radical such as super oxide and H₂O₂.
2. It is a lipophilic in character and is found in association with lipoproteins, fat deposits and cellular membranes. It protects polyunsaturated fatty acids from

peroxidation reactions.

Vitamin - E acts as a scavenger and gets itself oxidized by free radicals and spares unsaturated fatty acids.

Other function

1. Essential for membrane structure and integrity of cell.
2. It protects RBC from haemolysis by oxidizing agent.
3. It preserves and maintains germinal epithelium of gonads for proper reproduction.
4. It increases the synthesis of heme.
5. It prevents the oxidation of Vitamin - A.
6. It is required for proper storage of creatinine in skeletal muscle.

It protects liver from damage caused by CCl₄. Recent studies shows that excess in take of Vitamin - E protects against the development of heart disease.

Deficiency of vitamin E**In many animals the deficiency causes**

1. Sterility.
2. Degenerative changes in muscle.
3. Megaloblastic anaemia.
4. Changes in central nervous system.

9.2.3 VITAMIN K

(Antihemorrhagic vitamin)

It exists in a different form

1. Vitamin – K - Phylloquinone - present in plants.
2. Vitamin - K - Menaquinone - produced by intestinal bacteria.
3. Vitamin - K - Menadione is a synthetic form. All the three are naphtha quinone derivative.

Sources

Cabbage, cauliflower, tomatoes, egg yolk, meat, liver, cheese, etc.

Recommended Daily Allowances

It can be synthesized in the gut. Adult 70 -140 micro g /day.

Biochemical functions

The major function is involved in blood clotting. The Clotting factors II, VII, IX, X are synthesized as inactive precursors in the liver. The Vitamin - K acts as a coenzyme for the carboxylation of glutamic acid residues present in these proteins and this reaction is catalyzed by carboxylase.

Vitamin - K is also required for the carboxylation of glutamic acid residue of osteocalcin (calcium binding protein).

It is involved in oxidative processes taking place in the photosynthesis of the plant kingdom. It is involved in ETC and involved in oxidative phosphorylation.

Role in clotting

The carboxy glutamic acid (gla) residues of clotting factors are negatively charged (COO^-) and they combine with positively charged calcium ion (Ca^{2+}) to form a complex.

The prothrombin-calcium complex binds with phospholipids on the membrane surface of platelets. This leads to increased conversion of prothrombin to thrombin.

Mechanism of action of vitamin E.

Disease	Mechanism of action of vitamin E
1. Nocturnal muscle cramp	The precise mechanism not known. By virtue of its anti-oxidant property, vit. E. Prevents oxidation of certain radicals and better utilization of oxygen in muscle tissue, thereby improving muscle metabolism.
2. Intermittent claudication	Same as above. In addition A decrease in circulating lactate level and increase in pyruvate level noted after therapy. Improvement in blood supply due to opening of new vessels, improving circulation.
3. Fibrocystic breast disease	Precise mechanism of action in FBD remains obscure. It has been suggested that Vit. E probably acts by correcting the decreased progesterone/estrogen ratio in women of FBD.
4. atherosclerosis	Beneficial effects of vit. E in atherosclerosis are due to: (i) Inhibits the formation of lipid peroxides and restores PG-I₂ synthesis (ii) Inhibits platelets aggregation. (iii) Elevated HDL cholesterol level ↑ (increased scavenging action).

Deficiency of vitamin K.

It is uncommon, but the deficiency may be due to

1. Faulty absorption (lack of bile salts)
2. Loss of vitamin into feces (diarrhoea)
3. Administration of antibiotics (killing of intestinal flora).

Symptoms

The blood clotting time will be increased. Hypo prothrombonemia.

Hyper vitaminosis

Excessive Vitamin - K produces hemolytic anemia & Jaundice.

Antagonist of vitamin K

Heparin. Salicylates. Dicumarol.

Note

Bleeding disorder in infants due to hypo prothrombonemia and deficiency of other clotting factors. This is called hemorrhagic disease of new born.

Why new born baby is more prone to develop Vitamin - K deficiency and hypoprothrombinemia?

1. Because the placenta does not pass the vitamin K from maternal blood to foetus readily.
2. Because intestinal bacterial flora not yet developed fully in the newborn baby.

Treatment

By administering Vitamin - K to the mother before parturition or by giving the infant a small dose of vitamin - K.

9.3 : WATER-SOLUBLE VITAMINS**9.3.1****VITAMIN B₁**

(Thiamine (or) Aneurine, (or) Anti beri-beri factor (or) Anti - neuritic vitamin)

Chemistry.

1. Thiamine contains a pyrimidine ring and a thiazole ring held by a methylene bridge.
2. The alcohol group of thiamine is esterified with phosphate to form the coenzyme.

Thiamine pyrophosphate acts as a coenzyme for oxidative decarboxylation.

Sources

It is widely distributed in many plant and animal foods. Whole grains, rice bran, nuts and yeast, liver, egg and fish.

Recommended Daily Allowances

Adult - 1- 1.5 mg/day. Children - 0.7 - 1.2 mg/day Pregnancy and lactation --- 2 mg I day.

Biochemical functions

1. It is needed for good appetite.
2. Normal in takes of food and proper growth.
3. The active form of B1 is Thiamine pyrophosphate acts as a coenzyme for oxidativedecarboxylation.
4. In branched chain amino acid metabolism Dehydrogenase requires TPP.
5. Thiamine plays an important role in the transmission of Nerve impulse.

Deficiency of thiamine

Deficiency of thiamine Cause Beri - Beri. This is mainly seen the people who are takingexclusively polished rice as staple foods.

Symptoms

- 1) Loss of appetite (anorexia)
- 2) Weakness.
- 3) Constipation.
- 4) Nausea
- 5) Neuropathy
- 6) Irritability etc and numbness in the legs complaints of pins and needles sensations arereported.

Beri-Beri

It is classified into two types

Wet beri - beri and Dry beri – beri

Wet beri – beri

1. This is characterized by oedema in legs, face, trunk and serous cavities.
2. Breathlessness and palpitation are present.
3. Systolic blood pressure is elevated while diastolic is decreased.
4. The heart becomes weak and death may occur due to heart failure.

Dry beri – beri

1. This is associated with neurological manifestations resulting in peripheral neuritis.
2. Oedema will not be occurring.
3. The muscles become progressively weak and walking becomes difficult.
4. The affected individuals depend on support to walk and become bedridden, and even may die if not treated.

Infantile beri – beri

1. This is seen in infants born to mothers suffering from thiamine deficiency.
2. The breast milk of these mothers contains low thiamine content.

Symptoms of Infantile Beri – Beri

1. Sleeplessness
2. Restlessness
3. Vomiting.
4. Convulsions
5. Death may occur due to cardiac failure.

Wernicke korsakoff syndrome

1. This is seen in chronic alcoholics
2. This is due to insufficient intake or impaired intestinal absorption of thiamine will lead to this syndrome.
3. It is characterized by Loss of memory Apathy.
And arrhythmical to and fro motion of the eyeballs.

Thiaminase

1. It is present in certain sea foods.
2. So they destroy the thiamine in the diet (Cleave pyrimidine and thiazole rings are separated). So Thiamine deficiency is caused.

Thiamine antagonists

1. Pyri thiamine and oxy thiamine

Note

Repeated intravenous injection of thiamine leads to anaphylactic shock.

VITAMIN - B2

(Riboflavin (or) Lactoflavin)

Chemistry

1. It is an orange-yellow compound.
2. It contains 6, 7 di methyl iso alloxazine attached to D-Ribitol by a nitrogen atom.

Sources

1. Milk and milk product
2. Meat, egg, liver, kidney.
3. Cereals, fruits, vegetables, and fish.

Recommended Daily Allowances

Adult - 1.2 to 1.7 mg.

Pregnant & Lactating Women required more amounts.

Biochemical Functions

1. The active form of B2 is called FMN and FAD. They are called flavoproteins.
2. FMN /FAD are involved in the Dehydrogenase reaction.

Deficiency of riboflavin

1. Cheilosis (fissures at the corner of mouth)
2. Glossitis (inflammation on the tongue)

NIACIN

(Nicotinic acid or pellagra preventive factor of Gold berger)

Chemistry

1. It is a pyridine - 3 - carboxylic acid.
2. In tissues it is present in the form of nicotinamide

Source

1. Liver, yeast, whole grains, cereals, pulses, peanuts.
2. Milk, fish, egg and vegetables.
3. The essential amino acid tryptophan can serve as a precursor for synthesis of niacin that is 60 mg of tryptophan produces 1 mg of Niacin.

Recommended Daily Allowances

Adult - 15- 20 mg. Children - 10- 15 mg. Pregnant and lactating women require more.

Biochemical Functions

1. The active form of Niacin is NAD^+ and NADP^+ they acts as a coenzyme in oxidoreductase reaction.

Important enzymes are listed below

NAD^+	NADP^+
Alcohol dehydrogenase (Ethanol $\rightarrow$ Acetaldehyde) Lactate dehydrogenase (LDH)(P.A. L.A.) Malate dehydrogenase(Malate O.A.A.) Glyceraldehyde-3-P dehydrogenase(Gly-3-p $\rightarrow$ 1,3-phosphoglycerate) alpha -Glycero-P-dehydrogenase Pyruvate dehydrogenase Complex (P D H) (P.A $\rightarrow$ Acetyl CoA) alpha -Ketoglutarate dehydrogenase complex (alpha -Ketoglutarate $\rightarrow$ succinyl CoA)	Glucose 6-phosphate dehydrogenase(G-6-PD)(G-6-P $\rightarrow$ 6-Phosphogluconate) Glutathione reductase Either NAD^+ or NADP^+ Glutamate dehydrogenase (Glutamate $\rightarrow$ alpha -ketoglutarate + NH_3) Isocitrate-dehydrogenase(I C D) (Isocitrate $\rightarrow$ Oxalosuccinate)

Deficiency manifestation

Deficiencies of niacin cause pellagra.

Symptoms

1. Dermatitis.
2. Dementia
3. Diarrhea, if not treated may rarely lead to death.

Pellagra is mostly seen among the people whose staple diet is corn or maize, because tryptophan content is low in maize and Niacin is present in bound form it is not absorbed.

The amino acid leucine (high in Jowar) has been shown to inhibit the synthesis of

NAD⁺ and NADP⁺.

Therapeutic uses of niacin

1. Niacin inhibits lipolysis in adipose tissue and decreases the circulatory free fatty acids.
2. Triacylglycerol synthesis in the liver decreased.
3. The serum levels of LDL, VLDL, TGL and cholesterol are lowered. So Niacin is used for the treatment of hyper lipoproteinemia.

PYRIDOXINE (VIT B6)**Chemistry**

Since pyridoxine occurs in nature it is a mixture of three compounds (pyridoxine, pyridoxal and pyridoxamine). The more active derivatives are pyridoxal and pyridoxamine phosphates. Pyridoxine is 3-hydroxy-4, 5-dihydroxymethyl-2-methyl pyridine. Pyridoxal contains an aldehyde instead of hydroxy methyl in Number 4 and pyridoxamine contains a primary amine side chain in Number 4 of pyridine nucleus. These three compounds are interchangeable.

Sources

Rich sources: Yeast, rice polishings, certain seeds such as wheat and corn.

Good sources: Milk, meat, leafy vegetables, liver.

Fair sources: Fish, fruits, vegetables.

Biochemical functions

The active form of pyridoxine is called pyridoxal phosphate (PLP). It acts as a coenzyme in transamination, decarboxylation, deamination, transulfuration reactions.

Transamination

SGPT (or) ALT

Alanine + alpha- keto glutarate -----> Pyruvate + glutamate

SGOT (or) AST

Aspartate + alpha - keto glutarate ----- > O.A.A + glutamate

Decorboxylation

- PLP
1. Tryptophan $\longrightarrow$ Tryptamine

PLP

 2. Histidine $\longrightarrow$ Histamine

CO₂

PLP

 3. Glutamate $\longrightarrow$ GABA

PLP

 4. DOPA $\longrightarrow$ DOPAmine

PLP

 5. OH Tryptophan $\longrightarrow$ 5- OH Tryptamine

CO₂

Deamination

- PLP
- Serine $\longrightarrow$ Pyruvate + NH₃
- Dehydratase
- Threonine -----> alpha- keto glutarate + NH₃
- PLP

Deficiency symptoms

1. Neurological symptoms such as depression, irritability, nervousness and mental confusion.
2. Vitamin - B6 deficiency also causes hypochromic microcytic anaemia.

Drug induced B6 – deficiency

1. Isoniazide is a drug used for the treatment of tuberculosis it combines with pyridoxal phosphate to form inactive hydrazones derivatives that inhibit PLP dependent enzymes. So long-term use of Isoniazide patients are suffered by peripheral neuropathy.
2. Penicillamine is used for the treatment of rheumatoid arthritis; it also reacts with PLP and inhibits their action.

Pyridoxine Antagonist

Isoniazide, de-oxy pyridoxine.

Chemistry**BIOTIN****(Anti Egg white injury factor)**

Heterocyclic monocarboxylic acid, is a sulphur-containing vitamin.

Sources

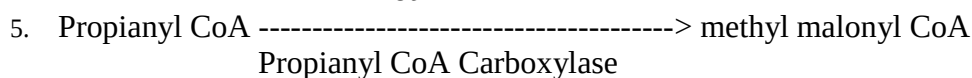
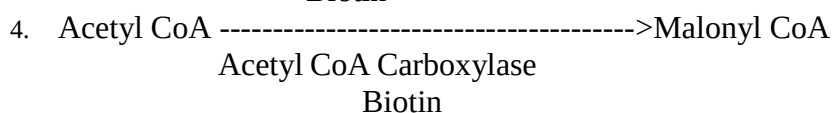
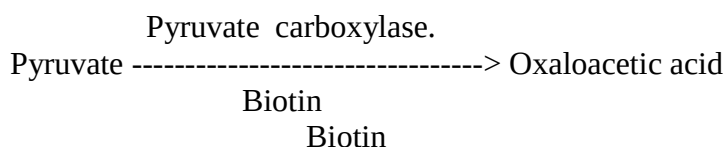
Liver, kidney, egg yolk, milk, tomatoes, grains, etc.

Recommended Daily Allowances

100 - 300 mg/ day.

Biochemical functions

1. It acts as a coenzyme in carboxylation reactions.
2. The biotin bound to the lysine of enzyme.
3. Then this complex reacts with CO₂ in presence of ATP to form carboxy biotin enzyme complex. This high-energy complex hands over the CO₂ to the substrate pyruvate carboxylase.

**Deficiency of biotin**

1. Anaemia, loss of appetite nausea, dermatitis, glossitis etc.
2. Biotin deficiency is uncommon, but it can be caused by the following reasons.
3. Destruction of intestinal flora due to prolonged use of drugs such as sulphonamides.
4. High consumption of raw egg The raw egg white contains a glycoprotein, avidin which tightly binds and blocks its absorption from the intestine.

PANTOTHENIC ACID**(Chick anti-dermatitis factor or filtrate factor)****Chemistry**

Pantothenic acid consists of two components pantoic acid and beta-alanine, held together by a peptide linkage.

Source

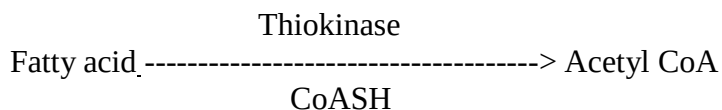
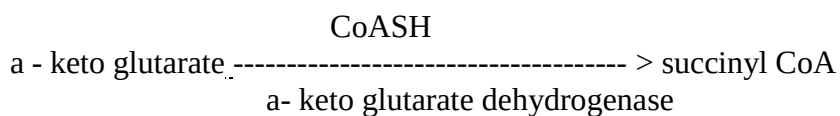
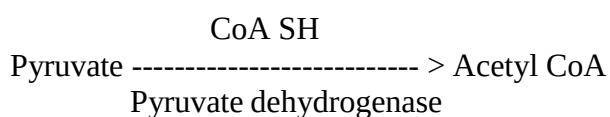
Egg, liver, meat, yeast, milk, etc.

Recommended Daily Allowances

Adult - 5 - 10 mg/day. Biochemical functions.

Biochemical functions

It is required for the formation of coenzyme A. Coenzyme A is required for acylation.

**Deficiency manifestations**

1. Burning feet syndrome (pain and numbness in the toes, sleeplessness, fatigue etc).
2. In experimental animal pantothenic deficiency causes anaemia, fatty liver etc

FOLIC ACID**Chemistry**

It contains three components.

Pteridine ring.

P-amino benzoic acid Glutamic acid.

Sources

1. It is widely distributed in nature, abundantly present in green plants.
2. It is yellow substance, slightly soluble in water.

3. Liver, kidney, yeast, cauliflower, cabbage etc.

Recommended Daily Allowances

Adult 100 micro g / day; Pregnant women - 300 micro g /day; Lactation - 150 micro g /day.

Biochemical function

The Active form of folic acid is called tetra hydro folate. It is mainly involved in one carbon metabolism.

Eg.

1. Purine(carbon 2,8) Which are incorporated into DNA and RNA
2. Pyrimidine nucleotide - deoxy thymidilic acid involved in the synthesis of DNA.
3. Glycine, serine, ethanolamine and choline are produced.
4. N-Formyl methionine, the initiator of protein synthesis is formed.

Deficiency of folic acid

This is most common vitamin deficiency in the pregnant women. Deficiency of folic acid causes macrocytic anaemia.

Four amino glutamic acid, it is an intermediate product of histidine metabolism.

Antagonist of folic acid

1. Aminopterin
2. Amithopterin.

Chemistry**CYANOCOBALMIN (Vitamin -B12)****(Anti pernicious anaemia factor)**

1. It is a red crystalline substance
2. It consists of
3. Four pyrrole ring surrounding a single cobalt atom, similar to that of porphyrins but differs from it in that ring.

Sources

Foods of animal origin are the only sources for vitamin B12. The rich sources are liver, kidney, milk, curd, egg, fish, pork and chicken. "Vitamin. B12" synthesized only

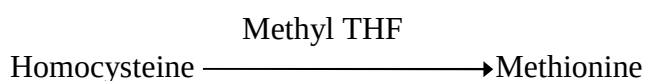
by microorganisms.

Recommended Daily Allowances

Adult = 3 micro g /day.

Biochemical functions

Synthesis of methionine from homocysteine

**Deficiency Symptoms**

subacute combined degeneration of the spinal cord low hemoglobin level (Megaloblastic anemia) folate trap pernicious anemia due to deficiency of intrinsic factor.

LIPOIC ACID

It is a sulphur containing vitamin.

Functions

Acts as a coenzyme for oxidation of alpha keto acids.

Alpha keto glutarate → Succinyl CoA

Pyruvate → acetyl CoA

INOSITOL

1. It is sugar alcohol derived from glucose.
2. It is a cyclic compound present mainly in muscles.

CHOLINE

It is a lipotropic factor prevents the accumulation of fat in the liver. It required for formation of lecithin, plasmalogen and sphingomyelin.

BIOFLAVONOIDS

They are found in peel and pulp of citrus fruits, tobacco leaves and many vegetables.

ANTIVITAMINS

Antivitamins are synthetic compounds, which are either structural analogues of the vitamins or compounds, which increase the requirement of a vitamin by inducing

artificial deficiency.

e.g.

1. Pyriithiamin and oxythiamine antagonist to thiamine
2. Iso-riboflavin is antivitamin of riboflavin.
3. Isoniazid is (drug used for tuberculosis) antagonist to pyridoxine
4. Aminopterin & amithopterin is antagonist to folic acid.
5. Dicumarol antagonist to Vit.K.

9.3.2: VITAMIN C

Chemistry

1. Ascorbic acid is a hexose derivative and closely resembles monosaccharides in structure.
2. It is a strong reducing agent.
3. L- ascorbic acid undergoes oxidation to form dehydroascorbate. Both ascorbic acid and dehydroascorbate are biologically active.
4. Oxidations of ascorbic acid are rapid in the presence of copper.

Hence vitamin - C becomes inactive if the foods are prepared in copper vessels.

Biosynthesis and metabolism

Many animals can synthesize ascorbic acid from glucose via uronic acid pathway.

However man and other primates like Guinea pig, bats cannot synthesize ascorbic acid due to the deficiency of enzyme L gulcanolactone oxidase.

Source

Citrus fruits, gooseberry, guava, green leafy vegetables, cabbage, spinach, tomatoes, potatoes.

Recommended Daily Allowances

60 -70 mg for adults and additional 20-40 % required more during pregnancy and lactation.

Biochemical functions

1. Collagen formation

Mainly required for the hydroxylation of proline and lysine. This hydroxy proline and hydroxylysine are required for the collagen cross-linking and strengthening the

fiber.

So Vitamin - C requires for the maintenance of normal connective tissue and the wound healing process.

2. Bone formation

Vitamin - C required for bone formation.

3. Iron and hemoglobin Metabolism

Vitamin - C enhances the absorption and keeps the iron in ferrous form.

4. Vitamin - C is essential for the hydroxylation of Tryptophan in the Tryptophan metabolism.
5. Required in Tyrosine metabolism for oxidation of P-hydroxy phenylpyruvate to homogentisic acid.
6. Synthesis of bile acid from cholesterol required.
1. Vitamin - C required in electron transport chain reaction. 8. It increases the synthesis of immunoglobulins.

Deficiency of vitamin C.

In the Humans, its deficiency produces a disease called “Scurvy”. The main defect is a failure to deposit intercellular cement substance.

1. Capillaries are fragile and there is tendency to haemorrhages –petechial,
2. subcutaneous, sub-periosteal and even internal haemorrhages can occur.
3. Wound healing is delayed due to deficient formation of collagen.
4. Gums are swollen and become spongy and bleed on slightest pressure. Hyperemia, swelling, sponginess and bleeding of gums are seen. In severe scurvy, may lead to secondary infection and loosening and falling of teeth.
5. Osteoid of bone is poorly laid and mineralization of bone is poor. The bones are weak and readily fracture.
6. Haemorrhages occurring below the periosteum and into the joints may cause extremely painful swellings of bones and joints.
7. Anemia may be associated which is hypochromic microcytic type.

“Bachelor” Scurvy

Elderly bachelors and widowers who may prepare their own foods are particularly prone to development of vitamin C deficiency.

Detection of Deficiency in Man

1. Prompt improvement following w.r.t administration of vitamin C.
2. Determination of concentration of ascorbic acid in blood.
3. Urine ascorbic acid – “Saturation” test. By administering a test dose of 5 mg per lb body wt, if more than 50% excreted in next 24 hours, it indicates no deficiency of the vitamin.
4. Intradermal test Consists of intradermal injection of 2, 6- dichlorophenol indophenol and determination.
5. Tourniquet test – capillary resistance or fragility test- A sphygmomanometer cuff is applied around the arm inflated so as it compresses the venous flow.

In a short time, appearance of several petechial haemorrhages on the forearm skin indicates.

9.4 SUMMARY

Vitamins are organic compounds required in the diet in small amount to perform specific biological functions for normal maintenance of optimum growth and health of the organism.

The term vitamin was coined from the word vital amine, since the earlier identified ones had amino groups; later works showed that most of them did not contain amino groups. Most of the vitamins are supplied by diet. Very few vitamins are synthesized in the intestine.

9.5 KEYWORDS**Vitamins**

Vitamins are organic compounds required in the diet in small amount to perform specific biological functions for normal maintenance of optimum growth and health of the organism.

Retinol (Vitamin - A alcohol)

It is a primary alcohol-containing β - ionone ring. The side chain has two isoprenoid units.

Four double bonds and one hydroxyl group.

It is not destroyed by heat due to the presence of double bonds.

Calciferol (Vitamin D)

Ergocalciferol (Vitamin - D₂) is formed from ergo sterol present in plants.

Cholecalciferol (Vitamin - D₃) is found in animals, ergocalciferol and cholecalciferol are sources for vitamin – D activity are called Provitamins.

Vitamin E.

1. Vitamin - E is the name given to a group of tocopherols, possessing vit E activity.
2. About 8 tocopherols are identified alpha, beta, gamma, delta etc. among these alpha –tocopherol is more active.
3. The tocopherols are derivatives of 6 hydroxy chromane (tocol) rings with isoprenoid side chain.
4. The antioxidant property is due to the chromane ring.

Vitamin K.

It exists in a different form

1. Vitamin - K₁ - Phylloquinone - present in plants.
2. Vitamin - K₂ - Menaquinone - produced by intestinal bacteria.
3. Vitamin - K₃ - Menadione is a synthetic form All three are naphtha quinone derivatives.

Vitamin C.

1. Ascorbic acid is a hexose derivative and closely resembles monosaccharides in structure.
2. It is a strong reducing agent.
3. L- ascorbic acid undergoes oxidation to form dehydroascorbate. Both ascorbic acid and dehydroascorbate are biologically active.
4. Oxidations of ascorbic acid are rapid in the presence of copper.

Hence vitamin - C becomes inactive if the foods are prepared in copper vessels.

EXERCISE

I; Essay Questions

1. Explain in detail the sources, structure, absorption, metabolism, functions, and deficiency manifestations of fat-soluble vitamins.
2. Give an account of the sources, metabolism, functions, and deficiency states of

water-soluble vitamins.

3. Why the newborn baby is more prone to develop vit K deficiency and hypoprothrombinemia?

II Multiple Choice Questions

1. Which of the following is the most important nutrient for a woman during her initial stages of pregnancy to prevent birth defects? ()
(a) Thiamin. (b) Vitamin. (c) Folic acid. (d) Vitamin.
2. Which of the following foods contains the highest concentrations of vitamin C? ()
(a) Parsley. (b) Broccoli. (c) Orange juice. (d) Black currants.
3. Which of the following vitamins is important in the process of blood clotting? ()
(a) Vitamin K. (b) Vitamin C. (c) Vitamin D. (d) Vitamin A.
4. Which one of the following is the most common cause of blindness in children all over the world?
(a) Glaucoma. (b) Vitamin A. (c) Colour blindness. (d) Cataracts. ()
5. It is caused by a lack of the following nutrients: ()
(a) Vitamin B12. (b) Vitamin B2. (c) Vitamin B6. (d) Vitamin B1.
6. Which of the following vitamins can act as both a visual pigment and a hormone at the same time? ()
(a) Thiamine. (b) Folic acid. (c) Riboflavin. (d) Retinal.
7. Megaloblastic anemia is caused by a deficiency in which of the following vitamins or minerals? ()
(a) Pyridoxine. (b) Niacin. (c) Folic acid. (d) Cobalamin
8. Which of the following is a fat-soluble vitamin? () (a) Vitamin B. (b) Vitamin K. (c) Vitamin B12. (d) Vitamin C.
9. Deficiency of niacin is caused due to the deficiency of () (a) Scurvy (b) Rickets. (c) pernicious anaemia (d) Pellagra.
10. Which of the following is a vitamin that acts as a hormone precursor? ()
(a) Vitamin A. (b) Vitamin C. (c) Vitamin K. (d) Vitamin D.

III Fill in the Blanks.

1. Coenzyme A is made up of several different components _____
2. Acute vitamin poisoning is caused due to _____
3. highest levels of vitamin C are present _____
4. Beriberi is caused due by the deficiency of _____
5. _____ vitamins function as both a visual pigment as well as a hormone

6. _____ nutrient deficiencies cause megaloblastic anemia
7. _____ is a fat-soluble vitamin
8. Deficiency of niacin is caused due to the deficiency of _____
9. _____ is a hormone precursor vitamin
10. A component of the coenzyme A is _____

II Answers: 1) c 2) d 3) a 4) b 5) d 6) d 7) c 8) b 9) d 10) b

III Answers: 1) Pantothenic acid 2) Eating the liver of a Polar bear 3) Black currants 4) Vitamin B1 5) Retinal 6) Folic acid 7) Vitamin K 8) Pellagra 9) Vitamin D 10) Pantothenic acid.



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