



**TAMIL NADU VETERINARY AND ANIMAL SCIENCES UNIVERSITY**

## **LECTURE NOTES ON**

### **UNIT – 1**

## **GENERAL VETERINARY BIOCHEMISTRY**

*(VCI MSVE syllabus 2016)*

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# **Scope and Importance of Bio-chemistry**

## **Bio-chemistry**

- Biochemistry is the science dealing with various molecules that are present in living cells and organisms and their chemical reactions.
- The term biochemistry was coined by Carl Alexander Neuberg (1877 –1956), an early pioneer in biochemistry who is often referred to as the "father of modern biochemistry".
- Life depends on biochemical reactions. The chemical processes that occur in living organisms are called as metabolism.
- Enzymes catalyze the metabolic reactions.
- Metabolism consists of two subdivisions called catabolism and anabolism.
- Catabolism refers to conversion of larger molecules into smaller molecules. Some of these reactions produce chemical energy that is captured as ATP.
- Anabolism refers to the conversion of small molecules into large ones during the process of biosynthesis. This process utilizes the ATP.
- The study of chemistry of life has shown that the basic principles of biochemistry are common to all living organisms.
- In general the same chemical compounds and the same metabolic processes are seen in bacteria and humans.
- The important aspects of biochemistry are
  - How a particular molecule is synthesized in the cell, its structural features, how the structure is related its function, the fate of those molecules in the body, how this molecule interacts with other molecules of the body and how the metabolism of these molecules is regulated.

## **LEVELS OF ORGANIZATION IN BIOLOGY AND MEDICINE**

- There are several levels of organization in humans and other animals.
- They are
  - Subatomic particles - Atom - Molecule - Macromolecule - Organelle - Cell - Organ or tissue - Whole animal - Population. Out of which biochemistry concentrates generally on molecules, macromolecules, organelles, cells, tissues and whole animals.

## **KNOWLEDGE OF BIOCHEMISTRY**

- It is a fundamental biological and medical science that provides an understanding of cell biology, microbiology, nutrition, pharmacology and physiology at the molecular level.
- Biochemistry of nucleic acid is the basis of genetics.
- Physiology, the study of body function, overlaps with biochemistry.
- Immunology employs several biochemical techniques.

- Biochemistry is the basis of pharmacology and pharmacy. Most of the drugs are metabolized by the enzyme-catalyzed reactions.
- Toxicology is the study of poisons interacting with the biochemical reactions.
- Biochemical approach is the basis for the elucidation of disease process- the pathology.
- Understanding nutrition depends on knowledge of biochemistry.
- Molecular Biology, a major branch of biochemistry is the study of gene structure, function and regulation.
- Knowledge of Biochemistry essential for Biotechnology, a multidisciplinary subject.
  - A specialized branch of the subject called Clinical Biochemistry is directly associated with clinical medicine. Health depends on the balance of biochemical reactions occurring in the body and disease reflects abnormalities in biomolecules. The determination of the concentration of a body chemical may be valuable in the prevention, diagnosis, and treatment and also to monitor the success of the treatment.
  - The technology of biochemistry may be applied to the study of any aspects of biology - humans, mammals, insects, plants, bacteria and viruses.

## CELL AND SUB CELLULAR COMPONENTS

The cell (from Latin cella) is the basic structural, functional, and biological unit of all known living organisms. A cell is the smallest unit of life that can replicate independently, and cells are often called the "building blocks of life". The study of cells is called cell biology

Cells are of two types, eukaryotic, which contain a nucleus, and prokaryotic, which do not. Prokaryotes are single-celled organisms, while eukaryotes can be either single-celled or multicellular

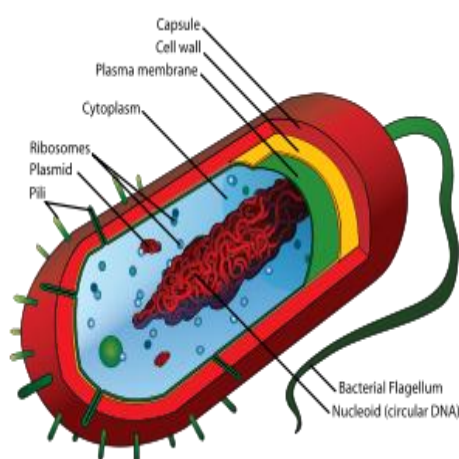


Fig: Prokaryotic cell

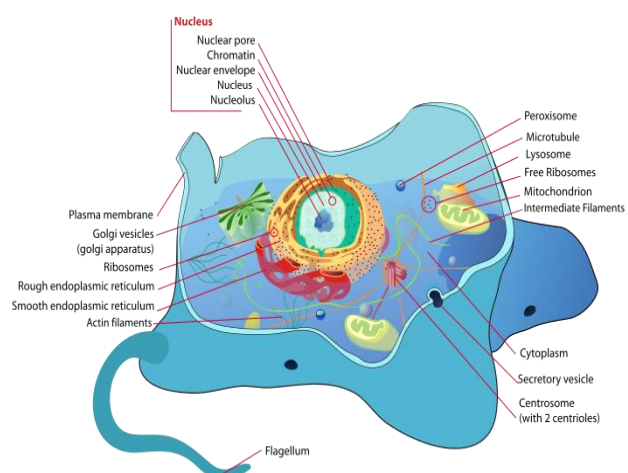


Fig: Eukaryotic cell

## **Sub cellular components**

### **Plasma Membrane**

- The cell membrane, or plasma membrane, is a biological membrane that surrounds the cytoplasm of a cell. In animals, the plasma membrane is the outer boundary of the cell, while in plants and prokaryotes it is usually covered by a cell wall.
- This membrane serves to separate and protect a cell from its surrounding environment and is made mostly from a double layer of phospholipids, which are amphiphilic (partly hydrophobic and partly hydrophilic). Hence, the layer is called a phospholipid bilayer, or sometimes a fluid mosaic membrane.
- Embedded within this membrane is a variety of protein molecules that act as channels and pumps that move different molecules into and out of the cell. The membrane is said to be 'semi-permeable', in that it can either let a substance (molecule or ion) pass through freely, pass through to a limited extent or not pass through at all.
- Cell surface membranes also contain receptor proteins that allow cells to detect external signaling molecules such as hormones.

### **Cell nucleus:**

- A cell's information center, the cell nucleus is the most conspicuous organelle found in a eukaryotic cell. It houses the cell's chromosomes, and is the place where almost all DNA replication and RNA synthesis (transcription) occur.
- The nucleus is spherical and separated from the cytoplasm by a double membrane called the nuclear envelope. The nuclear envelope isolates and protects a cell's DNA from various molecules that could accidentally damage its structure or interfere with its processing. During processing, DNA is transcribed, or copied into a special RNA, called messenger RNA (mRNA). This mRNA is then transported out of the nucleus, where it is translated into a specific protein molecule.
- The nucleolus is a specialized region within the nucleus where ribosome subunits are assembled. In prokaryotes, DNA processing takes place in the cytoplasm.

### **Mitochondria and Chloroplasts:**

- Generate energy for the cell.
- Mitochondria are self-replicating organelles that occur in various numbers, shapes, and sizes in the cytoplasm of all eukaryotic cells. Respiration occurs in the cell mitochondria, which generate the cell's energy by oxidative phosphorylation, using oxygen to release energy stored in cellular nutrients (typically pertaining to glucose) to generate ATP.
- Mitochondria multiply by binary fission, like prokaryotes.
- Chloroplasts can only be found in plants and algae, and they capture the sun's energy to make carbohydrates through photosynthesis.

### **Endoplasmic reticulum:**

- The endoplasmic reticulum (ER) is a transport network for molecules targeted for certain modifications and specific destinations, as compared to molecules that float freely in the cytoplasm.

- The ER has two forms: the rough ER, which has ribosomes on its surface that secrete proteins into the ER, and the smooth ER, which lacks ribosomes. The smooth ER plays a role in calcium sequestration and release.

#### **Peroxisomes:**

- Peroxisomes contain oxidative enzymes, such as catalase, D-amino acid oxidase, and uric acid oxidase
- Peroxisomes also contain the enzyme catalase, which decomposes hydrogen peroxide either by converting it to water or by using it to oxidize another organic compound.
- A major function of the peroxisome is the breakdown of very long chain fatty acids through beta-oxidation. In animal cells, the long fatty acids are converted to medium chain fatty acids, which are subsequently shuttled to mitochondria where they are eventually broken down to carbon dioxide and water. In yeast and plant cells, this process is carried out exclusively in Peroxisomes.

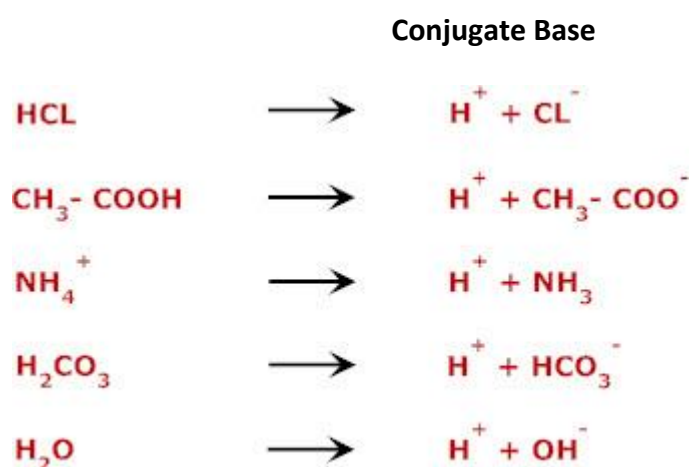
**Golgi apparatus:** The primary function of the Golgi apparatus is to process and package the macromolecules such as proteins and lipids that are synthesized by the cell.

**Lysosomes and Peroxisomes:** Lysosomes contain digestive enzymes (acid hydrolases). They digest excess or worn-out organelles, food particles, and engulfed viruses or bacteria. Peroxisomes have enzymes that rid the cell of toxic peroxides. The cell could not house these destructive enzymes if they were not contained in a membrane-bound system.

## **ACIDS, BASES, pH AND BUFFERS**

### **ACIDS AND BASES**

Some example of acids and bases



Some compounds can act as proton donor and proton acceptor and these compounds are known as ampholytes or are said to be amphoteric.

- As per the modern concept an acid is a proton donor and a base is a proton acceptor.
- On ionization, an acid donates a proton and a base (which is capable of accepting a proton). This base is known as a conjugate base.



- Acids and bases are classified into two groups depending on their tendency to lose proton or hydroxyl group respectively. They are strong acids and weak acids. Strong base and weak base.
- Strong acids or bases are those which are completely ionized in solution.

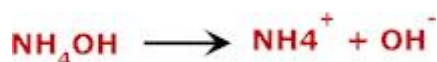
- Stronger acid
- Strong base



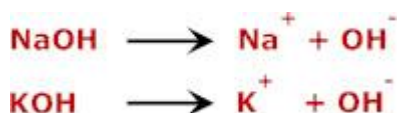
- The weak acid or base dissociates only to a limited extent and the concentration of  $\text{H}^+$  and  $\text{OH}^-$  depends on the dissociation constant of acid and base respectively
- Weak acid



- Weak base

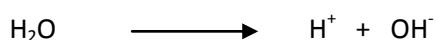


- The term alkali is reserved for those compounds that yield  $\text{OH}^-$  ions on dissociation



### DISSOCIATION OF WATER

Pure water consists almost entirely of  $\text{H}_2\text{O}$  molecules.



This process is termed the *auto-ionization* of water. The equilibrium expression for the auto-ionization reaction is equal to  $1.8 \times 10^{-16}$  moles /liter at  $25^\circ \text{C}$ .

$$K = \frac{[\text{H}^+][\text{OH}^-]}{[\text{H}_2\text{O}]}$$

The concentration of pure H<sub>2</sub>O is about 55M. The concentration of water is essentially unchanged during chemical reactions involving dilute aqueous.

$$K = \frac{[H^+][OH^-]}{(\text{constant})}$$

$$K(\text{constant}) = [H^+][OH^-]$$

$$K_w = K(\text{constant}) = [H^+][OH^-]$$

The constant,  $K_w$  is called as the *ion-product constant*. At 25°C, the value of  $K_w$  is  $1 \times 10^{-14}$

$$K_w = [H^+][OH^-] = 1 \times 10^{-14}$$

Based on this equilibrium constant, if we know the concentration of either  $H^+$  or  $OH^-$  ions in solution, then we can derive the concentration of the other ion

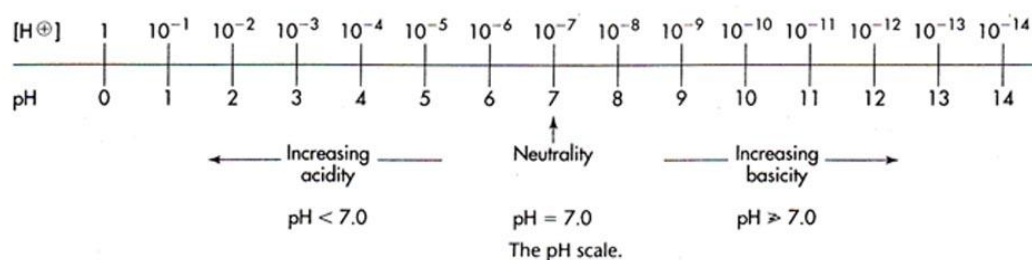
$$[H^+] = 1 \times 10^{-14} / [OH^-] \quad \text{or} \quad [OH^-] = 1 \times 10^{-14} / [H^+]$$

A solution for which  $[H^+] = [OH^-]$  is said to be *neutral*,  $[H^+][OH^-] = 1 \times 10^{-14}$

## pH

- It is a unit that describes the acidity and alkalinity of a solution.
- The biochemical processes such as transport of oxygen in the blood, the catalysis of reactions by enzymes and the generation of metabolic energy are strongly affected by the concentration of  $H^+$  ion.
- Many biological reactions are dependent on the charge on the molecules (+ ve or -ve charge).
- The charge on the molecule is determined by the ability of molecule to release or accept a proton, which in turn depends on the pH of the solution. So, in biological experiments it is necessary to measure the concentration of  $H^+$  ion.
- The  $H^+$  ion concentration of most of the biological solutions is very low and is in the range of  $10^{-1}$  to  $10^{-14}$  gram ions/L, which is very difficult to measure and express in conventional method of expression. Hence, Sorenson in 1909 introduced the term pH, to express the hydrogen ion concentration in a logarithmic manner, which is defined as **pH = -log of  $[H^+]$  or  $\log 1 / [H^+]$** . According to this the  $[H^+]$  concentration of  $10^{-8}$  g /L will be pH 8.0. The 'p' denotes 'negative logarithm of 'p' also stands for power. pH is the abbreviation of 'power of hydrogen'
- The pH scale is the useful way of expressing acidity, which in turn dependent on  $[H^+]$ . pH is generally in the range of 0 - 14, as the dissociation constant of water at 25°C is  $10^{-14}$ .
- An acidic solution has a pH below 7 and a basic solution has a pH above 7. Pure water is neither acidic nor basic and is said to be neutral  $[H^+] = [OH^-] = 10^{-7}$  M

- pH is inversely related to hydrogen ion concentration. That is lower the pH the higher the  $H^+$  ion concentration.
- The pH scale is logarithmic (exponential) not arithmetic (linear). This means that when a solution changes from pH 7 to pH 6, the  $H^+$  ion concentration increases by 10 fold. When it goes from pH 7 to pH 5 it increases by 100 fold. The pH of water is 7.0 that means water contains  $1 \times 10^{-7}$  g of  $H^+$  ions/L.
- The pH of an aqueous solution can be approximately measured using various indicator dyes, including litmus, phenolphthalein and phenol red, which undergo colour changes as a proton dissociate from the dye molecule.



- Accurate determination of pH in chemical and clinical laboratory is made with a glass electrode that is sensitive to  $H^+$  ion concentration. Measurement of pH sometimes used in the diagnosis of diseases. The normal pH of human plasma is 7.4, which is referred to as physiological pH. The blood of patients suffering from certain diseases such as diabetes can have a lower pH, a condition called acidosis. The condition in which the pH of the blood is higher than 7 is called alkalosis.

## BUFFER

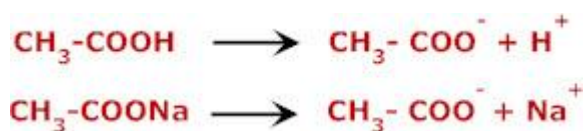
- A buffer solution is one that resists pH change on the addition of a small quantity of acid or alkali. Such solutions are used in biochemical experiments, where the pH is to be accurately controlled.
- Body fluids must be protected against change in pH. Because most enzymes are pH sensitive.
- During metabolism acids and bases are produced. In the long run, excess acids or base is eliminated via kidney and lungs. In the short run the body is protected against pH change by buffering systems. Acids produced by the body are carbonic acid, sulfuric acid, phosphoric acid, lactic acid, citric acid, ammonium ions Ketone bodies: acetoacetic acid and  $\beta$ -hydroxy butyric acid.
- Bicarbonate buffer is the major extracellular buffer and phosphate buffer is the major intracellular buffer in the body, which protects the body against the pH change.
- A buffer solution consists of a weak acid and its salt, the conjugate base (the proton acceptor). For e.g. acetic acid and sodium acetate or a weak base and its salts (e.g. ammonium hydroxide and ammonium chloride).



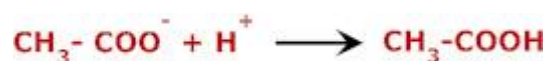
- During buffering strong acid or base is replaced by a weaker one, with a consequent reduction in the number of free hydrogen or hydroxyl ion.

### BUFFER PAIR

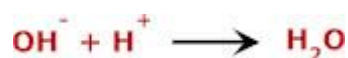
- Let us consider the buffer pair of acetic acid and sodium acetate.



- When an acid say HCL is added, the acetate ion of the buffer binds with  $\text{H}^+$  of HCL to form acetic acid, which is weakly ionized. Therefore the buffer resists the pH change due to acid.

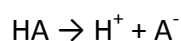


- When a base say NaOH is added the  $\text{H}^+$  ions of the buffer acetic acid combines with  $\text{OH}^-$  ions to form water, which is weakly ionized. Thus the pH change due to base addition is also prevented.



### HENDERSON - HASSELBALCH EQUATION

- A weak acid HA ionizes as follows



- The equilibrium constant for this reaction is written as follows

$$K_a = [\text{H}^+][\text{A}^-] / [\text{HA}]$$

The value of the dissociation constant ( $K_a$ ) indicates the tendency of the acid to lose its proton. Stronger acids have a greater tendency to dissociate and therefore have higher dissociation constant

- $[\text{HA}]$  = concentration of the undissociated acid,  $[\text{H}^+]$  = concentration of hydrogen ion and  $[\text{A}^-]$  = concentration of the conjugate base.
- Cross multiplying:

$$[\text{H}^+][\text{A}^-] = K_a [\text{HA}]$$

$$[H^+] = K_a * ([HA] / [A^-])$$

- Taking -log of both sides

$$-\log [H^+] = -\log K_a -\log ([HA] / [A^-])$$

- Substitute pH and pK for  $-\log [H^+]$  and  $-\log K$  respectively. The -log of the dissociation constant is defined as the pKa.

$$pH = pK_a -\log ([HA] / [A^-]) \quad (\text{The stronger the acid ,lower is its pKa})$$

- Then to remove the -sign invert the last term

$$pH = pK_a + \log ([A^-] / [HA])$$

- When the pH is equal to pKa, the concentration of conjugate base is equal to concentration of undissociated acid.

#### **FACTORS DETERMINING THE EFFECTIVENESS OF BUFFER**

- Two factors determine the effectiveness of buffer
  - Its pKa relative to pH of the solution
  - Concentration
- A buffer works best within 1 pH unit above or below its pKa
- A buffer is more effective the more concentrated it is. Because the more concentrated solution contains more buffer molecules
- A 1M solution of a given buffer has 1 million times more buffering molecules than 1  $\mu$ m solution
- Buffering capacity is the efficiency of the buffer solution to resists the change in pH when acid or base is added. It is the number of gram equivalents of either hydrogen ion or hydroxyl ion required to change the pH of 1L of 1M solutions by 1 unit
- Greater the buffering capacity, the less the change of pH

## DONNAN'S MEMBRANE EQUILIBRIUM

- Semi permeable membrane: A membrane that permits the passage of some solutes usually small molecules and solvent but not colloids.
- When two different concentrations of electrolyte solutions are separated from each other by a semi permeable membrane, if a non-diffusible ion (eg: protein) is present in one of the sides, then there is an unequal distribution of the diffusible ions between the compartments. This is referred to as Donnan's Membrane Equilibrium.

Consider the following experiment

- The two compartments A and B are separated by a semi permeable membrane, which is freely permeable to  $\text{Na}^+$  and  $\text{Cl}^-$  and impermeable to protein.
- Assume that at the start of the experiment the concentrations of the anion and cation on the two sides are equal.

A	B
90 $\text{Na}^+$	90 $\text{Na}^+$
90 Protein	90 $\text{Cl}^-$

**At start**

A	B
120 $\text{Na}^+$ 30 $\text{Cl}^-$ 90 Protein	60 $\text{Na}^+$ 60 $\text{Cl}^-$

**At equilibrium**

- Due to Donnan effect, the -ve charge of the non-diffusible anion hinders the diffusion of cation from 'A' side and favours the diffusion of anion from 'B' side. Hence,  $\text{Cl}^-$  diffuses from 'B' side to 'A' side down its concentration gradient along with some  $\text{Na}^+$  due to its opposite charge.
- According to Donnan membrane theory, the diffusible ions will pass through the membrane, until the products of concentration of the diffusible ions ie  $\text{Na}^+$  &  $\text{Cl}^-$  is same on both sides of the membrane.
- At equilibrium more osmotically active particles are present on 'A' side than 'B' side. Therefore the osmotic pressure on the 'A' side is greater than 'B' side (there is more ions on the 'A' side) because osmotic pressure depends on number of ions.

Donnan's equilibrium has the following effects in the body

- Proteins are present inside the cells. Due to Donnan effect more osmotically active particles are present in cells than in interstitial fluid.  $\text{Na}^+$ - $\text{K}^+$  pump counter balances the effect.
- Due to differences in the concentration of diffusible ions, an electrical difference is formed across the membrane (side 'A' is more -ve than side 'B'), which is balanced by opposite charges on the membrane.
- There are more proteins in the blood, due to which ionic movement occurs across the capillary walls.

# BIOCHEMISTRY OF CARBOHYDRATES

- Carbohydrates are the most abundant bio molecules, distributed widely in plants and animals where, they perform structural and functional roles. They are hydrated carbon molecules. Generally the hydrogen and oxygen will be present in the proportion of 2:1.
- Carbohydrates may be defined as poly hydroxy aldehydes or ketones, or any substances that yield one of these compounds on hydrolysis.
- Many carbohydrates have the empirical formula  $(\text{CH}_2\text{O})_n$ , where  $n$  is 3 or larger.
- Some carbohydrates contain nitrogen, phosphorous or sulphur also.

## BIOLOGICAL IMPORTANCE OF CARBOHYDRATES

- Carbohydrates provide the majority of energy in most organisms.
- Glucose is stored as glycogen in liver and muscle.
- Carbohydrates (**e.g.** cellulose) give structure to cell walls (in plants) and cell membranes.
- Carbohydrates serve as metabolic intermediates (**e.g.** glucose 6- phosphate, fructose –1,6 – biphosphate).The metabolic intermediates derived from glucose are used for the biosynthesis of amino acids, nucleic acid and nucleotides.
- Carbohydrates (**e.g.** ribose, deoxyribose) comprise large portions of the nucleotides that form DNA and RNA.
- Carbohydrates also play a role in lubrication, cellular intercommunication and immunity.
- It plays an important role in the metabolism of proteins and fatty acids.

## CLASSIFICATION OF CARBOHYDRATES

Based on the sugar units they contain, carbohydrates are classified into four groups, as

- Monosaccharides
- Disaccharides
- Oligosaccharides ( a small polymer of sugar)
- Polysaccharides

### Monosaccharides

- Simple sugars, consisting of single polyhydroxy aldehyde or ketone unit. They cannot be hydrolyzed to yield simpler forms of sugar.
- They can be subdivided into trioses, tetroses, pentoses, hexoses, heptoses and octoses, depending upon the number of carbon atoms they possess.
- Carbohydrates with an aldehyde as their functional group are called as Aldoses. Those with ketone group as functional group are called as ketoses.

Name	Empirical Formula	Aldoses	Ketoses
Trioses	C <sub>3</sub> H <sub>6</sub> O <sub>3</sub>	Glycerose	Dihydroxy acetone
Tetroses	C <sub>4</sub> H <sub>8</sub> O <sub>4</sub>	Erythrose	Erythrulose
Pentoses	C <sub>5</sub> H <sub>10</sub> O <sub>5</sub>	Ribose	Ribulose
Hexoses	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>	Glucose	Fructose

- Disaccharides Yield two molecules of the same or different monosaccharides when hydrolyzed. Examples are sucrose, lactose, and maltose.
- Oligosaccharides Yield 3–6 monosaccharide units on hydrolysis. Eg. maltotriose and raffinose
- Polysaccharides Yield more than 6 molecules of monosaccharide on hydrolysis. Examples of polysaccharides are starch, cellulose, glycogen and dextrans.
- The polysaccharides may be linear or branched eg. Cellulose is a linear polysaccharide and starch is a branched polysaccharide.
- Polysaccharides are sometimes called as hexosans or pentosans, depending upon the type of the monosaccharides they yield on hydrolysis eg., glycogen –hexosan

### PHYSIOLOGICALLY IMPORTANT MONOSACCHARIDES

Sugars	Occurrence	Functions
<b>Pentoses</b>		
D-Ribose	Nucleic acids	As an important component of DNA, RNA and also in NAD <sup>+</sup> , NADP <sup>+</sup> , FAD <sup>+</sup> and ATP  Ribose phosphates are intermediates in HMP pathway
D-Ribulose	Formed in metabolic processes	It is an intermediate in the HMP pathway
D-Arabinose	In wood gums	Constituent of glycoproteins
D-Xylose	Wood gums, proteoglycans and glycosaminoglycans	Constituent of glycoproteins
D-Lyxose	Heart muscle	A constituent of lyxoflavin isolated from human heart muscle
L- Xylulose	Intermediate in uronic acid	-

	pathway	
<b>Hexoses</b>		
D- Glucose (Blood sugar)	Fruit juice, hydrolysis of starch, cane sugar, maltose and lactose	The sugar of the body. The glucose is transported in the blood and oxidized in the cells to produce energy .In diabetes the glucose is present in the urine.
D-Fructose (Fruit sugar)	Found in fruit and honey	It is the sweetest of all the sugars. In seminal fluid it provides the energy source for the spermatozoa. It can be changed to glucose in the liver. In some hereditary disorder fructose is accumulated causing hypoglycaemia.
D- Galactose (Brain sugar)	Hydrolysis of lactose	It can be changed to glucose in the liver. It is used to synthesize lactose in the mammary gland. A constituent of glycolipids and glycoproteins Failure in the metabolism leads to galactosemia and cataract.
D-Mannose	Hydrolysis of plant mannans and gums	A constituent of many glycoproteins.

### **ISOMERISM IN CARBOHYDRATES**

**Isomers** are compounds having same molecular formulae but different structural form.

The presence of an asymmetric /chiral carbon atom (a carbon atom to which four different groups or atoms are attached) make possible the formation of isomers of a compound.

The isomers are of two types: structural isomers and stereoisomers.

- **Structural isomers** have same molecular formula but different structures due to difference in chain length (chain isomers), position of substituent groups (positional isomers) or different functional groups.  
Eg: Glucose and fructose are structural (functional) isomers as both have same molecular formula ( $C_6H_{12}O_6$ ) but have different functional groups, aldehyde and ketone respectively.
- **Stereoisomers** have same molecular formula; same structures but differ only in spatial configuration.
- The possible number of isomers formed by a monosaccharide depends upon the number of asymmetric or chiral carbon atoms.
- ✓ In general, a sugar with “n” number of asymmetric carbon atoms can have  $2^n$  stereo isomers.
- ✓ An aldose sugar with ‘n’ number of carbon atoms can have  $2^{n-2}$  stereoisomer.

- ✓ A ketose sugar with 'n' number of carbon atoms can have  $2^{n-3}$  stereo isomers.

Eg: Glucose has 4 asymmetric carbon atoms hence it can have  $2^4$  or 16 number of isomers.

**Optical isomers** are one type of stereoisomer where the isomers appear as mirror image of each other around the asymmetric carbon atom.

When a beam of plane polarised light is passed through the sugar many monosaccharides can rotate plane polarized light to right (d, dextrorotatory, +) or left side (l, levorotatory, -).

Eg: Glucose is dextrorotatory while Fructose is levorotatory. Dihydroxyacetone has no optical activity. Racemic mixture (+/- mixture) shows no optical activity.

### Enantiomers

The two stereoisomers that are non superimposable, mirror images of one another are called a pair of Enantiomers. (Greek: enantios + meros, oposit + part).

D and L-sugars are referred to as enantiomers. Their structures are mirror images of each other. D and L forms are termed depending on the arrangement of H and OH on the penultimate carbon atom and it is with reference to D and L - glyceraldehyde.

When the sugar has OH group on right, it is a D isomer and if OH group is on left side, then it is a L – isomer.

The majority of monosaccharides found in human body are D-type. Some sugars do occur naturally as L-forms such as L-arabinose. Only D- sugars are utilized by humans.

### Anomerism

Sugars in solution exist in ring form and not in straight chain form. The newly formed asymmetric carbon is called as Anomeric carbon.

If the two sugars which differ in the configuration at only C1 in case of aldoses and C2 in ketoses are known as anomers.

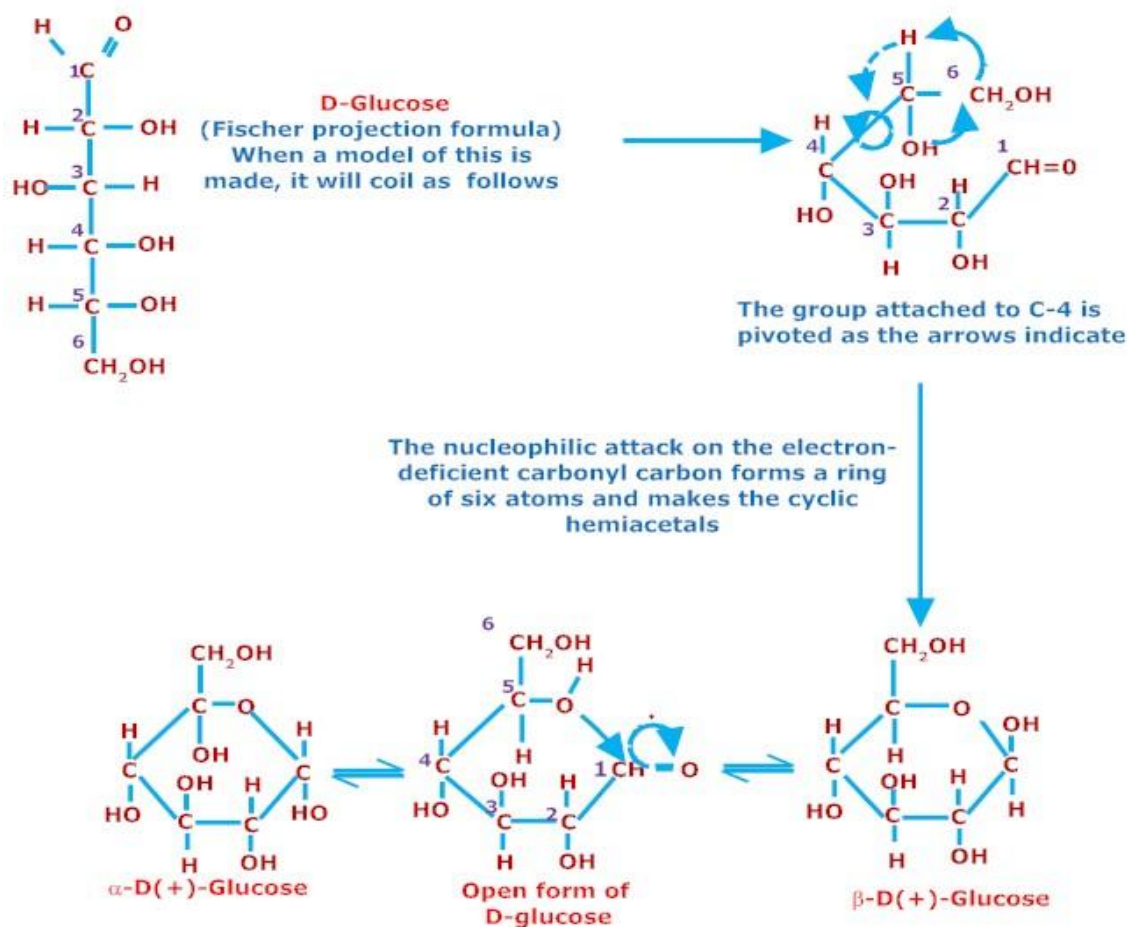
In ring forms, the orientation of -H and -OH groups around anomeric carbon atom will give rise to two isomers known as anomers. They are represented as alpha and beta sugars.

If the -OH group is below the plane of the ring it is referred to as  $\alpha$  -form (alpha) and if the -OH group is above the plane of the ring it is called the  $\beta$  – form (beta).

E.g.  $\alpha$ -D glucose and  $\beta$ -D-glucose,  $\alpha$ -D fructose and  $\beta$ -D-fructose

Aldose sugars forms mainly pyranose ring and ketose sugars forms furanose ring structure.

## Cyclic structure of carbohydrates



**Mutarotation** is the change in the specific optical rotation of an optically active compound without any change in other properties.

Mutarotation occurs because of the change in the equilibrium between two anomers.

The  $\alpha$ - and  $\beta$ - anomers of carbohydrates are typically stable solids. However, in aqueous solution, they quickly equilibrate to an equilibrium mixture of the two forms.

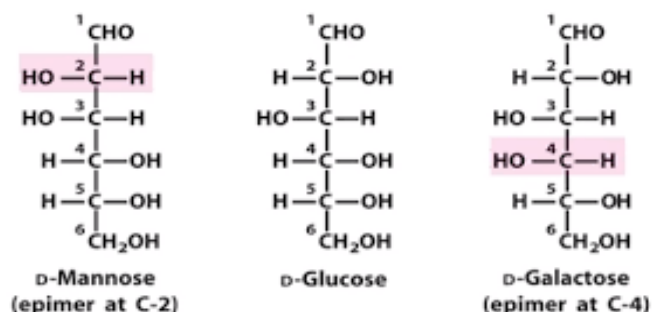
For example, in aqueous solution, glucopyranose exists as a mixture of 36%  $\alpha$ - D-Glucose (+112°) and 64%  $\beta$  -D-Glucose (+19°)

The equilibration occurs via the ring opening of the cyclic sugar at the anomeric center and it gradually changes to intermediate value of +53°.



## EPIMERISM:

Those stereoisomers which are differing in its configuration at only one chiral carbon atom are called as Epimers. For example, glucose and galactose are Epimers of each other, as they differ in only in the position of hydroxyl group at C4.



## REACTIONS OF MONOSACCHARIDES

### Oxidation:

- Mild oxidation conditions like under Bromine water, the aldehyde group gets oxidised to form corresponding Aldonic Acid. Eg.. Glucose forms Gluconic acid
- Strong oxidation- like  $\text{HNO}_3$  both the aldehyde and the primary alcoholic group gets oxidised to form corresponding Aldaric/saccharic Acid. Eg. Glucose forms Glucosaccharic acid, Galactose forms Mucic acid.
- Protective oxidation conditions (with acetylation) like  $\text{H}_2\text{O}_2$ , the aldehyde group remains intact but only the the primary alcoholic group oxidised to form corresponding Uronic Acid. Eg.. Glucose forms Glucuronic acid

### Reduction

- The aldehyde/Ketone group gets reduced to form corresponding Alcohols in presence of Sodium amalgam or enzymes.  
Eg.. Glucose forms Sorbitol, Galactose forms Dulcitol, Fructose forms Sorbitol or Mannitol.

### Dehydration

- Sugars are stable with Dilute acids. But when treated with strong mineral acids like  $\text{Con. H}_2\text{SO}_4$ , they lose water to form their corresponding Furfural.
- Pentoses form furfurals and hexoses form hydroxyl methyl furfurals.
- Enolisation

### ***Enolisation***

- When Sugars are treated with dilute alkali like  $\text{Ba(OH)}_2$ ,  $\text{Ca(OH)}_2$ , interconversion of sugars takes place due to ene-diol (Alkenes with two OH groups attached) formation.
- Interconversion of Glucose to Fructose and mannose and vice versa, takes place due to ene-diol formation is otherwise called as Lobry De Bryn von Ekenstein reaction or tautomerisation.
- Ene-diols formed acts as very powerful reducing agents.
- Treatment with strong alkali leads to caramalization/browning.

### ***Fischer –Kiliani synthesis***

- Reaction with Hydrogen cyanide (HCN) produces sugars with one carbon more than the parent molecule by forming the corresponding Nitrile derivative.
- eg: Erythrose (4C) forms Ribose (5C).

### ***Whols degradation***

- Reaction with Hydroxyl amine ( $\text{NH}_2\text{OH}$ ) produces sugars with one carbon less than the parent molecule by forming the corresponding Oxime derivative.
- eg: Ribose (5C) gives Erythrose (4C).

### ***Osazone reaction***

- All reducing sugars form Osazones when treated with excess of phenyl hydrazine and acetate buffer at boiling temperature.
- Each sugar produces its corresponding insoluble, yellow Osazone crystals which have a typical shape when seen under a microscope.
- Glucose, Fructose Mannose forms same Needle shaped crystals like sheaves of a corn because the structural differences of these sugars in their first and second carbon atoms get masked after formation of Osazones.
- Galactose forms Rhombic rod shaped crystals.
- Lactose – Hedge hog/ball badminton/powder puff shaped crystals.
- Maltose – Sunflower petal shaped crystals.
- Sucrose being a non reducing sugar does not form any Osazone crystal.

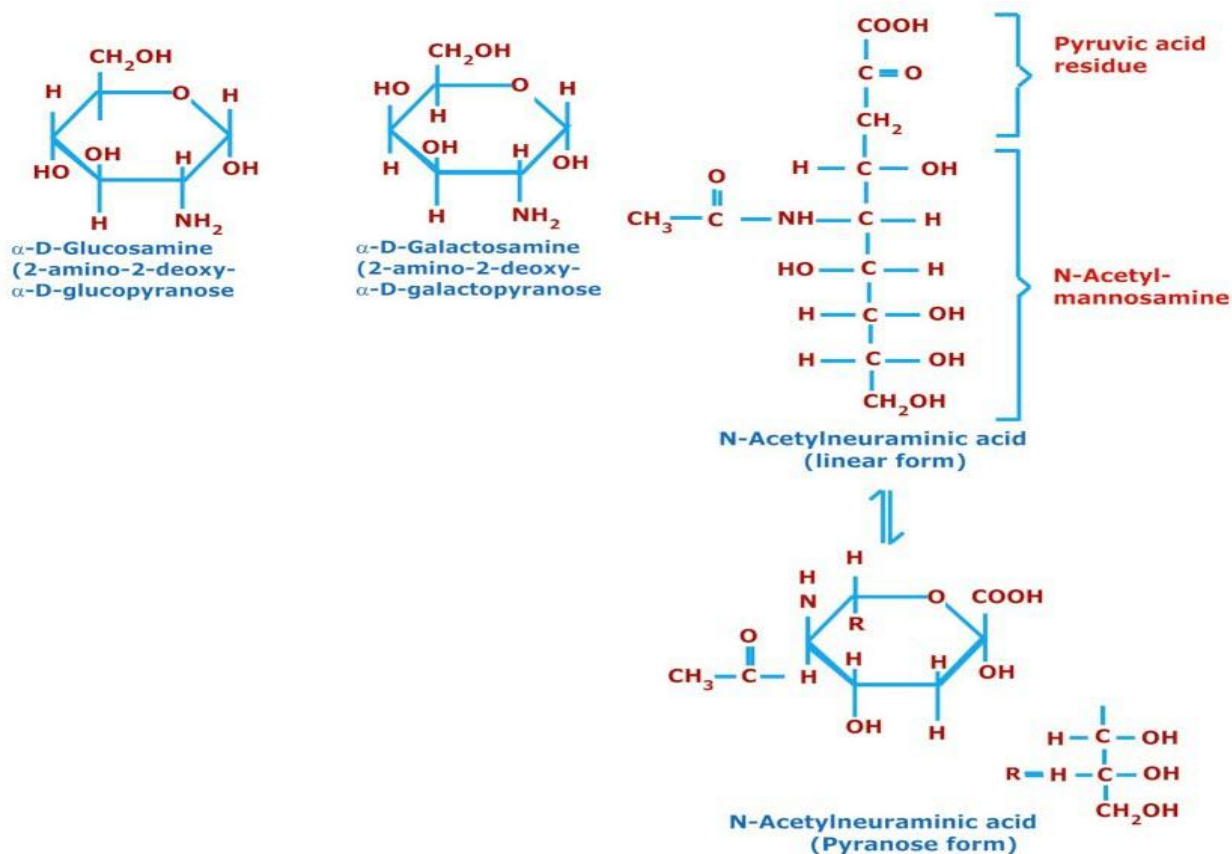
### ***Glycosides:***

- When the hydroxyl group on the anomeric carbon of a monosaccharides reacts with a OH or NH groups of other compounds like sterols, protein etc., glycosides are formed.

Eg: Ouabain- is a cardiac glycoside which inhibits NA-K ATPase pump.

## DERIVATIVES OF MONOSACCHARIDES

- **Phosphate esters:** When monosaccharides are used as fuel they are metabolized as phosphate esters. For e.g., triose phosphate, ribose 5-phosphate and glucose 6-phosphate.
- **Deoxy sugars:** In these derivatives, a hydrogen atom replaces one of the hydroxyl groups in the parent monosaccharide. An example is deoxyribose occurring in nucleic acid DNA, L-Fucose (6-deoxy-L-galactose) is widely distributed in plants, animals and microorganisms. L-rhamonose (6-deoxy-L-mannose) is also found as components of cell wall.
- **Amino sugars:** In a number of sugars, an amino group replaces one of the hydroxyl groups in the parent monosaccharide. Sometimes the amino group is acetylated. Examples of amino sugars are D-glucosamine, D-galactosamine and D- mannosamine. They commonly occur in glycoconjugates. Several antibiotics contain amino sugars.
- **Sugar alcohols:** In sugar alcohols, the carbonyl oxygen of the parent monosaccharide has been reduced producing a polyhydroxy alcohol. For example glycerol and myo-inositol are the important components of lipids. Ribitol is a component of FMN and FAD.
- **Sugar acids:** Sugar acids are carboxylic acids derived from aldoses, either by the oxidation of C-1 (the aldehyde carbon or by the oxidation of carbon bearing the primary alcohol). Sugar acids are important components of many polysaccharides.
- **Ascorbic acid or Vitamin C:** It is derived from D-glucuronate . It is an essential cofactor for many hydroxylation processes.



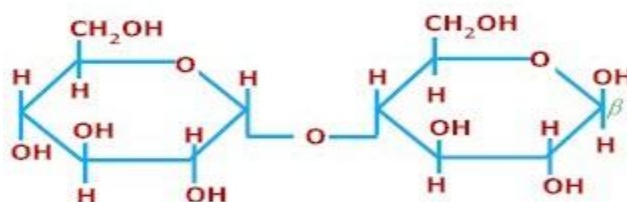
## DISACCHARIDES

### STRUCTURE AND FUNCTIONS OF DISACCHARIDES

- Disaccharides are sugars composed of 2 monosaccharide units joined by glycosidic linkage.
- The linkage may be either  $\alpha$  or  $\beta$ , depending on the position of the atoms (H and OH) attached to the anomeric carbon of the sugar.
- Disaccharides undergo mutarotation at the reducing end.
- The most common disaccharides produced by mammals are maltose, isomaltose and lactose.
- Maltose and isomaltose are generated during digestion of starch in the gut.
- Lactose is synthesized by the mammary glands and is an important component of milk.
- Common table sugar, sucrose is a non-reducing disaccharide.

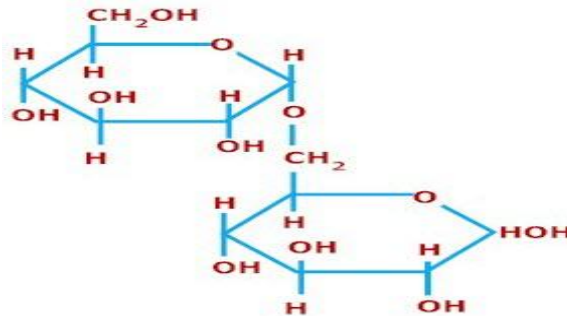
### MALTOSE

- Maltose is obtained as an intermediate in the hydrolysis of starch by enzymes known as amylases
- In maltose, one molecule of glucose is linked through the hydrogen group on the C – 1 carbon atom in a glycosidic bond to the hydroxyl group on the C– 4 of a second molecule of glucose
- The glycosidic linkage between the two glucose residues is designated as  $\alpha$  (1  $\rightarrow$  4) to specify that the anomeric carbon involved in the glycosidic bond has the  $\alpha$  - configuration and that it is linked to the hydroxyl group attached on carbon 4 of the second sugar
- In the second sugar, hydroxyl group can exist in either the  $\alpha$  or  $\beta$  configuration. This free anomeric hydroxyl group confers the property of mutarotation on maltose. The free anomeric carbon can act as a reducing agent and the disaccharide is a reducing disaccharide.



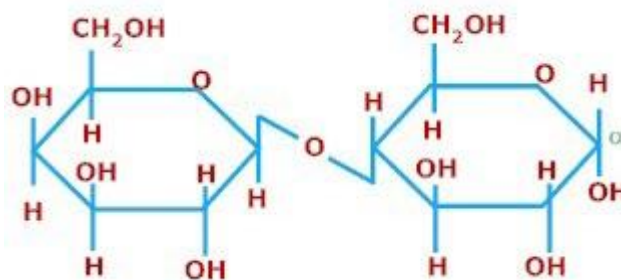
## ISOMALTOSE

- Isomaltose is another disaccharide obtained during the hydrolysis of certain polysaccharides (such as starch and glycogen) is similar to maltose except that it has  $\alpha$  (1  $\rightarrow$  6) linkage.



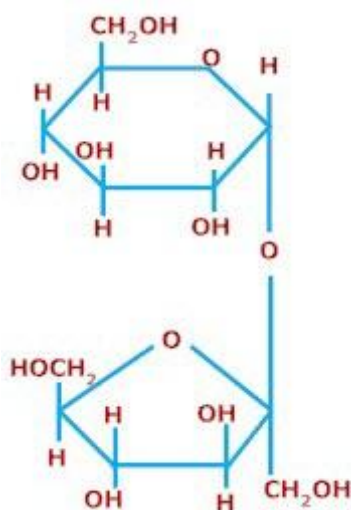
## LACTOSE

- Lactose is a disaccharide found in milk. On hydrolysis, it yields one molecule each of  $\beta$ -D-galactose and D-glucose.
- It possesses a  $\beta$ (1  $\rightarrow$  4) linkage in which C 1 of galactose is linked to the OH group attached on the carbon 4 of the glucose through O –glycosidic bond.
- It is a reducing sugar (the anomeric hydroxyl group of glucose is free) and can undergo mutarotation.
- Lactase is the enzyme that hydrolyses lactose in the system and its production decreases as the age increases.



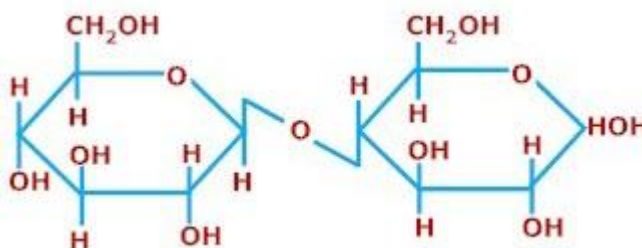
## SUCROSE

- Sucrose, the common table sugar is produced by plants but not by higher animals. Sugar beets and sugar canes are the common sources of sucrose.
- On hydrolysis, sucrose yields one molecule each of  $\alpha$ -D-glucose and  $\beta$ -D-fructose, but in contrast to other mono and disaccharides, sucrose is not a reducing sugar.
- This means that the reducing groups in both of the monosaccharide components (C-1 of glucose and C-2 of fructose) are linked through O – glycosidic bond.
- Sucrose is a dextrorotatory (+ 66.5°). When hydrolyzed with dil. HCl or enzyme sucrase, sucrose produces mixture containing glucose and fructose. The levo rotation of fructose is greater than the dextro rotation of glucose. Hence, the resultant mixture is levo-rotatory (-28.2°). This process of change in rotation is known as inversion. The mixture is known as invert sugar.



## CELLOBIOSE

- Cellobiose is a disaccharide formed during the acid hydrolysis of cellulose. It is identical with maltose except that the former compound has a  $\beta$  (1  $\rightarrow$  4) glycosidic linkage.



## PHYSIOLOGICALLY IMPORTANT DISACCHARIDES

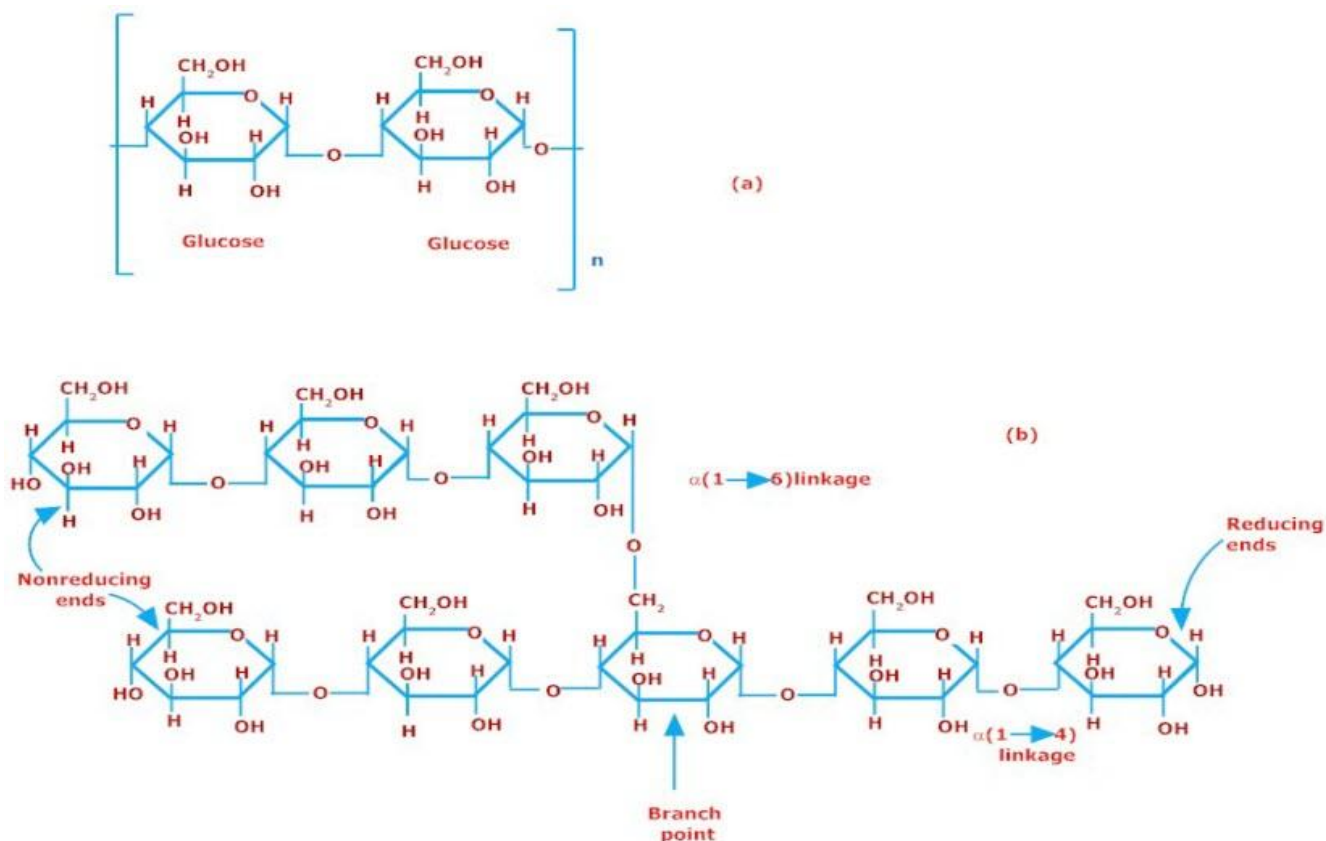
Sugars	Occurrence	PHYSIOLOGICAL IMPORTANCE
Maltose	Digestion of starch by amylase Germinating cereals and malt	On hydrolysis produces glucose for energy
Lactose	Milk	In lactase deficiency, malabsorption leads to diarrhoea and flatulence
Sucrose	Cane and beet sugar, sorghum, pine apple, carrot roots	In sucrase deficiency, malabsorption leads to diarrhoea and flatulence

## POLYSACCHARIDES

- Most of the carbohydrates found in nature occur as polysaccharides. They are also referred to as “glycans”. They contain ten to thousand monosaccharide units.
- Polysaccharides are composed of simple or derived sugars, joined by glycosidic linkages. They exist as linear chain or as branched structures.
- Homo polysaccharides contain only a single type of sugar unit. E.g. Cellulose, starch and glycogen.
- Hetero polysaccharides contain two or more kinds of sugar units. E.g. Hyaluronic acid, heparin and chondroitin sulphate.
- Some homopolysaccharides serve as storage function. They are used as fuels - starch in plant and glycogen in animal.
- Other homopolysaccharides such as cellulose and chitin serve as structural elements in plant cell walls and animal exoskeleton respectively.
- Hetero polysaccharides provide extracellular support for organisms of all kingdoms.

### STARCH

- Starch, the storage form of carbohydrate in plants contains 2 types of glucose polymers - amylose and amylopectin.
- Amylose consists of long, unbranched chains of glucose units linked by  $\alpha$  (1  $\rightarrow$  4) linkages (15 -20%), soluble in water.
- Amylopectin (85 – 80%) is also a higher molecular weight sugar, but is highly branched. It is composed of  $\alpha$  (1  $\rightarrow$  4) linked chains of glucose, that are joined together at branch points via  $\alpha$  (1  $\rightarrow$  6) linkages. Amylopectin is similar to glycogen except that, it has fewer branches. It is insoluble in water.



## DEXTRINS AND DEXTRANS

### Dextrins

- These are substances (smaller fragments) formed during the course of hydrolytic cleavage of starch either by enzymes or by HCl.
- The polysaccharides, starch and glycogen are polymers of glucose. These polysaccharides are hydrolyzed by amylases. There are two types of amylases called  $\alpha$  - amylase and  $\beta$ -amylase. They are present in the secretions of saliva and pancreas.
- $\alpha$  - amylase, which is present in both animals and plants is an endoamylase that hydrolyzes randomly the interior  $\alpha$  (1  $\rightarrow$  4) glycosidic bonds of amylose and amylopectin to yield a mixture of glucose, maltose and dextrins.
- Both amylases cannot hydrolyze the  $\alpha$  (1  $\rightarrow$  6) linkage. After the hydrolysis by amylase, highly branched dextrins are produced.
- On treatment with HCl, starch is converted to glucose. The following is the order of the production. Starch, which gives blue colour with iodine (negative for Benedict's) is hydrolyzed to amyloextrin (violet colour with iodine), erythroextrin (red colour with iodine) and achroextrin (no colour with iodine), which gives Benedict's positive.
- Another enzyme  $\beta$  - amylase is an exoamylase that catalyses the sequential hydrolysis from the non-reducing end of amylose or amylopectin to form maltose.



## Dextran

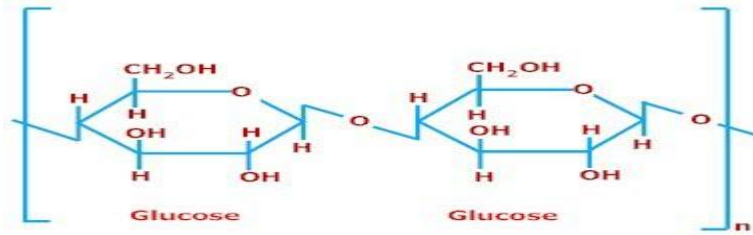
- A storage polysaccharide in yeast and bacteria also contains glucose residues but differ from glycogen and starch by having  $\alpha(1 \rightarrow 6)$  linkage and occasionally  $\alpha(1 \rightarrow 2)$ ,  $\alpha(1 \rightarrow 3)$  and  $\alpha(1 \rightarrow 4)$  linkages, which depends on species. It is used as plasma extenders. It is insoluble in water. In the case of blood loss It is given intravenously to increase the blood volume. Synthetic dextrans are used in the chromatography for the fractionation of proteins.

## GLYCOGEN

- It is the storage form of carbohydrate in animals. It is large, branched polysaccharides composed of glucose residues.
- Like amylopectin, glycogen consists of  $\alpha(1 \rightarrow 4)$  linkage in addition to  $\alpha(1 \rightarrow 6)$  branches. Glycogen is highly branched, and more compact than starch. Molecular weight varies, which depends on sources. For every 8-12 glucose units there is a branch point.
- It is readily soluble in water and gives red colour with iodine.
- It is present in liver and also in muscle tissues of animals. During starvation, glycogen from the liver is broken down to glucose and is transported to blood for the use by other tissues.

## CELLULOSE

- The other major polysaccharides of plants, which serve as a structural rather than a nutritional role.
- It is a linear, unbranched homopolysaccharides of D- glucose.
- It resembles the structure of amylose, but there is a very important difference, the glucose residues are in the  $\beta$  - configuration, whereas in amylose, amylopectin and glycogen, glucose is in a  $\alpha$  - configuration.
- The glucose residues in cellulose are linked by  $\beta(1 \rightarrow 4)$  linkages, which are not hydrolyzed by the amylases found in the digestive tract of humans or most other higher animals. Consequently, man and most animals cannot utilize the energy present in this glucose polymer.
- In ruminants and other herbivores, the bacteria that reside in the rumen secrete cellulase, - glucosidase, that catalyzes the hydrolysis of cellulose to glucose.
- The bacteria and others resident in the rumen then metabolize the glucose to volatile fatty acids that are beneficial to the host animals.

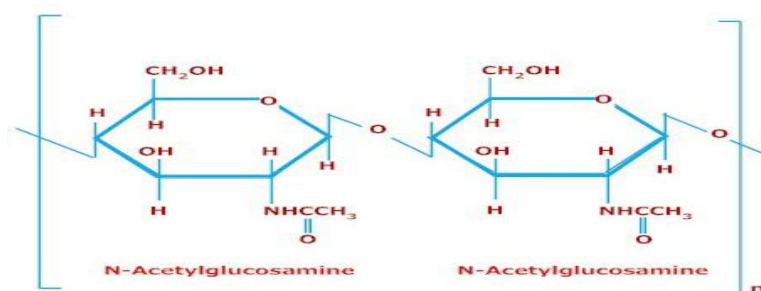


## INULIN

- It is a kind of starch found in tubers.
- It is a homoglycan composed of D- fructose units with repeating  $\beta(1 \rightarrow 2)$  linkages.
- It is clinically used to find renal clearance value and glomerular filtration rate.

## CHITIN

- It is an important structural polysaccharide of invertebrates. It is the principal component of hard exoskeleton of nearly a million species viz., insects, lobsters and crabs.
- It is the second most abundant polysaccharide, next to cellulose.
- It is a homopolymer of N-Acetyl – D – glucosamine linked by  $\beta(1 \rightarrow 4)$  linkage.
- The only chemical difference from cellulose is replacement of a hydroxyl group at C- 2 with an acetylated amino group.



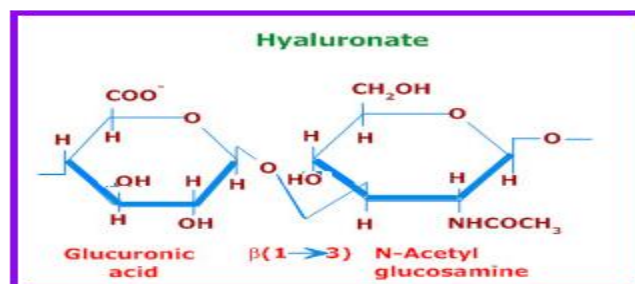
# HETEROPOLYSACCHARIDES

## HETEROPOLYSACCHARIDES / MUCOPOLYSACCHARIDES

- They are also known as glycosaminoglycans. They are complex carbohydrates containing amino sugars and uronic acids.
- When glycosaminoglycan chains are attached to a protein molecule, the compound is known as “proteoglycans”.
- They are components of extracellular matrix (also known as “ground substances”), which holds the cells of a tissue together and provides a porous pathway for the diffusion of the nutrients and oxygen to the individual cells.
- They are linear polymers, composed of repeating disaccharide units. One of the 2 monosaccharides is N – Acetylglucosamine or N – Acetylgalactosamine and the other is mostly an uronic acid usually glucuronic acid.
- Some of the examples are hyaluronic acid, chondroitin sulfate and heparin.

### Hyaluronic acid

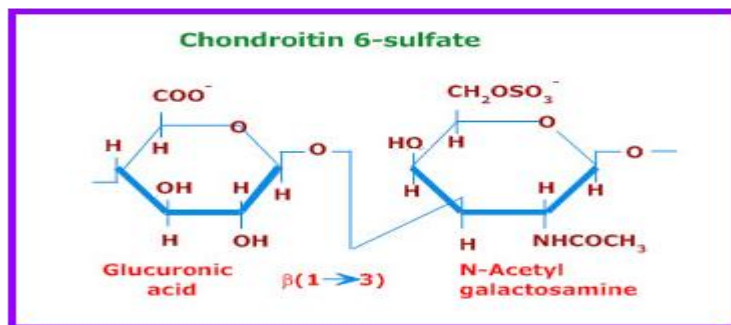
- The repeating unit of hyaluronic acid is a disaccharide containing D – Glucuronic acid and N-Acetyl glucosamine held by  $\beta$  - 1,3 linkage and repeating disaccharide by  $\beta$ -1,4-linkage.
- They form clear, highly viscous solutions, which serve as lubricants in the synovial fluids of joints and provide a cushioning effect.



### Chondroitin sulfate

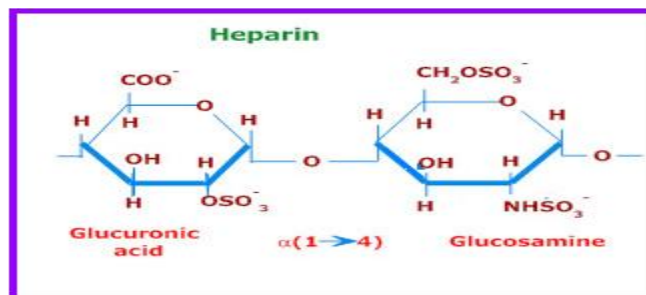
- Chondroitin – 4- sulfate is a major polysaccharide of cartilage, which contains alternating units of D-Glucuronic acid and N-Acetyl –D-galactosamine.
- The linkage is similar to hyaluronic acid and the N-acetyl galactosamine is sulphated at 4th position.

- The presence of the negatively charged groups at C-6 of D-glucuronic acid (carboxyl group) and the sulfate ester group at C-4 of N-Acetylgalactosamine, has two important consequences:
- The groups repel each other and cause the polysaccharides molecule to expand;
- They as well as the sugar hydroxyl groups are extensively hydrated. The net effect is that chondroitin-4-sulfate swells when placed in water and creates a gelatinous matrix that makes it as a good lubricant.



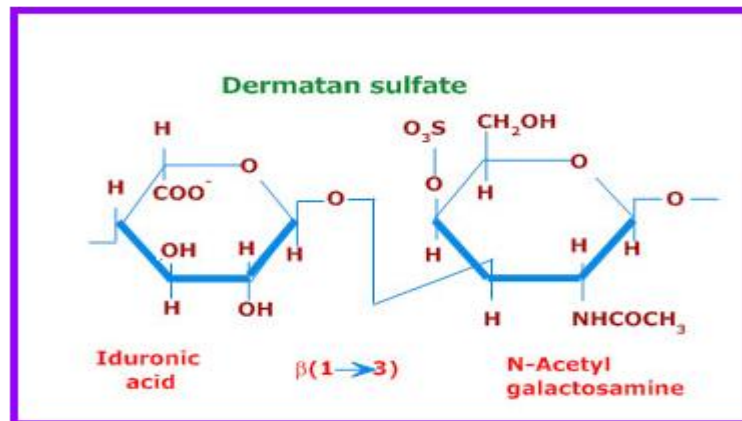
## Heparin

- It is made up of D-glucuronate sulphate / L-iduronate sulphate and N-sulphoglucosamine-6-sulfate linked by  $\alpha(1 \rightarrow 4)$  glycosidic bonds.
- 90% of uronic acids are iduronic acids.
- It is present in liver, lungs, spleen, monocytes etc. Commercial preparation of heparin is mainly from animal lung tissues.
- It is an anti-coagulant widely used when taking blood in vitro for clinical studies.
- It is also used in vivo to prevent intra vascular coagulation.



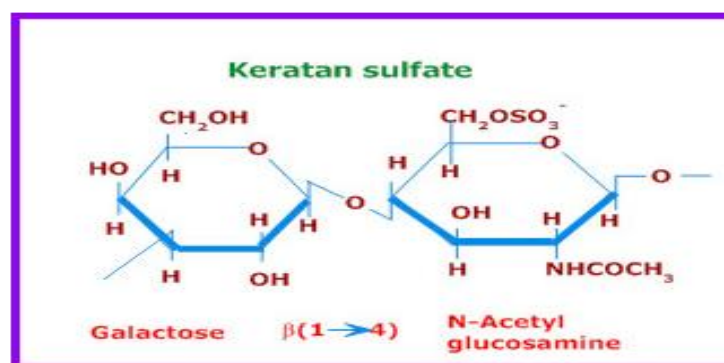
## Dermatan sulfate

- It is composed of alternating units of L- iduronic acid and N- acetyl galactosamine –4 – sulphate linked by  $\beta$  - 1,3 linkage and repeating disaccharide by  $\beta$  - 1,4 linkage .
- It is present in skin, blood vessels and heart valves.



## Keratan sulphate

- It is a heterogeneous GAG with variable sulphate content.
- It is made up of alternating units of D-galactosamine and N-acetyl glucosamine linked by  $\beta$  1,4 linkage.



## FUNCTIONS OF GLYCOSAMINOGLYCANS

Name of the sugar	Functions
Hyaluronic acid	It is not sulfated. Present in synovial fluid, vitreous humor and in the loose connective tissue. Serves as a lubricant and provides the cushioning effect.
Chondroitin 4 - and 6 - sulfates	Formation of bone, cartilage and cornea.
Heparin	It is present in mast cells. Serves as an anticoagulant. Causes the release of the lipoprotein lipase from the capillary walls.
Heparan sulfate	Component of skin fibroblast and aortic walls. Commonly found on all cell surfaces.
Keratan sulfate	Transparency of cornea. Found in loose connective tissues.
Dermatan sulfate	Found in skin, blood vessels and heart valves. Binds LDL to plasma walls.

## GLYCOPROTEINS

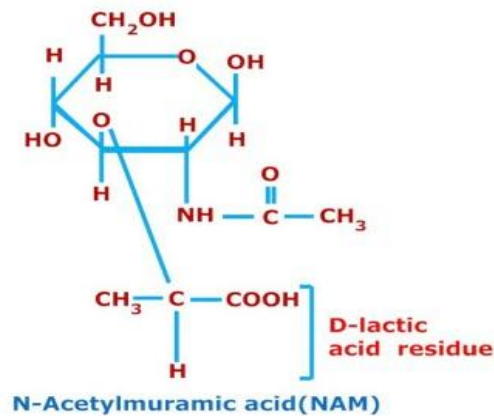
- They are proteins containing carbohydrates. The carbohydrate chain of a glycoprotein varies in length from 1 to 15 residues and is also called as oligosaccharide.
- The chain may be branched or unbranched. In mature glycoprotein glucose is absent. Uronic acids are absent.
- The carbohydrates present in glycoproteins are

Hexoses	Mannose and galactose
Acetylhexosamine	N-Acetylglucosamine and N-Acetylgalactosamine
Pentoses	Arabinose and Xylose
Methyl pentose	L-fucose
Sialic acid	N-Acetylneuraminic acid

- Functions of glycoproteins are
  - Structural molecule e.g., collagen
  - Transport proteins. E.g., Transferrin, ceruloplasmin
  - Enzymes. E.g. alkaline phosphatase.
  - Hormones. E.g., Thyroid stimulating hormone
  - Immunologic molecule. e.g., antibodies.
  - Antifreeze Proteins. e.g., Certain plasma proteins of cold water fish.
  - Cell attachment - recognition site.

## BACTERIAL CELL WALL POLYSACCHARIDES

- The cell walls of many bacteria are made of peptidoglycans, which are heteroglycan chains linked to peptides (murein is another name for peptidoglycan).
- The heteroglycan component is composed of alternating residues of N-acetylglucosamine and N-acetylmuramic acid joined by  $\beta(1\rightarrow4)$  linkage. N-acetylmuramic acid consists of N-acetylglucosamine in an ether link with D-lactic acid.
- Many such linear polymers lie side by side in the cell wall, cross-linked by short peptides.
- The antibacterial action of lysozyme (present in tears) is due to its ability to catalyze the hydrolysis of polysaccharide chain of peptidoglycan.



# LIPIDS

## LIPIDS

- Lipid is a general term for water- insoluble biological molecules.
- They are structurally and functionally diverse group of molecules.
- Lipids are organic substances soluble in organic solvents such as chloroform, ether, benzene, ethanol and other non-polar solvents.
- The lipids include fatty acids, fats, oils, waxes and related compounds such as phospholipids, eicosanoids, terpenes and steroids.

## Functions of lipids

- They are stored forms of energy in all living organisms. Fat molecules have maximum number of hydrogen atoms so, they have maximum of electrons, which produce more energy as ATP.
- They are structural components of the cell.
- The fatty acid, arachidonic acid is the precursor for all prostaglandins, leukotrienes and thromboxanes.
- The lipids are needed in the diet to solubilize the fat-soluble vitamins like A, D, E and K.
- The corticosteroids, sex hormones and Vitamin D are synthesized from cholesterol.
- Phosphatidylinositol triphosphate serves as the key precursor in the formation of second messenger.
- Lipids act as an insulating material, which prevents the dissipation of heat from the body.
- Lipids provide cushioning effect to the body which prevents the body against mechanical injuries.
- Because lipids are insoluble, they generate no osmotic pressure when stored in the cells.

## FATTY ACIDS

### What are fatty acids?

- Fatty acids are straight aliphatic chains with a methyl group at one end and a carboxyl group at the other end.
- They can be represented by the formula  $R-COOH$  where R is the alkyl group –  $CH_3(CH_2)_n$  (hydrocarbon chain). The hydrocarbon chain is hydrophobic and the carboxylate group is hydrophilic. Fatty acids occur primarily as esters of glycerol.
- Fatty acids are divided into
  - Short- (2 to 4 carbon atoms)
  - Medium - (6 to 10 carbon atoms)
  - Long - (12 to 26 or more carbon atoms).



- Most fatty acids present in human cells have an even number of carbon atoms, usually between 16 and 20.
- Fatty acid may contain no double bond (saturated) or contains one or more double bonds (unsaturated). Monounsaturated fatty acids contain one double bond and polyunsaturated fatty acids contain two or more double bonds. The double bonds of fatty acids are separated by three carbon interval.
- Cyclic fatty acids are also found - For e.g., chaulmoogric acid from chaulmoogra oil, which is used for the treatment of leprosy in humans and hydnocarpic acid.



### Functions of fatty acids

- They are the constituents of phospholipids and glycolipids (component of biological membranes).
- Fatty acids are fuel molecules. They are stored as triacylglycerols, which are uncharged esters of glycerol. They provide physical protection for the organ of the body.
- Fatty acid derivatives such as prostaglandins, leukotrienes and thromboxanes serve as local hormones and as an intracellular messenger in cell signalling mechanism.

### NOMENCLATURE OF FATTY ACID

- In the naming of fatty acids, saturated acids end in -anoic acid e.g. octanoic acid and the unsaturated fatty acids with double bonds end in -enoic acids, e.g. octadecenoic acid (oleic acid).
- Carbon atoms are numbered from the carboxyl carbon (carbon No.1). The carbon atom adjacent to the carboxyl carbon (No. 2) is also known as the  $\alpha$  - carbon. Carbon atom No.3 is the  $\beta$  - carbon and the distal methyl carbon is known as the  $\omega$  - carbon or n- carbon atom.
- The fatty acids are generally designated as follows ( $\Delta$ , delta system of numbering). For example, oleic acid is written as 18: 1, $\Delta$ 9. The number 18 indicates the number of carbon atoms, 1 indicates the number of double bond and the superscript 9 indicates the position of the double bonds i.e. the double bond is between carbon atoms 9 and 10 of the fatty acid. Some times the  $\Delta$  is omitted and oleic acid is indicated as (18: 1;9. )
- Alternatively, the position of a double bond can be denoted by counting from the distal end, with the carbon atom, (the methyl carbon) as number ( $\omega$ -system). For example  $\omega$  -3 fatty acid, linolenic acid (has a double bond between  $\omega$ -3 and  $\omega$ -4 C atom),  $\omega$ -6 fatty acid, linoleic and arachidonic (has a double bond between  $\omega$ -6 and  $\omega$ -7 C atom) and the  $\omega$  9 fatty acid, oleic acid (has a double bond between the C atoms  $\omega$ -9 and  $\omega$ -10 of the fatty acid).

- The pKa of fatty acid carboxyl group is about 4.8. Hence, fatty acids are ionized at physiologic pH and so it is appropriate to refer to them according to their carboxylate forms i.e. palmitate or hexadecanoate.
- The properties of fatty acids and the compounds that contain them are dependent on the chain length and the degree of unsaturation.
- Non- polar hydrocarbon chain of fatty acids accounts for the poor solubility of fatty acid in water. The carboxyl group is polar (ionized at neutral pH) and accounts for the slight solubility of fatty acids in water.
- Unsaturated fatty acids have lower melting points than saturated fatty acids of the same length. For eg. the melting point of stearic acid is 69.6o C, whereas that of oleic acid is 13.4o C. The melting point of polyunsaturated fatty acids of the C18 series is even lower. The melting point of arachidonic acid is - 49.5o C.
- The chain length also affects the melting point. The melting temperature of palmitic acid (C16) is 62.8o C. The melting point of stearic acid (C18) is 69.68o C.
- Fatty acids with short chain length and unsaturation enhance the fluidity of membranes.

#### SOME NATURALLY OCCURRING FATTY ACIDS

Number of carbons	Number of double bonds	Common name	Systematic name	Formula
12	0	Laurate	n-Dodecanoate	$\text{CH}_3(\text{CH}_2)_{10}\text{COO}^-$
14	0	Myristate	n-tetradecanoate	$\text{CH}_3(\text{CH}_2)_{12}\text{COO}^-$
16	0	Palmitate	n-hexadecanoate	$\text{CH}_3(\text{CH}_2)_{14}\text{COO}^-$
18	0	Stearate	n-Octadecanoate	$\text{CH}_3(\text{CH}_2)_{16}\text{COO}^-$
20	0	Arachidate	n-Eicosanoate	$\text{CH}_3(\text{CH}_2)_{18}\text{COO}^-$
22	0	Behenate	n-Docosanoate	$\text{CH}_3(\text{CH}_2)_{20}\text{COO}^-$
24	0	Lignocerate	n-Tetracosanoate	$\text{CH}_3(\text{CH}_2)_{22}\text{COO}^-$
16	1	Palmitoleate	Cis- $\Delta^9$ -Hexadecenoate	$\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7\text{COO}^-$
18	1	Oleate	Cis- $\Delta^9$ -Octadecenoate	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COO}^-$
18	2	Linoleate	Cis,Cis- $\Delta^9, \Delta^{12}$ -Octadecadienoate	$\text{CH}_3(\text{CH}_2)_4(\text{CH}=\text{CHCH}_2)_2(\text{CH}_2)_6\text{COO}^-$
18	3	Linolenate	all-Cis- $\Delta^9, \Delta^{12}, \Delta^{15}$ -Octadecatrienoate	$\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_3(\text{CH}_2)_6\text{COO}^-$
20	4	Arachidonate	all-Cis- $\Delta^5, \Delta^8, \Delta^{11}, \Delta^{14}$ -Eicosatetraenoate	$\text{CH}_3(\text{CH}_2)_4(\text{CH}=\text{CHCH}_2)_4(\text{CH}_2)_2\text{COO}^-$

## COMMON SATURATED AND UNSATURATED FATTY ACID

### Saturated fatty acid

Common name	Systematic name	No. of carbon	Occurrence
Formic	Methanoic	1	Involved in 1 'C' metabolism
Acetic	Ethanoic	2	Major end product of carbohydrate fermentation by rumen microbes
Propionic	Propanoic	3	End product of carbohydrate fermentation by rumen microbes
Butyric	Butanoic	4	Present in small quantities in butter. End products of carbohydrate fermentation by rumen microbes
Valeric	Pentanoic	5	
Caproic	Hexanoic	6	
Caprylic	Octanoic	8	Present in small amounts in butter and also in plant products
Capric	Decanoic	10	
Lauric	dodecanoic	12	Palm kernel and coconut oils
Myristic	Tetradecanoic	14	Nutmeg, coconut oils and butter
Palmitic	Hexadecanoic	16	Common in all animal and plant fats
Stearic	Octadecanoic	18	
Arachidic	Cicosanoic	20	Peanut oil
Behenic	Docosanoic	22	Seeds
lignoceric	Tetracosanoic	24	Cerebrosides and peanut oil.

### Unsaturated Fatty acids

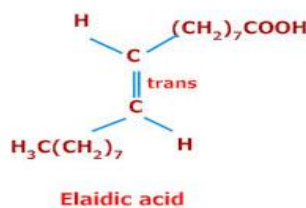
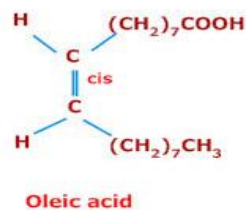
Common Name	Systematic Name	No of 'C' atom and position of double bond	Occurence
Palmitoleic	Hexadecenoic	16:1;9	In nearly all fats
Oleic	Octadecenoic	18:1;9	The most common fatty acid present in natural fats
Linoleic	Octadecadienoic	18:2;9,12	Present in many plant oils including corn, peanut, cottonseed and soybean.

$\gamma$ - Linolenic	Octadecatrienoic	18:3;9,12,15	Present in some plants
$\alpha$ - Linolenic	Octadecatrienoic	18:3;9,12,15	Linseed oil
Arachidonic	Eicosatetraenoic	20:4;5,8,11,14	Found in animal fats and in peanut oil. It is an important component of phospholipids in animals.

- CLA- Conjugated linoleic acid (18:2;9,11) Found in sea fish and milk.
- EPA - Eicosapentenoic acid (20:5; 5,8,11,14,17) found in sea fish.
- DHA-Docosahexaenoic acid (22:6; 4,7,10,13,16,19) found in sea fish.

### ISOMERISM IN UNSATURATED FATTY ACIDS

- Due to the presence of double bond, fatty acids exhibit geometrical isomerism, which depends on the orientation of groups around the double bond. The designation “cis” means that the acyl chains are on the same side. “Trans” means the acyl chains are on the opposite side of the double bond.
- The double bonds in most naturally occurring fatty acids are in the cis configuration. Cis and Trans isomers have different melting points and other physical constants. Trans fatty acids are stable but are injurious to health. Eg: The trans form of oleic acid (cis) is called elaidic acid.
- Trans fatty acids are formed when the vegetable oils are hydrogenated. For example in the manufacturing of margarine.
- Ruminant fat contains more trans long chain fatty acids than non-ruminants because rumen microbes isomerizes some plant cis long chain fatty acids to trans isomer.
- Trans fatty acids compete with essential fatty acids so there is reduction in the absorption of essential fatty acids, which may increase the symptoms of essential fatty acid deficiency.
- They have structures similar to saturated fatty acids. Hence, they increase cholesterol level and the formation of atherosclerosis.



## ESSENTIAL FATTY ACIDS

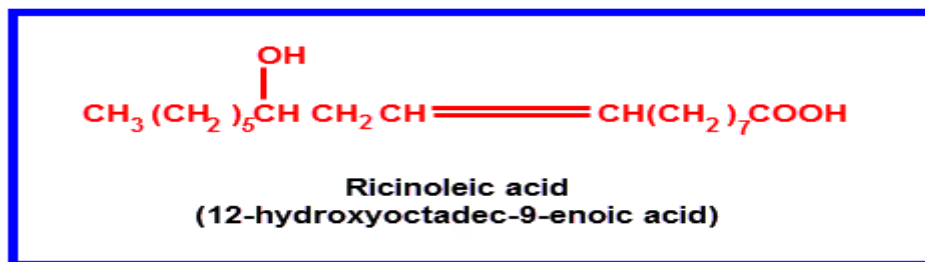
- Those polyunsaturated fatty acids that cannot be synthesized that must be supplied through the diet are called essential fatty acids.
- Essential fatty acids for many species including man are
  - Linoleic ( $\omega 6$ ) acid
  - linolenic( $\omega 3$ ) acid
  - Arachidonic acid is also an essential fatty acid and it can be formed from linoleic acid in most mammals but not in the cat family.
- Deficiency causes
  - Scaly dermatitis
  - Decreased availability of precursors for eicosanoid synthesis. (Eicosa is the Greek word for the number 20)
  - Affects fluidity of membrane structure.
  - The ordinary dietary oils are rich in linoleic acid ( $\omega 6$ ). The  $\omega$ -3 type of polyunsaturated fatty acids is produced by vegetation that grows in cold water. Fish that feeds on these organisms contain large amounts of  $\omega$ -3 fatty acids. Hence, fish oils are richer in  $\omega$ -3 type of fatty acids.

## DEFICIENCY DISORDERS OF FATTY ACIDS

- Different types of polyunsaturated fatty acids have different effects on lipid metabolism and on other risk factors for heart diseases.
- Recent clinical trials have shown that  $\omega$ -6 type decreases serum cholesterol level with a modest reduction in the level of TG.
- $\omega$ -3 types cause modest decrease in serum cholesterol and significantly lower TG levels.
- The biochemical mechanisms for these effects are not known.
- $\omega$ -3 types of fatty acids decrease the risk of heart disease by decreasing platelet aggregation.

## HYDROXY FATTY ACID

- The central nervous system contains hydroxy fatty acid. e.g., cerebronic acid, the hydroxyl group is attached on the  $\alpha$ - carbon atom. The other hydroxy fatty acid that occurs in nature is ricinoleic acid, the main fatty acid in castor oil.
- The hydroxyl group is attached to the C12. Hydroxy fatty acids are also formed in several tissues from arachidonic acid.
- Fatty acids that are normally present in mammalian tissues are generally straight chain type. Sebaceous gland produces branched chain fatty acids.



## LIPIDS

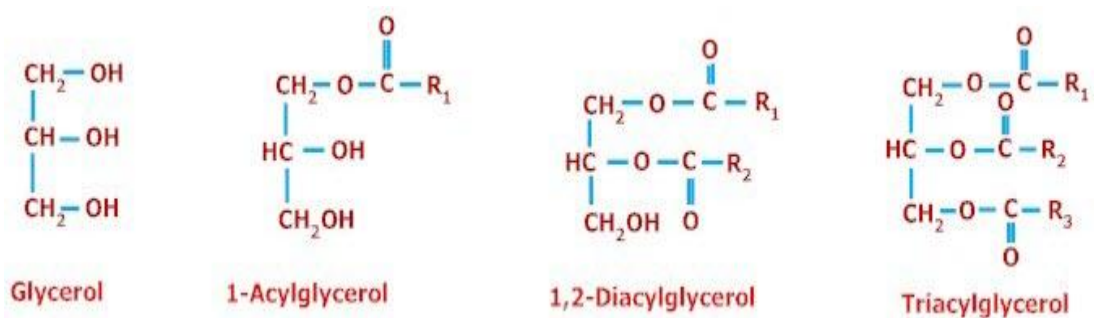
- Lipids are classified as
  - *Simple lipids*: These are esters of fatty acids with alcohols. Eg: triacylglycerols or neutral fats and waxes.
  - *Compound lipids*: These are esters of fatty acids with alcohol and containing some other groups like phosphoric acid, amino acid and carbohydrates. Eg: 1. Phospholipids 2. Sphingo lipids and 3. Glycolipids and 4. Other complex lipids.
  - *Derived lipids*: These are compounds obtained during the hydrolysis of simple and compound lipids. They are fatty acids, glycerol, steroids, alcohols fatty aldehydes and ketone bodies, lipid – soluble vitamins and steroid hormones.

### SIMPLE LIPIDS

#### TRIACYLGLYCEROLS

- Glycerol is a compound with three-carbon atom each of which contains an alcohol side chain. In the three dimensional configuration of glycerol, carbon 1 and 3 are not identical. For numbering the carbon atom of glycerol stereochemical numbering system is used (-sn). Enzymes in the system are more specific for C-1 or for C-3. Glycerol is generally phosphorylated on –sn –3 by the enzyme glycerol kinase to produce glycerol 3-phosphate and not glycerol 1-phosphate.
- Triacylglycerols are also referred to as triglycerides, fats or neutral fats. Fatty acid moiety in lipid ester is known as acyl group.
- Triglycerides are compounds that contain glycerol, to which 3 fatty acids are attached. The hydroxyl group of glycerol can react with fatty acids to form monoacylglycerols, diacylglycerols and triacylglycerols.
- In simple triacylglycerol, glycerol is attached to same kind of fatty acids in all the 3 positions. Eg: tristearin (made up of stearic acid) and tripalmitin (made up of palmitic acid).
- Mixed triacylglycerols contain 2 or more different types of fatty acids attached to the glycerol.

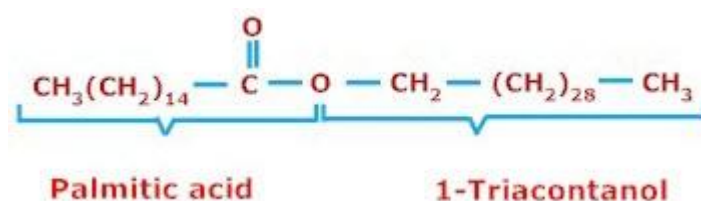
- Triacylglycerols are non polar and insoluble in water. They exist in solid (fats) or liquid form (oils) depending on the nature of the constituent fatty acids (saturated or unsaturated).
- Most plant triacylglycerols have low melting point and are liquid at room temperature, because they contain large proportion of unsaturated fatty acids such as oleic, linoleic and linolenic acids.
- The unsaturated fatty acids may be converted to saturated fatty acids by a process known as hydrogenation.
- In contrast, animal triacylglycerols contain higher proportion of saturated fatty acid such as palmitic and stearic acids resulting in higher melting points and are semisolid or solid at room temperature.
- Most naturally occurring fats such as butter and other food fats are made up of complex mixtures of simple and mixed triacylglycerols containing a variety of fatty acids differing in chain length and degree of saturation.
- Triacylglycerols undergo hydrolysis, when boiled with acids or bases or when acted upon by enzyme, lipase. Hydrolysis of triacylglycerols by KOH or NaOH is called as saponification (meaning soap formation) which yields a mixture of  $K^+$  or  $Na^+$  soaps and glycerol.
- Triacylglycerols with largely unsaturated fatty acids, which are thus liquid at room temperature, can be converted chemically into solid fats by hydrogenation of their double bonds.
- When exposed to air, TG containing highly unsaturated fatty acids tend to undergo a complex process called auto oxidation. Rancid fats contain appreciable amount of peroxidized fat.
- Large amount of TG can be stored in adipose tissue without the weight gain due to water as these molecules are hydrophobic in nature.



## WAXES

- These are esters of long chain fatty acids with higher molecular weight monohydric alcohols. Fatty acids may be saturated or unsaturated, having 14 to 36 carbon atoms. The long-chain alcohols will have usually 16 to 30 carbon atoms.
- Waxes are the chief storage form of metabolic fuel.

- Waxes serve as protective coat on fruits and leaves, which protects against parasites and prevents excessive evaporation of water.
- Waxes are insoluble in water and provide a water barrier for animals such as sheep. Birds such as waterfowl, secrete waxes from their preen glands to make their feathers water repellent.
- Biological waxes are used in the pharmaceutical industry in the manufacturing of lotions, ointments and polishes. The waxes used are lanolin (wool wax), bees wax, carnauba wax (palm tree wax) and spermaceti oil (from whales).



## FAT INDICES

The following are the fat indices

### Acid number

- It is the number of milligrams of KOH required to neutralize the free fatty acids present in 1 g of fat.
- The acid number thus tells us of the quantity of free fatty acid present in fat. Fat, which has been processed and stored properly, has a very low acid number.

### Saponification number

- It is the number of milligrams of KOH required to saponify 1 g of fat or oils.
- The saponification number thus provides information on the average chain length of the fatty acids in the fat. It varies inversely with the chain length of the fatty acids. The shorter the average chain length of the fatty acids, the higher is the saponification number.

### Iodine number or value

- It is the number of grams of iodine absorbed by 100 g of fat.
- The iodine number is a measure of the degree of unsaturation of the fatty acids in the fat.
- Oils like soybean, cotton seed oil have higher iodine number than the solid fats such as beef fat because, the former contain more unsaturated fatty acids in the fat molecule.



- However, iodine value gives no indication of the number of double bonds present in the fatty acid molecule.

#### **Acetyl number**

- It is the number of milligrams of KOH required to neutralize the acetic acid obtained by saponification of 1 g fat after it has been acetylated.
- This is a measure of the number of hydroxy- acid groups in the fat.
- Castor oil, because of its high content of ricinoleic acid, a fatty acid containing one OH group, has a high acetyl number (about 146).

#### **Polenske number**

- It is the number of millilitres of 0.1 N KOH required to neutralize the insoluble fatty acids (those not volatile with steam distillation) from 5g of fat.
- It is a measure of non-volatile (long chain) fatty acids present in a fat.

#### **Reichert – Meissl number**

- It is the number of millilitres of 0.1 N KOH required to neutralize the soluble volatile fatty acids derived from 5 g of fat.
- R.M. number thus measures the quantity of short chain fatty acids in the fat molecule.

### **RANCIDITY**

- Rancidity is a chemical change that results in an unpleasant odors and taste in a fat.
- The oxygen of the air attacks the double bond of fatty acids to form the peroxide linkage. Peroxidation of lipids exposed to oxygen is responsible not only for deterioration of foods (rancidity), but also for damage to tissues in-vivo, where it may be a cause of cancer, inflammatory diseases, atherosclerosis, ageing, etc.
- The iodine number of the fatty acid is thus reduced ( due to the reduction of double bonds) following the release of free fatty acid and glycerol.
- Lead or copper catalyzes rancidity. Exclusion of oxygen or the addition of antioxidant like Vit-C and E delays the process.
- Rancidity can be classified as
  - Oxidative rancidity
  - Hydrolytic rancidity

## Oxidative rancidity

- Oils that contain highly unsaturated fatty acids are spontaneously oxidized by atmospheric oxygen at ordinary temperatures.
- The oxidation takes place slowly resulting in the formation of short chain fatty acids and aldehydes, which gives rancid taste and odour to the fats.

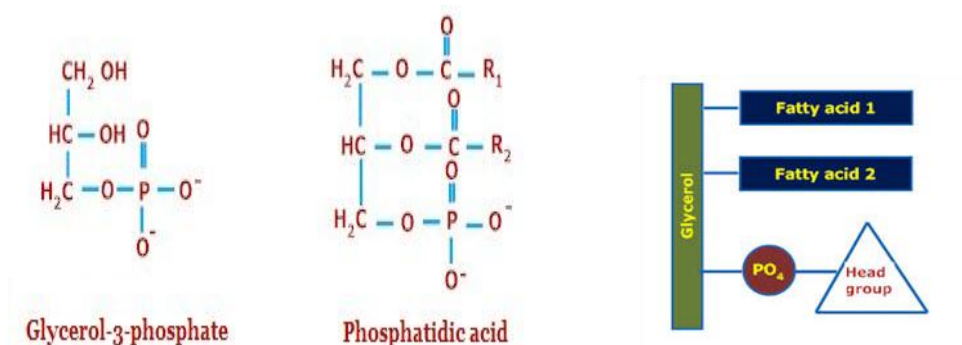
## Hydrolytic rancidity

- It involves partial hydrolysis of glycerides by lipase, which results in the release of free fatty acids. The process is hastened by the presence of moisture and increased temperature.
- In fats like butter, which contain a high percentage of volatile fatty acids, hydrolytic rancidity produces a disagreeable odour and taste due to the liberation of volatile fatty acids like butyric acid

# COMPOUND LIPIDS

## PHOSPHOLIPIDS

- They are so named because; they contain phosphorus in the form of phosphoric acid. In addition, they also contain glycerol, fatty acids and a nitrogenous base.
- Phospholipids contain fatty acids esterified to position 1 and 2 of glycerol and a phosphoryl group at position 3. If only a phosphate group is attached to position 3, then the compound is known as “Phosphatidic acid” which has no head alcohol. It is an intermediate in the synthesis of triacylglycerols and phospholipids.



- Phospholipids differ from triacylglycerol in possessing one or more highly polar head groups in addition to their non-polar hydrocarbon tail. For this reasons they are often called “polar lipids”. Due to the attachment of polar and non-polar groups in the same structure they are also known as amphipathic lipids.

- Fatty acids found in phosphoglycerides (or glycerophospholipids) have 16 or 18 C atoms.
- Usually one of the fatty acids is saturated and the other is unsaturated.
- The unsaturated fatty acid is always attached to the 2<sup>nd</sup> hydroxyl group of the glycerol.
- Different types of phosphoglycerides are named according to the alcohol attached on their polar head group of phosphatidic acid; all of these phosphoglycerides except sphingomyelins may be regarded as derivative of phosphatidic acid.
- There are two classes of phospholipids, viz. those that have glycerol as the back bone and those that contain sphingosine as back bone.
- The phospholipids containing glycerol as the back bone:
  - Phosphatidic acid and phosphatidyl glycerol
  - Phosphatidylcholine
  - Phosphatidylethanolamine
  - Phosphatidylinositol
  - Phosphatidylserine
  - Lysophospholipids
  - Plasmalogens
  - Cardiolipins.
- The phospholipids containing sphingosine as the back bone
  - Sphingomyelins.

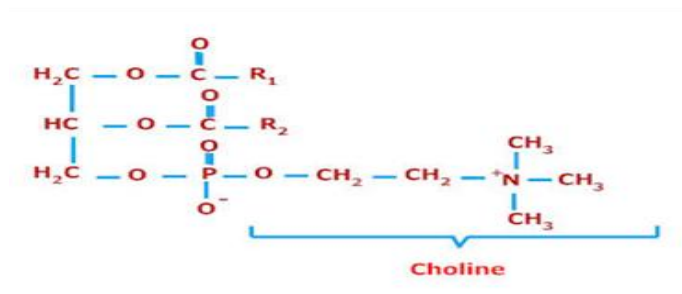
### **SIGNIFICANCE OF PHOSPHOLIPIDS**

- They are important components of cell membranes.
- Phosphatidylcholine is the major lipid component of lung surfactant- the extracellular fluid layer lining the alveoli. Surfactant serves to decrease the surface tension of this fluid layer, thereby preventing alveolar collapse.
- Respiratory distress syndrome: It is also called as hyaline membrane disease. It is due to the lack of surfactant production in the lungs. The major component of lung surfactant is dipalmitoylphosphatidylcholine. When the concentration of surfactant decreases, surface tension on the lung alveoli increases a portion of the lung collapse, which reduces the exchange of oxygen and carbon dioxide.
- Sphingomyelin is an important constituent of the myelin of nerve fibers. Being a component of lipoprotein, they are involved in the transport of triacylglycerols, from liver and intestine to various tissues.
- Choline acts as a lipotropic factor, prevents the accumulation of fats in liver.
- The detergent properties of phospholipids, especially phosphatidylcholine, play an important role in bile where they function to solubilize cholesterol.

- Phospholipids are involved in the process of blood coagulation.
- Arachidonic acid of phospholipids is the precursor for eicosanoids like prostaglandins, leukotrienes and thromboxanes.
- Hydrolysis of phosphatidylinositol produces a second messenger for the activity of hormones (diacylglycerol and inositol 1,4,5 triphosphate).
- Cardiolipin is a major lipid of inner mitochondrial membranes especially in cardiac tissues.

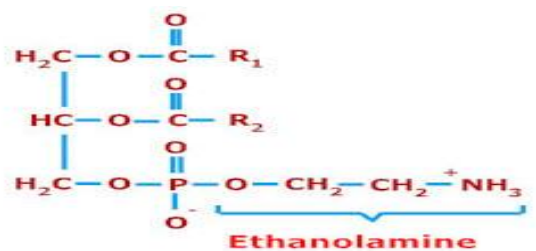
### PHOSPHATIDYLCHOLINE (Lecithin)

- It is a phosphoglyceride containing choline.
- Most of the phospholipids have a saturated acyl radical in the C1 position but an unsaturated radical in the C2 position of glycerol.
- They are soluble in ordinary fat solvents except acetone.
- It is an important lipotropic agent (prevents accumulation of lipids in tissues)



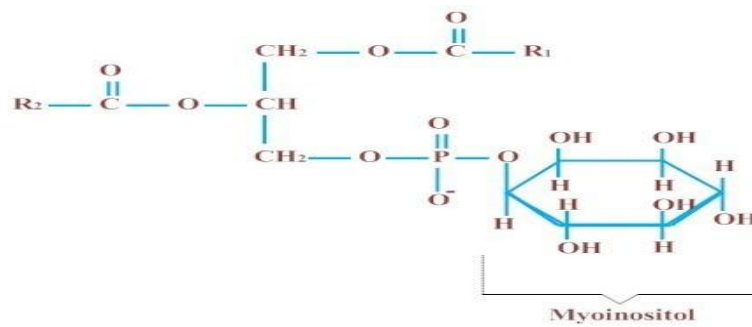
### PHOSPHATIDYLETHONALAMINE (Cephalin)

- It is otherwise called as cephalin
- It differs from phosphatidylcholine only in that ethanolamine replaces choline



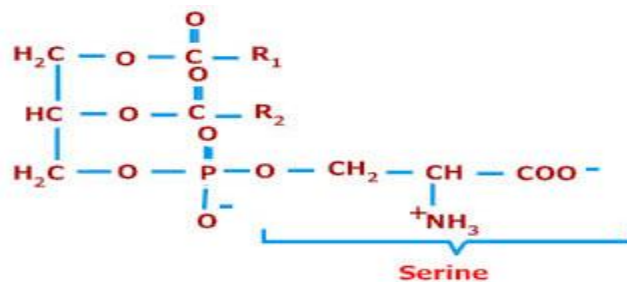
## PHOSPHATIDYLINOSITOL

- It contains the inositol in place of ethanolamine
- Inositol is a stereoisomer of myoinositol
- Phosphatidylinositol is a precursor for second messenger



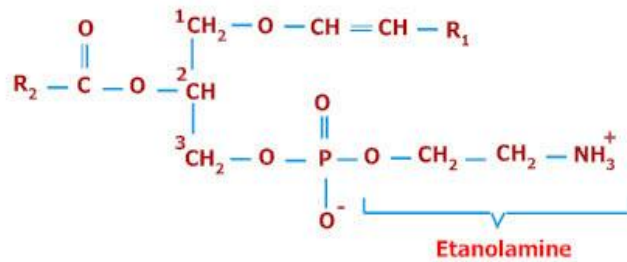
## PHOSPHATIDYLSERINE

- It contains amino acid serine rather than ethanolamine



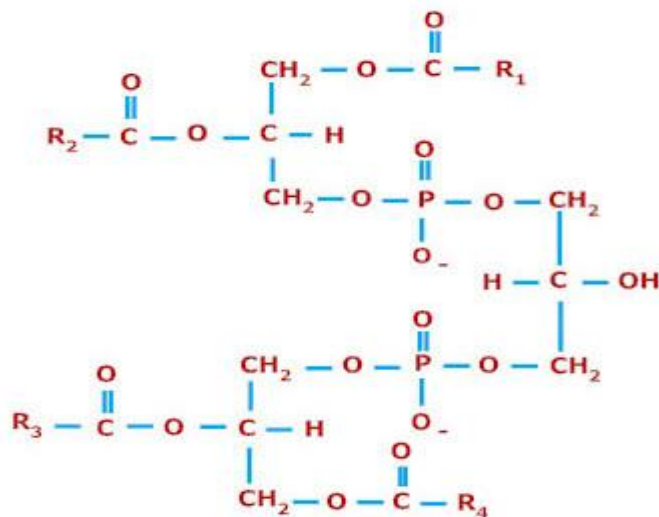
## PLASMALOGENS

- Some animal tissues are rich in ether lipids. When a fatty acid is attached by an ether rather than by an ester linkage at carbon 1 of the glycerol molecule, a plasmalogen is produced.
- For example, phosphatidylethanolamine is the plasmalogen that is similar in structure to phosphatidylethanolamine but containing an ether linkage at carbon-1.
- The fatty acid may be saturated or unsaturated. Plasmalogens constitute as much as 10% of the phospholipids of brain and muscle. The platelet activating factor is plasmalogen causing platelet aggregation and vascular dilation.



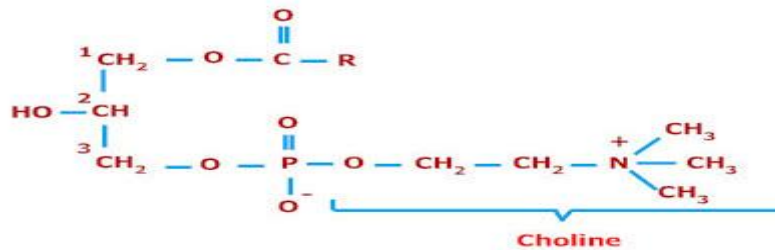
## CARDIOLIPIN

- They are made up of 2 molecules of phosphatidic acid linked by 1 molecule of glycerol
- It was isolated from heart muscle.
- It is present in the inner mitochondrial membrane and needed for the maintenance of ETC.
- Antigenic phospholipid.



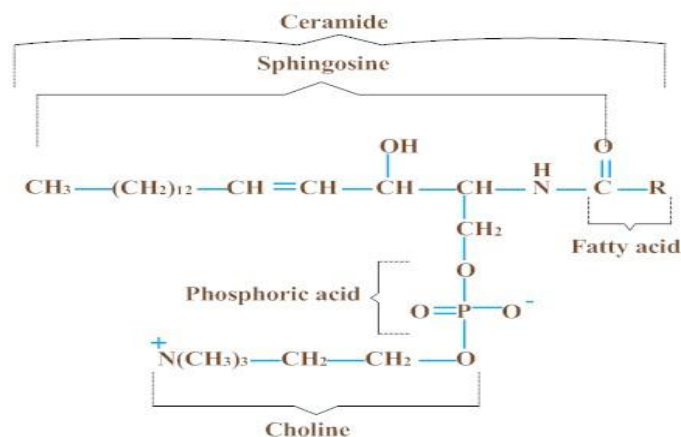
## LYSOPHOSPHOLIPIDS

- They are intermediates in the metabolism of phosphoglycerols. E.g., lysolecithine. It does not contain fatty acid at 2nd position.
- Snake venom contains an enzyme called phospholipase A<sub>2</sub>, which releases fatty acid from the C2 position producing lysolecithine.
- It acts as a detergent and is responsible for lysis of cell membrane particularly in erythrocytes causing hemolysis.



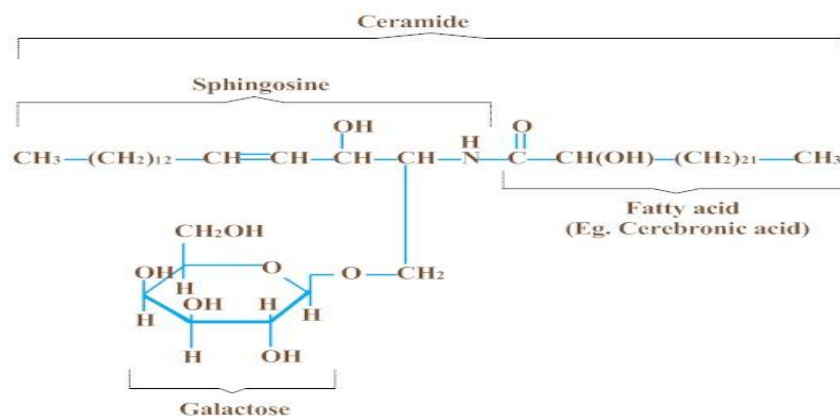
## SPHINGOPHOSPHOLIPIDS AND GLYCOLIPIDS

- There are 3 subclasses of sphingolipids. All are derivatives of ceramides, but differing in their head groups. Examples: sphingomyelins, glycolipids, and gangliosides.
- Sphingomyelins (Sphingophospholipid)**
  - The sphingomyelins contain amino alcohol, sphingosine (synthesized from serine and palmitic acid) rather than glycerol.
  - A fatty acid is attached to the amino group of sphingosine by an amide linkage, producing a ceramide. A structure also found in glycolipids
  - Ceramide can also serve as a precursor of glycolipids. The fatty acids found most frequently in sphingomyelins are palmitic, stearic, lignoceric and nervonic acid.
  - The alcohol group at carbon 1 of sphingosine is esterified to phosphorylcholine, producing sphingomyelin, an important constituent of myelin of nerve fibers, which insulates and protects the nerve fibres of CNS.
  - In Niemann-Pick disease, sphingomyelin accumulates in brain, liver and spleen, which are enlarged causing mental retardation and death occurs in early life.
  - On hydrolysis the sphingomyelin yields a fatty acid, phosphoric acid, choline and a complex amino alcohol sphingosine.



- **GLYCOLIPIDS**

- The glycolipids contain ceramide and one or more sugars.
- They do not contain phosphate group. The sugar containing sphingolipids are also called as "glycosphingolipids". Eg., Cerebrosides. These are, ceramide attached to single sugar, which may be either a molecule of galactose or glucose. As they contain neutral sugars, they are called as "Neutral Glycosphingolipids".
- Galactocerebrosides are present in cell membranes of the nervous tissue (particularly white matter of the brain) whereas glucocerebrosides are present in extra-neural tissues and only traces in brain.
- In humans diseases called Tay Sachs's disease and Gaucher's disease are seen. In these the lipids are not broken down properly and accumulate causing severe brain disorder.
- It is involved in myelin formation, nerve impulse conduction, and signal transduction and as receptor component for certain hormones / bacterial toxins.
- It is antigenic.
- A common fatty acid component of cerebroside is cerebronic acid. In addition, it may contain nervonic or lignoceric acid.
- **Psychosin:** When fatty acids are cleaved from cerebroside by alkaline hydrolysis with  $\text{Ba(OH)}_2$ , the remaining sphingosine is called as psychosin.



- **Gangliosides**

- They are also called as "Acidic glycosphingolipids", as they contain sialic acids.
- These are most complex glycosphingolipids, that contain ceramide and one or more molecules of sialic acid (N- acetyl neuraminic acid). Gangliosides are identified as GM1, GM2, GM3, etc, where G stands for gangliosides and M for mono sialic acid.
- They are predominantly found in ganglions. They are the components of cell surface membrane. It constitutes 6% of the brain lipid. They act as specific receptors for



some pituitary hormones and also involved in cell – cell recognition, growth and differentiation of tissues.

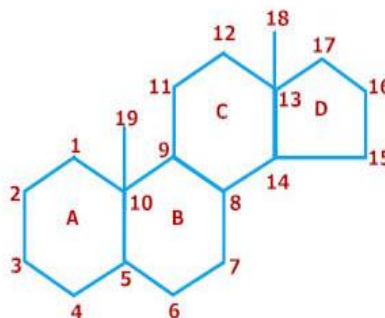
- **Sulfatides**

- Sulfoglycosphingolipids (sulfatides) are cerebroside that contain a galactosyl residue that has been sulfated and is therefore negatively charged at physiologic pH.
- Sulfatides are found predominantly in nervous tissue and in other tissues like liver, kidney and testes. Ratio of cerebroside to sulphatides in brain is normally 3:1.

## DERIVED LIPIDS

### STERIODS

- Steroids are generally considered along with lipids.
- All of the steroids have a common basic ring structure called **CPPP** nucleus or cyclopentanoperhydrophenanthrene.
- It contains 4 fused rings, 3 with six membered rings and one with five membered rings. The 3 six membered rings (A, B and C) resembles phenanthrene, attached to the 5 membered ring resembling cyclopentane ring (D).
- However, the rings are not uniformly unsaturated. The rings are also not benzene rings.
- The positions on the steroid nucleus are numbered as shown in the figure. Methyl side chains are shown by single bonds. These occur at positions 10 and 13 (constituting C atoms 19 and 18 respectively).
- A side chain is seen at position 17 (as in cholesterol).
- If the compound has one or more hydroxyl groups and no carbonyl or carboxyl groups, it is a sterol, and the name terminates in – ol.

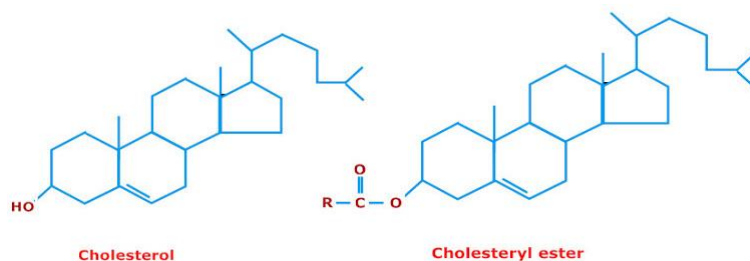


## CHOLESTEROL

- Cholesterol has steroid nucleus (cyclopentanoperhydrophenanthrene).
- The molecule contains 27 carbon atoms. There is one double bond between carbon atom 5 and 6 and a hydroxyl group at position 3. A branch consisting of 8-carbon unit is attached to the D -ring at position 17. Two methyl groups are attached at positions 10 and 13, constituting carbon atom 19 and 18 respectively.
- It occurs in animal fats but not in plant fats.
- Cholesterol is amphipathic with a polar head group (hydroxyl group at C3) and a non-polar hydrocarbon body.
- Much of the plasma cholesterol is in an esterified form, with a fatty acid attached at C3, which makes the structure more hydrophobic.

### Importance of cholesterol

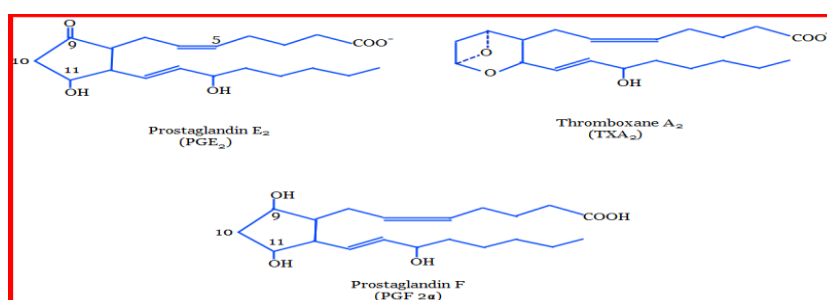
- It is a constituent of membrane.
- It is the precursor of bile acids, steroid hormones and vitamin D.
- It is a component of lipoproteins.
- It is a poor conductor of heat, hence acts as an insulating material.



## EICOSANOIDS

- Eicosanoids (twenty) are derivatives of the 20 carbon polyunsaturated fatty acid, arachidonic acid.
- There are three classes of eicosanoids: Prostaglandins, thromboxanes and leukotrienes.
- They are unstable and insoluble in water. These signaling molecules generally do not move far from the tissues that produced them.
- They act primarily on cells very nearer to the point of release. Unlike hormones, they are not transported between tissues in the blood.
- These compounds are known as autocooids (Local hormones), because they act primarily in the tissues in which they are produced.

- It is abbreviated as PGX<sub>2</sub> where X is the letter indicating the structure ( substituents attached to the ring) and n is the number of double bonds in the hydrocarbon side chains. Similar system is adopted for thromboxanes and leukotrienes.
- The main classes of prostaglandins are PGA, PGE and PGF.
- The prostaglandins are derived from prostanoic acid, a 20 carbon fatty acid that contains a five carbon saturated ring. A side chain containing seven carbon atom is attached to the ring at carbon 8 is denoted by dashed lines indicating that it projects below the plane of the ring. The another side chain containing 8 carbon is attached at carbon 12, which projects above the plane of the ring (indicated by solid line).
- Prostaglandins with one or three double bonds have weaker inflammatory effects than with 2 double bonds.
- Thromboxane differs from prostaglandins that it has a six membered ring containing 5 carbon atoms and one oxygen atom. They were discovered from blood platelets or thrombocytes. Leukotrienes were discovered in leukocytes.



### Prostaglandin Functions:

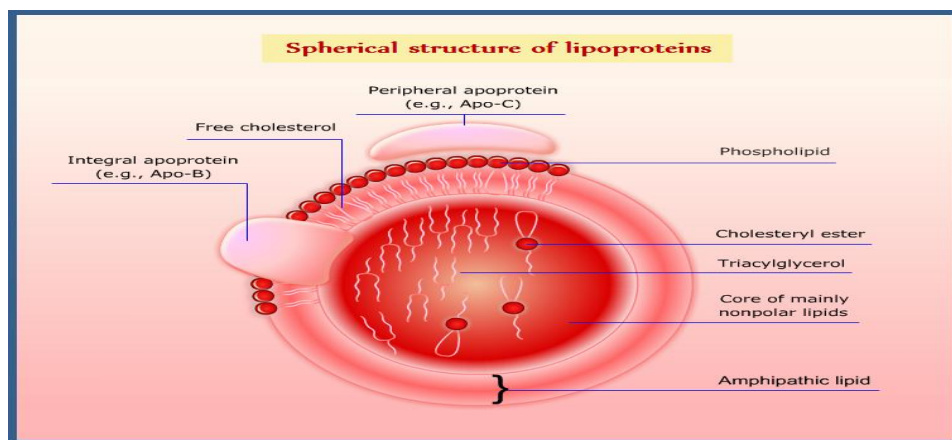
- They participate in many processes in the body. The biological actions are different in each organ.

Name of Prostaglandins	Actions
PGD <sub>2</sub>	Vasodilation, inhibition of platelet aggregation
PGF <sub>2</sub>	Myometrial contraction
PGI <sub>2</sub> (Prostacyclin)	Vasodilation, inhibition of platelet aggregation.
PGE <sub>2</sub>	Vasodilation, Contraction of broncho and gastrointestinal smooth muscle. Inhibition of gastric secretion.
TXA <sub>2</sub> (thromboxanes)	Vasoconstriction, platelet aggregation.
T <sub>4</sub>	Smooth muscle contraction, chemotaxis, inflammation and allergic reactions.

## LIPOPROTEINS AND BILE SALTS

### LIPOPROTEINS

- Lipids must bind to proteins to make them water soluble for transport.
- The lipids are transported in the form of lipoproteins in the blood.
- Larger the lipid content, the lower the density.
- Higher the protein content, higher the density.



### FUNCTIONS AND CLASSIFICATIONS OF LIPOPROTEINS

Lipoproteins perform three major functions:

- Transport of dietary fat from the intestine to other tissues
- Transport of endogenous triglycerides and cholesterol to other tissues
- Transfer of cholesterol from extra hepatic tissues to the liver.
- Based on the density lipoproteins are classified in
  - Chylomicrons
  - VLDL (or pre-beta lipoprotein)
  - LDL (or beta-lipoprotein)
  - HDL or (alpha - lipoprotein)
- These are, important physiologically and in clinical diagnosis.
- Triacylglycerol is the predominant lipid in chylomicrons and VLDL. Whereas cholesterol and phospholipids are predominant lipid in LDL and HDL respectively.

- A typical lipoprotein such as chylomicron or VLDL consists of mainly non-polar triacylglycerols and cholesteryl esters, surrounded by a single surface layer of amphipathic phospholipids and cholesterol.
- These are oriented in such a way that their polar groups face outward to the aqueous medium.
- The protein moiety of lipoprotein is known as apoprotein. (apo means detach), which controls the lipid transport in plasma and uptake of lipoproteins into the tissues.
- Chylomicrons transport dietary triacylglycerols (90%) and cholesteryl esters from the intestine to other tissues in the body. Due to the presence TG, it has low density.
- Very low-density lipoprotein transports triacylglycerols and cholesteryl esters synthesized in the liver to other tissues. It is similar to chylomicron.
- Lipoprotein lipase, which is located on the walls of blood capillaries acts on triacylglycerols present in the chylomicrons and VLDL and releases free fatty acids, which are then diffused into extrahepatic tissues.
- The fatty acids may be oxidized for producing energy or stored in the form of triacylglycerols.
- After the removal of triacylglycerols from VLDL the remnant is richer in the content of cholesteryl esters, which is known as LDL, which is finally returned to liver for uptake.
- Excess cholesterol is removed from the extra hepatic tissues by HDL for delivery to liver from there it is excreted in the form of bile salts in to the intestine.
- The transfer of cholesterol from extrahepatic tissues to the liver is called reverse cholesterol transport.

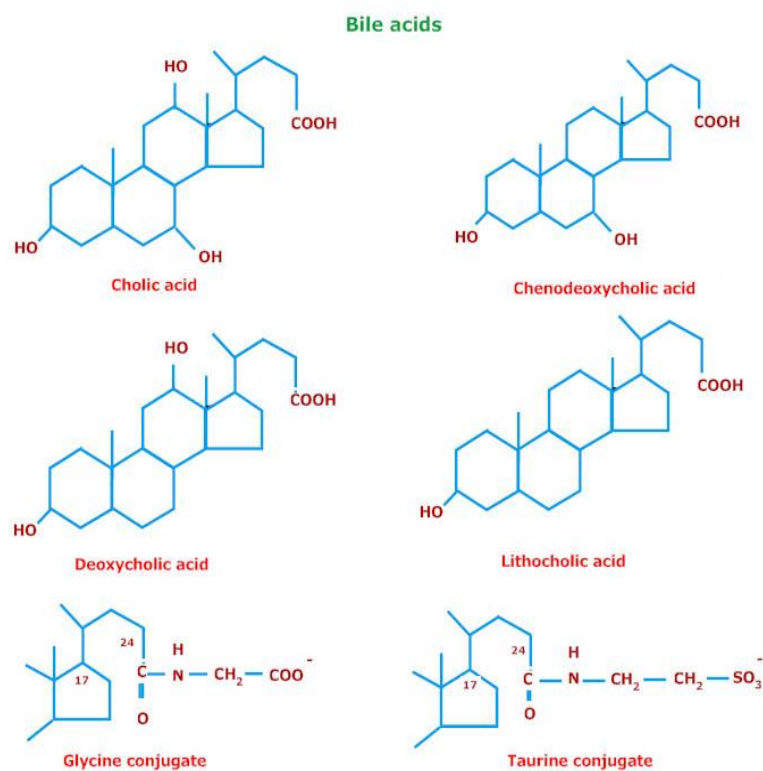
## **BILE SALTS**

- Cholesterol is not degraded to yield energy as there is no enzyme to degrade the ring structure of cholesterol.
- They are eliminated from the body mostly in the form of bile acids, in which the ring structure is intact.

## **Reactions**

- The first step and the rate limiting step is the catabolism of cholesterol is the conversion of cholesterol to 7 $\alpha$ -hydroxycholesterol by the enzyme 7- $\alpha$ -hydroxylase. This reaction needs NADPH, O<sub>2</sub> and Vit-C.
- In the subsequent step, the double bond is reduced; the side chain is oxidized and converted to a five carbon branch possessing a carboxyl group at the end, followed by hydroxylation to form two compounds. One has hydroxyl group at positions 3 and 7 and the other at positions 3, 7 & 12, namely chenodeoxycholic acid and cholic acid respectively. These compounds are called primary bile acids (they are produced in the liver).

- They are then conjugated to a molecule of glycine or taurine by an amide bond. These new structures are called as bile salts and they are
  - Glycocholic acid
  - Glychenodeoxycholic acid
  - Taurocholic acid
  - Taurochenodeoxycholic acid.
- The bile salts are then transported to the intestine via the bile.
- Bacteria in the intestine can remove glycine and taurine from the bile salts.
- Some of the primary acids are also converted into secondary bile acids by removing a hydroxyl group attached at carbon 7, producing deoxycholic acid from cholic acid and lithocholic acid from chenodeoxycholic acid.
- About 94% of the bile salts are reabsorbed and returns to liver by enterohepatic circulation and 6% is lost in the feces.
- Bile salts lower surface tension and are required for digestion and absorption of lipids.



## **STRUCTURE OF BIOLOGICAL MEMBRANE AND TRANSPORT ACROSS MEMBRANE**

- Biological membranes are thin sheet like structure composed of lipids and proteins. The ratio of proteins to lipids varies between different types of membranes. Generally 40-50% proteins are present. Myelin sheath has 80% lipids and 20% proteins. The inner membrane of mitochondria has 80% proteins and 20% lipids.
- Some membrane contains carbohydrates up to 10%. It is found on the external surface of the plasma membrane. They are attached to either proteins as glycoproteins and lipids as glycolipids.
- Three main groups of lipids are found in the membrane viz
  - phospholipids
  - sphingolipids
  - cholesterol

### **Functions of membranes**

- It is the boundary between the cell interior and exterior and maintains the shape of the cell.
- Transport of substances in and out of the cell. The lipid bilayer prevents the passage of hydrophilic ions and molecules.
- Cell cell interaction.
- Signal transduction.

### **Arrangements of proteins and lipids**

- Membrane is in the form of phospholipid bilayer, which is composed of two rows of phosphoglycerides that have hydrophobic fatty acyl groups oriented towards inward and the hydrophilic phosphate head group outward to the extracellular and cytoplasmic surface. Each lipid bilayer has different phospholipid composition.
- The arrangements of lipids and proteins in membranes are described by fluid mosaic model. According to this model the membrane is a dynamic structure (they are in constant motion) in which both proteins and lipids rapidly diffuse.
- Membrane proteins can be divided into two types. 1. peripheral and 2. integral.
- Peripheral proteins are bound loosely with the surface of either side of the membrane. They can be removed by mild treatment with solutions of high ionic strength.
- Integral proteins are tightly bound. The proteins may cross the membrane (called transmembrane proteins) or the proteins may be firmly embedded in the bilayer. These proteins can be removed by the use of powerful detergent. These membranes contain high portions of hydrophobic amino acids, which can interact with hydrophobic regions of lipid bilayer.
- Some proteins may be covalently attached to lipid prosthetic group.

- Membranes are asymmetric due to the irregular distribution of proteins

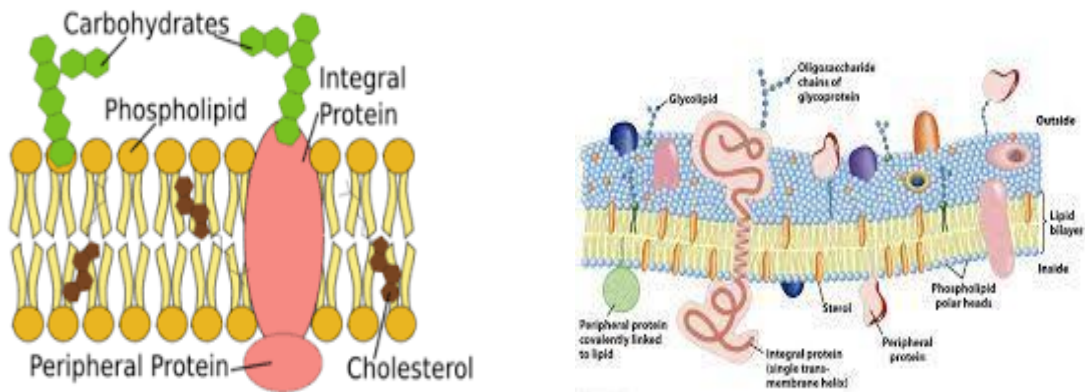


Fig: Fluid Mosaic Model / Lipid Bilayer

### Functions of membrane proteins

- Localization of enzyme activity
- Energy transduction
- Facilitated transport
- Receiving of extracellular signal

### Fluidity of membrane

- The degree of motion of the hydrocarbon chain within the lipid bilayer is called fluidity. Several factors influence the fluidity in membranes.
- Long chain saturated fatty acids decrease fluidity and permeability of membrane. They have straight tail, so that they are packed tightly.
- Unsaturated fatty acids present in the membrane phospholipids increase the fluidity and permeability of membrane. Greater the number of double bonds greater will be the fluidity and permeability. Unsaturated fatty acids exist in the cis form in the membrane, which makes kinked (twisted) tails. Membrane made of more unsaturated fatty acids has more kinks; hence, they are not highly packed and showing fluid nature.
- Presence of cholesterol prevents the movements of fatty acids and reduces fluidity. It prevents close packing of the hydrocarbon chains and thereby lowers the melting point.
- Fluidity increase with increase in temperature.
- Movements of proteins and lipids within the membrane are lateral. Neither protein nor lipids flip flop from one side to other ( passage of lipids from one leaflet of the bilayer to the other).



## **MEMBRANE TRANSPORT**

- The plasma membrane of the living cell not only covers the cytoplasm but also controls the transport of water and solutes between the external and internal environments.
- For the cell to survive it takes up the nutrients from outside and release the internal substances outside. This process occurs by means of transport either through cell membrane or through proteins in the lipid bilayer.
- This transport process can be divided into two types
  - Passive transport
  - Active transport

### **PASSIVE TRANSPORT**

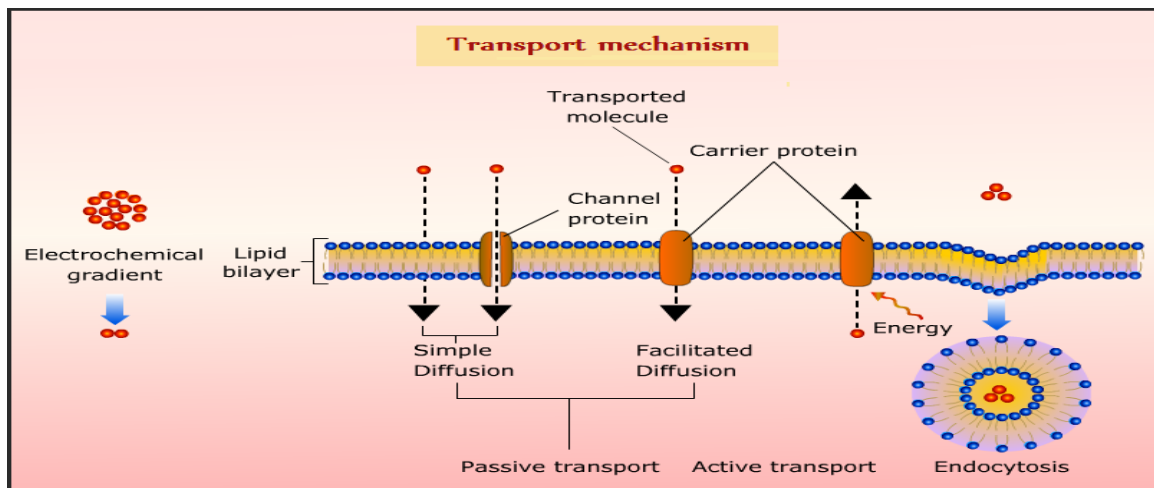
- Movements of molecules across the cell membrane in response to a concentration gradient.
- This type of transport may or may not be mediated by protein.
- The process requires no metabolic energy.
- Passive transport is of two types
  - Simple diffusion
  - Facilitated diffusion

### **SIMPLE DIFFUSION**

- A net movement (flux) of the solute from the region of higher concentration to one of lower concentration is called as diffusion.
- Diffusion occurs as downhill process. Higher the difference in concentration between two systems, the more rapid the rate of diffusion.
- Diffusion occurs either directly through the membrane or through membrane channels or through pores.
- Diffusion of molecules across the membrane depends on charge, size and lipid solubility of the substance. Smaller the molecules, greater the rate of diffusion and more the lipid solubility, more the rate of diffusion.
- Diffusion of ions depends on the charge and concentration of the ions inside and outside the membrane.
- Small, uncharged molecules (such as O<sub>2</sub>, CO<sub>2</sub> and H<sub>2</sub>O) and lipid soluble substances (Such as steroid hormones) cross membranes by simple diffusion.
- It does not exhibit saturation kinetics.

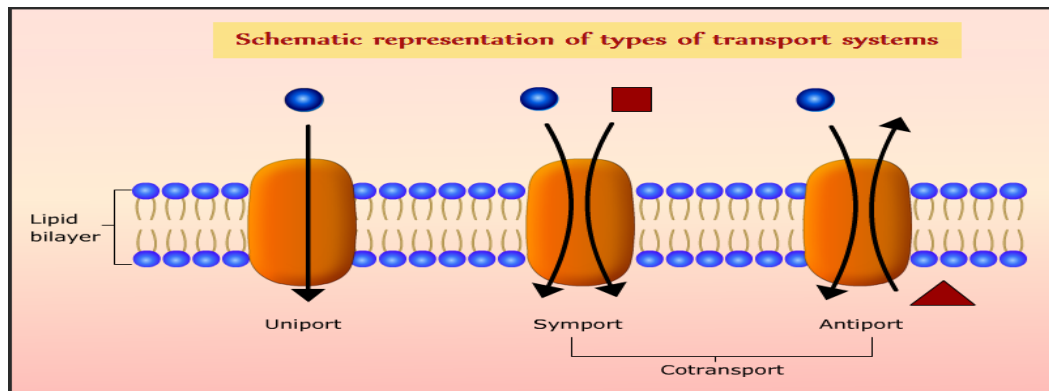
## FACILITATED DIFFUSION

- In facilitated diffusion, the movement of solute occurs through a carrier protein known as transporters also known as translocase, permeases. It is faster than simple diffusion. The process does not require expenditure of energy.
- This exhibits saturation kinetics.



## BIOLOGICAL SIGNIFICANCE

- Diffusion plays an important role in living cells. Some of the important examples are
  - Absorption of glucose from the intestine into blood.
  - Exchange of oxygen and carbon dioxide between air and blood.
  - Exchange of anions like chloride ( $\text{Cl}^-$ ) for bicarbonate ( $\text{HCO}_3^-$ ) in red blood cells.
  - Conduction of nerve impulses depends on the passive diffusion of ions.
  - Transport of a substance into a cell by facilitated diffusion is an example of uniport, in which there is a transport of only single molecule in one direction. For example: glucose is transported into erythrocytes by a uniport mechanism.
  - When two substances are co-transported from one side of the membrane to the other such as sodium and amino acid and sodium and glucose, the process is called symport.
  - When the transport of a substance in one direction is coupled to the transport of another substance in the opposite direction the process is called antiport. For example: the transport of  $\text{Cl}^-$  and  $\text{HCO}_3^-$ .
  - Another important passive transport system is gated pores or channels. These transport system is for specific ions and small molecules. (pore is used for bacteria and channel is used for animals) These gates or channels open and close on receipt of a signal.



## ACTIVE TRANSPORT

- In active transport, the cell uses energy to transport the substances. This process requires transport proteins.
- The solutes are usually transported from an area of lesser solute concentration to greater solute concentration. Hence, this process is an Uphill Process.
- Primary active transport
  - It is powered by direct source of energy. The transport of sodium and potassium by sodium/potassium ATPase in the plasma membrane is an example. ATP provides energy for the transport. The concentration of sodium in the outside of the cell is higher and that in the interior of the cell is lower. This enzyme is responsible for about one third of the basal energy requirement of the human.
  - Osmotic regulation (Acid – Base balance).
- Secondary active transport
  - It is driven by the concentration gradient. For example sodium moves into the cell where its concentration is lower, which facilitates the transport of amino acids or glucose into the cells.
- Endocytosis and Exocytosis
  - Cells need to import and export larger molecules that could not be transported via pores, channels or transport proteins. In eukaryotes, proteins and some other large substances are transported into and out of the cell by endocytosis and exocytosis respectively. In both cases transport is by the formation of specialized type of lipid vesicles.
- Endocytosis
  - It is the process by which macro molecules are engulfed by plasma membrane and brought into the cell within lipid vesicles. The receptor mediated endocytosis begins with the binding of macromolecules to specific receptor proteins in the plasma membrane of the cell. The membrane then invaginates forming a vesicle that contains the bound molecules. It fuses with the lysosome, which contains the

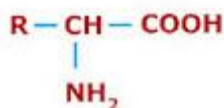
hydrolytic enzymes. Inside of lysosome the endocytosed material and the receptor may be degraded.

- There are two types of endocytosis
  - Phagocytosis
  - Pinocytosis
- Phagocytosis (cell eating) Phagocytosis involves the ingestion of large particles such as virus, bacteria, cells, or debris. Phagocytosis occurs only in specialized cells such as macrophages and granulocytes
- If the contents are liquid and smaller (protein) in size then the process is called Pinocytosis (cell drinking).
- Exocytosis
  - It is similar to endocytosis but the direction of transport is opposite. During exocytosis the materials to be secreted from the cell are enclosed in a vesicle the vesicles then fuse with the plasma membrane releasing the vesicles contents into the extracellular space. The zymogens of digestive enzymes are exported from the pancreatic cell in this manner.
- Group Translocation
  - Group translocation involves not only transport of substance across the membrane but also chemical modification of the substance during the process.
  - The substance is bound more tightly by the transporter prior to its modification. After modification, it is bound less tightly and is readily released.
  - The gama glutamyl cycle that transport amino acids into cells is an example.
- Transduction of extracellular signals
  - The transmission of chemical messages from hormones into cells is known as signal transduction. There are four methods of signal transduction
  - Production of cAMP by adenylate cyclase
  - Production of inositol phoshate and diacylglycerol from phosphatidyl inositol bisphosphate
  - Activation of tyrosine kinase
  - Transport to the cell nucleus.

# AMINO ACIDS

## AMINO ACIDS

- Amino acids are organic acids containing an amino group and a carboxyl group attached to the  $\alpha$  – carbon atom.
- In addition to the amino and carboxyl groups, each amino acid contains a side chain, called R group, which is also attached to  $\alpha$ - carbon atom. The side chain differs for each of the 20 amino acids.
- Amino acids are the monomers, which are used in the synthesis of proteins, which is a polymer. Each amino acid in a polypeptide chain is called as residue.
- The structure of an amino acid can be represented by the following formula



- The  $\alpha$ -carbon atom of all the amino acids except glycine is attached to four different chemical groups and is therefore asymmetric in nature.
- Because of this asymmetric nature, amino acids exist in either in D or L configuration. They cannot be superimposed because they are mirror image of each other.
- The two forms of each pair are termed as stereoisomers, optical isomers or enantiomers.
- All the amino acids found in protein are of L configuration. The functions of the amino acids and their roles in protein structure are related mainly to the chemical properties of their side chain.
- D-amino acid: amino acids having D-configuration are also present in nature. They are not normally present in living organisms. Some of the D- amino acids are found in bacterial cell walls and in some antibiotics produced by bacteria. The antibiotic gramicidin-S is an example, which contains two D- phenylalanine residues.

## IMPORTANCE OF AMINO ACIDS

- It is the component of proteins.
- Glycine and  $\gamma$  - aminobutyric acid (a glutamate decarboxylation product) and dopamine (a tyrosine product) are neurotransmitters.
- Histamine (the decarboxylation product of histidine), a vasodilator.
- Tyrosine is the precursor of the hormones thyroxine, epinephrine. The pigment melanin is also derived from tyrosine.
- Citrulline and ornithine are important intermediates in urea cycle.

- Homocysteine, an intermediate in the synthesis of methionine, s-adenosylmethionine a biological methylating agent
- Purines and pyrimidines are derived in part from amino acids.
- Sphingosine an intermediate in the synthesis of sphingolipids comes from serine.
- The neurotransmitter, 5- hydroxytryptamine (serotonin) and the nicotinamide ring of NAD are synthesized from tryptophan.
- Glutathione: ( $\gamma$  -Glu-Cys-Gly) a tripeptide containing sulfhydryl group protects red cell from oxidative damage. Glutathione also plays a key role in detoxification by reacting with the hydrogen peroxide and organic peroxides, the harmful byproduct formed during the metabolic reactions.
- Nitric Oxide (NO), a vasodilator is produced from the amino acid arginine.
- Heme of hemoglobin is synthesized from the amino acid glycine and succinyl CoA.
- Creatine phosphate (a high energy molecule) present in muscle is synthesized from glycine, arginine and methionine.

#### NOTATION FOR 20 STANDARD PROTEINS

AMINO ACID	THREE-LETTER SYMBOL	ONE-LETTER SYMBOL
Alanine	Ala	A
Valine	Val	V
Leucine	Leu	L
Isoleucine	Ile	I
Proline	Pro	P
Phenylalanine	Phe	F
Tryptophan	Trp	W
Methionine	Met	M
Glycine	Gly	G
Serine	Ser	S
Threonine	Thr	T
Cysteine	Cys	C
Glutamine	Gln	Q
Asparagine	Asn	N
Tyrosine	Tyr	Y
Lysine	Lys	K
Arginine	Arg	R

Histidine	His	H
Aspartate	Asp	D
Glutamate	Glu	E

### CLASSIFICATION OF AMINO ACIDS

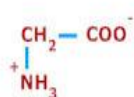
- There are several ways by which amino acids are classified
  - Classification based on the side chain group
  - Classification of amino acids based on polarity
  - Classification of amino acids based on nutritional requirement

### CLASSIFICATION BASED ON THE SIDE CHAIN (R) GROUP

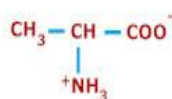
#### Simple amino acids

- These are amino acids, having no functional groups attached to the aliphatic side chain. Eg: glycine, alanine, valine, leucine and isoleucine.
- The amino acid valine, leucine and isoleucine contain branched aliphatic side chains. Hence, they are also known as branched chain amino acids.

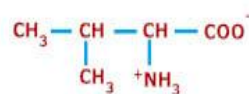
#### Simple amino acids



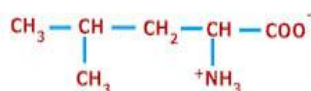
Glycine(Gly,G)



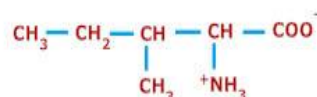
Alanine(Ala,A)



Valine(Val,V)



Leucine(Leu,L)

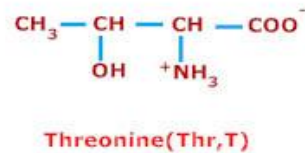
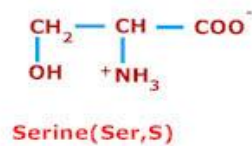


Isoleucine(Ile,I)

## Hydroxyl amino acids

- These are amino acids containing hydroxyl group in the side chain. Eg: serine and threonine.

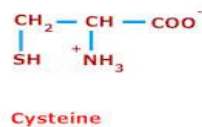
### Hydroxyl amino acids



## Sulphur containing amino acids

- These are amino acids, containing a sulphur atom attached on the aliphatic side chain. Eg: cysteine, cystine and methionine.

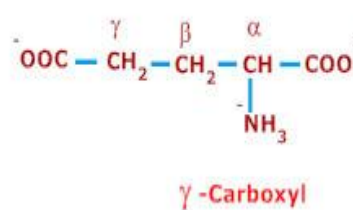
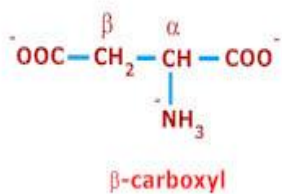
### Sulphur containing amino acids



## Acidic amino acids

- These amino acids will have one more carboxyl group attached on the side chain. Eg: aspartic acid and glutamic acid.

### Acidic amino acids



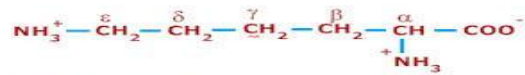
## Basic amino acids

- These amino acids will have some nitrogen containing basic group attached on the side chain. Eg: lysine and arginine.



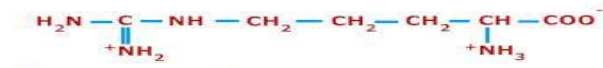
## Basic amino acids

### Lysine(Lys,K)



$\epsilon$ -Amino group

### Arginine(Arg,R)



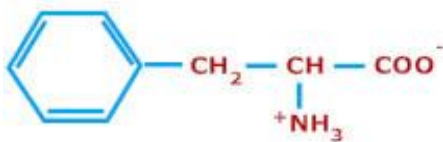
Guanidinium group

## Aromatic amino acids

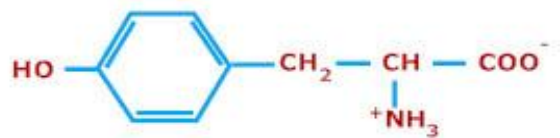
- These are amino acids containing the benzene nucleus attached on the side chain.  
Eg: phenylalanine, tyrosine and tryptophan.
- The nucleus present in tryptophan is known as indole. Presence of aromatic amino acids like tyrosine and tryptophan in protein, proteins absorb light at a wave length of 280nm

## Aromatic amino acids

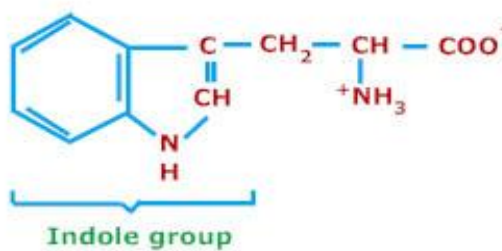
### Phenylalanine(Phe,F)



### Tyrosine(Tyr,y)

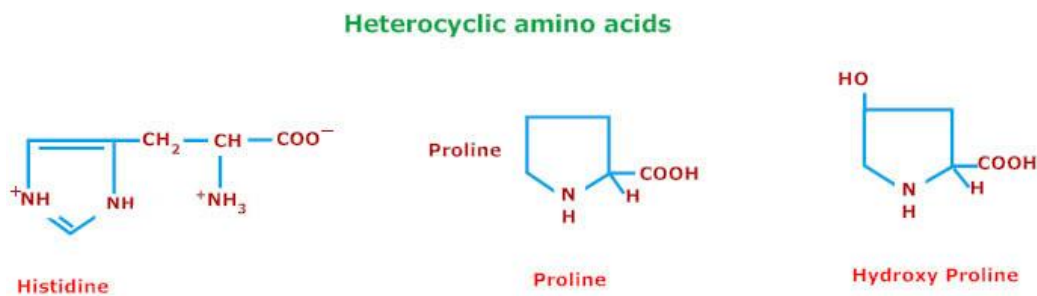


### Tryptophan(Trp,W)



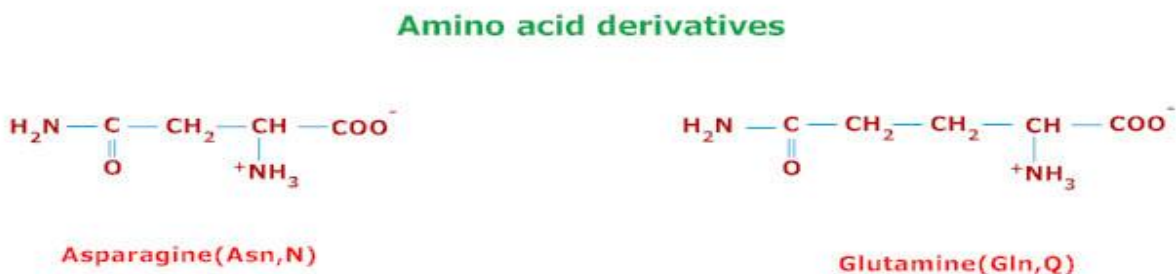
## Heterocyclic amino acids

- These amino acids contain a ring, which is having one atom other than carbon atom. E.g.: histidine, proline and hydroxyproline.
- The proline and hydroxyproline are also called as imino acids, because they contain an imino group instead of an amino group, as found in other amino acids.



## Amino acid derivatives

- Amino acid derivatives are formed as an intermediate in the metabolic reactions.
- Eg: asparagine and glutamine derived from aspartic acid and glutamic acid respectively.



## CLASSIFICATION OF AMINO ACIDS BASED ON POLARITY

There are 4 main classes of amino acids based on polarity, i.e. the interaction of the R group with water molecules at physiological pH.

### Amino acids with non-polar (hydrophobic) side chain

- These amino acids have a non-polar hydrophobic side chain. They do not provide protons or participate in hydrogen or ionic bonding. E.g.: alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan and methionine.

### **Amino acids with uncharged polar side chains (- R group)**

- These amino acids are more soluble in water. They are hydrophilic in nature. The functional groups can make hydrogen bonds with water. E.g.: glycine, serine, threonine, tyrosine, cysteine, asparagine and glutamine.
- The polarity of serine, threonine is due to the presence of hydroxyl groups; the polarity of asparagine and glutamine is due to the amide group and the sulfhydryl group (thiol group) is responsible for cysteine.

### **Amino acids with polar, negatively charged side chain**

- These amino acids will have a net negative charge at neutral pH. E.g.: aspartic acid and glutamic acid.
- These amino acids will have one more carboxyl group, which contribute to negative charge at neutral pH.

### **Amino acids with polar, positively charged side chain**

- These amino acids will have a net positive charge at pH 7. They accept protons. E.g.: lysine, arginine and histidine.
- Lysine contains a second amino group at  $\epsilon$  - position on the aliphatic side chain. Arginine contains a positively charged guanidino group and histidine contains an imidazole group.

## **CLASSIFICATION BASED ON NUTRITIONAL REQUIREMENT**

- There are two main classes based upon their requirement in the diet particularly for monogastric animals.

### **Essential amino acids**

- They cannot be synthesized in the body. They have to be supplied in the diet.
- Eg: methionine, arginine, threonine, tryptophan, valine, isoleucine, leucine, phenylalanine, histidine and lysine. Of the ten listed above, two amino acids namely arginine and histidine can be partly synthesized by adult humans – hence called as Semi-essential amino acids .

### **Non-essential amino acids**

- The other ten amino acids can be synthesized in the body – hence called as Non-essential amino acids.
- Eg: glycine, alanine, serine, tyrosine, cysteine, glutamic acid, aspartic acid, asparagine, glutamine and proline.

## PHYSICAL AND CHEMICAL PROPERTIES OF AMINO ACIDS

### Physical properties

- Amino acids are white, crystalline substances soluble in water and insoluble in organic solvents.
- They generally have high melting points, above 200°C.
- Amino acids may be tasteless (leucine), sweeter in taste (glycine and alanine) or bitter in taste (arginine).
- All amino acids except glycine possess asymmetric carbon atom and hence, optically active.
- Amino acids are amphoteric in nature, because, they contain both acidic (-COOH) and basic (-NH<sub>2</sub>) groups. They can donate or accept a proton. Hence, they are also known as ampholytes.
- Aromatic amino acids have an absorption maxima at 280 nm

### Chemical properties

- The chemical properties of amino acids are due to the presence of carboxyl and amino groups.

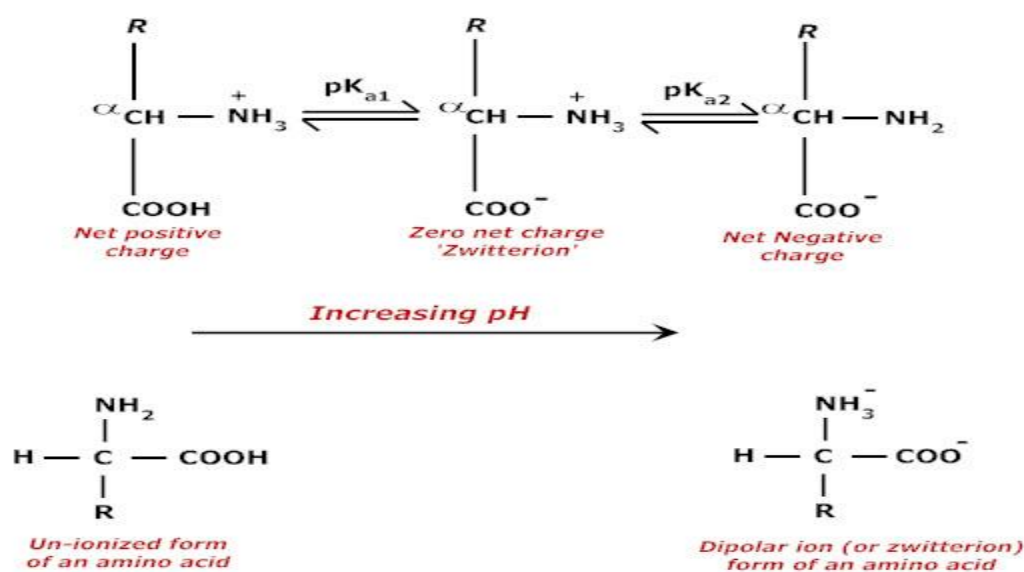
### Peptide Bonds

- The combination of an  $\alpha$  – amino group of one amino acid with the carboxyl group of a second amino acid, with the elimination of water, results in the formation of a peptide bond. The resulting compound is a dipeptide. A tri peptide contains three amino acid residues joined by two peptide bond, an oligopeptide contains around 20 amino acid (oligo=few) and a polypeptide contains more than 80 amino acid residues. The bonds are very stable. The distinction between peptide and protein is not clear. The term protein is used with more molecular weight and the polypeptide is for lower molecular weight.
- By convention, the amino acid sequence of a polypeptide is written from the amino terminus on the left and the carboxy terminus on the right. Each amino acid is abbreviated by a three letter symbol, usually the first three letters of its name or by a one letter symbol (this symbol is used for long sequence).
- Polypeptides are named as derivatives of amino acid with the free carboxyl group. The name of each amino acid in a polypeptide chain is changed to yl except the one at the C terminal. For example the tri-peptide glutathione, which is made up of glutamate, cysteine and glycine is represented by Gly---Cys-----Gly ( glutamyl –cysteinyl—glycine).

### ISOELECTRIC pH AND ZWITTER ION

- Charge of amino acid is dependent on pH.
- At acidic pH amino acids exists as cation (positively charged). The carboxyl group is unionized and the amino group is protonated.

- At alkaline pH as anion (negatively charged). The carboxyl group is ionized and the amino group is unionized.
- At neutral pH as dipolar ions (Zwitter ions) (dipolar means a molecule containing negatively charged and positively group in equal number). In the dipolar form of amino acid, the amino group is protonated and the carboxyl group is dissociated ( $-\text{NH}_3^+$ ,  $-\text{COO}^-$ ).
- The pH at which the amino acids possess equal number of negative and positive charges is the isoelectric pH (denoted as pI). At this pH the net charge of amino acid is '0', it is electrically neutral.



**Structure of the un-ionized and zwitterion forms of an  $\alpha$ -amino acid.**  
**The R group is the side chain.**

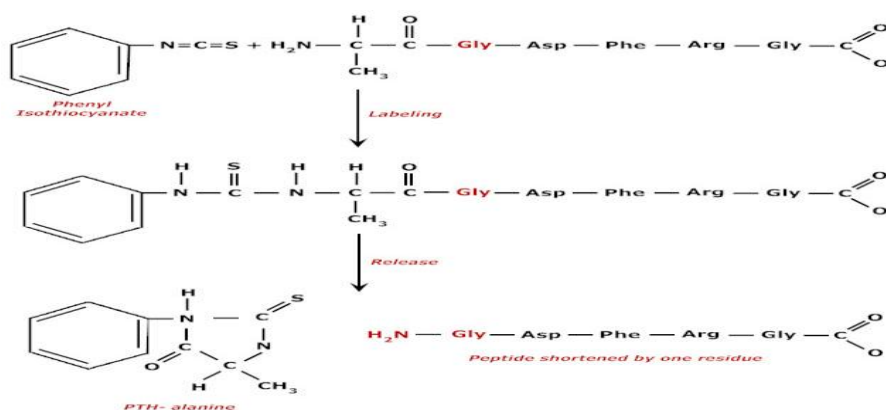
## REACTIONS OF AMINO ACIDS

### Reactions due to the presence of carboxyl group

- Esterification
  - Amino acids react with alcohol and forms ester in the presence of HCl.
- Decarboxylation reaction
  - Amino acids undergo decarboxylation to produce corresponding amines. This reaction is important, because, many biologically active amines are produced in the body involving this reaction.
- Reaction with amino group
  - The carboxyl group of amino acid reacts with the amino group to form an amide.

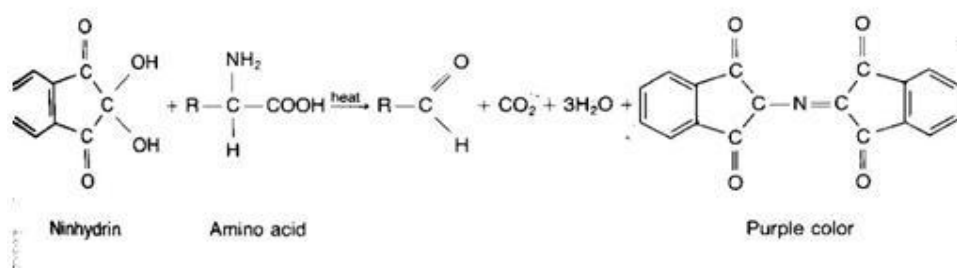
## Reactions due to the presence of amino group

- Formal titration
  - Formaldehyde reacts with the amino group ( $\text{-NH}_2$ ) of an amino acid and thereby prevents the formation of zwitter ions with carboxyl groups.
  - This permits the carboxyl group to exert maximum acidity, which may be measured by titration against a standard NaOH solution using phenolphthalein as indicator.
  - The amino group of amino acids behave as base while reacting with acids and forms corresponding salts.
- Reaction with nitrous acid
  - Amino groups of amino acids react with nitrous acid, to form the corresponding hydroxy acids liberating nitrogen gas.
  - The reaction is based on the Van Slyke's reaction.
- Sanger's reaction
  - Amino group of amino acid reacts with Sanger's reagent (1-Fluoro-2,4 - dinitrobenzene -FDNB) to yield 2,4- dinitrophenyl derivatives. This reaction is useful in determining the amino end of the polypeptide chain.
- Edman's reaction
  - Phenylisothiocyanate is known as Edman's reagent. It is used for the identification of N- terminal amino acid in a polypeptide.
  - Reaction of the free N- terminal amino group with the phenylisothiocyanate under mild alkaline conditions produces phenylthiohydantoin (PTH derivative).
  - This complex creates instability in the N – terminal peptide bond, which can be cleaved specifically without affecting the other peptide bonds.
  - This reagent can be applied repeatedly to determine the sequence of the polypeptide chain starting at the amino terminal of the polypeptide.
  - Dansyl chloride and dabsyl chloride are also used to identify the nature of amino acid present in the N- terminal side.



## Ninhydrin reaction

- Ninhydrin, a powerful oxidizing agent, causes oxidative decarboxylation of  $\alpha$  - amino acids, producing the  $\text{CO}_2$ ,  $\text{NH}_3$  and an aldehyde with one carbon atom lesser than the parent amino acid.
- The reduced ninhydrin (Hydrindantin) then reacts with the liberated ammonia and also with another molecule of ninhydrin forming a blue colored complex (Ruhemann's complex).
- This test is highly useful for the quantitative estimation of amino acids. Proline and hydroxy proline produce a yellow color rather than a purple color with ninhydrin.



## **PROTEINS AND THEIR BIOLOGICAL SIGNIFICANCE**

- Proteins are the main structural and functional molecules of all living organisms.
- Proteins are synthesized from amino acids, which are joined together by peptide bond to form a linear chain. Functions of proteins depend on the amino acid sequence.
- These chains are then folded into various ways to form the 3 – dimensional structures of proteins.

### **Functions of proteins**

- Most of the enzymes involved in the biochemical reactions in the body are protein in nature.
- Many hormones are proteins or peptides in nature. Eg: Insulin
- Proteins are seen in association with DNA molecules, where it controls the gene transcription and translation.
- Proteins are involved in the transport processes. Eg: Hemoglobin in erythrocytes involved in the transport of O<sub>2</sub>. Some transport proteins bind with steroid hormones and transport them to the other parts of the body for action. Lipoproteins transport lipids.
- Proteins have protective role in the body. Immunoglobulins and interferons are proteins that protect humans against bacterial and viral infections.
- Structural proteins like collagen and elastin provide structural strength and elasticity to organs and the vascular systems.
- Some proteins are used as nutrients eg: ovalbumin of egg white and casein of milk are used as nutrients.
- Proteins are involved in the maintenance of osmotic pressure of plasma.
- Some proteins like actin and myosin are involved in the contraction of skeletal muscles.

### **CLASSIFICATION OF PROTEINS**

- Based on the solubility and physical properties, proteins are classified into 3 major groups, namely,
  - Simple proteins
  - Conjugated proteins
  - Derived proteins
- Proteins can also be classified, according to their shapes
  - Globular protein
  - Fibrous protein



## SIMPLE PROTEINS

- These are proteins, which on complete hydrolysis yield only amino acids as an end product.
- They are further subdivided, into
  - *Albumins*: These proteins are soluble in water coagulated by heat and precipitated by saturated salt solution like ammonium sulphate. Eg: serum albumin and lactalbumin
  - *Globulins*: These proteins are insoluble in pure water, but soluble in dilute salt solutions. They may be coagulated by heat. Eg: Serum globulin, ovoglobulin.
  - *Glutelins*: These are soluble in dilute acids and alkalis and insoluble in water and neutral solvents. Eg: glutenin from wheat.
  - *Prolamine*: Soluble in 70 – 80 % alcohol, insoluble in water, absolute alcohol and other neutral solvents. Eg: zein of corn and gliadin of wheat.
  - *Histones*: These proteins are soluble in water and very dilute acids, insoluble in dilute ammonium hydroxide. These proteins are not coagulated by heat. They are strongly basic in nature due to the presence of excess amounts of arginine and lysine. Eg: Histones associated with nucleic acids.
  - *Protamines*: Basic polypeptide, soluble in water or ammonium hydroxide, not coagulated by heat. Basic amino acids will predominate in their structure. Eg: protamines of sperm cells.
  - *Albuminoids (Sclero proteins)*: Insoluble in all neutral solvents and in dilute acids and alkalis. These are the proteins of supportive tissue. Eg: Keratins and collagen.

## CONJUGATED PROTEINS

- These are simple proteins conjugated to non- protein substances known as prosthetic group.
- Further classification is based on the nature of the prosthetic group, attached to the simple proteins.

Nucleoproteins	Simple proteins associated with nucleic acids (DNA and RNA). Eg: chromatin of cell.
Glycoproteins	These are proteins having carbohydrates as prosthetic group. Glycoproteins contain less than 4% of carbohydrates whereas mucoproteins contain more than 4% of carbohydrates. Eg: mucin in saliva.
Lipoproteins	These are proteins associated with cholesterol, phospholipids and fatty acids.
Phosphoproteins	Phosphoric acid is the prosthetic group. Eg: casein in milk, vitelline in egg yolk.

Metalloproteins	These proteins are attached to various metal ions such as, copper, cobalt, iron, manganese and zinc. E.g. ceruloplasmin containing copper, carbonic anhydrase containing zinc.
Chromoproteins	Proteins are attached to colored pigments. Eg: hemoglobin, cytochromes and flavoproteins.

## **DERIVED PROTEINS**

- As the name implies that these proteins are formed from simple and conjugated proteins, from the action of heat, enzymes or chemicals.
- They are sub divided into,
  - Primary derived proteins
  - Secondary derived proteins

### **Primary derived proteins**

- The structure of these protein derivatives are slightly changed from original proteins. These are also called as denatured proteins. E.g. coagulated proteins. They are produced by action of alcohol and heat.
- Metaproteins: they are formed by the action of acid and alkali on proteins.

### **Secondary derived proteins**

- These are smaller molecules produced by the hydrolysis of proteins.
- They are generally water-soluble and not coagulated by heat. Eg: proteases, peptones and peptides are formed by the hydrolytic cleavage of proteins.

## **GLOBULAR AND FIBROUS PROTEINS**

### **Globular proteins**

- These are proteins, in which the polypeptide chain or chains are tightly coiled in three dimensions to form globular molecules. E.g., enzymes and plasma proteins.
- They are soluble in water.

### **Fibrous proteins**

- Fibrous proteins are those in which the polypeptide chains are either extended or coiled to form linear fibers.
- They are insoluble in water.
- They provide mechanical support to the cells or organism. E.g., keratin (the major component of hair and nail), collagen (component of skin, bones, teeth, blood vessel and connective tissues.) and elastin (structural component of skin and blood vessels).

# STRUCTURE AND PROPERTIES OF PROTEINS

## STRUCTURE ORGANISATION OF PROTEINS

Structure of protein is highly complex. There are 4 different levels of structures found in proteins; they are

- Primary
- Secondary
- Tertiary
- Quaternary structure.

### Primary structure

- The sequence of amino acids (the number, structure and order) in the chain of a protein is called the primary structure of proteins.
- Each chain has an amino group at one end, and a carboxyl group at the other end. By convention the peptide sequences are written with  $\text{NH}_2$  terminus on the left and the carboxy terminus on the right.
- Peptide bond between carbon and nitrogen shows partial double bond character.

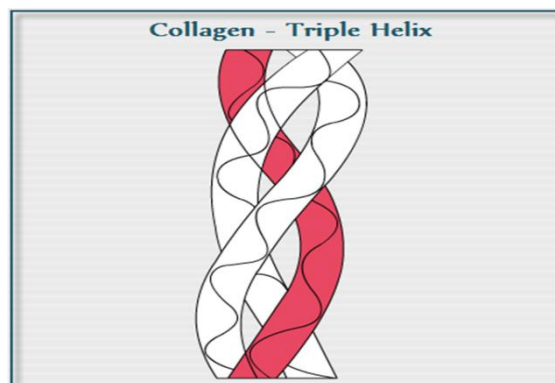
### Secondary structure

- Proteins are rarely found in an extended form. They tend to coil by themselves due to one part of a chain sticking to different parts of the chain by the formation of hydrogen bonds.
- The C=O and NH group of the peptide bond are capable forming hydrogen bonds. The Oxygen atom ( $\text{O}^-$ ) has a slight negative charge and the H has slight positive charge. Therefore, each can participate in the formation of hydrogen bonds. These hydrogen bonds result in 2 forms of secondary structures, in polypeptides. They are  $\alpha$  - helix and  $\beta$  - pleated structure.
- $\alpha$  - helices involve the amino acids in the same primary structural region,  $\beta$  - sheets involve stretches of 5 – 10 amino acids from different regions.
- **$\alpha$  - helix**
  - The simplest arrangement of the polypeptide chain could assume with its rigid peptide bond is a helical structure, which Pauling and Corey called as  $\alpha$  - Helix
  - The salient features of  $\alpha$  - Helix are given below,
    - The  $\alpha$  - helix is a tightly packed coiled (right hand helix) structure with amino acid side chains extending outward from the central axis. It was first found in  $\alpha$  - keratin, so the term  $\alpha$  - helix. The repeating unit is a single turn of the helix.
    - Each turn of  $\alpha$  - helix contains 3.6 amino acids, which permits the formation of intrachain hydrogen bonding between every fourth amino acid and travels a distance of 0.54 nm. The spacing of each amino acid is 0.15 nm.

- Portions of the polypeptide chains of many proteins are in the  $\alpha$  - helical configuration, but usually this form of secondary structure extends for only 10 or 15 amino acids and is then interrupted by non - helical stretches. Keratin (the protein of hair and wool), major proteins in muscle cells and hemoglobin (the protein of red blood cells) are examples of protein that contain large amount of  $\alpha$  - helical structure in their polypeptides.
- Charged R groups, bulky R groups and proline residues are unfavorable for the formation of the  $\alpha$  - helix.  
E.g., insoluble proteins of hair, feathers, hoofs and horns.

### Triple helix

- Collagen is the most abundant protein of the connective tissues. It is the fibrous protein. Collagen molecule consists of three polypeptides, called  $\alpha$  -helix, which wrap around each in a triple helix forming a rope like structure.
- The chains are held together by interchain hydrogen bonding. There are no intrachain hydrogen bonds. It is more extended than  $\alpha$  - helix. It has a repeating sequence as Gly-X-Y. Every third amino acid in the sequence is glycine, X-positions are occupied by proline and the Y-positions are occupied by hydroxyproline.



- **$\beta$  - pleated sheets**
  - It was first found after the elucidation of a structure hence, the name has been given.
  - In  $\beta$  - pleated structures, the hydrogen bonds are formed between 2 separate polypeptides or between 2 regions of the same polypeptide chains. It is in an extended form.
  - Interactions are between C= O of one peptide with NH of the other.
  - It is pleated because one  $\beta$ -carbon lies above and the other below the plane.
  - In the  $\beta$  - structures the R groups projects above and below the plane of the sheets.
  - The sheets are parallel, if the chains run in the same direction.

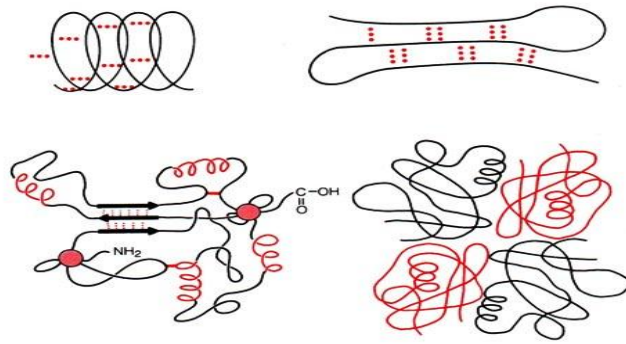
- If the chains run in the opposite direction, the sheets are anti parallel.
- This structure is less common in human protein. E.g. silk fibroin

### **Tertiary structure**

- It refers to the total three-dimensional structure of proteins.
- A polypeptide chain containing peptide bond and  $\alpha$  - helices and  $\beta$  - pleated structures undergoes further folding. This folding is called as tertiary structure.
- The R groups of amino acids, which project outward from  $\alpha$  - helical regions and  $\beta$  - structures play a major role in determining the tertiary structure of a protein. The R groups interact with one another.
- Because of the hydrophobic R groups interaction, a polypeptide chain tends to fold such that the hydrophobic R groups are concentrated inside the folded structure out of contact with H<sub>2</sub>O.
- The hydrophilic R groups are at the surface of a protein where they can interact with water.
- Other stabilizing forces are (a) presence of -SH groups of the amino acid cysteine (b) Ionic bond occurs when ionized acidic (-COO-) and basic groups(-NH<sub>3</sub><sup>+</sup>) are brought closure to each other.

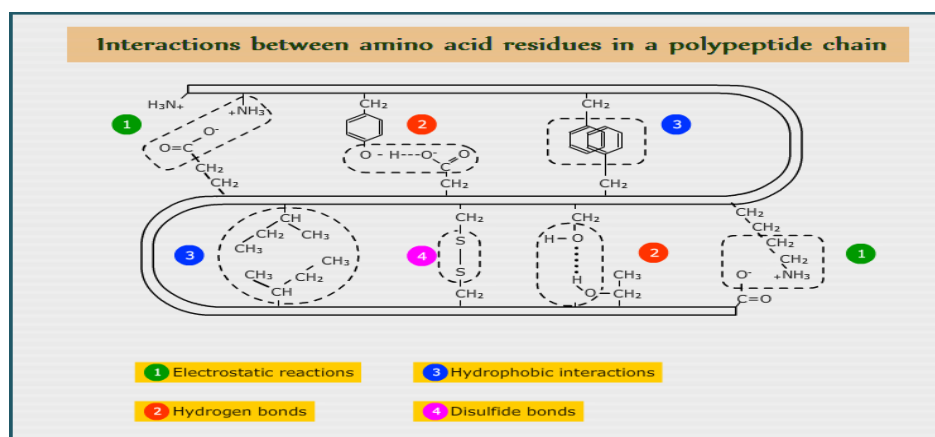
### **Quaternary structure**

- It is the 3 dimensional structures of proteins composed of more than one polypeptide chain (more subunits).
  - The individual polypeptides fold to yield secondary and tertiary structure but also bind to one another in a precise ways through hydrogen bonds, vander Waals forces, ionic bonds, disulfide bridges and hydrophobic interactions (non-covalent interactions). E.g. Hemoglobin and antibodies.
  - Many proteins contain more than one polypeptide chain (oligomeric protein), which may be identical or different in structure.
  - The individual single polypeptide chain of the oligomeric protein is called as Protomers or monomers or subunits.
  - If the subunits are of same type, then the protein is said to be homomeric and if the subunits are different, then, it is said to be heteromeric.



## INTERACTIONS STABILIZING THE PROTEINS

- Bonds that are stabilizing the proteins are
  - Hydrogen bonds
    - These bonds are formed when there is sharing of a hydrogen atom between two electronegative atoms. The hydrogen atom is covalently linked to one of the atoms and interact electro statically with second atom. It is formed in protein generally between amide nitrogen and the carbonyl oxygen.
  - Hydrophobic bonds
    - It is formed by the association of non-polar (hydrophobic side chains) side chains that come together to escape the water environment. These interactions are very important in forming protein structure.
  - Ionic interactions (electrostatic interaction or salt links)
    - This type of interaction occurs when oppositely charged groups such as  $\text{COO}^-$  and  $\text{NH}_3^+$  are brought closure. In the formation of nucleoproteins, ionic interactions are important in binding basic proteins with acidic molecules like phosphate.
  - Vander Walls forces
    - It is a non-specific attraction between atoms that are close but not overlapping and provide a weak attraction.



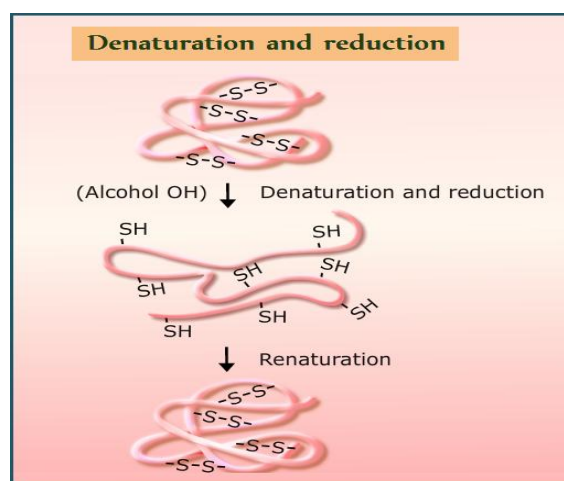
## CHEMICAL PROPERTIES OF PROTEINS

### Denaturation of proteins

- Secondary, tertiary and quaternary structure of proteins can be disrupted by chemicals like urea (6mol/L), 5M guanidinium hydrochloride, heat, high and low pH, detergents such as sodium dodecyl sulphate (1%) and sulfhydryl reagents such as mercaptoethanol.
- pH variations alter the ionization states of amino acid side chain, which changes protein charge resulting in the precipitation of proteins.
- High temperature disrupts a variety of interactions of proteins, hence, proteins lose the solubility and are then precipitated. An example is the denaturation of proteins caused by heating egg.
- Sodium dodecyl sulphate alters the protein structures by interacting with the non-polar residues of proteins thereby interfering with the hydrophobic interactions.
- High concentration of water-soluble organic substances such as aliphatic alcohols also interacts with hydrophobic forces.
- Solute such as urea can also precipitate proteins by disrupting hydrophobic interactions.
- The above conditions overcome the weak forces on which polypeptide chains are folded and disrupt hydrogen bonds causing polypeptides to unfold. Such unfolding is called denaturation. It is accompanied by loss of the normal biological function (e.g. enzyme activity) of the protein.
- Denatured proteins are usually not soluble in water, in part because denaturation exposes internal hydrophobic R groups. Denaturation does not break the primary structure of protein.

### Renaturation of proteins

- If a denatured protein returns to its native state, after the removal of denaturing agent, the process is called as renaturation.



## **Amphoteric nature**

- Due to the presence of  $\text{-NH}_2$  and  $\text{-COOH}$  group proteins are amphoteric.
- Charges on proteins are mainly due to the presence of the side chains of the amino acid residues, the N – terminal amino group and the C – terminal carboxyl group. The other carboxyl and amino group of each amino acids are involved in the peptide bond formation. Therefore, pI of proteins depends on the pH of the solution and pKa of amino acids forming the proteins.
- In solutions with pH values above the isoelectric point, the protein will have a net negative charge and at lesser pH values it will be positively charged.
- More proteins have pI values below 7 (due to presence of aspartate and glutamate), so that most proteins are negatively charged at neutral pH.

## **Precipitation of proteins**

- At isoelectric pH the proteins exist as zwitter ion (net charge is '0') at this pH the proteins are easily precipitated and it also shows no migration under an electric field.
- Proteins can be easily precipitated from solution by the addition of sulpho salicylic acid and phosphotungstic acid or by the addition of heavy metals like lead, silver and mercury. The principle behind this precipitation is by removing charges on the protein molecules.
- Alcohol also precipitates protein by reducing the dielectric constant of water (charges).

## **Salting out**

- Solubility of proteins is decreased by increasing the concentration of salt such as  $(\text{NH}_4)_2\text{SO}_4$ . The salts gradually dehydrate proteins by binding to water. The dehydrated proteins aggregate and precipitates.

## **Salting in**

- Solubility of some proteins is increased by increasing the concentration of neutral salts. This process is known as salting in.

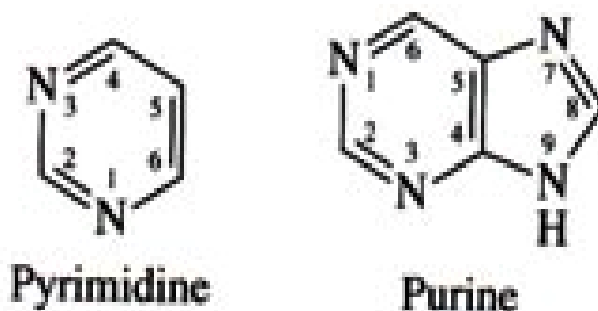


## **BIOCHEMISTRY OF NUCLEIC ACIDS**

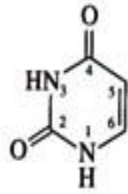
- Nucleic acids are nitrogen-containing compounds of higher molecular weight found in association with proteins in the cell.
- Nucleic acids account for 0.5 – 1 % of dry weight of the cell.
- They are conjugated proteins. The nucleic acid and protein complex is known as Nucleoproteins.
- The nucleoproteins can be separated into proteins and nucleic acids by treatment with acids or salts of higher concentration.
- The protein is usually a basic protein ( they contain more of arginine and lysine) such as protamines and histones.
- The nucleic acid is acidic due to the presence of phosphate group and are –vely charged under physiological condition.
- The 2 main groups of nucleic acids are Ribonucleic acid (RNA) and Deoxyribo nucleic acid (DNA).
- Hydrolysis of DNA and RNA under controlled conditions yield nucleotides and further hydrolysis of nucleotides yields nucleosides and phosphates.
- Hydrolysis of nucleosides then produces sugars, which may be either a ribose or a deoxyribose and a number of purine and pyrimidine bases. In nucleosides, the bases are linked to the pentose sugar by  $\beta$ -configuration.
- Purine bases are adenine and guanine. Pyrimidine bases are uracil, thymine and cytosine.
- A nucleic acid molecule is constructed of monomeric units called nucleotides joined together by phosphodiester bond.
- Nucleotides are composed of 3 parts, namely, nitrogenous base which may be a purine or a pyrimidine, a sugar molecule, which may be ribose or a deoxyribose and a phosphate group.
- Polynucleotide structure is written from the left to right ( $5' \rightarrow 3'$ ). The 5' end is phosphorylated and 3' end has a free hydroxyl group.
- At neutral pH, the purines and pyrimidines are hydrophobic and insoluble in water. At acidic or alkaline pH, the bases become charged and their solubility is increased in water.
- The length of the DNA is expressed as bp. Kb is a unit of 1000 bases in DNA or RNA sequence. In double stranded DNA it is kilo base pair ( kbp).

## CHEMISTRY OF DNA

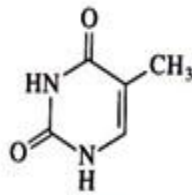
- DNA is built from 4 nucleotides. But, it is different from RNA in 2 important ways.
- The pyrimidine uracil in RNA is replaced in DNA by the pyrimidine - thymine. Uracil and thymine are very similar in structure. In thymine, the pyrimidine ring has a methyl group attached to the 5th carbon atom in the ring.
- The sugar ribose is present in RNA nucleotides and deoxyribose is present in DNA nucleotides.
- Deoxyribose is a ribose molecule that has a hydrogen atom on the 2nd carbon atom instead of a hydroxyl group.
- The attachment of deoxyribose to the two purines (adenine and guanine) and to the two pyrimidines (cytosine and thymine) yields 4 nucleosides.
- Because the sugar is deoxyribose instead of ribose, the nucleosides are called deoxy nucleosides.
- The 4 deoxynucleosides of DNA are deoxyadenosine, deoxyguanosine, deoxycytidine and thymidine (because, thymidine occurs only in DNA, it always contains the deoxy ribose and the deoxy – prefix is omitted).
- A deoxynucleotide is a deoxynucleoside with PO<sub>4</sub> group (deoxynucleotide = deoxynucleoside phosphate).
- The four deoxynucleotides are deoxyadenosine monophosphate (dAMP), deoxyguanosine monophosphate (dGMP), deoxycytidine monophosphate (dCMP) and thymidine monophosphate (TMP).
- The four deoxynucleotides are also called as deoxyadenylic acid, deoxyguanylic acid, deoxycytidylic acid and thymidylic acid.



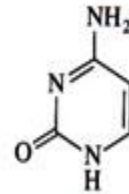
## PYRIMIDINES



Uracil  
(2,4-Dioxypyrimidine)

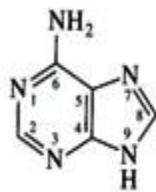


Thymine  
(2,4-Dioxo-5-methylpyrimidine)

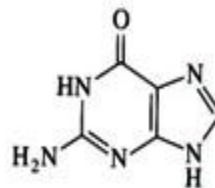


Cytosine  
(2-Oxo-4-aminopyrimidine)

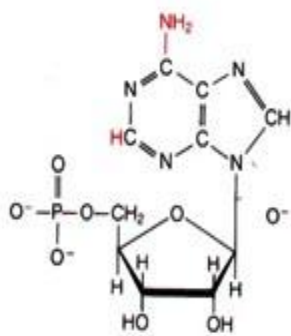
## PURINES



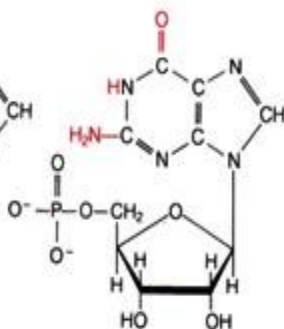
Adenine  
(6-Aminopurine)



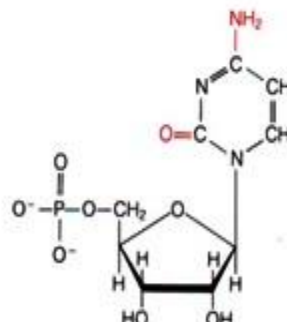
Guanine  
(2-Amino-6-oxopurine)



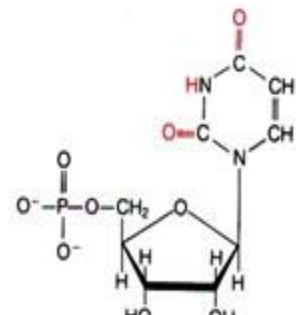
Adenosine  
monophosphate



Guanosine  
monophosphate



Cytidine  
monophosphate

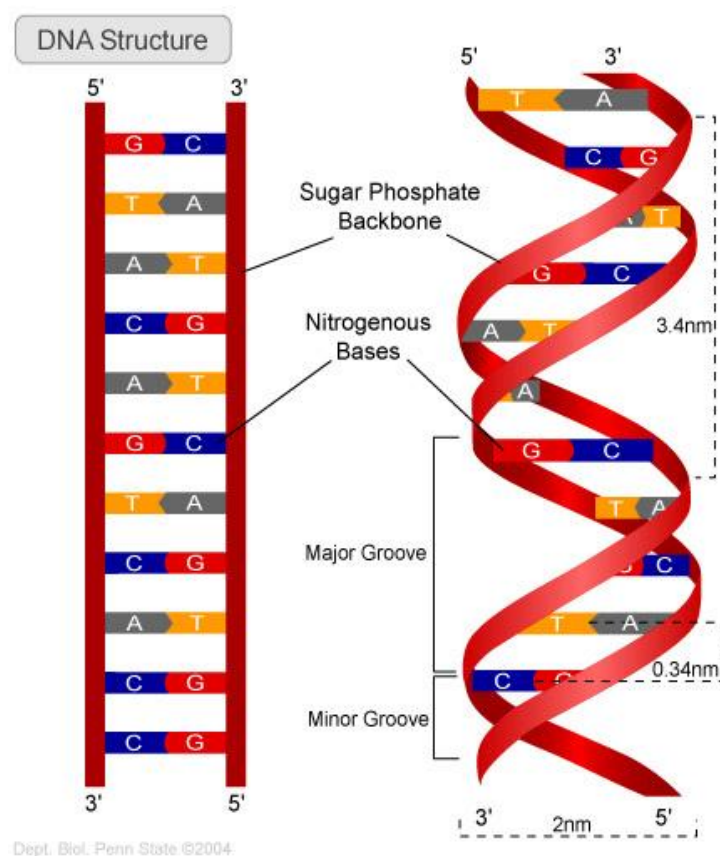


Uridine  
monophosphate

## STRUCTURE OF DNA

- The double helical structure of DNA was proposed by James Watson and Francis Crick in 1953.
- Each DNA molecule is made of 2 polynucleotide chains joined by hydrogen bonds between the bases.
- The mononucleotides are linked together by a phosphodiester linkage. Sugar and phosphate are seen around the helix, (outside surface). They are exposed to the aqueous environment. The hydrophobic bases are located in the interior of the helix.

- The position on the purine and pyrimidine rings are numbered and the position on the sugar carry a prime symbol to differentiate them from the ring position of the base.
- The purine or pyrimidine bases are attached to 1' carbon of the sugar. The phosphate is attached to 5' carbon. The 3' carbon is used for making bond with the phosphate of another nucleotide forming 3' → 5' phosphodiester bond.
- The 2 chains of DNA are antiparallel. One chain runs in 3' → 5' direction and the other runs in 5' → 3' direction.
- The base sequences of 2 strands are complementary.
- Adenine of one chain pairs with the thymine on the other chain. There are 2 hydrogen bonds between adenine and thymine.
- Guanine of one chain pairs with the cytosine of the other. There are 3 hydrogen bonds between guanine and cytosine.
- The double stranded molecule is twisted to form a helix with major and minor grooves.
- The diameter of the double helix is 20 Å (angstrom) units. Each turn of the helix is 34 Å units with 10 pairs of nucleotides.
- The purine or pyrimidine bases are bonded to carbon atom 1 of the sugar whereas the individual nucleotides are united by means of a phosphodiester linkage between carbon atom 3 of one sugar molecule and a terminal carbon 5 of the next.



## DNA DENATURATION

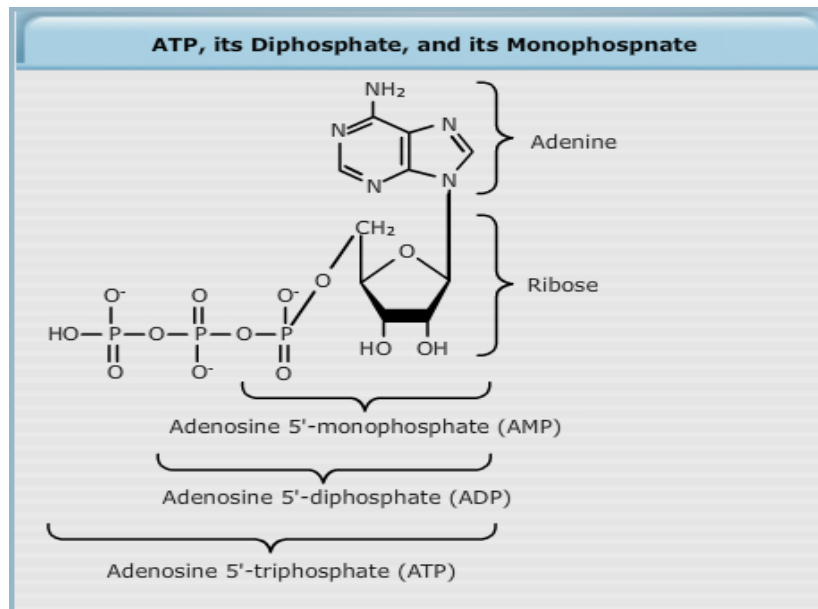
- Complete unwinding and separation of the complementary single strand is called denaturation. Denatured DNA consists of two single strands. Double stranded DNA is stable than the separated strands.
  - The strength of double strand DNA depends on ionic strength, its pH and base compositions. The double strand of DNA can be denatured by raising pH or temperature or drastically lowering the ionic strength. When a DNA solution is heated enough, the non-covalent forces that hold the two strands together weaken and finally break. When this happens, the two strands come apart in a process known as DNA denaturation, or DNA melting. The temperature at which the DNA strands are half denatured is called the melting temperature, or  $T_m$ . The amount of strand separation, or melting, is measured by the absorbance of the DNA solution at 260nm. When the two strands separate the absorbance rises 30% - 40%. This is called hyperchromic shift.
  - When the denatured DNA is brought to physiological condition, the single strands come back together to regenerate the B form of DNA.
  - Due to the  $-ve$  charge of phosphate groups, the two strands of DNA repel one another. When salt is added, The cations (monovalent or divalent) such as  $Na^+$ ,  $Mg^{2+}$ ,  $Mn^{2+}$  and  $Co^{2+}$  interact with the ionic phosphate groups of DNA and act as a shielding agent (repulsive force is reduced), hence the DNA is stabilized.
  - Enzymes that catalyze the hydrolysis of phosphodiester in nucleic acids are called nucleases. Ribonuclease acts on RNA and deoxyribonuclease acts on DNA. Nuclease can be further classified as exonuclease, which catalyzes the hydrolysis of phosphodiester linkage to release nucleotide residues from only one end of polynucleotide chain, or endonuclease, which catalyzes the hydrolysis of polynucleotide linkage at various sites within the polynucleotide chain.
  - At high temperature the phosphodiester bond may be broken into pieces but in the presence of alkali the DNA is unwound but not broken.
  - Presence of formaldehyde causes irreversible denaturation.
  - RNA is degraded under alkaline solution, whereas DNA is stable.
- ### Forms of DNA
- There are at least 6 forms of DNA. A, B, C, D, E and Z of which three are the major structural forms of DNA - the A, B and Z. The predominant form of DNA is the 'B' form, which is a right-handed helix with 10 base pairs per turn of the helix. This form is present in all the cells and its structure has been described above.
  - The 'A' form is produced by dehydrating the 'B' form. It is also a right-handed helix. 11 base pairs are present per turn.
  - The 'Z' form is a left-handed helix with 12 base pairs per turn. Due to its zigzag structure it is called as 'Z' form.

Characteristics	A-DNA	B-DNA	Z-DNA
Conditions	75% relative humidity; Na <sup>+</sup> , K <sup>+</sup> , Cs <sup>+</sup> ions	92% relative humidity; Low ion strength	Very high salt concentration
Shape	Broadest	Intermediate	Narrowest
Helix sense	Right-handed	Right-handed	Left-handed
Helix diameter	25.5Å°	23.7Å°	18.4Å°
Rise per base pair ('h')	2.3Å°	3.4Å°	3.8Å°
Base pairs per turn of helix ('n')	11	10.5	12
Glycosidic bond	anti	anti	anti for C, T & Syn for A, G
Major groove	Narrow and very deep	Wide and quite deep	Flat
Minor groove	Very broad and shallow	Narrow and quite deep	Very narrow and deep

### FUNCTIONS OF NUCLEOTIDES

- ATP is the principal form of chemical energy, available to cells. It is used as a phosphorylating agent and is also involved in muscle contraction, active transport and maintenance of ionic gradients. ATP is also a phosphate donor for the synthesis of other 5' – triphosphates.
- Nucleotides are the monomeric units of nucleic acids (DNA & RNA).
- Nucleotides are used in the synthesis of second messengers like cAMP and cGMP for the hormonal functions.
- Many of the regulated steps of metabolic pathways are controlled by intracellular concentrations of nucleotides, as allosteric regulator.
- They serve as a carrier of high-energy intermediates in the biosynthesis of carbohydrates, lipids and proteins. e.g.
  - GTP is involved in the synthesis of glucose (gluconeogenesis).
  - GDP is involved in the oxidation of α - Ketoglutaric acid to succinyl CoA to form GTP.
  - Uracil derivatives UTP is involved in the synthesis of glycogen and also in the epimerization of galactose and glucose (lactose biosynthesis).
  - Cytosine derivatives are involved in the biosynthesis of phosphoglycerides in animal tissues.

- Nucleotides are also structural components of several coenzymes of B complex vitamins. E.g. NAD, FAD, and pantothenic acid in Co- enzyme A.
- Biologically important nucleoside, S- adenosylmethionine (adenosyl derivative) is involved in several transmethylation processes.



## CHEMISTRY OF RNA

- RNA is built from 4 kinds of nucleotides. They contain the 4 nitrogenous bases, 2 purines (Adenine and Guanine) and 2 pyrimidines (Cytosine and Uracil).
- A purine or pyrimidine with a ribose molecule attached to it is called as Nucleoside.
- Attachment of phosphates to the sugar of a nucleoside makes a nucleotide.
- The 4 nucleotides of the RNA are adenylic acid, guanylic acid, cytidilic acid and uridylic acid.
- A nucleotide is also sometimes called as nucleoside monophosphate that is, adenosine monophosphate (AMP), guanosine monophosphate (GMP), cytidine monophosphate (CMP) and uridine monophosphate (UMP).
- In summary,
  - Purine or pyrimidine + ribose = nucleoside
  - Nucleoside +  $\text{PO}_4$  = nucleotide (nucleoside monophosphate)
- Or specifically,
  - Adenine + ribose = adenosine
  - Adenosine +  $\text{PO}_4$  = adenylic acid (adenosine monophosphate or AMP)

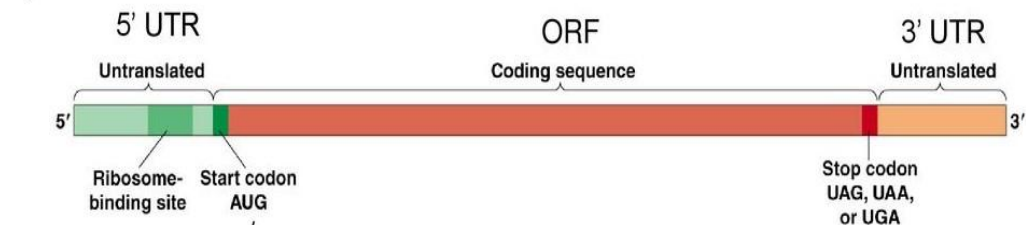
## TYPES OF RNA

- There are 3 major classes of RNA molecules viz.,
  - Messenger RNA (m RNA)
  - Transfer RNA (t RNA)
  - Ribosomal RNA (r RNA)
- All are synthesized from DNA base sequences.
- The RNA is involved in the process of protein biosynthesis. The RNAs differ from each other in terms of their size, function and some structural modifications.

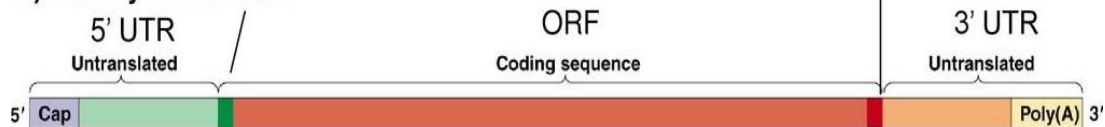
### MESENTER RNA (mRNA)

- They constitute 5 – 10% of the total RNA concentration in the cell.
- mRNA carries genetic information from the DNA to the cytoplasm, where it is used as a template for protein synthesis.
- mRNA has high molecular weight with a relatively short half – life.
- Two important features are,
  - The presence of a cap (7 – methyl guanosine triphosphate) at the 5' end,
  - A poly A tail at 3' end.
- The cap structure protects the 5' terminus from the action of exonuclease.
- The poly A tail is believed to have a role in transporting mRNA out of the nucleus. Note that not all mRNA have poly A tail.

#### a) Bacterial mRNA



#### b) Eukaryotic mRNA

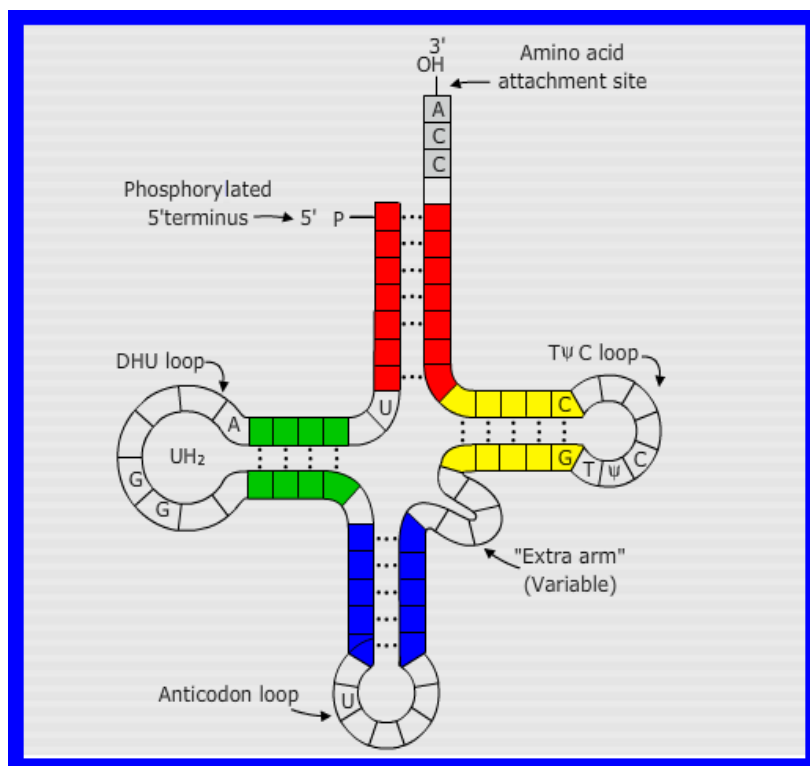


- *Functions of mRNA*
  - mRNA acts as a template for protein synthesis. The codons of mRNA determine the amino acid sequence of protein.
  - It interacts with the smaller subunit of the ribosome to initiate protein synthesis.
  - The anticodon of aminoacyl tRNA binds with the codon of mRNA by following the base pairing rule.
  - When synthesis is completed, the terminating codon, UAA, UGA and UAG causes the release of polypeptide chain.



## TRANSFER RNA (tRNA)

- They constitute about 15% of the total RNA in the cell. tRNA is also known as soluble RNA.
- tRNA is a small, single stranded molecule consists of approximately 75 – 90 nucleotides.
- It is the smallest of the 3 types of RNA.
- *Structure of tRNA*
  - All tRNA have a cloverleaf secondary structure, which is the arrangement that gives the maximum intramolecular complementary base pairing.
  - All tRNA molecule contains 4 main arms, viz.,
    - Amino acid acceptor arm consists of base sequences of CCA at the 3' end, which carries the amino acid.
    - Anticodon arm, which includes the triplet anticodon that interacts with mRNA in the process of protein synthesis.
    - D arm, so called because of its high content of modified base dihydrouridine.
    - T $\Psi$ C arm contains the T $\Psi$ C sequence, where the  $\Psi$  is the modified base, pseudouridine.
  - There is at least one specific tRNA for each of the amino acid.
- *Functions of tRNA*
  - tRNA molecules activate amino acids and bring them to ribosome for protein synthesis.
  - tRNA is to recognize the codon of the mRNA to ensure that the correct amino acid is incorporated.



## RIBOSOMAL RNA (r RNA)

- The ribosomal RNA accounts for 80% of the total cellular RNA.
- On the ribosomes the m RNA and t RNA molecules interact to form protein.
- All ribosomes contain 2 sub units of unequal size.
- Ribosomal RNA is found in association with a number of different proteins as components of ribosome.
- Prokaryotic ribosomes are smaller than eukaryotic ribosome.
- The intact prokaryotic ribosome is 70S ribosome (S- Svedberg unit, a measure of sedimentation rate). The intact eukaryotic ribosome is 80S.
- The prokaryotic ribosome 70S comprises 3 types: 16S, 23S and 5S rRNA. 16S r RNA, complexes with proteins and forms the 30S ribosomal subunit (smaller subunit), while 23S and 5S r RNAs complex with proteins and form the 50S ribosomal subunit (larger). The 30S and 50S ribosomal subunits join to form the 70S ribosome.
- The Eukaryotic ribosomes contain four types of r RNA molecules of 18, 28, 5, and 5.8S. 18S r RNA is complexed with proteins to form the 40S ribosomal subunit and the 28, 5 and 5.8S r RNAs are complexed with proteins to form the 60S ribosomal subunit. The 40 and 60S ribosomal subunits combine to form the 80S ribosome.

### Functions of ribosomal RNA

- The binding of m RNA to ribosome.
- In the formation of peptide bond, catalyzed by the larger subunit.
- The 70S ribosome contains 2 functional sites termed as A site and the P site. Aminoacyl – t RNA binds at the A site and peptidyl – t RNA binds at the P site. These sites are made of portions from both smaller and larger subunits.
- The enzyme that catalyses the peptide bond formation (Peptidyl transferase) occurs in the large subunit.

