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SCHOOL OF BIO AND CHEMICAL ENGINEERING
DEPARTMENT OF BIOTECHNOLOGY

UNIT – I – BIOCHEMISTRY AND BIOMOLECULES – SBTA1302

CHEMISTRY OF BIOMOLECULES

1. Hierarchy of the molecular organization of cells,

Life exhibits varying degrees of organization. Atoms are organized into molecules, molecules into organelles, and organelles into cells, and so on. According to the Cell Theory, all living things are composed of one or more cells, and the functions of a multicellular organism are a consequence of the types of cells it has. Cells fall into two broad groups: prokaryotes and eukaryotes. Prokaryotic cells are smaller (as a general rule) and lack much of the internal compartmentalization and complexity of eukaryotic cells. No matter which type of cell we are considering, all cells have certain features in common, such as a cell membrane, DNA and RNA, cytoplasm, and ribosomes. Eukaryotic cells have a great variety of organelles and structures.

Cell Size and Shape

The shapes of cells are quite varied with some, such as neurons, being longer than they are wide and others, such as parenchyma (a common type of plant cell) and erythrocytes (red blood cells) being equidimensional. Some cells are encased in a rigid wall, which constrains their shape, while others have a flexible cell membrane (and no rigid cell wall).

The size of cells is also related to their functions. Eggs (or to use the latin word, *ova*) are very large, often being the largest cells an organism produces. The large size of many eggs is related to the process of development that occurs after the egg is fertilized, when the contents of the egg (now termed a zygote) are used in a rapid series of cellular divisions, each requiring tremendous amounts of energy that is available in the zygote cells. Later in life the energy must be acquired, but at first a sort of inheritance/trust fund of energy is used.

Cells range in size from small bacteria to large, unfertilized eggs laid by birds and dinosaurs. The realtive size ranges of biological things is shown in Figure 1. In science we use the metric system for measuring. Here are some measurements and convesrions that will aid your understanding of biology.

1 meter = 100 cm = 1,000 mm = 1,000,000 µm = 1,000,000,000 nm

1 centimenter (cm) = 1/100 meter = 10 mm

1 millimeter (mm) = 1/1000 meter = 1/10 cm

1 micrometer (μm) = 1/1,000,000 meter = 1/10,000 cm

1 nanometer (nm) = 1/1,000,000,000 meter = 1/10,000,000 cm

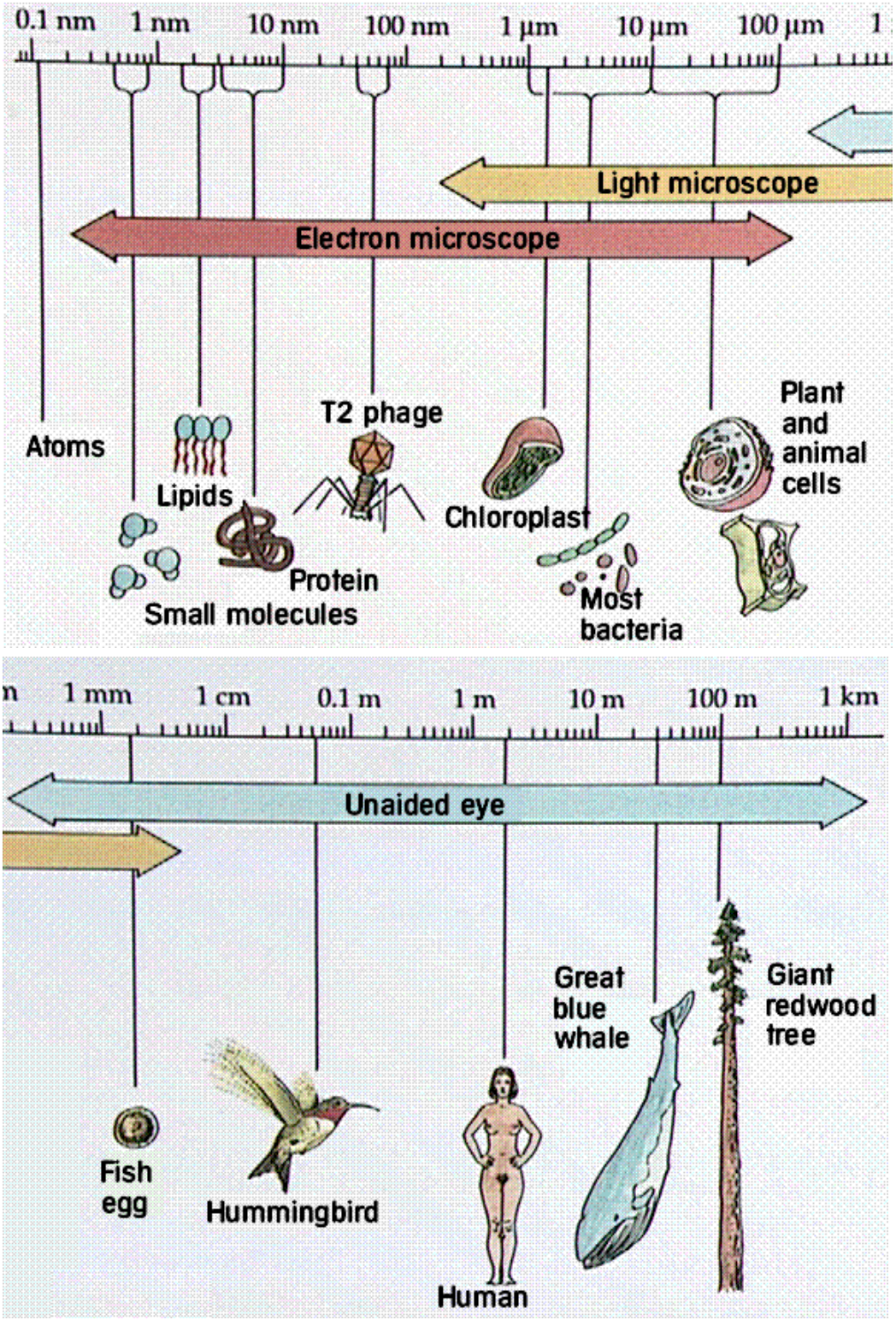


Figure 1. Sizes of viruses, cells, and organisms. Images from Purves et al., Life: The Science of Biology, 4th Edition.

2. THE CELL

The cell is the structural and functional unit of life. It may be also regarded as the basic unit of biological activity. The concept of cell originated from the contributions of Schleiden and Schwann (1838). However, it was only after 1940, the complexities of cell structure were exposed.

Prokaryotic and eukaryotic cells

The cells of the living kingdom may be divided into two categories

1. Prokaryotes (Greek : pro – before; karyon – nucleus) lack a well defined nucleus and possess relatively simple structure. These include the various bacteria.

2. Eukaryotes (Greek : eu – true; karyon – nucleus) possess a well defined nucleus and are more complex in their structure and function. The higher organisms (animals and plants) are composed of eukaryotic cells.

EUKARYOTIC CELL

The human body is composed of about 1014 cells. There are about 250 types of specialized cells in the human body e.g. erythrocytes, nerve cells, muscle cells, E cells of pancreas. An eukaryotic cell is generally 10 to 100 Pm in diameter. A diagrammatic representation of a typical rat liver cell is depicted in Fig.2.

The plant cell differs from an animal cell by possessing a rigid cell wall (mostly composed of cellulose) and chloroplasts. The latter are the sites of photosynthesis.

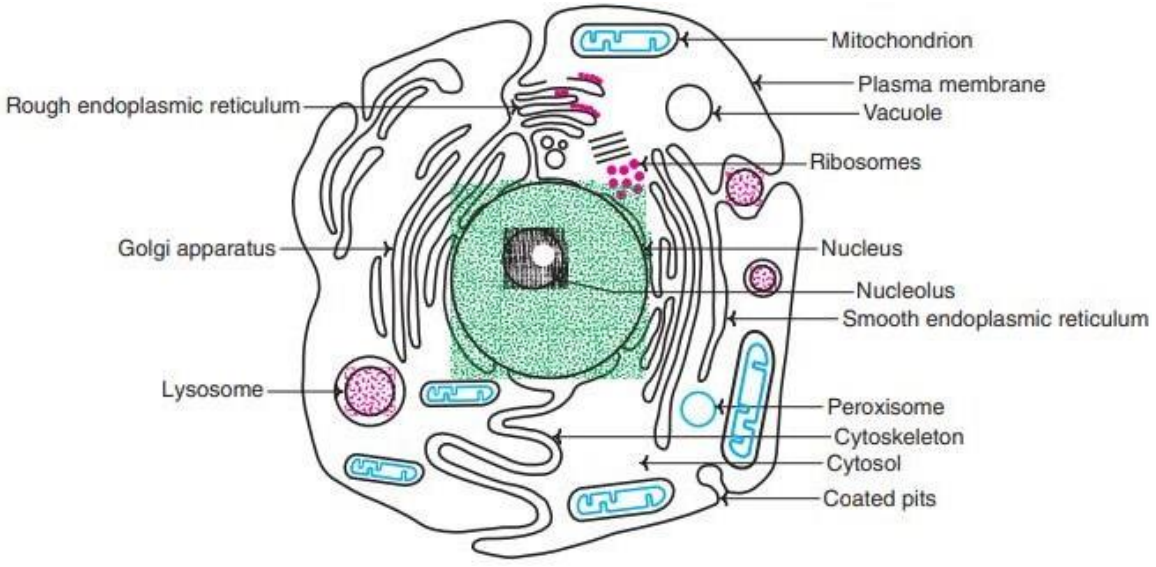


Fig:2 Diagrammatic representation of a rat liver cell.

Nucleus

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

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

The nucleus of the eukaryotic cell contains a dense body known as nucleolus. It is rich in RNA, particularly the ribosomal RNA which enters the cytosol through nuclear pores. The ground material of the nucleus is often referred to as nucleoplasm. It is rich in enzymes such as DNA polymerases and RNA polymerase.

Mitochondria

The mitochondria (Greek: mitos – thread; chondros – granule) are the centres for cellular respiration and energy metabolism. They are regarded as the **powerhouses of the cell** with variable size and shape. Mitochondria are rod-like or filamentous bodies, usually with dimensions of 1.0 u 3 Pm. About 2,000 mitochondria, occupying about 1/5th of the total cell volume, are present in a typical cell.

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

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

eukaryotes.

Endoplasmic reticulum

The network of membrane enclosed spaces that extends throughout the cytoplasm constitutes endoplasmic reticulum (ER). Some of these thread-like structures extend from the nuclear pores to the plasma membrane.

A large portion of the ER is studded with ribosomes to give a granular appearance which is referred to as rough endoplasmic reticulum. Ribosomes are the factories of protein biosynthesis. During the process of cell fractionation, rough ER is disrupted to form small vesicles known as microsomes. It may be noted that microsomes as such do not occur in the cell.The smooth endoplasmic reticulum does not contain ribosomes. It is involved in the synthesis of lipids (triacylglycerols, phospholipids, sterols) and metabolism of drugs, besides supplying Ca^{2+} for the cellular functions.

Golgi apparatus

Eukaryotic cells contain a unique cluster of membrane vesicles known as dictyosomes which, in turn, constitute Golgi apparatus (or Golgi complex). The newly synthesized proteins are handed over to the Golgi apparatus which catalyse the addition of carbohydrates, lipids or sulfate moieties to the proteins. These chemical modifications are necessary for the transport of proteins across the plasma membrane. Certain proteins and enzymes are enclosed in membrane vesicles of Golgi apparatus and secreted from the cell after the appropriate signals. The digestive enzymes of pancreas are produced in this fashion. Golgi apparatus are also involved in the membrane synthesis, particularly for the formation of intracellular organelles (e.g. peroxisomes, lysosomes).

Lysosomes

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

The lysosomal enzymes are responsible for maintaining the cellular compounds in a dynamic state, by their degradation and recycling. The degraded products leave the lysosomes, usually by diffusion, for reutilization by the cell. Sometimes, however, certain residual products, rich in lipids and proteins, collectively known as lipofuscin accumulate in the cell. Lipofuscin is the age pigment or wear and tear pigment which has been implicated in ageing process. As the cell dies, the lysosomes rupture and release hydrolytic enzymes that results in post-morteum autolysis.

The digestive enzymes of cellular compounds are confined to the lysosomes in the best

interest of the cell. Escape of these enzymes into cytosol will destroy the functional macromolecules of the cell and result in many complications. The occurrence of several diseases (e.g. arthritis, muscle diseases, allergic disorders) has been partly attributed to the release of lysosomal enzymes. Inclusion cell (I-cell) desease is a rare condition due to the absence of certain hydrolases in lysosomes. However, these enzyme are syntherized and found in the circulation. I-cell disease is due to a defect in protein targetting, as the enzymes cannot reach lysosomes.

Peroxisomes

Peroxisomes, also known as microbodies, are single membrane cellular organelles. They are spherical or oval in shape and contain the enzyme catalase. Catalase protects the cell from the toxic effects of H_2O_2 by converting it to H_2O and O_2. Peroxisomes are also involved in the oxidation of long chain fatty acids (> C18), and synthesis of plasmalogens and glycolipids. Plants contain glyoxysomes, a specialized type of peroxisomes, which are involved in the glyoxylate pathway.

Peroxisome biogenesis disorders (PBDs), are a group of rare diseases involving the enzyme activities of peroxisomes. The biochemical abnormalities associated with PBDs include increased levels of very long chain fatty acids (C24 and C26) and decreased concentrations of plasmalogens. The most severe form of PBDs is Zellweger syndrome, a condition characterized by the absence of functional peroxisomes. The victims of this disease may die within one year after birth.

Cytosol and cytoskeleton

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

3. Proteins and Amino Acids

Proteins are the most abundant organic molecules of the living system. They occur in every part of the cell and constitute about 50% of the cellular dry weight. Proteins form the fundamental basis of structure and function of life.

Functions of proteins

Proteins perform a great variety of specialized and essential functions in the living cells. These functions may be broadly grouped as *static* (structural) and *dynamic*.

Structural functions : Certain proteins perform brick and mortar roles and are primarily responsible

for structure and strength of the body. These include collagen and elastin found in bone matrix, vascular system and other organs and D-keratin present in epidermal tissues.

Dynamic functions : The dynamic functions of proteins are more diversified in nature. These include proteins acting as enzymes, hormones, blood clotting factors, immunoglobulins, membrane receptors, storage proteins, besides their function in genetic control, muscle contraction, respiration etc. Proteins performing dynamic functions are appropriately regarded as the working horses of the cell

AMINO ACIDS

Amino acids are a group of organic compounds containing two functional groups— amino and carboxyl. The amino group (—NH_2) is basic while the carboxyl group (—COOH) is acidic in nature.

General structure of amino acids

The amino acids are termed as D-amino acids, if both the carboxyl and amino groups are attached to the same carbon atom, as depicted below

The **α**-carbon atom binds to a side chain represented by R which is different for each of the 20 amino acids found in proteins. The amino acids mostly exist in the ionized form in the biological system (shown above).

Optical isomers of amino acids

If a carbon atom is attached to four different groups, it is asymmetric and therefore exhibits optical isomerism. The amino acids (except glycine) possess four distinct groups (R, H, COO–, NH_3^+) held by **α**-carbon. Thus all the amino acids (except glycine where R = H) have optical isomers. The structures of L- and D-amino acids are written based on the configuration of L- and **α**-glyceraldehyde as shown in Fig.3. The proteins are composed of L-**α**-amino acids.

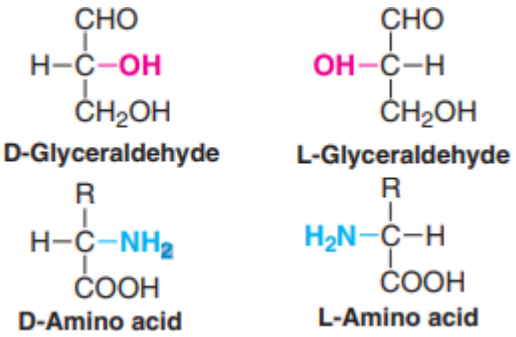
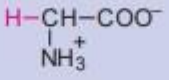
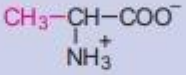
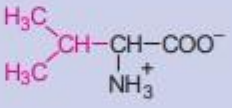
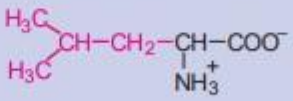
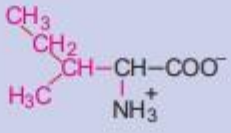
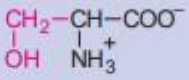
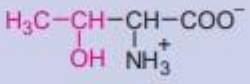
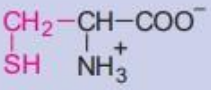
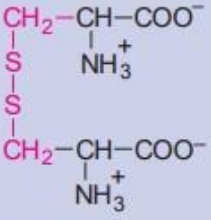
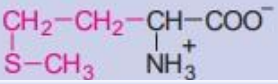
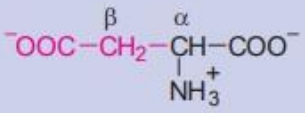
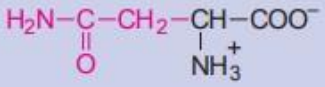
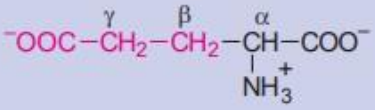
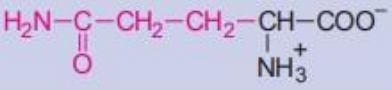
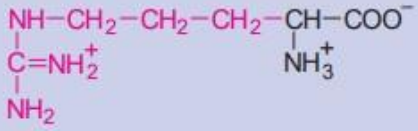
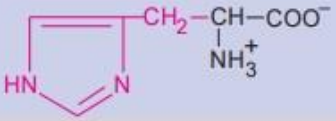
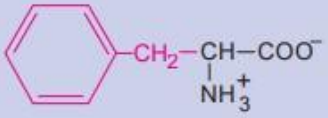
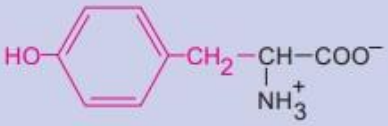
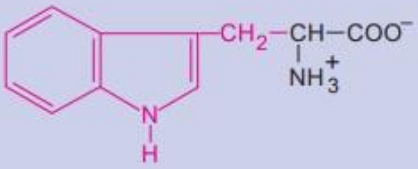
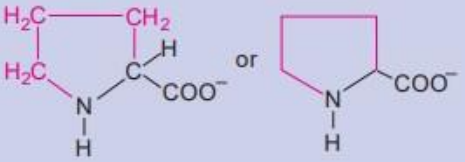


Fig. 3: D- and L-forms of amino acid based on the structure of glyceraldehyde.

Name	Symbol		Structure	Special group present
	3 letters	1 letter		
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
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)*

TABLE1: Structural classification of L-D-amino acids found in proteins.

Classification of amino acids

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. In Table 1, the different groups of amino acids, their symbols and structures are given. The salient features of different groups are described.

1. **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, Ile, Val) contain branched aliphatic side chains, hence they are referred to as branched chain amino acids.

2. **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.
3. **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 importa nt sulfur containing amino acid, is formed by condensation of two molecules of cysteine.
4. **Acidic amino acids and their amides :** Aspartic acid and glutamic acids are dicarboxylic monoamino acids while asparagine and glutamine are their respective amide derivatives. All these four amino acids possess distinct codons for their incorporation into proteins.
5. **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.
6. **Aromatic amino acids :** Phenylalanine, tyrosine and tryptophan (with indole ring) are aromatic amino acids. Besides these, histidine may also be considered under this category.
7. **Imino acids :** Proline containing pyrrolidine ring is a unique amino acid. It has an imino group (NH), instead of an amino group (NH2) found in other amino acids. Therefore, proline is an D-imino acid.

   Heterocyclic amino acids : Histidine, tryptophan and proline.

B. Classification of amino acids based on polarity : Amino acids are classified into 4 groups based on their polarity. Polarity is important for protein structure.

1. **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.
2. **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.
3. **Polar amino acids with positive 'R' group :** The three amino acids lysine, arginine and histidine are included in this group.
4. **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 20 amino acids (Table 1) are required for the synthesis of variety 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.

1. **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 (and also rats).

   Arginine, **V**aline, **H**istidine, **I**soleucine, **L**eucine, **L**ysine, **M**ethionine, **P**henylalanine, **T**hreonine, **T**ryptophan.

   The two amino acids namely arginine and histidine can be synthesized by adults and not by growing children, hence these are considered as semi–essential amino acids (remember Ah, to recall). Thus, 8 amino acids are absolutely essential while 2 are semi-essential.
2. **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, cysteine, aspartate, asparagine, 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 (glycogenic) or fat (ketogenic) or both. From metabolic view point, amino acids are divided into three groups.

1. **Glycogenic amino acids :** These amino acids can serve as precursors for the formation of glucose or glycogen. e.g. alanine, aspartate, glycine, methionine etc.
2. **Ketogenic amino acids** : Fat can be synthesized from these amino acids. Two amino acids leucine and lysine are exclusively ketogenic.
3. **Glycogenic and ketogenic amino acids :** The four amino acids isoleucine, phenylalanine, tryptophan, tyrosine are precursors for synthesis of glucose as well as fat.

Properties of amino acids

The amino acids differ in their physico– chemical properties which ultimately determine the characteristics of proteins.

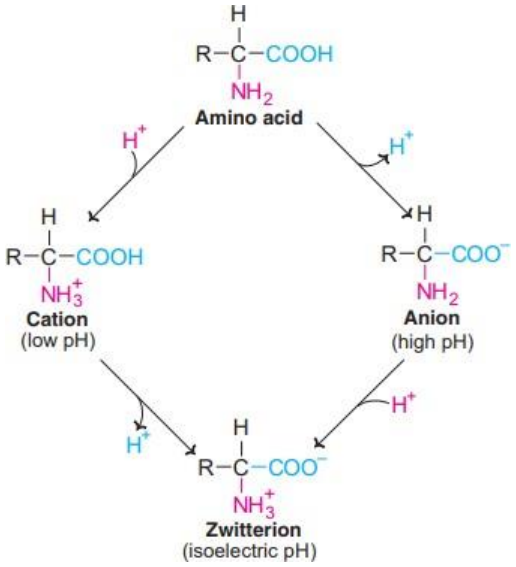
A. Physical properties

1. **Solubility** : Most of the amino acids are usually soluble in water and insoluble in organic solvents.
2. **Melting points :** Amino acids generally melt at higher temperatures, often above 200°C.
3. **Taste :** Amino acids may be sweet (Gly, Ala, Val), tasteless (Leu) or bitter (Arg, Ile). Monosodium glutamate (MSG; ajinomoto) is used as a flavoring agent in food industry, and Chinese foods to increase taste and flavor. In some individuals intolerant to MSG, Chinese restaurant syndrome (brief and reversible flulike symptoms) is observed.

4. **Optical properties :** All the amino acids except glycine possess optical isomers due to the presence of asymmetric carbon atom. Some amino acids also have a second asymmetric carbon e.g. isoleucine, threonine. The structure of L- and D-amino acids in comparison with glyceraldehyde has been give in figure 3.
5. **Amino acids as ampholytes :** Amino acids contain both acidic (COOH) and basic (NH_2) groups. They can donate a proton or accept a proton, hence amino acids are regarded as ampholytes.

Zwitterion or dipolar ion : The name zwitter is derived from the German word which means hybrid. Zwitter ion (or dipolar ion) is a hybrid molecule containing positive and negative ionic groups.

The amino acids rarely exist in a neutral form with free carboxylic (COOH) and free amino (NH2) groups. In strongly acidic pH (low pH), the amino acid is positively charged (cation) while in strongly alkaline pH (high pH), it is negatively charged (anion). Each amino acid has a characteristic pH (e.g. leucine, pH 6.0) at which it carries both positive and negative charges and exists as zwitterion (Fig.4).



Isoelectric pH (symbol pI) is defined as the pH at which a molecule exists as a zwitterion or dipolar ion and carries no net charge. Thus, the molecule is electrically neutral. The pI value can be calculated by taking the average pKa values corresponding to the ionizable groups. For the calculation of pI of amino acids with more than two ionizable groups, the pKas for all the groups have to be taken into account.

Fig:4. Existence of an amino acid as cation, anion and zwitterion.

Peptide bond

The amino acids are held together in a protein by covalent peptide bonds or linkages. These bonds are rather strong and serve as the cementing material between the individual amino acids (considered as bricks).

Formation of a peptide bond : When the amino group of an amino acid combines with the carboxyl group of another amino acid, a peptide bond is formed (Fig.5). Note that a dipeptide will have two amino acids and one peptide (not two) bond. Peptides containing more than 10 amino acids (decapeptide) are referred to as polypeptides. **Characteristics of peptide bonds :** The peptide bond is rigid and planar with partial double

bond in character. It generally exists in trans configuration. Both C O and NH groups of peptide bonds are polar and are involved in hydrogen bond formation.

Writing of peptide structures : Conventionally, the peptide chains are written with the free amino end (N-terminal residue) at the left, and the free carboxyl end (C-terminal residue) at the right. The amino acid sequence is read from N-terminal end to C-terminal end. Incidentally, the protein biosynthesis also starts from the N-terminal amino acid.

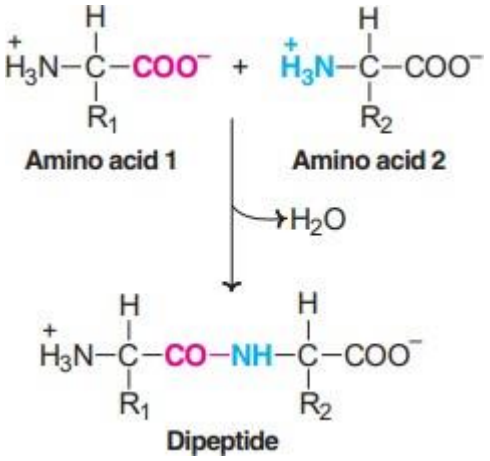


Fig.5 : Formation of a peptide bond.

PROPERTIES OF PROTEINS

1. **Solubility :** Proteins form colloidal solutions instead of true solutions in water. This is due to the huge size of protein molecules.
2. **Molecular weight :** The proteins vary in their molecular weights, which, in turn, is dependent on the number of amino acid residues. Each amino acid on an average contributes to a molecular weight of about 110. Majority of proteins/polypeptides may be composed of 40 to 4,000 amino acids with a molecular weight ranging from 4,000 to 440,000. A few proteins with their molecular weights are listed below :

   Insulin-5,700;
   Myoglobin-17,000;
   Hemoglobin-64,450;
   Serum albumin-69,000.

3. **Shape :** There is a wide variation in the protein shape. It may be globular (insulin), oval (albumin) fibrous or elongated (fibrinogen).
4. **Isoelectric pH :** Isoelectric pH (pI) as a property of amino acids has been described. The nature of the amino acids (particularly their ionizable groups) determines the pI of a protein. The acidic amino acids (Asp, Glu) and basic amino acids (His, Lys, Arg) strongly influence the pI. At isoelectric pH, the proteins exist as zwitterions or dipolar ions. They are electrically neutral (do not migrate in the electric field) with minimum solubility, maximum precipitability and least buffering capacity. The isoelectric pH(pI) for some proteins are given here

   Pepsin-1.1; Casein-4.6; Human albumin-4.7; Urease-5.0; Hemoglobin-6.7; Lysozyme-11.0.
5. **Acidic and basic proteins :** Proteins in which the ratio (H Lys + H Arg)/(H Glu + H Asp) is greater than 1 are referred to as basic proteins. For acidic proteins, the ratio is less than 1
6. **Precipitation of proteins :** Proteins exist in colloidal solution due to hydration of polar groups (COO–, NH3 +, OH). Proteins can be precipitated by dehydration or neutralization of polar groups.

   Precipitation at pI : The proteins in general are least soluble at isoelectric pH. Certain proteins (e.g. casein) get easily precipitated when the pH is adjusted to pI (4.6 for casein). Formation of curd from milk is a marvellous example of slow precipitation of milk protein, casein at pI. This occurs due to the lactic acid produced by fermentation of bacteria which lowers the pH to the pI of casein.

   Precipitation by salting out : The process of protein precipitation by the additional of neutral salts such as ammonium sulfate or sodium sulfate is known as salting out. This phenomenon is explained on the basis of dehydration of protein molecules by salts. This causes increased proteinprotein interaction, resulting in molecular aggregation and precipitation.
7. **Colour reactions of proteins** : The proteins give several colour reactions which are often useful to identify the nature of the amino acids present in them.

Reaction	Specific group or amino acid
1. Biuret reaction	Two peptide linkages
2. Ninhydrin reaction	α-Amino acids
3. Xanthoproteic reaction	Benzene ring of aromatic amino acids (Phe, Tyr, Trp)
4. Milllons reaction	Phenolic group (Tyr)
5. Hopkins-Cole reaction	Indole ring (Trp)
6. Sakaguchi reaction	Guanidino group (Arg)
7. Nitroprusside reaction	Sulfhydryl groups (Cys)
8. Sulfur test	Sulfhydryl groups (Cys)
9. Pauly's test	Imidazole ring (His)
10. Folin–Coicalteau's test	Phenolic groups (Tyr)

TABLE 2: Colour reactions of proteins/amino acids Reaction Specific group or amino acid.

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.

A. Functional classification of proteins

Based on the functions they perform, proteins are classified into the following groups (with examples)

1. **Structural proteins** : Keratin of hair and nails, collagen of bone.
2. **Enzymes or catalytic proteins** : Hexokinase, pepsin.
3. **Transport proteins** : Hemoglobin, serum albumin.
4. **Hormonal proteins :** Insulin, growth hormone.
5. **Contractile proteins** : Actin, myosin.
6. **Storage proteins** : Ovalbumin, glutelin
7. **Genetic proteins** : Nucleoproteins.
8. **Defense proteins** : Snake venoms, Immunoglobulins.
9. **Receptor proteins** for hormones, viruses

B.Protein classification based on chemical nature and solubility

This is a more comprehensive and popular classification of proteins. It is based on the amino acid composition, structure, shape and solubility properties. Proteins are broadly classified into 3 major groups.

1. **Simple proteins** : They are composed of only amino acid residues.
2. **Conjugated proteins** : Besides the amino acids, these proteins contain a non-protein moiety known as prosthetic group or conjugating group.

3. **Derived proteins** : These are the denatured or degraded products of simple and conjugated proteins.

1. Simple proteins

(a) Globular proteins : These are spherical or oval in shape, soluble in water or other solvents and digestible.

(i) Albumins : Soluble in water and dilute salt solutions and coagulated by heat. e.g. serum albumin, ovalbumin (egg), lactalbumin (milk).

(ii) Globulins : Soluble in neutral and dilute salt solutions e.g. serum globulins, vitelline (egg yolk).

(iii) Glutelins : Soluble in dilute acids and alkalies and mostly found in plants e.g. glutelin (wheat), oryzenin (rice).

(iv) Prolamines : Soluble in 70% alcohol e.g. gliadin (wheat), zein (maize).

(v) Histones : Strongly basic proteins, soluble in water and dilute acids but insoluble in dilute ammonium hydroxide e.g. thymus histones.

(vi) Globins : These are generally considered along with histones. However, globins are not basic proteins and are not precipitated by NH4OH.

(vii) Protamines : They are strongly basic and resemble histones but smaller in size and soluble in NH4OH. Protamines are also found in association with nucleic acids e.g. sperm proteins.

(viii) Lectins are carbohydrate-binding proteins, and are involved in the interaction between cells and proteins. They help to maintain tissue and organ structures. In the laboratory, lectins are useful for the purification of carbohydrates by affinity chromatography e.g. concanavalin A, agglutinin.

(b) Fibrous proteins : These are fiber-like in shape, insoluble in water and resistant to digestion. Albuminoids or scleroproteins are a predominant group of fibrous proteins.

(i) Collagens are connective tissue proteins lacking tryptophan. Collagens, on boiling with water or dilute acids, yield gelatin which is soluble and digestible.

(ii) Elastins : These proteins are found in elastic tissues such as tendons and arteries.

(iii) Keratins : These are present in exoskeletal structures e.g. hair, nails, horns. Human hair keratin contains as much as 14% cysteine.

2. Conjugated proteins

(a) Nucleoproteins : Nucleic acid (DNA or RNA) is the prosthetic group e.g. nucleohistones, nucleoprotamines.

(b) Glycoproteins : The prosthetic group is carbohydrate, which is less than 4% of protein. The term mucoprotein is used if the carbohydrate content is more than 4%. e.g. mucin (saliva), ovomucoid (egg white).

(c) Lipoproteins : Protein found in combination with lipids as the prosthetic group e.g. serum

lipoproteins.

(d) Phosphoproteins : Phosphoric acid is the prosthetic group e.g. casein (milk), vitelline (egg yolk).

(e) Chromoproteins : The prosthetic group is coloured in nature e.g. hemoglobins, cytochromes.

(f) Metalloproteins : These proteins contain metal ions such as Fe, Co, Zn, Cu, Mg etc., e.g. ceruloplasmin (Cu), carbonic anhydrase (Zn).

3. Derived proteins : The derived proteins are of two types. The primary derived are the denatured or coagulated or first hydrolysed products of proteins. The secondary derivatives are the degraded (due to breakdown of peptide bonds) products of proteins.

(a) Primary derived proteins

(i) Coagulated proteins : These are the denatured proteins produced by agents such as heat, acids, alkalies etc. e.g. cooked proteins, coagulated albumin (egg white).

(ii) Proteans : These are the earliest products of protein hydrolysis by enzymes, dilute acids, alkalies etc. which are insoluble in water. e.g. fibrin formed from fibrinogen.

(iii) Metaproteins : These are the second stage products of protein hydrolysis obtained by treatment with slightly stronger acids and alkalies e.g. acid and alkali metaproteins.

(b) Secondary derived proteins : These are the progressive hydrolytic products of protein hydrolysis. These include proteoses, peptones, polypeptides and peptides.

4. CARBOHYDRATES

Carbohydrates are the most abundant organic molecules in nature. They are primarily composed of the elements carbon, hydrogen and oxygen. The name carbohydrate literally means ‘hydrates of carbon’. Some of the carbohydrates possess the empirical formula $(C.H_2O)n$ where $n \leq 3$, satisfying that these carbohydrates are in fact carbon hydrates. However, there are several non-carbohydrate compounds (e.g. acetic acid, C2H4O2; lactic acid, $C_3H_6O_3$) which also appear as hydrates of carbon. Further, some of the genuine carbohydrates (e.g. rhamnohexose, $C_6H_{12}O_5$; deoxyribose, $C_5H_{10}O_4$) do not satisfy the general formula. Hence carbohydrates cannot be always considered as hydrates of carbon.

Carbohydrates may be defined as polyhydroxy aldehydes or ketones or compounds which produce them on hydrolysis. The term ‘sugar’ is applied to carbohydrates soluble in water and sweet to taste.

Structure of Carbohydrates

- Carbohydrates consist of carbon, hydrogen, and oxygen.
- The general empirical structure for carbohydrates is $(CH_2O)n$.
- They are organic compounds organized in the form of aldehydes or ketones with multiple hydroxyl groups coming off the carbon chain.

- The building blocks of all carbohydrates are simple sugars called monosaccharides.
- A monosaccharide can be a polyhydroxy aldehyde (aldose) or a polyhydroxy ketone (ketose).

The carbohydrates can be structurally represented in any of the three forms:

- Open chain structure.
- Hemi-acetal structure.
- Haworth structure.

Open chain structure – It is the long straight-chain form of carbohydrates.
Hemi-acetal structure – Here the 1st carbon of the glucose condenses with the -OH group of the 5th carbon to form a ring structure.

Haworth structure – It is the presence of the pyranose ring structure.

Properties of Carbohydrates

Physical Properties of Carbohydrates

- **Stereoisomerism –** Compound shaving the same structural formula but they differ in spatial configuration. Example: Glucose has two isomers with respect to the penultimate carbon atom. They are D-glucose and L-glucose.
- **Optical Activity** – It is the rotation of plane-polarized light forming (+) glucose and (-) glucose.
- **Diastereo isomers** – It the configurational changes with regard to C2, C3, or C4 in glucose. Example: Mannose, galactose.
- **Annomerism** – It is the spatial configuration with respect to the first carbon atom in aldoses and second carbon atom in ketoses.

Chemical Properties of Carbohydrates

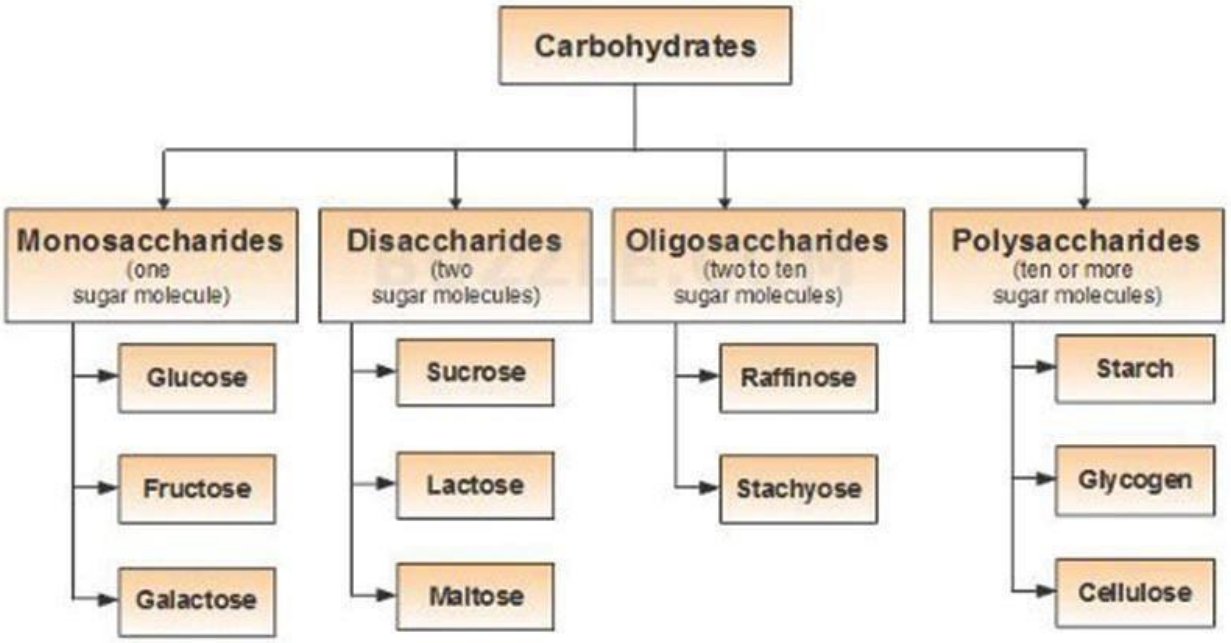
- **Osazone formation:** Osazone are carbohydrate derivatives when sugars are reacted with an excess of phenylhydrazine. eg. Glucosazone.
- **Benedict's test:** Reducing sugars when heated in the presence of an alkali gets converted to a powerful reducing species known as enediols. When Benedict's reagent solution and reducing sugars are heated together, the solution changes its color to orange-red/ brick red.
- **Oxidation:** Monosaccharides are reducing sugars if their carbonyl groups oxidize to give carboxylic acids. In Benedict's test, D-glucose is oxidized to D-gluconic acid thus, glucose is considered a reducing sugar.

- **Reduction to alcohols:** The C=O groups in open-chain forms of carbohydrates can be reduced to alcohols by sodium borohydride, NaBH4, or catalytic hydrogenation (H2, Ni, EtOH/H2O). The products are known as “alditols”.

Properties of Monosaccharides

- Most monosaccharides have a sweet taste (fructose is sweetest; 73% sweeter than sucrose).
- They are solids at room temperature.
- They are extremely soluble in water: – Despite their high molecular weights, the presence of large numbers of OH groups make the monosaccharides much more water-soluble than most molecules of similar MW.
- Glucose can dissolve in minute amounts of water to make a syrup (1 g / 1 ml H_2O).

Classification of Carbohydrates (Types of Carbohydrates)



The simple carbohydrates include single sugars (monosaccharides) and polymers, oligosaccharides, and polysaccharides.

Monosaccharides

- Simplest group of carbohydrates and often called simple sugars since they cannot be further hydrolyzed.
- Colorless, crystalline solid which are soluble in water and insoluble in a nonpolar solvent.

- These are compounds which possess a free aldehyde or ketone group.
- The general formula is Cn(H2O)nor CnH2nOn.
- They are classified according to the number of carbon atoms they contain and also on the basis of the functional group present.
- The monosaccharides thus with 3,4,5,6,7… carbons are called trioses, tetroses, pentoses, hexoses, heptoses, etc., and also as aldoses or ketoses depending upon whether they contain aldehyde or ketone group.
- Examples: Glucose, Fructose, Erythrulose, Ribulose.

Oligosaccharides

- Oligosaccharides are compound sugars that yield 2 to 10 molecules of the same or different monosaccharides on hydrolysis.
- The monosaccharide units are joined by glycosidic linkage.
- Based on the number of monosaccharide units, it is further classified as disaccharide, trisaccharide, tetrasaccharide etc.
- Oligosaccharides yielding 2 molecules of monosaccharides on hydrolysis is known as a disaccharide, and the ones yielding 3 or 4 monosaccharides are known as trisaccharides and tetrasaccharides respectively and so on.
- The general formula of disaccharides is $Cn(H_2O)n-1$and that of trisaccharides is $Cn(H_2O)n-2$ and so on.
- Examples: Disaccharides include sucrose, lactose, maltose, etc.
- Trisaccharides are Raffinose, Rabinose.

Polysaccharides

- They are also called “glycans”.
- Polysaccharides contain more than 10 monosaccharide units and can be hundreds of sugar units in length.
- They yield more than 10 molecules of monosaccharides on hydrolysis.
- Polysaccharides differ from each other in the identity of their recurring monosaccharide units, in the length of their chains, in the types of bond linking units and in the degree of branching.
- They are primarily concerned with two important functions ie. Structural functions and the storage of energy.
- They are further classified depending on the type of molecules produced as a result of hydrolysis.

- They may be homopolysaccharides, containing monosaccharides of the same type or heteropolysaccharides i.e., monosaccharides of different types.
- Examples of Homopolysaccharides are starch, glycogen, cellulose, pectin.
- Heteropolysaccharides are Hyaluronic acid, Chondroitin.

Functions

Carbohydrates are widely distributed molecules in plant and animal tissues. In plants and arthropods, carbohydrates from the skeletal structures, they also serve as food reserves in plants and animals. They are important energy sources required for various metabolic activities, the energy is derived by oxidation.

Some of their major functions include:

Living organisms use carbohydrates as accessible energy to fuel cellular reactions. They are the most abundant dietary source of energy (4kcal/gram) for all living beings.

- Carbohydrates along with being the chief energy source, in many animals, are instant sources of energy. Glucose is broken down by glycolysis/ Kreb's cycle to yield ATP.
- Serve as energy stores, fuels, and metabolic intermediates. It is stored as glycogen in animals and starch in plants.
- Stored carbohydrates act as an energy source instead of proteins.
- They form structural and protective components, like in the cell wall of plants and microorganisms. Structural elements in the cell walls of bacteria (peptidoglycan or murein), plants (cellulose) and animals (chitin).
- Carbohydrates are intermediates in the biosynthesis of fats and proteins.
- Carbohydrates aid in the regulation of nerve tissue and is the energy source for the brain.
- Carbohydrates get associated with lipids and proteins to form surface antigens, receptor molecules, vitamins, and antibiotics.
- Formation of the structural framework of RNA and DNA (ribonucleic acid and deoxyribonucleic acid).
- They are linked to many proteins and lipids. Such linked carbohydrates are important in cell-cell communication and in interactions between cells and other elements in the cellular environment.
- In animals, they are an important constituent of connective tissues.
- Carbohydrates that are rich in fiber content help to prevent constipation.
- Also, they help in the modulation of the immune system.

5. LIPIDS

Lipids (Greek: lipos–fat) are of great importance to the body as the chief concentrated storage form of energy, besides their role in cellular structure and various other biochemical functions. As such, lipids are a heterogeneous group of compounds and, therefore, it is difficult to define them precisely. Lipids may be regarded as organic substances relatively insoluble in water, soluble in organic solvents (alcohol, ether etc.), actually or potentially related to fatty acids and utilized by the living cells. Unlike the polysaccharides, proteins and nucleic acids, lipids are not polymers. Further, lipids are mostly small molecules.

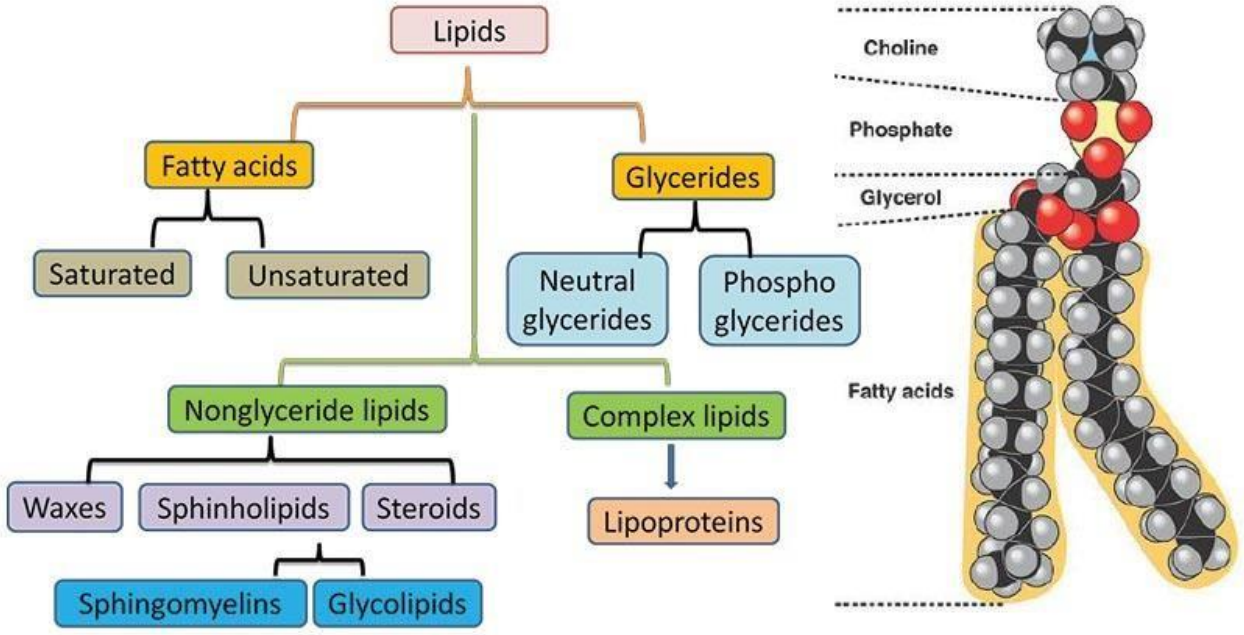
Structure of Lipids

- Lipids are made of the elements Carbon, Hydrogen and Oxygen, but have a much lower proportion of water than other molecules such as carbohydrates.
- Unlike polysaccharides and proteins, lipids are not polymers—they lack a repea•ting monomeric unit.
- They are made from two molecules: Glycerol and Fatty Acids.
- A glycerol molecule is made up of three carbon atoms with a hydroxyl group attached to it and hydrogen atoms occupying the remaining positions.
- Fatty acids consist of an acid group at one end of the molecule and a hydrocarbon chain, which is usually denoted by the letter 'R'.
- They may be saturated or unsaturated.
- A fatty acid is saturated if every possible bond is made with a Hydrogen atom, such that there exist no C=C bonds.
- Unsaturated fatty acids, on the other hand, do contain C=C bonds. Monounsaturated fatty acids have one C=C bond, and polyunsaturated have more than one C=C bond.

Structure of Triglycerides

- Triglycerides are lipids consisting of one glycerol molecule bonded with three fatty acid molecules.
- The bonds between the molecules are covalent and are called Ester bonds.
- They are formed during a condensation reaction.
- The charges are evenly distributed around the molecule so hydrogen bonds do not form with water molecules making them insoluble in water.

Classification (Types) of Lipids



Lipids are broadly classified (modified from Bloor) into simple, complex, derived and miscellaneous lipids, which are further subdivided into different groups

1. Simple lipids : Esters of fatty acids with alcohols. These are mainly of two types

A. **Fats and oils (triacylglycerols)** : These are esters of fatty acids with glycerol. The difference between fat and oil is only physical. Thus, oil is a liquid while fat is a solid at room temperature.

B. **Waxes :** Esters of fatty acids (usually long chain) with alcohols other than glycerol. These alcohols may be aliphatic or alicyclic. Cetyl alcohol is most commonly found in waxes. Waxes are used in the preparation of candles, lubricants, cosmetics, ointments, polishes etc.

2. Complex (or compound) lipids : These are esters of fatty acids with alcohols containing additional groups such as phosphate, nitrogenous base, carbohydrate, protein etc. They are further divided as follows.

A. **Phospholipids :** They contain phosphoric acid and frequently a nitrogenous base. This is in addition to alcohol and fatty acids.

(i) Glycerophospholipids : These phospholipids contain glycerol as the alcohol e.g., lecithin, cephalin.

(ii) Sphingophospholipids : Sphingosine is the alcohol in this group of phospholipids e.g., sphingomyelin.

B. **Glycolipids :** These lipids contain a fatty acid, carbohydrate and nitrogenous base. The alcohol is sphingosine, hence they are also called glycosphingolipids. Glycerol and phosphate are absent e.g cerebrosides, gangliosides.
C. **Lipoproteins :** Macromolecular complexes of lipids with proteins.
D. **Other complex lipids :** Sulfolipids, aminolipids and lipopolysaccharides are among the other complex lipids.

3.Derived lipids : These are the derivatives obtained on the hydrolysis of group 1 and group 2 lipids which possess the characteristics of lipids. These include glycerol and other alcohols, fatty acids, mono- and diacylglycerols, lipid (fat) soluble vitamins, steroid hormones, hydrocarbons and ketone bodies.

4. Miscellaneous lipids : These include a large number of compounds possessing the characteristics of lipids e.g., carotenoids, squalene, hydrocarbons such as pentacosane (in bees wax), terpenes etc.

NEUTRAL LIPIDS : The lipids which are uncharged are referred to as neutral lipids. These are mono-, di-, and triacylglycerols, cholesterol and cholesteryl esters.

Functions of lipids

Lipids perform several important functions

1. They are the concentrated fuel reserve of the body (triacylglycerols).
2. Lipids are the constituents of membrane structure and regulate the membrane permeability (phospholipids and cholesterol).
3. They serve as a source of fat soluble vitamins (A, D, E and K).
4. Lipids are important as cellular metabolic regulators (steroid hormones and prostaglandins).
5. Lipids protect the internal organs, serve as insulating materials and give shape and smooth appearance to the body.
6. Act as the structural component of the body and provide the hydrophobic barrier that permits partitioning of the aqueous contents of the cell and subcellular structures.

Lipids are major sources of energy in ani•mals and high lipid-containing seeds.

7. Activators of enzymes eg. glucose-6-phosphatase, stearyl CoA desaturase and ω-monooxygenase, and β-hydroxybutyric dehydrogenase (a mitochondrial enzyme) require phosphatidylcholine micelles for activation.

6. Purines & Pyrimidines

One of the important specialized pathways of a number of amino acids is the synthesis of purine and pyrimidine nucleotides. These nucleotides are important for a number of reasons. Most of them, not just ATP, are the sources of energy that drive most of our reactions. ATP is the most commonly used source but GTP is used in protein synthesis as well as a few other reactions. UTP is the source of energy for activating glucose and galactose. CTP is an energy source in lipid metabolism. AMP is part of the structure of some of the coenzymes like NAD and Coenzyme A. And, of course, the nucleotides are part of nucleic acids. Neither the bases nor the nucleotides are required dietary components. (Another perspective on this.) We can both synthesize them de novo and salvage and reuse those we already have.

The nitrogenous bases found in nucleotides (and, therefore, nucleic acids) are aromatic heterocyclic compounds. The bases are of two types—purines and pyrimidines. Their general structures are depicted in Fig.6. Purines are numbered in the anticlockwise direction while pyrimidines are numbered in the clockwise direction. And this is an internationally accepted system to represent the structure of bases.

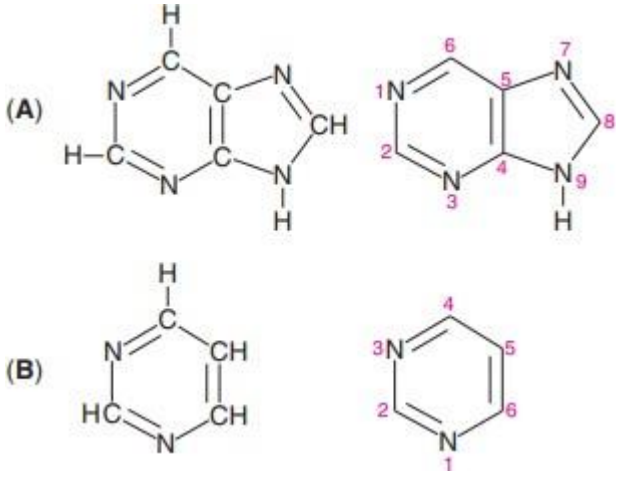


Fig. 6 : General structure of nitrogen bases (A) Purine (B) Pyrimidine (The positions are numbered according to the international system).

Major bases in nucleic acids

The structures of major purines and pyrimidines found in nucleic acids are shown in Fig.7. DNA and RNA contain the same purines namely adenine (A) and guanine (G). Further, the pyrimidine cytosine (C) is found in both DNA and RNA. However, the nucleic acids differ with respect to the second pyrimidine base. DNA contains thymine (T) whereas RNA contains uracil (U). As is observed in the Fig.6, thymine and uracil differ in structure by the presence (in T) or absence (in U) of a methyl group.

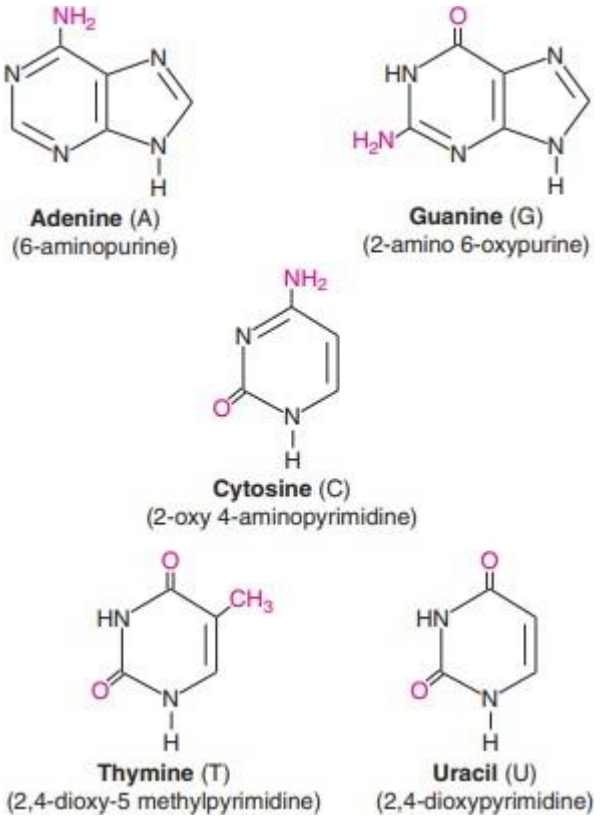
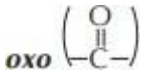


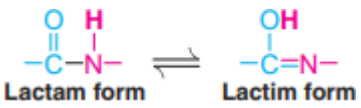
Fig.7: Structures of major purines (A, G) and pyrimidines (C, T, U) found in nucleic acids.

Tautomeric forms of purines and pyrimidines

The existence of a molecule in a keto (lactam) and enol (lactim) form is known as tautomerism. The heterocyclic rings of purines and pyrimidines



functional groups exhibit tautomerism as simplified below.



The purine—guanine and pyrimidinescytosine, thymine and uracil exhibit tautomerism. The lactam and lactim forms of cytosine are represented in Fig:8

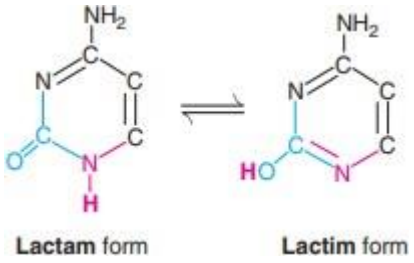


Fig. 8 : The tautomeric forms of cytosine.

At physiological pH, the lactam (keto) tautomeric forms are predominantly present

Minor bases found in nucleic acids : Besides the bases described above, several minor and unusual bases are often found in DNA and RNA. These include 5-methylcytosine, N^4-acetylcytosine, N^6-methyladenine, N^6, N^6-dimethyladenine, pseudouracil etc. It is believed that the unusual bases in nucleic acids will help in the recognition of specific enzymes.

Other biologically important bases : The bases such as hypoxanthine, xanthine and uric acid (Fig.9) are present in the free state in the cells. The former two are the intermediates in purine synthesis while uric acid is the end product of purine degradation.

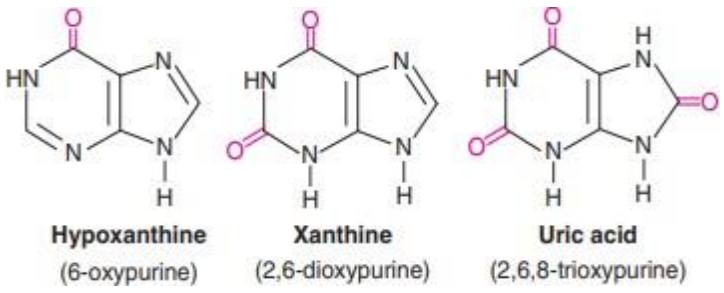


Fig. 9: Structures of some biologically important purines.

Purine bases of plants : Plants contain certain methylated purines which are of pharmacological interest. These include caffeine (of coffee), theophylline (of tea) and theobromine (of cocoa).

7. VITAMINS

Vitamins are a group of organic compounds that are extremely necessary and essential for normal growth and functioning of the human body. They are required in very small quantities but cannot be synthesized by the body itself, and can only be sourced from outside.

The organic compounds needed for the function of normal metabolic processes and for the growth and health of human beings and animals. These compounds, though required in very small amounts, are very vital to the life process and are termed as Vitamins.

Vitamins may be defined as a group of biomolecules (except fats, carbohydrates and proteins) most of which cannot be produced by the body and must be supplied in small amounts in diet to perform the specific biological function for the life, growth and health of human beings and animal organisms.

Example: Vitamin A, Vitamin K, Vitamin B etc.

Vitamins are a group of chemically diverse organic compounds that an organism requires for normal metabolism. Apart from a few exceptions (e.g., vitamin D), the human body cannot synthesize vitamins on its own in sufficient amounts and must, therefore, ensure a steady supply through the diet. Vitamins are micronutrients that do not provide energy (like macronutrients) but instead have very specific biochemical roles. They can be coenzymes in various reactions (B vitamins, vitamins A and K) and/or antioxidants that protect the cell and its membrane from free radicals (vitamins C and E). They can also enable cell signaling (vitamin A) and gene transcription (vitamins A and E) or function as hormones (e.g., vitamin D). Vitamins are classified into fat-soluble vitamins, which the body can store, and water-soluble vitamins, which, with the exception of vitamins B_9 (folate) and B_{12} (cobalamin), the body cannot store over significant periods of time and, therefore, require continuous intake. A balanced diet typically supplies the body with all vitamins it requires. Deficiencies occur mainly due to malnutrition, malabsorption disorders, or restrictive diets (e.g., vitamin B_{12} deficiency in a vegan diet).

Importance of Vitamins

Vitamins plays an important role in keeping good health of human beings. But vitamins neither supply energy nor help in building tissue of the cell but their deficiency in the body can cause serious diseases.

As we know that vitamins cannot be synthesized by our body, therefore, it must be supplied through food. Plants can synthesize all vitamins but animals can synthesize very few vitamins. Some vitamins are present in the nature also, like vitamin D which is either supplied by food or may be produced in the skin by the irradiation of ergosterol with ultraviolet light. Human body can also synthesize some vitamin A from carotenes, some components of vitamin B complex and vitamin K are synthesized by microorganisms present in the intestinal tract.

Sources of Vitamins

The main sources of vitamins are our food which consists of milk, butter, green vegetables, meat, eggs etc. Vitamins are also synthesized in the laboratory and are available in the form of tablets, capsules, which can be taken orally or as with injection as prescribed in the case of vitamin deficiency.

Classification of Vitamins

Vitamins are complex organic molecules. There are about 25 vitamins known till today. They are broadly classified into the following two categories: Water soluble and Fat Soluble

1. Water Soluble Vitamins: These vitamins are water soluble. These vitamins must be supplied regularly in diet because they are regularly excreted in urine and cannot be stored in our body.

Some important water soluble vitamins and their characteristics, sources and their deficiency diseases are given below:

1. **Vitamin B1:** Its chemical name is Thiamine, and commonly called as Aneurin or Antineuritic Vitamin.

   Characteristics: It is insoluble in oils and fats, and can be destroyed by heat above 313 K.

   Source: It is found in pulses, nut, whole cereals, rise polishing, yeast, egg yolk, milk, green vegetables and in fruits.

   Deficiency Diseases: Their deficiencies cause Beriberi disease, in which legs get paralysed and cause loss of appetite.

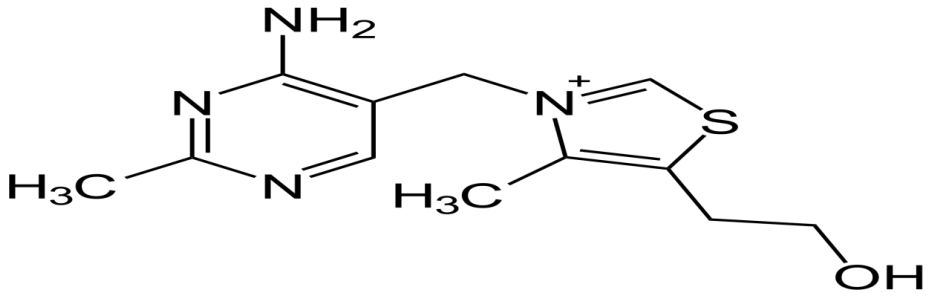


Fig 10. structure of Vitamin B1 (Thiamine)

2. **Vitamin B6:** Its chemical name is as Adermin or Pyridoxine. In fact it is a mixture of pyridoxine, pyridoxal and pyridoxamine.

   Characteristics: It is also insoluble in oil and fats.

Source: It is found in rice bran, yeast, molasses, meat, fish etc.

Deficiency Disease: It causes specific dermatitis in rats, pellagra and anaemia in human beings, affects central nervous system, causes general weakness, convulsions, weakness, nervousness, insomnia and irritability.

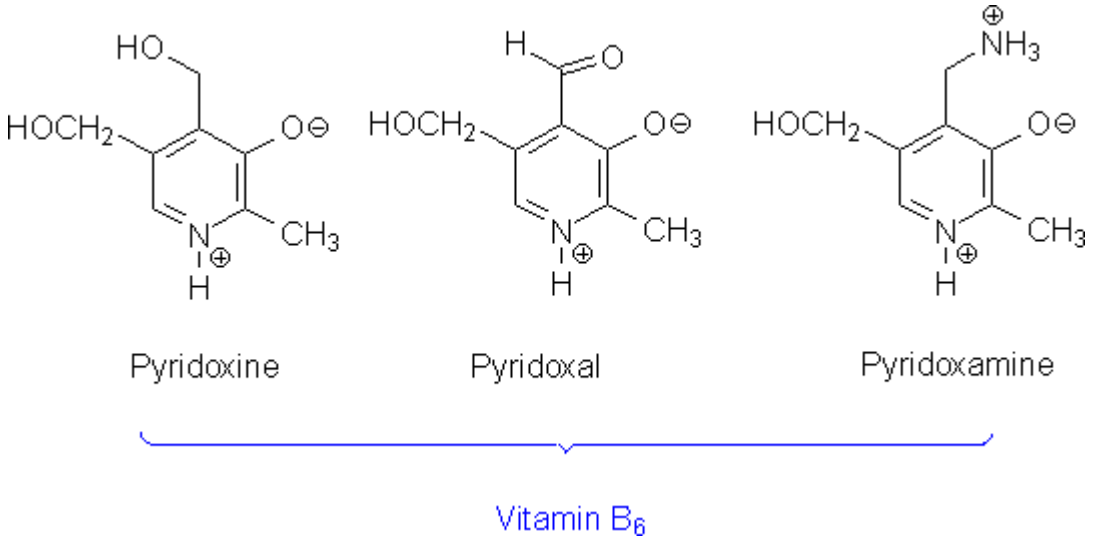


Fig 11. Structure of vitamin B6

3. **<u>Vitamin B12</u>**: Its chemical name is as cyanocobalamin. It contains cobalt.

Characteristics: It is insoluble in oils and fat.

Source: It is found in milk, eggs and liver of ox, sheep, pig etc.

Deficiency Disease: It causes pernicious anaemia, inflammation of tongue and mouth.

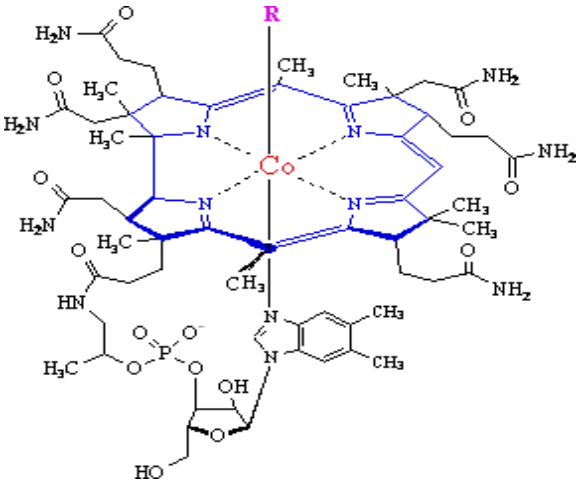
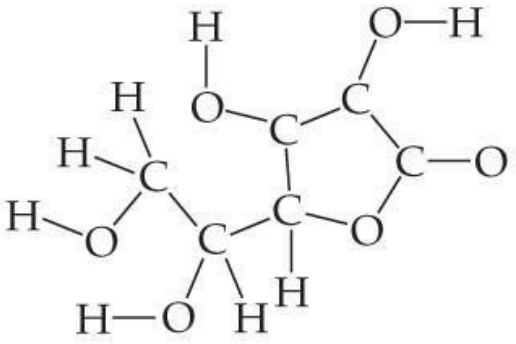


Fig 12. Structural detail of Vitamin B12

4. **Vitamin C:** Its chemical name is Ascorbic acid and commonly called as Antiscorbutic vitamin.
   Characteristics: It is destroyed by cooking and prolonged exposure to air. To avoid the loss, vegetables rich in vitamin C must be cooked in a closed pan and pressure cooker. Vitamin c increases resistance of the body towards diseases, maintains healthy skin and helps cuts and abrasion to heal properly.
   Source: It is found in citrus fruits, lemons, leafy vegetables, chillies, sprouted pulses and germinated grains.
   Deficiency Disease: It causes scurvy, pyorrhoea.



Vitamin C

Fig.13. Structural detail of Vitamin C

5. **Vitamin B_2:** Its chemical name is Riboflavin or Lactoflavin.
   Characteristics: It is sensitive to light but stable to heat. It is essential for the growth and health of animals.
   Sources: It is found in milk, yeast, green vegetables, meat, liver, kidney etc.
   Deficiency Diseases: Its deficiency retards growth, and causes general inflammation of tongue, dermatitis and cheilosis.

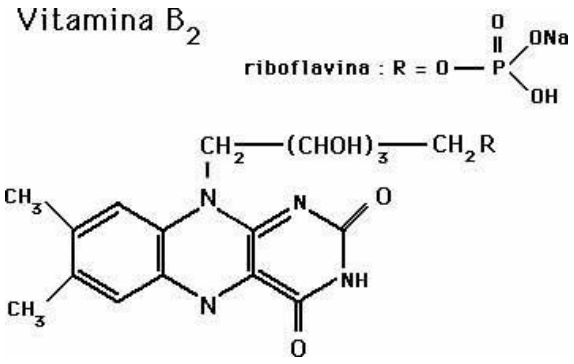


Fig 14. Structural detail of Vitamin B_2

2. Fat Soluble Vitamins: These vitamins are the oily substance and not readily soluble in water, however they are soluble in fat. Excess intake of these vitamins is harmful for health and may cause hypervitaminosis. Some important fat soluble vitamins and their characteristics, sources and disease caused by their deficiency is given below:

1. **Vitamin A**: Its chemical name is Retinol and also called Bright Eye Vitamin. Carotenoids are precursors of vitamin A.

   Characteristics: It is stable to heat. It promotes growth and vision in animals and increases resistance to diseases.

   Sources: It is present in milk, butter, eggs, fish liver oil, rice polishing, green vegetables etc.

   Deficiency Disease: Its deficiency causes Xerophthalmia, night blindness and xerosis.

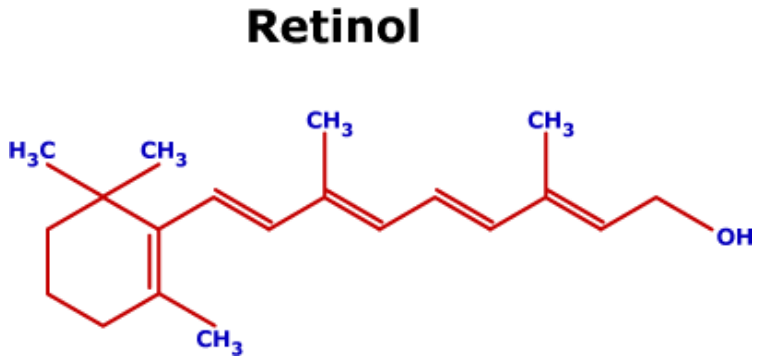


Fig 15. Structure of vitamin A or Retinol

2. **Vitamin D:** Its chemical name is ergocalciferol and commonly called as Antirachitic Vitamin or Sunshine Vitamin.

   Characteristics: It is also stable to heat and resistant to oxidation. It controls calcium and phosphorus metabolism.

   Sources: It is present in fish liver oils, butter, milk, eggs, liver and meat. Daily dose of vitamin D in the human body is about 0.025 mg.

   Deficiency Diseases: Its deficiency causes rickets in children and osteomalacia in adults.

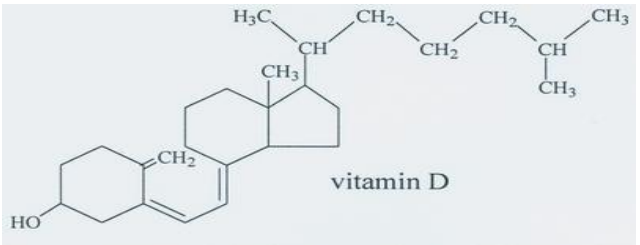


Fig 16. Structure of Vitamin D or Ergocalciferol

3. **Vitamin E**: It is a mixture of four vitamins called α, β, ϒ, $^{\delta}$- tocopherols.

Characteristics: It is stable to heat and oxidation.

Sources: Its sources are vegetable oils (like wheat germ oil, cotton seed oil, soybean oil, peanut oil etc.), eggs, milk etc. Its daily dose to the human body is about 5mg.

Deficiency Disease: Its deficiency causes sterility, increased fragility of RBCs and molecular weakness.

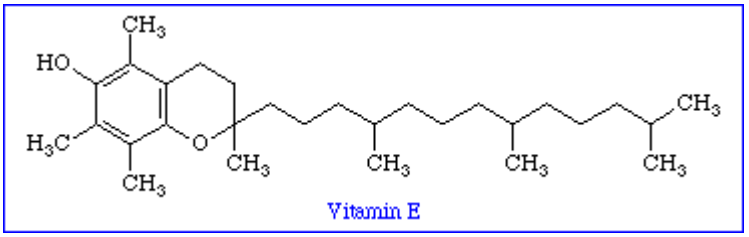


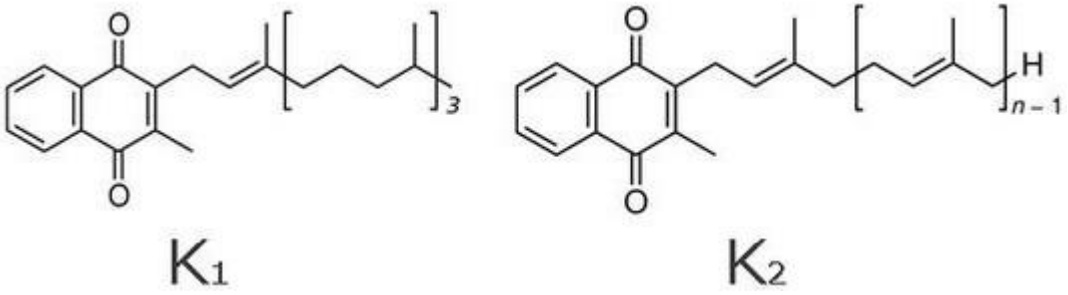
Fig 15. Vitamin E structure.

4. **Vitamin K**: Its chemical name is phylloquinone and commonly called as Antihemorrhagic Vitamin. And it is a mixture of two vitamins called K1 and K2.

Characteristics: It is sensitive to light and alkali.

Sources: Vitamin K1- alfalfa, leafy vegetables and spinach. Vitamin K2- occurs mainly in bacteria.

Deficiency Disease: Its deficiency causes haemorrhage, it lengthens the time of blood clotting.



Some vitamins are neither water soluble nor fat soluble. Example of such a vitamin is Vitamin H (biotin) . It is found in liver, yest, kidney and milk and its deficiency causes Dermatitis, loss of hair and paralysis.

Lack of a particular vitamin causes a specific deficiency disease. Multiple deficiencies caused by lack of more than one vitamin are called Avitaminoses. It is quite common in human beings.

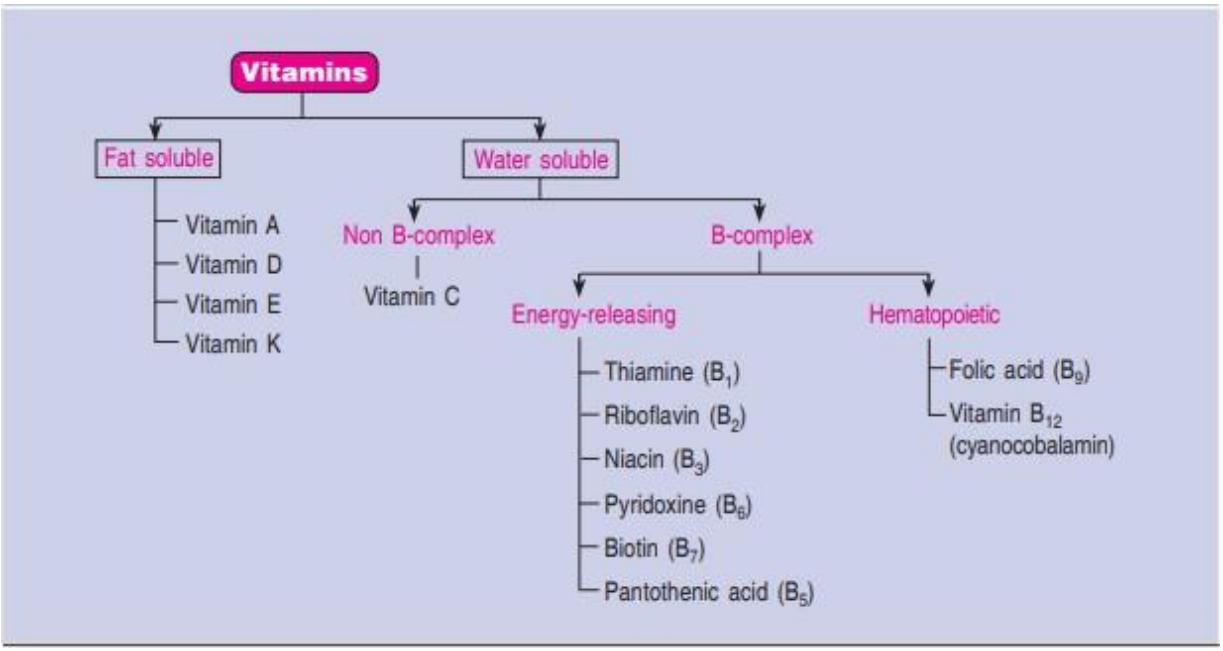


TABLE 3. Classification of vitamins

8. CHEMICAL BONDING

How elements interact with one another depends on how their electrons are arranged and how many openings for electrons exist at the outermost region where electrons are present in an atom. Electrons exist at energy levels that form shells around the nucleus. The closest shell can hold up to two electrons. The closest shell to the nucleus is always filled first, before any other shell can be filled. Hydrogen has one electron; therefore, it has only one spot occupied within the lowest shell. Helium has two electrons; therefore, it can completely fill the lowest shell with its two electrons. If you look at the periodic table, you will see that hydrogen and helium are the only two elements in the first row. This is because they only have electrons in their first shell. Hydrogen and helium are the only two elements that have the lowest shell and no other shells.

Chemical bonding is one of the most basic fundamentals of chemistry that explains other concepts such as molecules and reactions. Without it, scientists wouldn't be able to explain why atoms are attracted to each other or how products are formed after a chemical reaction has taken place. To understand the concept of bonding, one must first know the basics behind atomic structure.

A common atom contains a nucleus composed of protons and neutrons, with electrons in certain

energy levels revolving around the nucleus. In this section, the main focus will be on these electrons. Elements are distinguishable from each other due to their "electron cloud," or the area where electrons move around the nucleus of an atom. Because each element has a distinct electron cloud, this determines their chemical properties as well as the extent of their reactivity (i.e. noble gases are inert/not reactive while alkaline metals are highly reactive). In chemical bonding, only valence electrons, electrons located in the orbitals of the outermost energy level (valence shell) of an element, are involved.

IONIC BONDS

There are four types of bonds or interactions: ionic, covalent, hydrogen bonds, and van der Waals interactions. Ionic and covalent bonds are strong interactions that require a larger energy input to break apart. When an element donates an electron from its outer shell, as in the sodium atom example above, a positive ion is formed. The element accepting the electron is now negatively charged. Because positive and negative charges attract, these ions stay together and form an ionic bond, or a bond between ions. The elements bond together with the electron from one element staying predominantly with the other element. When Na^+ and Cl^- ions combine to produce NaCl, an electron from a sodium atom stays with the other seven from the chlorine atom, and the sodium and chloride ions attract each other in a lattice of ions with a net zero charge.

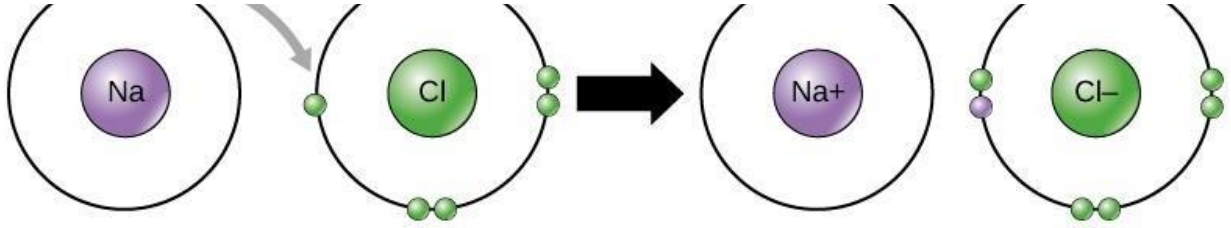


Figure 16 In the formation of an ionic compound, metals lose electrons and nonmetals gain electrons to achieve an octet.

COVALENT BONDS

Another type of strong chemical bond between two or more atoms is a covalent bond. These bonds form when an electron is shared between two elements and are the strongest and most common form of chemical bond in living organisms. Covalent bonds form between the elements that make up the biological molecules in our cells. Unlike ionic bonds, covalent bonds do not dissociate in water.

The hydrogen and oxygen atoms that combine to form water molecules are bound together by covalent bonds. The electron from the hydrogen atom divides its time between the outer shell of the hydrogen atom and the incomplete outer shell of the oxygen atom. To completely fill the outer shell of an oxygen atom, two electrons from two hydrogen atoms are needed, hence the subscript “2” in H_2O. The electrons are

shared between the atoms, dividing their time between them to “fill” the outer shell of each. This sharing is a lower energy state for all of the atoms involved than if they existed without their outer shells filled.

There are two types of covalent bonds: polar and nonpolar. Nonpolar covalent bonds form between two atoms of the same element or between different elements that share the electrons equally. For example, an oxygen atom can bond with another oxygen atom to fill their outer shells. This association is nonpolar because the electrons will be equally distributed between each oxygen atom. Two covalent bonds form between the two oxygen atoms because oxygen requires two shared electrons to fill its outermost shell. Nitrogen atoms will form three covalent bonds (also called triple covalent) between two atoms of nitrogen because each nitrogen atom needs three electrons to fill its outermost shell. Another example of a nonpolar covalent bond is found in the methane (CH_4) molecule. The carbon atom has four electrons in its outermost shell and needs four more to fill it. It gets these four from four hydrogen atoms, each atom providing one. These elements all share the electrons equally, creating four nonpolar covalent bonds.

In a polar covalent bond, the electrons shared by the atoms spend more time closer to one nucleus than to the other nucleus. Because of the unequal distribution of electrons between the different nuclei, a slightly positive (δ+) or slightly negative (δ–) charge develops. The covalent bonds between hydrogen and oxygen atoms in water are polar covalent bonds. The shared electrons spend more time near the oxygen nucleus, giving it a small negative charge, than they spend near the hydrogen nuclei, giving these molecules a small positive charge.

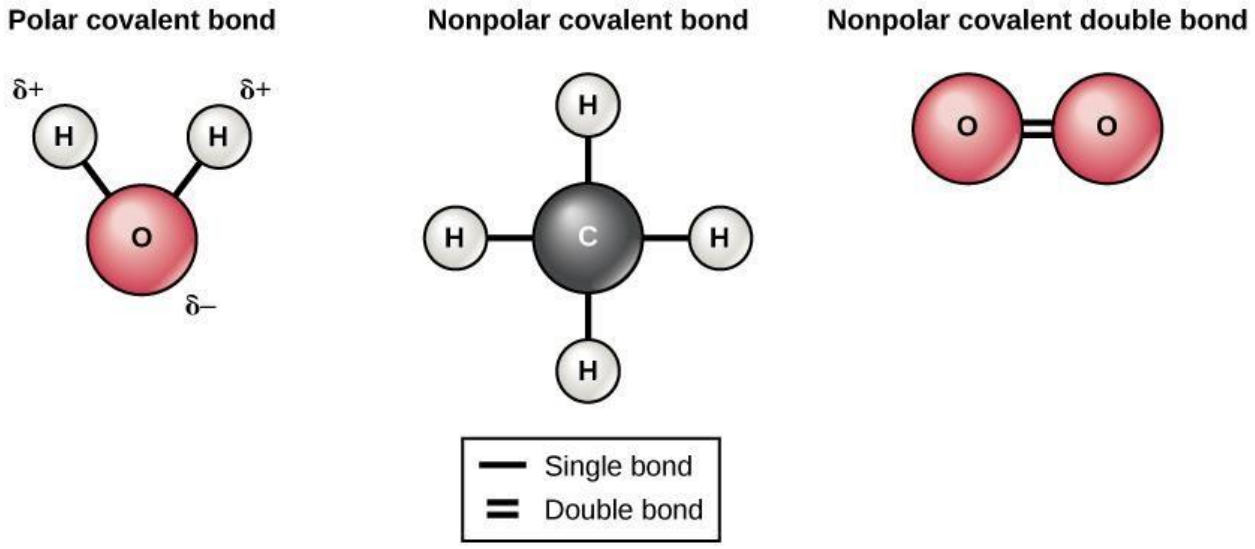


Figure 17 The water molecule (left) depicts a polar bond with a slightly positive charge on the hydrogen atoms and a slightly negative charge on the oxygen. Examples of nonpolar bonds include methane (middle) and oxygen (right).

HYDROGEN BONDS

Ionic and covalent bonds are strong bonds that require considerable energy to break. However, not

all bonds between elements are ionic or covalent bonds. Weaker bonds can also form. These are attractions that occur between positive and negative charges that do not require much energy to break. Two weak bonds that occur frequently are hydrogen bonds and van der Waals interactions. These bonds give rise to the unique properties of water and the unique structures of DNA and proteins. When polar covalent bonds contain a hydrogen atom form, the hydrogen atom in that bond has a slightly positive charge. This is because the shared electron is pulled more strongly toward the other element and away from the hydrogen nucleus. Because the hydrogen atom is slightly positive (δ^+), it will be attracted to neighboring negative partial charges (δ^-). When this happens, a weak interaction occurs between the δ^+ charge of the hydrogen atom of one molecule and the δ^- charge of the other molecule. This interaction is called a hydrogen bond. This type of bond is common; for example, the liquid nature of water is caused by the hydrogen bonds between water molecules. Hydrogen bonds give water the unique properties that sustain life. If it were not for hydrogen bonding, water would be a gas rather than a liquid at room temperature.

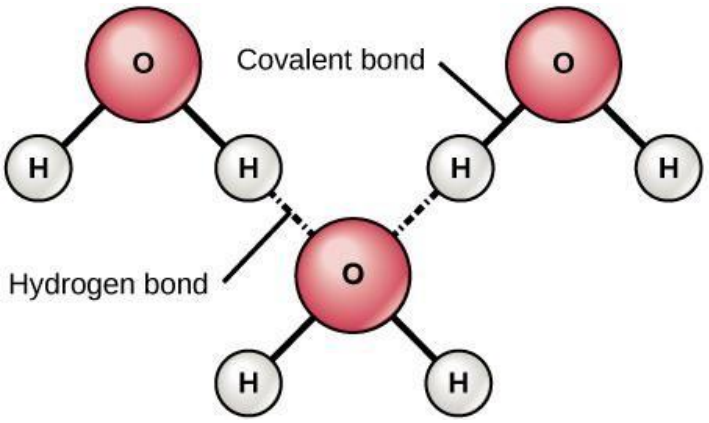


Figure 18: Hydrogen bonds form between slightly positive (δ+) and slightly negative (δ–) charges of polar covalent molecules, such as water.

Hydrogen bonds can form between different molecules and they do not always have to include a water molecule. Hydrogen atoms in polar bonds within any molecule can form bonds with other adjacent molecules. For example, hydrogen bonds hold together two long strands of DNA to give the DNA molecule its characteristic double-stranded structure. Hydrogen bonds are also responsible for some of the three-dimensional structure of proteins.

VAN DER WAALS INTERACTIONS

Like hydrogen bonds, van der Waals interactions are weak attractions or interactions between molecules. They occur between polar, covalently bound, atoms in different molecules. Some of these weak attractions are caused by temporary partial charges formed when electrons move around a nucleus. These weak interactions between molecules are important in biological systems.

Hydrophobic Interactions

Hydrophobic interactions describe the relations between water and hydrophobes (low water-soluble molecules). Hydrophobes are nonpolar molecules and usually have a long chain of carbons that do not interact with water molecules. The mixing of fat and water is a good example of this particular interaction. The common misconception is that water and fat doesn't mix because the Van der Waals forces that are acting upon both water and fat molecules are too weak. However, this is not the case. The behavior of a fat

droplet in water has more to do with the enthalpy and entropy of the reaction than its intermolecular forces.

Strength of Hydrophobic Interactions

Hydrophobic interactions are relatively stronger than other weak intermolecular forces (i.e., Van der Waals interactions or Hydrogen bonds). The strength of Hydrophobic Interactions depend on several factors including (in order of strength of influence):

1. **Temperature:** As temperature increases, the strength of hydrophobic interactions increases also. However, at an extreme temperature, hydrophobic interactions will denature.
2. **Number of carbons on the hydrophobes:** Molecules with the greatest number of carbons will have the strongest hydrophobic interactions.
3. **The shape of the hydrophobes:** Aliphatic organic molecules have stronger interactions than aromatic compounds. Branches on a carbon chain will reduce the hydrophobic effect of that molecule and linear carbon chain can produce the largest hydrophobic interaction. This is so because carbon branches produce steric hindrance, so it is harder for two hydrophobes to have very close interactions with each other to minimize their contact to water.

Biological Importance of Hydrophobic Interactions

Hydrophobic Interactions are important for the folding of proteins. This is important in keeping a protein stable and biologically active, because it allows the protein to decrease in surface area and reduce the undesirable interactions with water. Besides proteins, there are many other biological substances that rely on hydrophobic interactions for its survival and functions, like the phospholipid bilayer membranes in every cell of your body.

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DEPARTMENT OF BIOTECHNOLOGY

UNIT – II– BIOCHEMISTRY AND BIOMOLECULES – SBTA1302

1. Introduction

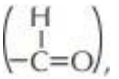
Carbohydrates are the most abundant organic molecules in nature. They are primarily composed of the elements carbon, hydrogen and oxygen. The name carbohydrate literally means ‘hydrates of carbon’. Some of the carbohydrates possess the empirical formula $(C.H_2O)n$ where $n \leq 3$, satisfying that these carbohydrates are in fact carbohydrates. However, there are several non-carbohydrate compounds (e.g. acetic acid, C2H4O2; lactic acid, $C_3H_6O_3$) which also appear as hydrates of carbon. Further, some of the genuine carbohydrates (e.g. rhamnohexose, $C_6H_{12}O_5$; deoxyribose, $C_5H_{10}O_4$) do not satisfy the general formula. Hence carbohydrates cannot be always considered as hydrates of carbon. Carbohydrates may be defined as polyhydroxy aldehydes or ketones or compounds which produce them on hydrolysis. The term ‘sugar’ is applied to carbohydrates soluble in water and sweet to taste.

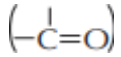
CLASSIFICATION OF CARBOHYDRATES

Carbohydrates are often referred to as saccharides (Greek: sakcharon–sugar). They are broadly classified into three major groups— monosaccharides, oligosaccharides and polysaccharides. This categorization is based on the number of sugar units. Mono- and oligosaccharides are sweet to taste, crystalline in character and soluble in water, hence they are commonly known as sugars.

Monosaccharides

Monosaccharides (Greek : mono-one) are the simplest group of carbohydrates and are often referred to as simple sugars. They have the general formula Cn(H2O)n, and they cannot be further hydrolysed. The monosaccharides are divided into different categories, based on the functional group and the number of carbon atoms

Aldoses : When the functional group in monosaccharides is an aldehyde  , they are known as aldoses e.g. glyceraldehyde, glucose.

Ketoses : When the functional group is a keto  group, they are referred to as ketoses e.g. dihydroxyacetone, fructose.

Based on the number of carbon atoms, the monosaccharides are regarded as trioses (3C), tetroses (4C), pentoses (5C), hexoses (6C) and heptoses (7C). These terms along with functional groups are used while naming monosaccharides. For instance, glucose is an aldohexose while fructose is a ketohexose (Table1).

Monosaccharides (empirical formula)	*Aldose*	*Ketose*
Trioses ($C_3H_6O_3$)	Glyceraldehyde	Dihydroxyacetone
Tetroses ($C_4H_8O_4$)	Erythrose	Erythrulose
Pentoses ($C_5H_{10}O_5$)	Ribose	Ribulose
Hexoses ($C_6H_{12}O_6$)	Glucose	Fructose
Heptoses ($C_7H_{14}O_7$)	Glucoheptose	Sedoheptulose

TABLE 1 Classification of monosaccharides with selected examples

Oligosaccharides

Oligosaccharides (Greek: oligo-few) contain 2-10 monosaccharide molecules which are liberated on hydrolysis. Based on the number of monosaccharide units present, the oligosaccharides are further subdivided to disaccharides, trisaccharides etc.

Polysaccharides

Polysaccharides (Greek: poly-many) are polymers of monosaccharide units with high molecular weight (up to a million). They are usually tasteless (non-sugars) and form colloids with water. The polysaccharides are of two types – homopolysaccharides and heteropolysaccharides.

MONOSACCHARIDES— STRUCTURAL ASPECTS

Stereoisomerism is an important character of monosaccharides. Stereoisomers are the compounds that have the same structural formula but differ in their spatial configuration. A carbon is said to be

asymmetric when it is attached to four different atoms or groups. The number of asymmetric carbon atoms (n) determines the possible isomers of a given compound which is equal to 2^n. Glucose contains 4 asymmetric carbons, and thus has 16 isomers.

Glyceraldehyde —the reference carbohydrate

Glyceraldehyde (triose) is the simplest monosaccharide with one asymmetric carbon atom. It exists as two stereoisomers and has been chosen as the reference carbohydrate to represent the structure of all other carbohydrates.

D- and L-isomers

The D and L isomers are mirror images of each other. The spatial orientation of H and OH groups on the carbon atom (C5 for glucose) that is adjacent to the terminal primary alcohol carbon determines whether the sugar is D- or L-isomer. If the OH group is on the right side, the sugar is of D-series, and if on the left side, it belongs to L-series. The structures of D- and L-glucose based on the reference monosaccharide, D- and L-glyceraldehyde (glycerose) are depicted in Fig.2

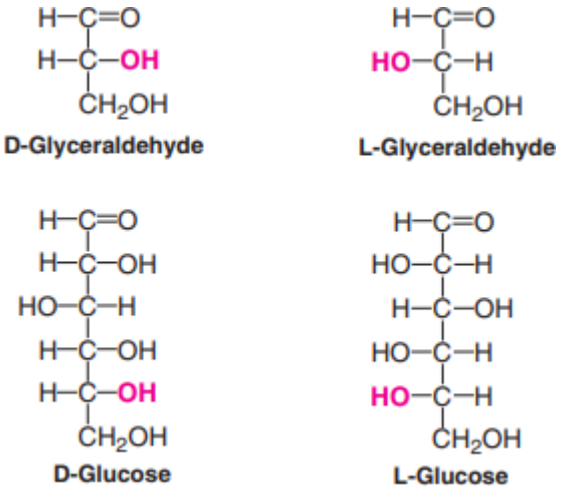


Fig. 1 : D-and-L- forms of glucose compared with D- and L- glyceraldehydes (the reference carbohydrate)

It may be noted that the naturally occurring monosaccharides in the mammalian tissues are mostly of D-configuration. The enzyme machinery of cells is specific to metabolise D-series of monosaccharides.

Optical activity of sugar

Optical activity is a characteristic feature of compounds with asymmetric carbon atom. When a beam of polarized light is passed through a solution of an optical isomer, it will be rotated either to the right or

left. The term dextrorotatory (d+) and levorotatory (l–) are used to compounds that respectively rotate the plane of polarized light to the right or to the left. An optical isomer may be designated as D(+), D(–), L(+) and L(–) based on its structural relation with glyceraldehyde. It may be noted that the D- and L-configurations of sugars are primarily based on the structure of glyceraldehyde, the optical activities however, may be different.

Racemic mixture : If d- and l-isomers are present in equal concentration, it is known as racemic mixture or dl mixture. Racemic mixture does not exhibit any optical activity, since the dextro- and levorotatory activities cancel each other. In medical practice, the term dextrose is used for glucose in solution. This is because of the dextrorotatory nature of glucose.

Configuration of D-aldoses

The configuration of possible D-aldoses starting from D-glyceraldehyde is depicted in Fig.2. This is a representation of Kiliani Fischer synthesis, by increasing the chain length of an aldose, by one carbon at a time. Thus, starting with an aldotriose (3C), aldotetroses (4C), aldopentoses (5C) and aldohexoses (6C) are formed. Of the 8 aldohexoses, glucose, mannose and galactose are the most familiar. Among these, D-glucose is the only aldose monosaccharide that predominantly occurs in nature.

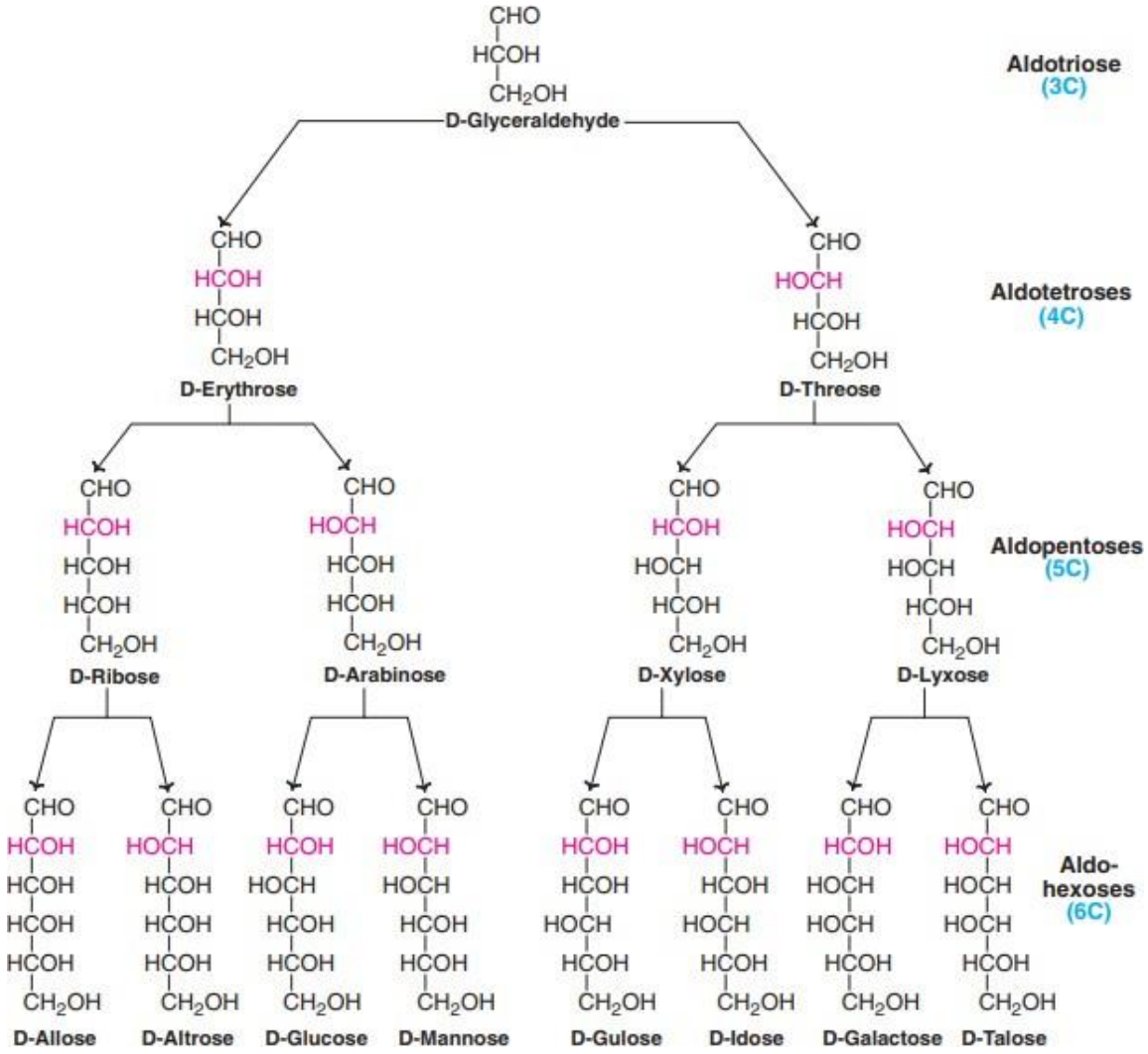


Fig. 2 : The structural relationship between D-aldoses shown in Fischer projection. (The configuration around C_2 (red) distinguishes the members of each pair).

Configuration of D-ketoses

Starting from dihydroxyacetone (triose), there are five keto-sugars which are physiologically important. Their structures are given in Fig.3.

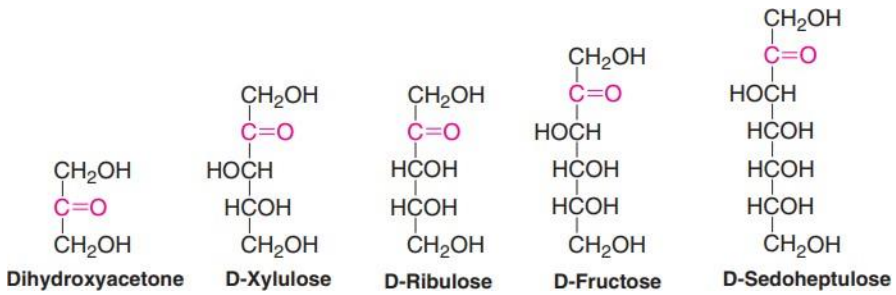


Fig. 3: Structures of ketoses of physiological importance.

Epimers

If two monosaccharides differ from each other in their configuration around a single specific carbon (other than anomeric) atom, they are referred to as epimers to each other (Fig.4). For instance, glucose and galactose are epimers with regard to carbon 4 (C_4-epimers). That is, they differ in the arrangement of OH group at C_4. Glucose and mannose are epimers with regard to carbon 2 (C_2-epimers).

The interconversion of epimers (e.g. glucose to galactose and vice versa) is known as epimerization, and a group of enzymes— namely—epimerases catalyse this reaction.

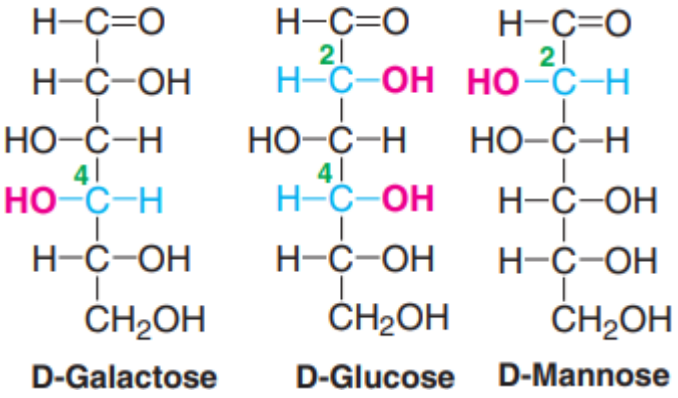


Fig. 4 : Structures of epimers (glucose and galactose are C_4-epimers while glucose and mannose are C_2-epimers).

Enantiomers

Enantiomers are a special type of stereoisomers that are mirror images of each other. The two members are designated as D- and L-sugars. Enantiomers of glucose are depicted in Fig.5. Majority of the sugars in the higher animals (including man) are of D-type (Fig.5). The term diastereomers is used to represent the stereoisomers that are not mirror images of one another.

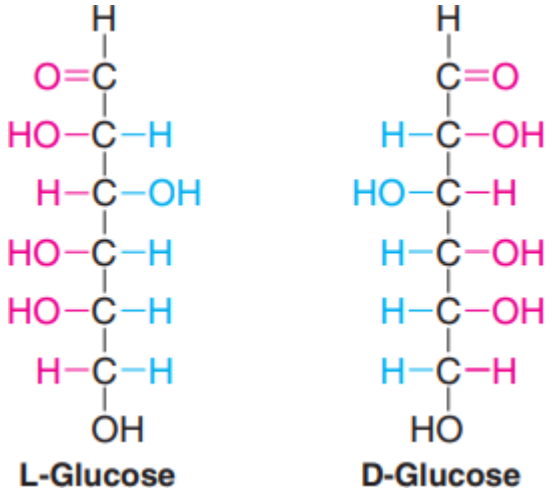


Fig. 5 : Enantiomers (mirror images) of glucose

STRUCTURE OF GLUCOSE

For a better understanding of glucose structure, let us consider the formation of hemiacetals and hemiketals, respectively produced when an aldehyde or a ketone reacts with alcohol. The hydroxyl group of monosaccharides can react with its own aldehyde or keto functional group to form hemiacetal and hemiketal. Thus, the aldehyde group of glucose at C1 reacts with the alcohol group at C5 to form two types of cyclic hemiacetals namely **α** and Beta, as depicted in Fig.6. The configuration of glucose is conveniently represented either by Fischer formulae or by Haworth projection formulae.

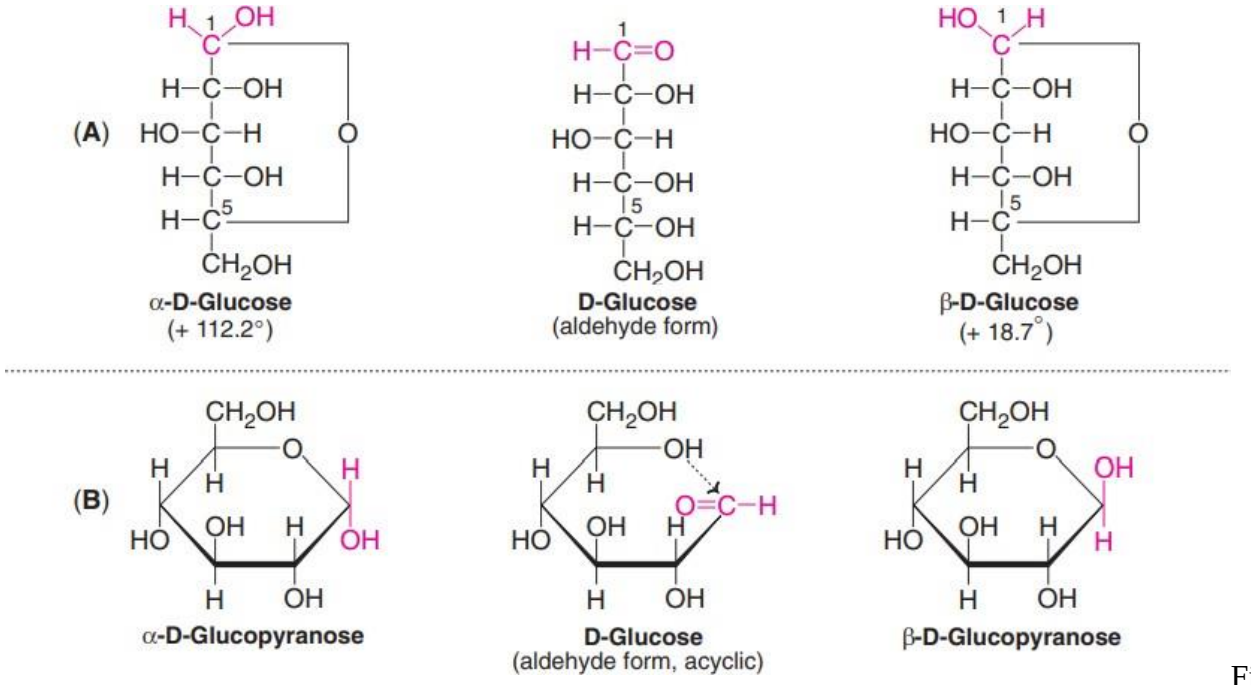


Fig. 6 : Mutarotation of glucose representing D and E anomers (A) Fischer projections (B) Haworth projections

Pyranose and furanose structures

Haworth projection formulae are depicted by a six-membered ring pyranose (based on pyran) or a five-membered ring furanose (based on furan). The cyclic forms of glucose are known as **α**-D-glucopyranose and **α**-D-glucofuranose (Fig.7).

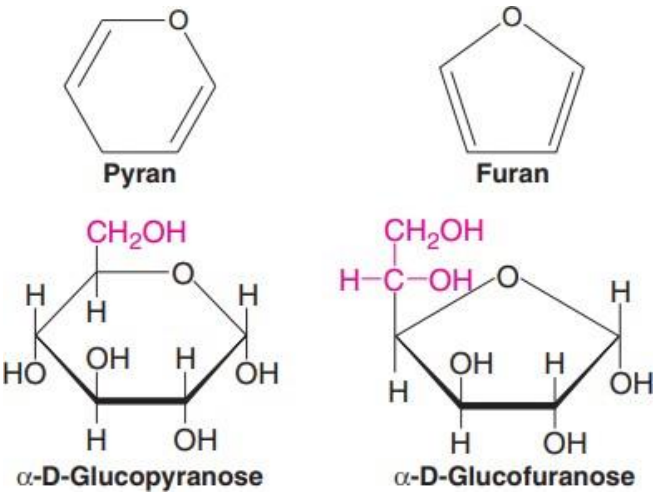


Fig. 7: Structure of glucose-pyranose and furanose forms.

Anomers—mutarotation

The **α** and **β** cyclic forms of D-glucose are known as anomers. They differ from each other in the configuration only around C1 known as anomeric carbon (hemiacetal carbon). In case of D anomer, the OH group held by anomeric carbon is on the opposite side of the group CH_2OH of sugar ring. The reverse is true for **β**-anomer. The anomers differ in certain physical and chemical properties.

Mutarotation : The **α** and **β** anomers of glucose have different optical rotations. The specific optical rotation of a freshly prepared glucose (**α** anomer) solution in water is +112.2° which gradually changes and attains an equilibrium with a constant value of +52.7°. In the presence of alkali, the decrease in optical rotation is rapid. The optical rotation of E-glucose is +18.7°. Mutarotation is defined as the change in the specific optical rotation representing the interconversion of **α** and **β** forms of D-glucose to an equilibrium mixture. Mutarotation depicted in Fig. 6, is summarized below

$$\underset{+\ 112.2^\circ}{\alpha\text{-D-Glucose}} \rightleftharpoons \underset{+\ 52.7^\circ}{\text{Equilibrium mixture}} \rightleftharpoons \underset{+\ 18.7^\circ}{\beta\text{-D-Glucose}}$$

$$\left(\text{Specific optical rotation } [\alpha]_D^{20}\right)$$

The equilibrium mixture contains 63% E-anomer and 36% **β**-anomer of glucose with 1% open chain form. In aqueous solution, the E form is more predominant due to its stable conformation. The **α** and **β** forms of glucose are interconvertible which occurs through a linear form. The latter, as such, is present in

an insignificant quantity. **Mutarotation of fructose** : Fructose also exhibits mutarotation. In case of fructose, the pyranose ring (six-membered) is converted to furanose (five-membered) ring, till an equilibrium is attained. And fructose has a specific optical rotation of –92° at equilibrium. The conversion of dextrorotatory (+) sucrose to levorotatory fructose is explained under inversion of sucrose.

DISACCHARIDES

Among the oligosaccharides, disaccharides are the most common (Fig.8). As is evident from the name, a disaccharide consists of two monosaccharide units (similar or dissimilar) held together by a glycosidic bond. They are crystalline, water-soluble and sweet to taste. The disaccharides are of two types.

1. **Reducing** disaccharides with free aldehyde or keto group e.g. maltose, lactose.
2. **Non-reducing** disaccharides with no free aldehyde or keto group e.g. sucrose, trehalose.

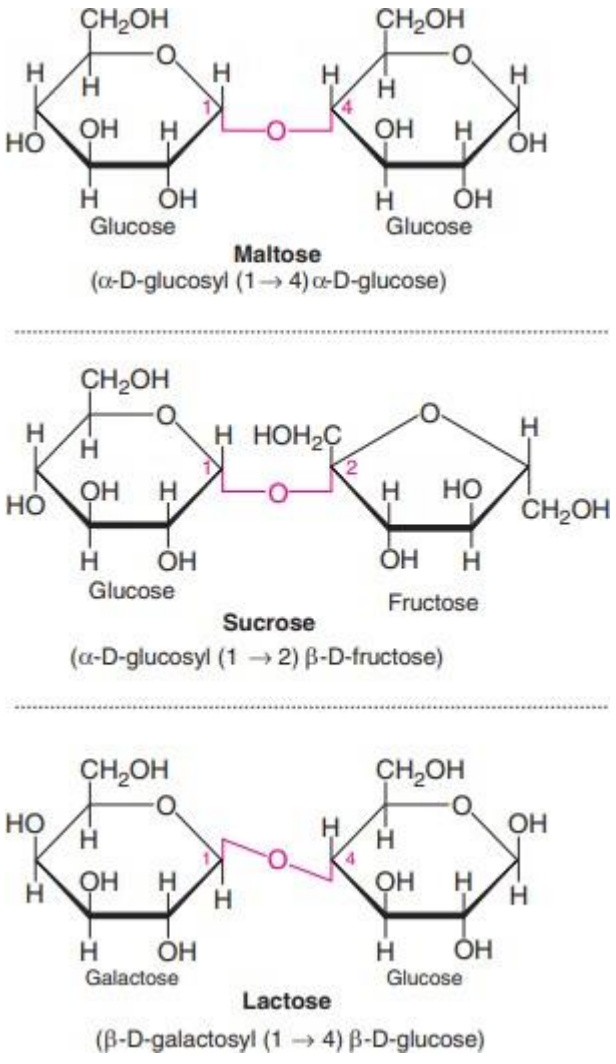


Fig. 8: Structures of disaccharides —maltose, sucrose and lactose.

Maltose

Maltose is composed of two **α** -D-glucose units held together by D (1→4) glycosidic bond. The free

aldehyde group present on C1 of second glucose answers the reducing reactions, besides the osazone formations (sunflower-shaped). Maltose can be hydrolysed by dilute acid or the enzyme maltase to liberate two molecules of α -D-glucose. In isomaltose, the glucose units are held together by **α** (1→ 6) glycosidic linkage.

Cellobiose is another disaccharide, identical in structure with maltose, except that the former has **β** (1→4) glycosidic linkage. Cellobiose is formed during the hydrolysis of cellulose.

Sucrose

Sucrose (cane sugar) is the sugar of commerce, mostly produced by sugar cane and sugar beets. Sucrose is made up of **α**-D-glucose and **β** D-fructose. The two monosaccharides are held together by a glycosidic bond (**α**$_1$ → β $_2$), between C1 of **α**-glucose and C2 of **α**-fructose. The reducing groups of glucose and fructose are involved in glycosidic bond, hence sucrose is a non-reducing sugar, and it cannot form osazones.

Sucrose is an important source of dietary carbohydrate. It is sweeter than most other common sugars (except fructose) namely glucose, lactose and maltose. Sucrose is employed as a sweetening agent in the food industry. The intestinal enzyme—sucrase—hydrolyses sucrose to glucose and fructose which are absorbed.

Inversion of sucrose

Sucrose, as such, is dextrorotatory (+66.5°). But, when hydrolysed, sucrose becomes levorotatory (–28.2°). The process of change in optical rotation from dextrorotatory (+) to levorotatory (–) is referred to as inversion. The hydrolysed mixture of sucrose, containing glucose and fructose, is known as invert sugar. The process of inversion is explained below.

Hydrolysis of sucrose by the enzyme sucrase (invertase) or dilute acid liberates one molecule each of glucose and fructose. It is postulated that sucrose (dextro) is first split into **α**-D glucopyranose (+52.5°) and β -D-fructofuranose, both being dextrorotatory. However, β -D fructofuranose is less stable and immediately gets converted to β -D-fructopyranose which is strongly levorotatory (–92°). The overall effect is that dextro sucrose (+66.5°) on inversion is converted to levo form (–28.2°).

Lactose

Lactose is more commonly known as milk sugar since it is the disaccharide found in milk. Lactose is composed of β -D-galactose and β - D- glucose held together by β (1 → 4) glycosidic bond. The anomeric carbon of C_1 glucose is free, hence lactose exhibits reducing properties and forms osazones (powder-puffor hedgehog shape). Lactose of milk is the most important carbohydrate in the nutrition of young mammals. It is hydrolysed by the intestinal enzyme lactase to glucose and galactose.

Lactulose

Lactulose is a synthetic disscharide containing galactose and fructose. It is neither digested nor absorbed in the inestine. Lactulose is useful for the treatment of hepatic encephalopathy, a disorder characterized by elevated plasma ammonium levels. Lactulose converts ammonia (NH_3) in the lumen to ammonium ion (NH_4^+). This results in a reduction in the plasma NH_3, since NH_4^+ ions are not easily absorbed.

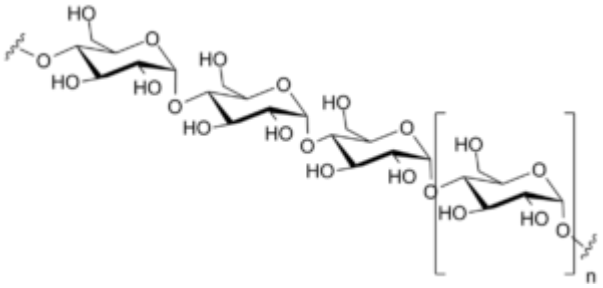
POLYSACCHARIDES

Polysaccharides (or simply glycans) consist of repeat units of monosaccharides or their derivatives, held together by glycosidic bonds. They are primarily concerned with two important functions-structural, and storage of energy. Polysaccharides are linear as well as branched polymers. This is in contrast to the structure of proteins and nucleic acids which are only linear polymers. The occurrence of branches in polysaccharides is due to the fact that glycosidic linkages can be formed at any one of the hydroxyl groups of a monosaccharide. Polysaccharides are of two types

1. Homopolysaccharides on hydrolysis yield only a single type of monosaccharide. They are named based on the nature of the monosaccharide. Thus, glucans are polymers of glucose whereas fructosans are polymers of fructose.

2. Heteropolysaccharides on hydrolysis yield a mixture of a few monosaccharides or their derivatives.

Structure of Polysaccharides



All polysaccharides are formed by the same basic process where monosaccharides are connected via glycosidic bonds. These glycosidic bonds consist of an oxygen molecule bridging two carbon rings. The bond is formed when a hydroxyl group is lost from the carbon of one molecule, while the hydrogen is lost by the hydroxyl group of another monosaccharide. Because two molecules of hydrogen and one of oxygen are expelled, the reaction is a dehydration reaction. The structure of the molecules being combined determines the structures and properties of the resulting polysaccharide. A polysaccharide used for energy storage will give easy access to the constituent monosaccharides whereas a polysaccharide used for support

is usually a long chain of monosaccharides that form fibrous structures.

Functions of Polysaccharides

Polysaccharides form a crucial part of cell function and structure.

Storage polysaccharides: Polysaccharides such as starch and glycogen are called storage polysaccharides because they are stored in the liver and muscles to be converted to energy later for body functions. Starch is found in plants whereas glycogen is found in animals.

Structural polysaccharides: Polysaccharides such as cellulose are structural polysaccharides which are found in the cell walls of plants. Another structural polysaccharide is chitin.

Class	Name	Source	Composition	Linkages
Structural polysaccharides	Cellulose	Plant cell walls	Glucose (beta linkage)	Unbranched 1→4
	Mannan	Yeast cell walls	Mannose (beta linkage)	Branched 1→2, 1→3, and 1→6
	Chitin	Arthropod shells, fungal cell walls	Acetylglucosamine and glucuronic acid (beta linkage)	Unbranched 1→4
	Hyaluronic acid	Synovial fluid (joints), subcutaneous tissue	Acetylglucosamine and glucuronic acid (beta linkage)	Unbranched 1→3 and 1→4
	Peptidoglycans	Bacterial cell walls	Acetylglucosamine and acetylmuramic acid	Unbranched 1→4
Nutrient polysaccharides	Inulin	Artichokes, dandelions	Fructose (beta linkage)	Unbranched 2→1
	Paramylum	Certain protozoa (e.g., *Euglena*)	Glucose (beta linkage)	Unbranched 1→3
	Glycogen	Certain protozoa (e.g., *Tetrahymena*) and most animals	Glucose (alpha linkage)	Branched 1→4 and 1→6
	Starch: Amylopectin	Plant cells and some protozoa (e.g., *Polytomella*)	Glucose (alpha linkage)	Branched 1→4 and 1→6
	Amylose		Glucose (alpha linkage)	Unbranched 1→4

Table 2: Polysaccharides

HOMOPOLYSACCHARIDES

Starch is the carbohydrate reserve of plants which is the most important dietary source for higher animals, including man. High content of starch is found in cereals, roots, tubers, vegetables etc. Starch is a homopolymer composed of D-glucose units held by **α**-glycosidic bonds. It is known as glucosan or glucan. Starch consists of two polysaccharide components-water soluble amylose (15-20%) and a water insoluble amylopectin (80-85%). Chemically, amylose is a long unbranched chain with 200–1,000 D- glucose units held by D (1 →4) glycosidic linkages. Amylopectin, on the other hand, is a branched chain with D (1 → 6) glycosidic bonds at the branching points and D (1→4) linkages everywhere else (Fig.9). Amylopectin molecules containing a few thousand glucose units look like a branched tree (20–30 glucose units per branch).

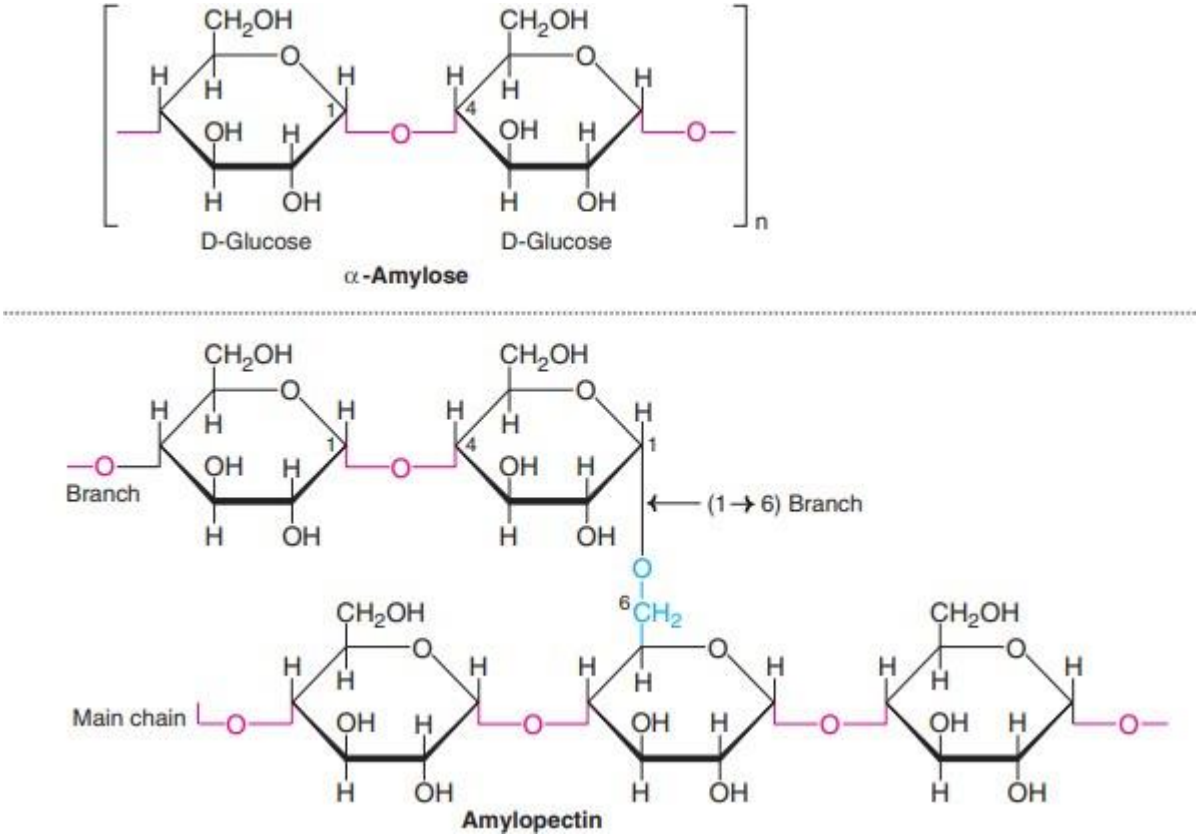


Fig. 9 : Structure of starch (D-amylose and amylopectin)

Starches are hydrolysed by amylase (pancreatic or salivary) to liberate dextrins, and finally maltose and glucose units. Amylase acts specifically on **α** (1 → 4) glycosidic bonds.

Dextrins

Dextrins are the breakdown products of starch by the enzyme amylase or dilute acids. Starch is sequentially hydrolysed through different dextrins and, finally, to maltose and glucose. The various intermediates (identified by iodine colouration) are soluble starch (blue), amylodextrin (violet), erythrodextrin (red) and achrodextrin (no colour).

Dextrans

Dextrans are polymers of glucose, produced by microorganisms. They are used as plasma volume expanders in transfusion, and chromatography (e.g. gel filtration).

Inulin I

nulin is a polymer of fructose i.e., fructosan. It occurs in dahlia bulbs, garlic, onion etc. It is a low molecular weight (around 5,000) polysaccharide easily soluble in water. Inulin is not utilized by the body. It is used for assessing kidney function through measurement of glomerular filtration rate (GFR).

Glycogen

Glycogen is the carbohydrate reserve in animals, hence often referred to as animal starch. It is present in high concentration in liver, followed by muscle, brain etc. Glycogen is also found in plants that do not possess chlorophyll (e.g. yeast, fungi). The structure of glycogen is similar to that of amylopectin with more number of branches. Glucose is the repeating unit in glycogen joined together by **α** (1→ 4) glycosidic bonds, and **α** (1→ 6) glycosidic bonds at branching points (Fig.10). The molecular weight (up to 1 u 108) and the number of glucose units (up to 25,000) vary in glycogen depending on the source from which glycogen is obtained.

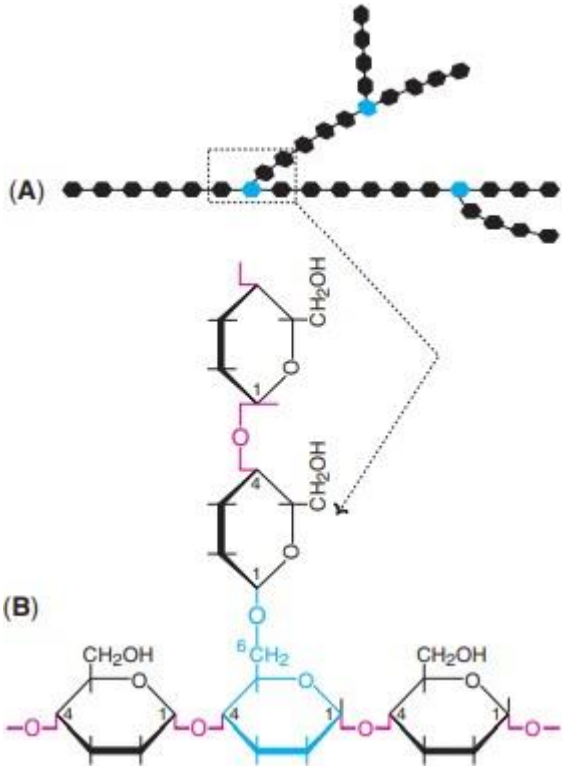


Fig.10: Structure of glycogen (A) General structure (B) Enlarged at a branch point.

Cellulose

Cellulose occurs exclusively in plants and it is the most abundant organic substance in the plant kingdom. It is a predominant constituent of plant cell wall. Cellulose is totally absent in the animal body.

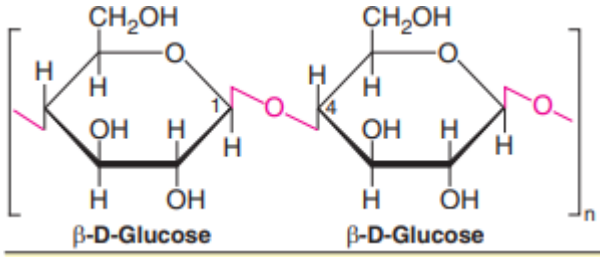


Fig. 11 : Structure of cellulose (The repeating unit 'n' may be several thousands).

Cellulose is composed of β -D-glucose units linked by β (1→4) glycosidic bonds (Fig.2.15). Cellulose cannot be digested by mammals— including man—due to lack of the enzyme that cleaves β -glycosidic bonds (α amylase breaks α bonds only). Certain ruminants and herbivorous animals contain microorganisms in the gut which produce enzymes that can cleave β -glycosidic bonds. Hydrolysis of cellulose yields a disaccharide cellobiose, followed by β -D-glucose.

Cellulose, though not digested, has great importance in human nutrition. It is a major constituent of fiber, the non-digestible carbohydrate. The functions of dietary fiber include decreasing the absorption of glucose and cholesterol from the intestine, besides increasing the bulk of feces.

CHITIN

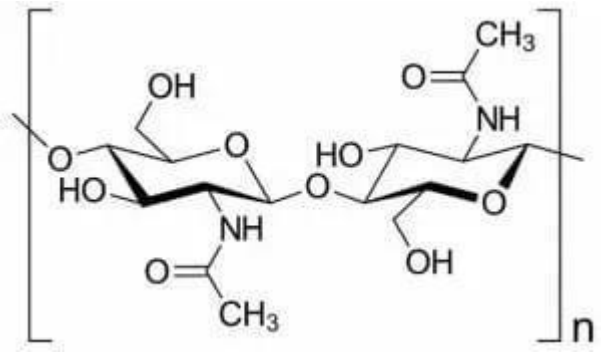
Chitin is a large, structural polysaccharide made from chains of modified glucose. Chitin is found in the exoskeletons of insects, the cell walls of fungi, and certain hard structures in invertebrates and fish. In terms of abundance, chitin is second to only cellulose. In the biosphere, over 1 billion tons of chitin are synthesized each year by organisms. This extremely versatile molecule can form solid structures on its own as in insect wings, or can combine with other components like calcium carbonate to make even stronger substances like the shell of a clam.

Like cellulose, no vertebrate animals can digest chitin on their own. Animals that eat a diet of insects often have symbiotic bacteria and protozoa which can break down the fibrous chitin into the glucose molecules that compose it. However, because chitin is a biodegradable molecule that dissolves over time, it is used in a number of industrial applications, such as surgical thread and binders for dyes and glues.

Function of Chitin

Chitin, like cellulose and keratin, is a structural polymer. Made from smaller monomers, or monosaccharides, structural polymers form strong fibers. When secreted inside or outside of cells in an organized way, the fibers form weak bonds between each other. This adds strength to the entire structure. Chitin and cellulose are both made from glucose monomers, while keratin is a fibrous protein. The various structural polymers arose early in the evolution of life, because they are seen only in certain groups. Cellulose is exclusive to plants, keratin to animals, and chitin to the arthropods, mollusks and fungi. Chitin and cellulose both evolved early-on in the history of life, while keratin arose in certain animals long after plants and fungi had branched off from the other eukaryotes.

Structure of Chitin



Chitin is made up of modified glucose monosaccharides. Glucose exists as a ring of carbon and oxygen molecules. Bonds between glucose molecules are known as glycosidic bonds. The oxygens that typically form hydroxyl groups bonded to the carbon ring can also form a bond with another carbon instead of a hydrogen. In this way, monosaccharides can be linked together in long chains. Chitin is formed by a series of glycosidic bonds between substituted glucose molecules.

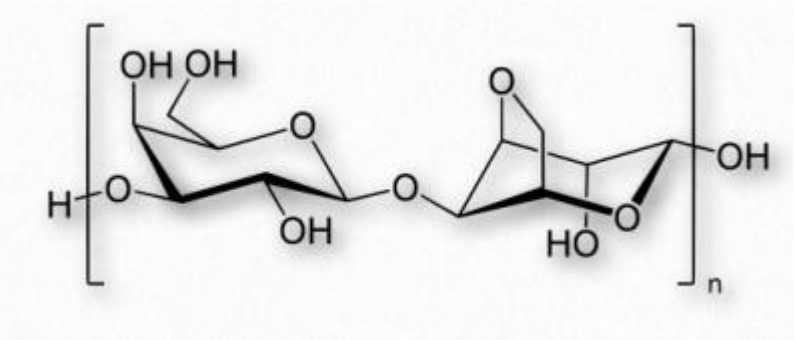
Chitin is different from cellulose because of the substitution that occurs on the glucose molecule. Instead of a hydroxyl group (OH), the glucose molecules in chitin have an amyl group attached that consists of carbon and nitrogen. Nitrogen is an electrically positive molecule, while the oxygen double bonded to the group is electrically negative. This produces a dipole in the molecule, which increases the hydrogen bonds that can formed between these molecules and the molecules around them. When combined in a matrix with

various compounds and other chitin molecules, the resulting structure can be very hard because of all the weak interactions between nearby molecules.

AGAR

Agar is a polymer made up of subunits of the sugar galactose .The word agar comes from the Malay word agar-agar (meaning jelly). It is also known as kanten or agal-agal (Ceylon agar).

Structure: It is an unbranched polysaccharide obtained from the cell walls of some species of red algae or seaweed. Agar polysaccharides serve as the primary structural support for the algae's cell walls.



Repeating unit of agarose - 3,6-anhydro-L-galactopyranose

Uses: Gracilaria, Gelidium, Pterocladia and other red algae are used in the manufacture of the all-important agar. Agar can be used as a vegetarian gelatin substitute, a thickener for soups, in jellies, ice cream and desserts, as a clarifying agent in brewing, a laxative and for paper sizing fabrics. It is used widely as a growth medium for microorganisms and for microbiological and biotechnological applications.

Sialic acids

N-Acetylneuraminic acid (NANA) is a derivative of N-acetylmannose and pyruvic acid. Itis an important constituent of glycoproteins and glycolipids. The term sialic acid is used to include NANA and its other derivatives. The carbohydrates found in glycoproteins include mannose, galactose, N-acetylglucosamine, N-acetylgalactosamine, xylose, L-fucose and N-acetylneuraminic acid (NANA). NANA is an important sialic acid

Blood group substances

The blood group antigens (of erythrocyte membrane) contain carbohydrates as glycoproteins or glycolipids. N-Acetylgalactosamine, galactose, fucose, sialic acid etc. are found in the blood group substances. The carbohydrate content also plays a determinant role in blood grouping.

The human ABO blood groups illustrate the effects of glycosyl- transferases. Carbohydrates are attached to glycoproteins and glycolipids on the surfaces of red blood cells. For one type of blood group, one of the three different structures, termed A, B, and O, may be present (Figure). These structures have in common an oligosaccharide foundation called the O (or sometimes H) antigen. The A and B antigens differ from the O antigen by the addition of one extra monosaccharide, either N-acetylgalactosamine (for A) or galactose (for B) through an α -1,3 linkage to a galactose moiety of the O antigen.

Specific glycosyltransferases add the extra monosaccharide to the O antigen. Each person inherits the gene for one glycosyltransferase of this type from each parent. The type A transferase specifically adds N-acetylgalactosamine, whereas the type B transferase adds galactose. These enzymes are identical in all but 4 of 354 positions. The O phenotype is the result of a mutation that leads to premature termination of translation and, hence, to the production of no active glycosyl transferase. These structures have important implications for blood transfusions and other transplantation procedures. If an antigen not normally present in a person is introduced, the person's immune system recognizes it as foreign. Adverse reactions can ensue, initiated by the intravascular destruction of the incompatible red blood cells.

Why are different blood types present in the human population? Suppose that a pathogenic organism such as a parasite expresses on its cell surface a carbohydrate antigen similar to one of the blood-group antigens. This antigen may not be readily detected as foreign in a person with the blood type that matches the parasite antigen, and the parasite will flourish. However, other people with different blood types will be protected. Hence, there will be selective pressure on human beings to vary blood type to prevent parasitic mimicry and a corresponding selective pressure on parasites to enhance mimicry. The constant "arms race" between pathogenic microorganisms and human beings drives the evolution of diversity of surface antigens within the human population.

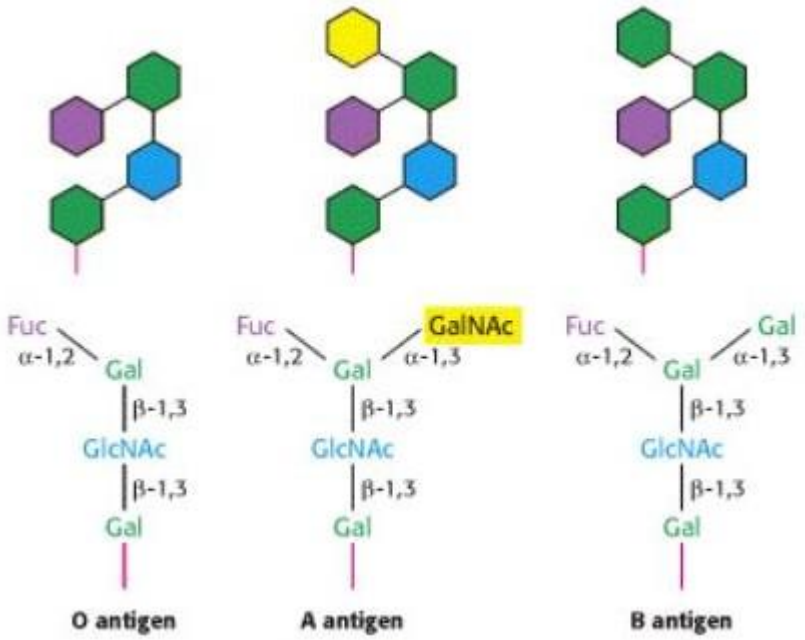


Fig 12: Structures of A, B, and O Oligosaccharide Antigens. Abbreviations: Fuc, fucose; Gal, galactose; GalNAc, N-acetylgalactosamine; GlcNAc, N-acetylglucosamine.

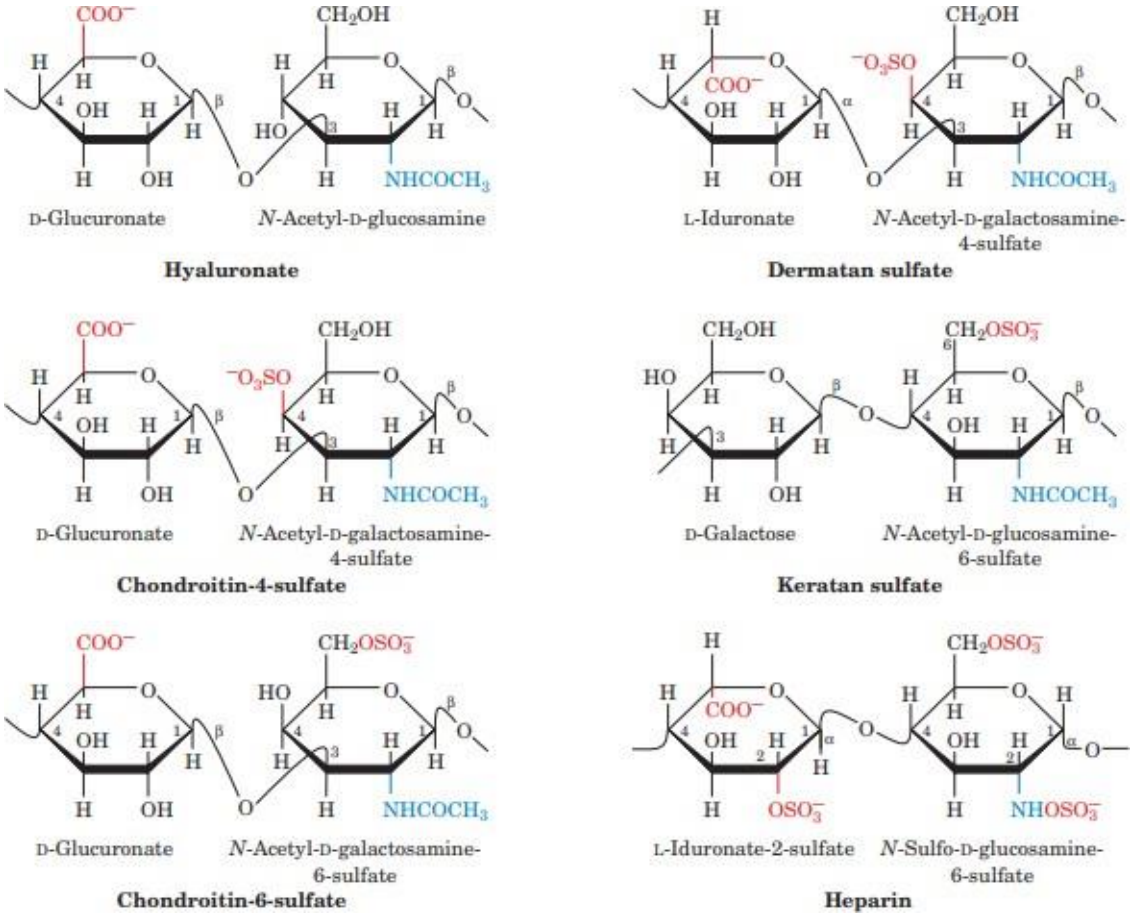
Hetero Polysaccharides

A polysaccharide that contains different types of monosaccharides is known as a heteropolysaccharide. Some of the important heteropolysaccharides are:

Hyaluronic Acid

Hyaluronic acid (also called hyaluronan) is an important GAG component of ground substance, synovial fluid (the fluid that lubricates the joints), and the vitreous humor of the eye. It also occurs in the capsules surrounding certain, usually pathogenic, bacteria. Hyaluronic acid molecules are composed of 250 to 25,000 β(1→4)-linked disaccharide units that consist of D-glucuronic acid and N-acetyl-Dglucosamine linked by a β (1 → 3) bond (Fig. 11-21). The anionic character of its glucuronic acid residues causes hyaluronic acid to bind cations such as K^+, Na^+, and Ca^{2+} tightly. X-ray fiber analysis indicates that Ca^{2+} hyaluronate forms an extended, left-handed, single-stranded helix with 3 disaccharide units per turn.

Hyaluronate's structural features suit it to its biological function. Its high molecular mass and numerous mutually repelling anionic groups make hyaluronate an extended, rigid, and highly hydrated molecule which, in solution, occupies a volume 1000 times that in its dry state. Hyaluronate solutions therefore have a viscosity that is shear dependent (an object under shear stress has equal and opposite forces applied across its opposite faces). At low shear rates, the hyaluronate molecules form tangled masses that greatly impede flow; that is, the solution is quite viscous. As the shear rate increases, the stiff rodlike hyaluronate molecules tend to line up with the flow and thus offer less resistance to it. This viscoelastic behavior makes hyaluronate solutions excellent biological shock absorbers and lubricants



Hyaluronic acid and other GAGs (see below) are degraded by hyaluronidase, which hydrolyzes their β(1→4) linkages. Hyaluronidase occurs in a variety of animal tissues, in bacteria (where it presumably expedites their invasion of animal tissue), and in snake and insect toxins.

1. **Chondroitin-4-sulfate** (Greek: chondros, cartilage), a major component of cartilage and other connective tissue has N-acetyl-D-galactosamine-4-sulfate residues in place of hyaluronate's N-acetyl-D-glucosamine residues.
2. **Chondroitin-6-sulfate** is instead sulfated at the C6 position of its N-acetyl-D-galactosamine residues. The two chondroitin sulfates occur separately or in mixtures depending on the tissue.

3. **Dermatan sulfate** (Greek: derma, skin), which is so named because of its prevalence in skin, differs from chondroitin-4-sulfate only by an inversion of configuration about C5 of the β-D-glucuronate residues to form α-L-iduronate.This results from the enzymatic epimerization of these residues after the formation of chondroitin. The epimerization is usually incomplete, so dermatan sulfate also contains glucuronate residues.
4. **Keratan sulfate** (Greek: keras, horn; not to be confused with the protein keratin) consists mainly of alternating β(1→4) linked D-galactose and N-acetyl-D-glucosamine-6- sulfate residues (and hence lacks uronic acid residues). It is a component of cartilage, bone, cornea, as well as hair, nails, and horn. Keratan sulfate is the most heterogeneous of the major GAGs in that its sulfate content is variable and it contains small amounts of fucose, mannose, N-acetylglucosamine, and sialic acid.
5. **Heparin** is a variably sulfated GAG that consists predominantly of alternating α (1→4) -linked residues of Liduronate-2-sulfate and N-sulfo-D-glucosamine-6-sulfate. It has an average of 2.5 sulfate residues per disaccharide unit, which makes it the most negatively charged polyelectrolyte in mammalian tissues. Heparin, in contrast to the above GAGs, is not a constituent of connective tissue, but occurs almost exclusively in the intracellular granules of the mast cells that line arterial walls, especially in the liver, lungs, and skin. It inhibits the clotting of blood, and its release, through injury, is thought to prevent runaway clot formation. Heparin is therefore in wide clinical use to inhibit blood clotting, for example, in postsurgical patients. Heparan sulfate, a ubiquitous cell surface component as well as an extracellular substance in blood vessel walls and brain, resembles heparin but has a far more variable composition with fewer N- and O-sulfate groups and more N-acetyl groups.

Stereochemistry of Carbohydrates

In the field of stereochemistry, the structure of a compound and the three-dimensional arrangement of its atoms needs to be known to understand the behavioural and chemical properties of the compound. Whilst molecular entities are by their nature three-dimensional, they are commonly depicted on two-dimensional media. Therefore, a certain degree of distortion is needed to accurately convey the three-dimensional structure.

In organic chemistry, there are conventions for illustrating bonds within a molecule. A wedge-dash notation is used to denote orientation of the structure. Dotted lines are used to represent an atomic bond

which is further away from the viewer, a wedge for the atoms nearer, and a solid line to show a bond in plane with the molecule.

Fischer and Haworth projections are two types of illustration which are used to represent the 3D arrangement of atoms in carbohydrates. They are also used to compare different carbohydrates.

Fischer projections

The Fischer projection was devised by German chemist Emil Fischer in 1891, who was the winner of the Nobel Prize in Chemistry in 1902. In a Fischer projection the carbohydrate is shown in its open chain form, rather than a cyclical one. Carbon atoms in the main chain of the carbohydrate molecule are connected vertically, whilst hydrogen atoms and hydroxyl groups are bonded horizontally. The horizontal lines illustrate the bonds which come out of the page, whereas the vertical lines show bonds that are in the page. Carbon atoms may or may not be shown in a Fischer projection.

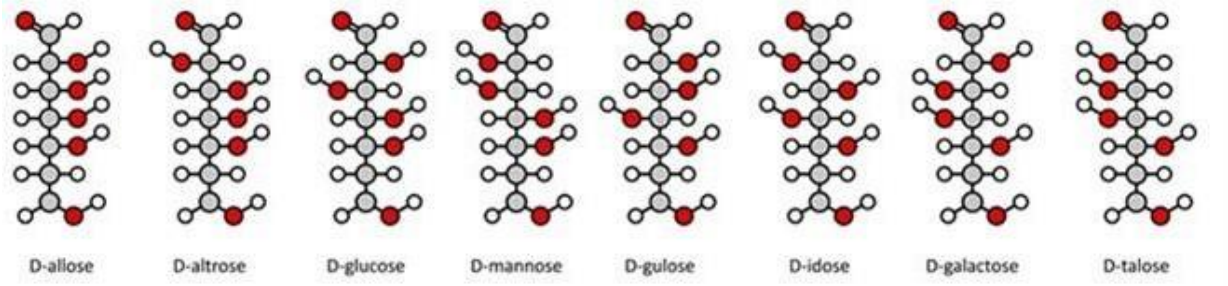


Fig 14. *D-aldohexose sugars: allose, altrose, glucose, mannose, gulose, idose, galactose, talose. Fischer-like projections. Image Credit: molekuul_be / Shutterstock.*

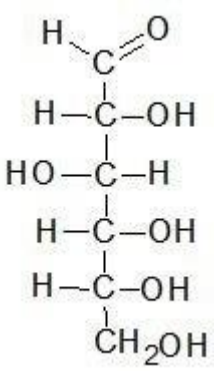
Fischer projections have one main advantage: it is easy to visually identify the stereochemical properties of a carbohydrate and compare the difference between two carbohydrates quickly and easily. For example, it is simple to tell the difference between two enantiomers (molecules that are a mirror image of each other.)

A Fischer projection can be rotated 180 degrees without affecting the molecule's stereoisomerism. If it were to be rotated 90 degrees then a different enantiomer would be illustrated, so therefore this is impossible with a Fischer projection. Small changes can affect a molecule's characteristics: therefore, care must be taken when using Fischer projections to illustrate a carbohydrate.

As Fischer projections can have a degree of ambiguity when confused with other types of drawing, their use to represent non-carbohydrates is discouraged. They are mainly used to illustrate monosaccharides. They can be used to represent other organic molecules including amino acids, but this is discouraged by the 2006 IUPAC recommendations. IUPAC rules also determine that the hydrogen atoms should be explicitly

drawn especially the hydrogen atoms of the end group of carbohydrates. Fischer projections are different to skeletal formulae.

Below is a Fischer projection of D-glucose in its open chain form.



Haworth projections

A Haworth projection differs from a Fischer projection in that it is used to represent the carbohydrate in its cyclical form. This is especially useful for sugars which have a ring structure. It was devised by the English chemist Sir Norman Haworth who expanded on the work of Fischer, characterizing many more carbohydrates. He developed the illustration technique after World War 1, and received the 1937 Nobel Prize for Chemistry for his work in investigating carbohydrates and Vitamin C.

In a Haworth projection, which is now the standard in organic chemistry for stereochemical carbohydrate illustrations, thicker bonds between carbon atoms represent those closest to the viewer, and the hydrogen/hydroxyl bonds below the plane of the carbon atoms represent those on the right in a Fischer projection. However, this rule does not apply to the groups on the two ring carbons bonded to the endocyclic oxygen atom.

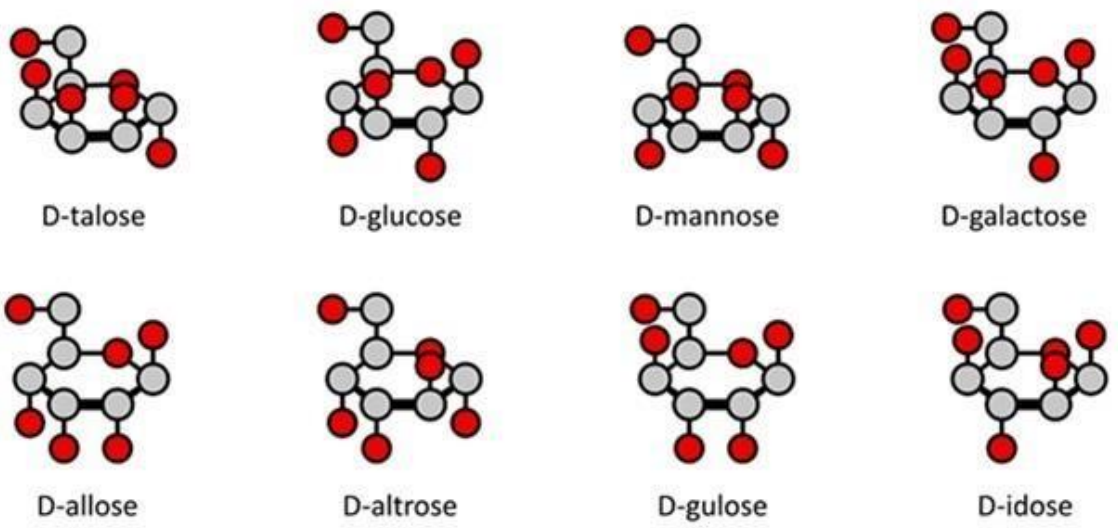
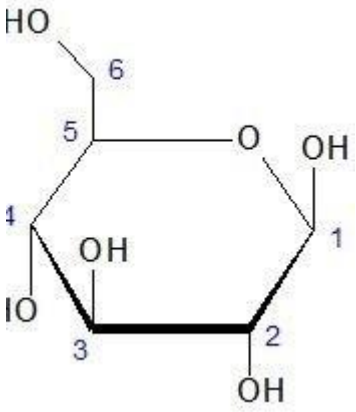


Fig 15; *D-aldohexose sugars: allose, altrose, glucose, mannose, gulose, idose, galactose, talose. Haworth-like projections. Image Credit: molekuul_be / Shutterstock.*

Compared to a Fischer projection, where carbon atoms may or may not be illustrated, in a Haworth projection they are implied. Carbon 1 is also referred to as the anomeric carbon. Hydrogen atoms which are bonded to a carbon atom are also implied, so are not shown. Hydroxyl groups and other atoms which may be bonded to the carbon ring are shown.

One drawback to Haworth projections is that they are not entirely accurate in representing the spatial positioning of all the atoms. A "chair" conformation can be used to more accurately depict the spatial positioning of the atoms; however, this conformation can make determination of the carbohydrate's basic stereochemistry more difficult.

Below is the Haworth projection for the cyclic form of D-glucose.



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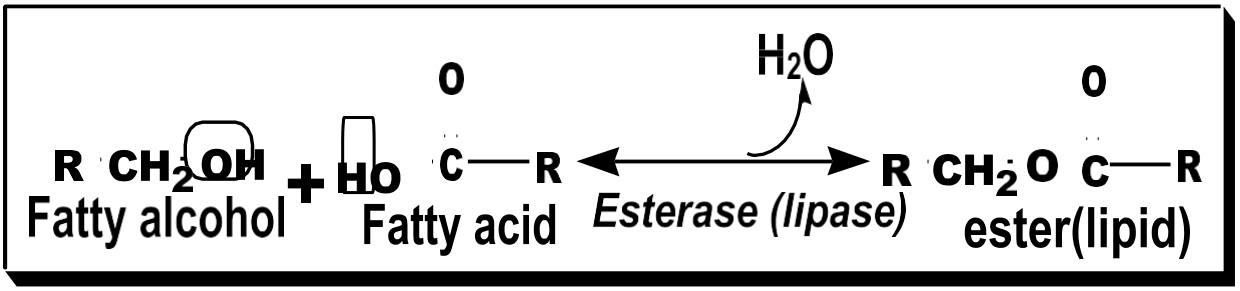
SCHOOL OF BIO AND CHEMICAL ENGINEERING
DEPARTMENT OF BIOTECHNOLOGY

UNIT – III – BIOCHEMISTRY AND BIOMOLECULES – SBTA1302

1. Introduction to lipids

Definition

Lipids are organic compounds formed mainly from alcohol and fatty acids combined together by ester linkage.



- Lipids are insoluble in water, but soluble in fat or organic solvents (ether, chloroform, benzene, acetone).
- Lipids include fats, oils, waxes and related compounds.
- They are widely distributed in nature both in plants and in animals.

Biological Importance of Lipids

1. They are more palatable and storable to unlimited amount compared to carbohydrates.
2. They have a high-energy value (25% of body needs) and they provide more energy per gram than carbohydrates and proteins but carbohydrates are the preferable source of energy.
3. Supply the essential fatty acids that cannot be synthesized by the body.
4. Supply the body with fat-soluble vitamins (A, D, E and K).
5. They are important constituents of the nervous system.
6. Tissue fat is an essential constituent of cell membrane and nervous system. It is mainly phospholipids in nature that are not affected by starvation.

7. Stored lipids “depot fat” is stored in all human cells acts as:

    - A store of energy.
    - A pad for the internal organs to protect them from outside shocks.
    - A subcutaneous thermal insulator against loss of body heat.

8. Lipoproteins, which are complex of lipids and proteins, are important cellular constituents that present both in the cellular and subcellular membranes.
9. Cholesterol enters in membrane structure and is used for synthesis of adrenal cortical hormones, vitamin D3 and bile acids.
10. Lipids provide bases for dealing with diseases such as obesity, atherosclerosis, lipid-storage diseases, essential fatty acid deficiency, respiratory distress syndrome,

Classification of Lipids

Bloor (1943) has proposed the following classification of lipids based on their chemical composition.

I. Simple lipids or Homolipids

These are esters of fatty acid with farious alcohols.

1. **Fats and oils** (triglycerides, triacylglycerols): These are esters of fatty acids with a trihydroxy alcohol, glycerol. A fat is solid at ordinary room temperature wheras an oil is liquid.
2. **Waxes:** These are esters of fatty acids with high molecular weight monohydroxy alcohols.

II. Compound lipids or Heterolipids.

These are esters of fatty acids with alcohol and possess additional group(s). e.g., sulfur, phosphorus, amino group, carbohydrate, or proteins beside fatty acid and alcohol.

Compound or conjugated lipids are classified into the following types according to the nature of the additional group

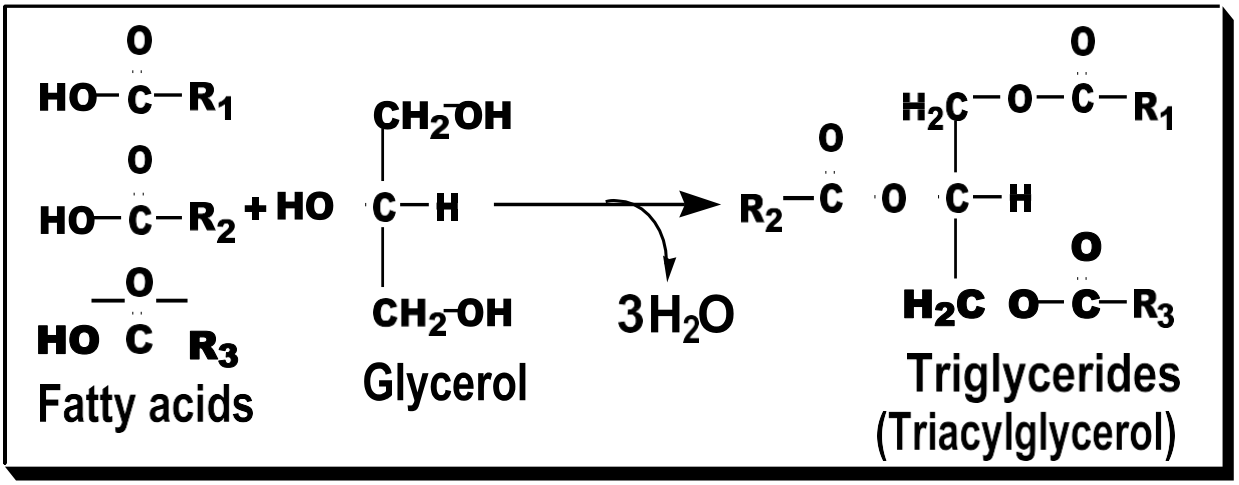
1. Phospholipids
2. Glycolipids.
3. Lipoproteins
4. Sulfolipids and amino lipids.

III. Derived lipids.

These are the substances derived from simple and compound lipids by hydrolysis. These include fatty acids, alcohols, mono- and diglycerides, steroids, terpenes and carotenoids.

Simple Lipids

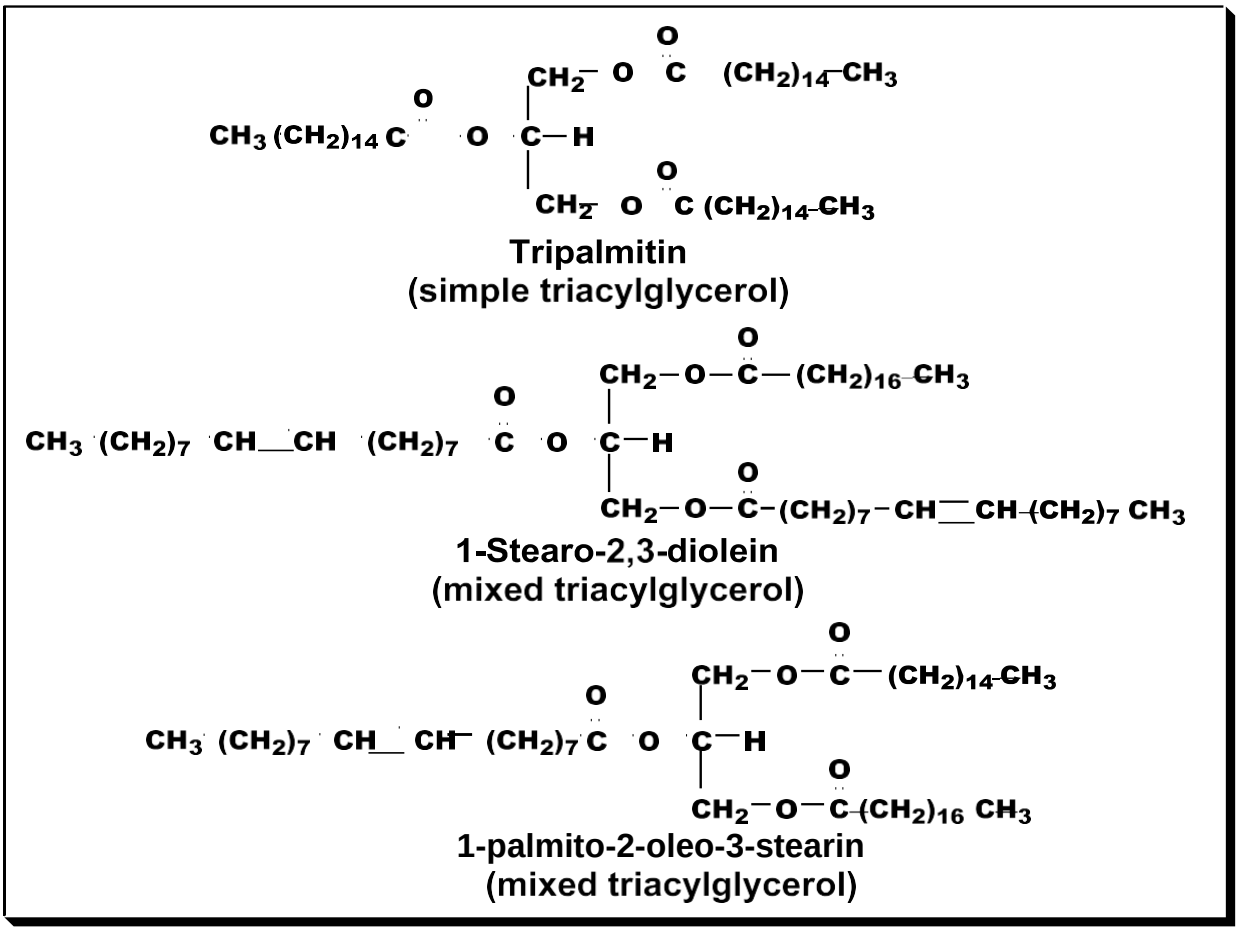
- They are called neutral because they are uncharged due to absence of ionizable groups in it.
- The neutral fats are the most abundant lipids in nature. They constitute about 98% of the lipids of adipose tissue, 30% of plasma or liver lipids, less than 10% of erythrocyte lipids.
- They are esters of glycerol with various fatty acids. Since the 3 hydroxyl groups of glycerol are esterified, the neutral fats are also called "Triglycerides".
- Esterification of glycerol with one molecule of fatty acid gives monoglyceride, and that with 2 molecules gives diglyceride.



Types of triglycerides

1. Simple triglycerides: If the three fatty acids connected to glycerol are of the same type the triglyceride is called simple triglyceride, e.g., tripalmitin.
2. Mixed triglycerides: if they are of different types, it is called mixed triglycerides, e.g., stearo-diolein and palmito-oleo-stearin.

- Natural fats are mixtures of mixed triglycerides with a small amount of simple triglycerides.



- The common fatty acids in animal fats are palmitic, stearic and oleic acids.
- The main difference between fats and oils is for oils being liquid at room temperature, whereas, fats are solids.

- This is mainly due to presence of larger percentage of unsaturated fatty acids in oils than fats that has mostly saturated fatty acids.

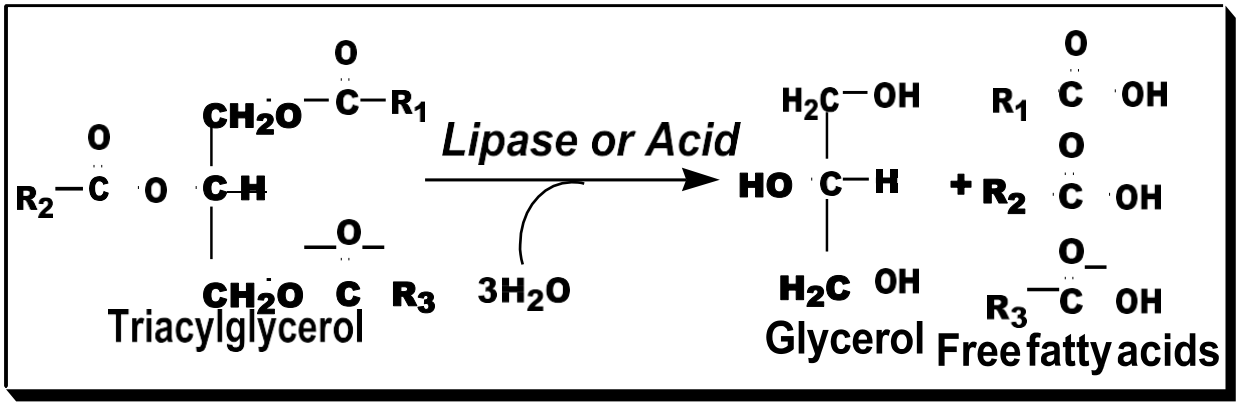
Physical properties of fat and oils

1. Freshly prepared fats and oils are colorless, odorless and tasteless. Any color, or taste is due to association with other foreign substances, e.g., the yellow color of body fat or milk fat is due to carotene pigments(cow milk).
2. Fats have specific gravity less than 1 and, therefore, they float on water.
3. Fats are insoluble in water, but soluble in organic solvents as ether and benzene.
4. Melting points of fats are usually low, but higher than the solidification point.

Chemical Properties of fats and oils

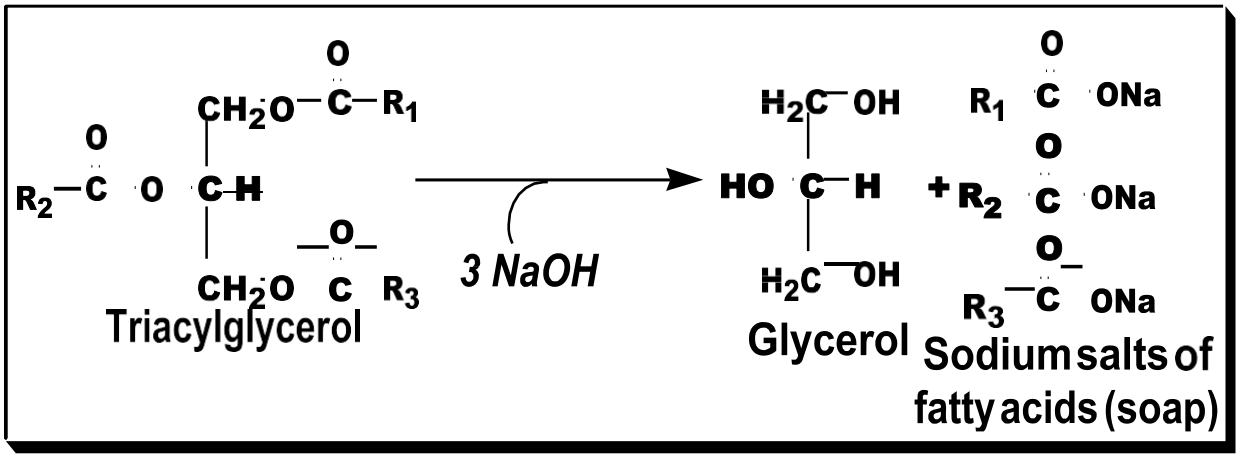
1. **Hydrolysis:**

- They are hydrolyzed into their constituents (fatty acids and glycerol) by the action of super heated steam, acid, alkali or enzyme (e.g., lipase of pancreas).
- During their enzymatic and acid hydrolysis glycerol and free fatty acids are produced.



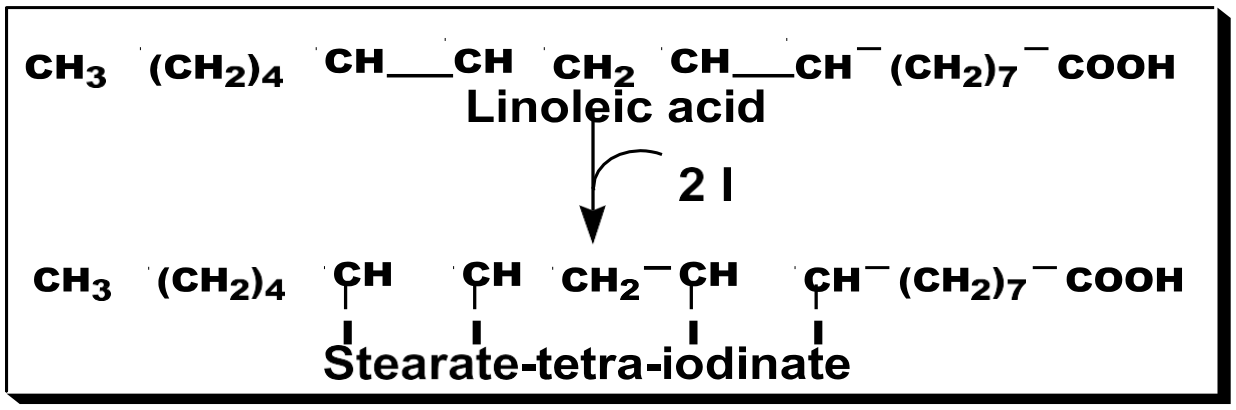
2. **Saponification**.

Alkaline hydrolysis produces glycerol and salts of fatty acids (soaps). Soaps cause emulsification of oily material this help easy washing of the fatty materials



3. **Halogenation**

- Neutral fats containing unsaturated fatty acids have the ability of adding halogens (e.g., iodine or iodination) at the double bonds.
- It is a very important property to determine the degree of unsaturation of the fat or oil that determines its biological value



4. Hydrogenation or hardening of oils:

- It is a type of addition reactions accepting hydrogen at the double bonds of unsaturated fatty acids.
- The hydrogenation is done under high pressure of hydrogen and is catalyzed by finely divided nickel or copper and heat.
- It is the base of hardening of oils (margarine manufacturing), e.g., change of oleic acid of fats (liquid) into stearic acid (solid).

5. Oxidation (Rancidty)

- This toxic reaction of triglycerides leads to unpleasant odour or taste of oils and fats developing after oxidation by oxygen of air, bacteria, or moisture.
- Also this is the base of the drying oils after exposure to atmospheric oxygen. Example is linseed oil, which is used in paints and varnishes manufacturing

Rancidity

- It is a physico-chemical change in the natural properties of the fat leading to the development of unpleasant odor or taste or abnormal color particularly on aging after exposure to atmospheric oxygen, light, moisture, bacterial or fungal contamination and/or heat.
- Saturated fats resist rancidity more than unsaturated fats that have unsaturated double bonds.

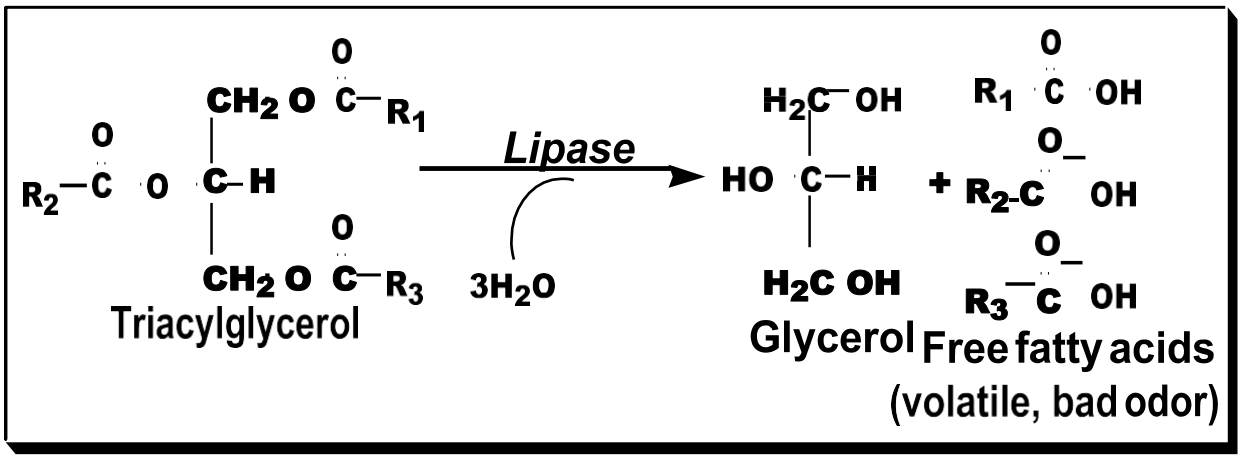
Types of Rancidity:

1. Hydrolytic rancidity
2. Oxidative rancidity
3. Ketonic rancidity

1. Hydrolytic rancidity:

- It results from slight hydrolysis of the fat by lipase from bacterial contamination leading to the liberation of free fatty acids and glycerol at high temperature and moisture.

- Volatile short-chain fatty acids have unpleasant odor.



2. Oxidative Rancidity:

- It is oxidation of fat or oil catalyzed by exposure to oxygen, light and/or heat producing peroxide derivatives which on decomposition give substances, e.g., peroxides, aldehydes, ketones and dicarboxylic acids that are toxic and have bad odor.
- This occurs due to oxidative addition of oxygen at the unsaturated double bond of unsaturated fatty acid of oils.

3. Ketonic Rancidity:

- It is due to the contamination with certain fungi such as *Asperigillus niger* on fats such as coconut oil.
- Ketones, fatty aldehydes, short chain fatty acids and fatty alcohols are formed.

- Moisture accelerates ketonic rancidity.

1. Iodine number (or value):

- Definition: It is the number of grams of iodine absorbed by 100 grams of fat or oil.

- Uses:It is a measure for the degree of unsaturation of the fat, as a natural property for it.

2. Saponification number (or value):

- Definition: It is the number of milligrams of KOH required to completely saponify one gram of fat.
- Uses: Since each carboxyl group of a fatty acid reacts with one mole of KOH during saponification, therefore, the amount of alkali needed to saponify certain weight of fat depends upon the number of fatty acids present per weight.
- Thus, fats containing short-chain acids will have more carboxyl groups per gram than long chain fatty acids and consume more alkali, i.e., will have higher saponification number

3. Acids Number (or value):

- Definition: It is the number of milligrams of KOH required to neutralize the free fatty acids present in one gram of fat.
- Uses: It is used for detection of hydrolytic rancidity because it measures the amount of free fatty acids present.

4. Reichert- Meissl Number (or value):

- Definition: It is the number of milliliters of 0.1 N KOH required to neutralize the water-soluble fatty acids distilled from 5 grams of fat. Short-chain fatty acid (less than 10 carbons) is distillated by steam.
- Uses: This studies the natural composition of the fat and is used for detection of fat adulteration.
- Butter that has high percentage of short-chain fatty acids has highest Reichert- Meissl number compared to margarine.

5. Acetyl Number (or value):

- Definition: It is number of milligrams of KOH needed to neutralize the acetic acid liberated from hydrolysis of 1 gram of acetylated fat (hydroxy fat reacted with acetic anhydride).
- Uses: The natural or rancid fat that contains fatty acids with free hydroxyl groups are converted into acetylated fat by reaction with acetic anhydride.
- Thus, acetyl number is a measure of number of hydroxyl groups present. It is used for studying the natural properties of the fat and to detect adulteration and rancidity.

Waxes

Waxes are solid simple lipids containing a monohydric alcohol (with a higher molecular weight than glycerol) esterified to long-chain fatty acids. Examples of these alcohols are palmitoyl alcohol, cholesterol, vitamin A or D.

Properties of waxes

- Waxes are insoluble in water, but soluble in fat solvents and are negative for acrolein test. (**Acrolein test** is used to detect the presence of glycerol or fat. When fat is treated strongly in the presence of a dehydrating agent like potassium bisulphate ($KHSO_4$), the glycerol portion of the molecule is dehydrated to form an unsaturated aldehyde, **acrolein** that has a pungent irritating odour.)

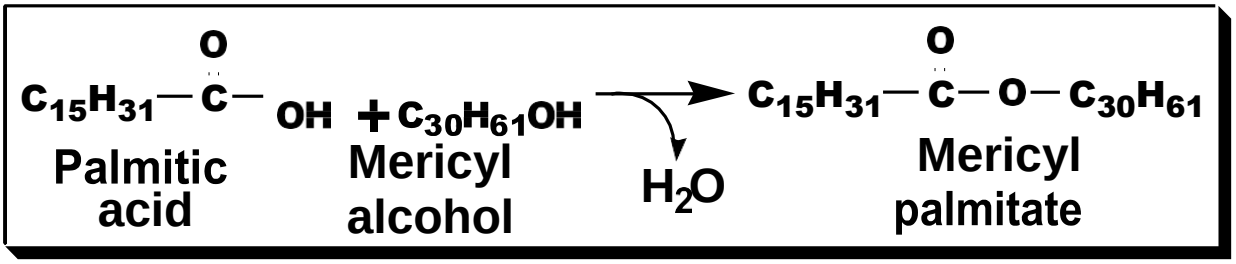
- Waxes are not easily hydrolyzed as the fats and are indigestible by lipases and are very resistant to rancidity.
- Thus they are of no nutritional value.

Type of Waxes

Waxes are widely distributed in nature such as the secretion of certain insects as bees- wax, protective coatings of the skins and furs of animals and leaves and fruits of plants. They are classified into true-waxes and wax-like compounds as follows:

1. True waxes

Bees-wax is secreted by the honeybees that use it to form the combs. It is a mixture of waxes with the chief constituent is mericyl palmitate.



2. Wax-like compounds

- Cholesterol esters: Lanolin (or wool fat) is prepared from the wool-associated skin glands and is secreted by sebaceous glands of the skin.
- It is very complex mixture, contains both free and esterified cholesterol, e.g., cholesterol-palmitate and other sterols.

Compound Lipids

They are lipids that contain additional substances, e.g., sulfur, phosphorus, amino group, carbohydrate, or proteins beside fatty acid and alcohol.

Compound or conjugated lipids are classified into the following types according to the nature of the additional group:

1. Phospholipids
2. Glycolipids.

3. Lipoproteins

4. Sulfolipids and amino lipids.

1. Phospholipids

Phospholipids or phosphatides are compound lipids, which contain phosphoric acid group in their structure.

Importance:

1. They are present in large amounts in the liver and brain as well as blood. Every animal and plant cell contains phospholipids.
2. The membranes bounding cells and subcellular organelles are composed mainly of phospholipids. Thus, the transfer of substances through these membranes is controlled by properties of phospholipids.
3. They are important components of the lipoprotein coat essential for secretion and transport of plasma lipoprotein complexes. Thus, they are lipotropic agents that prevent fatty liver.
4. Myelin sheath of nerves is rich with phospholipids.
5. Important in digestion and absorption of neutral lipids and excretion of cholesterol in the bile.
6. Important function in blood clotting and platelet aggregation.
7. They provide lung alveoli with surfactants that prevent its irreversible collapse.
8. Important role in signal transduction across the cell membrane.
9. Phospholipase A2 in snake venom hydrolyses membrane phospholipids into hemolytic lysolecithin or lysocephalin.
10. They are source of polyunsaturated fatty acids for synthesis of eicosanoids.

Sources: They are found in all cells (plant and animal), milk and egg-yolk in the form of lecithins.
Structure: phospholipids are composed of:

1. Fatty acids (a saturated and an unsaturated fatty acid).
2. Nitrogenous base (choline, serine, threonine, or ethanolamine).
3. Phosphoric acid.
4. Fatty alcohols (glycerol, inositol or sphingosine).

Classification of Phospholipids

They are classified into 2 groups according to the type of the alcohol present into two types:
A-Glycerophospholipids: They are regarded as derivatives of phosphatidic acids that are the simplest type of phospholipids and include:

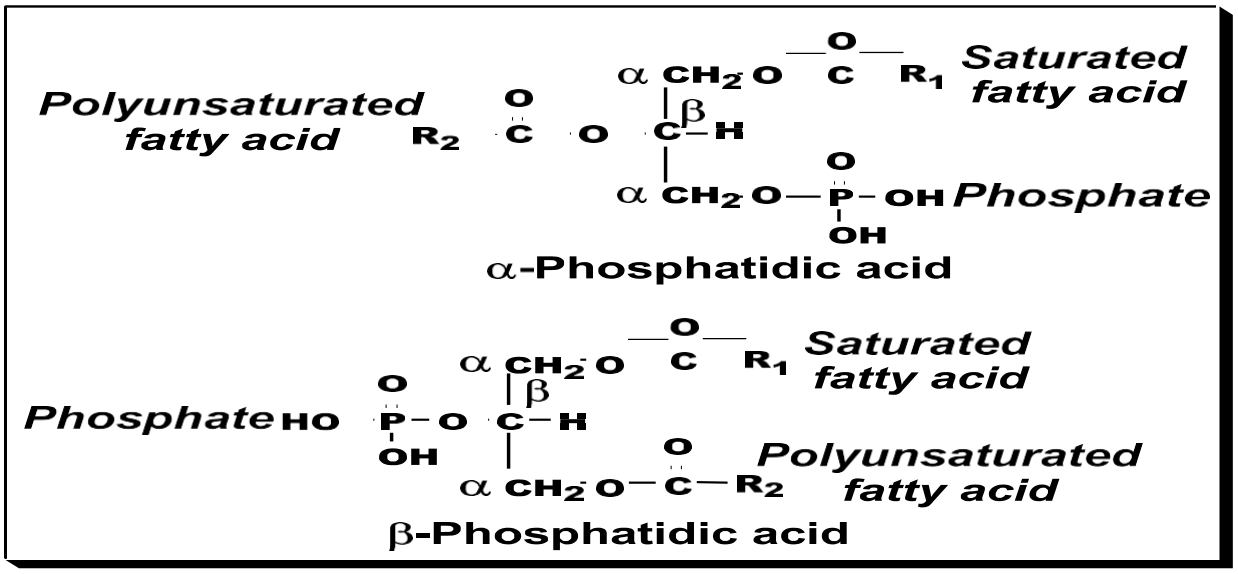
1. Phosphatidic acids.
2. Lecithins
3. Cephalins.
4. Plasmalogens.
5. Inositides.
6. Cardiolipin.

B-Sphingophospholipids: They contain sphingosine as an alcohol and are named Sphingomyelins.

A. Glycerophospholipids

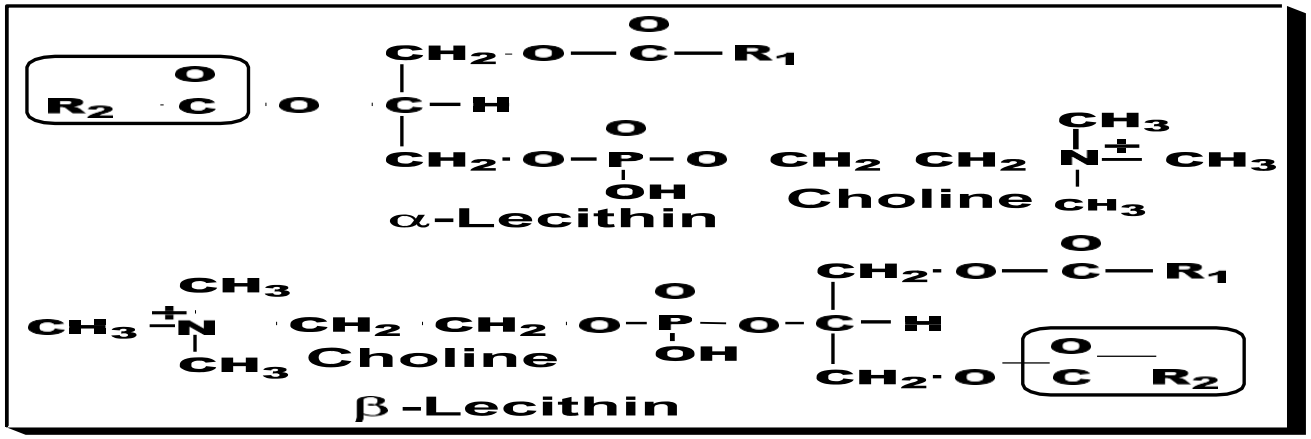
1. Phosphatidic acids:

They are metabolic intermediates in synthesis of triglycerides and glycerophospholipids in the body and may have function as a second messenger. They exist in two forms according to the position of the phosphate



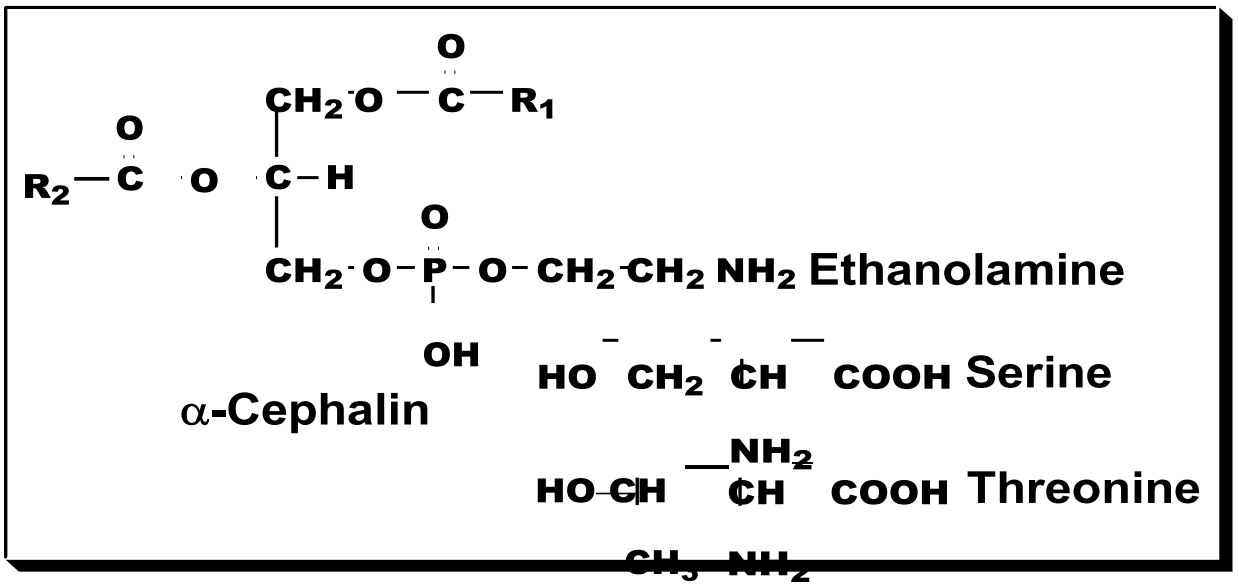
2. Lecithins:

- Lecithins are glycerophospholipids that contain choline as a base beside phosphatidic acid. They exist in 2 forms. Lecithins are a common cell constituent obtained from brain, egg yolk, or liver. Lecithins are important in the metabolism of fat by the liver.
- Structure: Glycerol is connected at C2 or C3 with a polyunsaturated fatty acid, at C1 with a saturated fatty acid, at C3 or C2 by phosphate to which the choline base is connected. The common fatty acids in lecithins are stearic, palmitic, oleic, linoleic, linolenic, clupandonic or arachidonic acids.



3.Cephalins (or Kephalins):

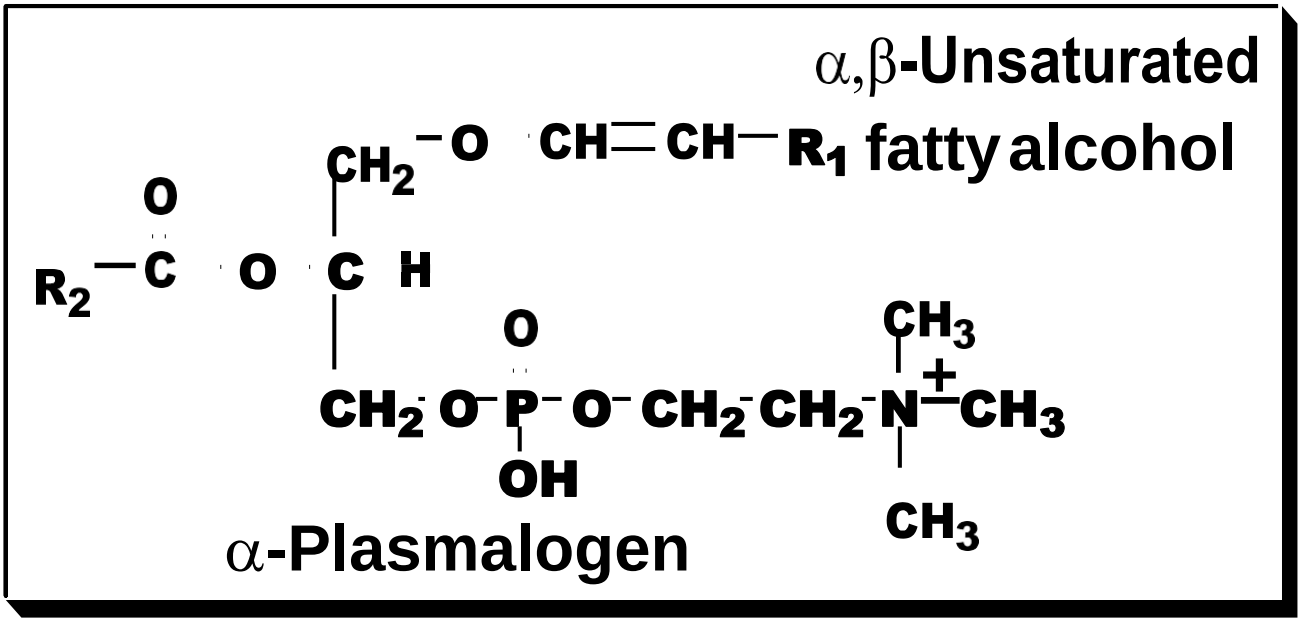
- They are phosphatidyl-ethanolamine or serine. Cephalins occur in association with lecithins in tissues and are isolated from the brain (Kephale = head).



- Structure: Cephalins resemble lecithins in structure except that choline is replaced by ethanolamine, serine or threonine amino acids.

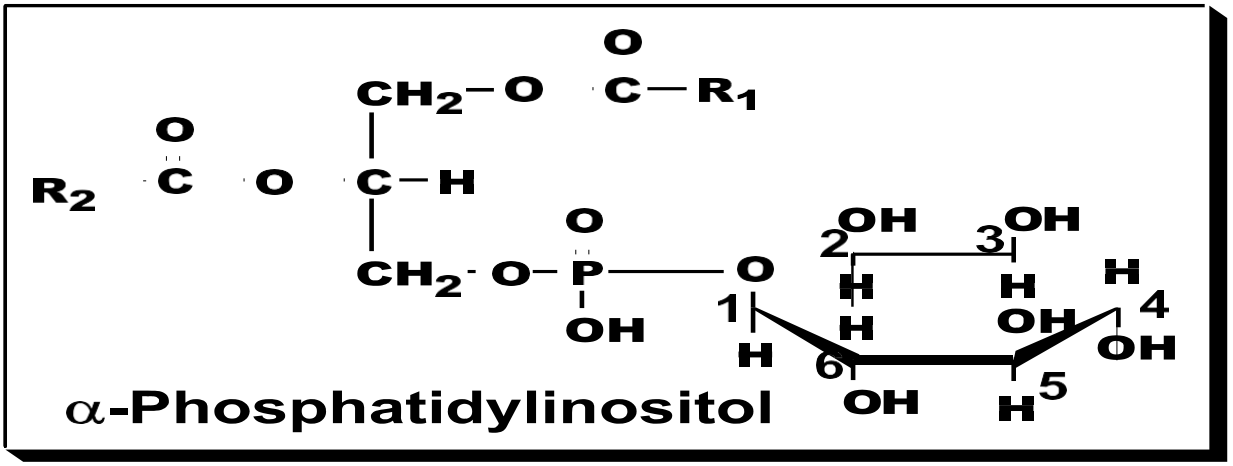
4. Plasmalogens:

- Plasmalogens are found in the cell membrane phospholipids fraction of brain and muscle (10% of it is plasmalogens), liver, semen and eggs.
- Structure: Plasmalogens resemble lecithins and cephalins in structure but differ in the presence of unsaturated fatty alcohol rather than a fatty acid at C1 of the glycerol connected by ether bond.
- At C2 there is an unsaturated long-chain fatty acid, however, it may be a very short-chain fatty acid



5. Inositides:

They are similar to lecithins or cephalins but they have the cyclic sugar alcohol, inositol as the base. They are formed of glycerol, one saturated fatty acid, one unsaturated fatty acid, phosphoric acid and inositol



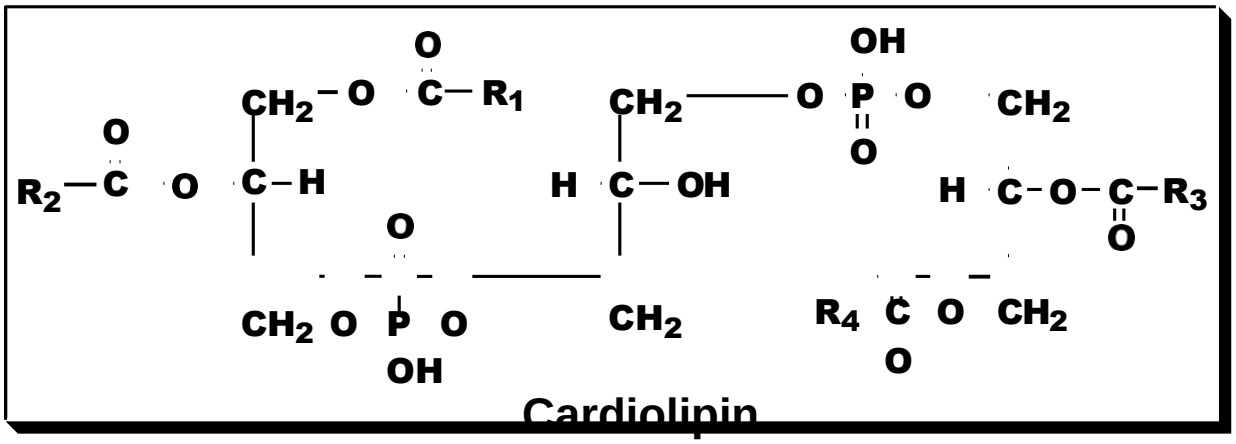
- Source: Brain, tissues. etc.,

Function:

- Phosphatidyl inositol is a major component of cell membrane phospholipids particularly at the inner leaflet of it.
- They play a major role as second messengers during signal transduction for certain hormone..
- On hydrolysis by phospholipase C, phosphatidyl-inositol-4,5-diphosphate produces diacyl-glycerol and inositol-triphosphate both act to liberate calcium from its intracellular stores to mediate the hormone effects.

6. Cardiolipins:

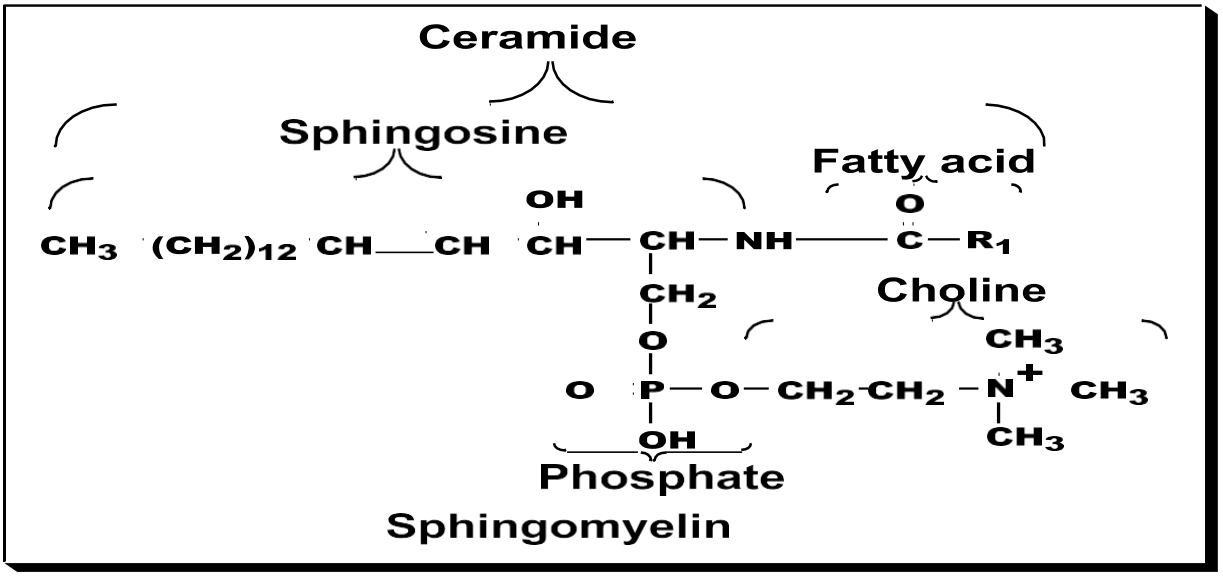
- They are diphosphatidyl-glycerol. They are found in the inner membrane of mitochondria initially isolated from heart muscle (cardio). It is formed of 3 molecules of glycerol, 4 fatty acids and 2 phosphate groups.
- Function: Used in serological diagnosis of autoimmunity diseases.



B. Sphingophospholipids

1. Sphingomyelins

- Sphingomyelins are found in large amounts in brain and nerves and in smaller amounts in lung, spleen, kidney, liver and blood.
- Structure: Sphingomyelins differ from lecithins and cephalins in that they contain sphingosine as the alcohol instead of glycerol, they contain two nitrogenous bases: sphingosine itself and choline.
- Thus, sphingomyelins contain sphingosine base, one long-chain fatty acid, choline and phosphoric acid.
- To the amino group of sphingosine the fatty acid is attached by an amide linkage.
- Ceramide This part of sphingomyelin in which the amino group of sphingosine is attached to the fatty acid by an amide linkage.
- Ceramides have been found in the free state in the spleen, liver and red cells.



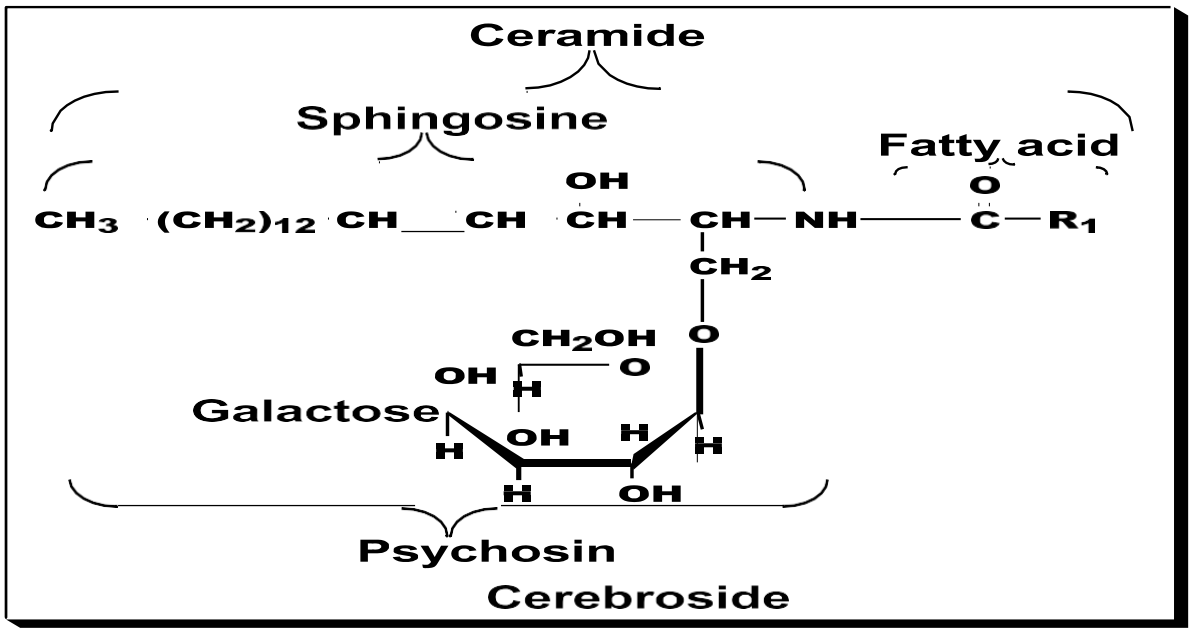
2. Glycolipids

- They are lipids that contain carbohydrate residues with sphingosine as the alcohol and a very long-chain fatty acid (24 carbon series).
- They are present in cerebral tissue, therefore are called cerebrosides
- Classification: According to the number and nature of the carbohydrate residue(s) present in the glycolipids the following are

1. Cerebrosides. They have one galactose molecule (galactosides).
2. Sulfatides. They are cerebrosides with sulfate on the sugar (sulfated cerebrosides).
3. Gangliosides. They have several sugar and sugaramine residues.

1. Cerebrosides:

- Occurrence: They occur in myelin sheath of nerves and white matter of the brain tissues and cellular membranes. They are important for nerve conductance.
- Structure: They contain sugar, usually □-galactose and may be glucose or lactose, sphingosine and fatty acid, but no phosphoric acid**.**

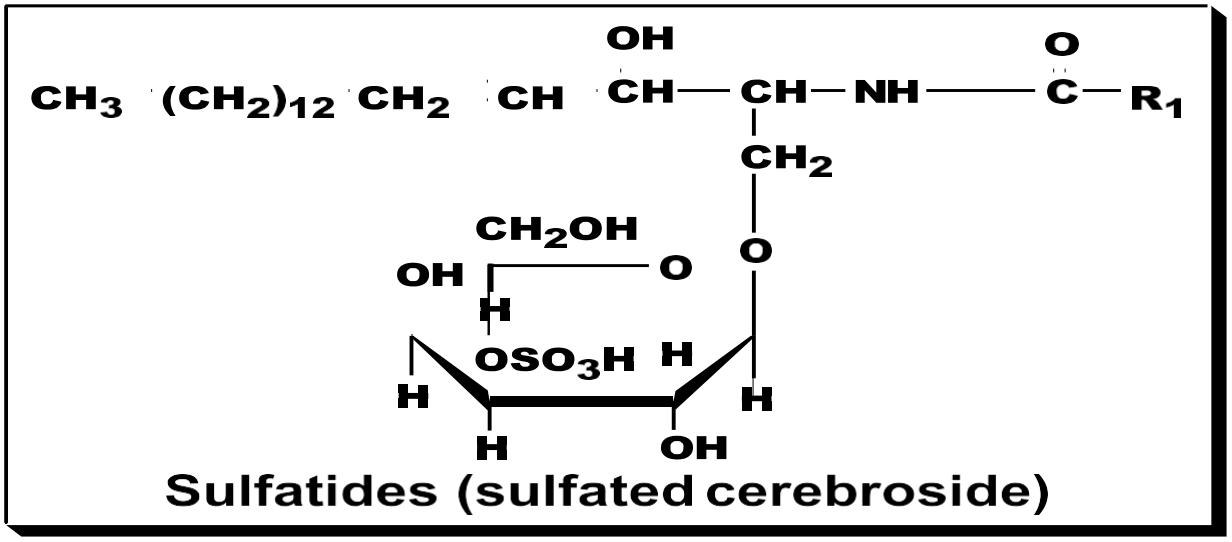


- Types: According to the type of fatty acid and carbohydrate present, there are 4 different types of cerebrosides isolated from the white matter of cerebrum and in myelin sheaths of nerves. Rabbit cerebrosides contain stearic acid.

1. Kerasin contains lignoceric acid (24 carbons) and galactose.
2. Cerebron (Phrenosin) contains cerebronic acid (2-hydroxylignoceric acid) and galactose.
3. Nervon contains nervonic acid (unsaturated lignoceric acid at C15) and galactose.
4. Oxynervon contains oxynervonic acid (2-hydroxynervonic acid) and galactose.

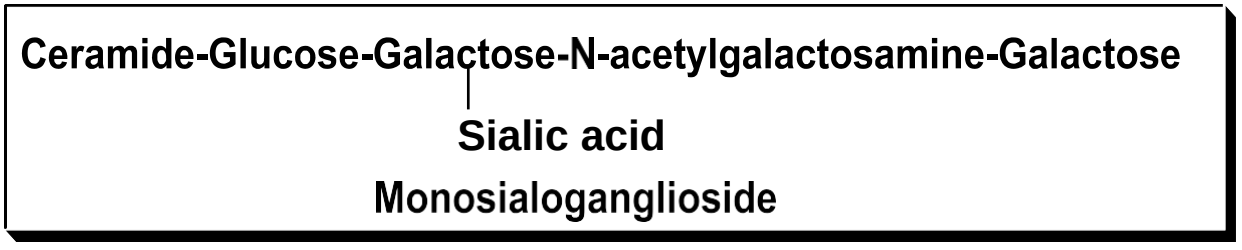
2. Sulfatides:

- They are sulfate esters of kerasin or phrenosin in which the sulfate group is usually attached to the –OH group of C3 or C6 of galactose. Sulfatides are usually present in the brain, liver, muscles and testes.



3. Gangliosides

- They are more complex glycolipids that occur in the gray matter of the brain, ganglion cells, and RBCs. They transfer biogenic amines across the cell membrane and act as a cell membrane receptor.
- Gangliosides contain sialic acid (N-acetylneuraminic acid), ceramide (sphingosine + fatty acid of 18-24 carbon atom length), 3 molecules of hexoses (1 glucose + 2 galactose) and hexosamine. The most simple type of it the monosialoganglioside,. It works as a receptor for cholera toxin in the human intestine.



3. Lipoproteins

Lipoproteins are lipids combined with proteins in the tissues. The lipid component is phospholipid, cholesterol or triglycerides. The holding bonds are secondary bonds.

1. Structural lipoproteins: These are widely distributed in tissues being present in cellular and subcellular membranes. In lung tissues acting as a surfactant in a complex of a protein and lecithin. In the eye, rhodopsin of rods is a lipoprotein complex.
2. Transport lipoproteins: These are the forms present in blood plasma. They are composed of a protein called apolipoprotein and different types of lipids. (Cholesterol, cholesterol esters, phospholipids and triglycerides). As the lipid content increases, the density of plasma lipoproteins decreases

Plasma lipoproteins

a) Chylomicrons:

They have the largest diameter and the least density. They contain 1-2% protein only and 98-99% fat. The main lipid fraction is triglycerides absorbed from the intestine and they contain small amounts of the absorbed cholesterol and phospholipids.

b) Very low-density lipoproteins (VLDL):

Their diameter is smaller than chylomicrons. They contain about 7-10% protein and 90-93% lipid. The lipid content is mainly triglycerides formed in the liver. They contain phospholipid and cholesterol more than chylomicrons.

c) Low-density lipoproteins (LDL):

They contain 10-20% proteins in the form of apolipoprotein B. Their lipid content varies from 80-90%. They contain about 60% of total blood cholesterol and 40% of total blood phospholipids. As their percentage increases, the liability to atherosclerosis increases.

d) High-density lipoproteins (HDL):

They contain 35-55% proteins in the form of apolipoprotein A. They contain 45- 65% lipids formed of cholesterol (40% of total blood content) and phospholipids (60% of total blood content). They act as cholesterol scavengers, as their percentage increases, the liability to atherosclerosis decreases. They are higher in females than in males. Due to their high protein content they possess the highest density.

Derived lipids

These are the substances derived from simple and compound lipids by hydrolysis. These include fatty acids, alcohols, mono- and diglycerides, steroids, terpenes and carotenoids.

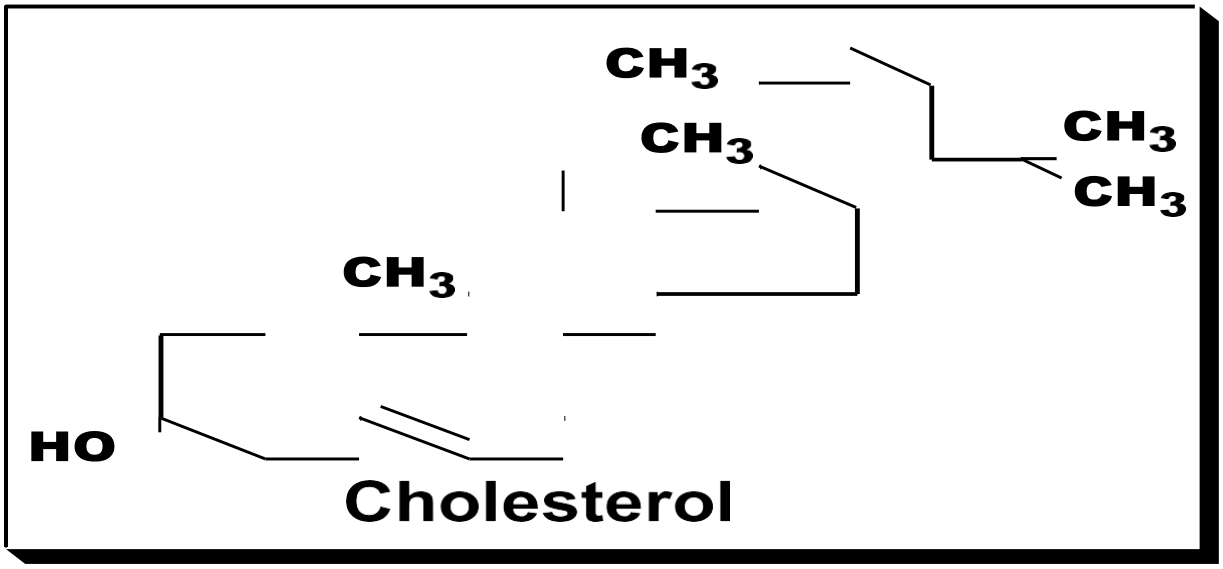
Cholesterol

- Importance: It is the most important sterol in animal tissues as free alcohol or in an esterified form (with linoleic, oleic, palmitic acids or other fatty acids).
- Steroid hormones, bile salts and vitamin D are derivatives from it.
- Tissues contain different amounts of it that serve a structural and metabolic role, e.g., adrenal cortex content is 10%, whereas, brain is 2%, others 0.2-0.3%.
- Source: It is synthesized in the body from acetyl-CoA (1gm/day, cholesterol does not exist in plants) and is also taken in the diet (0.3 gm/day as in, butter, milk, egg yolk, brain, meat and animal fat).

Physical properties

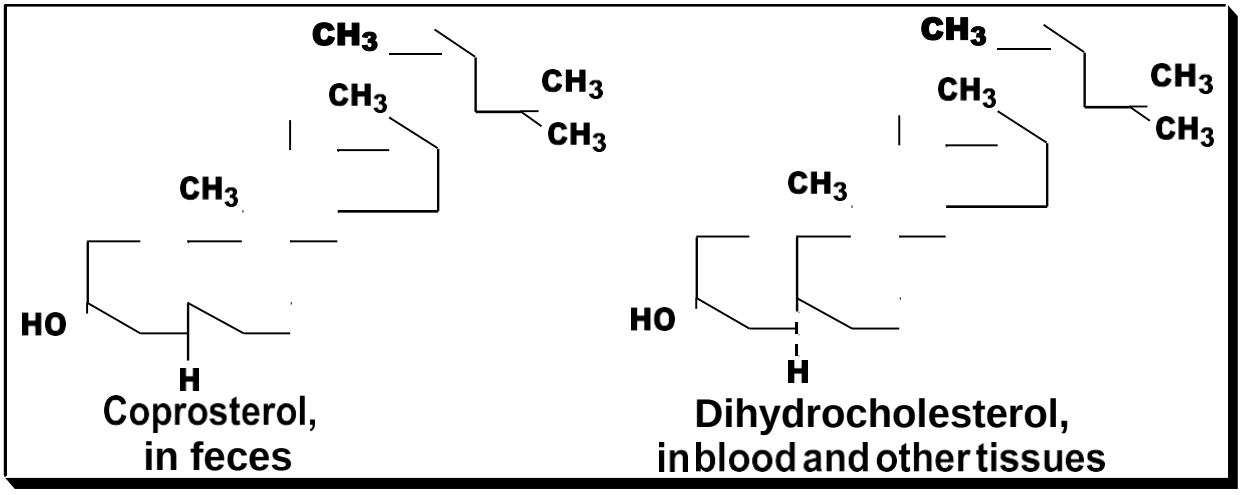
- It has a hydroxyl group on C3, a double bond between C5 and C6, 8 asymmetric carbon atoms and a side chain of 8 carbon atoms.
- It is found in all animal cells, corpus luteum and adrenal cortex, human brain (17% of the solids).

- In the blood (the total cholesterol amounts about 200 mg/dL of which 2/3 is esterified, chiefly to unsaturated fatty acids while the remainder occurs as the free cholesterol.

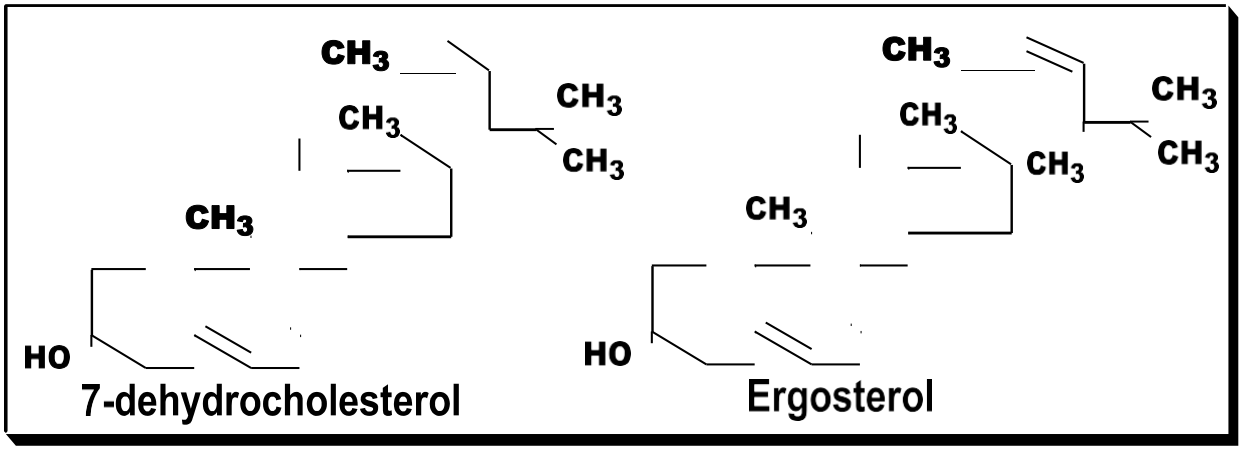


Chemical properties

- Intestinal bacteria reduce cholesterol into coprosterol and dihydrocholesterol.
- It is also oxidized into 7-Dehydrocholesterol and further unsaturated cholesterol with a second double bond between C7 and C8. When the skin is irradiated with ultraviolet light 7-dehydrocholesterol is converted to vitamin D3. This explains the value of sun light in preventing rickets.



- Ergosterol differs from 7-dehydrocholesterol in the side chain.
- Ergosterol is converted to vitamin D2 by irradiation with UV Ergosterol and 7-dehydrocholesterol are called Pro-vitamins D or precursors of vitamin D.
- It was first isolated from ergot, a fungus then from yeast. Ergosterol is less stable than cholesterol (because of having 3 double bonds).



TERPENES

Terpenes are a large and diverse class of organic compounds, produced by a variety of plants, particularly conifers, though also by some insects such as termites or swallowtail butterflies, which emit **terpenes** from their osmeteria (defensive organ).

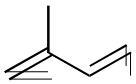
Terpenes and terpenoids are the most important constituents in essential oils

These hydrocarbons and their oxygenated derivatives have lesser than 40 carbon atoms.

The simplest terpenes are called monoterpenes with formula $C_{10}H_{16}$ those with the formula $C_{15}H_{24}$ are called as sesquiterpenes, with C20H32 as diterpenes and with $C_{30}H_{48}$ as triterpenes.

Terpenes with 40 carbon atoms (or tetraterpenes) include compounds called carotenoids

Terpenes are built from C_5 isoprene units



isoprene

(2-methyl-1,3-butadiene)

Terpenes are the building blocks for a number of molecules such as Phytol tail on chlorophyll, Ubiquinone tail, Gibberellins, Cytokinin and Steroids

Membrane Lipids

- Phospholipids are made up of a glycerol backbone with a hydrophilic head region containing a phosphate group and a hydrophobic tail region containing a saturated fatty acid and an unsaturated fatty acid.
- The fact that it has both types of fatty acids ensures the cell membrane is fluid.

- Cholesterol is interspersed throughout the cell membrane to add rigidity to it.

- It also allows the cell membrane to stay fluid over a wider range of temperatures.

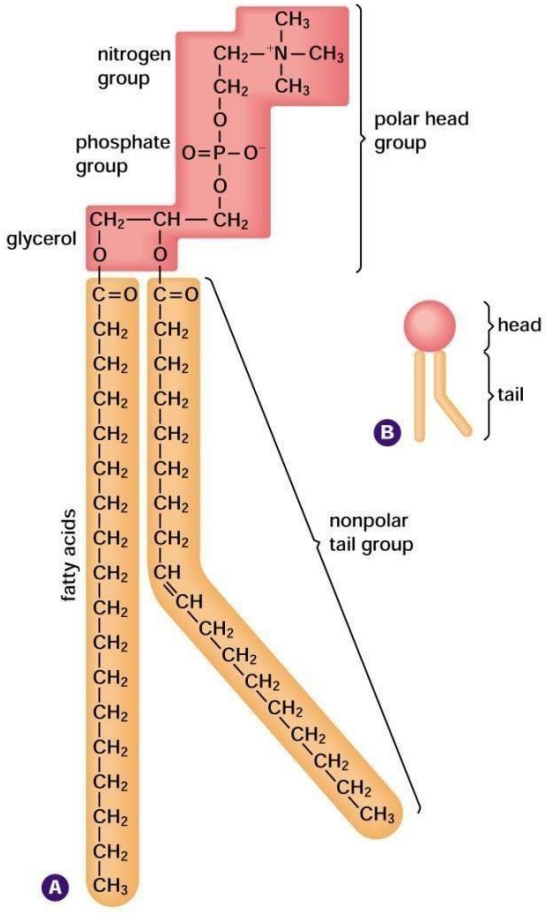


Fig. Head and tail region of phospholipid

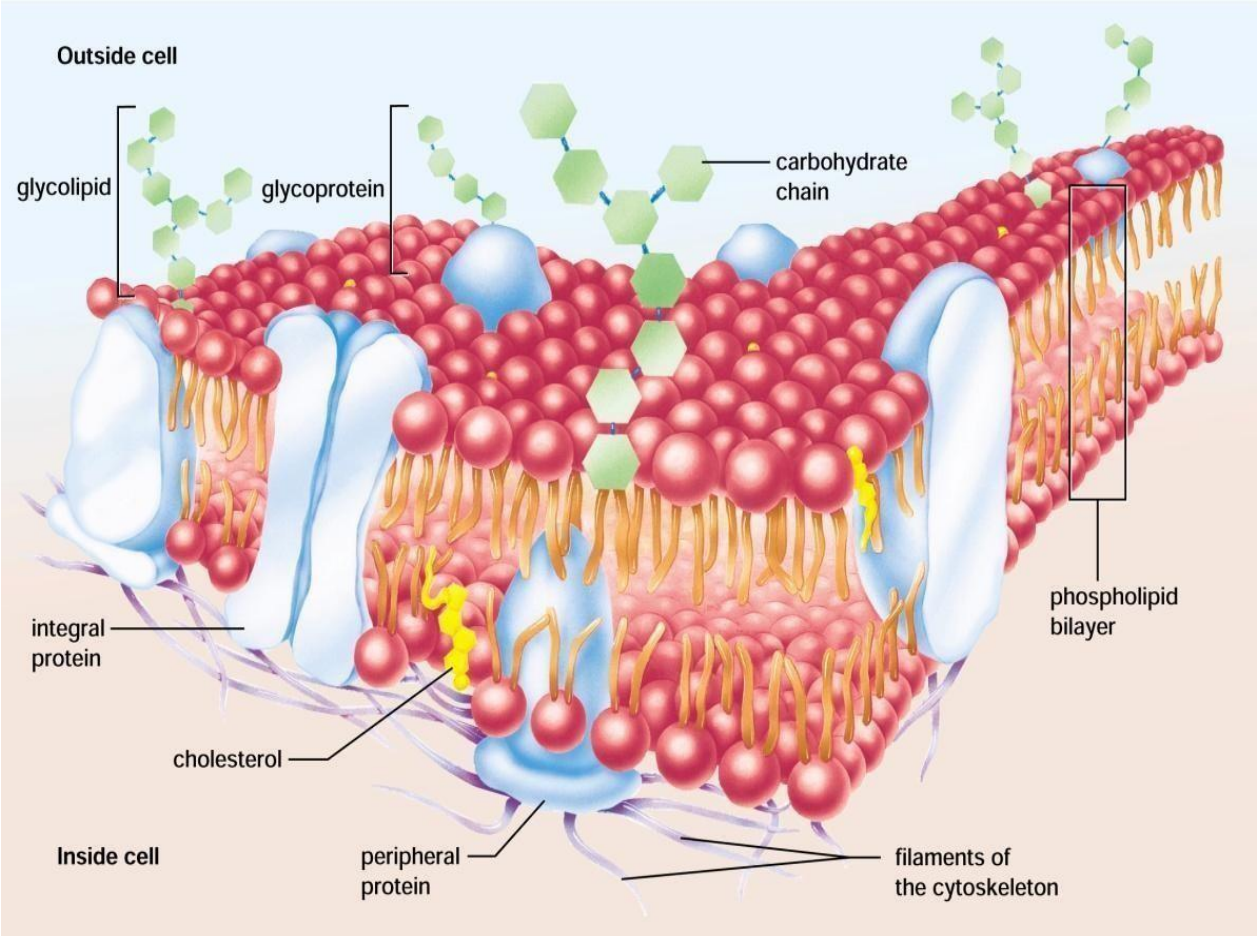


Fig. Lipid bilayer

Lipid membrane has four main functions:

1. Allow the transport of raw materials into the cell
2. Allow the transport of manufactured products and wastes out of the cell
3. Prevent the entry of unwanted material into the cell

Steroid Hormones

Steroid hormones are derived from cholesterol and differ only in the ring structure and side chains attached to it.

Types of steroid hormones

- Glucocorticoids - cortisol is the major representative in most mammals
- Mineralocorticoids - aldosterone being most prominent
- Androgens such as testosterone
- Estrogens including estradiol and estrone
- Progestogens (also known a progestins) such as progesterone

Functions of Steroid Hormones

Steroid hormones play important roles in:

1. carbohydrate regulation (glucocorticoids)
2. mineral balance (mineralocorticoids)
3. reproductive functions (gonadal steroids)

Steroids also play roles in inflammatory responses, stress responses, bone metabolism, cardiovascular fitness, behavior etc.,

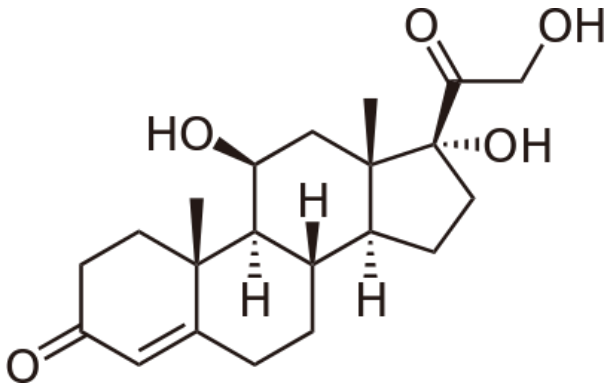
Steroid hormone synthesis

All steroid hormones are derived from cholesterol. A series of enzymatic steps in the mitochondria and endoplasmic reticulum of steroidogenic tissues convert cholesterol into all of the other steroid hormones and intermediates.

Glucocorticoids

The name glucocorticoid is composed from its role in regulation of glucose metabolism The primary glucocorticoid in humans is cortisol and produced in adrenal cortex.

Functions - promote gluconeogenesis; favor breakdown of fat and protein (fuel mobilization); anti-inflammatory

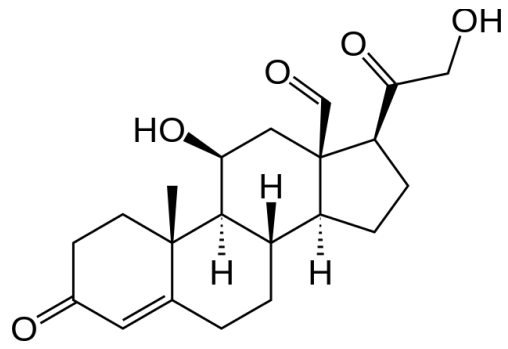


cortisol

Mineralocorticoids

Steroid hormones that affect electrolyte balance. The primary human mineralocorticoid, aldosterone is produced in adrenal cortex.

Functions - maintains blood volume and blood pressure by increasing sodium reabsorption by kidney



Aldosterone

Gonadal

steroids

Androgens

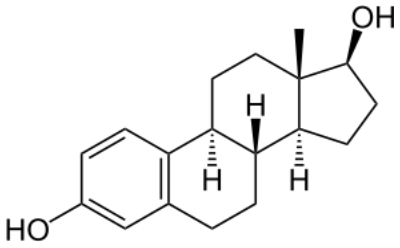
Produced in testes primarily but weak androgens in adrenal cortex.

Functions - Development of male secondary sex characteristics and prevents bone resorption

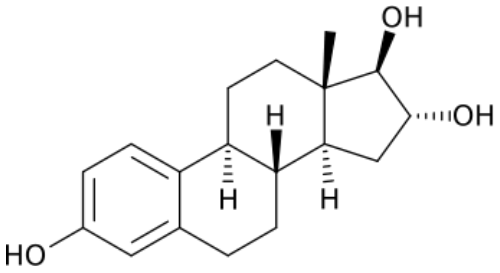
Estrogen

Produced in ovaries primarily but also in adipose cells of males and females

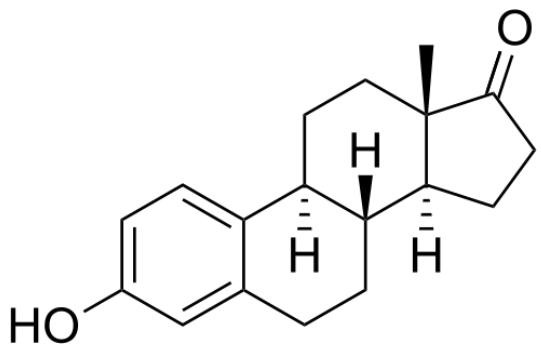
Functions - Development of female secondary sex characteristics; prevents bone resorption



Estradiol (E2)



Estriol (E3)



Estrone (E1)

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SCHOOL OF BIO AND CHEMICAL ENGINEERING
DEPARTMENT OF BIOTECHNOLOGY

UNIT – IV– BIOCHEMISTRY AND BIOMOLECULES – SBTA1302

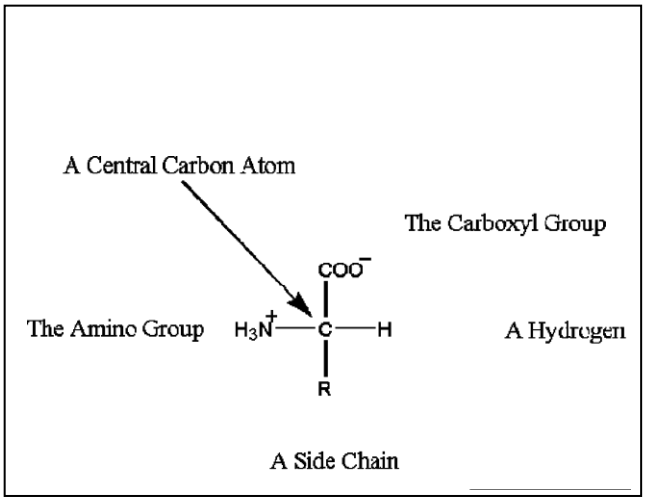
1. Introduction

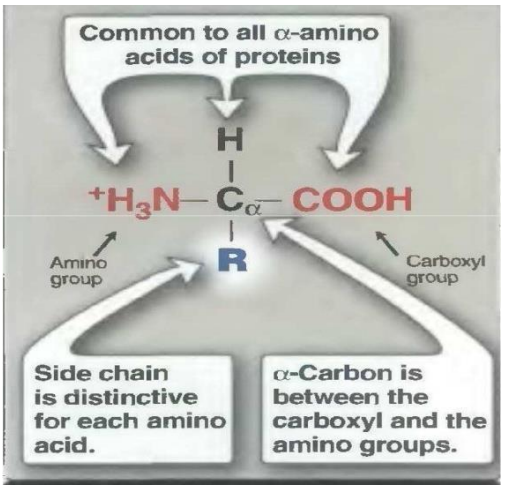
Amino acids

Amino acids are the building blocks of proteins. It has both an amino group ($-NH_2$) and an acid group (-COOH). There are more than 300 amino acids that occur in nature and many more yet to be characterized. Only 20 of the amino acids are found in the protein structure. The genetic code exists for only the 20 amino acids.

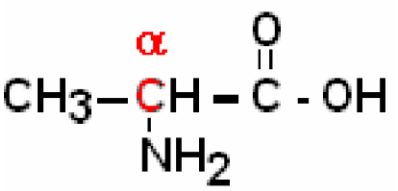
Structure of amino acids

Each amino acid has 4 different groups attached to α-carbon (which is carbon atom next to carboxylic group – COOH).

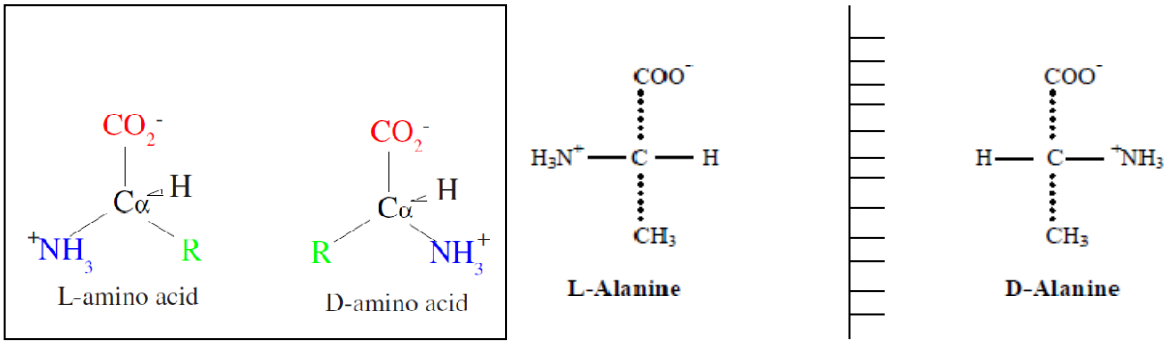




The properties of each amino acid are determined by its specific side chain (R-groups). R-groups vary in structure, size, electric charge and solubility in water from one amino acid to other. Amino acids found in proteins are α-amino acids. The amino group is always found on the carbon adjacent to the carboxyl group.



Chirality – amino acids (except glycine) have a tetrahedral C_α bonded to four different chemical groups. As a result of this, amino acids are optically active or chiral. Common amino acids are all L stereoisomers. "CO-R-N" mnemonic is used for distinguishing L and D stereoisomers. Looking down the H-C bond, CO-R-N spelled clockwise indicates the L stereoisomer.



There is no definitive answer on why the L isomer is found in proteins. Both D and L isomers have identical energies. Repetitive substructure in proteins (helices, sheets, turns) require all amino acids to have the same configuration. Apparently, living systems evolved from L amino acids based upon an initial random choice.

Amino acid names are often abbreviated as either 3 letters or single letter.

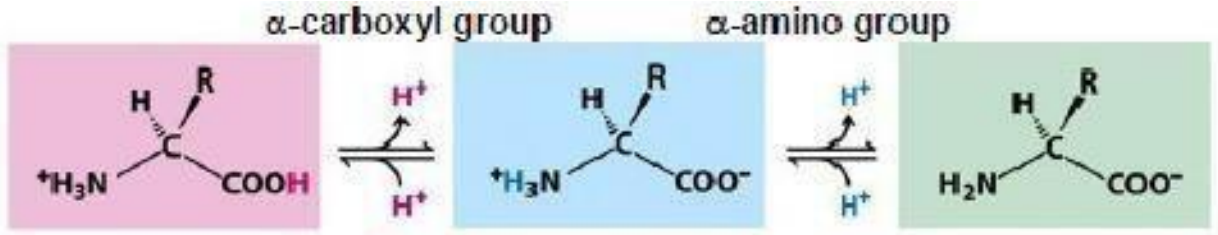
Names and Abbreviations of the Standard Amino Acids

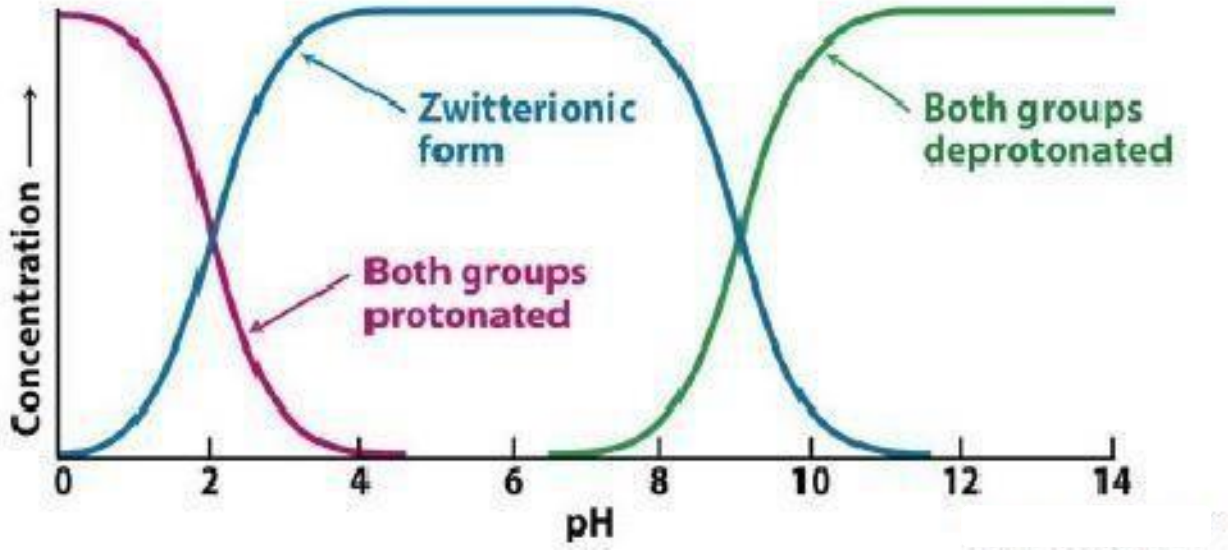
Amino Acid	Three-Letter Abbreviation	One-Letter Abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

Zwitter Ions

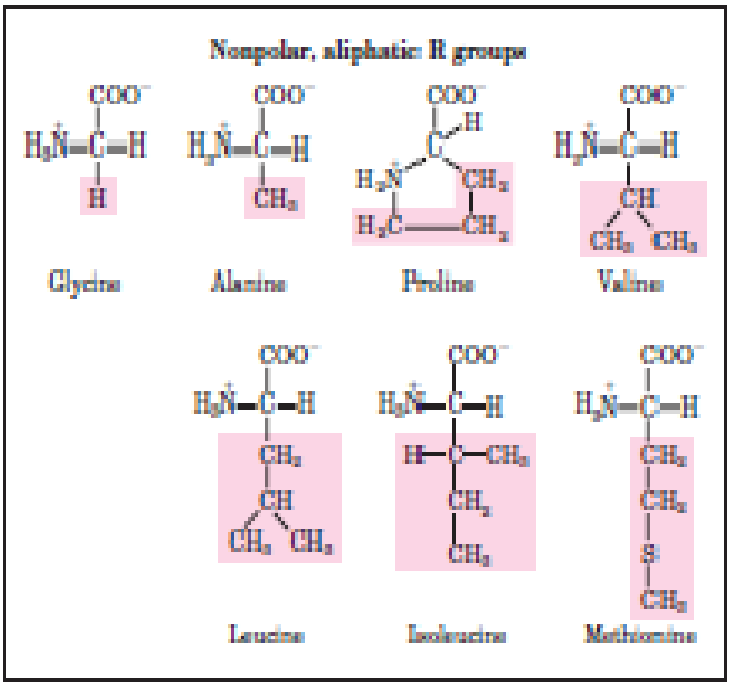
At physiological pH of 7, the carboxyl group of an amino acid is in its conjugate base form ($-COO^-$) and the amino group is in its conjugate acid form ($-NH_3^+$). Thus each amino acid can behave as either an acid or a base. Such molecules which can behave both like an acid and a base are termed amphoteric molecules. Also molecules that bear both positive and negative charges are called zwitter ions.

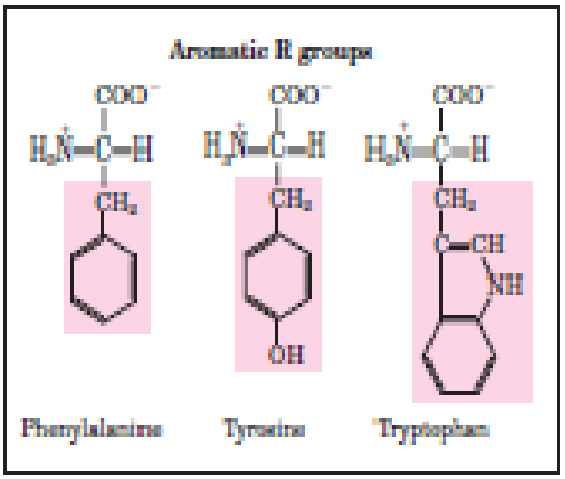
Amino acids contain ionizable groups. The predominant ionic form of these molecules in solution therefore depends on the pH. At acidic pH (pH <7) the carboxyl group (-COOH) is uncharged and the ammonium group ($-NH_3^+$) is protonated. Therefore the net charge on the amino acid is positive (+1). At basic pH (pH >7) the carboxyl group ($-COO^-$) loses its proton and becomes charged and the amino group ($-NH_2$) becomes uncharged by losing the proton. Therefore the net charge on the amino acid is negative (-1). The pH at which the amino acid has no net charge and is electrically neutral is called as the isoelectric point (pI).

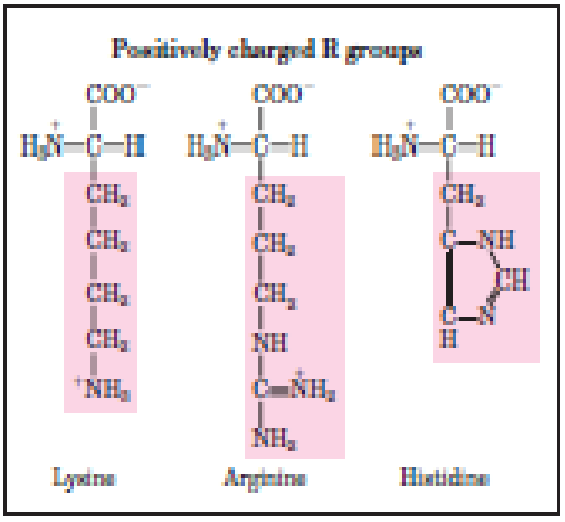


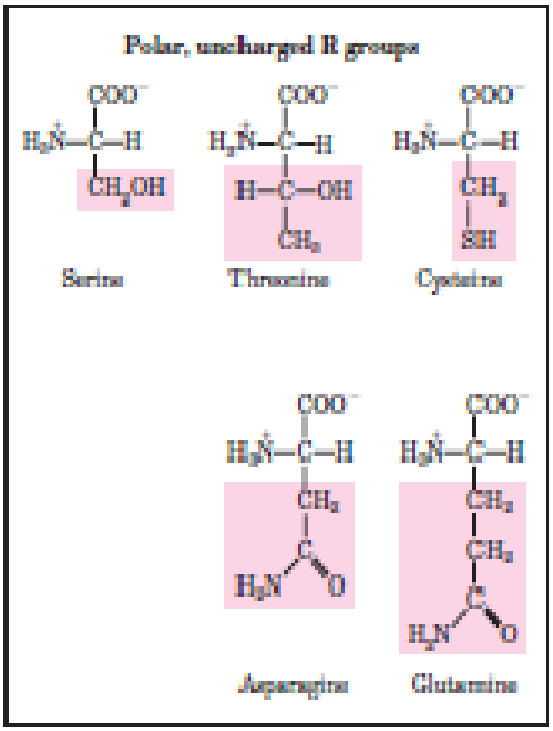


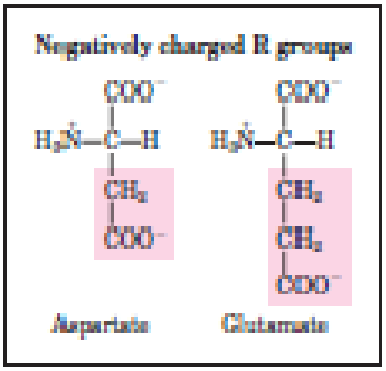
Structure of amino acids











Classification of amino acids

I) **Nutritional classification** – Based on the ability of the body to synthesize amino acids, they can be classified as essential and non-essential amino acids.

1. **Essential amino acids** – These amino acids cannot be formed (synthesized) in the body and so, it is essential to be included in the diet. Their deficiency in the body affects growth, health and protein synthesis. The following amino acids are essential:

1. Valine
2. Isoleucine
3. Lysine
4. Leucine
5. Methionine.
6. Tryptophan
7. Threonine
8. Phenyl alanine

2. **Semi-essential amino acids** – These amino acids are formed in the body but not in sufficient amount for body requirements especially in children. The semi-essential amino acids are:

1. Arginine
2. Histidine

3. **Non-essential amino acids** – The amino acids that can be synthesized in the body by regular metabolism in enough amounts are called as non-essential amino acids. They need not be included in the diet. They are:

1. Glycine
2. Alanine
3. Cysteine
4. Tyrosine
5. Proline
6. Serine
7. Asparagine
8. Glutamine
9. Aspartic acid
10. Glutamic acid.

II) Protein and Non-protein amino acids

1. **Proteinogenic amino acids –** The amino acids that are included in the genetic code are described as "proteinogenic". With a few exceptions only these amino acids can be included in the protein structure by translation. These amino acids are also called as the standard amino acids. They are

    - Alanine
    - Glycine
    - Proline
    - Valine
    - Leucine
    - Isoleucine
    - Tryptophan
    - Phenylalanine
    - Methionine
    - Serine
    - Threonine
    - Cysteine
    - Asparagine
    - Glutamine
    - Tyrosine
    - Histidine
    - Lysine
    - Arginine
    - Aspartic acid
    - Glutamic acid

2. **Non-protein amino acids –** The amino acids that are not found in protein structures are termed non-protein amino acids. More than 700 amino acids have been detected in living systems which belong to this class. They are also called as non-standard amino acids. These amino acids are formed as metabolic intermediates (eg., ornithine and citrulline). Non-standard amino acids arise from post translational modification.

    - Hydroxylysine
    - Hydroxyproline
    - Methylhistidine
    - Methylarginine
    - Phosphoserine
    - Formylmethionine

    Some amino acid derivatives also fall under these category (eg. Histamine, Catecholamine, Gamma amino butyric acid (GABA) and Dopamine).

Proteins – Introduction

Proteins are polypeptides, which are made up of many amino acids linked together as a linear chain. The structure of an amino acid contains a amino group, a carboxyl group, and a R group which is usually carbon based and gives the amino acid it's specific properties. These properties determine the interactions between atoms and molecules, which are: van der Waals force between temporary dipoles, ionic interactions between charged groups, and attractions between polar groups.

Proteins form the very basis of life. They regulate a variety of activities in all known organisms, from replication of the genetic code to transporting oxygen, and are generally responsible for regulating the cellular machinery and determining the phenotype of an organism. Proteins accomplish their tasks in the body by three-dimensional tertiary and quaternary interactions between various substrates. The functional properties depend upon the proteins three-dimensional structure. The (3D) structures arise because particular sequences of amino acids in a polypeptide chain fold to generate, from linear chains, compact domains with specific structures. The folded domains either serve as modules for larger assemblies or they provide specific catalytic or binding sites.

Protein Types and Functions

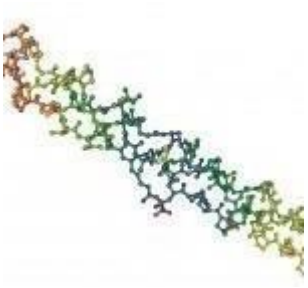
Role	Examples	Functions
Digestive enzyme	Amylase, lipase, pepsin	Break down nutrients in food into small pieces that can be readily absorbed
Transport	Hemoglobin	Carry substances throughout the body in blood or lymph
Structure	Actin, tubulin, keratin	Build different structures, like the cytoskeleton
Hormone signaling	Insulin, glucagon	Coordinate the activity of different body systems
Defense	Antibodies	Protect the body from foreign pathogens
Contraction	Myosin	Carry out muscle contraction
Storage	Legume storage proteins, egg white (albumin)	Provide food for the early development of the embryo or the seedling

Protein classification

Protein classification based on shape

On the basis of their shape, proteins may be divided into two classes: fibrous and globular.

Fibrous proteins



Collagen

They have primarily mechanical and structural functions, providing support to the cells as well as the whole organism. These proteins are insoluble in water as they contain, both internally and on their surface, many hydrophobic amino acids. The presence on their surface of hydrophobic amino acids facilitates their packaging into very complex supramolecular structures. In this regard, it should be noted that their polypeptide chains form long filaments or sheets, where in most cases only one type of secondary structure, that repeats itself, is found. In vertebrates, these proteins provide external protection, support and shape; in fact, thanks to their structural properties, they ensure flexibility and/or strength. Some fibrous proteins, such as α-keratins, are only partially hydrolyzed in the intestine.

Here are some examples.

- Fibroin

  It is produced by spiders and insects. An example is that produced by the silkworm, *Bombyx mori*.

- Collagen

  The term “collagen” indicates not a single protein but a family of structurally related proteins (at least 29 different types), which constitute the main protein component of connective tissue, and more generally, the extracellular scaffolding of multicellular

organisms. In vertebrates, they represent about 25-30% of all proteins. They are found in different tissues and organs, such as tendons and the organic matrix of bone, where they are present in very high percentages, but also in cartilage and in the cornea of the eye. In the different tissues, they form different structures, each capable of satisfying a particular need. For example, in the cornea, the molecules are arranged in an almost crystalline array, so that they are virtually transparent, while in the skin they form fibers not very intertwined and directed in all directions, which ensure the tensile strength of the skin itself. Note: the different types of collagen have low nutritional value as deficient in several amino acids (in fact, they contain no tryptophan and low amount of the other essential amino acids). The gelatin used in food preparation is a derivative of collagen.

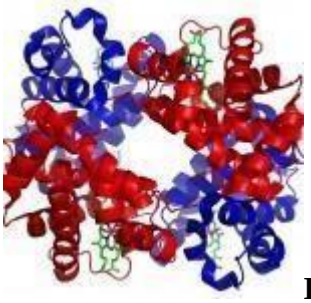
- α-Keratins

  They constitute almost the entire dry weight of nails, claws, beak, hooves, horns, hair, wool, and a large part of the outer layer of the skin. The different stiffness and flexibility of these structures is a consequence of the number of disulfide bonds that contribute, together with other binding forces, to stabilize the protein structure. And this is the reason why wool keratins, which have a low number of disulfide bonds, are flexible, soft and extensible, unlike claw and beak keratins that are rich in disulfide bonds.

- Elastin

  This protein provides elasticity to the skin and blood vessels, a consequence of its random coiled structure, that differs it from the structures of the α-keratins and collagens.

Globular proteins



Haemoglobin

Most of the proteins belong to this class. They have a compact and more or less spherical structure, more complex than fibrous proteins. In this regard, motifs, domains, tertiary and quaternary structures are found, in addition to the secondary structures. They are generally soluble in water but can also be found inserted into biological membranes (transmembrane proteins), thus in a hydrophobic environment. Unlike fibrous proteins, that have structural and mechanical functions, they act as:

- enzymes;
- hormones;
- membrane transporters and receptors;
- transporters of triglycerides, fatty acids and oxygen in the blood;
- immunoglobulins or antibodies;
- grain and legume storage proteins.

Examples of globular proteins are myoglobin, hemoglobin, and cytochrome c. At the intestinal level, most of the globular proteins of animal origin are hydrolyzed almost entirely to amino acids.

Protein classification based on solubility and chemical composition

On the basis of their chemical composition, proteins may be divided into two classes: simple and complex.

SIMPLE PROTEINS

Also known as homoproteins, they are made up of only amino acids. Simple proteins yield only amino acids on hydrolysis. Examples are plasma albumin, collagen, and keratin. These proteins are further classified based on their solubility in different solvents as well as their heat coagulability.

Albumins

- Albumins are readily soluble in water, dilute acids and alkalies, coagulated by heat.
- Seed proteins contain albumin in lesser quantities.
- Albumins may be precipitated out from solution using high salt concentration, a

process ‘called **'salting out'**.

- They are deficient in **glycine.**
- Serum albumin and ovalbumin (egg white) are examples.

Globulins

- Globulins are **insoluble or sparingly soluble in water**, but their solubility is greatly increased by the addition of neutral salts such as sodium chloride.
- These proteins are coagulated by heat.
- They are deficient in **methionine**.
- Serum globulin, fibrinogen, myosin of muscle and globulins of pulses are examples.

Prolamins

- Prolamins are insoluble in water but soluble in 70-80% aqueous alcohol.
- Upon hydrolysis they yield much proline and amide nitrogen, hence the name prolamin.
- They are deficient in **lysine**.
- Gliadin of wheat and zein of corn are examples of prolamins.

Glutelins

- Glutelins are insoluble in water and absolute alcohol but soluble in dilute alkalies and acids.
- They are plant proteins e.g., glutenin of wheat.

Histones

- Histones are small and stable basic proteins
- They contain fairly large amounts of basic amino acid, **histidine**.
- They are soluble in water, but insoluble in ammonium hydroxide.
- They are not readily coagulated by heat.
- They occur in **globin of hemoglobin and nucleoproteins**.

Protamines

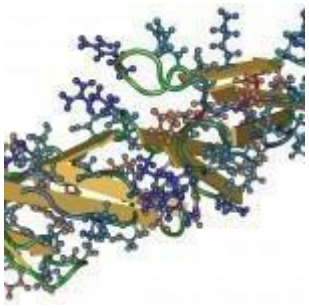
Protamines are the simplest of the proteins.

- They are soluble in water and are not coagulated by heat.
- They are basic in nature due to the presence of large quantities of arginine.
- Protamines are found in association with nucleic acid in the sperm cells of certain fish.
- Tyrosine and tryptophan are usually absent in protamines.

Albuminoids

- These are characterized by great stability and insolubility in water and salt solutions.
- These are called albuminoids because they are essentially similar to albumin and globulins.
- They are highly resistant to proteolytic enzymes.
- They are fibrous in nature and form most of the supporting structures of animals.
- They occur as chief constituent of exoskeleton structure such as hair, horn and nails.

CONJUGATED PROTEINS



Human Fibronectin

Sometimes also called heteroproteins, they contain in their structure a non-protein portion. These non-protein substances are known as prosthetic groups. The examples are glycoproteins, chromoproteins, nucleoproteins, mucoproteins, lipoproteins, metalloproteins and phosphoproteins.

Glycoproteins

They are proteins that covalently bind one or more carbohydrate units to the polypeptide backbone. Typically, the branches consist of not more than 15-20 carbohydrate units, where you can find arabinose, fucose (6-deoxygalactose), galactose, glucose, mannose, N-acetylglucosamine (GlcNAc, or NAG), and N-acetylneuraminic acid (Neu5Ac or NANA).

Examples of glycoproteins are: glycophorin, the best known among erythrocyte membrane glycoproteins; fibronectin, that anchors cells to the extracellular matrix through interactions on one side with collagen or other fibrous proteins, while on the other side with cell membranes; all blood plasma proteins, except albumin; immunoglobulins or antibodies.

Chromoproteins

They are proteins that contain colored prosthetic groups. Typical examples are: hemoglobin and myoglobin, which bind, respectively, one and four heme groups; chlorophylls, which bind a porphyrin ring with a magnesium atom at its centre; rhodopsins, which bind retinal.

Phosphoproteins

They are proteins that bind phosphoric acid to serine and threonine residues. Generally, they have a structural function, such as tooth dentin, or reserve function, such as milk caseins (alpha, beta, gamma and delta), and egg yolk phosvitin.

Nucleoproteins

- Nucleoproteins are simple basic proteins (protamines or histones) in salt combination with nucleic acids as the prosthetic group.
- They are the important constituents of nuclei and chromatin.

Mucoproteins

- These proteins are composed of simple proteins in combination with carbohydrates like mucopolysaccharides, which include hyaluronic acid and chondroitin sulphates.
- On hydrolysis, mucopolysaccharides yield more than 4% of amino-sugars, hexosamine and uronic acid e.g., ovomucoid from egg white.
- Soluble mucoproteins are neither readily denatured by heat nor easily precipitated by common protein precipitants like trichloroacetic acid or picric acid.
- The term glycoprotein is restricted to the protein that contains small amount of carbohydrate usually less than 4% hexosamine.

Lipoproteins

These are proteins conjugated with lipids such as neutral fat, phospholipids and cholesterol

Metalloproteins

- These are **metal-binding proteins**.
- A globulin, termed **transferrin** is capable of combining with **iron, copper and zinc.** This protein constitutes 3% of the total plasma protein.
- Another example is **ceruloplasmin**, which contains **copper.**

Derived proteins

These are proteins derived by partial to complete hydrolysis from the simple or conjugated proteins by the action of acids, alkalies or enzymes. They include two types of derivatives, primary-derived proteins and secondary-derived proteins.

Primary-derived proteins

These protein derivatives are formed by processes causing only slight changes in the protein molecule and its properties. There is little or no hydrolytic cleavage of peptide bonds.

Proteans

- Proteans are insoluble products formed by the action of water, dilute acids and enzymes.
- These are particularly formed from globulins but are insoluble in dilute salt solutions
- e.g., myosan from myosin, fibrin from fibrinogen.

Metaproteins

- These are formed by the action of acids and alkalies upon protein.
- They are insoluble in neutral solvents.

Coagulated proteins

Coagulated proteins are insoluble products formed by the action of heat or alcohol on natural proteins e.g., cooked meat and cooked albumin.

Secondary-derived proteins

- These proteins are formed in the progressive hydrolytic cleavage of the peptide bonds of protein molecule.
- They are roughly grouped into **proteoses, peptones and peptides according to average molecular weight.**
- Proteoses are hydrolytic products of proteins, which are soluble in water and are not coagulated by heat.
- Peptones are hydrolytic products, which have simpler structure than proteoses.
- They are soluble in water and are not coagulated by heat.
- Peptides are composed of relatively few amino acids.
- They are water-soluble and not coagulated by heat.
- The complete hydrolytic decomposition of the natural protein molecule into amino acids generally progresses through successive stages as follows:

Protein -----> Protean ------ >Metaprotein

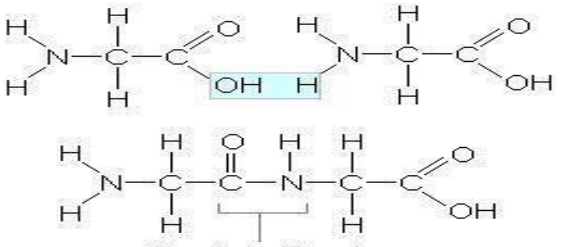
Proteoses ------>Peptones ------->Peptides ----- >amino acids

Protein Structures: Primary, Secondary, Tertiary, Quaternary

- Proteins are the largest and most varied class of biological molecules, and they show the greatest variety of structures. Many have intricate three-dimensional folding patterns that result in a compact form, but others do not fold up at all ("natively unstructured proteins") and exist in random conformations. The function of proteins depends on their structure, and defining the structure of individual proteins is a large part of modern Biochemistry and Molecular Biology. To understand how proteins fold, we will start with the basics of structure, and progress through to structures of increasing complexity.

Peptide Bonds

- To make a protein, amino acids are connected together by a type of amide bond called a “peptide bond”. This bond is formed between the alpha amino group of one amino acid and the carboxyl group of another in a condensation reaction. When two amino acids join, the result is called a dipeptide, three gives a tripeptide, etc. Multiple amino acids result in a polypeptide (often shortened to “peptide”). Because water is lost in the course of creating the peptide bond, individual amino acids are referred to as “amino acid residues” once they are incorporated. Another property of peptides is polarity: the two ends are different. One end has a free amino group (called the “N- terminal”) and the other has a free carboxyl group (“C-terminal”).

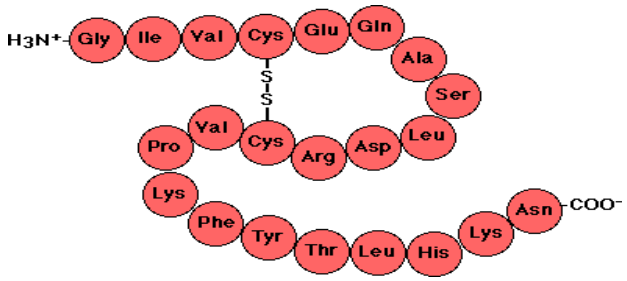


A molecule of water is removed from two glycine amino acids to form a peptide bond.

- In the natural course of making a protein, polypeptides are elongated by the addition of amino acids to the C-terminal end of the growing chain. Conventionally, peptides are written N-terminal first; therefore gly-ser is not the same as ser-gly or GS is not the same as SG. The connection gives rise to a repeating pattern of “NCC-NCC-NCC…” atoms along the length of the molecule. This is referred to as the “backbone” of the peptide. If stretched out, the side chains of the individual residues project outwards from this backbone.
- The peptide bond is written as a single bond, but it actually has some characteristics of a double bond because of the resonance between the C-O and C-N bonds.
- This means that the six atoms involved are coplanar, and that there is not free rotation around the C–N axis. This constrains the flexibility of the chain and prevents some folding patterns.

Primary Structure of Proteins

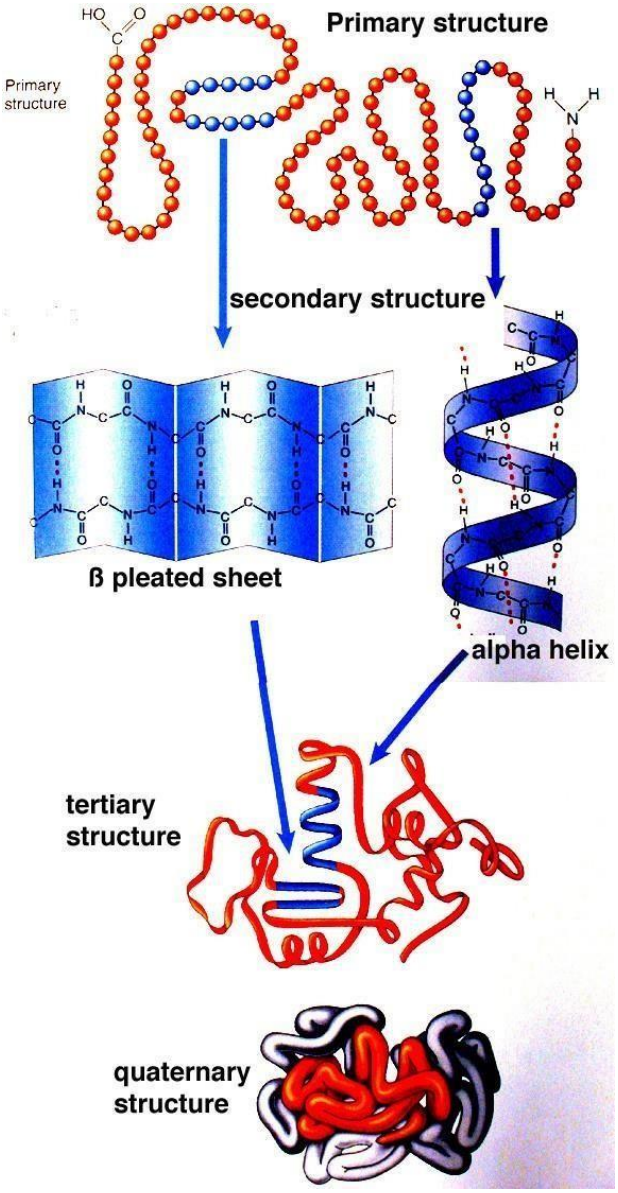
- It is convenient to discuss protein structure in terms of four levels (primary to quaternary) of increasing complexity. Primary structure is simply the sequence of residues making up the protein. Thus primary structure involves only the covalent bonds linking residues together.



- The minimum size of a protein is defined as about 50 residues; smaller chains are referred to simply as peptides. So the primary structure of a small protein would consist of a sequence of 50 or so residues. Even such small proteins contain hundreds of atoms and have molecular weights of over 5000 Daltons (Da). There is no theoretical maximum size, but the largest protein so far discovered has about 30,000 residues. Since the average molecular weight of a residue is about 110 Da, that single chain has a molecular weight of over 3 million Daltons.

Secondary Structure

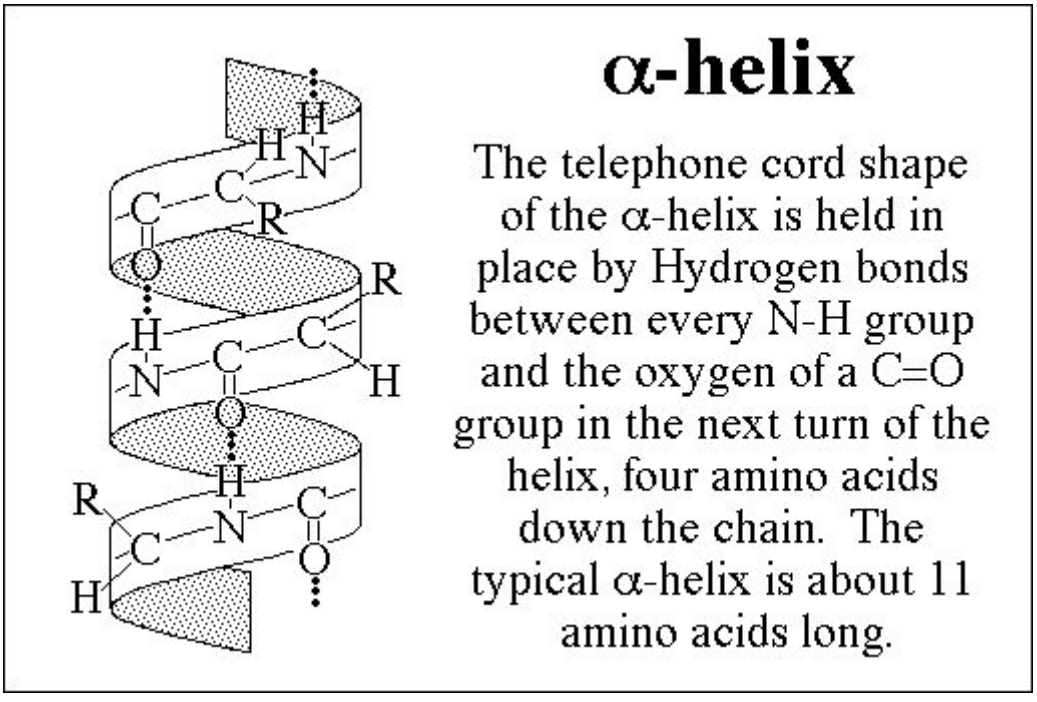
- This level of structure describes the local folding pattern of the polypeptide backbone and is stabilized by hydrogen bonds between N-H and C=O groups. Various types of secondary structure have been discovered, but by far the most common are the orderly repeating forms known as the alpha helix and the beta sheet.



- Alpha helix, as the name implies, is a helical arrangement of a single polypeptide chain, like a coiled spring. In this conformation, the carbonyl and N-H groups are oriented parallel to the axis. Each carbonyl is linked by a hydrogen bond to the N-H of a residue located 4 residues further on in the sequence within the same chain. All C=O and N-H groups are involved in hydrogen bonds, making a fairly rigid cylinder. The alpha helix has precise dimensions: 3.6 residues per turn, 0.54 nm per turn. The side chains project outward and contact any solvent, producing a structure something

like a bottle brush or a round hair brush. An example of a protein with many helical structures is the keratin that makes up human hair.

- The structure of a beta sheet is very different from the structure of an alpha helix. In a beta sheet, the polypeptide chain folds back on itself so that polypeptide strands like side by side, and are held together by hydrogen bonds, forming a very rigid structure. Again, the polypeptide N-H and C=O groups form hydrogen bonds to stabilize the structure, but unlike the alpha helix, these bonds are formed between neighbouring polypeptide (beta) strands. Generally the primary structure folds back on itself in either a parallel or antiparallel arrangement, producing a parallel or antiparallel beta sheet. In this arrangement, side chains project alternately upward and downward from the sheet. The major constituent of silk (silk fibroin) consists mainly of layers of beta sheet stacked on top of each another.



- Other types of secondary structure. While the alpha helix and beta sheet are by far the most common types of structure, many others are possible. These include various

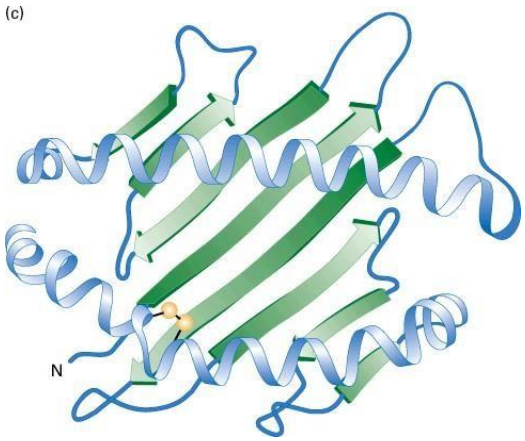
loops, helices and irregular conformations. A single polypeptide chain may have different regions that take on different secondary structures. In fact, many proteins have a mixture of alpha helices, beta sheets, and other types of folding patterns to form various overall shapes.

- What determines whether a particular part of a sequence will fold into one or the other of these structures? A major determinant is the interactions between side chains of the residues in the polypeptide. Several factors come into play: steric hindrance between nearby large side chains, charge repulsion between nearby similarly-charged side chains, and the presence of proline. Proline contains a ring that constrains bond angles so that it will not fit exactly into an alpha helix or beta sheet. Further, there is no H on one peptide bond when proline is present, so a hydrogen bond cannot form. Another major factor is the presence of other chemical groups that interact with each other. This contributes to the next level of protein structure, the tertiary structure.

Tertiary Structure

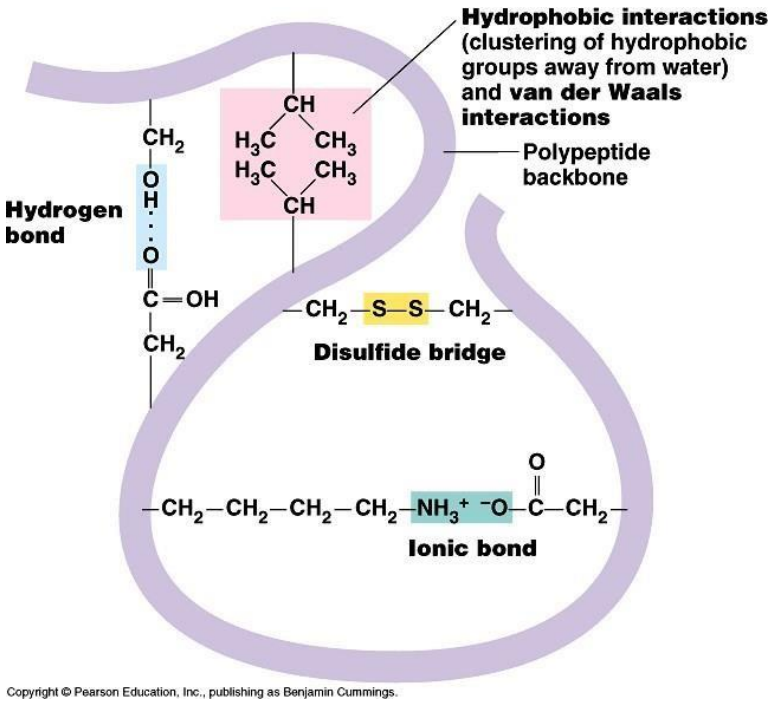
- This level of structure describes how regions of *secondary* structure fold together – that is, the 3D arrangement of a polypeptide chain, including alpha helices, beta sheets, and any other loops and folds. Tertiary structure results from interactions between side chains, or between side chains and the polypeptide backbone, which are often distant in sequence. Every protein has a particular pattern of folding and these can be quite complex.

- Whereas secondary structure is stabilized by H-bonding, all four “weak" forces contribute to tertiary structure. Usually, the most important force is hydrophobic interaction (or hydrophobic bonds). Polypeptide chains generally contain both hydrophobic and hydrophilic residues. Much like detergent micelles, proteins are most stable when their hydrophobic parts are buried, while hydrophilic parts are on



the surface, exposed to water. Thus, more hydrophobic residues such as trp are often surrounded by other parts of the protein, excluding water, while charged residues such as asp are more often on the surface.

- Other forces that contribute to tertiary structure are ionic bonds between side chains, hydrogen bonds, and van der Waals forces. These bonds are far weaker than covalent bonds, and it takes multiple interactions to stabilize a structure.
- There is one covalent bond that is also involved in tertiary structure, and that is the disulfide bond that can form between cysteine residues. This bond is important only in non-cytoplasmic proteins since there are enzyme systems present in the cytoplasm to remove disulfide bonds.
- Visualization of protein structures Because the 3D structures of proteins involve thousands of atoms in complex arrangements, various ways of depicting them so they are understood visually have been developed, each emphasizing a different property of the protein. Software tools have been written to depict proteins in many different ways, and have become essential to understanding protein structure and function.



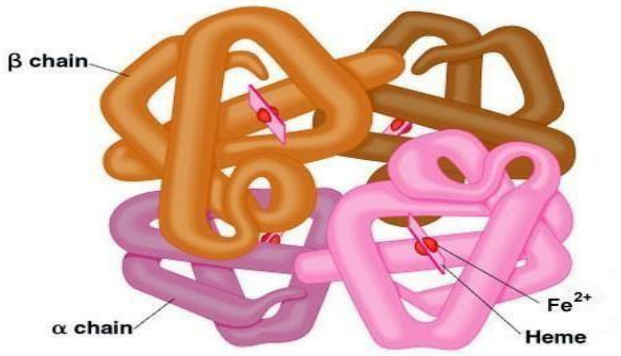
Structural Domains of Proteins

- Protein structure can also be described by a level of organization that is distinct from the ones we have just discussed. This organizational unit is the protein “domain" and the concept of domains is extremely important for understanding tertiary structure. A domain is a distinct region (sequence of amino acids) of a protein, while a structural domain is an independently-folded part of a protein that folds into a stable structure. A protein may have many domains, or consist only of a single domain. Larger proteins generally consist of connected structural domains. Domains are often separated by a loosely folded region and may create clefts between them.

Quaternary Structure

- Some proteins are composed of more than one polypeptide chain. In such proteins, quaternary structure refers to the number and arrangement of the individual polypeptide chains. Each polypeptide is referred to as a subunit of the protein. The same forces and bonds that create tertiary structure also hold subunits together in a stable complex to form the complete protein.
- Individual chains may be identical, somewhat similar, or totally different. As examples, CAP protein is a dimer with two identical subunits, whereas hemoglobin is a tetramer containing two pairs of non-identical (but similar)

subunits. It has 2 a subunits and 2 b subunits. Secreted proteins often have subunits that are held together by disulfide bonds. Examples include tetrameric antibody molecules that commonly have two larger subunits and two smaller subunits (“heavy chains" and “light chains") connected by disulfide bonds and noncovalent forces.



- In some proteins, intertwined a helices hold subunits together; these are called coiled- coils. This structure is stabilized by a hydrophobic surface on each a helix that is created by a heptameric repeat pattern of hydrophilic/hydrophobic residues. The sequence of the protein can be represented as “abcdefgabcdefgabcdefg…" with positions “a" and “d" filled with hydrophobic residues such as A, V, L etc. Each a helix has a hydrophobic surface that therefore matches the other. When the two helices coil around each other, those surfaces come together, burying the hydrophobic side chains and forming a stable structure. An example of such a protein is myosin, the motor protein found in muscle that allows contraction.

Protein Folding

- How and why do proteins naturally form secondary, tertiary and quaternary structures? This question is a very active area of research and is certainly not completely understood. A folded, biologically-active protein is considered to be in its “native" state, which is generally thought to be the conformation with least free energy.
- Proteins can be unfolded or “denatured" by treatment with solvents that disrupt weak bonds. Thus organic solvents that disrupt hydrophobic

interactions, high concentrations of urea or guanidine that interfere with H-bonding, extreme pH or even high temperatures, will all cause proteins to unfold. Denatured proteins have a random, flexible conformation and usually lack biological activity. Because of exposed hydrophobic groups, they often aggregate and precipitate. This is what happens when you fry an egg.

- If the denaturing condition is removed, some proteins will re-fold and regain activity. This process is called “renaturation." Therefore, all the information necessary for folding is present in the primary structure (sequence) of the protein. During renaturation, the polypeptide chain is thought to fold up into a loose globule by hydrophobic effects, after which small regions of secondary structure form into especially favorable sequences. These sequences then interact with each other to

  stabilize intermediate structures before the final conformation is attained.
- Many proteins have great difficulty renaturing, and proteins that assist other proteins to fold are called “molecular chaperones." They are thought to act by reversibly masking exposed hydrophobic regions to prevent aggregation during the multi-step folding process. Proteins that must cross membranes (eg. mitochondrial proteins) must stay unfolded until they reach their destination, and molecular chaperones may protect and assist during this process.

Nucleic Acids – Introduction

The first isolation of what we now refer to as **DNA** was accomplished by Johann Friedrich Miescher 1870. He reported finding a weakly acidic substance of unknown function in the nuclei of human white blood cells, and named this material "nuclein". A few years later, Miescher separated nuclein into protein and nucleic acid components. In the 1920's nucleic acids were found to be major components of chromosomes, small gene-carrying bodies in the nuclei of complex cells.

Elemental analysis of nucleic acids showed the presence of phosphorus, in addition to the usual C, H, N & O. Unlike proteins, nucleic acids contained no sulfur. Complete hydrolysis of chromosomal nucleic acids gave inorganic phosphate, 2-deoxyribose (a previously unknown sugar) and four different heterocyclic bases (shown in the following diagram). To reflect the

unusual sugar component, chromosomal nucleic acids are called deoxyribonucleic acids, abbreviated DNA. Analogous nucleic acids in which the sugar component is ribose are termed ribonucleic acids, abbreviated RNA. The acidic character of the nucleic acids was attributed to the phosphoric acid moiety.

Their functions include:

1. Serving as energy stores for future use in phosphate transfer reactions. These reactions are predominantly carried out by ATP.

2. Forming a portion of several important coenzymes such as NAD^+, $NADP^+$, FAD and coenzyme A.

3. Serving as mediators of numerous important cellular processes such as second messengers in signal transduction events. The predominant second messenger is cyclic-AMP (cAMP), a cyclic derivative of AMP formed from ATP.

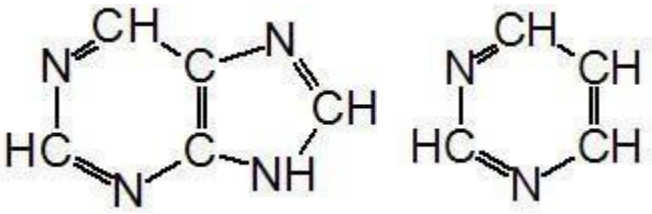
4. Serving as neurotransmitters and as signal receptor ligands. Adenosine can function as an inhibitory neurotransmitter, while ATP also affects synaptic neurotransmission throughout the central and peripheral nervous systems. ADP is an important activator of platelet functions resulting in control of blood coagulation.

5. Controlling numerous enzymatic reactions through allosteric effects on enzyme activity.

6. Serving as activated intermediates in numerous biosynthetic reactions. These activated intermediates include S-adenosylmethionine (S-AdoMet or SAM) involved in methyl transfer reactions as well as the many sugar coupled nucleotides involved in glycogen and glycoprotein synthesis.

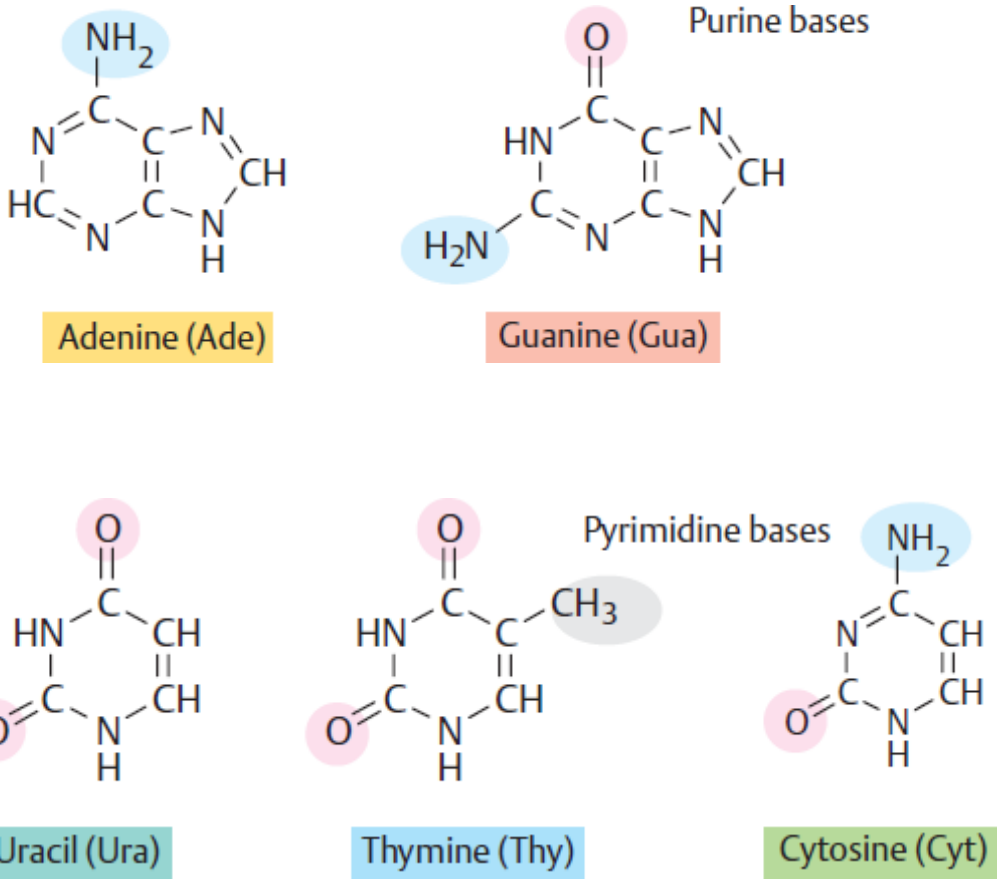
Nucleoside and Nucleotide Structure and Nomenclature

The nucleotides found in cells are derivatives of the heterocyclic highly basic, compounds, purine and pyrimidine.



Purine	Pyrimidine

Five of these bases are the main components of nucleic acids in all living creatures. The purine bases **adenine** and **guanine** and the pyrimidine base **cytosine** are present in both RNA *and* DNA. In contrast, **uracil** is only found in RNA. In DNA, **uracil** is replaced by thymine, the 5-methyl derivative of uracil.



When a nucleic acid base is N-glycosidically linked to ribose or 2-deoxyribose, it yields a **nucleoside**. The nucleoside **adenosine** (abbreviation: A) is formed in this way from adenine and ribose, for example. The corresponding derivatives of the other bases are called *guanosine* (G), *uridine* (U), *thymidine* (T) and *cytidine* (C). When the sugar component is 2- deoxyribose, the product is a **deoxyribonucleoside.**

In the cell, the 5’ OH group of the sugar component of the nucleoside is usually esterified with phosphoric acid. If the 5’ phosphate residue is linked via an acid– anhydride bond to additional phosphate residues, it yields nucleoside diphosphates

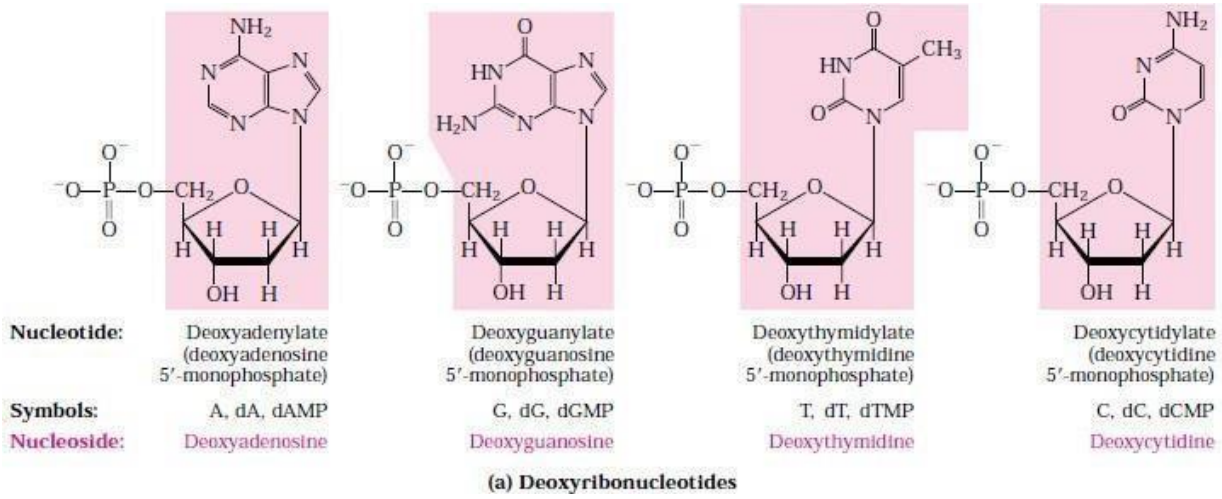
and triphosphates—e. g., ADP and ATP, which are important coenzymes in energy metabolism. All of these nucleoside phosphates are classified as **nucleotides**. In nucleosides and nucleotides, the pentose residues are present in the furanose form. The sugars and bases

are linked by an *N*-**glycosidic bond** between the C-1 of the sugar and either the N-9 of the purine ring or N-1 of the pyrimidine ring. This bond always adopts the β-configuration.

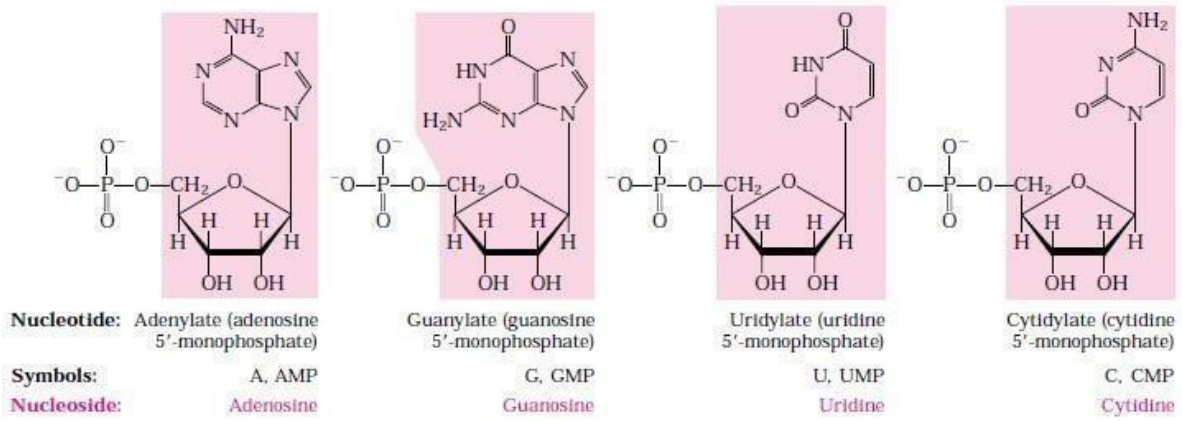
In the pentoses of nucleotidesand nucleosides the carbon numbers are given a prime (‘) designation to distinguish them from the numbered atoms of the nitrogenous bases.

The base of a nucleotide is joined covalently (at N-1 of pyrimidines and N-9 of purines) in an *N*-*β*-glycosyl bond to the 1_ carbon of the pentose, and the phosphate is esterified to the 5_ carbon. The *N*-*β*-glycosyl bond is formed by removal of the elements of water (a hydroxyl group from the pentose and hydrogen from the base), as in *O*-glycosidic bond formation.

Both DNA and RNA contain two major purine bases, **adenine** (A) and **guanine** (G), and two major pyrimidines. In both DNA and RNA one of the pyrimidines is **cytosine** (C), but the second major pyrimidine is not the same in both: it is **thymine** (T) in DNA and **uracil** (U) in RNA. Only rarely does thymine occur in RNA or uracil in DNA.



(a) Deoxyribonucleotides



(b) Ribonucleotides

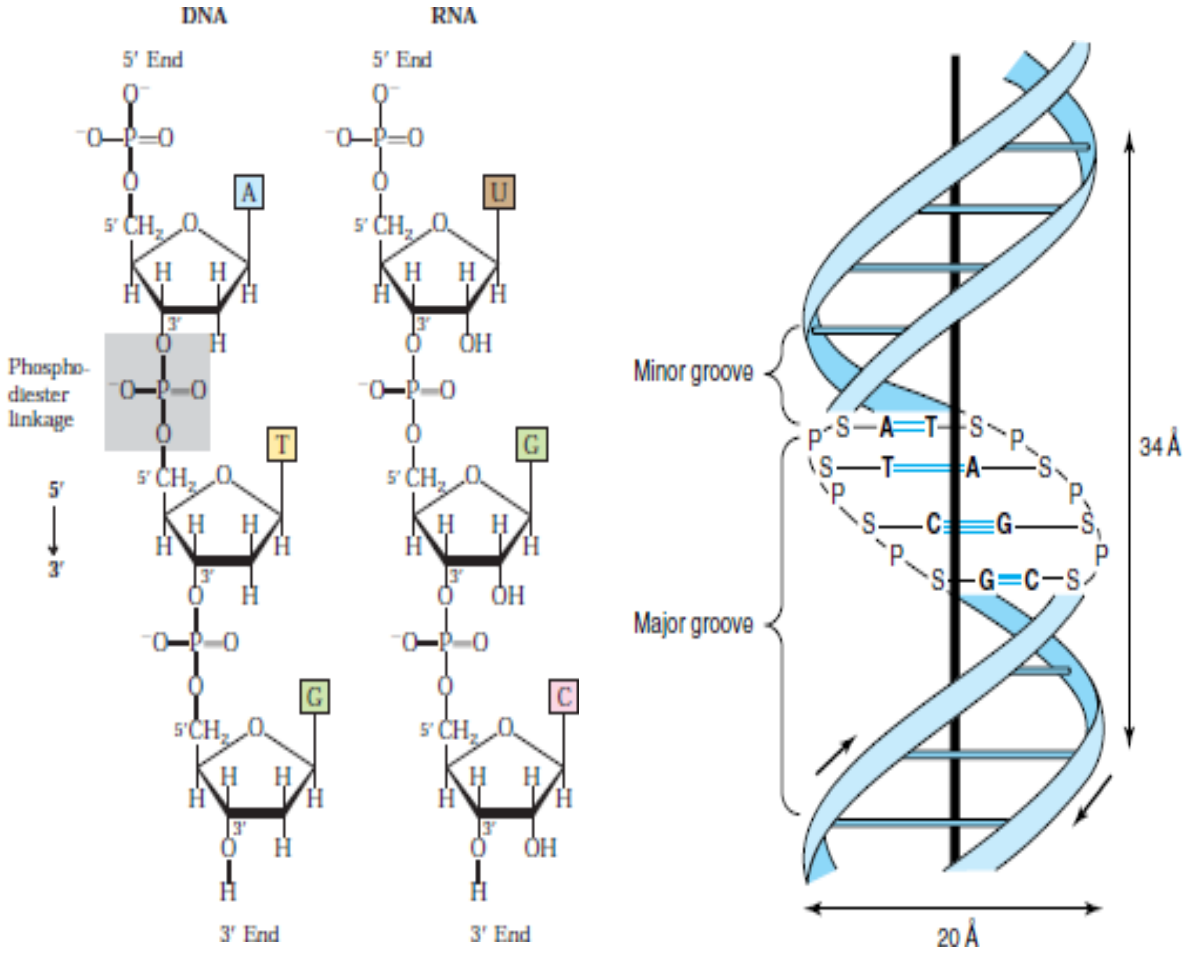
Nucleotide and Nucleic Acid Nomenclature

Base	*Nucleoside*	*Nucleotide*	*Nucleic acid*
Purines			
Adenine	Adenosine	Adenylate	RNA
	Deoxyadenosine	Deoxyadenylate	DNA
Guanine	Guanosine	Guanylate	RNA
	Deoxyguanosine	Deoxyguanylate	DNA
Pyrimidines			
Cytosine	Cytidine	Cytidylate	RNA
	Deoxycytidine	Deoxycytidylate	DNA
Thymine	Thymidine or deoxythymidine	Thymidylate or deoxythymidylate	DNA
Uracil	Uridine	Uridylate	RNA

The successive nucleotides of both DNA and RNA are covalently linked through phosphate-group “bridges,” in which the 5_-phosphate group of one nucleotide unit is joined to the 3_-hydroxyl group of the next nucleotide, creating a **phosphodiester linkage.** Thus the covalent backbones of nucleic acids consist of alternating phosphate and pentose residues, and the nitrogenous bases may be regarded as side groups joined to the backbone at regular intervals. The backbones of both DNA and RNA are hydrophilic.

By convention, the structure of a single strand of nucleic acid is always written with the 5’ end

at the left and the 3’ end at the right—that is, in the 5’ n 3’ direction. Some simpler representations of this pentadeoxyribonucleotide are pA-C-G-T-AOH, pApCpGpTpA, and pACGTA. A short nucleic acid is referred to as an **oligonucleotide.** The definition of “short” is somewhat arbitrary, but polymers containing 50 or fewer nucleotides are generally called oligonucleotides. A longer nucleic acid is called a **polynucleotide.**

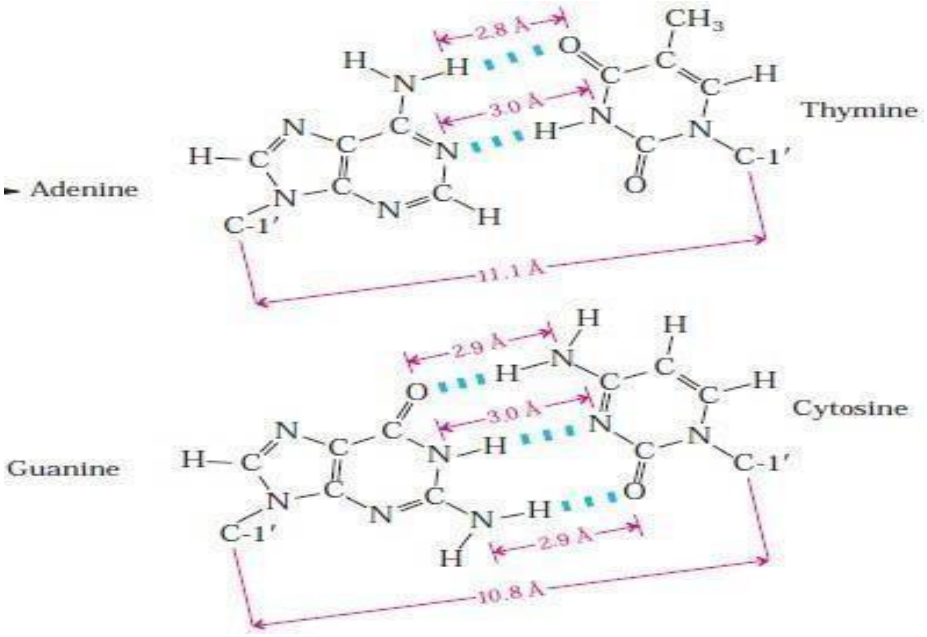


The existence of specific base-pairing interactions was discovered in the course of studies directed at determining the three-dimensional structure of DNA. Maurice Wilkins and Rosalind Franklin obtained x-ray diffraction photographs of fibers of DNA. The characteristics of these diffraction patterns indicated that DNA was formed of two chains that wound in a regular helical structure. From these and other data, James Watson and Francis Crick inferred a structural model for DNA that accounted for the diffraction pattern and was also the source of some remarkable insights into the functional properties of nucleic acids.

The features of the Watson-Crick model of DNA deduced from the diffraction patterns are:

1. Two helical polynucleotide chains are coiled around a common axis. The chains run in opposite directions.
2. The sugar-phosphate backbones are on the outside and, therefore, the purine and pyrimidine bases lie on the inside of the helix.
3. The bases are nearly perpendicular to the helix axis, and adjacent bases are separated by 3.4 Å. The helical structure repeats every 34 Å, so there are 10 bases (= 34 Å per repeat/3.4 Å per base) per turn of helix. There is a rotation of 36 degrees per base (360 degrees per full turn/10 bases per turn).
4. The diameter of the helix is 20 Å.

Watson and Crick discovered that guanine can be paired with cytosine and adenine with thymine to form base pairs that have essentially the same shape. These base pairs are held together by specific hydrogen bonds. This base-pairing scheme was supported by earlier studies of the base composition of DNA from different species. In 1950, Erwin Chargaff reported that the ratios of adenine to thymine and of guanine to cytosine were nearly the same in all species studied.



The meaning of these equivalences was not evident until the Watson-Crick model was proposed, when it became clear that they represent an essential facet of DNA structure. The spacing of approximately 3.4 Å between nearly parallel base pairs is readily apparent in the DNA diffraction pattern. The stacking of bases one on top of another contributes to the stability of the double helix.

DNA: structure ‘

Deoxyribonucleic acids (DNAs) are polymeric molecules consisting of nucleotide building blocks. Instead of ribose, however, DNA contains 2’-deoxyribose, and the *uracil* base in RNA is replaced by *thymine.* The spatial structure of the two molecules also differs. The first evidence of the special structure of DNA was the observation that the amounts of adenine and thymine are almost equal in every type of DNA. The same applies to guanine and cytosine. The model of DNA structure formulated in 1953 explains these *constant base ratios:* intact DNA consists of *two* polydeoxynucleotide molecules (“strands”).

Each base in one strand is linked to a *complementary* base in the other strand by H- bonds. Adenine is complementary to thymine, and guanine is complementary to cytosine. One purine base and one pyrimidine base are thus involved in each **base pair**. The complementarity of A with T and of G with C can be understood by considering the H bonds that are possible between the different bases. Potential donors are amino groups (Ade, Cyt, Gua) and ring NH groups. Possible acceptors are carbonyl oxygen atoms (Thy, Cyt, Gua) and ring nitrogen atoms. *Two* linear and therefore highly stable bonds can thus be formed in A–T pairs, and *three* in G–C pairs. Base pairings of this type are only possible, however, when the *polarity* of the two strands differs—i. e., when they run in opposite directions.

In addition, the two strands have to be intertwined to form a **double helix**. Due to steric hindrance by the 2’-OH groups of the ribose residues, RNA is unable to form a double helix. The structure of RNA is therefore less regular than that of DNA. The conformation of DNA that predominates within the cell is known as **B-DNA**. Along the

whole length of the DNA molecule, there are two depressions—referred to as the "minor groove" and the "major groove"—that lie between the strands.

DNA: conformation ‘

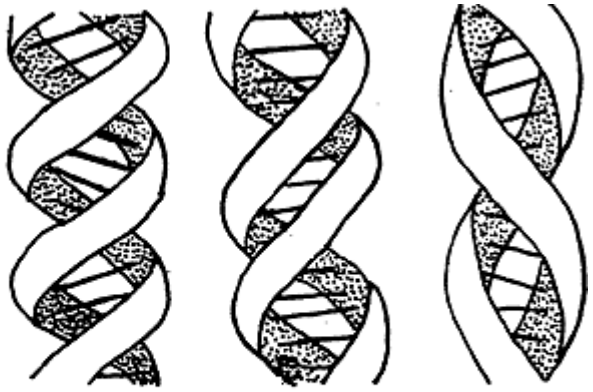
Investigations of synthetic DNA molecules have shown that DNA can adopt several different conformations. All of the DNA segments shown consist of 21 base pairs (bp) and have the same sequence. By far the most common form is **B-DNA**. This consists of two antiparallel polydeoxynucleotide strands intertwined with one another to form a **righthanded double helix**. The "backbone" of these strands is formed by deoxyribose and phosphate residues linked by phosphoric acid diester bonds.

In the B conformation, the aromatic rings of the nucleobases are stacked at a distance of 0.34 nm almost at right angles to the axis of the helix. Each base is rotated relative to the preceding one by an angle of 35°. A complete turn of the double helix (360°) therefore contains around 10 base pairs (abbreviation: bp), i.e., the *pitch* of the helix is 3.4 nm. Between the backbones of the two individual strands there are two grooves with different widths. The *major groove* is visible at the top and bottom, while the narrower *minor groove* is seen in the middle. DNA-binding proteins and transcription factors usually enter into interactions in the area of the major groove, with its more easily accessible bases.

In certain conditions, DNA can adopt the **A conformation**. In this arrangement, the double helix is still right-handed, but the bases are no longer arranged at right angles to the axis of the helix, as in the B form. As can be seen, the A conformation is more compact than the other two conformations. The minor groove almost completely disappears, and the major groove is narrower than in the B form. A-DNA arises when B- DNA is dehydrated. It probably does not occur in the cell.

In the **Z-conformation**, which can occur within GC-rich regions of B-DNA, the organization of the nucleotides is completely different. In this case, the helix is *left- handed,* and the

backbone adopts a characteristic *zig-zag* conformation (hence "Z-DNA"). The Z double helix has a smaller pitch than B-DNA. DNA segments in the Z conformation probably have physiological significance, but details are not yet known.



Form	A	B	Z
helical twist	right	right	left
base pairs per turn	11	10	12
occurrence	RNA, DNA	DNA	DNA

Feature	B-DNA	A-DNA	Z-DNA
Type of helix	Right-handed	Right-handed	Left-handed
Helical diameter (nm)	2.37	2.55	1.84
Rise per base pair (nm)	0.34	0.29	0.37
Distance per complete turn (pitch) (nm)	3.4	3.2	4.5
Number of base pairs per complete turn	10	11	12
Topology of major groove	Wide, deep	Narrow, deep	Flat
Topology of minor groove	Narrow, shallow	Broad, shallow	Narrow, deep

RNA

RNA differs from DNA in both structural and functional respects. RNA has two major structural differences: each of the ribose rings contains a 2´-hydroxyl, and RNA uses uracil in place of thymine. RNA molecules are capable of base pairing, but generally will not form large regions of stable RNA-RNA double helix. RNA can act as a genetic material (although this role, at least for current organisms, seems to be restricted to viruses). Unlike DNA, RNA

can form complex three-dimensional structures. As a result, RNA can also exhibit catalytic activity. The combination of the ability to store genetic information with the ability to catalyze reactions has resulted in a proposal for the origin of life: the “RNA World”. The RNA world hypothesis proposes that RNA molecules once filled all of the roles of protein and nucleic acid macromolecules, and acted in both an information storage capacity and as the source of the enzymatic activity required for metabolic reactions. In general, RNA is less suited to acting as genetic material than DNA, and is less suited to forming efficient catalysts than proteins. Assuming that the RNA world once existed, nearly all of its functions have been taken over by other biological molecules. However, some vestiges of the RNA world may still exist. The vast majority of RNA functions are concerned with protein synthesis.

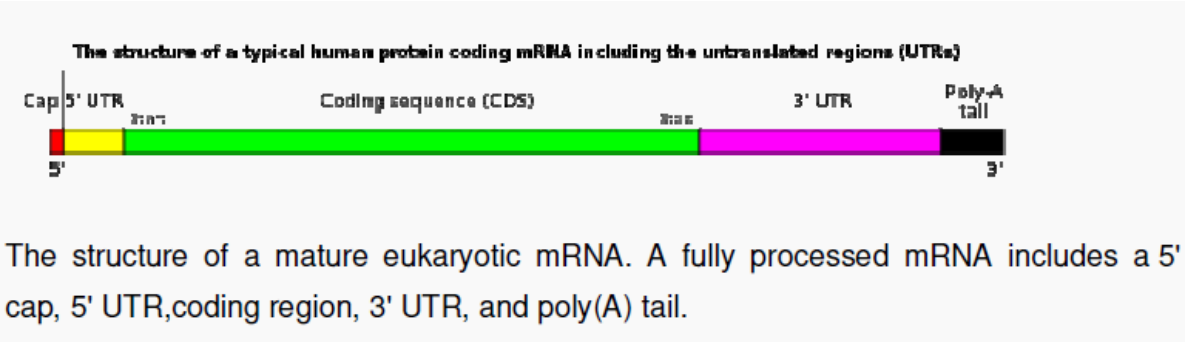
Characteristics

- RNA does not self replicate in order to multiply; instead it is encoded by DNA genes
- RNA is synthesized in order for the translation of DNA to be possible
- The DNA-RNA function is highly interdependable, i.e., if there is problem with DNA, there will be a problem with the RNA functions and vice versa (no RNA = no DNA translation can occur, thus DNA is useless without its RNA genes)

RNA genes of DNA encode for 3 major types of RNA:

- ribosomal RNA
- messenger RNA
- transfer RNA

mRNA – messenger RNA

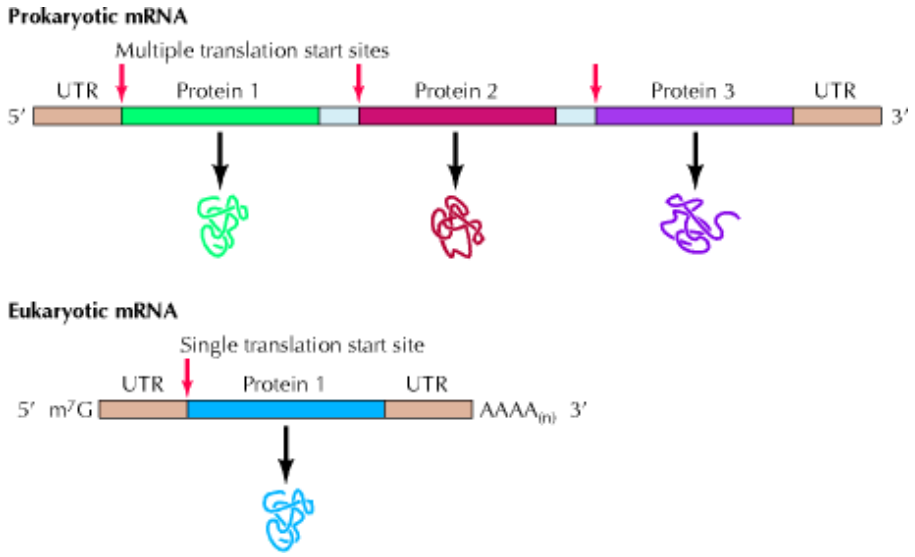


The structure of a mature eukaryotic mRNA. A fully processed mRNA includes a 5' cap, 5' UTR,coding region, 3' UTR, and poly(A) tail.

mRNA genes are the genes that encode only for proteins but this encoding has an RNA

intermediate. The DNA is firstly transcribed into mRNA and subsequently translated into a protein product. So the mRNA genes are the genes that encode for mRNA in order to synthesize proteins. mRNA constitutes only the 5% of the total RNA.

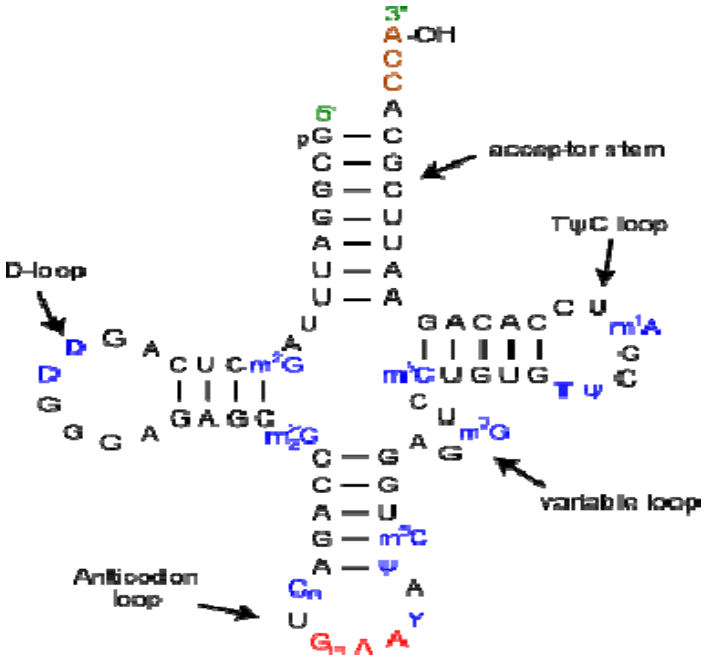
The DNA gives rise to nascent RNA in the nucleus. Addition of poly A tail to this nascent RNA makes this a pre-mRNA. The pre-mRNA has both introns and exons in it. Splicing removes the introns bringing the exons together to form the CDS. This is called the mature mRNA.



The prokaryotic mRNA is a polycistronic mRNA compared to the eukaryotic mRNA which is monocistronic mRNA. The prokaryotic mRNA has a leader sequence and a trailer sequence.

tRNA – transfer RNA

Transfer RNA is encoded by genes that also encode for the 5S size rRNA. RNA polymerase III is responsible for the transcription of these genes by binding on the promoter, situated about 100 base pairs downstream the Transcription Start Site -TSS, along with the Transcription Factors giving rise to the Transcription Initiation Complex. As soon as this complex is formed transcription process can begin and when the Transcription Complex faces an Adenine rich region transcription comes to an end as this area is an indication for the gene end. tRNA constitutes 15% of the total RNA and is directly involved in the translation of the mRNA. More specificaly tRNA binds onto a specific amino acid and brings it along the translation site so that it is bound on the newly synthesized peptide.



- tRNA binds to its specific amino acid recognized by its side R chain in presence of the aminoacyl tRNA synthetase enzyme. The synthetase binds the 5'-CCA-OH-3' acceptor arm with the —COOH group of the amino acid.
- When the small ribosomal subunit faces an AUG codon on the mRNA it indicates the commencing of the peptide formation. As soon as the AUG codon is recognized then the first tRNA binds on the small ribosomal subunit and on the mRNA through its anticodon arm, giving rise to the Translation Initiation Complex designated as $tRNA_i^{met}$. Eventually the large ribosomal subunit binds on the complex indicating the initiation of the translation process. Translation always begins with the methionine amino acid on the newly synthesized peptide.

Analysis of the tRNA sequence suggests a cloverleaf secondary structure formed by regions of base pairing between the sections of the RNA strand, with this cloverleaf folding into the three-dimensional structure.

rRNA – ribosomal RNA

In bacteria (prokaryotes) there are three different ribosomal RNAs called 5S, 16S, and 23S. Eukaryotes have homologous RNAs called 5S, 28S, and 18S ribosomal RNAs. In addition, they have a 5.8S RNA that is homologous to one end of the prokaryotic 23S RNA. Both prokaryotic and eukaryotic ribosomes can be broken down into two subunits (the S in 16S represents Svedberg units), nt= length in nucleotides of the respective rRNAs.

Note that the S units of the subunits (or the rRNAs) cannot simply be added because they represent measures of sedimentation rate rather than of mass. The sedimentation rate of each subunit is affected by its shape, as well as by its mass. The nt units can be added as these represent the integer number of units in the linear rRNA polymers (for example, the total length of the human rRNA = 7216 nt).

Prokaryotes

In prokaryotes a small 30S ribosomal subunit contains the 16S ribosomal RNA. The large

50S ribosomal subunit contains two rRNA species (the 5S and 23S ribosomal RNAs). Bacterial 16S ribosomal RNA, 23S ribosomal RNA, and 5S rRNA genes are typically organized as a co-transcribed operon. There may be one or more copies of the operon dispersed in the genome (for example, *Escherichia coli* has seven).

Archaea contains either a single rDNA operon or multiple copies of the operon. The 3' end of the 16S ribosomal RNA (in a ribosome) binds to a sequence on the 5' end of mRNA called the Shine-Dalgarno sequence.

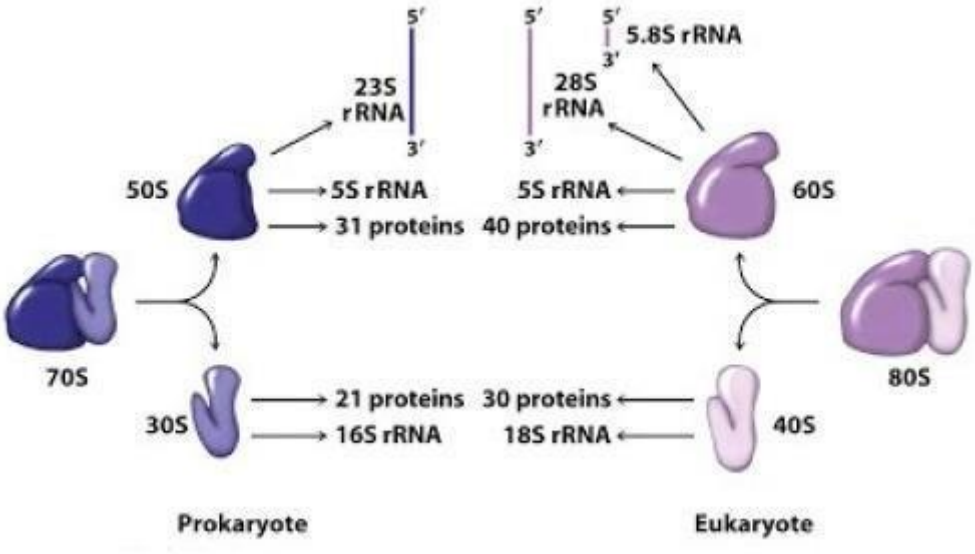
Eukaryotes

In contrast, eukaryotes generally have many copies of the rRNA genes organized in tandem repeats; in humans approximately 300–400 repeats are present in five clusters (on chromosomes13, 14, 15, 21 and 22). Because of their special structure and transcription behaviour, rRNA gene clusters are commonly called "ribosomal DNA" (note that the term seems to imply that ribosomes contain DNA, which is not the case).

The 18S rRNA in most eukaryotes is in the small ribosomal subunit, and the large subunit contains three rRNA species (the 5S, 5.8S and 28S in mammals, 25S in plants, rRNAs).

Mammalian cells have 2 mitochondrial (12S and 16S) rRNA molecules and 4 types of cytoplasmic rRNA (the 28S, 5.8S, 18S, and 5S subunits). The 28S, 5.8S, and 18S rRNAs are encoded by a single transcription unit (45S) separated by 2 internally transcribed spacers. The 45S rDNA is organized into 5 clusters (each has 30-40 repeats) on chromosomes 13, 14, 15, 21, and 22. These are transcribed by RNA polymerase I. 5S occurs in tandem arrays (~200-300 true 5S genes and many dispersed pseudogenes), the largest one on the chromosome 1q41-42. 5S rRNA is transcribed by RNA polymerase III.

The tertiary structure of the small subunit ribosomal RNA (SSU rRNA) has been resolved by X-ray crystallography. The secondary structure of SSU rRNA contains 4 distinct domains — the 5', central, 3' major and 3' minor domains.



16S rRNA – Significance

The 16S rRNA gene is a section of prokaryotic DNA found in all bacteria and archaea. This gene codes for an rRNA, and this rRNA in turn makes up part of the ribosome. The first 'r' in rRNA stands for ribosomal. The ribosome is composed of two subunits, the large subunit (LSU) and the small subunit (SSU). These two subunits sandwich the mRNA as it feeds through the ribosome for translation. While there are also associated proteins helping to make up the functional units of the ribosome, in general, in bacteria, the SSU is coded for by the the 16S rRNA gene, and the LSU is coded for by the 23S rRNA & 5S rRNA genes.

The 16S rRNA gene is a commonly used tool for identifying bacteria for several reasons. First, traditional characterization depended upon phenotypic traits like gram positive or gram negative, bacillus or coccus, etc. Taxonomists today consider analysis of an organism's DNA more reliable than classification based solely on phenotypes. Secondly, researchers may, for a number of reasons, want to identify or classify only

the bacteria within a given environmental or medical sample. While there is a homologous gene in eukaryotes, the 18S rRNA gene, it is distinct, thereby rendering the 16S rRNA gene a useful tool for extracting and identifying bacteria as separate from plant, animal, fungal, and protist DNA within the same sample. Thirdly, the 16S rRNA gene is relatively short at 1.5 kb, making it faster and cheaper to sequence than many other unique bacterial genes.

Ribosomes (and correspondingly the DNA that codes for them) have been mostly conserved over time, meaning that their structure has changed very little over time due to their important function, translating mRNA into proteins. But even within this gene there are parts that have been conserved more than others. This is due to the structure of the ribosome itself. With the way the ribosome folds, creating bonds with itself in some places (conserved regions) while other portions are looped and unbonded (hypervariable regions), the degree to which any portion of the gene is subject to mutations varies.

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DEPARTMENT OF BIOTECHNOLOGY

UNIT – V – BIOCHEMISTRY AND BIOMOLECULES – SBTA1302

UNIT - V

1. Vitamins - Introduction

Vitamins are essential nutrients that are required by the body. Since they were discovered and their positive effects became known for us, they became one of the most common products of the pharmaceutical industry. They all have a unique role in maintaining normal cell function, growth and development. Vitamins are classified into two categories:

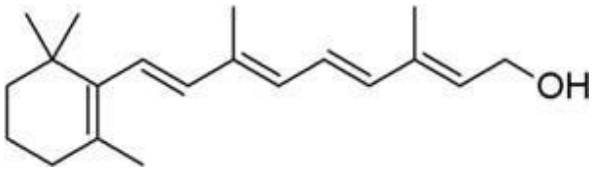
- Fat soluble vitamins (A, D, E and K),
- Water soluble vitamins (B and C).

Fat-soluble vitamins, once ingested, the body uses what it needs at the time and stores the rest in fat tissue. The vitamins can be stored and remain here until they are needed for future use. If too much is ingested this can cause hypervitaminosis, a potentially dangerous condition. Deficiencies can also occur when fat intake is low or if fat absorption is compromised in certain conditions (e.g. taking certain drugs, cystic fibrosis).

In contrast water-soluble vitamins are not stored in the body. The body uses the amount needed and any excess is excreted in urine. As they are not stored, the body requires a constant supply in order to stay healthy.

Fat Soluble vitamins

Vitamin A



Vitamin A structure

Role

- Good vision: It is a component of retinal pigments, which helps especially in low lighting.
- Reproduction, cell division and gene expression.
- Participates in bone and tooth development.
- Maintains mucous membranes of the mouth , nose, throat and lungs, by keeping them moist.
- Maintains healthy skin.
- Antioxidant, which may protect against cancer. Beta-carotene is an antioxidant.
- Supports immune function

Source

Vitamin A primarily comes from animal sources: eggs, meat, fortified milk, cheese, cream, liver, kidney, cod, and halibut fish oil. Beta-carotene comes from colourful fruits and vegetables, such as carrots, pumpkin, winter squash, dark green leafy vegetables and apricots. Usually the more intense the colour of the fruit or vegetable, the more beta-carotene it contains.

Excess

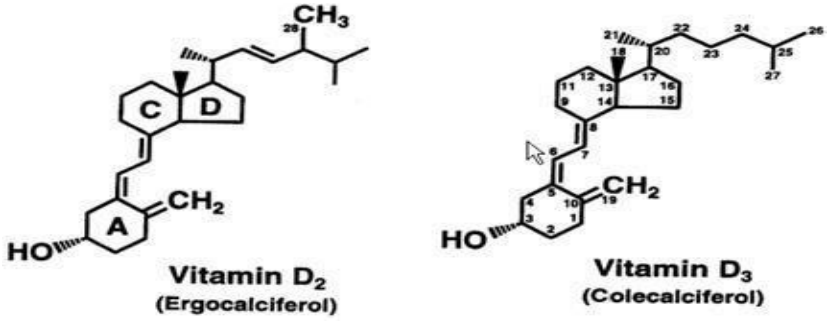
This can either be acute or chronic and can present with a number of symptoms. Acute toxicity causes dry, itchy skin, headache, nausea, loss of appetite and blurred vision. Severe toxicity can result in growth retardation, enlargement of the liver and spleen, loss of hair, bone pain, increased pressure in the skull and skin changes. Increased amounts of beta-carotene can turn the skin yellow or orange.

Deficiency

This is usually associated with strict diet restriction or excessive alcohol intake.

- **Mild:** night blindness, diarrhea, reduced resistance to infection, impaired vision.
- **Severe:** inflammation of the eyes, keratinisation of the skin and eyes and blindness in children.

Vitamin D



Role

Participates in metabolism of calcium and phosphate and maintains adequate serum concentrations of both. It also promotes calcium absorption in the gut. Vitamin D is especially important in growing children, as it is needed for strong bones and teeth. Research shows that it also provides protection against osteoporosis, hypertension, cancer, and some autoimmune diseases.

Source

Primary source is milk and other dairy products. It is also found in oily fish and cod liver oil. It is not only found in foods, it can be synthesized in the skin and is triggered by the exposure to UV rays from sunlight (it is recommended to get 10 to 15 minutes of sunshine three times weekly is enough to produce the body's requirement of vitamin D).

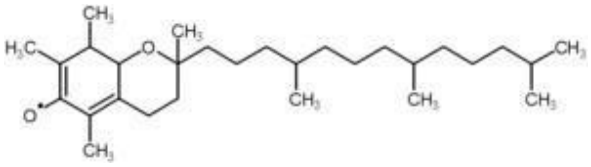
Excess

Toxicity causes elevated plasma concentration of calcium which can have some side effects: Blood vessel contract, high blood pressure and Calcium deposits in soft tissues such as the heart and lungs, Kidney stones, Nausea, vomiting, constipation, poor appetite, weakness, and weight loss.

Deficiency

The main diseases associated with vitamin D deficiency are Osteomalacia and rickets (in children). The symptoms that arise are nausea, weight loss and irritability for mild cases, and mental and physical growth retardation, kidney damage and movement of calcium from bones into soft tissues for the severe cases.

Vitamin E



Vitamin E structure

Role

It is an antioxidant that protects Vitamin A and C, red blood cells and essential fatty acids from becoming destroyed. It also prevents cell membranes from being damaged.

Source

It can be found in natural or synthetic forms. It is found in vegetable oils, cereals, meat, poultry, eggs, fruits, vegetables, legumes, wheat germ oil and whole grain and is also available as a supplement.

Excess

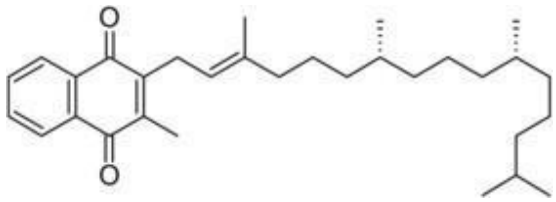
There is an increased risk of bleeding especially in patients taking blood-thinning agents such as heparin, warfarin or aspirin, and in patients with vitamin K deficiency. It can also cause nausea and digestive tract disorders.

Deficiency

This is very rare and impossible to produce without starvation. It generally occurs in infants and people unable to absorb fats.

Vitamin K

Vitamin K is group of compounds derivated from 2-methyl-1,4-naftochinon (IUPAC: 2-methylnaphthalene-1,4-dione)



Vitamin K_1 structure

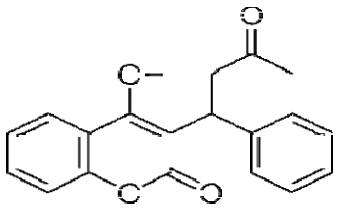
Role

It has an important role in normal blood clotting (**factors II, VII, IX and X** are vitamin K dependent, because it works as cofactor in carboxylation of glutamic acid to γ- carboxyglutamic acid which is essential for calcium binding on these factors), for synthesis of protein C and S and it is also needed to help build strong bones.

Role in coagulation therapy

The role of vitamin K is often exploited in anticoagulation treatments in patients with increased risk of thrombosis. These compounds used are referred to as vitamin K antagonists, many of these are coumarin based, the best know of which is warfarin. Coumarin based compounds function by preventing the conversion of the inactive epoxide form of vitamin K into its activated form by inhibiting the enzyme responsible for its reduction (vitamin K epoxide reductase).

Alternative anticoagulation treatments include heparin (via the inactivation of thrombin) in vivo and EDTA, oxalate and citrate (which remove Ca^{2+}) but can only be used in vitro due to the biological importance of Ca^{2+}.



Warfarin

Source

It is found in green leafy vegetables, such as broccoli and spinach, pulses, vegetable oils, cereals, milk, milk products, meat, eggs and fruit. Bacteria in the intestines can also synthesis Vitamin K and contribute to the available pool.

- Requirement: 1 µ/kg/day (except newborns)

Excess

This can cause the breakdown of red blood cells and also liver damage. Therefore if a person is taking blood-thinning agents, they may need to limit the amount of Vitamin K intake.

Note: Vitamin K is an antidote for warfarin.

Deficiency

It is very rare in adults, but can occur in individuals that cannot absorb it properly, due to lack of intestinal bacteria, as well as those being treated long term with antibiotics. It can cause excessive bleeding and increased tendency to bruise. It may also be the cause of haemorrhagic disease of newborn, because placental transfer of vitamin K is very low, its level in breast milk is low as well. (prevention: 1 mg of vit K intramuscularly 2–6 hours after birth and then 1 mg of vit K every week till age one month, and for exclusively breast fed children till age 6 months).

Water Soluble vitamins

Water-soluble vitamins consist of the B-group vitamins and vitamin C. Their deficiency is treated by administration of the deficient vitamin.

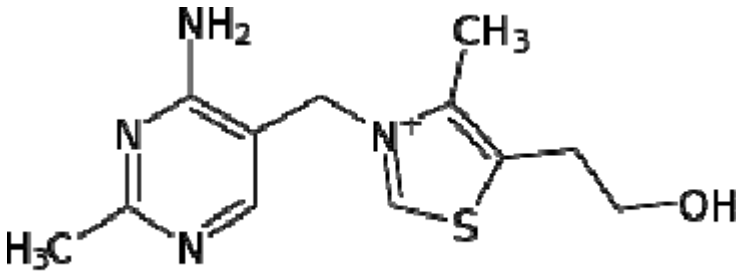
B Group Vitamins Features

- A common feature of group B vitamins is their occurrence in yeast (except vitamin B_{12}). However, if the yeast is included in the diet only as a means of rising bread, then yeast is not considered the major source of group B vitamins in humans; a small quantity of yeast does not contain nutritionally significant amount of B vitamins.
- Their metabolic effects are inter-linked.
- Deficiency of only a single vitamin occurs rarely.
- They are produced by the intestinal micro flora but the amount produced is generally

only a fraction of the daily recommended intake.

- Some are more frequently called by their name, others by number. Some vitamins may not have a number because it has been found that some substances, originally considered as vitamins, are NOT essential for humans, therefore they are not vitamins or are a mixture of substances.

Vitamin B_1 (thiamine)



Vitamin B1 - structure

Thiamine (vitamin B_1) is a coenzyme decarboxylase important for the metabolism of glucose and energy supply to nerve and muscle cells.

Source

Meat, fish, cereals, yeast, legumes.

Daily recommended intake for adults: 1-1.4 mg

Deficiency

The disease beri-beri from a lack of dietary vitamin B_1 is found today in very poor population groups (e.g. refugees) in countries where people live mostly on polished/white rice. It may also develop in people who live mostly on refined wheat flour products and among alcoholics and food faddists.

A typical image consists of nervous disorders, especially peripheral nerves (dry beri beri), edema and heart disease (beri beri wet). Impaired absorption of vitamin B_1 occurs in alcoholics and is manifested by Wernicke encephalopathy.

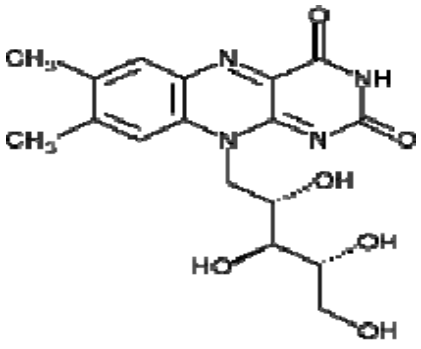
Suboptimal thiamine status based on biochemical criteria in Europe was detected only in 4- 6% of the population. Risk group are alcoholics.

Laboratory evaluation: thiamine excretion in the urine. In the absence of erythrocytes is reduced transketolase concentration in the blood and the sea is high concentrations of glycoxalate.

Excess

Signs of excess are not encountered.

Vitamin B_2 (riboflavin)



Vitamin B2 structure

Riboflavin or vitamin B_2 is part of coenzymes flavinadenine mononucleotide (FAD) and flavin mononucleotide (FMN), plays a key role in oxidative metabolism.

Source

A small amount is found in many foods. Main sources are meat, milk and milk products; good sources are also fish, offal (inner organs), eggs, and whole grain cereals. Milling of cereals removes most of vitamin B_2 - some countries (e.g. USA) fortify cereal products with riboflavin.

Recommended daily intake for adults: 1.2 to 1.5 mg

Deficiency

According to several population studies, the deficiency is widespread in developing countries, where diet is poor in animal foods, vegetables and fruits, and where cereals are milled (white flour). Frequently the deficiency is secondary due to malabsorption, enterocolitis, coeliac disease, chronic hepatitis; in children often after the use of broad- spectrum antibiotics. It may develop in cancer, cardiac disease, diabetes

Clinical picture: The description of the signs of riboflavin deficiency is somewhat inconsistent in various scientific publications. Riboflavin deficiency occurs almost always together with deficiencies of other group B vitamins, which may cause some of the signs. The signs most frequently described are: angular stomatitis, peeling lips (cheilosis), glossitis, and normocytic normochromic anemia.

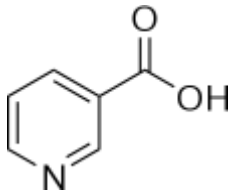
Laboratory evaluation: decreases secretion of vitamin B_2 in urine (normal values are 106– 638 nmol/l), decreased concentrations of glutathione and glutathione reductase in erythrocytes.

Excess

Signs of excess are not known.

Vitamin B_3 (niacin)

Niacin (vitamin B_3) is the name for nicotinamide and nicotinic acid. It is part of enzymes, oxido-reduction systems (nicotinamide adenine dinucleotide -NAD, nicotinamide adenine diphosphate -NADP). May form in the liver from tryptophan and its biosynthesis is very slow and it is needed vitamin B_6.



Structure of Niacin

Source

The source of most foods - meat, fish, cereals. The recommended daily dose for adults is by age and sex of 13-17 mg.

Deficiency

Disease pellagra is caused by the current lack of niacin and its precursor tryptophan. Today it has rarely occurs in a very poor population groups or for refugees in developing countries. Occurs in people who eat mostly corn/maize. The symptoms are as a mnemonic device used sometimes called "disease of three D" - dermatitis, diarrhea, dementia.

Surplus

Signs of excess food are not known. High doses of dietary supplements induce vasodilatation, warmth, gastritis, damage to liver cells. Income should not exceed 35 mg/ kg / day.

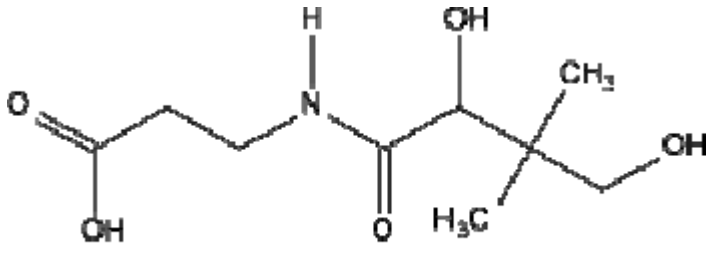
Pharmacological use

Nicotinic acid (niacin) and its derivatives are used to treat hyperlipidemia by inhibiting the secretion of VLDL from the liver and increasing the activity of peripheral lipoprotein lipase. This leads to a reduction in circulating VLDL (ie, TAG) and, consequently, LDL (cholesterol).

In contrast adipose tissueblocking the intracellular lipase, thus releasing the MK inventory, further reducing supply to the liver TAG and reduces VLDL synthesis.

- Adverse effects: harmless vasodilation (mediated release of prostaglandins) in the skin associated with subjective stream feeling hot - it can handle submitting aspirin; at 1 / 5 of patients treated with hyperuricemia; skin rash.

Vitamin B_5 (pantothenic acid)



Vitamin B5 structure

Pantothenic acid (vitamin B_5) is part of coenzyme A.

Source

Small amounts are in almost all foods contain a large amount of yeast, liver, meat, milk, whole grains and legumes. The daily recommended dose for adults: 6 mg

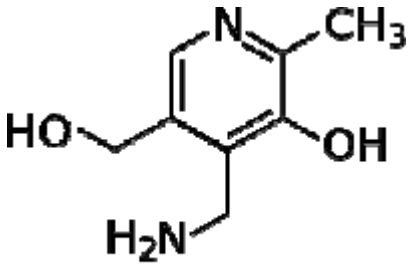
Deficit

Lack is not present - described only when administered pantothenic acid antagonists and extremely malnourished people with symptoms of deficiency of other nutrients, is manifested hair follicle atrophy, loss of pigmentation, dermatitis.

Surplus

Signs of excess are not known.

Vitamin B_6 (Pyridoxine)



Vitamin B6 structure

Pyridoxine the name vitamin B_6 comprises a group of compounds (pyridoxine, pyridoxamine, pyridoxal and phosphate). It is coenzyme for more than 50 enzymatic reactions - decarboxylase and transaminases, synthesis of acid nicotine and arachidonic acid, affects the function of the nervous system, immune reactions and synthesis of haemoglobin.

Source

It is abundant in food. The daily recommended dose for adults: 13-17 mg

Deficit

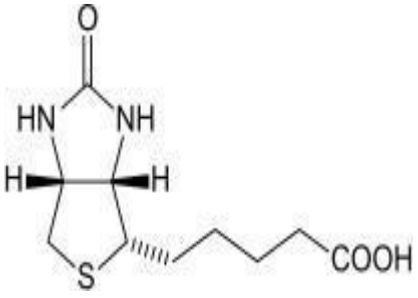
Deficiency with normal eating habits does not occur; manifested skin and mucosal changes, rhagades corners, peripheral neuropathy.

Surplus

Excess of food does not occur. After a prolonged intake of 50-500 mg - sensory neuropathy

Vitamin B_7 (Biotin)

Biotin Vitamin B_7, vitamin H, factor R - Several scholars have described it, only later discovered that it is the same substance) is important for the metabolism of amino acids and fatty acids, is a cofactor for carboxylases.



Biotin Structure

Source

At low concentrations in many foods. Rich sources are yeast, liver, egg yolk, nuts, lentils. The daily requirement (RDA cannot be estimated): 30-60 mg

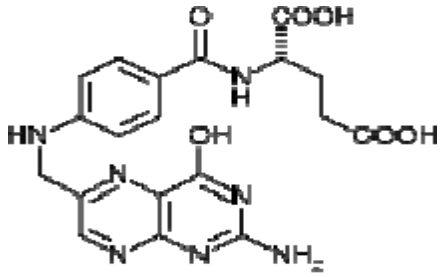
Deficit

Deficiency of food does not occur. Scientists described the people who long consumed a large amount of raw eggs (irreversibly binds to biotin with avidin contained in raw egg white) and improper parenteral nutrition. Symptoms: seborrheic dermatitis, fatigue, anorexia, nausea, hypercholesterolemia, vascular disorders.

Surplus

Signs of excess are not known.

Vitamin B_9 (Folic acid)



Vitamin B9 structure

Folic acid also is known as vitamin B_9, folate or folacin. Includes a group of compounds: Folic Acid (contains pterin, p-aminobenzoic acid and glutamic) and folic acid. Along with vitamin B_{12} is essential for the formation of nucleic acids and thus for synthesis of DNA, participate in the transfer radicals and in all processes of cell division, it is important for cell division and tissue with high mitotic activity.Absorbed in the proximal parts of the small intestine and when excess it is excreted in the urine.

Source

Liver, yeast, green leafy vegetables, as well as whole grain cereals, meat, milk, eggs and legumes. The recommended daily adult dose: 400mg. In pregnancy, 600mg for prevention of congenital malformations (mainly cleft neural tube).

Deficit

Deficiency of vitamin B_9 occurs in low supply, absorption or increased need during pregnancy. There is a megaloblastic anaemia, which is characterized by the presence of abnormal precursors of red blood cells in the bone marrow. Compared with normal cells are cells arising from these abnormal precursors of different shape, larger size, reduced viability and reduced ability to transport oxygen. Along with the lack of iron is its lack of a significant cause of anaemia in developing countries. Deficiency during pregnancy causes spina neural tube in the fetus.

- Laboratory evaluation: serum levels of folate, total homocysteine (increases in the absence, also in the absence of vitamin B_{12})

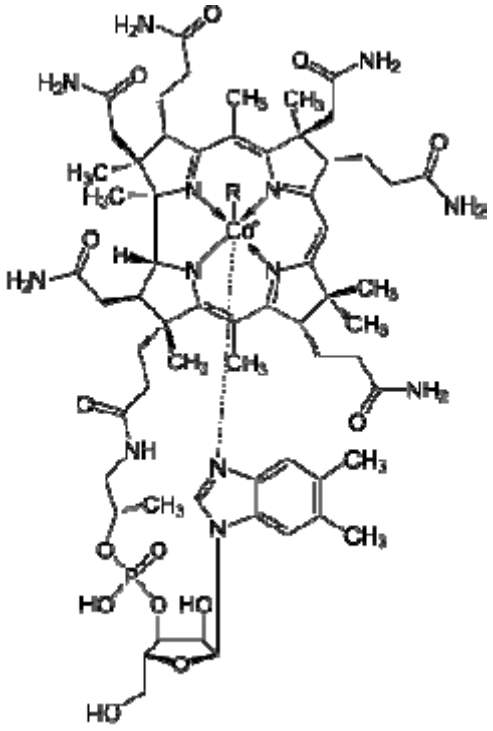
Surplus

High intake of folic acid can mask vitamin B_{12}, so the upper limit of the daily recommended intake of up to 1000 mg / day.

Vitamin B_{12} (cobalamin)

Vitamin B_{12}(cobalamin) is the collective name for several compounds that are in the center of porphyrin skeletal cobalt . Vitamin B_{12} has a number of biological functions - plays an important role in hematopoiesis, is essential for the development of the central nervous system in children, contributes to the formation of nucleic acids, transmethylation and has anabolic effect. Deficiency of vitamin B_{12} in adults causes macrocytic anemia, impaired rear and lateral

spinal cords, peripheral nerves and dementia or depression. Lack of vitamin B_{12} also affects secondary folate cycle resulting in impaired synthesis of purines and pyrimidines necessary for the formation of DNA and RNA.



Vitamin B12 structure

Source

In nutritionally significant quantities occurs only in animal foods. Rich sources are liver, kidney, meat warm-blooded animals (1-2 ig/100 g), fish, egg yolk and dairy products (milk ìg/100, 0.3 ml cheese ìg/100 0.2 to 0.6g). Plant foods contain trace amounts of vitamin B_{12} only if it has been processed by bacterial fermentation (e.g beer). Absorbed in the small intestine only if the stomach creates a complex with an internal factor. Therefore it is necessary to properly functioning stomach and large amounts of vitamin B_{12} are formed by the
intestinal flora in humans unusable. Cobalamine with an internal factor in the distal ileum bind to specific receptor cubilin and this complex then enters by endocytosis into enterocytes.

Inside the enterocyte cobalamin binds to other carriers and excreted into the plasma. 75-80% is bound to haptocorrin and goes to hepatocytes. The cells of other organs enter only vitamin B_{12} bound to transcobalamin II (the holotranscobalamin) after binding to specific receptors through endocytosis. The cell cobalamin is converted to active metabolites and adenosylcobalamine methylcobalamin, which serve as cofactors of enzymes. The daily recommended dose for adults: 3 mg. Minimal in infants: approximately 0.1 to 0.3 mg.

Function

Haemopoiesis; development of the central nervous system in childhood; cofactor of two metabolic reactions: conversion of homocysteine to methionine by methionine synthase (failure of this reaction leads to the accumulation of homocysteine); conversion methylmalonyl-CoA to succinyl-CoA action methylmalonyl-CoA mutase (failure of this reaction leads to an accumulation of methylmalonic acid and its increased urinary excretion).

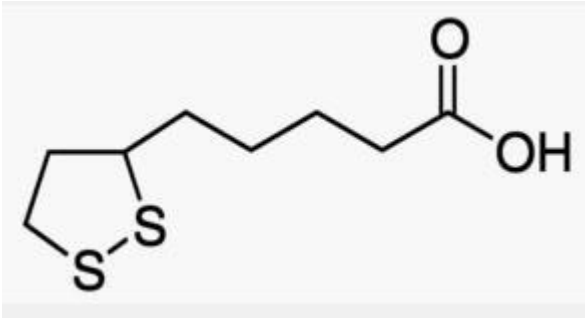
Deficit

Its deficiency is clinically manifested failure to thrive, macrocytic anemia and neurological symptoms. An adult is a stock (2-5 mg) of vitamin B_{12} in the liver, which cover the need for a period of 5-10 years. Stocks, which creates the infant in utero (approximately 25 micrograms), will be exhausted as early as 3-5 months. Among laboratory manifestations include mostly macrocytic anemia, elevated aminotransferases, hyperhomocysteinemia and increased acid secretion methylmalonic acid plasma concentrations of homocysteine and methylmalonic acid excretion increased in the urine. Metabolic changes precede clinical manifestations. Pernicious anemia is an autoimmune disease that leads to atrophy of the gastric mucosa and by the lack of intrinsic factor.

Surplus

Signs of excess were reported even after a high intake (5 mg) of the supplement.

Lipoic acid



Lipoic acid Structure

Alpha-lipoic acid is an antioxidant that's in many foods, and it's made naturally in our bodies. Yeast, liver, kidney, spinach, broccoli, and potatoes are good sources of alpha-lipoic acid.

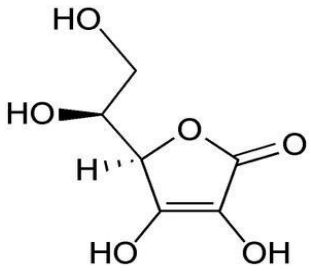
Alpha-lipoic acid is used for diabetes and nerve-related symptoms of diabetes including burning, pain, and numbness in the legs and arms.

Some people use alpha-lipoic acid for memory loss, chronic fatigue syndrome (CFS), HIV/AIDS, cancer, liver disease, diseases of the heart and blood vessels (including a disorder called cardiac autonomic neuropathy) and Lyme disease.

Alpha-lipoic acid is also used to treat eye-related disorders, such as damage to the retina, cataracts, glaucoma, and an eye disease called Wilson's disease.

600 milligrams daily for three weeks can be taken on symptoms of diabetic neuropathy

Vitamin C



Vitamin C structure

L-ascorbic acid, also known as vitamin C is water soluble strongly reducing effects. Man (as well as other primates and guinea pigs) cannot synthesize it, since it lacks L-gulonolactonexidase activity, therefore it must receive in food. L-ascorbate is involved in the hydroxylation of collagen, the synthesis of carnitine, the metabolism of tyrosine, acts as an antioxidant, supports immune system, iron absorption, has an effect on beta- oxidation of fatty acids, increases the activity of microsomal enzymes, accelerates the detoxification of xenobiotics. Reducing the effects of ascorbic acid is due to its easy oxidation to dehydroascorbate:

Source

Fruits, vegetables (including potatoes), liver. Average losses in cook foods are 30%. The daily recommended dose for adults: 100 mg. When the determination is considered, in addition to prevention of deficiency symptoms, as well as strengthening the immune system and prevention of degenerative diseases. Increased need for considerable physical exertion, psychological stress, alcohol abuse and drugs, some diseases (eg diabetes, renal insufficiency, infection). Intake of 150 mg / day is recommended for smokers.

Deficit

Ascorbic acid deficiency - scurvy (scurvy) - now appears only in extreme conditions. With a slight lack of preclinical manifestations we see in our country (fatigue, prolonged convalescence, impaired wound healing and decreased resistance to infection).

- Laboratory evaluation of the situation: the level of vitamin C in plasma. Clinical symptoms appear with values ≤ 10 μmol/L, an indicator of low intake of vitamin C are considered to values below 37 μmol/L. In terms of prevention of atherosclerosis and the tumors are regarded as desirable values ≥ 50 μmol / L.

Surplus

Signs of excess food are not. Approximately 1% of the unused vitamin C is converted to oxalate, the risk of urinary calculi, but low in healthy subjects. The daily intake should not exceed 1000 mg. Very high doses (5 g) can cause diarrhea. At high ascorbate intake (about grams per day), most of the substance is excreted in the urine.