Text Book of

BIOCHEMISTRY

BIOMOLECULE AND HUMAN PHYSIOLOGY

B.Sc. Part I - Semester I (Paper I)

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SYLLABUS
B.Sc.I Semester I Paper – II
BIOMOLECULES & HUMAN PHYSIOLOGY

Unit – I

Carbohydrates:

Structure of monosaccharides, sterioisomerism and optical isomerism of sugars, reactions of aldehydes and ketone groups, ring structure and anomeric forms, mutarotation. Chemical reactions of sugars, important derivatives of monosaccharides, di- and tri-saccarides Structure, occurrence and biological importance of monosaccharides, oligosaccharides and polysaccharides, e.g. glycogen, Starch, cellulose, blood group polysaccharides, inulin, chitin, glycosaminoglycans.

Unit – II

Lipids :

Definition and classification. Fatty acids: introduction, classification, nomenclature, structure and properties of saturated and unsaturated fatty acids. Essential fatty acids. Triacylglycerols: nomenclature, physical properties, chemical properties and characterization of fats - hydrolysis, saponification value, acid value, rancidity of fats, Reichert-Meissel number and reaction of glycerol. Biological significance of fats. Glycerophospholipids (lecithins, lysolecithins, cephalins. phosphatidyl serine, phosphatidyl inositol, plasmalogens), sphingomyelins, glycolipids -cerebrosides, gangliosides.

Unit – III

A) **Muscles:** Structure of striated muscle fiber. Molecular organization of contractile system, Sliding mechanism of muscle contraction, Neuromuscular Junction

B) **Digestion.** Digestion and absorption of carbohydrates, fats, proteins

Unit – IV

Membrane structure ftfntransport: Membrane composition, Fluid mosaic model of Singer & Nicolson. Active & passive transport, Na-K pump, Calcium Pump.

CHAPTER
1

CARBOHYDRATES

Carbohydrates are classified on the basis of their structures as -

(1) **Monosaccharides** - (Non-hydrolysable units)

- Aldoses → Polyhydroxy aldehydes (suffix - 'ose')
- Ketoses → Polyhydroxy ketones (suffix - 'ulose')

(2) **Derived Monosaccharides** - (Derivatives of monosaccharides)

(3) **Oligosaccharides** - (Hydrolysable and contain 2 to 10 units)

(4) **Polysaccharides** - (Hydrolysable and contain many units)

- Homopolysaccharides (same units)
- Heteropolysaccharides (different units)

Carbohydrates are defined as, polyhydroxyaldehydes or ketones or their derivatives or polymers.

(a) Aldoses :

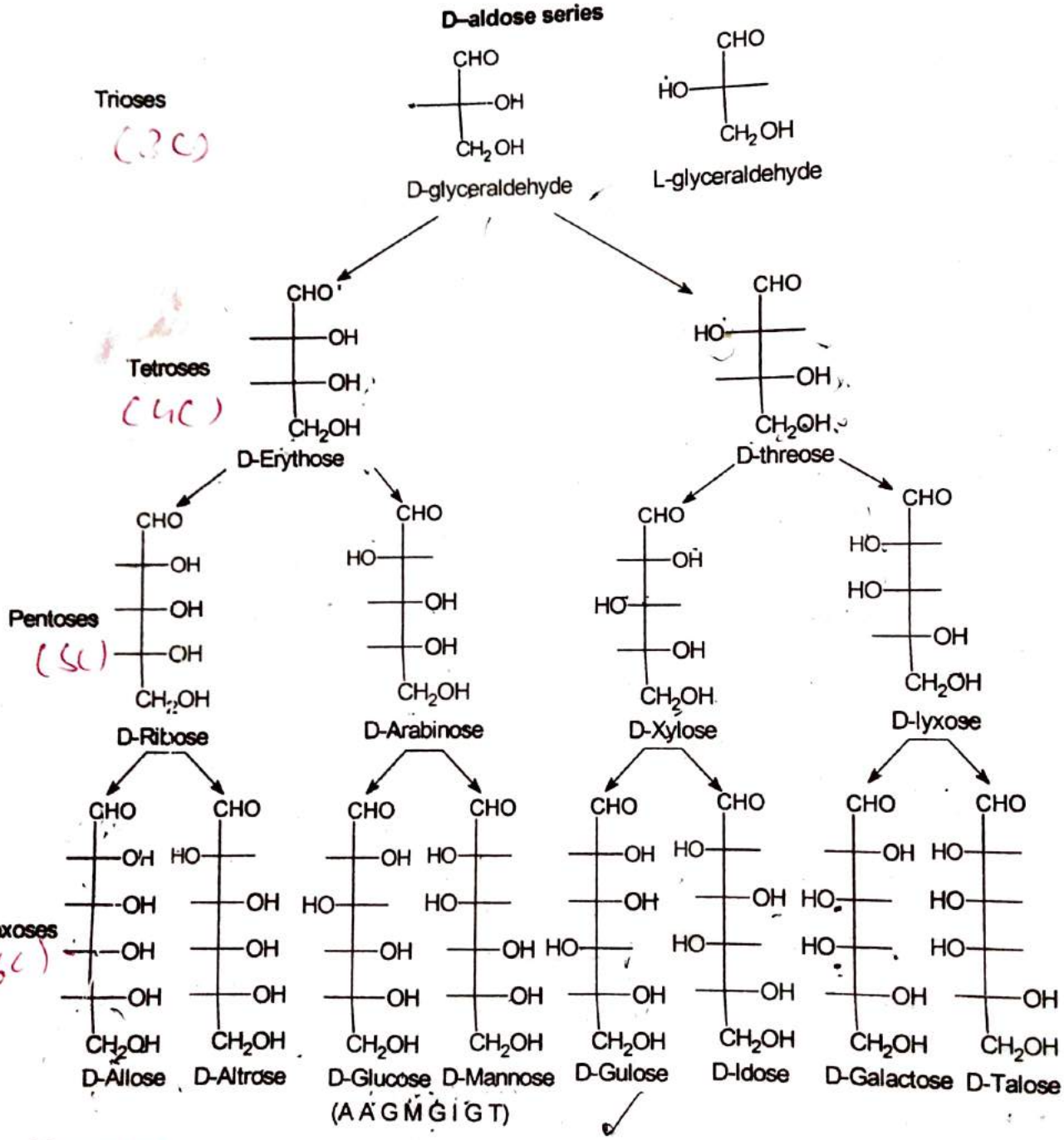
They can be represented by aldose series as follows :-

The series begins with three carbon containing triose, which has second carbon asymmentric carrying –OH group right then D– and if left then L–glyceraldehyde.

The series can be increased by adding one carbon (with –H and –OH) between first and second carbon forming two tetroses (due to different orientation of –H and –OH at added carbon).

All the members of D–aldose series are specified by D–conformation as penultimate (last but one) carbon atom has –OH group towards right.

Epimers : If any two sugar differ by orientation of –OH group at any one carbon atom except penultimate carbon then called epimer e.g. glucose and mannose are C–2 epimer since –OH group orientation differ at only carbon -2 while rest of the structure remain same. Similarly, glucose and galactose are C-4 epimer.

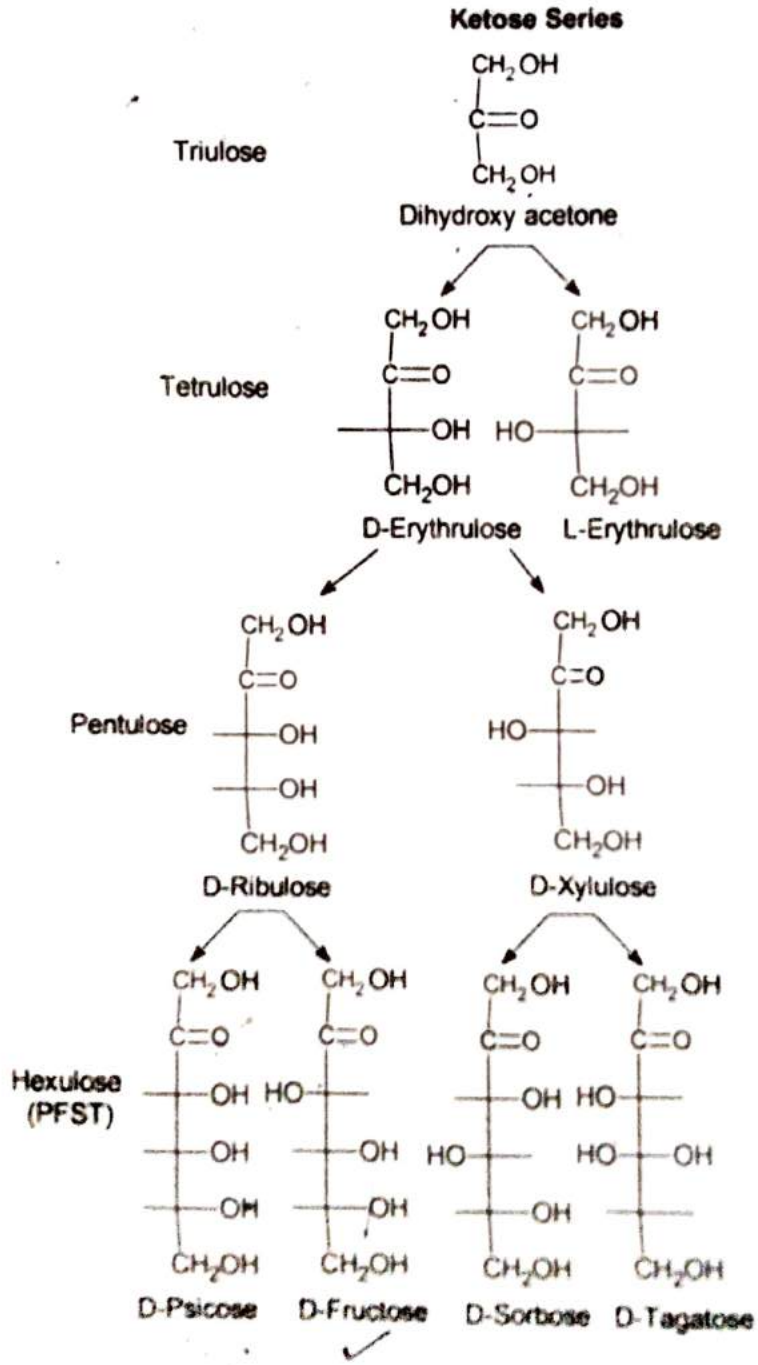


(b) Ketoses :

They can be represented by ketose series as follows :-

The series begins from three carbon containing triulose, which does not contain asyrnmetric carbon, to which one carbon with –H and –OH added in different orientation

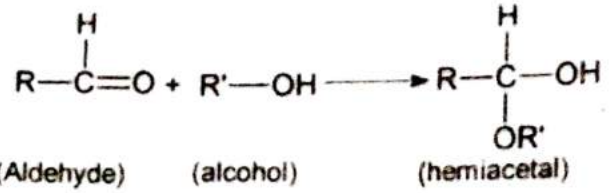
between second and third carbon forming two tetruloses as D-erythrulose and L-erythrulose. From D-erythrulose the D-Ketose series arises and all members of it has orientation of –OH group right, at last but one carbon atom. Again there are epimers in ketose series. E.g. D-ribulose and D-xylulose are C-3 epimers.



Glucose :

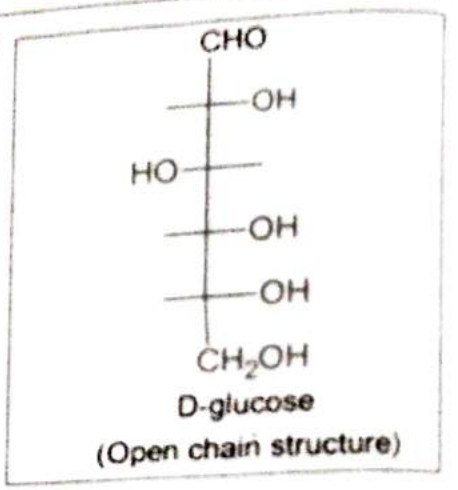
Open chain structure of glucose is unable to explain two forms of glucose as α - and β - forms. Which can be explained by **Fisher's formula** by forming hemiacetal structure

Aldehydic group reacts with alcoholic group forms hemiacetal by making carbonyl carbon asymmetric.

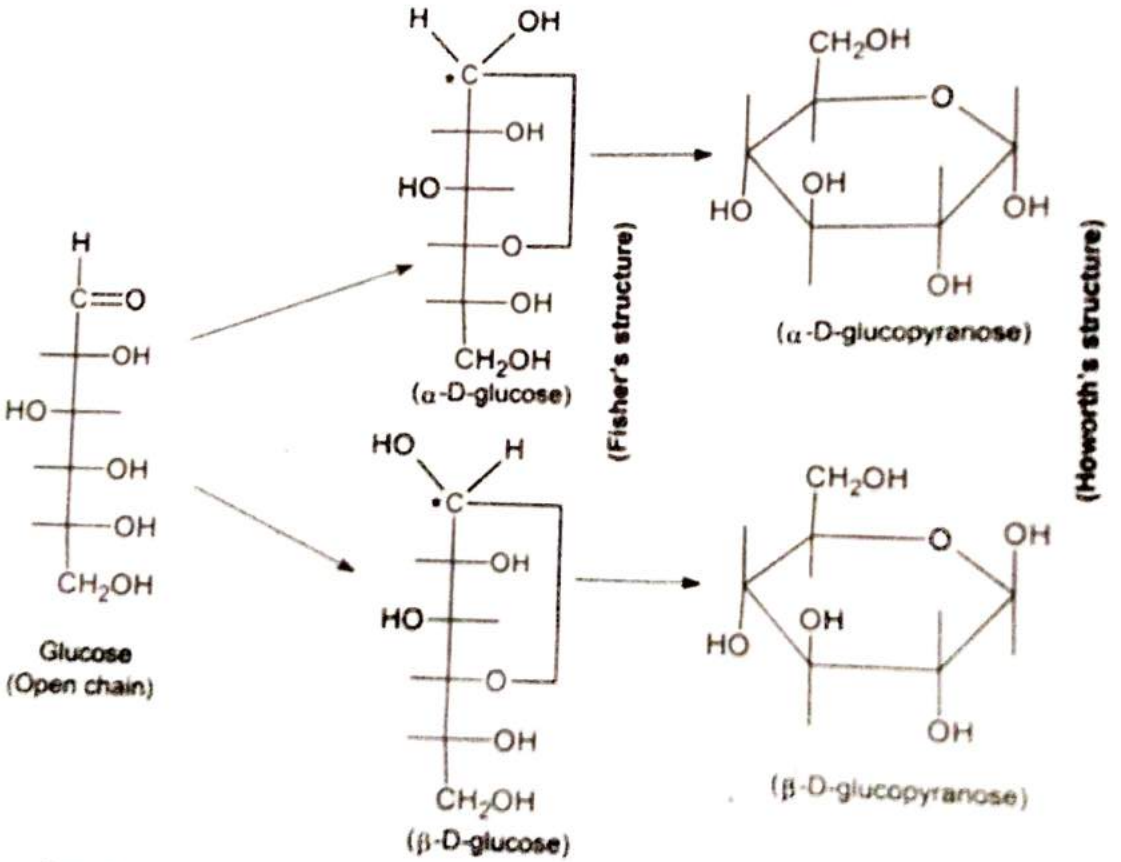


(Aldehyde) (alcohol) (hemiacetal)

When same reaction takes place intramolecularly hemiacetal forms as follows :-

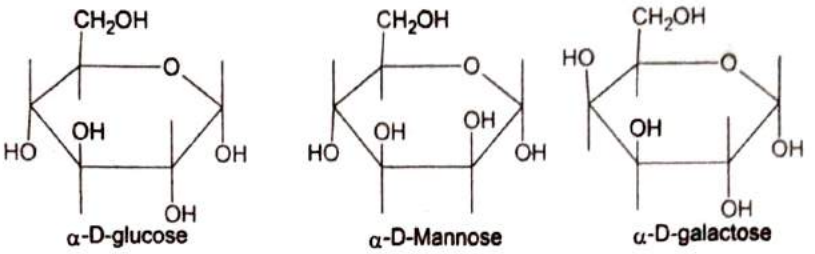


D-glucose
(Open chain structure)



- [on hemiacetal formation first carbon in glucose becomes asymmentric, called anomeric carbon, thus two forms of anomer α and β-D-glucose. Same can be shown in regular hexagonal ring in **Howorth projection formula**. Since ring is six membered with one oxygen atom called pyran, so in the name this term introduced as pyranose. If forming five membered with one oxygen atom containing ring like furan then term furanose.]

[During conversion of Fisher structure to Howorth structure, -OH group towards right in Fisher structure is shown below the plane of ring in Howrth structure and those towards left are shown above the ring.]



[α -D-glu and α -D-man are C-2 epimer and α -D-glu and α -D-gal are C-4 epimer (easy representation)]

Mutarotation :

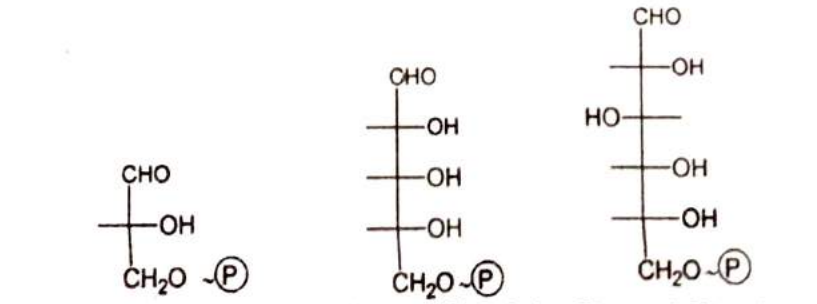
α -D-glucose ⟶ equilibrium Mixture ⟵ β -D-glucose

Sp. Rt. = +112·2° Sp. Rt. = + 52·7° Sp. Rt. = +18·7°

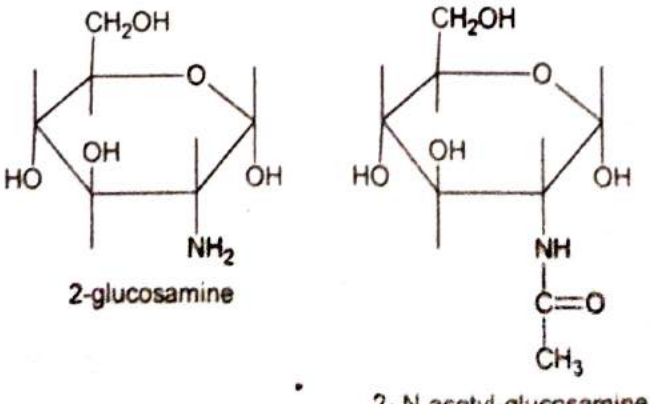
When both anomeric forms of glucose, in solution separately kept overnight, their specific rotation changes to +52·7° forming equilibrium mixture containing $\frac{1}{3}$ α -form and $\frac{2}{3}$ β -form.

Derived Monosaccharides :

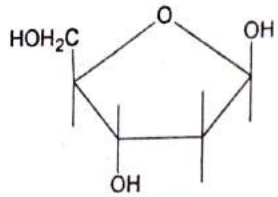
(a) Phosphate derivative



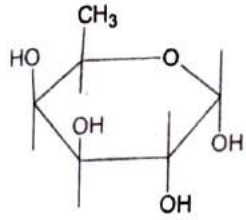
(b) Amino derivatives and amides



(c) Deoxy derivatives

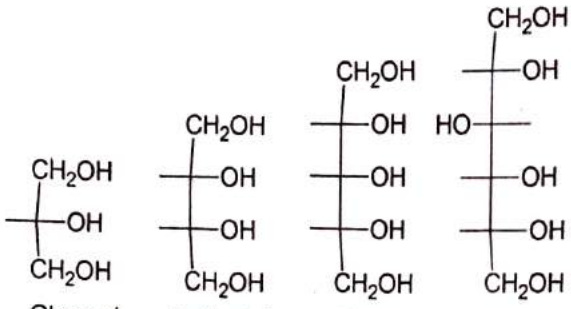


2-deoxyribose



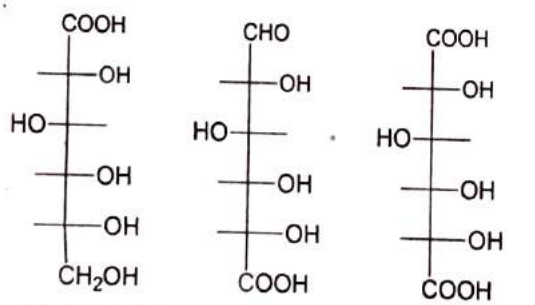
6-deoxygalactose (Fucose)

(d) Reduced derivative (carbonyl carbon get reduced to –OH group)

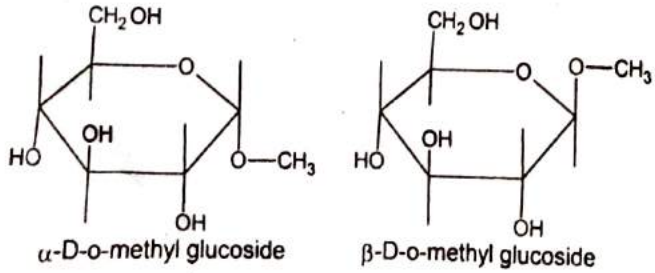


(e) Oxidized derivative (as acid derivative)

- Aldonic acid ⟶ (First carbon oxidized) ⟶ gluconic acid
- Alduronic acid ⟶ (Last carbon oxidized) ⟶ glucuronic acid
- Aldaric acid ⟶ (Both carbon oxidized) ⟶ glucaric acid



(f) Glycosides (at only anomeric carbon ether linkage)



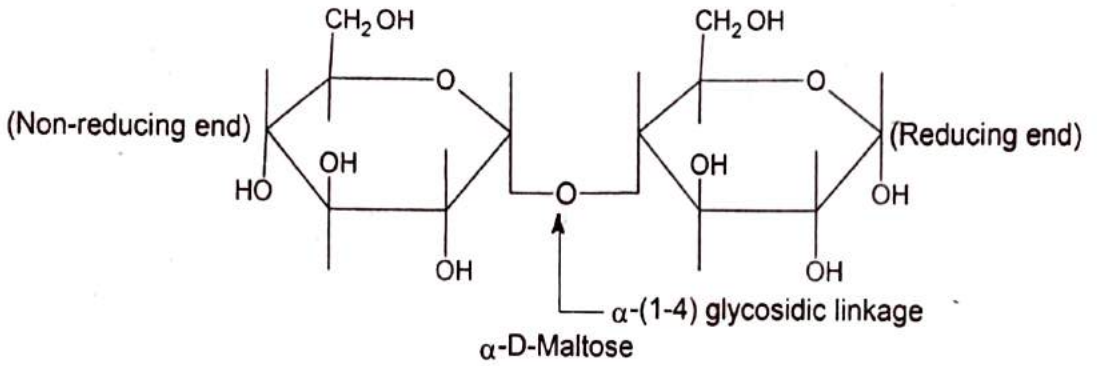
(Glycosidic linkage present also in oligosaccharide, polysaccharide, cerebroside, ganglioside, nucleoside etc.)

[specifically it is O-glycosidic linkage, in N-glycosidic OR S- glycosidic linkage, respectively O-replaced by N or S.]

Oligosaccharides :

- **Disaccharides** : Maltose, lactose, sucrose (glu-fru)
- **Trisaccharides** : Maltotriose, Raffinose (gal-glu-fru)
- **Tetrasaccharides** : Stachyose (gal-gal-glu-fru)
- **Pentasaccharides** : Verbascose (gal—gal-gal—glu—fru)

(a) Maltose - It is Disaccharide of α-D-glucose linked together by α-D-(1 $\longrightarrow$ 4) glucosidic linkage.



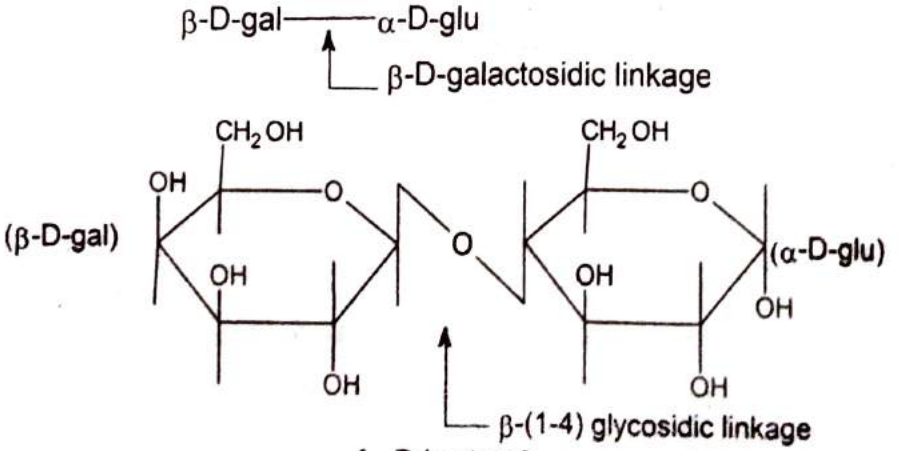
α-D-Maltose

O- α-D-glucopyranosyl - (1-4) -α-D-glucopyranose

[Systematic naming begins from non reducing end towards reducing end, if free then suffix 'ose']

Maltose is reducing sugar (since reducing end free). It also exist in two anomeric forms as α-D-Maltose and β-D-Maltose and exhibit mutarotation. Maltose is hydrolysed by maltase to glucose.

(b) Lactose (Milk sugar) - It is disaccharide of β-D-galactose and α-D-glucose linked together by β-D-galactosidic linkage.

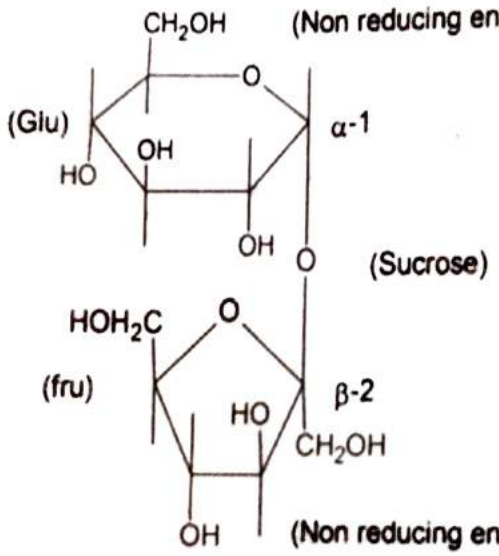


[α-D-Lactose]

O- β-D-galactopyranosyl-(1-4)-α-D-glucopyranose

Like maltose, the lactose is reducing sugar, exists in two anomeric forms as α-D-Lactose and β-D- Lactose and exhibit mutarotation. Lactose is hydrolysed by lactase to galactose and glucose.

(c) Sucrose (Table sugar) - It is disaccharide of β-D-fructose and α-D-glucose linked together by (β_2-α_1)-glycosidic linkage blocking anomeric carbon of both, i.e. blocking reducing carbon of both. Since anomeric carbon absent so no two forms, no mutarotation and sucrose is non reducing sugar.



O-β-D-fructofuranosyl - (2-1)-α-D-glucopyranoside

OR

O- α-D-glucopyranosyl-(1-2) -β-D-fructofuranoside

[Since both the ends are non reducing therefore name can begins from any end, so two names and since reducing end blocked so suffix is - 'side']

Sucrose is hydrolysed by sucrase enzyme as -

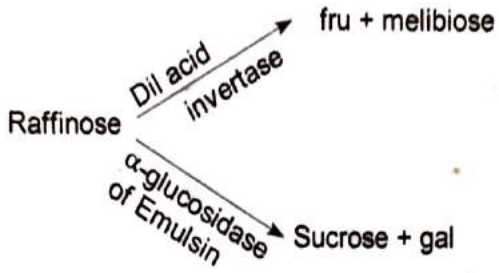
Sucrose ⟶ equilibrium Mix of glu + fructose (Furanose turns to pyranose)

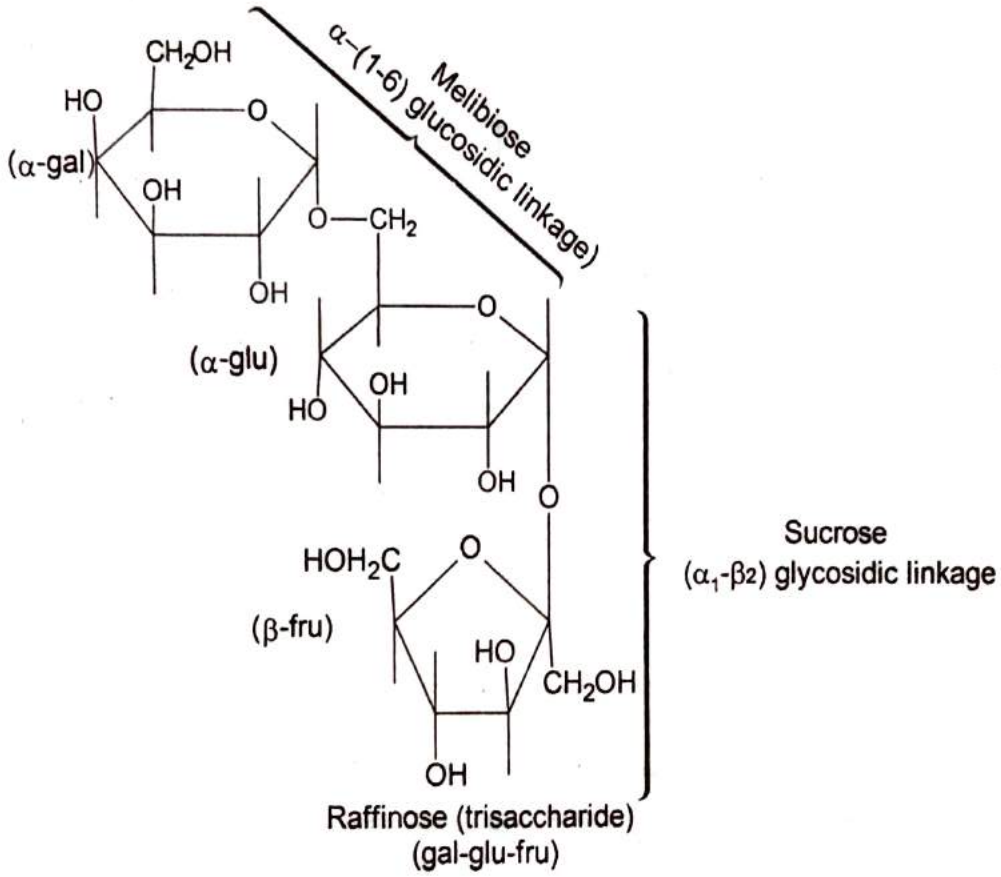
Sp. Rt. = +66.5° Sp. Rt. = +52.7° Sp. Rt. = −92°

This hydrolysis is accompanied by net change in optical rotation from dextro to levo. The product formed is called invert sugar, sucrase enzyme also called invertase and phenomenon inversion.

Raffinose -

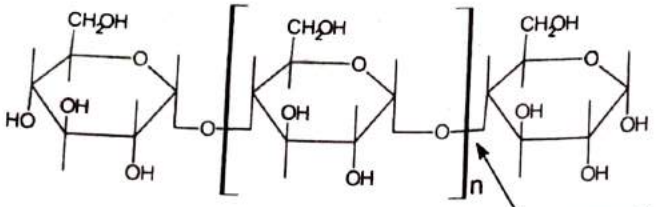
It is Trisaccharides, found in beet and in crystalline exudation on eucalyptis plant. It is also a non reducing sugar made up of α -D-gal, α -D-glu, β -D-fru. It is hydrolysed as follows-



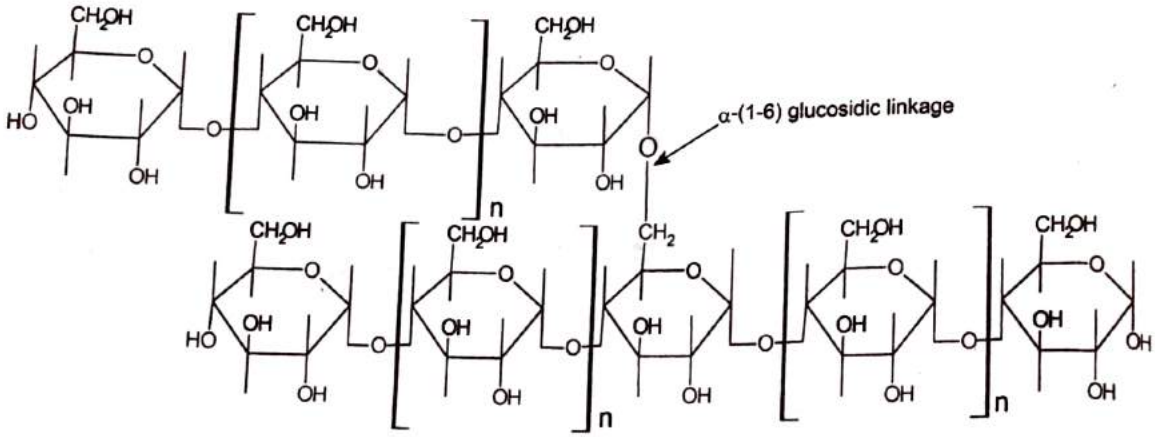


Homopolysaccharides ⟶ polymer of sugars with same monosaccharides like Starch, glycogen, cellulose, etc.

(a) **Starch** : It is energy shortage molecule, present in seeds, roots, tubers, etc. Chemically it is polymer of α -D-glucose, which has two types of components as linear amylose having α -(1-4) -glucosidic linkage and amylopectin which is branched by α -(1-6)-glucosidic linkage.



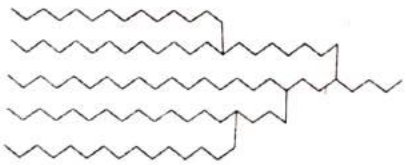
Amylose α-(1-4) -glucosidic linkage



Amylopectin

Enzyme amylase sequentially hydrolyze starch from nonreducing end forming erythrodextrin, achrodextrin, maltotriose, maltose, isomaltose and ultimately glucose. (Isomaltose isomer of maltose but having α-(1-6) glucosidic linkage between two glucose molecules.)

Non reducing end (Many)

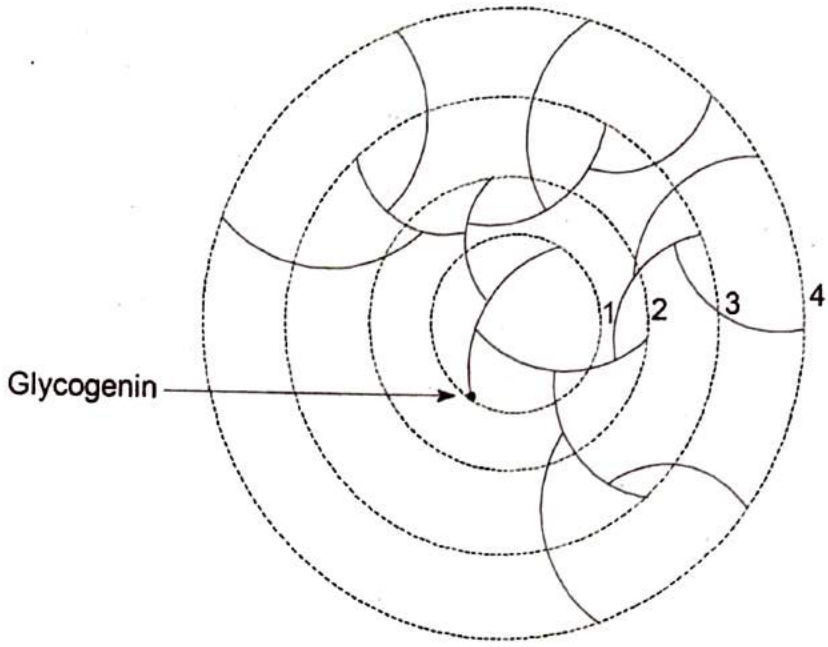


Reducing end (Single)

Amylopectin

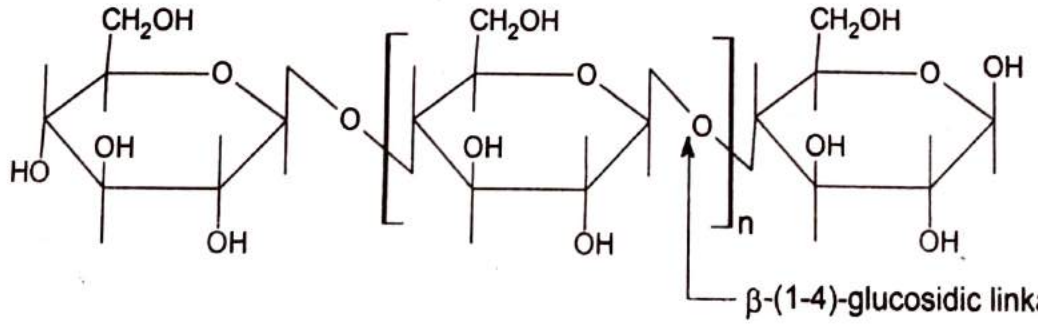
(b) **Glycogen** : It is also energy storage molecule, present only in animals, mainly in liver and skeletal muscles. It is structurally similar to amylopectin but amylose is absent, so also called animal starch. But differs from starch as it is more condensed than starch, branching in it occurs twice only after 6 to 8 glucose residues, while in starch branching after every 12 to 15 glucose residues.

Electron micrograph of glycogen granules reveals that it has twelve concentric rings. The glycogen chain initially develops on protein primer called glycogenin (which is essential), while 1st chain is in first ring and branched twice which enters in next ring, (in structure only 4-concentric rings are shown).



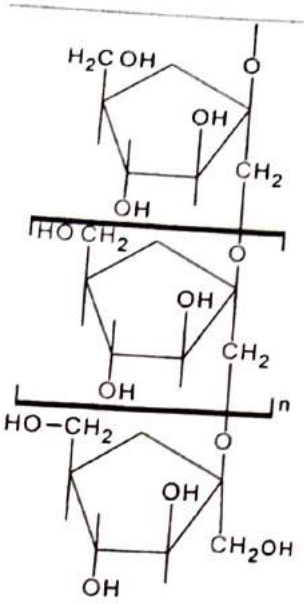
[Electron micrograph of glycogen]

(c) **Cellulose** : It is fibrous structure present in plant cell wall, chemically polymer of β-D-glucose linked by β-(1-4) glucosidic linkage, only linear polymer, while branching totally absent. On hydrolysis with cellulase it forms cellubiose (disaccharide) and ultimately glucose unit.

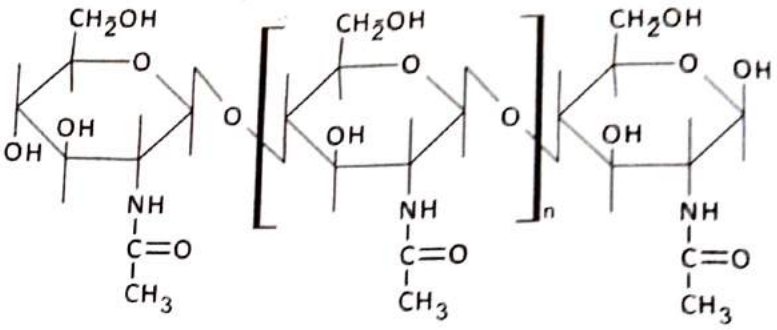


Inulin : It is homopolysaccharide of β -D-fructose with 30 – 35 units per molecule, joined together by β -2, a frutosidic linkage. It is a linear unbranched polymers (fructosan). It is found in tubes of dahlia and artichoke and roots of dandelion.

Chitin : It is linear homopolysachharide of N-acetyl-D-glucosamine, joined together by β -1-4-glucosidic linkage. Its structure is similar to cellulose where at second carbon of all rings –OH group replaced by acetylated amino groups. It is undigestible like cellulose, but hydrolysed by enzyme chitinases (produced in gastric juice of snail and bacteria). Chitin is present in exoskeleton of insects and crabs.



(Inulin)



(Chitin)

(Heteropolysaccharide : They are polymer of different sugars like hyaluronic acid.)

Glycosaminoglycans: These are heteropolysaccharides, made up of repatating disaccharide units as shown below, and containing amino sugars, therefore called glycosaminoglycan. They act as structural support material for connective tissues or mucous substances of a body and acting as lubricant and cementing substances and called mucopolysaccharide.

Mucopolysaccharide	Components of disaccharides	Linkage	
		within disaccharides	between disaccharides
Hyaluronic acid	D-glucuronic acid + N-acetyl-D-glucosamine	$\beta(1 \rightarrow 3)$	$\beta(1 \rightarrow 4)$
Chondroitin sulfate –A	D-glucuronic acid + N-acetyl -D-galactosamine -4-sulfate	$\beta(1 \rightarrow 3)$	$\beta(1 \rightarrow 4)$
Chondroitin sulfate-C	D-glucuronic acid + N-Acetyl D-galactosamine – 6- sulfate	$\beta(1 \rightarrow 3)$	$\beta(1 \rightarrow 4)$
Chondroitin sulfate – B (Dermatin sulfate)	L-iduronic acid + N-acetyl -D-galactosamine-4-sulfate	$\alpha(1 \rightarrow 3)$	$\beta(1 \rightarrow 4)$
Keratosulfate	D-galctose + N-acetyl-D-glucosamine-6-sulfate.	$\beta(1 \rightarrow 4)$	$\beta(1 \rightarrow 3)$

Human blood groups polysaccharides:

Human blood groups depends on the functioning of glycosyl-transferases, enzymes that catalyze the formation of glycosidic bond between monosaccharides. Specific-oligosaccharide antigens attach to the proteins and lipids on the surface of erythrocytes. Those attached to proteins have a serine or threonine residue or ceramide lipid intermediate.

The most basic oligosaccharide attached is called the O antigen (also referred to as the H antigen). This O antigen is the base oligosaccharide found in all three blood types O, A, and B.

1) Blood type O only has the O antigen attached to the red blood cells. The O antigen is of the following form:

   —Lipid—Glucose—Galactose—*N*-acetylglucosamine—Galactose—Fucose

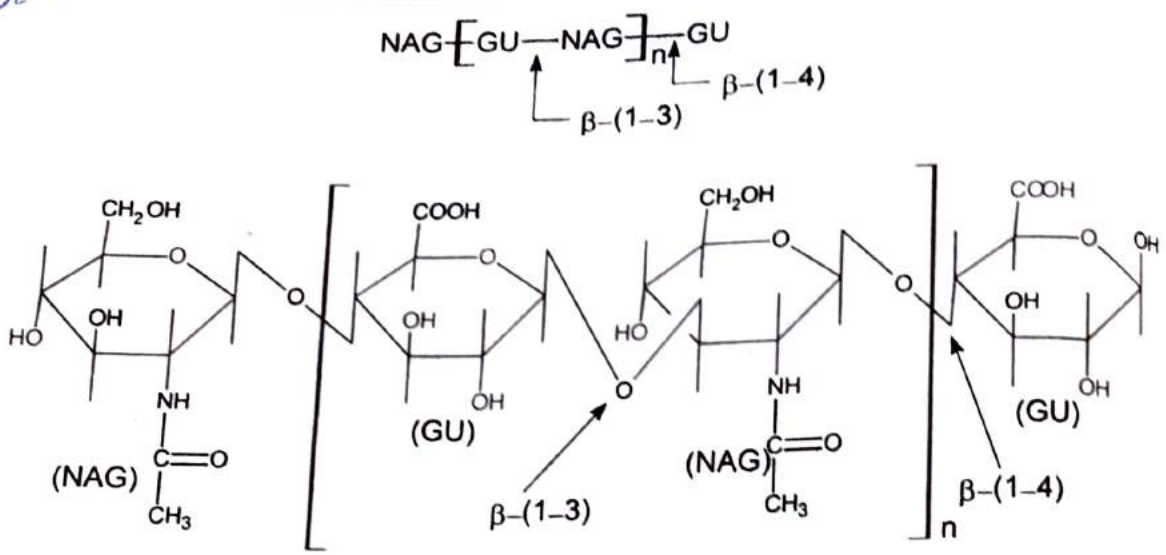
2) Blood type A is formed through the addition of the A antigen, which has *N*-acetylgalactosamine (GalNAc) glycosidically bonded to the O antigen.
3) Similarly for blood type B, the B antigen has an additional galactose forming a glycosidic bond to the O antigen.

In both the A and B blood types, the new antigen forms an α-1,3 linkage to the outermost galactose component of the O antigen through the help of specific glycosyl-transferases.

1) GalNAc-transferase adds the extra N-acetylgalactosamine for the A antigen.
2) While Gal-transferase adds the extra galactose for the B antigen.

Genes in a person's DNA code for the specific glycosyl-transferases to allow for the addition of antigens A and/or B to the O antigen. If a person's genes do not allow for the coding of type A or B transferase, then that person will have type O blood. The genes that code for GalNAc- and Gal-transferases are exactly similar, but differ for three amino acids. Thus this strong similarity between the two enzymes shows they are related through divergent evolution.

Hyaluronic acid : It is present in vitreous humor of eye, synovial fluid of joints (acting as viscous mucopolysaccharide-shock absorber), and covering over ovum. It is composed of alternate unit of Glucuronic acid (GU) and N-acetyl glucosamine (NAG) linked by β-(1-3) -glycosidic linkage -as disaccharide which is also repeated serveral times by β-(1-4) -glycosidic linkage, forming polymer. Thus there is alternate GU and NAG, similarly alternate β-(1-3) and β-(1-4) glycosidic linkage.



Hyaluronic acid is hydrolysed by enzyme hyaluronidase, secreted by bacteria, also present at tip of sperms. Since amino sugar (NAG) present, therefore it is a glycosaminoglycan (glycan/glucan means polymer of glucose, like fructan as polymer of fructose).

Reactions of Aldehyde and Keton groups

Oxidation of Carbonyl group:

Metal hydroxides like $Cu(OH)_2$, AgOH and $Bi(OH)_3$ oxidise free aldehydic or ketonic groups of mono and disacchacides and themselves reduced to lower oxides or to free metals. e.g. $Cu(OH)_2$ reduced to Cu_2O, AgOH to Ag and $Bi(OH)_3$ to Bi.

$$\text{Reducing sugar} + 2Cu(OH)_2 \rightarrow \text{oxidized sugar} + Cu_2O$$
$$\text{Blue} \qquad\qquad\qquad\qquad \text{red}$$

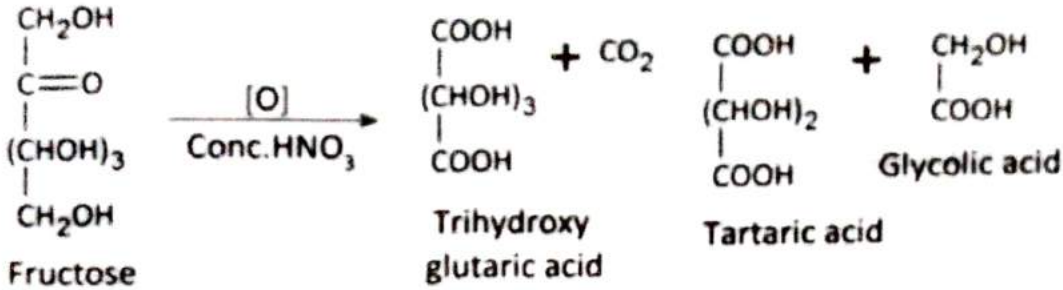
$Cu(OH)_2$ is present in oxidizing agents like Fehling, Benedict, and Barfoed's reagent.

Fehling's reagent : it consits of 2- solutions as solution A having $CuSO_4$ and solution B having KOH and sod. Pot. tartarate, when these two solutions are mixed, form a blue colored solution, containing $Cu(OH)_2$. Benedicts is a modified form of Fehling's reagent.

i) Glucose on oxidation with Fehling's reagent or HOBr (mild oxidizing agent) forms gluconic acid.

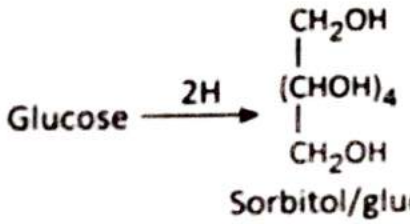
ii) Glucose on oxidation with conc. Nitric acid forms glucaric acid.

iii) Fructose on oxidation with conc. Nitric acid forms trihydroxy glutaric acid, tartaric acid and glycolic acid.



Reduction: Depending upon reducing agents different products have been formed in reduction of sugars.

a) Reduction with sodium amulgum:



Mannose $\xrightarrow{2H}$ Mannitol

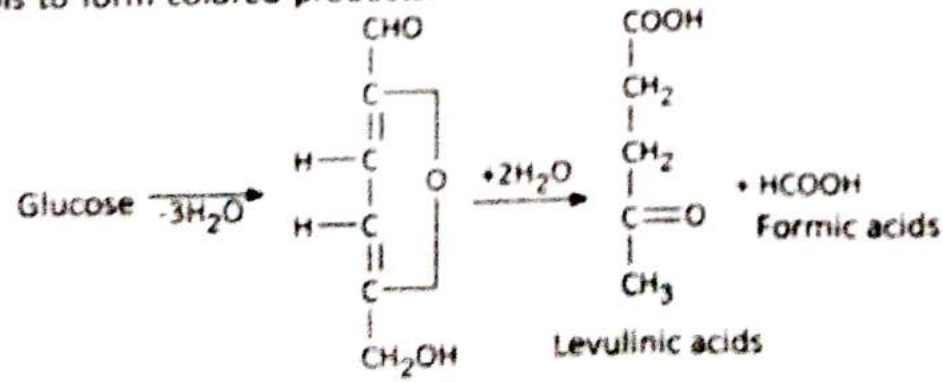
Fructose $\xrightarrow{2H}$ Sorbitol + mannitol (Mixture)

Xylose $\xrightarrow{2H}$ Xylitol

Ribose $\xrightarrow{2H}$ Ribitol

b) Reduction with strong mineral acids:

Hexoses form 5 – hydroxymethyl furfural, which on heating turns to levulinic acids. (It is a basis of Molisch test· where product of reduction condenses with organic phenols to form colored products.

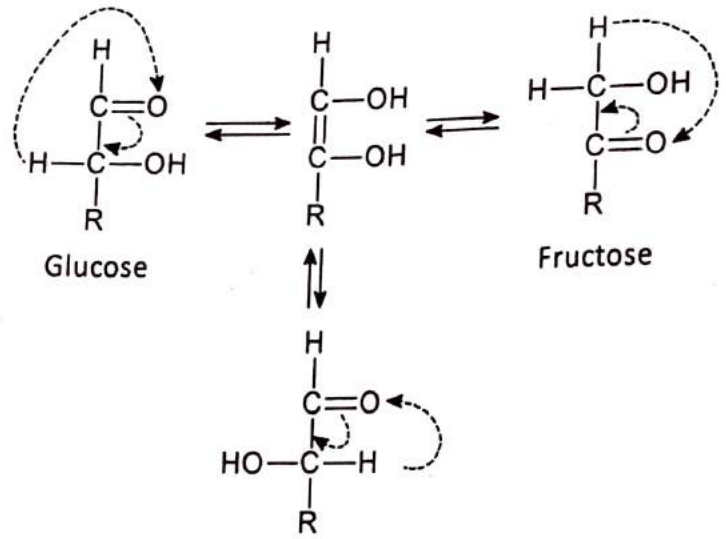


$$\text{Ribose} \xrightarrow[\text{(Heating)}]{\text{Reduction}} \text{Furfaral} + 3H_2O$$

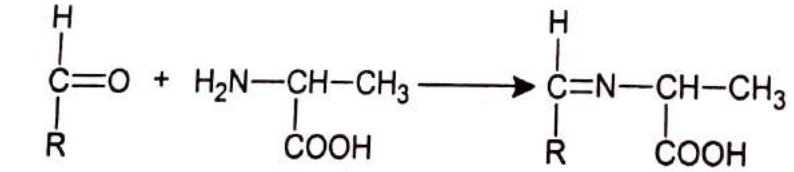
While tetrose and triose do not undergo reduction reaction to form furfural, since not having minimum 5- carbon atoms.

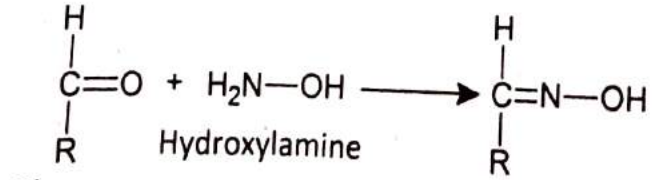
c) Reduction with dil. alkalies:

Glucose, fructose and mannose are inter convertible in presence of weak alkaline solution of $Ca(OH)_2$ or $Ba(OH)_2$ at low temperature. This reaction is called as Lobry de Bruyan – Alberda van Ekenstein transformation, which happens through enolisation (enol forming). Actual intermediate is enediol.

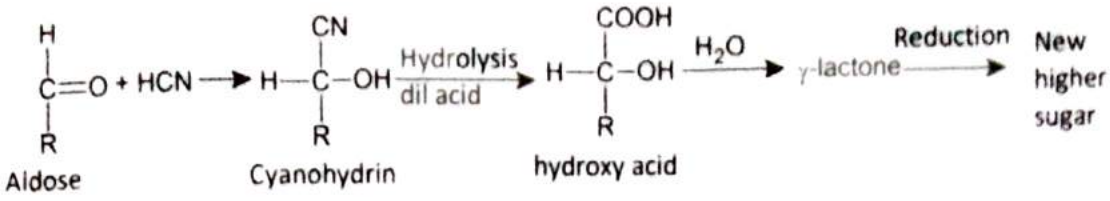


Reaction with alanine and Hydroxylamine:





Reaction with HCN (Kiliani synthesis)

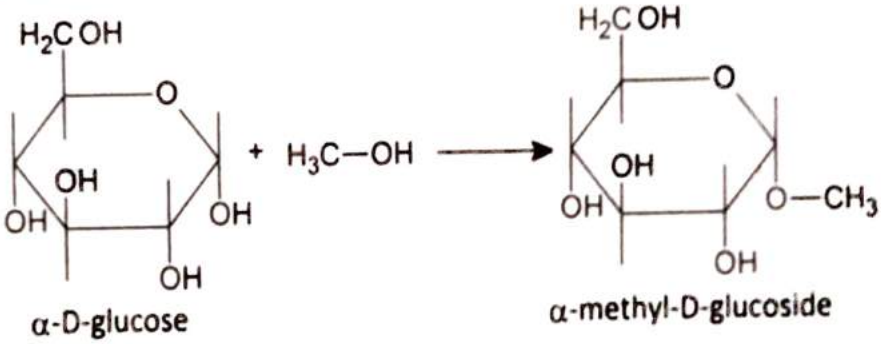


In this way higher sugars are synthesized by increasing one carbon.

Reaction with hydroxyl group:

a) Reaction with alcohol at anomeric carbon:

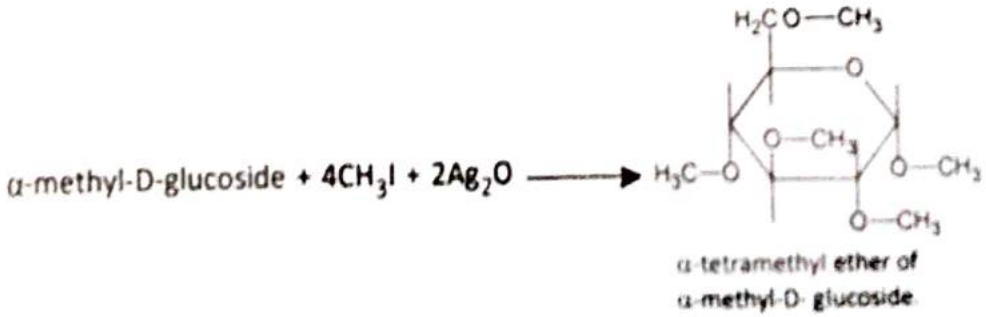
First carbon of aldose and second carbon of ketose i.e. carbonyl carbon are anomeric carbon of these sugars, have OH group in ring structure reacts with alcohol to form glycosides as α-and β-glycosides



b) Reaction with acetic anhydride:

All hydroxyl groups are acetylated in glucose to form penta-acetate

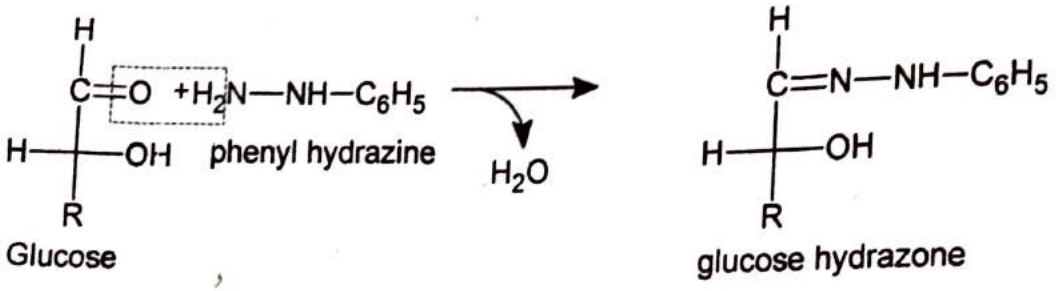
(c) Reaction with methyl iodide (etherification)



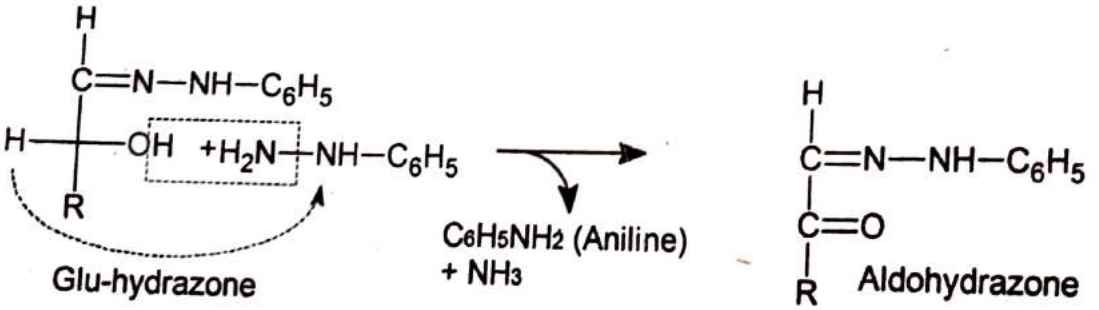
Osazone formation : Reaction of reducing sugar with phenyl hydrazine involves just two carbon atoms as carbonyl group (aldehydic or ketonic group) and the adjacent carbon.

During reaction one mole of phenyl hydrazine reacts with one mole of aldose/ ketose to form hydrazone, then second mole of phenyl hydrazine, oxidize hydrazone to aldohydhazone, while phenyl hydrazine itself reduced to aniline and ammonia. Then third mole of phenyl hydrazine reacts with carbonyl group of aldohydrazone to produce osazone. Similar, reaction takes place with ketose molecules.

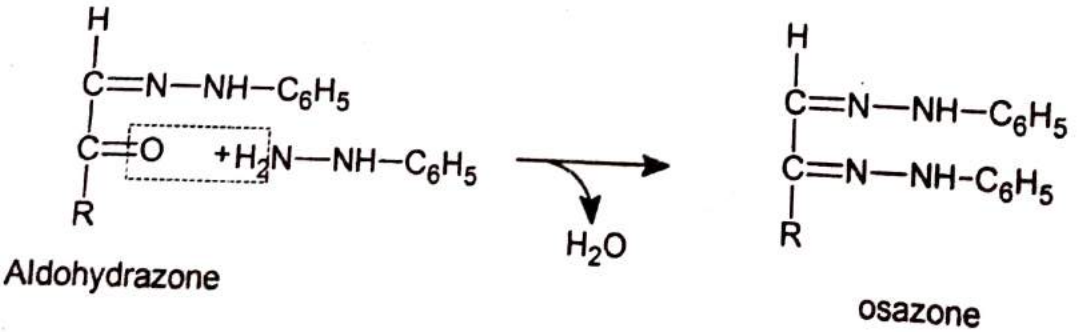
The glucose, fructose and mannose form same osazone due to their similar structure from C_3 to C_6 in these molecules. Different osazone have different shape e.g. glu, fru, mannose form needle shape, while lactosazone crystals have a typical hedgehog shape, maltosazone have petal - like appearance as sunflower like, while sucrose does not form osazone, as it does not contain free carbonyl group.



Glucose glucose hydrazone



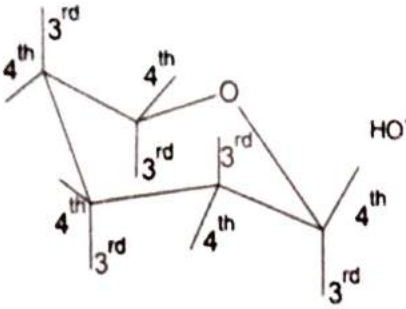
Glu-hydrazone Aldohydrazone



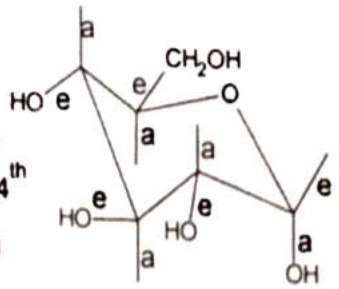
Aldohydrazone osazone

BOX-1 :

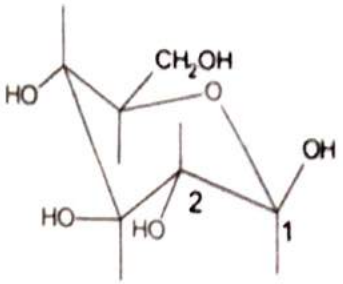
Fisher's form of structure have different bond length while Howorth structure have different bond angles therefore Boat and Chair conformations have been proposed taking equal bond length and equal angles of carbon atom. To which third bond is drawn from top carbon showing above and next carbons showing below, while 4^{th} bond is made making 109° approximate angle with 3^{rd} bond drawn.



3rd bond (axial)
4th bond (equatorial)

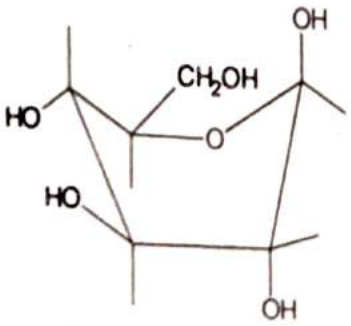


α-D-glucose

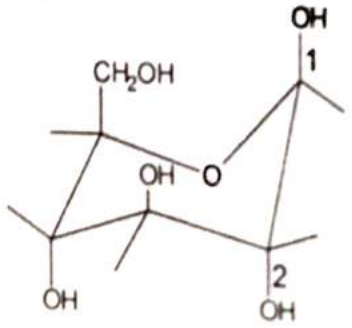


β-D-glucose

(chair conformation) ($^4C_1$ Chair)



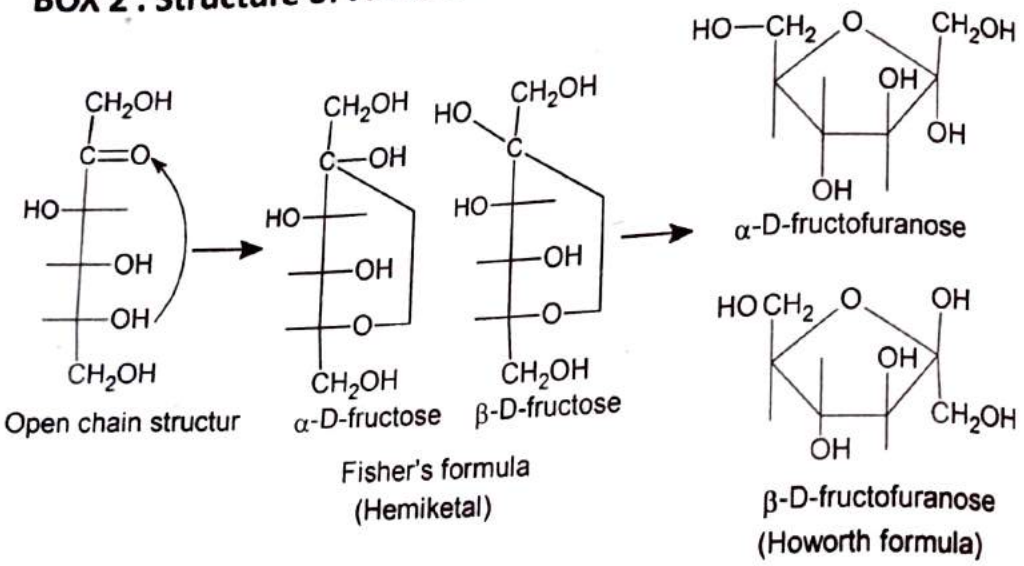
β-D-glucose (Boat form)



(Chair for $^1C_4$)

In β-glucose $^4C_1$chair form has all -OH groups equitorial but in α-glucose C-1 has -OH axial, therefore β-structure is more stable than α-structure, because in equitorial form of –OH group, going away from body of structure therefore more stable. β-glucose present $\frac{2}{3}$ while less stable $\frac{1}{3}$ α-glucose in equilibrium mixture, formed on mutarotation. Similarly polymer of β-D-glu i.e. cellulose is more in nature than starch (polymer of α). Cellulose is present in every cellwall of plant cell, while starch only in seeds. Similarly more stable cellulose undigestable while starch digestable.

BOX 2 : Structure of Fructose



[Two types of fructoses can be represented by Fisher's formula by hemiketal structure like hemiacetal structure of glucose. Similarly in Howorth formulas five membered furan ring formed.]

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CHAPTER 2

LIPIDS

Fatty Acids:

(I) Saturated Fatty Acids. (ABCCCLMPSABL)

Only even numbered fatty acids present in natural lipids.

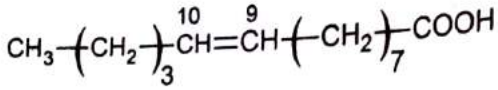
1)	Acetic Acid	2:0	Ethanoic Acid	$CH_3—COOH$
2)	Butyric Acid	4:0	ButanoicAcid	$CH_3-(CH_2)_2-COOH$
3)	Caproic Acid	6:0	Hexanoic Acid	$CH_3-(CH_2)_4-COOH$
4)	Caprylic Acid	8:0	Octanoic Acid	$CH_3-(CH_2)_6-COOH$
5)	CapricAcid	10:0	Decanoic Acid	$CH_3-(CH_2)_8-COOH$
6)	Lauric Acid	12:0	Dodecanoic Acid	$CH_3-(CH_2)_{10}-COOH$
7)	Myristic Acid	14:0	Tetradecanoic Acid	$CH_3-(CH_2)_{12}-COOH$
8)	Palmitic Acid	16:0	Hexadecanoic Acid	$CH_3-(CH_2)_{14}-COOH$
9)	StearicAcid	18:0	Octadecanoic Acid	$CH_3-(CH_2)_{16}-COOH$
10)	Arachidic Acid	20:0	Eicosanoic Acid	$CH_3-(CH_2)_{18}-COOH$
11)	Behenic Acid	22:0	Docosanoic Acid	$CH_3-(CH_2)_{20}-COOH$
12)	Lignoceric Acid	24:0	Tetracosanoic Acid	$CH_3-(CH_2)_{22}-COOH$

(II) Unsaturated Fatty Acids.

Only cis type of unsaturation present in natural form, though unstable.

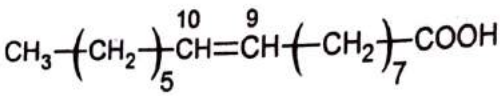
1) Monoenoic Acids

(i) Myristoleic Acid. 14:1;9



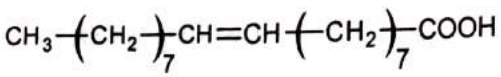
Δ^{9}–(cis)–Tetradecenoic acid

(ii) Palmitoleic Acid. 16:1;9



Δ^{9}–(cis)–Hexadecenoic acid

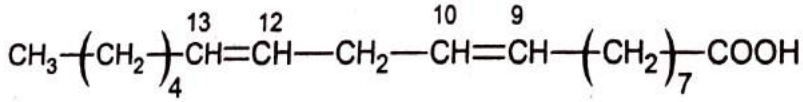
(iii) Oleic Acid. 18:1;9



Δ^{9}–(cis)–Octadecenoic acid

2) DienoicAcid.

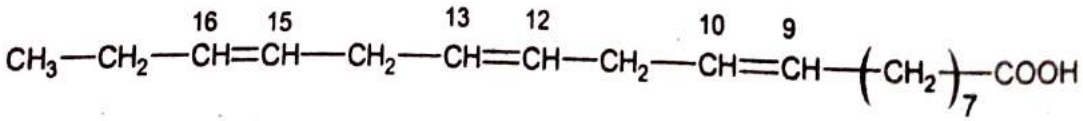
(i) Linoleic Acid - 18:2 ; 9,12



$\Delta^{9,12}$–(cis, cis)–octadecdienoic acid

3) Trienoic Acid.

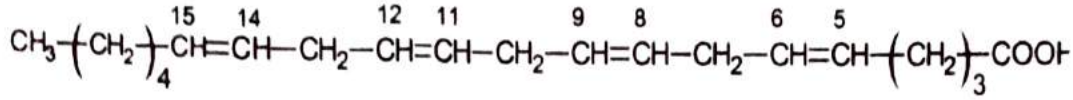
(i) Linolenic Acid - 18 :3 ; 9,12,15



$\Delta^{9,12,15}$–(all, cis)–octadectrienoic acid

4) Tetraenoic Acid

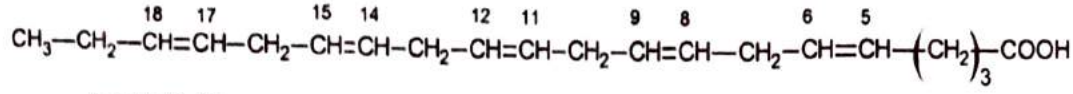
(i) Arachidonic Acid. 20:4 ; 5, 8,11, 14



$\Delta^{5,8,11,14}$–(all-cis)–Eicosatetraenoicacid

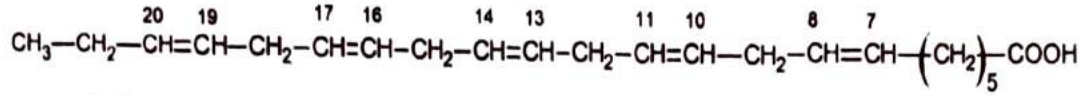
5) Pentaenoic Acid

(i) Eicosapentaenoic Acid (EPA). 20:5 ; 5,8,11,14,17



$\Delta^{5,8,11,14,17}$–(ali-cis)–Eicosapentaenoicacid (EPA)

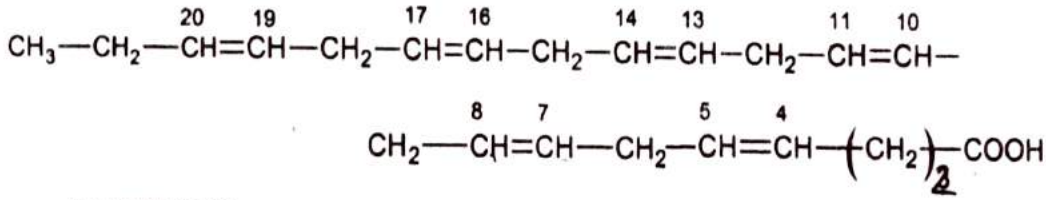
(ii) Docosapentaenoic Acid (DCPA). 22:5 ; 7,10,13,16,19



$\Delta^{7,10,13,16,19}$–(all-cis)–Docosapentaenoic acid(DCPA)

6) Hexaenoic Acid

(i) Docosahexanoic Acid. (DCHA) 22 :6 ; 4,7,10,13,16,19



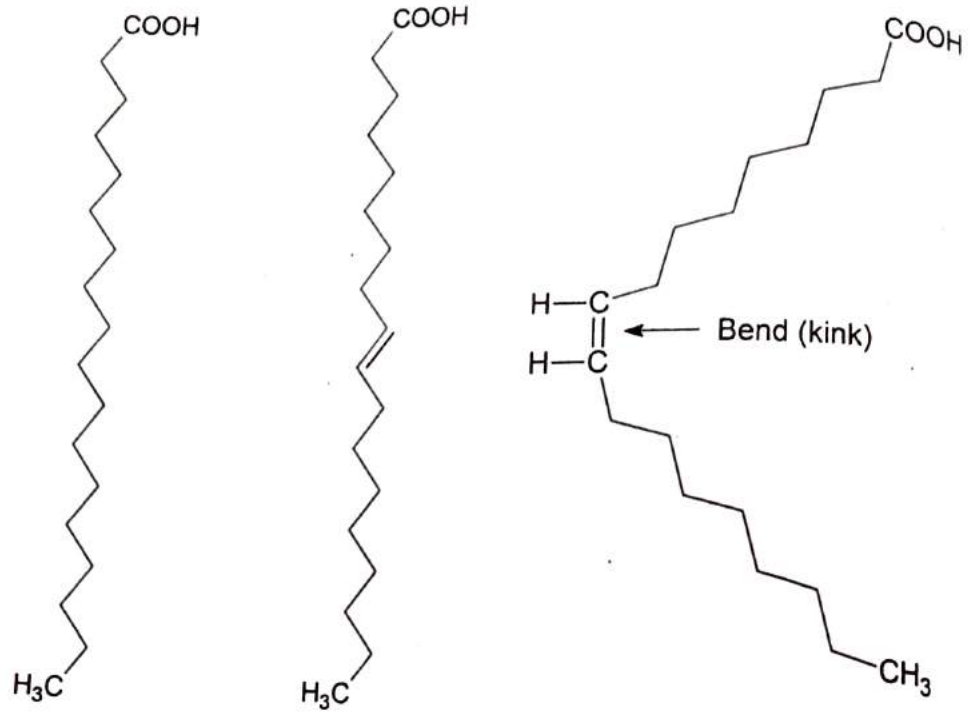
$\Delta^{4,7,10,13,16,19}$–(all-cis)–Docosahexaenoicacid(DCHA)

* **ω-numbering** :- ω-number begins from last C-atom where position of last double bond is mentioned. E.g. oleic acid ⟶ ω-9, Linoleic acid ⟶ ω-6, linolenic acid ⟶ ω-3.

Essential Fatty Acid : (EFA)

Linoleic acid and Linolenic acid are the two essential fatty acids. They must present in daily diet. Animal cannot synthesize, but only synthesized in chloroplast (green leaves).

1) Trans-isomer is solid in nature. It has zig-zag type of structure (Solid Nature).
2) Cis-isomer is liquid in nature because of rigid-kink (bend) in its structure (Liquid Nature).



18-C-Stearic acid

Trans-isomer (Elaidic acid)

Cis-isomer (Oleic acid) rigid-kink

Lipids are heterogenous group of substances which are soluble in organic solvents.

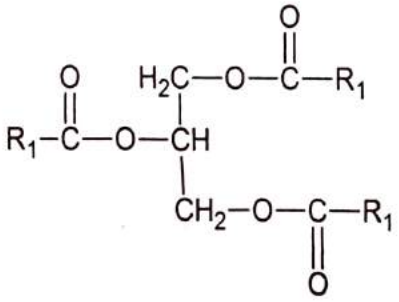
(Structural Classification of Lipids)

LIPID

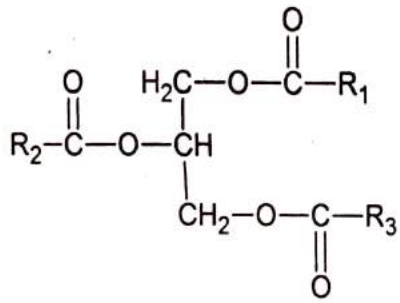
- Simple lipid (two types)
  - Triglycerides (Esters of glycerol) e.g. Oils and Fat
  - Waxes (Ester of higher alcohols)
- Compound/conjugated lipids
  - Phospholipids
    - Glycerophospolipids (glycerol as alcohol)
    - Sphingophospholipids (Sphingosine as alcohol)
  - Glycolipids (Non-phospholipid)
    - Cerebrosides (Single sugar)
    - Globoside (more than one sugar)
    - Gangliosides (Sialic acid derivative of globosides)
- Derived lipids
  - Alcohol
  - Fatty acids
  - Mono/di-glyceride
  - Steroids
  - Terpens
  - Eicosanoids

1. Simple lipids acts as a energy source
2. Compound lipids are structurally important so part of membrane.
3. Derived lipids are functional molecules acting as signaling mulecules - massage carriers.

* **Triglycerides** :- Naturally triglycerides have L-form of configuration. They are triesters of glycerol.

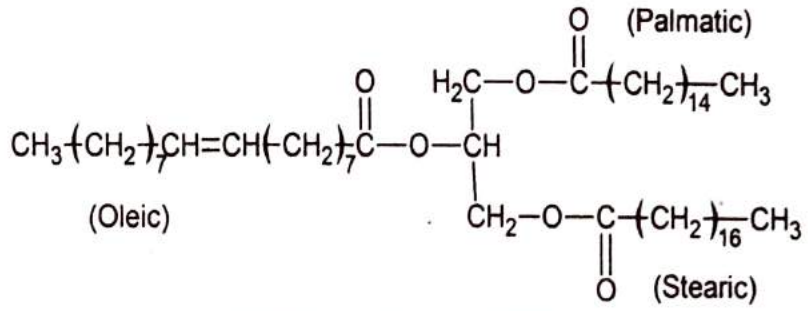


Homotriglyceride (Optically Inactive)
(Simple glycerides)



Heterotriglyceride (Optically Active)
(Mixed glycerides)

Generally unsaturated fatty acid present at 2nd C-atom in triglycerides.



L–α–palmityl, β–oleyl, γ–Stearyl glycerol

Enzymic hydrolysis:

* Triglyceride $\xrightarrow{\text{Lipase}}$ Glycerol + Fatty acids.

Triglycerides undergo hydrolysis by enzyme lipase to give glycerol and fatty acids.

Triglycerides $\xrightarrow{\text{Alkali}}$ Glycerol + salt of F.A. (Soap) ($RCOO^{-}NA^{+}$).

On treatment with alkali the soap i.e. salt of fatty acid is formed.

number of milligram of KOH needed to neutralize 1gm of fat.

Saponification number :-

Definition : mg of KOH required to saponify 1 gm of Fat is called as saponification number

(If Triglycerides $\xrightarrow{\text{has}}$ lower F.A. $\xrightarrow{\text{thus}}$ mol. Wt. low)

$\therefore$ 1gm fat contain more triglycerides $\xrightarrow{\text{so}}$ more F.A. $\xrightarrow{\text{so}}$ require more KOH

$\xrightarrow{\text{thus}}$ saponification number $\xrightarrow{\text{is}}$ ~~more~~ lower.

$$\text{(High) Saponification number } \alpha \frac{1}{\text{(lower) Fatty acids in triglycerides}}$$

(1) Saturation of triglycerides (Hydrogenation)

$$\text{Unsaturated fat} \xrightarrow[\text{Reduction}]{H_2/Ni} \text{saturated fat (Ghee)}$$

(2) Halogen can be added across double bonds forming dihalides.

Iodine number :- gms of Iodine absorbed by 100 gm of fat/oil is called as Iodine no.

Iodine no. α Unsaturation in fatty acid.

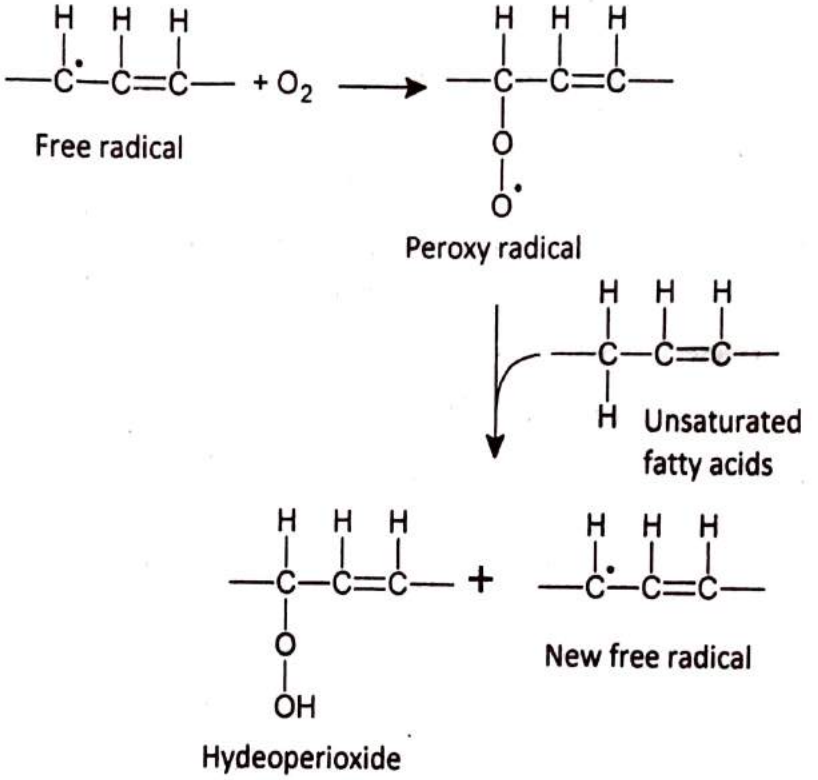
Acid value :- It is defined as mg of KOH required to neutralize free fatty acids present in 1 gm of fat. The fat which has been processed and stored properly has very low acid number.

Reichert – Meissl number :- It is defined as ml of 0.1 N KOH required to neutralize the soluble volatile fatty acids derived from 5 grams of fat. Thus it indicates quantity of short chain fatty acids (up to C_{10}) present in fat. Its value for coconut and palm oils are between 5 to 8, for butter fat between 17 to 35.

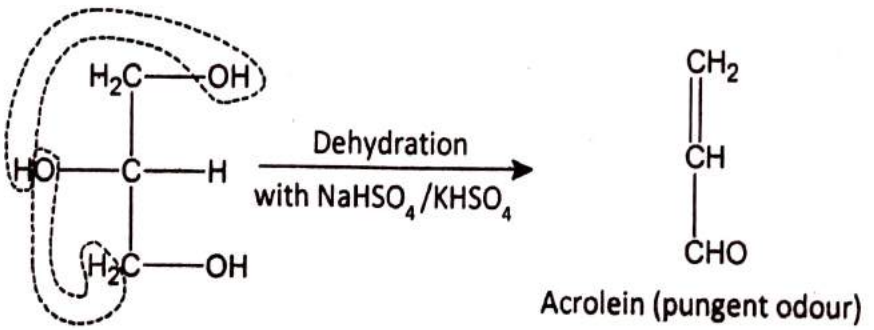
Rancidity of Fat:- Unsaturated fatty acids of oil are spontaneously oxidized by atmospheric oxygen forming short chain aldehydes and acids. It represents oxidative rancidity also called 'autoxidation'. It occurs generally in animals fats rather than vegetable fats. Which has natural antioxidants like vit E.

Linseed oil, a plant oil commonly used as a base for paints is rich in unsaturated fatty acids, on autoxidation followed by polymerization forms hard resins. Substances which promotes autoxidation called pro-oxidants like copper, iron and nickel salts of organic acids.

Autoxidation proceeds by a free radical mechanism where a methylene group is attacked, where a hydrogen atom has been removed from α-Methylene group, initiating chain reactions leading to oxidation.



Reaction of glycerol :

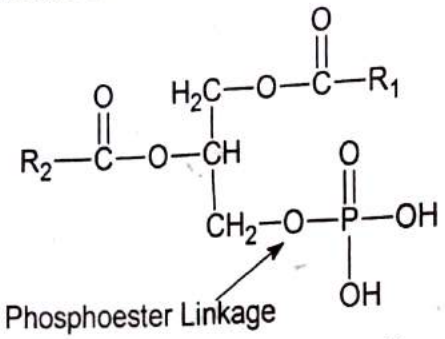


When fat is heated with dehydrating agents for unsaturated aldehydes, which is a characteristic reaction for presence of free glycerol in fat.

Conjugated Lipid

Phospholipid – Glycerophospholipid

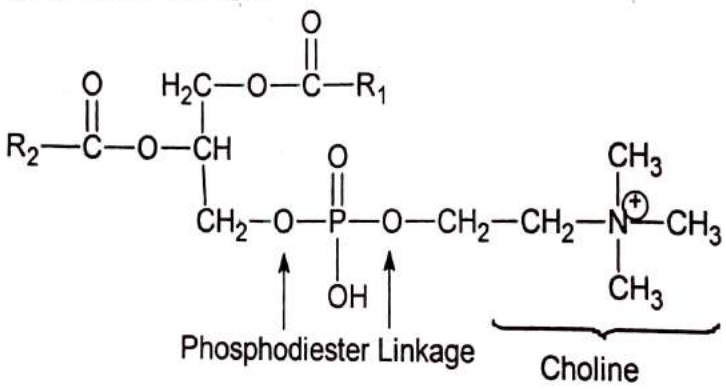
All glycerophospholipid are derivative L-α-phosphotidic acid.



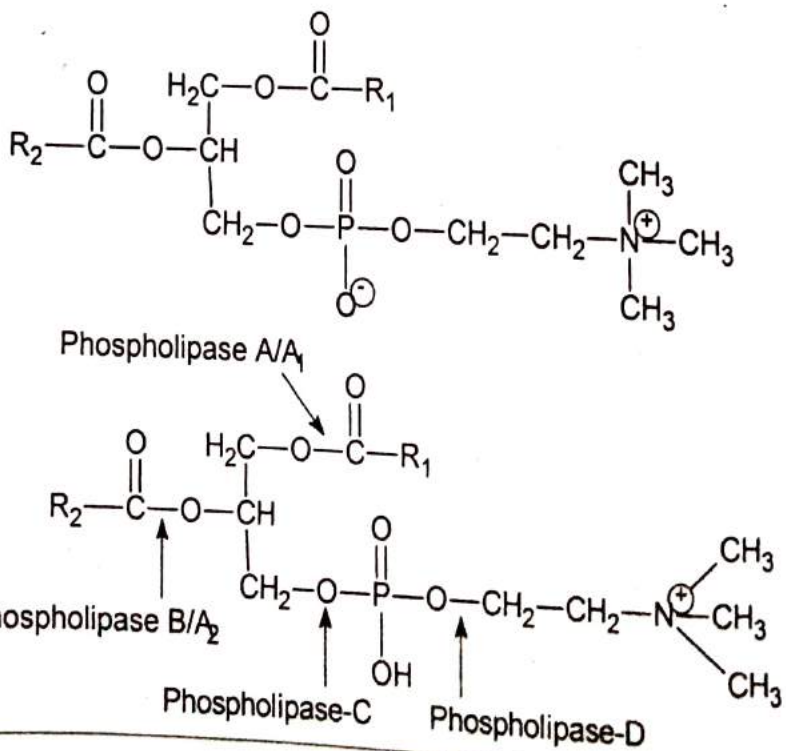
L-α-phosphotidic acid

e.g. (1) Lecithin (Phosphotidyl-choline)

It has phosphodiester linkage.



* **Zwiterionic form of lecithin**



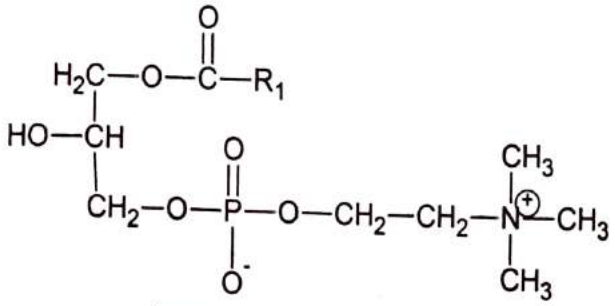
Enzymic hydrolysis :

(1) Phospholipase-B removes R_2COOH and form Lysolecithin.

(2) Phospholipase-A removes R_1COOH and once again form Lysolecithin by shifting fatty acid from C-2 to C-1.

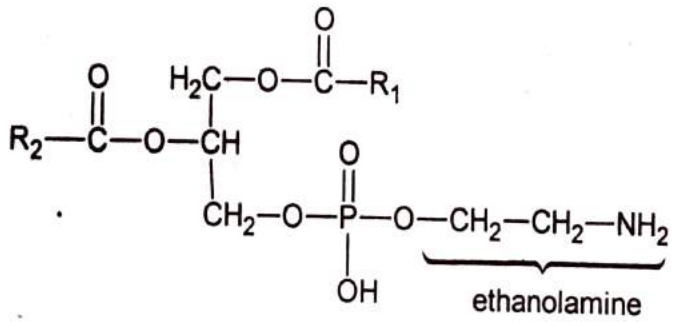
(3) Phospholipase-C forms Diglyceride + phosphocholine

(4) Phospholipase-D forms L-α-Phosphotidic acid + choline

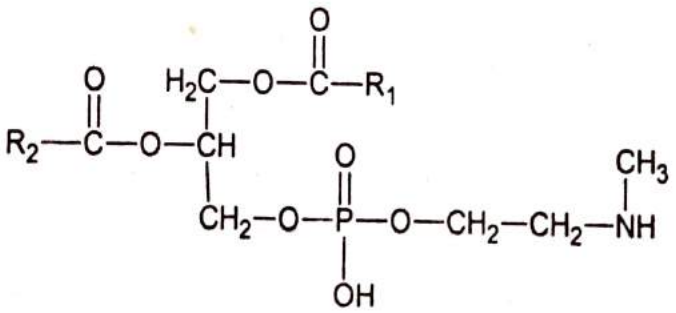


lyso–lecithine

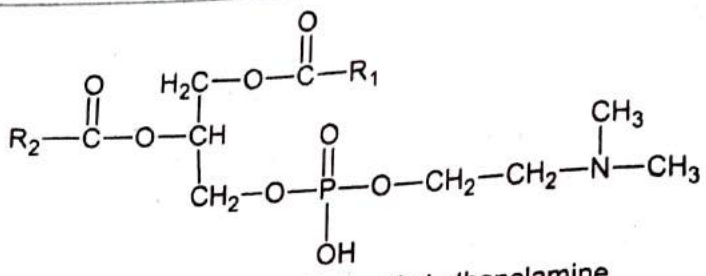
(2) Cephalin (Phosphatidyl ethanolamine)



It also forms lyso-cephalin, thus reacts similar to Lecithin.

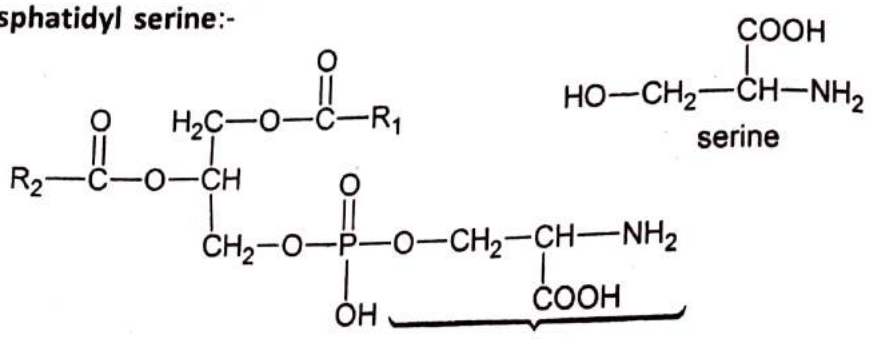


Phosphatidyl N-methyl ethanolamine

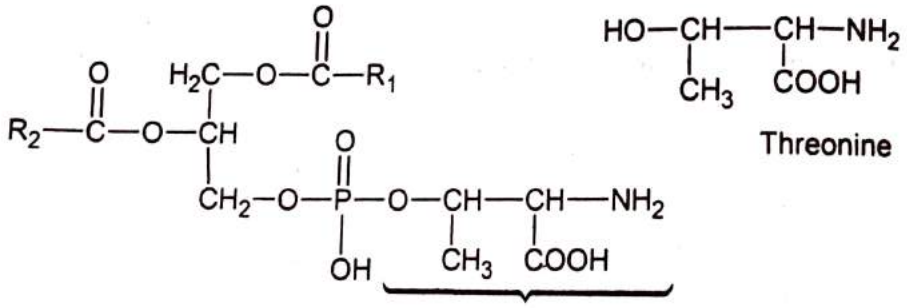


Phosphatidyl N, N-dimethyl ethanolamine

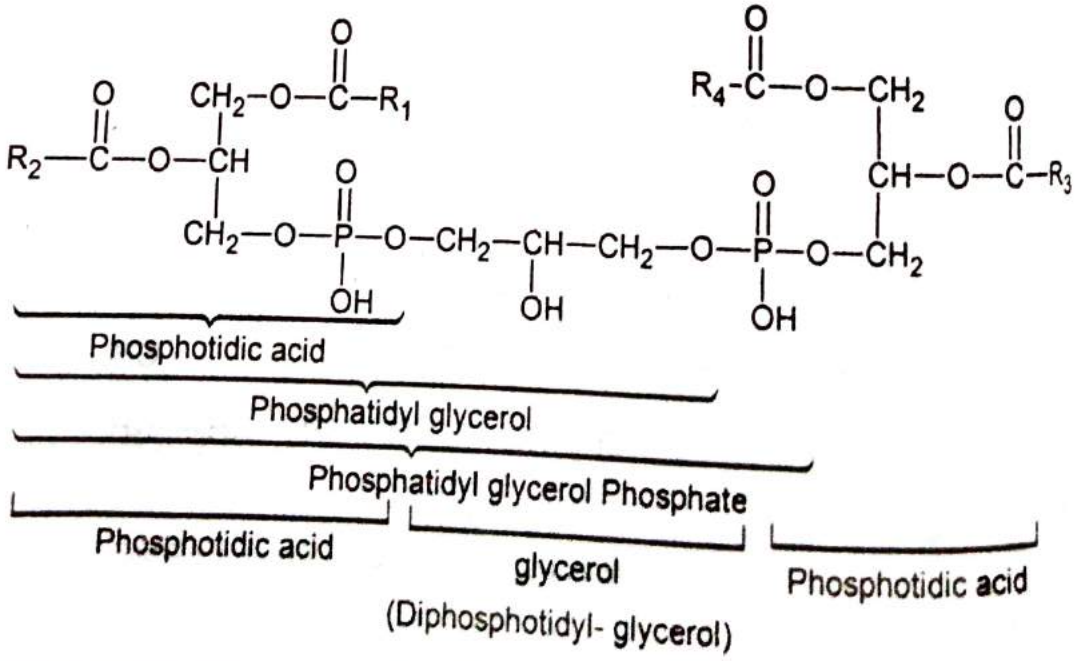
(3) Phosphatidyl serine:-



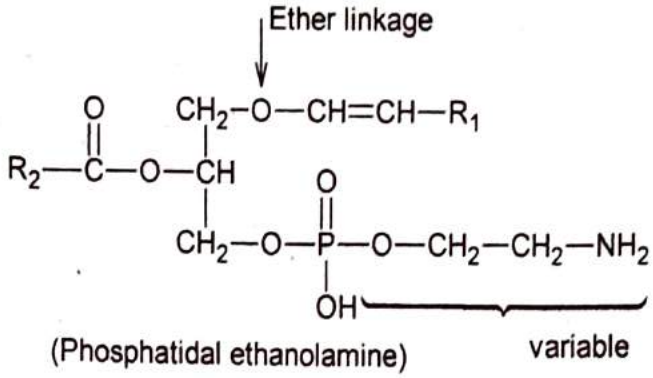
(4) Phosphatidyl Threonine:-

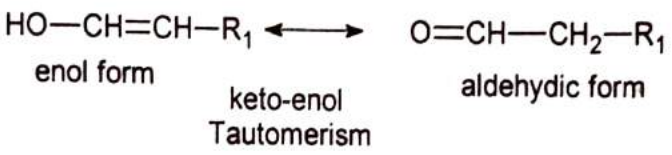


(5) Cardiolipin :- present in heart muscles.



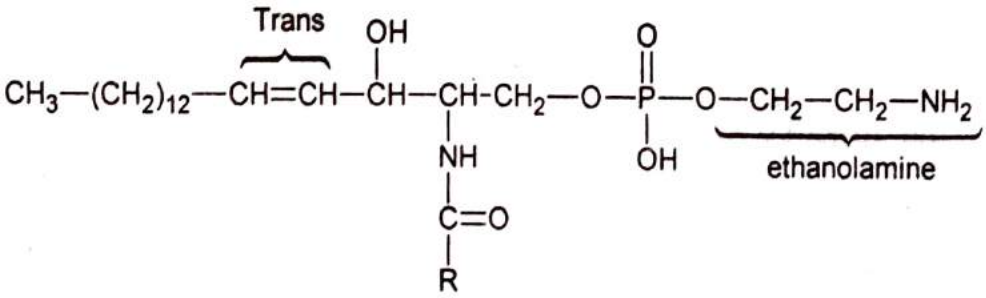
(6) Plasmalogen :-



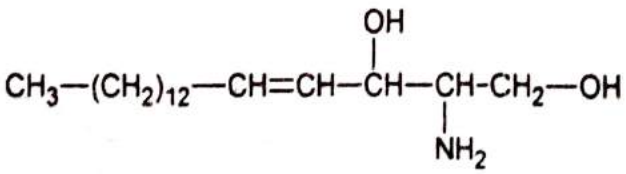


Aldehydic form ungergoes keto-enol tautomerism to form enol form that is linked by ether linkage to 1st C-atom of glycerol to form phosphatidal ethanolamine.

Sphingophospholipid :-

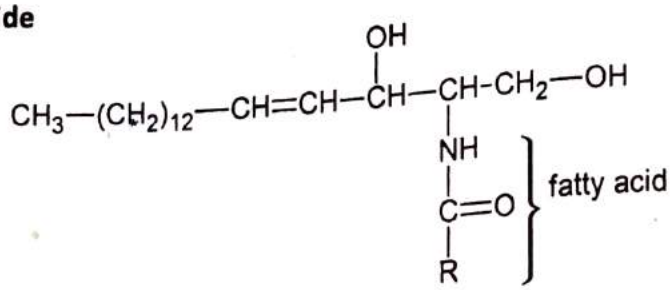


Sphingophospholipid has Δ^4–Sphingosine as amino alcohol.



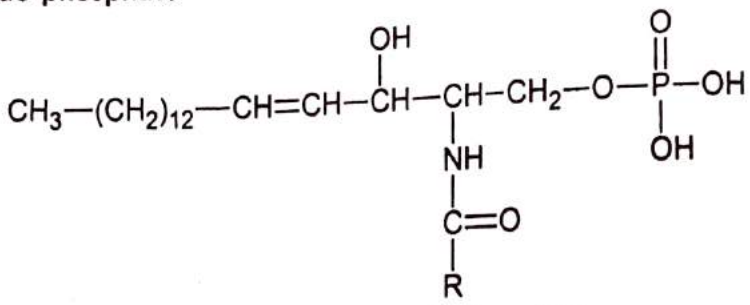
Δ^4 – sphingenine or Δ^4 – sphingosine

(ii) Ceramide



Ceramide has amide linkage

(iii) Ceramide phosphate



Ceramide phosphate

(1) Sphingosine + fatty acid ⟶ ceramide

(2) Ceramide + Phosphate ⟶ ceramide phosphate.

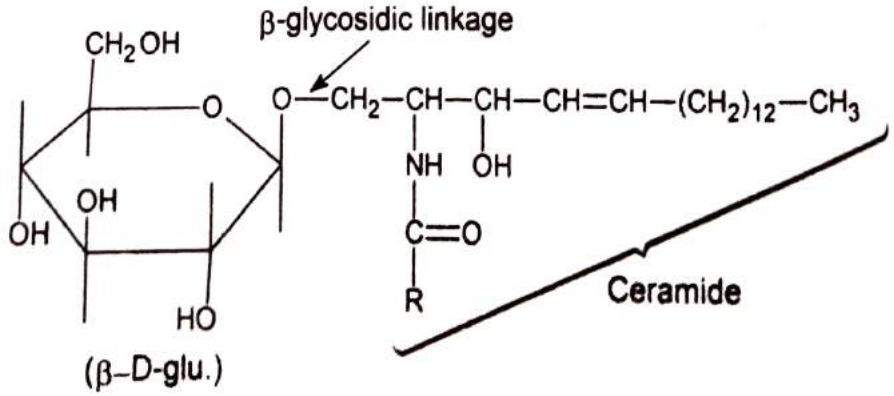
Non-phospholipids :- (Glycolipids)

(1) Cerebroside has single sugar may be glucose present in non-neural membrane or may be galactose present in neural membrane.

(2) Globoside has many sugars.

(3) Gangliosides are sialated globosides.

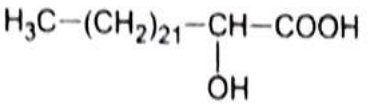
Cerebroside :-



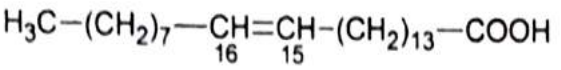
(β–D-glu.)

Cerebrosides have different fatty acids as- keracin has lignoceric acid (24:0) while phrenocin/cerebron has cerebronic acid i.e. 2-hydroxyl lignoceric acid.

Cerebronic Acid



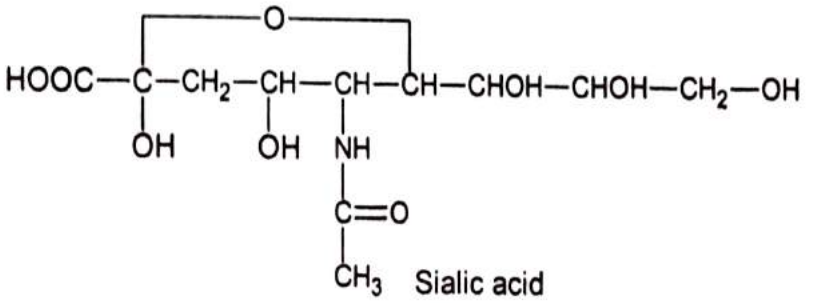
Nervon contains Nervonicacid (24:1;15)



Oxynervon contains oxynervonic acid (2-hydroxyl nervonic acid)

Globoside :- eg :- ceramide-glu-gal, ceramide-glu-gal-NA-gal

Ganglioside :- These are sialated globosides contain sialic acid (N-acetyl-neuraminic acid) (NANA)



Formula :- GM/D/T/Q (5-n) - where M/D/T/Q number of sialic acid

[G-stands for gangliosides, n-number of monosaccharides, M-monosialo, D-disialo, T-trisialo and Q-tetrasialo.]

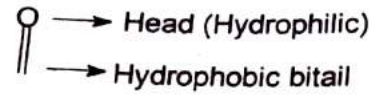
(1) ceramide–glu–gal (GM_3)
NANA

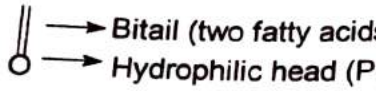
(2) ceramide–glu–gal–NAgal (GD_2)
NANA
NANA

(3) ceramide–glu–gal–NAgal–gal (GQ_1)
NANA NANA
NANA NANA

Amphipathic Nature of Conjugated Lipids

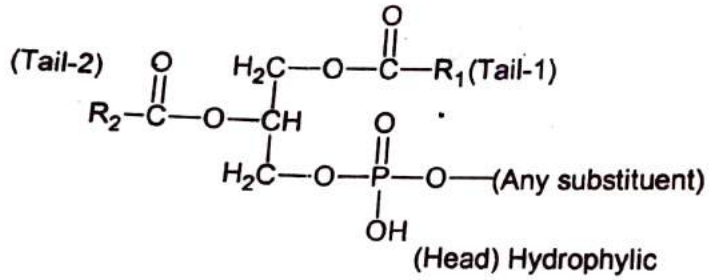
Conjugated Lipids are having hydrophilic group as well as hydrophobic group thus called as Amphipathic Nature.



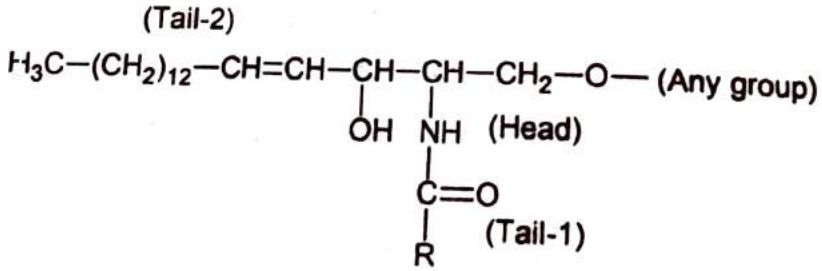


They are part of membrane, because of amphipathic nature.

BOX 3:



(Any-glycerophospholipid)



(Any-sphingolipid)

CHAPTER
3

MUSCLES

MUSCLE

Muscle is the major biochemical machine that converts chemical (potential) energy into mechanical (kinetic) energy. Muscle tissue is responsible for movement of the body and its parts and for changes in the size and shape of internal organ.

Muscle tissue is characterised by aggregates of specialised elongated cells arranged in parallel array, whose primary role is contraction.

STRUCTURE OF SKELETAL MUSCLE

Skeletal muscle is composed of fibrils surrounded by electrically excitable membrane, the sacrcolemma. The individual muscle fibre consist of a bundle of many myofibrils arranged in parallel. Myofibrils are immersed in the cytoplasm of a muscle fibre is called the sarcoplasm. Sarcoplasm is rich in mitochondria, glycogen, high energy compounds ATP and phosphocreatine and glycolytic enzymes. The functional unit of muscle is sarcomere. It exist along the axis of a fibril at distances of 2.5 μm [fig 3.1]

Alternating dark and light bands (A bands and I bands) are present in the microfibrils. The central region of the A band (the H zone) is less dense than the rest of the band. The I band is bisected by very dense and narrow Z line.

The cross section of myofibrils under electron microscope shows that the myofibrils are constructed of two types of longitudinal filaments. One type confined to the A band (the thick filament) contains mainly the protein myosin. These filaments are about 16nm in diameter. The other filament (the thin filament) lies in the I band but extends also into the A band. The thin filaments are smaller than those of myosin (about 6nm in diameter).

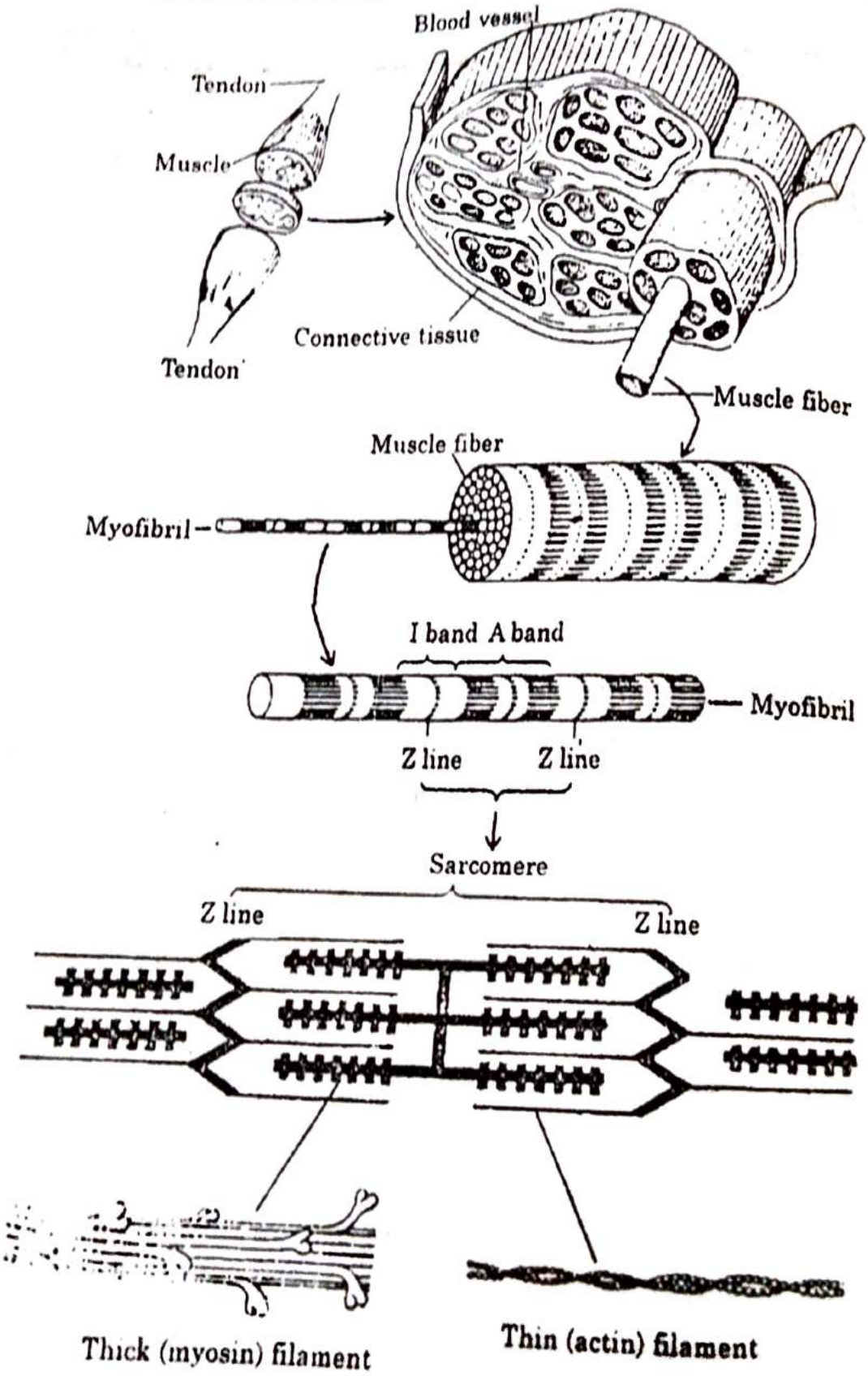


Fig. 3.1 : Levels of structural organization in a skeletal muscle.

The thin filaments contain the protein actin, tropomyosin and troponin. Each thin filament lies between 3 thick filaments.

When muscle contracts there is no change in the lengths of the thick filaments or of the thin filaments, but the H zone and the I bands shorten. Thus the arrays of inter digitating filaments must slide past one another during muscle contraction.

The cross bridges generate and sustain the tension. The tension develop during muscle contraction is proportionate to the filament overlap and thereby the number of cross-bridges, each cross-bridge head is connected to the thick filament via a flexible fibrous segment that can bend outward from the thick filament to accommodate the inter filament spacing.

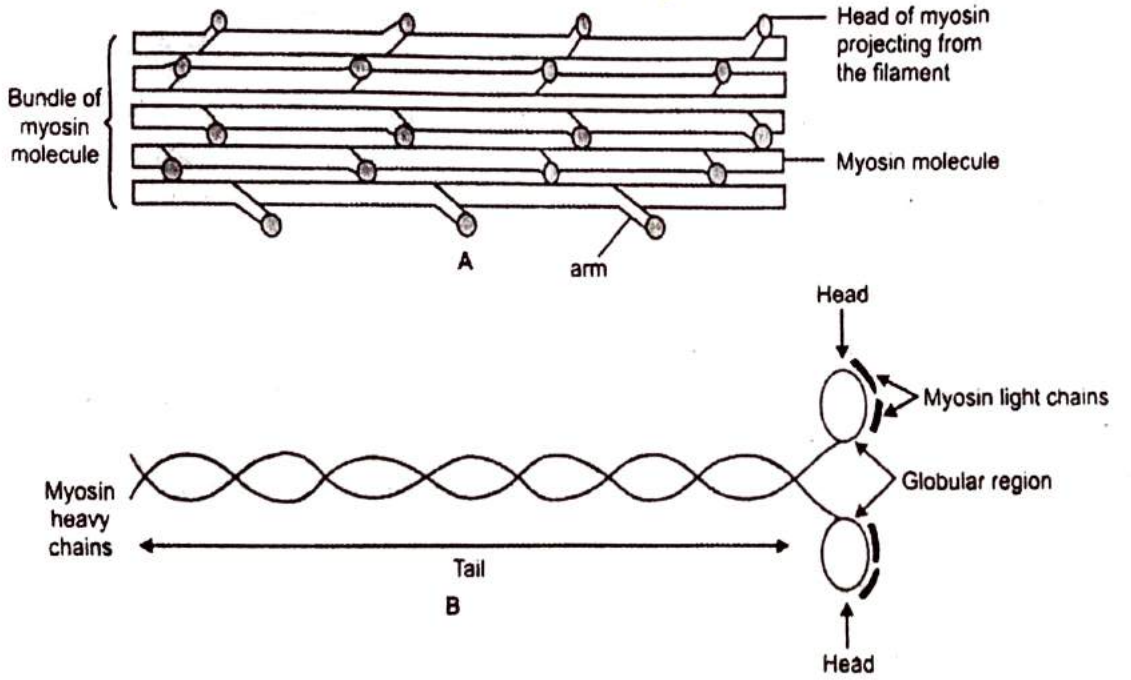


Fig. 3.2 : A = The thick filament consists of bundles of myosin molecules
B = Schematic structure of myosin molecules

Protein Composition of Muscle fibres

Skeletal muscle contains following types of proteins

- Contractile protein
  - Myosin II and
  - Actin
- Regulatory protein
  - Troponin and
  - Tropomyosin

- Minor or accessory protein involved in stabilising the structure of muscle contraction complex.

Contractile protein

Myosin

There are two types of myosin

- Myosin I and
- Myosin II

Myosin I is not found in muscle cells. Myosin II is the principal protein of thick filaments of muscle, required for muscle contraction, but it can also be found in non-muscular cells.

- Each myosin molecule is composed of six polypeptide chains, two identical heavy chains and four light chains. Light chains are of two types. [fig 3.2]
- Two identical heavy chains are wound around each other to form a double helix, which is called the tail of the myosin molecule.
- The amino terminal end of each of these chains is folded into a polypeptide structure called myosin head. Thus, there are two free heads lying side by side at on end of the double helix myosin molecule.
- One each type of light chain is associated with each of the myosin heavy chain heads.
- Globular head of myosin has two specific binding sites one for ATP and one for actin.

Myosin

1. Myosin is the richly abundant muscle protein and it is a globulin.
2. It is soluble in dilute salt solution and insoluble in water.
3. Its molecular weight is 5, 00,000 containing 2 major chains and 4 light chains.
4. The enzymes trypsin cleaves myosin into two components – meromyosins – of unequal size. Therefore, they are termed as light and heavy meromyosins.
5. It has adenosine triphosphates (ATPase) activity.
6. Myosin binds actin forming actomyosins.

Actin

1. Actin is a globulin of molecular weight 60,000.
2. It is the major constituents of thin filaments in striated muscle.
3. Actin is a polymer of globulin shaped sub unit called G-actin.
4. At physiological concentration of ATP, magnesium ions and at concentration of actin between 1mm and 10mm, G-actin polymerizes Spontaneously into a fibrous or filamentous form called F-actin [fig.3.3]
5. Each G-actin molecule of the thin filament has a binding site for myosin.
6. In association with myosin, actin plays a major role in muscle contraction.

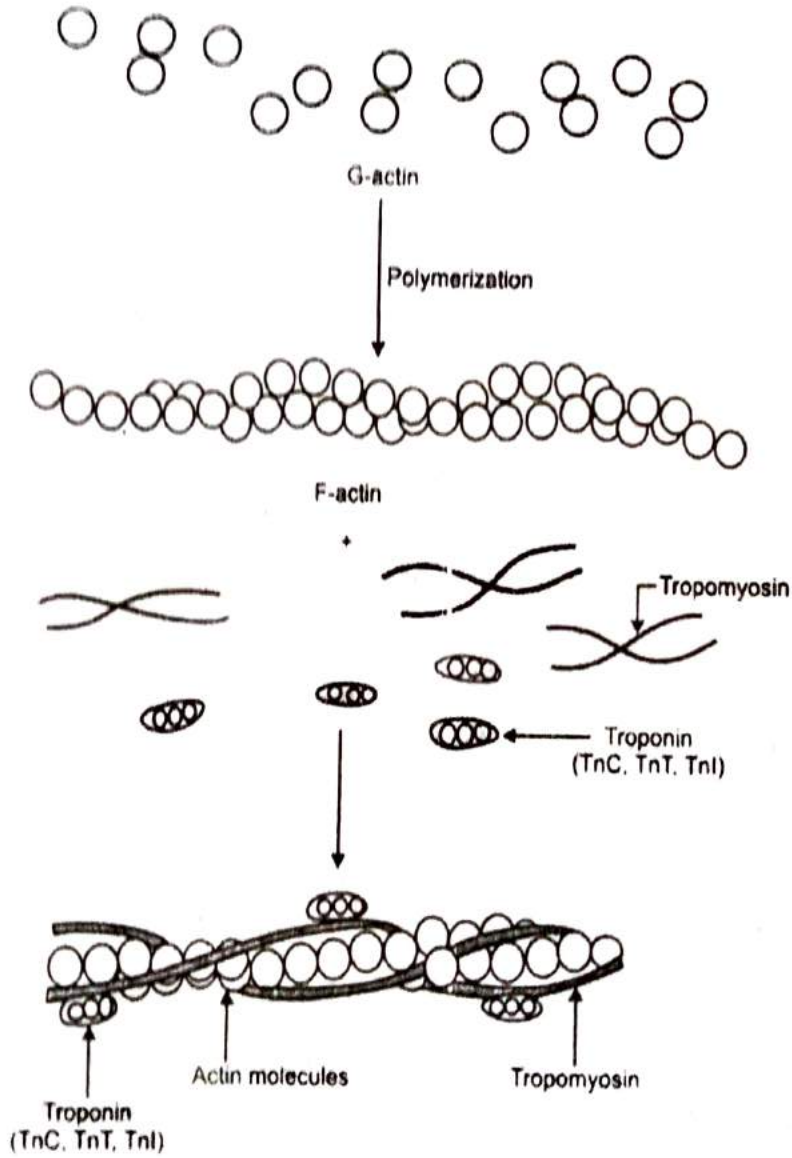


Fig. 3.3 : Assembly of proteins in thin filament.

Tropomyosin and Troponin

1. Tropomyosin and troponin complex are proteins present in the thin filaments of muscle.
2. Calcium ion has the effect on the interaction of actin and myosin which in turn is mediated by tropomyosin and troponin.
3. Tropomyosin is a double stranded α-helical rod of molecular weight 70,000 and present between the 2 strands of F actin.
4. Troponin is a complex of 3 polypeptide chains.
5. Troponin complex is present in the thin actin filaments at intervals of 38.5 nm.
6. A troponin complex with tropomyosin molecule regulates the activity of 7 actin monomers.

Sliding mechanism of muscle Contraction

The biochemical events occur during muscle contraction consist of five stages as follows [fig 3.4]

1. Binding of ATP to head myosin of thick filaments and detachment of myosin from actin (thin filament).
2. Confirmational changes in the myosin head as a result of hydrolysis of ATP to ADP + Pi.
3. Binding of myosin – ADP –Pi complex to actin.
4. Force generation on which myosin head releases inorganic phosphate and power stroke occurs.
5. Rettachment of myosin head to the new actin molecule of the thin filament and formation of rigor complex. [fig. 3.4].

Stage – 1

- In the first stage ATP binds to myosin head in the thick filament which is tightly bound to the actin molecule of the thin filament.
- Binding of ATP dissociates actomyosin into actin and myosin.
- Binding of ATP induces conformational changes in the actin binding site of myosin head. This change reduces the affinity of the myosin head for the actin molecule of the thin filament causing the myosin head to detach from the thin filament.

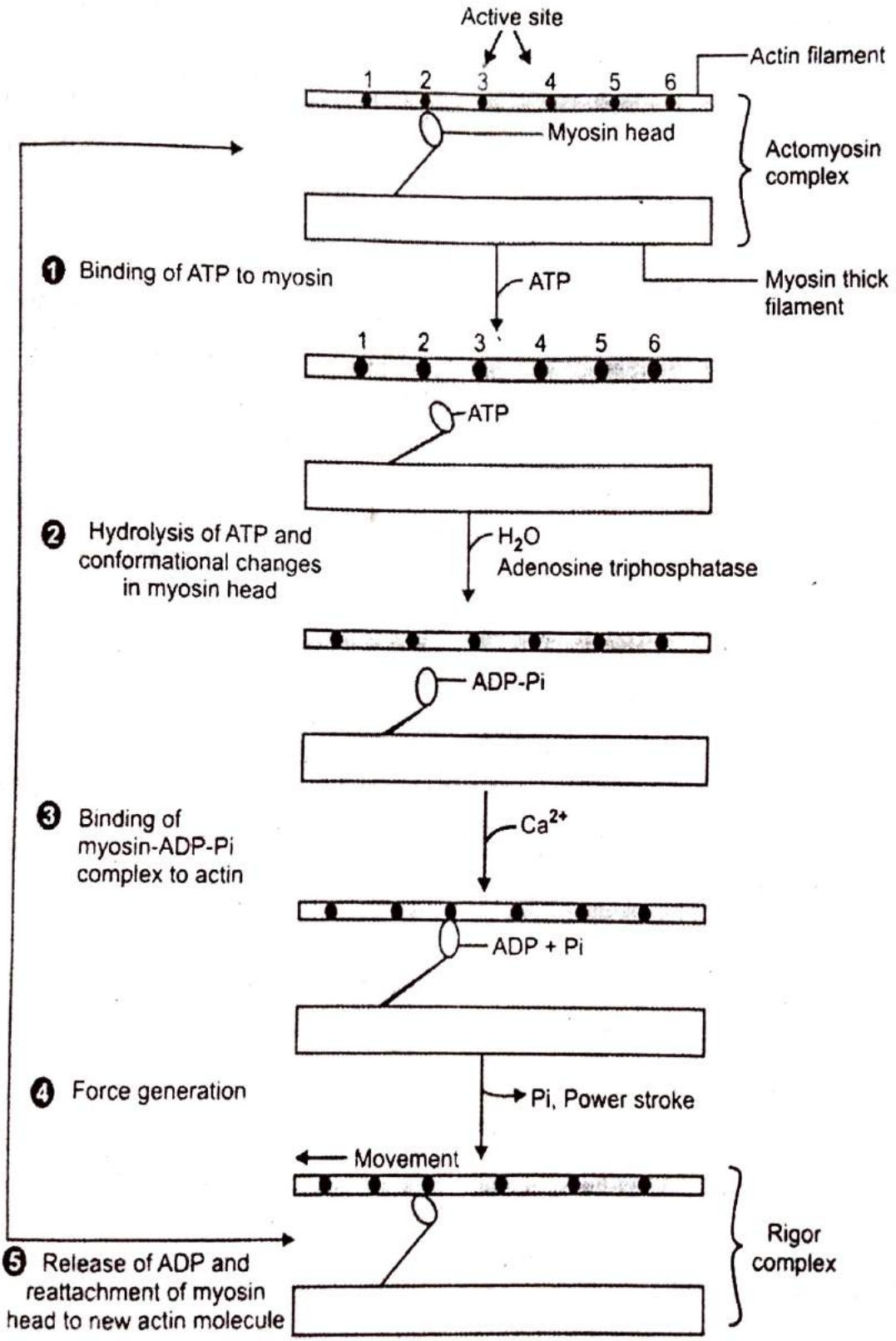


Fig. 3.4 : Schematic representation of myosin-actin interaction during contraction

Stage – 2

- Adenosin triphosphate (ATPase) of the myosin hydrolysis ATP to ADP and P_i and remain bound to myosin head.
- The energy released by the splitting of ATP is stored in the myosin molecule.
- Myosin in the form of myosin ADP-Pi complex is now in a high energy state. This is the predominant state at rest, i.e. relaxed state.

Stage – 3

- Upon muscle stimulation via calcium the inhibition of actin myosin interaction imposed by the regulatory proteins (tropomyosin and troponin) is lifted and consequently the myosin with bound ADP and Pi attaches to actin.

Stage – 4

- The actin myosin reassociation triggers the sequential release of inorganic phosphate (Pi) from myosin head; which results in increasing the strength of the myosin – actin interaction.
- This tight binding of myosin to actin results in a conformational change in myosin head tilting the angle 90° to 45°.
- This change in orientation of myosin head, relative to actin causes the generation of force called the "power Stroke" of muscle contraction which pulls the thin filament a distance about 70°A (10 nm) towards the centre of the sarcomere.

Stage – 5

- The myosin head is again tightly bound with release of ADP to a new actin molecule of the thin filament which is known as rigor configuration.
- The actin myosin complex remains intact until another molecule of ATP binds to the head of myosin molecule.
- With a new ATP, a new cycle may begin and the cycling may continue as long as regulatory calcium and ATP are present.

RIGOR MORTIS

- At the beginning of the contraction cycle, the myosin head is tightly bound to the actin molecule of the thin filament and ATP is absent. This arrangement in known as rigor configuration.

- ATP is needed for the detachment of myosin from the actin during muscle contraction. In case of ATP depletion the cycle is arrested and actin does not dissociate and relaxation does not occur.
- When actin and myosin are permanently bound in the absence of ATP, the muscle becomes rigid and stiff. The muscular rigidity and stiffening that begins at the moment of death due to lack of ATP is known as rigor mortis.

NEUROMUSCULAR JUNCTION

A neuromuscular junction is a place in the body where the axons of motor nerves meet the muscle, allowing them to transmit messages from the brain that cause the muscle to contract and relax. Every organism has thousands of these junctions that control the movements of the body and cause the heart to beat. It is only one example of many connections made between nerves and other parts of the body that result in a successfully functioning organism.

Neurons, or nerve cells, are specially designed cells that communicate using chemicals called neurotransmitters. Depending on the type of cell, specific neurotransmitters are designed to stimulate a response, assuming that a receptor is present. In addition to a body, neurons also have a long tail called an axon that terminates in dendrites, a bundle of fibers that can transmit chemicals to the next neuron. In the instance of a neuromuscular junction, one neuron can control many muscle cells, but each muscle cell only responds to one neuron.

At this junction, the motor neuron meets muscle tissue at an axion terminal. The surface of the muscle fiber forms small ridged folds for the end of axon to rest in. Inside these folds are depressions with acetylcholine receptors. The neuron forms synaptic vesicles that are filled with acetylcholine. They resemble small bulbs that will release the neurotransmitter when the muscle needs to contract.

The neuromuscular junction is crucial for life, and they begin forming early in fetal development. As motor neurons develop, a protein called agrin is made. This stimulates the formation of a muscle specific kinase, which will build receptors for acetylcholine on the surface of the muscle fiber. This is how the junction is formed, with the neuron itself emitting the needed chemical for development.

Several conditions can cause malfunctions at these sites, leading to loss of muscle control. Ultimately, lack of muscle control can cause death if it is severe, because the

body's largest muscle, the heart, could stop beating. The most common such illness is myasthenia gravis, which is caused by an autoimmune reaction against acetylcholine receptors. As a result, motor neurons cannot transmit the chemical to the muscles, which will cause muscle weakness and an ultimate loss of control.

Myasthenia gravis tends to be more concentrated along skeletal muscles and is especially noticeable in the face. Additionally, some toxins, such as botulinum, will inhibit the release of acetylcholine, which will lead to muscle paralysis.

Electron micrograph showing a cross section through the neuromuscular junction. T is the axon terminal, M is the muscle fiber. The arrow shows junctional folds with basal lamina. Active zones are visible on the tips between the folds.

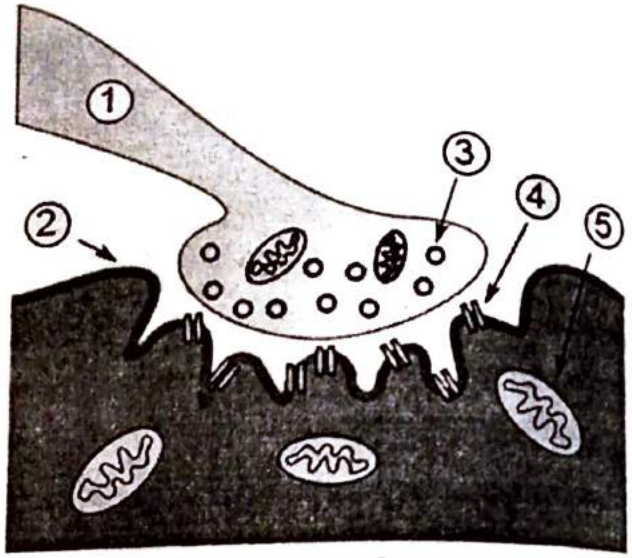


Fig. 3.5 : Detailed view of a neuromuscular junction: 1. Presynaptic terminal, 2. Sarcolemma, 3. Synaptic vesicle, 4. Nicotinic acetylcholine receptor 5. Mitochondrion

DIGESTION AND ABSORPTION

Food is the basic and essential requirement of man for his very existence. The food we eat consists of carbohydrates, proteins Lipids vitamins and minerals. The bulk of the food ingested is in a complex macromolecular from which cannot, as such be absorbed by the body.

Digestion is a process involving the hydrolysis of large and complex organic molecules of food stuff into smaller and preferably water soluble molecules which can be easily absorbed by the gastro intestinal tract for utilization by the organism. Digestion of macro molecules also promotes the absorption of fat soluble vitamins and certain minerals.

Cooking of the food and mastication (in the mouth) significantly improves the digestibility of foodstuffs by the enzymes.

Digestive juices

There are five digestive juices Saliva, gastric juice, pancreatic juice, succus entericus (intestinal juice) and bile. The necessity for so many digestive juices is that

1) One juice does not contain all the enzymes necessary for digesting all the different types of foodstuffs.
2) The second reason is that one particular digestive juice cannot digest a particular type of food up to completion. It will digest only up to a certain stage and then, the product will be handed over to the next digestive juice for further digestion.

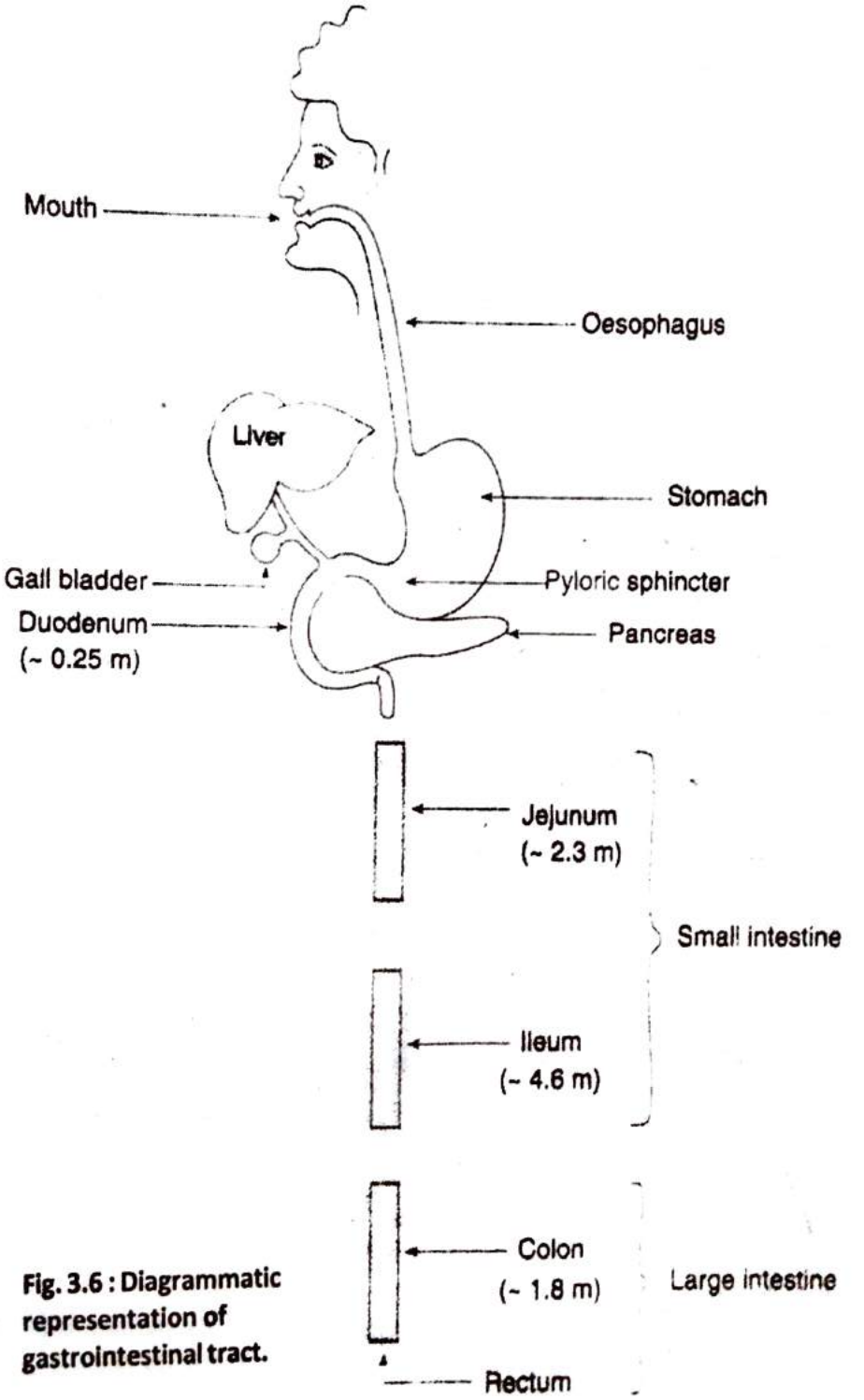


Fig. 3.6 : Diagrammatic representation of gastrointestinal tract.

One more interesting fact about the digestive juices is that their reactions are not all same. Saliva is slightly acid, gastric juice is strongly acid, but pancreatic juice is strongly alkaline. This more or less alternate acid and alkaline reaction, prevents any serious alteration of blood reactions. This is a special device for maintaining blood reaction constant.

The diagrammatic representation of GIT is depicted in fig. 3.6.

CARBOHYDRATES

The principal dietary carbohydrates are polysaccharides (starch, glycogen), disaccharides (lactose, sucrose) and, to a minor extent, mono-saccharides (glucose, fructose).

Starch is a plant polysaccharide composed of amylopectin and amylose. Amylose is the linear sequence of glucose units linked by α-1, 4 glyco-sidic bonds whereas amylopectin that constitutes 80-90% of starch is branched with α-1, 6-linkages at branching points. The ratio of 1, 4 to 1, 6-glycosidic linkages in starch is about 20 : 1. The structure of glycogen (animal starch) closely resembles that of amylopectin; however the branching is more frequent in glycogen.

Digestion

The digestion of carbohydrates occurs briefly in mouth and largely in the intestine. The polysaccharides get hydrated during heating which is essential for their efficient digestion. The hydrolysis of glycosidic bonds is carried out by a group of enzymes called *glycosidases* (Fig. 3.7). These enzymes are specific to the bond, structure and configuration of monosaccharide units.

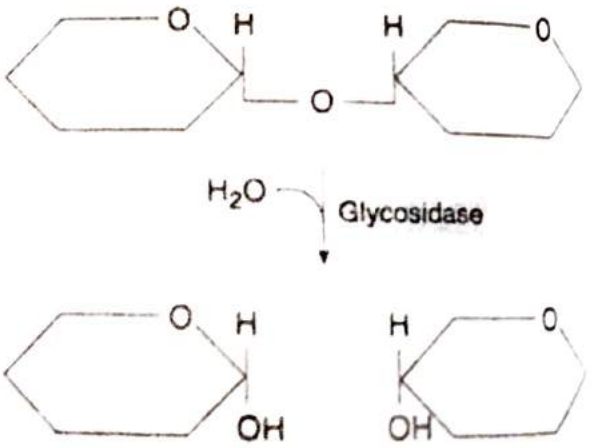


Fig.3.7 : Hydrolysis of a glycosidic bond.

Digestion in the mouth: Carbohydrates are the only nutrients for which the digestion begins in the mouth to a significant extent. During the process of mastication, *salivary α-amylase (ptyalin)* acts, on starch randomly and cleaves α-1, 4 glycosidic bonds. The products formed include α-limit dextrins, (containing about 8 glucose units with one or more α-1, 6 glycosidic bonds) maltotriose and maltose.

Carbohydrates not digested in the stomach:

The enzyme salivary amylase is inactivated by high acidity (low pH) in the stomach. Consequently, the ongoing degradation of starch is stopped.

Digestion in the small intestine:

The acidic dietary contents of the stomach, on reaching small intestine, are neutralized by bicarbonate produced by pancreas. The ***pancreatic α-amylase*** acts on starch and continues the digestion process. Amylase specifically acts on α-1, 4 glycosidic bonds and not on α-1, 6 bonds. The resultant products are disaccharides (maltose, isomaltose) and oligosaccharides (Fig. -3.8).

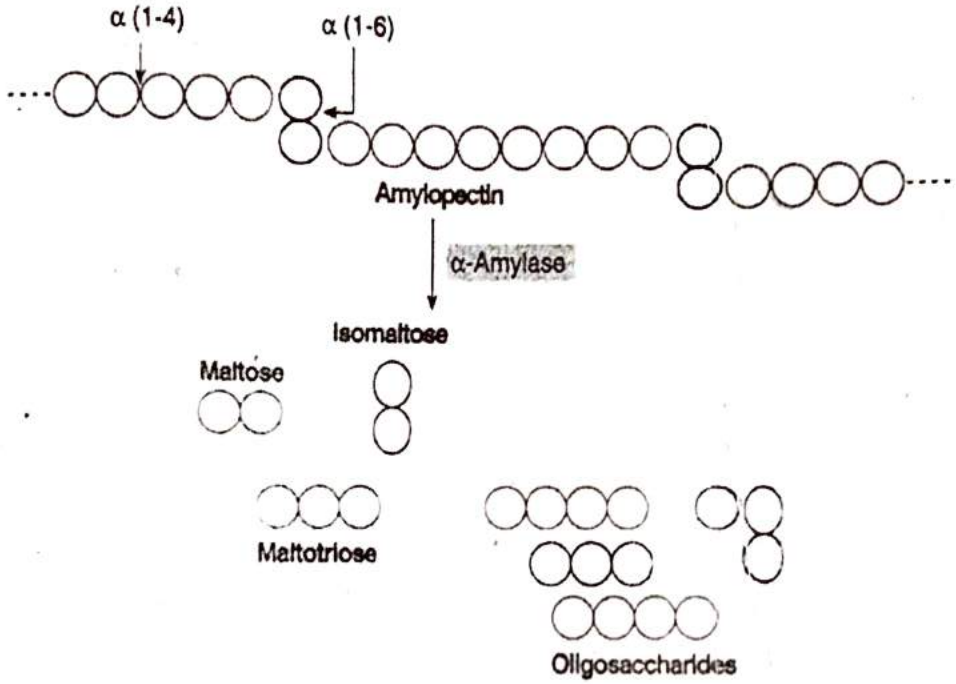


Fig. 3.8 : Degradation of amylopectin by salivary or pancreatic α-amylase.

The final digestion of di- and oligosaccharides to monosaccharides primarily occurs at the mucosal lining of the upper jejunum. This is carried out by ***oligosaccharidases and disaccharidases,*** the details of which are given in Table -1.2. The capacity of most of these enzymes is much greater than required for the digestion of the respective substrates. Particularly important is the enzyme ***sucrase*** which is capable of hydrolyzing a large quantity of table sugar (sucrose). In contrast, ***lactase (β-galactosidase)*** is the rate-limiting, and, consequently, the utilization of milk sugar (lactose) is limited in humans.

Table 1.2 Oligo- and disaccharidases with their respective substrates and products

Enzyme	Specificity	Substrate(s)	Product(s)
Glucoamylase	α-1, 4-Glucose	Amylose	Glucose
Isomaltase	α-1, 6-Glucose	Isomaltose, α-dextrin	Glucose
Maltase	α-1, 4-Glucose	Maltose, maltotriose	Glucose
Sucrase	α-Glucose	Sucrose	Glucose, fructose
Lactase	β-Galactose	Lactose	Glucose, galactose
Trehalase	α-1, 1-Glucose	Trehalose*	Glucose

* A non-reducing disaccharide found in young mushrooms

Absorption of monosaccharides

The principal monosaccharides produced by the digestion of carbohydrates are glucose, fructose and galactose. Of these, glucose accounts for nearly 80% of the total monosaccharides. The absorption of sugars mostly takes place in the *duodenum* and *upper jejunum* of small intestine.

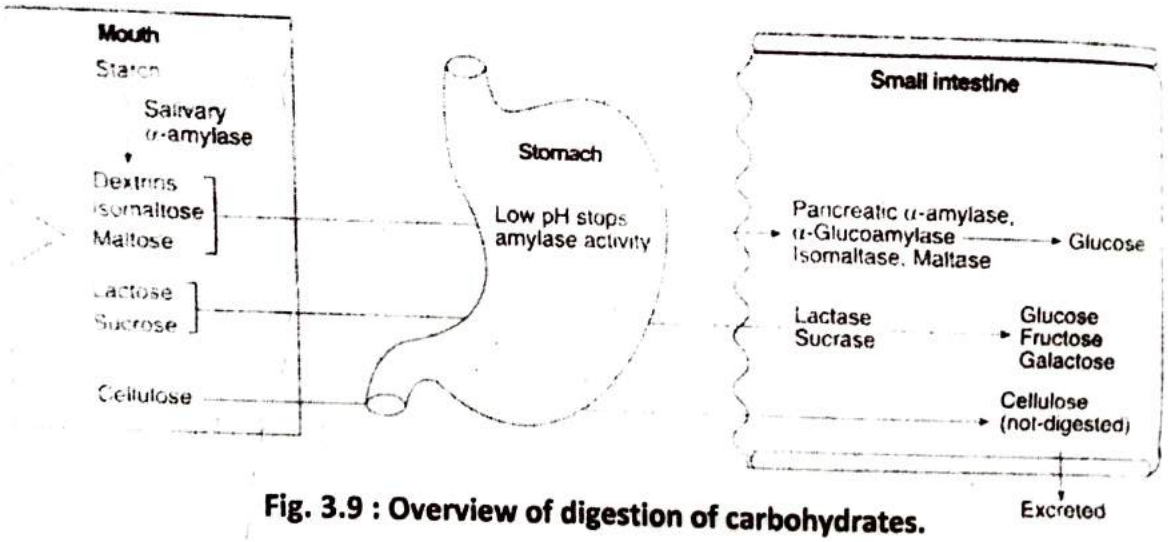


Fig. 3.9 : Overview of digestion of carbohydrates.

There exists a considerable variation in the absorption of different monosaccharides. The relative rates of absorption of important monosaccharides in comparison with glucose are given below

Glucose	–	100
Galactose	–	110
Fructose	–	43
Mannose	–	20
Xylose	–	15
Arabinose	–	09

It is observed that hexoses are more rapidly absorbed than pentoses. Further, among the monosaccharides, *galactose is most efficiently* absorbed followed by glucose and fructose. Insulin has no effect on the absorption of sugars.

Mechanism of absorption

Different sugars possess different mechanisms for their absorption. Glucose is transported into the intestinal mucosal cells by a carrier mediated and energy requiring process (Fig.3.10).

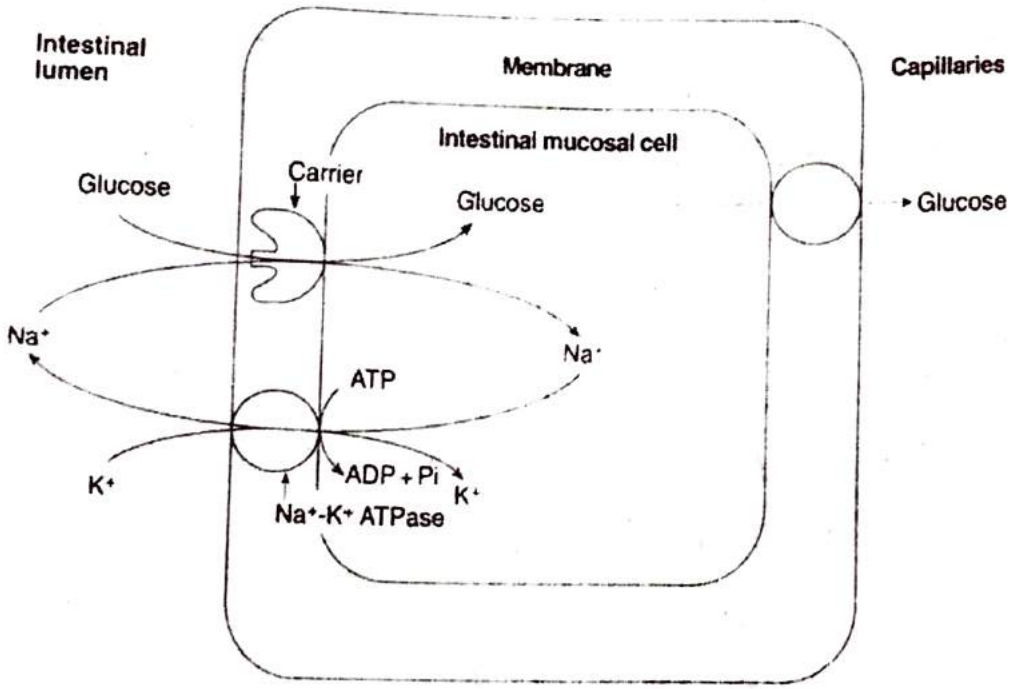


Fig. 3.10 : Transport of glucose across intestinal epithelium (Note: Transport amino acids also occurs by a similar mechanism; replace glucose in figure by amino acid).

Glucose* and Na** share the same transport system (symport). The concentration of Na^+ is higher in the intestinal lumen compared to mucosal cells. Na^+, therefore, moves into the cells along its concentration gradient and simultaneously glucose is transported into the intestinal cells. This is mediated by the same carrier system. Thus, Na^+ diffuses into the cell and it drags glucose along with it. The intestinal Na^+ gradient is the immediate energy source tor glucose transport. This energy is indirectly supplied by ATP since the reentry of Na^+ (against the concentration gradient) into the intestinal lumen is an energy-requiring active process. The enzyme ***Na^+-K^+ ATPase is involved in the transport of Na^+ in exchange of K^+ against the concentration gradient. The reabsorption of glucose that occurs in the proximal convoluted tubules of kidney is also mediated by Na^+ and the mechanism is similar to that described.

Once within the mucosal cell, glucose is transported into the capillaries by a facilitated passive diffusion. The maximum rate of glucose absorption is about 120 g/h.

The mechanism of absorption of galactose is similar to that of glucose. The inhibitor ***phlorizin*** blocks the Na^+ dependent transport of glucose and galactose.

Absorption of fructose :

Fructose absorption is relatively simple, It does not require energy and is independent of Na^+ transport- Fructose is transported by facilitated diffusion mediated by a carrier. Inside the epithelial cell, most of the fructose is converted to glucose. The latter then enters the circulation.

Pentoses are absorbed by a process of simple diffusion.

Non-digestive carbohydrates :

The plant foods are rich in fibrous material which cannot be digested either by the human enzymes or intestinal bacteria. The *fibers* are chemically complex carbohydrates which include cellulose, hemicellulose, pectins, lignin and gums.

Abnormalities of carbohydrate digestion :

In general, humans possess an efficient system of carbohydrate digestion and absorption. Since only the monosaccharides are absorbed, any defect in the activities of ***disaccharidases*** results in the passage of undigested disaccharides into the large intestine The disaccharides draw water from the intestinal mucosa by osmosis and cause osmotic diarrhea. Further, bacterial action of these undigested carbohydrates leads to flatulence

Disaccharidases are the intestinal brush border enzymes. Any alteration in the mucosa of the small intestine caused by severe diarrhea, malnutrition, intestinal diseases or drug therapy will lead to a temporary acquired deficiency of disaccharidases. The patients with such disorders are advised to restrict the consumption of sucrose and lactose.

Hereditary disorder with deficiency of individual disaccharidases in infants and children causes intolerance of specific disaccharides.

Lactose intolerance

Defect in the enzyme ***lactase*** (β-galactosidase) is the most common disaccharidase deficiency in humans. It is estimated that more than half of the world's adult population is affected by lactose intolerance. It is more commonly found in Africans (blacks) and Asians compared to Europeans. Surprisingly, according to a recent estimate, about 90%

of the adult Asians are lactase deficient. The mechanism of how lactase is lost in adults is not clear. It is however, known that there is a reduced production of lactase rather than an alteration in enzyme activity.

The treatment of lactose intolerance is quite simple. Elimination of lactose from the diet (severe restriction of milk and dairy products) will solve the problem.

Continued consumption of lactose by lactose intolerant individuals causes typical symptoms of ***flatulence***.

Sucrase deficiency

The deficiency of the enzyme sucrase causes intolerance to dietary sucrose. It is estimated that about 10% of Eskimos of Greenland and 2% of North Americans are affected by this disorder. The treatment is to remove sucrose from the diet.

The problem of flatulence

Flatulence is characterized by increased intestinal motility, cramps and irritation. This Occurs after ingestion of certain carbohydrates and is explained as follows.

The carbohydrates (di-, oligo-, and polysaccharides) not hydrolyzed by α-amylase and other intestinal enzymes cannot be absorbed. Lactose is not hydrolyzed in some individuals due to the deficiency of lactase. The di-, and oligosaccharides can be degraded by the bacteria present in ileum(lower part of small intestine) to liberate monosaccharides. The latter can be metabolized by the bacteria

During the course of utilization of monosaccharides by the intestinal bacteria, the gases such as ***hydrogen, methane and carbon dioxide***—

besides lactate and short chain fatty acids—are released. These compounds cause flatulence.

The occurrence of flatulence after the ingestion of ***leguminous*** seeds (bengal gram, redgram, beans, peas, soya bean) is very common. They contain several **non *digestible oligosaccharides*** by human intestinal enzymes. These compounds are degraded and utilized by intestinal bacteria causing flatulence ***Raffinose*** containing galactose, glucose and fructose is a predominant oligosaccharide found in leguminous seeds.

PROTEINS

The proteins subjected to digestion and absorption are obtained from two sources—dietary and endogenous.

The intake of dietary protein is in the range of 50-100 g/day. About 30-100 g/day of endogenous protein is derived form the digestive enzymes and worn out cells of the digestive tract. The digestion and absorption of proteins is very efficient in healthy humans, hence very little protein (about 5-10 g/day) is lost through feces. Dietary proteins are denatured on cooking and therefore, easily digested.

Proteins are degraded by a class of enzymes—namely ***hydrolases***—which specifically cleave the peptide bonds, hence known as peptidases. They are divided into two groups.

1. **Endopeptidases (proteases)** which attack the internal peptide bonds and release peptide fragments, e.g. pepsin, trypsin.
2. **Exopeptidases** which act on the peptide bonds of terminal amino acids. Exopeptidases are subdivided into ***carboxypeptidases*** (act on C- terminal ammo acid) and ***ammopeptidases*** (act on N-terminal amino acid).

The proteolytic enzymes responsible for the digestion of proteins are produced by the stomach, the pancreas and the small intestine. Proteins are not digested in the mouth due to the absence of proteases in saliva.

I. Digestion of protein's by gastric secretion

Protein digestion begins in the stomach. Gastric juice produced by stomach contains hydrochloric acid and a protease proenzyme namely pepsinogen.

Hydrochloric acid : The pH of the stomach is < 2 due to the presence of HCl, secreted by parietal (oxyntic) cells of gastric gland. This acid performs two important functions- denaturation of proteins and killing of certain microorganisms. The denatured proteins are more susceptible to proteases for digestion.

Pepsin : Pepsin *(Greek :* pepsis—digestion) is produced by the serous cells of the stomach as pepsinogen, the inactive zymogen or proenzyme, Pepsinogen is converted to active pepsin either by autocatalysis brought about by other pepsin molecules or by gastric HCl pH < 2). Removal of a fragment of polypeptide chain 44 ammo acids in case of pig enzyme) makes the inactive enzyme active after attaining a proper conformation.

Pepsin is an acid-stable endopeptidase optimally active at a very low pH (2.0). The active site of the enzyme contains two carboxyl groups, which are maintained at low pH. Pepsin A is the most predominant gastric protease which preferentially cleaves peptide bonds formed by amino groups of phenylalanine or tyrosine or leucine.

Pepsin digestion of proteins results in peptides and a few amino acids which act as stimulants for the release of the hormone cholecystokinin from the duodenum.

Rennin : This enzyme, also called chymosin, is found in the stomach of infants and children. Rennin is involved in the curdling of milk. It converts milk protein casein to calcium paracaseinate which can be effectively digested by pepsin. Rennin is absent in adults.

II. Digestion of proteins by pancreatic proteases

The proteases of pancreatic juice are secreted as zymogens (proenzymes) and then converted to active forms These processes are initiated by the release of two polypeptide hormones, namely ***cholecystokinin*** and **secretin** from the intestjne (Fig. 3.11)

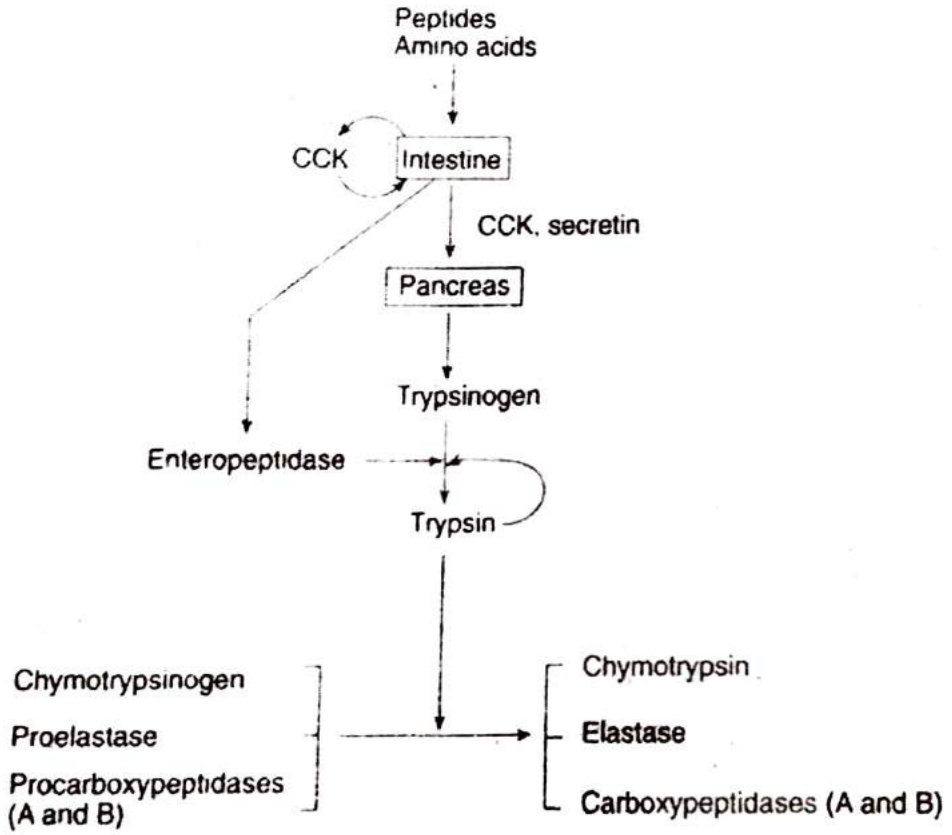


Fig. 3.11 : Formation and activation of pancreatic proteases (CCK-Cholecystokinin).

Release and activation of zymogens : The key enzyme for activation of zymogen is ***enteropeptidase*** (formerly enterokinase) produced by intestinal (mostly duodenal) mucosal epithelial cells. Enteropeptidase cleaves off a hexapeptide (6 amino acid fragment) from the N-terminal end of trypsinogen to produce trypsin, the active enzyme. ***Trypsin*** in turn, activates other trypsinogen molecules (autocatalysis). Further, trypsin is the common activator of all other pancreatic zymogens to produce the active proteases, namely chymotrypsin, elastase and carboxypeptidases (A and B).

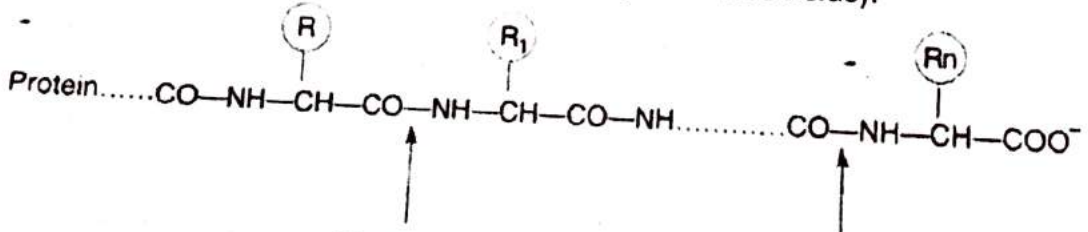
Specificity of pancreatic proteases : Trypsin, chymotrypsin and elastase are endopeptidases active at neutral pH. Gastric HCl is neutralized by pancreatic $NaHCO_3$ in the intestine and this creates favourable pH for the action of proteases.

The substrate specificity of pancreatic proteases is depicted in Fig.3.12 For instance, trypsin cleaves the peptide bonds, the carbonyl (—CO—) group of which is contributed by arginine or lysine.

Mechanism of action of pancreatic proteases : The amino acid serine is essential at the active centre to bring about the catalysis of all the three panacreatic proteases, hence these enzymes are referred to as ***serine proteases***. The enzyme inhibitor diisopropyl fluorophosphate binds with serine residue of the proteases and inactivates.

Action of carboxypeptidases : The pancreatic carboxypeptidases (A and B) are metalloenzymes (that are dependent on Zn^{2+}, for their catalytic activity, hence, they are sometimes called Zn-proteases. They also possess certain degree of substrate specificity in their action. For example, carboxypeptidase B acts on peptide bonds of COOH-terminal amino acid, the amino group of which is contributed by arginine or lysine (Fig.3.12).

The combined action of pancreatic proteases results in the formation of free amino acids and contains small peptides (2-8 amino acids).



Enzyme	Nature of R	Enzyme	Nature of Rn
Pepsin A	Tyr, Phe, Leu	Carboxypeptidase A	Ala, Ile, Leu, Val
Trypsin	Arg, Lys	Carboxypeptidase B	Arg, Lys
Chymotrypsin	Trp, Tyr, Phe, Leu, Met		
Elastase	Ala, Gly, Ser		

Fig.3.12 : Digestion of proteins—Specificity of enzyme cleavage of peptide bonds.

III. Digestion of proteins by small intestinal enzymes

The luminal surface of intestinal epithelial cells contains Aminopeptidases and dipeptidases- Aminopeptidase is a non-specific exopeptidase which repeatedly cleaves N-terminal amino acids one by one to produce free amino acids and smaller peptides. The dipeptidases act on different dipeptides to liberate amino acids.

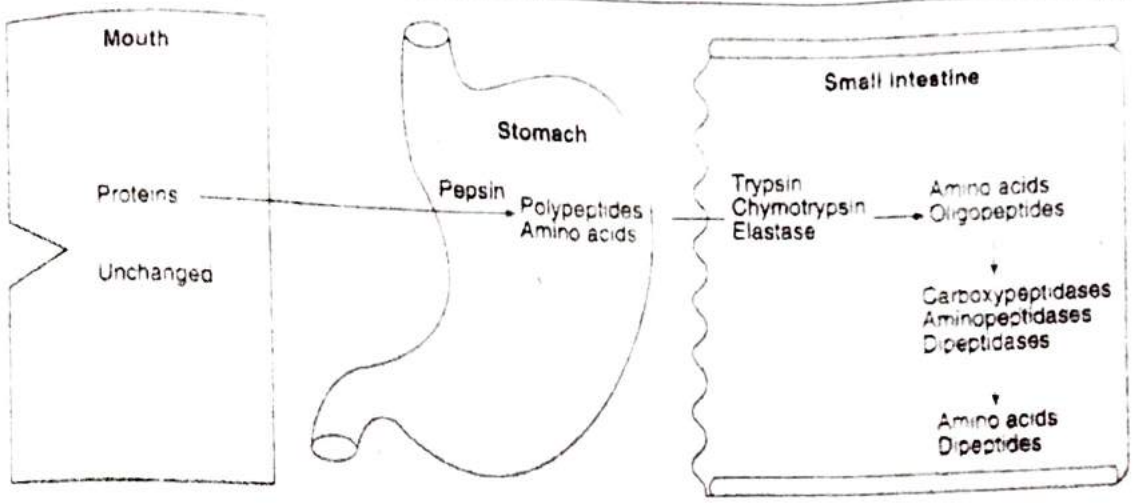


Fig.3.13 : Overview of digestion of proteins.

Absorption of amino acids and dipeptides

The free amino acids, dipeptides and to some extent tripeptides are absorbed by intestinal epithelial cells.

The di- and tripeptides, after being absorbed are hydrolysed into free amino acids in the cytosol of epithelial cells. The activities of dipeptidases are high in these cells. Therefore, ***after a protein meal, only the free amino acids are found in the portal vein.***

The small intestine possesses an efficient system to absorb free ammo acids. L-Amino acids are more rapidly absorbed than D-amino acids. The transport of L-amino acids occurs by an active process (against a concentration gradient), in contrast to D-amino acids which takes place by a simple diffusion.

Transport systems of amino acid absorption

At least six transport systems responsible for the absorption of amino acids have been identified

1. For short chain neutral amino acids (Ala, Ser, Thr).
2. For long chain, neutral and aromatic amino acids (Val, Leu, Ileu, Met, Phe, Tyr).
3. For acidic amino acids (Asp, Glu).
4. For basic amino acids and cystine (Lys, Arg, Cys-Cys).
5. For imino acids (Pro, Hyp).
6. For β-amino acids β-Ala, taurine),

Mechanism of amino acid absorption

Amino acids are primarily absorbed by a similar mechanism, as described for the transport of D-glucose. It is basically a Na^+-dependent active process linked with the transport of Na^+. As the Na^+ diffuses along the concentration gradient, the amino acid also enters the intestinal cell. Both Na^+ and amino acids share a common carrier and are transported together. The energy is supplied indirectly by ATP (for details, see absorption of monosaccharides and Fig. 3.10).

A Na^+-independent system of amino acid transport across intestinal cells has also been identified. The compound ***cytochalasin B*** inhibits Na^+-independent transport system.

Another transport system to explain the mechanism of amino acid transfer across membrane in the intestine and kidney has been put forth. This is known as ***γ-glutamyl cyle or Meister cycle*** (Fig. 3.13) and involves a tripeptide namely glutathione (γ-glutamylcysteinylglycine).

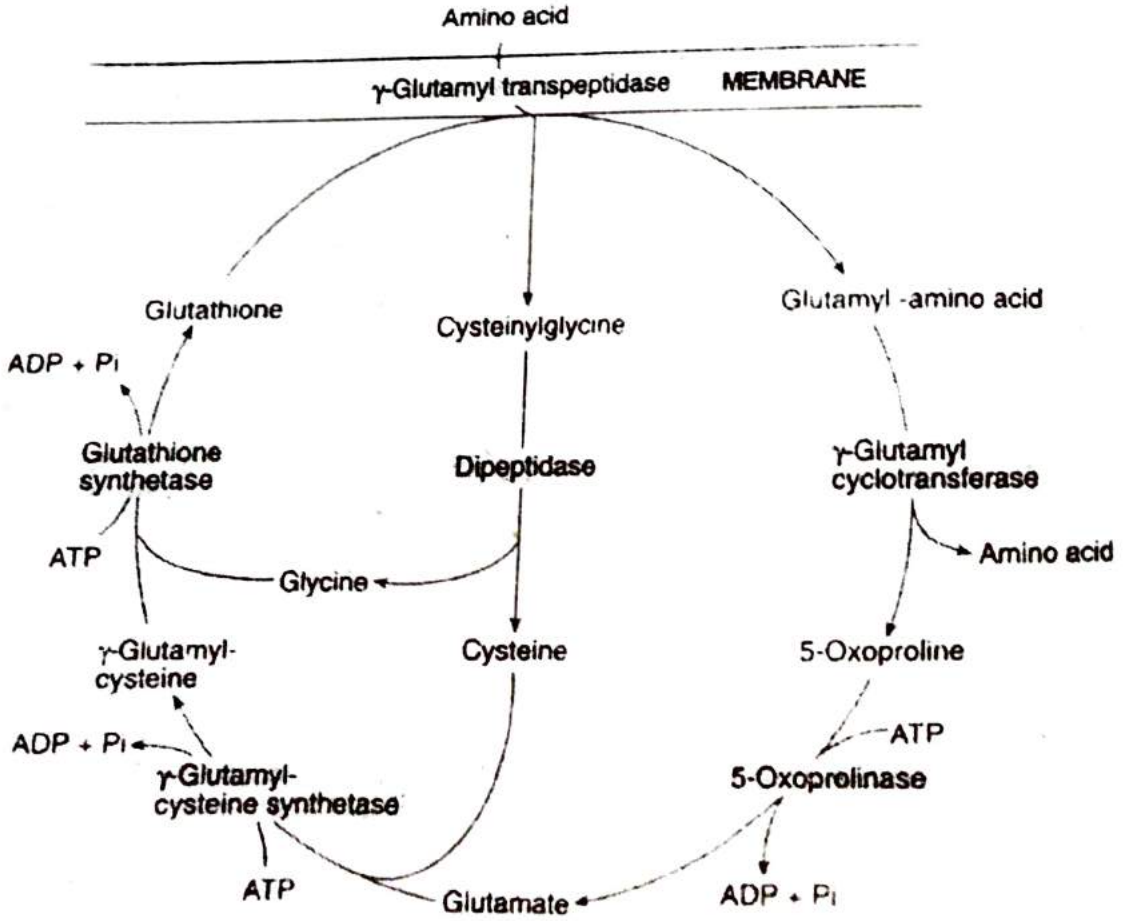


Fig.3.13 : The γ-glutamyl cycle (Meister cycle).

The free ammo acid is transferred to glutathione to form γ-glutamyl amino acid and cysteinyl-glycine. This reaction is catalysed by a membrane bound enzyme

γ-glutamyl transpeptidase. In the cytosol, bound amino acid (being transported) is freed and the γ-glutamyl residue is converted to 5-oxoproline which then forms glutamate by an ATP dependent reaction. Glutamate is converted back to glutathione by first combining with cysteine and then with glycine. Both these reactions require ATP. Thus 3 ATP are utilized for the transport of a single molecule of an amino acid which appears to be a needless extravagance. However, this may be necessary for the rapid transport of certain amino acids, namely cysteine and glutamine.

The γ-glutamyl cycle appears to be important for the metabolism of glutathione, since this tripeptide undergoes rapid turnover in the cells. There may be more physiological significance of γ-glutamyl cycle.

Absorption of intact proteins and polypeptides

For a short period, immediately after birth, the small intestine of infants can absorb intact proteins and polypeptides. The uptake of proteins occurs by a process known as ***endocytosis*** *or* ***pinocytosis***. The macromolecules are ingested by formation of small vesicles of plasma membrane followed by their internalization. The direct absorption of intact proteins is very important for the transfer of maternal immunoglobulins (γ-globulins) to the offspring.

The intact proteins and polypeptides are not absorbed by the adult, intestine However, the macromolecular absorption in certain individuals appears to be responsible for antibody formation that often causes **food allergy**.

Abnormalities of protein digestion and amino acid absorption

Any defect in the pancreatic secretion impairs protein and fat digestion. This causes the loss of undigested protein in the feces along with the abnormal appearance of lipids. Deficiency of pancreatic secretion may be due to pancreatitis, cystic fibrosis or surgical removal of pancreas.

Hartnup's disease (neutral amino aciduria)

Hartnup is the name of the family in whom this disease was first discovered. It is characterized by the inability of intestinal and renal epithelial cells to absorb neutral amino acids. Tryptophan absorption is most severely affected with a result that typical symptoms of *pellagra* are observed in the patients of Hartnup's disease. This is related to the impairment in the conversion of tryptophan to NAD^+ and $NADP^+$ the coenzymes of niacin.

LIPIDS

There is considerable variation in the daily consumption of lipids which mostly depends on the economic status and dietary habits. The intake of lipids is much less (often < 60 g/day) in poorer sections of the society, particularly in the less developed countries. In the developed countries, an adult ingests about 60-150 g of lipids per day. Of this, more than 90% is fat (triacylglycerol). The rest of the dietary lipid is made up of phospholipids, cholesterol, cholesteryl esters and free, fatty acids. In addition to the dietary intake, about 1-2 g cholesterol and 3-5 g. phospholipids (mostly lecithin) are daily secreted into the intestine through bile.

Lipids are insoluble *or* sparingly soluble in aqueous solution. The digestive enzymes, however, are present in aqueous medium. This poses certain problems for the digestion and absorption of lipids. Fortunately, the digestive tract possesses specialized machinery to

1. Increase the surface area of lipids for digestion;
2. Solubilize the digested products for absorption.

Minor digestion of lipids in the stomach

The digestion of Lipids is initiated in the stomach, catalysed by ***acid-stable lipase***. This enzyme (also called lingual lipase) is believed to originate from the glands at the back of tongue. Stomach contains a separate *gastric lipase* which can degrade fat containing short chain fatty acids at neutral pH. The digestion of lipids in the stomach of an adult is almost negligible, since lipids are not emulsified and made ready for lipase action. Further, the low pH in the stomach is unfavourable for the action of gastric lipase.

In case of infants, the milk fat (with short chain fatty acids) can be hydrolysed by gastric lipase to some extent This is because the stomach pH of infants is close to neutrality, ideal for gastric lipase action.

Emulsification of lipids in the small intestine

Emulsification is the phenomenon of dispersion of lipids into smaller droplets due to reduction in the surface tension. This is accompanied by increase in the surface area of lipid droplets. Emulsification is essential for effective digestion of lipids, since the enzymes can act only on the surface of lipid droplets. More correctly, lipases act at the interfacial area between the aqueous and lipid phase.

The process of emulsification occurs by three complementary mechanisms

1. Detergent action of bile salts;
2. Surfactant action of degraded lipids;
3. Mechanical mixing due to peristalsis.

1. **Bile salts** : The terms bile salts and bile acids are often used interchangeably. At physiological pH, the bile acids are mostly present as anions. Bile salts are the biological detergents synthesized from cholesterol in the liver. They are secreted with bile into the duodenum. Bile salts possess steroid nucleus, the side chain of which is attached to either ***glycine (glycocholic acid)*** or ***taurine (taurocholic acid)***.

Bile salts are the most effective biological emulsifying agents. They interact with lipid particles and the aqueous duodenal contents and convert them into smaller particles (emulsified droplets). Further, bile salts stabilize the smaller particles by preventing them from coalescing.

2. **Surfactant action of degraded lipids** : The initial digestive products of lipids (catalysed by lipase) namely free fatty acids, monoacylglycerols promote emulsification. These compounds along with phospholipids are known as surfactants, They are characterized by possessing polar and non-polar groups. Surfactants get absorbed to the water-lipid interfaces and increase the interfacial area of lipid droplets. Thus the initial action of the enzyme lipase helps in further digestion of lipids.

3. Besides the action of bile salts and surfactants, the ***mechanical mixing due to peristalsis*** also helps in the emulsification of lipids. The smaller lipid emulsion droplets are good substrates for digestion.

Digestion of lipids by pancreatic enzymes

The pancreatic enzymes are primarily responsible for the degradation of dietary triacyl-glycerols, cholesteryl esters and phospholipids.

Degradation of triacylglycerols (fat)

Pancreatic lipase is the major enzyme that digests dietary fats. This enzyme preferentially cleaves fatty acids (particularly long chain, above 10 carbons! at position 1 and 3 of triacylglycerols. The products are ***2-monoacylglycerol*** (formerly 2-monoglyceride) and ***free fatty acids*** (Fig. 3.14). The activity of pancreatic lipase is inhibited by bile acids which are present along with the enzyme in the small intestine. This

problem is overcome by a small protein, ***colipase*** (mol. wt. 12,000). It is also secreted by pancreas as procolipase and converted to active form by trypsin. Colipase binds at the lipid-aqeuous interface and helps to anchor and stabilize lipase.

Lipid esterase is a less specific enzyme present in pancreatic juice. It acts on monoacylglycerols, cholesteryl esters, vitamin esters etc. to liberate free fatty acids. The presence of bile acids is essential for the activity of lipid esterase.

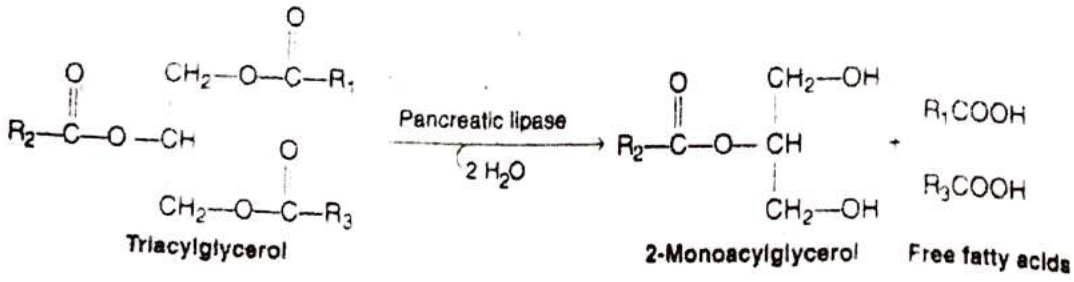


Fig.3.14 : Enzymatic cleavage of dietary fat.

Degradation of cholesteryl esters

A specific enzyme namely pancreatic *cholesterol esterase* (cholesteryl ester hydrolase) cleaves cholesteryl esters to produce cholesterol and free fatty acids (Fig. 3.15).

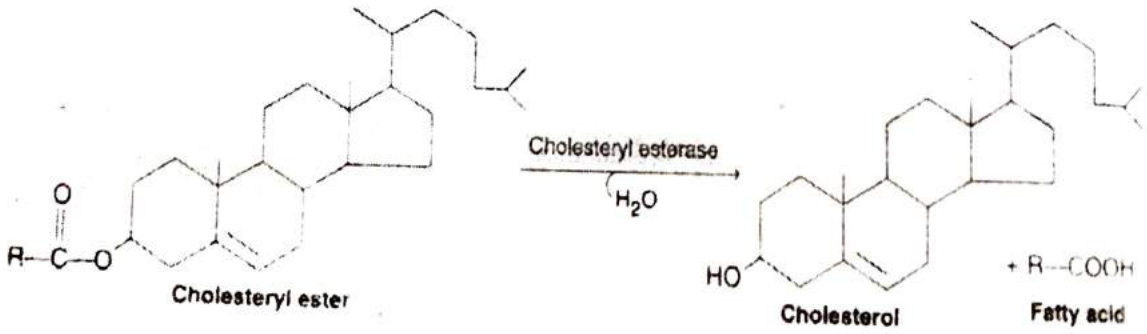


Fig.3.15 : Enzymatic cleavage of cholesteryl ester.

Degradation of phospholipids

Phospholipases are enzymes responsible for the hydrolysis of phospholipids. Pancreatic juice is rich in phospholipase A_2 which cleaves the fatty acid at the 2nd position of phospholipids. The products are a .free fatty acid and a lysophospholipid. Phospholipase A_2 is secreted as a zymogen which is activated in the intestine by the action of trypsin.

The degradation of lecithin (phosphotidyl-choline) is depicted in Fig. 3.16. The product lysolecithin is further hydrolysed by lysophospholipase to fatty acid and glycerylphosphoryl choline. The latter may be further degraded and absorbed or secreted in the feces.

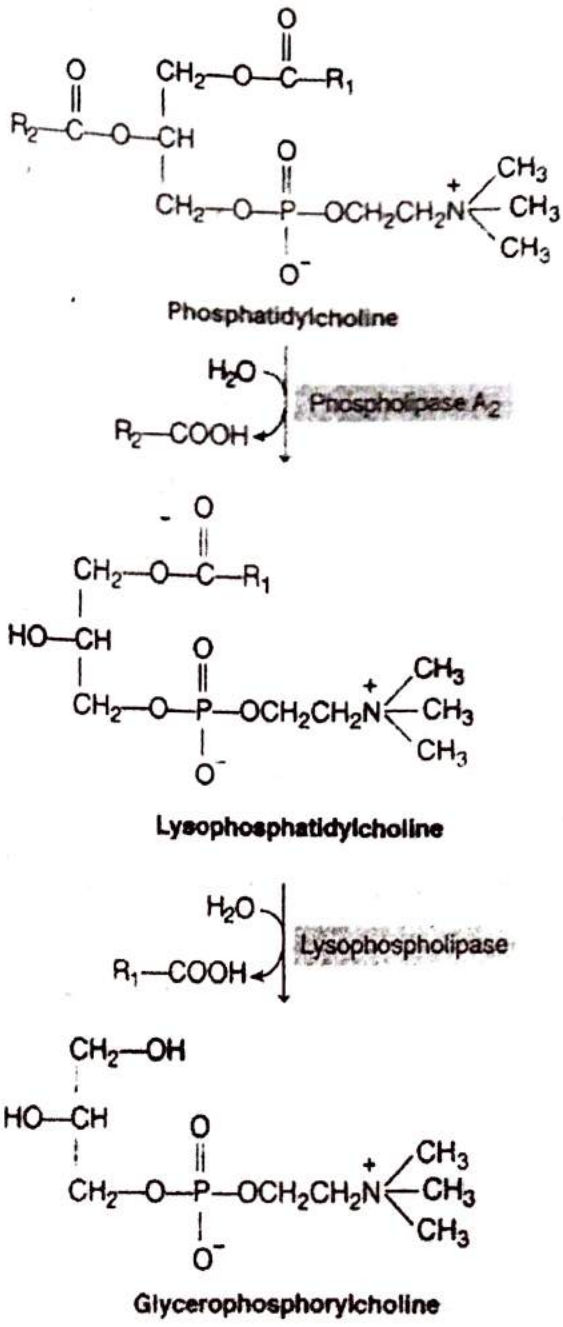


Fig.3.16 : Enzymatic cleavage of phosphotipid.

Absorption of lipids

The former and present theories to explain the absorption of lipids are briefly described hereunder

1 **Lipolytic theory put forth by Verzar** : According to this, fats are completely hydrolysed to glycerol and free fatty acids. The latter are absorbed either as soaps or in association with bile salts.

2 **Partition theory proposed by Frazer** : This theory states that the digestion of triacylglycerols is partial and not complete. The partially digested triacylglycerols, in association with bile salts, form emulsions. The lipids are taken up by the intestinal mucosal cells. As per this theory, resynthesis of lipids is not necessary for their entry into the circulation.

3. **Bergstrom theory :** This is a more recent and comprehensive theory to explain lipid absorption. It has almost replaced the earlier theories and is briefly described hereunder

The primary products obtained from the lipid digestion are 2-monoacylglycerol, free fatty acids and free cholesterol.

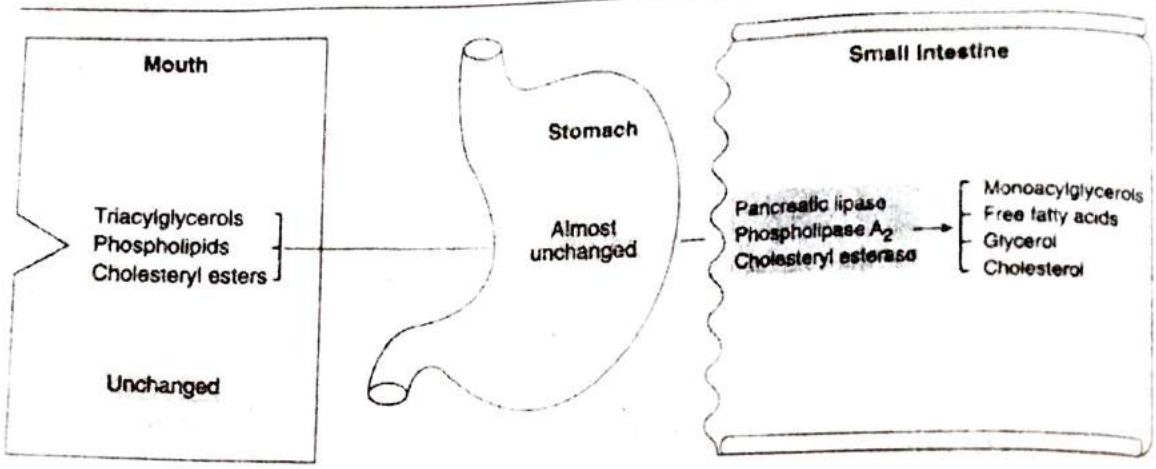


Fig. 3.17 : Overview of digestion of lipids.

Role of bile salts in lipid absorption

Besides their participation in digestion, bile salts are essential for absorption of lipids. Bile salts form ***mixed micelles*** with lipids. These micelles are smaller in size than the lipid emulsion droplets (utilized for digestion, described above). The micelles have a disk like shape with lipids (monoacylglycerol, fatty acids, cholesterol and phospholipids) at the interior and bile salts at the periphery. The hydrophilic groups of the lipids are oriented to the outside (close to the aqueous environment) and the hydrophobic groups to the inside. In this fashion, the bile salt micelles exert a solubilizing effect on the lipids.

Mechanism of lipid absorption

The mixed micelles serve as the major vehicles for the transport of lipids from the intestinal lumen to the membrane of the intestinal mucosal cells, the site of lipid absorption. The lipid components pass through the unstirred fluid layer and are absorbed through the plasma membrane by diffusion (Fig. 3.18). Absorption is almost complete for monoacylglycerols and free fatty acids which are slightly water soluble. However (for water insoluble lipids, the absorption is incomplete. For instance, less than 40% of the dietary cholesterol is absorbed.

The micelle formation is also essential for the absorption of fat soluble vitamins, particularly vitamins A and K.

The efficiency of lipid absorption is dependent on the quantity of bile salts to solubilize digested lipids in the mixed micelles. It may, however, be noted that in the absence of bile salts, the lipid absorption occurs to a minor extent. This is mostly due to

the slightly water soluble nature of monoacylglycerols and free fatty acids. Further, short and medium chain fatty acids are not dependent on micelle formation for the absorption.

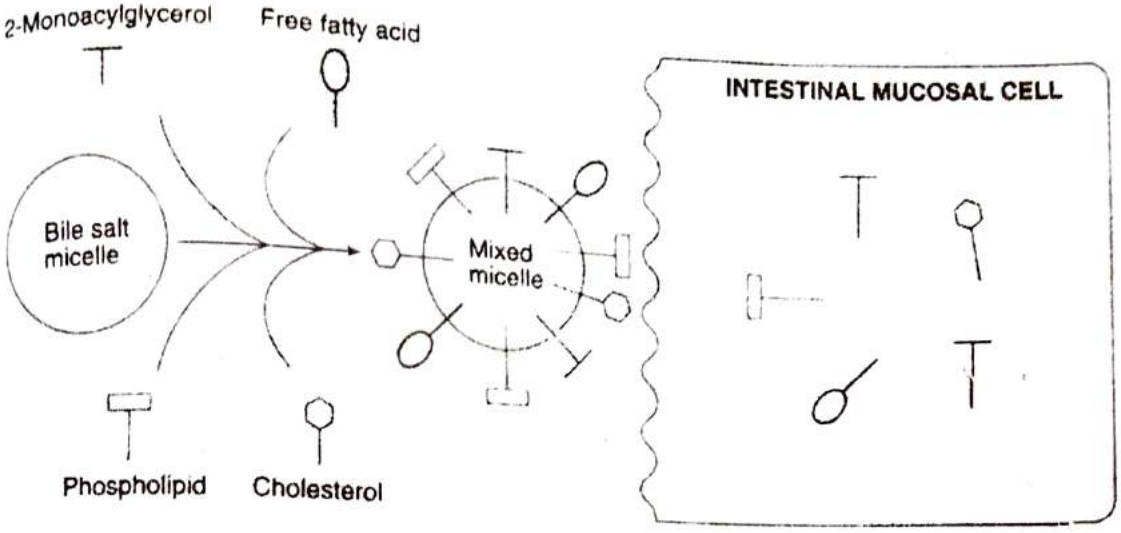


Fig. 3.18 : Absorption of lipids by intestinal mucosal cells.

Synthesis of lipids in the intestinal mucosal cells

The fatty acids of short and medium chain length (< 10 carbons), after their absorption into the intestinal cells, do not undergo any modification. They enter the portal circulation and are transported to the liver in a bound form to albumin.

The long chain fatty acids are activated by ***thiokinase*** (fatty acyl CoA synthetase) in the intestinal cells. The acyl CoA derivatives so formed combine with 2-monoacyl-glycerols to produce ***triacylglycerols***. These reactions are catalysed by a group of enzymes, namely acyltransferases. Further, within the intestinal cells, cholesterol is converted to cholestrylester and phospholipids are regenerated from the absorbed lysophospholipids. The newly synthesized lipids are usually different from those consumed in the diet.

Secretion of lipids from the intestinal mucosal cells

The lipids that are resynthesized in the intestinal cells are hydrophobic in nature. They are put together as lipid droplets and surrounded by a thin layer consisting of mostly apolipoproteins (A_1 and B-48) and phospholipids. This package of lipids enveloped in the layer stabilizes the droplets and increases their solubility. These particles are known as ***chylomicrons***.

Chylomicrons migrate to the plasma membrane of intestinal mucosal cells. They are released into the lymphatic vessels by exocytosis. The presence of chylomicrons *(Greek:* chylos—juice) gives the lymph a milky appearance, which is observed after a lipid-rich meal. Chylomicrons enter the large body veins via the thoracic duct. Blood from here flows to the lungs and then to the peripheral tissues (muscle, adipose tissue) and, finally, to the liver. Adipose tissue and muscle take up a large proportion of dietary lipids from chylomicrons for storage and transport. It is believed that this bypass arrangement (passage of chylomicrons through peripheral tissues) protects the liver from a lipid overload after a meal.

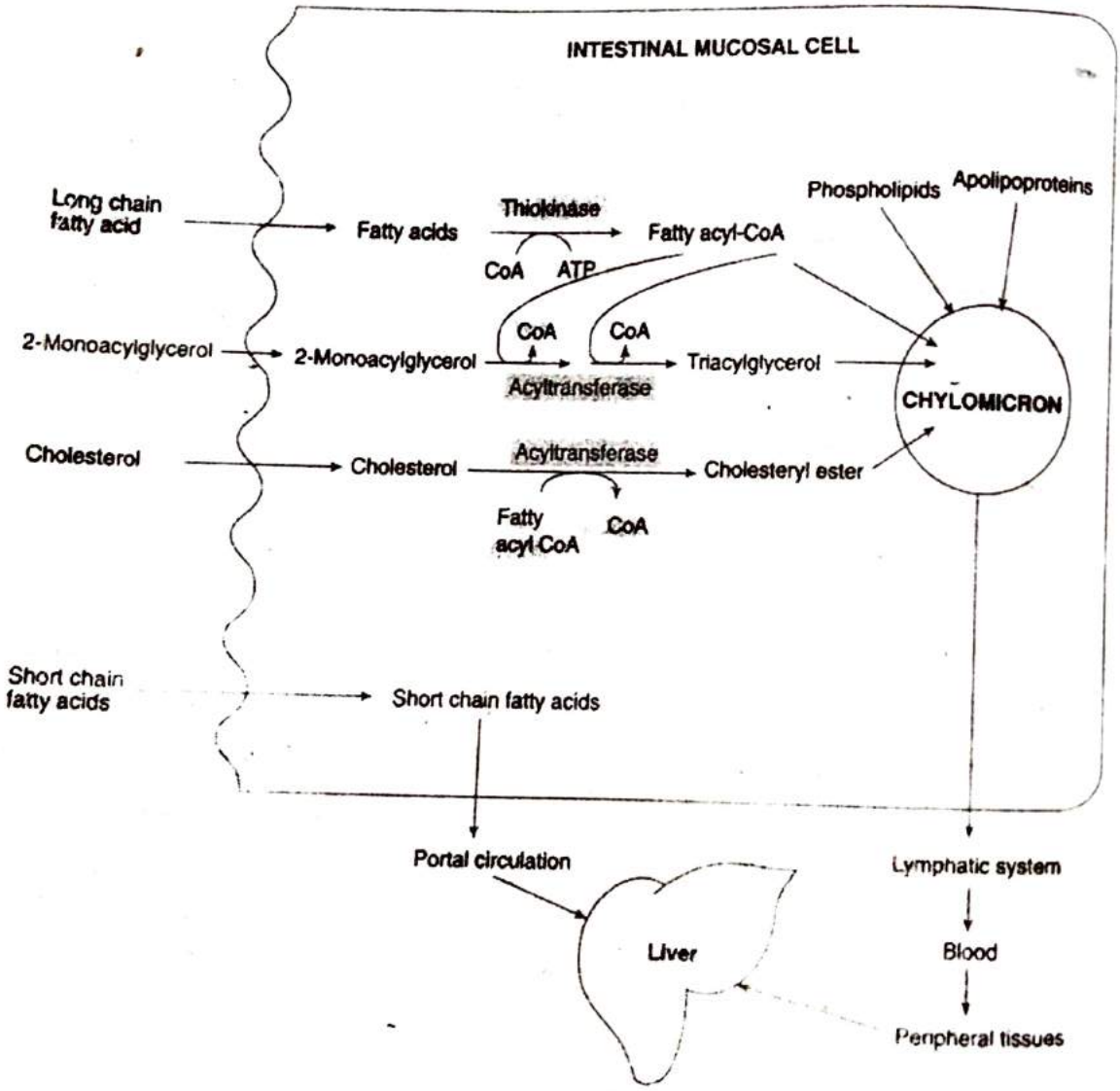


Fig. 3.19 : Formation and secretion of chylomicrons by intestinal mucosal cells.

CHAPTER 4

MEMBRANE STRUCTURE & TRANSPORT

CELL MEMBRANE (PLASMA MEMBRANE)

- The cell is enveloped by a thin membrane called cell membrane or plasma membrane.
- Cell membrane separate cells from their external environment and divide the interior of the cell into compartments. They are 75 to 90 $\mathring{A}$ thick.
- The fluid outside the cell membrane is called extra cellular fluid (ECF), while that inside the cell, covered by the cell membrane is called intracellular fluid (ICF).

STRUCTURE AND CHEMICAL COMPOSITION

- Cell membranes mainly consist of lipids, proteins and smaller proportion of carbohydrates that are linked to lipids and proteins. The ratio of protein to lipid can vary widely from 1:4 to 4:1
- The chemical composition of cell membranes varies widely as shown in Table 4.2
- The basic organisation of biologic membranes is illustrated in Figure 4.3
- Electron microscopy has revealed the cell membrane as an organized structure consisting of a lipid bilayer primarily of phospholipids and penetrated protein molecules forming a mosaic like pattern (see Figure 4.6)
- Membranes are asymmetric. The two faces of a membrane are different. The outer and inner surfaces of all known biological membranes have different components and different enzymatic activities
- **Membranes are fluid structures**. The unsaturated **fatty** acids bound to phospholipids contribute to the fluid state of the membrane. At body temperature lipids are in a fluid state and this fluidity of the membrane is essential for the normal functioning to occur, e.g. **exocytosis and endocytosis and lysosomal activity**

- The cell membrane is anchored to the **cytoskeleton** which is a network of **microfilaments** and **microtubules** that interact with each other and with the components of the plasma membrane.

Table 4.1 : Biochemical functions of subcellular organelles of the eukaryotic cell

Subcellular organelles	*Function*
Plasma membrane	Transport of molecules in and out of cell, receptors for hormones and neurotransmitters
Lysosome	Intracellular digestion of macromolecules and hydrolysis of nucleic acid, protein, glycosami-noglycans, glycolipids, sphingolipids
Golgi apparatus	Post-transcriptional modification and sorting of proteins and export of proteins
Rough endoplasmic reticulum	Biosynthesis of protein and secretion
Nucleus	Storage of DNA. replication and repair of DNA, transcription and post-transcriptional processing
Peroxisomes	Metabolism of hydrogen peroxide and oxidation of long chain fatty acids
Nucleolus	Synthesis of r-RNA and formation of ribosomes
Mitochondrion	ATP synthesis, site for tricarboxylic acid cycle, fatty acid oxidation, oxidative phospho-rylation, part of urea cycle and part of haem synthesis.
Smooth endoplasmic reticulum	Biosynthesis of steroid hormones and phospholipids, metabolism of foreign compounds (cytochrome P_{450} detoxification)
Cytosol	Site for glycolysis. pentose phosphate pathway, part of gluconeogenesis, urea cycle and haem synthesis, purine and pyrimidine nucleotide synthesis.

Table 4.2: Chemical composition of cell membranes

Membrane	*Chemical composition by weight*		
	Protein%	Lipid%	Carbohydrate
Erythrocyte	49	43	8
Outer mitochondrial membrane	50	46	4
Inner mitochondrial membrane	75	23	2
Myelin	20	75	5

MEMBRANE LIPIDS

- The major classes of membrane lipids are,
  - **phospholipids,**
  - **glycolipids** and
  - **cholesterol**

They are all **amphipathic** molecules, that is they have both hydrophobic and hydrophilic ends.

- Membrane lipids spontaneously form bilayer in aqueous medium, burrying their hydrophobic tails and leaving their hydrophilic ends exposed to the water (Figure 4.3).

Phospholipids

- Each phospholipid molecule consists of head in which phosphoric acid group is located and two thin tails which consists of fatty acid portion (Figure 4.4)
- The head end is polar and hydrophilic while the tail end is the non-polar and hydrophobic

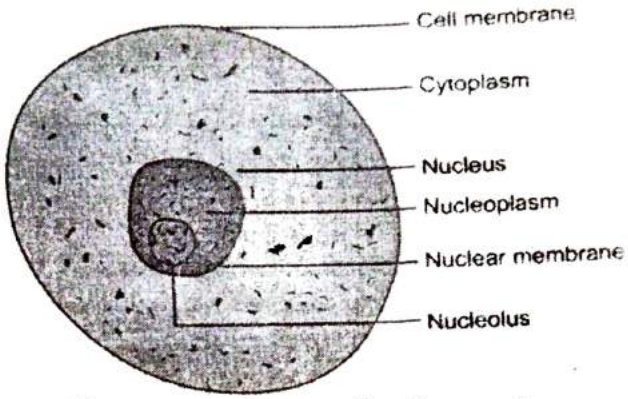


Fig. 4.1 : Structure of cell, seen in light microscope

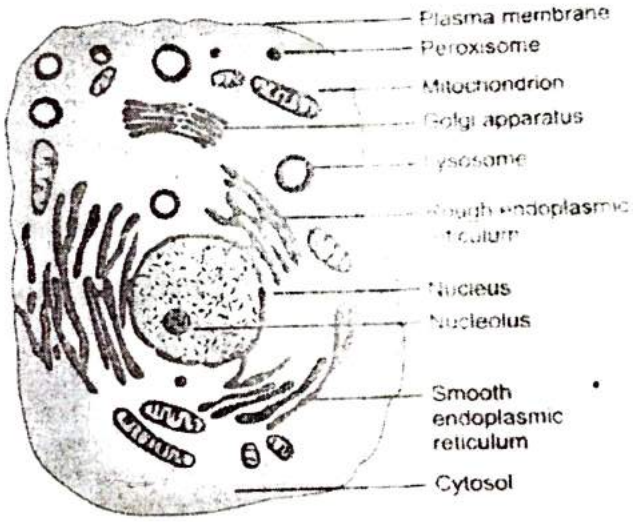


Fig. 4.2 : A diagrammatic representation of a typical eukaryotic cell

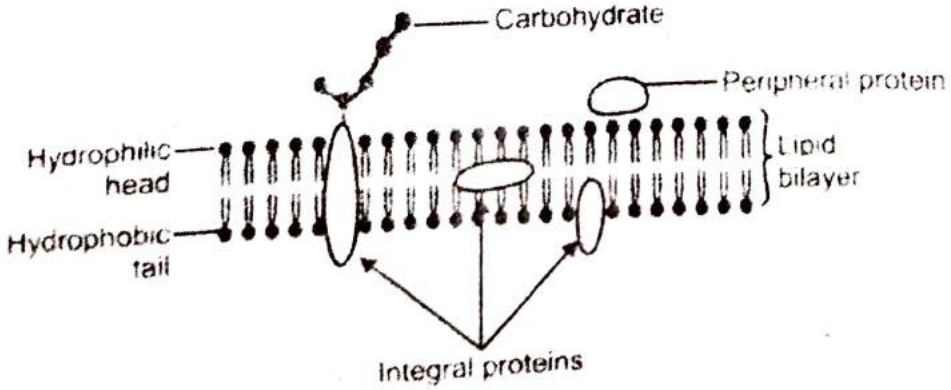


Fig. 4.3: The basic organisation of biological membrane

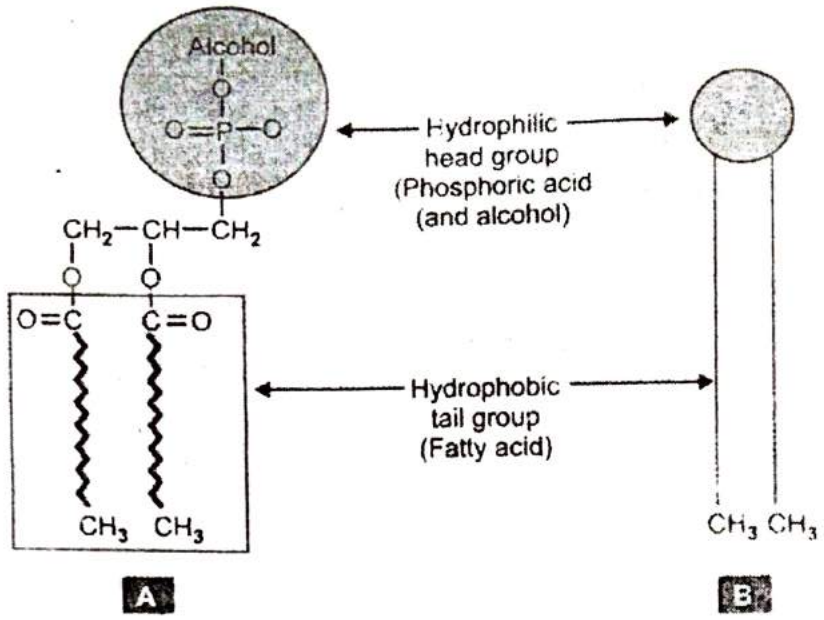


Figure 4.4 : Structure of phospholipid
A = A common glycerophospholipid
B = Diagrammatic representation of phospholipid

- Two major classes of phospholipid present in membranes are
  - Phosphoglycerides and
  - Sphingomyelins
- Phosphoglycerides consists of glycerol backbone to which two fatty acids and phosphorylated alcohol is attached (Figure 4.4). The common alcohol moieties of phosphoglycerides are **serine, ethanolamine, choline, glycerol** and **inositol**
- Sphingomyelins which contain a sphingosine back bone rather than glycerol to which fatty acids and phosphorylcholine is attached. Sphingomyelins are prominent in myelin sheaths.
- The phospholipid molecules are not attached to their neighbouring phospholipid molecule by any chemical bonds. Thus to some extent, they can move laterally and such movement are called **lateral movements**. Also a phospholipid molecule can be twisted or folded to some extent. This is how a cell can alter its shape.
- The **lipid bilayer** in the middle of the membrane is **impermeable** to the usual water soluble substances, such as ions, glucose and urea
- Conversely, fat soluble substances such as oxygen, carbon dioxide and alcohol can penetrate this portion of the membrane with ease.

Functions of cell membrane phospholipids

Functions of phospholipid molecule of cell membrane are as follows:

- Provide the membrane its form
- Provide a selective barrier that prevents movement of water and water soluble substances from one cell component to other.

Glycolipids

- Glycolipids are sugar containing lipids derived from sphingosine, e.g. **cerebroside** and **gangliosides**
- Glycolipids are present only over the outer surface of the cell membrane
- Glycolipids are inserted into the membrane lipid bilayer in such a manner that the carbohydrate group projects out from the surface of the bilayer, interacting with the phospholipid head groups.

Cholesterol

- Cholesterol exists almost **exclusively in the plasma membranes of mammalian cells.**
- It provides stability to the membrane
- Cholesterol is inserted into the lipid bilayer between phospholipid molecules in both leaflets of the lipid bilayer. Its hydroxyl group is oriented towards the aqueous interface and interacts with the polar head groups of the phospholipids and remainder of the molecule interact with the hydrocarbon chains of the phospholipid fatty acyl groups (Figure 4 .5).

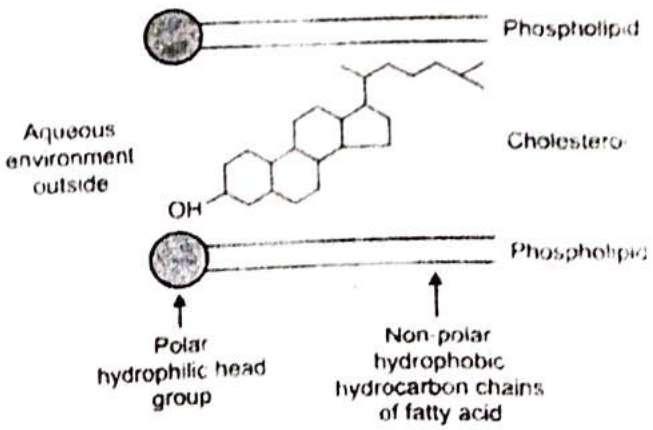


Fig. 4.5 : Inseration of cholesterol into the membrane lipid bilayer.

PROTEINS OF THE CELL MEMBRANE

- Proteins of the membrane are classified into two major categories
  - Integral proteins or intrinsic proteins or transmembrane proteins and
  - Peripheral or extrinsic proteins.

Ankyrine & spectrin.

Peripheral proteins are attached to the surface of the lipid bilayer by electrostatic and hydrogen bonds. They bound loosely to the polar head groups of the membrane phospholipid bilayer (see Figure 4.3). Peripheral proteins comprise about 30% of the membrane proteins.

GPCR
Band-3-P
Glycoprotein

Integral proteins are either partially or totally immersed in the lipid bilayer. Many integral membrane proteins span the lipid bilayer from one side to the other and are called **transmembrane protein** whereas others are partly embedded in either the outer or inner leaflet of the lipid bilayer (see Figure 4.3).

- **Transmembrane proteins** are exposed on both the extracellular fluid and the cytoplasmic surfaces of the bilayer and in this way can form a **channel** or **pore** through the lipid phase. They act as enzymes and transport carriers for ions as well as water soluble substances such as glucose.
- Most of these membrane proteins are **glycoproteins**. They bear short chains of carbohydrates on the exterior side of the membrane (see Figure 4.3).

Functions of Integral Proteins

Integral protein serve as:

Channels (pores) : Through which water, and water soluble substances especially ions diffuse between extracellular and intracellular fluid. These protein channels also have selective properties, that allow preferential diffusion of some substances more than others.

Carrier proteins : Transport substances that otherwise could not penetrate the lipid bilayer. Sometimes these even transport substances in the direction opposite to their natural direction of diffusion, which is called *"active transport"*.

Enzymes: That activate or inactivate various metabolic processes.

Functions of Peripheral Proteins

Peripheral proteins function almost entirely as:

- **Enzymes** and
- **Receptors**. Receptors bind neurotransmirters and hormones initiating physiological and biochemical changes inside the cell.

MEMBRANE CARBOHYDRATES (The Cell Glycocalaxy)

Membrane carbohydrate is not free. It occurs in combination with proteins or lipids in the form of ***glycoproteins*** or ***glycolipids***. Most of the integral proteins are glycoproteins and about one tenth of the membrane lipid molecules are glycolipids.

The carbohydrate (Glyco) portion of these molecules protrude to the outside of the cell, dangling outward from the cell surface (Figure 4.3).

Many other carbohydrate compounds called proteoglycans which are mainly carbohydrate substances bound to small protein cores. These ***proteoglycans*** are loosely attached to the outer surface of the cell as well. ***Thus, the entire outer surface of the cell which has a loose carbohydrate layer called "the glycocalaxy".*** The carbohydrate moieties attached to the outer surface of the cell have several important functions.

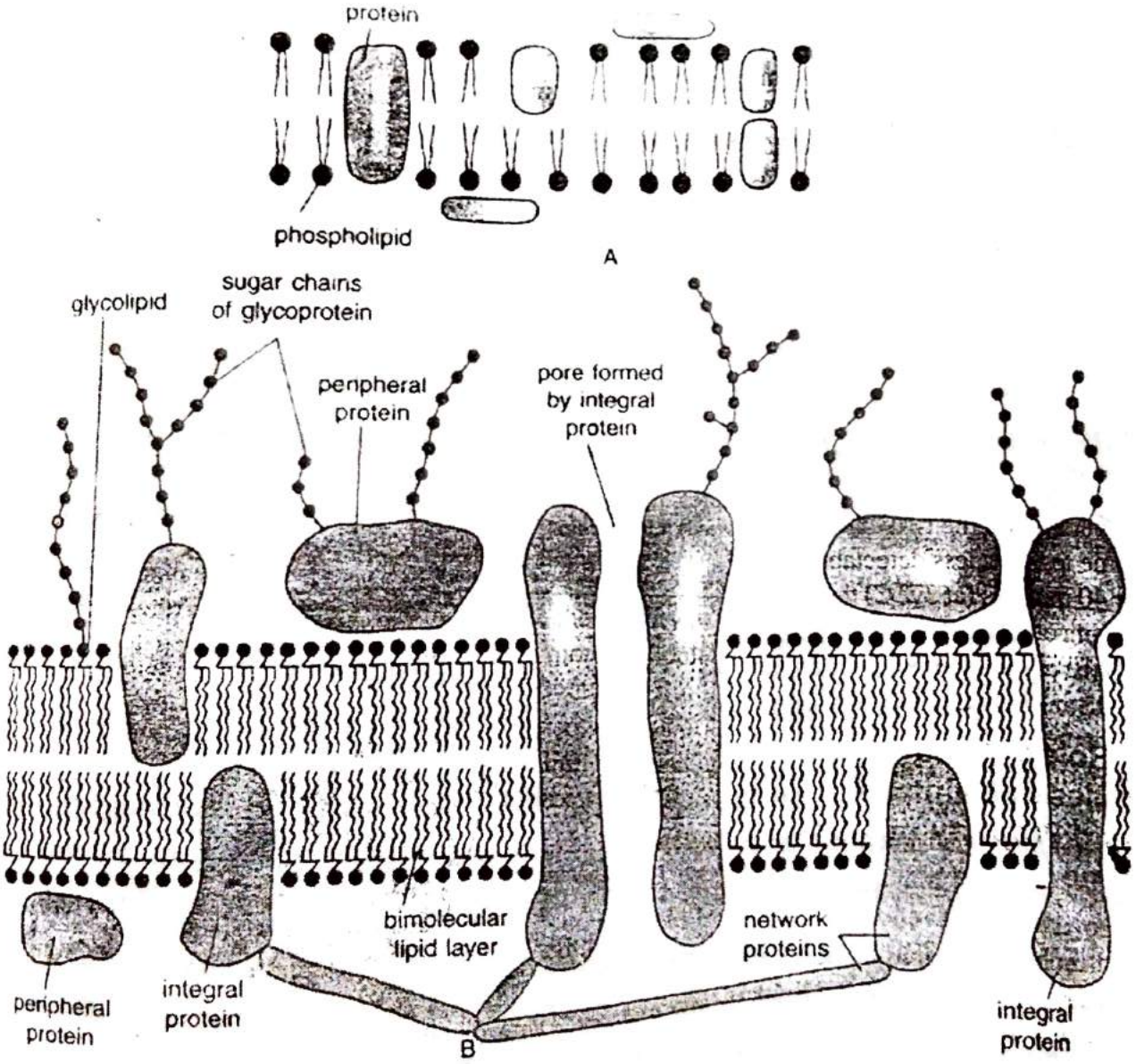


Fig. 4.6 : Fluid mosaic model of the plasma membrane. A. - Simplistic view, B. - Complex view (after Berns, 1983, Sheeler and Bianchi, 1987).

Functions of Membrane Carbohydrate

- Many of them are electrically negatively charged, which gives most cells an overall negatively charged surface that repels other negative compounds and do not permit the negatively charged particles of the cell to move outside
- Glycocalaxy of some cells is attached to the glycocalayx of other cells thus attaching cells to one another
- Many of the carbohydrates act as receptor substances for binding hormones such as insulin
- Some carbohydrate moieties enter into immune reactions and functions in antibody processing.

FUNCTIONS OF CELL MEMBRANE

- Protective function: The cell membrane protects the cytoplasm and the organelles of the cytoplasm.
- Maintenance of shape and size of the cell.
- **As a semipermeable membrane**: The cell membrane permits only some substances to pass in either direction, and it forms a barrier for other substances.

THE FLUID-MOSAIC MODEL OF THE CELL PLASMA MEMBRANE

The fluid-mosaic model describes the plasma membrane of animal cells. The plasma membrane that surrounds these cells has two layers (a *bilayer*) of*phospholipids* (fats with phosphorous attached), which at body temperature are like vegetable oil (fluid). And the structure of the plasma membrane supports the old saying, "Oil and water don't mix."

Each phospholipid molecule has a head that is attracted to water (*hydrophilic: hydro* = water; *philic* = loving) and a tail that repels water (*hydrophobic: hydro*= water; *phobic* = fearing). Both layers of the plasma membrane have the hydrophilic heads pointing toward the outside; the hydrophobic tails form the inside of the bilayer.

Because cells reside in a watery solution (extracellular fluid), and they contain a watery solution inside of them (cytoplasm), the plasma membrane forms a circle around each cell so that the water-loving heads are in contact with the fluid, and the water-fearing tails are protected on the inside.

Proteins and substances such as cholesterol become embedded in the bilayer, giving the membrane the look of a mosaic. Because the plasma membrane has the

consistency of vegetable oil at body temperature, the proteins and other substances are able to move across it. That's why the plasma membrane is described using the *fluid-mosaic model.*

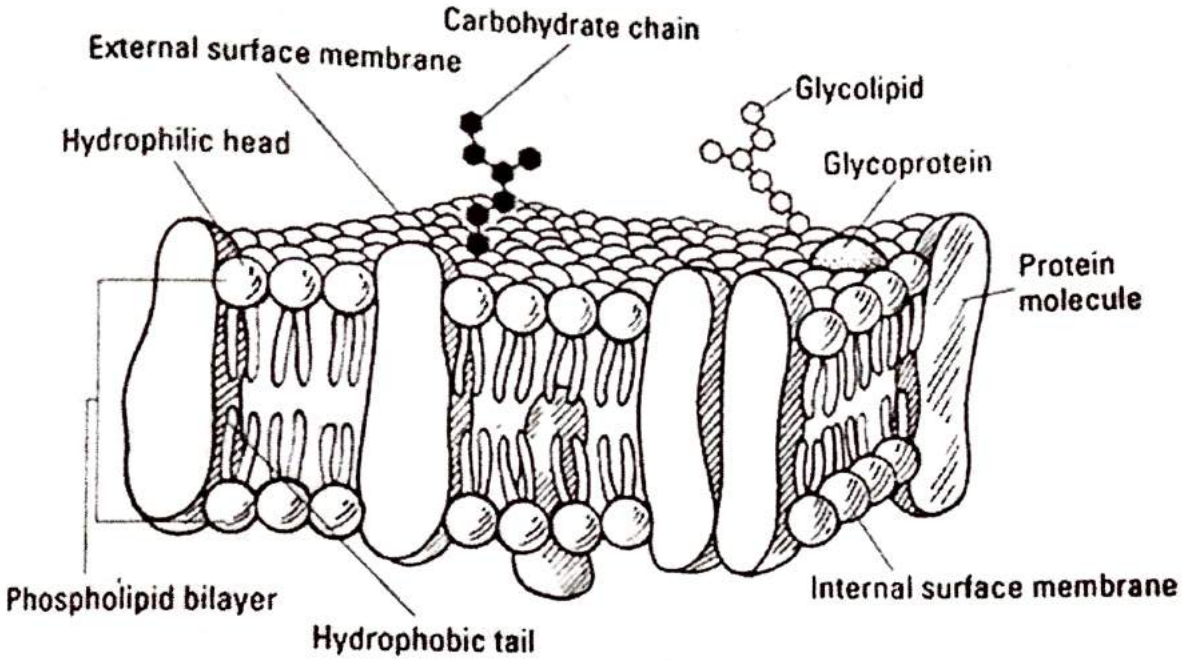


Fig. 4.7 : The fluid-mosaic model of plasma membranes.

The molecules that are embedded in the plasma membrane also serve a purpose. For example, the cholesterol that is stuck in there makes the membrane more stable and prevents it from solidifying when your body temperature is low. (It keeps you from literally freezing when you're "freezing.") Carbohydrate chains attach to the outer surface of the plasma membrane on each cell. These carbohydrates are specific to every person, and they supply characteristics such as your blood type.The fluid mosaic model was first proposed by S. J. Singer and G. L. Nicolson in 1972 to describe the structure of cell membranes (Singer and Nicolson 1972). In this now-accepted theory about cell structure, phospholipid molecules, each with one hydrophobic, and one hydrophilic end, make up most of the membrane. The hydrophilic heads form the inner and outer surfaces the membraneand the hydrophobic tails, which are repelled by the water within and outside the cell, are sandwiched in between (see figure). This is known as the lipid bilayer.

ACTIVE AND PASSIVE TRANSPORT

Active and passive transport are biological processes that move oxygen, water and nutrients into cells and remove waste products. Active transport requires chemical energy because it is the movement of biochemicals from areas of lower concentration

to areas of higher concentration. On the other hand, passive trasport moves biochemicals from areas of high concentration to areas of low concentration; so it does not require energy.

Comparison chart (Fig. 4.8 and 4.9)

	Active Transport	Passive Transport
Definition	Active Transport uses ATP to pump molecules AGAINST/UP the concentration gradient. Transport occurs from a low concentration of solute to high concentration of solute. Requires cellular energy.	Movement of molecules DOWN the concentration gradient. It goes from high to low concentration, in order to maintain equilibrium in the cells. Does not require cellular energy.
Types of Transport	Endocytosis, cell membrane/sodium-potassium pump & exocytosis	Diffusion, facilitated diffusion, and osmosis.
Types of Particles Transported	proteins, ions, large cells, complex sugars.	Anything soluble (meaning able to dissolve) in lipids, small monosaccharides, water, oxygen, carbon dioxide, sex hormones, etc.
Examples	phagocytosis, pinocytosis, sodium/potassium pump, secretion of a substance into the bloodstream (process is opposite of phagocytosis & pinocytosis)	diffusion, osmosis, and facilitated diffusion.
Importance	In eukaryotic cells, amino acids, sugars and lipids need to enter the cell by protein pumps, which require active transport. These items either cannot diffuse or diffuse too slowly for survival.	It maintains equilibrium in the cell. Wastes (carbon dioxide, water, etc.) diffuse out and are excreted; nutrients and oxygen diffuse in to be used by the cell.
Functions	Transports molecules through the cell membrane against the concentration gradient so more of the substance is inside the cell (i.e. a nutrient) or outside the cell (i.e. a waste) than normal. Disrupts equilibrium established by diffusion.	Maintains dynamic equilibrium of water, gases, nutrients, wastes, etc. between cells and extracellular fluid; allows for small nutrients and gases to enter/exit. No NET diffusion/osmosis after equilibrium is established.

Process

There are two types of active transport: primary and secondary. In primary active transport, specialized trans-membrane proteins recognize the presence of a substance that needs to be transported and serve as pumps, powered by the chemical energy ATP, to carry the desired biochemicals across. In secondary active transport, pore-forming proteins form channels in the cell membrane and force the biochemicals across using an electromagnetic gradient. Often, this energy is gained by simultaneously moving another substance down the concentration gradient.

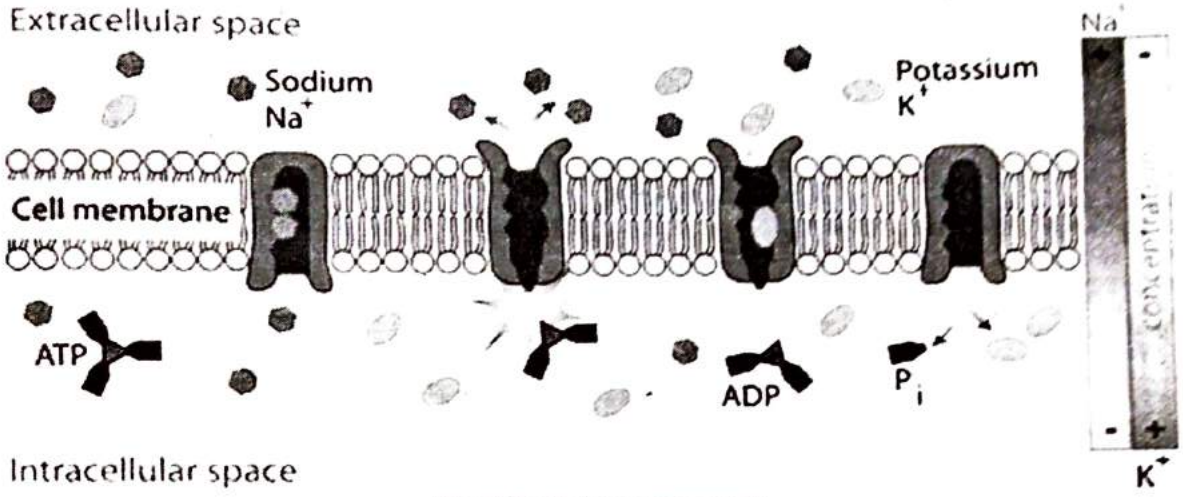


Fig. 4.8 : Active Transport

Example of primary **active transport**, where energy from hydrolysis of ATP is directly coupled to the movement of a specific substance across a membrane independent of any other species.

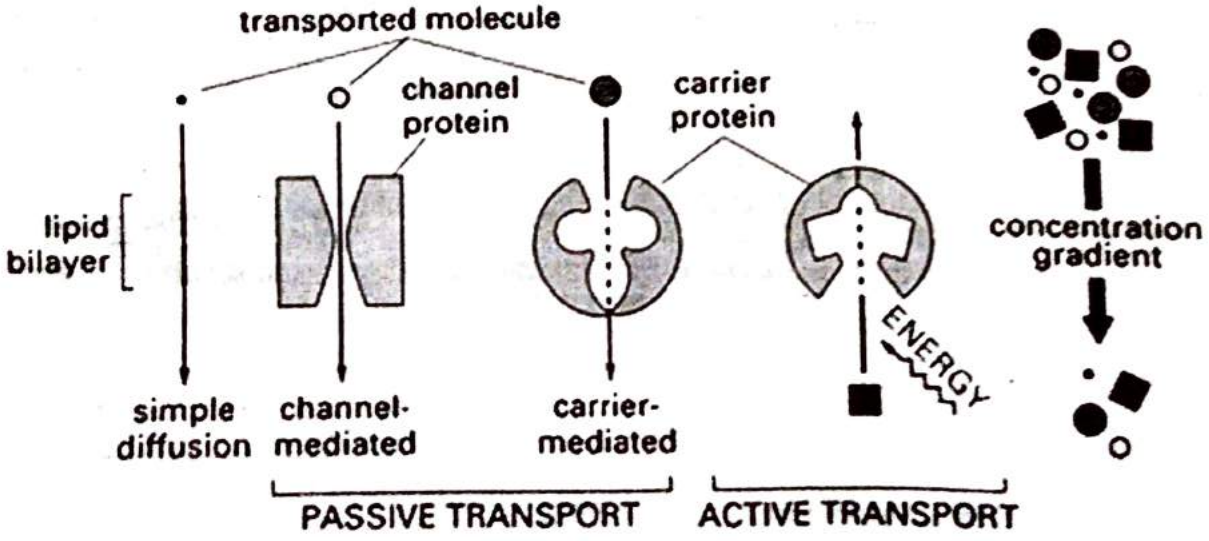


Fig. 4.9 : Active and Passive Transport.

There are four main types of passive transport : osmosis, diffusion, facilitated diffusion and filtration. Diffusion is the simple movement of particles through a permeable membrane down a concentration gradient (from a more concentrated solution to a less concentrated solution) until the two solutions are of equal concentration. Facilitated diffusion uses special transport proteins to achieve the same effect. Filtration is the movement of water and solute molecules down the concentration gradient, e.g. in the kidneys, and osmosis is the diffusion of water molecules across a selectively permeable membrane. None of these processes require energy.

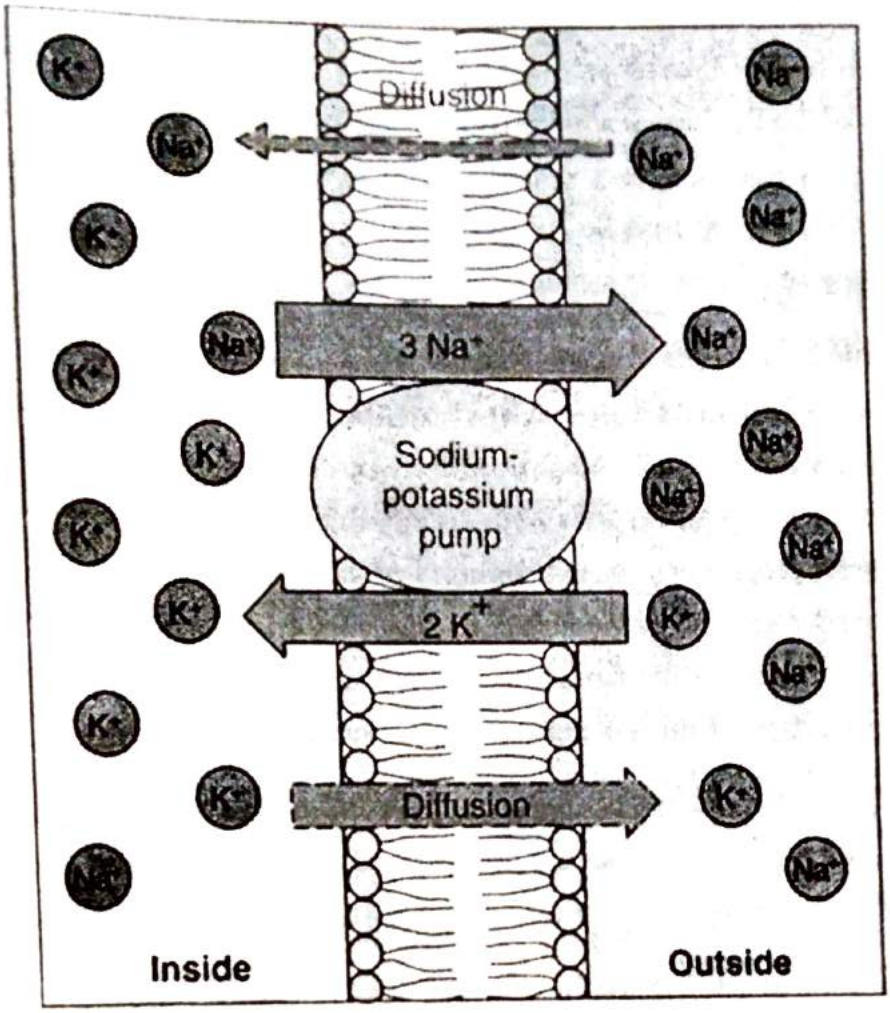
The Sodium-Potassium Pump

In order to maintain the cell potential, cells must keep a low concentration of sodium ions and high levels of potassium ions within the cell (intracellular). Outside cells (extracellular), there are high concentrations of sodium and low concentrations of potassium, so diffusion occurs through ion channels in the plasma membrane. In order to keep the appropriate concentrations, the sodium-potassium pump pumps sodium out and potassium in through active transport.

The mechanism is : The pump, with bound ATP, binds 3 intracellular Na+ ions, ATP is hydrolyzed, leading to phosphorylation of the pump at a highly conserved aspartate residue and subsequent release of ADP.

A conformational change in the pump exposes the Na+ ions to the outside. The phosphorylated form of the pump has a low affinity for sodium ions, so they are released. The pump binds 2 extracellular K+ ions, leading to the dephosphorylation of the pump. ATP binds, and the pump reorients to release potassium ions inside the cell so the pump is ready to go again.

As the plasma membrane is far less permeable to sodium than it is to potassium ions, an electric potential (negative intracellularly) is the eventual result. The electrical and concentration gradient established by the sodium-potassium ATPase supports not only the cell resting potential but the action potentials of nerves and muscles. Export of sodium from the cell provides the driving force for several facilitated transporters, which import glucose, amino acids and other nutrients into the cell. Translocation of sodium from one side of an epithelium to the other side creates an osmotic gradient that drives the absorption of water.



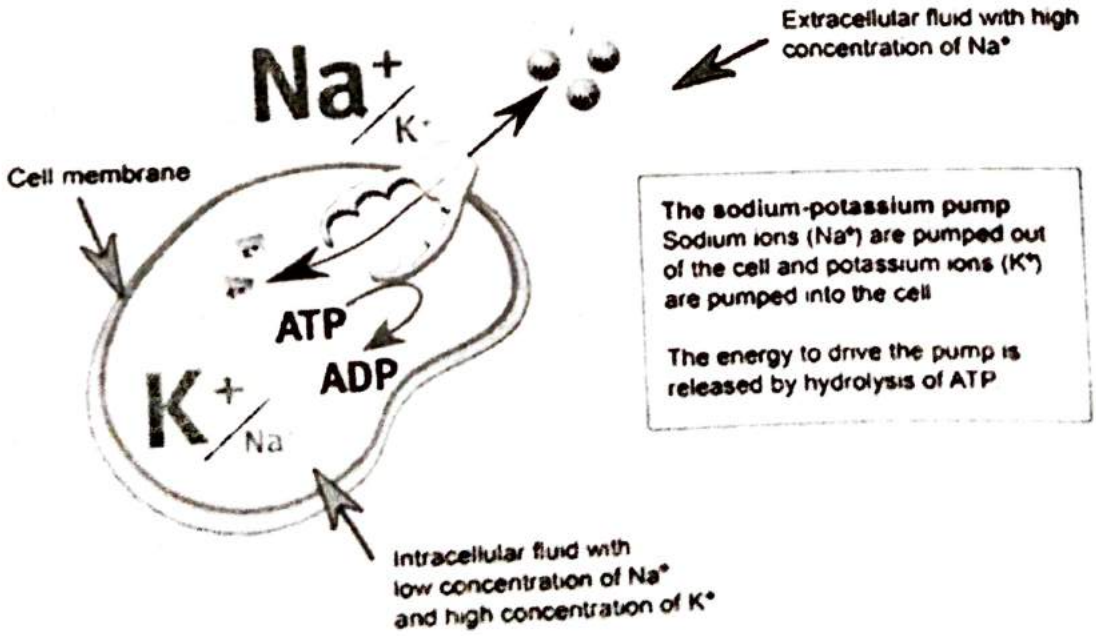


Fig. 4.10 : The Sodium-Potassium Pump

Another important task of the Na+-K+ pump is to provide a Na+ gradient that is used by certain carrier processes. In the gut, for example, sodium is transported out of the resorbing cell on the blood side via the Na+-K+ pump, whereas, on the resorbing side, the Na+-Glucose symporter uses the created Na+ gradient as a source of energy to import both Na+ and Glucose, which is far more efficient than simple diffusion. Similar processes are located in the renal tubular system. (Fig. 4.10)

Calcium Pump

Active transport mechanism is the calcium pump. Calcium ions are normally maintained at extremely low concentration in the intercellular cytosol of virtually all cells in the body, at a concentration about 10,000 times less than that in the extracellular fluid. This is achieved mainly by two primary active transport Calcium Pumps. One is the cell membrane and, pumps Calcium to the outside of the cell. The other pumps calcium ions into one or more of the intracellular vesicular organelles of the cell, such as sarcoplasmic reticulam of muscle cells and mitochondria in all cells.

In each of these instances, the carrier protein penetrates the membrane and functions as an enzyme ATPase having the same capability to cleave ATP as the ATPase of the sodium carrier protein. The difference is that this protein has a highly specific binding site for calcium instead of for sodium.

Intercellular → occuring between cell.