19BTU101 BIOCHEMISTRY AND METABOLISM SEMESTER - I 4H - 4C

Instruction Hours / week: L: 4 T: 0 P: 0 **Marks:** Internal: **40** External: **60** Total: **100**

End Semester Exam: 3 Hours

Course Objectives

- This course is designed to provide clear understanding on the underlying principles of structures and functions of biomolecules to the students of the subjects.

Course Outcomes (CO's)

1. The learners will acquire knowledge on the structure and functions relationship of proteins nucleic acid carbohydrates and as well their roll in various biological process.

UNIT- I Introduction to macromolecules:

Amino acids & Proteins: Structure, properties and function of Amino acids and Protein, Amino acid and protein classification. Protein Purification. Denaturation and renaturation of proteins. Fibrous and globular proteins.

UNIT-II Carbohydrates and Metabolism:

Carbohydrates: Structure, Function and properties of Monosaccharides, Disaccharides and Polysaccharides. Bacterial cell wall polysaccharides, Glycoprotein's and their biological functions; Glycolysis: Fate of pyruvate under aerobic and anaerobic conditions. Pentose phosphate pathway and its significance, Gluconeogenesis, Glycogenolysis and glycogen synthesis. TCA cycle.

UNIT-III Enzymes:

Nomenclature and classification of Enzymes, Holoenzyme, apoenzyme, Cofactors, coenzyme, groups, metalloenzymes, monomeric & oligomeric enzymes, activation energy and transition state, enzyme activity, specific activity, common features of active sites, Role of: NAD+, NADP+, FMN/FAD, coenzymes A, Thiamine pyrophosphate, Pyridoxal phosphate, lipoic-acid, Biotin vitamin B12, Tetrahydrofolate and metallic ions. Photosynthesis – Photosystem I and II.

UNIT-IV Lipids:

Structure and functions –Classification, nomenclature and properties of fatty acids, essential fatty acids. Phospholipids, sphingolipids, glycolipids, cerebrosides, gangliosides, Prostaglandins, Cholesterol. ß-oxidation of fatty acids.

UNIT-V Nucleic acids:

Structure and functions: Physical & chemical properties of Nucleic acids, Nucleosides & Nucleotides, purines & pyrimidines, Biologically important nucleotides, Double helical model of DNA structure, A, B & Z – DNA, denaturation and renaturation of DNA

SUGGESTED READINGS

1. Buchanan B, Gruissem W, and Jones R. (2015). Biochemistry and Molecular Biology of Plants. 2nd edition. American Society of Plant Biologists.
2. Nelson DL, and Cox MM. (2013). Lehninger: Principles of Biochemistry. 6th edition. New York: W.H. Freeman and Company.
3. Murray RK, Bender DA, Botham KM, and Kennelly P.J. (2012). Harper's illustrated Biochemistry. 29th edition. London : McGraw-Hill Medical.
4. Berg JM, Tymoczko JL, and Stryer L. (2011). Biochemistry. 7th edition. Newyork : W.H. Freeman & Company.
5. Hopkins WG, and Huner P.A. (2008). Introduction to Plant Physiology. 4nd edition. John Wiley & Sons.

LECTURE PLAN

S.No	Lecture Duration (hr)	Topics	Support materials
		UNIT I	
1.	1	Amino acids – Essential amino acids Structures	T1: 67-80
2.	1	Classification of Amino acids based on properties and functions	T1: 70-71
3.	1	Protein structure – linear –Primary structure	T1: 1130-1135
4.	1	Protein structure – helical –secondary structure	T1: 1145-1148
5.	1	Protein structure – Tertiary structure	T1: 1149-1153
6.	1	Protein structure – Quaternary structure	T1: 1153-1160
7.	1	Classification of proteins - Fibrous and Globular Protein	T1: 232-240; T1: 281-282
8.	1	Protein purification – Size exclusion chromatography	T1: 138-140
9.	1	Revision	-
10.	1	Revision	-
		UNIT II	
11.	1	Carbohydrates structure, and functions - Monosaccharaides	T1:359-363
12.	1	Disaccharides and sugar derivaties	T1:367-369
13.	1	Polysaccharides – Homo and Hetero	T1:365-366

14.	1	Bacterial cell wall polysaccharides and glycoproteins	T1:375-383
15.	1	Glycolysis – fate of pyruvate under aerobic and anaerobic condition	T1:593-600
16.	1	Hexose mono phosphate pathway	T1:892-895
17.	1	Glycogenesis – Glycogen synthesis;	T1:660-665
18.	1	Glycogenolysis – Glycogen breakdown	T1:660-665
19.	1	Gluconeogenesis	T1:667-670
20.	1	TCA cycle	T1:789-795
21.	1	Revision	-
22.	1	Revision	-
UNIT III			
23.	1	Classification of Enzymes	T1: 479-480
24.	1	Holoenzyme, apoenzyme, Cofactors, coenzyme, metalloenzymes	T1: 482-483
25.	1	Activation energy and transition state, enzyme activity, specific activity, Active sites	T1: 483-485
26.	1	Role of: NAD+, NADP+, FMN/FAD, coenzymes A, Thiamine pyro phosphate	T1: 485-487
27.	1	Pyridoxal phosphate, lipoic-acid, Biotin	T1: 487-490
	1	vitamin B12,Tetrahydrofolate and metallic ions	T1: 492-493

28.			
29.	1	Photosynthesis – Photosystem I	T1: 914-917
30.	1	Photosynthesis – Photosystem II	T1: 917-921
31.	1	Revision	-
32.	1	Revision	-
UNIT IV			
33.	1	Classification of lipids – Simple, conjugated and derived	T1: 386-394
34.	1	Essential fatty acids, Phospholipids	T1: 389-390
35.	1	Sphingolipids, Glycolipids, cerebrosides	T1: 390-391
36.	1	Gangliosides, Prostaglandins	T1: 392-393
37.	1	Cholesterol – Structure and Functions	T1: 392-394
38.	1	ß-oxidation of fatty acids	T1: 951-953
39.	1	Revision	-
40.	1	Revision	-
UNIT V			

41.	1	Structure and functions of Nucleic acids - purines & pyrimidines	R1: 82-84
42.	1	Biologically important nucleotides, Double helical model of DNA structure	T1: 85-90
43.	1	Watson and Crick model of DNA	T1: 88-90
44.	1	A, B & Z – DNA	R1: 281-282
45.	1	Denaturation and renaturation of DNA	T1: 89-92
46.	1	Revision	-
47.	1	Revision	-

UNIT-I

SYLLABUS

Introduction to Biochemistry: Amino acids & Proteins**:** Structure, properties and function of Amino acids and Protein, Amino acid and protein classification. Protein Purification. Denaturation and renaturation of proteins. Fibrous and globular proteins.

UNIT I

Introduction to Biochemistry: Amino acids & Proteins**:** Structure, properties and function of Amino acids and Protein, Amino acid and protein classification. Protein Purification. Denaturation and renaturation of proteins. Fibrous and globular proteins.

Amino acids

Definition

Amino acids are a group of organic compounds containing two functional groups amino and carboxyl. The amino group (-NH2) is basic while the carboxyl group (-COOH) is acidic in nature.

Classification

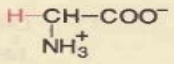
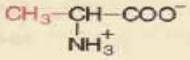
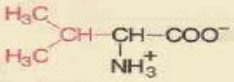
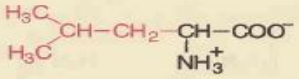
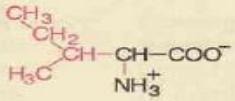
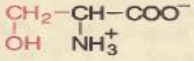
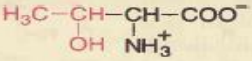
There are different ways of classifying the amino acids based on the structure and chemical nature nutritional requirement, metabolic fate etc.

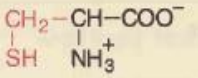
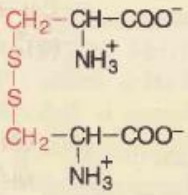
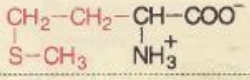
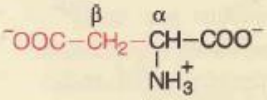
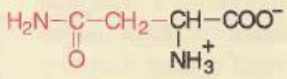
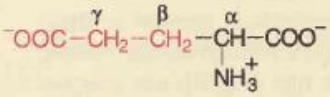
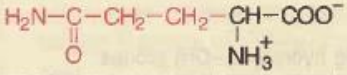
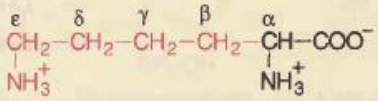
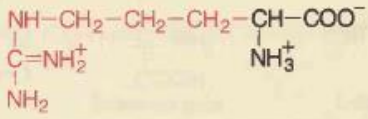
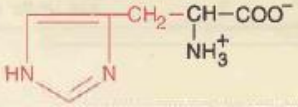
a. Amino acid classification based on the structure:

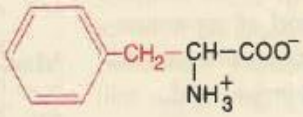
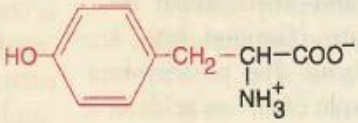
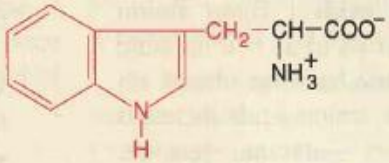
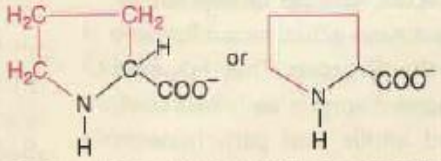
- A comprehensive classification of amino acids is based on their structure and chemical nature.
- Each amino acid is assigned a 3 letter or 1 letter symbol.
- These symbols are commonly used to represent the amino acids in protein structure.

- The 20 amino acids found in proteins are divided into seven distinct groups.
- The different groups of amino acids, their symbols and structures are given.
- The salient features of differen groups are described next.

TABLE 4.1 Structural classification of L-α-amino acids found in proteins

Name	Symbol (3 letters)	Symbol (1 letter)	Structure	Special group present
I. Amino acids with aliphatic side chains				
1. Glycine	Gly	G		
2. Alanine	Ala	A		
3. Valine	Val	V		Branched chain
4. Leucine	Leu	L		Branched chain
5. Isoleucine	Ile	I		Branched chain
II. Amino acids containing hydroxyl (—OH) groups				
6. Serine	Ser	S		Hydroxyl
7. Threonine	Thr	T		Hydroxyl
Tyrosine	Tyr	Y	See under aromatic	Hydroxyl

Name	Symbol		Structure	Special group present
	3 letters	1 letter		
III. Sulfur containing amino acids				
8. Cysteine	Cys	C		Sulfhydryl
Cystine	—	—		Disulfide
9. Methionine	Met	M		Thioether
IV. Acidic amino acids and their amides				
10. Aspartic acid	Asp	D		β-Carboxyl
11. Asparagine	Asn	N		Amide
12. Glutamic acid	Glu	E		γ-Carboxyl
13. Glutamine	Gln	Q		Amide
V. Basic amino acids				
14. Lysine	Lys	K		ε-Amino
15. Arginine	Arg	R		Guanidino
16. Histidine	His	H		Imidazole

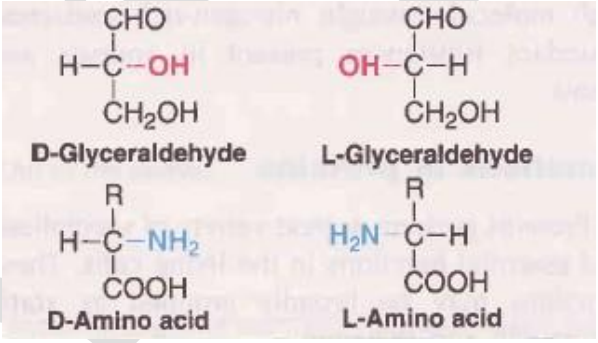
Name	Symbol		Structure	Special group present
	3 letters	1 letter		
VI. Aromatic amino acids				
17. Phenylalanine	Phe	F		Benzene or phenyl
18. Tyrosine	Tyr	Y		Phenol
19. Tryptophan	Trp	W		Indole
VII. Imino acid				
20. Proline	Pro	P		Pyrrolidine

(Note : R group is shown in red)

- **Amino acids with aliphatic side chains:** These are monoamino monocarboxylic acids. This group consists of the most simple amino acids-glycine, alanine, valine, leucine and isoleucine. The last three amino acids (Leu, lle, Val) contain branched aliphatic side chains, hence they are referred to as branched chain amino acids.
- **Hydroxyl group containing amino acids:** Serine, threonine and tyrosine are hydroxyl group containing amino acids. Tyrosine-being aromatic in nature-is usually considered under aromatic amino acids.
- **Sulfur containing amino acids**: Cysteine with sulfhydryl group and methionine with

thioether group are the two amino acids incorporated during the course of protein synthesis. Cystine, another important sulfur containing amino acid, is formed by condensation of two molecules of cysteine.

- **Acidic amino acids and their amides:** Aspartic acid and glutamic acids are dicarboxylic monoamino acids while asparagine and glutamine are their resoective amide derivatives. All these four amino acids possess distinct codons for their incorporation into proteins.
- **Basic amino acids**: The three amino acids lysine, arginine (with guanidino group) and histidine (with imidazole ring) are dibasic monocarboxylic acids. They are highly basic in character.
- **Aromatic amino acids:** Phenylalanine, tyrosine and tryptophan (with indole ring) are aromatic amino acids. Besides these, histidine may also be considered under this category.
- **lmino acids:** Proline containing pyrrolidine ring is a unique amino acid. It has an amino group (=NH), instead of an amino group (-NH2) found in other amino acids. Therefore proline is an amino acid.



b. Classification of amino acids based on polarity:

- Amino acids are classified into 4 groups based on their polarity. The polarity in turn reflects the functional role of amino acids in protein structure.
- **Non-polar amino acids :** These amino acids are also referred to as hydrophobic (water hating). They have no charge on the 'R' group. The amino acids included in this group are - alanine, leucine, isoleucine, valine, methionine, phenylalanine, tryptophan and proline.

- **Polar amino acids with no charge on 'R' group:** These amino acids, as such, carry no charge on the 'R'group. They however possess groups such as hydroxyl, sulfhydryl and amide and participate in hydrogen bonding of protein structure. The simple amino acid glycine (where R = H) is also considered in this category. The amino acids in this group are glycine, serine, threonine, cysteine, glutamine, asparagine and tyrosine.
- **Polar amino acids with positive 'R' group:** The three amino acids lysine, arginine and histidine are included in this group.
- **Polar amino acids with negative 'R'group:** The dicarboxylic monoamino acids aspartic acid and glutamic acid are considered in this group.

c. Nutritional classification of amino acids:

- The twenty amino acids are required for the synthesis of variety of proteins, besides other biological functions.
- However, all these 20 amino acids need not be taken in the diet. Based on the nutritional requirements amino acids are grouped into two classes essential and nonessential.
- **Essential or indispensable amino acids:** The amino acids which cannot be synthesized by the body and, therefore, need to be supplied through the diet are called essential amino acids. They are required for proper growth and maintenance of the individual. The ten amino acids listed below are essential for humans.
- **Non - essential or dispensable amino acids :** The body can synthesize about '10 amino acids to meet the biological needs, hence they need not be consumed in the diet. These are-glycine, alanine, serine, cystein e, aspartate,a sparagnie, glutamate, glutamine, tyrosine and proline.

d. Amino acid classification based on their metabolic fate:

- The carbon skeleton of amino acids can serve as a precursor for the synthesis of glucose.
- From metabolic view point, amino acids are divided into three
- **Glycogenic amino acids:** These amino acids can serve as precursors for the formation of glucose or glycogen. e.g. alanine, aspartate, glycine, methionine etc.
- **Ketogenic amino acids**: Fat can be synthesized from these amino acids. Two amino acids leucine and lysine are exclusively ketogenic.

- **Glycogenic and ketogenic amino acids:** The four amino acids isoleucine, phenylalanine, tryptophan, tyrosine are precursors for synthesis of glucose as well as fat.

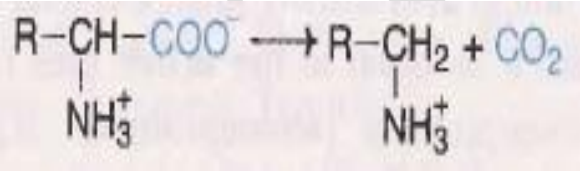
iii. Chemical reactions of amino acids:

- The general reactions of amino acids are mostly due to the presence of two functional groups namely carboxyl (-COOH) group and amino (-NH2) group.

iv. Reactions due to –COOH group:

1. Amino acids form salts (-COONa) with bases and esters (-COOR') with alcohols.

2. **Decarboxylation:** Amino acids undergo decarboxylation to produce corresponding amines.



- This reaction assumes significance in the living cells due to the formation of many biologically important amines.
- These include histamine, tyramine and y-amino butyric acid (CABA) from the amino acids histidine, tyrosine and glutamate, respectively.

3. **Reaction with ammonia:** The carboxyl group of dicarboxylic amino acids reacts with NH3 to form amide

- Aspartic acid + NH, ------- Asparagine
- Glutamic acid + NH ------ Glutamine

v. Reactions due to -nh_2 group:

4. The amino groups behave as bases and combine with acids (e.g. HCI) to form salts (-NHiCl-).

5. **Reaction with ninhydrin :** The a-amino acids react with ninhydrin to form a purple, blue or pink colour complex (Ruhemann's purple).

- Amino acid + Ninhydrin ----- Keto acid + NH_3+CO_2+Hydrindantin

- Hydrindantin + NH_3 + Ninhydrin ------ Ruhemann's purple
- Ninhydrin reaction is effectively used for the quantitative determination of amino acids and proteins.

6. **Colour reactions of amino acids:** Amino acids can be identified by specific colour reactions

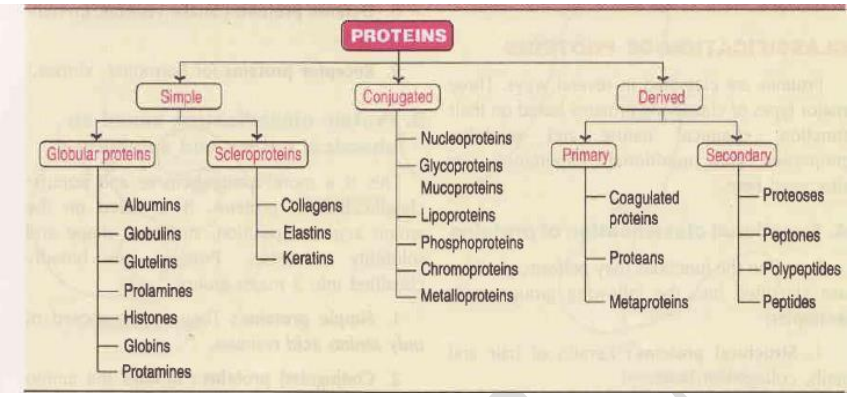
7. **Transamination:** Transfer of an amino group from an amino acid to a keto acid to form a new amino acid is a very important reaction in amino acid metabolism.

8. **Oxidative deamination:** The amino acids undergo oxidative deamination to liberate free ammonia.

PROTEINS

i. Classification of proteins:

- Proteins are classified in several ways.
- Three major types of classifying proteins based on their function, chemical nature and solubility properties and nutritional importance are discussed here.
- **Simple proteins:** On hydrolysis they yield only the amino acids and occasional small carbohydrate compounds. Examples are: albumins, globulins, glutelins, albuminoids, histones and protamines.
- **Conjugated proteins:** These are simple proteins combined with some non-protein material in the body. Examples are: nucleoproteins, glycoproteins, phosphoproteins, haemoglobins and lecithoproteins.
- **Derived proteins:** These are proteins derived from simple or conjugated proteins by physical or chemical means. Examples are: denatured proteins and peptides

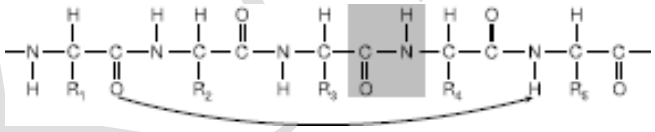


ii. Properties:

- Proteins can also be characterized by their chemical reactions. Most proteins are soluble in water, in alcohol, in dilute base or in various concentrations of salt solutions.
- Proteins have the characteristic coiled structure which is determined by the sequence of amino acids in the primary polypeptide chain and the stereo configuration of the radical groups attached to the alpha carbon of each amino acid.
- Proteins are heat labile exhibiting various degrees of lability depending upon type of protein, solution and temperature profile.
- Proteins can be reversible or irreversible, denatured by heating, by salt concentration, by freezing, by ultrasonic stress or by aging. Proteins undergo characteristic bonding with other proteins in the so-called plastein reaction and will combine with free aldyhyde and hydroxy groups of carbohydrates to form Maillard type compounds.

Primary Structure of Proteins

- The α-carboxyl group of one amino acid is covalently linked to the α –amino group of the next amino acid by an amide bond, commonly known as a peptide bond when in proteins. When two amino acid residues are linked in this way the product is a dipeptide. Many amino acids linked by peptide bonds form a polypeptide.
- The repeating sequence of α -carbon atoms and peptide bonds provides the backbone of the polypeptide while the different amino acid side chains confer functionality on the protein. The amino acid at one end of a polypeptide has an unattached α -amino group while the one at the other end has a free α -carboxyl group. Hence, polypeptides are directional, with an N terminus and a C terminus. Sometimes the N terminus is blocked with, for example, an acetyl group.
- The sequence of amino acids from the N to the C terminus is the primary structure of the polypeptide. Typical sizes for single polypeptide chains are within the range 100–1500 amino acids, though longer and shorter ones exist.



Section of a polypeptide chain. The peptide bond is boxed. In the α -helix, the CO group of amino acid residue n is hydrogen-bonded to the NH group of residue n + 4 (arrowed).

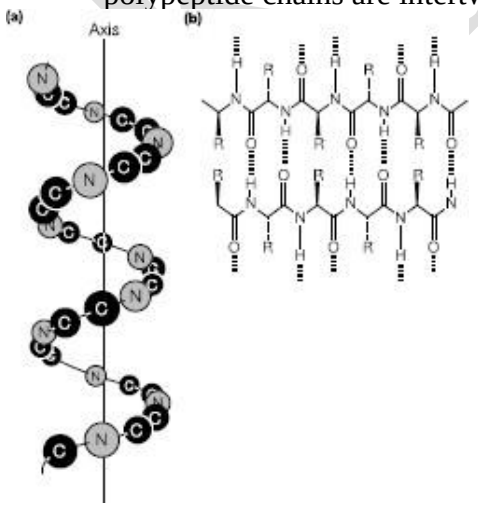
Secondary structure of Proteins

- The highly polar nature of the C=O and N–H groups of the peptide bonds gives the C–N bond partial double bond character. This makes the peptide bond unit rigid and planar, though there is free rotation between adjacent

peptide bonds.

- This polarity also favors hydrogen bond formation between appropriately spaced and oriented peptide bond units. Thus, polypeptide chains are able to fold into a number of regular structures which are held together by these hydrogen bonds. The best known **secondary structure** is the α **-helix**. The polypeptide backbone forms a right-handed helix with 3.6 amino acid residues per turn such that each peptide N–H group is hydrogen bonded to the C=O group of the peptide bond three residues away. Sections of α -helical

  secondary structure are often found in globular proteins and in some fibrous proteins. The β- **pleated sheet** (β- sheet) is formed by hydrogen bonding of the peptide bond N–H and C=O groups to the complementary groups of another section of the polypeptide chain.
- Several sections of polypeptide chain may be involved side-by side, giving a sheet structure with the side chains (R) projecting alternately above and below the sheet. If these sections run in the same direction (e.g. N terminus→C terminus), the sheet is **parallel**; if they alternate N→C and C→N, then the sheet is **antiparallel**. β-Sheets are strong and rigid and are important in structural proteins, for example silk fibroin. The connective tissue protein **collagen** has an unusual **triple helix** secondary structure in which three polypeptide chains are intertwined, making it very strong.



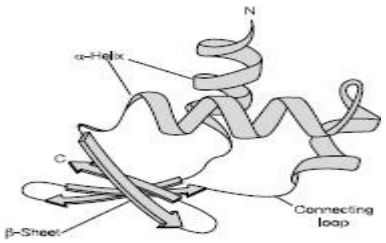
α - Helix secondary structure. Only the α-carbon and peptide bond carbon and nitrogen atoms of the polypeptide backbone are shown for clarity. (b) Section of aβsheet secondary structure.

Tertiary structure of Proteins

- The way in which the different sections of α-helix, β-sheet, other minor secondary structures and connecting loops fold in three dimensions is the tertiary structure of the polypeptide.
- The nature of the tertiary structure is inherent in the primary structure and, given the right conditions, most polypeptides will fold spontaneously into the correct tertiary structure as it is generally the lowest energy conformation for that sequence. However, *in vivo*, correct folding is often assisted by proteins called chaperones which help prevent mis- folding of new polypeptides before their synthesis (and primary structure) is complete.

- Folding is such that amino acids with hydrophilic side chains locate mainly on the exterior of the protein where they can interact with water or solvent ions, while the hydrophobic amino acids become buried in the interior from which water is excluded. This gives overall stability to the structure.
- Various types of non-covalent interaction between side chains hold the tertiary structure together: van der Waals forces, hydrogen bonds, electrostatic salt bridges between oppositely charged groups (e.g. the NH3 + group of lysine and the side chain COO– groups of aspartate or glutamate) and hydrophobic interactions between the nonpolar side chains of the aliphatic and aromatic amino acids.
- In addition, covalent disulfide bonds can form between two cysteine residues which may be far apart in the primary structure but close together in the folded tertiary structure. Disruption of secondary and tertiary structure by heat or extremes of pH leads to denaturation of the protein and formation of a random coil conformation.

  Schematic diagram of a section of protein tertiary structure.



Quaternary structure of Proteins

- Many proteins are composed of two or more polypeptide chains (subunits). These may be identical or different. Hemoglobin has two α-globin and two β-globin chains ($\alpha_2\beta_2$). The same forces which stabilize tertiary structure hold these subunits together, including disulfide bonds between cysteines on separate polypeptides. This level of organization is known as the quaternary structure and has certain consequences.
- First, it allows very large protein molecules to be made. Tubulin is a dimeric protein made up of two small, non-identical α and β subunits. Upon hydrolysis of tubulin-bound

  GTP, these dimers can polymerize into structures containing many hundreds of α and β subunits. These are the microtubules of the cytoskeleton.
- Secondly, it can provide greater functionality to a protein by combining different activities into a single entity, as in the fatty acid synthase complex. Often, the interactions between the subunits are modified by the binding of small molecules and this can lead to the allosteric effects seen in enzyme regulation.

Globular or Corpuscular Proteins

- These have an axial ratio (length : width) of less than 10 (usually not over 3 or 4) and, henceforth, possess a relatively spherical or ovoid shape.
- These are usually soluble in water or in aqueous media containing acids, bases, salts or alcohol, and diffuse readily. As a class, globular proteins are more complex in conformation than fibrous proteins, have a far greater variety

of biological functions and are dynamic rather than static in their activities.

- Tertiary and quaternary structures are usually associated with this class of proteins. Nearly all enzymes are globular proteins, as are protein hormones, blood transport proteins, antibodies and nutrient storage proteins.
- A simple functional classification of globular proteins is not possible because of 2 reasons : (a) Firstly, these proteins perform a variety of different functions. (b) Secondly, many widely-differing globular proteins perform almost similar functions.
- However, Conn and Stumpf (1976) have classified globular proteins as follows:

Globular Proteins
- Cytochrome C
- Blood proteins
  - Serum albumin
  - Glycoproteins
  - Antibodies (= Immunoglobulins)
  - Hemoglobin
  - Hormones
- Enzymes
- Nutrient proteins

Fibrous or Fibrillar Proteins

- These have axial ratios greater than 10 and, henceforth, resemble long ribbons or fibres in shape.
- These are mainly of animal origin and are insoluble in all common solvents such as water, dilute acids, alkalies and salts and also in organic solvents. Most fibrous proteins serve in a structural or protective role.
- The fibrous proteins are extremely strong and possess two important properties which are characteristic of the elastomers.
- These are:

(a) They can *stretch* and later recoil to their original length.

(b) They have a tendency to *creep*, *i.e.*, if stretched for a long time, their basic length increases and equals the stretched length but, if the tension on the two ends of the fibril is relaxed, they creep to their shorter and shorter length. A large scar, for

example, creeps to a smaller size if there is no tension on the scar. On the contrary, if the scar is in a region of high tension, the scar becomes larger and larger as happens in the skin of a person gradually becoming obese. It is a heterogeneous group and includes the proteins of connective tissues, bones, blood vessels, skin, hair, nails, horns, hoofs, wool and silk.

- The important examples are:

I. **Collagens.** These are of mesenchymal origin and form the major proteins of white connective tissues (tendons*, cartilage) and of bone. More than half the total protein in mammalian body is collagen; acted upon by boiling in water, dilute acids or alkalies to produce the soluble gelatins; unique in containing high contents (12%) of hydroxyproline; poor in sulfur since cysteine and cystine are lacking.

II. **Elastins.** Also of mesenchymal origin; form the major constituents of yellow elastic tissues (ligaments, blood vessels); differ from collagens in not being converted to soluble gelatins.

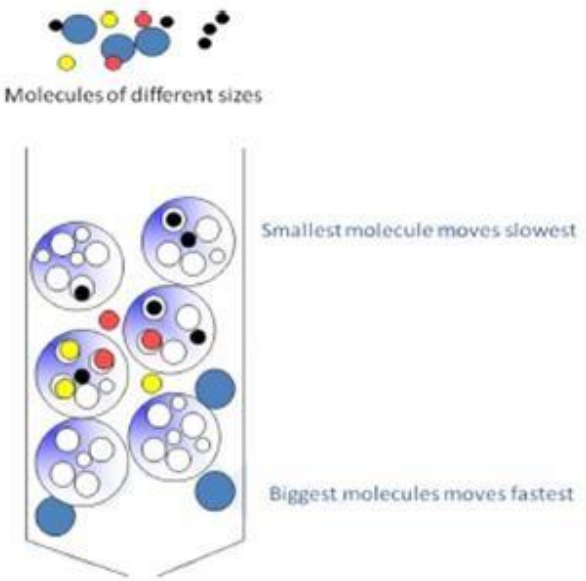
III. **Keratins.** These are of ectodermal origin; form the major constituents of epithelial tissues (skin, hair, feathers, horns, hoofs, nails); *usually contain large amounts of sulfur in the form of cystine*– human hair has about 14% cystine.

IV. **Fibroin.** It is the principal constituent of the fibres of silk; composed mainly of glycine, alanine and serine units.

Size Exclusion (Gel Filtration) Chromatography

- Size exclusion chromatography is used for semi-preparative purifications and various analytical assays. It is a separation technique which takes the advantage of the difference in size and geometry of the molecules. The molecules are separated based on their size. Grant Henry Lathe and Colin R Ruthven was the pioneer of size exclusion chromatography who started this technique for separation of analytes of different size with starch gels as the matrix, later Jerker Porath and Per Flodin introduced dextran gels. Other gel filtration matrices include agarose and polyacrylamide.

- **Principle:** Size exclusion chromatography (SEC) is the separation of mixtures based on the molecular size (more correctly, their hydrodynamic volume) of the components. Separation is achieved by the differential exclusion or inclusion of solutes as they pass through stationary phase consisting of heteroporous (pores of different sizes) cross linked polymeric gels or beads. The process is based upon different permeation rates of each solute molecule into the interior of gel



particles. Size exclusion chromatography involves gentle interaction with the sample, enabling high retention of biomolecular activity. For the separation of biomolecules in aqueous systems, SEC is referred to as gel filtration chromatography (GFC), while the separation of organic polymers in non-aqueous systems is called gel permeation chromatography (GPC).

- The basic principle of size exclusion chromatography is quite simple. A column of gel particles or porous matrix is in equilibrium with a suitable mobile phase for the molecules to be separated. Large molecules are completely excluded from the pores will pass through the space in between the gel particles or matrix and will come first in the effluent. Smaller

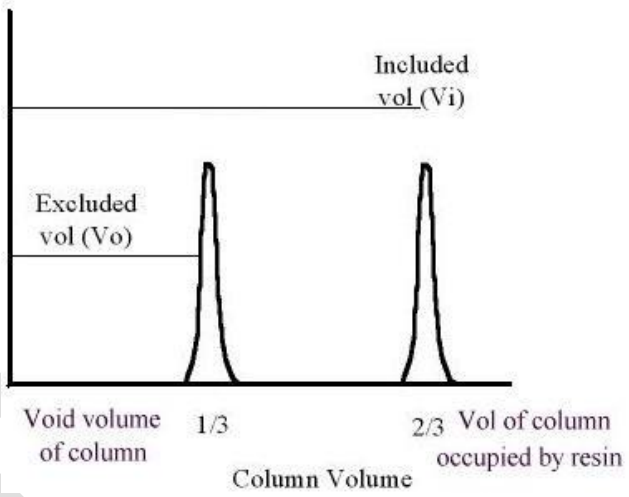
molecules will get distributed in between the mobile phase of in and outside the molecular sieve and will then pass through the column at a slower rate, hence

appear later in effluent.

- There are two extremes in the separation profile of a gel filtration column. There is a critical molecular mass (large mass) which will be **completely excluded** from the gel filtration beads. All solutes in the sample which are equal to, or larger, than this critical size will behave identically: they will all eluted in the excluded volume of the column. There is a critical molecular mass (small mass) which will be **completely included** within the pores of the gel filtration beads. All solutes in the sample which are equal to, or smaller, than this critical size will behave identically: they will all eluted in the included volume of the column Solutes between these two ranges of molecular mass will elute between the excluded and included volumes (Fig. 2) Thus, while deciding a size exclusion matrix for protein purification, included and excluded range should be considered. For example: Sephadex G 75 matrix has fractionation range 3-80. This tells that the matrix has included volume range 3 kDa and excluded volume range 80kDa. If protein of interest and impurities both are close to 80 kDa or above they are likely to co-elute in excluded volume. Thus purification will not work. Now you can think what is the use of a size exclusion matrix Sephadax G25 (range 1- 5kDa)? This is generally used for desalting as all proteins are above 5kDa and comes in excluded volume and salts are eluted late in included volume.
- In gel filtration **the resolution is a function of column length** (the longer the better). However, one drawback is related to the maximum sample volume which can be loaded. The larger the volume of sample loaded, the more the overlap between separated peaks. Generally speaking, the sample size one can load is limited to about ***3-5% of the total column volume*.** Thus, gel filtration is best saved for the end stages of a purification, when the sample can be readily concentrated to a small volume. Gel filtration can also be used to remove salts from the sample, due to its ability to separate "small" from "large" components. Finally, gel filtration can be among the most "gentle" purification methods due to the lack of chemical interaction with the resin.

Mechanism of Size Exclusion Chromatography

- Size exclusion (also known as gel filtration chromatography) is a case of liquid-liquid partition chromatography, in which the solute molecules are get distributed in between two liquid phases, (i) liquid in the gel pores and (ii) liquid outside the gel. The size exclusion may be explained by **Steric Exclusion Mechanism.** As the gel particles contains range of pore sizes, small molecules can enter in large number of pores while the large molecules will get small number of pores into which they can enter. Thus the different fractions of total pore volume are accessible to molecules of different sizes. Thus, molecules with different sizes will differ in distribution coefficient between these two liquid phases [As the small molecules can enter in more pores while larger molecules can enter in pores only larger than the molecular size]



The excluded volume (Vo) is approximately equal to one third of the column volume, the included volume is approximately equal to two thirds of the column volume.

The total volume (Vt) of a column packed with a gel that has been swelled by solvent is given by

$$Vt = Vg + VI + Vo$$

Where Vg is the volume occupied by the solid matrix of gel, Vi is the volume of

solvent held in the pores or interstices and Vo is the free volume outside the gel particles. When mixing or diffusion occurs, the diffusion equilibrium and the retention volume (VR) of the given species is given by

VR = V(int.) + Kd V(int.)

where distribution coefficient (Kd) is given by

Kd = Vi(acc) / V(total)

where Vi(acc) is the accessible pore volume.

V(total) is the total pore volume and V(int.) is the interstitial volume. The other proposed mechanism is **Secondary Exclusion Mechanism.** This mechanism states that when a sample containing a mixture of small and large molecules is applied to a gel filtration column, the small molecules diffuse rapidly into the pores of gel, whereas large molecules will find relatively few unoccupied pores and move further down the column till they find the unoccupied pores. This results in the enhancement of separation of small and large molecules.

iii. Denaturation:

- The phenomenon of disorganization of native protein structure is known as denaturation.
- Denaturation results in the loss of secondary, tertiary and quaternary structure of proteins.
- This involves a change in physical, chemical and biological properties of protein molecules.

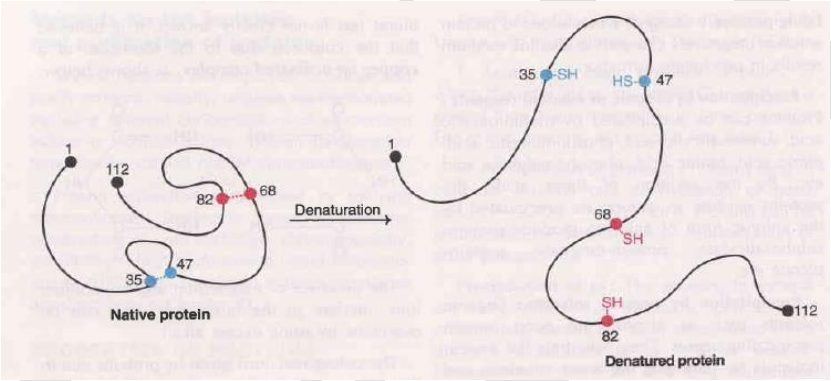
a. Agents of denaturation:

- **Physical agents**: Heat, violent shaking, X-rays, UV radiation.
- **Chemical agents:** Acids, alkalies, organic solvents (ether, alcohol), salts of heavy metals (Pb, Hg), urea, salicylate.

b. Characteristics of denaturation:

- The native helical structure of protein is lost.

- The primary structure of a protein with peptide linkages remains intact i.e., peptide bonds are not hydrolyzed.
- The protein loses its biological activity.
- Denatured protein becomes insoluble in the solvent in which it was originally soluble.
- The viscosity of denatured protein (solution) increases while its surface tension decreases.
- Denaturation is associated with increase in ionizable and sulfhydryl groups of protein.
- Denatured protein is more easily digested.
- Denaturation is usually irreversible.



- Careful denaturation is sometimes reversible (known as renaturation).
- Denatured protein cannot be crystallized.

Denaturation of protein

iv. Renaturation:

- The original structure of a protein is a three-dimensional structure.
- The process of returning a denatured protein structure to its original structure and normal level of biological activity, or simply the remodification or folding of an unfolded polypeptide chain of proteins to its normal three-dimensional structure is known as reconstitution of protein.
- This reconstitution of a protein structure is also known as renaturation of protein.
- In other words, renaturation of proteins is technically the opposite of denaturation of proteins.
- In a renatured protein, the primary structure of the biopolymer remains the same,

but the protein which had been denatured (with the help of such agents as chaotropic agents, detergents, heat or reagents) gets restored back to its former native structure (that is the native structure of the protein before it was denatured) and is able to function as effectively as before, because a renatured protein merely undergoes the process of reversal of a denatured protein.

- In fact, a renatured protein is able to carry out its functions better, faster and more efficiently, because it is able to pinpoint the level of biological activity that it was going through prior to the process of denaturation.

UNIT-I

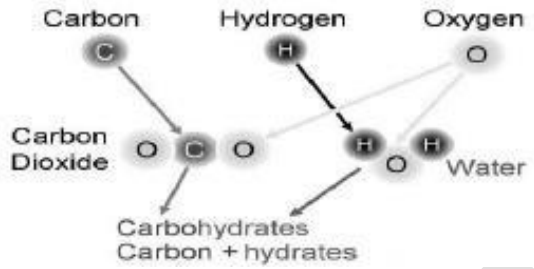
Amino acids & Proteins**: Carbohydrates and Metabolism: Carbohydrates: Structure, Function and properties of Monosaccharides, Disaccharides and Polysaccharides. Bacterial cell wall polysaccharides, Glycoprotein's and their biological functions; Glycolysis: Fate of pyruvate under aerobic and anaerobic conditions. Pentose phosphate pathway and its significance, Gluconeogenesis, Glycogenolysis and glycogen synthesis. TCA cycle.**

CARBOHYDRATES

Definition

Carbohydrates are polyhydroxylated aldehydes or ketones and their derivatives.

- The word "carbohydrate" includes polymers and other compounds synthesized from polyhydroxylated aldehydes and ketones.
- They can be synthesized in the laboratory or in living cells. Simple carbohydrates or the entire carbohydrate family may also be called saccharides.
- In general carbohydrates have the empirical formula $(CH_2O)_n$. The term generated from carbon and hydrate; though some also contain nitrogen, phosphorus, or sulfur. Chemically, carbohydrates are molecules that are composed of carbon, along with hydrogen and oxygen - usually in the same ratio as that found in water (H_2O).



They originate as products of photosynthesis, an endothermic reductive condensation of carbon dioxide requiring light energy and the pigment chlorophyll.

$nCO_2 + nH_2O$ + energy $C_nH_{2n}O_n + nO_2$

Typical carbohydrates are composed of strings or chains of monosaccharides - that is, chains of individual sugars.

Importance of carbohydrates

- Carbohydrates are of great importance in biology. The unique reaction, which makes life possible on the Earth, namely the assimilation of the green plants, produces sugar, from which originate, not only all carbohydrates but, directly or indirectly, all other components of living organisms.
- The carbohydrates are a major source of metabolic energy, both for plants and for animals that depend on plants for food.
- Aside from the sugars and starch that meet this vital nutritional role, carbohydrates also serve as a structural material (cellulose), a component of the energy transport compound ATP, recognition sites on cell surfaces, and one of three essential components of DNA and RNA.

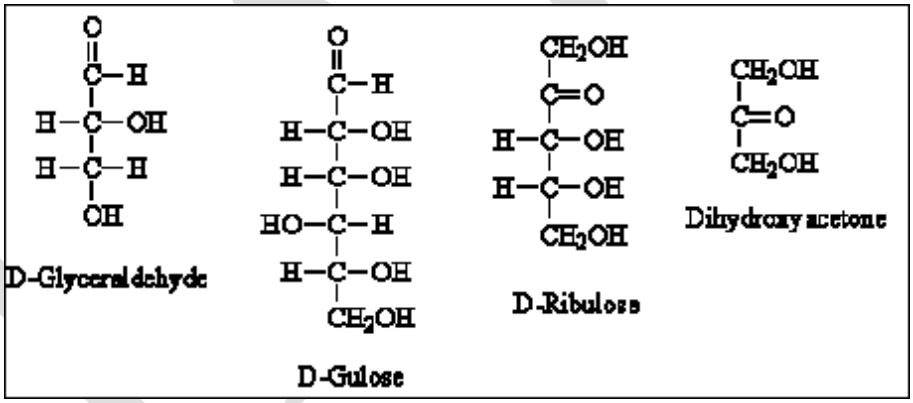
Classification

Carbohydrates are called saccharides or, if they are relatively small, sugars. Classifications of carbohydrates are outlined in the following table.

Table 1

Complexity	Simple Carbohydrates monosaccharides		Complex Carbohydrates disaccharides, oligosaccharides & polysaccharides			
Size	Tetrose C4 sugars	Pentose C5 sugars	Hexose C6 sugars	Heptose C7 sugars	Octose C8 sugars	Nonose C9 sugars
CHO Function	Aldose: sugars having an aldehyde function or an acetal equivalent.					
C=O Function	Ketose: sugars having a ketone function or an acetal equivalent.					

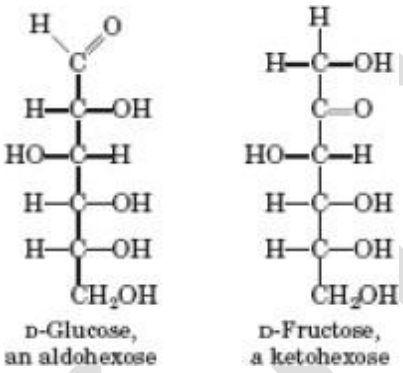
The compounds carbohydrates have common same functional groups, glyceraldehydes and gulose are classifed as aldoses and ribulose and dihydroxyacetone as ketoses. All of these compounds are alcohols with many hydroxyl groups. They are polyhydroxylated and either aldehydes or ketones.



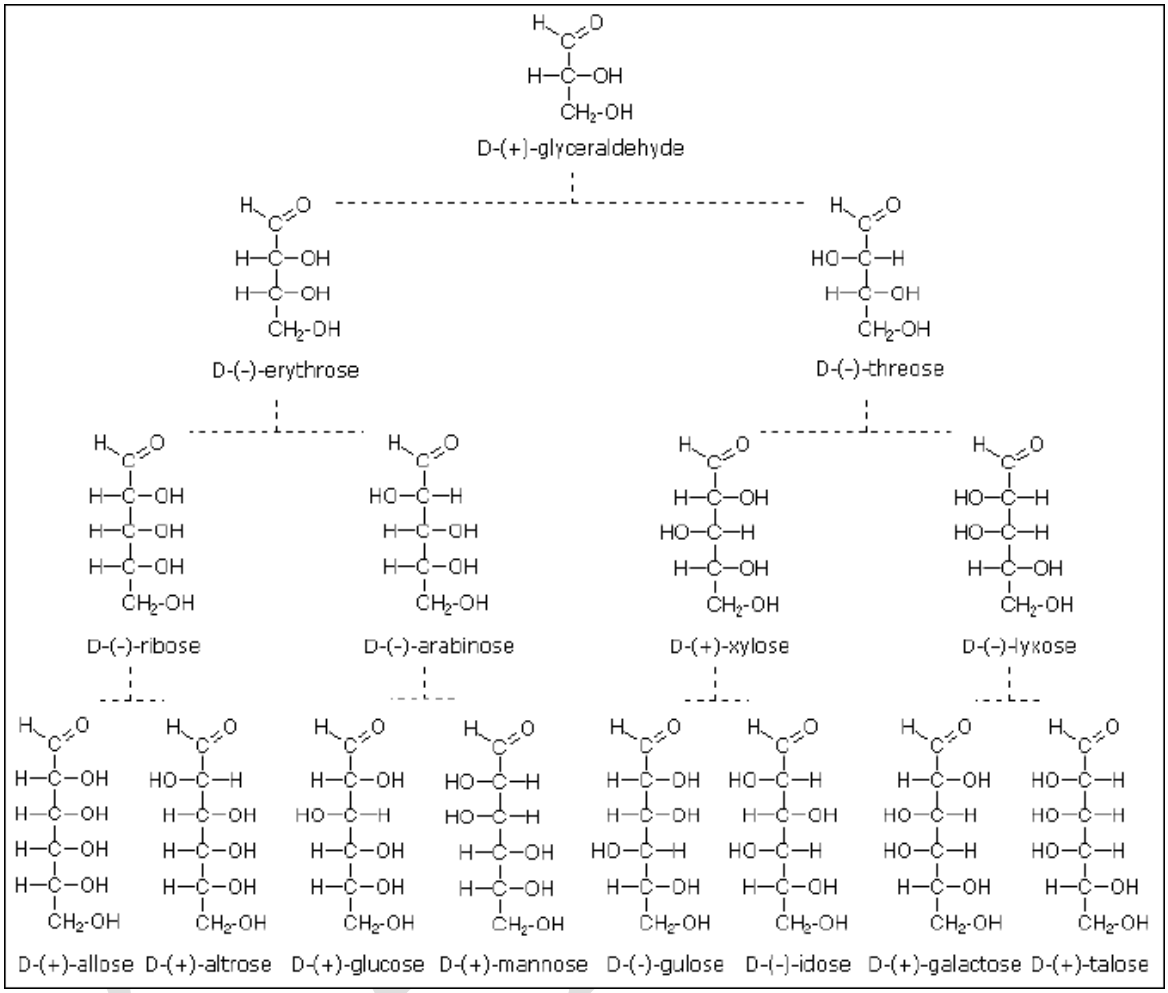
Monosaccharides

- The simplest and smallest unit of the carbohydrates is the monosaccharide, (mono = one, saccharide = sugar) from which disaccharides, oligosaccharides, and polysaccharides are constructed.
- Monosaccharides are either aldehydes or ketones, with one or more hydroxyl groups;

- the six-carbon monosaccharides glucose (an aldohexose) and fructose (a keto hexose) have five hydroxyl groups.
- The carbon atoms, to which hydroxyl groups are attached, are often chiral centers, and stereoisomerism is common among monosaccharides.

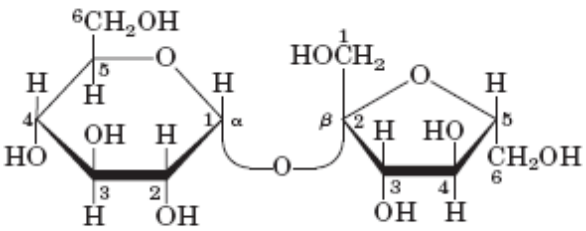


- Because these molecules have multiple asymmetric carbons, they exist as diastereoisomers, isomers that are not mirror images of each other, as well as enantiomers.
- In regard to these monosaccharides, the symbols D and L designate the absolute configuration of the asymmetric carbon farthest from the aldehyde or keto group.
- D-Ribose, the carbohydrate component of RNA, is a five-carbon aldose.
- D-Glucose, D-mannose, and D-galactose are abundant six-carbon aldoses.
- It may be noted that D-glucose and D-mannose differ in configuration only at C-2.
- Sugars differing in configuration at a single asymmetric center are called epimers.
- Thus, D-glucose and D-mannose are epimeric at C-2; D-glucose and D-galactose are epimers with respect to C-4**.**



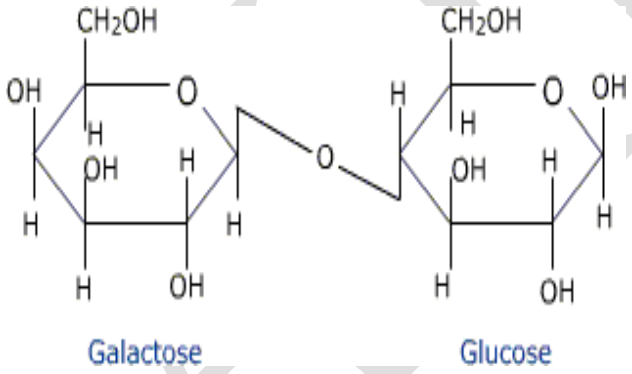
B. Disaccharides

- A disaccharide consists of two monosaccharides joined by an O-glycosidic bond.
- Disaccharides can be homo- and heterodisaccharide.
- Three most abundant disaccharides are sucrose, lactose, and maltose.
- In sucrose the anomeric carbon atoms of a glucose unit and a fructose unit are joined.



Sucrose
α-D-glucopyranosyl β-D-fructofuranoside
Glc(α1↔2β)Fru

- Lactose, the disaccharide of milk, consists of galactose joined to glucose by a β (1→4) glycosidic linkage.



- In maltose, α (1→4) glycosidic linkage joins two glucose units.
- Sucrose and lactose are heterosaccharides and maltose is homosaccharide.

C. Oligosaccharide

- An oligosaccharide is a saccharide polymer containing a small number (typically three to ten) of component sugars, and is also known as simple sugars.
- They are generally found either O- or N-linked to compatible amino acid side chains in proteins or to lipid moieties.
- They (homo-and hetero-oligosaccharides) are also liberated as intermediate products of saccharification by action of glycosidases on polysaccharides.

D. Polysaccharides

They consist of repeat units of monosaccharides or their derivatives. These units are held by glycosidic bonds. These carbohydrates liberate large number of monosaccharide molecules on hydrolysis. They are colorless and tasteless. So, they are called non-sugars. They are concerned with two important functions - structural and storage of energy. Some examples of polysaccharides are starch, cellulose, glycogen and dextrins. However starch and cellulose are the most important of these.

Polysaccharides are linear as well as branched polymers. The general formula is $(C_6H_{10}O_5)_n$, where 'n' stands for a very large number. The occurrence of branches in polysaccharides is due to the glycosidic linkages formed at any one of the hydroxyl groups of a monosaccharide.

Classification of polysaccharides

Polysaccharides are divided into two types:

Homopolysaccharides and Heteropolysaccharides.

- Homopolysaccharides

These are composed of only one type of monosaccharide molecules. Some e.g., of these are: starch, cellulose, glycogen, insulin and chitin.

- Heteropolysaccharides

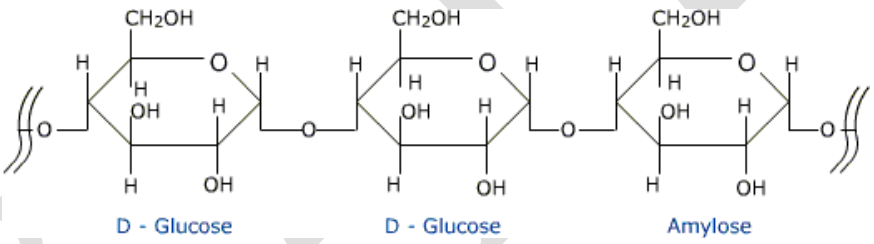
These are composed of different types of monosaccharide molecules. They are also called as heteroglycans.

- Mucopolysaccharides are the heteroglycans made of repeating units of sugar derivatives like amino sugars and uronic acids. These are known as glycosamino glycans (GAG). Important mucopolysaccharides are hyaluronic acid, chondroitin sulphate and heparin.

Structure of starch and cellulose

Starch

- Starch occurs in all plants, particularly in their seeds. The main sources are wheat, maize, rice, potatoes, barley and sorghum.
- Starch is a white amorphous powder, insoluble in cold water. It solution in water gives a blue color with iodine solution. The blue color disappears on heating and reappears on cooling. On hydrolysis with dilute acids or enzyme, starch breaks down into molecules of variable complexity and finally D-Glucose.
- Starch does not reduce Fehlingss solution or Tollens reagent and does not form an osazone indicating that all the hemiacetal hydroxyl group of glucose units (C-1) are linked with glycosidic linkages.
- Starch consists of two polysaccharide components. They are amylose (20% - 80%) and amylopectin (80% - 90%).

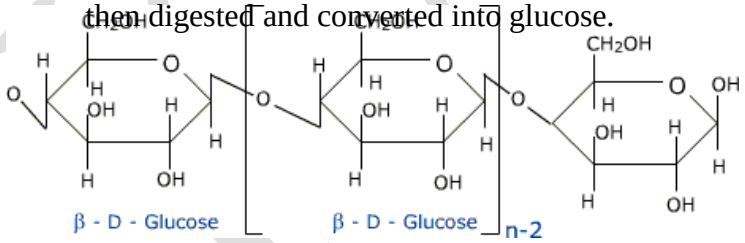


- Amylose is water soluble, long unbranched (linear) chain with 200-1000 D-glucose units. These units are joined together by a(1 4) glycosidic linkage involving C-1 of one glucose unit and C-4 of the other glucose unit. Its molecular weight can range from 10,000 to 500,000. Amylose gives blue color with iodine.
- Amylopectin is water insoluble, branched chain with 20-30 glucose units per branch. These units are held with two types of glycosidic bonds, a(1 6) glycosidic bonds at branching points and a(1 4) bonds in the linear chain. Amylopectin does not give blue color with iodine.
- Amylase (present in saliva), is the enzyme that hydrolyses starch. It acts

specifically on a(1 4) linkages. The end product of hydrolysis of starch is glucose which is an essential nutrient.

Cellulose

- It is the chief constituent of the cell walls of plants wood contains 45-50% while cotton contains 90-95% cellulose. It is a colourless amorphous solid which decomposes on heating. It is largely linear and its individual strands align with each other through multiple hydrogen bonds. This lends rigidity to its structure. It is thus used effectively as a cell wall material. Cellulose does not reduce Tollens reagent or Fehlings solution. It does not from osazone and is not fermented by yeast. It is not hydrolyzed so easily as starch but on heating with dilute H_2SO_4 under pressure yields only D-glucose.
- Cellulose is composed of b-D-glucose units linked by b(1 4) glycosidic bonds. It is a linear chain cellulose on hydrolysis yields a disaccharide cellobcose and then produces b- D-glucose. Due to the lack of an enzyme that can cleave b-glycosidic bonds, all mammals cannot digest cellulose. Large population of cellulolytic bacteria present in the stomach of ruminant mammals like cattle, sheep etc., breaks down the cellulose with the help of enzyme cellulose. It is then digested and converted into glucose.



Structure of Cellulose

B: Sugars in the cell wall of

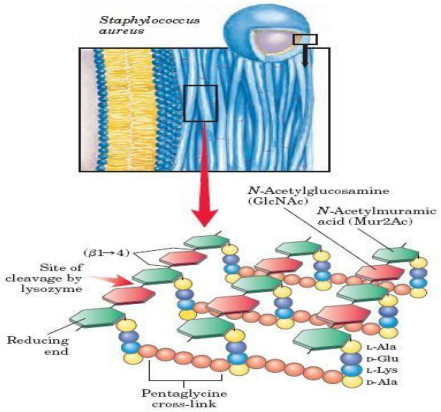
bacteria

In contrast to eukaryotic cells, bacterial cells have a cell wall in addition to a lipid bilayer membrane. These are essentially carbohydrate polymers, which offer protection from exterior hypotonic condition and the high internal osmotic pressures,

preventing swelling and bursting of the cells. The membrane consists of a peptidoglycan.

a) In Gram positive bacteria

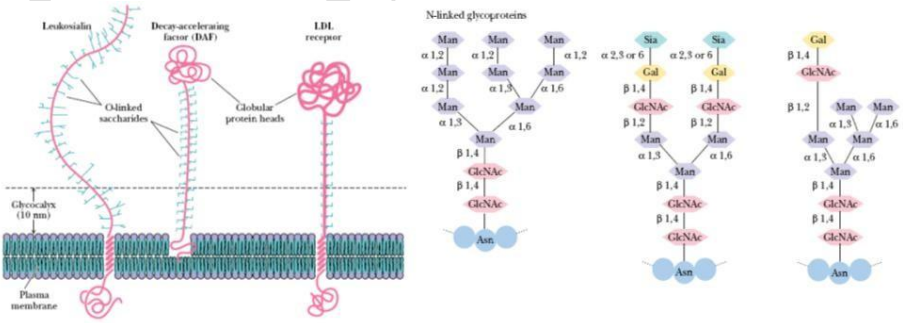
- Gram-positive bacteria can be stained with Gram stain. The wall consists of a GlcNAc (b 1->4) MurNAc repeat (like that in chitin which is a polymer of GlcNAc in (b 1->4) links, but in which the OH of lactate is in ether-linkage to C3 to form N-Acetylmuramic acid). A tetrapeptide (Ala-D-isoGlu-Lys-D-Ala) is attached in amide link to the carboxyl group of the lactate in MurNAc. The GlcNAc (b 1->4) MurNAc strands are covalently connected by a pentaglycine bridge through the epsilon amino group of the tetrapeptide Lys on one strand and the D-Ala of a tetrapeptide on another strand.
- Techioic acids are often attached to the C6 of MurNAc. Teichoic acid is a polymer of glycerol or ribitol to which alternative GlcNAc and D-Ala are linked to the middle C of the glycerol. Multiple glycerols are linked through phosphodiester bonds. These teichoic acids often make up 50% of the dry weight of the cell wall, and present a foreign (or antigenic) surface to infected hosts. These often serve as receptors for viruses that infect bacteria (bacteriophages)



GLYCOPROTEINS- Glycoproteins are proteins that contain carbohydrate. Proteins destined for an extracellular location are characteristically glycoproteins. For example,

fibronectin and proteoglycans are important components of the extracellular matrix that surrounds the cells of most tissues in animals. Immunoglobulin G molecules are the principal antibody species found circulating free in the blood plasma. Many membrane proteins are glycosylated on their extracellular segments. Many proteins found in nature are glycoproteins because they contain covalently linked oligo- and polysaccharide groups. The list of known glycoproteins includes structural proteins, enzymes, membrane receptors, transport proteins, and immunoglobulins, among others.

- Carbohydrate groups may be linked to polypeptide chains via the hydroxyl groups of serine, threonine, or hydroxylysine residues (in **O-linked saccharides**) or via the amide nitrogen of an asparagine residue (in **N-linked saccharides**). The carbohydrate residue linked to the protein in O-linked saccharides is usually an *N*-acetylgalactosamine, but mannose, galactose, and xylose residues linked to protein hydroxyls are also found. Oligosaccharides O-linked to glycophorin involve *N*acetylgalactosamine linkages and are rich in sialic acid residues. N-linked saccharides always have a unique core structure composed of two *N*-acetyl glucosamine residues linked to a branched mannose triad. Many other sugar units may be linked to each of the mannose residues of this branched core. O-Linked saccharides are often found in cell surface glycoproteins and in **mucins,** the large glycoproteins that coat and protect mucous membranes in the respiratory and gastrointestinal tracts in the body. Certain viral glycoproteins also contain O-linked sugars. O-Linked



The O-linked saccharides of glycoproteins appear in many cases to adopt extended conformations that serve to extend the functional domains of these proteins above the membrane surface. *(Adapted from Jentoft, N., 1990, Trends in Biochemical Sciences 15:291–294.)*

saccharides in glycoproteins are often found clustered in richly glycosylated domains of the polypeptide chain.

The Functions of Carbohydrates in the Body

There are five primary functions of carbohydrates in the human body. They are energy production, energy storage, building macromolecules, sparing protein, and assisting in lipid metabolism.

Carbohydrate functions as Bio Fuel

Carbohydrate functions as an energy source of the body and acts as Bio fuel.Step wise details for the process of production of energy are discussed below.

- Polysaccharides such as starch and glycogen are first hydrolyzed by enzymes to Glucose.
- Glucose is the transported from one cell to another by blood in case of animals and cell sap in case of plants.
- Glucose is then oxidized to produce carbon dioxide and water.
- Energy is released in this process which is used for functioning of the cells.

Carbohydrate functions as Primary Source of Energy

The process of production of energy by carbohydrates is described in above steps. Now it is important to note, that fats and proteins can also be burned to provide energy but **carbohydrate functions as primary source of energy**. Fats are only burned if there is non-availability of carbohydrates. When fat is burned in absence of carbohydrates, toxic compounds like called **ketone bodies** are produced. Accumulation of these ketone bodies over long period causes a condition called **Ketosis**. In this condition blood becomes unable to carry oxygen properly and this can be fatal. Thus, one of important function of carbohydrate is help burn fat properly.

Carbohydrate functions as storage food

Different forms of Carbohydrate are stored in living organism as storage food.

- Polysaccharide starch acts as storage food for plants.

- Glycogen stored in liver and muscles acts as storage food for animals.
- Inulin acts as storage food of dahlias,

onion and garlic. Thus carbohydrate performs the

function of storing food.

Carbohydrate functions as framework in body

Different Carbohydrates especially Polysaccharides act as framework in living organism.

- Cellulose forms cell wall of plant cell along with hemicelluloses and Pectin
- Chitin forms cell wall of fungal cell and exoskeleton of arthropods
- Peptidoglycan forms cell wall of bacteria and cyanobacteria.

Thus carbohydrates function as contributing material to the cellular structure.

Carbohydrate functions as Anticoagulant

Heparin is a polysaccharide (carbohydrate) which acts as anticoagulant and prevents intravascular clotting.

Carbohydrate functions as Antigen

Many antigens are glycoprotein (which contains oligosaccharide) in nature and give immunological properties to the blood.

Carbohydrate functions as Hormone

Many Hormones like FSH (Follicular Stimulating Hormone which takes part in ovulation in females) and LH (Leutinizing Hormone) are glycoprotein and help in reproductive processes.

Carbohydrates provide raw material for industry

Carbohydrates are an important component of many industries like textile, paper, lacquers and breweries.

Other Functions

Agar is polysaccharide used in culture media, laxative and food.
Cellulose acts as roughage of food. It stimulates peristalsis movement and secretion of digestive enzymes.

Hyaluronic acid found in between joints acts as synovial fluid and provides frictionless movement.

Carbohydrate

Metabolism

Introduction

Glucose is the major form of sugar moiety present in blood and other body fluids. The digestion of food carbohydrates, such as starch, sucrose, and lactose produces the monosaccharides glucose, fructose and galactose, which pass into the blood stream. The study of synthesis (Anabolism) and degradation (Catabolism) of biomolecules is biochemically termed as metabolism.

Anabolism + Catabolism = Metabolism

(Synthesis) (Degradation)

Since glucose is the most important carbohydrate existing in physiological amounts in the body and is easily absorbed from the diet, the metabolism of carbohydrate resolves itself to the study of the metabolism of glucose and its main derivatives. The monosaccharides galactose and fructose are converted to glucose in the liver. All the monosaccharides are completely absorbed in the small intestine.

The glucose in the circulating blood and tissue fluids is drawn upon by all the cells of the body and used for the production of energy. Normally carbohydrate metabolism supplies more than half of the energy requirements of the body. In fact the brain largely depends upon carbohydrate metabolism as a source of energy and quickly ceases to function properly when the blood glucose level falls much below normal.

Carbohydrate as a source of energy

The major function of carbohydrate in metabolism is to serve as fuel and get oxidised to provide energy for other metabolic processes. The metabolic intermediates are

used for various biosynthetic reactions. For this purpose, carbohydrate is utilized by the cells mainly in the form of glucose. A major part of dietary glucose is converted to glycogen for storage in liver. Glucose is degraded in the cell by way of a series of phosphorylated intermediates mainly via two metabolic pathways.

1. Glycolysis
2. Tricarboxylic acid cycle

Glycolysis

- Oxidation of glucose to pyruvate is called glycolysis.
- It was first described by Embden-Meyerhof and Parnas. Hence it is also called as Embden-Meyerhof pathway.
- Glycolysis occurs virtually in all tissues.
- Erythrocytes and nervous tissues derive the energy mainly from glycolysis.
- This pathway is unique in the sense that it can proceed in both aerobic (presence of O2) and anaerobic (absence of O2) conditions.
- All the enzymes of glycolysis are found in the extra mitochondrial soluble fraction of the cell, the cytosol.

Reactions of glycolytic pathway

Series of reactions of glycolytic pathway which degrades glucose to pyruvate are represented below. The sequence of reactions occurring in glycolysis may be considered under four stages.

Stage I

This is a *preparatory phase*. Before the glucose molecule can be split, the rather asymmetric glucose molecule is converted to almost symmetrical form, fructose 1,6-diphosphate by donation of two phosphate groups from ATP.

1. Uptake of glucose by cells and its phosphorylation

Glucose is freely permeable to liver cells, intestinal mucosa and kidney tubules where glucose is taken up by 'active' transport. In other tissues insulin facilitates the uptake

of glucose. Glucose is phosphorylated to form glucose 6-phosphate. The enzyme involved in this reaction is glucokinase. This reaction is irreversible.

2. Conversion of glucose 6-phosphate to fructose 6-phosphate

Glucose 6-phosphate is converted to fructose 6-phosphate by the enzyme phosphogluco isomerase.

3. Conversion of fructose 6-phosphate to fructose 1,6 diphosphate

Fructose 6-phosphate is phosphorylated irreversibly at 1 position catalyzed by the enzyme phosphofructokinase to produce fructose 1,6-diphosphate

Stage II

1. Actual splitting of fructose 1,6 diphosphate

Fructose 1,6 diphosphate is split by the enzyme aldolase into two molecules of triose phosphates, an aldotriose-glyceraldehyde 3-phosphate and one ketotriose - dihydroxy acetone phosphate. The reaction is reversible. There is neither expenditure of energy nor formation of ATP.

2. **Interconvertion of triose**

phosphates Both triose

phosphates are inter-convertible

Stage III

It is the energy yielding stage. Reactions of this type in which an aldehyde group is oxidised to an acid are accompanied by liberation of large amounts of potentially useful energy.

1. Oxidation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate

Glycolysis proceeds by the oxidation of glyceraldehyde 3-phosphate to form 1,3-bisphosphoglycerate. The reaction is catalyzed by the enzyme glyceraldehyde 3-phosphate dehydrogenase

2. Conversion of 1,3-bisphosphoglycerate to 3-phosphoglycerate

The reaction is catalyzed by the enzyme phosphoglycerate kinase. The high energy phosphate bond at position - 1 is transferred to ADP to form ATP molecule.

Stage IV

It is the recovery of the phosphate group from 3-phosphoglycerate. The two molecules of 3- phosphoglycerate, the end-product of the previous stage, still retains the phosphate group, originally derived from ATP in

Stage I.

1. Conversion of 3-phosphoglycerate to 2-phosphoglycerate.

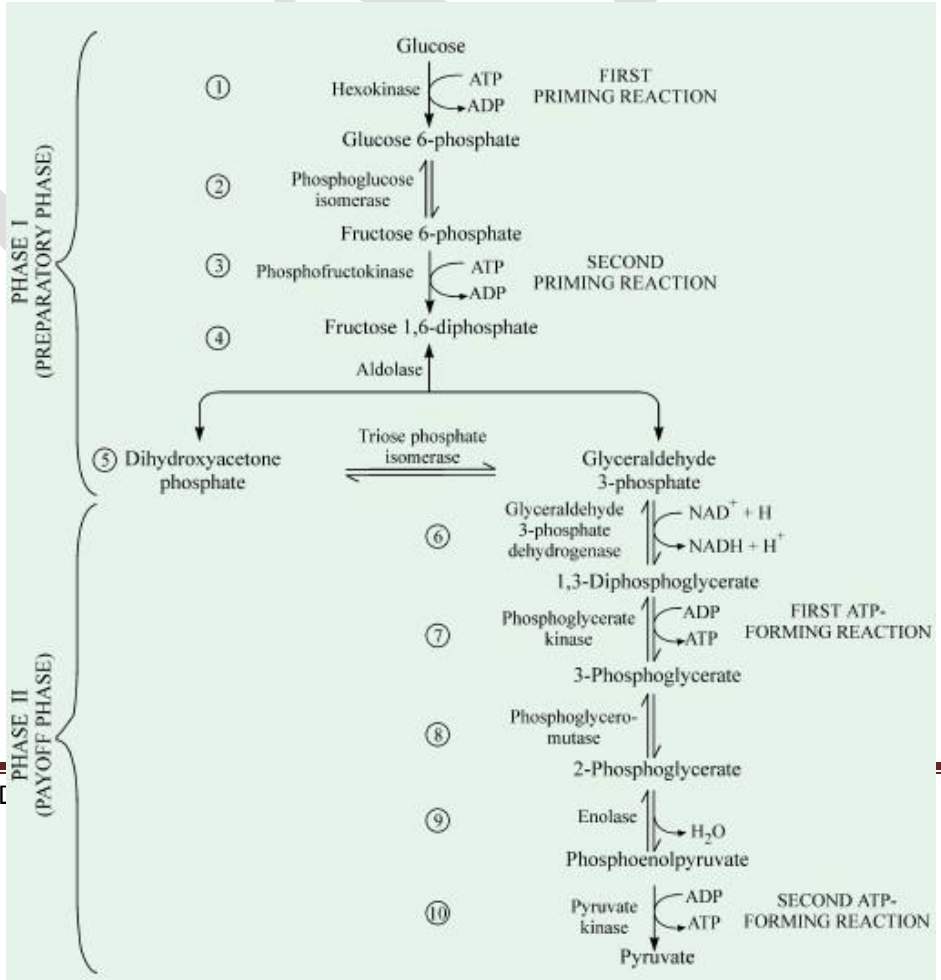
3-phosphoglycerate formed by the above reaction is converted to 2-phosphoglycerate, catalyzed by the enzyme phosphoglycerate mutase.

2. Conversion of 2-phosphoglycerate to phosphoenol pyruvate

The reaction is catalyzed by the enzyme enolase, the enzyme requires the presence of either Mg2+ or Mn2+ ions for activity.

3. Conversion of phosphoenol pyruvate to pyruvate

Phosphoenol pyruvate is converted to pyruvate, the reaction is catalysed by the enzyme pyruvate kinase. The high energy phosphate group of phosphoenol pyruvate



is directly transferred to ADP, producing ATP. The reaction is irreversible.

Summary of glycolysis

During glycolysis NAD+ is reduced to NADH. At the same time, glyceraldehyde 3-phosphate is oxidized to 1,3-bisphosphoglycerate. To conserve the coenzyme NAD+, NADH must be reoxidized. Under anaerobic conditions this is done when pyruvic acid is converted to lactic

acid. In the presence of oxygen, NADH, can be oxidized to NAD+ with the help of the respiratory enzymes.

Energy yield per glucose molecule oxidation

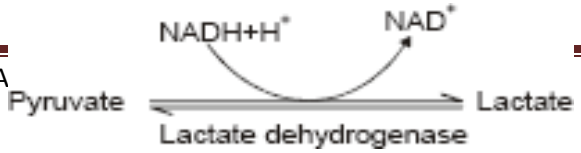
During glycolysis ATP molecules are used and formed in the following reactions (aerobic phase).

Reactions Catalyzed	ATP used	ATP formed
Stage I 1. Glucokinase (for phosphorylation)	1	
2. Phosphofructokinase I (for phosphorylation)	1	
Stage II 3. Glyceraldehyde 3-phosphate dehydrogenase (oxidation of 2 NADH in respiratory chain)		6
4. Phosphoglycerate kinase (substrate level phosphorylation)		2
Stage IV 5. Pyruvate kinase (substrate level phosphorylation)		2
Total	2	10

Net gain = 8 ATP

Anaerobic phase

In the absence of O2, reoxidation of NADH at glyceraldehyde 3-phosphate dehydrogenase stage cannot take place in respiratory chain. But the cells have limited coenzyme. Hence to continue the glycolysis **NADH must be reoxidized to NAD**+. This is achieved by reoxidation of NADH by conversionof pyruvate to lactate

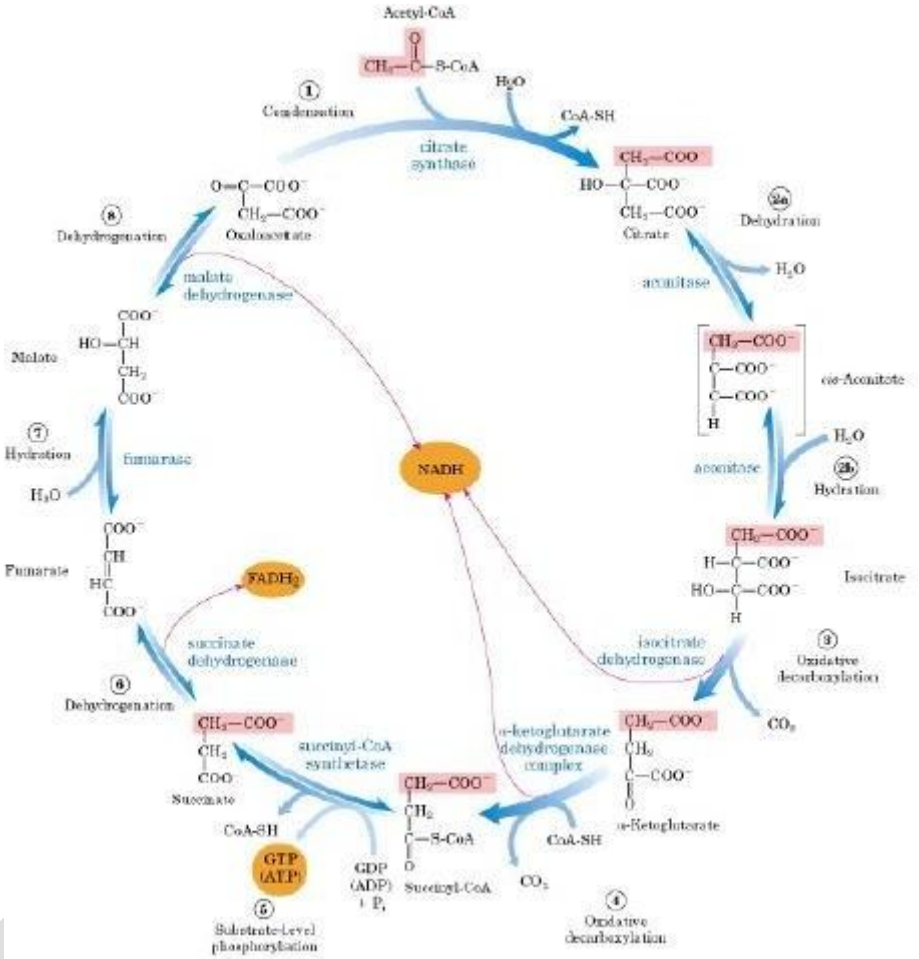


(without producing ATP).

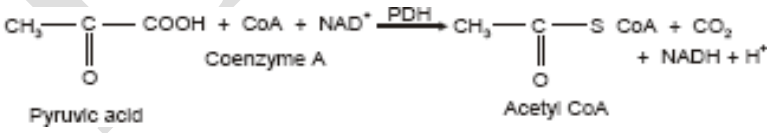
It is to be noted that in the reaction catalyzed by glyceraldehyde 3-phosphate dehydrogenase, therefore, no ATP is produced.
In the anaerobic phase oxidation of one glucose molecule produces 4 - 2 = 2 ATP.

Tricarboxylic acid cycle (TCA cycle)

This cycle is the aerobic phase of carbohydrate metabolism and follows the anaerobic pathway from the stage of pyruvate and is called as citric acid cycle or TCA cycle. The name citric acid cycle stems from citric acid which is formed in the first step of this cycle. This cycle is also named "Kerbs cycle" after H.A. Krebs, an English biochemist who worked on it.



Under aerobic conditions, pyruvate is oxidatively decarboxylated to acetyl coenzyme A (active acetate) before entering the citric acid cycle. This occurs in the mitochondrial matrix and forms a link between glycolysis and TCA cycle.



PDH-pyruvate dehydrogenase

This reaction is catalysed by the multienzyme complex known as pyruvate dehydrogenase complex.

Reactions of the citric acid cycle

There are eight steps in the cycle and the reactions are as follows.

1. Formation of citrate - The first reaction of the cycle is the condensation of acetyl CoA with oxaloacetate to form citrate, catalyzed by citrate synthase. This is an irreversible reaction.

2. Formation of isocitrate via cis aconitate - The enzyme aconitase catalyzes the reversible transformation of citrate to isocitrate, through the intermediary formation of cis aconitate.

3. Oxidation of isocitrate to a-ketoglutarate and CO2 - In the next step, isocitrate dehydrogenase catalyzes oxidative decarboxylation of isocitrate to form a-ketoglutarate.

4. Oxidation of a-keto glutarate to succinyl CoA and CO2 - The next step is another oxidative decarboxylation, in which a-ketoglutarate is converted to succinyl CoA and CO2 by the action of the a-ketoglutarate dehydrogenase complex. The reaction is irreversible.

5. Conversion of succinyl CoA to succinate - The product of the preceding step, succinyl CoA is converted to succinate to continue the cycle. GTP is formed in this step (substrate level phosphorylation).

6. Oxidation of succinate to fumarate - The succinate formed from succinyl CoA is oxidized to fumarate by the enzyme succinate dehydrogenase

7. Hydration of fumarate to malate - The reversible hydration of fumarate to malate is catalyzed by fumarase.

8. Oxidation of malate to oxaloacetate - The last reaction of the citric acid cycle is, NAD linked malate - dehydrogenase which catalyses the oxidation of malate to oxaloacetate.

Energy yield from TCA cycle

If one molecule of the substrate is oxidized through

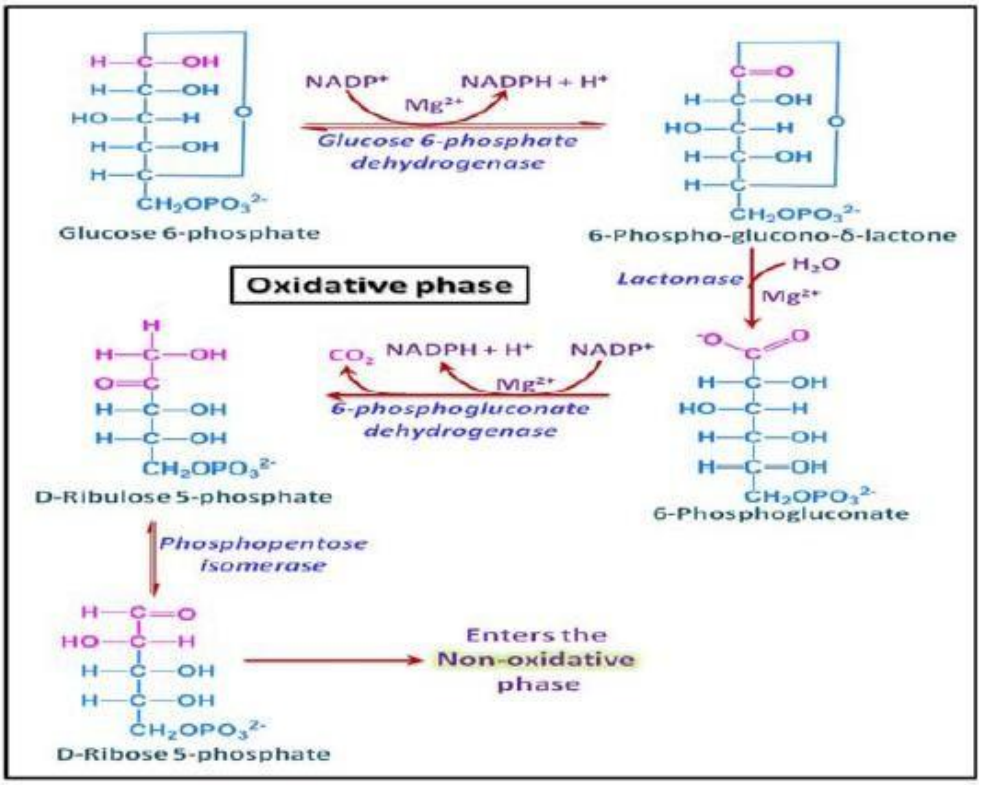
Reactions	No.of ATP formed
1. 2 isocitrate → 2 α-ketoglutarate (2 $NADH + 2H^+$) (2 × 3)	6
2. 2 α-ketoglutarate → 2 succinyl CoA (2 $NADH + 2H^+$) (2 × 3)	6
3. 2 succinyl CoA → 2 succinate (2 GTP = 2ATP)	2
4. 2 succinate → 2 Fumarate (2 $FADH_2$) (2 × 2)	4
5. 2 malate → 2 oxaloacetate (2 $NADH + 2H^+$) (2 × 3)	6

NADH in the electron transport chain three molecules of ATP will be formed and through FADH2, two ATP molecules will be generated.

As one molecule of glucose gives rise to two molecules of pyruvate by glycolysis, intermediates of citric acid cycle also result as two molecules.

HMP shunt pathway

Although glycolysis and citric acid cycle are the common pathways by which animal tissues oxidise glucose to CO2 and H2O with the liberation of energy in the form of ATP, a number of alternative pathways are also discovered. The most important one is Hexose Monophosphate Shunt Pathway (HMP shunt). The pathway occurs in the extra mitochondrial soluble portion of the cells. Unlike glycolysis and Krebs cycle which are primarily concerned with the generation of ATP, HMP shunt generates a different type of metabolic energy - the reducing power. Some of the electrons and hydrogen atoms of fuel molecules are conserved for biosynthetic purposes rather than ATP formation. This reducing power of cells is NADPH (reduced nicotinamide adenine dinucleotide phosphate).



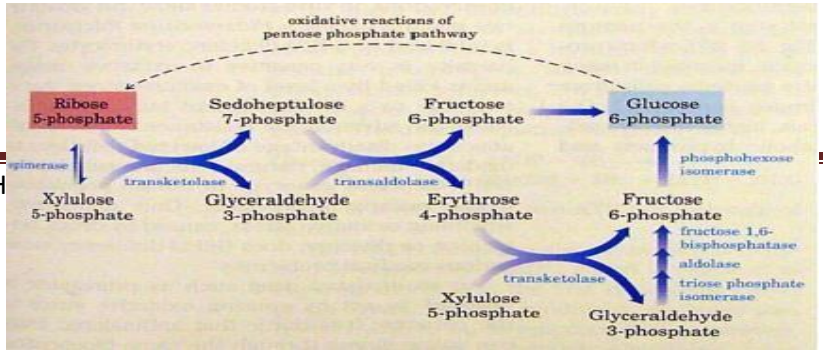
HMP Pathway Figure

The fundamental difference between NADPH and NADH (reduced nicotinamide adenine dinucleotide) is that NADH is oxidised by the respiratory chain to generate ATP whereas NADPH serves as a hydrogen and electron donor in reductive biosynthesis, for example in the biosynthesis of fatty acids and steroids.

The first reaction of the pentose phosphate pathway is the dehydrogenation of glucose 6- phosphate by glucose 6-phosphate dehydrogenase to form 6-

phosphoglucono d-lactone.

Step 1 - Glucose 6-phosphate in the presence of NADP and the enzyme glucose 6-



phosphate dehydrogenase, forms 6-phospho glucono-d-lactone.

The first molecule of NADPH is produced in this step.

Step 2 - The 6-phospho glucono d-lactone is unstable and the ester spontaneously hydrolyses to 6-phosphogluconate. The enzyme that catalyses the reaction is lactonase

Step 3 - 6-phospho gluconate further undergoes dehydrogenation and decarboxylation by 6- phosphogluconate dehydrogenase to form the ketopentose, D-ribulose 5-phosphate. This reaction generates the second molecule of NADPH.

Step 4 - The enzyme phosphopentose isomerase converts ribulose 5-phosphate to its aldose isomer, D-ribose 5-phosphate.

In some tissues, the hexose phosphate pathway ends at this point, and its overall equation is

$$\text{Glucose 6-phosphate} + 2NADP^{+} + H_2O \longrightarrow \text{Ribose 5-phosphate} + CO_2 + 2NADPH + 2H^{+}$$

The net result is the production of NADPH, a reductant for biosynthetic reactions, and ribose 5- phosphate, a precursor for nucleotide synthesis.

Glycogen

Glycogen is the major storage form of carbohydrate in animals and corresponds to starch in plants.It occurs mainly in liver.

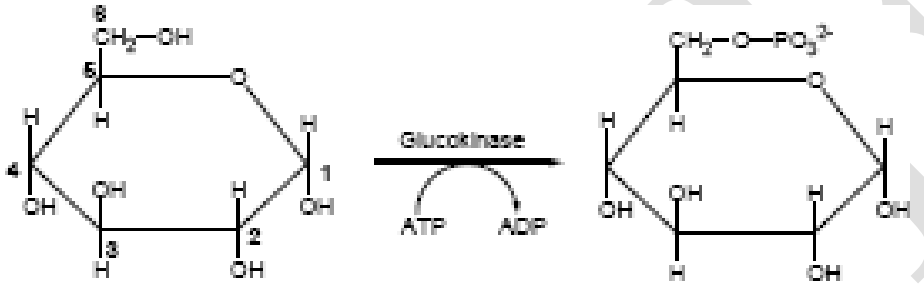
Glycogen biosynthesis

- The process of biosynthesis of glycogen from glucose is known as glycogenesis. This occurs in all the tissues of the body but the major sites are liver and muscles. A considerable amount is synthesised in kidney also.
- Glycogenesis is a very essential process since the excess of glucose is converted and stored up as glycogen which could be utilised at the time of requirement. In the absence of this process the tissues are exposed to excess

of glucose immediately after a meal and they are starved of it at other times. The following are the various reactions of glycogenesis.

Step 1

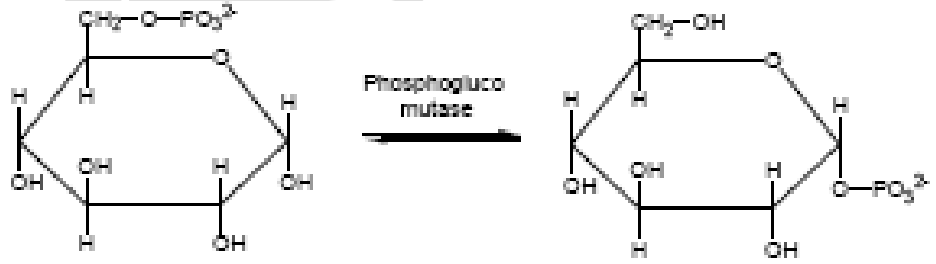
Glucose is phosphorylated to glucose 6-phosphate, a reaction that is common to the first reaction in the pathway of glycolysis from glucose. This reaction is catalysed by hexokinase in muscle and glucokinase in liver in the presence of ATP.



Glucose Glucose 6-phosphate

Step 2

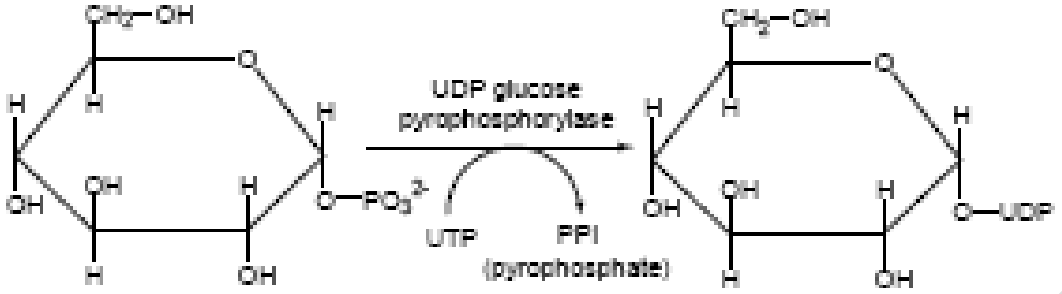
Glucose 6-phosphate is then reversibly converted to glucose 1-phosphate in a reaction catalysed by enzyme phosphogluco mutase. This process requires Mg2+ and a small amount of glucose 1,6-diphosphate as coenzyme.



Glucose 6-phosphate Glucose 1-phosphate

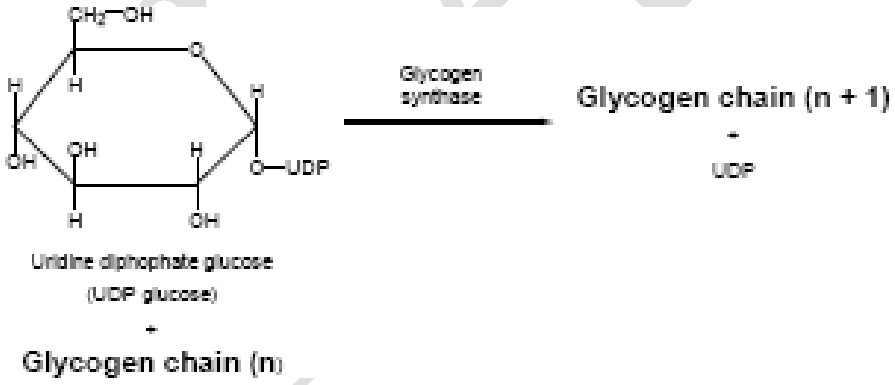
Step 3

The glucose 1-phosphate is then activated by the energy produced by the hydrolysis of uridine triphosphate (UTP) in the presence of uridine diphosphate glucose pyrophophosrylase. This is a key reaction in glycogen biosynthesis.



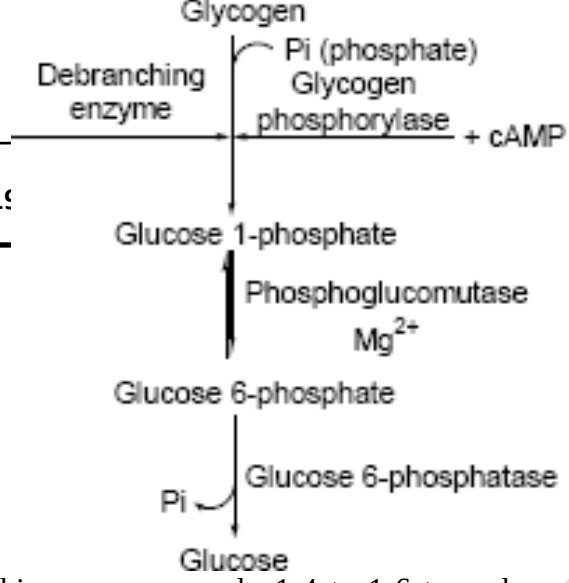
Step 4

UDP-glucose is the immediate donor of glucose residues in the reaction catalyzed by glycogen synthase, which promotes the transfer of the glucose residue from UDP-glucose to a nonreducing end of a branched glycogen chain.



Step 5

When the chain has become long with more than 8 glucose units, a second enzyme,



namely branching enzyme amylo 1-4 to 1-6 transglycosylase acts on the glycogen and helps in joining of 1,4 glycogen chain with a similar neighbouring chain to form a 1-6 linkage, thus forming a branching point in the molecule. Glycogen thus formed may be stored in liver, muscles and tissues.

Degradation of glycogen (Glycogenolysis)

When the blood sugar level falls (Hypoglycemia), glycogen stored in the tissues specially glycogen of liver and muscles may be broken down and this process of breakdown of glycogen is called glycogenolysis.

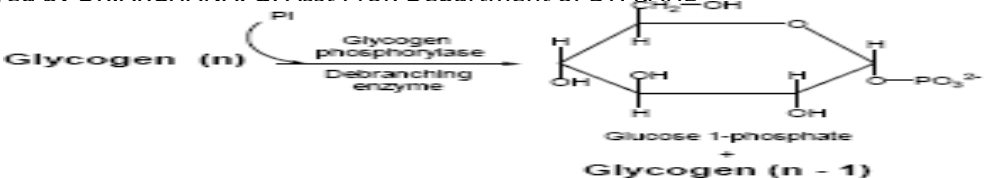
Glycogenolysis

The following are the various steps of glycogenolysis.

Step 1

The first step in the breakdown of glycogen is catalyzed by two enzymes which act independently. The first enzyme, namely glycogen phosphorylase with inorganic phosphate catalyses the cleavage of a terminal a 1-4 bond of glycogen to produce glycogen with one

molecule less and a molecule of glucose 1-phosphate. The enzyme glycogen phosphorylase cannot cleave a 1-6 linkage. This is carried out by another enzyme

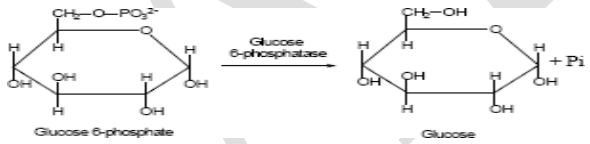


called the debranching enzyme (a 1-6 glucosidase) which hydrolyses these bonds and thus make more a 1-4 linkage accessible to the action of glycogen phosphorylase. The combined action of glycogen phosphorylase and the debranching enzyme converts glycogen to glucose 1-phosphate.

Step 2

The glucose 1-phosphate is then reversibly converted to glucose 6-phosphate by the action of the enzyme phosphoglucomutase

Step 3 The next reaction namely the conversion of glucose 6-phosphate to glucose takes



place in the liver and kidney by the action of the enzyme glucose 6-phosphatase.

Glucose 6-phosphatase removes phosphate group from glucose 6-phosphate enabling the free glucose to diffuse from the cell into the extra cellular spaces including blood. This reaction does not occur in the muscles because muscles lack the enzyme glucose 6-phosphatase.

Gluconeogenesis

- The synthesis of glucose from non-carbohydrate precursors is known as gluconeogenesis.
- The major site of gluconeogenesis is liver.

- It usually occurs when the carbohydrate in the diet is insufficient to meet the demand in the body, with the intake of protein rich diet and at the time of starvation, when tissue proteins are broken down to amino acids.

Gluconeogenesis and glycolysis

Gluconeogenesis and glycolysis are opposing metabolic pathways and share a number of enzymes. In glycolysis, glucose is converted to pyruvate and in gluconeogenesis pyruvate is converted to glucose. However gluconeogenesis is not exact reversal of glycolysis.

There are three essentially irrevesible steps in glycolysis which are

1. Glucose + ATP $\xrightarrow{\text{Glucokinase}}$ Glucose 6-phosphate + ADP
2. Fructose 6-phosphate + ATP $\xrightarrow{\text{Phosphofructo kinase}}$ Fructose 1,6-diphosphate + ADP
3. Phosphoenol pyruvate + ADP $\xrightarrow{\text{Pyruvate kinase}}$ Pyruvate + ATP

In gluconeogenesis these three reactions are bypassed or\ substituted by the following news ones.

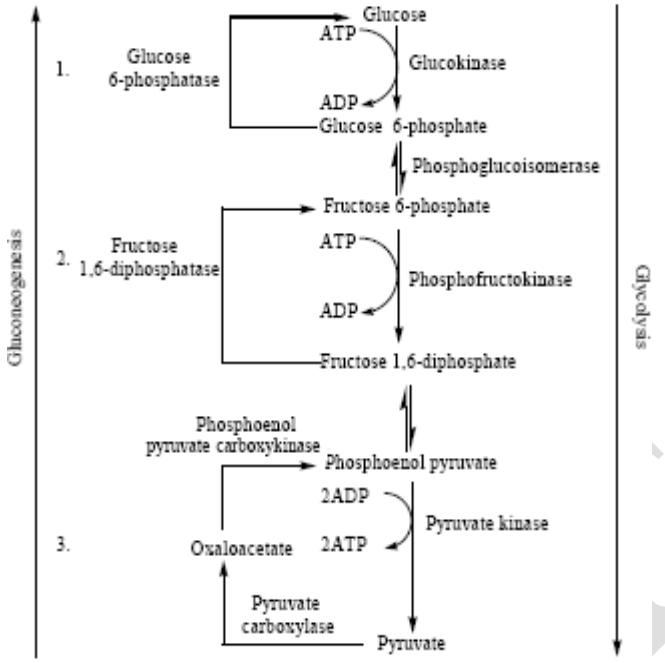


Fig. 3.5 Gluconeogenesis and Glycolysis

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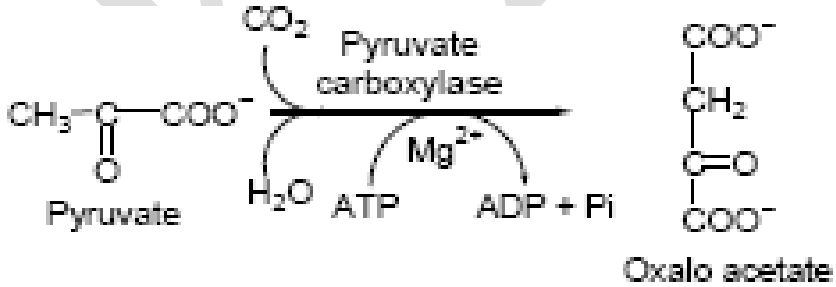
Gluconeogenesis and glycolysis

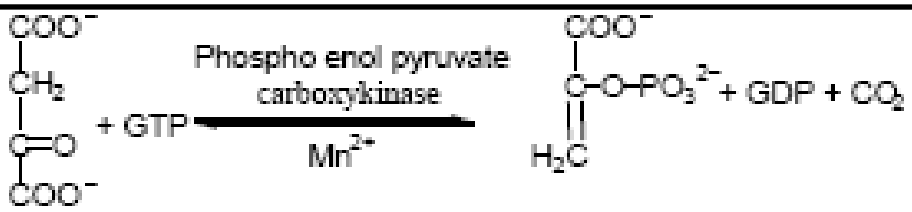
Reactions of gluconeogenesis

1. The formation of phosphoenol pyruvate begins with the carboxylation of pyruvate at the expense of ATP to form oxalo acetate. Oxaloacetate is converted to phosphoenolpyruvate by phosphorylation with GTP, accompanied by a simultaneous



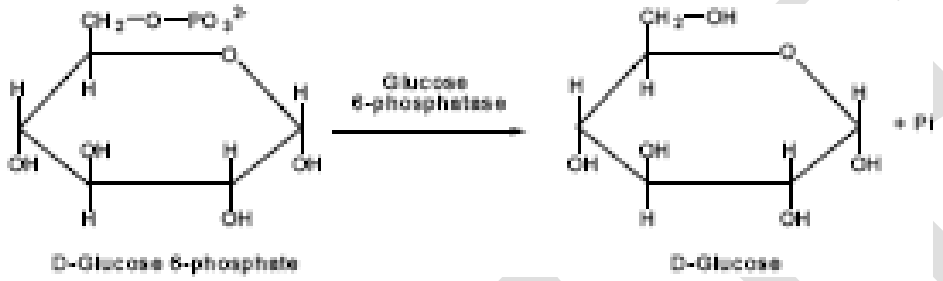
decarboxylation.

2. Fructose 6-phosphate is formed from fructose 1,6-diphosphate by hydrolysis and the enzyme fructose 1,6-diphosphatase catalyses this reaction.



Oxalo acetate Phospho enol pyruvate

3. Glucose is formed by hydrolysis of glucose 6-phosphate catalysed by glucose 6-phosphatase.



D-Glucose 6-phosphate D-Glucose

Gluconeogenesis of amino acids

Amino acids which could be converted to glucose are called glucogenic amino acids. Most of the glucogenic amino acids are converted to the intermediates of citric acid cycle either by transamination or deamination.

Gluconeogenesis of Propionate

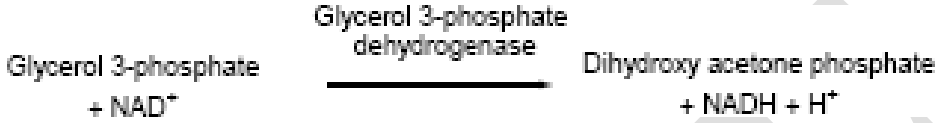
Propionate is a major source of glucose in ruminants, and enters the main gluconeogenic pathway via the citric acid cycle after conversion to succinyl CoA.

Gluconeogenesis of Glycerol

At the time of starvation glycerol can also undergo gluconeogenesis. When the triglycerides are hydrolysed in the adipose tissue, glycerol is released. Further metabolism of glycerol does not take place in the adipose tissue because of the lack of glycerol kinase necessary to phosphorylate

it. Instead, glycerol passes to the liver where it is phosphorylated to glycerol 3-phosphate by the enzyme glycerol kinase.

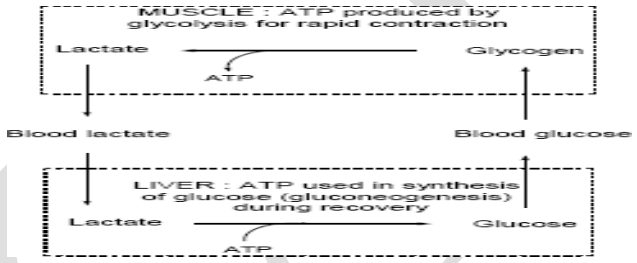
This pathway connects the triose phosphate stage of glycolysis, because glycerol 3-phosphate is oxidized to dihydroxy acetone phosphate in the presence of NAD+ and glycerol 3-phosphate dehydrogenase.



This dihydroxy acetone phosphate enters gluconeogenesis pathway and gets converted to glucose. Liver and kidney are able to convert glycerol to blood glucose by making use of the above enzymes.

Gluconeogenesis of lactic acid (Cori cycle)

The liver and skeletal muscles exhibit a special metabolic cooperation as far as carbohydrates are concerned by the way of a cycle of conversions known as Cori



cycle.

Cori cycle

In this cycle liver glycogen may be converted into muscle glycogen and vice versa and the major raw material of this cycle is lactate produced by the active skeletal muscles. At the time of heavy muscular work or strenuous exercise, O2 supply is inadequate in active muscles but the muscles keep contracting to the maximum. Hence, glycogen stored up in the muscle is converted into lactic acid by glycogenolysis followed by anaerobic glycolysis and thus
lactate gets accumulated in the muscle. Muscle tissue lacks the enzyme glucose 6-phosphatase hence it is incapable of synthesizing glucose from lactic acid and the conversion take place only in the liver.

Lactate diffuses out of the muscle and enters the liver through blood. In the liver lactate is oxidised to pyruvate which undergoes the process of gluconeogenesis resulting in the resynthesis of glucose. The glycogen may be once again converted to

glucose (glycogenolysis) and may be recycled to the muscle through the blood. The process of gluconeogenesis completes the cycle by converting glucose once again to muscle glycogen.

UNIT-III

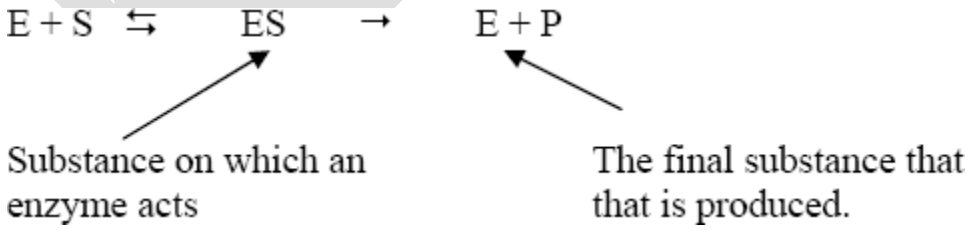
SYLLABUS

Enzymes : Nomenclature and classification of Enzymes, Holoenzyme, apoenzyme, Cofactors, coenzyme, groups, metalloenzymes, monomeric & oligomeric enzymes, activation energy and transition state, enzyme activity, specific activity, common features of active sites, Role of: NAD+, NADP+, FMN/FAD, coenzymes A, Thiamine pyrophosphate, Pyridoxal phosphate, lipoic-acid, Biotin vitamin B12, Tetrahydrofolate and metallic ions. Photosynthesis – Photosystem I and II.

ENZYME

Enzymes are protein specialized to catalyse biological reactions (Biocatalyse):

- Increase the speed of reactions
- They do not changes in the reaction.
- Very important to life as life depends on biological reactions (example digestion).
- Any change in a single enzyme can have very harmful effects.
- Very specific
- Enzymes (E) act on certain substances which are known as substrate(s). They form an Enzyme-Substance Complex (ES) which is broken or changed to give the Product (P).



CLASSIFICATION/ NOMENCLATURE OF ENZYMES

The most generally accepted classification of enzymes is in term of the reactions they catalyse.

Individual enzymes are named by adding the suffix „„ase"" to the name of the substrate acted upon or to the reaction brought about.

In the 1960"s the International union of Biochemists and molecular Biologist (IUBMB) established a commission on enzyme nomenclature for the ever increasing number enzymes being identified. The commission identified enzymes by the type of reactions they catalysed and defined six major classes.

(1) OXIDOREDUCTASES

These groups of enzyme catalyse the oxidation reduction reaction. They are subdivided into

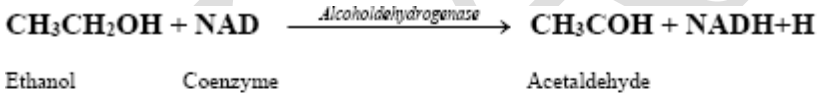
(a)Dehydrogenases: which catalyse the removal of 2 atoms of hydrogen from substrates and their transference to a co-acceptor. (They remove 2H to form double bonds).

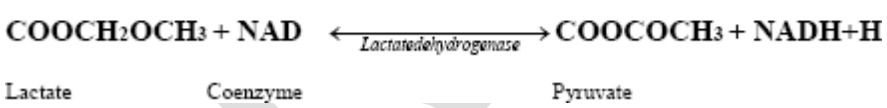
(b) Oxidases: They catalyse the direct reduction of oxygen (oxidation).

(c) Oxygenases: They catalyse the incorporation of oxygen into substrate molecule.

(d) Oxidative deaminates: they catalyse the oxidation of amino compounds with elimination of a molecule of NH3 ammonia.

Examples:





(2) TRANSFERASES

They bring about the exchange of groups between substrates i.e. group transfer reactions.

AB+CD □□□□AC+BD

They are subdivided into:

(a) Amino transferases: These bring about the exchange of amino and keto group between an amino acid and a keto acid.

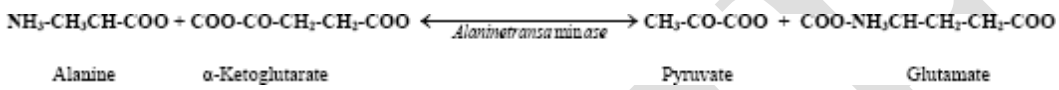
(b) Kinases: These bring about the transfer of a phosphate radical using ATP as the donor or ADP acceptor.

(c) Phosphorylases: which are phosphorylytic analog of the hydrolytic enzymes. They catalyse the splitting of the substrates with a molecule of phosphoric acid instead of water.

(d)Glycosyltransferases: These bring about the transfer of a glycosyl group.

(e) C1-Transferases: These bring about the transfer of an acyl group.

Example:



(3) HYDROLASES

These groups of enzymes employ water to cleave covalent bonds. They include the digestive enzymes. They catalyze the hydrolytic cleavage of C-C, C-O, C-N, P-O, and certain other bonds, including acid anhydride bonds They catalyse this type of reaction.

$$AB + H_2O \longrightarrow AOH + HB$$

This group may be subdivided according to the type of substrates acted on.

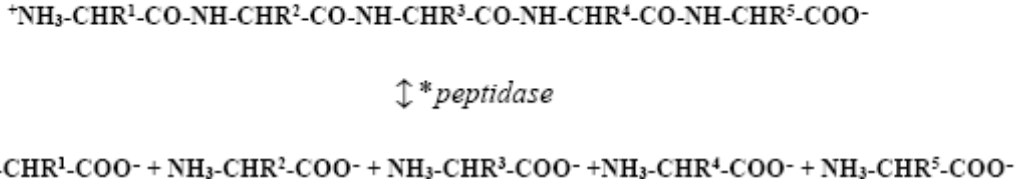
(a) Peptidases are enzymes (proteolytic enzymes or peptide hydrolase) which catalyse the hydrolysis of the peptide bonds.

(b) Amylases are enzymes (carbohydrases or glycoside hydrolases) which catalyse the hydrolysis of glycosidic bonds.

(c) Lipases are enzymes which catalyse the hydrolysis of lipids.

(d)Phosphatases catalyse the hydrolysis of phosphoric acids.

Examples:



(4) LYASES:

These are enzymes which remove or add groups to substrates non-hydrolytically by electron rearrangement therefore leaving double bond or adding groups to double bonds. They catalyse the cleavage of C-C, C-O, C-N, and other bonds by elimination, leaving double bonds, and also add groups to double bonds. The group however also include the decarboxylases, deaminases, deamidases, dehydrases. It involves the addition of groups to a double bond or formation of double bonds by the removal of group. The reaction may be represented by this scheme:

$$AB \longleftrightarrow A + B$$

For example

$$CH_3\text{-}CO\text{-}COO + H \xleftrightarrow[Pyruvatedecarboxylase]{} CH_3\text{-}COH + CO_2$$

Pyruvate **Acetaldehyde**

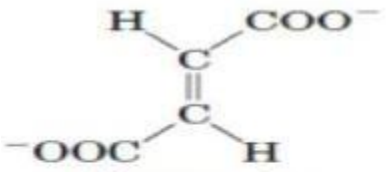
(5) ISOMERASES

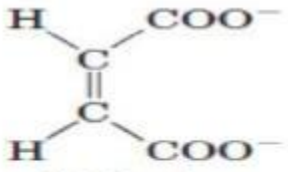
They are enzymes which catalyse the internal rearrangement within a substrate and therefore do not involve the addition or removal of group. These group include; (1)Epimarases which catalyse the inter-conversion of the D and L isomers.

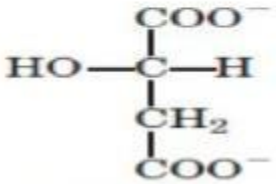
(2) Cis and trans-isomarases which catalyse group transfer between the cis and Trans location of a substrate

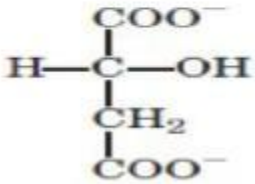
(3) mutases are enzymes which catalyse the intramolecular transfer of a group, specifically a phosphate group.

Example: Fumarate occurs as the Trans isomer while its cis form is called maleate, the enzyme that converts fumarate to malate catalyzes the hydration of the Trans double bond of fumarate but not the cis double bond of maleate (the cis isomer of fumarate). In the reverse direction (from L-malate to fumarate), fumarase is equally stereospecific: D-malate is not a substrate.


Fumarate


Maleate


L-Malate


D-Malate

(6)LIGASES

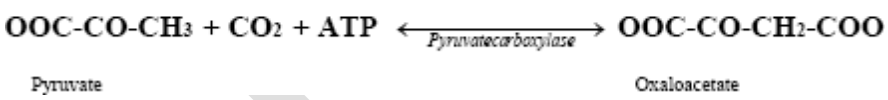
Which brings about the formation of different types of covalent bonds to synthesize bio molecules and it requires an input of chemicaal energy which is provided by simultaneously breaking down bio molecules such as ATP. It uses energy from hydrolysis of ATP. Classes include

(1) synthetases

(2) synthases

(3) carboxylases

Example:



Because of the ever increasing number of newly discovered enzymes, biochemists, by international agreement, have adopted a system for naming and classifying enzymes in the official nomenclature every enzyme is distinctly identifiable by its formal name and by a four component number.

This system divides enzymes into six classes, each with subclasses, based on the type of

reaction catalyzed.

Each enzyme is assigned a four-part classification number and a systematic name, which identifies the reaction it catalyzes .e.g Alcohol dehydrogenase in scientific report. It is identified as alcohol NAD+ oxidoreductase, (E.C.1.1.1.1.)

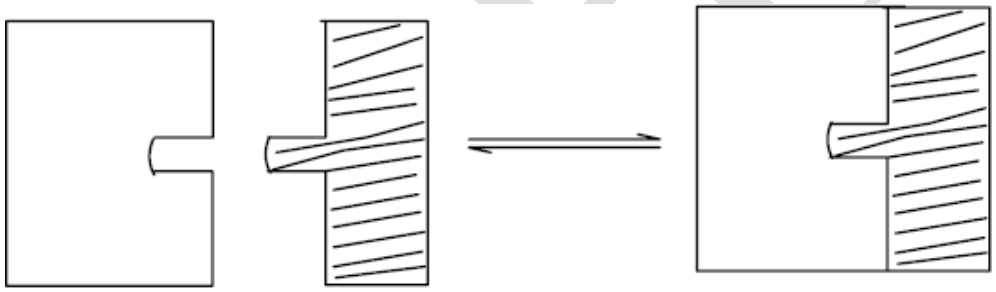
EC means enzyme commission, the lot 1 refers to class 1 (oxidoreductase) and the 2nd 1 refers to the type of group oxidized (1=alcohol) the 3rd 1 the oxidizing agent (1-coenzyme NAD+) and the 4th (1-alcohol dehydrogenase) because official names are often lengthy.

Properties of enzymes

- All enzymes are large and highly specialized globular proteins synthesized in cells.
- Their molecular weight generally ranges from 14000 to 400,000 Da.
- Enzymes are mostly water soluble colloids but some enzymes remain tightly bound to cell membranes.
- An enzyme is a catalyst which speeds up the rate of a specific reaction and while doing so it remains chemically unchanged and without loss of activity at the end of the reaction.
- It is to be emphasized that in an enzyme catalyzed reaction, the chemical equilibrium remains unchanged and the enzyme only speeds up the approach of this equilibrium. As such, enzymes can enhance reaction rates in cells as much as 10^{16} times the uncatalyzed rate.
- As stated earlier each type of enzyme has a specific „active site conformation‟ that is essential for its catalytic activity.
- The „active site conformation‟ includes the presence of some specific amino acid(s) at the active site besides the three dimensional structure of protein.
- These amino acids are involved in binding of substrate and catalysis of the reaction. In addition, there are some other amino acids at the active site whose side chains help in creating microenvironments at the site.
- Thus, the function of an enzyme depends on spatial arrangement of binding sites, catalytic sites and their microenvironment.

In the process of catalysis, enzyme (E) binds substrate (S) to form enzyme-substrate (ES) complex.

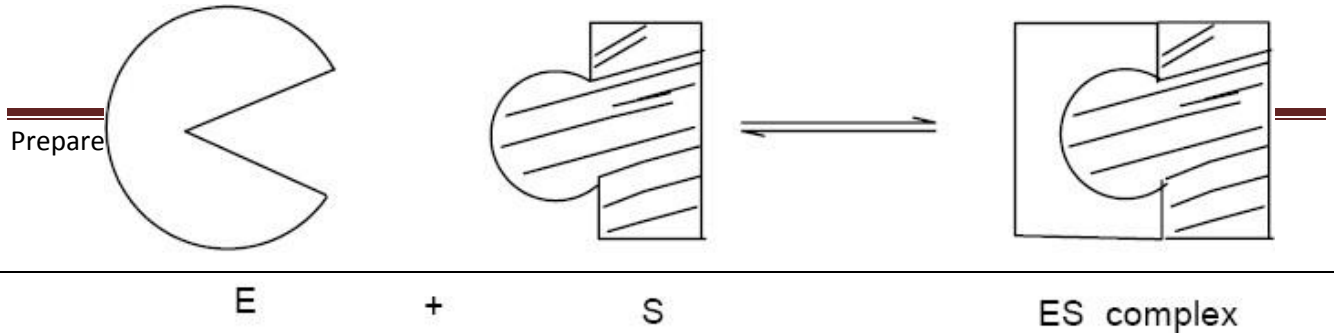
- „Lock- and- Key‟ hypothesis: Fischer in 1890 put forward this concept to explain that complementary structural features between E and S are responsible for the formation of ES complex (Fig. 2).
- According to this concept, the structure (or conformation) of enzyme is rigid. The substrate nicely fits into the active site (earlier called binding site) just as key fits into a lock. This model, however, failed to explain many other behavioural features of enzymes such as, high enzyme specificity.



Enzyme-substrate interaction after 'lock-and-key' hypothesis

„Induced- Fit‟ hypothesis: Koshland in 1958 suggested that the structures of E and S are complementary to each other only when they exist together in ES form, but not in their free existence (Fig.3).

Accordingly, during the binding of substrate to enzyme a conformational change takes place in enzyme and this allows the enzyme to make a grip around substrate. This type of mechanism helps to achieve high degree of specificity for the enzyme.



Enzyme-substrate interaction after 'Induced-Fit' hypothesis

There are some enzymes which require a „non-protein" part attached to their molecules for the activity. These are called ***cofactors***.

The cofactor could be metallic ions or some small molecular weight organic molecules. The nature of binding of a cofactor with the enzyme could be either loose or very tight.

In case the binding is loose the „non-protein" organic part is called ***coenzyme***. On the other hand when binding is very tight (sometimes even covalent) then this is called *prosthetic group*. When metal ions are tightly bound to the enzyme then it is called „**metalloenzyme**". The examples of some metalloenzymes are given in Table 2.

Table 2: Some metalloenzymes

Name Enzyme	Metal
Carbonic anhydrase	Zinc
Phenol oxidase	Copper
Carbooxypeptidase A	Zinc
Nitrogenase	Iron and molybdenum
Xanthine oxidase	Molybdenum
Superoxide dismutase	Manganese
Glutathione peroxidase	Selenium
Alcohol dehydrogenase	Zinc
Arginase	Manganese
Ascorbic acid oxidase	Copper
Cytochrome oxidase	Iron, copper
Nitrate reductase	Vanadium
Pyruvate kinase	Potassium, magnesium
Urease	Nickel
Xanthine	Molybdenum, iron
Glutathione peroxidase	Cobalt
Creatine kinase	Manganese
Nickel hydrogenase	Nickel

A fully functional enzyme is called ***holoenzyme*** which is frequently constituted of a proteinpart and a non-protein part. The protein-part of enzyme is called ***apoenzyme*** and, as stated above, the non-protein (organic) part is coenzyme.

Apoenzyme + coenzyme ==== Holoenzyme

- Table 3 shows the various coenzymes and prosthetic groups involved with enzymes.
- There are certain substances which modulate the activity of a holoenzyme. If the activity of enzyme is increased the substance is called *activator.*
- Generally, metal ions are involved in activation of enzyme activity.
- The activation is caused either by binding with substrate or helping in formation of effective ES-complex by bringing suitable conformational changes in enzyme protein. In addition, the metal ion may participate directly in catalytic process. Some of the enzymes requiring metal ions are listed in Table 3.
- Example of non-metal ion as activator of enzyme is that of Cl- for amylase. On the other hand, if the substance decreased the enzyme activity then it is called *inhibitor*. There are a number of substances which cause inhibition of an enzyme catalyzed reaction.

Table 3: Cofactors, coenzymes and prosthetic groups

Cofactor	Enzyme
Mg^{2+}	Hexokinase
Ni^{2+}	Urease
Mo	Nitrate reductase
Cu^{2+}	Cytochrome oxidase
Mn^{2+}	Arginase
Zn^{2+}	Alcoholic dehydrogenase
Coenzyme	
Nicotinamide adenine dinucleotide (NAD^{+})	Alcohol dehydrogenase, Lactate dehydrogenase
Thiamine pyrophosphate (TPP)	Pyruvate dehydrogenase
Flavin adenine dinucleotide (FAD)	Succinate dehydrogenase
Prosthetic group	
Pyridoxal phosphate	Amino transferases, Glycogen phosphorylase
Heme	Cytochrome oxidase

Active site of enzyme

- Enzyme proteins are large molecules and a very small region of the enzyme protein is involved in substrate binding and subsequent catalysis of the reaction. This region is called „active site" or „active centre".
- This site contains certain amino acid residues whose side chains are in specific conformation and participate in the catalyzed reaction.
- At the end of the reaction these side chains assume their original conformation. The amino acids
- present at the active site may appear very close to each other but most often they are widely separated from each other in their location at the level of primary structure of protein.
- The amino acids are brought close to each other due to folding of protein structure. The side chains of these amino acids are involved to form a part of the pocket located either on the surface of enzyme molecule or forming a deep opening in the enzyme (Fig. 4).

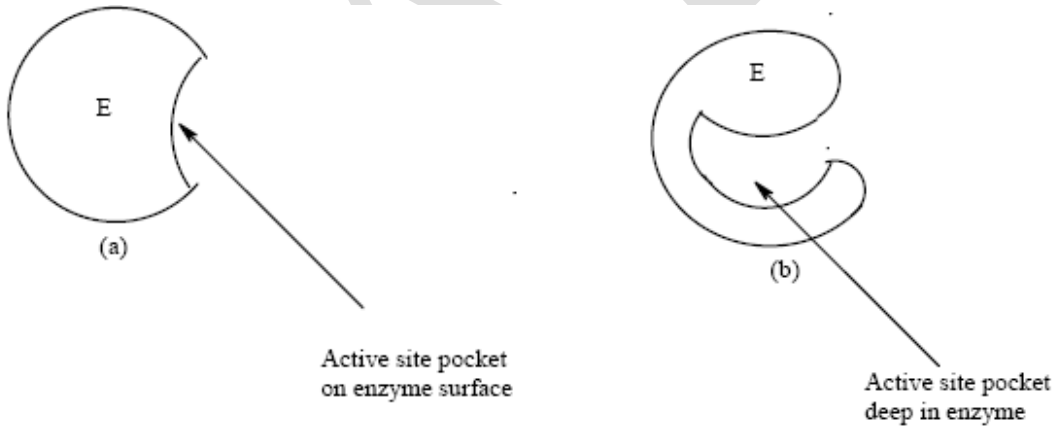


Fig. 4: Location of active sites in enzyme (a) on enzyme surface and (b) deep inside enzyme molecule

- The binding of substrate molecule at this site is facilitated by the flexible nature of this site so that an effective ES-complex is formed.

- The cofactors or coenzymes which are present at the active site facilitate the formation of ES-complex and subsequent catalysis.
- The binding of substrate to enzyme at the active site is due to weak interactions (non-covalent bonds) between the two. The amino acids that are generally involved at the active site for substrate binding and catalysis in various enzymes are cysteine, serine, histidine, aspartate, glutamate, tyrosine, arginine, lysine, etc.

Unit of enzyme activity

- he activity of an enzyme catalyzed reaction is defined in some quantity which indicates an estimate of the rate of that reaction. Thus, activity is expressed as units in many ways because measurement of number of enzyme molecules or its mass is difficult.According to International Commission on Enzymes, „One International Unit" of enzyme is defined as the amount of enzyme protein that catalyzes formation of one micromole of product in one minute under the conditions of assay (pH, temperature and ionic strength).
- Another definition of unit is in Katal. One Katal is that amount of enzyme which catalyzes the conversion of one mole of substrate into product in one second under the experimental conditions.
- Arbitrary Unit has a definition and according to which it is that amount of enzyme which transforms one micromole or nano mole or pico mole of substrate into product per minute under the assay conditions.
- However, when enzyme activities are expressed in units as per these definitions then sometimes it is difficult to compare the activities of various enzymes. This becomes relatively a lot easier when activity unit is expressed as specific activity.
- Accordingly, the specific activity is activity units per mg protein i.e. specific activity of enzyme
- A general agreement on expression of specific activity is micromoles per min per mg protein.

Turnover number: When enzyme is fully saturated with substrate then number of substrate molecules converted into product in unit time by one molecule of enzyme is called *turnover over number* (k*cat*). This is also referred to as *„molecular activity'*.

Isoenzymes

- In a number of organisms the existence of different molecular forms of an enzymic protein catalyzing same reaction has been shown. These are called *isoenzymes* or *isozymes*.
- The presence of these isozymes in different organs of an organism suggests the different roles of these enzymic proteins. For example, lactate dehydrogenase (LDH) present in different human tissues has five isozymic forms. These forms are commonly separated by gel electrophoresis in five different bands.
- Every band catalyzes the same reaction and these five forms are known as LDH1, LDH2, LDH3, LDH4 and LDH5. These proteins vary in their quaternary structure. All these forms are tetrameric proteins. Two different types of subunits, called H and M are present in variable numbers in each case. There are separate genes for the synthesis of H and M subunits. The relative predominance of these two forms varies in different tissues. For example, H subunits predominate in heart while M subunits predominate in liver and skeletal muscle. The five isozymic forms have the composition as H4, MH3, M2H2 and M3H and M4.

There are enzymes having only one polypeptide unit as structural entity and there are enzymes having more than one polypeptide units. These are named accordingly as;

- **Monomeric enzymes*:*** If an enzyme is made up of a single polypeptide unit it is called monomeric enzyme.
- **Oligomeric enzymes:** These enzymes having quaternary structure are made up of two or more polypeptide chains which are linked to each other by non-covalent interactions.

These proteins are also called *multimeric* proteins having high molecular weight (usually more than 40,000 Da) and their component polypeptides are called *subunits*.

- All regulatory enzymes are oligomeric enzymes showing the property of *allosteric regulation*.
- If these enzymes are made up of single type of monomer subunits, they are called *homooligomers*.

When subunits are of different kinds then they are termed *hetero-oligomers*. Most of the oligomeric enzymes are made up of either 2 or 4 subunits. The following are examples of some exceptions:

(i) Glutamine synthetase in *E. coli* has 12 identical subunits.

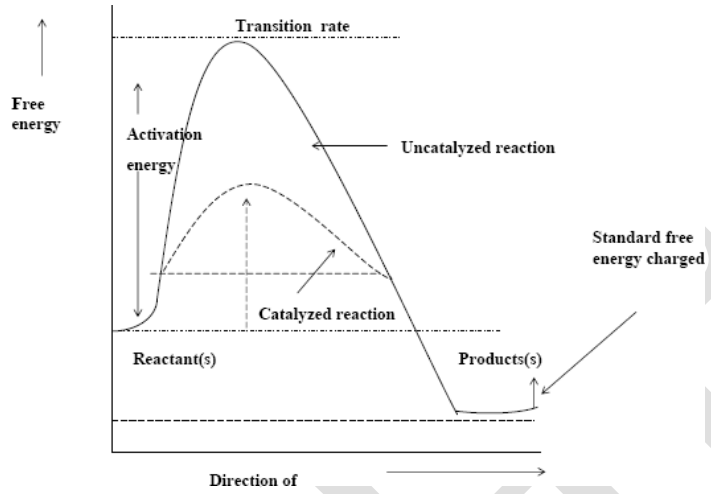
(ii) Aspartate transcarbamoylase has two types of subunits in 6+6 numbers.

Difference between catalysed and uncatalysed reaction:

How enzymes help to increase the reaction rate to such a level by functioning as catalyst could be explained with the help of Fig. 1 which illustrates the energy changes that take place during the conversion of reactants into products.

- The equilibrium of such a reaction is determined by the energy states of reactants and products. These energy states remain unaffected by enzyme action. If the reaction has to proceed then the reactants (or substrate) must be brought to a higher energy level (or state), which is called *transition state.*
- The amount of energy required to bring the substrate to transition state is called *energy of activation.*
- This energy acts as a barrier for the progress of the reaction, thereby limiting the rate of the
- reaction. Catalysts (including enzymes) function by bringing down the activation energy and
- hence increase the rate of reaction.

- The rate of catalyzed reaction both in forward and reverse direction remains same because both these rates have to undergo through same transition state.



Need for energy in case of uncatalyzed and enzyme catalyzed reactions

The enzyme catalyzed reactions involve binding of substrate(s) to enzyme (E) at the specific site, called *active site*, to form enzyme substrate complex (ES). This interaction lowers the energy of activation and facilitates formation of new transition state (Fig. 1 broken curve). The substrate while bound to enzyme is converted into product and then released from enzyme. This whole process is represented by following equation:

$$E+S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E+P$$

- Each enzyme is very specific in its function though it is generally depicted by the above equation.
- There are three types of enzyme specificities viz., substrate, stereo chemical and reaction specificity.
- The extent of substrate specificity varies from enzyme to enzyme.

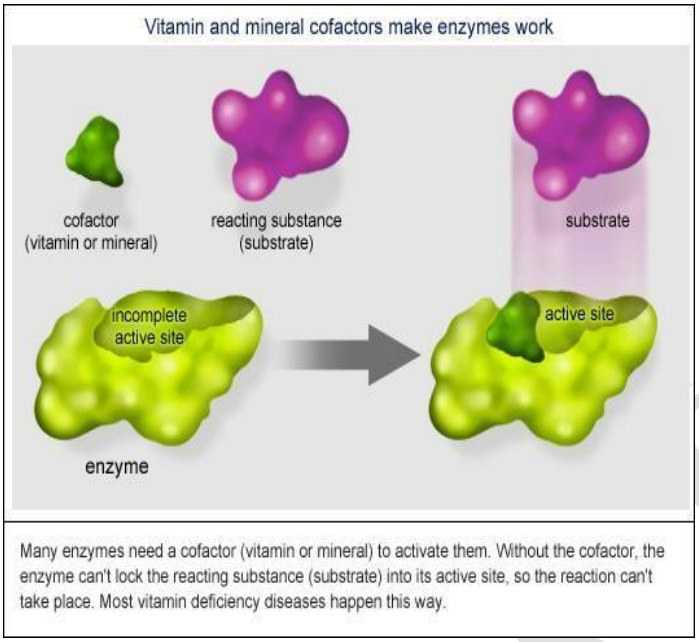
What are Enzymes, Coenzymes and Cofactors?

➨ **Enzymes** are large biological molecules responsible for thousands of metabolic

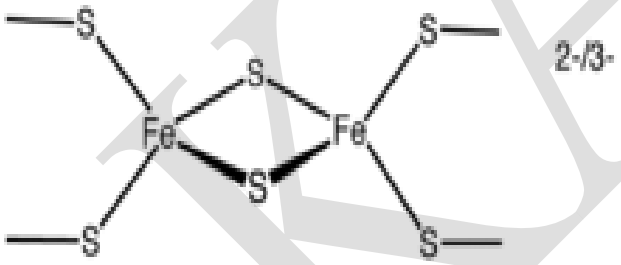
processes that sustain life.

- They are highly selective catalyst, greatly accelerating both the rate and specificity of metabolic reactions.
- Some enzymes require no chemical groups for activity other than their amino acid residues. Other requires an additional chemical component called a **cofactor** for the required activity.
- A **cofactor** is a non-protein chemical compound that is required for the protein's biological activity. These proteins are commonly enzymes, and cofactors can be considered "helper molecules" that assist in biochemical transformations.
- Cofactors can be divided into two broad groups: **organic cofactors**, such as flavin or heme, and **inorganic cofactors**, such as the metal ions Mg^{2+}, Cu^{+}, Mn^{2+}, or iron-sulfur clusters.

Inorganic cofactor - Metal Ions



Ion	Examples of enzymes containing this ion
Cupric	Cytochrome oxidase
Ferrous or Ferric	Catalase Cytochrome (via Heme) Nitrogenase Hydrogenase
Magnesium	Glucose 6-phosphatase Hexokinase DNA polymerase



A simple [Fe $_2$ S $_2$] cluster containing two iron atoms and two sulfur atoms, coordinated by four protein cysteine residues.

Organic cofactor

- Organic cofactors are small organic molecules (typically a molecular mass less than 1000 Da) that can be either loosely or tightly bound to the enzyme and directly participate in the reaction.

Cofactor	Vitamin	Additional component	Chemical group(s) transferred	Distribution
NAD^+ and $NADP^+$	Niacin (B_3)	ADP	Electrons	Bacteria, archaea and eukaryotes
Coenzyme A	Pantothenic acid(B_5)	ADP	Acetyl group and other acyl groups	Bacteria, archaea andeukaryotes
Ascorbic acid	Vitamin C	None	Electrons	Bacteria, archaea andeukaryotes
Flavin mononucleotide	Riboflavin (B_2)	None	Electrons	Bacteria, archaea andeukaryotes

Coenzyme

- loosely bound cofactors termed **coenzymes**
- Any of a number of freely diffusing organic compounds that function as cofactors with enzymes in promoting a variety of metabolic reactions.
- Coenzymes are a type of cofactor and they are bound to enzyme's active sites to aid with their proper functioning.
- Coenzymes which are directly involved and altered in the course of chemical reactions are considered to be a type of secondary substrate.

Coenzymes as vitamins

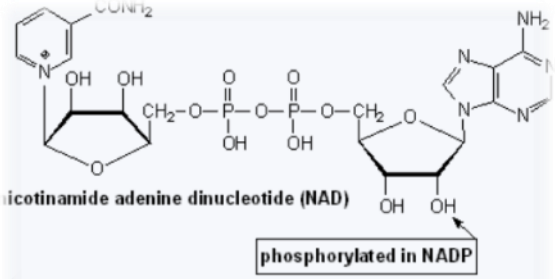
- Many coenzymes are closely related to vitamins. Some of them are important growth factors.
- Coenzymes are the precursors of vitamins.
- A vitamin is a main component of an coenzyme endowed with bio catalytic functions.
- Coenzymes involved in transfer of hydrogens are called hydrogen transferring enzymes and

those which transfer a specific group are known as group transferring coenzymes.

Coenzymes in Hydrogen transfer reaction

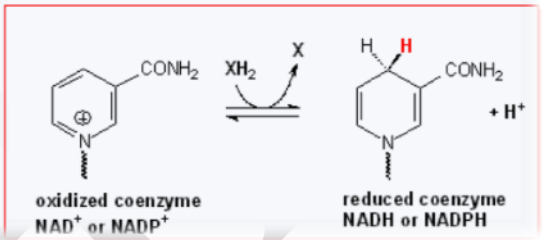
❖ **Nicotinamide nucleotide**

- These coenzyme involved in hydrogen transfer reaction and form essential components of dehydrogenase.



Biochemical function

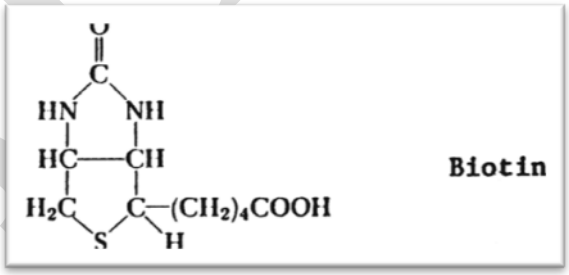
- These NAD+ and NADP are coenzymes of a number of dehydrogenases catalyzing oxidation-reduction reaction.
- All reaction catalyzed by them reversible



Coenzymes involved in group transfer

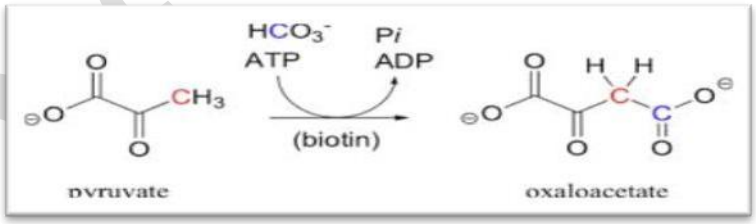
❖ **BIOTIN**

biotin is a coenzyme belonging to vitamin B2 group which is an essential growth factor for yeast and other microorganism, but is also required by higher organism.



Biochemical function

Biotin is a water soluble vitamin and participate in transfer of carboxyl group.



Role of coenzyme

- The function of coenzymes is to transport groups between enzymes.
- Chemical groups include hydride ions which are carried by coenzymes such as NAD,
- phosphate groups which are carried by coenzymes such as ATP
- acetyl groups which are carried by coenzymes such as coenzyme A.
- Coenzymes which lose or gain these chemical groups in the course of the reaction are often reformed in the same metabolic pathway.

For example NAD+ used in glycolysis and the citric acid cycle is replaced in the electron transport chain

Function of coenzyme

- The coenzyme is essential for the biological activity of the enzyme.
- A coenzyme is a low molecular weight organic substance, without which the enzyme cannot exhibit any reaction.
- One molecule of the coenzyme is able to convert a large number of substrate molecules with the help of enzyme.

Salient features of coenzyme

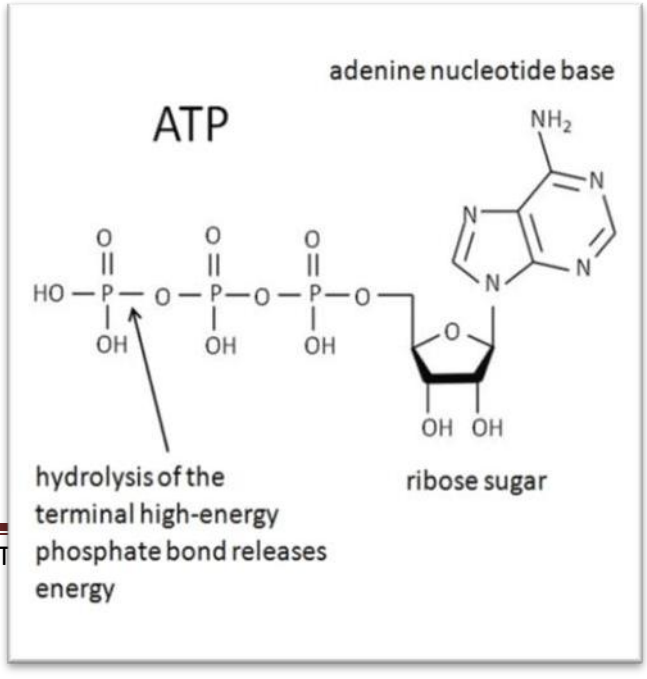
- Coenzymes are heat stable.
- They are low-molecular weight substances.
- The coenzymes combine loosely with the enzyme molecules and so, the coenzyme can be separated easily by dialysis.
- When the reaction is completed, the coenzyme is released from the apo-enzyme, and goes to some other reaction site.

Important coenzyme

- **Alcohol dehydrogenase**
- **Coenzyme A**
- **Flavin adenine dinucleotide (FAD)**
- **Nicotinamide adenine dinucleotide (NAD)**
- **Adenosine triphosphate (ATP)**

Adenosine triphosphate (ATP)

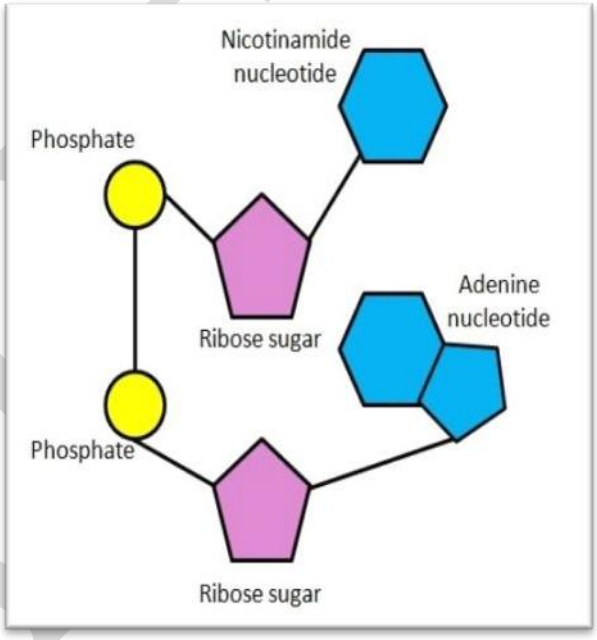
- The function of ATP is to transport chemical energy within cells for metabolism.
- ATP is often referred to as the energy currency of cells.
- Adenosine triphosphate is composed of an adenine nucleotide base, a ribose sugar and three phosphate groups.
- Energy can be released from ATP when the terminal phosphate group is



released in a hydrolysis reaction. This is because the energy of ATP is held in the bonds between the phosphate groups and when the bonds are broken it is accompanied by a release of energy.

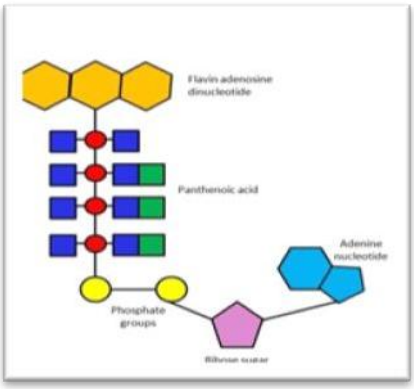
Nicotinamide adenine dinucleotide (NAD)

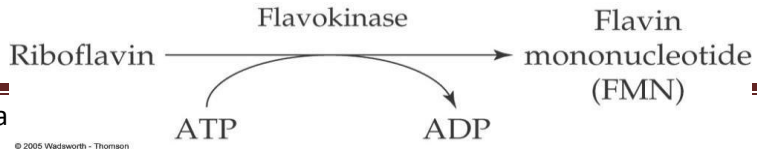
- NAD is composed of two nucleotides, adenine and nicotinamide.
- The nucleotides are held together by a pair of phosphate groups which act as a bridge and are also bonded to a ribose sugar each.
- The function of NAD is to carry electrons from one enzyme controlled reaction to another.
- NAD is involved with redox reactions because substrates are either oxidized , in which they lose electrons or are reduced in which they gain electrons.
- NAD is either found as NAD+, which is an oxidizing agent and is involved with accepting electrons from other molecules.
- NADH which is used as a reducing agent to donate electrons to other molecule

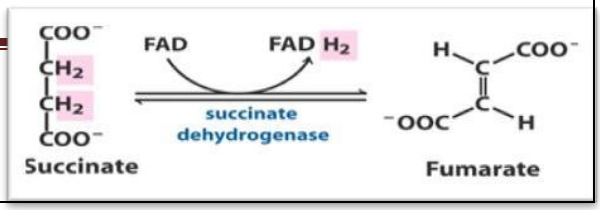


Flavin adenine dinucleotide (FAD)

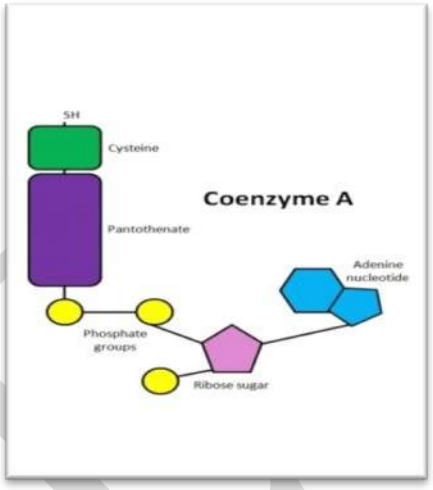
- FAD is composed of an adenine nucleotide, a ribose sugar and two phosphate groups.
- FAD can also exist as a monophosphate and is called flavin adenine monophosphate (FMN).
- FAD is involved with redox reactions.
- like NAD, FAD can exist in two redox states FAD and FADH.





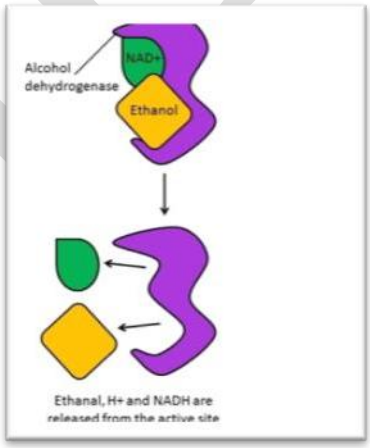


Coenzyme A



- Coenzyme A is a prominent coenzyme of living organism which transfers the acyl group of carboxylic acid.
- It plays an important role in the metabolism of proteins, carbohydrates and fats which are important reactions that allow the energy from food to be released. For example coenzyme A is required for the oxidation of pyruvate in the citric acid cycle.
- Coenzyme A is also important in the synthesis of cholesterol and steroid hormones, and is required for the detoxification of a range of harmful drugs that can accumulate in the liver.

Alcohol dehydrogenase



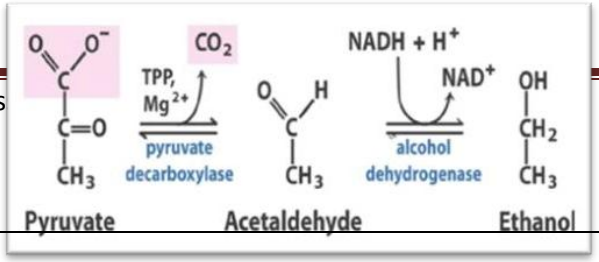
- Alcohol dehydrogenase (ADH) is an enzyme which uses NAD+ as a coenzyme.
- ADH has two binding regions, one where the primary substrate, ethanol binds and one where the coenzyme, NAD+ is able to bind.
- The enzyme is responsible for the conversion of ethanol to ethanal. The reaction is an oxidation- reduction reaction and results in the removal of two hydrogen ions and two electrons from ethanol. The hydrogen ions and electrons are added to NAD+ which converts the coenzyme to NADH + H+. This is the first reaction involved with the metabolism of ethanol.

Vitamin B_1 – Thiamine

The active form is thiamin pyrophosphate (TPP)

- Thiamin is rapidly converted to thiamin pyrophosphate (TPP) in small intestine, brain and liver.
- TPP is formed from thiamin by the action of thiamine diphosphotransferase.
- TPP coenzyme is required by enzymes in the decarboxylation of α-keto acids.
- Entity Transferred; Aldehydes

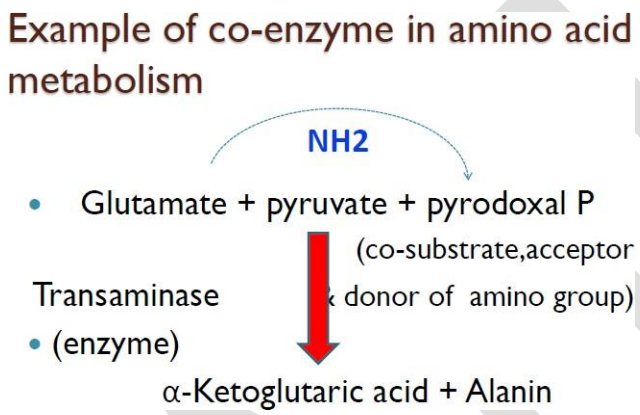
TPP as co-enzymes



Pyridoxal Phosphate

PLP is Derivative of Pyridoxine (Vit. B6) involved in.

1. Transamination reactions required for the synthesis and catabolism of the amino acids.
2. Decarboxylation reactions.
3. Entity Transferred; Amino Groups(-NH_2)



The Two Parts of Photosynthesis Photosystem I and II

Light-dependent and light-independent reactions are two successive reactions that occur during photosynthesis.

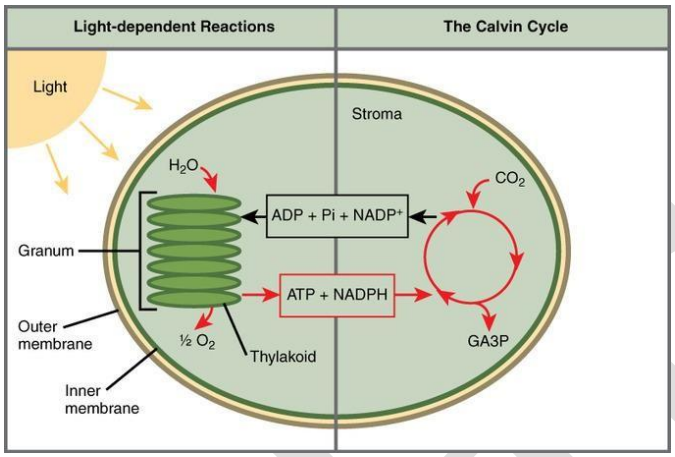
Photosynthesis takes place in two sequential stages:

1. The light-dependent reactions;
2. The light-independent reactions, or Calvin Cycle.

Light-Dependent Reactions

Just as the name implies, light-dependent reactions require sunlight. In the light-dependent reactions, energy from sunlight is absorbed by chlorophyll and converted into stored chemical energy, in the form of the electron carrier molecule NADPH (nicotinamide adenine dinucleotide phosphate)

and the energy currency molecule ATP (adenosine triphosphate). The light-dependent reactions take place in the thylakoid membranes in the granum (stack of thylakoids), within the chloroplast.



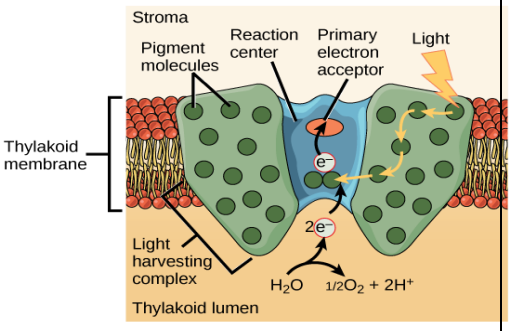
The two stages of photosynthesis: Photosynthesis takes place in two stages: light-dependent reactions and the Calvin cycle (light-independent reactions). Light-dependent reactions, which take place in the thylakoid membrane, use light energy to make ATP and NADPH. The Calvin cycle, which takes place in the stroma, uses energy derived from these compounds to make GA3P from CO2.

Photosystems

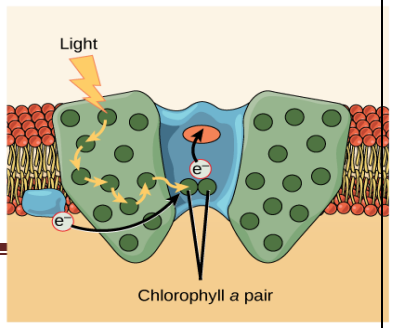
Photosystems I & II: As explained above, the photosystems manipulate electrons with energy harvested from light.

The process that converts light energy into chemical energy takes place in a multi-protein complex

(a) Photosystem II (P680)



(b) Photosystem I (P700)



called a photosystem. Two types of photosystems are embedded in the thylakoid membrane: photosystem II (PSII) and photosystem I (PSI). Each photosystem plays a key role in capturing the energy from sunlight by exciting electrons. These energized electrons are

ransported by "energy carrier" molecules, which power the light-independent reactions.

Photosystems consist of a light-harvesting complex and a reaction center. Pigments in the light- harvesting complex pass light energy to two special chlorophyll *a* molecules in the reaction center. The light excites an electron from the chlorophyll *a* pair, which passes to the primary electron acceptor. The excited electron must then be replaced. In photosystem II, the electron comes from the splitting of water, which releases oxygen as a waste product. In photosystem I, the electron comes from the chloroplast electron transport chain.

The two photosystems oxidize different sources of the low-energy electron supply, deliver their energized electrons to different places, and respond to different wavelengths of light.

Light-Independent Reactions

In the light-independent reactions or Calvin cycle, the energized electrons from the light- dependent reactions provide the energy to form carbohydrates from carbon dioxide molecules. The light-independent

reactions are sometimes called the Calvin cycle because of the cyclical nature of the process.

Although the light-independent reactions do not use light as a reactant (and as a result can take place at day or night), they require the products of the light-dependent reactions to function. The light-independent molecules depend on the energy carrier molecules, ATP and NADPH, to drive the construction of new carbohydrate molecules. After the energy is transferred, the energy carrier molecules return to the light-dependent reactions to obtain more energized electrons. In addition, several enzymes of the light-independent reactions are activated by light.

UNIT-IV

SYLLABUS

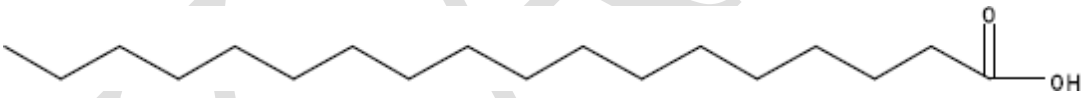
Lipids: Structure and functions –Classification, nomenclature and properties of fatty acids, essential fatty acids. Phospholipids, sphingolipids, glycolipids, cerebrosides, gangliosides, Prostaglandins, Cholesterol, ß-oxidation of fatty acids.

Lipids

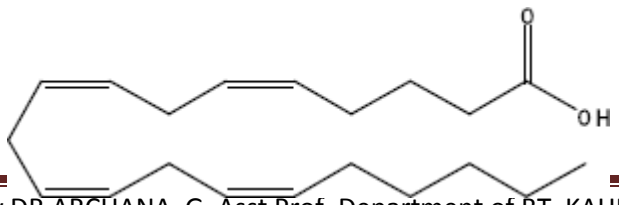
Lipids

Biomolecules that have the common property of being soluble in organic (nonpolar) solvents, but not in water.

Fatty Acids – most lipids contain fatty acids (the simplest type of lipids) in their structures. They are carboxylic acids with an even number of carbon atoms, usually between 10 and 20 (memorize their common names).



Prostaglandins are formed from arachidonic acid (*all-cis*-5,8,11,14-eicosatetraenoic acid), which is an unsaturated fatty acid with 20 carbons. These hormone like substances increase or lower the blood pressure, inflammation and pain when tissues are injured. Prostaglandins are potent but have a short half-life before being inactivated and excreted. Therefore, they exert only a paracrine (locally active) or autocrine (acting on the same cell from which it is synthesized) function.



- ***Lipids*** are naturally occurring molecules from plants or animals that are soluble in nonpolar organic solvents.
- Lipid molecules contain large hydrocarbon portion and not many polar functional group, which accounts for their solubility behavior.

FATTY ACIDS (FAs)

two major physiological roles:

(i) building blocks of *phospholipids* and *glycolipids* in *biological membranes*

(ii) fuel molecules

CLASSIFICATION OF FAs

• According to the chain length

- *short-chain fatty acid* - SCFA < 6 carbon atoms
- *medium-chain fatty acid* - MCFA 6-12 carbon atoms
- *long-chain fatty acid* - LCFA 14-20 carbon atoms
- *very-long chain fatty acid* - VLCFA > 20 carbon atoms

• According to the degree of saturation (presence or absence of double bonds)

– *saturated*

– *unsaturated cis/trans isomers*

Name	Type	Number of carbon atoms	Number of double bonds	Symbol
Palmitic acid	Saturated	16	0	16:0
Stearic acid	Saturated	18	0	18:0
Oleic acid	Monounsaturated	18	1	18:1n-9
α-linolenic acid (ALA)	ω-3 polyunsaturated	18	3	18:3n-3
Eicosapentaenoic acid (EPA)	ω-3 polyunsaturated	20	5	20:5n-3
Docosapentaenoic acid (DPA) n-3	ω-3 polyunsaturated	22	5	22:5n-3
Docosahexaenoic acid (DHA)	ω-3 polyunsaturated	22	6	22:6n-3
Linoleic acid (LNA)	ω-6 polyunsaturated	18	2	18:2n-6
DPA n-6	ω-6 polyunsaturated	22	5	22:5n-6
Arachidonic acid (ARA)	ω-6 polyunsaturated	20	4	20:4n-6

UNSATURATED: with one double bond: + *enoic*

e.g. C18: Octadec*enoic* acid

- with two double bonds: + *dienoic*

e.g.C18: Octadeca*dienoic* acid

- with three double bonds: + *trienoic*

e.g. C18: Octadeca*trienoic* acid

Most common in animal and plant fats

Less easily digestible

Common name Systematic name Formula

C14:0 Myristic acid Tetradecanoic acid CH3(CH2)12COOH

C16:0 Palmitic acid Hexadecanoic acid CH3(CH2)14COOH

C18:0 Stearic acid Octadecanoic acid CH3(CH2)16COOH

C20:0 Arachidic acid Eicosanoic acid CH3(CH2)18COOH

MONOUNSATURATED FAs

16:1(9) Palmitooleic a. *cis*-9-Hexadecenoic acid

18:1(9) Oleic a. *cis*-9-Octadecenoic acid

18:1(9) Elaidic a. *trans*-9-Octadecenoic acid

22:1(13) Erucic a. *cis*-13-Docosenoic acid

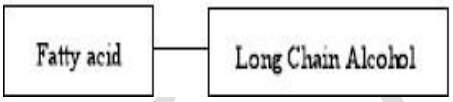
Classification of Lipids

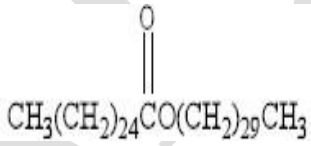
Lipids are classified as follows:

1. Simple lipids: Esters of fatty acids with various alcohols.

(a) Fats: Esters of fatty acids with glycerol. Oils are fats in the liquid state.

(b) Waxes: are carboxylic acid esters where both R groups are long straight hydrocarbon chain. Performs external protective functions.





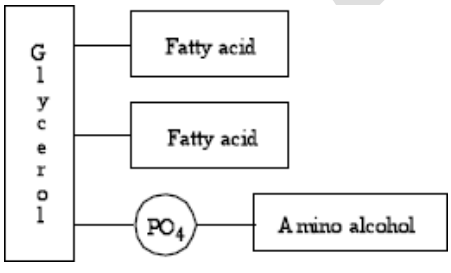
$CH_3(CH_2)_{24}CO(CH_2)_{29}CH_3$ Beeswax (found in honeycombs)

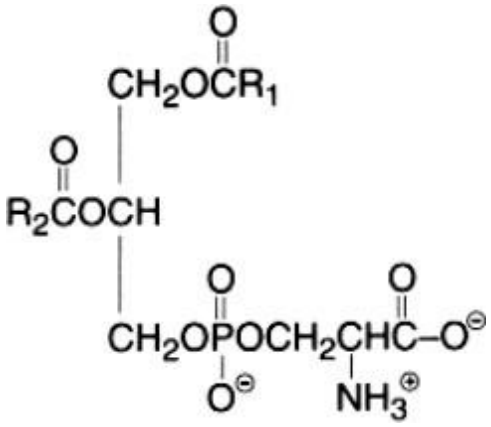
- The leaves and fruits of many plants have waxy coatings, which may protect them from dehydration and small predators. The feathers of birds and the fur of some animals have similar coatings which serve as a water repellent.
- Waxes are also used in wax polishes for furniture and other wood products, footwear and vehicles, as mold release agents in mold making, as a coating for Edam and Gouda cheeses, and to waterproof leather and fabric.

2. Complex lipids: Esters of fatty acids containing groups in addition to an alcohol and a fatty acid.

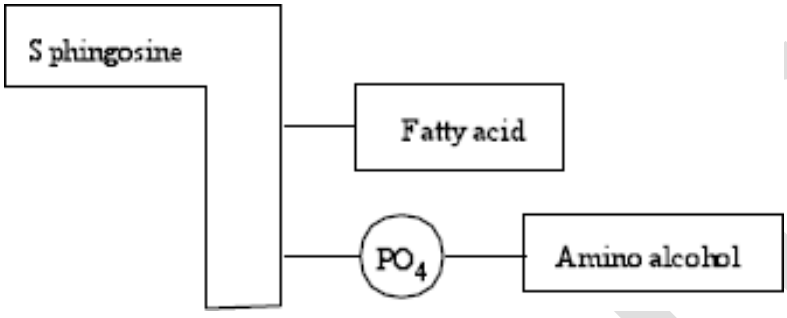
- *Triacylglycerol* – are carboxylic acid triesters of glycerols. They are a major source of biochemical energy.
- *Glycerophopholipids* - triesters of glycerols that contain charged phosphate diesters. They help to control the flow of molecules into and out of cells.

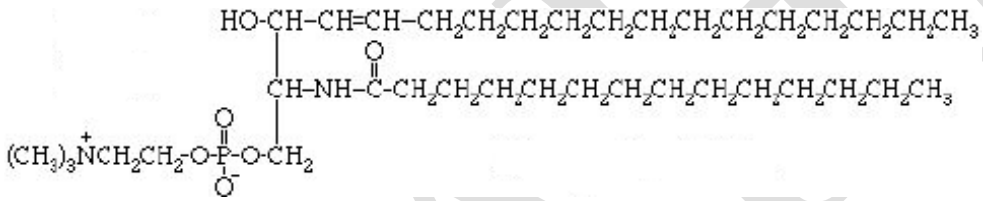
Glycerophospholipids (or phospholipids) are a family of lipids similar to TAG's except that one hydroxyl group of glycerol is replaced by the ester of phosphoric acid and an amino alcohol, bonded through a phosphodiester bond. Depending on the amino alcohol, these can be **Lecithins** (containing choline) or **Cephalines** (containing ethanolamine or serine). These are the most abundant lipids in cell membranes.





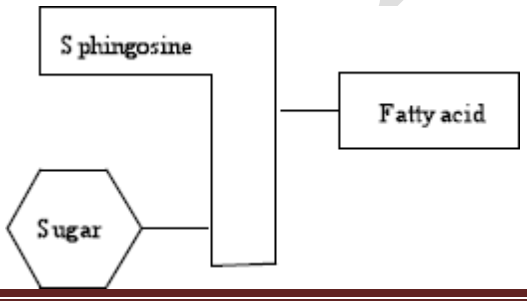
- *Sphingomyelins* – amides derived from an amino alcohol, also contain charged phosphate diester groups. They are essential to the structure of cell membranes.

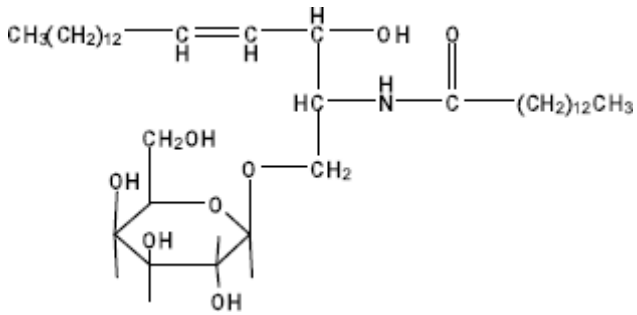




- *Glycolipids* – amides derived from sphingosine, contain polar carbohydrate groups. On the cell surface, they connect with by intracellular messengers.

Glycosphingolipids. **Cerebrosides** contain a monosaccharide and **Gangliosides** are similar, but they contain two or more monosaccharides.



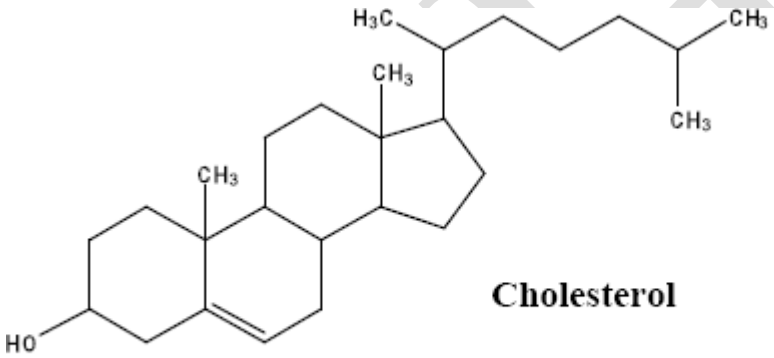


3. Precursor and derived lipids: These include fatty acids, glycerol, steroids, other alcohols, fatty aldehydes, and ketone bodies, hydrocarbons, lipidsoluble vitamins, and hormones. Because they are uncharged, acylglycerols (glycerides), cholesterol, and cholesteryl esters are termed neutral lipids.

Lipids that are not esters or amides:

Steroids – They performs various functions such as hormones and contributes to the structure of cell membranes.

Eicosanoids – They are carboxylic acids that are a special type of intracellular chemical messengers.



Cholesterol

This is one of the most important and abundant steroids in the body.

Fatty Acids

Fatty acids can be said to be carboxylic acids, and come in two major varieties.

- Saturated fatty acids do not have any double bonds. A fatty acid is saturated when every carbon atom in the hydrocarbon chain is bonded to as many hydrogen atoms as possible (the carbon atoms are saturated with hydrogen). Saturated fatty acids are solids at room temperature. Animal fats are a source of saturated fatty acids. In addition, fatty acids pack easily and form rigid structures (e.g., fatty acids are found in membranes).

- Unsaturated fatty acids can have one or more double bonds along its hydrocarbon chain. A

fatty acid with one double bond is called monounsaturated. If it contains two or more double bonds, we say that the fatty acid is polyunsaturated. The melting point of a fatty acid is

influenced by the number of double bonds that the molecule contains and by the length of the hydrocarbon tail. The more double bonds it contains, the lower the melting point. As the length of the tail increases, the melting point increases. Plants are the source of unsaturated fatty acids

–CH = CH – CH = CH –

Unsaturated fatty acid chain

–CH – CH – CH –

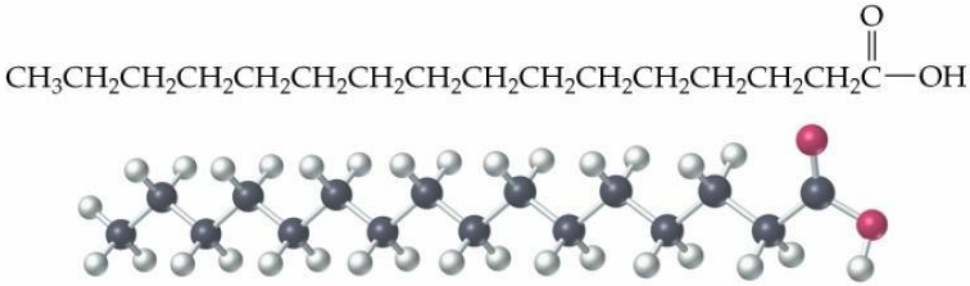
Saturated fatty acid chain

Aliphatic chain showing structure of unsaturated fatty acid chain with double bonds and saturated fatty acid chain with single bonds.

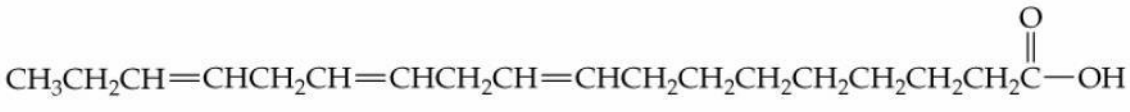
Properties of Fats and Oils

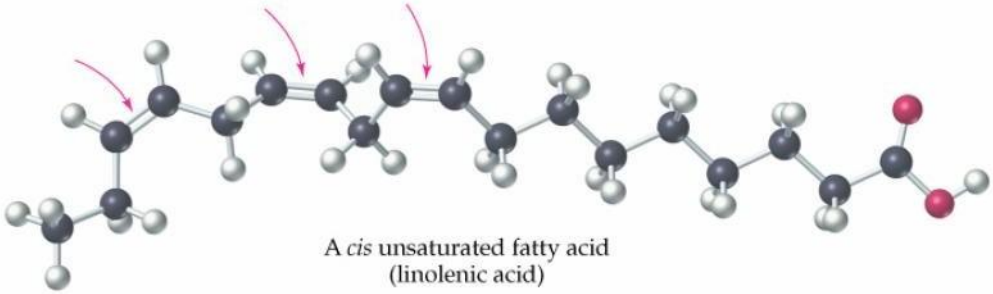
Oils: A mixture of triglycerols that is liquid because it contains a high proportions of unsaturated fatty acids.

Fats: A mixture of triglycerols that is solid because it contains a high proportions of saturated fatty acids.



A saturated fatty acid
(palmitic acid)





A *cis* unsaturated fatty acid
(linolenic acid)

Properties of triglycerols in natural fats and oils:

- Nonpolar and hydrophobic
- No ionic charges
- Solid triglycerols (Fats) - high proportions of saturated fatty acids.
- Liquid triglycerols (Oils) - high proportions of unsaturated fatty acids.

Chemical Reactions of Triglycerols

Hydrogenation: The carbon-carbon double bonds in unsaturated fatty acids can be hydrogenated by reacting with hydrogen to produce saturated fatty acids. For example, margarine is produced when two thirds of the double bonds present in vegetable oil is hydrogenated.

Hydrolysis of triglycerols: Triglycerols like any other esters react with water to form their carboxylic acid and alcohol – a process known as hydrolysis. - In body, this hydrolysis is catalyzed by the enzyme hydrolase and is the first step in the digestion of dietary fats and oils.

- In the laboratory and commercial production of soap, hydrolysis of fats and oils is usually

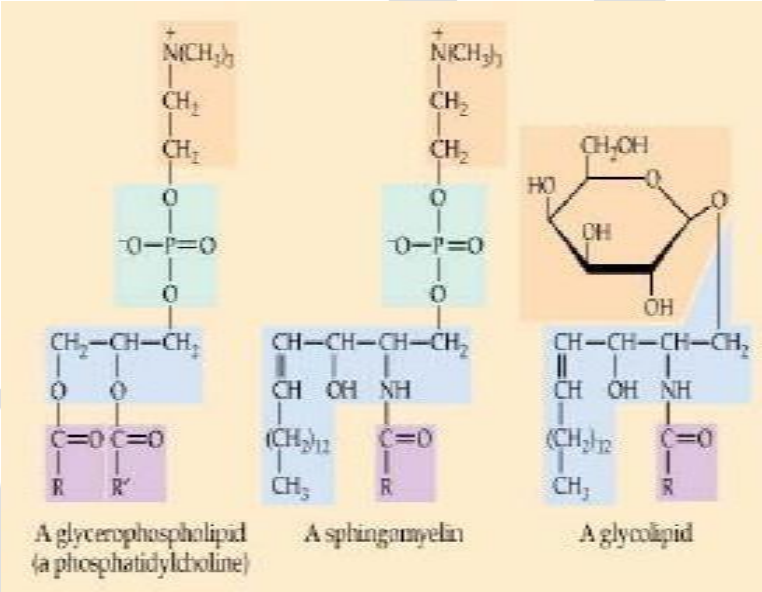
carried out by strong aqueous bases such as

NaOH and KOH and is called saponification.

Cell Membrane Lipids:

Phosphilipids and Glycolipids

- Cell membranes establish a hydrophobic barrier between the watery environment in the cell and outside the cell. Lipids are ideal for this function.
- The three major kinds of cell membrane lipids in animals are *phospholipids*, *glycolipids*, and *cholesterol*.

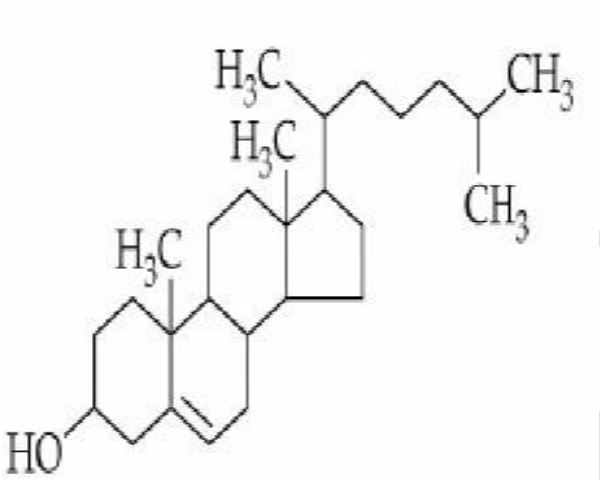


- Phosphoilipids contain an ester link between a phosphoric acid and an alcohol. The alcohol is either a glycerol to give a glycerophopholipid or a sphingosine to give sphingomyelins.
- Glycolipids: Glycolipids are derived from sphingosine. They differ from sphingomyelins by having a carbohydrate group at C1 instead of a phosphate bonded to a choline.

Cell Membrane Lipids:

Cholesterol

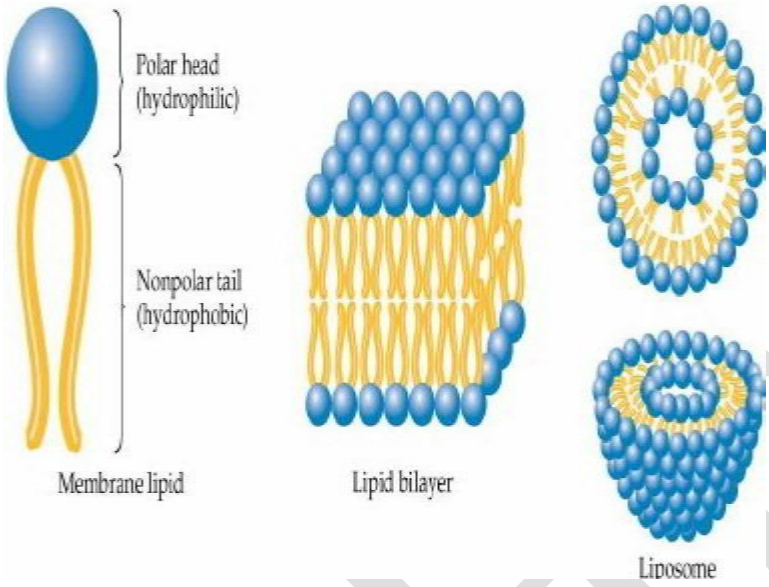
Animal cell membranes contain significant amount of cholesterol.

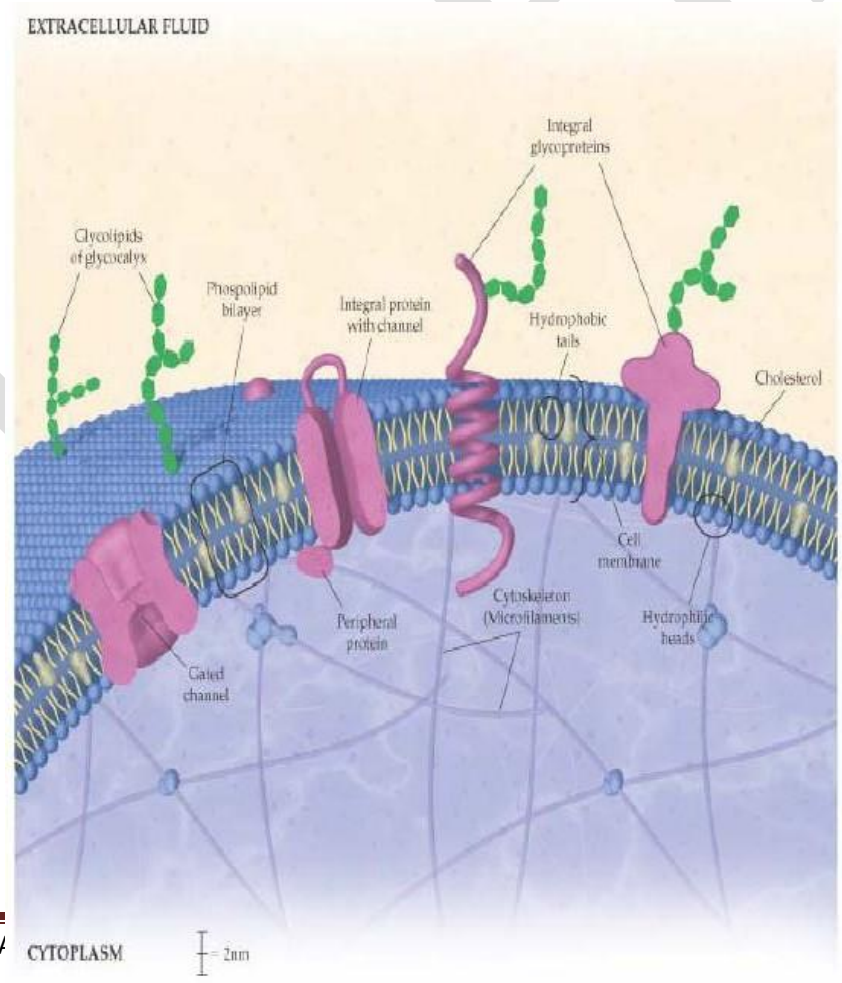


- Cholesterol is a steroid, a member of the class of lipids that all contain the same four ring system.
- Cholesterol serves two important purposes: as a component of cell membranes and as a starting materials for the synthesis of all other steroids.

Structure of Cell Membranes

The basic structural unit of cell membrane is lipid bilayer which is composed of two parallel sheets of membrane lipid molecules arranged tail to tail. Bilayers are highly ordered and stable, but still flexible.


Polar head
(hydrophilic)
Nonpolar tail
(hydrophobic)
Membrane lipid
Lipid bilayer
Liposome

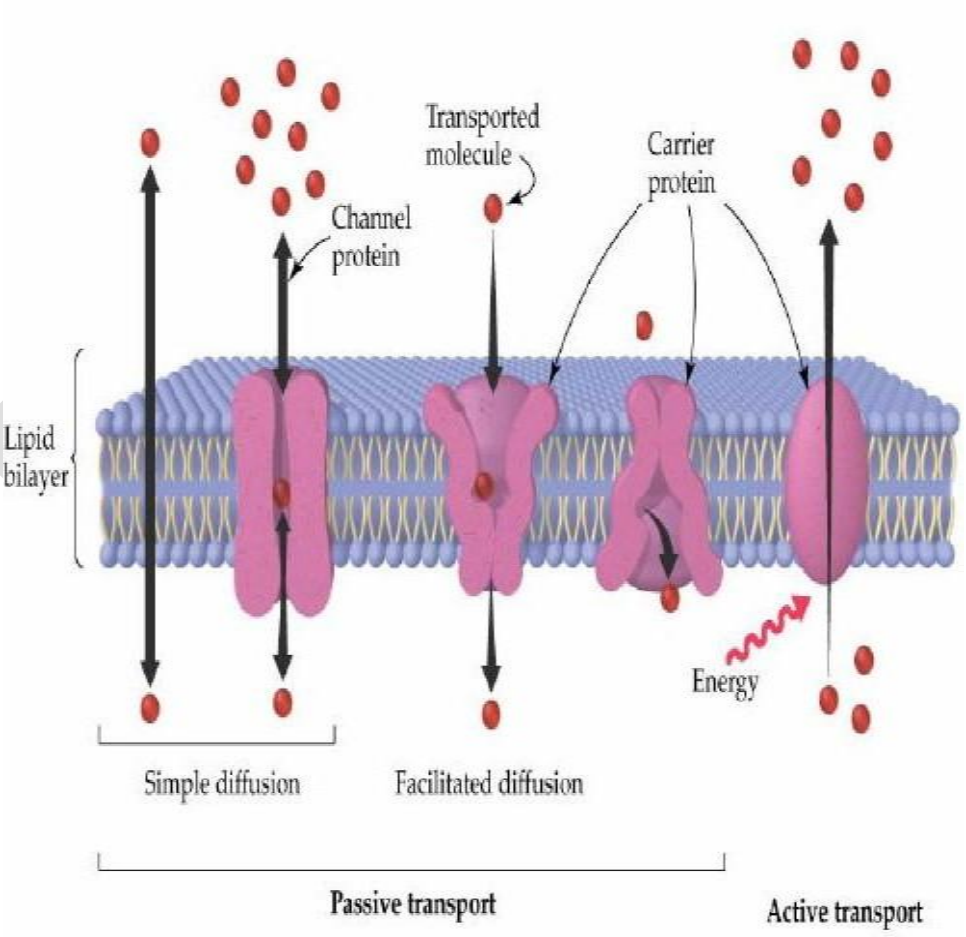

EXTRACELLULAR FLUID
Integral glycoproteins
Glycolipids of glycocalyx
Phospolipid bilayer
Integral protein with channel
Hydrophobic tails
Cholesterol
Cell membrane
Cytoskeleton (Microfilaments)
Peripheral protein
Hydrophilic heads
Gated channel
CYTOPLASM
2nm

When phospholipids are shaken vigorously with water, they spontaneously form liposome – small spherical vesicle with lipid bilayer surrounding an aqueous center. Water soluble substances can be trapped in the center of the liposome, and lipid-soluble substances can be incorporated into the bilayer.

Transport Across Cell Membranes

The cell membranes allow the passage of molecules and ions into and out of a cell by two modes; passive transportation and active transportation.

- *Passive transport* – substances move across the cell membrane freely by diffusion from regions of higher concentration to regions of lower concentration. Glucose is transported into many cells



in this way.

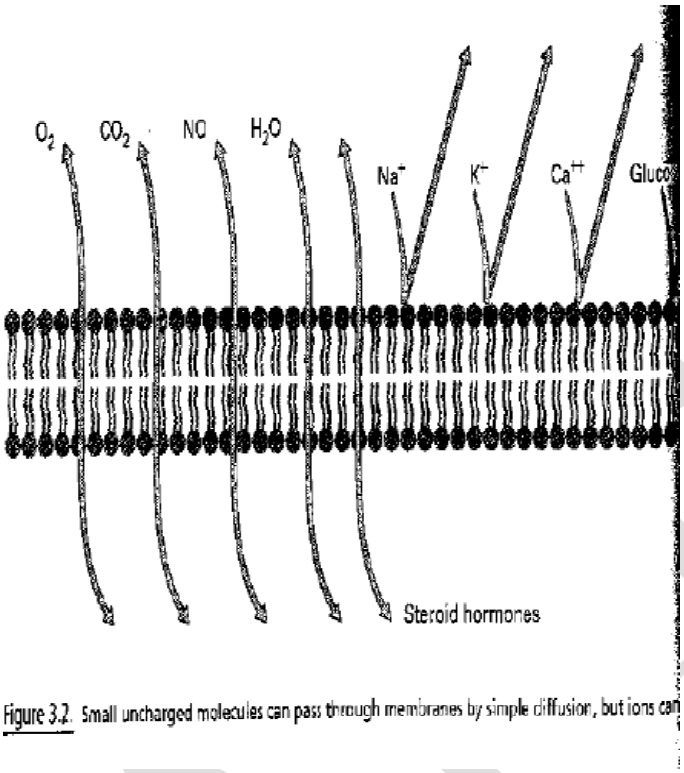
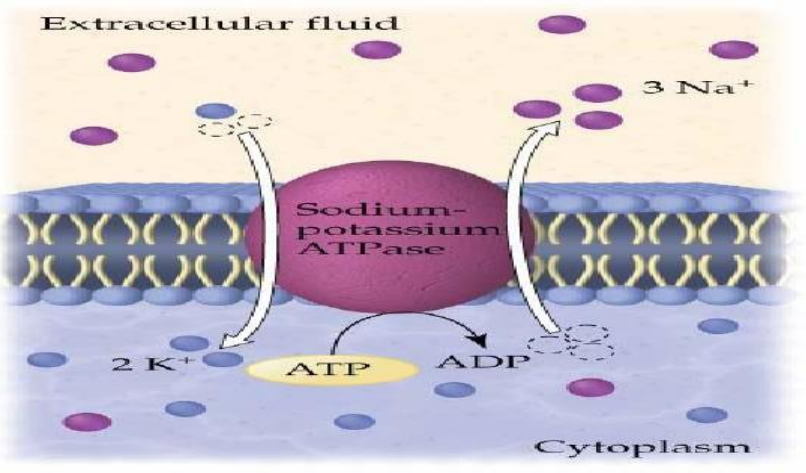


Figure 3.2. Small uncharged molecules can pass through membranes by simple diffusion, but ions can

Active transport - substances move across the cell membrane only when energy is supplied because they must go in the reverse direction from regions of lower to regions of higher concentration. Only by this method, cells maintain lower Na+ concentration within cells and higher Na+ concentration in extracellular fluids, with the opposite concentration ratio for K+.



Properties of cell membranes:

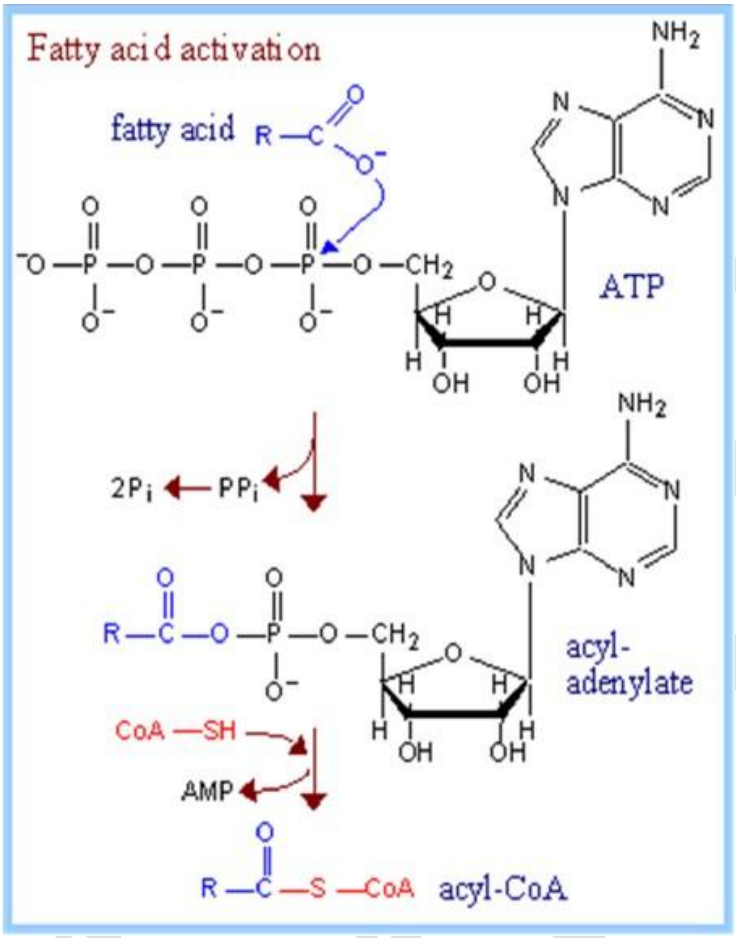
- Cell membranes are composed of a fluid like phospholipid bilayer.
- The bilayer incorporates cholesterol, proteins, and glycolipids.
- Small nonpolar molecules cross by diffusion through the lipid bilayer.
- Small ions and polar molecules diffuse through the aqueous media in protein pores.
- Glucose and certain other substances cross with the aid of proteins without energy input.
- Na+, K+, and other substances that maintain concentration gradients inside and outside the cell cross with expenditure of energy and the aid of proteins.

OXIDATION OF FATTY ACIDS

Although fatty acids are both oxidized to acetyl-CoA and synthesized from acetyl-CoA, fatty acid oxidation is not the simple reverse of fatty acid biosynthesis but an entirely different process taking place in a separate compartment of the cell. The separation of fatty acid oxidation in mitochondria from biosynthesis in the cytosol allows each process to be individually controlled and integrated with tissue requirements. Each step in fatty acid oxidation involves acyl-CoA derivatives catalyzed by separate enzymes, utilizes NAD+ and FAD as coenzymes, and generates ATP. It is an aerobic process, requiring the presence of oxygen.

OXIDATION OF FATTY ACIDS OCCURS IN MITOCHONDRIA

Fatty acids must first be converted to an active intermediate before they can be catabolized. This is the only step in the complete degradation of a fatty acid that requires energy from ATP. In the presence of ATP and coenzyme A, the enzyme **acyl-CoA synthetase (thiokinase)** catalyzes the conversion of a fatty acid (or free fatty acid) to an ‒active fatty acid‖ or acyl-CoA, which uses one high-energy phosphate with the formation of AMP and Ppi

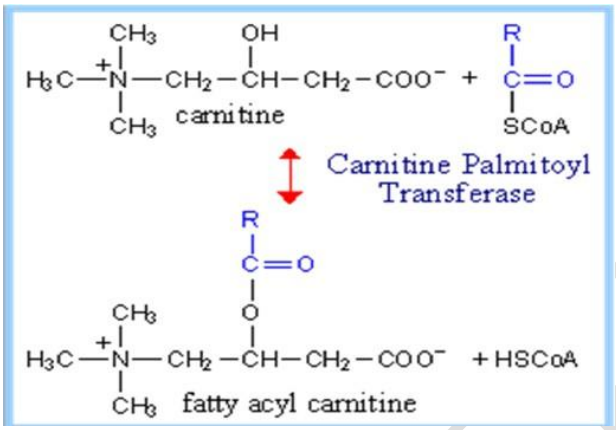


Long-Chain Fatty Acids Penetrate the Inner Mitochondrial Membrane as Carnitine Derivatives

Carnitine is widely distributed and is particularly abundant in muscle. Long-chain acyl-CoA (or FFA) will not penetrate the inner membrane of mitochondria. However, **carnitine palmitoyltransferase-I,** present in the outer mitochondrial membrane, converts long-chain acyl- CoA to acylcarnitine, which is able to penetrate the inner membrane and gain access to the β- oxidation system of enzymes (Figure 22–1). **Carnitine-acylcarnitine translocase** acts as an inner membrane exchange transporter. Acylcarnitine is transported in, coupled with the transport out of one molecule of carnitine. The acylcarnitine then reacts with CoA, catalyzed by **carnitine palmitoyltransferase-II,** located on the inside of

the inner membrane. Acyl-CoA is reformed

in the mitochondrial matrix, and carnitine is liberated.



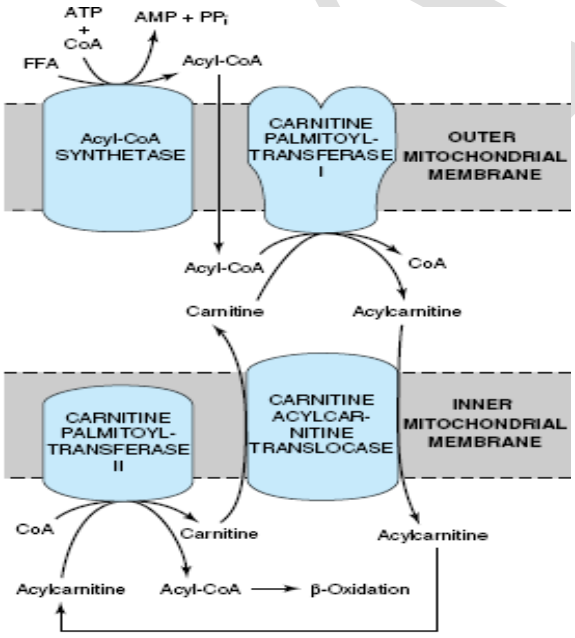


Figure 22–1. Role of carnitine in the transport of long-chain fatty acids through the inner mitochondrial membrane. Long-chain acyl-CoA cannot pass through the inner mitochondrial membrane, but its metabolic product, acylcarnitine, can.

β-OXIDATION OF FATTY ACIDS INVOLVES SUCCESSIVE CLEAVAGE WITH RELEASE OF ACETYL-CoA

In β-oxidation (Figure 22–2), two carbons at a time are cleaved from acyl-CoA molecules, starting at the carboxyl end. The chain is broken between the α(2)- and β(3)-carbon atoms— hence the name β-oxidation. The two-carbon units formed are acetyl-CoA; thus, palmitoyl- CoA forms eight acetyl-CoA molecules.

The Cyclic Reaction Sequence Generates FADH2 & NADH

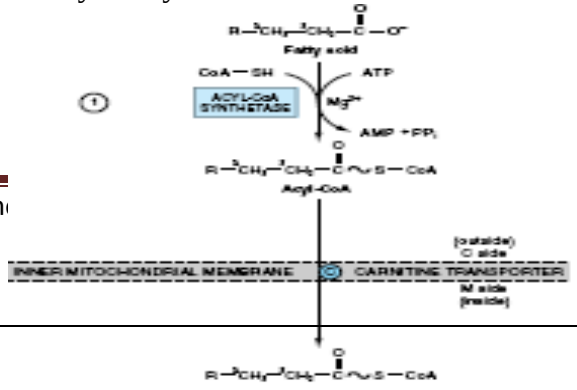
- Several enzymes, known collectively as ‒fatty acid oxidase,‖ are found in the mitochondrial matrix or inner membrane adjacent to the respiratory chain. These catalyze the oxidation of acyl-CoA to acetyl-CoA, the system being coupled with the phosphorylation of ADP to ATP (Figure 22–3).

- The first step is the removal of two hydrogen atoms from the 2(α)- and 3(β)-carbon atoms, catalyzed by **acyl-CoA dehydrogenase** and requiring FAD. This results in the formation of Δ2-*trans*-enoyl-CoA and FADH2. The reoxidation of FADH2 by the respiratory chain requires the mediation of another flavoprotein, termed **electron- transferring flavoprotein.**

- Water is added to saturate the double bond and form 3-hydroxyacyl-CoA, catalyzed by

  _2-enoyl-CoA hydratase. The 3-hydroxy derivative undergoes further dehydrogenation on the 3-carbon catalyzed by

  L(+)-3-hydroxyacyl-CoA



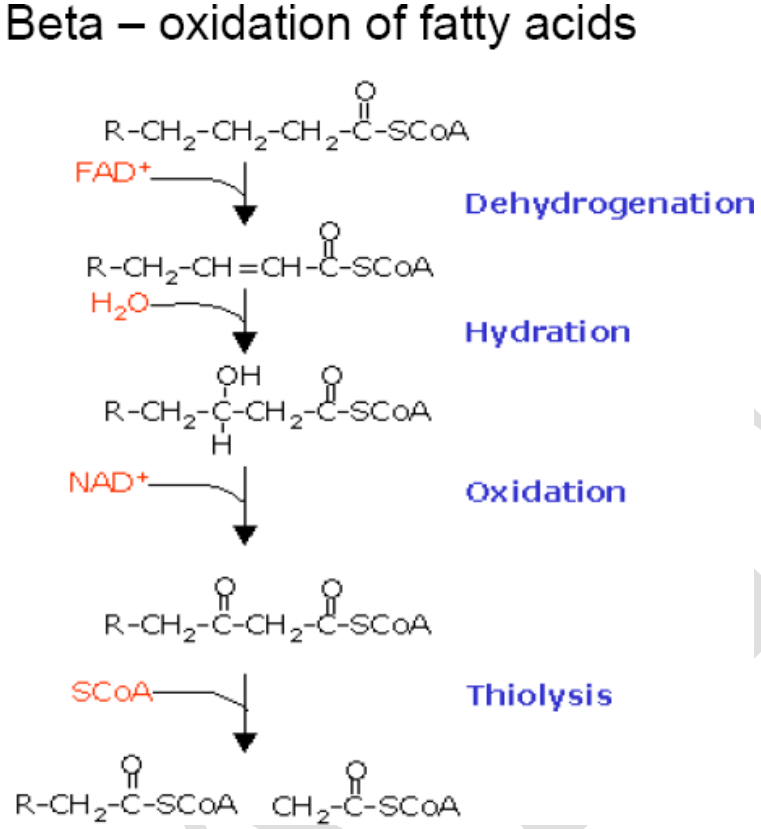
dehydrogenase to form the corresponding 3-ketoacyl-CoA compound. In this case, NAD+ is the coenzyme involved.

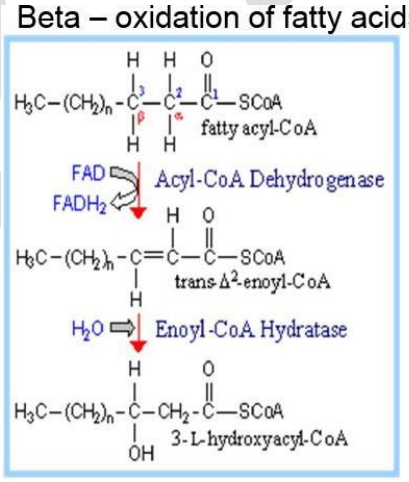
- Finally, 3-ketoacyl- CoA is split at the 2,3- position by **thiolase** (3-ketoacyl- CoA-thiolase), forming acetyl-CoA and a new acyl- CoA two carbons shorter than the original acyl-CoA molecule. The acyl-CoA formed in the cleavage reaction reenters the

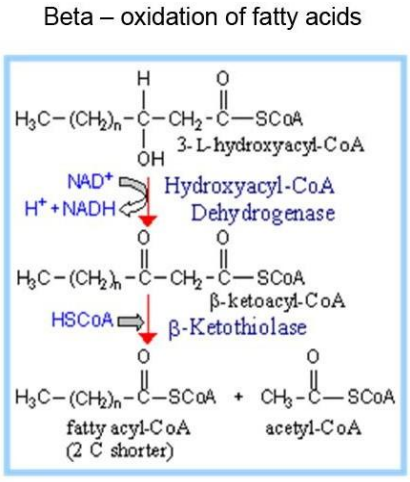
oxidative pathway at reaction 2 (Figure 22–3).

- In this way, a long-chain fatty acid may be degraded completely to acetyl-CoA (C2 units). Since acetyl-CoA can be oxidized to CO2 and water

via the citric acid cycle (which is also found within the mitochondria), the complete oxidation of fatty acids is achieved.





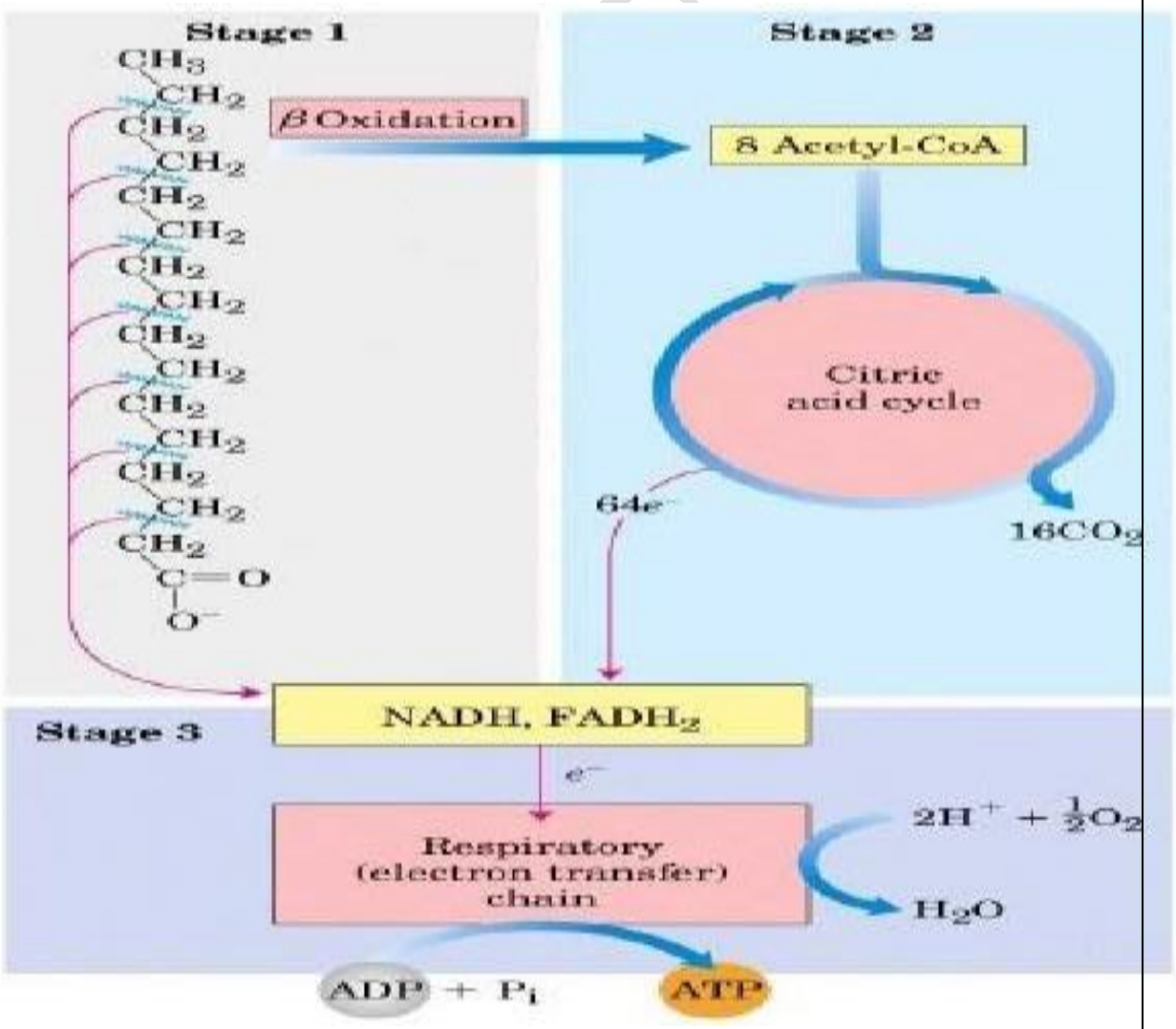


For example for a 16 carbon fatty acid, Palmityl-CoA, it will take 7 cycle of β-oxidation

to generate 8 acetyl-CoA.

Thus there will be production of 7 FADH2, 7 NADH molecules during the β-oxidation cycles.

Oxidation of 8 acetyl-CoA in TCA cycle will produce 8 ATPs, 8 FADH2, 24 NADH



Though it produces more energy, it does not directly produce ATP during the oxidation steps(no substrate level phosphorylation)

β-Oxidation yields Acetyl CoA,NADH & FADH,requiring TCA

cycle and Respiratory chain for further metabolism

TCA cycle and Respiratory chain requires O2

So Fatty acid cannot be used as an energy source in the absence of O2

Oxidation of a Fatty Acid With an Odd Number of Carbon Atoms Yields Acetyl- CoA Plus a Molecule of Propionyl-CoA

Fatty acids with an odd number of carbon atoms are oxidized by the pathway of β-oxidation, producing acetyl- CoA, until a three-carbon (propionyl-CoA) residue remains.

This compound is converted to succinyl-CoA, a constituent of the citric acid cycle. Hence, the propionyl residue from an odd-chain fatty acid is the only part of a fatty acid that is glucogenic.

Oxidation of Fatty Acids Produces a Large Quantity of ATP

Transport in the respiratory chain of electrons from FADH2 and NADH will lead to the synthesis of five high-energy phosphates for each of the first seven acetyl-CoA molecules formed by β- oxidation of palmitate (7 . 5 = 35).

A total of 8 mol of acetyl- CoA is formed, and each will give rise to 12 mol of ATP on oxidation in the citric acid cycle, making 8 . 12 = 96 mol.

Two must be subtracted for the initial activation of the fatty acid, yielding a net gain of 129 mol of ATP per mole of palmitate, or 129 . 51.6* = 6656 kJ. This represents 68% of the free energy of

Energy yield from palmitic acid

	ATP
• From palmitoyl CoA to acetyl CoA:	
Acyl CoA dehydrogenase 7 FADH2	14
Beta-OH dehydrogenase 7 NADH	21
• From 8 acetyl CoA	96
• Total energy yield	131
ATP are used for activation of FA	-2
Hence net gain of ATP	129

combustion of palmitic acid.

UNIT-V

SYLLABUS

Nucleic acids: Structure and functions: Physical & chemical properties of Nucleic acids, Nucleosides & Nucleotides, purines & pyrimidines, Biologically important nucleotides, Double helical model of DNA structure, A, B & Z – DNA, denaturation and renaturation of DNA.

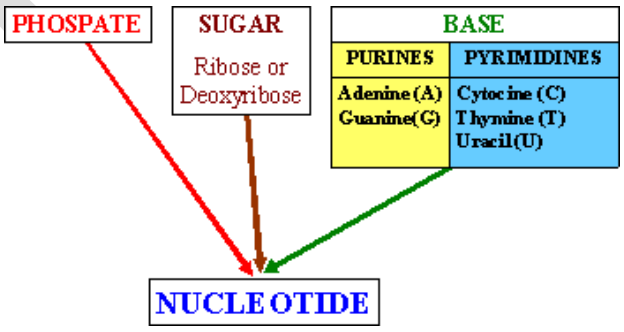
Nucleic acids

Nucleic acids consist of nucleotides that have a sugar, nitrogen base, and phosphate. Two types of nucleic acid are found.

- Deoxyribonucleic acid (DNA)
- Ribonucleic acid (RNA)

Physical & chemical properties of Nucleic acids

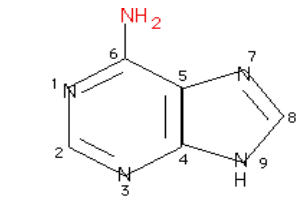
- Polymers of four nucleotides
- Linked by alternating sugar-phosphate bonds
- RNA: ribose and A, G, C, U
- DNA: deoxyribose and A,G,C,T
- Nucleic acids are polynucleotides
- Their building blocks are nucleotides

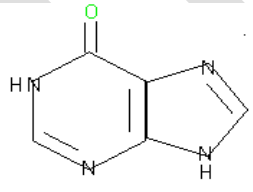


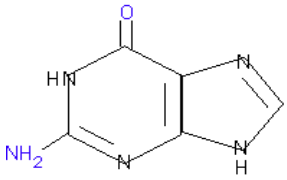
Structure of purines and pyrimidines:

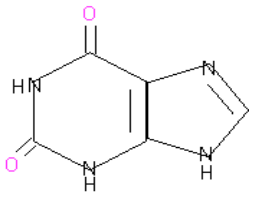
Purines:

- A purine is a heterocyclic aromatic organic compound, consisting of a pyrimidine ring fused to an imidazole ring.
- Adenine = 6-amino purine
- Guanine = 2-amino-6-oxy purine
- Hypoxanthine = 6-oxy purine
- Xanthine = 2,6-dioxy purine







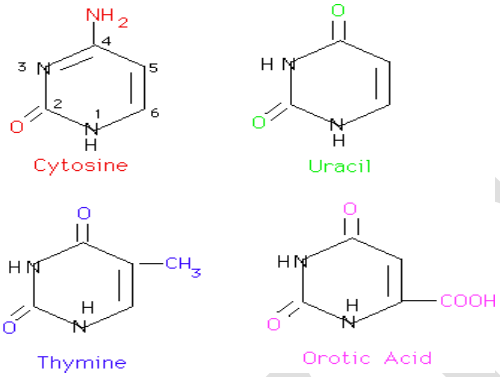


- Adenine and guanine are found in both DNA and RNA.
- Hypoxanthine and xanthine are not incorporated into the nucleic acids as they are being synthesized but are important intermediates in the synthesis and degradation of the purine nucleotides.

Pyrimidines:

- Uracil = 2,4-dioxy pyrimidine
- Thymine = 2,4-dioxy-5-methyl pyrimidine
- Cytosine = 2-oxy-4-amino pyrimidine

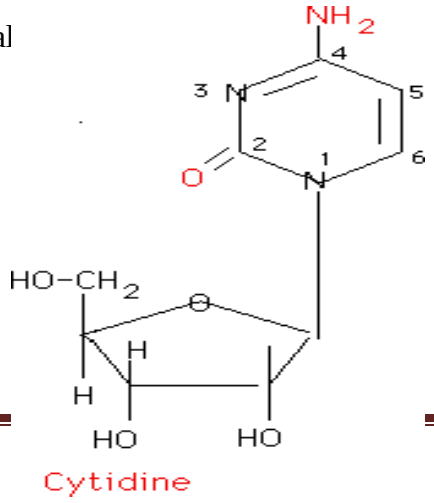
- Orotic acid = 2,4-dioxy-6-carboxy pyrimidine



- Cytosine is found in both DNA and RNA.
- Uracil is found only in RNA.
- Thymine is normally found in DNA.
- Sometimes tRNA will contain some thymine as well as uracil.

Nucleosides:

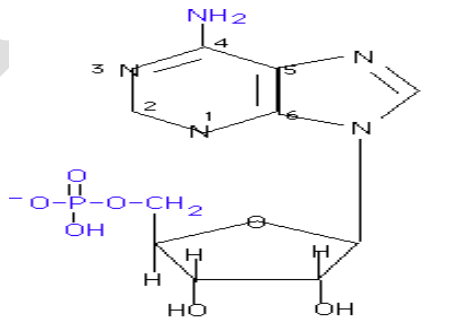
- If a sugar, either ribose or 2-deoxyribose, is added to a nitrogen base, the resulting compound is called a nucleoside.
- Carbon 1 of the sugar is attached to nitrogen 9 of a purine base or to nitrogen 1 of a pyrimidine base.
- The names of purine nucleosides end in -osine and the names of pyrimidine nucleosides end in -idine.
- The convention is to number the ring atoms of the base normal distinguish the ring atoms of the sugar.
- Unless otherwise specified, the sugar is assumed to be ribose.
- To indicate that the sugar is 2'-deoxyribose, a d- is placed before the name.
  - Adenosine
  - Guanosine



- Inosine - the base in inosine is hypoxanthine
- Uridine
- Thymidine
- Cytidine

Nucleotides:

- Adding one or more phosphates to the sugar portion of a nucleoside results in a nucleotide.
- Generally, the phosphate is in ester linkage to carbon 5' of the sugar.
- If more than one phosphate is present, they are generally in acid anhydride linkages to each other.
- If such is the case, no position designation in the name is required.
- If the phosphate is in any other position, however, the position must be designated.
- For example, 3'-5' cAMP indicates that a phosphate is in ester linkage to both the 3' and 5' hydroxyl groups of an adenosine molecule and forms a cyclic structure.
- 2'-GMP would indicate that a phosphate is in ester linkage to the 2' hydroxyl group of a guanosine. Some representative names are:
  - AMP = adenosine monophosphate = adenylic acid
  - CDP = cytidine diphosphate
  - dGTP = deoxy guanosine triphosphate
  - dTTP = deoxy thymidine triphosphate (more commonly designated TTP)
  - cAMP = 3'-5' cyclic adenosine monophosphate



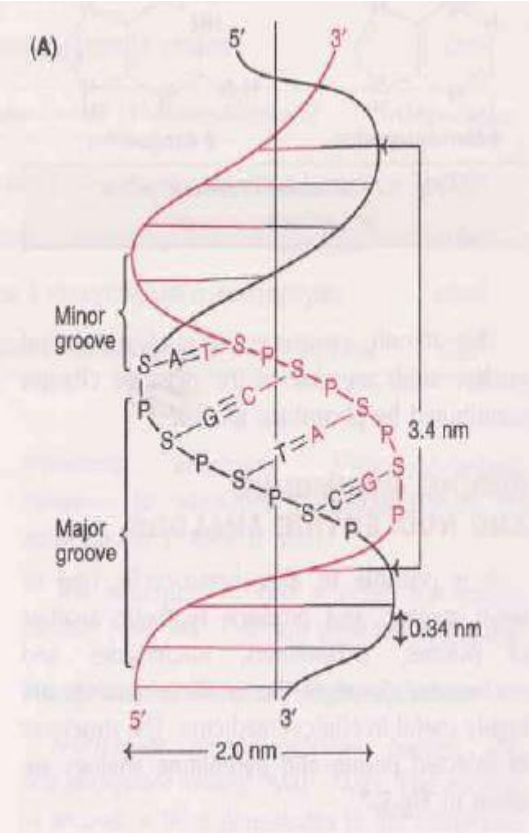
Adenosine Monophosphate
AMP

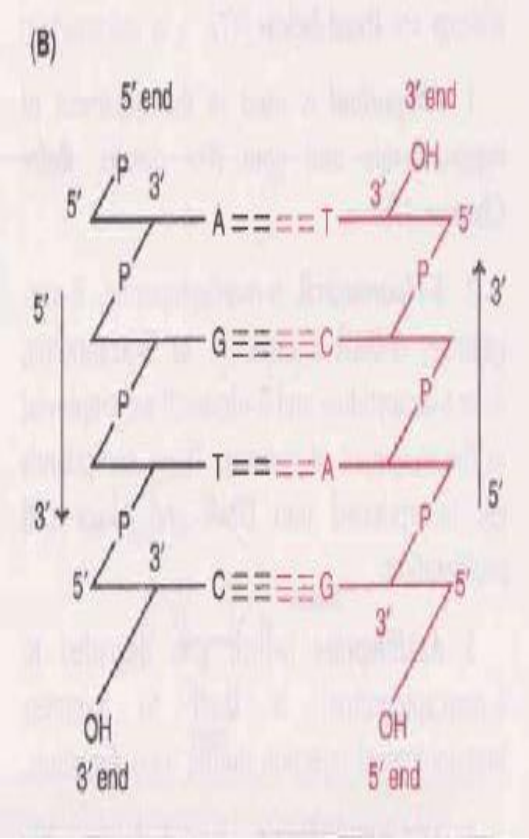
DNA

- DNA is a polymer of deoxyribonucleotides (or simply deoxynucleotides).
- It is composed of monomeric units namely deoxyadenylate (dAMP), eoxyguanylate (dGMP), deoxycytidylate(dCMP) and deoxythymidylate(d TMP) (It may be noted here that some authors prefer to use TMP for deoxythymidylate, since it is found only in DNA).
- The details of the nucleotide structure are given above.

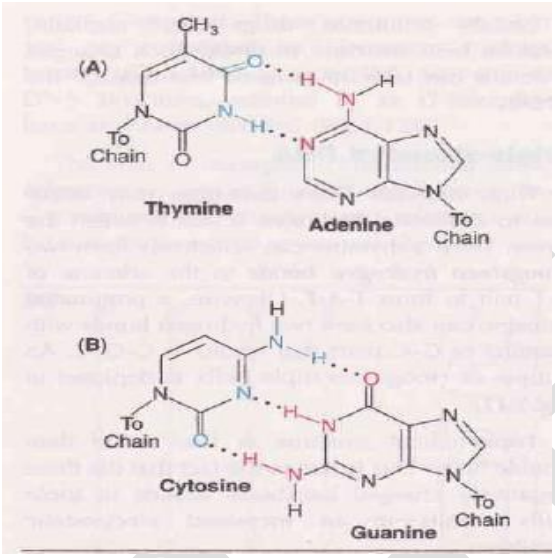
DNA double helix:

- The double helical structure of DNA was proposed by lames Watson and Francis Crick in 1953 (Nobel Prize, 1962).
- The elucidation of DNA structure is considered as a milestone in the era of modern biology.
- The structure of DNA double helix is comparable to a twisted ladder.
- The salient features of Watson - Crick Model of DNA (now known as B-DNA) are described next.





(A) Watson-Crick model of DNA helix (B) Complementary base pairing in DNA helix.



Complementary base paring in DNA

(A) Thymine pairs with adenine by 2 hydrogen bonds

(B) Cytosine pairs with guanine by 3 hydrogenbonds.

The DNA is a right handed double helix.

- It consists of two polydeoxyribonucleotide chains (strands) twisted around each other on a common axis.
- The two strands are antiparallel, i.e., one strand runs in the 5' to 3' direction while the other in 3'to 5'direction. This is comparable to two parallel adjacent roads carrying traffic in opposite direction.
- The width (or diameter) of a double helix is 20 A^o (2 nm).
- Each turn (pitch) of the helix is 34 A" (3.4 nm) with 10 pairs of nucleotides each pair placed at a distance of about 3.4 A^o.
- Each strand of DNA has a hydrophilic deoxyribose phosphate backbone (3'-5' phosphor diester bonds) on the outside (periphery) of the molecule while the hydrophobic bases are stacked inside (core).

- The two polynucleotide chains are not identical but complementary to each other due to base pairing.
- The two strands are held together by hydrogen bonds formed by complementary base pairs.
- The A-T pair has 2 hydrogen bonds while G-C pair has 3 hydrogen bonds. The G = C is stronger by about 50% than A=T.
- The hydrogen bonds are formed between a purine and a pyrimidine only.
- If two purines face each other, they would not fit into the allowable space. And two pyrimidines would be too far to form hydrogen bonds.
- The only base arrangement possible in DNA structure, from spatial considerations is A-T, T-A, G-C and C-C.
- The complementary base pairing in DNA helix proves Chargaffs rule.
- The content of adenine equals to that of thymine (A = T) and guanine equals to that of cytosine (G = C). 10.
- The genetic information resides on one of the two strands known as template strand or sense strand.
- The opposite strand is antisense strand.
- The double helix has (wide) major grooves and (narrow) minor grooves along the phosphodiester backbone.
- Proteins interact with DNA at these grooves, without disrupting the base pairs and double helix.

Forms of DNA

B-Form, A-Form, Z-Form of DNA

Three major forms of DNA are double stranded and connected by interactions between complementary base pairs. These are terms A-form, B-form,and Z-form DNA.

B-form DNA

The information from the base composition of DNA, the knowledge of dinucleotide structure, and the

insight that the X-ray crystallography suggested a helical periodicity were combined by Watson and Crick in 1953 in their proposed model for a double helical structure for DNA. They proposed two strands of DNA -- each in a right-hand helix -- wound around the same axis. The two strands are held together by H-bonding between the bases (in anti conformation) as shown in Fig. 2.13.

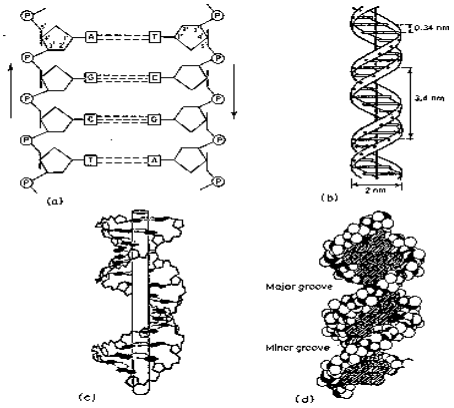
Major groove Major groove

Minor groove Minor groove

An A:T base pair and (right) a G:C base pair

Bases fit in the double helical model if pyrimidine on one strand is always paired with purine on the other. From **Chargaff's rules**, the two strands will pair A with T and G with C. This pairs a keto base with an amino base, a purine with a pyrimidine. Two H-bonds can form between A and T, and three can form between G and C. This third H-bond in the G:C base pair is between the additional exocyclic amino group on G and the C2 keto group on C. The pyrimidine C2 keto group is not involved in hydrogen bonding in the A:T base pair.

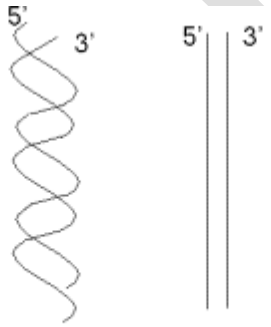
These are the complementary base pairs. The base-pairing scheme immediately suggests a way to replicate and copy the the genetic information.



Antiparallel (a), plectonemically coiled (b, c, d) DNA strands. The arrows in a are pointed 3' to 5', but they illustrate the antiparallel nature of the duplex. The two strands of the duplex are antiparallel and plectonemically

coiled. The nucleotides arrayed in a 5' to 3' orientation on one strand align with complementary nucleotides in the the 3' to 5' orientation of the opposite strand.

The two strands are not in a simple side-by-side arrangement, which would be called a paranemic joint (Fig. 2.15). Rather the two strands are coiled around the same helical axis and are intertwined with themselves (which is referred to as a plectonemic coil). One consequence of this intertwining is that the two strands cannot be separated without the DNA rotating, one turn of the DNA for every "untwisting" of the two strands.



In a plectonemic coil, the two strands wrap around each other.
In a paranemic joint, the two strands align side-by-side.

Duplex DNA has the two strands wrapped around each other in a plectonemic coil (left), not a paranemic duplex (right).

Dimensions of B-form (the most common) of DNA

- 0.34 nm between bp, 3.4 nm per turn, about 10 bp per turn
- 1.9 nm (about 2.0 nm or 20 Angstroms) in diameter

Major and minor groove

The major groove is wider than the minor groove in DNA (Fig. 2.14d), and many sequence specific proteins interact in the major groove. The N7 and C6 groups of purines and the C4 and C5 groups of pyrimidines face into the major groove, thus they can make specific contacts with amino acids in DNA-binding proteins. Thus specific amino acids serve as H-bond donors and acceptors to form H-

bonds with specific nucleotides in the DNA. H-bond donors and acceptors are also in the minor groove, and indeed some proteins bind specifically in the minor groove. Base pairs stack, with some rotation between them.

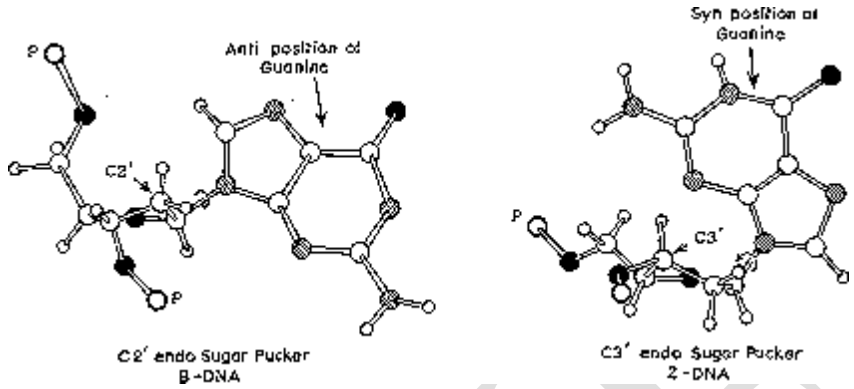
A-form nucleic acids and Z-DNA

Three different forms of duplex nucleic acid have been described. The most common form, present in most DNA at neutral pH and physiological salt concentrations, is B-form. That is the classic, right-handed double helical structure we have been discussing. A thicker right-handed duplex with a shorter distance between the base pairs has been described for RNA-DNA duplexes and RNA-RNA duplexes. This is called A-form nucleic acid.

A third form of duplex DNA has a strikingly different, left-handed helical structure. This Z DNA is formed by stretches of alternating purines and pyrimidines, e.g. GCGCGC, especially in negatively supercoiled DNA. A small amount of the DNA in a cell exists in the Z form. It has been tantalizing to propose that this different structure is involved in some way in regulation of some cellular function, such as transcription or regulation, but conclusive evidence for or against this proposal is not available yet.

Differences between A-form and B-form nucleic acid

The major difference between A-form and B-form nucleic acid is in the conformation of the deoxyribose sugar ring. It is in the C2' endoconformation for B-form, whereas it is in the C3' endoconformation in A-form. As shown in Fig. 2.16, if you consider the plane defined by the C4'-O-C1' atoms of the deoxyribose, in the C2' endoconformation, the C2' atom is above the plane, whereas the C3' atom is above the plane in the C3' endoconformation. The latter conformation brings the 5' and 3' hydroxyls (both esterified to the phosphates linking to the next nucleotides) closer together than is seen in the C2' endoconfromation (Fig. 2.16). Thus the distance between adjacent nucleotides is reduced by about 1 Angstrom in A-form relative to B-form nucleic acid (Fig. 2.17).

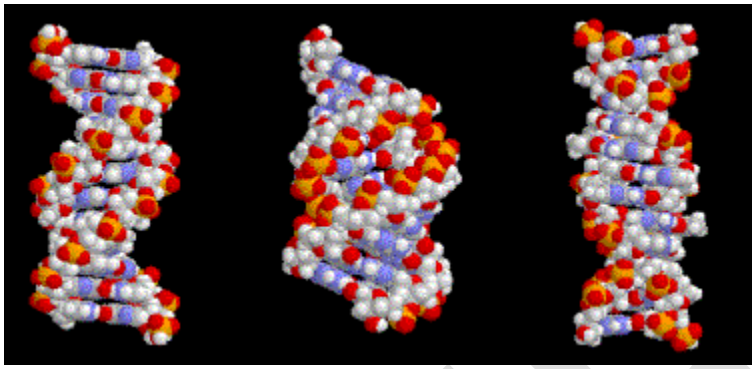


.Syn and anti conformations of the base relative to the sugar in nucleotides

A second major difference between A-form and B-form nucleic acid is the placement of base-pairs within the duplex. In B-form, the base-pairs are almost centered over the helical axis (Fig. 2.15), but in A-form, they are displaced away from the central axis and closer to the major groove. The result is a ribbon-like helix with a more open cylindrical core in A-form.

Z-form DNA

Z-D NA is a radically different duplex structure, with the two strands coiling in left-handed helices and a pronounced zig-zag (hence the name) pattern in the phosphodiester backbone. As previously mentioned, Z-DNA can form when the DNA is in an alternating purine-pyrimidine sequence such as GCGCGC, and indeed the G and C nucleotides are in different conformations, leading to the zig-zag pattern. The big difference is at the G nucleotide. It has the sugar in the C3' endoconformation (like A-form nucleic acid, and in contrast to B-form DNA) and the guanine base is in the synconformation. This places the guanine back over the sugar ring, in contrast to the usual anticonformation seen in A- and B-form nucleic acid. Note that having the base in the anticonformation places it in the position where it can readily form H-bonds with the complementary base on the opposite strand. The duplex in Z-DNA has to accomodate the distortion of this G nucleotide in the synconformation. The cytosine in the adjacent nucleotide of Z-DNA is in the "normal" C2' endo, anticonformation.



B-form (left), A-form (middle) and Z-DNA (right).

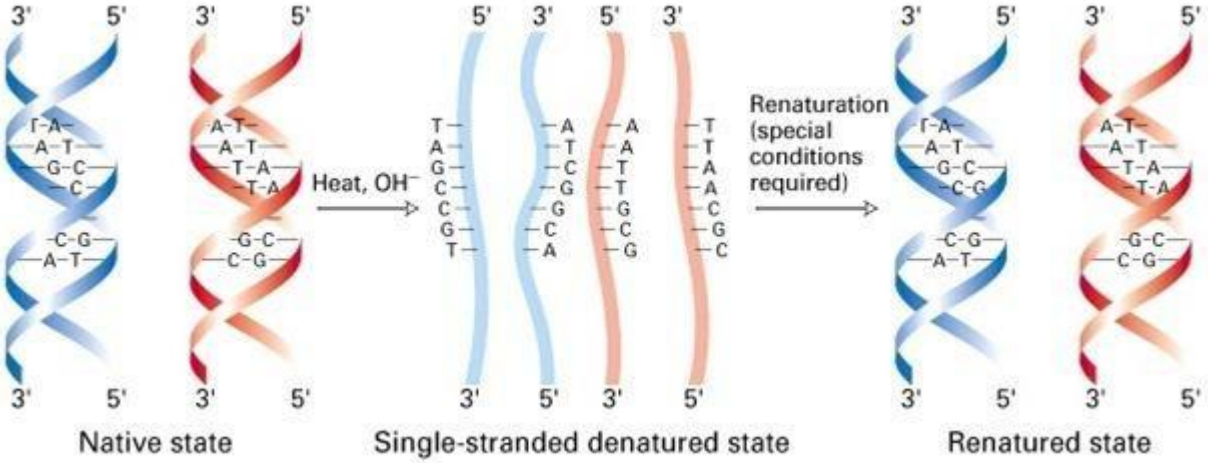
Comparisons of B-form, A-form and Z-DNA

	B	A	Z
helix sense	RH	RH	LH
bp per turn	10	11	12
vertical rise per bp	3.4	2.56	3.7 Angstroms
rotation per bp	+36	+33	-30 degrees
helical diameter	19	23	18 Angstroms

Even classic B-DNA is not completely uniform in its structure. X-ray diffraction analysis of crystals of duplex oligonucleotides shows that a given sequence will adopt a distinctive structure. These variations in B-DNA may differ in the propeller twist (between bases within a pair) to optimize base stacking, or in the 3 ways that 2 successive base pairs can move relative to each other: twist, roll, or slide.

Denaturation of DNA strands:

- The two strands of DNA helix are held together by hydrogen bonds.
- Disruption of hydrogen bonds (by change in pH or increase in temperature) results in the separation of polynucleotide strands.
- This phenomenon of loss of helical structure of DNA is known as denaturation.
- The phosphodiester bonds are not broken by denaturation.
- Loss of helical structure can be measured by increase in absorbance at 260 nm (in a spectrophotometer).



Denaturation and renaturation of DNA.

- Melting temperature (Tm) is defined as the temperature at which half of the helical structure of DNA is lost.
- Since C-C base pairs are more stable (due to 3 hydrogen bonds) than A-T base pairs(2 hydrogen bonds), the Tm is greater for DNAs with higher C-C content.
- Thus, the Tm is 65□C for 35% G-C content while it is 70□C for 50% G-C content.
- Formamide destabilizes hydrogen bonds of base pairs and, therefore, lowers Tm.
- This chemical compound is effectively used in recombinant DNA experiments.

Renaturation:

- Renaturation or reannealing is the process in which the separated complementary DNA strands can form a double helix.
- It is effected by cooling
- It involves reannealing or formation of hydrogen bond between complimentary base pairs.
- Upon renaturation viscocity increases
- The rate renaturation is directly proporotional to the concentration of complementary strands.

Application of denaturation and renaturation

- To understand genome size and complexity
- To understand Genetic relatedness
- To understand Relative proportion of single copy and repetitive sequence

UNIT-1	opt 1	opt 2	opt 3	opt 4	Answer
An atom is made up of central _____ containing positively charged protons	nucleus	molecule	nucleolus	shells	nucleus
The number of electrons that can be present in the L shell is _____.	12	8	18	17	8
The simplest atom consisting of positively charged proton and an single negatively charged electron orbit is	oxygen	hydrogen	nitrogen	sulphur	hydrogen
The mass of a proton or neutron is called _____.	amu	aum	anm	aun	aum
The number of protons on an atom is called the _____ of the atom.	atomic number	mass number	molecular number	proton number	atomic number
The amount of energy required to remove loosely held electron from a normal gaseous atom is called _____.	gaseous potential	ionization potential	molecular potential	proton potential	gaseous potential
A chemical bond that involves the sharing of electron pairs between atoms is called_____.	covalent	coordinate	electrovalent	non covalent	covalent
Chemical bond formed between two atoms due to transfer of electron(s) from one atom to the other. atom is called ..	coordinate	electrovalent	noncovalent	covalent	electrovalent
_____ of an atom is a measure of its power to attract electrons that it is sharing in a covalent bond.	electronegativity	electronaffinity	electropositivity	electroavidity	electroavidity
The distance between two atomic nuclei in a covalent molecule is called _____.	Bond angle	Bond circle	Bond distance	Bond area	Bond distance
_____ Cytochrome oxidase is	a3	aa3	a	a8	aa3
Ionization of water can be described by an _____ constant.	molecular	ionization	equilibrium	ionizable	ionization
The number of H+ ions present in a solution is a measure of _____ of the solution.	Alkalinity	basicity	acidity	avidity	acidity
The _____ of a solution is dependent upon the number for hydroxyl ions present.	basicity	acidity	alkalinity	neutrality	alkalinity
_____ is defined as the negative logarithm of hydrogen ion concentration.	pH	[–H]	[-OH]	H+	pH
The pH of pure water at 25°C is _____.	6	8	7	10	7
A condition called _____ occurs when pH of the blood is higher than normal.	acidosis	alkalosis	basidosis	avidosis	alkalosis
A ____ is defined as a substance that has a greater tendency to lose its proton and completely dissociates.	strong acid	weak acid	strong base	weak base	strong acid
A compound which can accept a pair of electrons from a base is called _____.	electrophile	nucleophile	extremophile	acidophile	electrophile
_____ is used to determine the amount of an acid in a given solution.	centrifugation	separation	titration	neutralization	titration
_____ resists changes in pH on the addition of acid or base.	buffer	pH paper	acidohile	electrophile	buffer
The pK_a of the weak acid is given by a simple expression called _____ equation.	Lowry-Bronsted	Lowry-Hasselbach	Henderson-Hasselbach	Hasselbach	Henderson-Hasselbach
The principal buffer for erythrocytes is _____.	bicarbonate	phosphate	protein	hemoglobin	hemoglobin
The pH of blood is maintained at _____.	7.8	7.4	6.4	7.1	7.4
_____ is the number of isomers of glucose.	4	8	12	16	16
The human heart muscle contains	D- Arabinose	Galactose	D- Lyxose	D- Xylose	D- Lyxose
Epimers of Glucose _____.	Fructose	Galactose	Ribose	Deoxyribose	Galactose
Cellulose is made up of the molecules of_____.	α- Glucose	β-Glucose	Mannose	non of the above	β-Glucose
Glucose absorption may be decreased in _____.	Oedema	Nephritis	Rickets	Osteomyelitis	Oedema
Fructose 1, 6- bisphosphate is activated by----?	ATP	AMP	UTP	ADP	ATP
Glucose -6 - phosphate is absent from _____.	Adipose tissue	Kidney	Intestine	Heart	Adipose tissue
The number of high energy phosphates contained by ATP is _____.	1	3	5	2	2
The synthesis of adenylate cyclase is increased by.	parathyroid	pituitary	thyroid	insulin	thyroid
The toxicity of oxygen is due to its conversion to _____.	metaloxide	superoxide	hyperoxide	hypooxide	superoxide
Keratin the protein of hair is synthesized from the aminoacid	glycine	Serine	Proline	Methionine	Methionine
Most aminoacids are substrates for transamination except_____.	Alanine	Threonine	Serine	Valine	Threonine
In the liver Glyceroldehyde 3 phosphate is converted into	Glycol	Formaldehyde	Formic acid	Glycerol	Glycerol
Cytochrome Oxidase is poisoned by _____.	cyanide	sulphide	sulphite	sulphate	cyanide
The respiratory chain is folded in to _____ oxidation/reduction loops in the membrane.	2	4	3	1	2
Glutamic dehydrogenase is a	monomer	Tetramer	Dimer	polymer	monomer

The energy released in the formation of noncovalent bond is _____	0 kcal/mol.	1-5 kcal/mol	6-9 kcal/mol	0.1-0.5 kcal/mol	1-5 kcal/mol
An example for hydrophobic molecule is _____	water	heat	rosewater	oils	oils
_____ bond is considered to be a very weak bond.	ionic	covalent	hydrophobic	vanderwaals	vanderwaals
Primary atomic bond is _____ bonds.	covalent	hydrogen	vanderwaals	all	all
Secondary atomic bond is _____ bonds.	Covalent	ionic	metallic	hydrogen	hydrogen
The bond formed between atoms or groups carrying opposite charges is known as____	covalent	electrostatic	hydrogen	metallic	electrostatic
Ionic bond is otherwise known as _____ bond.	covalent	metallic	hydrogen	electrovalent	electrovalent
Increasing salt concentration _____ the strength of ionic bonding.	increases	stabilizes	regulates	reduces	reduces
pH of hydrochloric acid secreted by stomach lining is _____	6	7	3	1	1
Grape fruit is in the pH of _____	6	7	3	1	1
_____ is a substance which produces hydrogen ions(H^+) by dissociation.	Base	water	liquid	acid	acid
The pH scale ranges between _____	0 & 14	-114	-115	0 & 15	0 & 14
An acid dissociation constant is denoted by _____	K_a	K_{da}	Kb	a & b	K_a
The bicarbonate ion is the conjugate base of _____	2 carbon atom	Carbonic acid	Carbamides	Carbondioxide	Carbonic acid
Retinol exists as an ester with higher fatty acids in the_____	Head	Kidney	Brain	Nose	Kidney
Carotenes are transported with the _____	Protein	Lipoprotein	Minerals	Lipids	Lipoprotein
The perccentage of Vitamin A in the form of esters is stored in the liver _____	80	85	90	95	95
_____ a pH indicator composed of a solution of several compounds that exhibits several smooth colour changes over a pH value range from 1-14 to indicate the acidity or alkalinity of solutions.	pH indicator	Acid indicator	Alkali indicator	Universal indicator	Universal indicator
pKa value of phenol is _____	1.99	1.99	8.99	10	10
Amphetamine has pK_A of _____	1.8	2.28	3.38	9.9	9.9
UNIT-2	opt 1	opt 2	opt 3	opt 4	
Glycosides are found in many _____.	drugs	vitamins	minerals	nucleoproteins	drugs
Iodine solution produces no colour with _____.	cellulose	glycogen	starch	dextrin	cellulose
The distinguishing test between monosaccharides and dissacharides is _____.	barfoed's test	seliwanoff's test	fehling's test	benedict's test	barfoed's test
Barfoed's solution is not reduced by ____.	glucose	mannose	sucrose	ribose	sucrose
The non-protein part of rhodopsin is	Retinal	Retinol	Carotene	Repsin	Retinal
Heparin has a molecula weight of _____.	14000	14500	17000	17500	17000
The component of cartilage and cornea is _____.	dermatosulphate	keratosulphate	hyaluronic acid	chondroitin sulphate.	keratosulphate
In place of glucuronic acid chondroitin sulphate B contains _____.	gluconic acid	gulonic acid	iduronic acid	sulphonic acid	iduronic acid
UDPG is essential for the synthesis of_____.	Lactose	galactose	ribose	deoxtribose	Lactose
Cellulose is made up of molecules of _____.	a glucose	b-glucose	d- glucose	g- glucose	b-glucose
Continued intake of excesive amounts of vitamin A especailly in children produces _____.	Irritability	Nausea	Vomiting	Redness	Irritability
Each branch of amylopectin is at an interval of _____glucose units.	14-20	24-30	34-40	44-50	24-30
Hexokinase has high affinity for glucose than_____.	Protease	Glucokinase	Cellolase	Aldolase	Glucokinase
Glucose is removed from the blood following a meal by _____.	hexokinase	glucokinase	heparinkinase	phosphokinase	hexokinase
Cyclic AMP is formed from ATP by the enzyme adenylate cyclase which is activated by the hormone.	insulin	epinephrine	glucagons	progesterone	epinephrine
The absorption of glucose is interfered by the deficiency of _____.	vitamin A	thiamine	riboflavin	pyridoxine	thiamine
The branching enzyme acts on the glycogen chain between glucose units of _____.	1 and 6	2 and 7	3 and 9	6 and 11	6 and 11
The synthesis of adenylate cyclase is increased by _____.	thyroid hormones	growth hormones	ACTH	FTH	thyroid hormones
Inulin is a _____.	glucosan	fucosan	fructosan	pyranosan	fructosan
Glucose on treatment with strong mineral acids produces _____.	levulinic acid	levunyl acid	gluconic acid	glucuronic acid	levulinic acid
Heparin is used as an _____	anticoagulant	coagulant	depressent	antidepressent	anticoagulant
Each turn of the helix amylose consists of _____ glucose units.	2	4	6	7	6
The method of formation of glucose and glycogen from non-carbohydrate sources is called as _____.	glycogenesis	glucogenesis	Gluconeogenesis	Glycolysis	glucogenesis

Enzymes are destroyed by	formaldehyde	sucrose	dextrose	fructose	formaldehyde
The enzyme _____ splits fructose 1,6 diphosphate in to two triose phosphate.	enolase	glucokinase	aldolase	hexokinase	aldolase
Pyruvate dehydrogenase is inhibited by _____.	fluoride	sulphide	arsenite	sulphate	arsenite
Hypoglycemia occurs due to the inhibition of phosphoglucomutase by _____.	fructose -1 phosphate	glucose-6 phosphate	fructose	glucose 1,6 phosphate	fructose -1 phosphate
The approximate number of mols of pyruvate dehydrogenase in pyruvate dehydrogenase complex is _____.	19	29	34	24	29
The carrier of citric acid cycle is _____.	succinate	fumarate	malate	oxaloacetate	oxaloacetate
When equal amounts of dextro and levo rotatory isomers are present in a mixture it is said to be _____.	isomeric	epimeic	racemic	enantiomeric	racemic
Cis-trans isomerism occurs in compounds with _____ bonds.	single	double	sugar	phosphate	sugar
Fructokinase is present in _____.	intestine	adipose tissue	heart	brain	intestine
Pyruvate is accumulated by the dietary deficiency of _____.	folic acid	B6	b12	thiamine	thiamine
The glycogen content of _____ is more than in muscle.	liver	brain	kidney	intestine	liver
In galactosemic individual UDP galactose is formed by epimerization from _____.	glucose	UDP glucose	CDP glucose	ITP glucose	UDP glucose
In the liver glyceraldehyde –3 phosphate is converted to _____.	glycol	formaldehyde	formic acid	glycerol	glycerol
The reaction involving the conversion of succinyl CoA to succinate requires _____.	CDP	GDP	ADP	ATP	ADP
The heptose ketose sugar formed as a result of chemical reactionin HMP shunt is _____.	glucoheptose	galactoheptose	sedoheptulose	manooheptose	sedoheptulose
On boiling benedict's solution is not reduced by _____.	sucrose	lactose	maltose	fructose	sucrose
The general formula for polysaccharide is _____.	$(C_6H_{10}O_5)n$	$(C_6H_{12}O_6)n$	$(C_6H_{12}O_5)n$	$1(C_6H_{10}O_6)n$	$(C_6H_{10}O_5)n$
Human heart muscle contains_____	D-Arabinose	D-Ribose	D-Lyxose	D-Xylose	D-Lyxose
The intermediate n hexose monophosphate shunt is _____	D-Ribulose	D-Arabinose	D-Lyxose	D-Xylose	D-Ribulose
Honey contains the hydrolytic product of _____	Lactose	Maltose	Inulin	Starch	Inulin
Iodine solution produces no colour with _____	Cellulose	Starch	Dextrin	Glycogen	Cellulose
Amylose contains glucose units _____	100-200	200-300	300-400	500-600	300-400
Blood group substances consist of _____	Lactose	Maltose	Fucose	Mucose	Fucose
Salivary amylase is activated by _____	Na^+	K^+	HCO_3^-	Cl^-	Cl^-
Glucose absorption may be decreased in _____	Oedema	Nephritis	Rickets	Osteomyelitis	Oedema
Sugars forming five-membered rings are called _____	Epimerases	Furanoses	Racemases	Hydrolases	Furanoses
A method for synthesis of monosaccharides was first proposed by _____	Michaelis Menton	Firoz	Kiliani	Persoz	Kiliani
Galactose on reduction yields _____	Dulcitol	Mannitol	Sorbitol	Aldol	Dulcitol
Sucrose is refered as _____	Sugar	Simple sugar	Fructosan	Invert sugar	Invert sugar
Starch is formed by _____chain	α-glucosidic	β- glucosidic	γ- glucosidic	All	α-glucosidic
Gluconeogenesis is a reversal of _____	Krebs cycle	HMP shunt	PMP	Glycolysis	Glycolysis
_____ cannot be synthesized in man.	Lactic acid	HCl	Protein	Ascorbic acid	Ascorbic acid
Insulin is destroyed by _____	Lyases	Ligases	Aldolases	Peptidase	Peptidase
_____ are antagonists to insulin.	Pyruvic acid	Glucokinase	Glycogenin	Glucagon	Glucagon
In Juvenile Diabetes, the _____ of pancreas are exhausted.	α-cells	β-cells	γ-cells	All	β-cells
The key enzyme of TCA cycle is _____	Citrate synthase	Isocitrate dehydrogenase	α -ketoglutarate	All	All
UNIT-3	opt 1	opt 2	opt 3	opt 4	
In diabetic individuals, Sorbitol level is _____ in eye lens.	Decreased	Increased	Moderate	Highly decreased	Increased
Fats are solids at	10° C	20° C	30°	40° C	20° C
Lecithin contains a nitrogenous base named as	ethanolamine	choline	inositol	phospholipids	ethanolamine
Postaglandins increase intestinal motility and cause_____	Constipation	Loose motion	Diarrhoea	Dysentery	Loose motion
Esters of fatty acids with higher alcohols other than glycerol are said to be	waxes	fats	both of the above	lipoprotein	waxes
Gangliosides are the glycolipids occurring in	liver	brain	kidney	muscle	brain
The prostaglandins are synthesized from	arachidonic acid	oleic acid	linoleic acid	linolenic acid	arachidonic acid
The essential fatty acids retard	atherosclerosis	diabetes mellitus	nephritis	edema	atherosclerosis

Eicasonoids are formed from	arachidonate	palmitate	stearate	butyrate	arachidonate
The principal organ for cholesterol synthesis is	brain	thyroid	liver	lungs	liver
LDL contains the apoprotein	C-I	C-II	C-III	B	B
Fats are esters of _____ with glycerol	fatty acids	waxes	Phospholipids	Cholesterol	fatty acids
PG3 and TX3 inhibit the release of	oleic acid	palmitoleic acid	palmitic acid	arachidonic acid	arachidonic acid
Serum LDL has been found to be increased in	obstructive jaundice	hepatic jaundice	hemolytic jaundice	septicemia	obstructive jaundice
HDL is synthesized and secreted from	pancreas	liver	kidney	muscle	liver
Liebermann- Burchard reaction is performed to detect	cholesterol	glycerol	fatty acid	vitamin D	cholesterol
Sulpholipids have been isolated from	heart	liver	brain	intestine	brain
Lecithins are soluble in ordinary fat solvents except	benzene	ethylalcohol	methylalcohol	acetone	acetone
Cardiolipin found in mitochondria is formed from	lipositol	phosphatidyl ethanolamine	phosphadityl glycerol	inositol	phosphadityl glycerol
w- oxidation takes place by the hydrolysis in microsomes involving	cytochrome b	cytochrome c	cytochrome p-450	cytochrome a3	cytochrome p-450
Prostaglandins are liberated in the circulation by the stimulation of	posterior pituitary	anterior pituitary	adrenal gland	thyroid	adrenal gland
The great majority of absorbed fat appears in the form of	HDL	chylomicrons	VLDL	LDL	chylomicrons
The fatty acids containing even and odd numbers of carbon atoms and aiso unsaturated fatty acids are oxidized by_____	a- Oxidation	b - Oxidation	w- Oxidation	g- Oxidation	b - Oxidation
Long chain fatty acids are first activated to acyl- CoA in the	cytosol	mitochondria	microsomes	lysosomes	cytosol
Phospholipids help the oxidation of	glycerol	fatty acids	glycerophosphates	glycophosphates	fatty acids
Cyclooxygenase is termed as	inhibiting enzyme	suicide enzyme	oxidizing enzyme	reducing enzyme	suicide enzyme
The synthesis of prostaglandins is inhibited by	aspirin	arsenite	fluoride	cyanide	aspirin
Faty acids synthesis takes place in the presence of the coenzyme	NAD+	reduced F+	reduced NAD	reduced NADP	reduced NADP
The concentration of sphingomyelins are inceased in	Gaucher's disease	Fabry's disease	Febrile disease	Niemann pick disease	Niemann pick disease
The protein moiety of lipoproteins is known as	apoprotein	preprotein	post protein	pseudoprotein	apoprotein
In adipose tissue prostaglandins decrease	lipogenesis	ketogenesis	lipolysis	ketolysis	lipogenesis
The beta lipoprotein fraction increases in severe	diabetes mellitus	uremia	.nephritis	muscular dystrophy	diabetes mellitus
An example of cardiac glycoside is	digitoxin	strobanthin	lycophyll	digitalis	strobanthin
Acyl-CoA dehydrogenase converts acyl-CoA to a, b-unsaturated acyl-CoA in the presence of the coenzyme	NAD+	NADP	ATP	FAD+	FAD+
Before the action of lipase the fat is emulsified by	lipoproteins	phospholipids	ergosterols	digitoxin	phospholipids
Leukotrienes are not formed in	leukocytes	mastocytoma	platelets	brain cells	brain cells
Leukotriene C4 is formed by the addition of	ascorbic acid	glutathione	glutamate	1aspartate	glutathione
The blood cholesterol level is increased in the deficiency of	vitamin D	vitamin B2	pyridoxine	aspartate	pyridoxine
Phosphadityl inositol is found in	cabbages	soyabeans	cauliflower	apples	soyabeans
Ketone bodis are utilized in	mitochondria	extrahepatic tissues	nuclei	chromosomes	extrahepatic tissues
Carboxylation of acetyl coA to malonyl CoA takes place bin the presence of	FAD+	biotin	NAD+	NADP+	biotin
Hydrolysis of fat by alkali is known as _____	Saponification number	Saponification	esterification	purification	Saponification
The concentration of sphingomyelins are increased in _____ disease.	Gauchers	Fabrys	Febrile	Niemann-Picks	Niemann-Picks
Lignouric acid present in peanut oil contain _____ carbon atom.	18	20	22	24	24
Arachidonic acid contain _____ number of double bond.	2	3	4	5	4
The shape of arachidonic acid is _____	L	M	U	V	U
Waxes contains higher alcohols known as _____	Methyl	Ethyl	Phytyl	Cetyl	Cetyl
The synthesis of prostaglandins is inhibited by _____	Aspirin	Arsenite	Fluoride	Cyanide	Aspirin
Prostaglandins increase Cyclic AMP in _____	Kidney	Liver	Platelets	Heart	Platelets
Sterilized milk is devoid of	vitamin A	Vitamin B2	Vitamin D	Vitamin C	Vitamin C
The example of saponin is _____	Oxygenin	Deoxygenin	Digitonin	a & b	Digitonin
_____ is essential for cholesterol absorption.	Bile	Fattyacids	Protein	Carbohydrates	Bile
Physical exercise _____ the serum cholesterol level.	Increase	Decrease	Stimulates	Regulates	Decrease

Tay Sachs disease is characterized by increased accumulation of _____ in brain and spleen.	GM_1 Ganglioside	GM_2 Ganglioside	GM_3 Ganglioside	GM_4 Ganglioside	GM_2 Ganglioside
Krabbes disease is due to the deficiency of _____.	Lipase	Permease	Transacetylase	B-galactosidase	B-galactosidase
Butyryl –Co A is the primer molecule in _____	Liver	Pancreas	Gall bladder	Mammary gland	Mammary gland
Alcoholism leads to _____	Liver cirrhosis	Splenomegaly	Kidney stone	b & c	Liver cirrhosis
_____ in the dosage of ½ to 1 gm thrice daily lowers cholesterol.	Streptomycin	Niacin	Gentamicin	Kanamycin	Niacin
Sunflower oil contains a high proportion of _____fattyacids.	Monounsaturated	Polyunsaturated	Monosaturated	Polysaturated	Polyunsaturated
UNIT-4	opt 1	opt 2	opt 3	opt 4	
_____ is the poor source of vitamin D.	Egg	Butter	Milk	Liver	Milk
_____ are found both inside and outside of mitochondria.	Streptokinases	Thiokinases	Lipokinases	Thyrokinases	Thiokinases
Out of 200 different aminoacids found in nature the number of aminoacids present in protein is _____	20	23	22	24	20
Enzyme catalyzed hydrolysis of proteins produces aminoacids of the form _____.	D-	DL-	LD	L-	L-
The ph of arginine is _____.	10.5	10.6	10.8	11	10.8
The neutral aminoacid is _____.	leucine	lysine	proline	Histidine	leucine
The aminoacid containing hydroxyl group is _____.	alanine	isoleucine	arginine	threonine	threonine
The basic aminoacid is _____.	glycine	Histidine	proline	serine	Histidine
All aminoacids are optically active except _____.	glycine	serine	threonine	tryptophan	glycine
The aminoacid which synthesizes many hormones is _____.	valine	phenylalanine	alanine	Histidine	phenylalanine
Aminoacids are insoluble in _____.	acetic acid	chloroform	ethanol	benzene	benzene
The sulphur containing aminoacid is _____.	glycine	methionine	valine	homoserine	methionine
The melting point of aminoacids is above _____.	100° C	180° C	200° C	220° C	200° C
From two aminoacids peptide bond formation involves the removal of one molecule of _____.	ammonia	water	carbondioxide	carboxylic acid	water
Normal daily output of urea through urine in grams is_____.	10 to 20	15 to 25	20 to 30	25 to 35	20 to 30
An example of globulin is _____.	leucosin	tuberin	oryzenin	legunelin	tuberin
An example of scleroprotein is _____.	glutenin	gliadin	salmine	elastin	elastin
An example of metalloprotein is _____.	elastin	siderophilin	mucin	glutenin	siderophilin
Many globular proteins are stable in water solution although they lack in _____.	hydrogen bond	covalent bond	salt bonds	disulphide bonds	disulphide bonds
Each turn of a-helix consists of _____ aminoacids.	3.2	3	2.8	3.6	3.6
The distance traveled per turn of a–helix is _____.	0.34 nm	0.44nm	0.54nm	0.64nm	0.54nm
a–helix is stabilized by _____ bonds.	hydrogen	disulphide	nonpolar	polar	hydrogen
The milk protein in the stomach of infants is digested by _____.	pepsin	trypsin	chymotrypsin	rennin	rennin
The half life of antibody proteins is _____	4 weeks	2 weeks	3 weks	1 week	2 weeks
Carboxy peptidase B in the small intestine hydrolyses peptides containing _____.	Leucine	Isoleucine	Arginine	Cysteine	Arginine
Protein anabolism is stimulated by _____.	ACTH	Testosterone	Glucagons	Epinephrine	Testosterone
The metabolism of protein is integrated with that of carbohydrate and fat through _____.	Oxaloacetate	Citrate	Isocitrate	Malate	Oxaloacetate
In the small intestine trypsin hydrolyzes peptide linkages containing _____.	Arginine	Histidine	Serine	Aspartate	Arginine
Chymotrypsin in the small intestine hydrolyzes peptide linkages containing _____.	Phenylalanine	Alanine	Methionine	Valine	Phenylalanine
The building up and breakdown of protoplasm are concerned with the metabolism of _____.	Carbohydrates	Fats	Protein	Minerals	Protein
Aminoacids provide the nitrogen for the synthesis of _____.	the bases of the Phospholipids	uric acid	glycolipids	chondroitin sulphates	the bases of the Phospholipids
The end product of aminoacid nitrogen metabolism in uricotelic organisms is _____.	bilirubin	urea	uric acid	biliverdin	uric acid
Oxidative conversion of many aminoacids to their corresponding a-keto acids occurs in mammalian _____.	liver and kidney	adipose tissue	pancreas	intestine	liver and kidney
Synthesis of glutamine is accompanied by the hydrolysis of _____.	ATP	ADP	TPP	creatinine phosphate	ATP
The biosynthesis of urea occurs mainly in the liver_____.	cytosol	mitochondria	microsomes	nuclei	mitochondria

One molecule of urea is synthesized at the expense of _____ number of ATP.	1	2	4	3	3
The symptom of ammonia intoxication includes _____.	blurring of vision	constipation	mental confusion	diarrhea	blurring of vision
The pH at which the aminoacid has no net charge and does not move in the electric field is called as _____.	Isobarric	piezoelectric	isoelectric	isothermic	isoelectric
The only ketogenic amino acid is _____.	leucine	isoleucine	alanine	glycine	leucine
In severe acidosis, the output of urea is_____	Decreased	Slightly decreased	Highly increased	Moderately increased	Decreased
Glutathione is a _____.	dipeptide	tripeptide	polypeptide	pentapeptide	tripeptide
The transaminase activity requires the coenzyme _____.	ATP	B6-PO_4	NAD+	FAD+	B6-PO_4
Polymers of more than 100 aminoacids are termed as _____	Proteins	Polypeptides	Aminoacids	Glucoprotein	Proteins
The example of Phosphoprotein is _____	Mucin	Ovovitellin	Ovomucoid	Tendomucoid	Ovovitellin
Each hydrogen bond is quite_____	Strong	Weak	Non of the above	Both of the above	Weak
Glutamic dehydrigenase is a _____	Monomer	Tetramer	Dimer	Polymer	Tetramer
Aldolase molecule is a _____	Monomer	Tetramer	Trimer	Dimer	Trimer
Foetal haemoglobin contains _____	Two α and two γ chains	Two α and two β chains	Two α and two α chains	Two β and two β chains	Two α and two γ chains
Both valine and isoleucine on catabolism produce_____	Alanine	Succinyl-CoA	Methionine	Valine	Succinyl-CoA
By overheating, the nutritional value of cereal protein is _____	Increased	Lowered	Unchanged	Changed	Lowered
Transamination is a _____ process.	Irreversible	Reversible	Inhibition	a & c	Reversible
Most aminoacid are substrates for transamination except _____	Alanine	Threonine	Serine	Valine	Threonine
In brain, the mechanism for the removal of ammonia is the formation of _____	Glutamate	Aspartate	Asparagine	Glutamine	Glutamine
The Competitive inhibitor of arginine is _____	Citrulline	Malate	Lysine	Serine	Lysine
Uremia occurs in _____	Cirrhosis	Nephritis	Diabetes	Thrombosis	Nephritis
Cysteine is formed from _____	Serine	Valine	Glutamine	Methionine	Methionine
_____on metabilism yields acetoacetate and acetyl -CoA	Serine	Leucine	Valine	Glutamine	Leucine
_____ mg of tryptophan produce I mg of niacin.	20	40	80	60	60
UNIT-5	opt 1	opt 2	opt 3	opt 4	
_____ highly concentrated in muscle and brain tissues	Carnosine	Ornithine	Ergothionine	Kynurenine	Carnosine
_____ test is positive for the aminoacid Cysteine.	Millon	Ninhydrin	Nitroprusside	Catalase	Nitroprusside
The Protein act as the defense against infection by _____	Protein antigen	Protein antibodies	Amyloprotein	Glucoprotein	Protein antibodies
The first incoming NTP binds _____ at the start point of initiation	DNApolymerase I	polymerases	DNA polymerase	RNA polymerase	RNA polymerase
The word enzyme is derived from the Greek meaning in _____	Bacteria	Microbes	Yeast	Fungi	Yeast
The term enzyme was first used by _____ in 1878.	Watson	Alan Ferst	Kuhne	Stevens	Kuhne
Non-protein chemical compound that is required for the protein's biological activity_____	Coenzyme	Cofactor	Isomerases	Synthetases	Cofactor
The 40S subunit contains 18S rRNA and about about_____ polypeptide chains.	10	20	40	30	30
The reactions in which two molecules are joined at the expense of an energy source are catalyzed by _____	Ligases	Isomerases	Transferases	Hydrolases	Ligases
Michaelis-Menton equation is _____	$\nu = Vmax [S]/[S] + Km$	$\nu = [S]+Km/Vmax [S]$	$\nu = Vmax [S]+Km/[S]$	$\nu = Km/Vmax [S]+[S]$	$\nu = Vmax [S]/[S] + Km$
Retinal is reduced to retinol by retinene reductase in presence of the coenzyme_____	NAD+	NADP+	NADH+H+	NADPH+H+	NADH+H+
The unit of genetic information is the_____ or cistron.	Genom	Codon	Gene	Anticodon	Gene
Chromatin consists of a long double stranded _____molecules	RNA	DNA	Subunit	tDNA	DNA
The mammalian ribosome contains the number of major nucleoprotein subunits.	1	2	4	5	2
Each transfer RNA molecule contains the number of nucleotides _____	J.B.Sumner	Koshland	Menten	Fisher	J.B.Sumner
Gene is a segment of the DNA molecule containing base pairs about	300	400	500	600	600

The sequences recognized by RNA polymerase are called	Terminator	Promoter	Both of the above	Non of the above	Promoter
Optimum temperature for an enzyme-catalyzed reaction is_____	30°C-40°C	25°C-40°C	35°C-45°C	85°C-90°C	25°C-40°C
DNA is refered as _____	Transforming factor	Range constants	Transplantation factor	Heterogenous factor	Transforming factor
Messenger RNA has a molecuar weight of_____	15000 to 30000	20000 to 35000	25000 to 40000	30000 to 50000	30000 to 50000
The 60S subunit contains 5s rRNA a 5.8S;rRNA and a_____	18S	30S	28S	40S	28S
DNA is denatured by _____	Salt	Water	Heat	Cold	Heat
A chemical bond formed between two molecules when the carboxyl group of one molecule reacts with the amino group of the other molecule, releasing a molecule of water (H2O)	Hydrophobic interaction	Hydrophilic interaction	Disulphide bonds	Peptide bonds	Peptide bonds
cGMP is antagonistic to _____	cAMP	CTP	ATP	PHB	cAMP
Michaelis-Menten model describes _____	Enzyme stability	Enzyme specificity	Enzyme kinetics	Enzyme degradation	Enzyme kinetics
cGMP is formed from _____by the enzyme adenyl cyclase	ATP	GDP	CTP	CDP	ATP
An example for a semipermeable membrane is _____	cytosol	Cell wall	Dialysis membrane	Biofilm	Dialysis membrane
_____ a procedure to remove waste products and excess fluid from the blood when the kidneys stop working properly	Osmosis	Reverse osmosis	Electrophoresis	Dialysis	Dialysis
_____is a functional group consisting of the formula RN=C=NR.	Gluteraldehyde	Carbodiimides	Carbondioxide	Glycoside	Carbodiimides
The lactam form is the predominant tautomer of	Uracil	Cytosine	Adenine	Xanthine	Uracil
The chemical name 2-amino-6-oxypurine is said to be_____	Adenine	Xanthine	Guanine	Hypoxinthine	Guanine
All catalysts are enzymes, but not all enzymes are catalysts.	TRUE	FALSE	Non of the above	both of the above	TRUE
_____ is an important molecule in metabolism, used in many biochemical reactions	Pyruvate	Carboxide	Acetyl Co A	Acetamide	Acetyl Co A
An important antioxidant in plants, animals, fungi, and some bacteria and archaea, preventing damage to important cellular components caused by reactive oxygen species such as free radicals, peroxides, lipid peroxides and heavy metals_____	Gluteraldehyde	Glutamate	Glycerol	Glutathione	Glutathione
A dicarboxylic acid with structure $CH_2(COOH)_2$_____	Succinic acid	Malonic acid	Pyruvic acid	Formic acid	Malonic acid
In Vmax, [Et] denotes _____	Enzyme at time t	Total Enzyme	Substrate	Product	Total Enzyme
Lyases belongs to _____ class in the major classes of Enzymes.	3^{rd}	2^{nd}	5^{th}	4^{th}	4^{th}
The _____ is a molecule upon which an enzyme acts	Substrate utilized	Substrate involved	Substrate oxidized	Substrate recovered	Substrate oxidized
The initiation of DNA synthesis requires priming by a short length of	RNA	DNA	Hydroxyl group	Alkyl group	RNA
International system of units is _____	SI	Anson	Katal	Newton	SI
A biochemically active compound formed by the combination of an enzyme with a oenzyme_____	Apoenzyme	Isoenzyme	Holoenzyme	Heyteroenzyme	Holoenzyme
Enzymes can be precipitated by _____	Ammonium Sulphate	Ammonium Oxalate	Ammonium Chloride	Ammonium oxide	Ammonium Sulphate
_____ is the inorganic chemical component that is required for enzyme activity	Coenzyme	Protein	Aminoacids	Cofactor	Cofactor
In _____ the international union of Biochemistrty gave the classification and naming system of enzymes on the basis of overall reaction catalysed.	1923	1961	1941	1963	1961
An active group of cysteine is _____	Alcoholic	Imidazole	Sulfhydryl	Phenolic	Sulfhydryl
Induced Fit Mechanism was proposed by _____	Fisher	Michael	Kunhe	Koshland	Koshland
The Substrate is specific towards _____ of the enzyme.	Active site	Allosteric group	Hydroxyl group	Inactive group	Active site
For entrapping enzymes instead of cellulose acetate fibres _____ is used	Calcium Chloride	Calcium oxalate	Calcium alginate	Cellulose oxide	Calcium alginate
DNA gyrases act to relieve the stress generated by_____	Gyrases	Helicases	Polymerases	Esterase	Helicases
The enzyme involved in hydrolysis is _____	Reductases	Lyases	Ligases	Hydrolases	Hydrolases
IUPAC is _____	International	International	Indian Unit of	Indian Union	International

	Unit of Pure and Applied Chemistry	Union of Pure and Applied Chemistry	Pure and Applied Chemistry	of Pure and Applied Chemistry	Union of Pure and Applied Chemistry
Fifth class enzyme is _____	Oxidoreductase	Lyases	Hydrolases	Isomerases	Lyases
Last digit number of E.C.Number represents the _____ of enzyme within the subsub class	Register number	Code number	Serial number	Account number	Serial number
Systematic code number is otherwise known as _____	Enzyme cofactor number	Enzyme coenzyme number	Enzyme coordinate number	Enzyme Commission number	Enzyme Commission number
An example for yeast enzymes having E.C.number 3.2.1.23 is _____	Invertase	Raffinase	Lactase	Lipase	Lactase
E.C.number of α-amylase is _____	3.2.1.1	3.2.1.3	1.1.3.4	3.2.1.2	3.2.1.1
β-amylase is an _____ enzyme.	intracellular	toxic	heterogenous	extracellular	extracellular
An example for intracellular enzyme is _____	Pectinase	Aminoacylase	Lipase	Papain	Aminoacylase
E.C number of Raffinase is _____	3.2.1.23	3.2.1.22	3.2.1.15	3.2.1.1	3.2.1.22
An example for extracellular enzyme is _____	Aminoacylase	Lipase	Raffinase	Catalase	Lipase
E.C.number of α-amylase is _____	3.2.1.1	3.2.1.3	1.1.3.4	3.2.1.2	3.2.1.1
An example for a animal enzyme is _____	Rennet	α-amylase	Pullulanase	Raffinase	Rennet
An example for a plant enzyme is _____	Rennet	Lipoxygenase	Lipase	Pullulanase	Lipoxygenase
An example for a yeast enzyme is _____	Rennet	Lipase	Lactase	Raffinase	Lipase