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CARBOHYDRATE CHEMISTRY

Topics Discussed

- Classification of Carbohydrates.
- Monosaccharides: Cyclic structure, isomerism, optical activity, mutarotation, properties.
- Oligosaccharides:
    - Disaccharides: Maltose, lactose, cellobiose, sucrose, trehalose.
    - Trisaccharides: Rhafinose.
    - Tetrasaccharides: Stacchyose.
- Polysaccharides:
    - Homopolysaccharides: Starch, dextran, glycogen, cellulose, inulin, agar-agar, chitin.
    - Heteropolysaccharides: Plant gums and mucilages, pectins, glycoproteins, mucopolysaccharides.
- Mucopolysaccharidosis.

Learning Objectives:

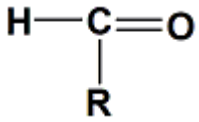
After understanding this part the student should be able to:

- Compare and contrast the structure and medical importance of the different types of carbohydrates.
- Describe the bases of the different characteristics of monosaccharides.
- Describe the medical importance of the different types of oligosaccharides and their disease implications.
- Describe the medical importance of the different types of oligosaccharides and their disease implications.

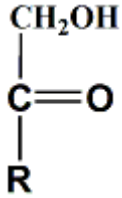
Carbohydrate Chemistry

Definition:

Carbohydrates are polyhydroxy aldehydes or polyhydroxy ketones or substances that yield these compounds on hydrolysis. Many, but not all carbohydrates have the common formula: $C_x(H_2O)_y$, where x and y are whole numbers that differ depending on the specific carbohydrate to which we are referring. Nevertheless, carbohydrates may also contain nitrogen, phosphorous and sulfur.



Polyhydroxy aldehyde



Polyhydroxy kettone

Importance:

1. Oxidation of carbohydrates constitutes the main energy source for the human body.
2. Insoluble carbohydrate polymers serve a structural and protective role in the walls of bacteria and plants and connective tissue of animal.
3. Some carbohydrate polymers are concerned with the lubrication of joints and tendons.
4. Carbohydrates participate in the recognition and adhesion between cells.
5. Some carbohydrate polymers are attached covalently to proteins or lipids to form complex molecules called glycoconjugates which include glycoproteins , proteoglycans & glycolipids. They serve as mediators of specific cell-to-cell

interaction and adhesion and interaction between cells and extracellular matrix.

Classification of carbohydrates:

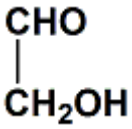
Depending on the basic units contributing to the carbohydrate, they are classified into:

1. **Monosaccharides:** They are the simplest sugars that consist of a single saccharide unit (polyhydroxy aldehyde or ketone) and cannot be further hydrolyzed.
2. **Oligosaccharides:** They are carbohydrates that are formed of 2-10 monosaccharides linked by glycosidic linkage and give monosaccharides on acid hydrolysis
3. **Polysaccharides:** They are carbohydrates that are formed of more than10 monosaccharides linked by glycosidic linkage and give monosaccharides on acid hydrolysis.

I. Monosaccharides

- They are classified according to the number of carbon atoms into five important groups. Each of these groups is subdivided according to the type of functional chemical group into: **Aldoses "aldo-sugars**"(sugars containing aldehyde group) and **Ketoses"keto-sugars"** (sugars containing ketone group).

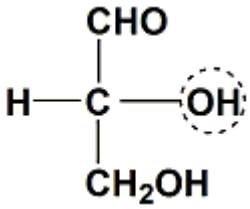
1. Bioses: are monosaccharides containing **2** carbon atoms, e.g.,



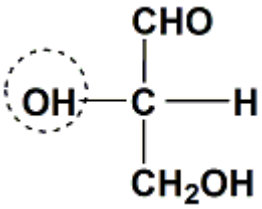
Glycolaldehyde

2. Trioses: are monosaccharides containing 3 carbon atoms, e.g.

A- Aldotriose: Example is



D-Glyceraldehyde



L-Glyceraldehyde

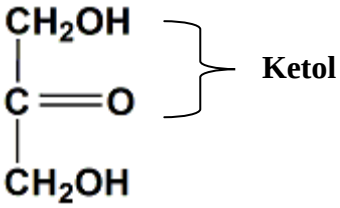
- The mother compound of all aldoses is the glyceraldehydes and so called the ***reference sugar***.

- D- Glyceraldehyde differs from L-form only in one carbon atom (before the last one i.e.subterminal carbon atom), where the OH (hydroxyl group) in the D-form at the right, while in the L-form at the left.

- An aldose is formed at C1 of aldehyde group (H—C=O), at the end, it contains a terminal alcohol group (primary alcohol group, CH_2OH) , and in between the secondary alcohol groups are arranged (H—C—OH) according to the total number of carbons present in each molecule.

- Aldoses, which are present in nature, are mainly derivatives of D-glyceraldehyde, so all contain the OH group at the carbon atom before the last on the right side.

B- Ketotriose: e.g.



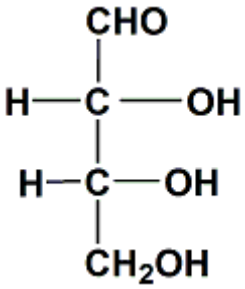
Dihydroxyacetone, the hydroxylated form of acetone

- The mother compound of all ketoses is the dihydroxyacetone and is present only in one form.

- All ketoses have two terminal primary alcohol groups (CH_2OH) and have one ketone (C=O).
- If the number of carbons exceeds 3 (4 or more), the secondary alcohol groups are arranged between the ketol (or carbonyl) group and the terminal alcohol group.

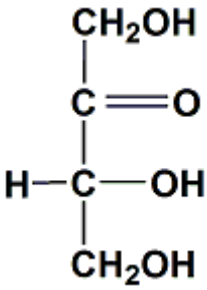
3. Tetroses*:* are monosaccharides containing 4 carbon atoms:

A- Aldotetroses:



D-Erythrose

B- Ketotetroses:



D-Erythr*ulose*

- Erythrulose contains 4 carbon atoms.
- It present in D and L forms,where the OH group may be present on the right or on the left side of the carbon atom before the last.
- Most of naturally occurring ketoses are mainly in the D-form , and considered as derivatives of D-erythrulose.

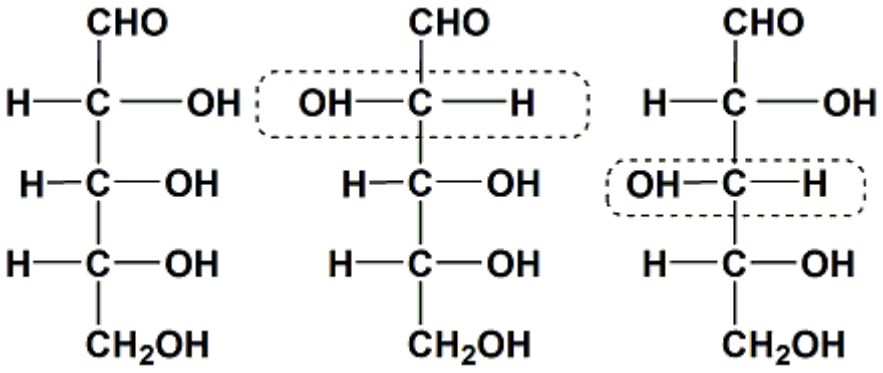
4. Pentoses:

Importance (Function):

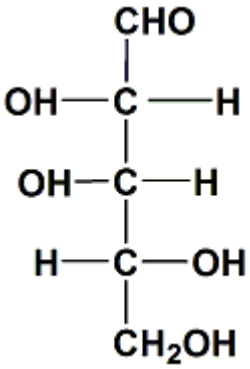
- They are monosaccharides that contain 5 carbon atoms.
- Ribose is the most important pentose because it enters in the structure of DNA and RNA and important free nucleotides such as ATP,GTP and other high energy phosphate compounds and coenzymes NAD , NADP and flavoproteins.
- Ribose phosphate and ribulose phosphate are intermediates in the pentose phosphate shunt (a minor pathway for glucose oxidation).
- Arabinose and xylose are constituents of glycoproteins in plants and animals.
- Xylulose is an intermediate in uronic acid pathway (a minor pathway for glucose oxidation).

Examples:

A- Aldopentoses:

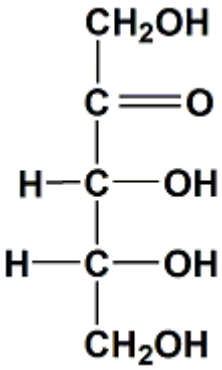


D-Ribose **D-Arabinose** **D-Xylose**

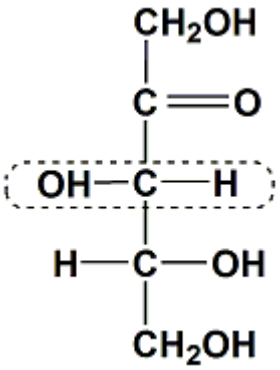


D-Lyxose

B- Ketopentose:



D-Rib*ulose*



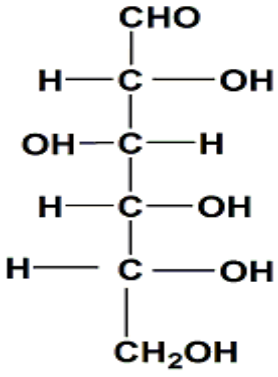
D-Xyl*ulose*

5. Hexoses*:*

Are monosaccharides containing 6 carbon atoms. They are the most important monosaccharides particularly glucose:

A- Adohexoses:

1.Glucose:

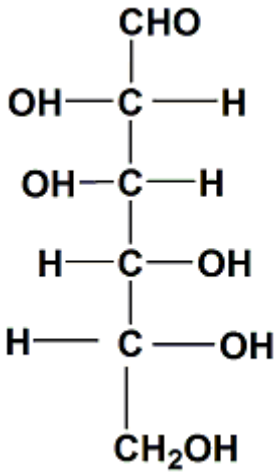


D-glucose

- It is called grape sugar, blood sugar or **Dextrose** because it is dextrorotatory.
- It is fermentable, reducing and gives needle-like osazone crystals.
- It gives soluble saccharic acid with concentrated nitric acid.
- It is produced by hydrolysis of starch, dextrins, glycogen, sucrose, maltose and lactose.
- All monosaccharides have to be converted into glucose to be utilized in the body.
- In cases of diabetes mellitus , it is raised in the blood (hyperglycemia) and appears in urine(glucosuria).

2.Mannose:

- It is a subunit in glycoproteins and sialic acid
- It is obtained by hydrolysis of some plant carbohydrates such as plant mannosans and gums.
- It is reducing and gives needle-like osazone crystals.

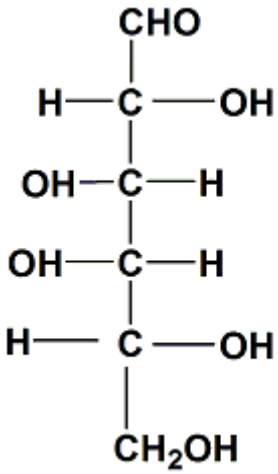


D-Mannose

3. Galactose:

- It is a subunit of the milk sugar, lactose that is synthesized in the lactating mammary glands.

- It is produced by hydrolysis of lactose.
- It is synthesized from glucose in the body and converted into glucose in the liver to be used as source of energy.

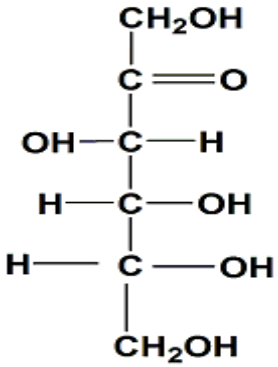


D-Galactose

- It enters in structure of glycoproteins, glycolipids, sulfolipids and mucopolysaccharides in its amine form.
- It accumulates in blood and appears in urine with cataract in a disease called galactosemia. It is reducing and gives sun-like osazone crystals.
- It is non-fermentable except with a special type of yeast.
- It gives insoluble galactic acid (mucic acid) when reacts with concentrated nitric acid.

B- Ketohexoses:

1. Fructose:



D-Fructose

- Also called levoulose because it is levorotatory. It is called also the semen sugar.
- It is the main sugar in bee's honey and fruits.
- It is the sweetest sugar known and can be converted into glucose in the liver to be used as source of energy.
- It is reducing and gives needle-like osazone crystals.
- It is obtained from inulin and sucrose hydrolysis.
- It is fermentable and gives cherry red color with Seliwanoff's reagent.
- Accumulation of fructose in case of fructose intolerance leads to hypoglycemia.

Asymmetric carbon atoms and their relation to isomerism and optical activity

Definition:

Asymmetric carbon atom is the carbon atom to which is attached 4 different groups or atoms.

Example: Sub-terminal carbon atom or the one before the last carbon atom or carbon atom just above the primary alcohol group (CH2OH) is an asymmetric carbon atom (the central carbon in glyceraldehyde).

- The presence of a double bond (as in the aldehyde group), or two or more similar atoms or groups (as in the terminal primary alcohol group) makes the carbon atom symmetric.
- All monosaccharides contain one or more asymmetric carbon atoms except dihydroxyacetone.

Importance of asymmetric carbon atom:

Any compound containing asymmetric carbon atom has the following two properties:

1. Isomerism is created around it.
2. It makes the compound optically active.

Isomerism

Definition:

Isomers are substances which have the same molecular formula but differ in distribution of their atoms into groups or distribution of these groups and atoms in the space around carbon atoms.

Types:

I- Structural isomerism.

II- Stereo-isomerism.

I. Structural isomerism:

Definition:

They are isomers that have different structure due to different ways of arrangement of atoms and groups forming the molecule.

Types:

a. Chain isomerism.

b. Positional isomerism:

c. Functional group isomerism:

d. Ring isomerism:

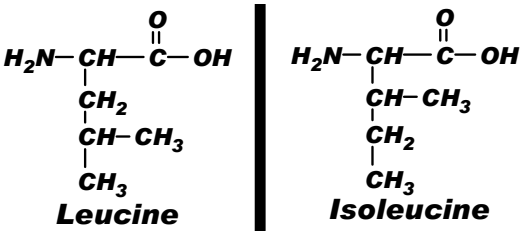
Each of them will be discussed in some details:

a. Chain isomerism.

Definition:

These are isomers that have different structures due to different ways of attachment of carbon atoms forming the molecule.

Example: Leucine and iso-Leucine .

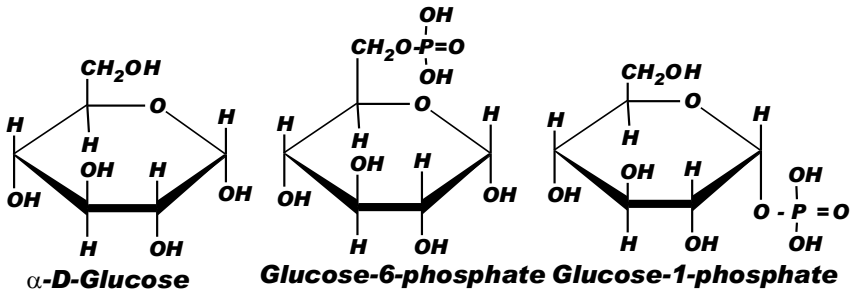


b. Positional isomerism:

Definition:

These are isomers that have the same carbon skeleton but differ in the position of the substituent groups.

Example: Glucose, glucose-6-phosphate, glucose-1-phosphate.



c. Functional group isomerism:

Definition:

These are isomers that have the same carbon skeleton, the same position of substituent group but have different functional group (aldo-/keto-).

Examples of aldo/keto isomers include:

- Trioses: glyceraldehyde/dihydroxyacetone.
- Tetroses: erythrose/erythrulose.
- Pentoses: ribose/ribulose, xylose/xylulose.
- Hexoses: glucose/fructose, mannose/fructose.

d. Ring isomerism:

- Pyran/furan forms such as glucopyranose and glucofuranose, and fructopyranose and fructofuranose.

II. Stereo-isomerism:

Definition:

- They are molecules having the same structure but differ in position of their different groups and atoms in the space, i.e., in spatial configuration.
- The number of stereoisomers = 2^n, where n is the number of asymmetric carbon atoms in the molecule. So:
    1. Aldotrioses contain one asymmetric carbon atom, so they have two isomers.
    2. Aldotetroses contain two asymmetric carbon atoms, so they have four isomers.
    3. Aldopentoses contain three asymmetric carbon atoms, so they have 8 isomers.
    4. Aldohexoses contain four asymmetric carbon atoms, so they have 16 isomers.
- The number of asymmetric carbon atoms in case of ketoses is less by one compared to the corresponding group of aldoses as follows:

Group	Number of asymmetric carbon atoms	Number of streoisomers
• Aldotrioses.	1	2
• Aldotetroses.	2	4
• Ketotetroses.	1	2
• Aldopentoses.	3	8
• Ketopentoses.		4
• Aldohexoses.	2	16
• Ketohexoses.	4 3	8

Types:

There are four types of stereo-isomerism as follows:

a. D and L isomerism (enantiomers).

b. Epimers.

c. Anomers.

d. Geometric isomerism.

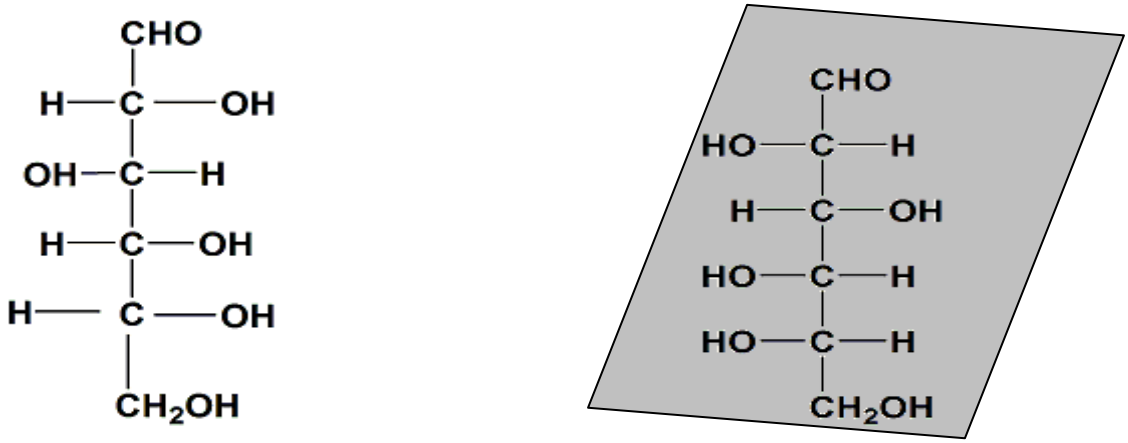
a. D and L isomerism (enantiomers):

Definition:

- They differ in distribution of H and OH groups around the sub-terminal asymmetric carbon atoms (the one before the last carbon).
- D form has the OH group to the right of the sub-terminal carbon atom whereas; it is on the left in L form.
- This difference makes the two forms (D and L) mirror image to each other due to the change of the position of all H and OH groups into the opposite direction of D form in L form.
- Metabolizable sugars in human body are D-forms only.

Examples:

1. D-glucose and L-glucose.



D-Glucose is mirror image to its isomer L-Glucose

2. Other examples include: D-mannose & L-mannose, D-galactose& L-galactose &D-fructose & L-fructose, D-ribose & L-ribose,D-erythrose &Lerythrose , D-erythrulose&L-erythrulose and so on.

Optical isomerism:

- These are isomers created due to the presence of an asymmetric carbon.
- D- or L-sugar can be dextrorotatory (+) or levorotatory (-), i.e., D (+) and D (-), and L(+) and L(-). D (+) and D (-).
- Most of natural D-forms are + (d) and L-forms are – (l).
- **Racemic mixture** is a mixture of equal amounts of dextrorotatory and levorotatory isomers of a compound, or DL mixture. This mixture is optically inactive because they antagonize the effect of one another e.g. solution contains equal amounts of D & L glucose.

b. Epimers:

Definition:

They are stereoisomers which differ in distribution of H and OH groups around a single asymmetric carbon atom other than the anomeric and DL-form creating carbon before the last i.e., without difference on other carbon atoms.

Examples:

- Ribose is an epimer to each of arabinose and xylose.
- Glucose is an epimer to each of mannose and galactose.
- Arabinose and xylose as well as galactose and mannose are not epimers to each other because they differ around

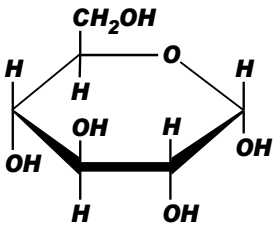
distribution of H and OH in more than one asymmetric center.

c. Anomers:

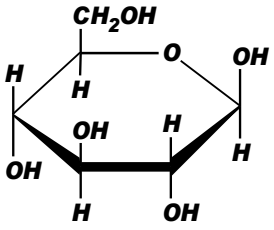
Definition:

They are stereoisomers which differ in distribution of H and OH group around the asymmetric anomeric carbon atom which is C1 in aldoses or C2 in ketoses after cyclization of the molecule. If the OH on the right side of the anomeric carbon atom , it is called α-form while if the OH on the left side it is called β-form.

Examples: α- and β-glucose are anomers.



α-D-Glucose



β-D-Glucose

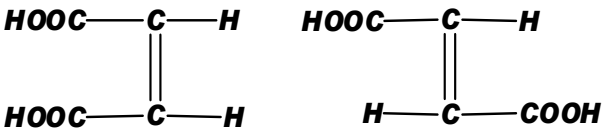
d. Geometric isomerism:

Definition:

It involves distribution of atoms or groups around the axis of a double bond in the space.

Example:

cis-Maleic acid and trans-Fumaric acid. Maleic acid is a cis-form that means that the two radicals of the molecule around the double bond are on one side. Whereas, its trans-form isomer, Fumaric acid, has the two radicals of the molecule around the double bond are located on opposite sides.



Maleic acid
(cis form)

Fumaric acid
(trans form)

Optical activity

Definition:

- It is the ability of the sugar to rotate the plane polarized light either to the right, so it is called **dextrorotatory** (d or +) such as glucose, galactose and starch or to the left and so it is called **levorotatory** (l or -) such as fructose and invert sugar.
- It is due to the presence of at least one asymmetric carbon atom in the molecule. Therefore, all monosaccharides are optically active except dihydroxyacetone , which has no asymmetric carbons in its structure .
- Ordinary light vibrates in all directions. Ordinary light can be changed to plane polarized light by passing it through a prism made of calcium carbonate (calcite, CaCO3, Nicole's prism or polarizer). Plane polarized light vibrates in one plane and direction.

Measurement of the optical activity:

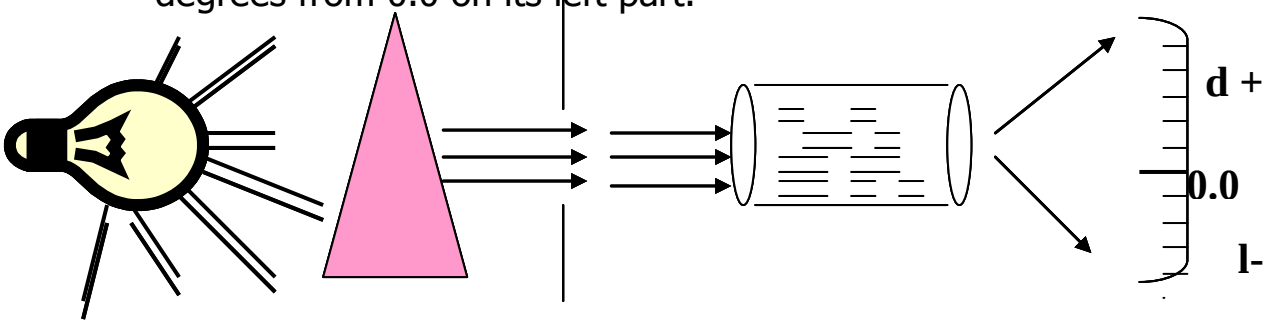
- Optical activity is determined by **polariscope or polarimeter** .
- ***Structure of the polariscope:***

It is consists of:

1. A light source (usually a sodium lamp).

2. A polarizer that is a prism made of calcite.

3. A narrow slit path to bring forth a parallel beam of light.
4. A polarimeter tube to contain the solution of the substance tested (mostly 1.0 decimeter in length).
5. A scale graduated in increasing positive (+) degrees from 0.0 on its right part and in increasing negative (-) degrees from 0.0 on its left part.



Factors affecting optical activity:

1. Type and Concentration of the substance and type of solvent.
2. Type of light used and temperature.
3. Length of polarimeter tube in decimeters.(1 decimeter=10 cm).

Specific rotation:

- It is the observed angle of deviation of the plane polarized light in degrees from the straight path. It is measured when the solution of the substance or the sugar dissolved in water is introduced in the path of the plane polarized light under the following conditions. The light source used is sodium light, the temperature is 20°C, the concentration is 1 gm/ml and the polarimeter tube is one decimeter in length.
- It is calculated by the following equation,

$$[\alpha]_D^{20\,^{\circ}C} = \frac{a \times 100}{L \times c}$$

Where: a = Angle observed in degrees, C = Concentration in gm/ml, and L = length of polarimeter tube in decimeters, D=sodium light source, α=observed rotation

- Each optically active substance has a characteristic specific rotation such as, α-glucose is +112, β-glucose is +19 and fructose is - 92.5.

Uses of polariscope and importance of optical activity:

1. Identify an unknown optically active substance.
2. Identify whether the substance is optically active or not.
3. Identify whether the substance is levorotatory or dextrorotatory.
4. Determine the concentration of the substance.
5. Differentiate between glucosuria and lactosuria. This is important in late pregnancy to differentiate between diabetes mellitus (glucose) and the normal appearance of lactose produced by the mammary glands in urine.

Mutarotation

Definition:

- It is a temporary change in the specific rotation of an optically active sugar when it is freshly prepared. Mutarotation is due to the presence of a free anomeric carbon (C1 in aldoses, C2 in ketoses).
- With time, it tends to stabilize at the specific rotation of the compound. Therefore, it is recommended not to measure specific rotation of the solution directly after its preparation.

Examples:

- For example, α-glucose when freshly prepared has a specific rotation of +112, then the specific rotation

decreases gradually till it stabilizes at +52.5 (point of equilibrium).

- β-glucose when freshly prepared has a specific rotation of +19, then the specific rotation gradually increases till it is stabilizes at +52.5 (point of equilibrium).

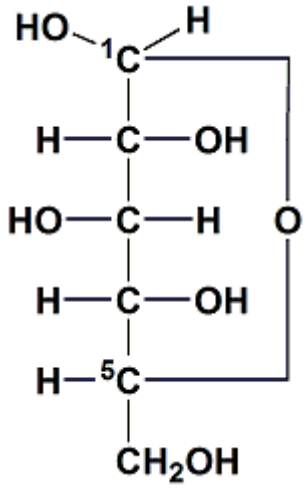
Ring (Cyclic) structure of sugars

- Sugars exist in solutions mostly in a cyclic form and a very small fraction is transiently present in the open chain form.
- Two forms for aldo- or keto-sugars to be in a ring or cyclic structure: Furan, so the sugar will called furanose & pyran , so the sugar will called pyranose.
- For aldo-sugars, Furan means condensation of the carbon atom no.1 (which is the aldehyde group) to the carbon no.4, while pyran means condensation of the carbon no. 1 to the carbon no.5. Aldo-sugars are more stable in pyranose form.
- For keto-sugars, Furan means condensation of the carbon atom no.2 (which is the ketone group) to the carbon no.5, while pyran means condensation of the carbon no. 2 to the carbon no.6. Keto-sugars are more stable in furanose form.
- The OH group formed on the aldehyde or keto group due to cyclization of the sugar molecule makes C1 in aldoses or C2 in ketoses an asymmetric carbon atom. Therefore, the two α and β forms of the sugar are isomers and called anomers because that carbon atom is called the anomeric carbon atom (i.e., C1 in aldoses and C2 in ketoses).The α-form where the OH group is present on the right side and the β-form where the OH group is on the left side of the anomeric carbon atom.

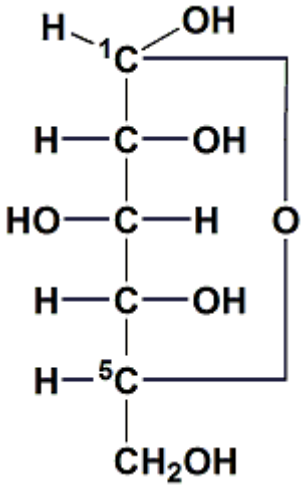
- Furanose & pyranose can be written in two methods:
  I. Old formula and called Fisher's projection formula.
  II. New method and called Haworth projection formula.

I. Fisher projection formulae:

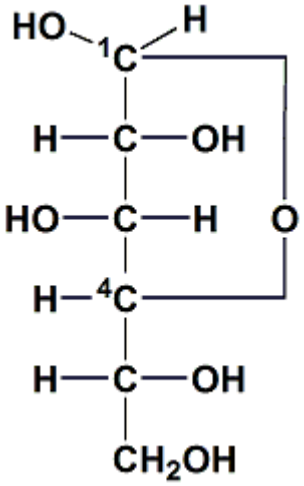
1. For aldo-sugars: e.g. Glucose:



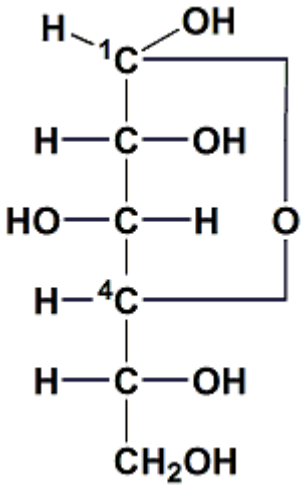
β-D-Glucopyranose



α-D-Glucopyranose

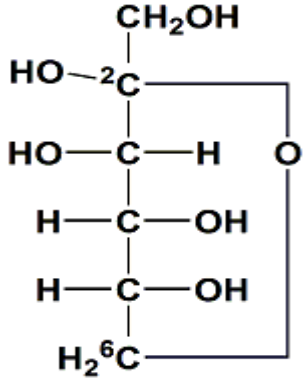


β-D-Glucofuranose

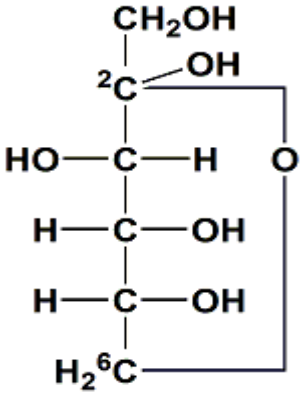


α-D-Glucofuranose

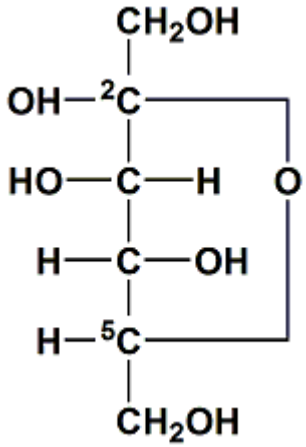
2. For keto-sugars: e.g. Fructose:



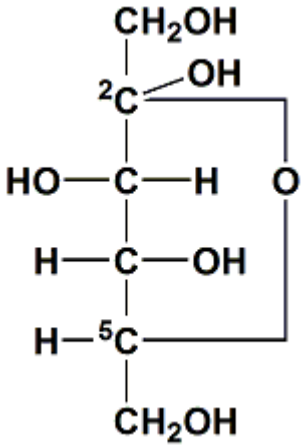
β-D-Fructopyranose



α-D-Fructopyranose



β-D-Fructofuranose

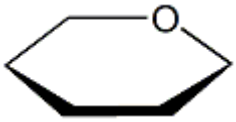


α-D-Fructofuranose

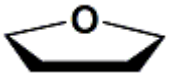
II. Haworth projection formulae:

Because Fisher's formula could not explain some of the chemical and physical characteristics of sugars, Haworth put forth his projection formula to describes the furanose or pyranose rings as follow:

1. C and O atoms of the ring are drawn in the plane of the page.
2. H and OH or other side groups are written on perpendicular plane.
3. All groups located on the left side of fisher's are written upwards. All groups located on the right side of fisher's are written downwards.
4. After the last carbon atom participating in the ring which is C4 in furanose & C5 in pyranose, this role is oppositely applied, i.e., right groups are written upwards and left groups are written downwards.
5. The radical of the molecule (the extra-cyclic part) is written upwards in D sugar and inside the ring (or down wards) in L-sugar.
6. The edge of the ring nearest to the reader is presented by thick lines.



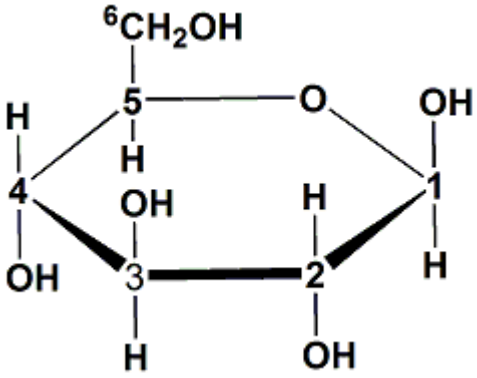
Pyran



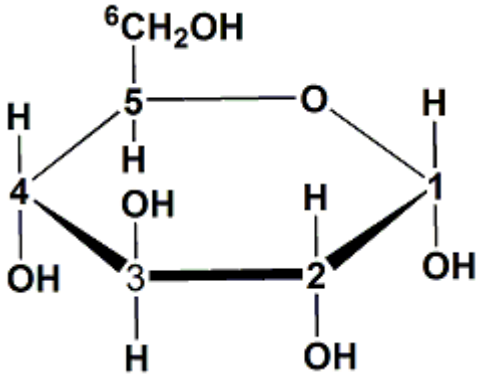
Furan

Examples:

1. Aldo-sugars: e.g. glucose:

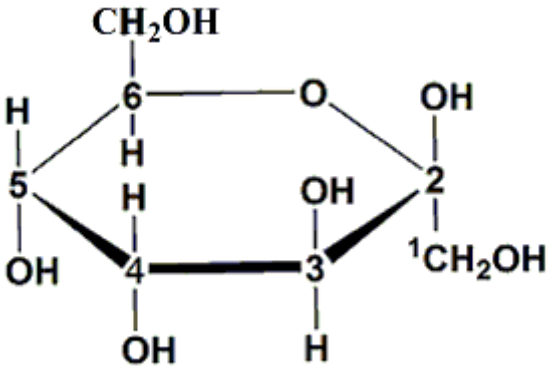


β-D-Glucopyranose

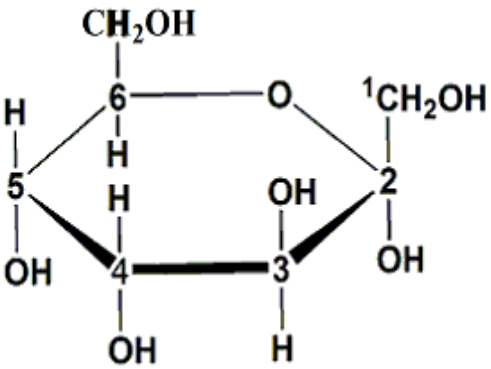


α-D-Glucopyranose

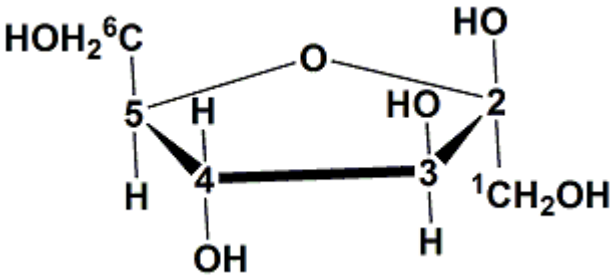
2.Keto-sugars e.g. Fructose:



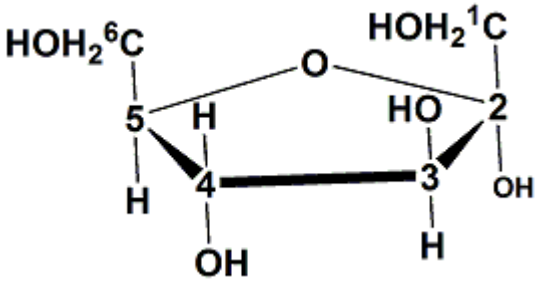
β-D-Fructopyranose



α-D-Fructopyranose



β-D-Fructofuranose



α-D-Fructofuranose

Chemical reactions of monosaccharides

- **They include the followings:**
  - **I- Oxidation of sugars (Sugar acids).**
  - **II- Reduction of monosaccharides (sugar alcohols).**

I- Oxidation of sugars (sugar acids):

- Aldose (-CHO) is oxidized into Acid (-COOH).
- The product of oxidation depends on the nature of oxidizing reagent.

1. Oxidation by mild or weak oxidizing reagent:

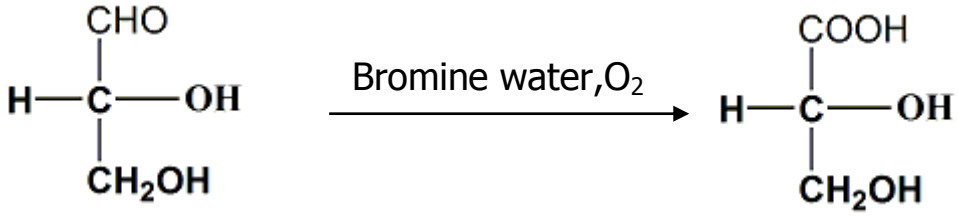
Effect:

- It oxidizes the first carbon and gives **Aldonic acid**, e.g., Bromine water.

Examples include:

A- Glyceric acid:

- It is sugar acid resulting from oxidation of the glyceraldehyde by mild oxidizing reagent as follow:

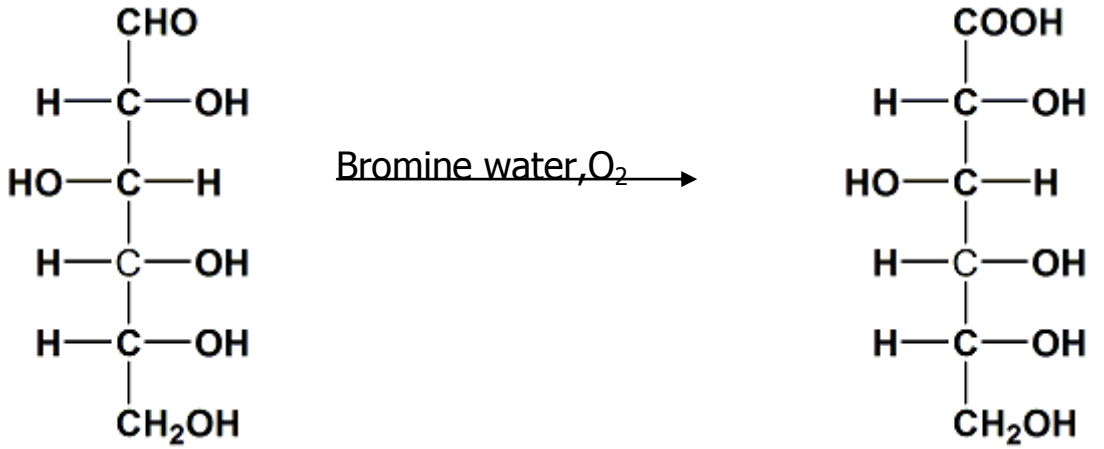


D-Glyceraldehyde **D-Glyceric acid**

- It is important in metabolism of carbohydrates in the body.

B- Gluconic acid:

- It is sugar acid resulting from oxidation of the glucose by mild oxidizing reagent as follow:



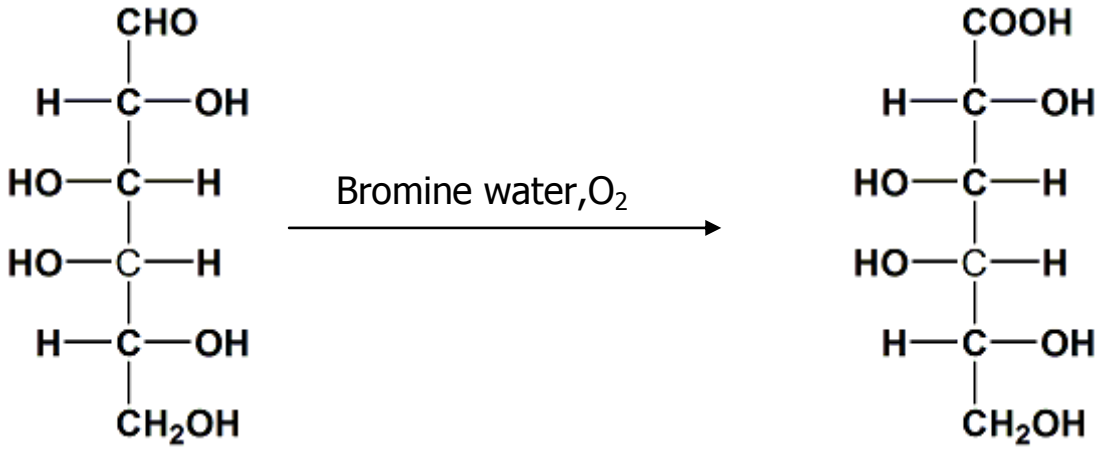
D-Glucose **D-Gluconic acid**

Biological importance of gluconic acid:

- It is an intermediate in carbohydrate metabolism.
- It is used as Ca^{2+} gluconate for intravenous supplementation of calcium for slow dissociation.

C- Galactonic acid:

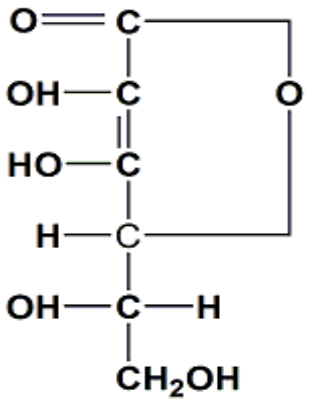
It is sugar acid resulting from oxidation of the galactose by mild oxidizing reagent as follow:



D-Galactose **D-Galactonic acid**

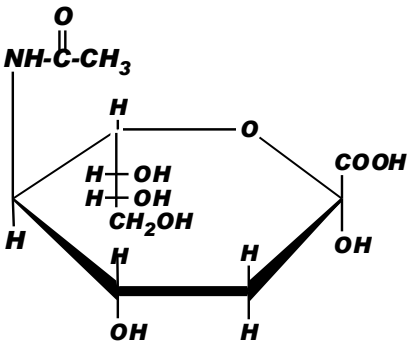
D-L-ascorbic acid (vitamin C):

- It is synthesized from gulose (a glucose derivative) in plants and animals except human, primates and guinea pig. It is an antioxidant. Its deficiency produces a disease in human termed scurvy.



L-Ascorbic acid

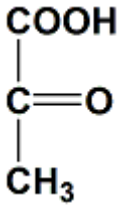
E- Sialic acid:



Sialic acid

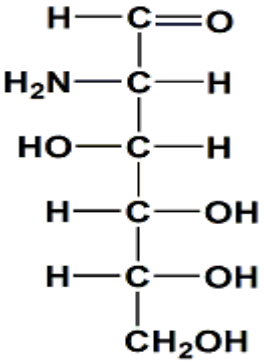
- It is amino-sugar as it contains an amino group (-NH2).
- It is sugar acid as it contains a carboxylic group (-COOH).
- It is deoxy sugar as it contains two hydrogen atoms at C3.
- It enters in the structure of glycoproteins & glycolipids.

- It is formed as follow:

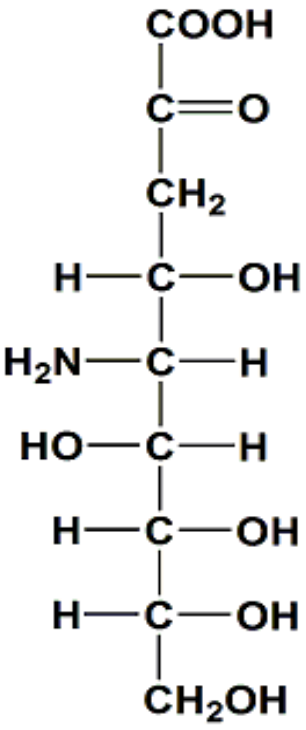


Pyruvic acid

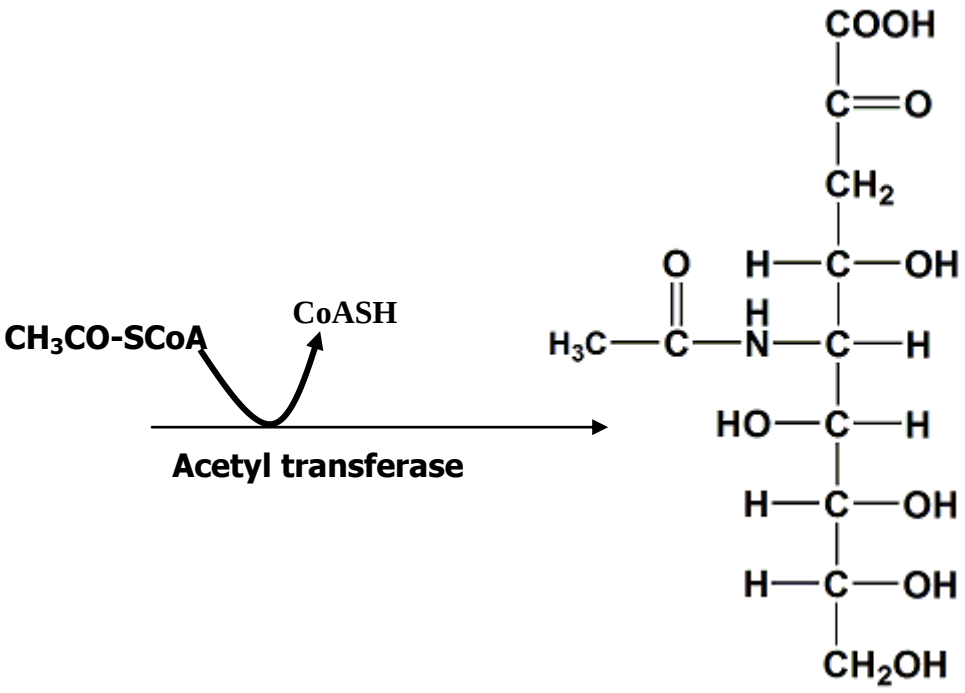
+



Mannosamine



Neuraminic acid



Sialic acid"N-acetyl neuraminic acid"

2. Oxidation by moderate oxidizing reagents:

- Such as H_2O_2 (hydrogen peroxide) or diluted HNO_3.

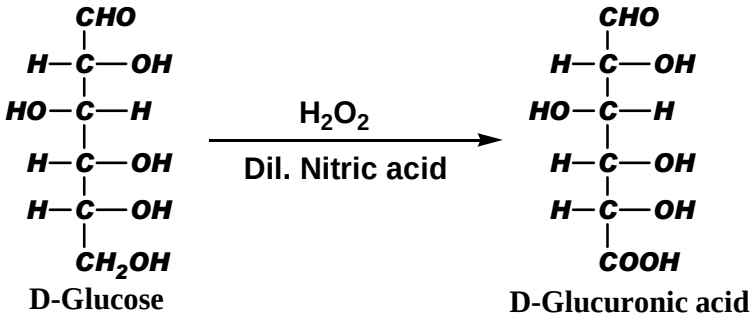
Effect:

- They oxidize the last carbon (CH_2OH) to give uronic Acids.

Examples include:

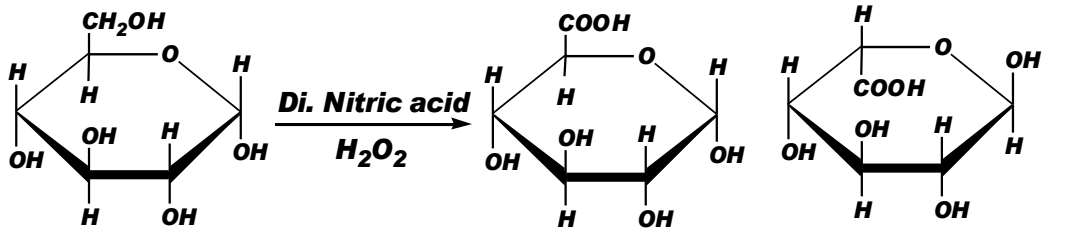
A-Glucuronic acid:

- It is sugar acid resulting from oxidation of the glucose by moderate oxidizing reagent as follow:



Biological importance of glucuronic acid:

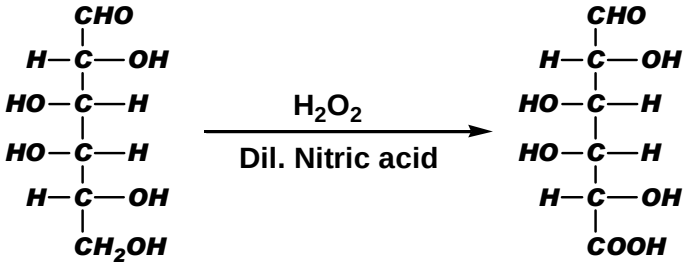
- It is synthesized in the liver.
- Glucuronic acid enters in the structure of very important molecules such as mucopolysaccharides.
- It is used for excretion of bilirubin and steroid hormones and detoxication of certain drugs and several toxins by conjugation.
- L-iduronic acid is the 5-epimer of D-glucuronic acid and it enters in structure of mucopolysaccharides.



α-D-Glucose α-D-Glucuronic acid β-L-Iduronic acid

B- Glacturonic acid:

- It is sugar acid resulting from oxidation of the glucose by moderate oxidizing reagent as follow:



D-Galactose D-Galacuronic acid

- Galactose gives galacturonic acid that enters in plant gums and pectin structure.

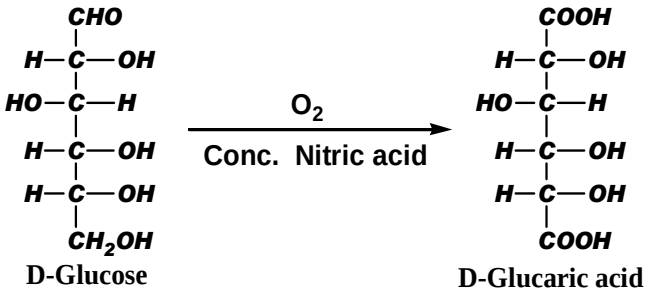
3. Oxidation by strong oxidizing reagent:

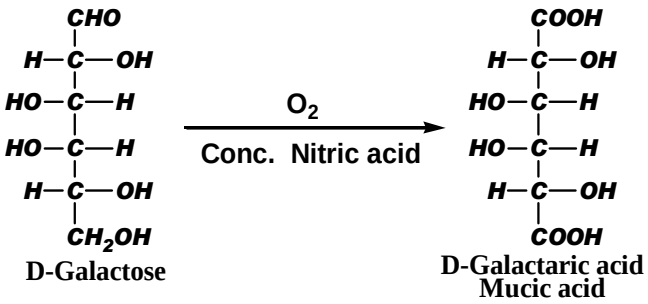
Effects:

- Concentrated HNO3 oxidizes the first and last carbon to give aldaric acid.

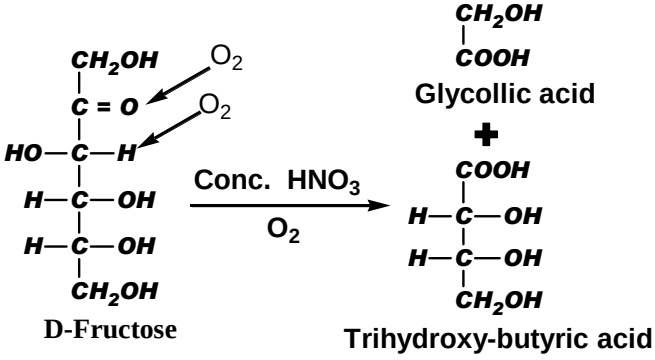
Examples:

A) This reaction is important to differentiate between glucose and galactose since **glucaric acid** is water soluble, whereas, **mucic acid** is insoluble in water and precipitates in the form of insoluble crystals of specific shape.



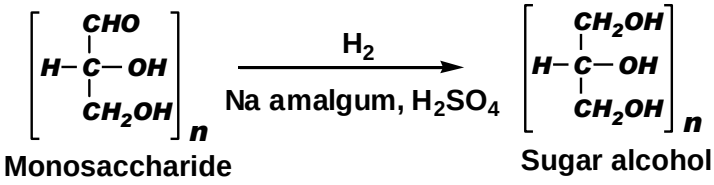


B) Oxidation of fructose: The molecule is cleaved into two acids with shorter chain length. One is **glycollic acid** and the other is **trihydroxy-butyric acid** a derivative of Butyric acid (CH3 – CH2 – CH2 – COOH), as follows:



II. Reduction of monosaccharides (sugar alcohols):

- Reduction of monosaccharides using Na amalgum, H_2SO_4 & H_2 will gives sugar alcohols as follow:

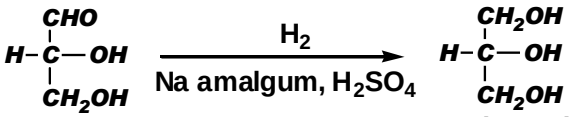


Monosaccharide — Sugar alcohol

Examples:

A) - Glycerol:

- It is a sugar alcohol resulting from reduction of Glyceraldehyde into glycerol as follow:

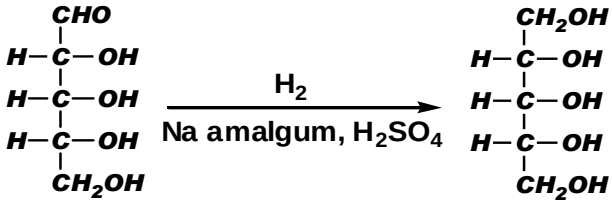


Glyceraldehyde — Glycerol

- It enters in structure of lipids, creams and explosives

B) - Ribitol:

- It is a sugar alcohol resulting from reduction of ribose into ribitol as follow:



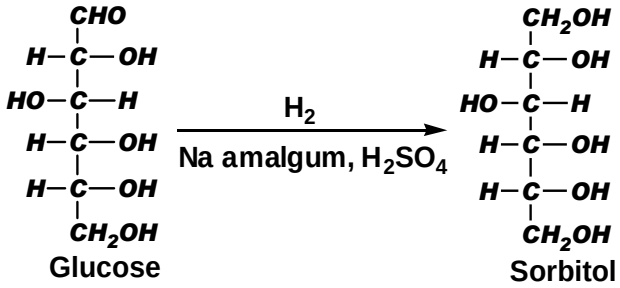
Ribose — Ribitol

- Ribose reduction gives Ribitol that is a part of the structure of vitamin B2

(Riboflavin),

C)-Sorbitol:

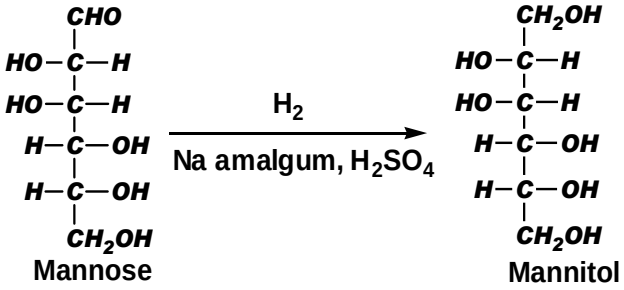
- It is a sugar alcohol resulting from reduction of glucose into sorbitol or glucitol as follow:



- Sorbitol enters in medical industries.

D) - Mannitol:

- It is a sugar alcohol resulting from reduction of mannose into mannitol as follow:

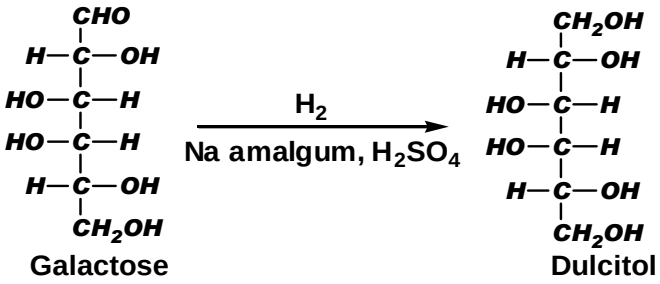


Biological importance of mannitol:

- It is injected intravenously to reduce intracranial hypertension in cases of meningitis, cerebral hemorrhage or thrombosis.
- It is used in kidney function testing.

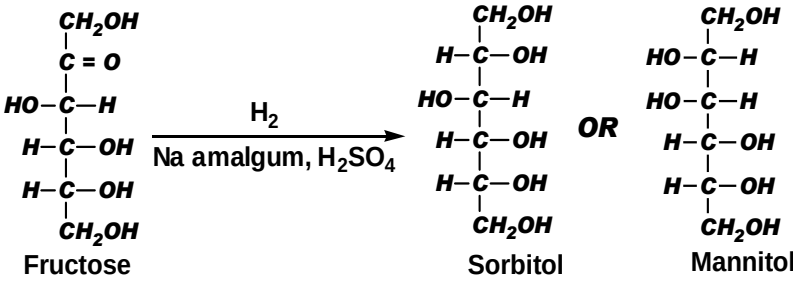
E) - Galacitol:

- It is a sugar alcohol resulting from reduction of galactose into Dulcitol or galacitol as follow:



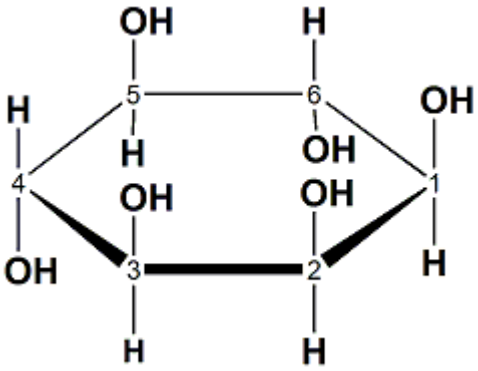
F) - Fructose reduction:

- Fructose reduction gives Sorbitol or Mannitol as follow:



G)-Inositol:

- It is a hexahydric alcohol (6 OH groups).
- It has more than 14 isomers.
- It presents in high concentration in bran (outer coat of cereals) where it combines with 6 molecules of phosphoric acid to give phytic acid. Phytic acid forms insoluble iron, calcium and magnesium salts (phytate) which hinder absorption of Ca2+, Mg2+ and iron in the intestine.



- It presents in high concentration in heart and muscles tissues, so it is called muscle sugar.
- It is considered a member of vitamin B complex because it is essential for synthesis of phospholipids, phosphatidylinositol that mediates hormonal action inside the cells.

Monosaccharide derivatives of biological importance

They include the followings:

1. **Amino sugars or sugaramines.**
2. **Deoxy sugars.**
3. **Sugar acids (product of oxidation of sugars, see above).**
4. **Sugar alcohols (product of reduction of sugars, see above).**

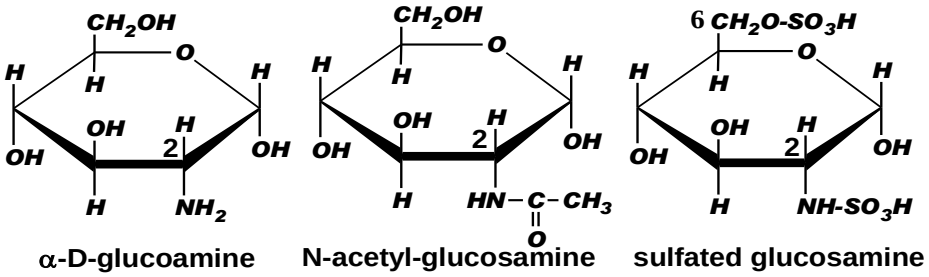
1- Amino sugars (sugaramines)

- Replacing OH group on C2 by an amino group (NH2) produces them.

Examples:

1. Glucosamine:

- Glucosamine is also called chitosamine because is the only sugar derivative in chitin. It can be acetylated into N-acetyl glucosamine and sulfated into sulfated glucosamine,



α-D-glucoamine N-acetyl-glucosamine sulfated glucosamine

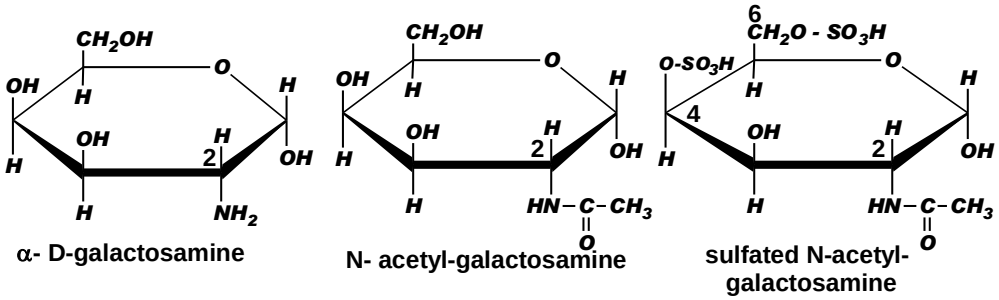
Biochemical importance:

1. Enters in the structure of exoskeleton of insects (chitin, so it is called chitosamine).

2. Enters in the structure of mucopolysaccharides, hyaluronic acid contain N-acetyl glucosamine and heparin contain sulfated glucosamine.

2. Galactosamine (Chondrosamine)

- The major sugar derivative in chondroitin sulfate, it may be acetylated or sulfated,

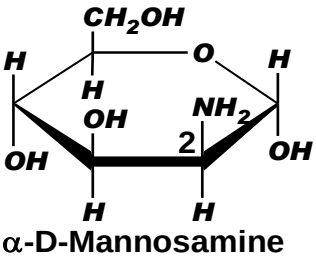


α- D-galactosamine N- acetyl-galactosamine sulfated N-acetyl-galactosamine

Biochemical importance:

- It enters in the structure of the sulfate-containing mucopolysaccharides (chondroitin sulfate) and glycolipids.

3. Mannosamine:



α-D-Mannosamine

Biochemical importance:

- Mannosamine enters in the structure of antibiotics, e.g., erythromycin together with other amino sugars where they are required for the activity of these antibiotics.

4. Sialic acid: Discussed before.

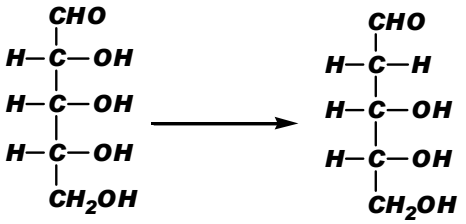
2. Deoxysugars:

- These are sugars in which OH group is replaced by H. This may occur at either of three places:

I. At C2 gives Deoxy sugar proper.

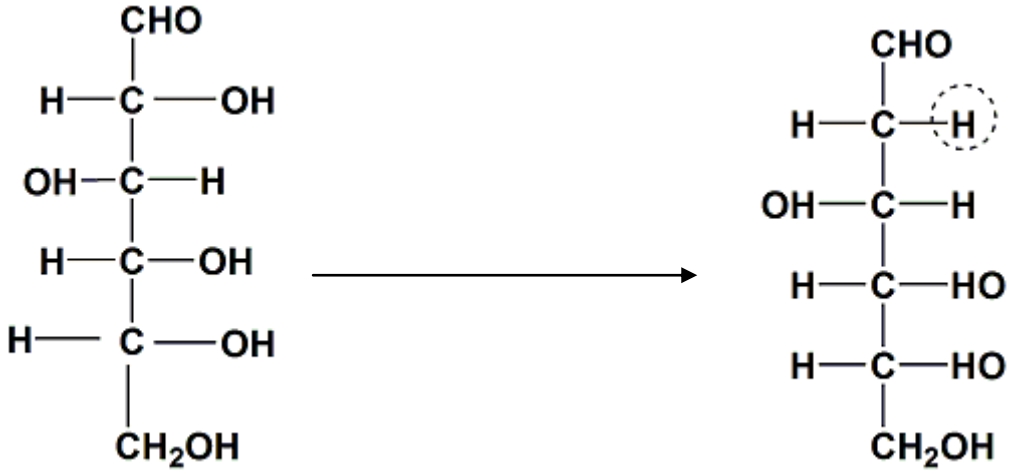
Examples:

1. Deoxyribose: It enters in structure of DNA**.**



Ribose Deoxyribose

2**. 2-deoxy-glucose**: this acts as invitro inhibitors of glycolysis and recently used as anti-cancer chemotherapeutic agent.

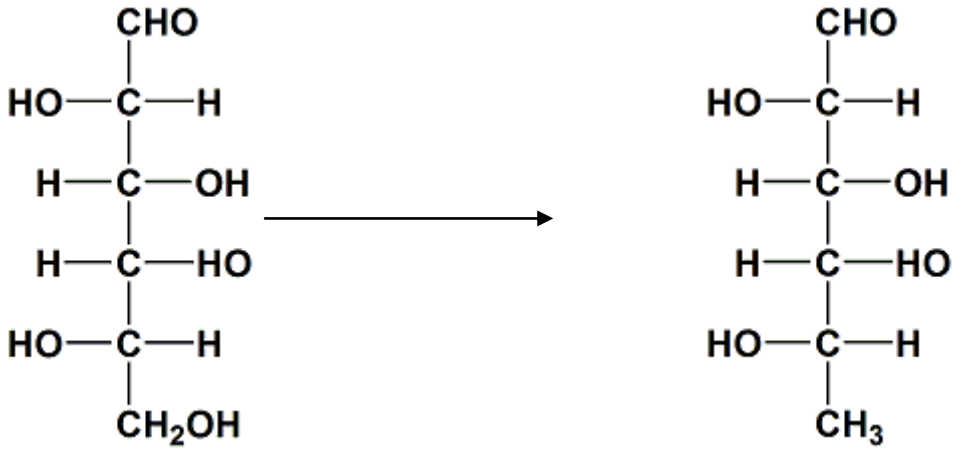


D-Glucose **2-deoxy-D-Glucose**

II. At C6 gives methyl pentoses (Methylose).

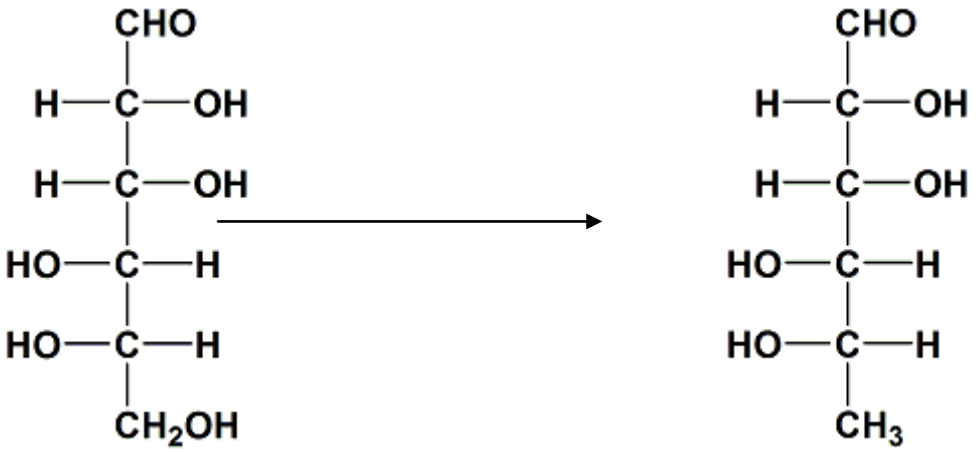
Examples:

1. L-galactose gives **L-fucose.**



L-Galactose **L-Fucose**

2. L-Mannose gives **L-rhamnose.**



L-Mannose **L-Rhamnose**

- L-rhamnose and L-fucose enter in the structure of glycoproteins, e.g., blood group substance.

III. at C3, as in Sialic acid. Previuosly discussed**.**

II- Disaccharides

Definition:

- These are formed by condensation of two molecules of monosaccharides , bound together by glycosidic linkage .

Classification:

- They are classified according to the presence of free ketone or aldehyde group or not into two major classes:

A) Reducing disaccharides:

- They contain free ketone or aldehyde group that making them to have reducing properties**.**
- **Examples** include:
  1. **Maltose.**
  2. **Isomaltose.**
  3. **Lactose.**
  4. **Cellobiose.**

B) Non-reducing disaccharides:

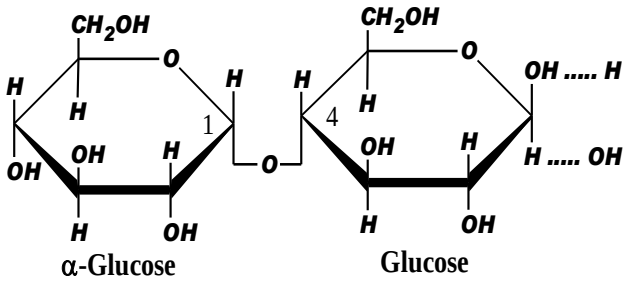
- They don't contain free ketone or aldehyde group that making them not to have reducing properties**.**
- **Examples** include:
  1. **Sucrose.**
  2. **Trehalose.**

I- Reducing disaccharides:

1. Maltose "Malt sugar"

Structure:

- It consists of 2 α-glucose units linked by α-1, 4-glycosidic linkage.



α– and β–Maltose

Sources:

a. Malt.
b. It is also produced during digestion of starch by amylase.

Properties:

Since maltose contains free aldehyde group, so having the following properties:

1. It exists in α and β- forms.
2. It exhibits mutarotation.
3. It is reducing disaccharide.
4. It gives osazone called maltosazone that have rosette shaped.
5. It is fermentable.

2. Isomaltose

Structure:

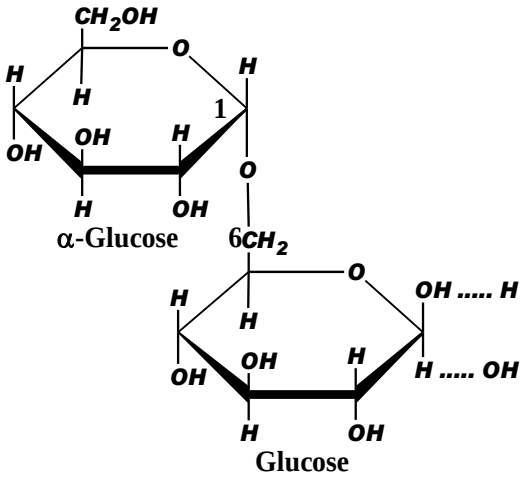
- It consists of 2 α-glucose units linked by α-1, 6-glycosidic linkage.

Sources:

- It is produced during digestion of starch and glycogen by amylase.

Properties:

- The same as maltose.

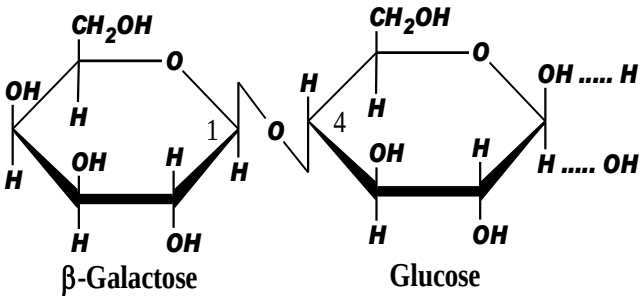


α– and β–isomaltose

3. Lactose"Milk sugar"

Structure:

- It is formed of β-galactose and α or β-glucose linked by β-1,4-glycosidic linkage β-galactose participates by C1 while α-glucose participates by C4.



α– and β–Lactose

Sources:

- It is the sugar present in milk. It may appear in urine in late pregnancy and during lactation.

Properties:

- Since lactose contains free aldehyde group, so having the following properties:

1. It exists in α and β- forms.
2. It exhibits mutarotation.
3. It is reducing disaccharide.
4. It gives osazone called lactosazone that have sun-shaped.
5. It is non-fermentable due to absence of lactase enzyme in the yeast.

Lactose is the most suitable sugar for baby feeding because:

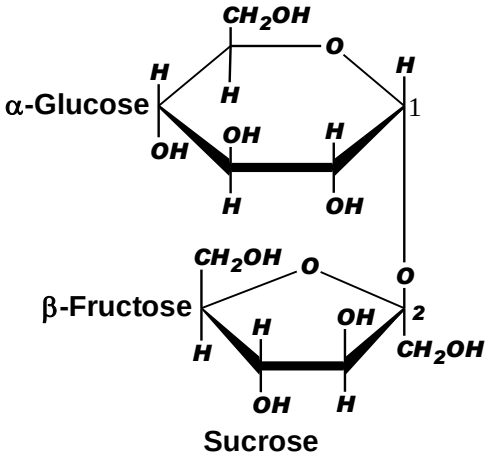
1. It is least sweet sugar, so the baby can take large amounts without loss of his appetite.
2. Non-fermentable, so no gas formation and not cause colic or distension.
3. Has laxative effect, preventing constipation, no irritation to stomach so no vomiting.
4. Easily digestible and help the absorption of minerals present in milk.
5. Unabsorbed sugar used as food for the large intestinal bacteria that form number of vitamins that benefits the baby.

II- Non-reducing disaccharides:

1. Sucrose"Cane and beet sugar"

Structure:

- It is formed of α-glucose linked to β-fructose by α-1,β-2 glycosidic linkage.



Properties:

- The 2 anomeric carbons (C1 of glucose and C2 of fructose) are involved in the linkage, therefore it is:
    1. Non-reducing sugar.
    2. Non osazone forming.
    3. Not mutarotating.
    4. Not having α or β-forms.
- It is a dextrorotatory sugar but when it is hydrolyzed by sucrase enzyme or by acid hydrolysis (HCl) the mixture of sugars produced is levorotatory. This is because the levorotatory power of fructose (-92.5) cancels the dextrorotatory power of glucose (+52.5) since they are at equal proportions in the product. This is why this sugar is called invert sugar. Sucrase enzyme is therefore, also called invertase enzyme.

Differences between sucrose and invert sugar:

	Sucrose	**Invert sugar**
1. Other names	• Cane sugar • Table sugar. • Beet sugar.	• Bees honey sugar
2. Formed of	α-glucose &β-fructose linked by α-1,β-2 glycosidic linkage	Equal mixture of α-glucose &β-fructose
3. Optical activity	Dextrorotatory (d) (+)	Levorotatory (l)(-)
4. α &β forms	Abscent	Present
5. Mutarotation	Abscent	Present
6. Reducing power	Abscent	Present
7. Osazone formation	Abscent	Present " needle shaped"

8. Digestion	Need digestion by sucrase	No further digestion
9. Structure	α-Glucose β-Fructose Sucrose	**α-Glucose** **β- Fructose**

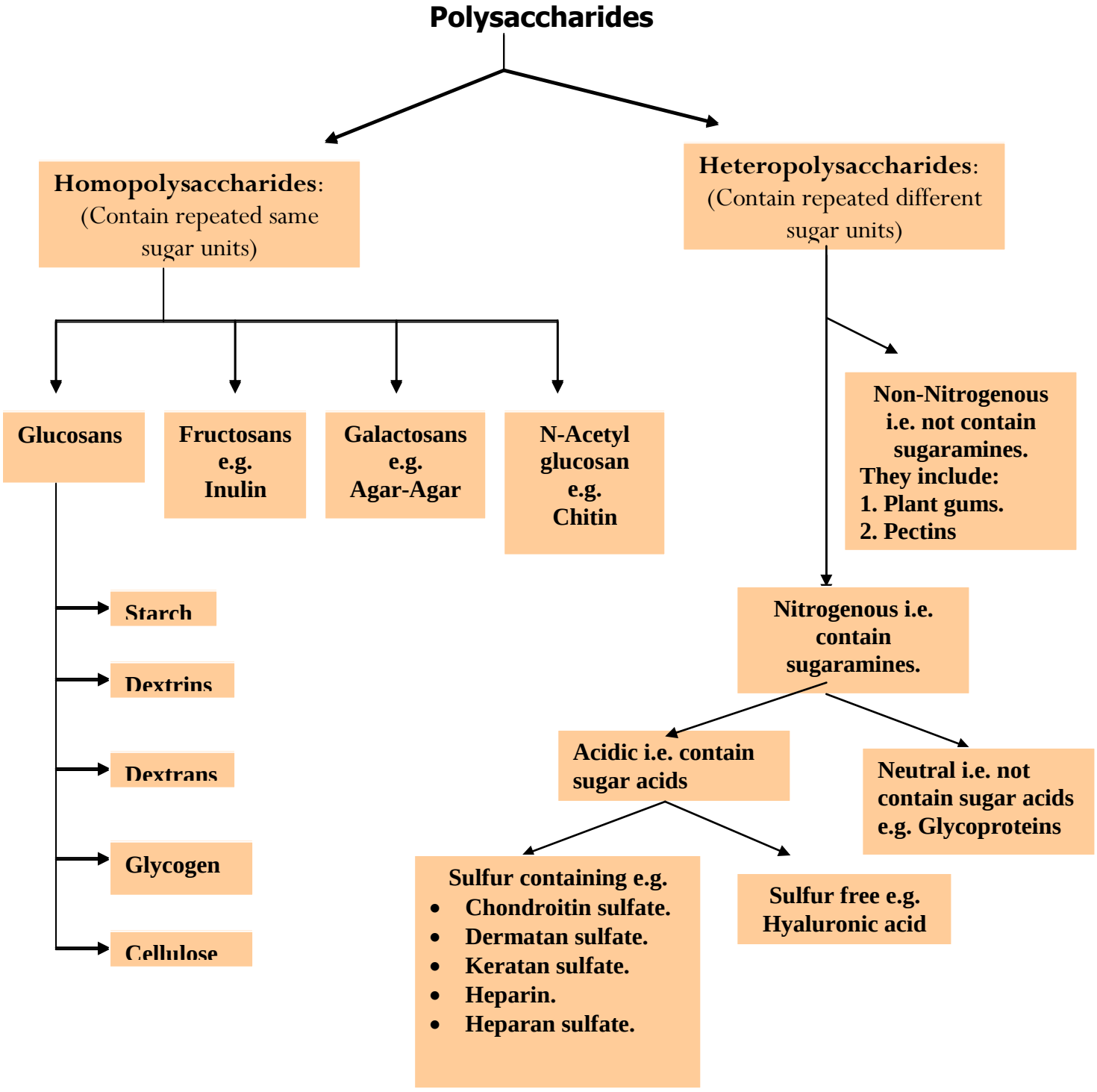
III- Polysaccharides

Definition:

- These are carbohydrates formed of more than 10 sugar units

.

Classification:



I-Homopolysaccharides:

Starch

Nature:

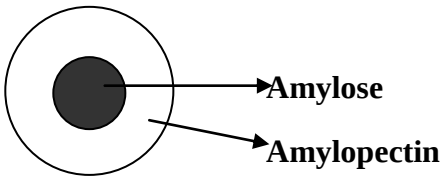
- Stored form of carbohydrates in plants never in animals.

Sources:

- Cereals e.g. rice, potatoes, wheat.

Structure:

- It is in the form of starch granules.
- Each starch granule consists of :
  - The core is amylase.
  - The shell is amylopectin.

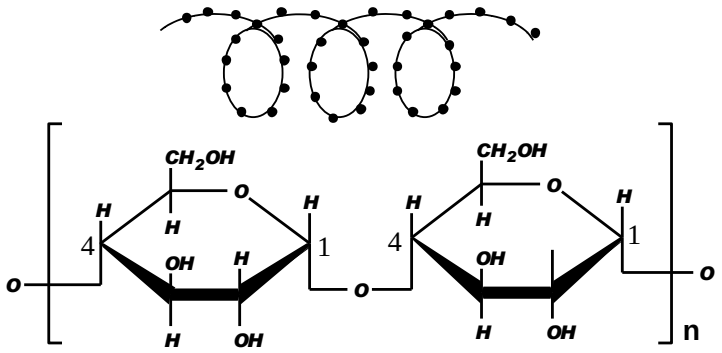


Amylose

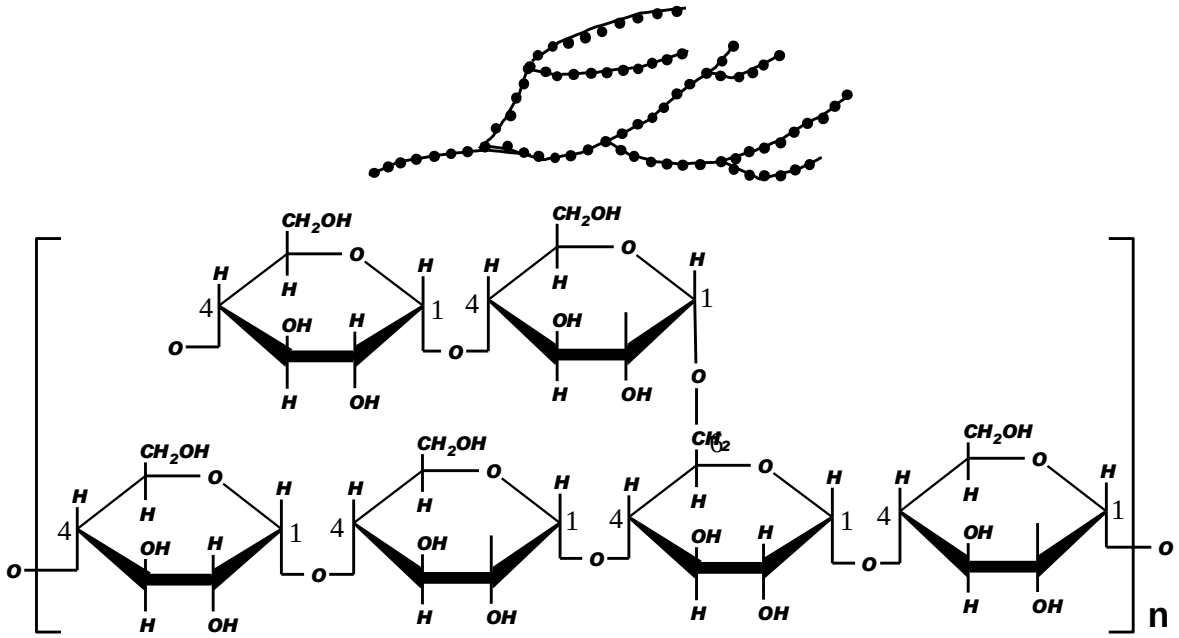
- Form the inner part of the starch granule.
- Water soluble.
- Gives blue color with iodine.
- Formed of straight helical chains of α-glucose units linked by α-1,4 glycosidic linkage.

Amylopectin

- Form the outer coat of the starch granule.
- Water insoluble.
- Gives red color with iodine.
- Formed of branched chains that consist of α-glucose units linked by α-1, 4 glycosidic linkage along the branch and α-1, 6 glycosidic linkage at the branching point.



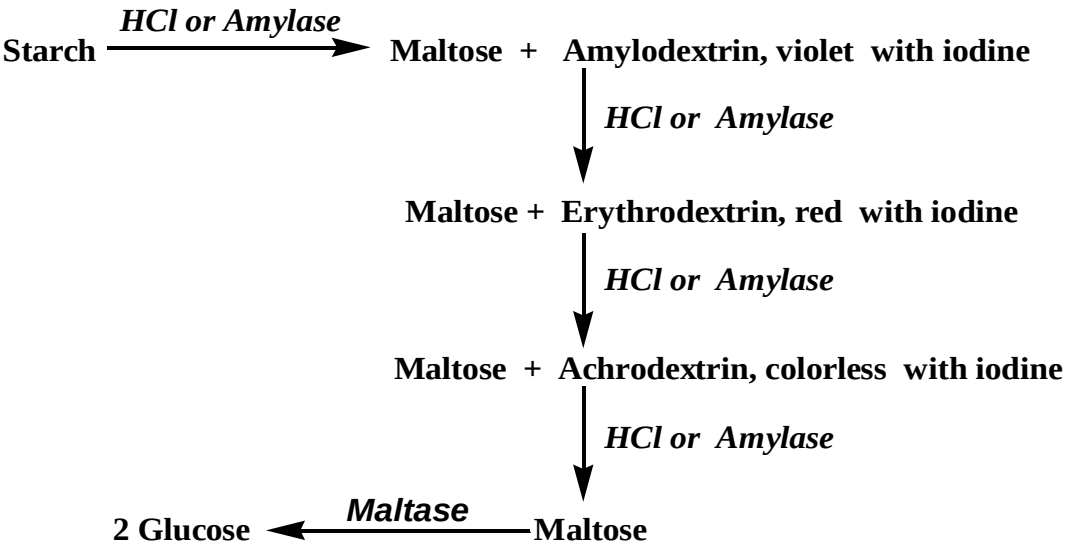
Amylose



Amylopectin

Hydrolytic products of starch:

- Starch can be hydrolyzed by HCl or amylase. Hydrolysis produces dextrins that can be distinguished by their reaction with iodine as below.



Differences between Starch, Glycogen & cellulose:

Starch	Glycogen	Cellulose
1. Nature: Stored form of carbohydrate in plants.	Stored form of carbohydrates in animals.	Structural form of carbohydrate in plant cells.
2. Source: Cereals, e.g., wheat, rice, and tubers, e.g., potatoes.	Muscles and liver	Linen and cotton are nearly pure cellulose.
3. Solubility: Amylose is water soluble and amylopectin is insoluble.	Water soluble forming colloidal solution.	Water insoluble.
4. Nature of the chains: Amylose is helical straight chain (α-glucose units linked by α-1,4-glucosidic bonds). Amylopectin is branched chain (α-glucose units linked by α-1,4- and α-1,6-glucosidic bonds).	Branched chain similar to amylopectin but its trees are shorter and have more branches than amylopectin tree.	Straight chain (large number of β-glucose units linked by β-1,4-glucosidic bonds).
5. Reaction with iodine: Amylose gives blue color and amylopectin gives red color.	Gives red color.	No color.
6. Digestibility: Is hydrolyzed by HCl or amylase into dextrins and maltose.	Digestible by amylase into dextrins and maltose.	Non-digestible but HCl hydrolysis gives cellobiose.

N.B: Cellulose is a major food for herbivorous, although it is not digestible in humans but it is essential in human foods for:
1. Prevent constipation by increasing the bulk of stool.
2. Adsorbs toxins in foods and prevent their absorption into the body.

3. Fermented by the large intestinal bacteria giving volatile fatty acids which give some water soluble vitamins and has anticancer effect for colon cells

Differences between Dextrins & Dextrans:

	Dextrins	Dextrans
They belong to glucosan homopolysaccharides		
Source	Products of starch hydrolysis.	Synthesized by the bacteria that have sucrose in their media.
Types	It has three types: • Erythrodextrin (red with iodine). • Amylodextrins (violet with iodine). • Achrodextrin (colorless with iodine).	Only one type.
Structure	α-glucose units linked by α-1, 4 & α-1, 6 glycosidic linkages.	α-glucose units linked by α-1, 3, α-1, 4 & α-1, 6 glycosidic linkages.
Uses	It has sweet test , so used as demulcent	• It used as plama substitute to restore blood pressure in shock. • It complexes with iron for intramuscular injection to treat iron deficiency anemia. • Sodium dextran sulfate is used as anticoagulant.

Inulin

- It is formed of fructose units.
- It is type of fructosans which is type of homopolysaccharides.
- It is present in onions & tubers.
- It is not metabolizable in human body , so used in evaluation of kidney function as a part of inulin clearance test

Agar-Agar

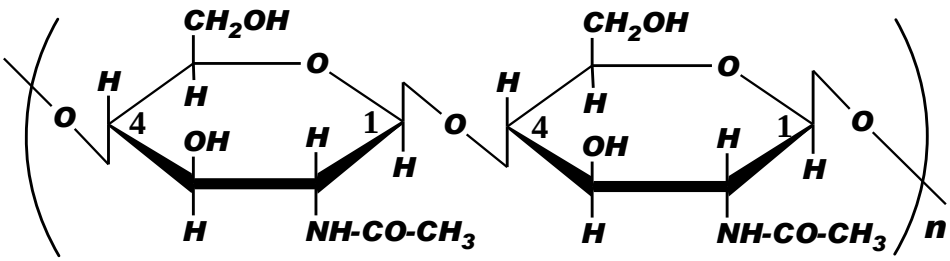
- It is galactosan i.e. formed of galactose as the building units.
- It presents in seaweed.

Biochemical importance:

- It is used for growth of bacteria and mammalian cells in culture.
- It imbibes water and increases intestinal contents to prevent and treat constipation.
- Some electrophoresis gels are formed of it.

Chitin

It is a homopolysaccharide formed of β-N-acetyl-glucosamine units linked by β-1,4-glucosidic linkage .



β-N-acetyl-glucosamine β-N-acetyl-glucosamine

Chitin

- It presents in the exoskeleton of insects and crustaceans.

Plant gums and mucilage:

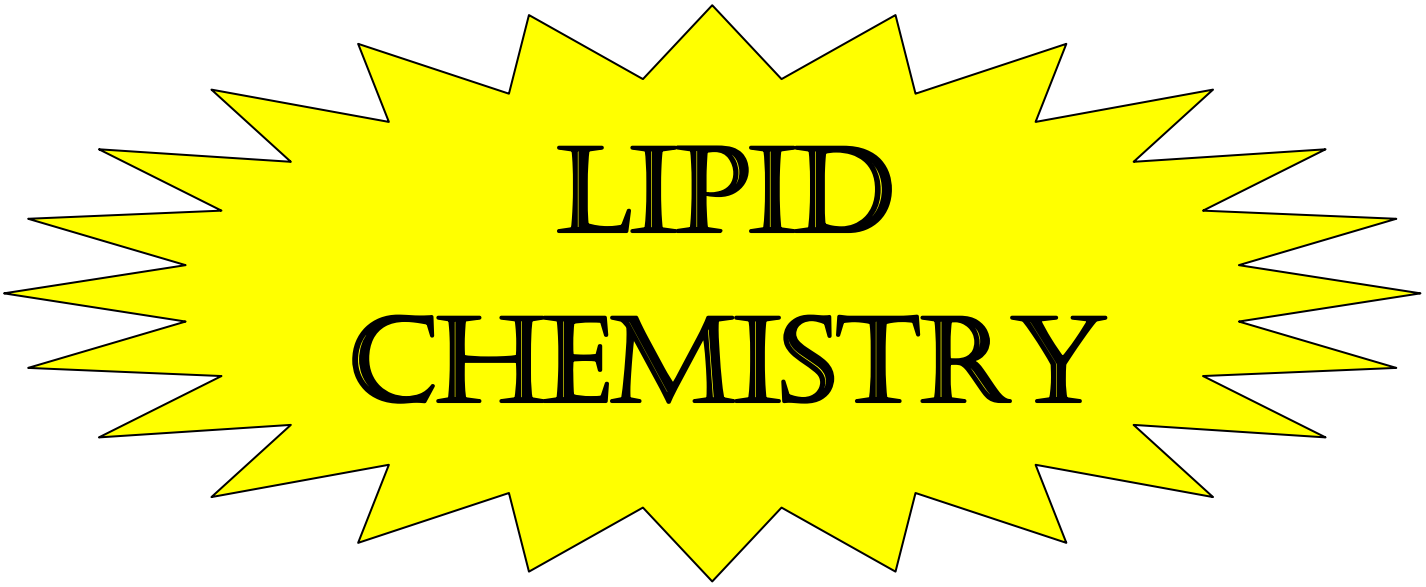
- They are exudates of plants that do not contain amino sugars.
- They contain pentoses, hexoses and uronic acids, e.g., Arabic gum which is rich in arabinose.
- They are emulsifying agents and demulcents.

Pectin:

- They are present in fruits and are responsible for settling of jams.
- They are formed of pentoses, hexoses, uronic acids mainly galacturonic acid.
- They are water soluble formed from a water insoluble compound called pectose present in raw fruits which is transformed into pectin by the action of sunlight, heat and pectase enzyme when fruits are ripened.

Biochemical importance:

1. Emulsifying reagents.
2. Demulcents.
3. Responsible for settling of jams.
4. They increase in size when they absorb water forming a jell and so they are used in the treatment of infantile diarrhea.


LIPID
CHEMISTRY

Topics Discussed

- Classification of lipids and their components: Fatty acids and fatty alcohols.
- Eicosanoids as derived lipid and their structural and physiological characteristic and related diseases.
- Neutral lipids and their characteristics.
- The mechanisms causing rancidity.
- Applications of fat constants.
- Classification, structural properties, and medical importance of the different types of compound lipids and related diseases.
- Classification, structural properties, and medical importance of the different types of steroids and related diseases.

Learning Objectives:

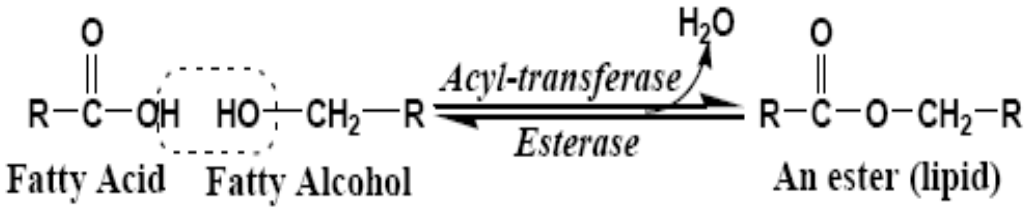
After understanding this part the student should be able to:

- Compare and contrast the structure and medical importance of the different types of lipids.
- Describe the bases of the biochemical and medical importance of the essential fatty acids.
- Describe the biochemical and medical importance of the eicosanoids.
- Compare the different types and fates of rancidity.
- Compare and contrast the uses and importance of different fat numbers
- Describe the medical importance of the different types of compound lipids and their disease implications.

Lipid Chemistry

Definition of lipids:

- They are organic compound which have the following common properties:
- Formed mainly from alcohol and fatty acids combined together by ester linkage (-o-) As follows:



- Lipids are insoluble in water, but soluble in non-polar (fat) or organic solvents (ether, chloroform, benzene, acetone, etc.).

Biological Importance of Lipids:

I- Membranes:-

A biological membrane is a form of lipid bilayer. The formation of lipid bilayers is an energetically preferred process when the glycerophospholipids are in an aqueous environment.

II- Energy storage:-

Triacylglycerols, stored in adipose tissue, are a major form of energy storage in animals.

III- Signaling:-

In recent years, evidence has emerged showing that lipid signaling is a vital part of the cell signaling.

IV- Other functions

The "fat-soluble" vitamins (A, D, E and K) – which are isoprene-based lipids – are essential nutrients stored in the liver and fatty tissues, with a diverse range of functions. Acyl-carnitines are involved in the transport and metabolism of fatty acids in and out of mitochondria, where they undergo beta oxidation.

Classification of Lipids:

I)- Simple lipids:

- **They are esters of fatty acids with alcohols.**
- **Sub classified according to the type of alcohol into :-**
  - ***A)- Neutral fats.***
  - ***B)- Waxes.***

II- Compound or conjugated lipids:

- They contain additional group(s) beside the alcohol and fatty acids
- They are classified according to the additional group(s) into:-

1)- Phospholipids: the additional groups are nitrogenous base & phosphoric acid.
2)- Glycolipids: the additional groups are carbohydrate sugars alone or with sugar derivatives.
3)- **Lipoproteins**: the additional groups are proteins.
4)- Sulfolipids: the additional group is sulfur.
5)-Aminolipids: the additional groups are amino acids.

III- Derived Lipids:

- They are products of hydrolysis of simple and compound lipids and/or their derivatives that still possess the general characteristics of lipids.

- Examples:
1)-Fatty acids.
2)- Fatty Alcohols: which are alcohols associated with lipids.
3)- Steroids.
4)- Eicosanoids.

Two examples from derived lipids will be discussed now & the remaining examples will be discussed later on in this chapter:
I)- Alcohols.
II)- Fatty acids.

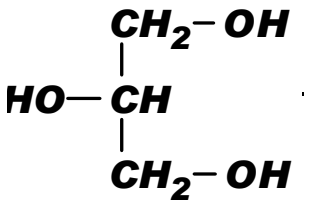
I)- Fatty Alcohols:

- Examples:
a)- Glycerol.
b)- Sphingosine.
c)- Alcohols that have higher molecular weight than glycerol such as :
1) Cholesterol.
2)- Vitamin D.
3)- Vitamin A.
4)- Cetyl alcohol.
5)- Mericyl alcohol.

a)- Glycerol:

Definition: It is trihydric alcohol containing three hydroxyl groups "OH" with popular name Glycerin.

Chemical structure:



Glycerol

Properties:-

1)- Physical properties:-

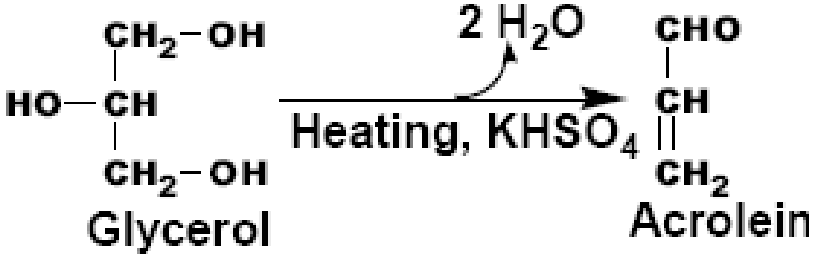
a)- Colorless viscous oily liquid with sweet taste.

b)- Soluble in water and alcohols and insoluble in non polar fat solvents.

2)- Chemical properties:-

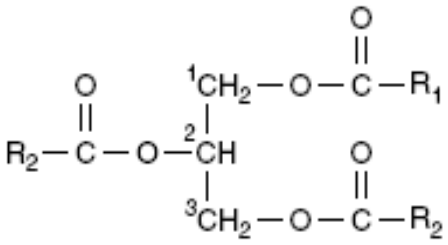
a)-It gives positive Acrolein test:-

- On heating with sulfuric acid or KHSO4 "potassium hydrogen sulfate" , it will dehydrated (two molecules of water are removed) and the resulting compound is called acrolein which has bad odour.
- This reaction can be used for detection of free glycerol or any glycerol containing compounds.
- The reaction occurs as follows:-



b)- On esterification with fatty acids , it gives:-

1)- Monoglyceride or monoacyl-glycerol: one fatty acid + glycerol.
2)- Diglyceride or diacyl-glycerol: two fatty acids + glycerol .
3)- Triglyceride or triacyl-glycerol: three fatty acids + glycerol:



Triacylglycerol

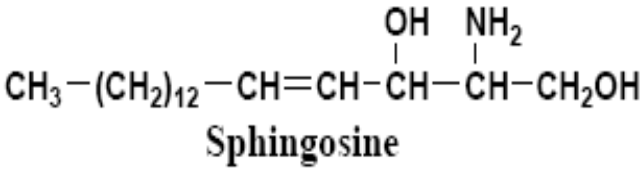
N.B: If the three fatty acids connected to glycerol are of the same type , the triglyceride is called "***Simple triglyceride***" & if the three fatty acids connected to glycerol are of different types , the triglyceride is called "***mixed triglyceride***" .
c)- It is synthesized in the body from glucose & has nutritive value
by conversion into glucose and enters in structure of phospholipids " Glycerophospholipids".

Uses of Glycerol:

1)- Glycerol enters in pharmaceutical and cosmetic preparations e.g. creams as it is hygroscopic.
2)- Nitroglycerin (glyceryl trinitrate) is used as a vasodilator especially for the coronary arteries, thus it is used in treatment of angina pectoris.
3)- Glycerol is used in treatment of glaucoma (increased intraocular pressure) due to its ability to dehydrate the tissue from its water content.

b)- Sphingosine:

1)- It is the alcohol present in sphingolipids.
2)- It is synthesized in the body from serine and palmitic acid.



Sphingosine

II)- Fatty acids:

-They have the general formula R-(CH_2)n-COOH.
- The carbon skeleton of fatty acids are numbered either from the carboxylic group or from the terminal methyl group (termed omega carbon, ω) as follows:

1- Starting from the carboxylic group:

There are two systems as follows::
a- By giving the carboxylic group the number 1 and proceed toward the – $CH_{3:-}$

6 5 4 3 2 1
CH_3-CH_2-CH_2-CH_2-CH_2-COOH
ξ δ γ β α

b- By using α ,β ,γ system , where α-carbon is the first carbon adjacent to the carboxylic group.

2- ***Starting from the terminal methyl group*** or omega carbon (ω):-

ω1 ω2 ω3 ω4 ω5
CH_3-CH_2-CH_2-CH_2-CH_2-COOH

- In cases of unsaturated fatty acids i.e. there is /are double bond(s), we can say that this fatty acid for example is ω7 fatty acid, this mean that the number of carbon atoms by which the first double bond located from the methyl "CH_3" end of that fatty acid considering carbon

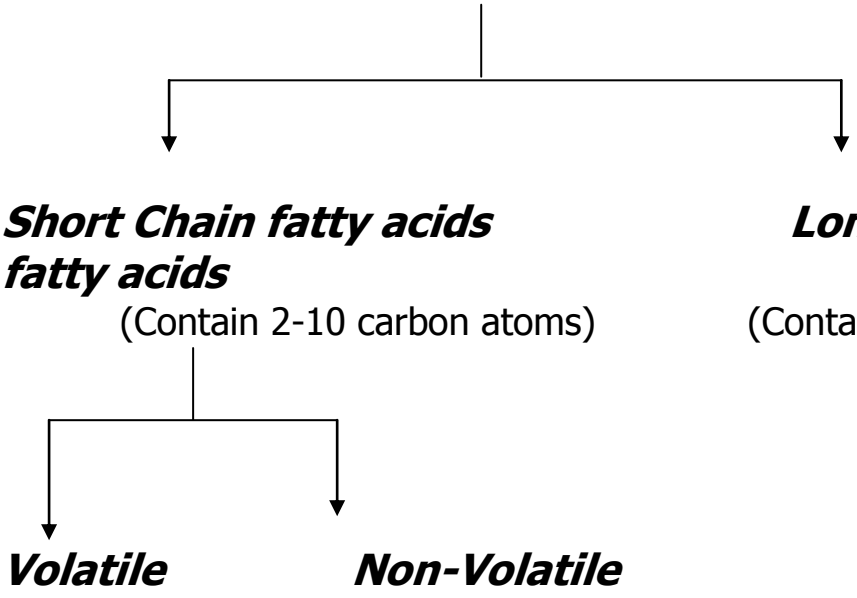
atom of CH_3 as the carbon number 1 " ω1" equal 7 carbon atoms & so on.

-Fatty acids can be classified into:

A- Saturated Fatty Acids.
B- Unsaturated Fatty Acids.
C- Hydroxy Fatty Acids.
D- Branched chain fatty acids.

A-Saturated Fatty acids:

- They contain no double bonds.
- The number of their carbon atoms range from 2-24 carbon atoms or more.
- They are subclassified according to the number of carbon atoms into:



1)- Volatile short chain fatty acids:-

- Liquid at room temprature.
- Volatile.
- Water soluble.

Examples:-

* **Acetic acid :** contains two carbon atoms.

CH_3-COOH

* **Propionic acid:** contains three carbon atoms.

CH_3-CH_2-COOH

***Butyric acid:** contains four carbon atoms, so called as it is commonly present in butter.

CH_3-$(CH_2)_2$-COOH

***Valeric acid:** contains five carbon atoms.

CH_3-$(CH_2)_3$-COOH

***Caproic acid:** contains six carbon atoms.

CH_3-$(CH_2)_4$-COOH

2)- Non-volatile short chain fatty acids:-

- Solid at room temperature .
- Non-volatile.
- Partially water soluble.

Examples:-

* **Caprylic acid:** contains eight carbon atoms.

CH_3-$(CH_2)_6$-COOH

***Capric acid:** contains ten carbon atoms.

CH_3-$(CH_2)_8$-COOH

3)-Long chain fatty acids:-

- Solid at room temperature .
- Non-volatile.
- Water insoluble.

Examples:-

***Palmitic acid:** contains 16 carbon atoms.

CH_3-$(CH_2)_{14}$-COOH

* **Stearic acid:** contains 18 carbon atoms.

CH_3-$(CH_2)_{16}$-COOH

Palmitic & stearic acids are widely distributed in animal fats.

***Arachidic acid:** which contains 20 carbon atoms.

CH_3-$(CH_2)_{18}$-COOH

* **Lignoceric acid:** contains 24 carbon atoms.

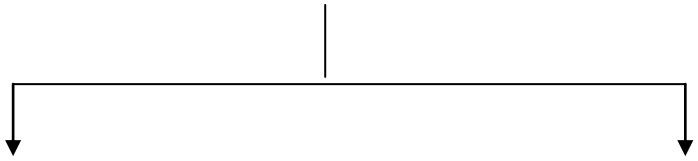
CH_3-$(CH_2)_{22}$-COOH

B-Unsaturated Fatty acids:

Definition: These are fatty acids which contain double bonds that affect their physical properties e.g. decrease melting temperature , therefore all unsaturated fatty acids are liquid at room temperature.

Classification:-

They are classified according to the number of double bonds into:-



A)-Monounsaturated fatty acids ***B)-Polyunsaturated fatty acids***

A) - Monounsaturated fatty acids

Definition: They are unsaturated fatty acids which contain one double bond.

Examples:

* **Palmitoleic acid** : which is unsaturated palmitic acid, it is

$C16:1\Delta^9$ i.e. this fatty acid has 16 carbon atoms & one double bond located between carbon atom number 9 & 10 from carboxylic end of fatty acid as follows:

$$CH_3\text{-}(CH_2)_5\text{-}CH{=}CH\text{-}(CH_2)_7\text{-}COOH$$

- Palmitoleic acid is ω7 fatty acid. , indicates a double bond on the 7th carbon counting from the methyl end of the fatty acid (ω -carbon).

* **Oleic acid:**it is the most common fatty acid in natural fat , it is $C18:1\Delta^9$ i.e. this fatty acid has 18carbon atoms

& one double bond located between carbon atom number 9 & 10 from carboxylic end of fatty acid as follows:

$$CH_3\text{-}(CH_2)_7\text{-}CH{=}CH\text{-}(CH_2)_7\text{-}COOH$$

So it is ω9 fatty acid.

*** Nervonic acid :** which is unsaturated lignoceric acid": it is
$C24{:}1\Delta^{15}$ i.e. this fatty acid has 24 carbon atoms & one double bond located between carbon atom number 15 & 16 from carboxylic end of fatty acid as follows:

$$CH_3\text{-}(CH_2)_7\text{-}CH{=}CH\text{-}(CH_2)_{13}\text{-}COOH$$

So it is ω9 fatty acid.

b)-Polyunsaturated fatty acids(PUFAs):

Definition: They are fatty acids which contains more than one double bond & some of them are essential i.e. can't be synthesized in our bodies & must be supplied in diet as they are important for normal growth & metabolism.

Sources:

* Plant " vegetable " oils: such as corn oil, linseed oil, peanut oil, olive oil, cottonseed oil, soybean oil.
* Code liver oils & animal fats.

Properties:

They are liquid at room temperature.

Functions "Importance":-

1-Prevention & treatment of fatty liver:-

As they are needed for triacylglycerol &phospholipids synthesis which bind different apolipoproteins in liver ,

forming lipoproteins which secreted in the plasma " plasma lipoprotein" , so decrease the fot content of the liver.

2- Prevention & treatment of Atherosclerosis hypercholestreamia & coronary artery diseases:- because PUFAs lead to:-

- Enhance mobilization of cholesterol esters to the liver.
- Increase hepatic Low Density Lipoproteins "LDL" receptors & decrease LDL uptake by vascular macrophages.
- Enhance conversion of cholesterol into bile acids.

3- They have an important role in blood clotting (intrinsic factor).

4- They have an important role in the health of the retina and vision.

5- They have an important role in the health of the skin i, normal growth and reproduction.

6- They are precursors for Eicosanoids biosynthesis.

7- Energy production via their oxidation.

8- Enter in structure of ceelular & subcellular membranes & help plasma phospholipids transport.

Deficiency Lead to:-

1) - Fatty liver.

2) - impaired lipid transport.

3) - Dermatitis, poor growth, decrease reproductive capacity.

4) - decrease resistance to stress & increase susceptibility to infections

Examples:

*** Linoleic acid :** $C18:2\Delta^{9,12}$

- It is essential fatty acid , ω6 fatty acid.

$CH_3\text{-}(CH_2)_4\text{-}CH{=}CH\text{-}CH_2\text{-}CH{=}CH\text{-}(CH_2)_7\text{-}COOH$

*** Linolenic acids:**

1- α-Linolenic acid : $C18:3\Delta^{9,12,15}$
- It is essential fatty acid , ω3 fatty acid.

CH_3-CH_2-CH=CH-CH_2-CH=CH-CH_2-CH=CH-$(CH_2)_7$-COOH

2- γ-Linolenic acid: $C18:3\Delta^{6,9,12}$
- It is semi-essential fatty acids , ω6 fatty acid

CH_3-$(CH_2)_4$-CH=CH-CH_2-CH=CH-CH_2-CH=CH-$(CH_2)_4$-COOH

*** Arachidonic acid:** $C20:4\Delta^{5,8,11,14}$
- It is semi-essential fatty acid , ω6 fatty acid

CH_3-$(CH_2)_4$-CH=CH-CH_2-CH=CH-CH_2-CH=CH-CH_2-CH=CH-$(CH_2)_3$-COOH

*** Clupandonic acid:** $C22:5\Delta^{7,10,13,16,19}$
- It is semi-essential fatty acid, ω3 fatty acid.

CH_3-CH_2-CH=CH-CH_2-CH=CH-CH_2-CH=CH-CH_2-CH=CH-CH_2-CH=CH-$(CH_2)_5$-COOH

C- Hydroxy Fatty Acids:

Definition:
- These are fatty acids which contain one or more hydroxyl group(s) (OH) in their structure.

Examples:-

1- Ricinoleic acid.
2- Dihydroxy srearic acid.
3- Oxynervonic acid.
4- Cerebronic acid

} Found in brain glycolipids.

D- Branched chain fatty acids:

- These are fattyacids with branched chains.
- Example: Phytanic acid, that is a by-product of chlorophyll catabolism in human and animal . If it is not oxidized in the body by initial α- oxidation followed by β-oxidation, it will be toxic resulting in Refsum's disease.

I)- Simple lipids:

- **They are esters of fatty acids with alcohols.**
- **Sub classified according to the type of alcohol into :-**

A)- Neutral fats:-

- They are called neutral due to absence of ionizable groups i.e. they are uncharged.
- They are esters of three fatty acids with glycerol in the form of triglycerides "Triacylglycerols" i.e contain three acyl groups .
- Neutral fats are classified into two subgroups:

A) Oils : They are liquid due to their high content of unsaturated fatty acids(USFAs) , they have lower melting points e.g. corn , cottonseed oils & soybean oils.

B) Solid fats: they have higher melting points due to their high content of saturated fatty acids(SFAs) e.g. margarine

B) - Waxes:-

- They are esters of fatty acids and long chain monohydric alcohols but not glycerol.
- Examples:

1. Esters of fatty acids with cetyl or mericyl alcohol.
2. Esters of fatty acids with cholesterol.
3. Esters of fatty acids with vitamin A or D.

Types of Waxes:-

A) - True Waxes: which include:

1) - Bees wax: secreted by hony bees that use it to form combs.

- It is formed of ester of palmitic acidwith mericyl alcohol.

2) - Spermaceti wax:

- Present in skull of certain whales & dolphin.
- Used in manufacture of candles.
- It is formed of ester of of palmitic acid with cetyl alcohol.

3) - Carnuba wax:

- Plant wax derived from carnuba leaves.
- used in manufacture of polishes.

B) - Waxes like compounds & include:

1**) - Cholesterol esters "Lanolin or wool fat":**

- Present in wool fats secreted by sebaceous glands.
- Formed of ester of fatty acid with alcohol which is cholesterol.
- Used in pharmaceutical & cosmotic preparations as it is hygroscopic i.e. absorb much water.

2) - Esters of vitamin A & D:

- Formed of ester of fatty acid with vitamin A or D as alcohols.
- They act as storable form of these vitamins.

Table showing the main differences between Neutral fats& Waxes:

	Neutral fats	***Waxes***
Number of fatty acids	3 fatty acids	One fatty acid
Type of fatty acids	Long & short chain fatty acids e.g. palmitic , stearic & oleic acids	Fatty acid is mainly palmitic or srearic acid i.e. long chain fatty acids
Type of alcohol	Glycerol which is trihydric alcohol	Long chain monohydric alcohol e.g. cetyl alcohol , mericyl alcohol , cholesterol , vitamin A , vitamin D
Acrolein test	Positive due to presence of glycerol	Negative due to absence of glycerol.
Nature at room temperature	Soft , liquid or solid	Hard & solid
Digestibility	Digestible (hydrolyzed by lipase)	Indigestible (not hydrolysable by lipase) .
Nutritive value	nutritive	Of no nutritional value
Rancidity	Rancidible	Never get rancid
Examples	Butter & vegetable oils	A)- True Waxes: which include: 1)- Bees wax. 2)- Spermaceti wax. 3)- Carnuba wax.

		B)- Waxes like compounds & include: 1)- Cholesterol esters " Lanolin or wool fat". 2)- Esters of vitamin A & D.

Properties of neutral fats:-

I- Physical properties.
II-Chemical properties.

I- Physical properties:

A) - Freshly prepared fats & oils are colorless, odorless, & tastless.
B)-They have specific gravity less than 1 & so they float on water.
C) - They are insoluble in water & soluble in organic solvents e.g ether & benzene.
D) - Their melting points are usually low but higher than solidification point.

- Melting point depend on:

1- The length of chain of the fatty acid.
2- The degree of unsaturation .

- Neutral fats that have shorter chain fatty acids & or more unsaturated fatty acids e.g. oils posse lower melting point than those having longer chain fatty acids & or more saturated fatty acids e.g. solid fats. , this mean that there is inverse relationship between the melting point & the degree of unsaturation as

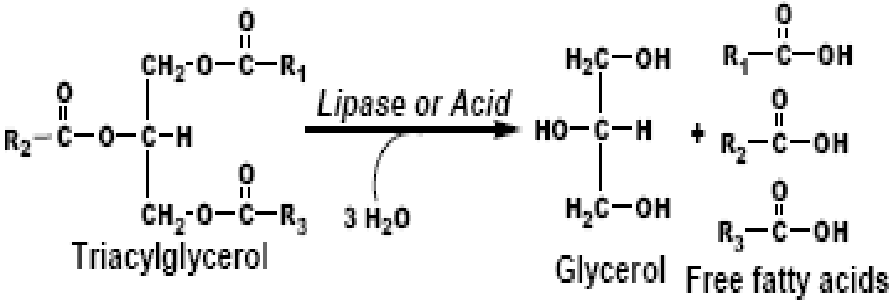
increasing the degree of unsaturation leads to decrease the melting point & vice versa ,but the melting point is directly proportionate with the length of the chain of fatty acids as increasing the length of chain of fatty acids leads to increasing the melting point & vice versa.

II-Chemical properties

1-Hydrolysis.
2-Hydrogenation or hardening of oils.
3-Oxidation.

1-Hydrolysis:

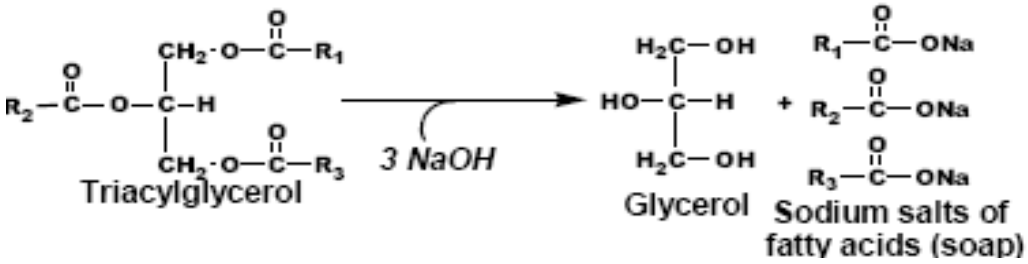
- Hydrolysis occurs by acids , alkalis , heat or lipase enzyme that gives fatty acids & glycerol as follows:



Saponification:

- **Definition:** it is the process of hydrolysis of neytral fats by addition of an alkali such as NaoH (Sodium hydroxide) or KoH (Potassium hydroxide) that gives glycerol & salts of fatty acids , these salts of fatty acids are called **Soap.**

- **Example:**



- **Saponifiable fraction:**

It is the portion of total lipids that after treatment with alkali become soluble in water & insoluble in ether.

- **Unsaponifiable fraction:**

It is the portion of total lipids that can't be saponified by alkali & insoluble in water & soluble in ether & they include:-

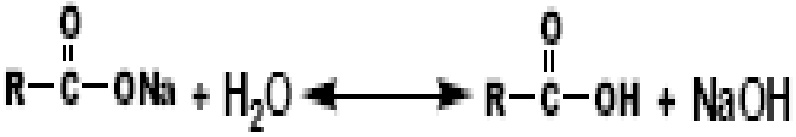
1- High molecular weight alcohols.
2- Hydrocarbons.
3- Waxes, ketones & steroids.

Types of Soaps:-

1- Ordinary Soaps (hard Soaps) : formed as a result of saponification of neutral fat with NaoH.
2- Green soft soaps: formed as a result of saponification of neutral fat with KoH.
3- Calcium & magnesium Soaps: which are useless insoluble soap.
4- Germicidal soap: soap with high molecular weight unsaturated fatty acids or with sodium ricinoleate, these types of soaps have germicidal action & can detoxify diphtheria & tetanus toxins.

Cleaning action of soap:

- Due to their ability to reduce surface tension , thus facilitate emulsification of oils & grease so can be easily washed by water.
- Reverse hydrolysis of soap into free NaOH resulting on washing of skin with soap & water has irritative effect on skin which is called irritative alkaline reaction as shown :



- Can be decreased by adding excess fatty acid or Na-oleate added to soap especially toilet soap as sodium oleate is not easily hydrolyzed.

Saponification number or value:

Definition: It is number of milligrams of KoH required to completely saponofy one gram of fat.

N.B: Fats containing more short chain fatty acids will have more carboxyl groups "COOH" per gram than fat containing more long chain fatty acids , each carboxyl group of a fatty acid will reacts with one molecule of KoH during saponification.

Uses of saponification number or value:

1. Choose the most suitable type of fat for soap manufacture as toilet soap is manufactured from fat with low saponification number (as it will consume less irritating KoH) while cleaning soap manufactured from fat with high saponification number.
2. Detection of natural characteristics & adulteration of fat.

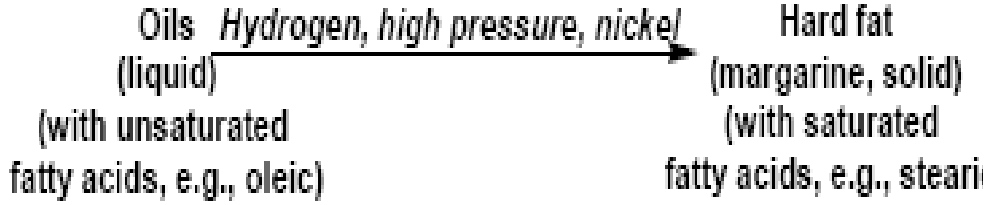
2-Hydrogenation or hardening of oils:

Definition:-

- It is accepting hydrogen at the double bonds of unsaturated fatty acids under high pressure of hydrogen & it is catalyzed by addition of nickel or copper & heat, it is the base of hardening of oils (Margarine manufacturing).

Example:

- Change of oleic acid of fat (liquid) into stearic acid (solid).



- It is advisable not to saturate all double bonds otherwise margarine produced will be very hard, difficult to digest & so of very low biological value.

Advantages for hydrogenated fats :-

1. It is more pleasant and digestible fat.
2. It is less liable to cause gastric or intestinal irritation.
3. It is easily stored and transported and less liable to rancidity.

Disadvantages of hydrogenated fats:-

1-lack of fat-soluble vitamins (A, D, E and K).
2- Lack of essential fatty acids due to their saturation.

3-Oxidation:

Definition:

- It is oxidation "addition of oxygen" of unsaturated fatty acids at double bonds to give peroxide derivatives that decompose into aldehydes, ketones & short chain fatty acids.

- It is the base of drying oils after exposure to atmospheric oxygen & so can be used in paints & varnishes manufacturing .

Rancidity

Definition:

- It is physiochemical changes in natural properties of fat leading to development of abnormal taste , odor or color particularly after aging following exposure to :
  1. Atmospheric oxygen.
  2. heat , light & moisture.
  3. bacterial , fungal contamination .

N.B: saturated fat resist rancidity more than unsaturated fat that have unsaturated double bonds.

Types & Causes:

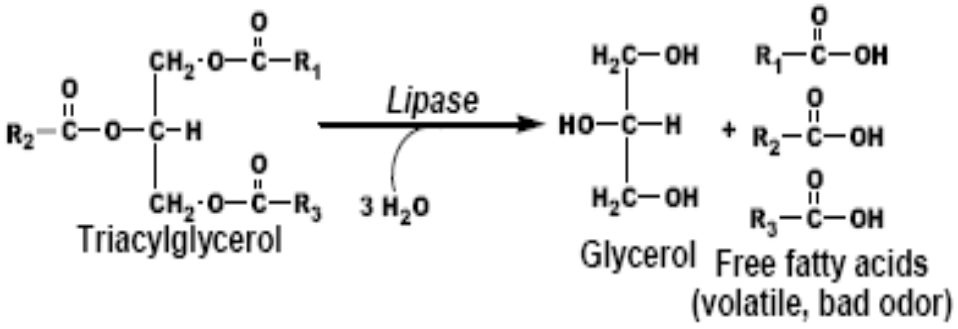
I-Hydrolytic rancidity.
II-Oxidative rancidity.
III-ketonic rancidity.

I-Hydrolytic rancidity:

Lipase from bacterial contamination in presence of
High temperature & moisture

Fat ⟶ volatile short chain fatty acids that have bad odor

as follow:



II-Oxidative rancidity:

- It occurs as a result of exposure of fat to oxygen light & or heat leading to addition of oxygen to the double bond of unsaturated fatty acids with production of peroxide derivatives.

- Peroxide derivatives will decompose to toxic bad odor substances such as peroxides, aldehydes, ketones, hydroxyl fatty acids & dicarboxylic acids.

III-ketonic rancidity:

- It occurs as a result of contamination of fat with certain fungi e.g. aspergillus niger in presence of moisture , leading to formation of ketones , aldehydes short chain fatty acids & alcohols.

Hazards of rancidity:

1. The products of rancidity are toxic, i.e., causes food poisoning and cancer.
2. Rancidity destroys the fat-soluble vitamins (vitamins A, D, K and E).
3. Rancidity destroys the polyunsaturated essential fatty acids.
4. Rancidity causes economical loss because rancid fat is inedible.

How to prevent Rancidity? By:

1- Avoidance of causes of rancidity such as light , temperature , oxygen , moisture , bacteria & fungi.

2- Removal of catalysts of rancidity e.g. copper & lead.

3- Addition of antioxidants such as vitamin E (tocopherols), phenols, naphthols, hydroquinones & tannins.

Fat Numbers or Fat Values or Fat Constants

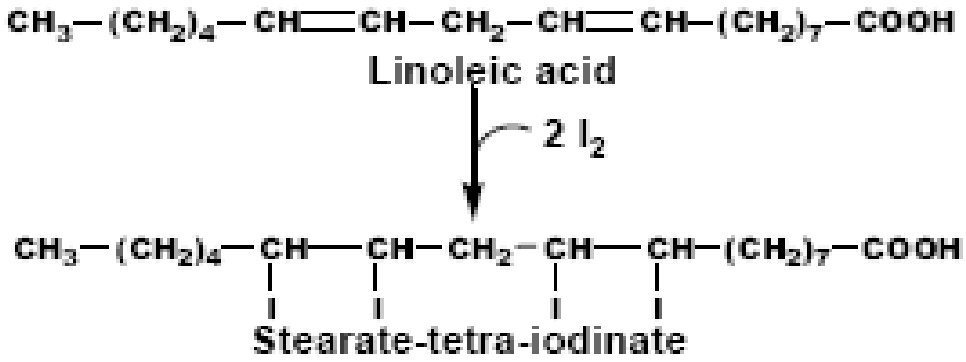
These tests or numbers depend on:
1- Physical properties e.g. melting & solidification points.
2- Chemical properties e.g. degree of unsaturation , liability to saponification.

Uses:
1- Checking the purity of fat & detect fat adulteration.
2- Identify biological value & natural characteristics of fats.
3-Detect fat rancidity & presence of toxic hydroxyl fatty acids.

- They include the followings:

I- Iodine number or value:

Definition:
It is the number of grams of iodine absorbed by 100 grams of fat or oil.
Uses:
1-Measures the degree of unsaturation of fat as unsaturated fatty acids absorb iodine at their double bonds as follows:



2- Checking the purity of fat & detect fat adulteration.
3- Identify the biological value & natural characteristics of fats

II-Saponification number:

- Previously discussed in Saponification.

III- Acid number:

Definition:

- It is the number of milligrams of KoH required to neutralize the free fatty acids present in one gram of fat.

Uses:

- It measures the amount of free fatty acids present (products of hydrolytic rancidity which are alcohol & free fatty acids) so it is used for detection of hydrolytic rancidity.

IV- Acetyl number:

Definition:

- It is number of milligrams of KoH required to neutralize the acetic acid liberated from hydrolysis of one gram of acetylated fat.

N.B: fat containing hydroxyl fatty acids able to be acetylated due to presence of free hydroxyl groups , so acetyl number measure number of hydroxyl groups present .

Uses:

1-Checking the purity of fat & detect fat adulteration.
2- Identify natural characteristics of fats.
3- Detection of fat rancidity & presence of toxic hydoxy fatty acids

V- Reichert- Meissl Number (or value):

Definition:

- It is the number of milliliters of 0.1 N KoH required to neutralize the water soluble fatty acids (volatile short chain fatty acids less than 10 carbon atoms) in 5 grams of fat , so this number measures the percentage of short chain fatty acids in fat.

Uses:

1- Detection of fat adulteration.
2- Detection of natural composition of fat.

II- Compound Lipids:

Definition: They are lipids that contain additional groups beside alcohol & fatty acids.

- They are classified according to the additional groups into :-

I-Phospholipids (phosphatides).
II- Glycolipids.
III- Lipoproteins.
IV- Sulfolipids .

V- Aminolipids.

I- Phospholipids (phosphatides):

Definition: They are compound lipids which contain phosphoric acid group in their structure.

Sources:

- Present in every cell (plant or animal cells) especially in the cell membranes as the major lipids of biological membranes are phospholipids & cholesterols.
- milk , egg yolk.

Classification:

- Phspholipids can be classified into two main groups according to the type of alcohol present:

A- Glycerophospholipids:-

- also called phosphatidic acid derivatives.
- they contain ***glycerol*** as an alcohol.
- they include the followings:-

1- Phosphatidic acids.
2- Lecithins.
3- Cephalins.
4- Plasmalogens.
5- Inositides.
6- Cardiolipins.

B- Sphingophospholipids:

- They contain ***sphingosine*** as an alcohol.
- They include sphingomyelens.

1- Phosphatidic acids:

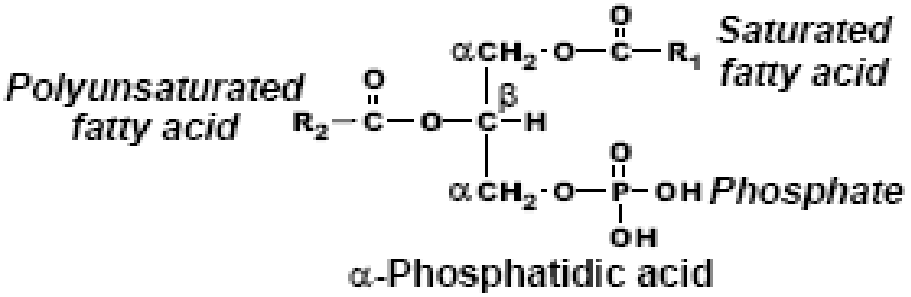
Structure:

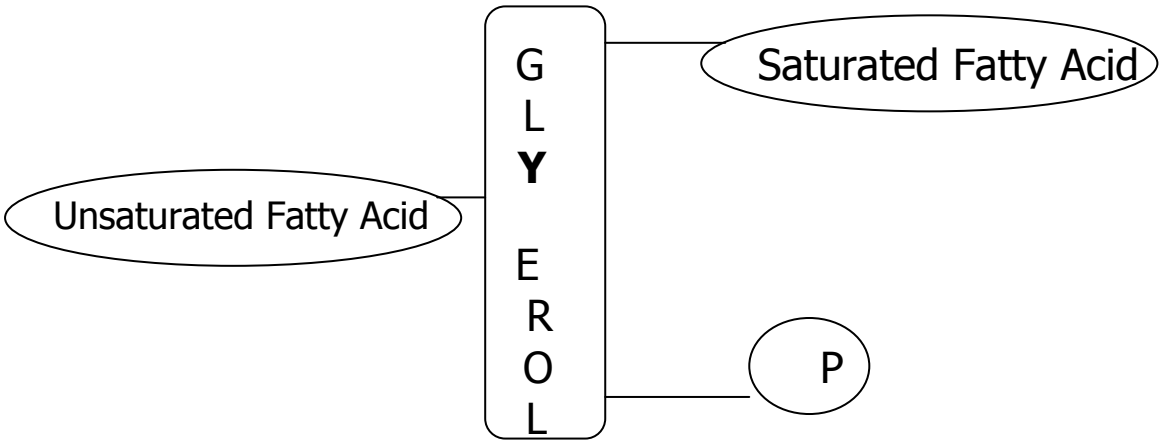
It is formed of:

- *Diacylglycerol (DAG) :*one of the two fatty acids is saturated attached to C1 of glycerol & the other fatty acid is unsaturated attached to C2 or C3 of glycerol.

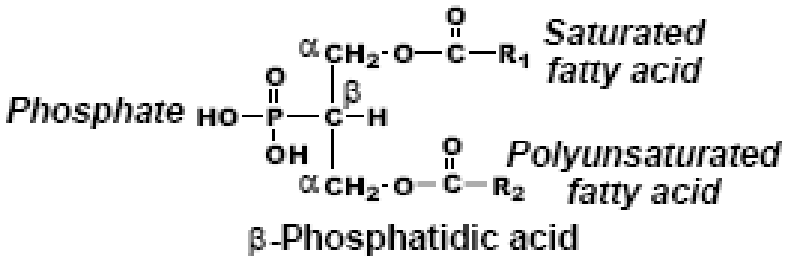
- *Phosphoric acid group.*

* If the phosphoric acid group attached to C3 of the DAG , it is called α-phosphatidic acid as follows:





* If phosphoric acid group attached to C2 of the DAG & the unsaturated fatty acid attached to C3 of the DAG it is called β-phosphatidic acid as follows:



β-Phosphatidic acid

Importance:

1- They are metabolic intermediates during bisynthesis of triacylglycerols & glycerophospholipids in the body.
2- Signaling function as it acts as second messenger.

2- Lecithins (Phosphatidyl Choline):

Structure:

- It is formed of:phosphatidic acid & choline as a base, so lecithin is called phosphatidyl choline i.e. it is formed of:

- *Diacylglycerol (DAG)* : which contains one saturated fatty acidattached to it's C1 & the other is unsaturated fatty acid attached to it's C2 or C3.

Common fatty acids in lecithins are :

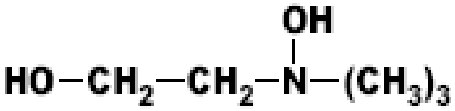
* Saturated fatty acids : - Stearic acid.
  - Palmitic acid.

*Unsaturated Fatty acids:

Monounsaturated: - Oleic acid.

Polyunsaturated: - Lenoleic acid.
- Lenolenic acid.
- Arachidonic acid.
- Clupandonic acid.

- *Phosphoric acid* attached to C2 or C3 of DAG .
- *Choline base* attached to phosphoric acid:

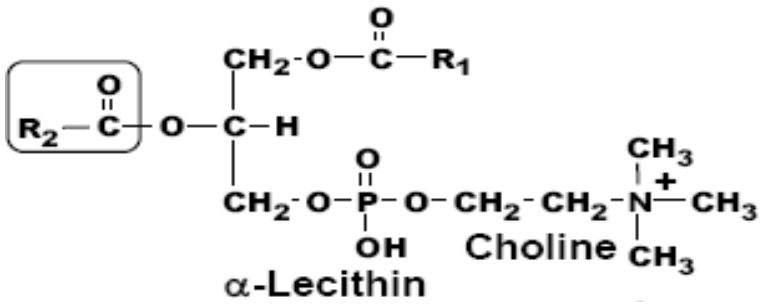


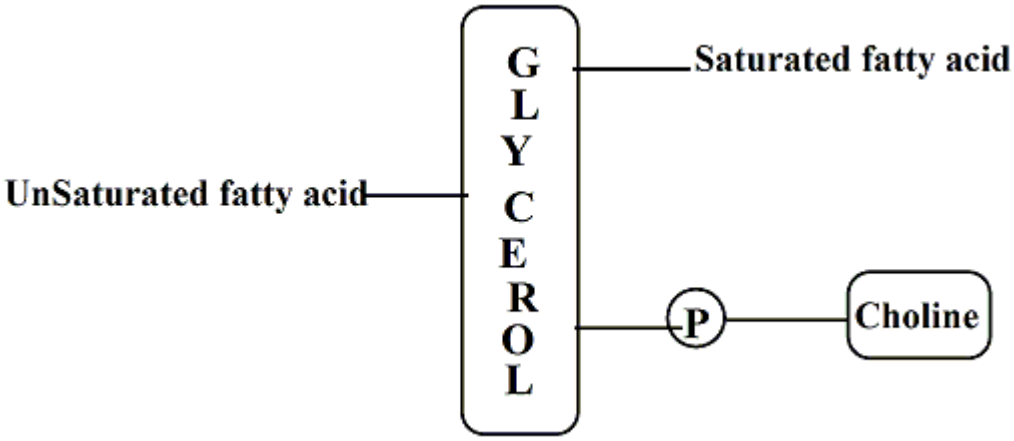
Choline

- Lecithins present in two forms : α- form & β- form.

* α- form: Present in brain.

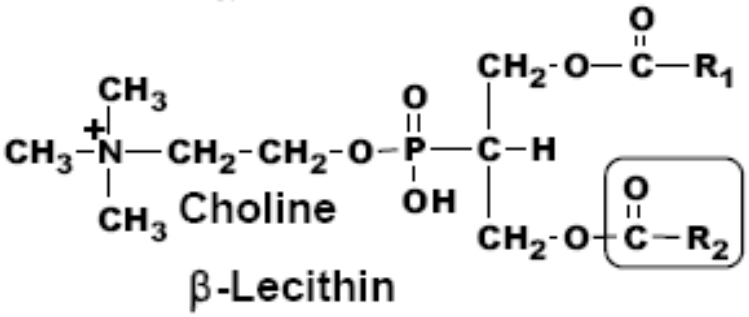
- In which the the phosphoric acid attached to C3 of DAG & the unsaturated fatty acid attached to C2 of DAG as follows:





* β- form: Present in egg yolk.

- In which phosphoric acid attached to C2 & the unsatyrated fatty acid attached to C3 of DAG as follows:



β-Lecithin

* Both forms α and β are present in liver.

The Greek word for egg yolk — lekithos — is the basis for the name lecithin.

Properties of Lecithins:

1- White to pale yellow waxy solids when freshly prepared & on exposure to air , they darken due to oxidation of polyunsaturated fatty acids.

2- Soluble in fat solvents including alcohol but insoluble in acetone (used for separation of lecithins from fats).

3- Form emulsion in water (Colloidal solution) & extremely hygroscopic.

4- They are hydrolyzed by alkali (i.e. saponifiable) producing phosphoric acid , choline , glycerol & soap.

Importance:-

1- Lysolecithin formation :

Snake & Scorpion venom contain lecithinase enzyme which hydrolyzes the polyunsaturated fatty acid converting lecithin into lysoleithin which cause heamolysis of R.B.Cs . This partially explains the toxic effect of snake or scorpion venom, poisonous spiders and stinging insects.

2- Eicosanoid synthesis:

- Through hydrolysis of the polyunsaturated fatty acids from cell membrane phospholipids including lecithin by phospholipase A2.

3- Lung surfactant:

- It is formed of : a)- Dipalmitoyl – lecithin.

b)- Sphingomyelin.

c)- Apoprotein A,B,C &D

- It is produced by type-II alveolar cells.

- It's function :

a)- Lower alveolar surface tension , so prevent their collapse during expiration & improve gas exchange .

b)- Activate macrophages to kill pathogens .

- Premature babies lacking lung surfactant suffer from Respiratory Distress Syndrome , there is artificial surfactant that can be given to these babies .

- Natural or synthetic glucocorticoids increase surfactant production.

4- Lipotropic activity : lecithins prevent accumulation of fat in liver & prevent fatty liver as they play a role in lipid transport from liver through formation of lipoproteins.

5- Lecithins present in bile to solubilize cholesterol presents in bile so lecithins deficiency lead to cholesterol gall bladder stones (cholelithiasis).

6- Enter in structure of cell membranes affecting their permeability and also present on the outer leaflet of plasma membrane so:

a- participate in intercellular contact & communication.

b- acts as receptors for bacterial toxins .

7- Due to their content of choline , lecithins act as **neurotransmitter & methyl donor in transmethylation** reactions.

8- Nutritional lecithin: which is lecithin taken in Diet **has been called "brain food"** : Because of its high concentration of choline ,choline, in the form of acetylcholine, is a neurotransmitter within the brain. In fact, the brain contains the body's single largest store of acetylcholine. Without acetylcholine, primary brain

functions such as memory, thought, and muscular control would not be possible. Acetylcholine is so critical to healthy brain function that if sufficient amounts are not supplied by the diet, the body will "cannibalize" body tissue and extract the needed choline or choline building blocks.

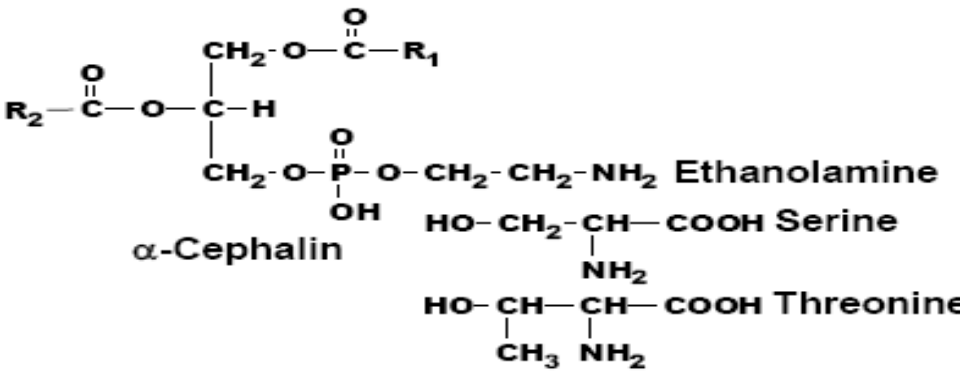
3- Cephalins (or Kephalins):

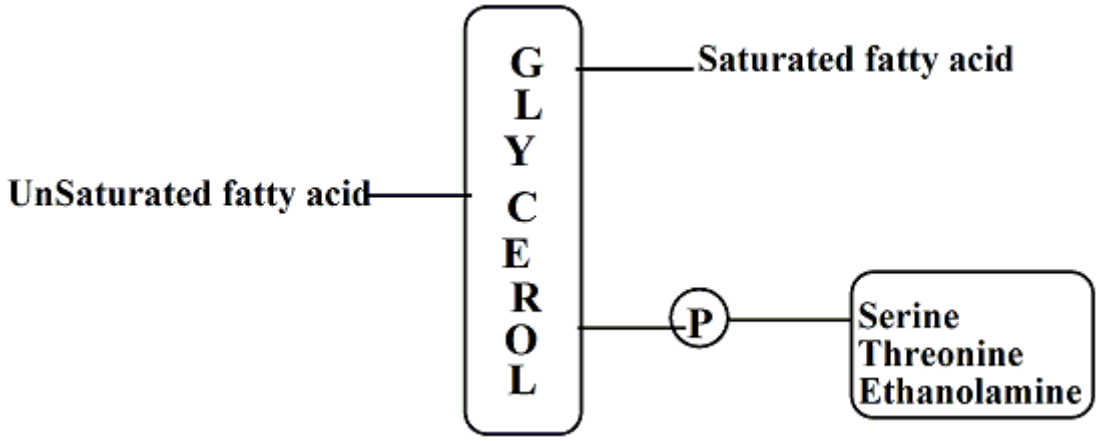
Location:

1- They are occurring in association with lecithins in tissues.
2- They are isolated from the brain (Kephale = head).

Structure:

- They are called phosphatidyl-Serine or phosphatidyl-Ethanolamine or phosphatidyl-Thereonine i.e. resemble lecithin in structure except that choline is replaced by :
1- Ethanolamine.
2- Serine.
3- Threonine.
- It is presents in two forms:- α – form & β- form as follows:





Properties of Cephalins:

- As lecithins (1,2,3,4) except that they are insoluble in alcohol.

Importance:-

1- Lysocephalin formation:

- Lysocephalin produced by action of cephalinase enzyme present in snake & scorpion venom which hydrolyzes the PUFAs present in cephalins producing lysocephalin.
- Lysocephalin has hemolytic action on R.B.Cs.

2- Eicosanoid synthesis :

- Through hydrolysis of the polyunsaturated fatty acids from cell membrane phospholipids including cephalins by phospholipase A2.

3- Used in **early diagnosis of Apoptosis** (programmed cell death)

as it is usually kept on the inner leaflet (cytosolic side) of cell membranes by an enzyme called flippase , when the cell undergoes apoptotic cell death , cephalin is no longer restricted to the cytosolic part of the membrane but becomes exposed on the surface of the cell.

4- Enter in structure of Thromboplastin (Thrombokinase) which accelerate **blood clotting** as follows:

$$\text{Prothrombin} \xrightarrow[Ca^{++}]{\text{Thromboplastin}} \text{Thrombin}$$

5- Enter in structure of cell membranes affecting their permeability and also present on the outer leaflet of plasma membrane so:

a- participate in intercellular contact & communication.

b- acts as receptors for bacterial toxins .

6- Lipotropic activity : cephalins prevent accumulation of fat in liver & prevent fatty liver as they play a role in lipid transport from liver through formation of lipoproteins.

4- Plasmalogens:

Location:

- Present in cell membrane phospholipids of liver , brain , muscle , semen & eggs.

.

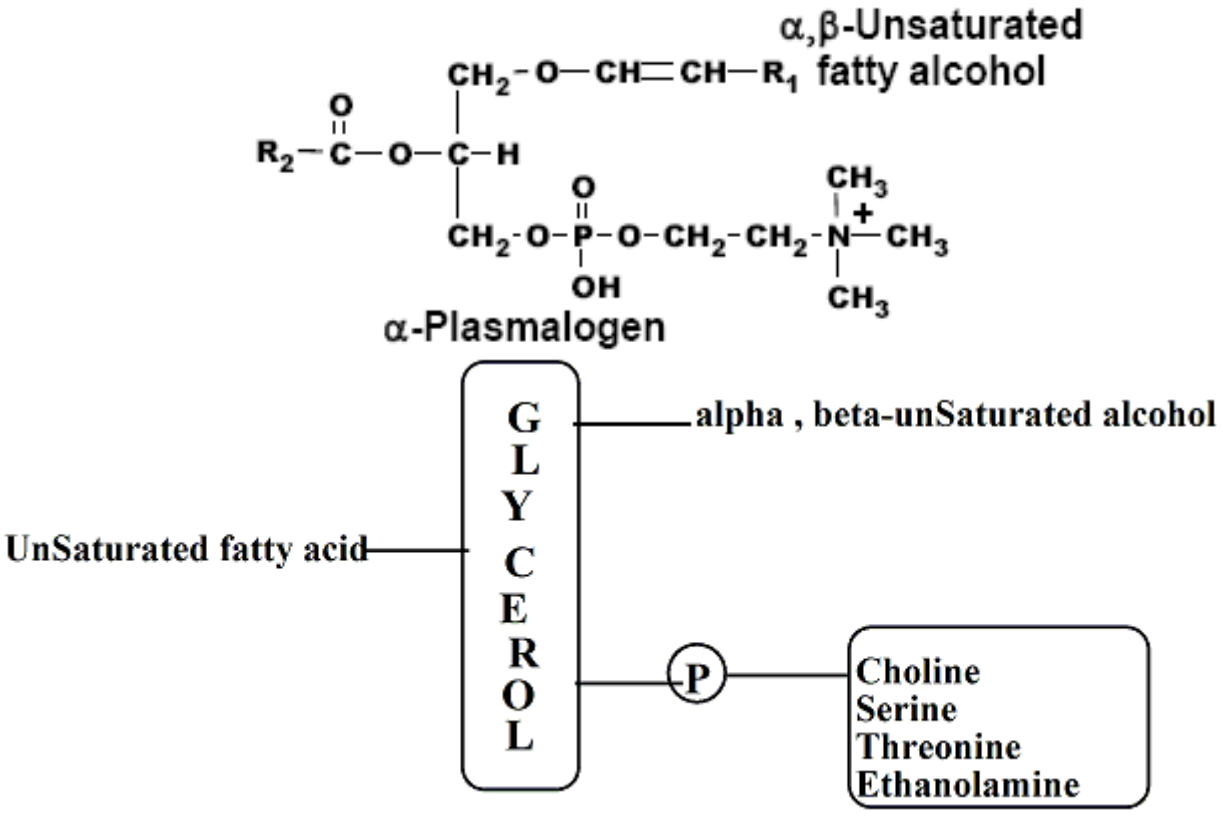
Structure:

*** Plasmalogens** resemble lecithins & cephalins in structure except :-

1- At C1 of Glycerol : Presence of α , β unsaturated alcohol rather than a fatty acid connected by ether linkage (R-O-R).

2- At C1 of Glycerol: There is an unsaturated long chain fatty acid , however it may be short chain fatty acid (e.g. acetic acid in platlet aggregating factor).
3- At C3 of Glycerol: Phosphoric acid attached to which any of the followings is attached:
a- Choline .
b- Serine.
c- Threonine.
d- Ethanolamine.

* **Plasmalogens** present in two forms: α-form (if the unsaturated fatty acid attached to C2) & β-form (if the unsaturated fatty acid attached to C3)as follows:



Properties of Plasmalogens:
- Similar to lecithins (1,2,3 &4).

Importance:-

1-Eicosanoid synthesis :
- Through hydrolysis of the polyunsaturated fatty acids from cell membrane phospholipids including plamalogens by phospholipase A2.

2- Promote **platlet aggregation**: as Platlet Aggregating Factor **(PAF)** is a plasmalogen.

3- Enter in structure of cell membranes affecting their permeability and also present on the outer leaflet of plasma membrane so:
a- participate in intercellular contact & communication.
b- acts as receptors for bacterial toxins .
4- Due to their content of choline , plasmalogens act as **neurotransmitter & methyl donor in transmethylation** reactions.
5- Lipotropic activity : plasmalogens prevent accumulation of fat in liver & prevent fatty liver as they play a role in lipid transport from liver through formation of lipoproteins.

5- Inositides (Phosphatidyl inositol) (Lipositol):

Location:
- Brain tissues.

Structure:
- They are phosphatidyl inositol .
- Formed of glycerol , one saturated fatty acid , one unsaturated fatty acid , phosphoric acid & inositol (which is sugar alcohol).

- Inositol : it is hexahydric alcohol that has more than 14 isomer.

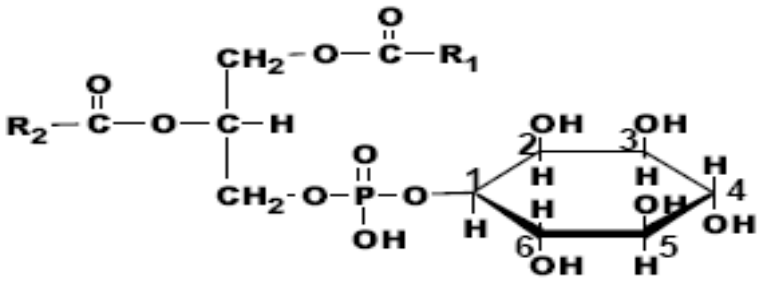
1- It is present in high concentration in Bran (outer coat of cereals where it combines with 6 molecules of phosphoric acid to give phytic acid. Phytic acid forms insoluble iron, calcium and magnesium salts (phytate) which hinder absorption of Ca2+, Mg2+ and iron in the intestine.

2- It presents in high concentration in heart and muscles tissues, so it is called muscle sugar.

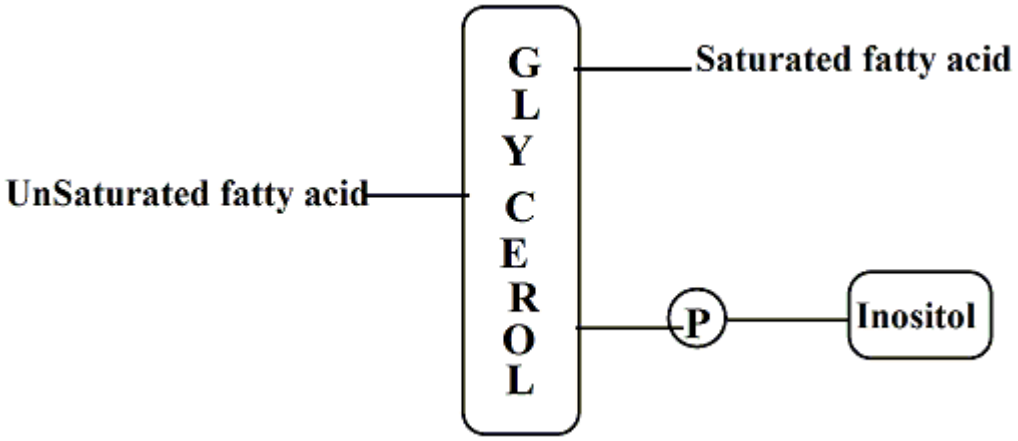
3- It is considered a member of vitamin B complex.

4- It is essential for synthesis of phospholipids, phosphatidylinositol.

- Phosphatidylinositol. is present in two forms:α - form & β-form.



α-Phosphatidylinositol



Properties of phosphatidylinositol :

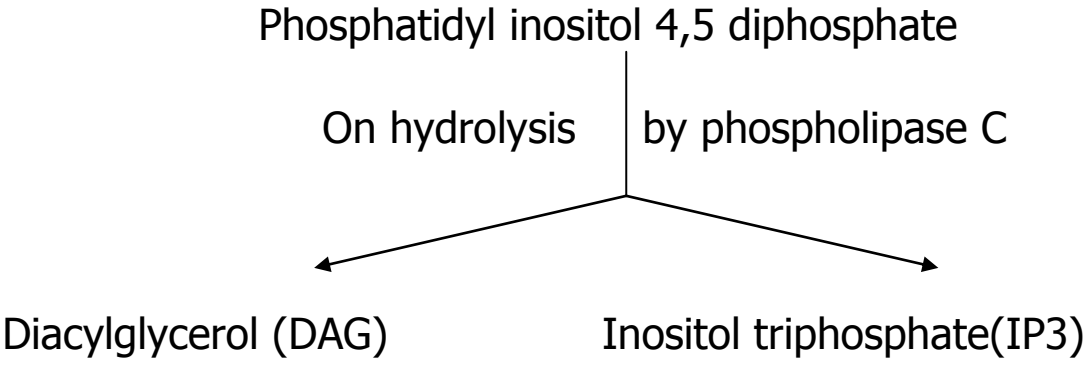
- Similar to lecithins (1,2,3 &4).

Importance:-

1-Eicosanoid synthesis :
- Through hydrolysis of the polyunsaturated fatty acids from cell membrane phospholipids including phosphatidylinositol by phospholipase A2.

2- Enter in structure of cell membranes affecting their permeability and also present on the outer leaflet of plasma membrane so:
a- participate in intercellular contact & communication.
b- acts as receptors for bacterial toxins .

3- Acts as second messenger : during signal transduction of certain hormones:
- Phosphorylation of phosphatidyl inositol at 4 & 5 positions by specific kinase leads to formation of phosphatidyl inositol 4,5 diphosphate .



- Both DAG & IP3 cause increase cytoplasmic Ca++ through :
a- Increase Na+/Ca++ & H+/Ca++ exchange.
b- Increase release of Ca++ from intracellular stores.
To mediate the hormonal actions
4- Lipotropic activity : phosphatidyl inositols prevent accumulation of fat in liver & prevent fatty liver as they

play a role in lipid transport from liver through formation of lipoproteins.

6- Cardiolipins:

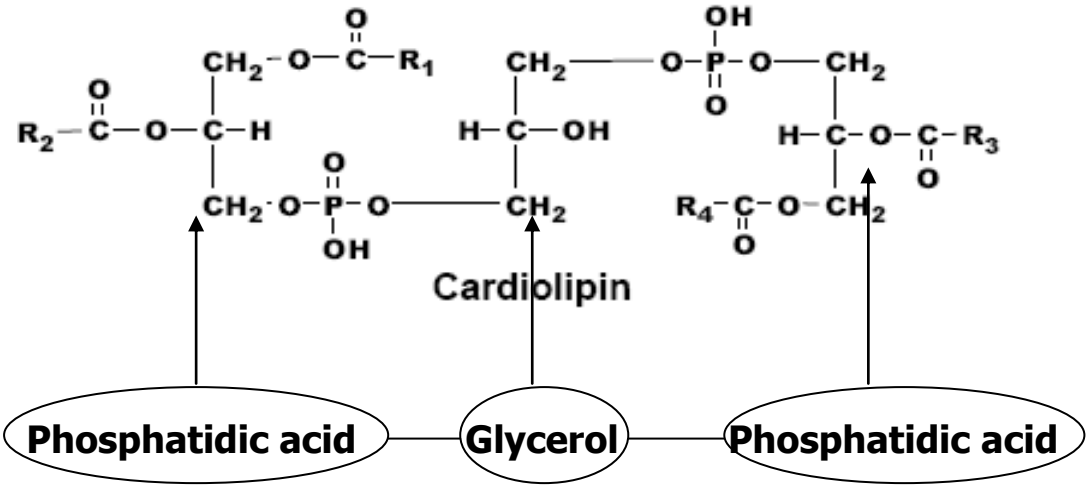
Location:

- In inner membrane of mitochondria.
- Initially isolated from heart muscle (Cardio).

Structure:

- It is diphosphatidyl glycerol.
- Formed of two phosphatidic acids with glycerol in between.
- So it is totally composed of:

a- three molecules of glycerols.
b- four fatty acids.
c- two phosphoric acid groups. As follows:



Importance:-

1**- Enter in structure of cell membranes** affecting their permeability and also present on the outer leaflet of plasma membrane so:

a- Participate in intercellular contact & communication.
b- Acts as receptors for bacterial toxins.

2- Used in **serological diagnosis** of syphilis & autoimmune diseases.

3-Eicosanoid synthesis :
- Through hydrolysis of the polyunsaturated fatty acids from cell membrane phospholipids including cardiolipins by phospholipase A2.

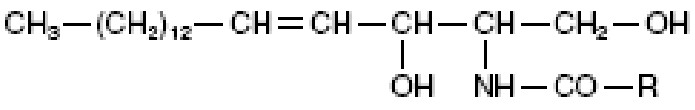
4- Lipotropic activity : cardiolipins prevent accumulation of fat in liver & prevent fatty liver as they play a role in lipid transport from liver through formation of lipoproteins.

Sphingomyelins

Location:
- Present in large amounts in brain & nerves.
-Presents in small amounts in liver , spleen , lungs, kidneys& blood.

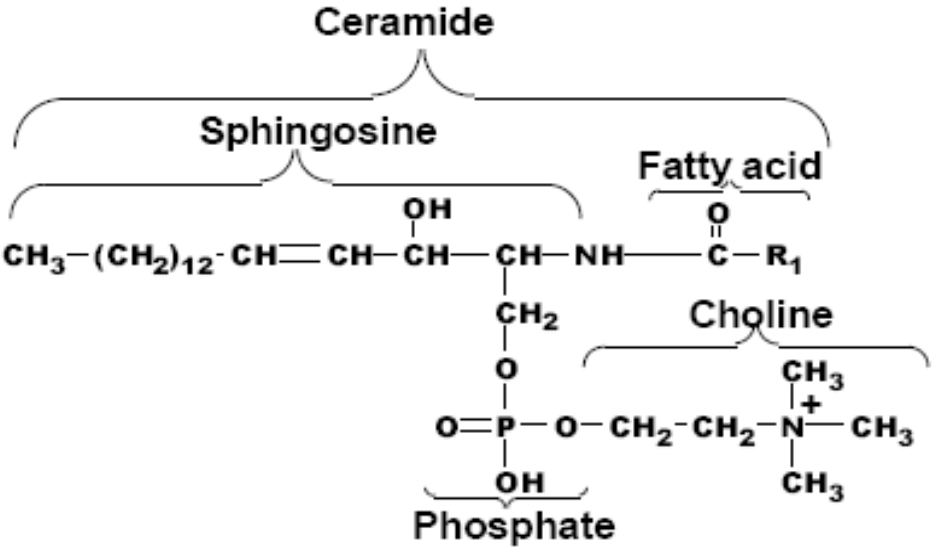
Structure:

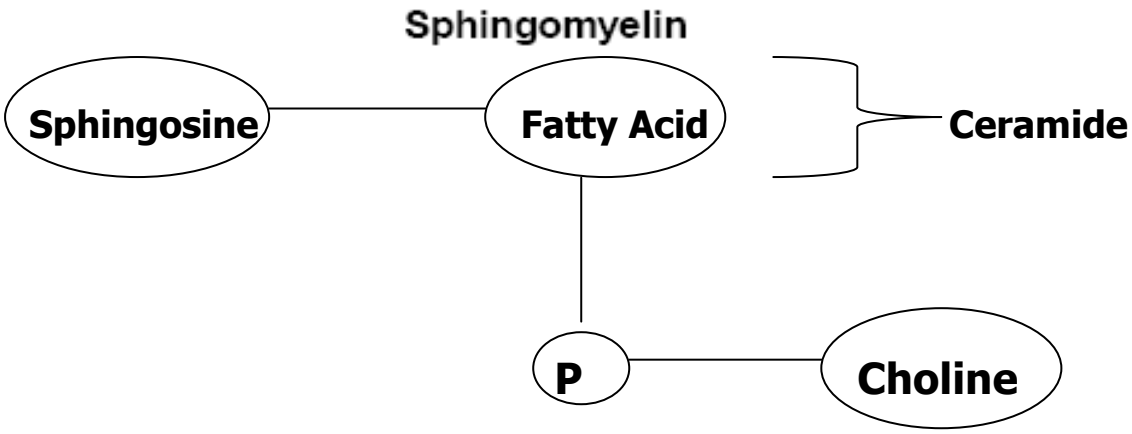
They are consist of :
1- Ceramide: which is sphingosine alcohol linked with fatty acid by amide linkage as follows:



Ceramide

N.B: Fatty acids commonly enter in it's structure are: Palmitic , stearic , lignoceric & nervonic acid.
2- Phosphoric acid attached to sphingosine .
3- Choline attached to phosphoric acid forming phosphocholine.





Properties of Sphingomyelines:

-They are white crystals stable to light and air .
-They are insoluble in ether or cold alcohol but soluble in chloroform, benzene or hot alcohol.

Importance:-

1-Eicosanoid synthesis :

- Through hydrolysis of the polyunsaturated fatty acids from cell membrane phospholipids including phosphatidylinositol by phospholipase A2.

2- Enter in structure of cell membranes affecting their permeability and also present on the outer leaflet of plasma membrane so:

a- participate in intercellular contact & communication.

b- acts as receptors for bacterial toxins .

3- Due to their content of choline , plasmalogens act as **neurotransmitter & methyl donor in transmethylation** reactions.

4- Ceramide of sphingomyelines **mediate apoptosis** or programmed cell death.It is presents in free state in spleen , liver & R.B.Cs

5- Sphingomyelines act as **electric insulators** , so play important role in nerve impulse conduction.

6. Lipotropic activity : sphingomyelines prevent accumulation of fat in liver & prevent fatty liver as they play a role in lipid transport from liver through formation of lipoproteins.

Lipids Containing Choline:-

1- Lecithins.

2- Plasmalogenes.

3- Sphingomyelins.

III-Lipoproteins:

Definition:

They are lipids combined with proteins, lipid component may be:-

- Phospholipids.
- Triglycerides.
- Cholesterol : free cholesterol or cholesterol esters.

Types of lipoproteins:

1- Structural lipoproteins: presents in cellular & subcellular membranes e.g. in lungs (they are presents in surfactant) , in eyes (they are present in rhodopsin).

2- Plasma lipoproteins (Transport lipoproteins). Will discussed in some details now.

Plasma lipoproteins

Location:

- They are presents in blood plasma.

Importance:

- Lipids are water insoluble substances that can't be transported in aqueous medium e.g. plasma.
- For lipids to be transported between plasma & different tissues , they have to be associated with a protein forming water soluble complexes called "plasma lipoproteins".
- The protein fraction called apolipoprotein or apoprotein which is synthesized by liver & intestinal mucosa.
- Failure of the liver to synthesizes apolipoproteins will prevent mobilization of fat from liver leading to " Fatty liver".

Apolipoproteins " apoproteins":

Definition:

- They are the protein meioty of plasma lipoproteins account from 0.5-2% in chylomicrons up to 60% in HDL"high density lipoprotein" , synthesized by liver & intestinal mucosa.
- Some are integral i.e. can't be removed or transferred & others are peripheral that can be removed and transferred freely between lipoproteins.

Types of apolipoproteins:

- **Apo-A (I, II, IV):** they are present in Chylomicrons & HDL.
- **Apo-D**: presents only in HDL.
- **Apo-B**:

*** Apo-B100** presents in VLDL, IDL and LDL.
*** Apo-B48** (which is the truncated form of Apo-B): presents in Chylomicrons & chylomicron remnants..
- Apo-C(I,II,III): they are present in chylomicrons , VLDL &HDL.
- Apo-E: presents in chylomicrons , chylomicron remnants, VLDL &HDL.

Importance of apolipoproteins:

1- Form water soluble complexes with lipids for their transport between blood & different tissues forming part of structure of lipoproteins..
2- Some of them act as activators for enzymes e.g. Apo-CII activates lipoprotein lipase & Apo-AI activates Lecithin-Cholesterol Acyl Transferase (LCAT).
3- Some of them act as inhibitors for enzymes e.g.Apo-AII & Apo-CIII for lipoprotein lipase inhibits lipoprotein lipase& Apo-CI inhibits cholesteryl ester transfer protein.
4- Some are important for receptor mediated uptake of plasma lipoproteins as they act as ligands for interaction

with lipoprotein receptors in tissues e.g, apo B-100 and apo E for the LDL receptor . Apo-B48 & Apo-E for hepatic uptake of chylomicron remnants.

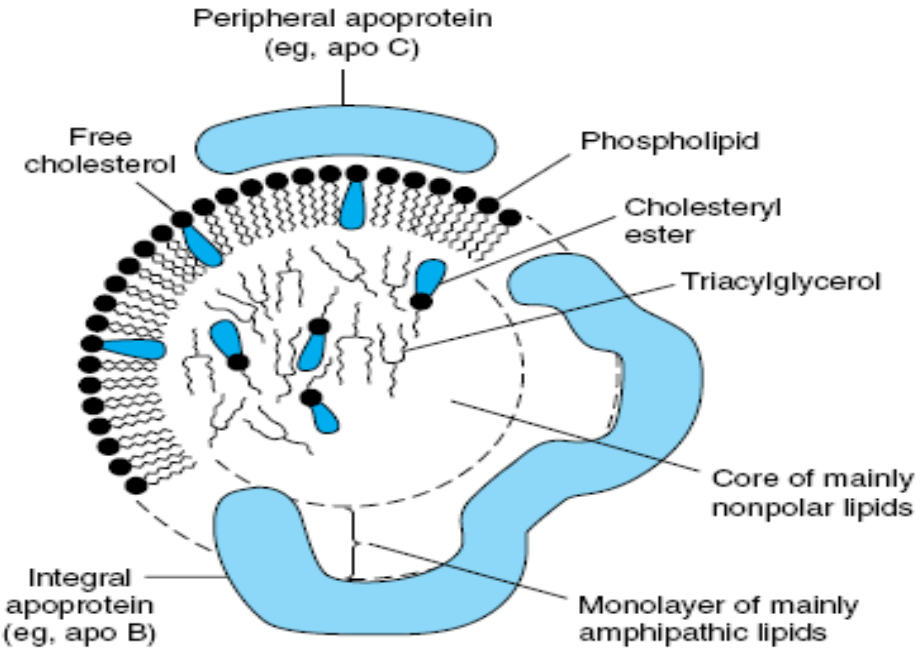
N.B: The functions of apo A-IV and apo D, however, are not yet
clearly defined.

General structure of plasma lipoproteins :

A- Central core of Non-polar lipids including TAGs & Cholesteryl esters.

B- Outer layer of :

* More polar lipids including free cholesterol & phospholipids.
* Apolipoproteins : integral & peripheral ones.



Methods of separation of plasma lipoproteins:

1-By Ultracentrifugation:

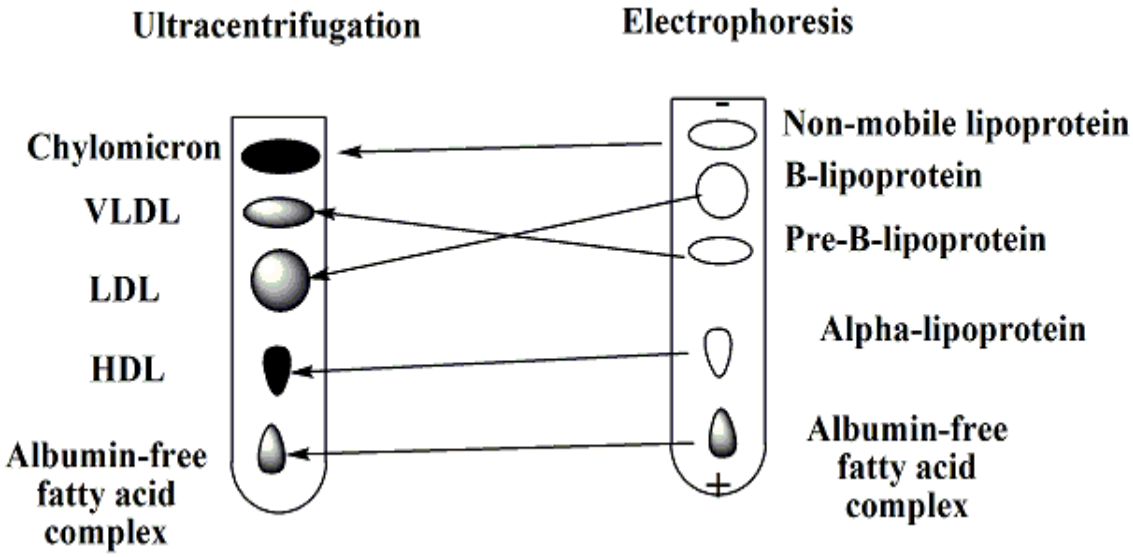
- They are separated into five fractions according to their density in NaCL solution:

1)- Chylomicrons.
2)- Very Low Density Lipoproteins (VLDL).
3)- Low Density Lipoproteins(LDL).
4)-High Density Lipoproteins (HDL).
5)- Albumin – free fatty acid complex.

2- By Electrophoresis:

- They are separated into five fractions depending on their rate of migration in an electric field towards oppositely charged electrodes:

1)- Non-mobile lipoprotein {Chylomicrons}.
2)- β-lipoprotein {Low Density Lipoproteins(LDL)}.
3)- Pre-β-lipoprotein {Very Low Density Lipoproteins (VLDL)}.
4)-α-lipoprotein {High Density Lipoproteins (HDL)}.
5)- Albumin – free fatty acid complex.



Comparison between Ultracentrifugation fractions & Electrophoresis fractions

- Each group nearly contains all types of lipids (triacylglycerols, phospholipids, cholesterol, cholesterol esters and F.F.A.) but they differ in:

a) The main lipid content of each type.

b) Site of synthesis.

c) Type and amount of the associated protein.

d) Functions.

	Chylomicrons	VLDL (Pre-β-lipoprotein)	LDL (β-Lipoprotein)	HDL (α-Lipoprotein)	Albumin-free fatty acid complex
Site of synthesis	**Intestinal mucosal cells**	**Liver**	**From VLDL in circulation & also in liver**	**Liver & intestine**	**Adipose tissue**
The main lipid content	**- 98-99%. - TAGs are the main lipids .But also contain phospholipids , cholesterol & cholesteryl esters.**	**- 90-93%. - TAGs are the main lipids .But also contain phospholipids , cholesterol & cholesteryl esters.**	**-80-90%. -60% cholesterol & 40 % phospholipids.**	**- 45-65%. -60% phospholipids & 40 % cholesterol.**	**-1%. -free fatty acids.**
Type and amount of the associated protein	**- 1-2%. - Apo-B48 , Apo-E ,Apo-CI,II,III & Apo-AI,II,IV.**	**-7-10%. - Apo-B100 , Apo-E &Apo-CI,II,III**	**-10-20%. -Apo-B100**	**-35-55% . - Apo-CI,II,III ,Apo-AI,II,IV & Apo-E**	**- 99%. albumin**

Functions	**Transfer of dietary lipids from intestine to blood & different tissues.**	**Transport lipids mainlt TAGs from liver to extrahepatic tissues.**	**Transfer cholesterol from blood to liver ,suprarenal cortex & gonads (testes & ovaries) which contain LDL receptors**	**1- Acts as reservoir for Apo-C & Apo-E transferring them to chylomicrons & VLDL. 2- It binds cholesterol in tissues & blood & esterified it by LCAT (which is activated by Apo-AI) , then transport it to liver & steroidogenic tissues i.e reverse cholesterol transport**	**Albumin acts as carrier for long chain free fatty acids in plasma.**

N.B:

-As the percentage of **LDL** increases in the blood, the liability to atherosclerosis increases.

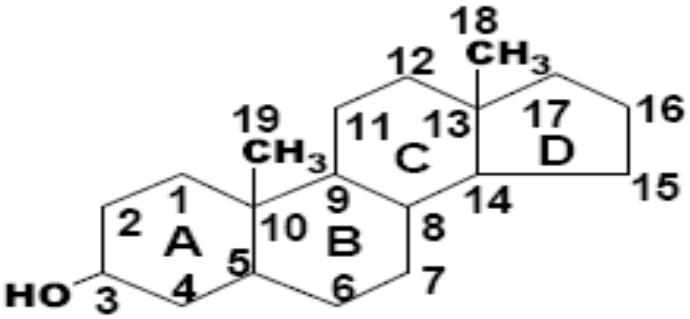
- As the percentage of **HDL** increases, the liability to atherosclerosis decreases.

Steroids

Definition:

- They are cyclic compounds which contain steroid nucleus or ring called "Cyclo –pentano-perhydro-phenan-threne".

Structure:



Steroid ring

- They consists of :

1- Four rings A,B,C,D with 17 carbon atoms.
2- Oxygen presents at C3 in the form of (OH) or (C=O).
3- CH3 at C10 &C13.
4- Side chain (R) at C17.
5- C &D rings are always saturated while A &B rings may be unsaturated.

Examples of Steroids:

1- Sterols: e.g. cholesterol & ergosterol.
2- Adrenal cortical hormones: glucocorticoids , mineralocorticoids & adrenal androgens.
3- Male & female sex hormones: testosterone , estrogen & progesterone.
4- bile acids & bile salts.
5- Vitamin D group e.g Vit.D2 , Vit.D3.
6- Cardiac glycosides.

Sterols:

Definition:

- They are complex monohydric alcohols present in animals & plants either in free state or in the form of esters.

Structure:

Steroid nucleus with:

- 8 -10 Carbon atoms in the side chain at C17.

- Hydroxyl group (OH) at C3.
- Methyl group at C10 & C13.
- Double bond in ring B .

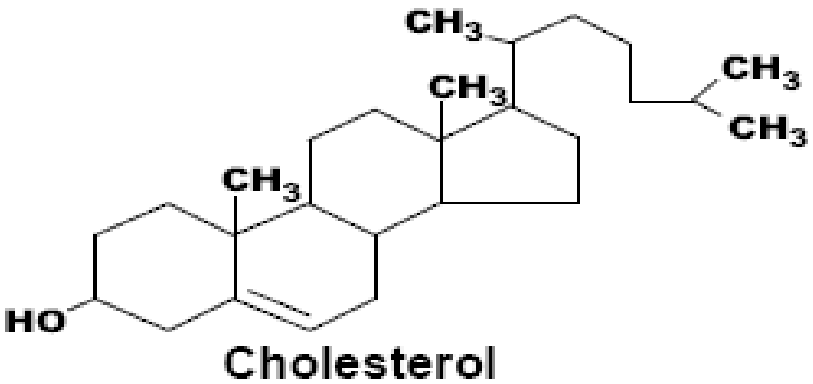
Types:

1- Zoosterols: in animals e.g.Cholesterol.
2- Mycosterols: in lower plants e.g. Ergosterol.
3- Phytosterols : in higher plants e.g Stigmasterol , Sitosterol & they have no nutritional value.

Cholesterol:

Structure:

Steroid nucleus with:
- 8 Carbon atoms in the side chain at C17.
- Hydroxyl group (OH) at C3 which is the site of attachement of fatty acid for formation of choleseryl esters..
- Methyl group at C10 & C13.
- Double bond in ring B between C5 &C6.



Cholesterol

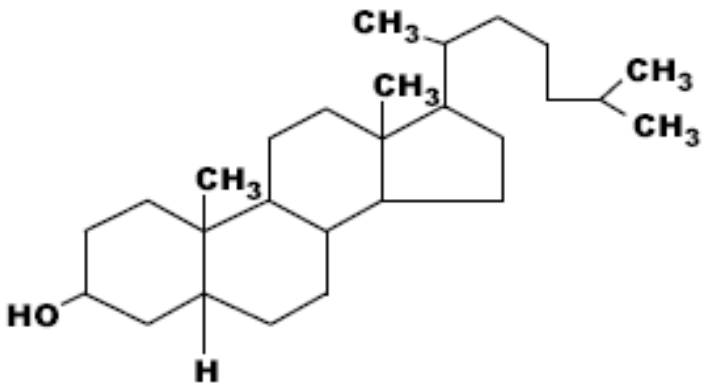
Physical properties:

1- Odorless white to yellow crystalline rhombic plates.

2- Insoluble in water.
3- Soluble in ether , benzene & chloroform.
4- Melting point at 147 - 150ºC.

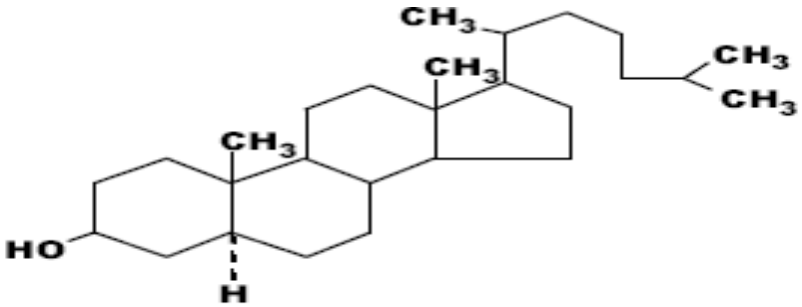
Chemical properties:
1- Unsaponifiable (not hydrolyzed by alkali).
2- Contain double bond , so absorb 2 halogen atoms e.g. Iodine.
3- Reduction & oxidation in the body as follows:-

*** Reduction :**
1- Cholesterol reduced by action of intestinal bacteria produce ***Coprostanol*** " fecal sterol" :



Coprostanol

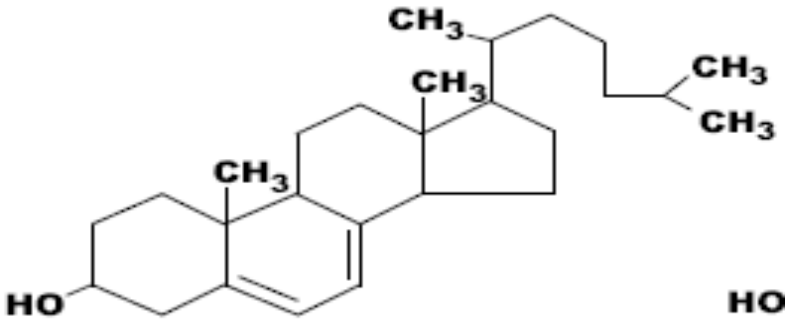
2- Cholesterol also can be reduced in the blood & other tissues to form ***dihydrocholesterol*** (β – Cholestanol).
- ***Plasma cholesterol*** level is 150-220 mg/dl (2/3 is esterified & 1/3 is in free form.



Dihydrocholesterol,
in blood and other tissues

*** Oxidation** :

1- Cholesterol oxidized in the fat under the skin into ***7-dehydrocholesterol*** which is Vitamin D3 precursor.



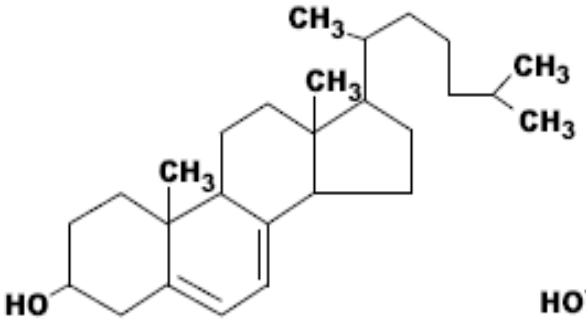
7-dehydrocholesterol

2. Cholesterol also oxidized in the liver to form bile acids.

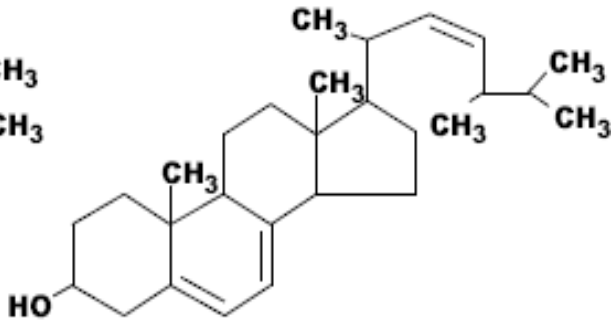
Ergosterol:

* First discovered & isolated from ergot which is fungus of Rye , then then from yeast

* Ergosterol differs from 7-dehydrocholesterol in the side chain as ergosterol contain extra methyl group & double bond).



7-dehydrocholesterol



Ergosterol

*Ergosterol is less stable than cholesterol (because of having 3 double bonds).

* It is Vitamin D 2 precursor as is it is oxidized by UV light into ergocalciferol which is vitamin D2.

Protein Chemistry

Topics Discussed

- Classification of amino acids.
- Physical and chemical properties of amino acids.
- Properties of proteins.
- Separation techniques for proteins and amino acids.
- Bonds participating in the protein structure and the different levels of the protein structure.
- Protein denaturation.
- Classification and the role of proteins.

Learning Objectives:

After understanding this part the student should be able to:

- Compare and contrast the different types of amino acids and the biochemical bases and uses of their properties.
- Describe the biochemical and medical importance of the essential amino acids.
- Compare and contrast the different approaches used to separate proteins/amino acids from a solution mixture.
- Describe the different properties of proteins and their medical applications.
- Describe the biochemical and medical importance of the protein denaturation.
- Compare the different types and role of bonds participating in the protein structure.
- Describe the protein structure and diseases implicating amino acid changes and/or misfolding.
- Describe the different type and function of proteins.

Definition:

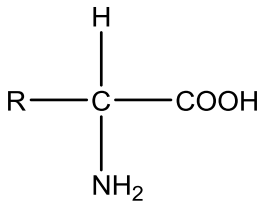
- Proteins are organic compounds with a high molecular weight formed of carbon, oxygen, hydrogen and nitrogen and may also contain sulfur, phosphorus coloring non-protein organic groups and metal ions. They are polymers formed of subunits called amino acids linked together by peptide linkage. The term protein is applied to describe molecules greater than 50 amino acids.

Biological importance of proteins:

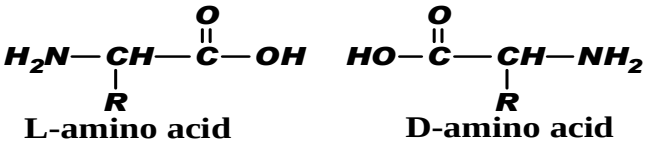
1. **Nutritional role:** Provide the body with essential amino acids, nitrogen and sulfur.
2. **Catalytic role:** All enzymes are proteins in nature.
3. **Hormonal role:** Most of hormones and all cellular receptors are protein in nature.
4. **Defensive role:** The antibodies (immunoglobulins) that play an important role in the body's defensive mechanisms are proteins in nature.
5. Plasma proteins are responsible for most effective **osmotic pressure** of the blood. This osmotic pressure plays a central role in many processes, e.g., urine formation. Thus help in maintenance of electrolytes and water balance in the body.
6. **Transport role:** Proteins carry lipids in the blood forming lipoprotein complexes. Proteins also carry, hormones, e.g., thyroid hormones and minerals, e.g., calcium, iron and copper. **Hemoglobin** (a chromo-protein) carries O_2 from the lung to tissues is a protein.
7. **Structural role:** Proteins are the main structural component in bone, muscles, cyto-skeleton and cell membrane.
8. **Blood clotting:** coagulation factors are proteins.
9. **Control of gene expression:** Most factors required for DNA replication, transcription and mRNA translation are protein in nature.

Amino Acids

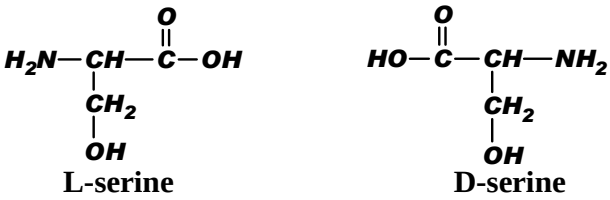
- Amino acids are organic acids that contain NH_2 group. They are the structural units of proteins and are obtained from them by hydrolysis. The general formula of any amino acid is as follows:



- Although about 300 amino acids exist in nature, only 20 of them can polymerize in protein structure. For each a specific codon exists in the genetic code.
- The metabolizible form of them are L-amino acids (amino group is on the left side configuration). All amino acids present in mammals are L-amino acids.



- D-amino acids are found in the cell walls of bacteria.
- Serine is an amino acid taken as a reference for configuration of amino acids.



- Each amino acid (except proline and hydroxyproline) has a carboxyl group (COOH), an amino group (NH_2) and a characteristic side chain (R).
- In α-amino acids, both-COOH and $-NH_2$ groups are attached to the same (α-) carbon atom. This why they are amphoteric, i.e., react as acid (by COOH) and as alkali (by NH_2).
- At physiological PH (approximately PH 7.4) the carboxyl group is dessociated forming a negatively charged carboxylate ion ($-COO^-$) and the amino group is protonated forming positively charged ion ($-NH_3^+$).

- All amino acids (except glycine) are optically active, i.e., can rotate plane polarized light. This is because the 4 groups attached to α-carbon are different. In glycine, the α-carbon is attached to 2 hydrogen atoms, therefore, is optically inactive.

Classification of Amino Acids

Amino acids can be classified by one of three methods:

1. **Chemical classification**: Based upon the number of amino groups or carboxyl groups in the amino acid:
   - **Neutral amino acids** (mono-amino, mono-carboxylic).
   - **Acidic amino acids** (mono-amino, dicarboxylic).
   - **Basic amino acids** (diamino, mono-carboxylic).
2. **Biological classification**: Based upon whether the amino acids can be synthesized in human body or not:
   - Indispensable or essential amino acids: Not synthesized in the body and must be supplied in the diet.
   - Dispensable or non-essential amino acids: Can be synthesized in the body and hence is not essential to be present in diet.
3. **Metabolic Classification**: Based upon the fate of amino acid inside the body:
   - **Glucogenic amino acids**, that can be converted to glucose.
   - **Ketogenic amino acids**, that can be converted to ketone bodies.
   - **Mixed amino acids,** i.e., can be converted to both glucose and ketone bodies.

A. Chemical classification

(According to number of carboxyl and amino groups)

- Amino acids can be classified into:

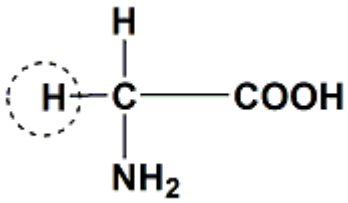
a) Neutral amino acids. b) Acidic amino acids. c) Basic amino acids.

I) Neutral amino acids

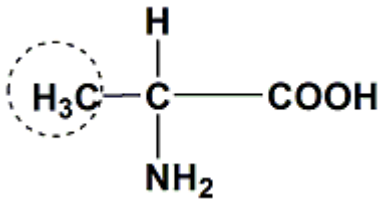
- They contain one amino group and one carboxyl group. They have **5** types:

1. Aliphatic amino acids: e.g.,

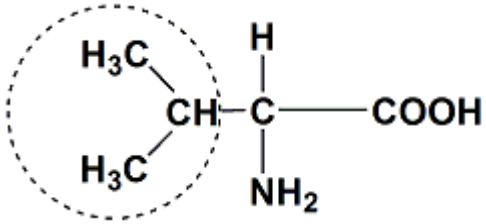
- **Glycine**



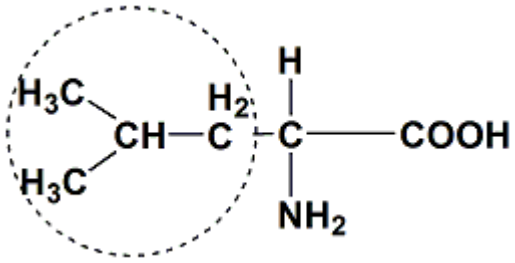
- **Alanine**



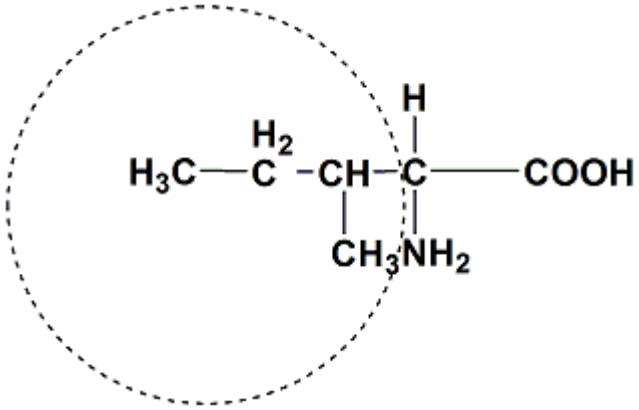
- **Valine**



- **Leucine**

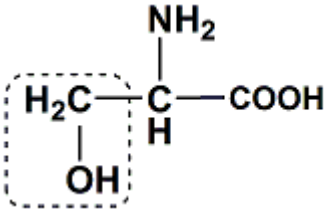


- **Isoleucine**

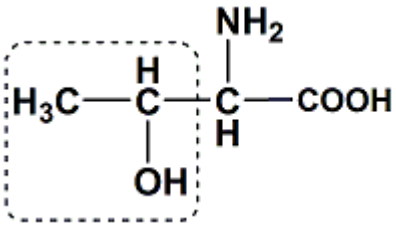


2. Hydroxy amino acids: contain –OH group in their side chain e.g., serine, threonine, tyrosine, hydroxyproline and hydroxy-lysine.

- **Serine**



- **Threonine**

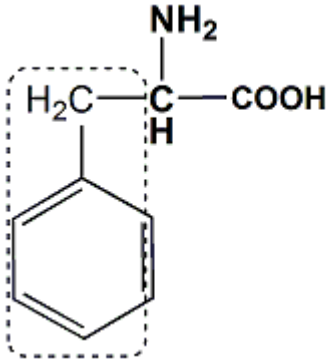


- Serine gives choline for phospholipid and acetylcholine synthesis and the OH groups of threonine and serine are site for protein glycosylation and phosphorylation.

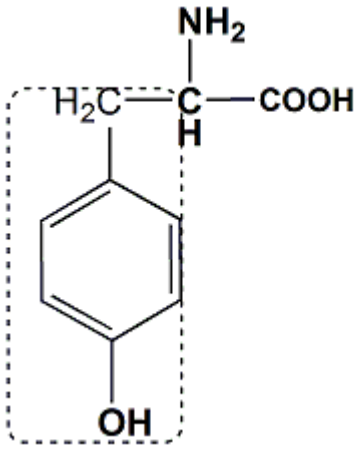
3. Aromatic amino acids: e.g., phenylalanine, tyrosine and tryptophan.

- Tyrosine is synthesized from phenyl alanine and both give triiodothyronine and thyroxin, adrenaline and noradrenaline, melanin pigment and cresol and phenol in the body, e.g.,

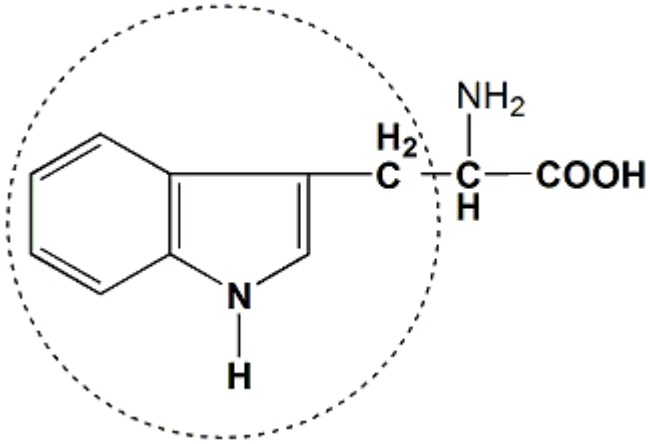
- **Phenyl alanine**



- **Tyrosine**



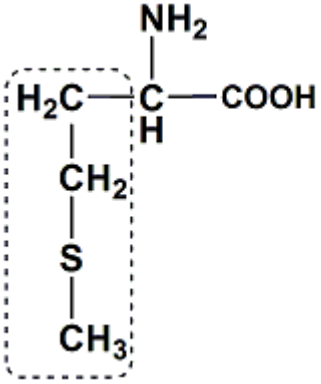
- **Tryptophan**



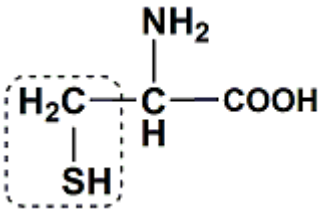
4. Sulfur-containing amino acids: e.g.,

- Cysteine gives cystine and its SH group is very essential in activity of many proteins particularly the active sites of enzymes. It is also important for intra- and inter-polypeptide disulfide bond formation that stabilizes protein structure. S-adenosylmethionine (see nucleic acids) is active form of methionine and is the main methyl donor.

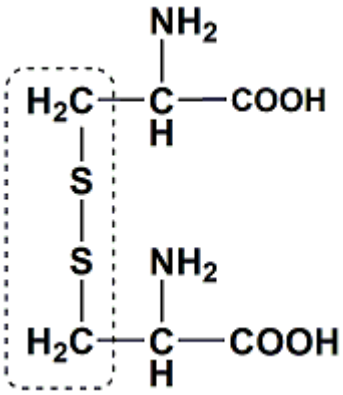
- **Methionine**



- **Cysteine**

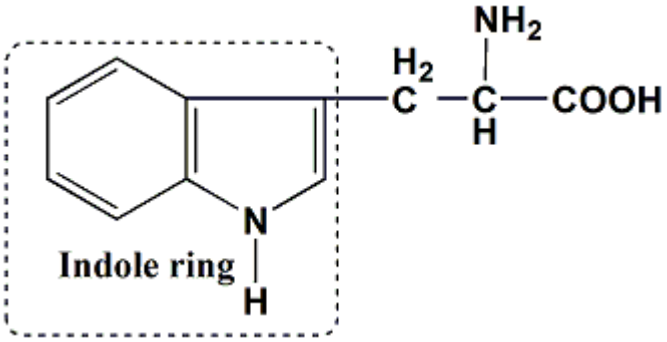


- **Cystine**

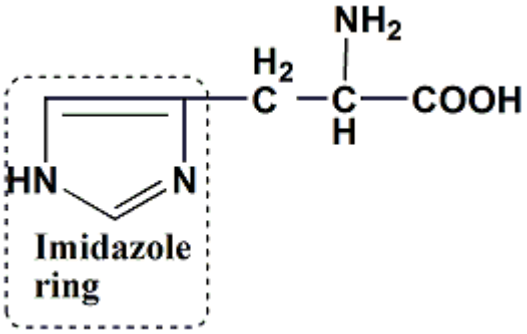


5. Heterocyclic amino acids: e.g.,

1. Tryptophan



2. Histidine

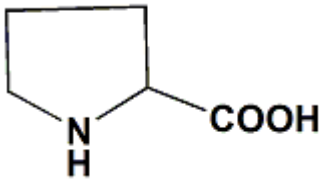


- Histidine is basic in solution on account of the imidazole ring and often considered as basic amino acids. Histidine gives histamine a very important inflammatory mediator. Tryptophan is often considered as aromatic amino acids

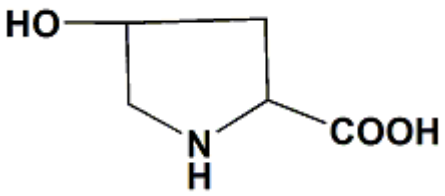
since it has aromatic ring in its structure. Tryptophan gives nicotinic acid, melatonin, serotonin and indican in the body.

3. **Imino acids:** They not contain Free amino group "NH2" & instead they contain an imino group "NH" in its pyrrilidone ring that still functioning in the peptide formation . They include proline and hydroxyproline. Proline gives hydroxyproline that is essential for collagen cross-linking.

- **Proline**



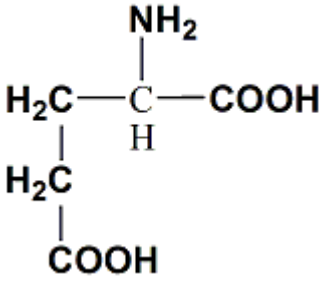
- **Hydroxy praline**



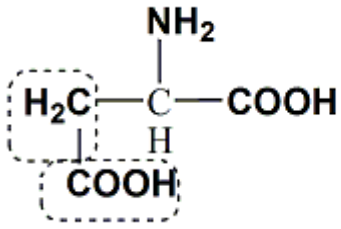
II) Acidic amino acids

- They contain 2 carboxyl groups and one amino group, e.g., glutamic acid and asparatic acid.

1. Glutamic acid

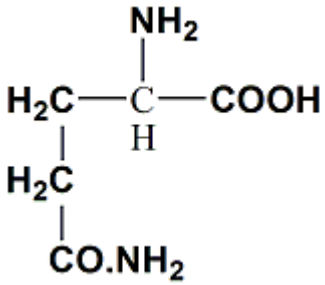


2. Aspartic acid

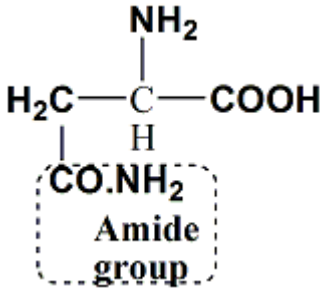


- These acidic amino acids can occur in the tissue in the form of amides, e.g., glutamic acid ⇒ **glutamine** and asparatic acid ⇒ **asparagine.**

- Glutamic acid ⟶ Glutamine



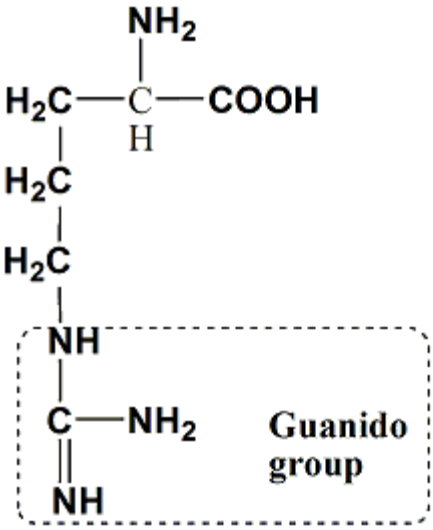
- Aspartic acid ⟶ Aspargine



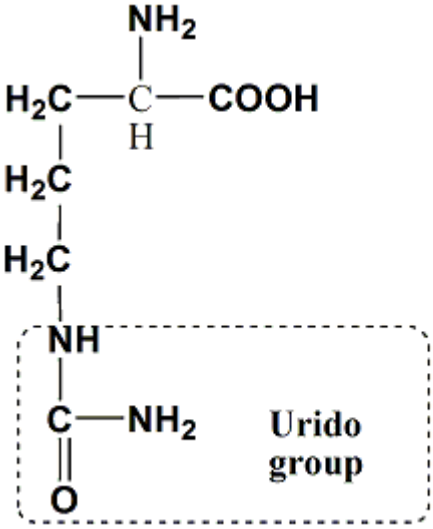
III) Basic amino acids

- They contain 2 amino groups and one carboxyl group, e.g**.,**
  1. Arginine.
  2. Citrulline.
  3. Ornithine.
  4. Lysine.
  5. Hydroxylysine.
- **Ornithine and Arginine. Ornithine** does not enter in the synthesis of proteins and is usually present in the free form. It is synthesized from **arginine**. Both are participate in urea synthesis from ammonia. Arginine is used in synthesis of nitric oxide, a hormonal second messenger. Ornithine is used for synthesis of polyamines that are important for cell cycle control. **Citrulline** is formed from ornithine during urea synthesis but does not participate in protein structure. **Lysine and Hydroxy lysine:** They participate in protein cross-linking.

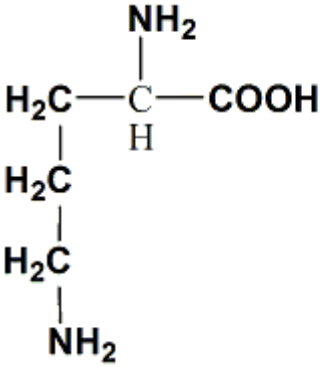
1. Arginine:



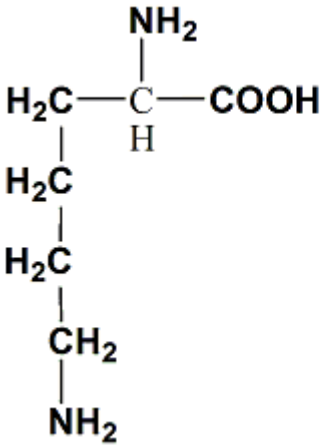
2. Citrulline:



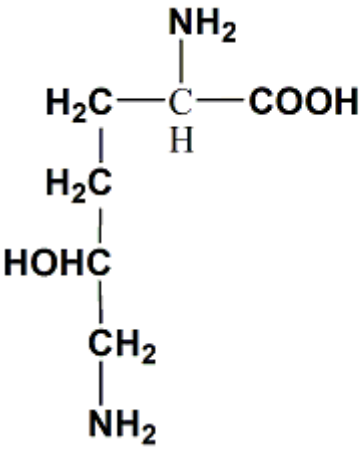
3. Ornithine:



4. Lysine:



5. Hydroxylysine:



Other classifications of amino acids

⇒ **Metabolic classification**:

- Amino acids may be classified into glucogenic amino acids, i.e., those which can be converted into glucose, ketogenic amino acids, i.e., those which can be converted into ketone bodies and mixed amino acids, i.e., those which can be converted into both glucose and ketone bodies.

Ketogenic	***Ketogenic & glucogenic***	***Glucogenic***
Leucine	**Lysine**	**Rest of amino acids**
	Isoleucine	

	Tyrosine	
	Tryptophan	
	Phenyl alanine	

⇒ **Biological or Nutritional Classification**:

- Some amino acids can not be synthesized inside the body. If these amino acids are not taken in diet they will affect the growth and the health. Thus, amino acids may be classified into:

A- Essential amino acids: (Indispensable amino acids):

- These are amino acids that can not be synthesized in the human body and should be taken in the diet, otherwise their deficiency will lead to a nutrition deficiency disease that affect both growth and health.
- Arginine and histidine are semi-essential, i.e., they are mainly required in growing children, pregnant and lactating women and convalescent patients. The main source for these amino acids are animal proteins (milk, egg, meat, liver, fish, chicken) and a few plant proteins (bean and lintels). They are as follows (**VITTAL LyMPH**):

Valine	**Isoleucine**	**Threonine**	**Tryptophan**	**Arginine**
Leucine	**Lysine**	**Methionine**	**Phenylalanine**	**Histidine**

Or

I	***Left***	***Home***	***To***	***Make***
Isoleucine	Leucine	Histidine	Tryptophan	Methionine
Visit	***Through***	***London***	***Philippine***	***Argentin***
Valine	Threonine	Lysine	Phenylalanine	Arginine

B- Non essential amino acids: (Dispensable amino acids):

- The rest of amino acids can be synthesized inside the human body and their deficiency in diet does not affect the growth or the health.

Isoelectric point "I.E.P"

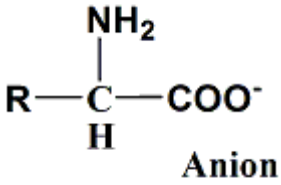
I- For the amino acid:

- It is the PH at which zwitterion is formed.

⇒ **Zwitterion formation:**

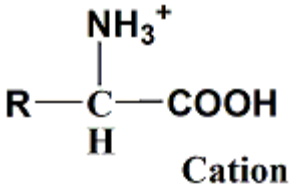
Monoamino – Monocarboxylic amino acids present in the aqueous solutions as follow:

1. At alkaline PH: The carboxylic group "COOH" dissociated i.e. loss of its proton and become negatively charged "COO^-", so the amino acid will carry negative charge and called **anion**.



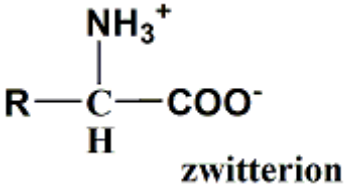
Anion

2. At acidic PH: The amino group "NH_2" protonated i.e. acquire a proton and become positively charged "NH_3^+" and the amino acid will carry positive charge and called **cation.**



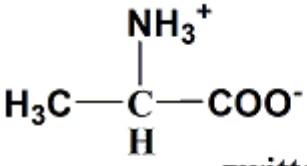
Cation

3. At certain PH "I.E.P": The amino acid will carry equal positive & negative charge and becomes :
   - Electrically neutral.
   - Least soluble.
   - & not migrate in an electric field & called zwitterion.



zwitterion

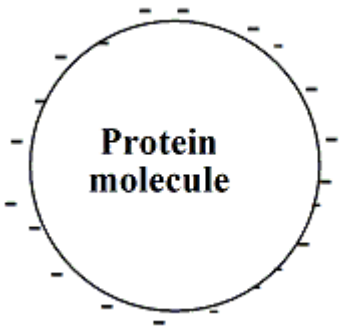
Example: zwitterion of alanine:



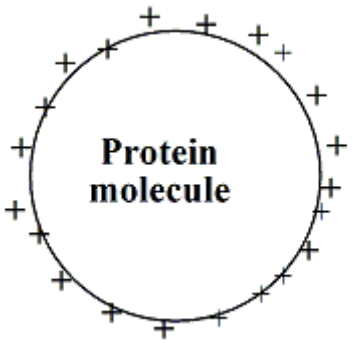
zwitterion of alanine

II- For protein molecule:

1. **In alkaline medium:** The protein molecule will be negatively charged & will migrates towards the anode if put in an electric field.



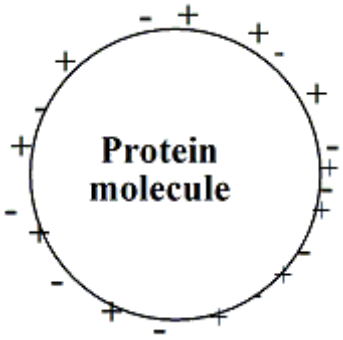
2. **In acidic medium:** The protein molecule will be positively charged & will migrates towards the cathode if put in an electric field.



3. At I.E.P "certain PH" : The protein molecule will carry equal positive & negative charges & become :

- Electrically neutral.
- Least soluble.

& not migrate in an electric field



Importance of I.E.P:

1. Used to identify the compound.
2. For amino acids & protein separation , avoid the I.E.P.
3. For amino acids & protein precipitationn , use the I.E.P.

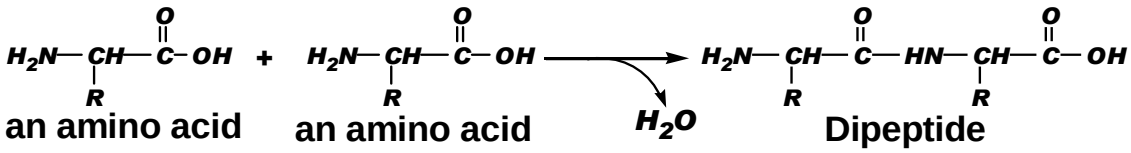
Peptides

Definition:

- Peptides are compounds, formed of less than 50 amino acids linked together by peptide bonds.

Formation of the peptide bond:

- The reaction between –COOH group of an amino acid and $-NH_2$ group of another amino acid leads to the formation of peptide bond. It is formed by removal of water.



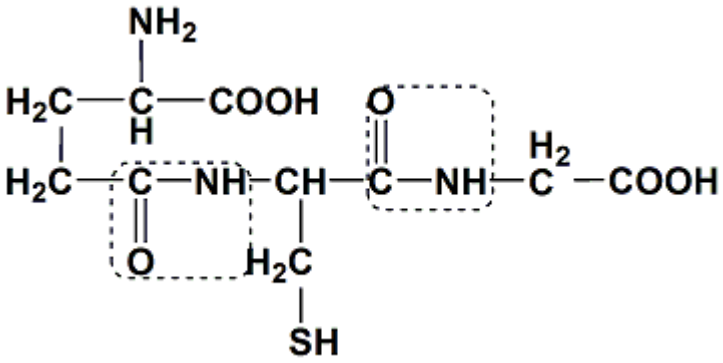
- **Classification of peptides:**

1- Dipeptide (2 amino acids and one peptide bond).
2- Tripeptide (3 amino acids and 2 peptide bonds).
3- Oligopeptide (3-10 amino acids).
4- Polypeptide (10-50 amino acids).

Glutathione

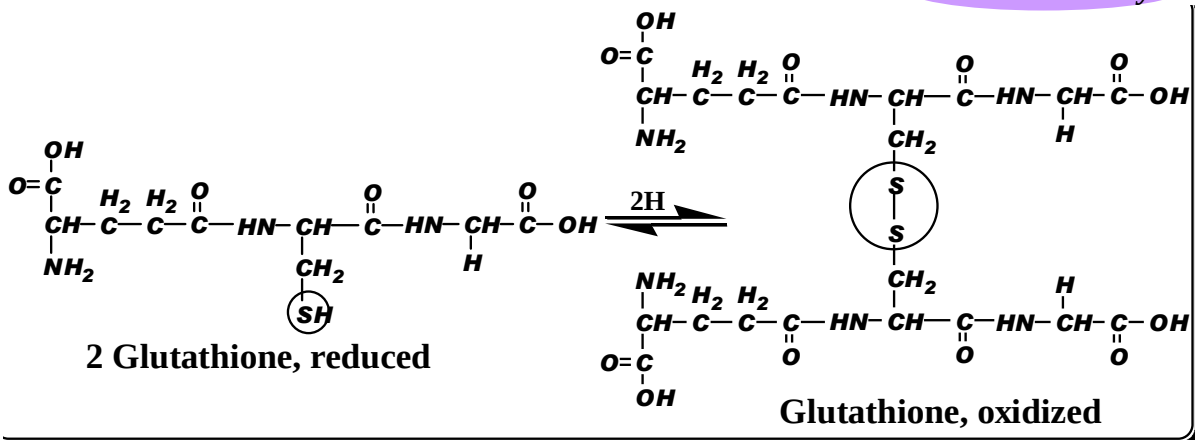
- An example for physiologically active short peptides.

- It is a tripeptide formed of 3 amino acids: γ-glutamate, cysteine and glycine , so it is called γ **-glutamyl, cysteinyl, Glycine**.
- It use the $NADP^+$ as coenzyme .



- It presents in two forms:
  1. Reduced form "G-SH" : which contains free SH " sulfhydyryl group".
  2. Oxidized form "G-S-S-G" : which contains a disulfide bond with no free SH group.

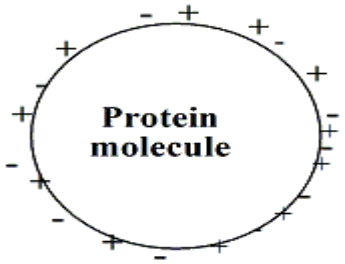
$$2G\text{-}SH \overset{2H}{\leftrightarrow} G\text{-}S\text{-}S\text{-}G$$

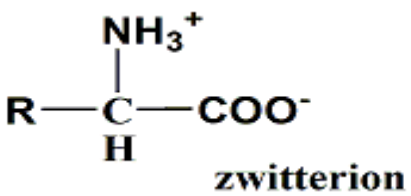


- **Functions of glutathione:**
  1) It has a role in absorption of amino acids.
  2) It activates many enzymes.
  3) It inactivates insulin hormone, by breaking its disulfide bonds.
  4) It protects the cell membrane from damage, e.g., prevents hemolysis of erythrocytes.
  5) It prevents rancidity of fat (or lipid peroxidation) as it acts as antioxidant.

Methods of precipitation of proteins

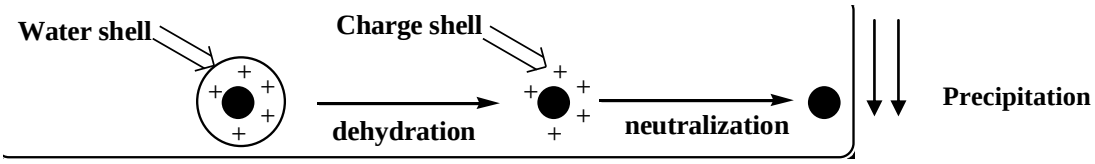
1. At the iso-electric point: At which the amino acid or protein carries an equal positive and negative charges & so it becomes electrically neutral , least soluble & not migrates in an electric field.





2. By various concentration of neutral salts solutions:,

- Neutral salts Such as: sodium chloride "Nacl", ammonium sulfate "NH_4SO_4", magnesium sulfate "$MgSO_4$".
- With different concentration of salts different proteins can be precipitated.
- Fibrinogen is precipitated by 20% saturation. Globulins are precipitated by 50% saturation. Albumins are precipitated by 100% saturation.
- Mechanism is called " ***salting out phenomenon***":
  - ⇒ Definition: It is the process of decreasing the solubility of the proteins by adding large amount of salts , How??
  - ⇒ Salt ions will cause :
    1. Dehydration i.e. removal of the water shell surrounding the protein molecules.
    2. Followed by neutralization of protein molecules leading to their precipitation as follow:



3. By heavy metals: e.g., mercury and silver salts, etc. Heavy metals combine with protein forming insoluble metalloproteins. They precipitate proteins carrying negative charges.

4. By alkaloidal reagents: e.g., trichloroacetic acid , picric acid and tannic acid. Alkaloidal reagents form insoluble complex with protein. They precipitate proteins carrying positive charges.

5. Alcohol precipitation: Different proteins are precipitated with various concentrations of alcohol at temperature below O ^{o}C due to dehydration. At such low temperature denaturation of protein does not occur.

6. Heat coagulation: Heating of some proteins causes their coagulation. The coagulated protein is a denatured and insoluble protein. Only albumins, globulins and glutelins are heat coagulable.

7. **Immunoprecipitation:** using specific protein antibodies.

8. Precipitation by **nitric acid.**

Separation techniques for proteins and amino acids

⇒ **They include:**

1. **Precipitation: previously discussed.**
2. **Chromatography.**
3. **Electrophoresis.**
4. **Dialysis.**
5. **Ultracentrifugation.**
6. **Immunoprecipitation.**

Chromatography:

- Chromatography is a group of separation techniques, where a mixture of molecules are separated. The separated molecules are divided between a stationary sold phase and liquid mobile phase.
- The separation process depends on the tendency of one type of molecules in the mixture to associate more strongly with one phase than the other.

Electrophoresis:

⇒ It is movement of charged particles in an electric field towards the oppositely charged electrode.

⇒ A filter paper or cellulose acetate or gel is dipped in an alkaline buffer , then put in an electric field , then the protein mixture is applied towards the cathode (the negative electrode) side.

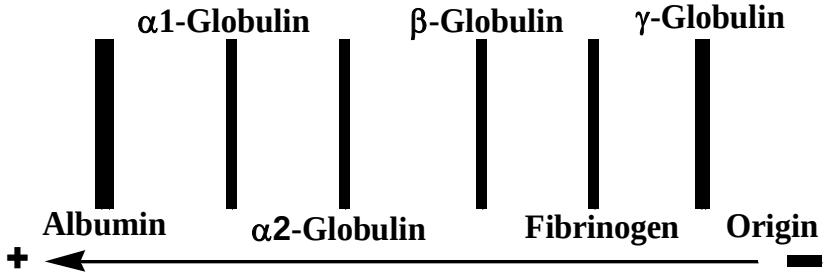
⇒ As the proteins will carry negative charges in the alkaline medium , they will migrate towards the anode (the positive electrode).

⇒ The rate of their migration using the samecurrent & buffer depend on :

1. The charges carried by each protein.
2. The molecular weight and shape of protein.
3. Voltage of the current.
4. pH.
5. Temperature.

& So , they will separated into bands with their density directly proportionate to their concentration in the mixture.

⇒ Example: Plasma proteins electrophoresis:



- As the albumin has the highest concentration among the plasma proteins , it shows the densest band in the electrophoresis.

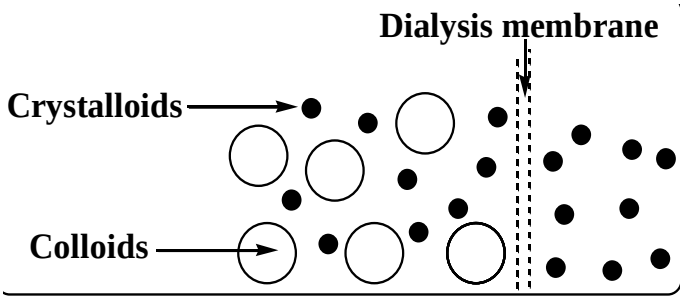
⇒ ***Diagnostic importance of plasma protein electrophoresis:***

- Plasma proteins electrophoresis may be used in diagnosis of certain diseases, e.g.,

1. In hypoalbuminemia as in advanced liver disease, the albumin band becomes less dense.
2. In hypogamma-globulinemia, there is a defective production of γ-globulins and its band appears faint.
3. Hypergamma-globulinemia , the γ-globulins band appears more dense . This occurs in some malignant diseases e.g. multiple myeloma.

Dialysis:

- Definition: It is separation of colloids (which has large molecular weight e.g. proteins) from crystalloids (which has small molecular weight e.g. salts) , using semi-permeable membrane.
- Protein mixture put in a cellophane bag & immersed in water. the cellophane contains pores that allow the passage of water and small sized molecules e.g. salts but not the large sized molecules e.g. proteins.
- The products of the dialysis are called dialyzate.



⇒ ***Clinical importance:***

- It can be used in patients with renal failure in which blood passes through dialyzing machine to get rid of waste products and preserving the plasma proteins and blood cells.

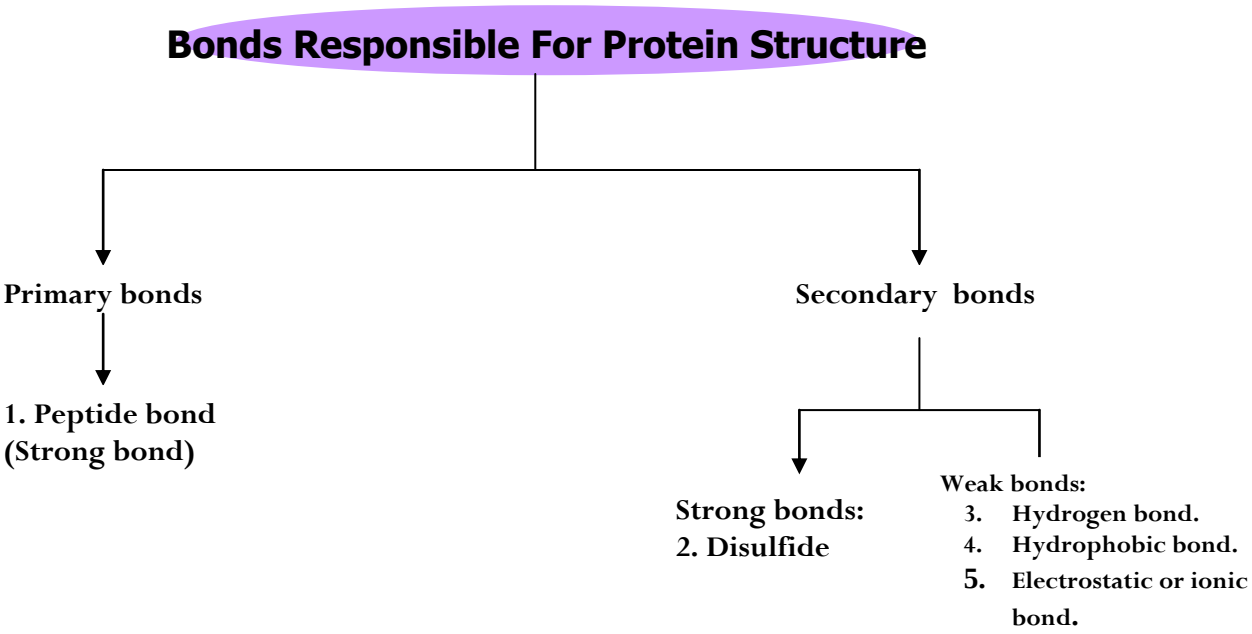
Ultracentrifugation:

- By this method, a mixture of proteins is separated into different fractions according to their densities.

Precipitation by antibodies (Immunoprecipitation):

- Antibodies to specific proteins can often be prepared and can be used to react with the desired protein in a mixture. The interaction of protein and antibody may produce an antigen-

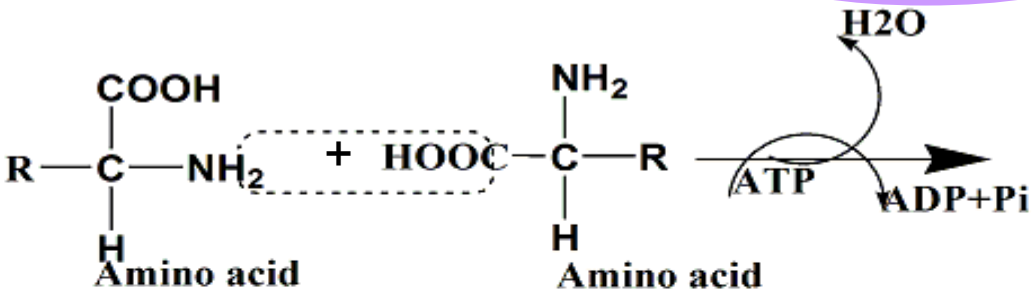
antibody complex large enough to be centrifuged out of solution allowing recovery of protein.

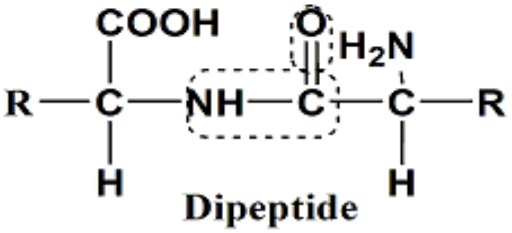


- Two classes of strong bonds (peptide and disulfide) and 3 classes of weak bonds (hydrogen, hydrophobic and electrostatic bonds) generally stabilize Protein structure.

1. Peptide bond (primary bond):

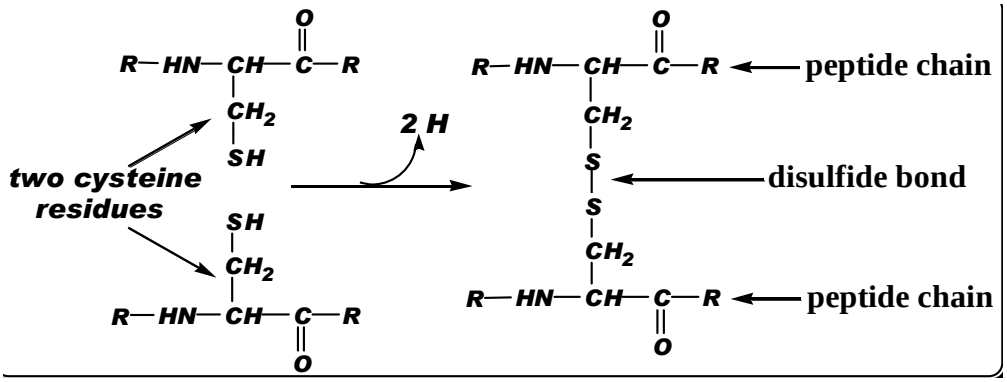
- It is primary strong bond.
- It resists the denaturation.
- It is the only bond responsible for primary structure formation of the proteins & so it is called primary bond.
- It is formed between the NH2 of an amino acid & the COOH group of the next amino acid with loss of water & this require ATP as follow:





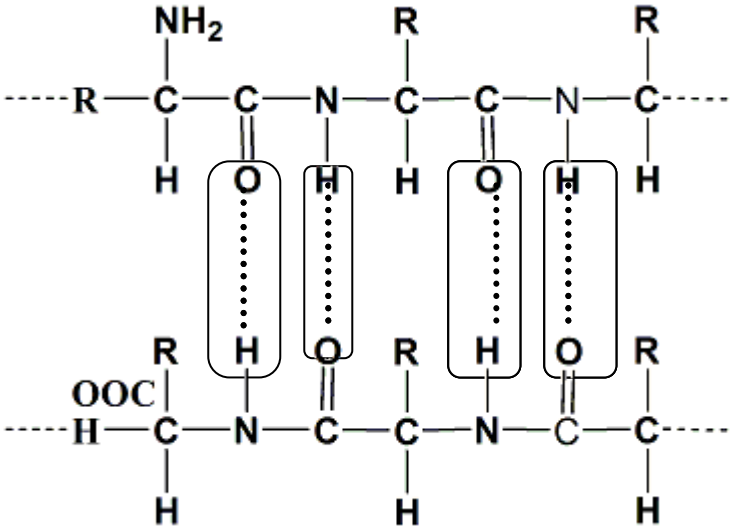
2. Disulfide bonds (secondary bond):

- It is secondary strong bond.
- It is liable to denaturation.
- It is formed between the SH "sulfhydryl "groups of two cysteine residues within the same (intra-chain) or two different polypeptide chains (inter-chain).



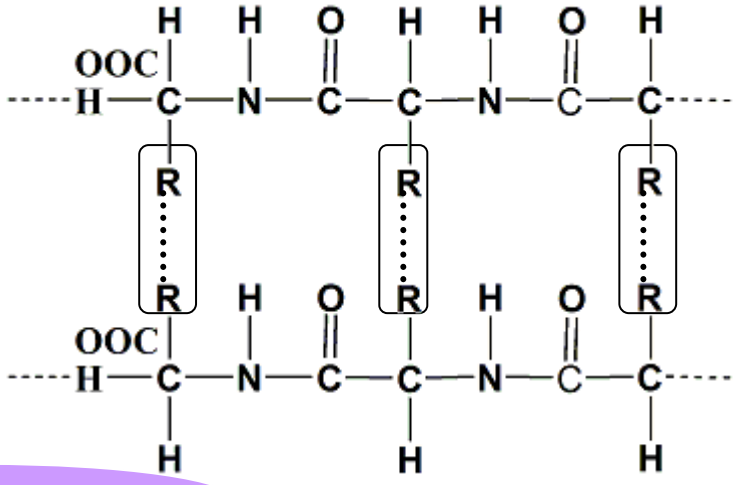
3. Hydrogen bond:

- It is secondary weak bond.
- It is formed between the hydrogen atom of –NH of a peptide bond on one peptide chain and the oxygen of C=O of another peptide bond on an adjacent peptide chain or a loop belongs to same peptide chain as follow:



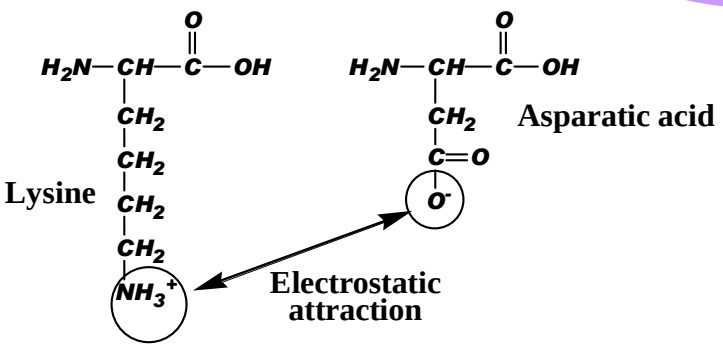
4. Hydrophobic bond:

- It is secondary weak bond.
- It is formed between the non polar side chains (R) of neutral amino acids which tend to fold to the interior of the protein molecule away from the solvent.



5. Electrostatic bond:

- It is secondary weak bond.
- It is formed between oppositely charged groups in the side chains of amino acids e.g. ε-amino group of lysine and the carboxyl group of asparatic acid.



Denaturation of proteins

Definition:

- It is unfolding of proteins with loss of its secondary , tertiary & quaternary structure resulting from exposure to:

1. Physical agents: such as shaking , high temprature , X-ray & γ-radiation.
2. Chemical agents: such as solvents , strong acids & alkalies.
3. Enzymes: such as digestive enzymes.

- Leading to :

1. Physical changes: in the form of :
    a. Increased viscosity.
    b. Decreased solubility.
    c. Decreased diffusibility.
2. Chemical changes: in the form of:

a. Disruption of weak secondary bonds resulting in loss of secondary , teriary & quaternary structure.

3. Loss of biological activities.

- Denaturation does not affect primary structure i.e not accompained by hydrolysis of peptide bonds.

Significance & applications of Denaturation:

1. Denatured proteins, e.g., cooked meat are easily digested.

2. Avoidance of denaturation is important for biological samples used for determination of enzymatic, hormonal or protein contents. This

is done by proper sample collection and storage because if denaturation occurs false results will be obtained.

3. Blood samples to be analyzed for small molecules, e.g., uric acid and glucose are first treated with acid such as trichloroacetic acid or phosphotungestic acid to precipitate the plasma proteins (by denaturation).
4. Detection of albumin in urine by heat coagulation test is based on denaturation by heat.
5. Several approaches for stoppage of bleeding and treatment of burns is based on precipitation and denaturation of a superficial protein layer by tannic acid & picric acid.

Classification of Proteins

I. According to the biological importance of the protein:

1) Proteins of high biological value:

- These are all proteins of animal origin (with a few exceptions) and some proteins of plant origin, that contain all the 10 essential amino acids in well balanced amounts and are easily digestible.
- Examples of animal proteins include; milks and its products, egg, liver, fishes, red and white meats. Examples of the few plant proteins of high biological value are lentils and broad beans.

2) Proteins of low biological value:

- These are proteins that is deficient in one or more of the essential amino acids or containing very little amount of one of them or are indigestible.
- Most of plant protein are of low biological value and a very few animal proteins are also of low biological value such are collagen because is deficient in tryptophan and cysteine and keratins because they are indigestible.
- This does not imply that a person should eat only a protein of high biological value to avoid deficiency of essential amino acids, but this can

be also avoided by eating two or more proteins of low biological value that complete each other's deficiency.

II. According to the axial ratio of the protein molecule:

- Studies on the shape of the protein molecule using ultramicroscope indicates that there are two types of proteins in nature:

1. Fibrous proteins. 2. Globular proteins.

1. Fibrous proteins:

- They have an axial ratio of more than 10. Axial ratio = Length/*Width* of the protein molecule. They are fairly stable proteins in which the straight polypeptide chains lie parallel (or antiparallel) to one another along a single axsis forming fibers or sheets. They are usually insoluble and non motile.

Examples**:-**

a. Keratin proteins in hairs, wool and skin. In its native state, it is present in the form of coiled polypeptide chains called ∝-keratin. It can be stretched by denaturation forming β-keratin.

b. Myosin is the major protein of muscles. During muscle relaxation it is called ∝-myosin but during muscle contraction, it undergoes a change in its structure and it becomes β-myosin.

2. Globular proteins: Their axial ratio is less than 10. Their one or more peptide chains are folded or coiled on themselves in a very compact manner. They are less stable than fibrous proteins and they are usually soluble and motile. Examples are albumin, globulins, and insulin.

III. According to the composition of the Protein:

- There are 3 main groups (on the basis of their solubility and physical properties):

A) Simple proteins. B) Conjugated proteins. C) Derived proteins.

A) Simple Proteins

- These are proteins which on hydrolysis produce amino acids only.
- They include:

1. Albumins & globulins.
2. Protamines.
3. Histones.
4. Gliadins "Prolamines".
5. Glutelins.
6. Scleroproteins.

1- Albumin & Globulins:

Albumin	**Globulins**
1. They are heat coagulable: this test is used to detect albumin in urine of patients with kidney diseases.	
2. They are of high biological value as they contain all the 10 essential amino acids in a well balanced amounts and in an easily digested form .	
3. Soluble in water and salt solution	- Soluble in salt solution only.
4. Precipitated by full saturated ammonium sulfate solution.	-Precipitated by half saturated ammonium sulfate solution.
5. M.W.: 68 KDa.	- M.W.: 150 KDa.
6. Presents in :	Presents in :
• Egg→ovalbumin.	Egg→egg globulins
• Serum→serum albumin.	Serum→serum globulins: By electrophoresis , there are α,β &γ-globulins.
• Milk→lactalbumin.	
7. Functions:	

- Both function as protein carrier for transport of different elements , vitamins & hormones.
- Albumin keep blood osmosis. Antibodies are γ-globulins.

	2.Protamines	**3.Histones**	**4.Gliadins "Prolamines"**	**5.Glutelins**
Location	• Sperms & ova. • In association with nucleic acids. • In tuna & salmon fishes.	• In globin of hemoglobin. • In association with nucleic acids.	• Only in plants such as : 1. Wheat. 2. Zein of maize. بـــــروتين الذرة	• Only in plants such as :Wheat.
Structure	Classified into: • Mono-protamines: Which contain argenine amino acid only. • Di-protamines: which contain lysine & histidine amino acids. • Tri-protamines:	Contain argenine & histidine , but less lysine.	Rich in proline & deficient in tryptophan & lysine	Rich in glutamic acid.

	which contain Argenine,lysine & histidine amino acids.			
Characters	1. Strong basic proteins. 2. Ammonia soluble. **3.** Non-heat coagulable**.**	1. Less basic proteins. 2.Ammonia insoluble. 3.Heat coagulable& the coagulum soluble by addition of diluted acids while the coagulum of albumin & globulin increases by addition of diluted acids.		

6) Scleroproteins (Albuminoids):

General characters for scleroproteins:

- They are extracellular proteins never exist intracellularly.
- Non-digestible , of low biological value , non-coagulable.
- Extremly insoluble in all protein solvents e.g. water , saline ,diluted acids , alkalies & 70% ethanol .
- On prolonged boiling in water , diluted acids or alkalies, they are hydrolyzed into free amino acids.

- Their function is body protection.

Examples:

a. Keratins b. Collagens c. Elastins d. Reticulins

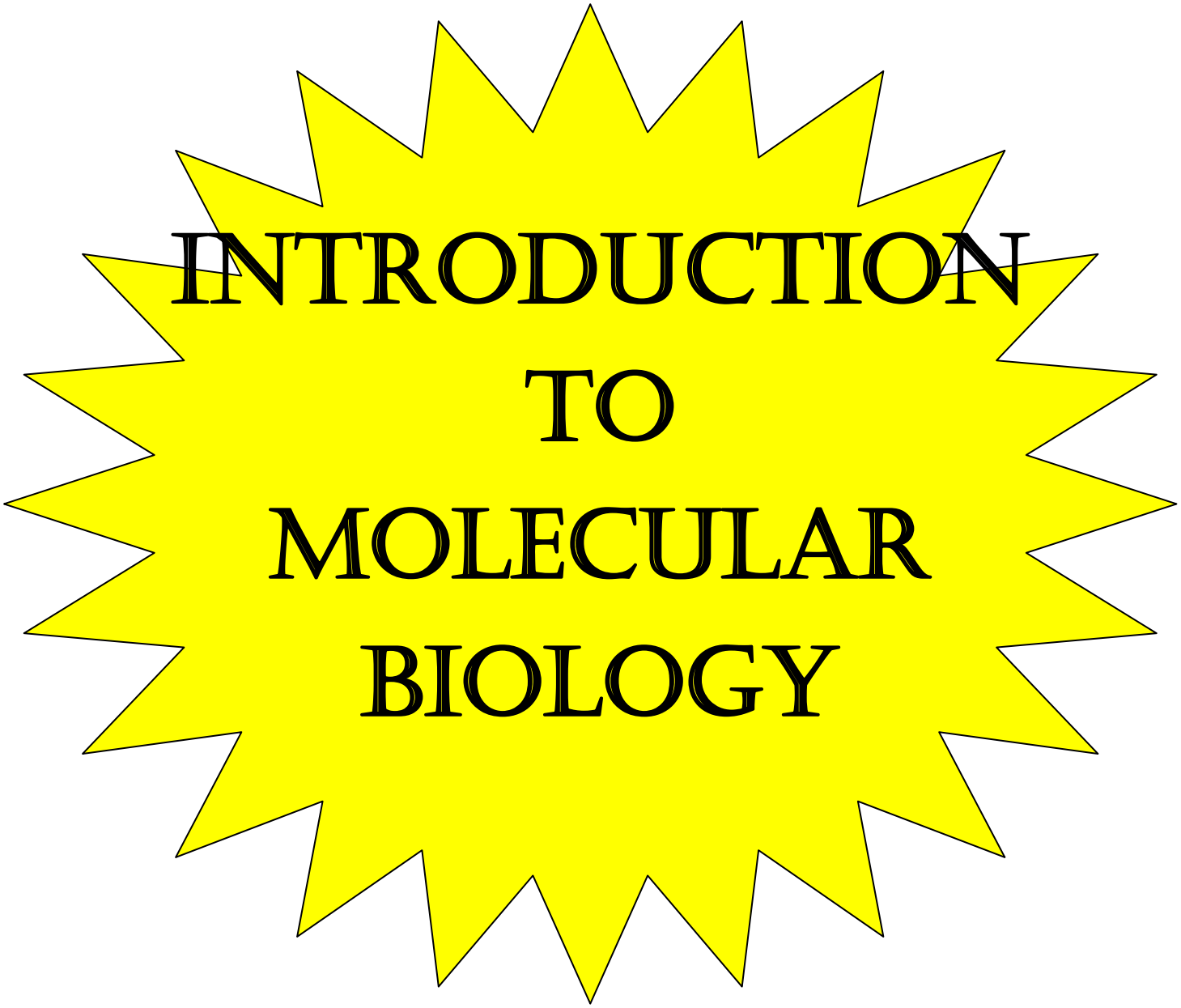
	Keratins	**Collagens**	**Elastins**	**Reticulins**
Location	Hair , nails & superficial layers of the skin	Bone & tendons	Elastic tissue of tendons , big arteries & uterine wall during pregnancy	Reticular tissues e.g. liver , splee & lymph nodes
Structure	• Mainly of cystine i.e. rich in sulfur. • Present as α-keratin & β-keratin	Rich in proline , hydroxy proline & glycine.	Rich in alanine , valine & leucine.	

Characters	Barium disulfide dissolve keratin , so it enters in cosmotics that deal with removal of hair.	On prolonged boiling in water , diluted acids or alkalies , it hydrolyzed into ***Gelatin***: • It is derived protein from collagen, of low biological value , used as apeptizer , easily digestible . • It is rich in proline , hydroxyprolin e & glycine but it is deficient in tryptophan. • Gel formation on cooling.		

Differences between albumin & albuminoids:

Albumin	Albuminoids
1. Heat coagulable. 2. Of high biological value. 3. Contain all the 10 essential amino acids. 4. Poor in glycine. 5. Soluble in protein solvents. 6. Precipitated by fully saturated ammonium sulfate solution.	1. Heat coagulable. 2. Of low biological value. 3. Defective. 4. Rich in glycine. 5. Extremly insoluble in protein solvents. 6. Not.

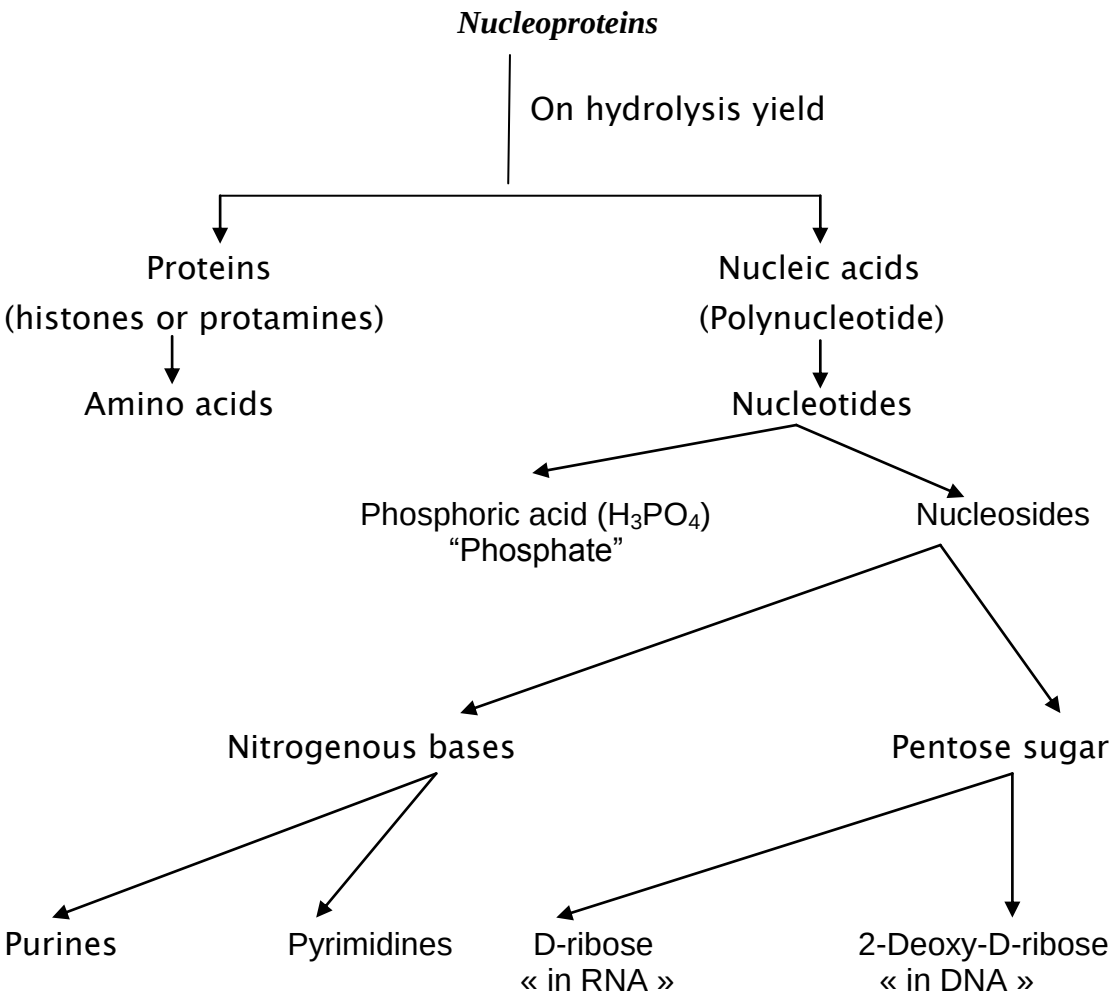
7. Present intracellularly & extracellularly. 8. Examples: a. Ovalbumin. b. Serum albumin. c. Lactalbumin.	7. Present extracellularly & never exists intracellularly. 8. Examples: a. Keratins. b. Collagen. c. Elastins. d. Reticulins.


INTRODUCTION
TO
MOLECULAR
BIOLOGY

Nucleoproteins

Definition:- nucleoproteins are conjugated proteins formed of non-protein prosthetic group (Nucleic acid) & one or more simple protein molecules, this simple protein is usually basic protein e.g. histones or protamines.

Hydrolytic products of nucleoproteins



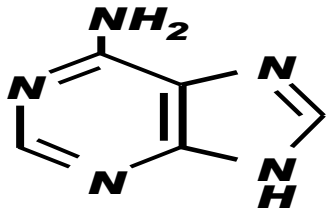
Nitrogenous Bases

- Nitrogenous bases that enter in RNA & DNA structure are either purines or pyrimidines.

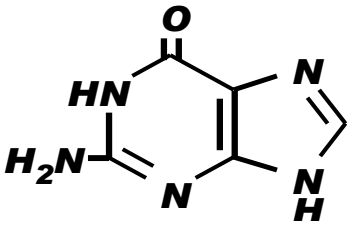
I-Purines:-

- There are two purine bases:-

a)- Adenine = (6-amino purine) = **(A)**:-

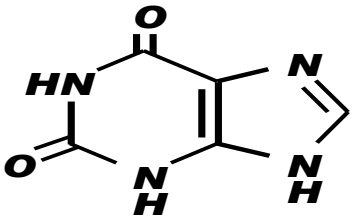


b)- Guanine = (2-amino,6-oxy purine) = **(G):-**

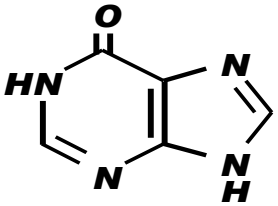


- Both (A) & (G) enter in the structure of DNA & RNA.
- There are a number of derived metabolic products from adenine & guanine which are:-

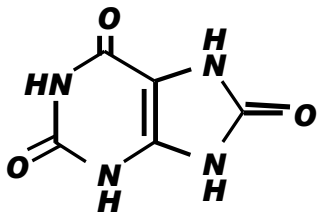
a)- Xanthine = (2,6 – dioxypurine):-



b)- Hypoxanthine = (6-oxypurine):-



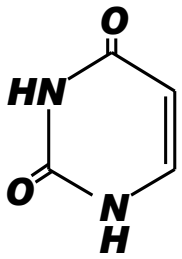
c)- Uric acid = (2,6,8-trioxypurine):-



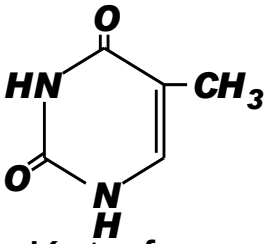
II- Pyrimidines:-

- There are three pyrimidines:-

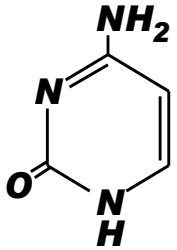
a)- Uracil = (2,4-dioxy-pyrimidine) = (U):-



b)-Thymine = (5 methyl-uracil)= (T):-



c)-Cytosine = (2-oxy,4-amino-pyrimidine) = (C) :-

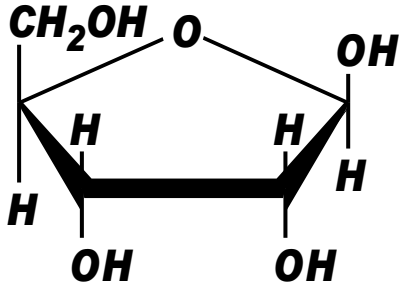


- Uracil enters in structureof RNA only.
- Thymine enters in structure of DNA only.
- Cytosine enters in structure of DNA &RNA.

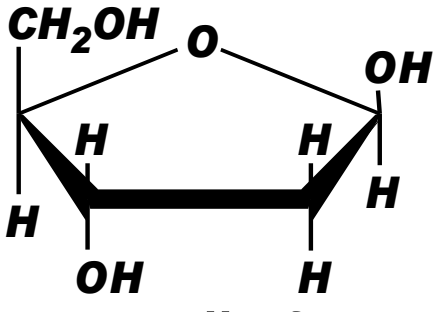
Pentoses that enter in nucleic acid structure

- They are either :-

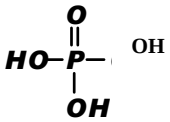
a)- **D-ribose**:- which enters in structure of RNA:-



b)- **2-Deoxy-D-ribose** :- which enters in structure of DNA.



Phosphoric acid "Phosphate"(H_3PO_4)



Nucleotides & Nucleosides

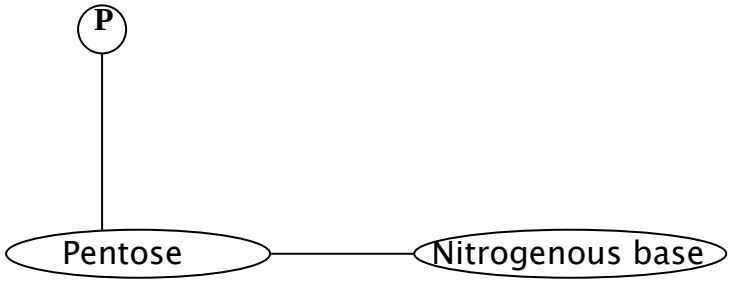
I-Nucleotides:-

- Each nucleotide is formed of:-

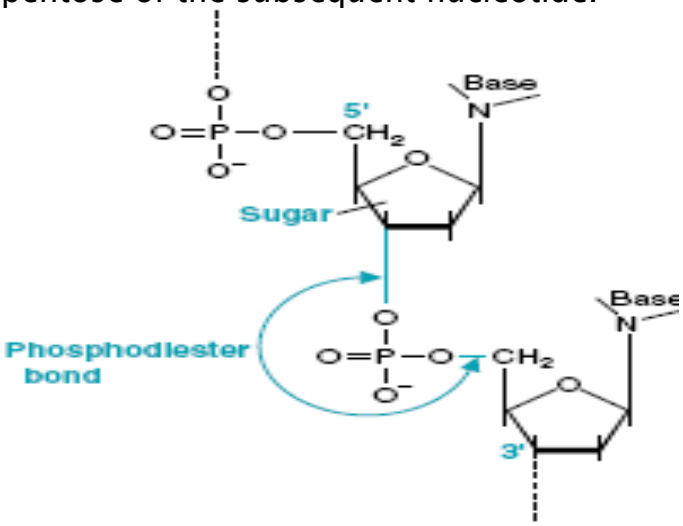
a)- Nitrogenous base(purine or pyrimidine).

b)- Pentose sugar (D-ribose in RNA & 2-Deoxy-D-ribose in DNA).

c)- Phosphoric acid "Phosphate"(H3PO4).



- C1 of the pentose sugar attaches to N9 of purine bases or N1 of pyrimidine bases.
- The "OH" group of phosphoric acid attaches to the "OH" group on C5 or C3 of the pentose sugar.
- Phosphodiester bonds:- they connect the nucleotides with each others & formed between "OH" group on C3 of pentose sugar of a nucleotide & "OH" group of phosphate attached to "OH" group on C5 of pentose of the subsequent nucleotide.



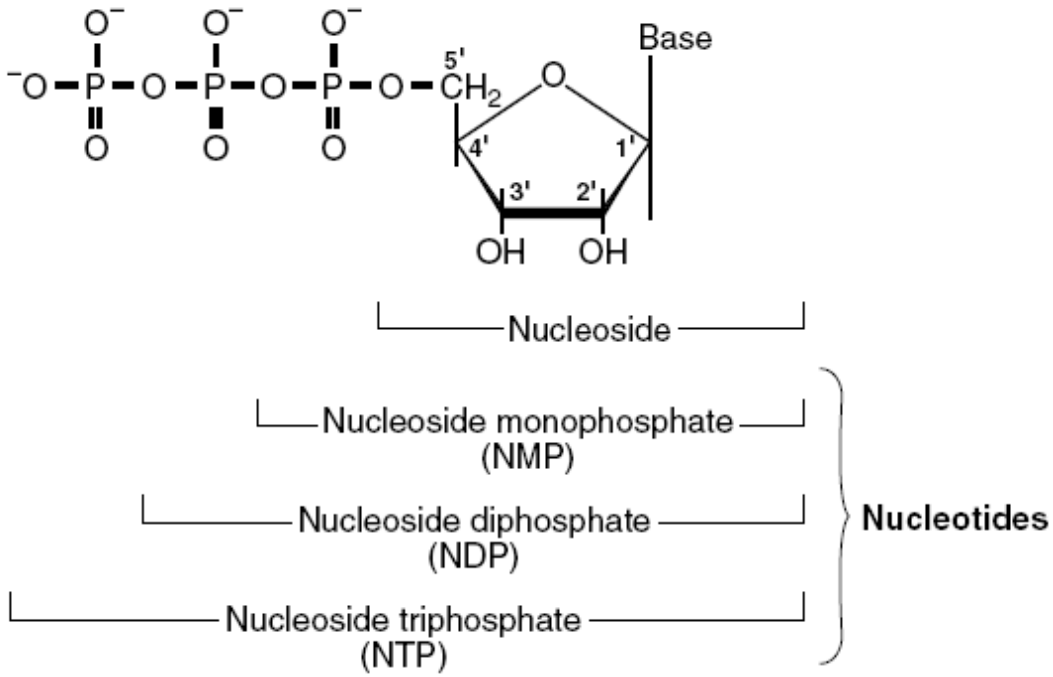
II- Nuclosides:-

- Each nucleoside is formed of a nitrogenous base & a pentose sugar attached to each other as previously mentioned.
- So Nucleoside = Base + Pentose.
- Each nucleotide or nucleoside is named according to the nitrogenous base as follows:-

Base	Nucleoside	Nucleotide
Adenine	Adenosine(A)	Adenosine Monophosphate (AMP, Adenylic acid)
	2-Deoxy-adenosine (dA)	2-deoxyAdenosine Monophosphate (dAMP, 2-deoxyadenylic acid)
Guanine	Guanosine (G)	Guanosine Monophosphate (GMP, Guanylic acid)
	2-deoxy-guanosine (dG)	2-deoxyGuanosine Monophosphate (dGMP, 2-deoxyguanylic acid)

Cytosine	Cytidine (C)	Cytidine Monophosphate (CMP, Cytidylic acid)
	2-deoxycytidine (dC)	2-deoxyCytidine Monophosphate (dCMP, 2-deoxycytidylic acid)
Uracil	Uridine (U)	Uridine Monophosphate (UMP, Uridylic acid)
Thymine	2-deoxy - thymidine (dT)	2-deoxyThymidine Monophosphate (dTMP 2-deoxythymidylic acid)

- N.B: Deoxynucleosides or Deoxynucleotides contain 2-deoxy ribose as pentose , their names are preceeded by the term deoxy (or letter d) e.g. dCMP.



Nucleoside and nucleotide structures. Shown with ribose as the sugar. The corresponding deoxyribonucleotides are abbreviated dNMP, dNDP, and dNTP. N= any base (A, G, C, U, or T).

Nucleic Acids

Nucleic acids are high molecular weight molecules formed of thousands of nucleotides as building units. Nucleotides are the monomeric units of the nucleic acids, DNA (deoxyribonucleic acid) and RNA (ribonucleic acid). Each nucleotide consists of a heterocyclic nitrogenous base, a sugar, and phosphate. DNA contains the purine bases adenine (A) and guanine (G) and the pyrimidine bases cytosine (C) and thymine (T). RNA contains A, G, and C, but it has uracil (U) instead of thymine. In DNA, the sugar is deoxyribose, whereas in RNA it is ribose.

Differences between DNA & RNAs:

	DNA	***RNAs***
Name	Deoxyribonucleic acid	Ribonucleic acid
Occurance	Nucleus & mitochondria	Synthesized in the nucleus and functions in the cytoplasm
Number of strands	Double stranded but single strand DNA may present e.g. in bacteriophages	Single stranded
Type of nucleotides And bases	Deoxyribonucleotides - Adenine (A) - Guanine (G) - Thymine **(T)** - Cytosine (C)	Ribonucleotides - Adenine (A) - Guanine (G) - Uracil **(U)** - Cytosine (C)

	DNA	***RNAs***
Effect of alkali	Not hydrolysable by alkali	Hydrolysable by alkali
Type of sugar	Deoxyribose	Ribose
Types	One type	It is mainly of three types (rRNA , mRMA , tRNA), beside the small nuclear RNA, snRNA, used in mRNA splicing) that differ in: gene of origin, function, size and structural modification.
Synthesis Process	It is called replication	It is called transcription.
Functions	Storage of genetic information, cell division, DNA replication & RNA transcription.	RNAs play a central role in the process of protein synthesis

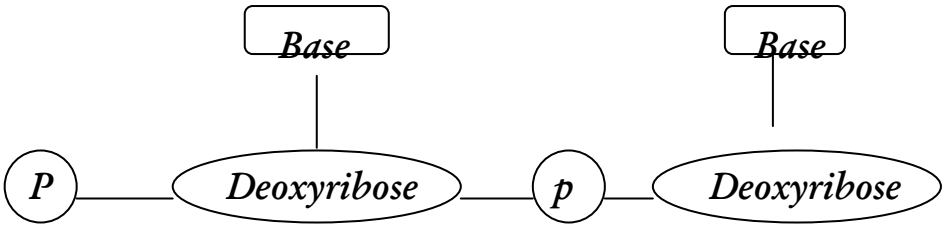
1-Chemistry or Structure of Deoxy Ribonucleic Acid (DNA):

I) - Primary structure (level) of DNA (single strand formation):

- It is the linear sequence of its building deoxyribo-nucleotides units as follow:

1. Deoxy-adenylic acid (adenine – deoxyribose – phosphoric acid).
2. Deoxy-guanylic acid (guanine – deoxyribose – phosphoric acid).
3. Deoxy-cytidylic acid (cytosine – deoxyribose – phosphoric acid).
4. Deoxy-thymidylic acid (thymine-deoxyribose – phosphoric acid).

- The deoxy-nucleotides are interconnected together by phosphodiester bonds.



II) - Secondary structure of DNA (double stranded helix formation):

- In which the DNA presents in the form of a double helix coiled around common longitudinal axis.
- This conformation leads to formation of two unequal grooves :

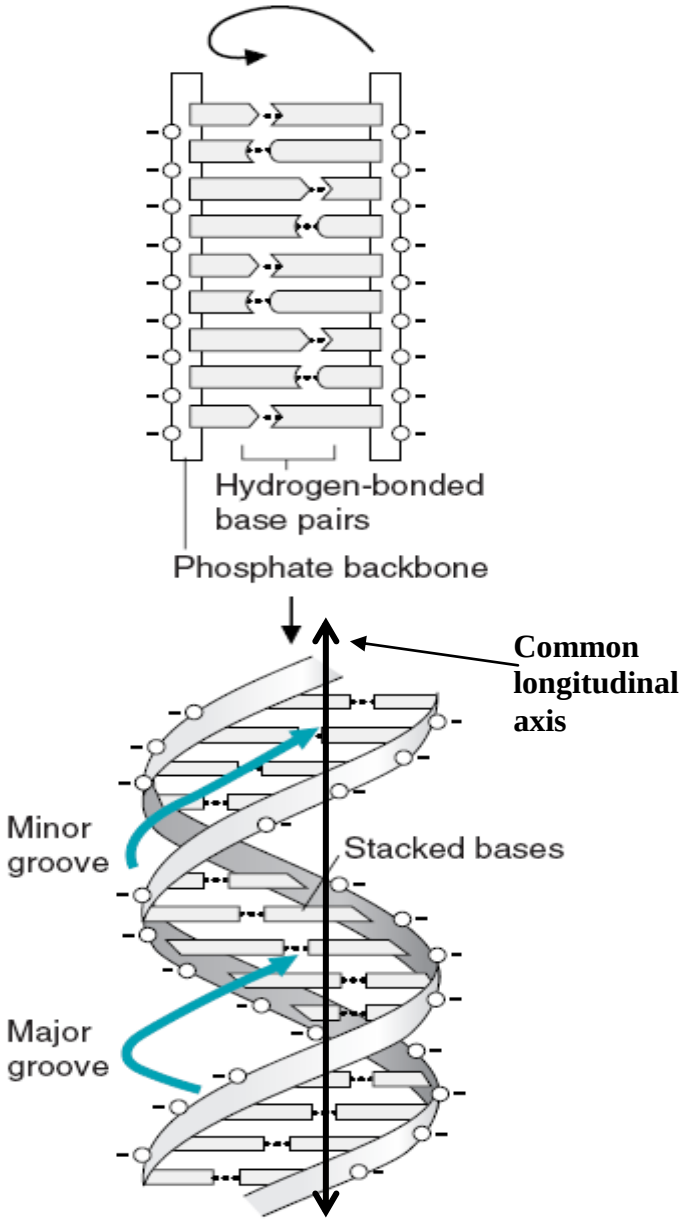
1. Major groove: Where regulatory proteins interact with DNA & it occurs at A=T dimmer.

2. Minor groove: Where histones interact with DNA & it occurs at G≡C dimmer.

- The two grooves are unequal due to the space filling of A=T dimmer is not the same as for G≡C dimmer.

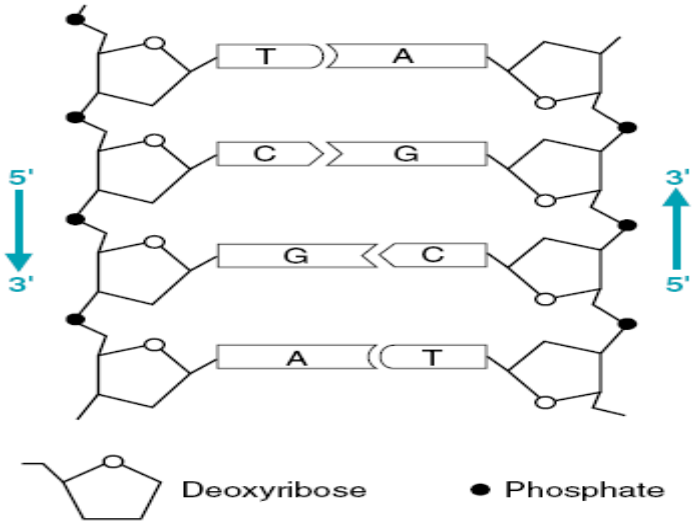
The two strands of the DNA show the following criteria:

- 1. The hydrophilic deoxyribose and phosphoric acid project outwards forming the backbone of the DNA molecule while the hydrophobic nitrogenous bases are hidden the core of the DNA.
- 2. The plane of the nitrogenous bases is perpendicular to the helix axis and the bases are partially overlapping with each other i.e. base stacking.



Two DNA strands twist to form double helix.The hydrogen-bonded base pairs, shown in blue, create stacking forces with adjacent base pairs. Because of the twisting of the helix, grooves are formed along the surface, the larger one being the major groove, and the smaller one the minor groove.

3. They are anti-parallel: One strand runs in 3'→5' direction and called anti-sense or non-coding or template strand and the other runs in 5'→3' direction and called sense or coding strand.See the following figure.

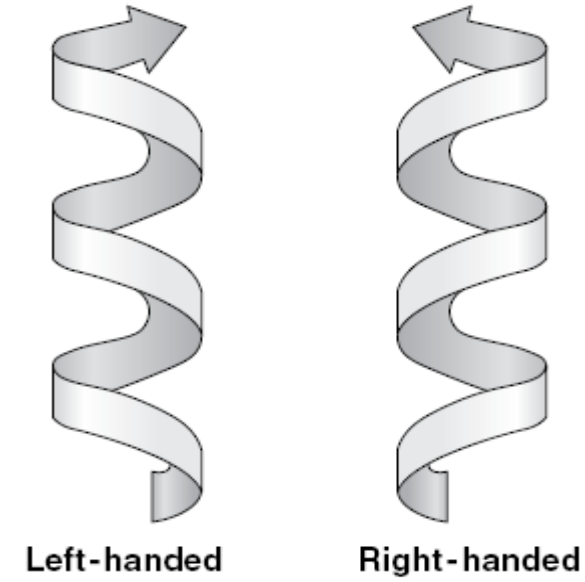


4. They have 3'-5' polarity: The two free ends of the DNA strands are different with free phosphate attaches to the (OH) group of C5 of the deoxy ribose of the first nucleotide forming 5' end (top end or left terminal) & free (OH) group of c3 of the deoxy ribose of the last nucleotide forming 3' end (bottom end or right terminal) . This polarity is very important for reading of the code sequence in 3'→5' direction for DNA replication & RNA transcription and in 5'→3' direction on mRNA for protein synthesis.

5. They are complementary to each others i.e. knowing the sequence of one strand determine the sequence of the other strand , depending on the base pairing rule in which adenine binds thymine by double hydrogen bonds (A=T) & Guanine binds cytosine by triple hydrogen bonds (G≡C) , so the ratio of purines (A & G) to pyrimidine (T & C) in the DNA ~ 1.

6. It may be right handed or left handed:

If you look up through the bottom of a helix along the central axis and the helix spirals away from you in a clockwise direction (toward the arrowhead in the drawing), it is a right-handed helix. If it spirals away from you in a counterclockwise direction, it is a left handed helix as shown in the following figure:



III) - Structural forms or classification of the DNA:

DNA is classified into:

A) – According to the physical conditions and the base composition.

B) – According to the number and the shape of DNA strands.

A) – According to the physical conditions and the base composition:

B-form	A-form	Z-form
It is right-handed helix	It is right-handed helix	It is left handed
Contains 10 base pair per turn	Contains 11 base pair per turn	Contains 12 base pair per turn

It is the most common form at physiological conditions (low salt and high degree of hydration).	It is the dehydrated form of B-form under lower hydration and higher salt content with, thicker helix than B-form and has shorter turn height but does not exist under physiological conditions	It is zigzag-like helix that is thinner than B-form. It is formed in areas rich in G and C under high cation or salt concentration that lead to disappearance of the major groove and deepening of the minor one

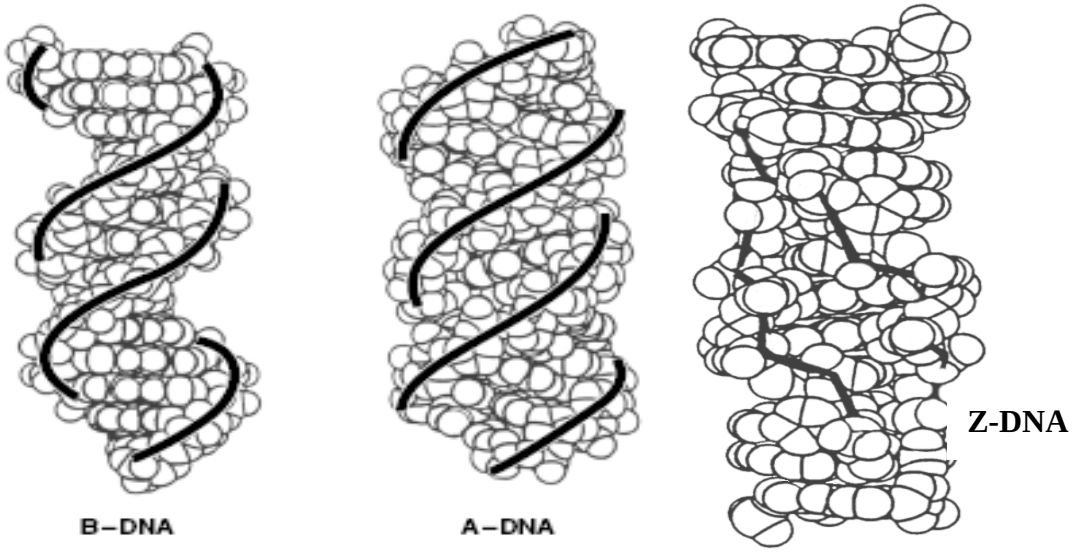


Figure showing B, A, and Z forms of DNA.

B) – According to the number and the shape of DNA strands:

DNA is either single or double stranded and each is either circular or opened linear;

a. Double stranded linear DNA, present in nucleus of human cells & some DNA viruses.
b. Double stranded circular DNA, present in mitochondria and bacterial nuclear and plasmid DNA. It is also present in plants and some DNA viruses.
c. Single stranded circular DNA, present in some bacteriophages.

IV) - Chromatin organization (Tertiary structure of DNA):

Chromatin:

- The DNA formed in the human cell has a length of 1 meter and the typical human cell diameter is 20μm and the nucleus diameter is 5-10μm, so for the DNA to be packed into such small space should be condensed by organizing proteins into a compact structure (DNA –protein complex) which is called chromatin.
- Chromatin consists of:
    1. Very long double stranded DNA molecule.
    2. Small quantity of RNA.
    3. Histones which are basic proteins.
    4. Protamines which are basic proteins.

Nucleosomes:

- They are the packaging units of chromatin arranged in a manner resemble beads on string of DNA.

Histones:

- They are simple basic proteins that are positively charged at physiological PH (7.4) forming ionic bonds with the negatively charged DNA.
- They are 5 major classes (H1 , H2 {H2A & H2B} , H3 & H4) that fall into two main groups:
    - The first group: which form the nucleosome core and is formed of octamer i.e. 8 subunits which are 2 H2A , 2 H2B , 2 H3, 2 H4 surrounded on their surface by DNA segment of 146 nucleotide in length that coil 1.75 turn.
    - The second group: This is formed of H1 only which binds to the linker DNA which is the DNA segment between nucleosomes and has length of 30 nucleotides thus completing two DNA turns.

- Further compaction of chromatin occurs as the strings of nucleosomes wind into helical, tubular coils called solenoid structures. See the following figure:

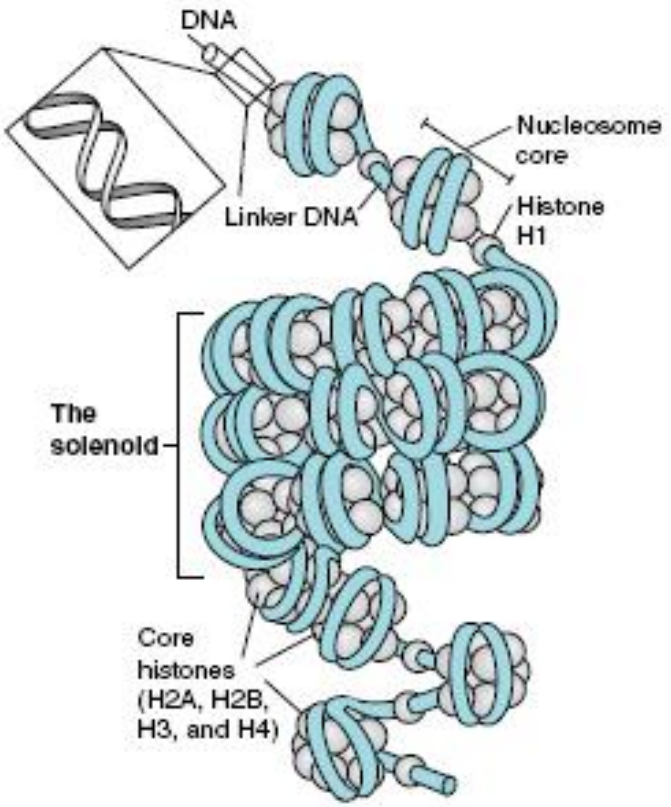
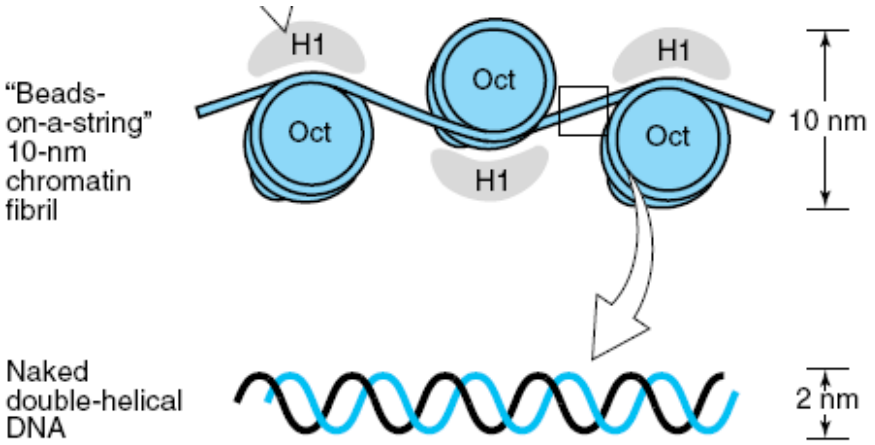


Figure showing A polynucleosome, indicating the histone cores and linker DNA.

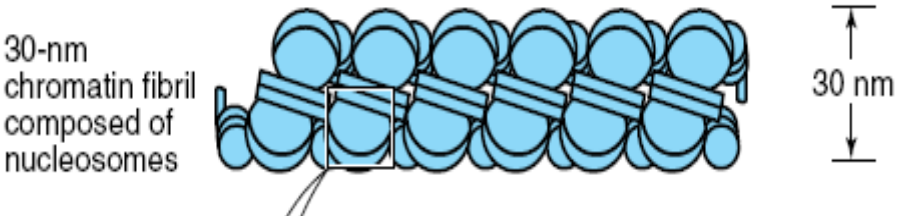
- Functions of histones:
    1. Structural role.
    2. Regulatory role (increase or decrease the rate of transcription) as they subject to covalent modifications such as phosphorylation , acetylation & methylation.
    3. Affect the chromosomal condensation during the DNA replication and repair.

Organization of DNA into chromatin occurs as follows:

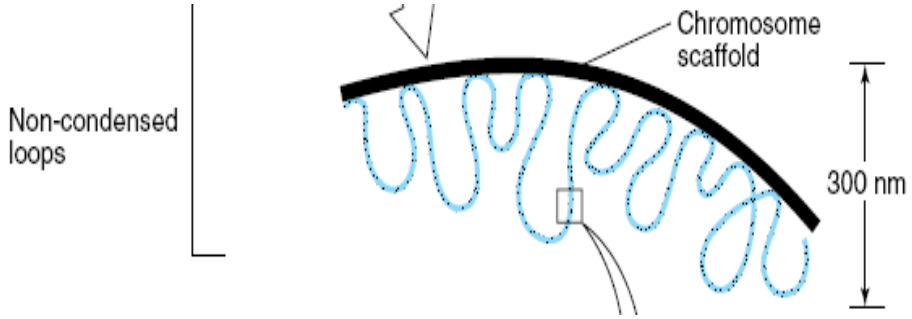
1. The DNA coiled around histone octamer resembling beads on string forming 10 nm chromatin fibers.



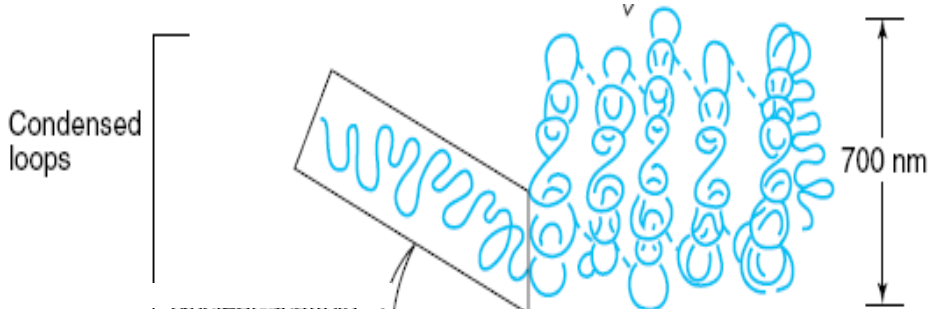
2. The 10nm chromatin fiber coils more around a linear axis forming 30nm chromatin fiber.



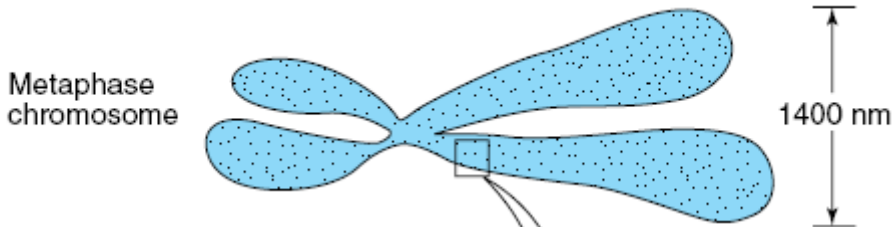
3. The 30 nm chromatin fiber undergoes extensive coiling around scaffold protein to be just a spot in 300 nm non-condensed chromosomal loop.



4. The 300 nm non-condensed chromosomal loop coils more and more to be just a spot in a 700 nm condensed chromosomal loop.



5. The 700 nm condensed chromosomal loop coils more and more to be just a spot in 1400 nm metaphase chromosome.



Types of chromatin:

1. Euchromatin: This has the following characters:

a. Active.

b. Stained light.

c. DNase-1 hypersensitive.

2. Heterochromatin: This has the following characters:

a. Inactive.

b. Stained dark.

c. Not sensitive to DNase-1 .

3. Facultative Heterochromatin: If heterochromatin turned to euchromatin at specific developmental phase.

V) - Mitochondrial DNA:

- It is double stranded circular DNA composed of heavy (H) and a light (L) chains or strands.
- It is 16.569 kilobase pair in length.
- It codes for:
  1. 2 types of mt rRNA molecules.
  2. 22 types of mt tRNA molecules.
  3. Many protein subunits of four enzymes involved in energy production in mitochondrial respiratory chain which are ATP synthase (complex V) , Cytochrome oxidase (complex IV) , NADH-Q reductase (complex I) & Coenzyme-Q – cytochrome C reductase (complex III).

- Genetic code differs slightly from the standard code: UGA (which is standard stop codon) is read as tryptophan and AGA & AGG (which are standard codons for arginine) are read as stop codons.

- All mitochondrial DNA in the zygote are maternally derived as the sperm not carries any mitochondria in the fertilized egg & mutations of mitochondrial DNA result in **MELAS** syndrome which are group of maternally inherited diseases include myopathy, encephalopathy, lactic acidosis & stroke like episodes.

2-Chemistry or Structure of Ribonucleic Acids (RNAs):

There are four types of RNAs which are:

(1) Messenger RNA (mRNA).

(2) Ribosomal RNA (rRNAs).

(3) Transfer RNA (tRNA).

These types will be discussed in some details:

I- The messenger RNA (mRNA):

- The blue script (primary transcript) of mRNA is **h**eterogenous **n**uclear RNA "hnRNA" , also called immature mRNA which differ from other types of RNA in that it never leave the nucleus and functions as a precursor from which mRNA is processed.

- **Length:** It is single RNA strand with 400-4000 nucleotides in length.

- **Function:** It carries codons for protein synthesis which are recognized by anticodons of tRNAs.

- **Structure:** It is single strand formed of linear sequence of its building ribonucleotides units (AMP , UMP , GMP , CMP) with:

1. **7-methyl guanosine triphosphate cap** at 5' end:

Functions:

a- Facilitates initiation of translation of mRNA.

b- Protects the 5'end from attack by 5' to 3' exonucleases & phosphatases.

2. Poly adenylate tail (poly A tail) at 3' end:
It is formed of 25-250 adenine nucleotide base .
Functions:

a- Facilitates the mRNA transport.
b- Protect the 3'end from attack by 3' to 5' exonucleases.

3. 3' & 5' terminal un translated regions:
Functions:

a- Contain regulatory sequence to which regulatory proteins bind to regulate translation of mRNA.
b- Control the mRNA half life.

Table showing the differences between the prokaryotic & eukaryotic mRNA

	Prokaryotic mRNA	*eukaryotic mRNA*
Half life	Short	Long
Numbers of cistrons " translation units"	Polycistronic	Monocistronic
Introns	No	Yes
Post-transcriptional processing (splicing , capping , tailing & editing)	No	Yes
Separation of transcription from translation	No	Yes

II)-The transfer RNA (tRNA) "Soluble RNA":

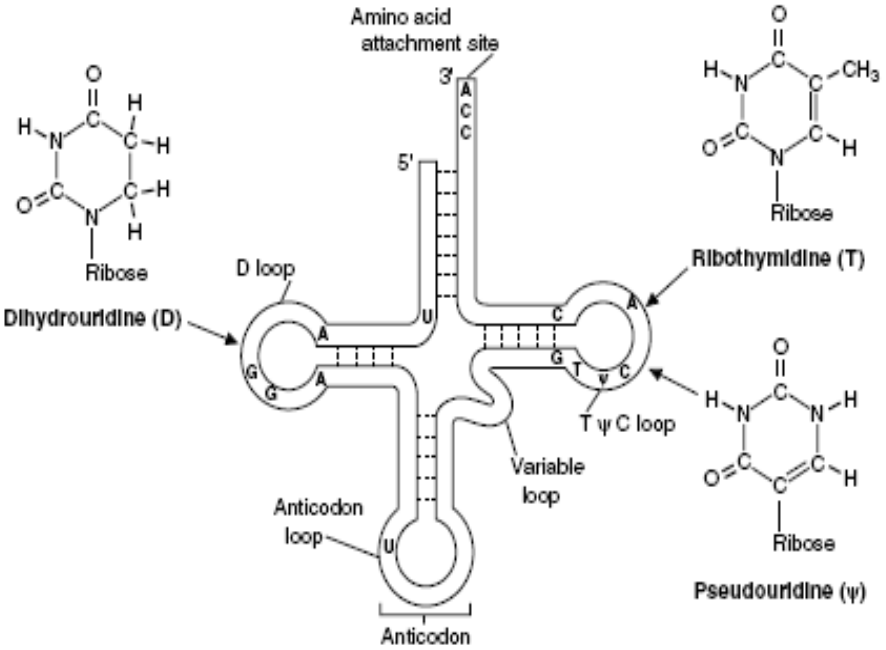
Length: It is the smallest RNA, having less than 100 nucleotides length.

Structure:

It resembles clover leaf in shape with the appearance of intra-chain hydrogen bonding with four loops:

1. T ΨC loop.
2. D- loop.

   Most tRNA molecules contain ribothymidine (T), in which a methyl group is added to uridine to form ribothymidine. They also contain dihydrouridine (D), in which one of the double bonds of the base is reduced; and pseudouridine (ψ), in which uracil is attached to ribose by a carbon–carbon bond rather than a nitrogen–carbon bond.
3. Variable loop.
4. Anticodon loop: This is a nucleotide base sequence complementary to its specific codon carried on the mRNA.
5. 3' ACC terminus: This is the site of amino acid attachment.



The tRNA clover leaf. Bases that commonly occur in a particular position are indicated by letters. Base-pairing in stem regions is indicated by lines between the strands. The locations of the modified bases dihydrouridine (D), ribothymidine (T), and pseudouridine (ψ) are indicated.

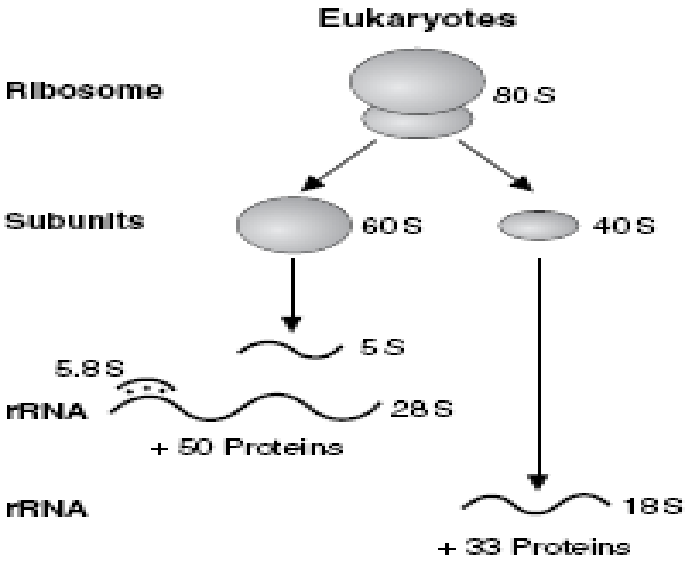
Function:

- It carries the amino acids connected to the 3'-end and transfers them to the ribosomes for protein synthesis through binding by it's anticodon loop to it's complementary codon on mRNA. There is at least one tRNA for each amino acid, so there are at least 20 types of tRNA.

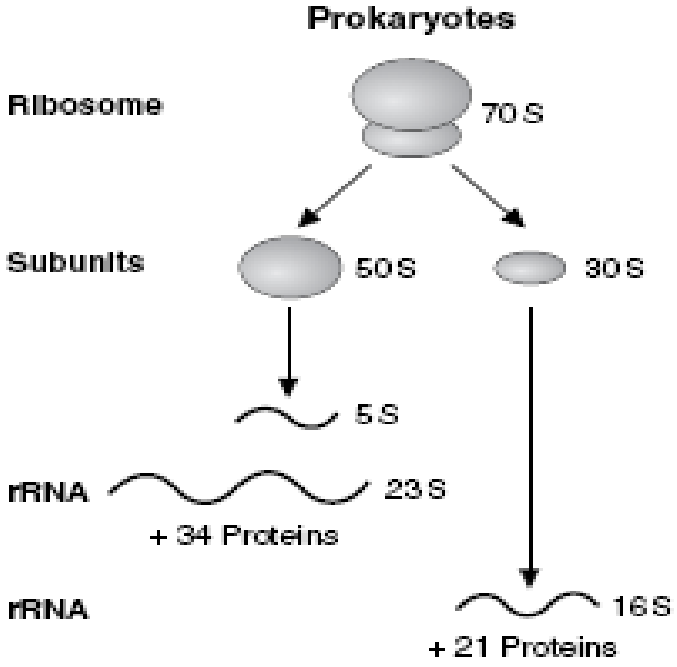
III)-The ribosomal RNA (rRNA):

- The rRNAs assemble with certain proteins to form the ribosomes,
- **Function**: They are the site of protein synthesis.
- **Svedberg unit "S unit"**: It is the rate of floatation of rRNA in NaCl solution during centrifugation.
- **Structure:**
  - The complete ribosome has two binding sites for the amino acyl- tRNA, "**A**" site & "**P**" site. Each of them extend over both ribosomal subunits & both cover the two neighboring codons on mRNA.
  - The eukaryotic ribosome differ in it's structure from the prokaryotic ribosome.

1. Eukaryotic ribosome is 80S in size that is formed of two subunits; 60S and 40S. 60S subunit is composed of ~50 protein molecules and 5S, 5.8S and 28S rRNAs. The 40S subunit is composed of ~33-35 protein molecules and 18S rRNA.



2. Prokaryotic ribosome is 70S in size and formed of 50S and 30S subunits. The 50S is formed of ~34 protein molecules and 23S and 5S rRNA. The 30S subunit is formed of ~21 proteins and 16S rRNA .

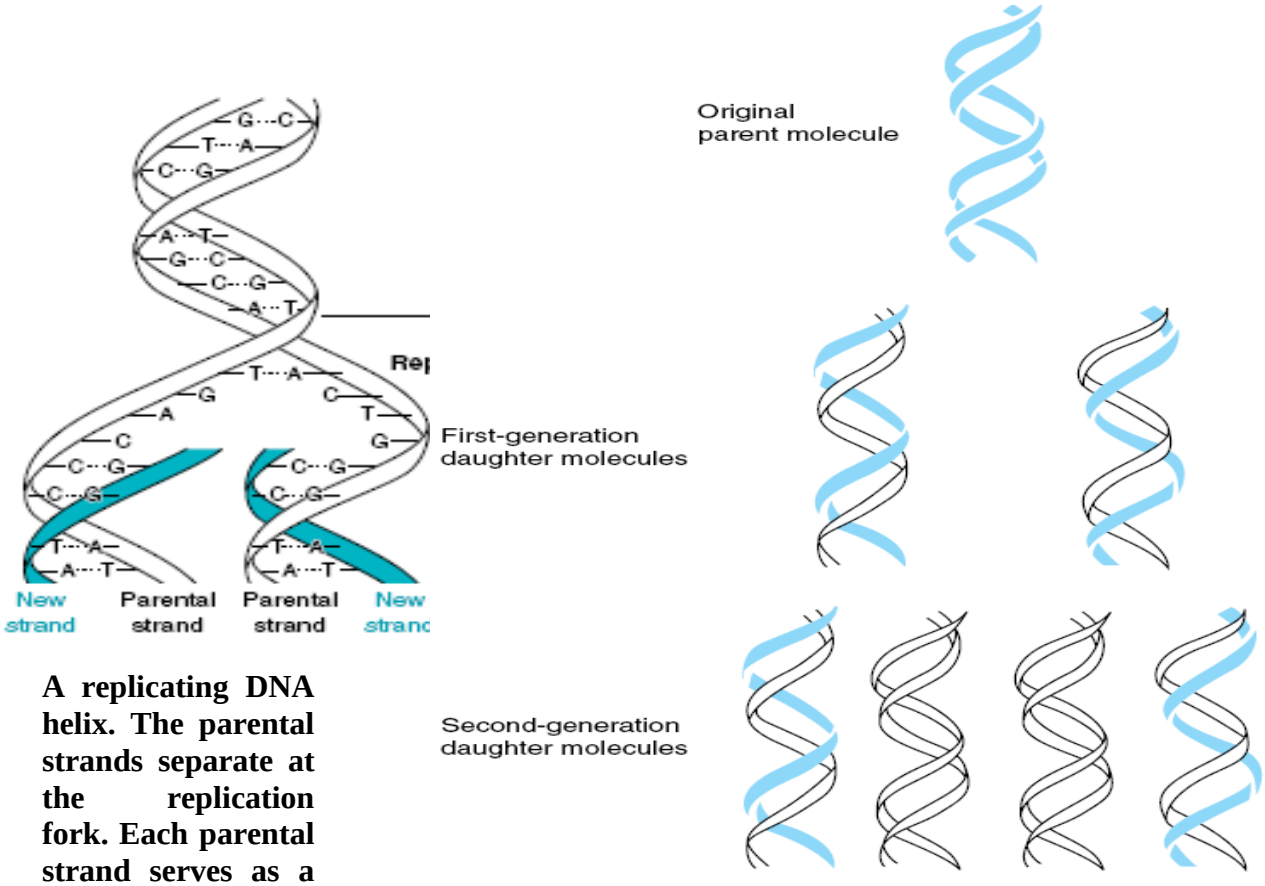


N.B: Prokaryotes and Eukaryotes:

Living things are divided into two broad categories: prokaryotes and eukaryotes. Bacteria (Archaea and Eubacteria) are prokaryotes, which mean they lack a nucleus or other membrane-bound organelles. Eukaryotes include all protozoans, fungi, plants, and animals (including humans), and these cells are characterized by a nucleus (which houses the chromosomes) as well as a variety of other organelles.

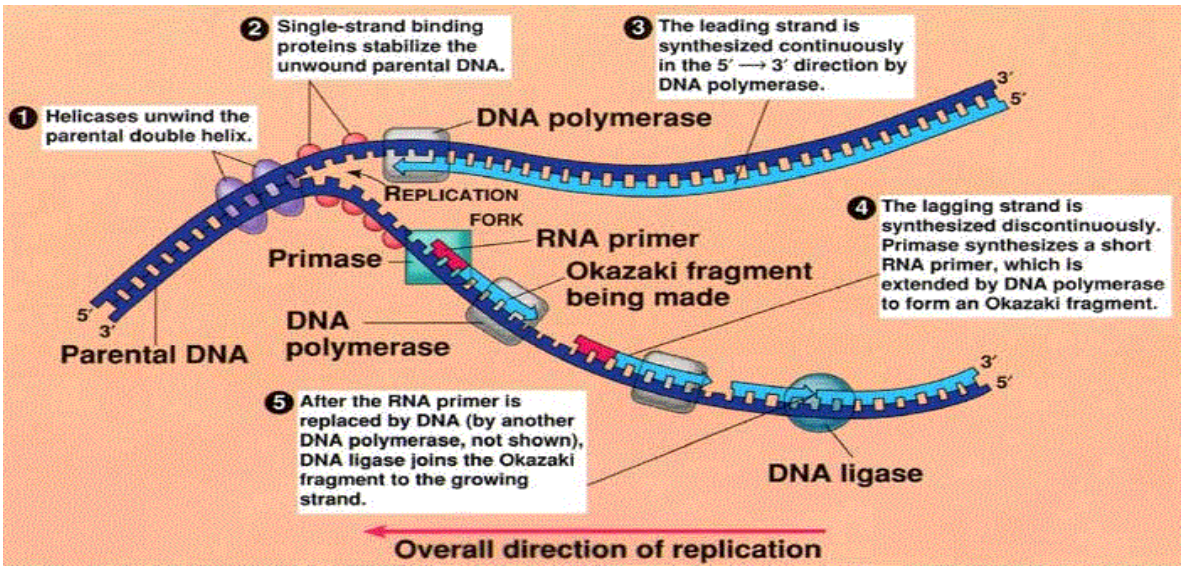
DNA Replication

Definition: It is the process of synthesis of DNA from DNA & it is semi conservative process. As during replication, separation of the parental double stranded DNA helix into two single DNA strands , each of them acts as a template upon which a new strand will be synthesized & each of the resulting two daughter DNA molecules will contains an old strand (one parent strand conserved) & new strand synthesized from the free nucleotides present in the nucleus. See the following figure:



A replicating DNA helix. The parental strands separate at the replication fork. Each parental strand serves as a template for the synthesis of a new strand.

Steps of replication:

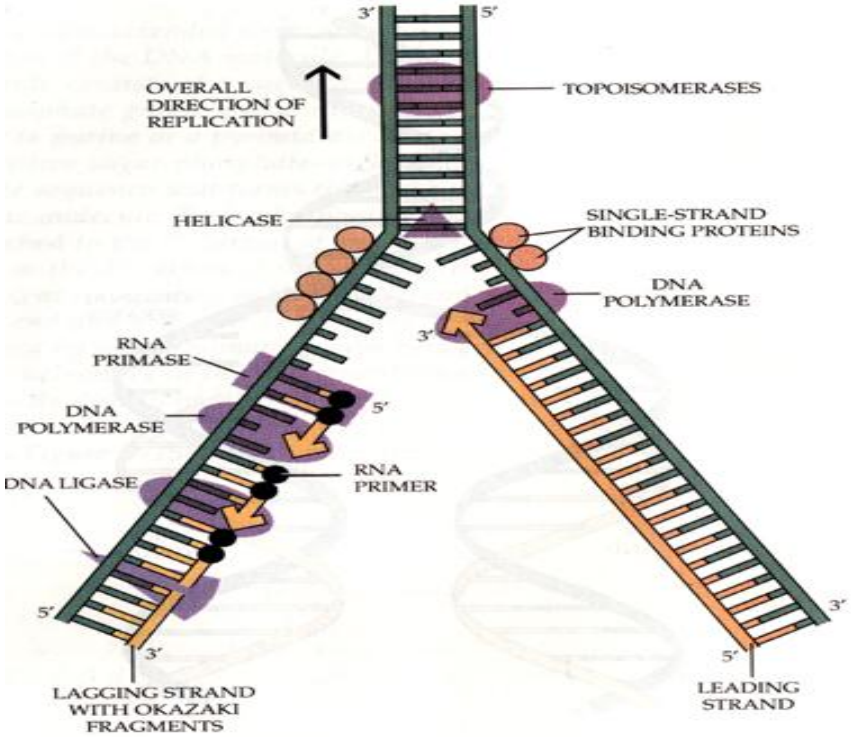


Leading strand:

- It is built in 5'→3' direction towards the replication fork.
- It is built in a continuous manner.
- It contains few RNA primers.

Lagging strand:

- It is built in 5'→3' direction away from the replication fork.
- It is built in a discontinuous manner forming okazaki fragments which are small fragments of DNA.
- It contains many RNA primers.



Reverse transcriptase "R.T" (RNA dependent DNA polymerase)

The normal flow of genetic information (*central dogma*) occurs as follow:

Transcription *Translation* *Modifications*

DNA ⇒ mRNA ⇒ Protein ⇒ Enzymes, Hormones, Receptors

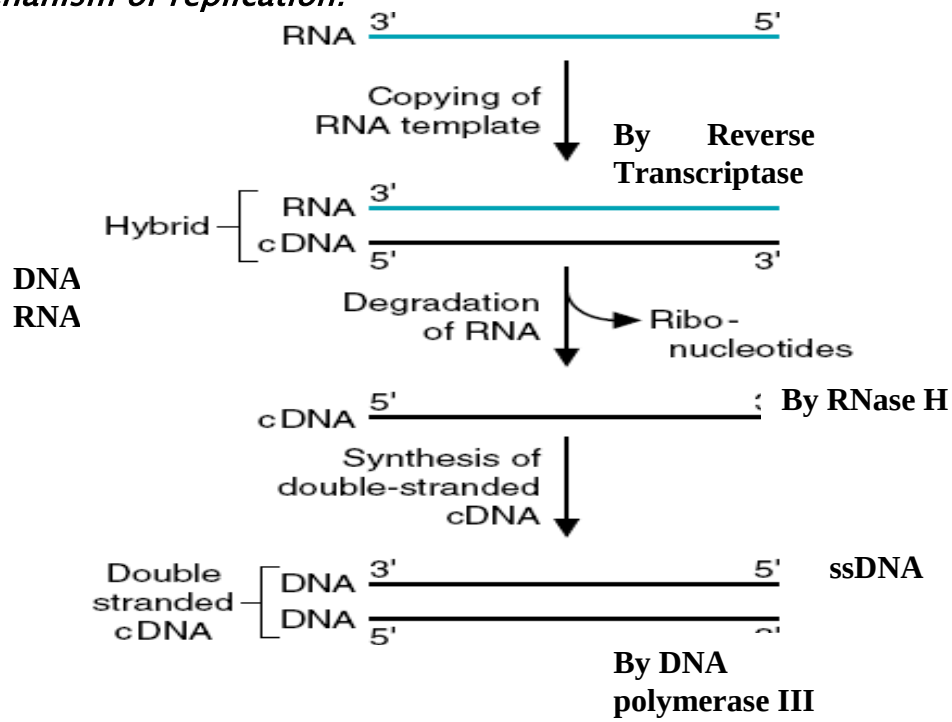
One species of viruses called retroviruses has a mechanism for reversing the first step in the central dogma i.e.

Reverse transcription *Transcription* *Translation*

RNA ⇒ dcDNA ⇒ mRNA ⇒ Protein

These viruses has single stranded RNA & an enzyme called Reverse Transcriptase , that is, an RNA-dependent DNA polymerase that makes a complementary DNA (cDNA) copy of the genetic RNA template, complementary DNA (because it is complementary to the RNA template), or cDNA ,This is why it is called reverse transcriptase,).

Mechanism of replication:



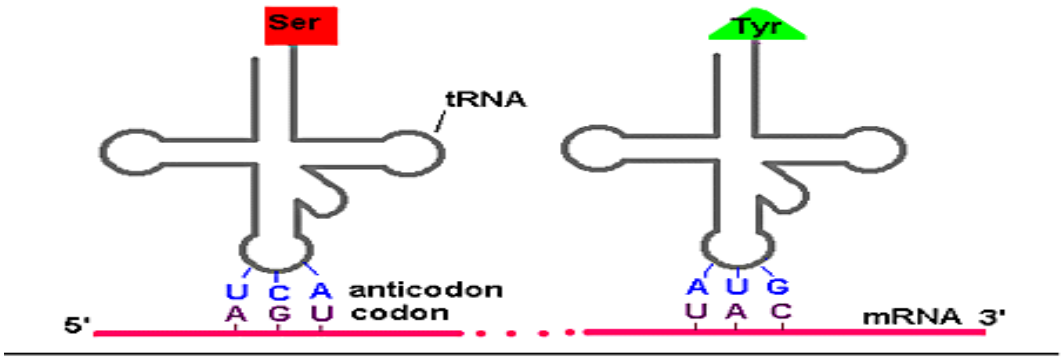
Action of reverse transcriptase. This enzyme catalyzes the production of a DNA copy from an RNA template. The RNA of a DNA-RNA hybrid is degraded, and the single DNA strand is used as a template to make double-stranded DNA.

- Then the newly synthesized viral double stranded cDNA enters the nucleus of the infected cell and integrates itself by recombination into a host chromosome.
- The viral genes are then transcribed into mRNAs to be translated into viral proteins and also into genomic RNA that combines with the viral proteins to form new viruses.
- Examples of retroviruses are HIV, HAV "Hepatitis A virus", some tumor viruses.
- R.T is very important in recombinant DNA technology.

The Genetic Code

Definition:

It is the dictionary that gives the relationship between the nucleotide base sequence on the DNA, transcribed mRNA & amino acids sequences on the protein molecule. It is made up of collection of codons.



2nd base in codon

1st base in codon	U	C	A	G	3rd base in codon
U	Phe Phe Leu Leu	Ser Ser Ser Ser	Tyr Tyr STOP STOP	Cys Cys STOP Trp	U C A G
C	Leu Leu Leu Leu	Pro Pro Pro Pro	His His Gln Gln	Arg Arg Arg Arg	U C A G
A	Ile Ile Ile Met	Thr Thr Thr Thr	Asn Asn Lys Lys	Ser Ser Arg Arg	U C A G
G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G

The genetic code

Codons: They are the individual words of the genetic code dictionary , each codon is a sequence of three nucleotide bases on the mRNA which determine the type & position of the amino acid that will enter in the structure of the protein molecule.

- The sequence of the nucleotide bases on the mRNA are always read in 5'→3' direction by the anticodons of the tRNAs carrying specific amino acids.
- There are four nucleotide bases enter in the structure of mRNA (A,U,G,C) used to form three-base codons , so there are $4^3 = 4X4X4 = 64$ different codons.
- Three of these codons are called stop codons or (Non-sense codons as they not code for amino acids) or (termination codons as their appearance on the sequence of the mRNA indicate that the synthesis of the polypeptide chain coded by that mRNA has been completed. They are UAA, UAG & UGA.

Characters of the genetic code:

1. Specificity (Unambiguous).
2. Degeneracy.
3. Universality.
4. Non-overlapping.
5. Comma-less.
6. Co linearity of gene & product.

1. ***Specificity:*** This means that each codon is specific for single amino acid & not code for any other amino acid e.g. UUU codes for phenylalanine.
2. ***Degeneracy:*** As there are 64 codons , three of them are non-sense or termination codons so that there are 61 codons that

indicate multiple codons must code for the same amino acid e.g. Arginine has six different codons .

- Codons specifying for the same amino acid are called *Degenerate* or *synonyms* or *nick names.*

3. ***Universality:*** This means that the same codon code for the same amino acid in plants , animals , humans ,bacteria & viruses with the exception of some mitochondrial codons as follow:

   - Mitochondrial AUA codes for methionine while nuclear AUA codes for isoleucine.

   - Mitochondrial UGA codes for tryptophan while nuclear UGA is a non-sense or stop codon.

4. ***Non-overlapping***: This means that there is no base can functions as a common member in two consecutive codons:

---------- X

AUG CCG ---------- √

5. ***Comma-less:*** This means that the codons are continuous structure without interruption i.e. codons are written without interruption.

A,U,G,C,C,G ---------- X

AUG CCG ---------- √

6. ***Co linearity of gene & product***: There is linear correspondence between the nucleotide base sequence on the DNA, transcribed mRNA & amino acid sequence on the protein molecule.

Codon – Anticodon recognition "Crick's wobble or shaking hypothesis":

- Reduced specificity of the third base of the codon is called 3rd base degeneracy or wobbling phenomenon.
- Because change in the third base of the codon not makes any difference in codon translation:
    - UUU codes for phenylalanine.
    - UUC codes for phenylalanine also.
- If change in the first or second base of the codon , change in the codon translation will occurs that may results in a different amino acid:
    - UUU codes for phenylalanine.
    - CUU codes for leucine.
- *Significance of Wobbling phenomenon*: This enable one tRNA with specific anticodon to complement several codons on the mRNA code for the same amino acid , so no need for 61 tRNA to identify 61 codonsi.e. Decrease the number of tRNA molecules needed.

DNA mutations or Alterations

Definition:

These are changes in the nucleotide base sequence of the genetic code due to replacement, addition (insertion) or removal (deletion) of one or more nucleotide base.

Types:

1. ***Single base alterations*** (point mutation) which include:

a. Depurination (removal of purine) or depyrimidination (removal of pyrimidine).

b. Deamination of nucleotide base (removal of amino group):

   - Cytosine →Uracil
   - Adenine→Hypoxanthine
   - Guanine→Xanthine

c. Alkylation of a nucleotide base (mostly guanine) i.e. covalent addition of an alkyl radical.

d. Deletion or insertion of a nucleotide base.

e. Base analog incorporation.

2. ***Two base alteration***s: which include U.V induced thymine-thymine dimmer.

3. ***Chain break*** by breakage of phosphodiester bond & it includes:

   - Single strand break.
   - Double strand break.

4. *Cross links:*

- DNA-DNA cross links: they occur between bases of the same or opposite strand.
- DNA-protein cross links: they occur between the DNA & protein molecules e.g. histones.

Causes:

1. Physical agents: e.g. γ-rays, X-rays, U.V rays & ionizing radiation.
2. Chemical agents: e.g. anticancer base analog & alkylating agents.
3. Environmental pollutants & free radicals e.g. nitrous oxide which cause deamination of adenine into hypoxanthine.
4. Errors of DNA replication with absent repair.

Point Mutation

Definition:

It is single base change or alteration.

Mechanisms:

1. *Substitution of one base for another :* which may be either:

- *Transition mutation:* If one purine base changed to the other purine base or if one pyrimidine base changed to the other pyrimidine base.

- *Transversion mutation*: If one purine base changed to either of the two pyrimidines or if one pyrimidine base changed to either of the two purines

2. Deletion or insertion of the first nucleotide base of the genetic codon leading to *frame-shift mutation*.

Types & causes: Previously mentioned.

Fate "Effects" of point mutation:

1. **Silent mutation:**

Definition: The codon containing the changed base still codes for the same amino acid.

- The change occurs in the 3rd base (3rd base degeneracy or Wobbling phenomenon).
- Codons specifying for the same amino acid are called degenerate or synonyms or nicknames.
- Example: UUU codes for phenylalanine & UUC still codes for phenylalanine.

2. **Miss-sense mutation**:

Definition: The codon containing the changed base may codes for different amino acid.

- The change occurs in the 1st or the 2nd base.
- Example: UUU codes for phenylalanine while CUU codes for leucine.

- The effect of the mistaken amino acid on the function of the protein molecule depends on the nature & position of the substituted amino acid in that protein molecule.
- Protein may be normally functioning, partially functioning, non-functioning or not produced at all due to defective RNA processing.
- Example: Sickle cell anemia : In which the R.B.Cs contain abnormal hemoglobin called Hb S which contains two normal α-chains & two mutant β- chains in which the amino acid number 6th which is glutamate is replaced by valine.

3. Non-sense mutation:

Definition: The codon containing the changed base becomes one of the three termination codons (UAA,UAG,UGA).

- The change occurs in the 1st or 2nd nucleotide base:
  - UCA→ codes for serine amino acid.
  - UGA→ is a termination codon.
- This leads to:
  - Premature stoppage or termination of protein synthesis resulting in truncated protein. Or
  - No protein synthesized at all.

4. Frame-shift mutation:

Definition: Deletion of the first nucleotide base in the genetic codon leading to disturbance of the reading frame with:

1. *Truncated protein*: in which premature termination of protein synthesis occurs if a termination codon develop as a result of the shift. Or
2. *Garbled protein*: due to translation into a totally different protein after the shift point. Or
3. *No protein produced* at all due to degradation of the mRNA.

N.B: Deletion or insertion of three nucleotide bases produces protein with extra or lack an amino acid & this has moderate effect on the produced protein.

RNA Synthesis (Transcription)

Definition

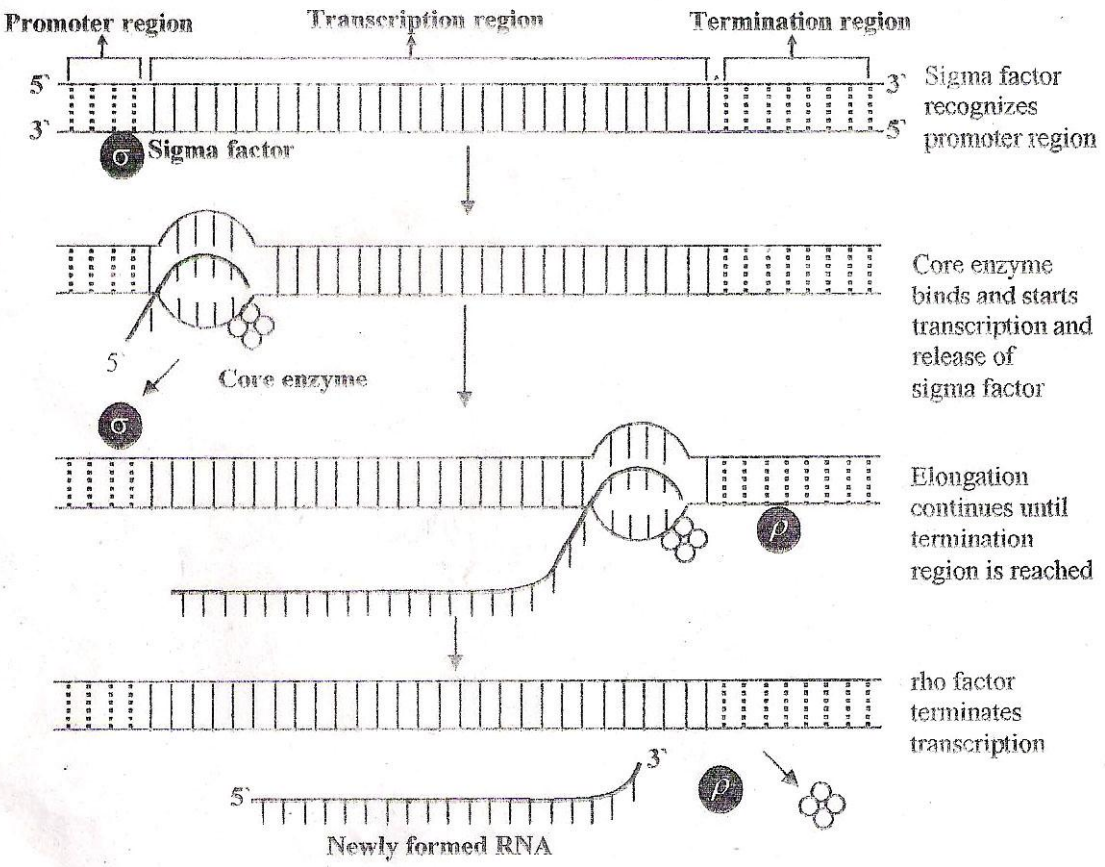
It is the process of production of RNA copy from a specific region along the length of DNA, i.e., the gene proper. RNA is formed & modified in the nucleus then pass to the cytoplasm to performs its function

Steps of transcription of RNAs in the prokaryotes:

As said before that in the prokaryotes only one type of the RNA polymerase is required for synthesis of the three types of the RNAs. This because that the prokaryotic RNA polymerase is a multisubunit enzyme that recognizes the promoter region for initiation of the transcription process; next, it makes a complementary RNA copy of the DNA template, and then recognizes the end of the DNA or the termination region.

The process of prokaryotic gene transcription can be divided into three phases:

1. Initiation.
2. Elongation.
3. Termination.


Promoter region
Transcription region
Termination region
5`
3`
3`
5`
σ Sigma factor
Sigma factor recognizes promoter region
5`
Core enzyme
Core enzyme binds and starts transcription and release of sigma factor
σ
ρ
Elongation continues until termination region is reached
rho factor terminates transcription
3`
5`
ρ
Newly formed RNA

Protein Synthesis (Translation)

Requirements for protein synthesis:

1. Amino acids.
2. tRNAs.
3. Amino-acyl tRNA synthetase enzyme.
4. mRNA.
5. Ribosomes.
6. Protein factors.
7. High energy compounds.

1.Amino acids:

All amino acids that will enter in the structure of the finished protein must be present at the time of the protein synthesis.

2. tRNAs:

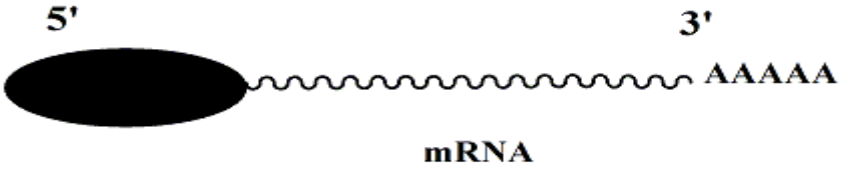
- About 31 types enter in the process of protein synthesis.
- When the tRNA binds covalently to its corresponding amino acid at its 3'ACC terminus , it is called charged tRNA , while if it is not attached to the amino acid , it is called uncharged tRNA.
- The amino acid when binds to the tRNA molecule , it is called activated.
- Because of the ability of the tRNA molecule both to binds to specific amino acid & to recognizes the codon codes for that amino acid on the mRNA by its anticodon loop , it is called adaptor molecule.

3.Amino-acyl tRNA synthetase enzyme:

It catalyzes the covalent binding of the amino acid to its corresponding tRNA with hydrolysis of ATP into AMP +PPi at its 3' ACC terminus.

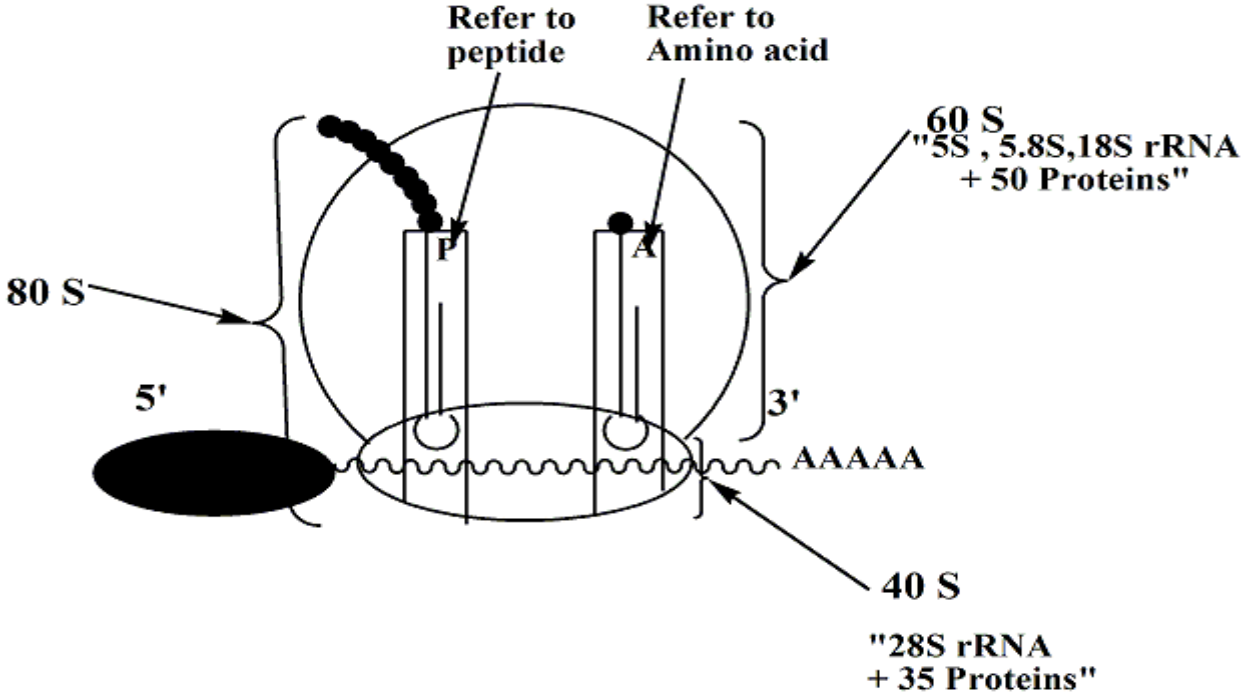
4.mRNA:

Which acts as a template for synthesis of the desired particular protein.



5.Ribosomes:

- They are the factories of protein synthesis.
- The complete ribosome have two binding sites for the amino acyl-tRNAs , P site & A site.



- The "P" site is for the preceding amino acyl tRNA & the "A" site is for the following amino acyl tRNA . Each of them extends over both ribosomal subunits & they cover the two neighboring codons on the mRNA.

6. Protein factors:

They include:

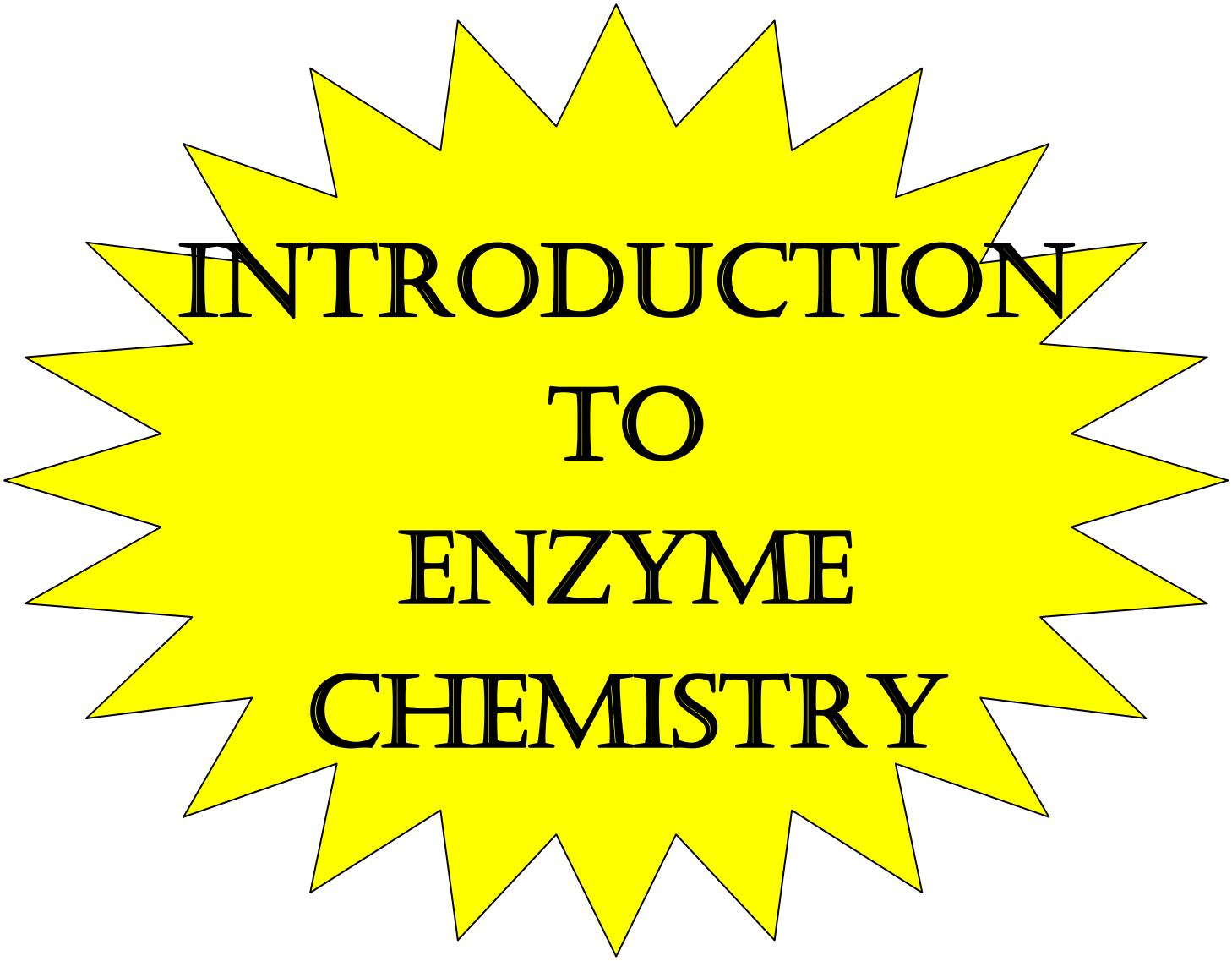
- Eukaryotic initiation factors "eIFs".
- Eukaryotic elongation factors "eEFs".
- Eukaryotic releasing factors "eRFs".

7. High energy compounds:

They are used as a source of energy e.g. GTP & ATP.

Steps of protein synthesis:

I. Activation of the amino acids.
II. Initiation step of protein synthesis.
III. Elongation step of protein synthesis.
IV. Termination step of protein synthesis.


INTRODUCTION
TO
ENZYME
CHEMISTRY

Enzyme Chemistry

Definition

Enzymes are proteins that act as **catalysts for** chemical reactions in the body. A catalyst is a material that speeds up a chemical reaction but is itself unchanged at the end of the reaction.

Enzyme Components

Enzymes are chemically classified into:-

1- Simple protein enzymes:-Which consists only of protein e.g. Pepsin.
2- Conjugated protein enzymes (**Holozymes** or **Holoenzymes**):- which consist of :-
   a- Protein part:- called **apoenzyme**.
   b- Non-protein part which is either :-
      1- Loosely attached (i.e., non-covalently) to apoenzyme and called **Coenzyme or Cofactor.** **Or**
      2- Firmly attached (i.e., covalently) to apoenzyme and called **Prosthetic group**.

Table showing the differences between components of holoenzyme:-

	Apoenzyme	Coenzyme	Prosthetic Group	Cofactor
Nature	Protein	Organic, non-protein	Organic and inorganic	Inorganic
Source	Specific gene	Vitamins or nucleotides	Heme and inorganic metals, Selenium in GSHPX, Cu2+ in SOD	Inorganic elements
Examples	All enzymes	NAD, FAD, TPP, ATP, UTP	FMN, iron-Heme	Mg2+, Ca2+ Cu2+, Mn2+
Attachment to the apoenzyme		Loose (non-covalent)	Very tight (covalent/noncovalent)	Loose
Heat stability	labile	Fairly stable	Stable	Very stable
MW	largest	Smaller	Smaller	Smallest
Determine substrate specificity	Yes	No	No	No
Determine chemical nature of the reaction	Yes	Yes	Yes	Yes & No

Functional sites of enzyme system

1- Catalytic (active site):-

It is the site on the enzyme surface that catalyzes the chemical reaction i.e. carries out the chemical reaction .

2- Substrate binding site:-

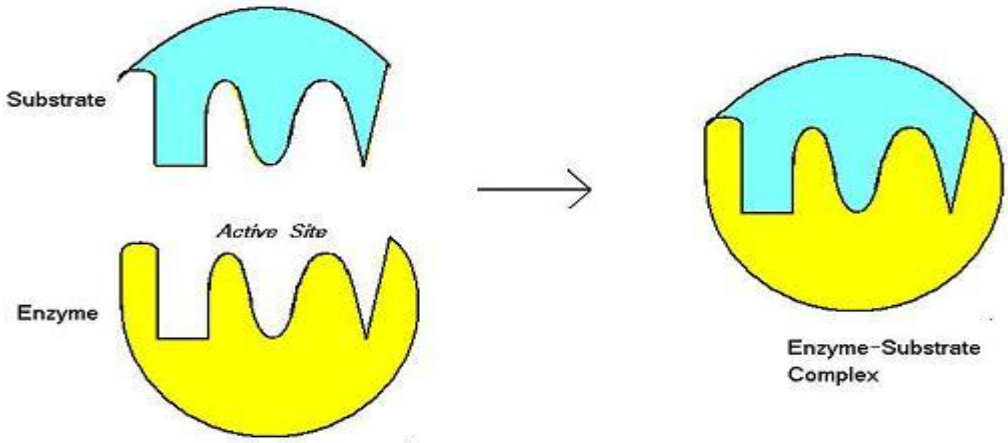
It is the site on the enzyme surface at which the substrate specifically binds.

Both sites :-

A- Either separated by large or small distance or combined into one site.

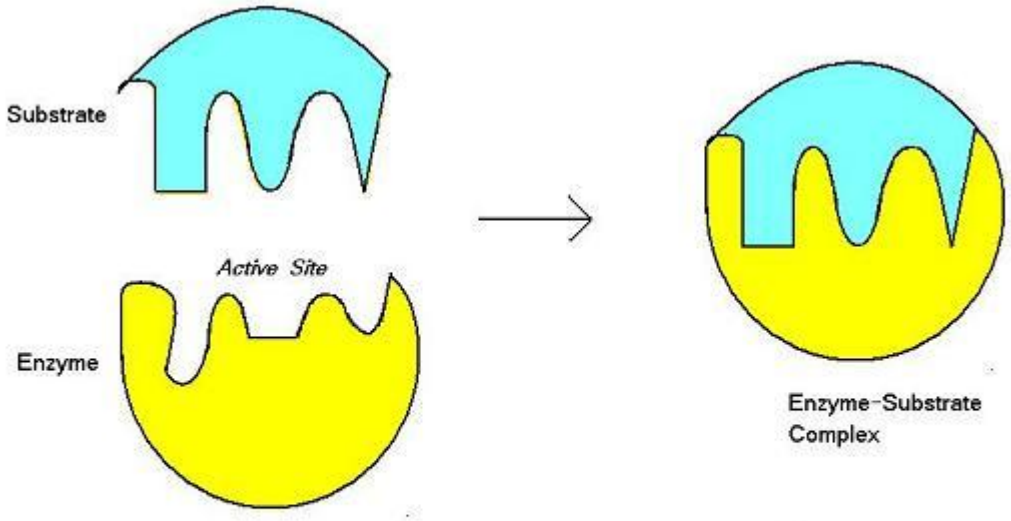
B- They are either:-

A- Rigid model " Lock & key model": No changes in their shape after binding of the substrate .



Lock-and-key Model.- The substrate and enzyme active site have complementary shapes

B- Flexible model " Induced fit model": After binding of substrate , it induce conformational changes in their shapes to induce proper fitting of the enzyme- substrate complex.



Induced-fit Model. - The enzyme active site forms a complementary shape to the substrate after binding.

3- Allosteric site:-

Allosteric means other site (from the greek word "allos") , allosterity means change in shape . It is site on the enzyme surface other than the catalytic and substrate binding site to which allosteric effector binds.

Enzymes regulated by allosteric effector are called allosteric enzymes. Allosteric activators or inhibitors are compounds that bind at sites other than the active catalytic site and regulate the enzyme through conformational changes affecting the catalytic site. The plot of V versus [S] for an allosterically regulated enzyme is a sigmoid saturation curve

Allosteric effector(regulatory molecule) is a low molecular weight substance with no or little structural similarities to the substrate and may be end product of enzyme catalyzed reaction & leads to one of the following effects:-

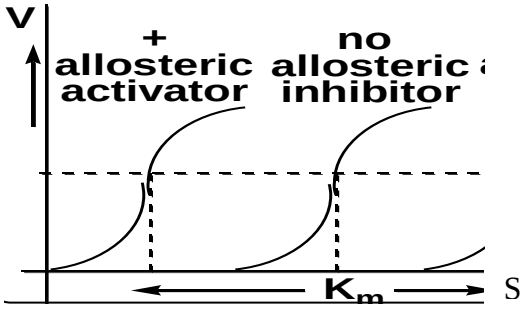
A)- Conformational changes mainly in catalytic site , leading to increase the rate of enzyme catalyzed reaction i.e. increase enzyme activity through:-

1- Increase enzyme substrate affinity.

2- Decrease Km.

And called *Allosteric activator* or " *feed back activator*" or " *positive allosteric effector*".

Example: glucose-6-phosphate is an allosteric activator to glycogen synthase.

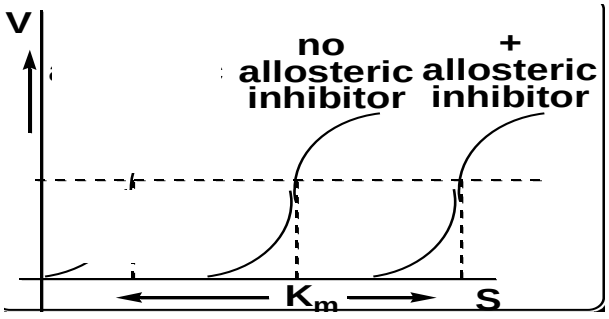


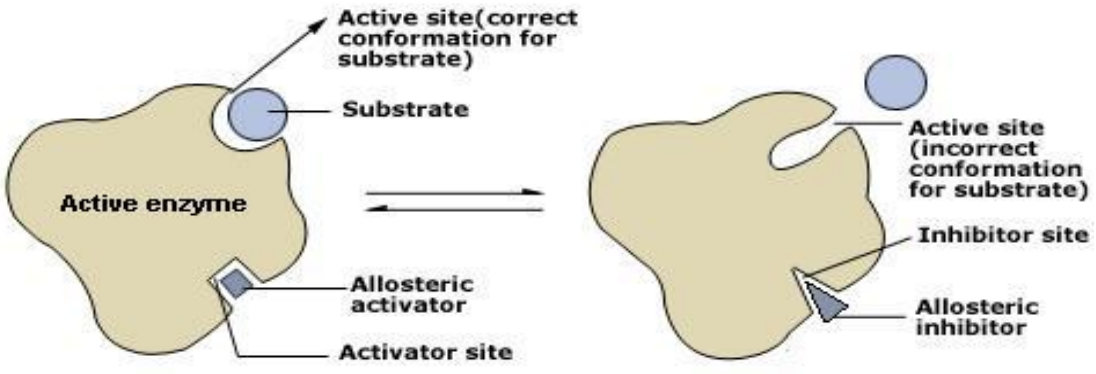
B)- Conformational changes mainly in catalytic site , leading to decrease the rate of enzyme catalyzed reaction i.e. decrease enzyme activity through:-

1- Decrease enzyme substrate affinity.

2- Increase km.

And called *Allosteric inhibitor* or " *feed back inhibitor* " or " *negative allosteric effector*" . They are non-competitive inhibitors.



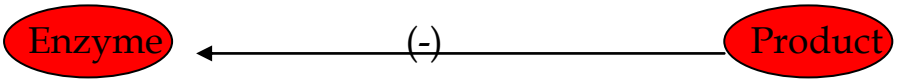


Schematic representation of allosteric enzyme activity

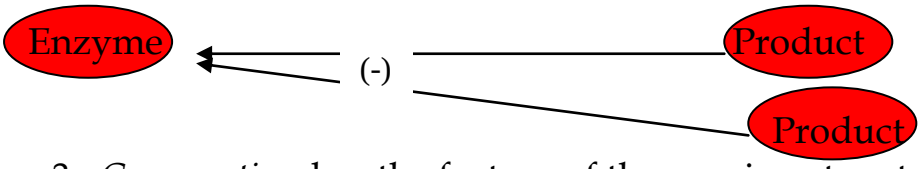
Example: glucose-6-phosphate is an allosteric inhibitor of hexokinase.

Allosteric inhibitor may be one of the following types:-

1- *Cumulative* :- in which single product presents in enough amount inhibit the enzyme.

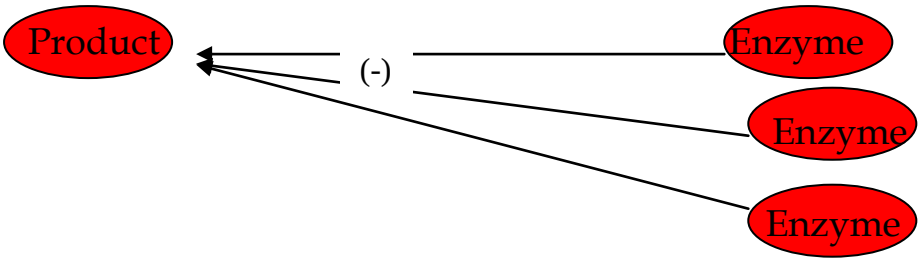


2- *Concerted*:- in which two or more products have to be present simultaneous and in enough amount to inhibit the enzyme.



3- *Co-operative*: has the feature of the previous two types.

4- *Enzyme multiplicity*: multiple enzymes having different catalytic actions , all are inhibited by single product.



Enzyme Specificity (Substrate specificity of the enzymes)

Enzymes show different degrees of specificity:-

1- *Relative , low or bond specificity*: In this type the enzyme acts on substrates that are similar in structure and contain the same type of bonds e.g.

a. Amylase : which acts on α 1,4 glycosidic bonds in starch , dextrins and glycogen.

b. Lipase : that hydrolyzes ester bonds in different triglycerides.

c. Protease: that catalyzes the hydrolysis of peptide bonds of different proteins.

2- *Moderate , structural or group specificity:* In this type of specificity , the enzyme is specific not only to the type of bond but also to the structure (chemical groups or atoms) surrounding it e.g.

a. Pepsin : is an endopeptidase that hydrolyzes central peptide bonds in which the amino group belongs to aromatic amino acids e.g. phenylalanine , tyrosine and tryptophan.

b. Trypsin : is an endopeptidase that hydrolyzes central peptide bonds in which the amino group belongs to basic amino acids e.g. arginine , lysine and histidine.

c. Chymotrypsin : is an endopeptidase that hydrolyzes central peptide bonds in which the carboxyl group belongs to aromatic amino acids.

d. Aminopeptidase : is an exopeptidase that hydrolyzes peripheral peptide bond at the amino terminal (end) of the polypeptide chain.

e. Carboxypeptidase: is an exopeptidase that hydrolyzes peripheral peptide bond at the carboxyl terminal (end) of the polypeptide chain.

3- *Absolute , high or substrate specificity*: In this type of specificity the enzyme acts only on one substrate e.g.

a) Uricase , which acts only on uric acid.

b) Arginase , which acts only on arginine.

c) Carbonic anhydrase , which acts only on carbonic acid.

d) Lactase , which acts only on lactose.

e) Sucrase , which acts only on sucrose.

f) Maltase , which acts only on maltose.

4- *Optical or Streo-specificity:* In this type of specificity , the enzyme is specific not only to the substrate but also to it's optical configuration i.e. acts on a specific isomer of the substrate and not acts on another isomers e.g.

a. L- amino acid oxidase acts only on L-amino acids.

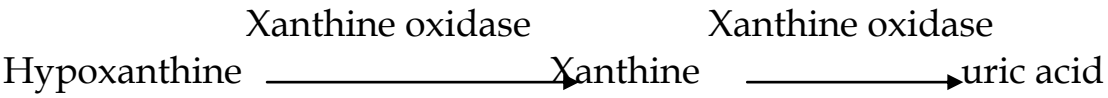
b. D- amino acid oxidase acts only on D- amino acids.

c. α- glycosidase acts only on α-glycosidic bonds , which are present in starch , dextrin and glycogen.

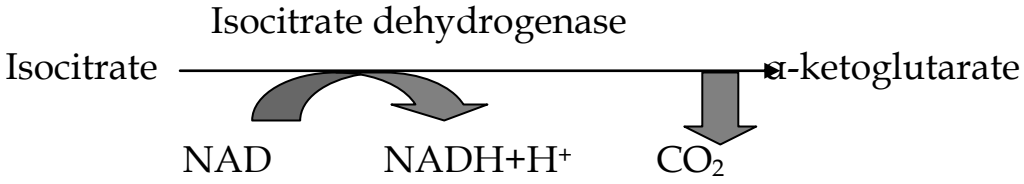
d. β- glycosidase acts only on β- glycosidic bonds , which are present in cellulose.

N.B: We can digest glycogen and starch due to presence of α-glycosidase but we can't digest cellulose due to absence of . β-glycosidase.

5- ***Dual specificity****:* There are two types of dual specificity:
A- The enzyme may acts on two substrate by one reaction type e.g. xanthine oxidase enzyme acts on xanthine and hypoxanthine (two substrates) by oxidation (one reaction type):



B- The enzyme may acts on one substrate by two different reaction types e.g. isocitrate dehydrogenase enzyme acts on isocitrate (one substrate) by oxidation followed by decarboxylation (two different reaction types):

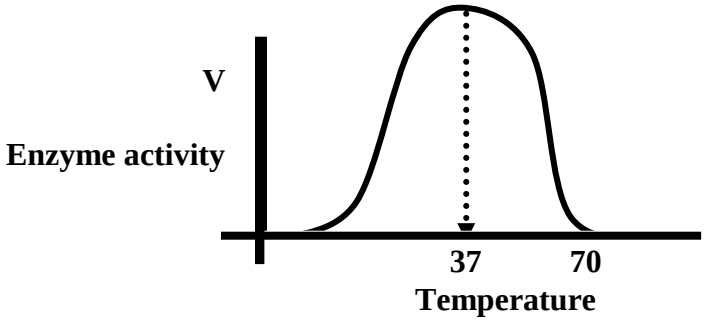


Factors affecting the rate of enzyme catalyzed reaction(Regulation of enzyme activity)

1- Temperature:

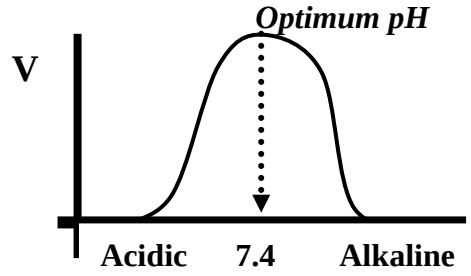
There is optimal temperature at which the enzyme attains its maximal activity (37^0C-40^0C) . Low temperature inactivate the enzyme and high temperature cause denaturation of the enzyme. The rise in the temperature from low temperature to optimal temperature causes an increase in the rate of the reaction due to :
A)- The rise in the temperature increases the initial energy of substrate leading to a decrease in the activation energy and lower the energy barrier of the reaction.

B)- Also , the rise in temperature increases collision of the molecules i.e. more molecules become in the bond forming or bond breaking distance.



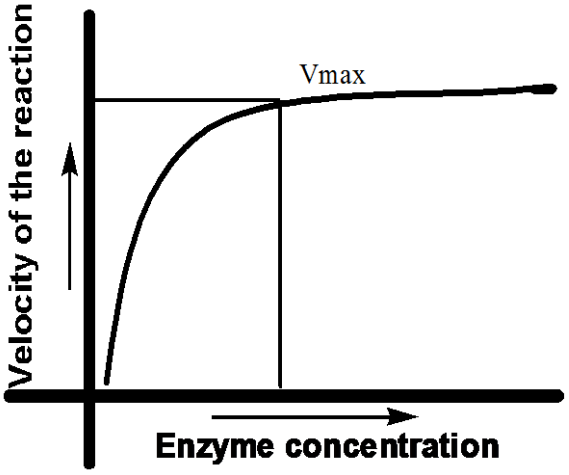
2- PH:

There is optimal PH at which the enzyme acts maximally .For example, blood enzymes act at 7.4 & pancreatic amylase acts at 7.2.The PH affects the state of ionization of amino acid residues at the active sites of the enzyme and ionization of the substrate. Extreme pH caused by strong acids or strong alkalis lead to denaturation and destroy the enzyme



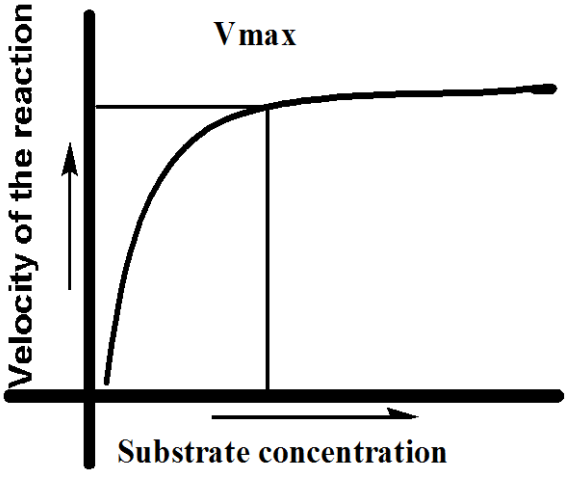
3- Concentration of the enzyme:

Increase the enzyme concentration leads to increase the rate of enzyme catalyzed reaction till certain point , after which increase in the enzyme concentration is not associated with further increase in the rate of the enzyme catalyzed reaction due to inhibition of the enzyme by the accumulated products.



4- Concentration of the substrate

Increase the substrate concentration leads to increase the rate of enzyme catalyzed reaction till certain point , after which increase in the substrate concentration is not associated with further increase in the rate of the enzyme catalyzed reaction due to saturation of all the active sites of the enzyme by the accumulated products and so the excess substrate will find no free enzyme to interact with.



5- Protecting the enzymes from light and radiation which lead to their denaturation.

6- Protecting the enzymes from oxidation or inhibitors.

7-Concentration of cofactors or coenzymes:

In the conjugated protein enzymes that need coenzymes or cofactors , the increase in the coenzymes or cofactors concentration causes an increase in the rate of the enzyme action.

8- Effect of time:

At the beginning the rate of the reaction increases , but by time the rate of the reaction decreases due to:-

1- Depletion of substrates.
2- Accumulation of end products.
3- Change in PH of the reaction , which becomes different from the optimum PH of the enzyme.

9- Allosteric regulation for allosteric enzymes: see functional sites of the enzyme.

10- Compartmentation of the enzymes:

Each enzyme is active only in specific compartment of the cell e.g. cytosol , mitochondria …….etc.

11- Covalent modification:

This mean that covalent removal or addition of foreign group will affect enzyme activity e.g. phosphorylation-dephosphorylation mechanism , glycogen phosphorylase responsible for glycogen breakdown is activated when phosphorylated with protein kinase and inactivated when dephosphorylated with protein phosphates.

12- Hormonal control:

Enzyme activity is regulated by hormones through:

A)- They affect the rate of synthesis and degradation of the enzymes.

B)- They affect the concentration of allosteric effectors through covalent modification .

13- Enzyme induction and repression :

Enzyme induction occurs by presence of an inducer , which is a compound that increase the low level of the rate of synthesis of an

enzyme. While enzyme repression occurs by presence of a repressor , which is a compound that decrease the high level of the rate of synthesis of an enzyme.

14- Constitutional Enzymes:

Housekeeping enzymes are constitutive enzymes because their rate of synthesis in a cell is constant and does not depend on an inducer. The level of the enzyme is only controlled by its rate of degradation.

15- Lysosomal enzymes:

Lysosomes are membrane- bounded specialized sac like compartements within most cells play critical role in metabolic functions .

- One of their primary role is to pick up substances such as carbohydrates , lipids & proteins , breaking them down into smaller molecules so can be used again in metabolic processes.
- They contain granules that are aggregates of digestive enzymes,these enzymes are often referred to as "acid hydrolases" because they require an acidic environment to function properly & they use water molecules to split large molecules into fragments.
- Lysosomes function in intracellular & extracellular digestion.

16- Zymogens (proenzymes):

Definition:

It is the inactive form of the enzyme e.g. pepsinogen , trypsinogen , blood clotting factors.

Zymogens are inactive due to:

1- Presence of an inhibitory extra-polypeptide chain.
2- Presence of conformational changes.
3- Presence of inhibitory subunit.
4- Presence of regulatory covalent modification.
5- The enzyme require for it's activity cofactor or allosteric activator or activating protein or coenzyme.

Zymogens are activated by:

1- Removal of the inhibitory extra-polypeptide chain by:
   a. Specific enzymes.
   b. Non-specific proteolytic enzymes.
   c. Autoactivation.
   d. Acid hydrolysis.
2- Changing the conformational changes.
3- Removal of the inhibitory subunit.
4- Covalent modification.
5- Association with cofactor or allosteric activator or activating protein or coenzyme.

Significance:

1- To protect the secretory cells and transporting duct system from the enzyme.
2- To keep the enzyme in a storable form till time of use, e.g., blood clotting.
3- To provide mechanisms for regulating the enzymes activity.

Isoenzymes (Isozymes)

Definition:

Isoenzymes are isomers of the same enzyme. They are physically (structurally electrophoretically and immunologically) distinct forms of the same enzyme that catalyze the same chemical reaction(s) and differ in their catalytic activity , optimum PH , Km and in distribution between different tissues and subcellular compartments. The physical differences between isoenzymes are due to different genes (alleles), different subunits and/or modified subunits used in synthesizing isoenzyme forms.

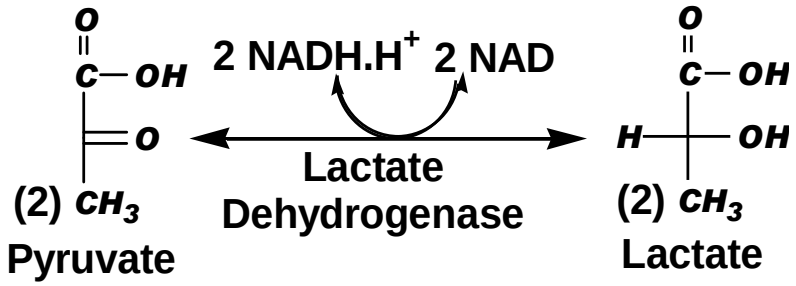
Biochemical significance of isoenzymes:

1. They explain metabolic differences between subcellular organelles of a cell.
2. They explain metabolic differences between different tissues.
3. They explain metabolic differences between individuals.
4. They explain differences in drug metabolism between different individuals.
5. They explain genetic bases of metabolic differences between individuals.
6. They have laboratory clinical diagnostic application.
7. They are example of the tissue-specific differential expression of genes.
8. They explain structure-function relationship of proteins.

Examples:

<u>1- Lactate dehydrogenase « LDH » :-</u>

- Which catalyzes reversible conversion of pyruvate into lactate with oxidation of $NADH+H^+$ into NAD^+ as follows:-



- LDH has 5 isoenzymes ,LDH-1,-2,-3,-4 &-5 .
- There is a six LDH isoenzyme presents in male genital tissue named LDH-X.
- LDH isoenzymes differ in structure & tissue distribution as follows:-

- Isoenzyme	- Structure	- Tissue distribution
- LDH-1	- HHHH	- Heart & R.B.Cs.
- LDH-2	- HHHM	

- LDH-3	- HHMM	- Brain & kidneys.
- LDH-4	- HMMM	- Liver & muscles.
- LDH-5	- MMMM	

- ***Diagnostic importance***:

a)- In myocardial infarction there is increase in the total LDH with increase in LDH-1 &-2.

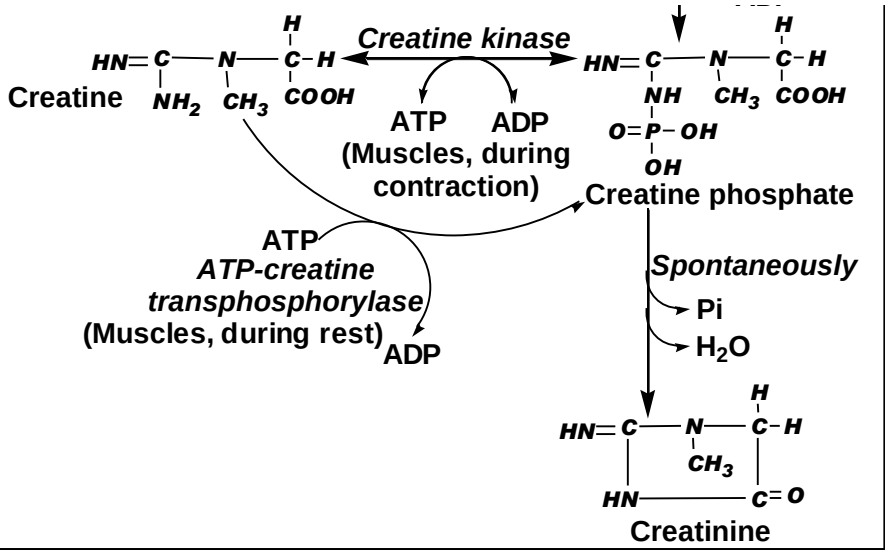
B)- In acute viral hepatitis , there is increase in the total LDH with increase in LDH-4&-5.

2- Alkaline phosphatase (ALP):-

- At least there are six isoenzymes for ALP.
- The most common types are:-
  - **1)- Hepatic ALP .**
  - **2)- Intestinal ALP.**
  - **3)- Placental ALP .**
  - **4)- Bone ALP.**
- Hepatic & intestinal ALP show major bands between different ALP isoenzymes during electrophoresis.
- Intestinal ALP is inhibited by phenylalanine & resist neuroaminidase.
- Hepatic ALP shows 2 bands , one lies close to β-lipoprotein band & the other derived from biliary system & lies between β-lipoprotein band & α- globulins band ,so bouble increase in both bands of diagnostic importance in extrahepatic jaundice.
- Placental ALP increase during the last six months of pregnancy & it is heat stable.
- Bone ALP increase with osteoblastic activity during growth period of childrens & also increase pathologically in rickets & hyperparathyroidism.

3- Creatine phosphokinase "CPK":-

Creatine phosphate (phosphagen) is the main store of high energy in muscles. During muscular contraction, creatine phosphate regenerates ATP from ADP catalyzed by *creatine kinase "CPK"* and during rest ATP regenerates creatine phosphate catalyzed by ATP-creatine transphosphorylase and the kinase as follows:



In human CPK exists as three different isozymes. Each isozyme is a dimer composed of two protomers (polypeptides) "M" (for muscle) and "B" (for brain).

- Thus, three possible isozymes as follows:

Type	*Structure*	*Electrophoretic mobility*	*Tissues distribution*
CPK-1	BB	Fastest (highly -ve)	Brain
CPK-2	MB	Follows	Myocardium
CPK-3	MM	Slowest (highly +ve)	Skeletal muscle

In myocardial infarction, increase of CPK-2 (MB) occurs within 4 hours maximum in 24 hours, then falls rapidly , while in brain cell damage , increase of CPK-1 (BB) occurs and in cases of muscular dystrophies e.g Duchenne dystrophy , increase of CPK-3 (MM) occurs.