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INSTITUTE OF SCIENCE AND TECHNOLOGY
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**SCHOOL OF BIO AND CHEMICAL ENGINEERING**

**DEPARTMENT OF BIOTECHNOLOGY**

# UNIT – I – BASIC BIOCHEMISTRY – SMB1102

| **SMB1102** | **BASIC BIOCHEMISTRY** | **L** | **T** | **P** | **Credits** | **Total Marks** |
|---|---|---|---|---|---|---|
| | | 5 | 0 | 0 | 3 | 100 |

COURSE OBJECTIVES:

This course is aimed give an understanding about the basics of biochemistry dealing carbohydrates, Amino acids, Lipids, nucleic acid and vitamins.

UNIT 1: CARBOHYDRATES 12 Hrs. Carbohydrate – Definition, Classification, biological significance, structure of glucose, digestion and absorption of carbohydrates.

UNIT 2: PROTEINS 12 Hrs. Amino acids – structure, classification (Essential and non-essential, protein and non-protein amino acids). Proteins – definition, classification and structure (primary, secondary, tertiary and quaternary).

UNIT 3: LIPIDS 12 Hrs. Lipids – definition, classification and biological significance. Structure, properties and functions of fatty acids.

UNIT 4 NUCLEIC ACIDS 12 Hrs. Nucleic acids – Structure of DNA and its functions. Different forms of DNA. Different types of RNA and its functions.

UNIT 5 VITAMINS 12 Hrs. Vitamins – Source, biological function, daily requirement and deficiency symptoms of fat soluble vitamins (A, D, E and K) and water soluble vitamins (Ascorbic acid, thiamine, riboflavin, pyridoxine, niacin, pantothenic acid, lipoic acid, biotin, folic acid and vitamin B12).

TEXT / REFERENCE BOOKS:

1. Lehninger Principles of Biochemistry-David L. Nelson, Michael M. Cox, Macmillan Worth Publishers.
2. Harper―s Biochemistry-Rober K. Murray, Daryl K. Grammer, McGraw Hill, Lange Medical Books. 25th edition.
3. Fundamentals of Biochemistry-J.L. Jain, Sunjay Jain, Nitin Jain, S. Chand & Company.
4. Biochemistry-Dr. Amit Krishna De, S. Chand & Co., Ltd. END SEMESTER EXAMINATION QUESTION

PAPER PATTERN Max. Marks: 100 Exam Duration: 3 Hrs.

PART A: 10 questions of 2 marks each - No choice 20 Marks

PART B: 2 questions from each unit of internal choice; each carrying 16 marks 80 Marks

# UNIT: 1

# CARBOHYDRATES

Carbohydrates are the most abundant biomolecules on earth. Oxidation of carbohydrates is the central energy-yielding pathway in most non-photosynthetic cells.

**Definition:** Carbohydrates are polyhydroxy aldehydes or ketones, or substances that yield such compounds on hydrolysis.

Carbohydrates have the empirical formula $(CH_2O)_n$.

There are three major classes of carbohydrates:

1. **Monosaccharides**

Monosaccharides, or simple sugars, consist of a single polyhydroxy aldehyde or ketone unit. The most abundant monosaccharide in nature is the six-carbon sugar D-glucose, sometimes referred to as dextrose.

2. **Oligosaccharides**

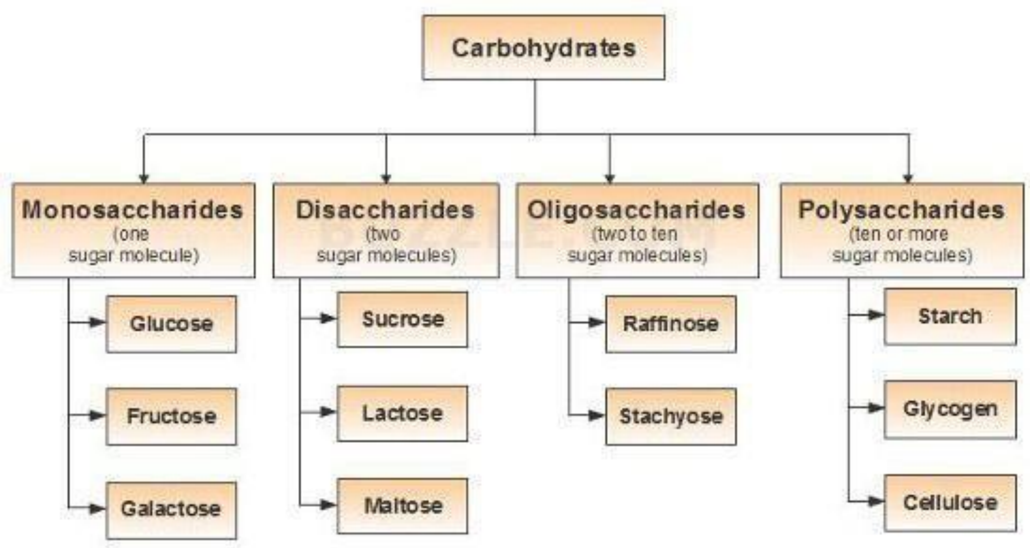
Oligosaccharides consist of short chains of monosaccharide units, or residues, joined by characteristic linkages called glycosidic bonds. The most abundant are the disaccharides, with two monosaccharide units. Example: sucrose (cane sugar).

3. **Polysaccharides**

The polysaccharides are sugar polymers containing more than 20 or so monosaccharide units, and some have hundreds or thousands of units. Example: starch.

Polysaccharides are of two types based on their function and composition. Based on function, polysaccharides are of two types: storage and structural.

A. Storage polysaccharide - starch.
B. Structural polysaccharide - cellulose.



## General properties of carbohydrates

- Carbohydrates act as energy reserves, also stores fuels, and metabolic intermediates.
- Ribose and deoxyribose sugars forms the structural frame of the genetic material, RNA and DNA.
- Polysaccharides like cellulose are the structural elements in the cell walls of bacteria and plants.
- Carbohydrates are linked to proteins and lipids that play important roles in cell interactions.
- Carbohydrates are organic compounds, they are aldehydes or ketones with many hydroxyl groups.

## Physical Properties of Carbohydrates:

- Stereoisomerism - Compound shaving same structural formula but they differ in spatial configuration. Example: Glucose has two isomers with respect to penultimate carbon atom. They are D-glucose and L-glucose.

- Optical Activity - It is the rotation of plane polarized light forming (+) glucose and (-) glucose.

- Diastereoisomerism - It is the configurational change with respect to C2, C3, or C4 in glucose. Example: Mannose, galactose.
- Anomerism - It is the spatial configuration with respect to the first carbon atom in aldoses and second carbon atom in ketoses.

## Biological Importance

- Carbohydrates are chief energy source, in many animals, they are instant source of energy. Glucose is broken down by glycolysis/ Kreb's cycle to yield ATP.
- Glucose is the source of storage of energy. It is stored as glycogen in animals and starch in plants.
- Stored carbohydrates act as energy source instead of proteins.
- Carbohydrates are intermediates in biosynthesis of fats and proteins.
- Carbohydrates aid in regulation of nerve tissue and as the energy source for brain.
- Carbohydrates get associated with lipids and proteins to form surface antigens, receptor molecules, vitamins and antibiotics.
- They form structural and protective components, like in cell wall of plants and microorganisms.
- In animals they are important constituent of connective tissues.
- They participate in biological transport, cell-cell communication and activation of growth factors.
- Carbohydrates rich in fibre content help to prevent constipation.
- Also they help in modulation of immune system.

## Monosaccharides

- The word “Monosaccharides” derived from the Greek word “Mono” means Single and “saccharide” means sugar
- Monosaccharides are polyhydroxy aldehydes or ketones which cannot be further hydrolysed to simple sugar.

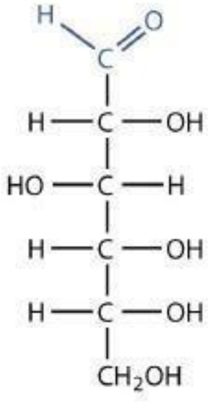
- Monosaccharides are simple sugars. They are sweet in taste. They are soluble in water. They are crystalline in nature.

- They contain 3 to 10 carbon atoms, 2 or more hydroxyl (OH) groups and one aldehyde (CHO) or one ketone (CO) group.

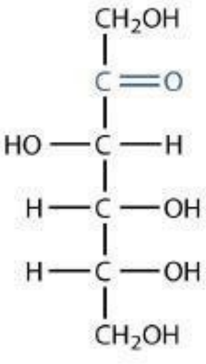
## Classification of Monosaccharides

Monosaccharides are classified in two ways. (a) First of all, based on the number of carbon atoms present in them and (b) secondly based on the presence of carbonyl group.

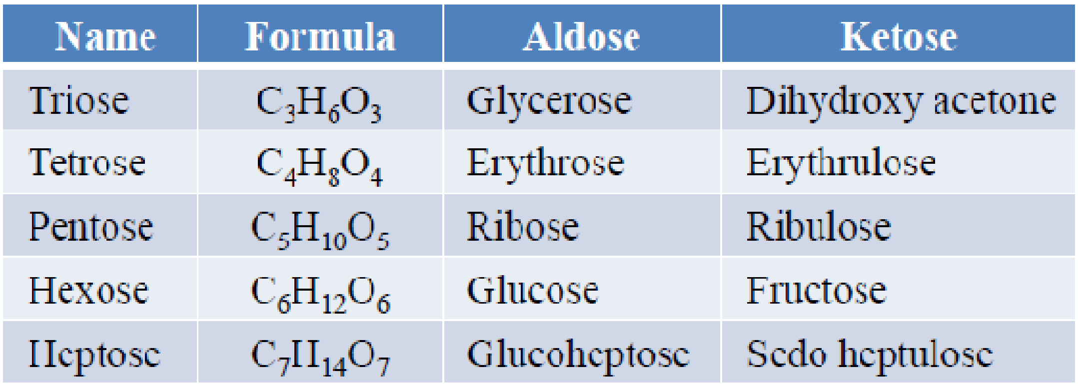
The naturally occurring monosaccharides contain three to seven carbon atoms per molecule. Monosaccharides of specific sizes may be indicated by names composed of a stem denoting the number of carbon atoms and the suffix -*ose*. For example, the terms *triose*, *tetrose*, *pentose*, and *hexose* signify monosaccharides with, respectively, three, four, five, and six carbon atoms. Monosaccharides are also classified as aldoses or ketoses. Those monosaccharides that contain an aldehyde functional group are called aldoses; those containing a ketone functional group on the second carbon atom are ketoses. Combining these classification systems gives general names that indicate both the type of carbonyl group and the number of carbon atoms in a molecule. Thus, monosaccharides are described as aldotetroses, aldopentoses, ketopentoses, ketoheptoses, and so forth. Glucose and fructose are specific examples of an aldohexose and a ketohexose, respectively.



Glucose
(an aldohexose)



Fructose
(a ketohexose)



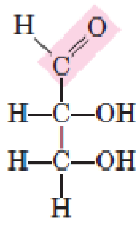
| Name | Formula | Aldose | Ketose |
|---|---|---|---|
| Triose | $C_3H_6O_3$ | Glycerose | Dihydroxy acetone |
| Tetrose | $C_4H_8O_4$ | Erythrose | Erythrulose |
| Pentose | $C_5H_{10}O_5$ | Ribose | Ribulose |
| Hexose | $C_6H_{12}O_6$ | Glucose | Fructose |
| Heptose | $C_7H_{14}O_7$ | Glucoheptose | Sedo heptulose |

**Trioses**

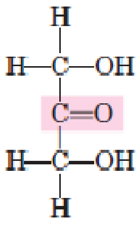
Trioses are “Monosaccharides” containing 3 carbon atoms. The molecular formula of triose is C3H6O3

Characteristics:

- Trioses are simple sugars
- They are soluble in water
- They are sweet in taste.
- The triose may contain an aldehyde group (aldotriose) or a ketone group (ketotriose). Example: Glyceraldehyde and Dehydroxyacetone



Glyceraldehyde, an aldotriose



Dihydroxyacetone, a ketotriose

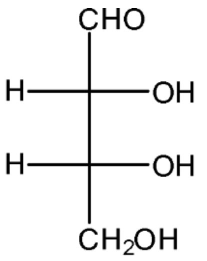
**Tetroses**

Tetroses are “Monosaccharides” containing 4 carbon atoms. The molecular formula of tetrose is C4H8O4

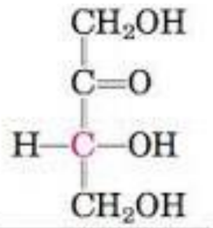
Characteristics:

- Tetroses are simple sugars

- Tetroses are soluble in water
- They are sweet in taste.
- They are crystalline forms.
- The tetroses may contain an aldehyde group (aldotetrose) or a ketone group (ketotetrose).



**Erythrose**



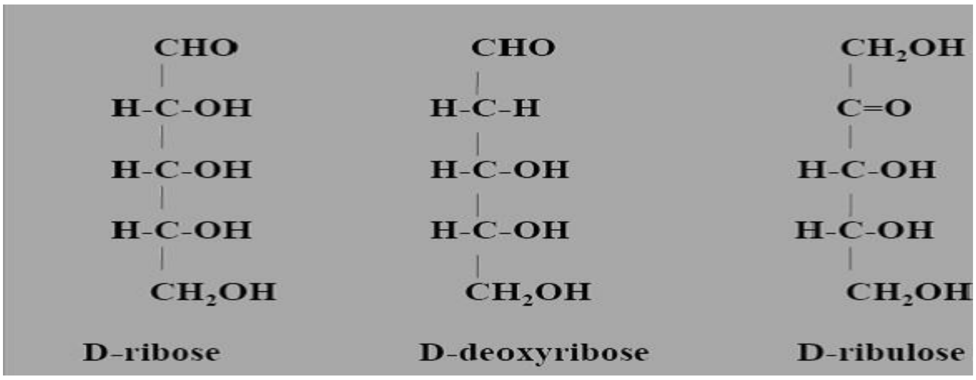
D-Erythrulose

**Pentoses**

Pentoses are "Monosaccharides" containing 5 carbon atoms. It is an important component of "nucleic acid". The molecular formula of Pentose is $C_5H_{10}O_5$

Characteristics:

- Pentoses are simple sugars
- Pentoses are soluble in water
- They are sweet in taste.
- They are crystalline forms.
- The pentoses may contain aldehyde group (aldopentose) or ketone group (ketopentose).


CHO
H-C-OH
H-C-OH
H-C-OH
CH2OH
D-ribose
CHO
H-C-H
H-C-OH
H-C-OH
CH2OH
D-deoxyribose
CH2OH
C=O
H-C-OH
H-C-OH
CH2OH
D-ribulose

## Hexoses

Hexoses are “Monosaccharides” containing 6 carbon atoms. The molecular formula of Hexose is C6H12O6

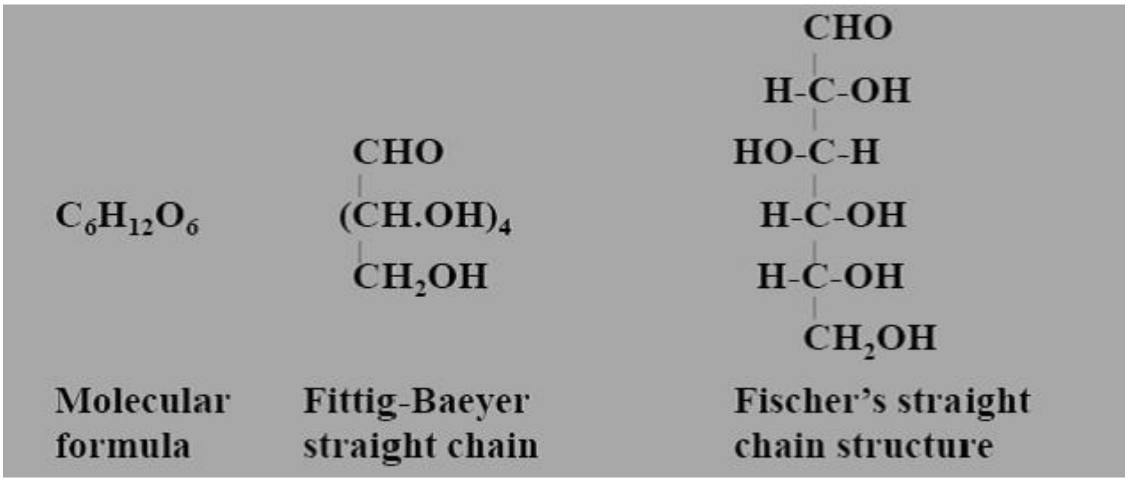
Characteristics:

- Hexoses are simple sugars
- Hexoses are soluble in water
- They are sweet in taste.
- They are crystalline forms.
- The hexoses may contain an aldehyde group (aldohexose) or a ketone group (ketohexose).

## Structure of Monosaccharides

1. **Straight or Open Chain Structure**: Here 6 carbon atoms of glucose are arranged in a straight line. It is also called open chain structure because the two ends remain separate and they are not linked. Open chain structures are of two types:

- (a)Structure proposed by Fittig and Baeyer
- (b)Structure proposed by Fischer known as Fischer’s Projection Formula.



2. **Cyclic or Ring Structure:** Here the atoms are arranged in the form of a ring. Haworth (1929) proposed this formula and hence the name Haworth's Projection Formula. The sugar molecules exist in two type of rings which are as follows – (a)Furanose Ring – 5 membered ring

(b)Pyranose Ring- 6 membered ring

Furanose Ring

Pyranose Ring

## Properties of Monosaccharides

1. Colour - colourless
2. Shape - crystalline
3. Solubility – in Water
4. Taste - sweet
5. Optical activity – Optically active. (a) Dextrorotatory ('d' form) and (b) Levorotatory ('l' form)
6. **Mutarotation** – The change in specific rotation of an optically active compound is called mutarotation. +1120 +52.50 +190 α-D-glucose β -D-glucose
7. **Reducing agents –**

Monosaccharides reduce oxidizing agent such as hydrogen peroxide. In such reaction, sugar is oxidized at the carbonyl group and oxidizing agent becomes reduced.

$C_6H_{12}O_6 + 2\ Cu(OH)_2 \rightarrow C_6H_{12}O_7 + Cu_2O + 2H_2O$

## Chemical properties of carbohydrates

Monosaccharides

Reactions of monosaccharides are due to the presence of hydroxyl (-OH) and the potentially free aldehyde (-CHO) or keto ( >C=O) groups.

### Reaction with alkali

Dilute alkali

Sugars in weak alkaline solutions undergo isomerization to form 1,2-enediol followed by the formation of a mixture of sugars.

### Strong alkali

Under strong alkaline conditions sugar undergo caramelization reactions.

### Reducing property of sugars

- **Sugars are classified as either reducing or non-reducing depending upon the**
    - Presence of potentially free aldehyde or keto groups.
    - Petal ions such as copper or silver to form insoluble cuprous oxide, under alkaline condition.

and Nylander's tests) and quantitative reactions.

- All monosaccharides are reducing. In the case of oligosaccharides, if the molecule possesses a free aldehyde or ketone group it belongs to reducing sugar (maltose and lactose).
- If the reducing groups are involved in the formation of glycosodic linkage.,

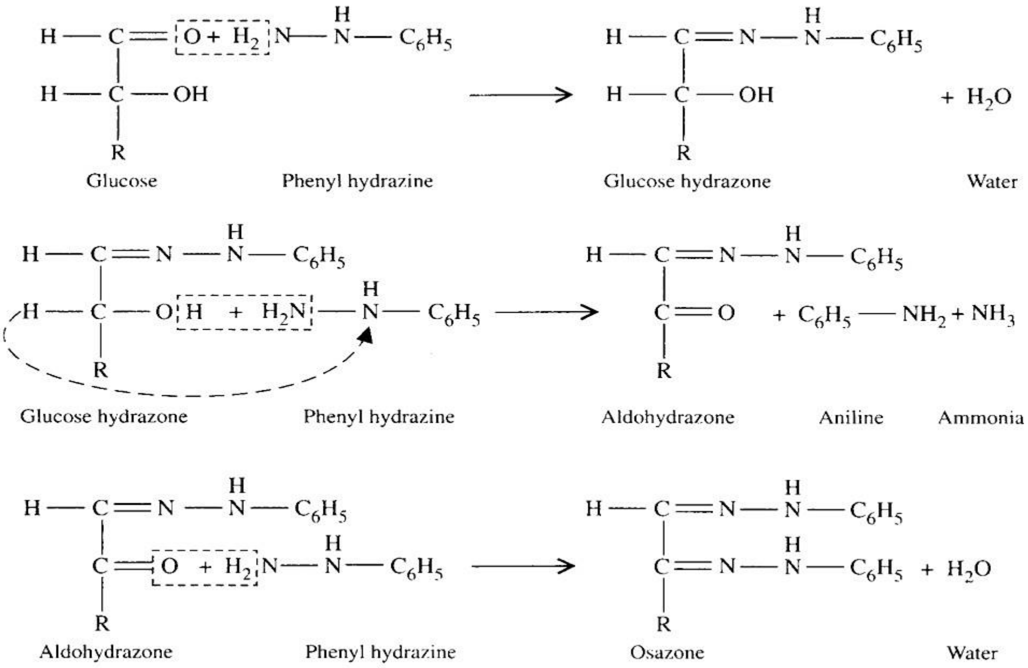
the sugar belongs to the non- reducing group (trehalose, sucrose, raffinose and

stachyose).

### Reaction with phenylhydrazine Formation of Osazone:

When reducing sugars are heated with phenylhydrazine at pH 4.7 a yellow

precipitate is obtained.



- The precipitated compound is called as osazone.
- One molecule of reducing sugar reacts with three molecules of phenylhydrazine.
- D-mannose and D-fructose form same type of osazone as that of D-glucose since the configuration of C-3, C-4, C-5 and C-6 is same for all the three sugars. (Glucose osazone looks Sheaves of corn or broomstick), lactose is powder puff shape.
- This reaction serves to distinguish between aldose and ketose sugars.

Disaccharides - like sunflowers

Lactose - like tight balls of needles

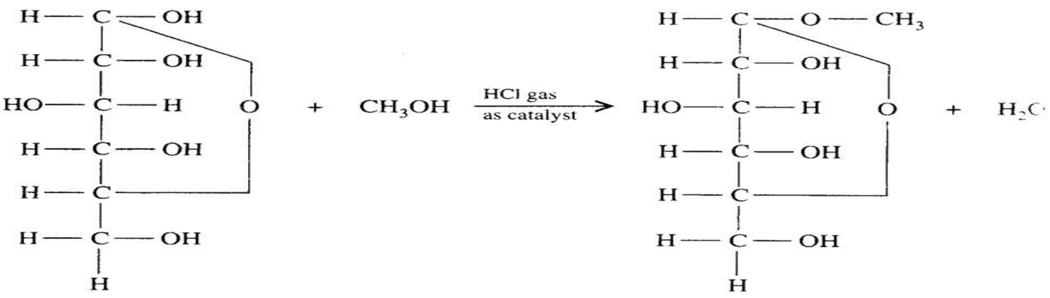
Monosaccharides - like needle-shaped or broomstick

**Reaction with acids**

Heating a solution of hexoses in a strong non-oxidising acidic conditions, hydroxyl methyl furfural is formed.

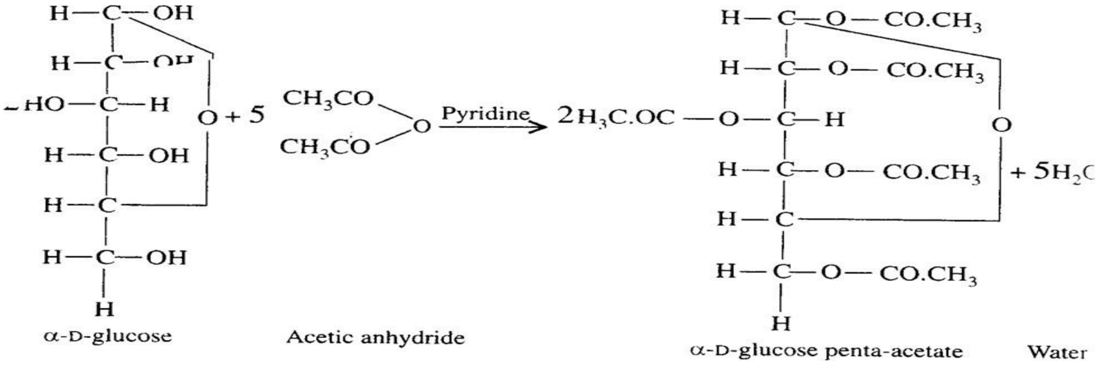
The hydroxymethyl furfural from hexose is usually oxidized further to other products.
When phenolic compounds such as resorcinol, α-naphthol or anthrone are added, mixture of coloured compounds are formed.

**Glucoside formation -**



Glucose + Methyl alcohol = Methyl glucoside

**Esterification** – The chemical reaction that takes place during the formation of the ester is called esterification. Esterification is the process of combining an organic acid (RCOOH) with an alcohol (ROH) to form an ester (RCOOR) and water; or a chemical reaction resulting in the formation of at least one ester product.



- The precipitated compound is called as osazone.
- One molecule of reducing sugar reacts with three molecules of phenylhydrazine.
- D-mannose and D-fructose form same type of osazone as that of D-glucose

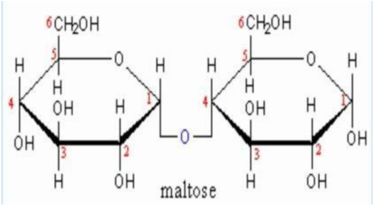
### Disaccharides

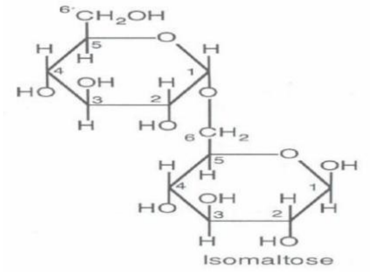
Disaccharides consist of two sugars joined by an O-glycosidic bond. The most abundant disaccharides are sucrose, lactose and maltose. Other disaccharides include isomaltose, cellobiose and trehalose.

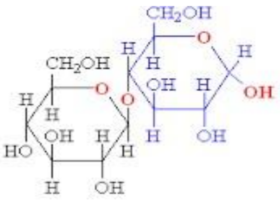
The disaccharides can be classified into:

1. **Homodisaccharides**
2. **Heterodisaccharides**.

| **Homodisaccharides** | **Maltose**<br>**(malt sugar )** | **Isomaltose** | **Cellobiose** |
|---|---|---|---|
| **Structure** | 2α-glucose | 2 α-glucose | 2β-D-glucose |
| **Type of bond** | α-1-4 glucosidicbond | α1-6 glucosidicbond | β1-4 glucosidicbond. |
| **Anomeric Carbon** | Free | Free | Free |
| **Reducing Property** | Reducing | Reducing | Reducing |
| **Produced by** | It is produced from starch by the action of amylase | by the hydrolysis of some polysaccharides such as dextran | by the acid hydrolysis of cellulose |



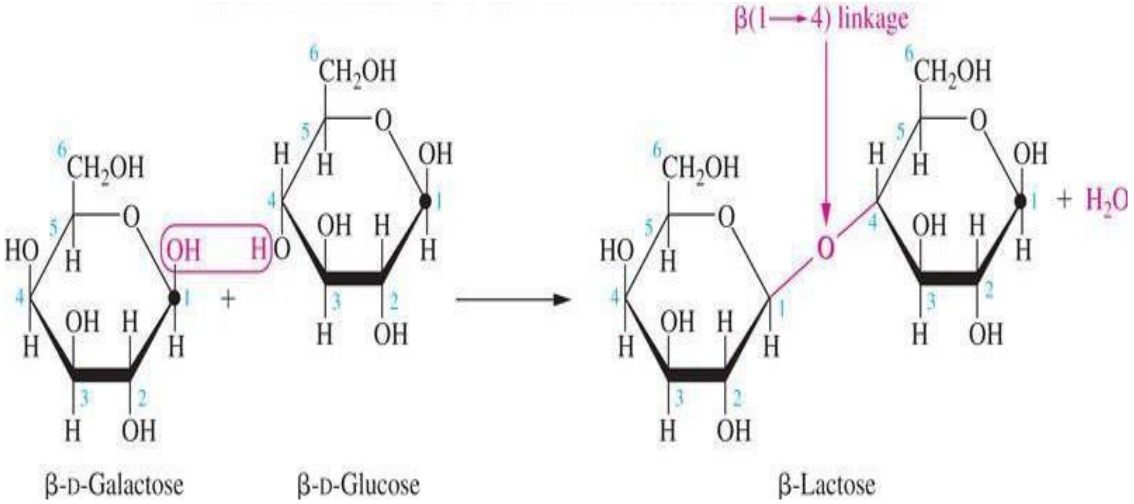


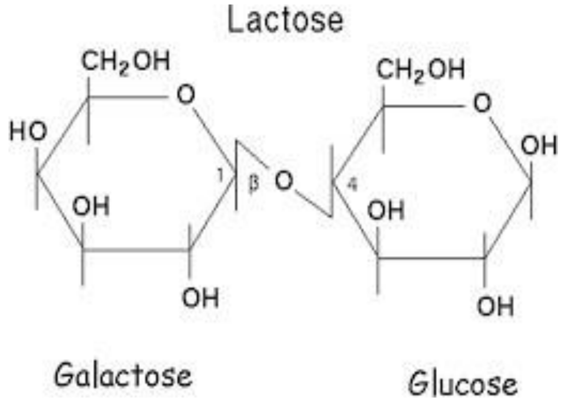


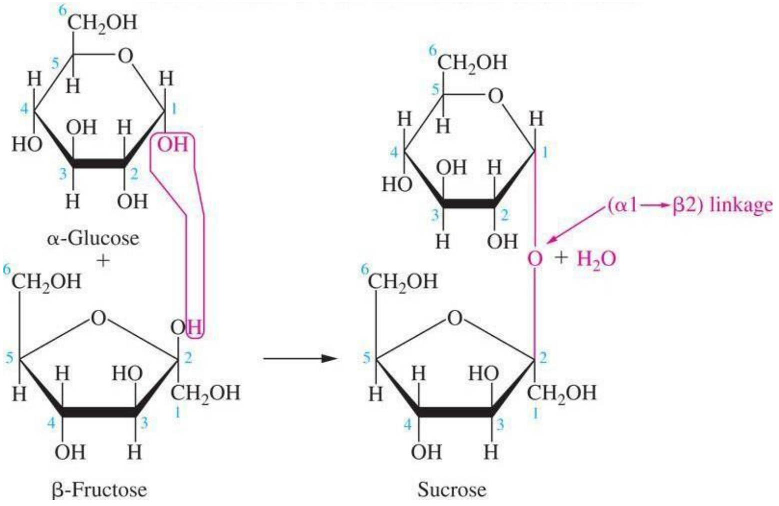
cellobiose
4-(β-D-glucopyanosyl)-D-glucose

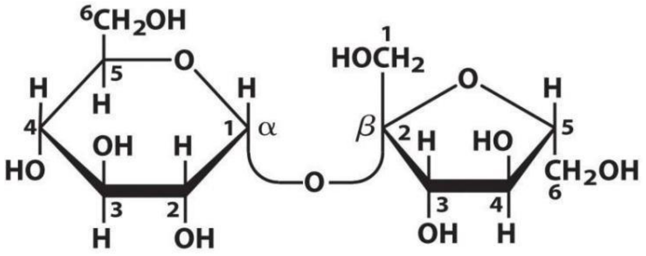
**Heterodisaccharides**: are formed of 2 different monosaccharide units

| **Heterodisaccharides** | **Sucrose** | **Lactose** |
|---|---|---|
| **Composition** | α-D-glucose+ β–D-fructose | β-D-galactose and β-D-glucose |
| **Type of bond** | α-1-β-2 glucosidic bond OR β 2-α-1 fructosidic bond | a β (1→4) galactosidic bond |
| **Anomeric C** | no free aldehyde or ketone group | Free |
| **Reducing property** | is not a reducing sugar | Reducing |
| **Composition** | α-D-glucose+β–D-fructose | β-D-galactose and β-D-glucose |
| **Effect of hydrolysis** | The hydrolysis of sucrose to glucose and fructose is catalysed by sucrase (also called invertase) | Hydrolysed by the intestinal lactase enzyme into galactose and glucose |
| **Present in** | Table sugar<br>Cane sugar,<br>beet sugar | Milk sugar<br>It may appear in urine in late pregnancy and during lactation |


β(1⟶4) linkage
$CH_2OH$
O
OH
H
HO
1
2
3
4
5
6
+
$H_2O$
β-D-Galactose
β-D-Glucose
β-Lactose


Lactose
$CH_2OH$
O
HO
OH
1
β
4
Galactose
Glucose





**Sucrose**
**$\alpha$-D-glucopyranosyl $\beta$-D-fructofuranoside**
**Glc($\alpha$1↔2$\beta$)Fru**

## Polysaccharides

Polysaccharides contain hundreds or thousands of carbohydrate units.

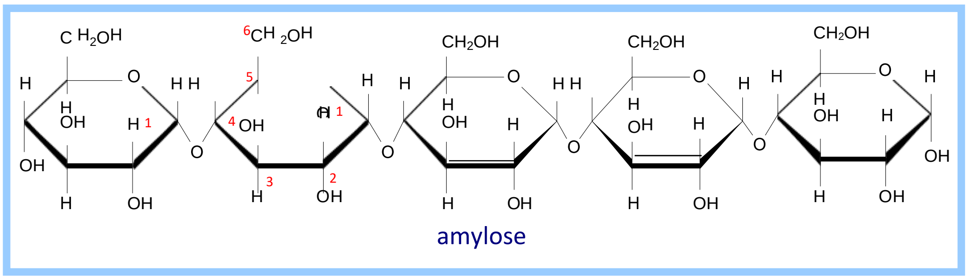
- Polysaccharides are *not* reducing sugars, since the anomeric carbons are connected through glycosidic linkages.
- **Nomenclature:**

**Homopolysaccharide-** a polysaccharide is made up of **one type** of monosaccharide unit

**Heteropolysaccharide-** a polysaccharide is made up of more than **one type** of monosaccharide unit

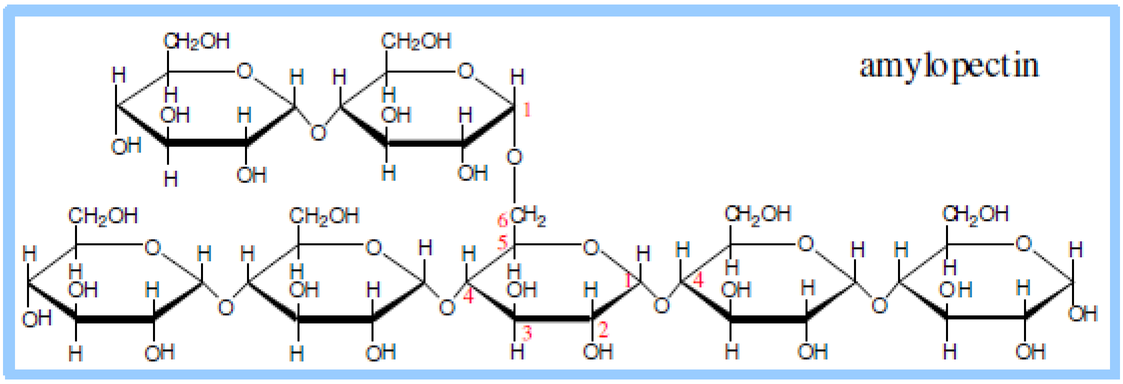
## Starch

- Starch is a polymer consisting of D-glucose units.
- Starches (and other glucose polymers) are usually insoluble in water because of the high molecular weight, but they can form thick colloidal suspensions with water.
- Starch is a **storage** compound in plants, and made of glucose units
- It is a homopolysaccharide made up of two components: **amylose** and **amylopectin**.
- Most starch is 10-30% amylose and 70-90% amylopectin.
- **Amylose** – a straight chain structure formed by **1,4 glycosidic bonds** between **α-D-glucose** molecules.



**Structure of Amylose Fraction of Starch**

- The amylose chain forms a helix.
- This causes the blue colour change on reaction with iodine.
- Amylose is poorly soluble in water, but forms micellar suspensions
- Amylopectin-a glucose polymer with mainly α -(1→4) linkages, but it also has branches formed by α -(1→6) linkages. Branches are generally longer than shown above.

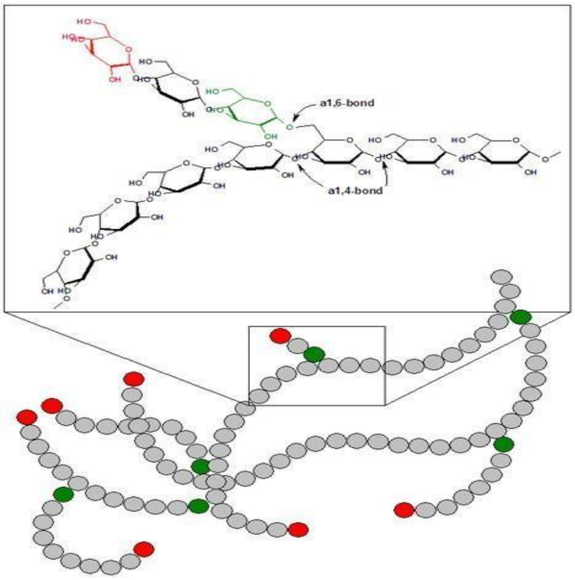


**Structure of Amylopectin Fraction of Starch**

- Amylopectin causes a red-violet colour change on reaction with iodine.
- This change is usually masked by the much darker reaction of amylose to iodine.

## Glycogen

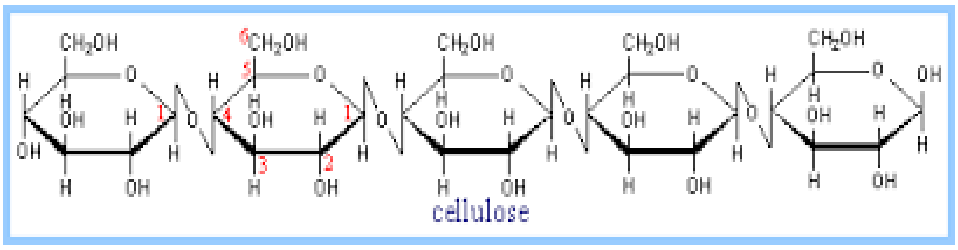
- Storage polysaccharide in animals
- Glycogen constitutes up to 10% of liver mass and 1-2% of muscle mass
- Glycogen is stored energy for the organism
- Similar in structure to amylopectin, only difference from starch: number of branches
- Alpha(1,6) branches every 8-12 residues
- Like amylopectin, glycogen gives a red-violet color with iodine



## Cellulose

- The β-glucose molecules are joined by condensation, i.e. the removal of water, forming β-(1,4) glycosidic linkages.
- The glucose units are linked into straight chains each 100-1000 units long.
- Weak hydrogen bonds are formed between parallel chains binding them into cellulose microfibrils.
- Cellulose microfibrils arrange themselves into thicker bundles called macrofibrils. (These are usually referred to as fibres.)
- The cellulose fibres are often “glued” together by other compounds such as hemicelluloses and calcium pectate to form complex structures such as plant cell walls.
- Because of the β-linkages, cellulose has a different overall shape from amylose, forming extended straight chains which hydrogen bond to each other, resulting in a very rigid structure.

- Cellulose is an important structural polysaccharide, and is the single most abundant organic compound on earth. It is the material in plant cell walls that provides strength and rigidity; wood is 50% cellulose.
- Most animals lack the enzymes needed to digest cellulose, although it does provide needed roughage (dietary fiber) to stimulate contraction of the intestines and thus help pass food along through the digestive system
- Some animals, such as cows, sheep, and horses, can process cellulose through the use of colonies of bacteria in the digestive system which are cap able of breaking cellulose down to glucose; ruminants use a series of stomachs to allow cellulose a longer time to digest. Some other animals such as rabbits reprocess digested food to allow more time for the breakdown of cellulose to occur.
- Cellulose is also important industrially, from its presence in wood, paper, cotton, cellophane, rayon, linen, nitrocellulose (gun cotton), photographic films (cellulose acetate), etc.



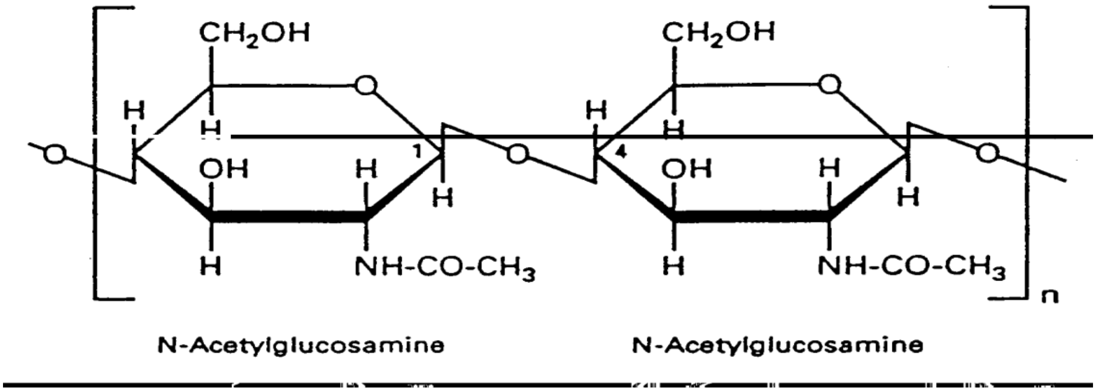
## Chitin

- Chitin is a polymer that can be found in anything from the shells of beetles to webs of spiders. It is present all around us, in plant and animal creatures.
- It is sometimes considered to be a spinoff of cellulose, because the two are very molecularly similar.
- Cellulose contains a hydroxy group, and chitin contains acetamide.
- Chitin is is a "natural polymer," or a combination of elements that

exists naturally on earth.

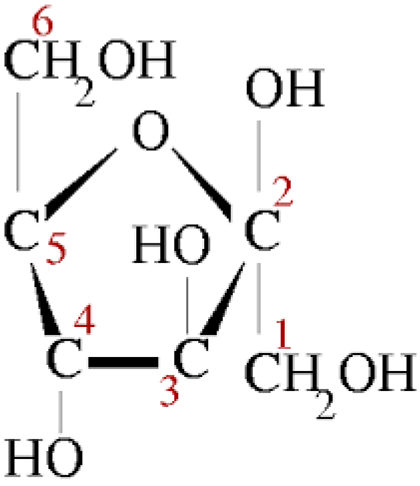
- Crabs, beetles, worms and mushrooms contain large amount of chitin.
- Chitin is a very firm material, and it helps to protect an insect against harm and pressure.

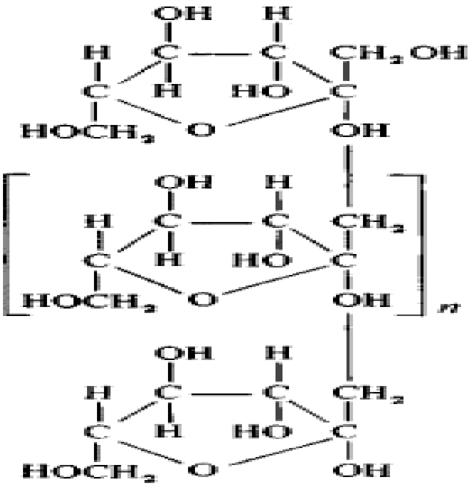
Chitin



## Inulin

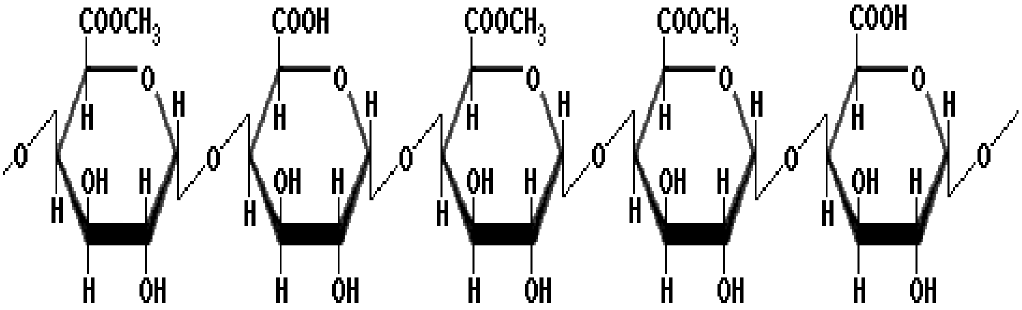
- Inulin is stored in the tubers of the dahlia and artichoke and in the roots of dandelion. It is also found in onion and garlic.
- Inulin has a molecular weight of about 5,000 kd and consists of about 30–35 fructose units per molecule.
- It is formed in the plants by eliminating a molecule of water from the glycosidic OH group on carbon atom 2 of one β-D-fructose unit and the alcoholic OH group on carbon atom 1 of the adjacent β-D-fructose unit.





## Pectin

- Pectins are found as intercellular substances in the tissues of young plants and are especially abundant in ripe fruits such as guava, apples and pears.
- Pectin is a polysaccharide of α-D-galacturonic acid where some of the free carboxyl groups are, either partly or completely, esterified with methyl alcohol and others are combined with calcium or magnesium ions. Chemically, they are called polygalacturonides.



## Mucopolysaccharides

Polysaccharides that are composed not only of a mixture of simple sugars but also of derivatives of sugars such as amino sugars and uronic sugars are called mucopolysaccharides.

## Hyaluronic acid

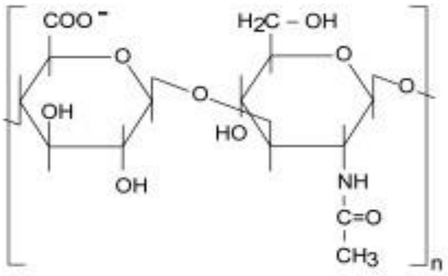
- It is the most abundant member of mucopolysaccharides and is found in higher animals as a component of various tissues such as the vitreous body of the eye, the umbilical cord and the synovial fluid of joints.
- It is a straight-chain polymer of D-glucuronic acid and N-acetyl-D-glucosamine (NAG) alternating in the chain. Its molecular weight approaches approximately, 5,000,000. Linkages involved are β-1 → 3 and β-1 → 4.

## Chondroitin

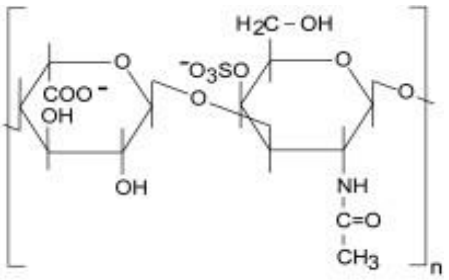
- Chondroitin is of limited distribution. It is found in cartilage and is also a component of cell coats. It is a parent substance for two more widely distributed mucopolysaccharides, chondroitin sulfate A and chondroitin sulfate B.
- Chondroitin is similar in structure to hyaluronic acid except that it contains galactosamine rather than glucosamine. It is, thus, a polymer of β- D glucuronido- 1, 3-N-acetyl-D-galactosamine joined by β- 1 → 4 linkages.
- The two chondroitin sulfate A and C are widely distributed and form major

structural components of cartilage, tendons and bones.

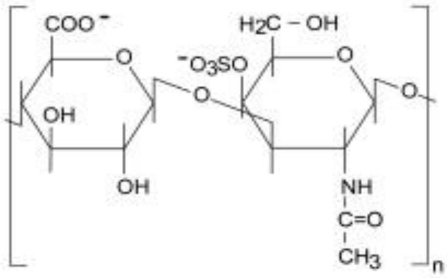
- Chondroitin sulfates may be regarded as derivatives of chondroitin where, in the galactosamine moiety, a sulfate group is esterified either at carbon 4 as in chondroitin sulfate A or at carbon 6 as in chondroitin sulfate C
- The two linkages involved in both types of chondroitin sulfate would, obviously, be the same. These are β-1 → 3 and β-1 → 4.



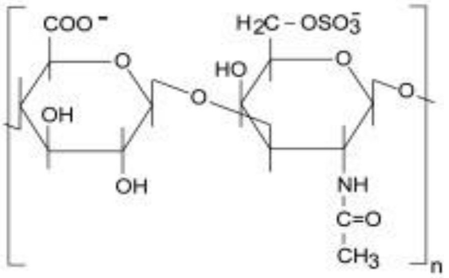
Hyaluronan



Dermatan Sulfate



Chondroitin-4-Sulfate



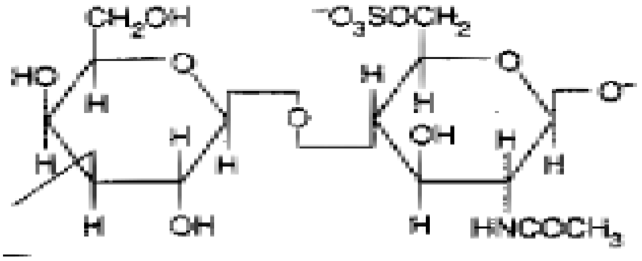
Chondroitin-6-Sulfate

## Dermatan Sulfate

- Dermatan sulfate is a mucopolysaccharide structurally similar to chondroitin sulfate A except that the D-glucuronic acid is replaced by L-iduronic acid
- The two linkages involved are α-1 → 3 and β-1 → 4. Dermatan sulfate is also known by its conventional name, **chondroitin sulfate B**.

## Keratosulfate

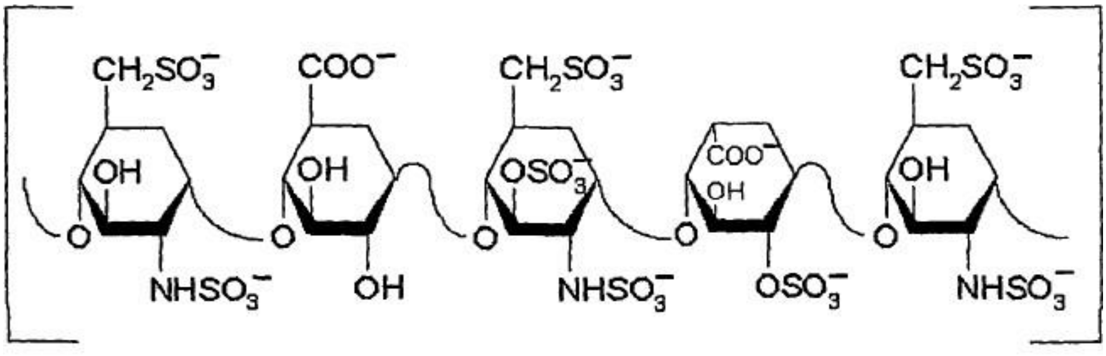
- Keratan sulfate is a sulfated glycosaminoglycans that have been found especially in the cornea, cartilage, and bone. Keratan sulfates are large, highly hydrated molecules which in joints can act as a cushion to absorb mechanical shock. Keratosulfate differs from other mucopolysaccharides in that the uronic acid component is replaced by D-galactose. Here, the second acetylated amino sugar component (which is N-acetyl-D-glucosamine in this case) is esterified by a sulfate group at carbon 6. Although, the two alternating linkages involved are β-1 → 4 and β-1 → 3, in this case the linkage between the repeating disaccharide units is β-1 →3 rather than β-1 → 4.



## Heparin

- It is composed of D-glucuronic acid units, most of which (about 7 out of every 8) are esterified at C2 and D-glucosamine-N-sulfate (= sulfonylaminoglucose).

- Heparin acts as an anticoagulant. It prevents coagulation of blood by inhibiting the prothrombin thrombin conversion. This eliminates the effect of thrombin on fibrinogen.



**Heparin**

**The following table is the list of biologically important polysaccharides and their functions:**

| Name of the Polysaccharide | Composition | Occurrence | Functions |
|---|---|---|---|
| Starch | Polymer of glucose containing a straight chain of glucose molecules (amylose) and a branched chain of glucose molecules (amylopectin) | In several plant species as main storage carbohydrate | storage of reserve food |
| Glycogen | Polymer of glucose | Animals (equivalent of starch) | Storage of reserve food |
| Cellulose | Polymer of glucose | Different regions of plant, in sieve tubes of phloem | Cell wall matrix |
| Insulin | Polymer of fructose | In roots and tubers (like Dahlia) | Storage of reserve food |

| | | | |
|---|---|---|---|
| Pectin | Polymer of galactose and its derivatives | Plant cell wall | Cell wall matrix |
| Hemi cellulose | Polymer of pentoses and sugar acids | Plant cell wall | Cell wall matrix |
| Lignin | Polymer of glucose | Plant cell wall (dead cells like sclerenchyma) | Cell wall matrix |
| Chitin | Polymer of glucose | Bodywall of arthropods. In some fungi also | Exoskeleton Impermeable to water |
| Murein | Polysaccharide cross linked with amino acids | Cell wall of prokaryotic cells | Structural protection |
| Hyaluronic acid | Polymer of sugar acids | Connective tissue matrix, Outer coat of mammalian eggs | Ground substance, protection |
| Chrondroitin Sulphate | Polymer of sugar acids | Connective tissue Matrix | Ground substance |
| Heparin | Closely related to Chrondroitin | Connective tissue Cells | Anticoagulant |
| Gums and mucilages | Polymers of sugars and sugar acids | Gums - bark or trees. Mucilages - Flower | Retain water in dry seasons |

## Digestion and Absorption of Carbohydrates

### From the Mouth to the Stomach

The mechanical and chemical digestion of carbohydrates begins in the mouth. Chewing, also known as mastication, crumbles the carbohydrate foods into smaller and smaller pieces. The salivary glands in the oral cavity secrete saliva that coats the food particles. Saliva contains the enzyme, salivary amylase. This enzyme breaks the bonds between the monomeric sugar units of disaccharides, oligosaccharides, and starches. The salivary amylase breaks down amylose and amylopectin into smaller chains of glucose, called dextrins and maltose. The increased concentration of maltose in the mouth that results from the mechanical and chemical breakdown of starches in whole grains is what enhances their sweetness. Only about five percent of starches are broken down in the mouth. (This is a good thing as more glucose in the mouth would lead to more tooth decay.) When carbohydrates reach the stomach no further chemical breakdown occurs because the amylase enzyme does not function in the acidic conditions of the stomach. But the mechanical breakdown is ongoing—the strong peristaltic contractions of the stomach mix the carbohydrates into the more uniform mixture of chyme.

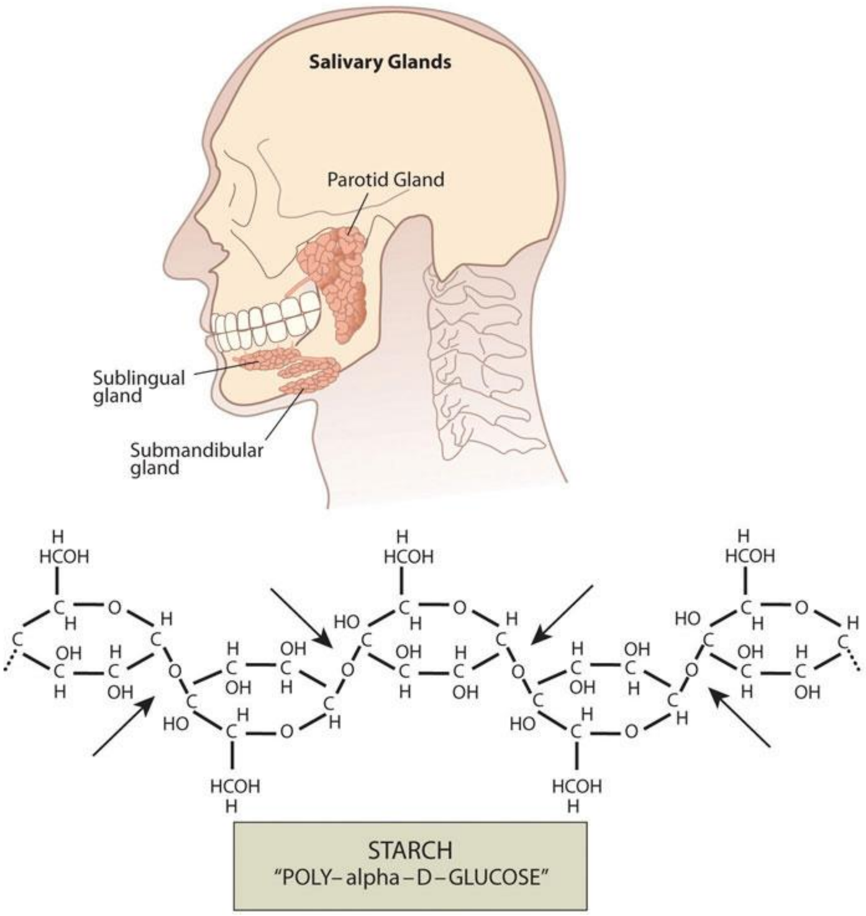


***Figure 3.3.2:*** *Salivary glands secrete salivary amylase, which begins the chemical breakdown of carbohydrates by breaking the bonds between monomeric sugar units.*

## From the Stomach to the Small Intestine

The chyme is gradually expelled into the upper part of the small intestine. Upon entry of the chyme into the small intestine, the pancreas releases pancreatic juice through a duct. This pancreatic juice contains the enzyme, pancreatic amylase, which starts again the breakdown of dextrins into shorter and shorter carbohydrate chains. Additionally, enzymes are secreted by the intestinal cells that line the villi. These enzymes, known collectively as disaccharides, are sucrase, maltase, and lactase. Sucrase breaks sucrose into glucose and fructose molecules. Maltase breaks the bond between the two glucose units of maltose, and lactase breaks the bond between galactose and glucose. Once carbohydrates are chemically broken down into single sugar units they are then transported into the inside of intestinal cells.

When people do not have enough of the enzyme lactase, lactose is not sufficiently broken down resulting in a condition called lactose intolerance. The undigested lactose moves to the large intestine where bacteria are able to digest it. The bacterial digestion of lactose produces gases leading to symptoms of diarrhea, bloating, and abdominal cramps. Lactose intolerance usually occurs in adults and is associated with race. Most people with lactose intolerance can tolerate some amount of dairy products in their diet. The severity of the symptoms depends on how much lactose is consumed and the degree of lactase deficiency.

## Absorption: Going to the Blood Stream

The cells in the small intestine have membranes that contain many transport proteins in order to get the monosaccharides and other nutrients into the blood where they can be distributed to the rest of the body. Fructose is absorbed by facilitated diffusion while glucose and galactose are actively transported. The first organ to receive glucose, fructose, and galactose is the liver. The liver takes them up and converts galactose to glucose, breaks fructose into even smaller carbon-containing units, and either stores glucose as glycogen or exports it back to the blood. How much glucose the liver exports to the blood is under hormonal control and you will soon discover that even the glucose itself regulates its concentrations in the blood.

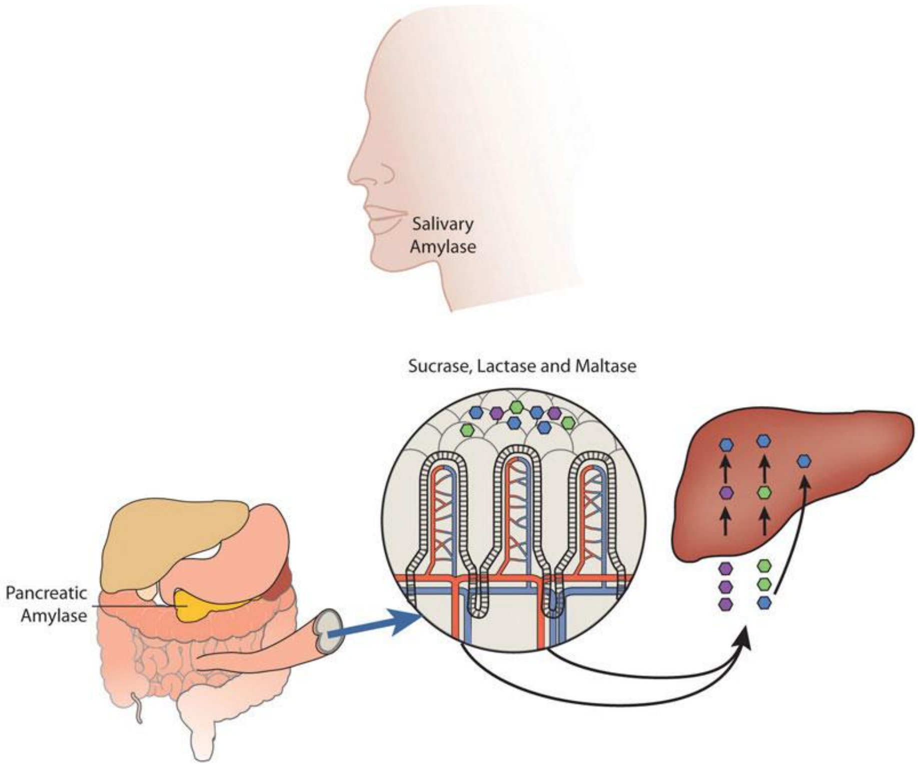


***Figure 3.3.3:*** *Carbohydrate digestion begins in the mouth and is most extensive in the small intestine. The resultant monosaccharides are absorbed into the bloodstream and transported to the liver.*

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**SCHOOL OF BIO AND CHEMICAL ENGINEERING**

**DEPARTMENT OF BIOTECHNOLOGY**

# UNIT – II – BASIC BIOCHEMISTRY – SMB1102

| **SMB1102** | **BASIC BIOCHEMISTRY** | **L** | **T** | **P** | **Credits** | **Total Marks** |
|---|---|---|---|---|---|---|
| | | 5 | 0 | 0 | 3 | 100 |

COURSE OBJECTIVES:

This course is aimed give an understanding about the basics of biochemistry dealing carbohydrates, Amino acids, Lipids, nucleic acid and vitamins.

UNIT 1: CARBOHYDRATES 12 Hrs. Carbohydrate – Definition, Classification, biological significance, structure of glucose, digestion and absorption of carbohydrates.

UNIT 2: PROTEINS 12 Hrs. Amino acids – structure, classification (Essential and non-essential, protein and non-protein amino acids). Proteins – definition, classification and structure (primary, secondary, tertiary and quaternary).

UNIT 3: LIPIDS 12 Hrs. Lipids – definition, classification and biological significance. Structure, properties and functions of fatty acids.

UNIT 4 NUCLEIC ACIDS 12 Hrs. Nucleic acids – Structure of DNA and its functions. Different forms of DNA. Different types of RNA and its functions.

UNIT 5 VITAMINS 12 Hrs. Vitamins – Source, biological function, daily requirement and deficiency symptoms of fat soluble vitamins (A, D, E and K) and water soluble vitamins (Ascorbic acid, thiamine, riboflavin, pyridoxine, niacin, pantothenic acid, lipoic acid, biotin, folic acid and vitamin B12).

TEXT / REFERENCE BOOKS:

1. Lehninger Principles of Biochemistry-David L. Nelson, Michael M. Cox, Macmillan Worth Publishers.
2. Harper―s Biochemistry-Rober K. Murray, Daryl K. Grammer, McGraw Hill, Lange Medical Books. 25th edition.
3. Fundamentals of Biochemistry-J.L. Jain, Sunjay Jain, Nitin Jain, S. Chand & Company.
4. Biochemistry-Dr. Amit Krishna De, S. Chand & Co., Ltd. END SEMESTER EXAMINATION QUESTION

PAPER PATTERN Max. Marks: 100 Exam Duration: 3 Hrs.

PART A: 10 questions of 2 marks each - No choice 20 Marks

PART B: 2 questions from each unit of internal choice; each carrying 16 marks 80 Marks

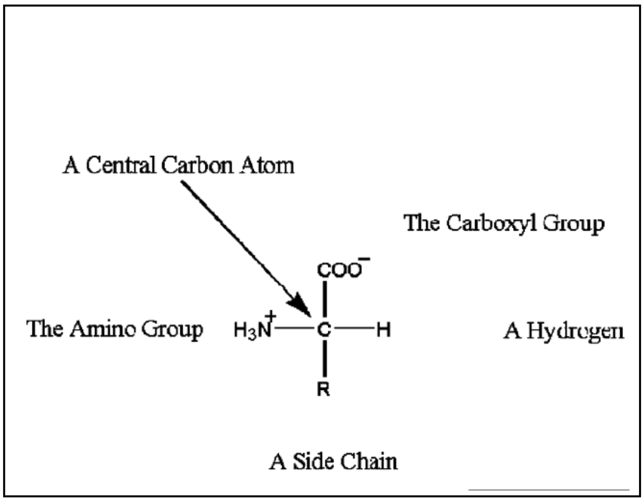
# UNIT - II

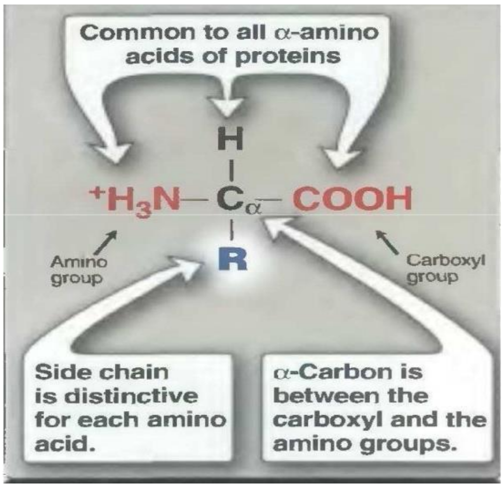
## Amino acids

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. 21. Selenocysteine, 22. pyrrolysine were discovered recently.

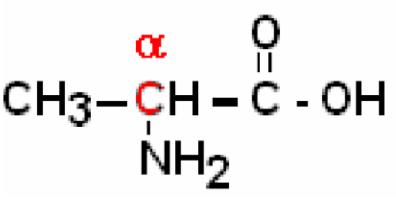
## Structure of amino acids

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



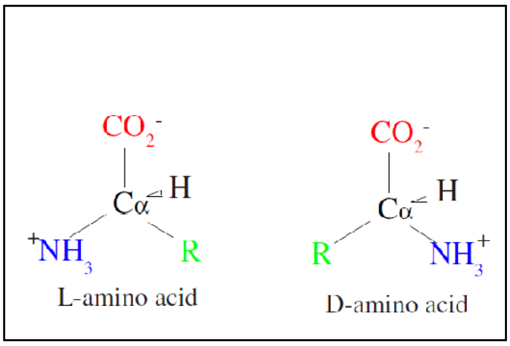


The properties of each amino acid are determined by its specific side chain (R-groups). R-groups vary in structure, size, electric charge and solubility in water from one amino acid to other. Amino acids found in proteins are α-amino acids. The amino group is always found on the carbon adjacent to the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



$$CH_3-\underset{NH_2}{\overset{\alpha}{C}H}-\overset{O}{\overset{\|}{C}}-OH$$

Chirality – amino acids (except glycine) have a tetrahedral $C_\alpha$ 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.



L-amino acid

D-amino acid

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.

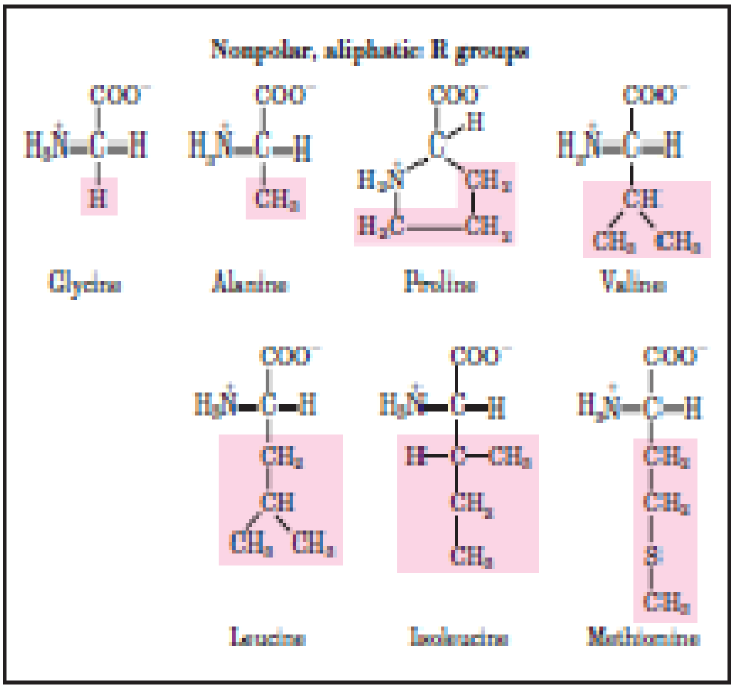
Amino acid names are often abbreviated as either 3 letters or single letter.

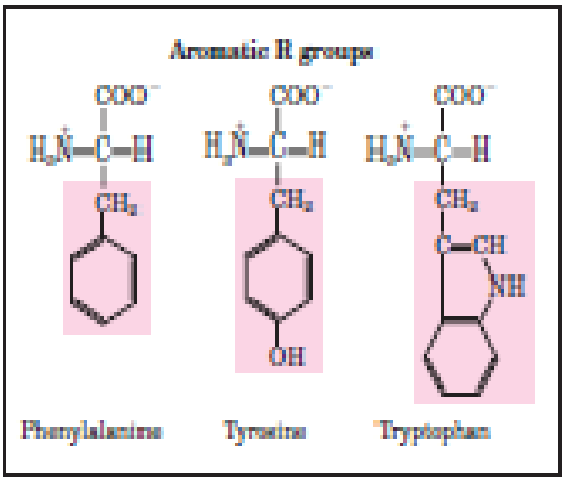
## Zwitter Ions

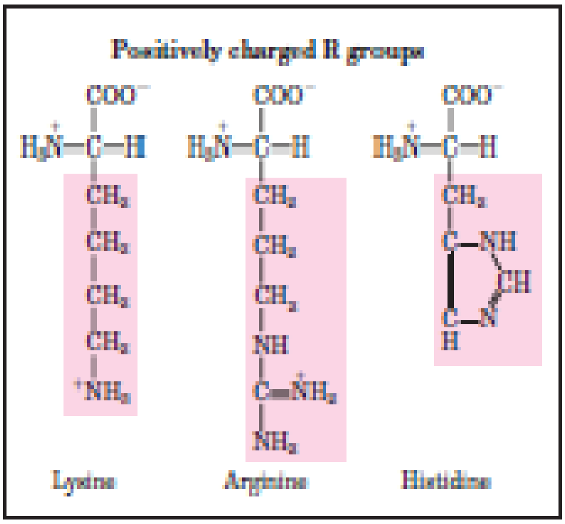
At physiological pH of 7, the carboxyl group of an amino acid is in its conjugate base form ($-COO^-$) and the amino group is in its conjugate acid form ($-NH_3^+$). Thus each amino acid can behave as either an acid or a base. Such molecules which can behave both like an acid and a base are termed amphoteric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

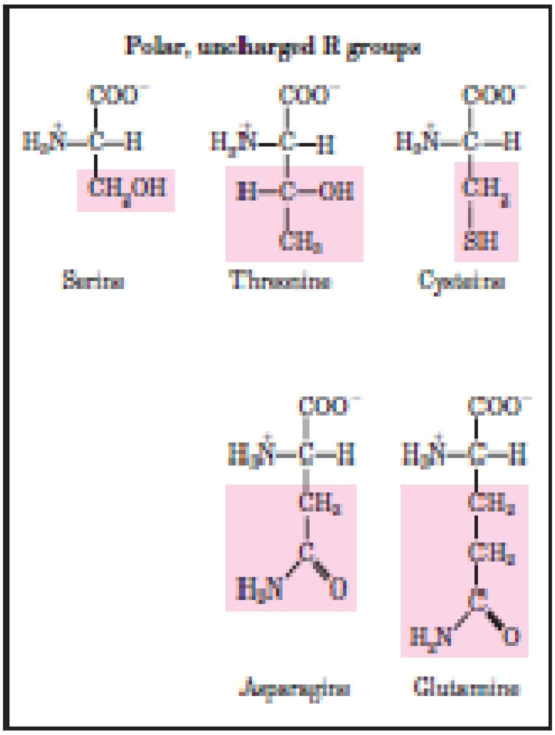
Amino acids contain ionizable groups. The predominant ionic form of these molecules in solution therefore depends on the pH. At acidic pH ($pH < 7$) the carboxyl group ($-COOH$) is uncharged and the ammonium group ($-NH_3^+$) is protonated. Therefore the net charge on the amino acid is positive (+1). 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).

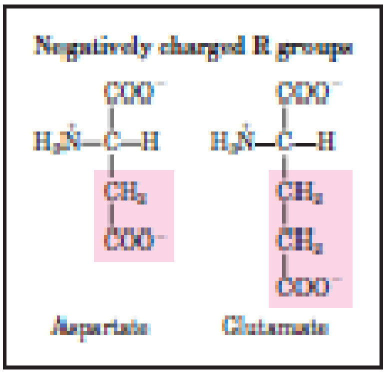
## Structure of amino acids











## Classification of amino acids

1. **Structural classification**
2. **Polarity**
3. **Nutritional Requirements**
4. **Metabolic fate**

**1. Structural classification**

i. Aliphatic side chain Aminoacids

a. Simple aminoacids: Glycine and Alanine

b. Branched aminoacids: VIL valine, Leucine and Isoleucine

c. OH group: OH! Tie Saree thread : tyrosine, serine, theronine

d.Sulfur containing:Methionine, Cysteine

e. Acidic amino acids: Aspartic acid, Glutamic acid

f. Amide group: Aspargine, Glutamine

g. Basic aminoacids: His basics are loose : Histidine, Arginine, Lysine

ii. AROMATIC AMINOACIDS

Phenylalanine

tyrosine

- **Aromatic Aminoacids with heterocyclic ring**

**Tryptophan**

Histidine

**iii. Iminoacid**

Proline

**2. Polar aminoacids**

**charged: negative charged : Aspartic acid, Glutamic acid**

**Positive: Histidine, Lysine, Arginine,**

**Uncharged: Glycine, serine, theronine, aspargine, glutamine**

**Non-Polar AA: Aromatic: Phe, Tyr, Trypt, Valine, Leucine, Isoleucine, Methionine.**

**3. 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:

**Mnemonic: Try This VIP MALL**

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

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. Mnemonic; Almost All Girls Go Crazy After Getting Taken Prom Shopping,

Which stands for Alanine, Asparagine, Glutamate, Glutamine, Cysteine, Aspartate, Glycine, Tyrosine, Proline and Serine.

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

4. Metabolic facts:
i. ketogenic AA: : Leucine, and lysine
ii. Glucose and keto:  Phe, Tyr, Tryp, Isoleucine
iii. Gucogenic: all remaining aminoacids

**I) 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 the class of macromolecules containing nitrogen that are essential for the survival of life. It is now well known that proteins are at the center of action in biological processes and are essential structural components of the cells.

Proteins are composed of smaller units called amino acids linked together as a linear chain. All proteins from whatever sources these may be are composed of 20 different amino acids. 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 |

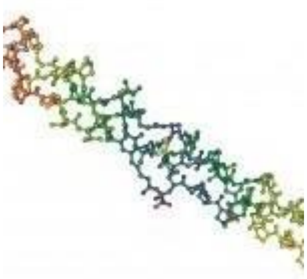
| | | |
|---|---|---|
| Defense | Antibodies | Protect the body from foreign pathogens |
| Contraction | Myosin | Carry out muscle contraction |
| Storage | Legume storage proteins, egg white (albumin) | Provide food for the early development of the embryo or the seedling |

## Protein classification

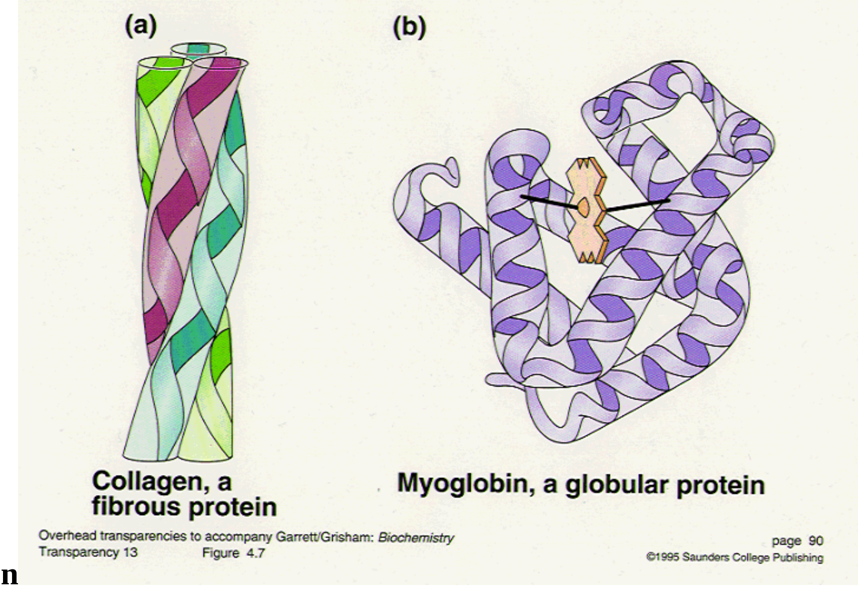
### Protein classification based on shape

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

*Fibrous proteins*



**Collagen**



Collagen, a fibrous protein

Myoglobin, a globular protein

Overhead transparencies to accompany Garrett/Grisham: *Biochemistry*
Transparency 13 Figure 4.7
page 90
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Fibrous proteins are scleroproteins. Fibrous proteins are elongated strand-like structures and are usually present in the form of rods or wires. They are made up of a single unit or structure which is repeated multiple times. Fibrous proteins are highly resistant to digestion by enzymes and are extremely tensile.

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 by its 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.

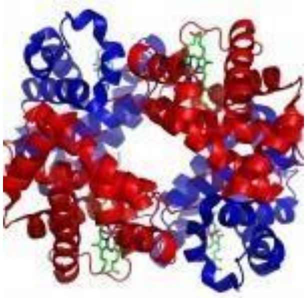
- α-Keratins

  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**

A Globular protein is spherical in shape and has the property of forming colloids with water. It gets dissolved in water. Globular proteins are also called as spheroproteins owing to their shape. 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.

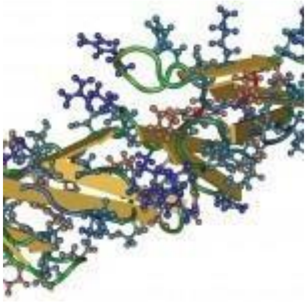
❑

- 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

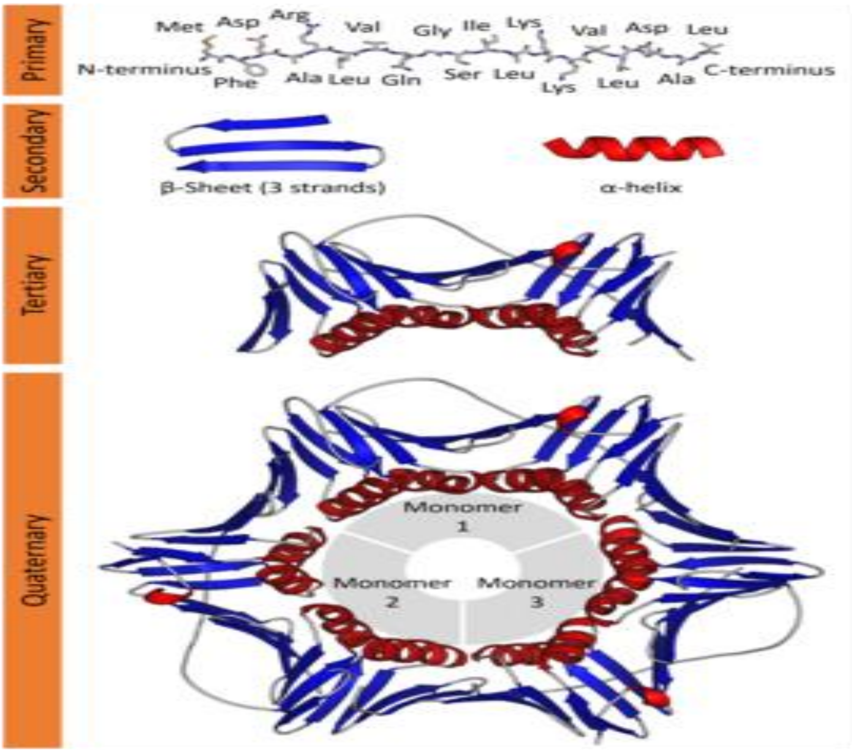
- These proteins are formed in the progressive hydrolytic cleavage of the peptide bonds of protein molecule.
- They are roughly grouped into **proteoses, peptones and peptides according to average molecular weight.**

- 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

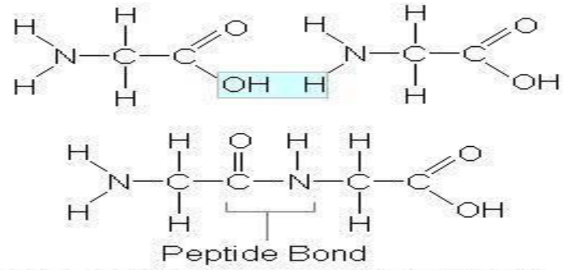
### Protein Structures: Primary, Secondary, Tertiary, Quaternary

- 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”).

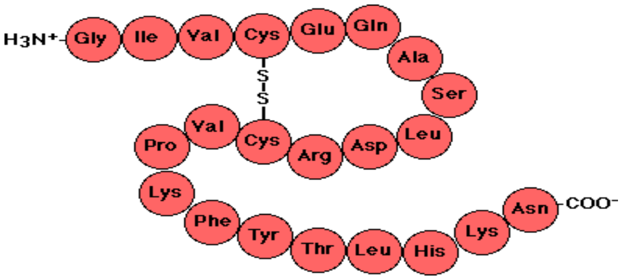


A molecule of water is removed from two glycine amino acids to form a peptide bond.

- 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.

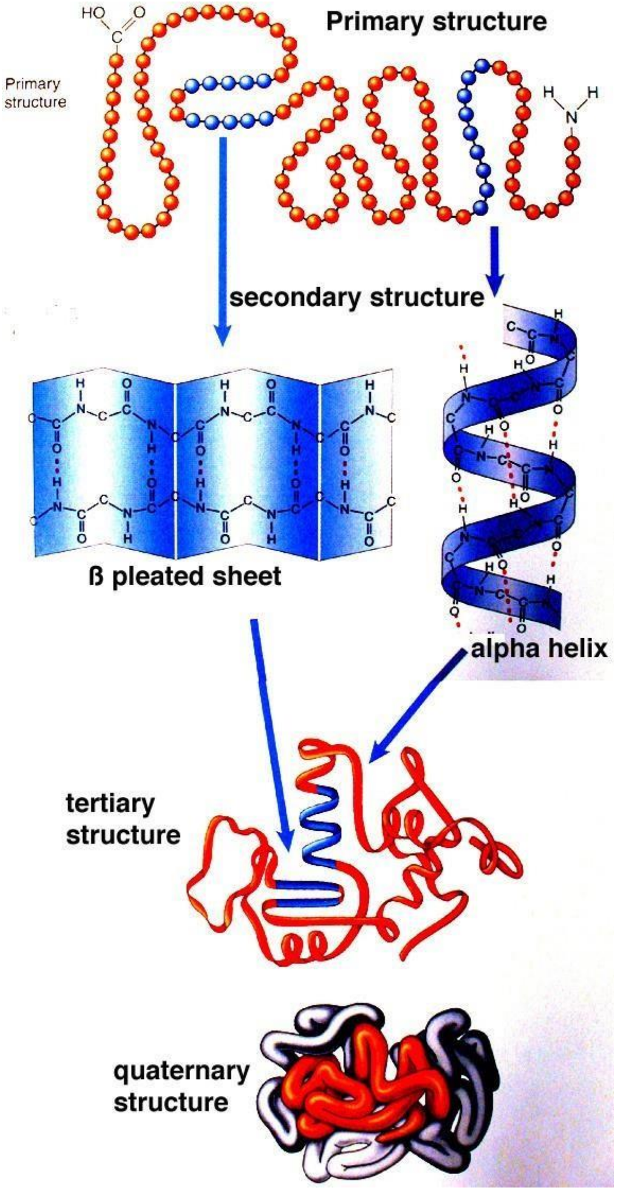


$H_2N\text{-CH-CO-NH-CH-CO-NH-CH-CO----------NH-CH-COOH}$

- 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.

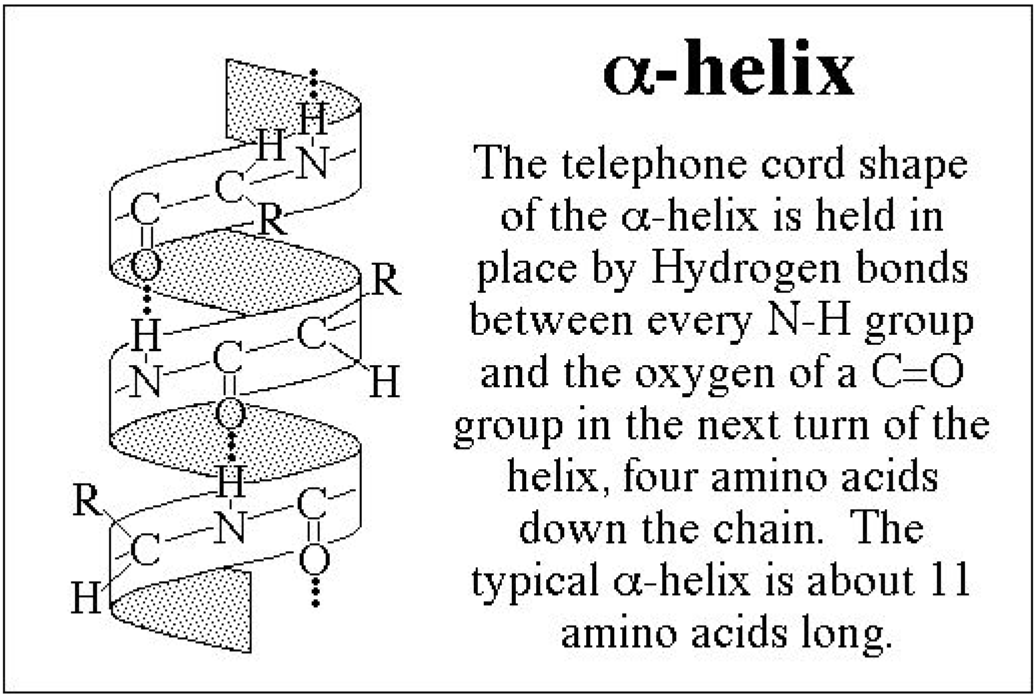
## Secondary Structure

- 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.



**α-helix**

The telephone cord shape of the α-helix is held in place by Hydrogen bonds between every N-H group and the oxygen of a C=O group in the next turn of the helix, four amino acids down the chain. The typical α-helix is about 11 amino acids long.

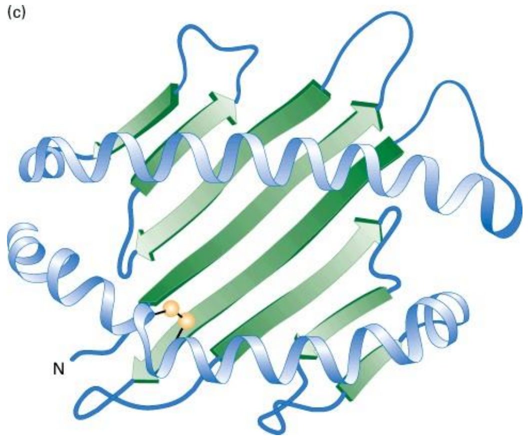
- Other types of secondary structure. While the alpha helix and beta sheet are by far the most common types of structure, many others are possible. These include various loops, helices and irregular conformations. A single polypeptide chain may have different regions that take on different secondary 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.

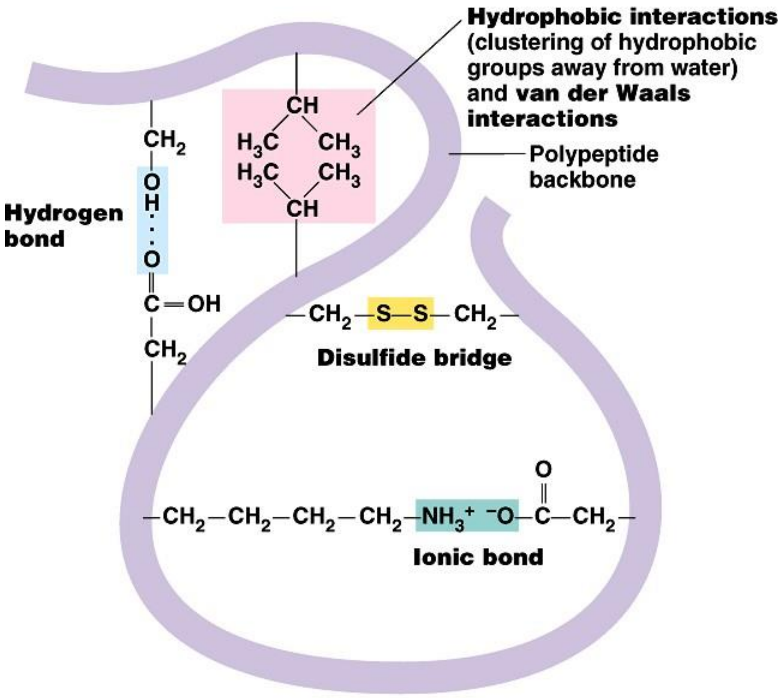
- Whereas secondary structure is stabilized by H-bonding, all four “weak" forces contribute to tertiary structure. Usually, the most important force is hydrophobic interaction (or hydrophobic bonds). Polypeptide chains generally contain both hydrophobic and hydrophilic residues. Much like detergent micelles,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



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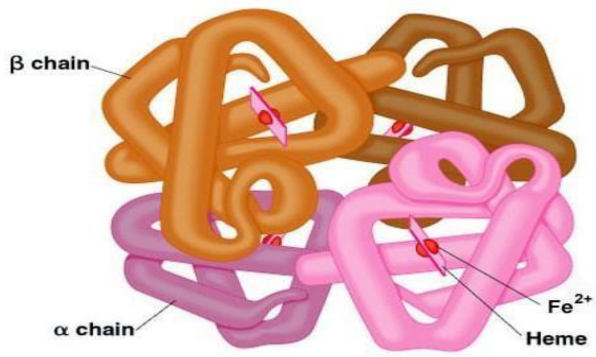
## Structural Domains of Proteins

- 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.

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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

# UNIT – III – BASIC BIOCHEMISTRY – SMB1102

| | | L | T | P | Credits | Total Marks |
|---|---|---|---|---|---|---|
| **SMB1102** | **BASIC BIOCHEMISTRY** | **L** | **T** | **P** | **Credits** | **Total Marks** |
| | | 5 | 0 | 0 | 3 | 100 |

COURSE OBJECTIVES:

This course is aimed give an understanding about the basics of biochemistry dealing carbohydrates, Amino acids, Lipids, nucleic acid and vitamins.

UNIT 1: CARBOHYDRATES 12 Hrs. Carbohydrate – Definition, Classification, biological significance, structure of glucose, digestion and absorption of carbohydrates.

UNIT 2: PROTEINS 12 Hrs. Amino acids – structure, classification (Essential and non-essential, protein and non-protein amino acids). Proteins – definition, classification and structure (primary, secondary, tertiary and quaternary).

UNIT 3: LIPIDS 12 Hrs. Lipids – definition, classification and biological significance. Structure, properties and functions of fatty acids.

UNIT 4 NUCLEIC ACIDS 12 Hrs. Nucleic acids – Structure of DNA and its functions. Different forms of DNA. Different types of RNA and its functions.

UNIT 5 VITAMINS 12 Hrs. Vitamins – Source, biological function, daily requirement and deficiency symptoms of fat soluble vitamins (A, D, E and K) and water soluble vitamins (Ascorbic acid, thiamine, riboflavin, pyridoxine, niacin, pantothenic acid, lipoic acid, biotin, folic acid and vitamin B12).

TEXT / REFERENCE BOOKS:

1. Lehninger Principles of Biochemistry-David L. Nelson, Michael M. Cox, Macmillan Worth Publishers.
2. Harper―s Biochemistry-Rober K. Murray, Daryl K. Grammer, McGraw Hill, Lange Medical Books. 25th edition.
3. Fundamentals of Biochemistry-J.L. Jain, Sunjay Jain, Nitin Jain, S. Chand & Company.
4. Biochemistry-Dr. Amit Krishna De, S. Chand & Co., Ltd. END SEMESTER EXAMINATION QUESTION

PAPER PATTERN Max. Marks: 100 Exam Duration: 3 Hrs.

PART A: 10 questions of 2 marks each - No choice 20 Marks

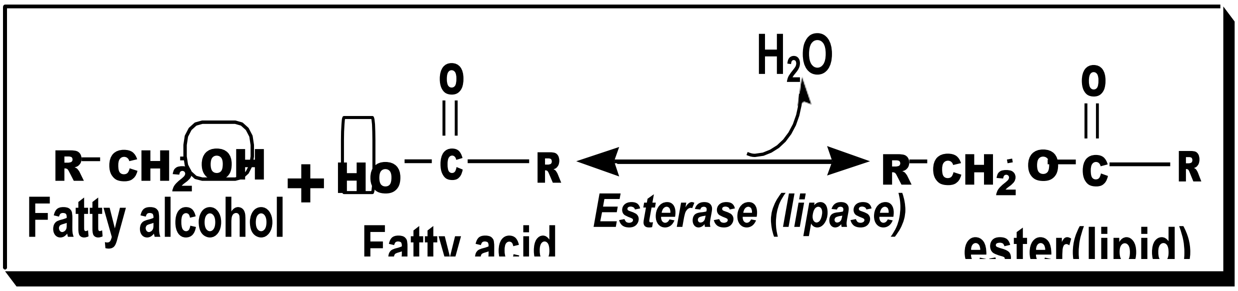
PART B: 2 questions from each unit of internal choice; each carrying 16 marks 80 Marks

# UNIT -III

## LIPDS

### Definition

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



$$R-CH_2-OH + HO-\overset{O}{\overset{\|}{C}}-R \xleftrightarrow[\text{Esterase (lipase)}]{H_2O} R-CH_2-O-\overset{O}{\overset{\|}{C}}-R$$

- 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

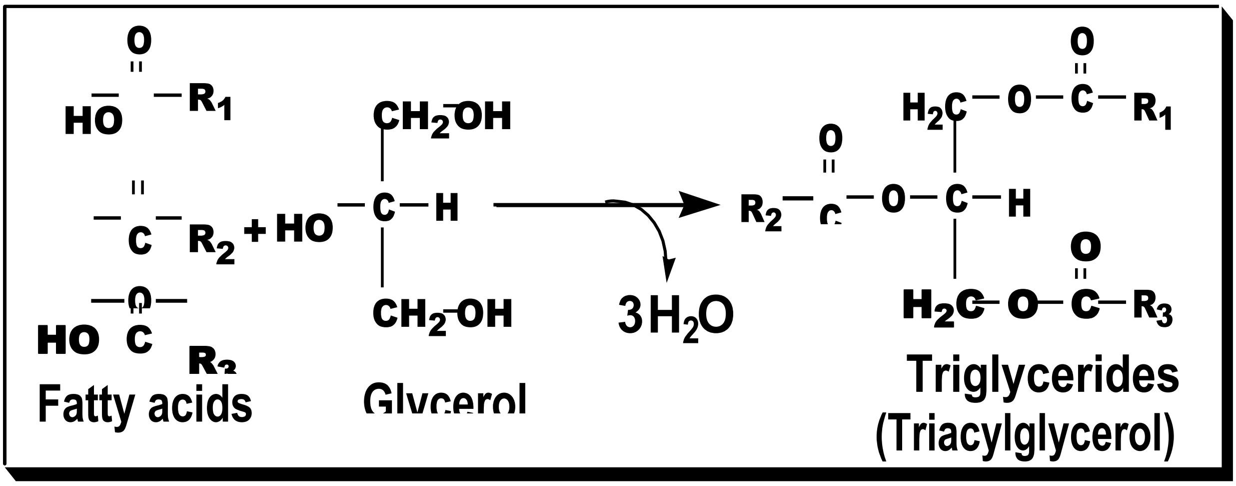
1. Phospholipids
2. Glycolipids.
3. Lipoproteins
4. Sulfolipids and amino lipids.

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**

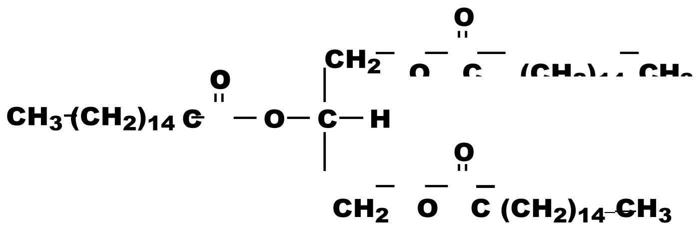
- They are called neutral because they are uncharged due to absence of ionizable groups in it.
- The neutral fats are the most abundant lipids in nature. They constitute about 98% of the lipids of adipose tissue, 30% of plasma or liver lipids, less than 10% of erythrocyte lipids.
- They are esters of 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.



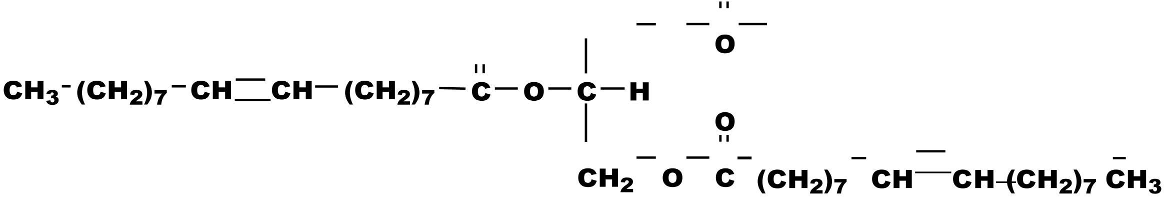
**Types of triglycerides**

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.

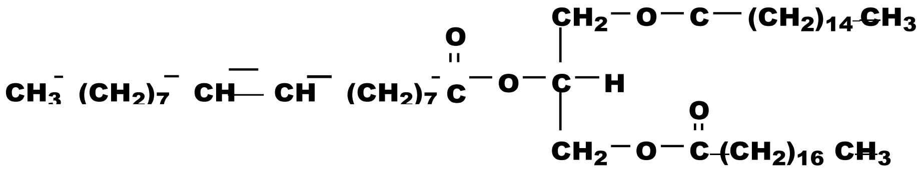
- Natural fats are mixtures of mixed triglycerides with a small amount of simple triglycerides.



**Tripalmitin**
**(simple triacylglycerol)**



**1-Stearo-2,3-diolein**
**(mixed triacylglycerol)**



**1-palmito-2-oleo-3-stearin**

- 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.

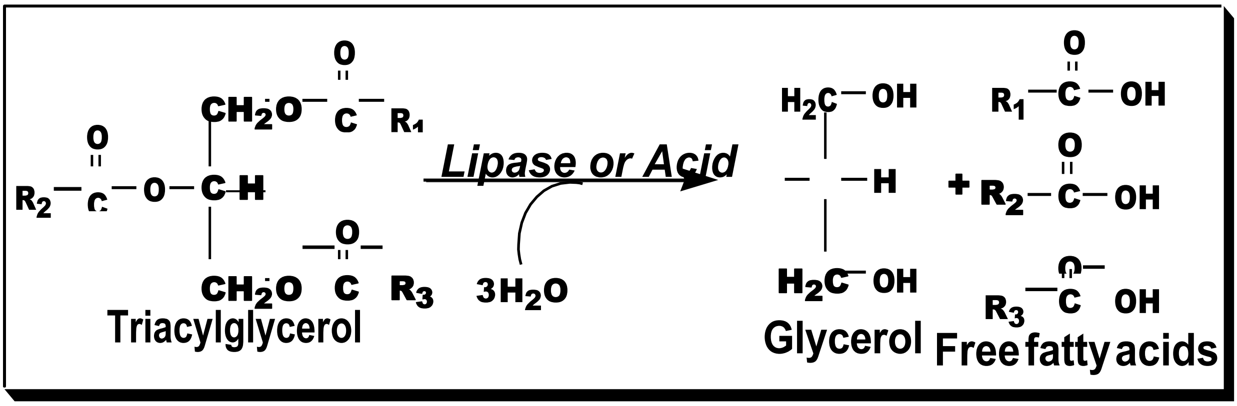
## Physical properties of fat and oils

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

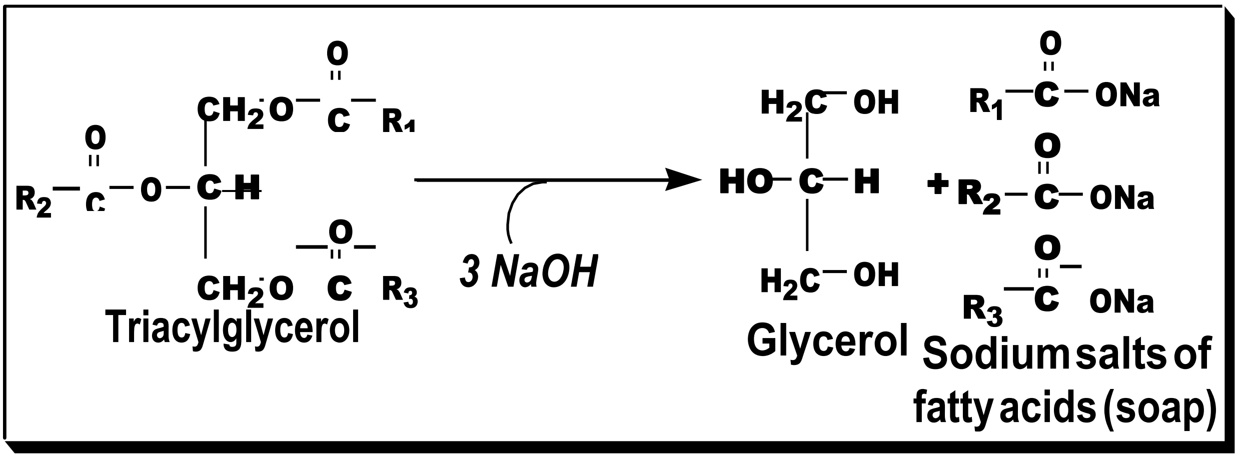
1. **Hydrolysis:**

- 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.



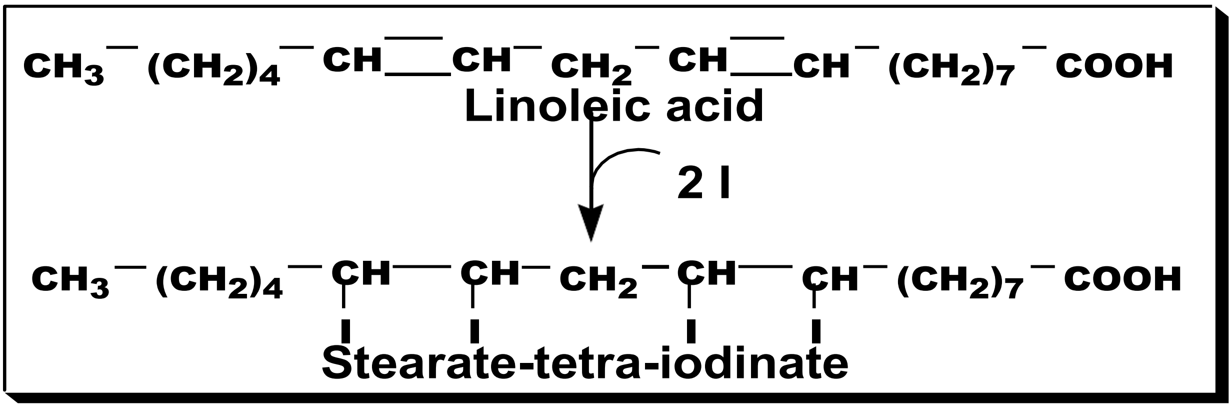
2. **Saponification**.

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.

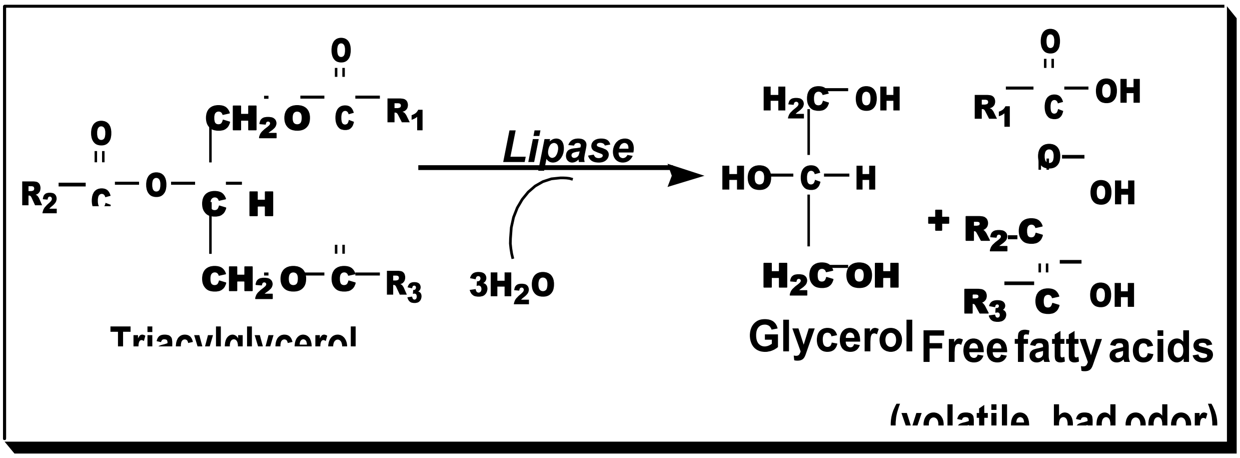
- Saturated fats resist rancidity more than unsaturated fats that have unsaturated double bonds.

**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.)

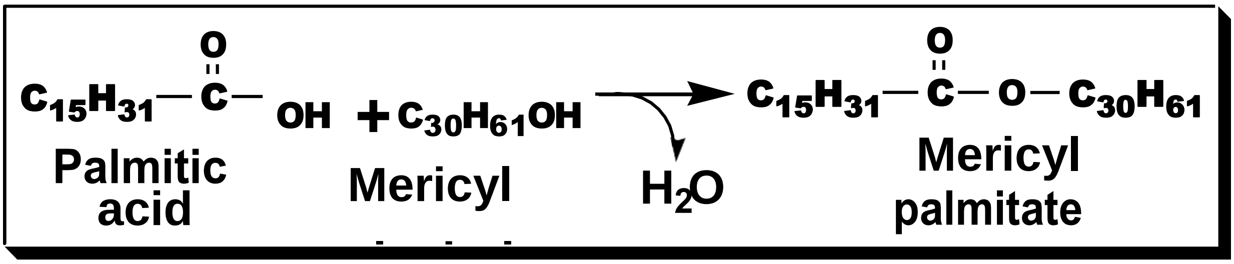
- Waxes are not easily hydrolyzed as the fats and are indigestible by lipases and are very resistant to rancidity.
- Thus they are of no nutritional value.

### 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.



$$C_{15}H_{31}-\overset{O}{\overset{\|}{C}}-OH + C_{30}H_{61}OH \xrightarrow[H_2O]{} C_{15}H_{31}-\overset{O}{\overset{\|}{C}}-O-C_{30}H_{61}$$

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-<u>Glycerophospholipids:</u>** They are regarded as derivatives of phosphatidic acids that are the simplest type of phospholipids and include:

1. <u>Phosphatidic acids</u>.

2. <u>Lecithins</u>

3. <u>Cephalins</u>.

4. <u>Plasmalogens</u>.

5. <u>Inositides</u>.

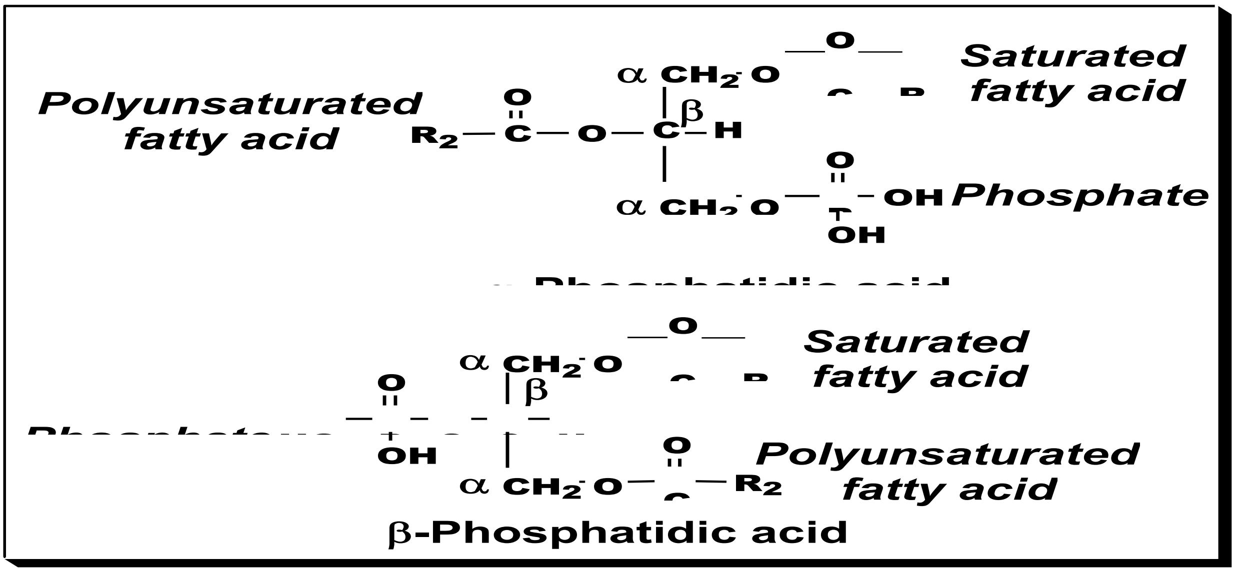
6. <u>Cardiolipin</u>.

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

A. Glycerophospholipids

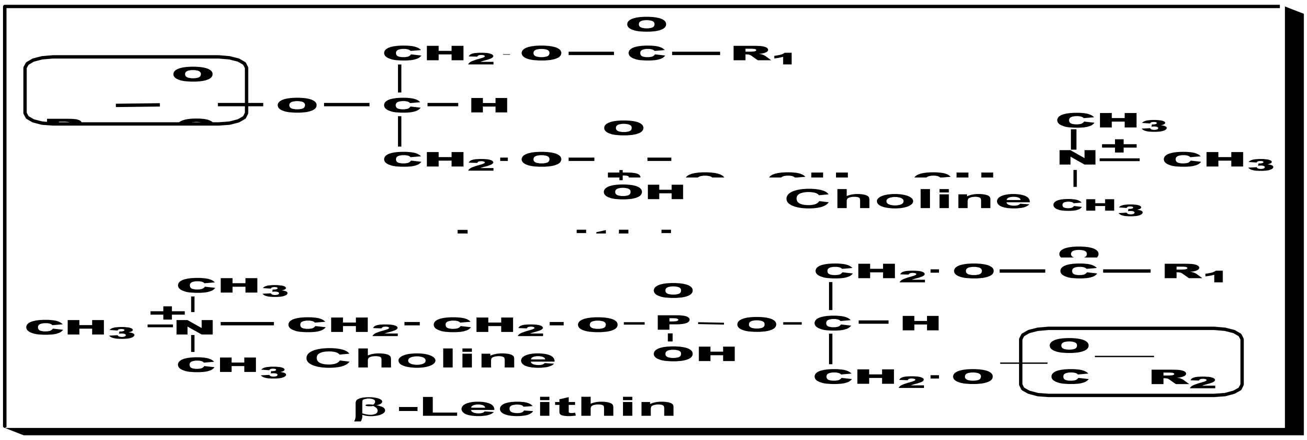
1. Phosphatidic acids:

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



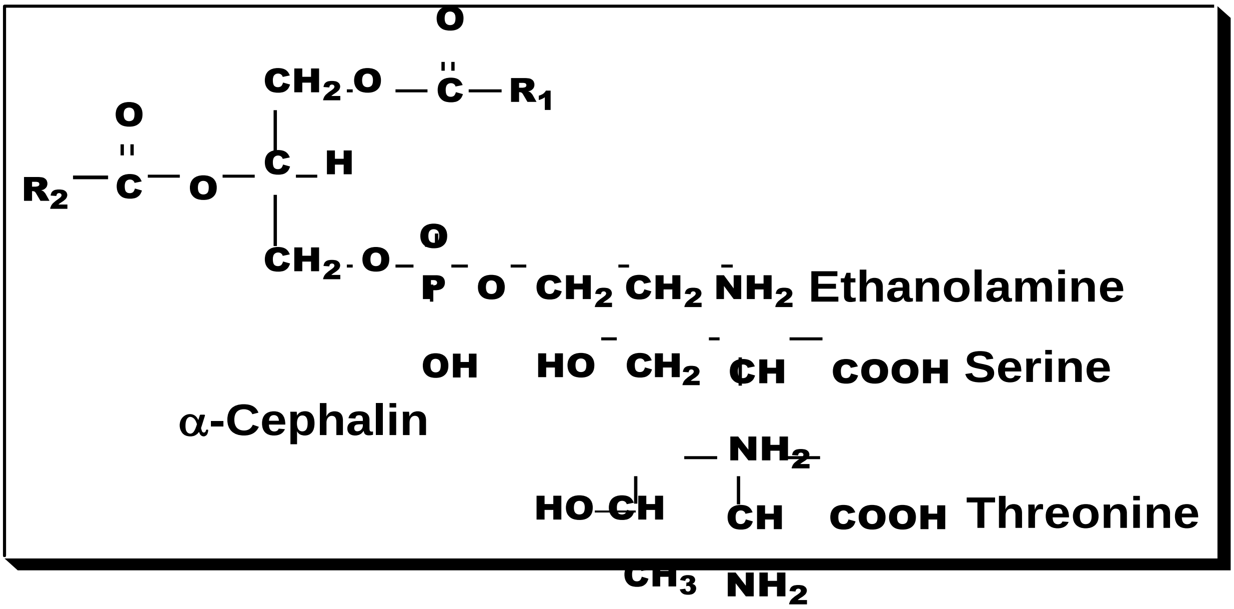
2. Lecithins:

- 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).

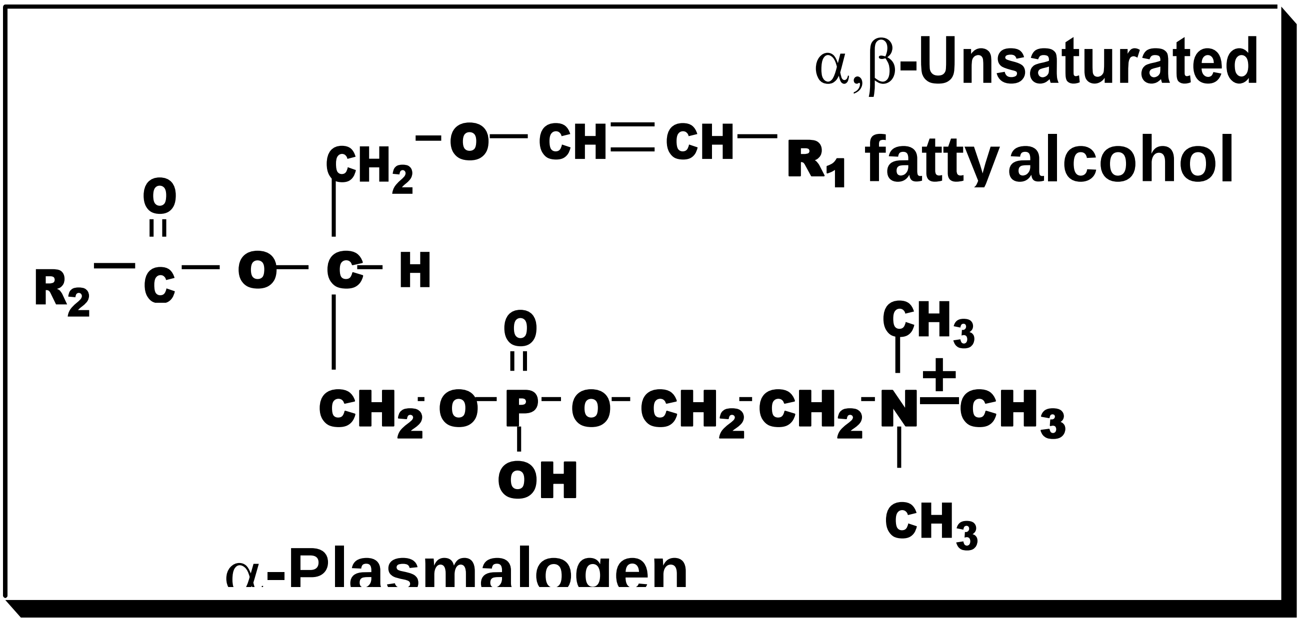


- Structure: Cephalins resemble lecithins in structure except that choline is replaced by ethanolamine, serine or threonine amino acids.

– - -

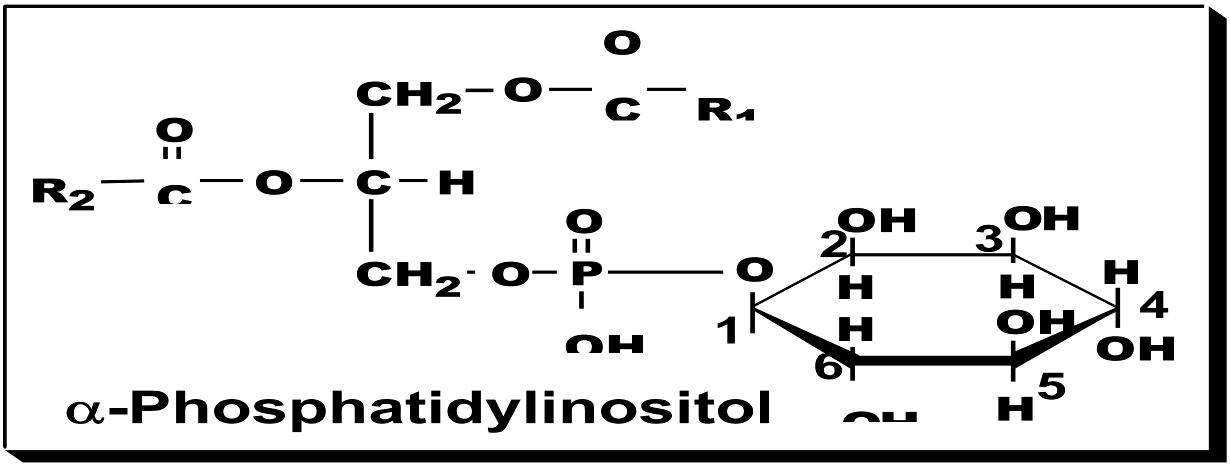
4. Plasmalogens:

- 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



α-Phosphatidylinositol

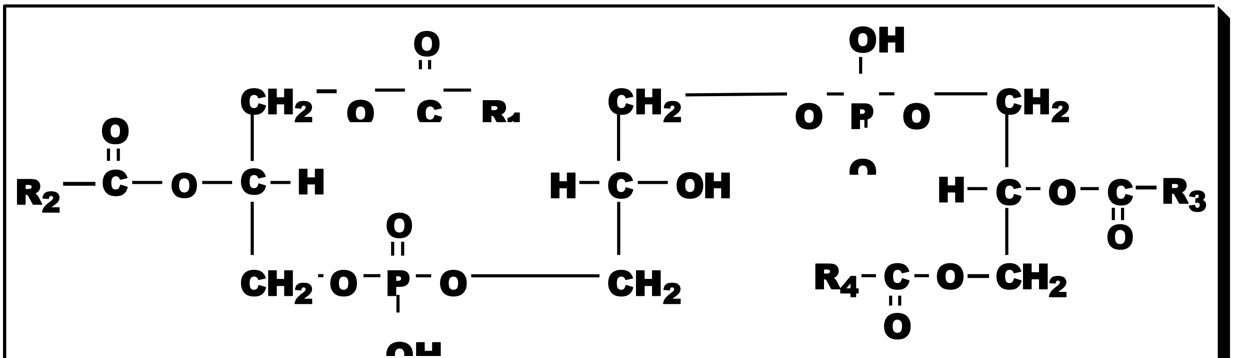
- Source: Brain, tissues. etc.,

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.

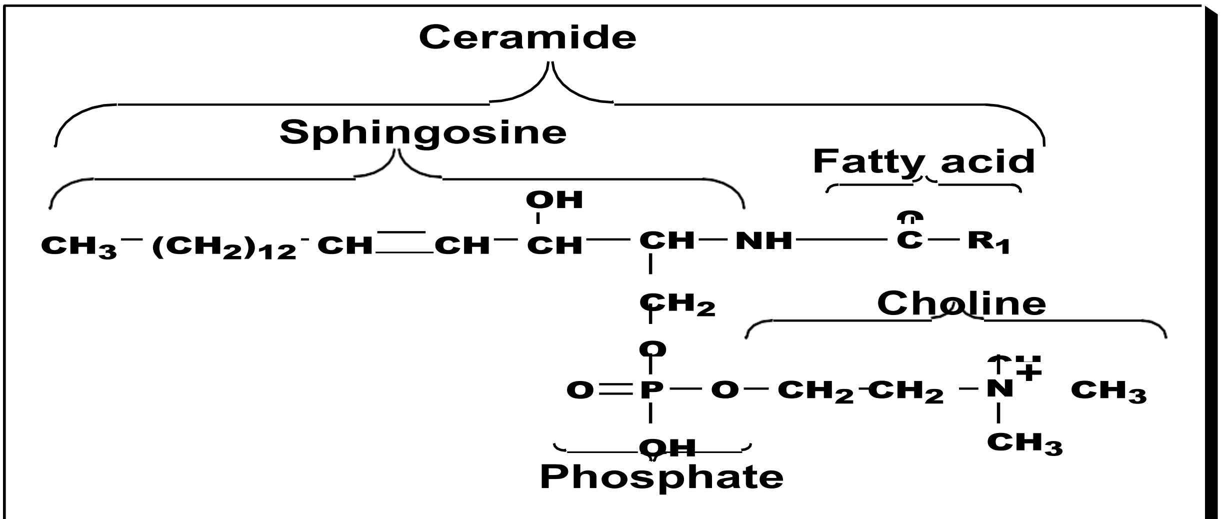


Cardiolipin

**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.



Sphingomyelin

## 2. Glycolipids

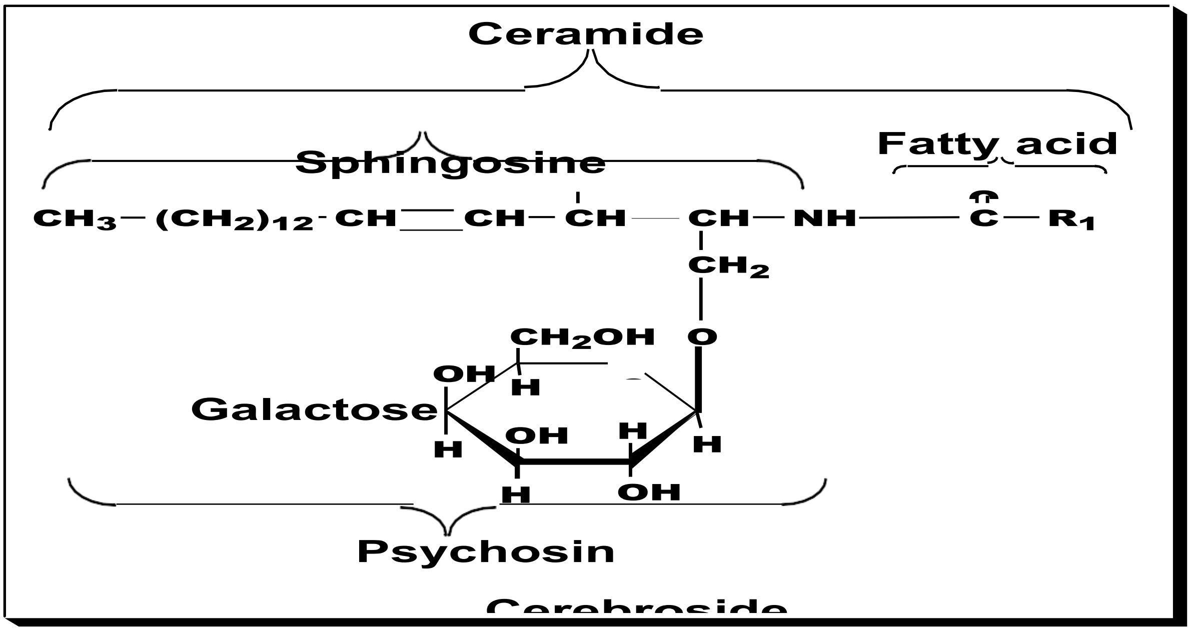
- They are lipids that contain carbohydrate residues with sphingosine as the alcohol and a very long-chain fatty acid (24 carbon series).
- They are present in cerebral tissue, therefore are called cerebrosides
- Classification: According to the number and nature of the carbohydrate residue(s) present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

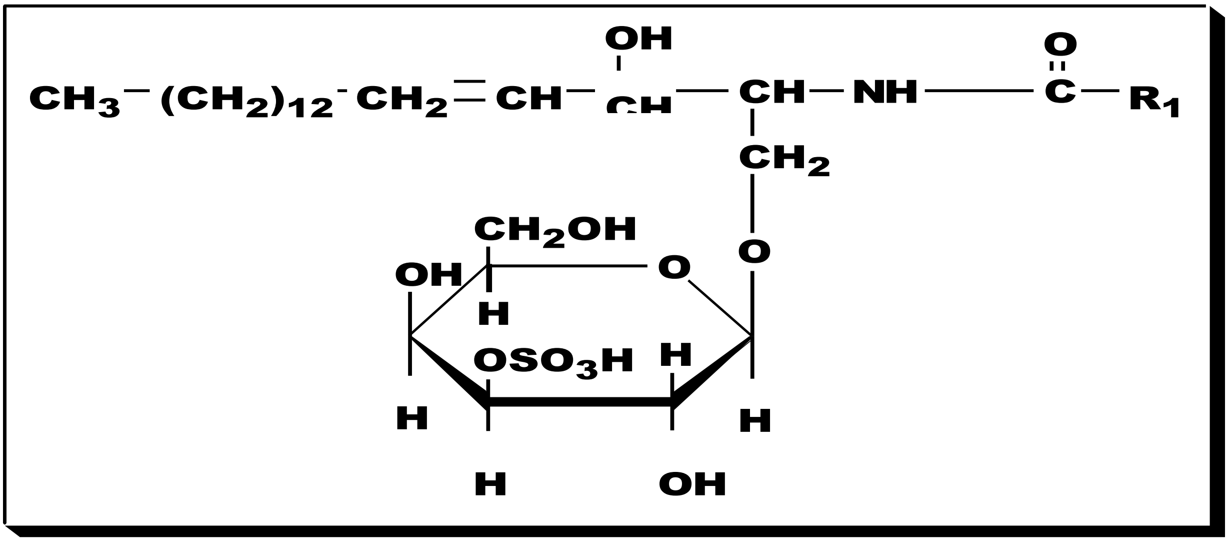


- Types: According to the type of fatty acid and carbohydrate present, there are 4 different types of cerebrosides isolated from the white matter of cerebrum and in myelin sheaths of nerves. Rabbit cerebrosides contain stearic acid.

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.

**Ceramide-Glucose-Galactose-N-acetylgalactosamine-Galactose**
**|**
**Sialic acid**
**Monosialoganglioside**

### 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.

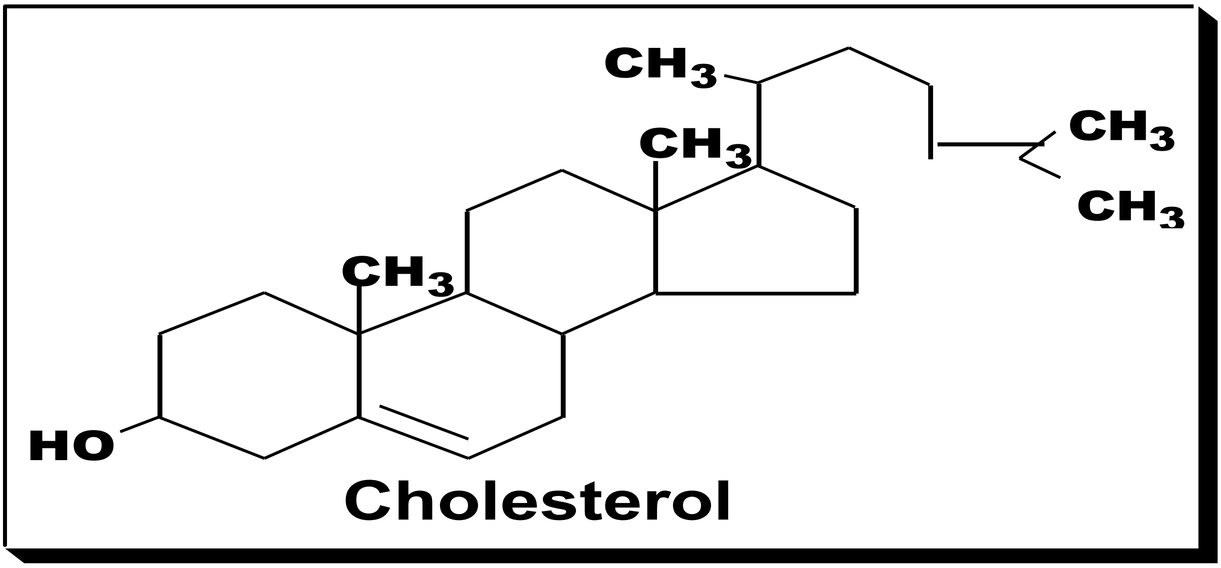
## Cholesterol

- 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

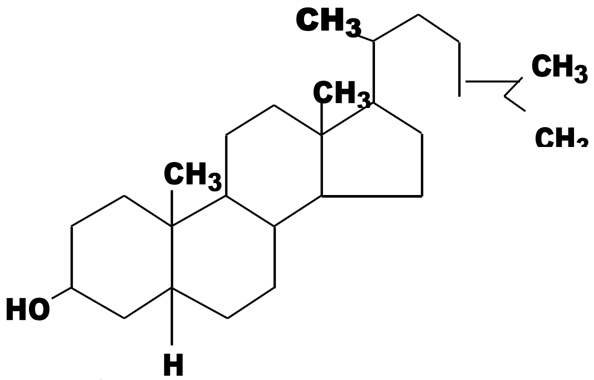
- It has a hydroxyl group on C3, a double bond between C5 and C6, 8 asymmetric carbon atoms and a side chain of 8 carbon atoms.
- It is found in all animal cells, corpus luteum and adrenal cortex, human brain (17% of the solids).

- 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.

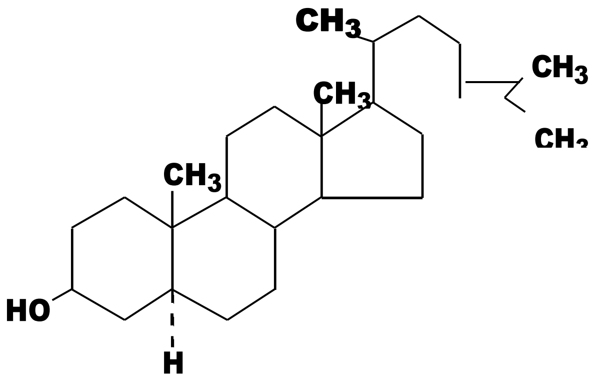


**Chemical properties**

- 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.

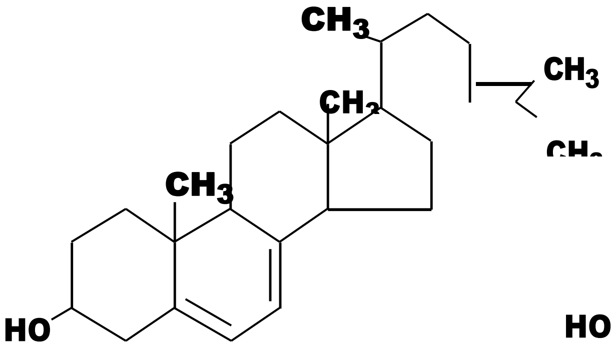


Coprosterol,
in feces

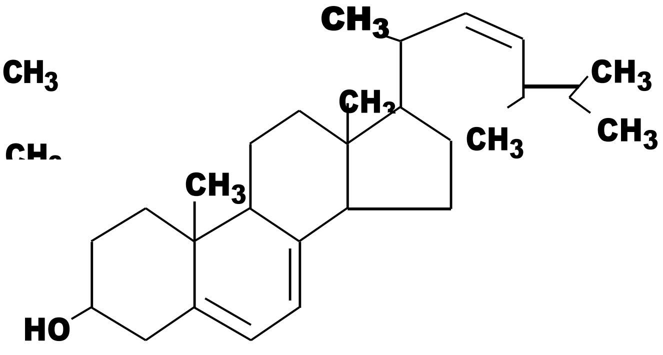


Dihydrocholesterol,
in blood and other tissues

- <u>Ergosterol</u> 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).



7-dehydrocholesterol



Ergosterol

## TERPENES

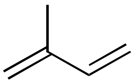
**Terpenes** are a large and diverse class of organic compounds, produced by a variety of plants, particularly conifers, though also by some insects such as termites or swallowtail butterflies, which emit **terpenes** from their osmeteria (defensive organ).

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.

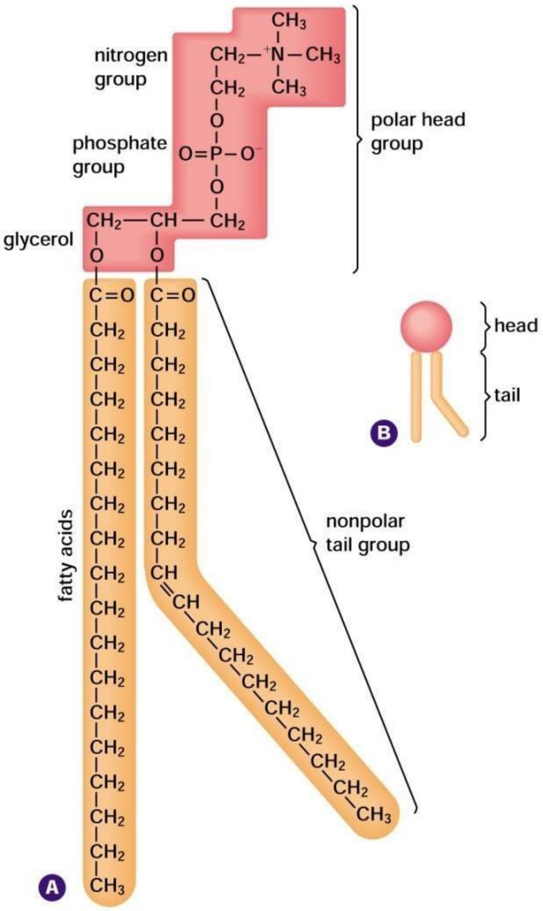


**Fig. Head and tail region of phospholipid**

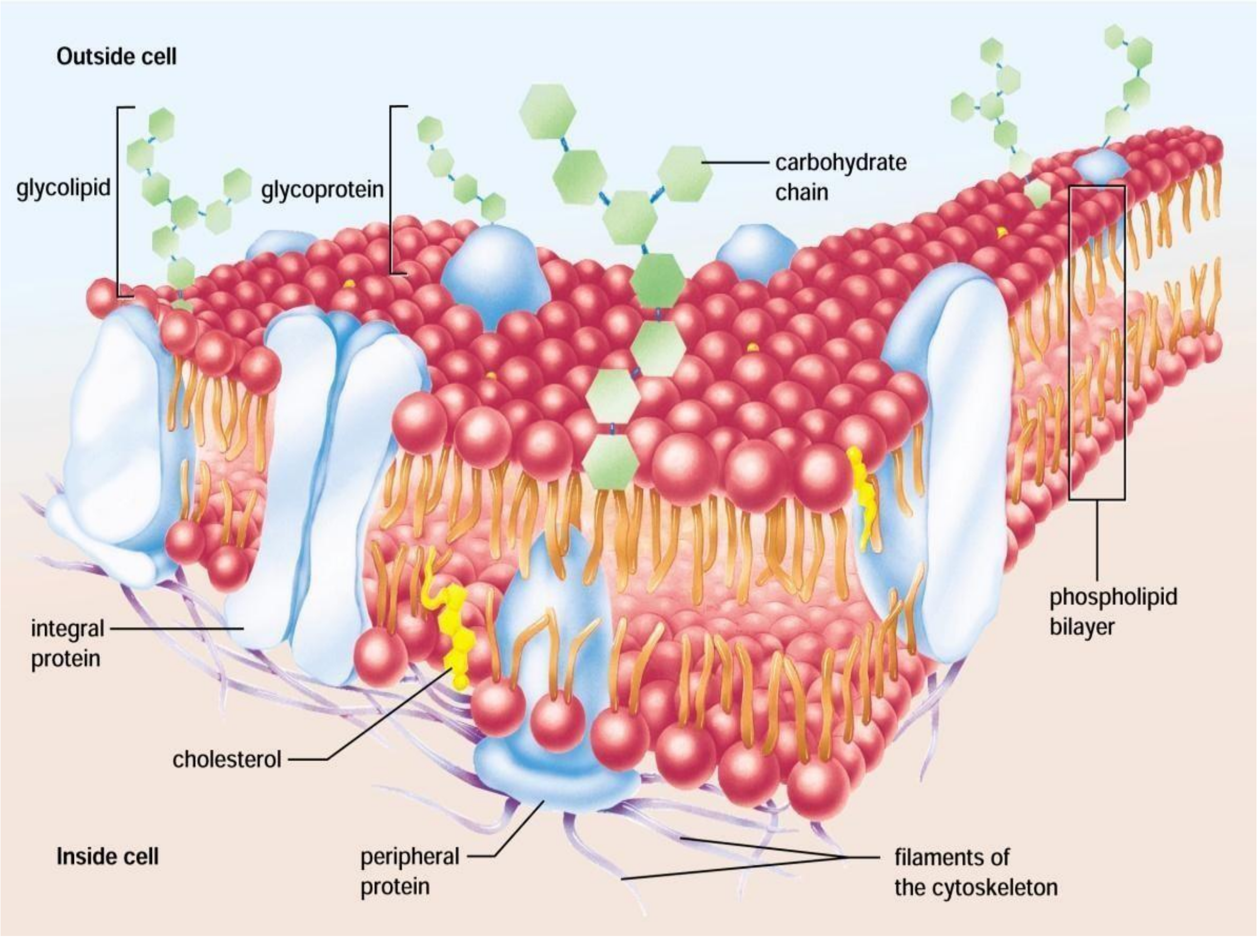


**Fig. Lipid bilayer**

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.,

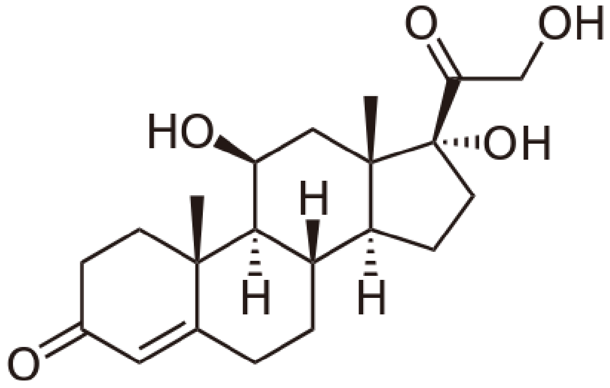
## Steroid hormone synthesis

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

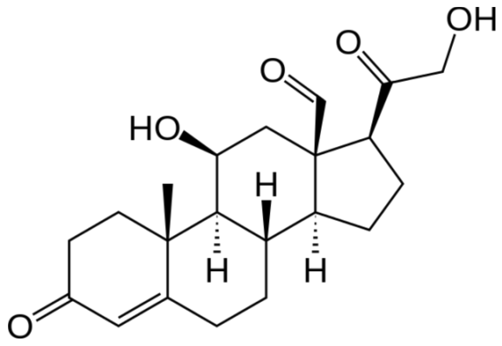


cortisol

## 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**

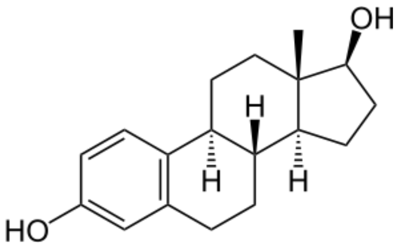
Produced in testes primarily but weak androgens in adrenal cortex.

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

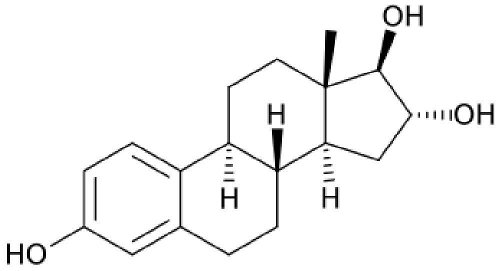
## Estrogen

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

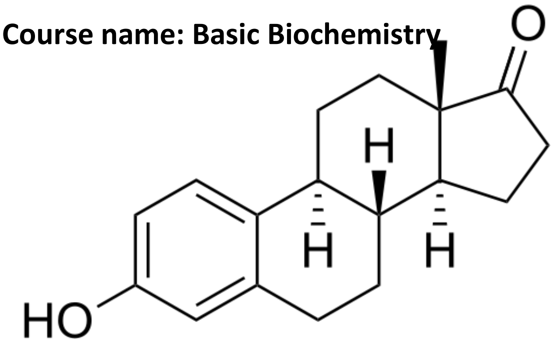
Functions - Development of female secondary sex characteristics; prevents bone resorption



**Estradiol (E2)**



**Estriol (E3)**



**Estrone (E1)**

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**SCHOOL OF BIO AND CHEMICAL ENGINEERING**

**DEPARTMENT OF BIOTECHNOLOGY**

# UNIT – IV – BASIC BIOCHEMISTRY – SMB1102

| **SMB1102** | **BASIC BIOCHEMISTRY** | **L** | **T** | **P** | **Credits** | **Total Marks** |
|---|---|---|---|---|---|---|
| | | 5 | 0 | 0 | 3 | 100 |

COURSE OBJECTIVES:

This course is aimed give an understanding about the basics of biochemistry dealing carbohydrates, Amino acids, Lipids, nucleic acid and vitamins.

UNIT 1: CARBOHYDRATES 12 Hrs. Carbohydrate – Definition, Classification, biological significance, structure of glucose, digestion and absorption of carbohydrates.

UNIT 2: PROTEINS 12 Hrs. Amino acids – structure, classification (Essential and non-essential, protein and non-protein amino acids). Proteins – definition, classification and structure (primary, secondary, tertiary and quaternary).

UNIT 3: LIPIDS 12 Hrs. Lipids – definition, classification and biological significance. Structure, properties and functions of fatty acids.

UNIT 4 NUCLEIC ACIDS 12 Hrs. Nucleic acids – Structure of DNA and its functions. Different forms of DNA. Different types of RNA and its functions.

UNIT 5 VITAMINS 12 Hrs. Vitamins – Source, biological function, daily requirement and deficiency symptoms of fat soluble vitamins (A, D, E and K) and water soluble vitamins (Ascorbic acid, thiamine, riboflavin, pyridoxine, niacin, pantothenic acid, lipoic acid, biotin, folic acid and vitamin B12).

TEXT / REFERENCE BOOKS:

1. Lehninger Principles of Biochemistry-David L. Nelson, Michael M. Cox, Macmillan Worth Publishers.
2. Harper―s Biochemistry-Rober K. Murray, Daryl K. Grammer, McGraw Hill, Lange Medical Books. 25th edition.
3. Fundamentals of Biochemistry-J.L. Jain, Sunjay Jain, Nitin Jain, S. Chand & Company.
4. Biochemistry-Dr. Amit Krishna De, S. Chand & Co., Ltd. END SEMESTER EXAMINATION QUESTION

PAPER PATTERN Max. Marks: 100 Exam Duration: 3 Hrs.

PART A: 10 questions of 2 marks each - No choice 20 Marks

PART B: 2 questions from each unit of internal choice; each carrying 16 marks 80 Marks

# UNIT- IV

## 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:

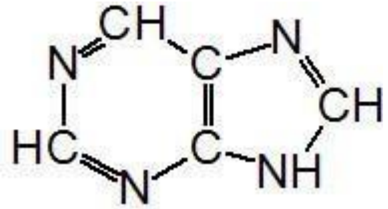
1. Serving as energy stores for future use in phosphate transfer reactions. These reactions are predominantly carried out by ATP.
2. Forming a portion of several important coenzymes such as $NAD^+$, $NADP^+$, FAD and coenzyme A.
3. Serving as mediators of numerous important cellular processes such as 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.

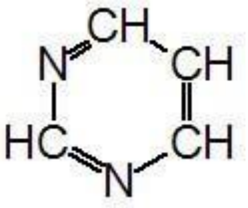
**6.** Serving as activated intermediates in numerous biosynthetic reactions. These activated intermediates include S-adenosylmethionine (S-AdoMet or SAM) involved in methyl transfer reactions as well as the many sugar coupled nucleotides involved in glycogen and glycoprotein synthesis.

## Nucleoside and Nucleotide Structure and Nomenclature

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



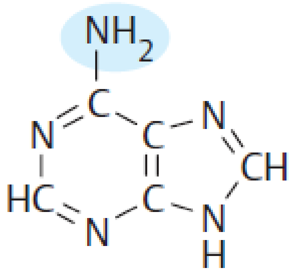
**Purine**



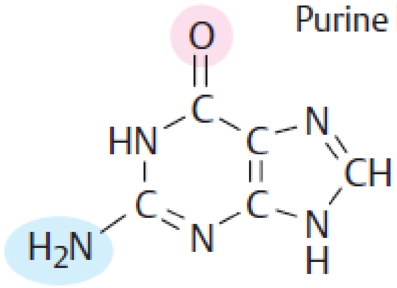
**Pyrimidine**

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.

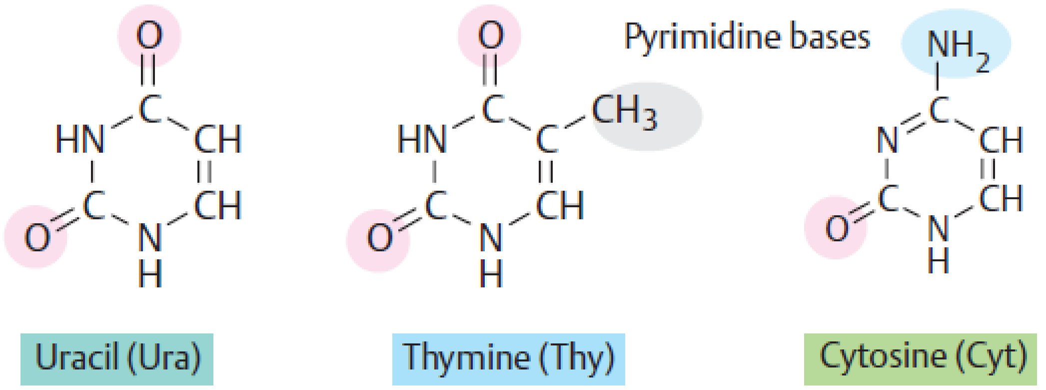
Purine bases



Adenine (Ade)



Guanine (Gua)



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.**

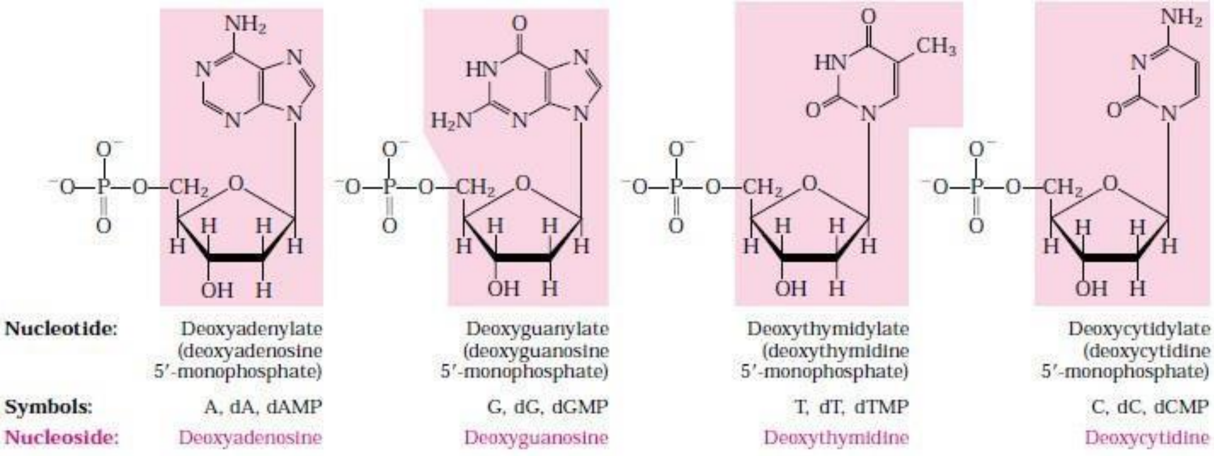
In the cell, the 5’ OH group of the sugar component of the nucleoside is usually esterified with phosphoric acid. If the 5’ phosphate residue is linked via an acid– anhydride bond to additional phosphate residues, it yields nucleoside diphosphates

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.

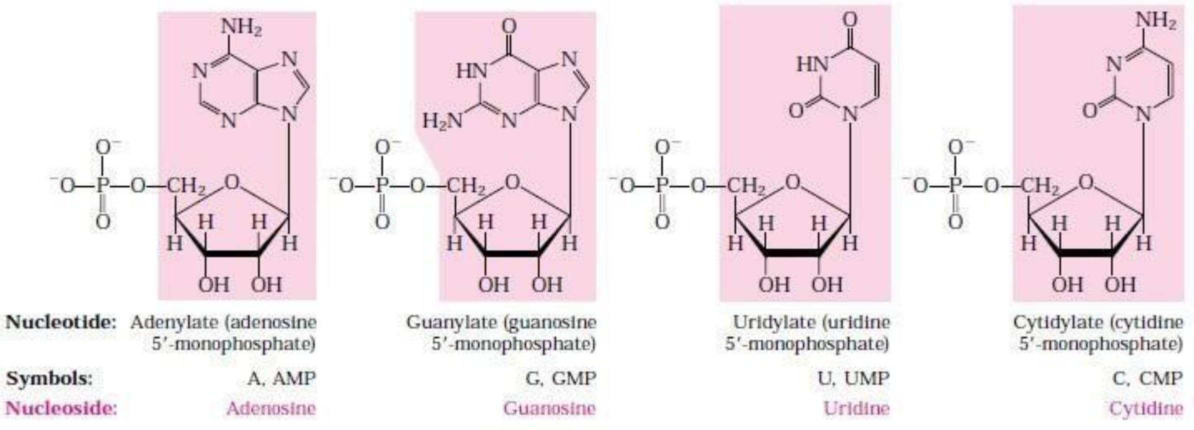
In the pentoses of nucleotidesand nucleosides the carbon numbers are given a prime (‘) designation to distinguish them from the numbered atoms of the nitrogenous bases.

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.

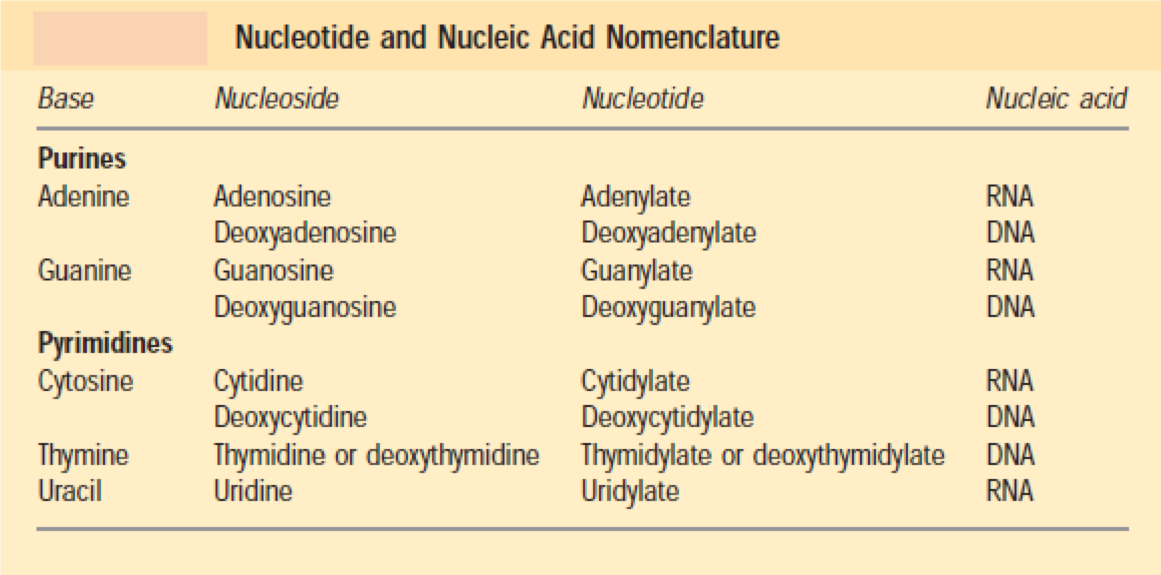
Both DNA and RNA contain two major purine bases, **adenine** (A) and **guanine** (G), and two major pyrimidines. In both DNA and RNA one of the pyrimidines is **cytosine** (C), but the second major pyrimidine is not the same in both: it is **thymine** (T) in DNA and **uracil** (U) in RNA. Only rarely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



**(a) Deoxyribonucleotides**



**(b) Ribonucleotides**

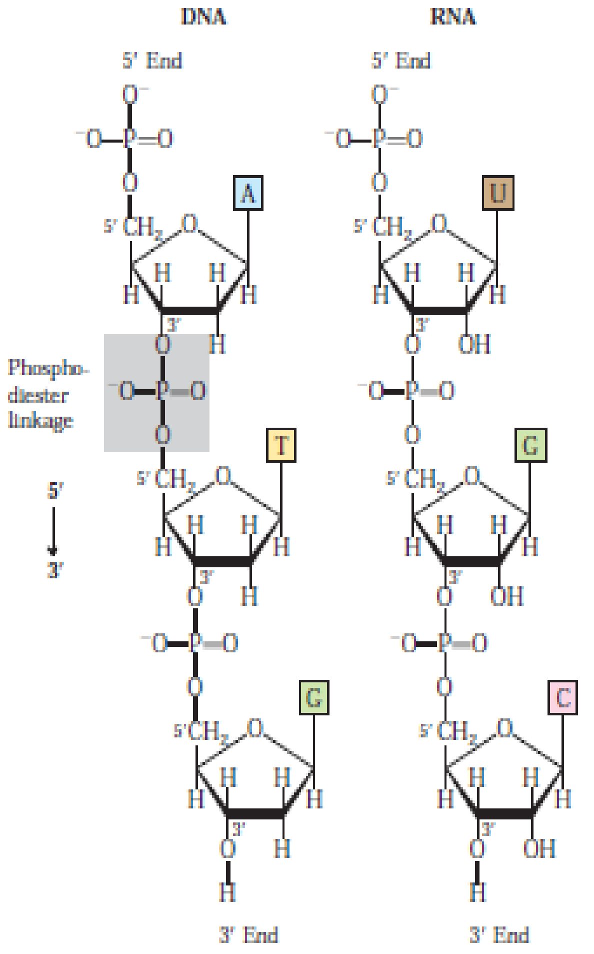


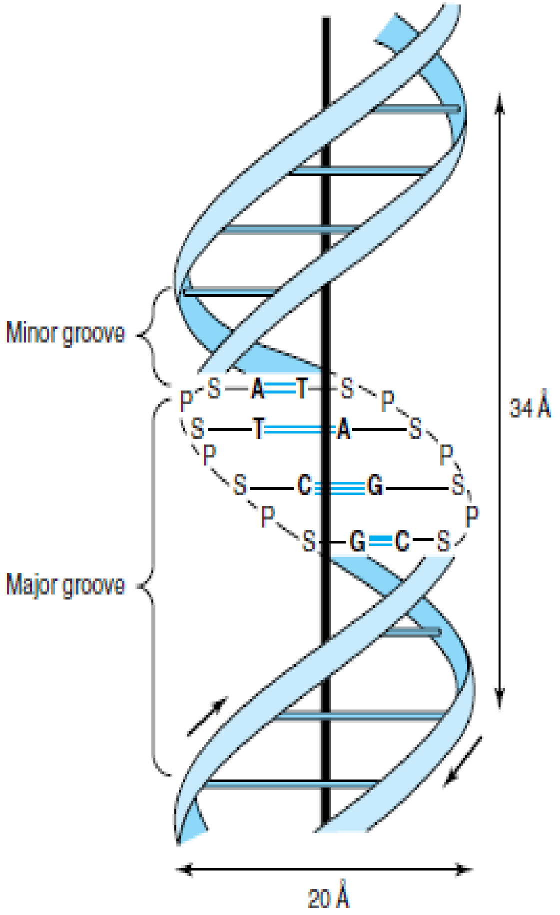
Nucleotide and Nucleic Acid Nomenclature

| *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:

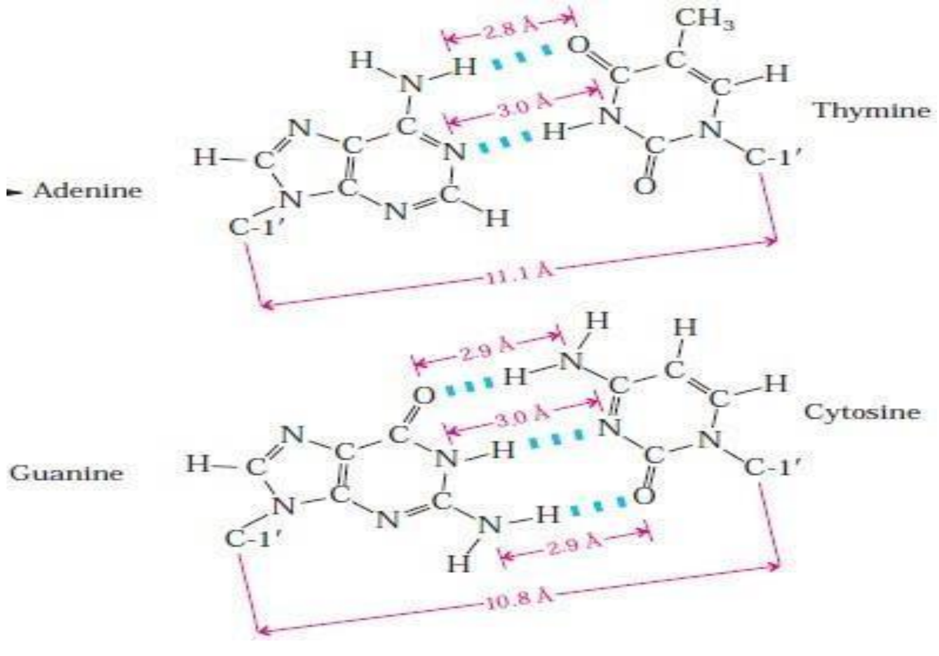
1. Two helical polynucleotide chains are coiled around a common axis. The chains run in opposite directions.

**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.

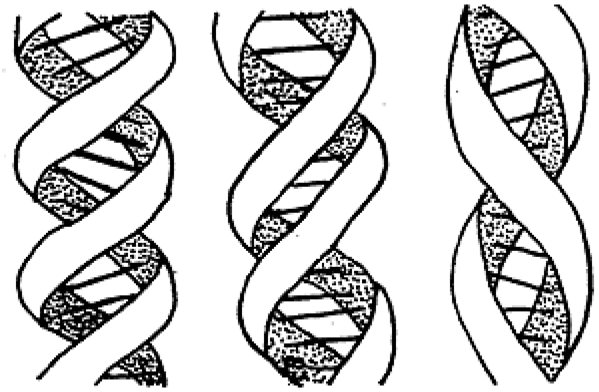
**DNA: conformation '**

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.

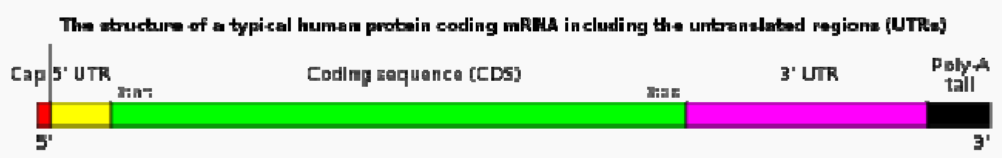
**Characteristics**

- 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

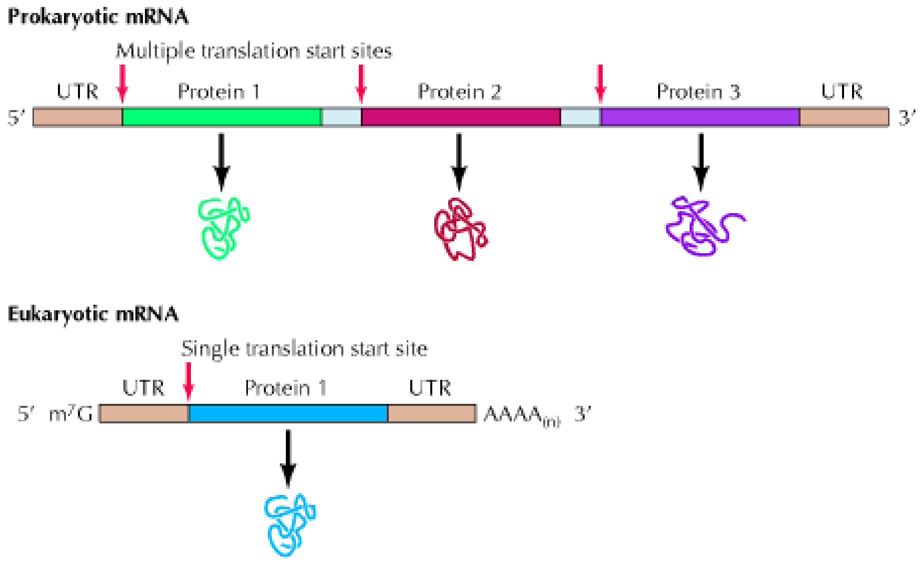
## mRNA – messenger RNA



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.

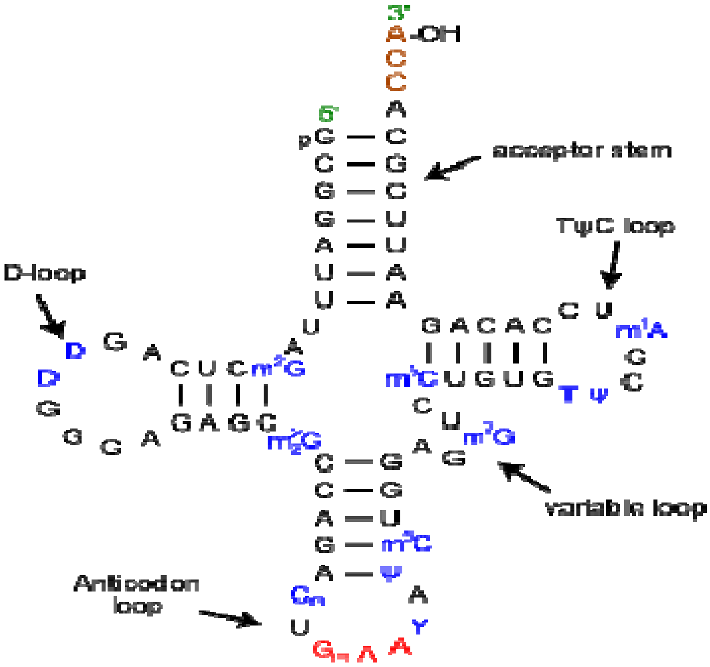
The DNA gives rise to nascent RNA in the nucleus. Addition of poly A tail to this nascent RNA makes this a pre-mRNA. The pre-mRNA has both introns and exons in it. Splicing removes the introns bringing the exons together to form the CDS. This is called the mature mRNA.



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.

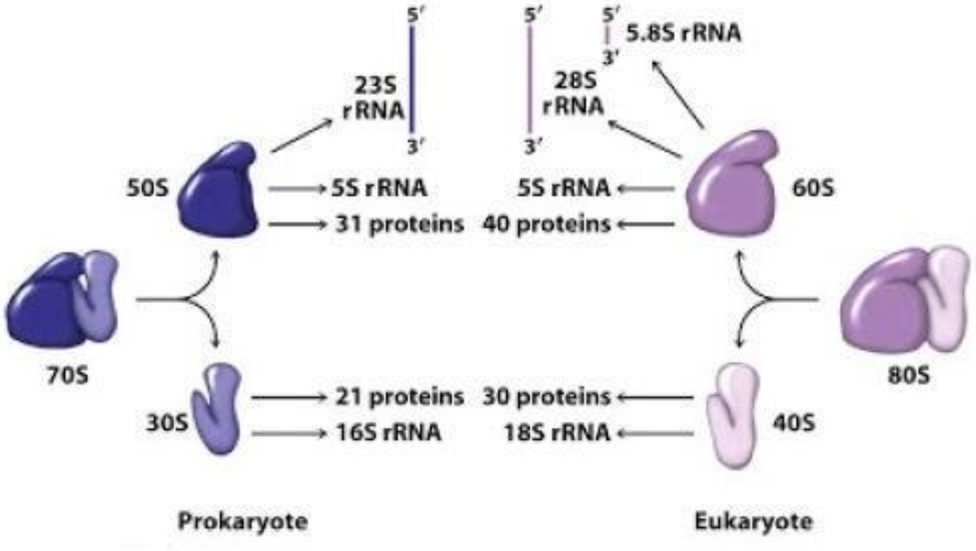
### Eukaryotes

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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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

# UNIT – V – BASIC BIOCHEMISTRY – SMB1102

| **SMB1102** | **BASIC BIOCHEMISTRY** | **L** | **T** | **P** | **Credits** | **Total Marks** |
|---|---|---|---|---|---|---|
| | | 5 | 0 | 0 | 3 | 100 |

COURSE OBJECTIVES:

This course is aimed give an understanding about the basics of biochemistry dealing carbohydrates, Amino acids, Lipids, nucleic acid and vitamins.

UNIT 1: CARBOHYDRATES 12 Hrs.
Carbohydrate – Definition, Classification, biological significance, structure of glucose, digestion and absorption of carbohydrates.

UNIT 2: PROTEINS 12 Hrs. Amino acids – structure, classification (Essential and non-essential, protein and non-protein amino acids). Proteins – definition, classification and structure (primary, secondary, tertiary and quaternary).

UNIT 3: LIPIDS 12 Hrs. Lipids – definition, classification and biological significance. Structure, properties and functions of fatty acids.

UNIT 4 NUCLEIC ACIDS 12 Hrs. Nucleic acids – Structure of DNA and its functions. Different forms of DNA. Different types of RNA and its functions.

UNIT 5 VITAMINS 12 Hrs.
Vitamins – Source, biological function, daily requirement and deficiency symptoms of fat soluble vitamins (A, D, E and K) and water soluble vitamins (Ascorbic acid, thiamine, riboflavin, pyridoxine, niacin, pantothenic acid, lipoic acid, biotin, folic acid and vitamin B12).

TEXT / REFERENCE BOOKS:

1. Lehninger Principles of Biochemistry-David L. Nelson, Michael M. Cox, Macmillan Worth Publishers.

2. Harper―s Biochemistry-Rober K. Murray, Daryl K. Grammer, McGraw Hill, Lange Medical Books. 25th edition.

3. Fundamentals of Biochemistry-J.L. Jain, Sunjay Jain, Nitin Jain, S. Chand & Company.

4. Biochemistry-Dr. Amit Krishna De, S. Chand & Co., Ltd. END SEMESTER EXAMINATION QUESTION

PAPER PATTERN Max. Marks: 100 Exam Duration: 3 Hrs.

PART A: 10 questions of 2 marks each - No choice 20 Marks

PART B: 2 questions from each unit of internal choice; each carrying 16 marks 80 Marks

# UNIT - V

## 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:

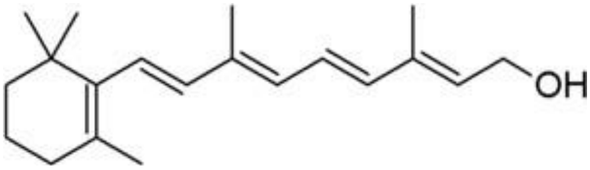
- Fat soluble vitamins (A, D, E and K),
- Water soluble vitamins (B and C).

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**

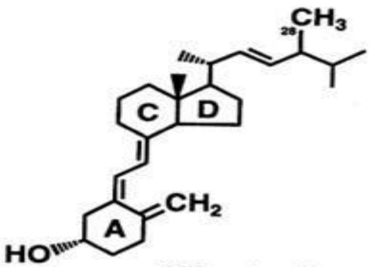
This can either be acute or chronic and can present with a number of symptoms. Acute toxicity causes dry, itchy skin, headache, nausea, loss of appetite and blurred vision. Severe toxicity can result in growth retardation, enlargement of the liver and spleen, loss of hair, bone pain, increased pressure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

**Deficiency**

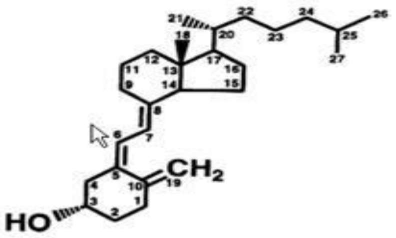
This is usually associated with strict diet restriction or excessive alcohol intake.

- **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



Vitamin $D_2$
(Ergocalciferol)



Vitamin $D_3$
(Colecalciferol)

### 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).

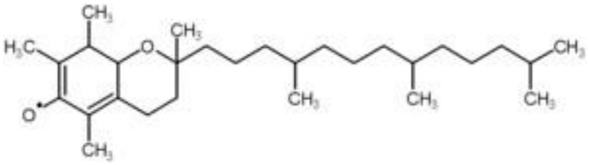
### Excess

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

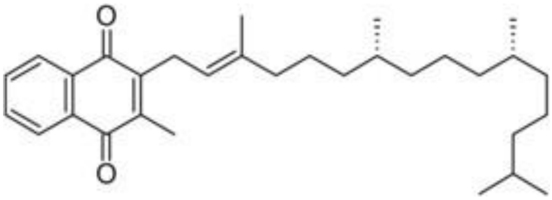
There is an increased risk of bleeding especially in patients taking blood-thinning agents such as heparin, warfarin or aspirin, and in patients with vitamin K deficiency. It can also cause nausea and digestive tract disorders.

### 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

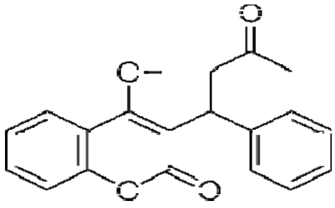
### Role

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.

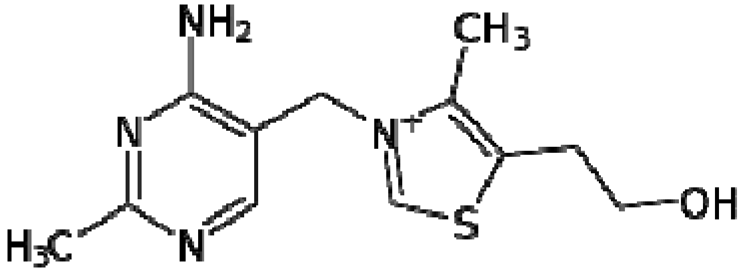
### B Group Vitamins Features

- 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.

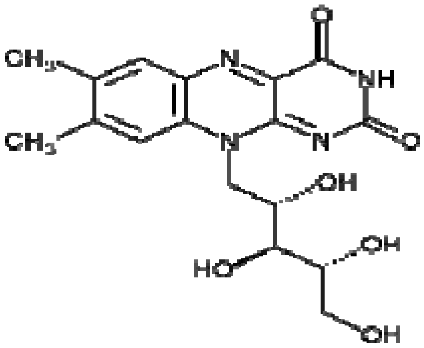
Suboptimal thiamine status based on biochemical criteria in Europe was detected only in 4-6% of the population. Risk group are alcoholics.

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.

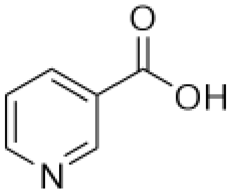
**Laboratory evaluation:** decreases secretion of vitamin $B_2$ in urine (normal values are 106–638 nmol/l), decreased concentrations of glutathione and glutathione reductase in erythrocytes.

### 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.

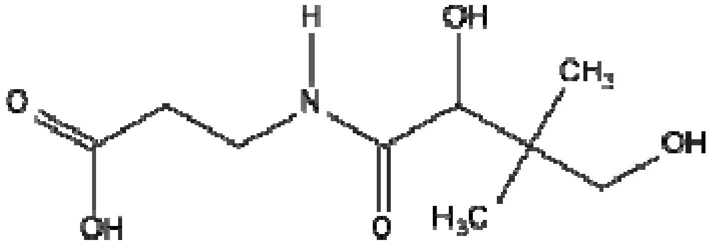
**Pharmacological use**

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

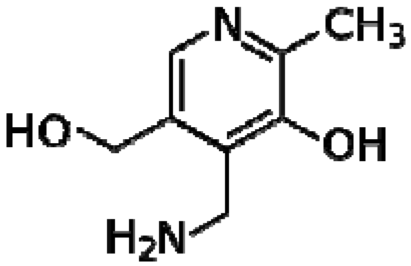
### Deficit

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

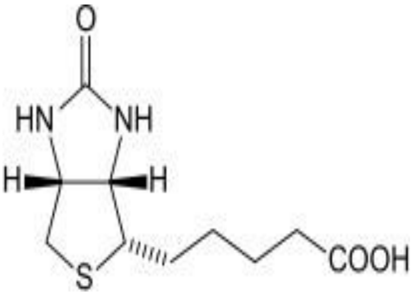
Deficiency with normal eating habits does not occur; manifested skin and mucosal changes, rhagades corners, peripheral neuropathy.

## 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

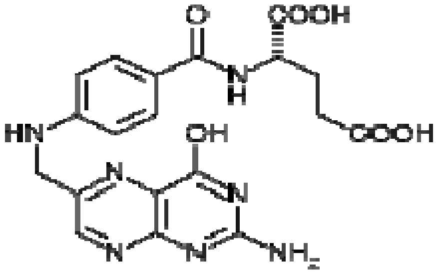
### Deficit

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.

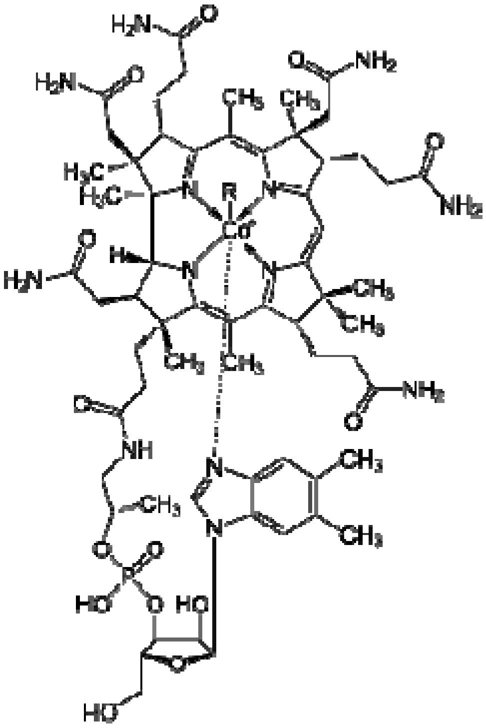
- Laboratory evaluation: serum levels of folate, total homocysteine (increases in the absence, also in the absence of vitamin $B_{12}$)

### 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).

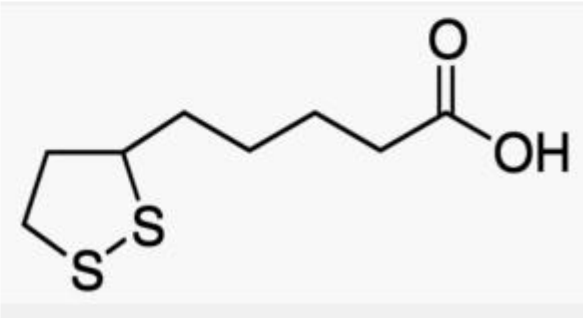
**Deficit**

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.

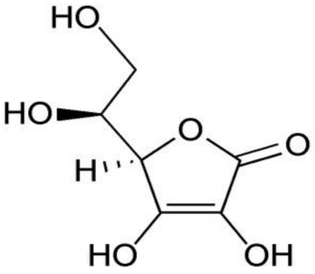
Alpha-lipoic acid is used for diabetes and nerve-related symptoms of diabetes including burning, pain, and numbness in the legs and arms.

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.