KARPAGAM ACADEMY OF HIGHER EDUCATION

(*Deemed to be University Established Under Section 3 of UGC Act 1956*)
Pollachi Main Road, Eachanari Post, Coimbatore – 641 021. INDIA
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DEPARTMENT OF MICROBIOLOGY
(For the candidates admitted from 2015 onwards)

Subject	**:**	**Biochemistry**	**Semester**	**:**	**I**
Subject code	**:**	**17MBU103**	**Class**	**:**	**I B.Sc Microbiology**

SYLLABUS

Program Objective

- To provide the basics of Biochemistry and its applications.
- To learn structures and functions of enzymes, proteins, carbohydrates, fats, process of metabolism and the molecular basis of the action of genes also form a part of Biochemistry.
- It serves as a good research techniques and the ability to combine and analyze information.

Program Outcome

By the end of the course, the students will:

1. Acquire knowledge and understand the molecular machinery of living cells.
2. Understand the basic awareness about Biomolecules and their importance in human life.
3. Understand the general structure and properties of lipids, carbohydrates, proteins and their functions in the cell.
4. Understand the basic concepts of enzymes and its applications.
5. Understand the importance of Vitamins in our body.

Unit I

Atoms and molecules, cell structure, cell organelles, developing membrane structure, transport of molecules, Beer and Lambert's Law, Colorimeter, Anabolism and catabolism and standard for energy change.

Unit II

Monosaccharides-families, stereo isomerism, epimers, Mutarotation and anomers. Forms of glucose and fructose, Haworth projection. Sugar derivatives. Disaccharides- occurrence, concept of reducing and non-reducing sugars and Haworth projections. Polysaccharides-storage and structural polysaccharides.

Unit III

Classification and functions of lipids. Storage lipids- structure and function of fatty acids. Triacylglycerols. Saponification. Structural lipids- structure, functions and properties of phosphoglycerides and sphingolipids.

Unit IV

Classification and functions of proteins and amino acids, Structure of amino acids and concept of zwitterion. Ninhydrin reaction. Natural modifications of amino acids in proteins. Non protein amino acids, Oligopeptides: Structure and functions of glutathione, insulin and aspartame. Primary and Secondary structure of proteins-alpha helix, beta pleated sheet. Tertiary and quaternary structures of proteins. Human haemoglobin structure.

Unit V

Structure and classification of enzymes, mechanism of action of enzymes. Km equation and enzyme activity. Allosteric enzyme and its mechanism. Multienzyme complex. Enzyme inhibition. Vitamins-classification and characteristics, sources and importance.

SUGGESTED READINGS

1. Campbell, M.K. (2012) Biochemistry, 7th edition. Published by Cengage Learning.
2. Campbell, P.N., and Smith, A.D., (2011) Biochemistry Illustrated, 4th edition. Published by Churchill Livingstone.
3. Tymoczko, J.L., Berg, J.M., and Stryer, L. (2012) Biochemistry: A short course, 2nd edition. W.H.Freeman.
4. Berg, J.M., Tymoczko, J.L., and Stryer, L. (2011) Biochemistry, W.H.Freeman and Company.
5. Nelson, D.L and Cox, M.M. (2008) Lehninger Principles of Biochemistry, 5th edition. W.H. Freeman and Company.
6. Willey, M.J., Sherwood, L.M., &Woolverton, C. J. (2013) Prescott, Harley and Klein's Microbiology. 9th edition. McGrawHill.

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LECTURE PLAN

Sl. No	LECTURE DURATION	TOPICS	BOOK REFERENCE	PAGE NO
UNIT-I				
1	1	Atoms and Molecules	T1	1
2	2	Cell Structure, Cell organelles	T1	2-7
3	1	Developing membrane structure	T1	2-7
4	2	Transport of molecules	T2	2
5	1	Beer and Lambert's law	T1	76
6	1	Colorimeter		
7	1	Anabolism and catabolism	T2	27
8	1	Standard for energy change	T2	23-26
9	1	Revision and Possible QP discussion		
10	1	Revision and Possible QP discussion		
Total: 12 hours				
UNIT-II				
1	1	Families of Monosaccharide	T1	235-236
2	2	Stereoisomerism of Monosaccharide, epimers, Mutarotation and anomers	T1	237-238
3	1	Forms of glucose and fructose and Haworth projection formulae	T1	239

4	1	Sugar derivatives	T1	240
5	2	Disaccharides: concept of reducing and non-reducing sugars, Haworth projections	T1	239 ,243-249
6	2	Polysaccharides; storage polysaccharides, starch and glycogen	T1	244-247
7	1	Structural polysaccharides cellulose and chitin	T1	247-252
8	1	Revision and Possible QP discussion		
9	1	Revision and Possible QP discussion		
Total: 12 hours				
UNIT-III				
1	1	Classification of lipids		
2	1	Functions of Lipids	T1	357-363
3	1	Storage lipids; structure of fatty acids	T1	343-346
4	1	Functions of storage lipids	T1	343-346
5	1	Triacylglycerols & Saponification	T1	346
6	2	Structural lipids-structure, functions and properties of phosphoglycerides	T1	350-352
7	1	Structure, functions and properties of sphingolipids	T1	349, 354
8	1	Revision and Possible QP discussion		
9	1	Revision and Possible QP discussion		
Total: 10 hours				
UNIT-IV				
1	1	Classification and functions of proteins and amino acids	T1	74-76
2	2	Structure of amino acids and concept of zwitterions, Ninhydrin reaction	T1	72-79
3	1	Natural modifications of amino acids in proteins	T1	114-116
4	1	Non protein aminoacids	T1	114-116
5	2	Oligopeptides: Structure and functions of glutathione, insulin and aspartame	T1	876-878, 439, 559
6	1	Primary and secondary structure of proteins-alpha helix, beta pleated sheet	T1	113-123
7	1	Tertiary and quaternary structures of proteins	T1	113-123

8	1	Human haemoglobin structure	T1	154-158
9	2	Revision and Possible QP discussion		
10	1	Revision and Possible QP discussion		
Total: 12 hours				
UNIT-V				
1	1	Structure and classification of enzymes	T1	183-185
2	1	Mechanism of action of enzymes	T1	186-194
3	2	Km equation and enzyme activity	T1	197-201
4	2	Allosteric enzyme and its mechanism	T1	220-223
5	1	Multienzyme complex	T1	223-225
6	1	Enzyme inhibition	T1	226-236
7	2	Vitamins-classification and characteristics, Sources and importance	T1	360-362
8	1	Revision and Possible QP discussion		
9	1	Revision and Possible QP discussion		
Total: 12 hours				
PREVIOUS YEAR END SEMESTER EXAMINATION QUESTION PAPER DISCUSSION				
1	1	Previous year ESE question paper discussion		
2	1	Previous year ESE question paper discussion		
Total: 2 hours				
Grand Total: 60 hours				

REFERENCES

T1	David L Nelson and Michael M. Cox (2008). Lehninger Principles of Biochemistry (5th edition), Freeman and Company.

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UNIT-I: COURSE MATERIAL

Unit-I

Atoms and molecules, cell structure, cell organelles, developing membrane, structure, transport of molecules, Beer and Lambert's Law, Colorimeter, Anabolism and catabolism and standard for energy change.

Suggest Readings

1. Berg, J.M., Tymoczko, J.L., and Stryer, L. (2011). Biochemistry, W.H. Freeman and Company.
2. Nelson, D.L and Cox, M.M. (2008). Lehninger Principles of Biochemistry, 5th edition. W.H. Freeman and Company.

Atoms and Molecules

An atom is the smallest unit of matter that has the properties of an element. It is composed of a dense core called the nucleus and a series of outer shells occupied by orbiting electrons. Elements are substances consisting of one type of atom, for example Carbon atoms make up diamond, and also graphite. Pure (24K) gold is composed of only one type of atom, gold atoms.

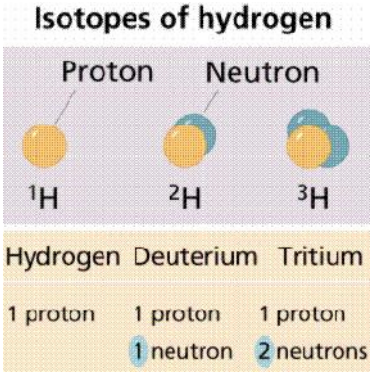
Subatomic particles were discovered during the 1800s. There are three main subatomic particles viz., Proton, Neutron and Electron.

- The **proton** is located in the center (or nucleus) of an atom, each atom has at least one proton. Protons have a charge of +1, and a mass of approximately 1 atomic mass unit (amu). Elements differ from each other in the number of protons they have, e.g. Hydrogen has 1 proton; Helium has 2.
- The **neutron** also is located in the atomic nucleus (except in Hydrogen). The neutron has no charge, and a mass of slightly over 1 amu.
- The **electron** is a very small particle located outside the nucleus. Because they move at speeds near the speed of light the precise location of electrons is hard to pin down. Electrons occupy orbital's, or areas where they have a high statistical probability of occurring. The charge on an electron is -1. Its mass is negligible (approximately 1800 electrons are needed to equal the mass of one proton).

Name	Charge	Location	Mass
Proton	+1	atomic nucleus	1.6726×10^{-27} kg
Neutron	0	atomic nucleus	1.6750×10^{-27} kg
Electron	-1	electron orbital	9.1095×10^{-31} kg

The **atomic number** is the number of protons an atom has. It is characteristic and unique for each element. The **atomic mass** (also referred to as the atomic weight) is the number of protons and neutrons in an atom. Atoms of an element that have differing numbers of neutrons (but a constant atomic number) are termed isotopes.

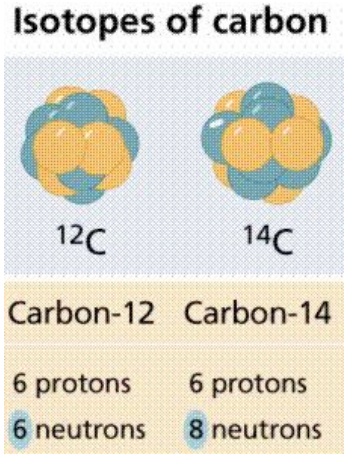
Example 1: Isotopes of Hydrogen



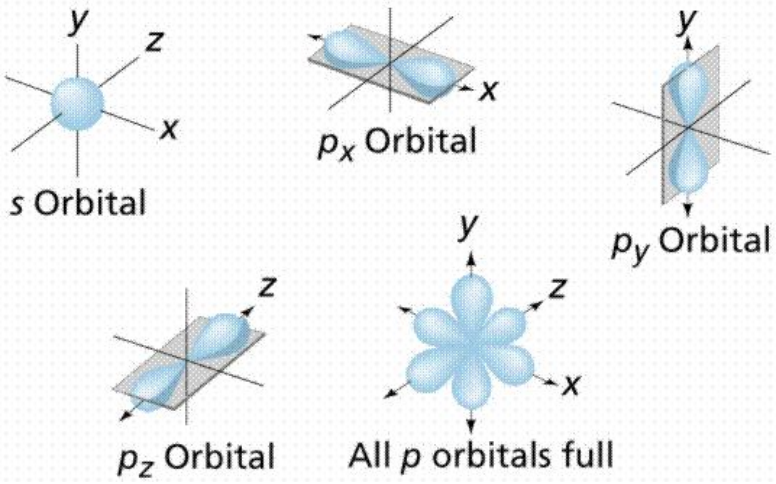
Note that each of these isotopes of hydrogen has only one proton. Isotopes differ from each other in the number of neutrons, not in the number of protons.

Some isotopes are radioisotopes, which spontaneously decay, releasing radioactivity. Other isotopes are stable. Examples of radioisotopes are Carbon-14 (symbol ^{14}C), and deuterium (also known as Hydrogen-2; ^{2}H). Stable isotopes are ^{12}C and ^{1}H.

Example 2: Isotopes of carbon



An orbital is also an area of space in which an electron will be found 90% of the time. Orbitals have a variety of shapes. Each orbital has a characteristic energy state and a characteristic shape. The *s* orbital is spherical. Since each orbital can hold a maximum of two electrons, atomic numbers above 2 must fill the other orbitals. The p_x, p_y, and p_z orbitals are dumbbell shaped, along the x, y, and z axes respectively.



Energy levels (also referred to as electron shells) are located a certain "distance" from the nucleus. The major energy levels into which electrons fit, are (from the nucleus outward) K, L, M, and N. Sometimes these are numbered, with electron configurations being: $1s^2 2s^2 2p^1$, (where the first shell K is indicated with the number 1, the second shell L with the number 2, etc.). This nomenclature tells us that for the atom mentioned in this paragraph, the first energy level (shell) has two electrons in its *s* orbital (the only orbital it can have), and second energy level has a maximum of two electrons in its *s* orbital, plus one electron in its *p* orbital.

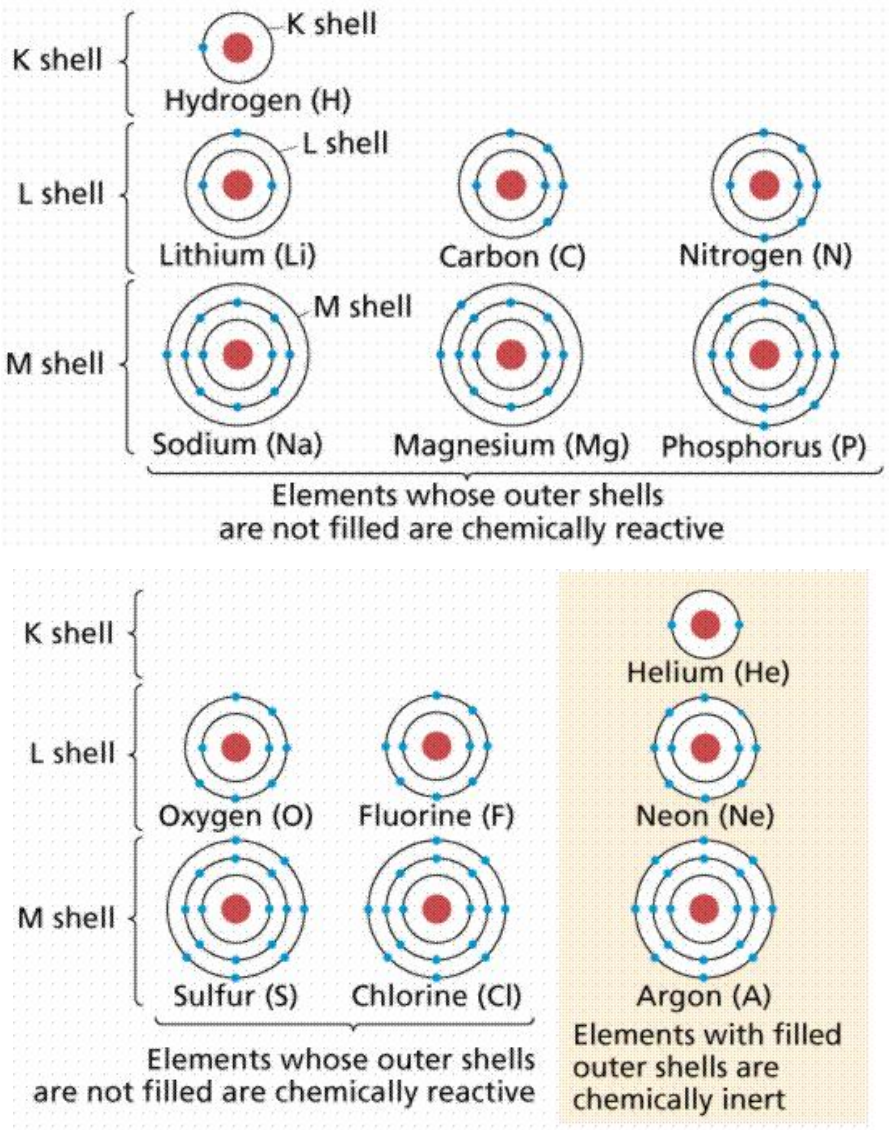
As a general rule, for the atoms we are likely to encounter in biological systems, atoms tend to gain or lose their outer electrons to achieve a Noble Gas outer electron shell configuration of two or eight electrons. The number of electrons that are gained or lost is characteristic for each element, and ultimately determines the number and types of chemical bonds atoms of that element can form.

Chemical Bonding

Ionic bonds

Ionic bonds are formed when atoms become ions by gaining or losing electrons. Chlorine is in a group of elements having seven electrons in their outer shells. Members of this group tend to gain one electron, acquiring a charge of -1. Sodium is in another group with elements having one electron in their outer shells. Members of this group tend to lose that outer electron, acquiring a charge of +1. Oppositely charged ions are attracted to each other, thus Cl^- (the symbolic representation of the chloride ion) and Na^+ (the symbol for the sodium ion, using the Greek word *natrium*) form an ionic bond, becoming the molecule

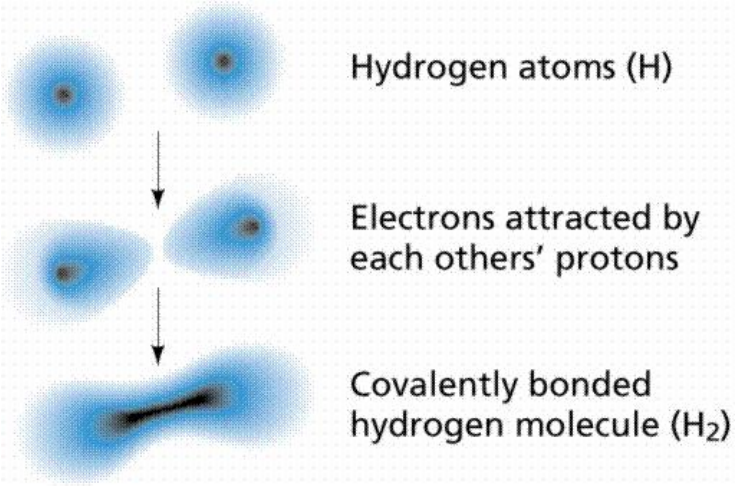
sodium chloride. Ionic bonds generally form between elements in Group I (having one electron in their outer shell) and Group VII a (having seven electrons in their outer shell). Such bonds are relatively weak, and tend to disassociate in water, producing solutions that have both Na and Cl ions.



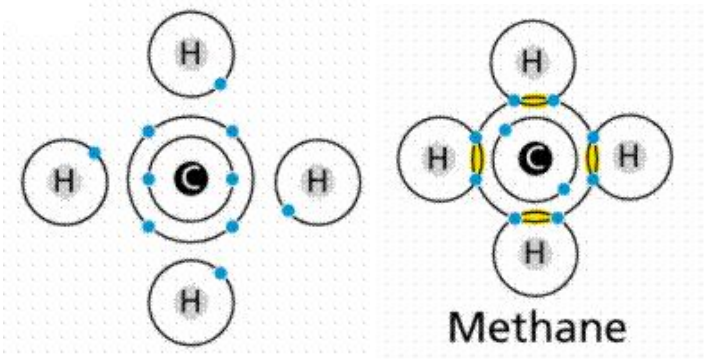
Covalent bonds

Covalent bonds form when atoms share electrons. Since electrons move very fast they can be shared, effectively filling or emptying the outer shells of the atoms involved in the bond. Such bonds are referred to as electron-sharing bonds. An analogy can be made to child custody: the children are like electrons, and tend to spend some time with one parent

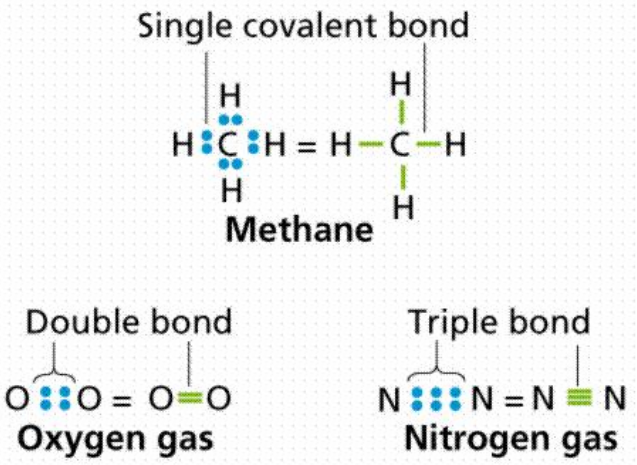
and the rest of their time with the other parent. In a covalent bond, the electron clouds surrounding the atomic nuclei overlap.



Carbon (C) is in Group IV a, meaning it has four electrons in its outer shell. Thus to become a "happy atom", Carbon can either gain or lose four electrons. By sharing the electrons with other atoms, Carbon can become a happy atom, alternately filling and emptying its outer shell, as with the four hydrogen's.



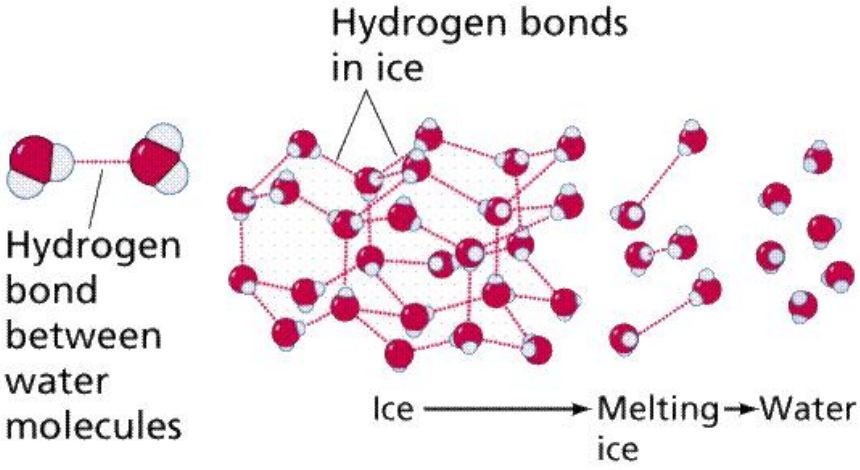
The molecule methane (chemical formula CH_4) has four covalent bonds, one between Carbon and each of the four Hydrogens. Carbon contributes an electron, and Hydrogen contributes an electron. The sharing of a single electron pair is termed a single bond. When two pairs of electrons are shared, a double bond results, as in carbon dioxide. Triple bonds are known, wherein three pairs (six electrons total) are shared as in acetylene gas or nitrogen gas. The types of covalent bonds are:



Hydrogen bonds

Hydrogen bonds result from the weak electrical attraction between the positive end of one molecule and the negative end of another. Individually these bonds are very weak, although taken in a large enough quantity, the result is strong enough to hold molecules together or in a three-dimensional shape.

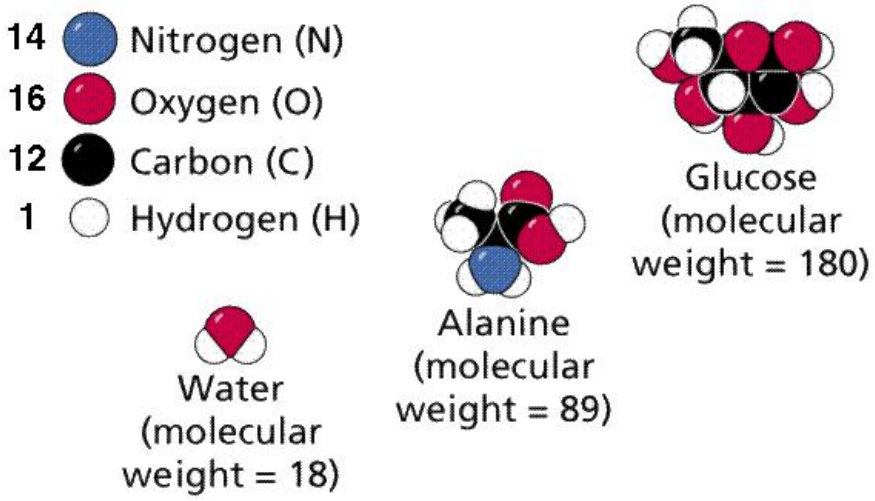
Example: Formation of a hydrogen bond between the hydrogen side of one water molecule and the oxygen side of another water molecule.



Molecules

Molecules are compounds in which the elements are in definite, fixed ratios. Those atoms are held together usually by one of the three types of chemical bonds discussed above. For example: water, glucose, ATP. Mixtures are compounds with variable formulas/ratios of their components. For example: soil. Molecular formulas are an expression in the simplest whole-number terms of the composition of a substance. For

example, the sugar glucose has 6 Carbons, 12 hydrogens, and 6 oxygens per repeating structural unit. The formula is written $C_6H_{12}O_6$.



Chemical reactions occur in nature, and some also can be performed in a laboratory setting. One chemical equation are linear representations of how these reactions occur. Combination reactions occur when two separate reactants are bonded together, e.g. A + B ----> AB. Disassociation reactions occur when a compound is broken into two products, e.g. AB -----> A + B.

The cell and cell theory

Soon after Anton van Leeuwenhoek invented the microscope, Robert Hooke in 1665 observed a piece of cork under the microscope and found it to be made of small compartments which he called "cells" (Latin cell = small room). In 1672, Leeuwenhoek observed bacteria, sperm and red blood corpuscles, all of which were cells. In 1831, Robert Brown, an Englishman observed that all cells had a centrally positioned body which he termed the nucleus.

The cell theory

In 1838 Matthias Schleiden and Theodor Schwann formulated the "Cell Theory." The cell theory maintains that

- All organisms are composed of cells.
- Cell is the structural and functional unit of life, and
- Cells arise from pre-existing cells.

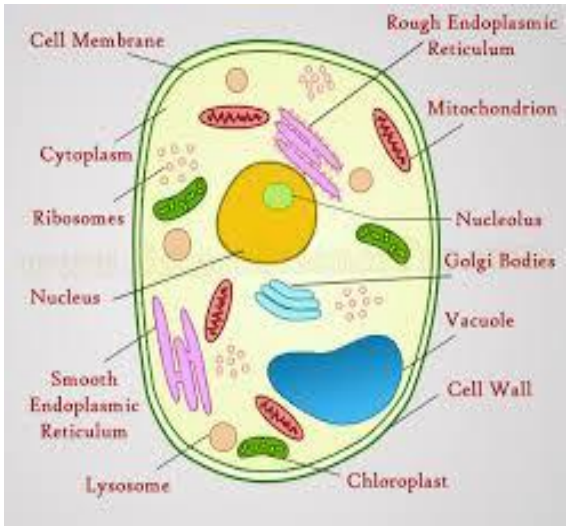
The cells vary considerably, in shape and size

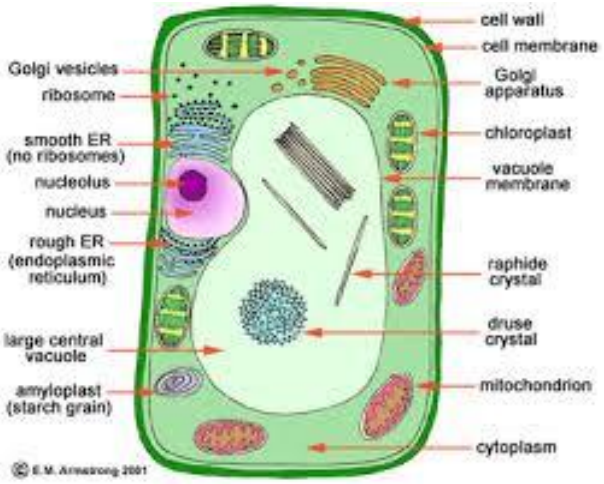
Cell Structure, cell organelles and developing membrane structure

A cell may be defined as a unit of protoplasm bounded by a plasma or cell membrane and possessing a nucleus. Protoplasm is the life giving substance and includes the cytoplasm and the nucleus. The cytoplasm has in it organelles such as ribosomes, mitochondria, Golgi bodies plastids, lysosomes and endoplasmic reticulum. Plant cells have in their cytoplasm large vacuoles containing non-living inclusions like crystals, pigments etc. The bacteria have neither organelles nor a well formed nucleus. But every cell has three major components

- plasma membrane
- cytoplasm
- DNA (naked in bacteria and covered by a membrane in all other organisms

Animal Cell **Plant Cell**





Plasma membrane

Each cell has a limiting boundary, the cell membrane, plasma membrane or plasmalemma. It is a living membrane, outermost in animal cells but next to cell wall in plant cells.

The plasma membrane is made of proteins and lipids and several models were proposed regarding the arrangement of proteins and lipids. The fluid mosaic model proposed by Singer and Nicholson (1972) is widely accepted.

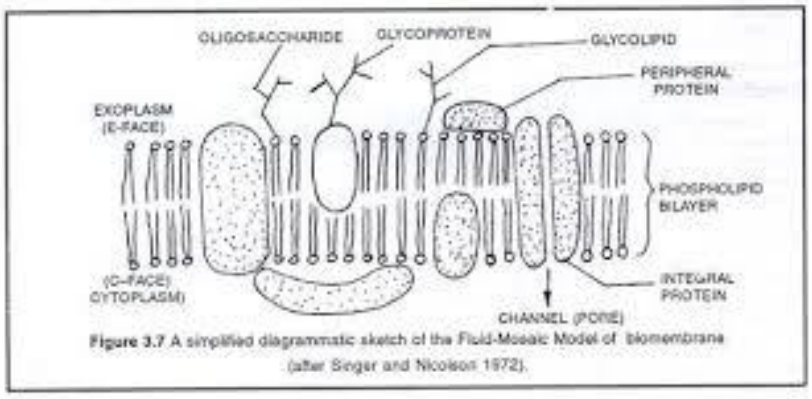


Figure 3.7 A simplified diagrammatic sketch of the Fluid-Mosaic Model of biomembrane (after Singer and Nicolson 1972).

According to the fluid mosaic model,

(i) The plasma membrane is composed of a lipid bilayer of phospholipid molecules into which a variety of globular proteins are embedded.

(ii) Each phospholipid molecule has two ends, an outer head hydrophilic i.e. water attracting, and the inner tail pointing centrally hydrophobic, i.e. water repelling

(iii) The protein molecules are arranged in two different ways:

a. Peripheral proteins or extrinsic proteins: these proteins are present on the outer and inner surfaces of lipid bilayer.

b. Integral proteins or intrinsic proteins: These proteins penetrate lipid bilayer partially or wholly.

Functions

(i) The plasma membrane encloses the cell contents.

(ii) It provides cell shape (in animal cells) e.g. the characteristic shape of red blood cells, nerve cells, bone cells, etc

(iii) It allows transport of certain substances into and out of the cell but not all substance, so it is termed selectively permeable.

Cell wall

In bacteria and plant cells the outermost cell cover, present outside the plasma membrane is the cell wall. Bacterial cell wall is made of peptidoglycan.

Structure

- Outermost non-living, layer present in all plant cells.
- Secreted by the cell itself.

- In plant, made of cellulose but may also contain other chemical substance such as pectin and lignin.
- The substance constituting the cell is not simply homogenous but it consists of fine threads or fibres called microfibrils.
- It may be thin (1 micron) and transparent as in the cells of onion peel. In some cases it is very thick as in the cells of wood.

Functions

- The cell wall protects the delicate inner parts of the cell.
- Being rigid, it gives shape to the cell.
- Being rigid, it does not allow distension of the cell, thus leading to turgidity of the cell that is useful in many ways.
- It freely allows the passage of water and other chemicals into and out of the cells.
- There are breaks in the primary wall of the adjacent cells through which cytoplasm of one cell remains connected with the other. These cytoplasmic strands which connect one cell to the other one are known as plasmodesmata.
- Walls of two adjacent cells are firmly joined by a cementing material called middle lamella made of calcium pectate.

Mitochondria and chloroplast - the energy transformers

Mitochondria (found in plant and animal cells) are the energy releasers and the chloroplasts (found only in green plant cells) are the energy trappers.

Mitochondria (Singular = mitochondrion)

Appear as tiny thread like structure under light microscope. Approximately 0.5 - 1.00 μm (micrometer) Number usually a few hundred to a few thousand per cell (smallest number is just one as in an alga (Micromonas).

Structure

The general plan of the internal structure of a mitochondria observed by means of electron microscope. It consists of the following parts:

- Wall made of double membrane
- The inner membrane is folded inside to form projections called cristae which project into the inner compartment called matrix.

Function

Oxidizes pyruvic acid (breakdown product of glucose) to release energy which gets stored in the form of ATP for ready use. This process is also called cellular respiration.

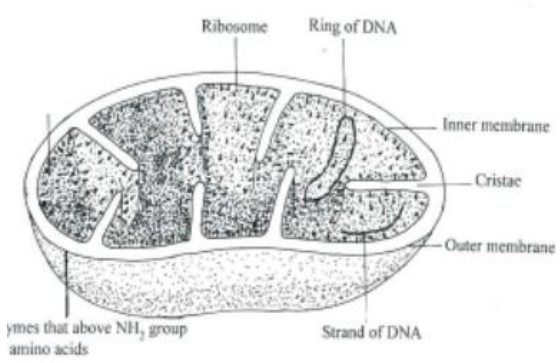
Plastids

Plastids are found only in a plant cell. They may be colorless or with color. Based on this fact, there are three types of plastids.

(i) Leucoplast-white or colorless

(ii) Chromoplast – blue, red, yellow etc.

(iii) Chloroplast – green

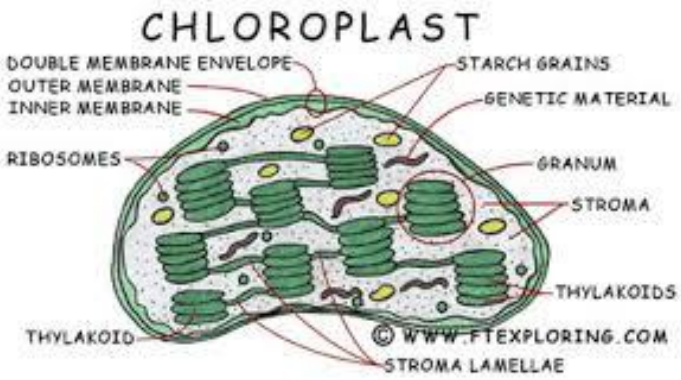


Chloroplast

- Found in all green plant cells in the cytoplasm.
- Number 1 to 1008
- Shape: Usually disc-shaped or spherical as in most plants around you. In some ribbon - shaped as in an alga spirogyra or cup - shaped as in other algae *Chlamydomonas*.
- Structure: the general plan of the structure of a single chloroplast is given below:
    - Wall made of double membrane i.e. outer membrane and inner membrane numerous stack-like (piles) groups or grana (singular = granum) are interconnected by lamellae.
    - Sac like structures called thylakoids placed one above the other constitute granum.
    - Inside of the chloroplast is filled with a fluid medium called stoma.

Function

Chloroplasts are the seat of photosynthesis (production of sugar, from carbon dioxide and water in the presence of sunlight).



Chloroplast versus mitochondria

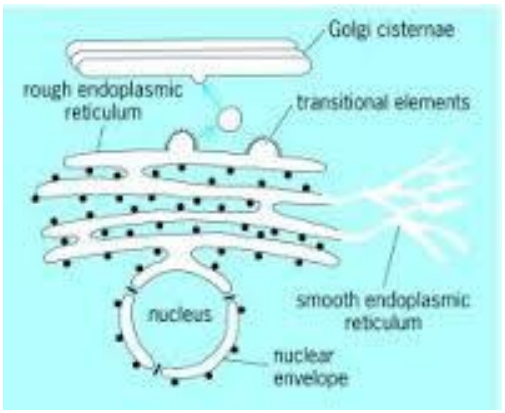
These two organelles are opposite to each other, one traps the solar energy locking it in a complex molecule (by photosynthesis), the other releases the energy by breaking the complex molecule (by respiration).

Similarities between mitochondria and chloroplasts

Both contain their own DNA (the genetic material) as well as their own RNA (for protein synthesis). Thus, they can self duplicate to produce more of their own kind without the help of nucleus. Since chloroplasts and mitochondria contain their own DNA the hereditary molecule and also their own ribosomes, they are termed semi-autonomous only because they are incapable of independent existence though they have ribosomes and DNA.

Endoplasmic reticulum (ER), Golgi body and ribosomes

Endoplasmic reticulum (ER) and Golgi body are single membrane bound structures. The membrane has the same structure (lipid-protein) as the plasma membrane but ribosomes do not have membranes Ribosomes are involved in synthesis of substances in the cell, Golgi bodies in secreting and the ER in transporting and storing the products. These three organelles operate together. The figure below shows the diagram of ER and Golgi body. Note the ribosomes present in ER.



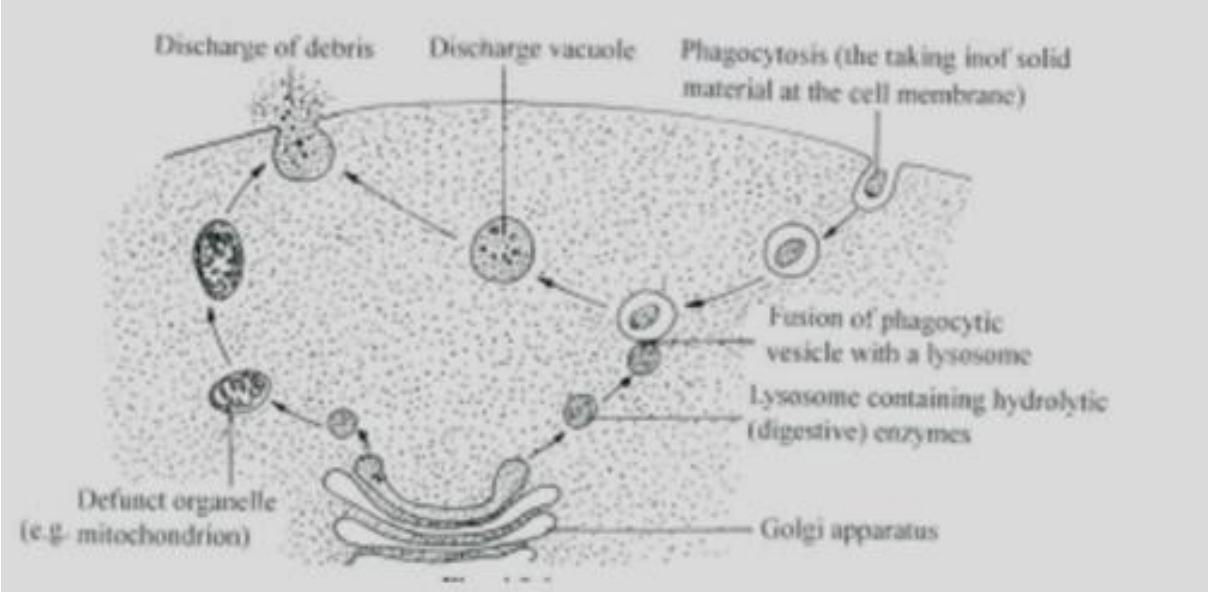
Endoplasmic reticulum (ER)	Gogli body	Ribosomes
Structure		
A network of membranes with thickness between 50 - 60A°. It is of two types– rough endoplasmic reticulum (RER) i.e. when ribosomes are attached to it and Smooth-endo-plasmic reticulum (SER) when no ribosomes are present.	Is a stack of membranous sacs of the same thickness as ER. Exhibit great diversity in size and shape.	Spherical about 150 - 250 Å in diameter, made up of large molecules of RNA and proteins (ribonucleo proteins)
Throughout the cytoplasm and is in contact with the cell membrane as well as the nuclear membrane.	In animal cells present around the nucleus, 3 to 7 in number. In plant cells, many and present scattered throughout the cell called dictyosomes.	Present either as free particles in cytoplasm or attached to ER. Also found stored in nucleolus inside the nucleus. 80S types found in eukaryotes and 70S in prokaryotes (S-svedberg unit of measuring ribosomes).
Function		
Provides internal framework, compartment and reaction surfaces, transports enzymes and other materials through out the cell. RER is the site for protein synthesis and SER for steroid synthesis, stores carbohydrates.	Synthesis and secretion as enzymes, participates in transformation of membranes to give rise to other membrane structure such as lysosome, acrosome, and dictyosomes, synthesize wall element like pectin, mucilage.	Site for protein synthesis.

The micro bodies (tiny but important)

These are small sac-like structures bounded by their membranes. These are of different kinds and the most important ones are lysosomes, peroxisomes and glyoxysomes.

Lysosomes (lysis = breaking down; soma = body)

Lysosomes are present in almost all animal cells and some non - green plant cells (Fig 4.9). They perform intracellular digestion.



Some main features of lysosomes are as follows

- Membranous sacs budded off from Golgi body.
- May be in hundreds in single cell.
- Contain several enzymes (about 40 in number)
- Materials to be acted upon by enzymes enter the lysosomes.
- Lysosomes are called "suicidal bags" as enzymes contained in them can digest the cell's own material when damaged or dead.
- Importance of intracellular digestion by the lysosomes
- Help in nutrition of the cell by digesting food, as they are rich in various enzymes which enable them to digest almost all major chemical constituents of the living cell.
- Help in defense by digesting germs, as in white blood cells.
- Help in cleaning up the cell by digesting damaged material of the cell.
- Provide energy during cell starvation by digestion of the cells own parts (autophagic, auto: self; phagos: eat up).
- Help sperm cells in entering the egg by breaking through (digesting) the egg membrane.
- In plant cells, mature xylem cells lose all cellular contents by lysosome activity.
- When cells are old, diseased or injured, lysosomes attack their cell organelles and digest them. In other words lysosomes are autophagic, i.e. self devouring.

Peroxisomes

Found both in plant and animal cells. Found in the green leaves of higher plants. They participate in oxidation of substrates resulting in the formation of hydrogen peroxide.

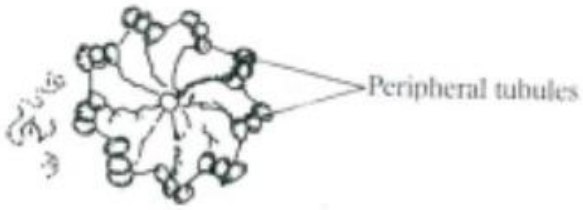
- They often contain a central core of crystalline material called nucleoid composed of urate oxidase crystals.
- These bodies are mostly spherical or ovoid and about the size of mitochondria and lysosomes. They are usually closely associated with E.R.
- They are involved in with photorespiration in plant cells.
- They bring about fat metabolism in cells.

Glyoxysomes

- The micro bodies present in plant cells and morphologically similar to peroxisomes.
- Found in the cell of yeast and certain fungi and oil rich seeds in plants.
- Functionally they contain enzyme of fatty acid metabolism involved in the conversion of lipids to carbohydrates during germination.

Centriole

It is present in all animal cells, located just outside the nucleus. It is cylindrical, 0.5 μm in length and without a membrane. It has 9 sets of peripheral tubules but none in the centre. Each set has three tubules arranged at definite angles. It has its own DNA and RNA and therefore it is self duplicating. Function: Centrioles are involved in cell division. They give orientation to the 'mitotic spindle' which forms during cell division.



Nucleus

General structure of nucleus

- It is the largest organelle seen clearly when the cell is not dividing.
- It stains deeply, is mostly spherical, WBC have lobed nuclei.
- It is mostly one in each cell (uni-nucleate, some cells have many nuclei; (multinucleate).

- Double layered nuclear membrane enclosing nucleoplasm which contains chromatin network and a nucleolus.

Functions

- Maintains the cell in a working order.
- Co-ordinates the activities of organelles.
- Takes care of repair work.
- Participates directly in cell division to produce genetically identical daughter cells, this division is called mitosis.
- Participates in production of gametes through another type of cell division called meiosis.

The part of a nucleus are given here

Nuclear membrane

- Double layered membrane is interrupted by large number of pores.
- Membrane is made up of lipids and proteins (like plasma membrane) and has ribosomes attached on the outer membrane which make the outer membrane rough.
- The pores allow the transport of large molecules in and out of nucleus, and the membranes keep the hereditary material in contact with the rest of the cell.

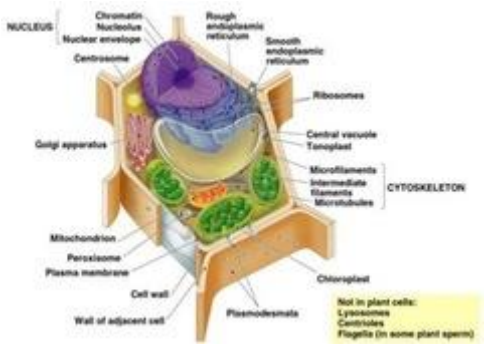
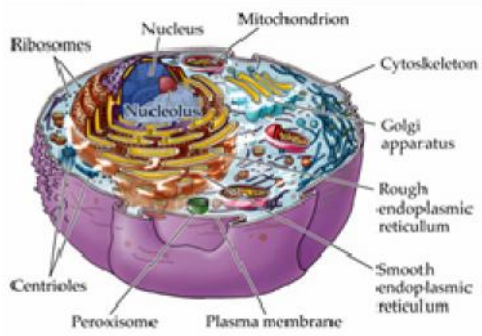
Chromatin

- Within the nuclear membrane there is jelly like substance (karyolymph or nucleoplasm) rich in proteins.
- In the karyolymph, fibrillar structures form a network called chromatin fibrils, which gets condensed to form distinct bodies called chromosomes during cell division. On staining the chromosomes, two regions can be identified in the chromatin material heterochromatin dark and autromaticn (light). Heterochromatin has less DNA and genetically less active than euchromatin which has more DNA and genetically more active.
- Number of chromosomes is fixed in an organism. During cell division chromosomes divide in a manner that the daughter cells receive identical amounts of hereditary matter.

Nucleolus

- Membraneless, spheroidal bodies present in all eukaryotic cells except in sperms and in some algae.
- Their number varies from one to few, they stain uniformly and deeply. It has DNA, RNA and proteins.
- Store house for RNA and proteins; it disappears during cell division and reappears in daughter cells.
- Regulates the synthetic activity of the nucleus.
- Thus nucleus and cytoplasm are interdependent, and this process is equal to nucleo–cytopalsmic interaction.

Differences between animal cell and plant cell

	Plant Cell		Animal Cell
1	A plant cell is usually larger in size.		An animal cell is comparatively smaller in size.
2	 **Plant Cell** It is enclosed by a rigid cellulose cell wall in addition to plasma membrane.		 **Animal Cell** It is enclosed by a thin, flexible plasma membrane only.
3	It cannot change its shape.		An animal cell can often change its shape.
4	Plastids are present. Plant cells exposed to sunlight contain chloroplast.		Plastids are usually absent.

5	A mature plant cell contains a large central vacuole.		An animal cell often possesses many small vacuoles.
6	Nucleus lies on one side in the peripheral cytoplasm.		Nucleus usually lies in the centre.
7	Centrioles are usually absent except in motile cells of lower plants.		Centrioles are practically present in animal cells
8	Lysosomes are rare.		Lysosomes are always present in animal cells.
9	Glyoxysomes may be present.		They are absent.
10	Tight junctions and desmosomes are lacking. Plasmodesmata are present.		Tight junctions and desmosomes are present between cells. Plasmodesmata are usually absent.
11	Reserve food is generally in the form of starch.		Reserve food is usually glycogen.
12	Plant cell synthesize all amino acids, coenzymes and vitamins required by them.		Animal cell cannot synthesize all the amino acids, co enzymes and vitamins required by them.
13	Spindles formed during cell divisions in anastral i.e. without asters at opposite poles.		Spindle formed during cell division is amphiastral i.e. has an ester at each pole.
14	Cytokinesis occurs by cell plate method.		Cytokinesis occurs by construction or furrowing.
15	Plant cell does not burst if placed in hypotonic solution due to the presence of the cell wall.		Animal cell lacking contractile vacuoles usually burst, if placed in hypertonic solution.

Transport of Molecules across Cell Membrane

Some endogenous substances and many drugs easily diffuse across the lipid bilayer. However, the lipid bilayer presents a formidable barrier to larger and more hydrophilic molecules (such as ions). These substances must be transported across the membrane by special proteins. The following are the types of transport:

There are two ways in which substances can enter or leave a cell:

1) Passive

a) Simple Diffusion

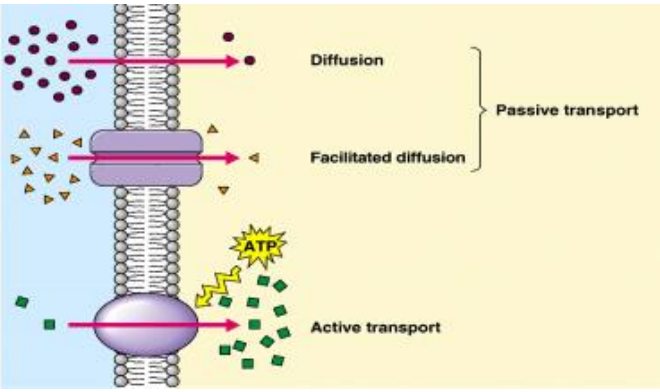
b) Facilitated Diffusion

c) Osmosis (water only)

2) Active

a) Molecules

b) Particles



Simple Diffusion across the lipid bilayer

Since membranes are held together by weak forces, certain molecules can slip between the lipids in the bilayer and cross from one side to the other. This spontaneous process is termed diffusion. Diffusion is the movement of particles down their gradient. A gradient is any imbalance in concentration, and moving down a gradient just means that the particle is trying to be evenly distributed.

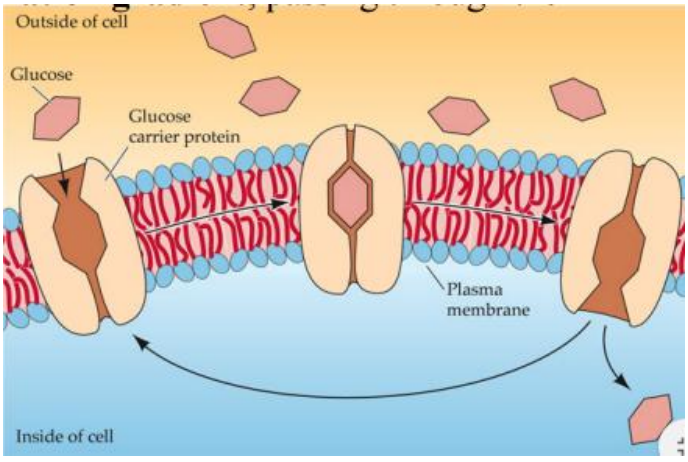
We call this evening-out moving “downhill”, and it doesn’t require energy. The molecule most likely to be involved in simple diffusion is water - it can easily pass through cell membranes.

Some major examples of diffusion in biology:

- Gas exchange at the alveoli — oxygen from air to blood, carbon dioxide from blood to air.
- Gas exchange for photosynthesis — carbon dioxide from air to leaf, oxygen from leaf to air.
- Gas exchange for respiration — oxygen from blood to tissue cells, carbon dioxide in opposite direction.
- Transfer of transmitter substance — acetylcholine from presynaptic to postsynaptic membrane at a synapse.
- Osmosis — diffusion of water through a semi-permeable membrane.

Facilitated Diffusion

This is the movement of specific molecules down a concentration gradient, passing through the membrane via a specific carrier protein. Thus, rather like enzymes, each carrier has its own shape and only allows one molecule (or one group of closely related molecules) to pass through. Selection is by size; shape; charge. Common molecules entering/leaving cells this way include glucose and amino-acids. It is passive and requires no energy from the cell. If the molecule is changed on entering the cell (glucose + ATP → glucose phosphate + ADP), then the concentration gradient of glucose will be kept high, and there will a steady one-way traffic.



When water undergoes simple diffusion, it is known as **osmosis**.

Osmosis is a special example of diffusion. It is the diffusion of water through a partially permeable membrane from a more dilute solution to a more concentrated

solution – down the water potential gradient) Note: diffusion and osmosis are both passive, i.e. energy from ATP is not used. A partially permeable membrane is a barrier that permits the passage of some substances but not others; it allows the passage of the solvent molecules but not some of the larger solute molecules. Cell membranes are described as selectively permeable because not only do they allow the passage of water but also allow the passage of certain solutes.

The presence of particular solutes stimulates the membrane to open specific channels or trigger active transport mechanisms to allow the passage of those chemicals across the membrane.

Some major examples of osmosis

- Absorption of water by plant roots.
- Re-absorption of water by the proximal and distal convoluted tubules of the nephron.
- Re-absorption of tissue fluid into the venule ends of the blood capillaries.
- Absorption of water by the alimentary canal — stomach, small intestine and the colon.

There are 3 types of solutions that involve water and how they affect the cell. They are:

Hypertonic Solution: the solution the cell is placed in has less water than the cell

In a hypertonic solution, there is a higher concentration of water inside the cell than outside the cell. A hypertonic solution has more solute (salt, sugar, etc.) than the cell and this cause there to be less water in the solution. Water flows from an area of high concentration to an area of low and leaves the cell. This loss of water causes the cell to shrivel. In animal cells, the shriveling is called crenating. The red blood cells in the picture to the left have crenated.

In plant cells, plasmolysis occurs and the cell membrane shrinks away from the cell wall. Death will result in both cells.

Hypotonic Solution: the solution the cell is placed in has more water than the cell

In a hypotonic solution, the solution contains a higher percentage of water than the cell. A hypotonic solution has less solute than the cell and this causes the solution to have more water than the cell. When a cell is placed in a hypotonic solution, water flows from

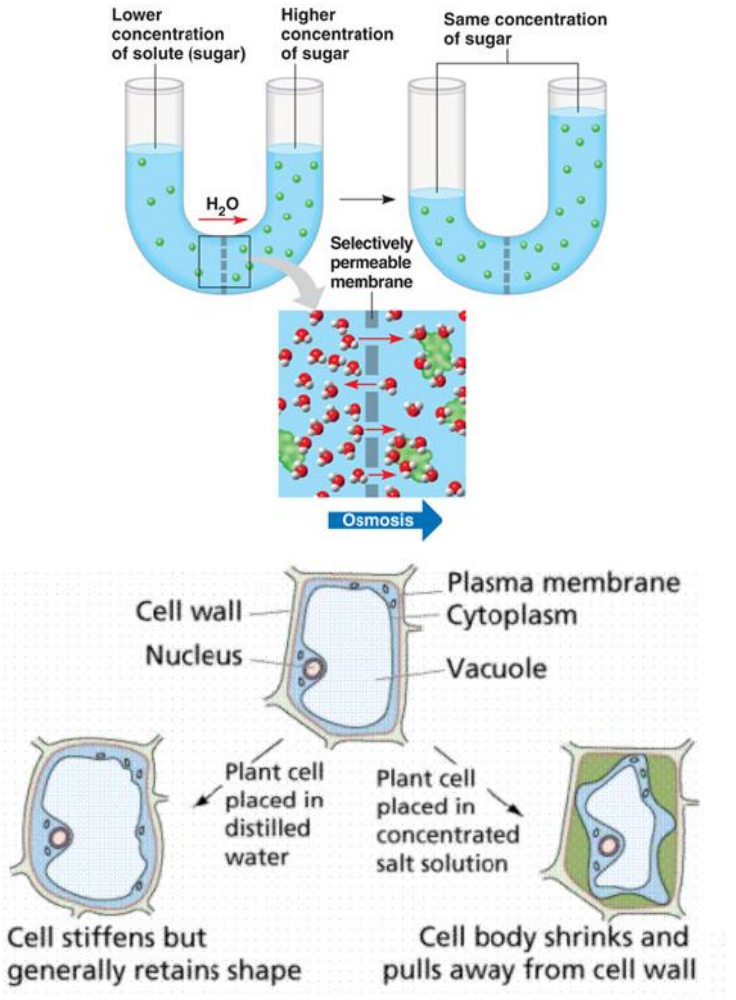
an area of high concentration to an area of low and rushes into the cell. This causes the cell to expand and possibly burst. In animal cells, the cell bursts or will lyse, killing the cell.

In plant cells, the cell membrane is pressed up against the cell wall but the cell wall does not allow the cell to expand anymore and the plant cell does not die.

Isotonic Solution: the solution the cell is placed in has equal amount of water as the cell

In an isotonic solution, there is the same percentage of water on the outside of the cell as the inside of the cell. An isotonic solution has the same amount of solute as the inside of the cell. Water moves at a constant rate in and out of the cell and the cell maintains its original shape.

In animal and plant cells, the cell keeps its shape when in an isotonic solution. Most cells live in an isotonic environment and they are able to maintain their shape and survive.

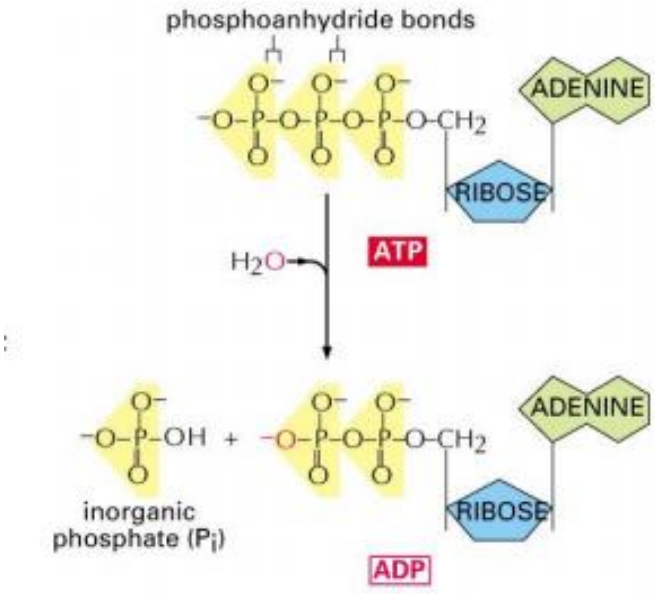


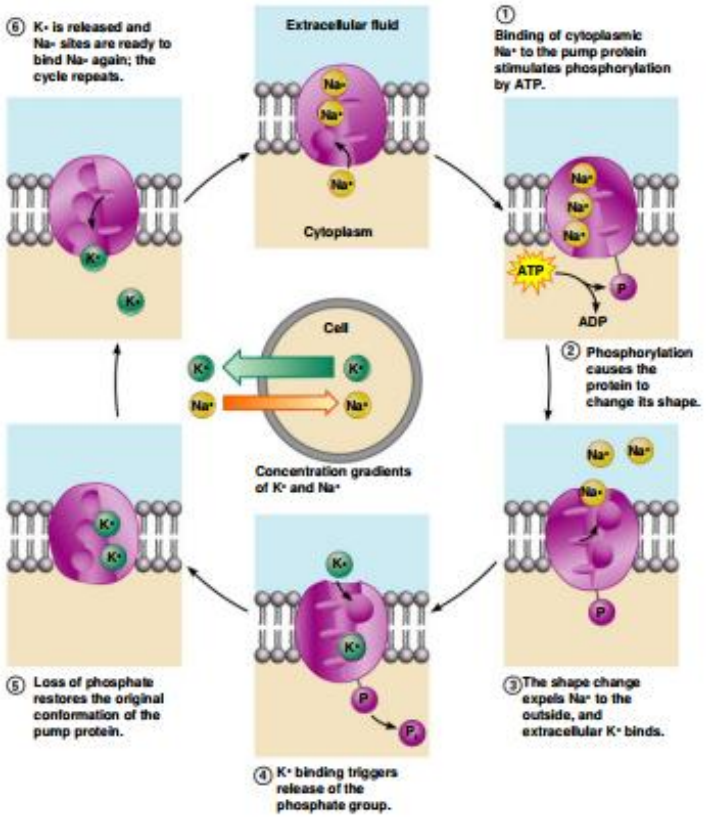
Active Transport

Transport method that moves particles from an area of low concentration to an area of high concentration or against a concentration gradient. Active transport relies on energy from the breakdown of ATP to move substances across the membrane. ATP, or adenosine triphosphate, is the main source of energy in the cell. The hydrolysis of the end phosphate group from an ATP molecule releases energy. The use of energy from ATP in active transport can be direct or indirect. Direct use of ATP is called primary active transport, and indirect use is called secondary active transport.

Primary active transport:

An example of primary active transport is the sodium-potassium pump or sodium-potassium ATPase (Na/K ATPase). The function of these pumps is to move 3 sodium ions from inside the cell to the extracellular fluid outside. It also pumps 2 potassium ions into the cell. So the concentration of K (potassium) is higher on the inside of the cell as it is on the outside and the concentration of Na (Sodium) is higher on the outside of the cell. Many cells use 1/3 to 1/2 of their ATP to run these ion pumps.





Secondary active transport

Secondary active transport does not use ATP. Instead it uses the energy stored from ion gradients. This is where an understanding of the sodium-potassium pump comes in handy. Let me explain. The Na/K ATPase moves Na actively by using ATP. It moves Na across its concentration gradient towards the outside of the cell, and in turn brings in K ions inside the cell.

As an example as to why this is important let's look at sugar, glucose. There is generally more glucose inside of the cell than outside. So, moving glucose across its gradient will generally take a lot of energy but not if we use sodium. Since we actively pump Na outside of the cell, the environment has more sodium than the inside of the cell.

This means that the sodium will want to move from outside the cell to inside. The cell uses this property to move glucose. It will allow sodium to re-enter the cell, but the sodium allows glucose to piggyback a ride into the cell alongside it. This is what is called co-transport and it uses a symporter.

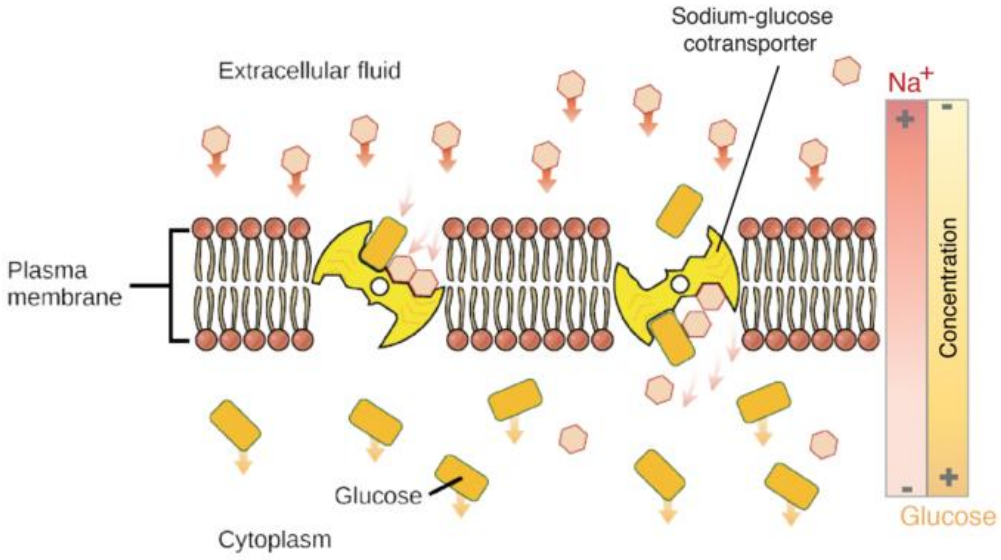
Symporter (Co-transport) is co-transport going in the same direction.

The other type of co-transport is exchange. This uses what is called an antiporter. The cell will move a solute of higher concentration outside the cell to inside the cell (Same example as Na). While the solute moves from higher to lower concentration, it generates energy. This is needed to move a molecule from the cell to outside of the cell, against its concentration gradient.

An example of this is Na/Ca exchanger (Sodium-Calcium). This is an example of an antiporter. It exchanged 3 sodium ions by moving it into the cell. For this exchange it will transport 1 calcium ion to the environment. This is similar to going to a store and exchanging money for items.

Antiporter (Exchange) is co-transport moving in the opposite direction.

The last type of port that uses the ion gradient is a **uniporter**. This is a simple port that moves an ion through a channel across its gradient. This is a type of diffusion and can be considered passive transport.



Differences between active and passive transport

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

	Active Transport	Passive Transport
Importance	In eukaryotic cells, amino acids, sugars and lipids need to enter the cell by protein pumps, which require active transport. These items either cannot diffuse or diffuse too slowly for survival.	It maintains equilibrium in the cell. Wastes (carbon dioxide, water, etc.) diffuse out and are excreted; nutrients and oxygen diffuse in to be used by the cell.

Beer and Lambert's Law

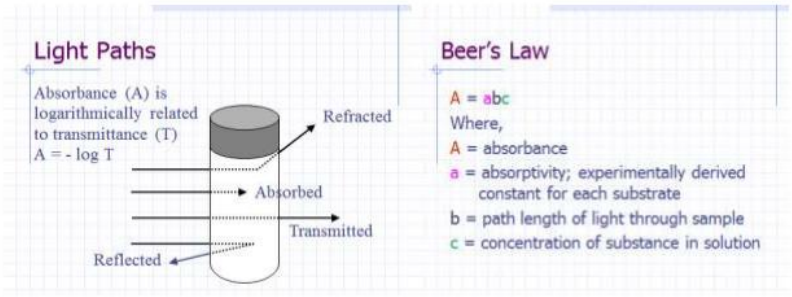
There are following possibilities when light is targeted to an object:

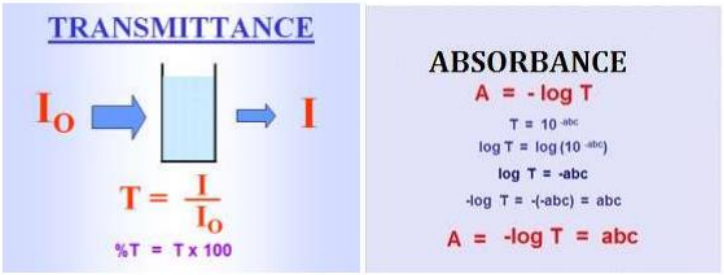
1. Transmission (if the object is transparent)
2. Absorption (if the object is liquid/gas or capable of absorption)
3. Reflection (if an opaque material is placed)
4. Refraction (if medium is changed during the path of light)
5. Scattering (if the surface is rough)

The Beer-Lambert law (also called the Beer-Lambert-Bouguer law or simply Beer's law) is the linear relationship between absorbance and concentration of an absorber of electromagnetic radiation. The general Beer-Lambert law is usually written as:

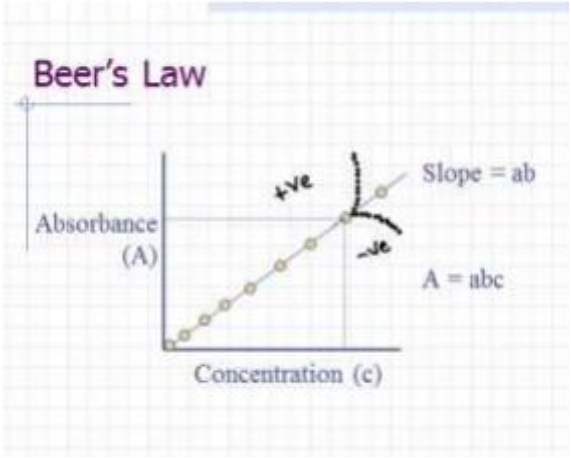
$$\mathbf{A = a_\lambda \cdot b \cdot c}$$

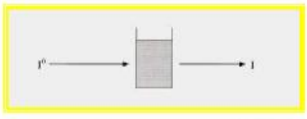
Where A is the measured absorbance, a_λ is a wavelength-dependent absorptivity coefficient, b is the path length, and c is the analyte concentration

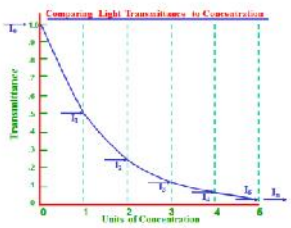




As given in the above figure, as the transmission increases, absorbance decreases exponentially.

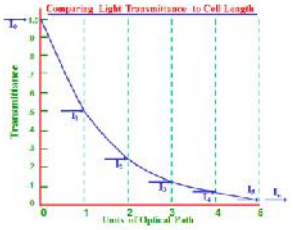






Graph for Beer's Law

$I_T = I_o e^{-kc}$



Graph for Lambert's Law

$I_T = I_o e^{-kT}$

Colorimeter

A colorimeter is a device used to test the concentration of a solution by measuring its absorbance of a specific wavelength of light.

Principle

The colorimeter is based on Beer-Lambert's law, according to which the absorption of light transmitted through the medium is directly proportional to the medium concentration.

Instrumentation

The instrument use for colorimetry is colorimeter. This apparatus will comprise of the following parts:

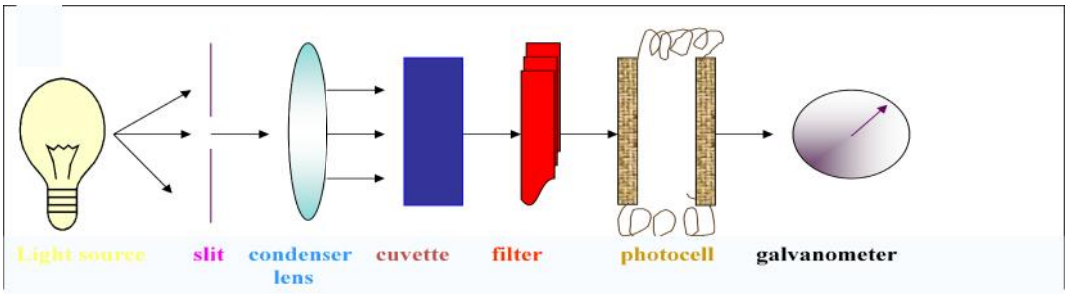
1. Light source
2. Filter (the device that selects the desired wavelength to obtain monochromatic light)
3. Cuvette chamber (the transmitted light passes through compartment wherein the solution containing the colored solution are kept in cuvette, made of glass or disposable plastic)
4. Detector (this is a photosensitive element that converts light into electrical signals)
5. Galvanometer (measures electrical signal quantitatively)

White light from a tungsten lamp passes through a slit, then a condenser lens, to give a parallel beam which falls on the solution under investigation contained in an absorption cell or cuvette. The cell is made of glass with the sides facing the beam cut parallel to each other.

Beyond the absorption cell is the filter, which is selected to allow maximum transmission of the color absorbed. If a blue solution is under examination, then red is absorbed and a red filter is selected.

The light then falls on to a photocell which generates an electrical current in direct proportion to the intensity of light falling on it.

This small electrical signal is increased by the amplifier which passes to a galvanometer of digital readout to give absorbance reading directly.



Anabolism and Catabolism

Metabolism is the set of life-sustaining chemical transformations within the cells of living organisms. These enzyme-catalyzed reactions allow organisms to grow and reproduce, maintain their structures, and respond to their environments. The word metabolism can also refer to all chemical reactions that occur in living organisms, including digestion and the transport of substances into and between different cells, in which case the set of reactions within the cells is called intermediary metabolism or intermediate metabolism.

The term metabolism is derived from the Greek – "Metabolismos" for "change", or "overthrow". The history of the scientific study of metabolism spans several centuries and has moved from examining whole animals in early studies, to examining individual metabolic reactions in modern biochemistry. The first controlled experiments in human metabolism were published by Santorio Santorio in 1614 in his book Ars de statica medicina. He described how he weighed himself before and after eating, sleep, working, sex, fasting, drinking, and excreting. He found that most of the food he took in was lost through what he called "insensible perspiration".

Catabolism is the metabolic process by which molecules are broken down and energy is produced. The body then receives the energy it needs to perform the most basic functions. Catabolism breaks down carbohydrates, amino acids, and lipids in the following stages.

- Step 1: Digestion

  First, large molecules are digested into smaller counterparts. This occurs for all molecules too large to be directly absorbed. ATP then pumps everything back into cells.
- Step 2: Energy Release Begins

  Acetyl coenzyme A further breaks down molecules, beginning the release of energy.

- Step 3: Energy Produced

  The molecules are oxidized, releasing all stored energy.

  Carbohydrates are broken down into simple sugars, protein into amino acids, amino acids into keto acids, and fat lipids into free fatty acids and glycerol.

  Anabolism is the process by which the body utilizes the energy released by catabolism to synthesize complex molecules.

  These complex molecules form cellular structures and act as the building blocks of the body. Though working in an opposite fashion to catabolism, anabolism similarly has 3 steps.

- Step 1: Precursor Production

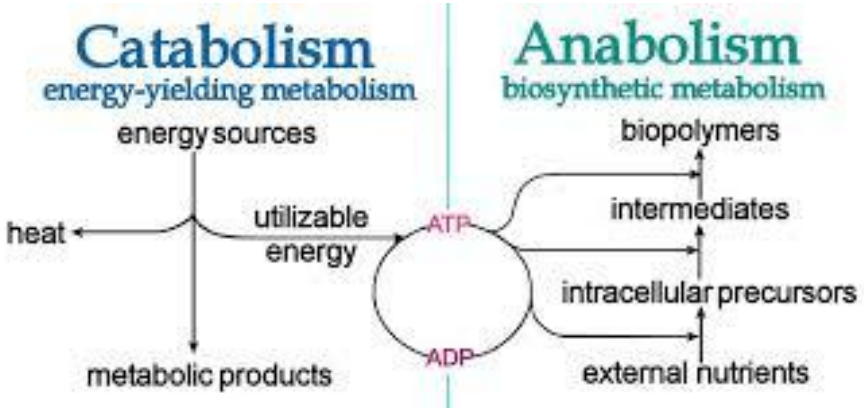
  Precursors of complex molecules are produced, such as amino acids and monosaccharide's.

- Step 2: Activation

  These precursors are activated into reactive forms by ATP.

- Step 3: Assembly

  Proteins, polysaccharides, lipids, and nucleic acids are assembled and ready for use.



Standard for energy change

The free energy change of a chemical process under standard state conditions, ΔG^o, can be determined four different ways:

- From Free Energies of Formation
- From Enthalpy Changes and Entropy Changes
- From Equilibrium Constants

- From Cell Potentials

Using Free Energies of Formation to Determine Standard State Free Energy Changes

If we know the standard free energy changes of formation, ΔG^o_f, of each species in a change we can determine the standard state free energy change, ΔG^o, for the change using the following equation:

$$\Delta G^o = \Sigma\ \Delta G^o_{f(products)} - \Sigma\ \Delta G^o_{f(reactants)}$$

Using Enthalpy Changes and Entropy Changes to Determine Standard State Free Energy Changes

If we know the enthalpy change, ΔH^o, and the entropy change, ΔS^o, for a chemical process, we can determine the standard state free energy change, ΔG^o, for the process using the following equation:

$$\Delta G^o = \Delta H^o - T\Delta S^o$$

In this equation T is the temperature on the Kelvin scale. In introductory courses we make the assumption that ΔH^o and ΔS^o, do not change as the temperature changes.

Using Equilibrium Constants to Determine Standard State Free Energy Changes

If we know the equilibrium constant, K_{eq}, for a chemical change (or if we can determine the equilibrium constant), we can calculate the standard state free energy change, ΔG^o, for the reaction using the equation:

$$\Delta G^o = - RT \ln K_{eq}$$

In this equation

- R = 8.314 J mol^{-1} K^{-1} or 0.008314 kJ mol^{-1} K^{-1}.
- T is the temperature on the Kelvin scale.
- K_{eq} is the equilibrium constant at the temperature T.

Using Cell Potentials to Determine Standard State Free Energy Changes

If we know the standard state cell potential, E^o, for an electrochemical cell (or if we can determine the standard state cell potential), we can calculate the standard state free energy change, ΔG^o, for the cell reaction using the equation:

$$\Delta G^o = -nFE^o$$

In this equation

- n is the number of moles of electrons exchanged in the cell reaction.
- F is 96.485 kJ $volt^{-1}$ $mole^{-1}$ (the "Faraday").

E^o is the cell potential under standard state conditions.

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DEPARTMENT OF MICROBIOLOGY
(For the candidates admitted from 2015 onwards)

Subject	**:**	**Biochemistry**	**Semester**	**:**	**I**
Subject code	**:**	**17MBU103**	**Class**	**:**	**I B.Sc Microbiology**

UNIT-I: COURSE MATERIAL

Unit-I

Atoms and molecules, cell structure, cell organelles, developing membrane, structure, transport of molecules, Beer and Lambert's Law, Colorimeter, Anabolism and catabolism and standard for energy change.

Suggest Readings

1. Berg, J.M., Tymoczko, J.L., and Stryer, L. (2011). Biochemistry, W.H. Freeman and Company.
2. Nelson, D.L and Cox, M.M. (2008). Lehninger Principles of Biochemistry, 5th edition. W.H. Freeman and Company.

Atoms and Molecules

An atom is the smallest unit of matter that has the properties of an element. It is composed of a dense core called the nucleus and a series of outer shells occupied by orbiting electrons. Elements are substances consisting of one type of atom, for example Carbon atoms make up diamond, and also graphite. Pure (24K) gold is composed of only one type of atom, gold atoms.

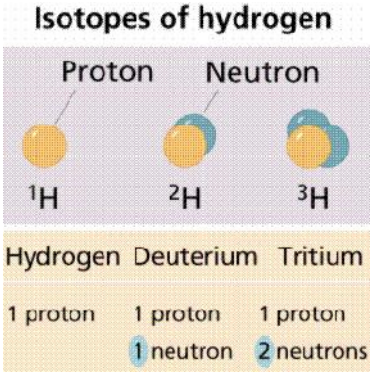
Subatomic particles were discovered during the 1800s. There are three main subatomic particles viz., Proton, Neutron and Electron.

- The **proton** is located in the center (or nucleus) of an atom, each atom has at least one proton. Protons have a charge of +1, and a mass of approximately 1 atomic mass unit (amu). Elements differ from each other in the number of protons they have, e.g. Hydrogen has 1 proton; Helium has 2.
- The **neutron** also is located in the atomic nucleus (except in Hydrogen). The neutron has no charge, and a mass of slightly over 1 amu.
- The **electron** is a very small particle located outside the nucleus. Because they move at speeds near the speed of light the precise location of electrons is hard to pin down. Electrons occupy orbital's, or areas where they have a high statistical probability of occurring. The charge on an electron is -1. Its mass is negligible (approximately 1800 electrons are needed to equal the mass of one proton).

Name	Charge	Location	Mass
Proton	+1	atomic nucleus	1.6726×10^{-27} kg
Neutron	0	atomic nucleus	1.6750×10^{-27} kg
Electron	-1	electron orbital	9.1095×10^{-31} kg

The **atomic number** is the number of protons an atom has. It is characteristic and unique for each element. The **atomic mass** (also referred to as the atomic weight) is the number of protons and neutrons in an atom. Atoms of an element that have differing numbers of neutrons (but a constant atomic number) are termed isotopes.

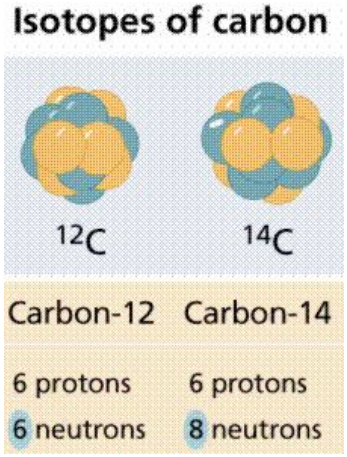
Example 1: Isotopes of Hydrogen



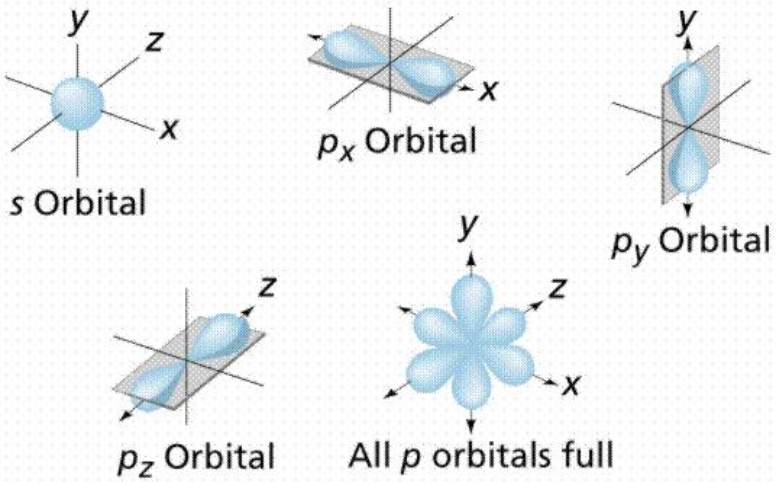
Note that each of these isotopes of hydrogen has only one proton. Isotopes differ from each other in the number of neutrons, not in the number of protons.

Some isotopes are radioisotopes, which spontaneously decay, releasing radioactivity. Other isotopes are stable. Examples of radioisotopes are Carbon-14 (symbol ^{14}C), and deuterium (also known as Hydrogen-2; ^{2}H). Stable isotopes are ^{12}C and ^{1}H.

Example 2: Isotopes of carbon



An orbital is also an area of space in which an electron will be found 90% of the time. Orbitals have a variety of shapes. Each orbital has a characteristic energy state and a characteristic shape. The *s* orbital is spherical. Since each orbital can hold a maximum of two electrons, atomic numbers above 2 must fill the other orbitals. The p_x, p_y, and p_z orbitals are dumbbell shaped, along the x, y, and z axes respectively.



Energy levels (also referred to as electron shells) are located a certain "distance" from the nucleus. The major energy levels into which electrons fit, are (from the nucleus outward) K, L, M, and N. Sometimes these are numbered, with electron configurations being: $1s^2 2s^2 2p^1$, (where the first shell K is indicated with the number 1, the second shell L with the number 2, etc.). This nomenclature tells us that for the atom mentioned in this paragraph, the first energy level (shell) has two electrons in its *s* orbital (the only orbital it can have), and second energy level has a maximum of two electrons in its *s* orbital, plus one electron in its *p* orbital.

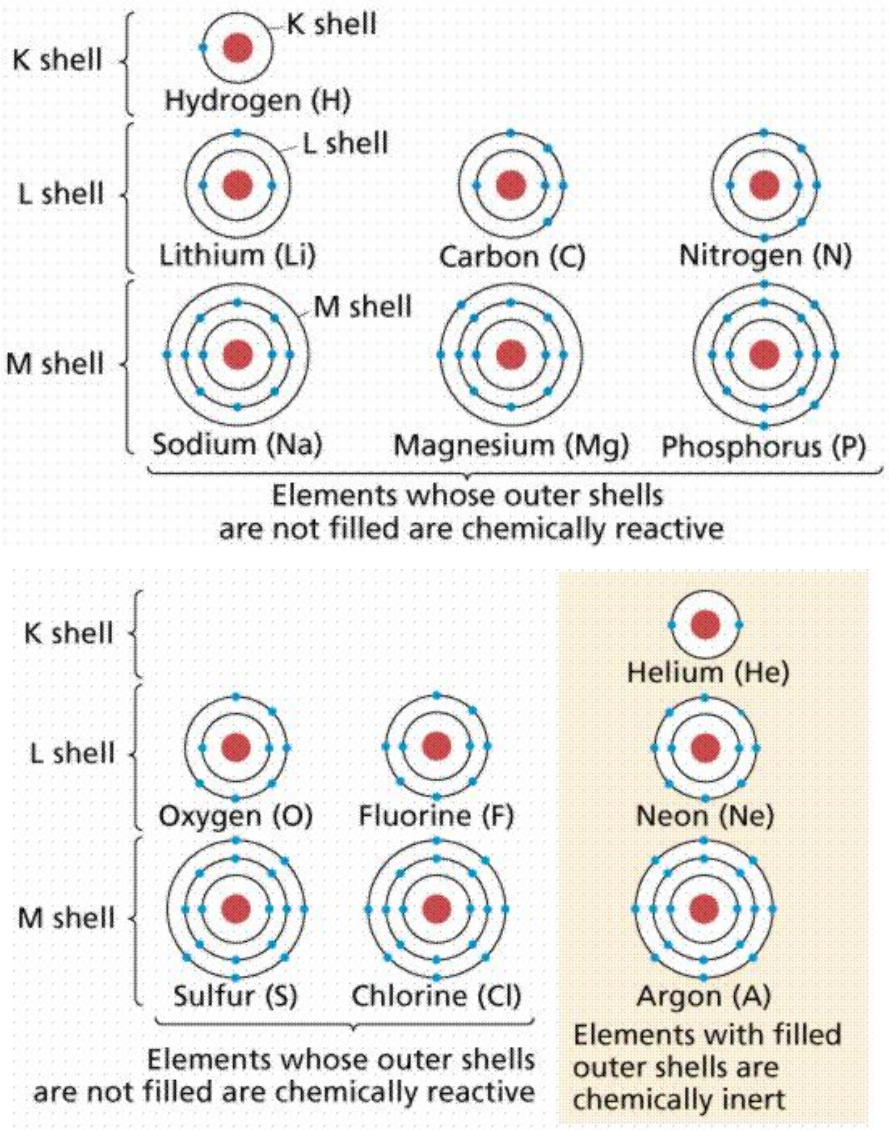
As a general rule, for the atoms we are likely to encounter in biological systems, atoms tend to gain or lose their outer electrons to achieve a Noble Gas outer electron shell configuration of two or eight electrons. The number of electrons that are gained or lost is characteristic for each element, and ultimately determines the number and types of chemical bonds atoms of that element can form.

Chemical Bonding

Ionic bonds

Ionic bonds are formed when atoms become ions by gaining or losing electrons. Chlorine is in a group of elements having seven electrons in their outer shells. Members of this group tend to gain one electron, acquiring a charge of -1. Sodium is in another group with elements having one electron in their outer shells. Members of this group tend to lose that outer electron, acquiring a charge of +1. Oppositely charged ions are attracted to each other, thus Cl^- (the symbolic representation of the chloride ion) and Na^+ (the symbol for the sodium ion, using the Greek word *natrium*) form an ionic bond, becoming the molecule

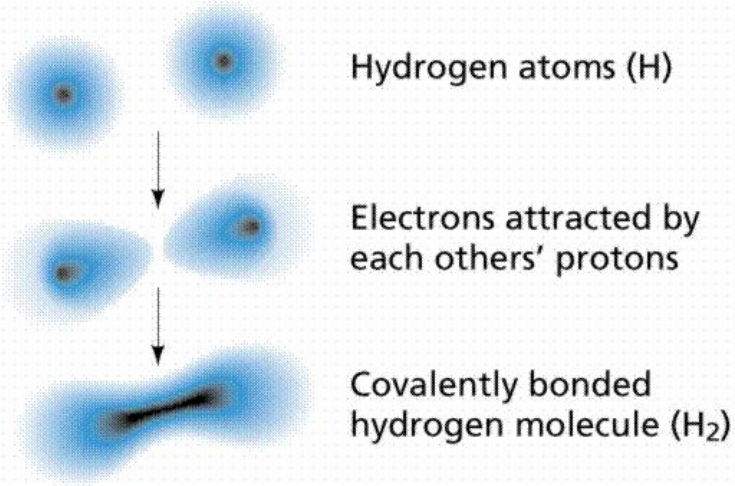
sodium chloride. Ionic bonds generally form between elements in Group I (having one electron in their outer shell) and Group VII a (having seven electrons in their outer shell). Such bonds are relatively weak, and tend to disassociate in water, producing solutions that have both Na and Cl ions.



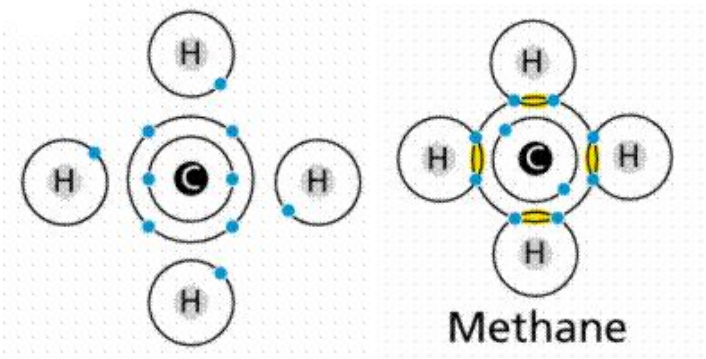
Covalent bonds

Covalent bonds form when atoms share electrons. Since electrons move very fast they can be shared, effectively filling or emptying the outer shells of the atoms involved in the bond. Such bonds are referred to as electron-sharing bonds. An analogy can be made to child custody: the children are like electrons, and tend to spend some time with one parent

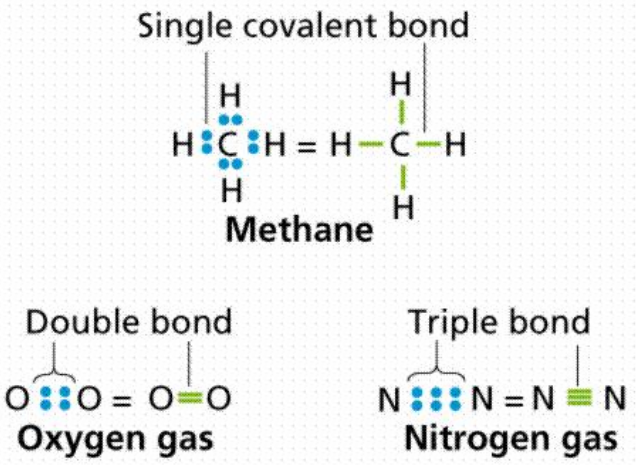
and the rest of their time with the other parent. In a covalent bond, the electron clouds surrounding the atomic nuclei overlap.



Carbon (C) is in Group IV a, meaning it has four electrons in its outer shell. Thus to become a "happy atom", Carbon can either gain or lose four electrons. By sharing the electrons with other atoms, Carbon can become a happy atom, alternately filling and emptying its outer shell, as with the four hydrogen's.



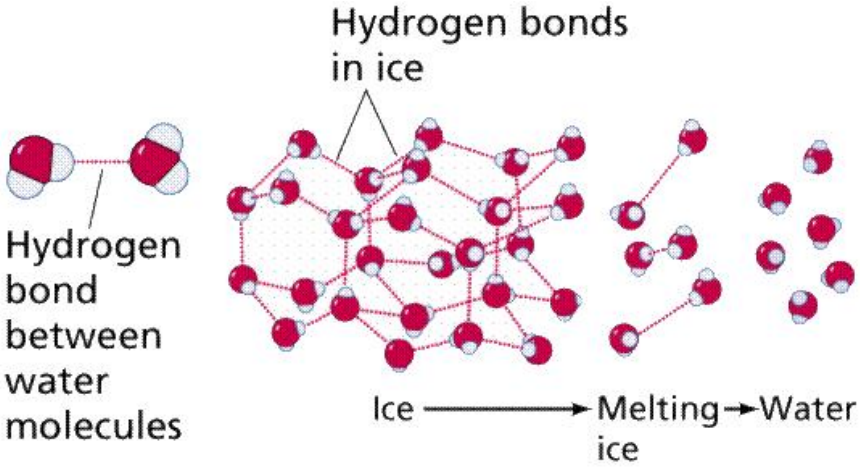
The molecule methane (chemical formula CH_4) has four covalent bonds, one between Carbon and each of the four Hydrogens. Carbon contributes an electron, and Hydrogen contributes an electron. The sharing of a single electron pair is termed a single bond. When two pairs of electrons are shared, a double bond results, as in carbon dioxide. Triple bonds are known, wherein three pairs (six electrons total) are shared as in acetylene gas or nitrogen gas. The types of covalent bonds are:



Hydrogen bonds

Hydrogen bonds result from the weak electrical attraction between the positive end of one molecule and the negative end of another. Individually these bonds are very weak, although taken in a large enough quantity, the result is strong enough to hold molecules together or in a three-dimensional shape.

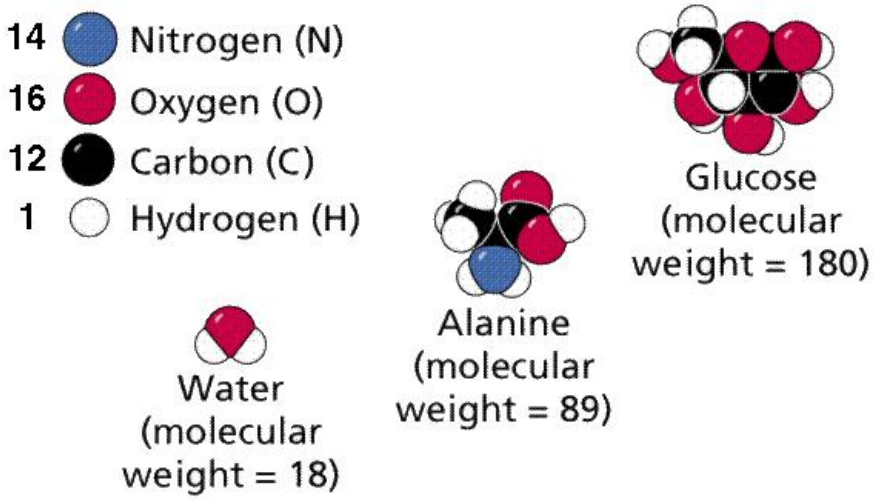
Example: Formation of a hydrogen bond between the hydrogen side of one water molecule and the oxygen side of another water molecule.



Molecules

Molecules are compounds in which the elements are in definite, fixed ratios. Those atoms are held together usually by one of the three types of chemical bonds discussed above. For example: water, glucose, ATP. Mixtures are compounds with variable formulas/ratios of their components. For example: soil. Molecular formulas are an expression in the simplest whole-number terms of the composition of a substance. For

example, the sugar glucose has 6 Carbons, 12 hydrogens, and 6 oxygens per repeating structural unit. The formula is written $C_6H_{12}O_6$.



Chemical reactions occur in nature, and some also can be performed in a laboratory setting. One chemical equation are linear representations of how these reactions occur. Combination reactions occur when two separate reactants are bonded together, e.g. A + B ----> AB. Disassociation reactions occur when a compound is broken into two products, e.g. AB -----> A + B.

The cell and cell theory

Soon after Anton van Leeuwenhoek invented the microscope, Robert Hooke in 1665 observed a piece of cork under the microscope and found it to be made of small compartments which he called "cells" (Latin cell = small room). In 1672, Leeuwenhoek observed bacteria, sperm and red blood corpuscles, all of which were cells. In 1831, Robert Brown, an Englishman observed that all cells had a centrally positioned body which he termed the nucleus.

The cell theory

In 1838 Matthias Schleiden and Theodor Schwann formulated the "Cell Theory." The cell theory maintains that

- All organisms are composed of cells.
- Cell is the structural and functional unit of life, and
- Cells arise from pre-existing cells.

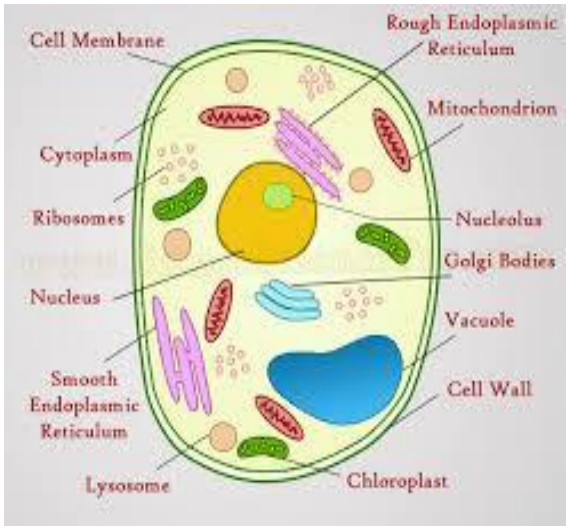
The cells vary considerably, in shape and size

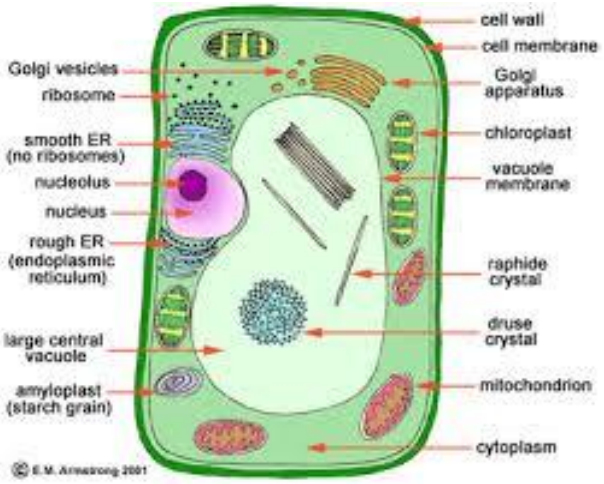
Cell Structure, cell organelles and developing membrane structure

A cell may be defined as a unit of protoplasm bounded by a plasma or cell membrane and possessing a nucleus. Protoplasm is the life giving substance and includes the cytoplasm and the nucleus. The cytoplasm has in it organelles such as ribosomes, mitochondria, Golgi bodies plastids, lysosomes and endoplasmic reticulum. Plant cells have in their cytoplasm large vacuoles containing non-living inclusions like crystals, pigments etc. The bacteria have neither organelles nor a well formed nucleus. But every cell has three major components

- plasma membrane
- cytoplasm
- DNA (naked in bacteria and covered by a membrane in all other organisms

Animal Cell **Plant Cell**





Plasma membrane

Each cell has a limiting boundary, the cell membrane, plasma membrane or plasmalemma. It is a living membrane, outermost in animal cells but next to cell wall in plant cells.

The plasma membrane is made of proteins and lipids and several models were proposed regarding the arrangement of proteins and lipids. The fluid mosaic model proposed by Singer and Nicholson (1972) is widely accepted.

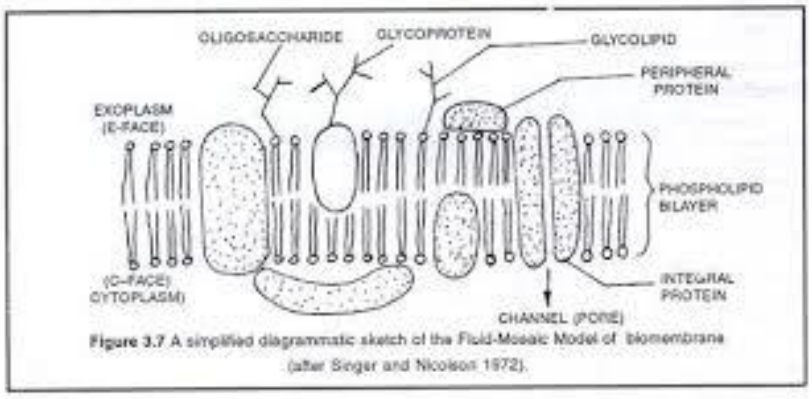


Figure 3.7 A simplified diagrammatic sketch of the Fluid-Mosaic Model of biomembrane (after Singer and Nicolson 1972).

According to the fluid mosaic model,

(i) The plasma membrane is composed of a lipid bilayer of phospholipid molecules into which a variety of globular proteins are embedded.

(ii) Each phospholipid molecule has two ends, an outer head hydrophilic i.e. water attracting, and the inner tail pointing centrally hydrophobic, i.e. water repelling

(iii) The protein molecules are arranged in two different ways:

a. Peripheral proteins or extrinsic proteins: these proteins are present on the outer and inner surfaces of lipid bilayer.

b. Integral proteins or intrinsic proteins: These proteins penetrate lipid bilayer partially or wholly.

Functions

(i) The plasma membrane encloses the cell contents.

(ii) It provides cell shape (in animal cells) e.g. the characteristic shape of red blood cells, nerve cells, bone cells, etc

(iii) It allows transport of certain substances into and out of the cell but not all substance, so it is termed selectively permeable.

Cell wall

In bacteria and plant cells the outermost cell cover, present outside the plasma membrane is the cell wall. Bacterial cell wall is made of peptidoglycan.

Structure

- Outermost non-living, layer present in all plant cells.
- Secreted by the cell itself.

- In plant, made of cellulose but may also contain other chemical substance such as pectin and lignin.
- The substance constituting the cell is not simply homogenous but it consists of fine threads or fibres called microfibrils.
- It may be thin (1 micron) and transparent as in the cells of onion peel. In some cases it is very thick as in the cells of wood.

Functions

- The cell wall protects the delicate inner parts of the cell.
- Being rigid, it gives shape to the cell.
- Being rigid, it does not allow distension of the cell, thus leading to turgidity of the cell that is useful in many ways.
- It freely allows the passage of water and other chemicals into and out of the cells.
- There are breaks in the primary wall of the adjacent cells through which cytoplasm of one cell remains connected with the other. These cytoplasmic strands which connect one cell to the other one are known as plasmodesmata.
- Walls of two adjacent cells are firmly joined by a cementing material called middle lamella made of calcium pectate.

Mitochondria and chloroplast - the energy transformers

Mitochondria (found in plant and animal cells) are the energy releasers and the chloroplasts (found only in green plant cells) are the energy trappers.

Mitochondria (Singular = mitochondrion)

Appear as tiny thread like structure under light microscope. Approximately 0.5 - 1.00 μm (micrometer) Number usually a few hundred to a few thousand per cell (smallest number is just one as in an alga (Micromonas).

Structure

The general plan of the internal structure of a mitochondria observed by means of electron microscope. It consists of the following parts:

- Wall made of double membrane
- The inner membrane is folded inside to form projections called cristae which project into the inner compartment called matrix.

Function

Oxidizes pyruvic acid (breakdown product of glucose) to release energy which gets stored in the form of ATP for ready use. This process is also called cellular respiration.

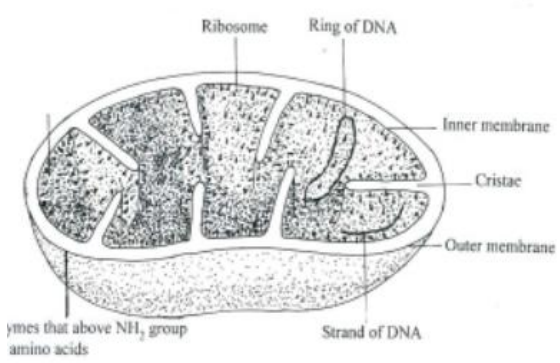
Plastids

Plastids are found only in a plant cell. They may be colorless or with color. Based on this fact, there are three types of plastids.

(i) Leucoplast-white or colorless

(ii) Chromoplast – blue, red, yellow etc.

(iii) Chloroplast – green

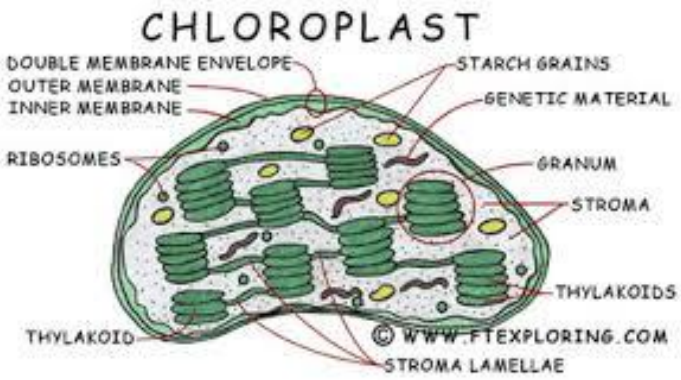


Chloroplast

- Found in all green plant cells in the cytoplasm.
- Number 1 to 1008
- Shape: Usually disc-shaped or spherical as in most plants around you. In some ribbon - shaped as in an alga spirogyra or cup - shaped as in other algae *Chlamydomonas*.
- Structure: the general plan of the structure of a single chloroplast is given below:
    - Wall made of double membrane i.e. outer membrane and inner membrane numerous stack-like (piles) groups or grana (singular = granum) are interconnected by lamellae.
    - Sac like structures called thylakoids placed one above the other constitute granum.
    - Inside of the chloroplast is filled with a fluid medium called stoma.

Function

Chloroplasts are the seat of photosynthesis (production of sugar, from carbon dioxide and water in the presence of sunlight).



Chloroplast versus mitochondria

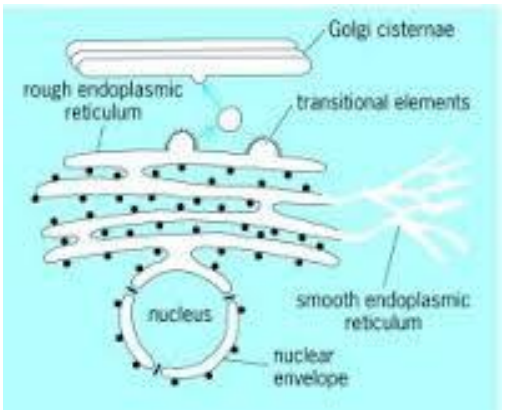
These two organelles are opposite to each other, one traps the solar energy locking it in a complex molecule (by photosynthesis), the other releases the energy by breaking the complex molecule (by respiration).

Similarities between mitochondria and chloroplasts

Both contain their own DNA (the genetic material) as well as their own RNA (for protein synthesis). Thus, they can self duplicate to produce more of their own kind without the help of nucleus. Since chloroplasts and mitochondria contain their own DNA the hereditary molecule and also their own ribosomes, they are termed semi-autonomous only because they are incapable of independent existence though they have ribosomes and DNA.

Endoplasmic reticulum (ER), Golgi body and ribosomes

Endoplasmic reticulum (ER) and Golgi body are single membrane bound structures. The membrane has the same structure (lipid-protein) as the plasma membrane but ribosomes do not have membranes Ribosomes are involved in synthesis of substances in the cell, Golgi bodies in secreting and the ER in transporting and storing the products. These three organelles operate together. The figure below shows the diagram of ER and Golgi body. Note the ribosomes present in ER.



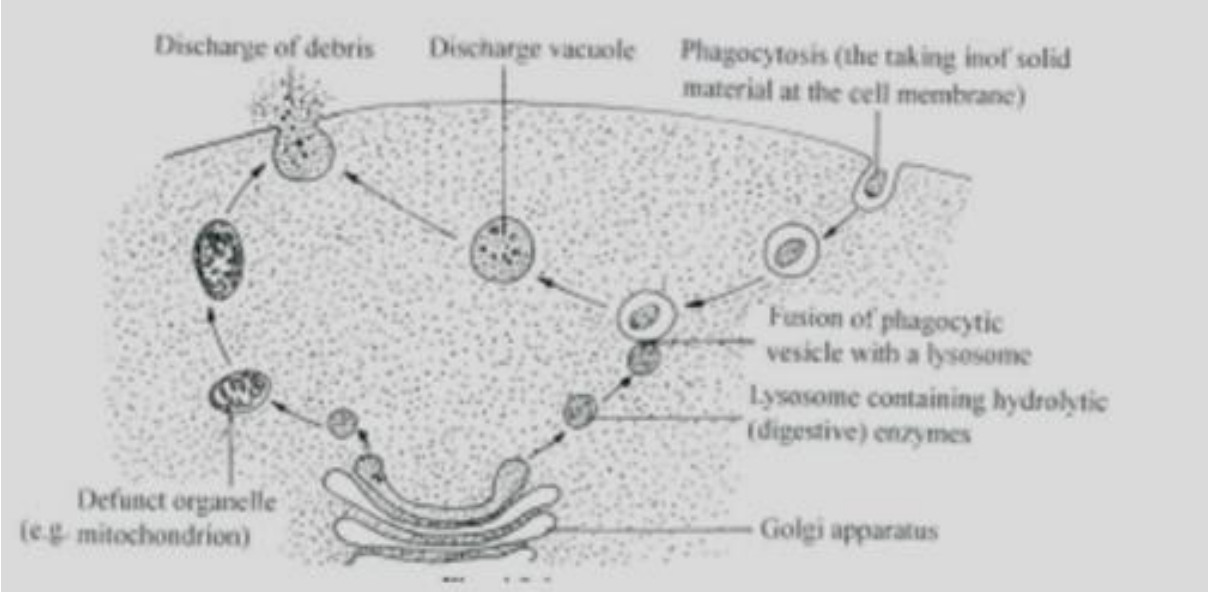
Endoplasmic reticulum (ER)	Gogli body	Ribosomes
Structure		
A network of membranes with thickness between 50 - 60A°. It is of two types– rough endoplasmic reticulum (RER) i.e. when ribosomes are attached to it and Smooth-endo-plasmic reticulum (SER) when no ribosomes are present.	Is a stack of membranous sacs of the same thickness as ER. Exhibit great diversity in size and shape.	Spherical about 150 - 250 Å in diameter, made up of large molecules of RNA and proteins (ribonucleo proteins)
Throughout the cytoplasm and is in contact with the cell membrane as well as the nuclear membrane.	In animal cells present around the nucleus, 3 to 7 in number. In plant cells, many and present scattered throughout the cell called dictyosomes.	Present either as free particles in cytoplasm or attached to ER. Also found stored in nucleolus inside the nucleus. 80S types found in eukaryotes and 70S in prokaryotes (S-svedberg unit of measuring ribosomes).
Function		
Provides internal framework, compartment and reaction surfaces, transports enzymes and other materials through out the cell. RER is the site for protein synthesis and SER for steroid synthesis, stores carbohydrates.	Synthesis and secretion as enzymes, participates in transformation of membranes to give rise to other membrane structure such as lysosome, acrosome, and dictyosomes, synthesize wall element like pectin, mucilage.	Site for protein synthesis.

The micro bodies (tiny but important)

These are small sac-like structures bounded by their membranes. These are of different kinds and the most important ones are lysosomes, peroxisomes and glyoxysomes.

Lysosomes (lysis = breaking down; soma = body)

Lysosomes are present in almost all animal cells and some non - green plant cells (Fig 4.9). They perform intracellular digestion.



Some main features of lysosomes are as follows

- Membranous sacs budded off from Golgi body.
- May be in hundreds in single cell.
- Contain several enzymes (about 40 in number)
- Materials to be acted upon by enzymes enter the lysosomes.
- Lysosomes are called "suicidal bags" as enzymes contained in them can digest the cell's own material when damaged or dead.
- Importance of intracellular digestion by the lysosomes
- Help in nutrition of the cell by digesting food, as they are rich in various enzymes which enable them to digest almost all major chemical constituents of the living cell.
- Help in defense by digesting germs, as in white blood cells.
- Help in cleaning up the cell by digesting damaged material of the cell.
- Provide energy during cell starvation by digestion of the cells own parts (autophagic, auto: self; phagos: eat up).
- Help sperm cells in entering the egg by breaking through (digesting) the egg membrane.
- In plant cells, mature xylem cells lose all cellular contents by lysosome activity.
- When cells are old, diseased or injured, lysosomes attack their cell organelles and digest them. In other words lysosomes are autophagic, i.e. self devouring.

Peroxisomes

Found both in plant and animal cells. Found in the green leaves of higher plants. They participate in oxidation of substrates resulting in the formation of hydrogen peroxide.

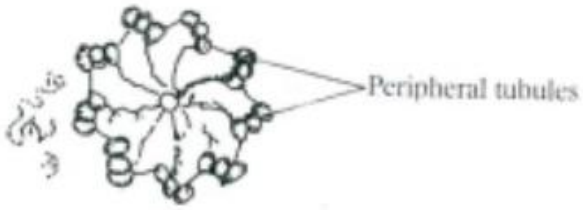
- They often contain a central core of crystalline material called nucleoid composed of urate oxidase crystals.
- These bodies are mostly spherical or ovoid and about the size of mitochondria and lysosomes. They are usually closely associated with E.R.
- They are involved in with photorespiration in plant cells.
- They bring about fat metabolism in cells.

Glyoxysomes

- The micro bodies present in plant cells and morphologically similar to peroxisomes.
- Found in the cell of yeast and certain fungi and oil rich seeds in plants.
- Functionally they contain enzyme of fatty acid metabolism involved in the conversion of lipids to carbohydrates during germination.

Centriole

It is present in all animal cells, located just outside the nucleus. It is cylindrical, 0.5 μm in length and without a membrane. It has 9 sets of peripheral tubules but none in the centre. Each set has three tubules arranged at definite angles. It has its own DNA and RNA and therefore it is self duplicating. Function: Centrioles are involved in cell division. They give orientation to the 'mitotic spindle' which forms during cell division.



Nucleus

General structure of nucleus

- It is the largest organelle seen clearly when the cell is not dividing.
- It stains deeply, is mostly spherical, WBC have lobed nuclei.
- It is mostly one in each cell (uni-nucleate, some cells have many nuclei; (multinucleate).

- Double layered nuclear membrane enclosing nucleoplasm which contains chromatin network and a nucleolus.

Functions

- Maintains the cell in a working order.
- Co-ordinates the activities of organelles.
- Takes care of repair work.
- Participates directly in cell division to produce genetically identical daughter cells, this division is called mitosis.
- Participates in production of gametes through another type of cell division called meiosis.

The part of a nucleus are given here

Nuclear membrane

- Double layered membrane is interrupted by large number of pores.
- Membrane is made up of lipids and proteins (like plasma membrane) and has ribosomes attached on the outer membrane which make the outer membrane rough.
- The pores allow the transport of large molecules in and out of nucleus, and the membranes keep the hereditary material in contact with the rest of the cell.

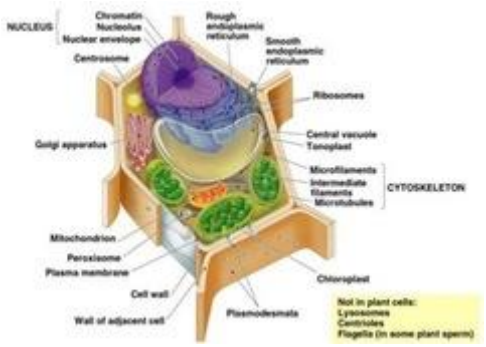
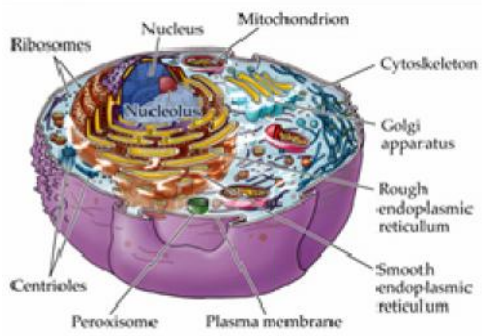
Chromatin

- Within the nuclear membrane there is jelly like substance (karyolymph or nucleoplasm) rich in proteins.
- In the karyolymph, fibrillar structures form a network called chromatin fibrils, which gets condensed to form distinct bodies called chromosomes during cell division. On staining the chromosomes, two regions can be identified in the chromatin material heterochromatin dark and autromaticn (light). Heterochromatin has less DNA and genetically less active than euchromatin which has more DNA and genetically more active.
- Number of chromosomes is fixed in an organism. During cell division chromosomes divide in a manner that the daughter cells receive identical amounts of hereditary matter.

Nucleolus

- Membraneless, spheroidal bodies present in all eukaryotic cells except in sperms and in some algae.
- Their number varies from one to few, they stain uniformly and deeply. It has DNA, RNA and proteins.
- Store house for RNA and proteins; it disappears during cell division and reappears in daughter cells.
- Regulates the synthetic activity of the nucleus.
- Thus nucleus and cytoplasm are interdependent, and this process is equal to nucleo–cytopalsmic interaction.

Differences between animal cell and plant cell

	Plant Cell		Animal Cell
1	A plant cell is usually larger in size.		An animal cell is comparatively smaller in size.
2	 **Plant Cell** It is enclosed by a rigid cellulose cell wall in addition to plasma membrane.		 **Animal Cell** It is enclosed by a thin, flexible plasma membrane only.
3	It cannot change its shape.		An animal cell can often change its shape.
4	Plastids are present. Plant cells exposed to sunlight contain chloroplast.		Plastids are usually absent.

5	A mature plant cell contains a large central vacuole.		An animal cell often possesses many small vacuoles.
6	Nucleus lies on one side in the peripheral cytoplasm.		Nucleus usually lies in the centre.
7	Centrioles are usually absent except in motile cells of lower plants.		Centrioles are practically present in animal cells
8	Lysosomes are rare.		Lysosomes are always present in animal cells.
9	Glyoxysomes may be present.		They are absent.
10	Tight junctions and desmosomes are lacking. Plasmodesmata are present.		Tight junctions and desmosomes are present between cells. Plasmodesmata are usually absent.
11	Reserve food is generally in the form of starch.		Reserve food is usually glycogen.
12	Plant cell synthesize all amino acids, coenzymes and vitamins required by them.		Animal cell cannot synthesize all the amino acids, co enzymes and vitamins required by them.
13	Spindles formed during cell divisions in anastral i.e. without asters at opposite poles.		Spindle formed during cell division is amphiastral i.e. has an ester at each pole.
14	Cytokinesis occurs by cell plate method.		Cytokinesis occurs by construction or furrowing.
15	Plant cell does not burst if placed in hypotonic solution due to the presence of the cell wall.		Animal cell lacking contractile vacuoles usually burst, if placed in hypertonic solution.

Transport of Molecules across Cell Membrane

Some endogenous substances and many drugs easily diffuse across the lipid bilayer. However, the lipid bilayer presents a formidable barrier to larger and more hydrophilic molecules (such as ions). These substances must be transported across the membrane by special proteins. The following are the types of transport:

There are two ways in which substances can enter or leave a cell:

1) Passive

a) Simple Diffusion

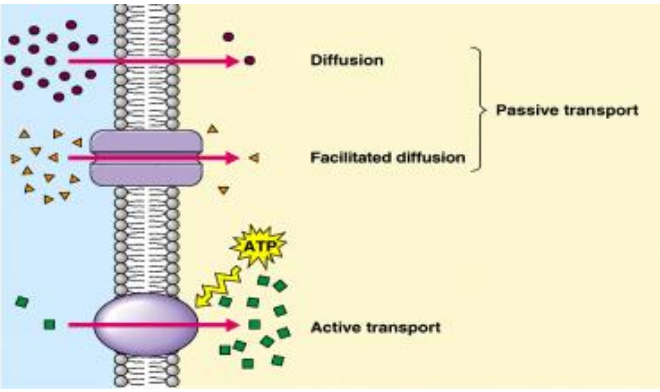
b) Facilitated Diffusion

c) Osmosis (water only)

2) Active

a) Molecules

b) Particles



Simple Diffusion across the lipid bilayer

Since membranes are held together by weak forces, certain molecules can slip between the lipids in the bilayer and cross from one side to the other. This spontaneous process is termed diffusion. Diffusion is the movement of particles down their gradient. A gradient is any imbalance in concentration, and moving down a gradient just means that the particle is trying to be evenly distributed.

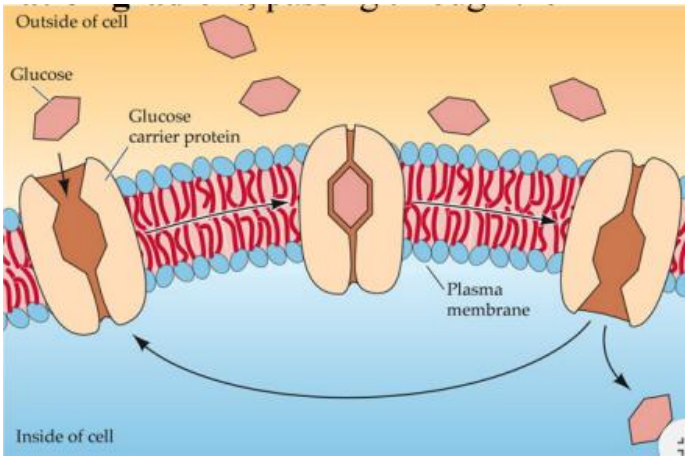
We call this evening-out moving “downhill”, and it doesn’t require energy. The molecule most likely to be involved in simple diffusion is water - it can easily pass through cell membranes.

Some major examples of diffusion in biology:

- Gas exchange at the alveoli — oxygen from air to blood, carbon dioxide from blood to air.
- Gas exchange for photosynthesis — carbon dioxide from air to leaf, oxygen from leaf to air.
- Gas exchange for respiration — oxygen from blood to tissue cells, carbon dioxide in opposite direction.
- Transfer of transmitter substance — acetylcholine from presynaptic to postsynaptic membrane at a synapse.
- Osmosis — diffusion of water through a semi-permeable membrane.

Facilitated Diffusion

This is the movement of specific molecules down a concentration gradient, passing through the membrane via a specific carrier protein. Thus, rather like enzymes, each carrier has its own shape and only allows one molecule (or one group of closely related molecules) to pass through. Selection is by size; shape; charge. Common molecules entering/leaving cells this way include glucose and amino-acids. It is passive and requires no energy from the cell. If the molecule is changed on entering the cell (glucose + ATP → glucose phosphate + ADP), then the concentration gradient of glucose will be kept high, and there will a steady one-way traffic.



When water undergoes simple diffusion, it is known as **osmosis**.

Osmosis is a special example of diffusion. It is the diffusion of water through a partially permeable membrane from a more dilute solution to a more concentrated

solution – down the water potential gradient) Note: diffusion and osmosis are both passive, i.e. energy from ATP is not used. A partially permeable membrane is a barrier that permits the passage of some substances but not others; it allows the passage of the solvent molecules but not some of the larger solute molecules. Cell membranes are described as selectively permeable because not only do they allow the passage of water but also allow the passage of certain solutes.

The presence of particular solutes stimulates the membrane to open specific channels or trigger active transport mechanisms to allow the passage of those chemicals across the membrane.

Some major examples of osmosis

- Absorption of water by plant roots.
- Re-absorption of water by the proximal and distal convoluted tubules of the nephron.
- Re-absorption of tissue fluid into the venule ends of the blood capillaries.
- Absorption of water by the alimentary canal — stomach, small intestine and the colon.

There are 3 types of solutions that involve water and how they affect the cell. They are:

Hypertonic Solution: the solution the cell is placed in has less water than the cell

In a hypertonic solution, there is a higher concentration of water inside the cell than outside the cell. A hypertonic solution has more solute (salt, sugar, etc.) than the cell and this cause there to be less water in the solution. Water flows from an area of high concentration to an area of low and leaves the cell. This loss of water causes the cell to shrivel. In animal cells, the shriveling is called crenating. The red blood cells in the picture to the left have crenated.

In plant cells, plasmolysis occurs and the cell membrane shrinks away from the cell wall. Death will result in both cells.

Hypotonic Solution: the solution the cell is placed in has more water than the cell

In a hypotonic solution, the solution contains a higher percentage of water than the cell. A hypotonic solution has less solute than the cell and this causes the solution to have more water than the cell. When a cell is placed in a hypotonic solution, water flows from

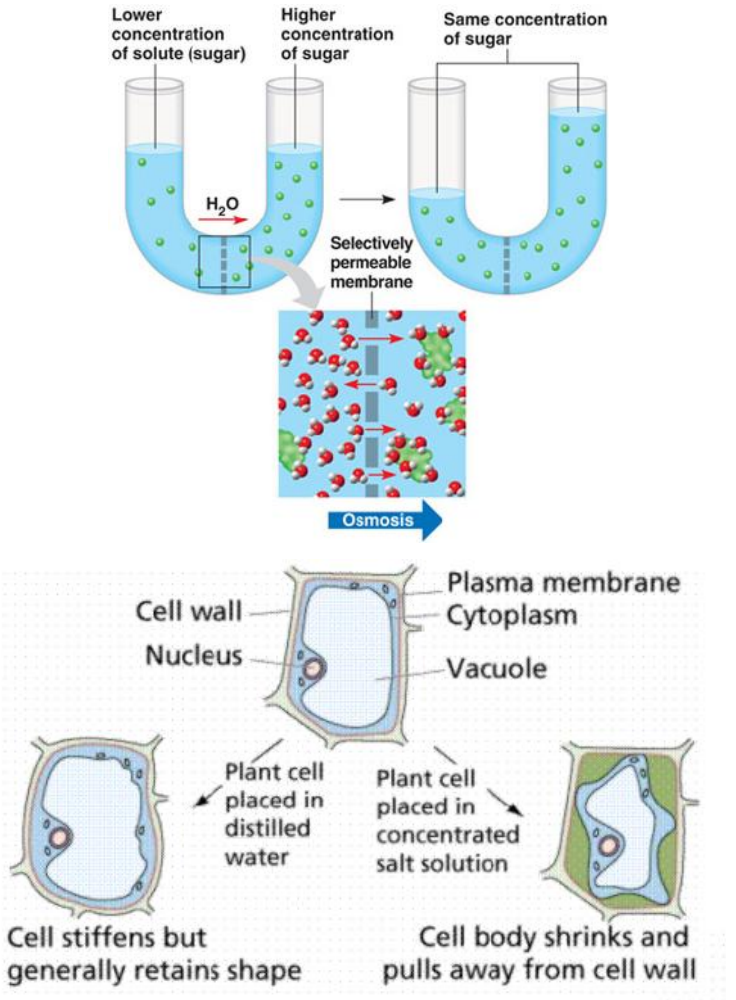
an area of high concentration to an area of low and rushes into the cell. This causes the cell to expand and possibly burst. In animal cells, the cell bursts or will lyse, killing the cell.

In plant cells, the cell membrane is pressed up against the cell wall but the cell wall does not allow the cell to expand anymore and the plant cell does not die.

Isotonic Solution: the solution the cell is placed in has equal amount of water as the cell

In an isotonic solution, there is the same percentage of water on the outside of the cell as the inside of the cell. An isotonic solution has the same amount of solute as the inside of the cell. Water moves at a constant rate in and out of the cell and the cell maintains its original shape.

In animal and plant cells, the cell keeps its shape when in an isotonic solution. Most cells live in an isotonic environment and they are able to maintain their shape and survive.

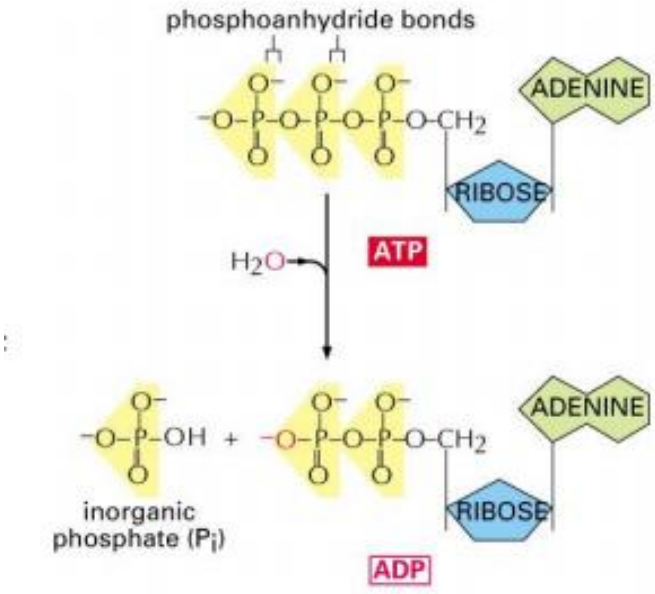


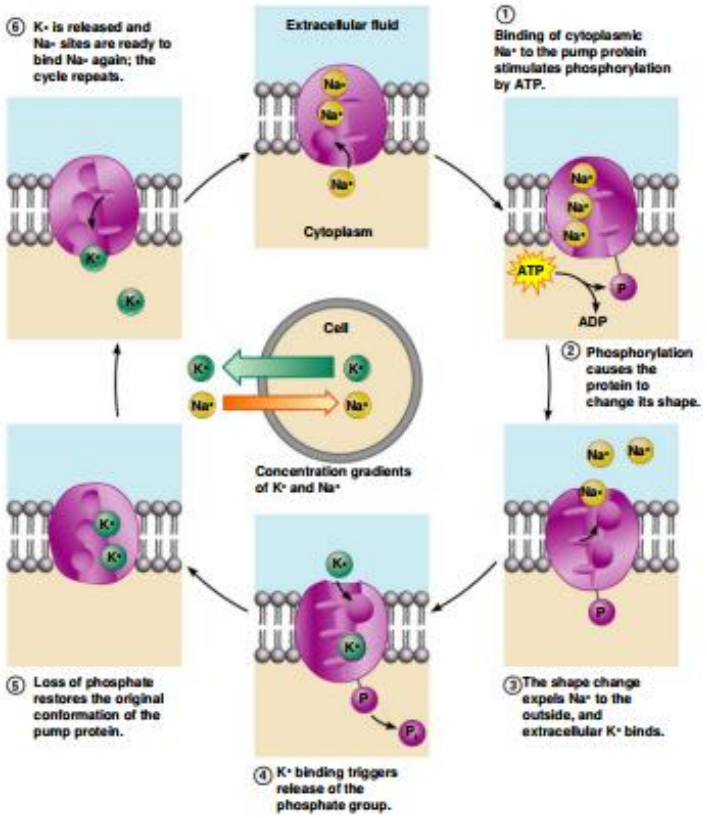
Active Transport

Transport method that moves particles from an area of low concentration to an area of high concentration or against a concentration gradient. Active transport relies on energy from the breakdown of ATP to move substances across the membrane. ATP, or adenosine triphosphate, is the main source of energy in the cell. The hydrolysis of the end phosphate group from an ATP molecule releases energy. The use of energy from ATP in active transport can be direct or indirect. Direct use of ATP is called primary active transport, and indirect use is called secondary active transport.

Primary active transport:

An example of primary active transport is the sodium-potassium pump or sodium-potassium ATPase (Na/K ATPase). The function of these pumps is to move 3 sodium ions from inside the cell to the extracellular fluid outside. It also pumps 2 potassium ions into the cell. So the concentration of K (potassium) is higher on the inside of the cell as it is on the outside and the concentration of Na (Sodium) is higher on the outside of the cell. Many cells use 1/3 to 1/2 of their ATP to run these ion pumps.





Secondary active transport

Secondary active transport does not use ATP. Instead it uses the energy stored from ion gradients. This is where an understanding of the sodium-potassium pump comes in handy. Let me explain. The Na/K ATPase moves Na actively by using ATP. It moves Na across its concentration gradient towards the outside of the cell, and in turn brings in K ions inside the cell.

As an example as to why this is important let's look at sugar, glucose. There is generally more glucose inside of the cell than outside. So, moving glucose across its gradient will generally take a lot of energy but not if we use sodium. Since we actively pump Na outside of the cell, the environment has more sodium than the inside of the cell.

This means that the sodium will want to move from outside the cell to inside. The cell uses this property to move glucose. It will allow sodium to re-enter the cell, but the sodium allows glucose to piggyback a ride into the cell alongside it. This is what is called co-transport and it uses a symporter.

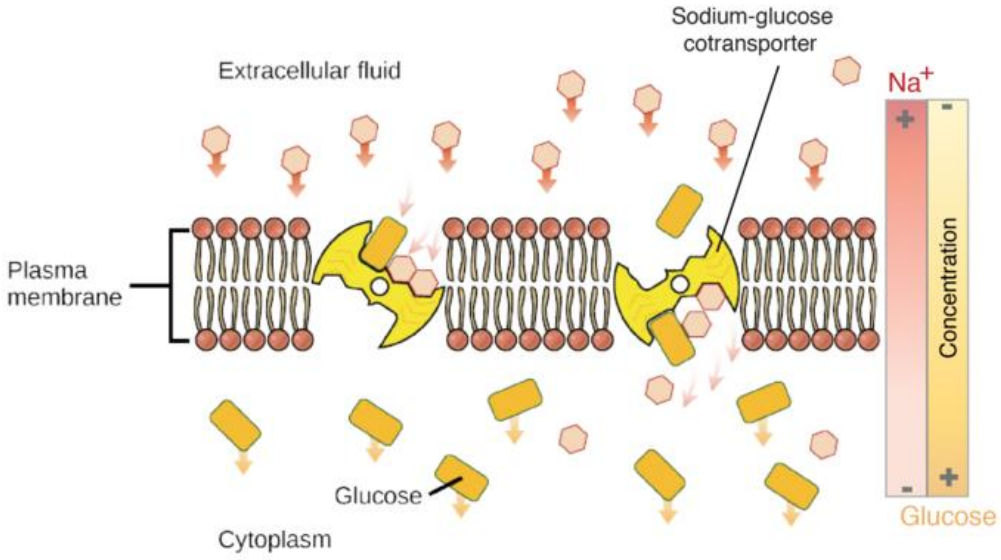
Symporter (Co-transport) is co-transport going in the same direction.

The other type of co-transport is exchange. This uses what is called an antiporter. The cell will move a solute of higher concentration outside the cell to inside the cell (Same example as Na). While the solute moves from higher to lower concentration, it generates energy. This is needed to move a molecule from the cell to outside of the cell, against its concentration gradient.

An example of this is Na/Ca exchanger (Sodium-Calcium). This is an example of an antiporter. It exchanged 3 sodium ions by moving it into the cell. For this exchange it will transport 1 calcium ion to the environment. This is similar to going to a store and exchanging money for items.

Antiporter (Exchange) is co-transport moving in the opposite direction.

The last type of port that uses the ion gradient is a **uniporter**. This is a simple port that moves an ion through a channel across its gradient. This is a type of diffusion and can be considered passive transport.



Differences between active and passive transport

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

	Active Transport	Passive Transport
Importance	In eukaryotic cells, amino acids, sugars and lipids need to enter the cell by protein pumps, which require active transport. These items either cannot diffuse or diffuse too slowly for survival.	It maintains equilibrium in the cell. Wastes (carbon dioxide, water, etc.) diffuse out and are excreted; nutrients and oxygen diffuse in to be used by the cell.

Beer and Lambert's Law

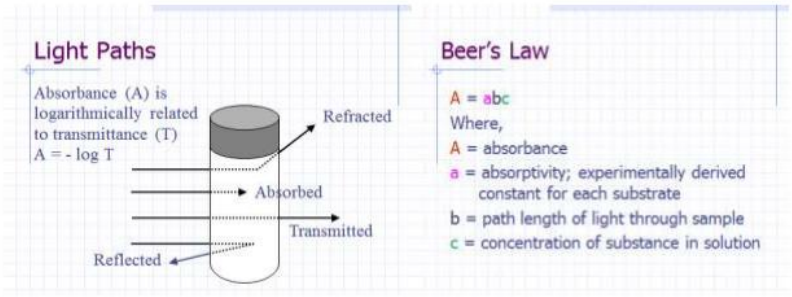
There are following possibilities when light is targeted to an object:

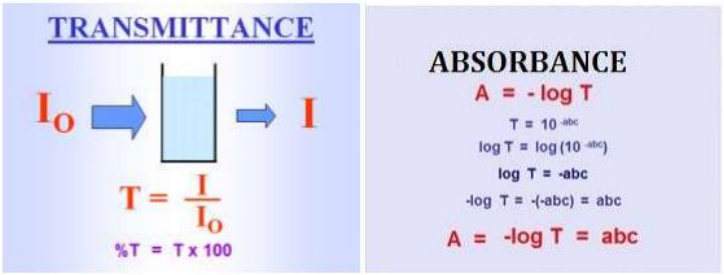
1. Transmission (if the object is transparent)
2. Absorption (if the object is liquid/gas or capable of absorption)
3. Reflection (if an opaque material is placed)
4. Refraction (if medium is changed during the path of light)
5. Scattering (if the surface is rough)

The Beer-Lambert law (also called the Beer-Lambert-Bouguer law or simply Beer's law) is the linear relationship between absorbance and concentration of an absorber of electromagnetic radiation. The general Beer-Lambert law is usually written as:

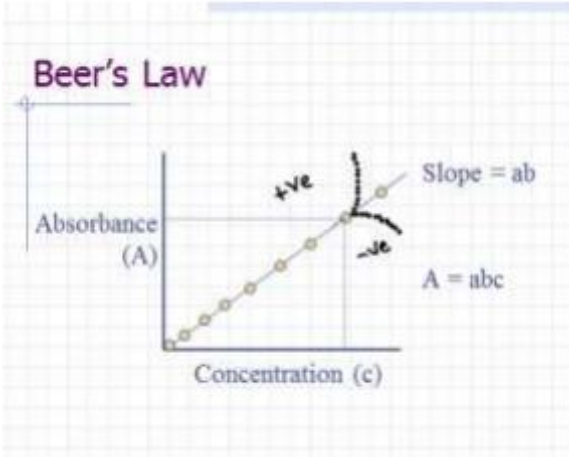
$$\mathbf{A = a_\lambda \cdot b \cdot c}$$

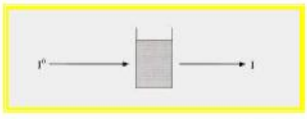
Where A is the measured absorbance, a_λ is a wavelength-dependent absorptivity coefficient, b is the path length, and c is the analyte concentration

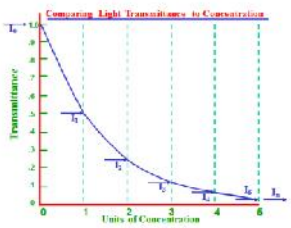




As given in the above figure, as the transmission increases, absorbance decreases exponentially.

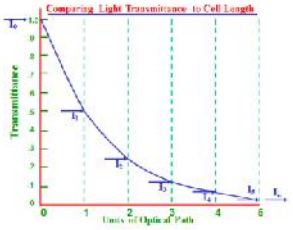






Graph for Beer's Law

$I_T = I_o e^{-kc}$



Graph for Lambert's Law

$I_T = I_o e^{-kT}$

Colorimeter

A colorimeter is a device used to test the concentration of a solution by measuring its absorbance of a specific wavelength of light.

Principle

The colorimeter is based on Beer-Lambert's law, according to which the absorption of light transmitted through the medium is directly proportional to the medium concentration.

Instrumentation

The instrument use for colorimetry is colorimeter. This apparatus will comprise of the following parts:

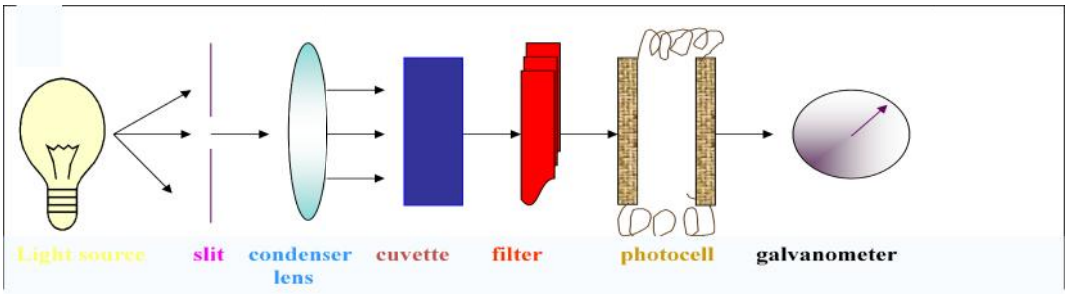
1. Light source
2. Filter (the device that selects the desired wavelength to obtain monochromatic light)
3. Cuvette chamber (the transmitted light passes through compartment wherein the solution containing the colored solution are kept in cuvette, made of glass or disposable plastic)
4. Detector (this is a photosensitive element that converts light into electrical signals)
5. Galvanometer (measures electrical signal quantitatively)

White light from a tungsten lamp passes through a slit, then a condenser lens, to give a parallel beam which falls on the solution under investigation contained in an absorption cell or cuvette. The cell is made of glass with the sides facing the beam cut parallel to each other.

Beyond the absorption cell is the filter, which is selected to allow maximum transmission of the color absorbed. If a blue solution is under examination, then red is absorbed and a red filter is selected.

The light then falls on to a photocell which generates an electrical current in direct proportion to the intensity of light falling on it.

This small electrical signal is increased by the amplifier which passes to a galvanometer of digital readout to give absorbance reading directly.



Anabolism and Catabolism

Metabolism is the set of life-sustaining chemical transformations within the cells of living organisms. These enzyme-catalyzed reactions allow organisms to grow and reproduce, maintain their structures, and respond to their environments. The word metabolism can also refer to all chemical reactions that occur in living organisms, including digestion and the transport of substances into and between different cells, in which case the set of reactions within the cells is called intermediary metabolism or intermediate metabolism.

The term metabolism is derived from the Greek – "Metabolismos" for "change", or "overthrow". The history of the scientific study of metabolism spans several centuries and has moved from examining whole animals in early studies, to examining individual metabolic reactions in modern biochemistry. The first controlled experiments in human metabolism were published by Santorio Santorio in 1614 in his book Ars de statica medicina. He described how he weighed himself before and after eating, sleep, working, sex, fasting, drinking, and excreting. He found that most of the food he took in was lost through what he called "insensible perspiration".

Catabolism is the metabolic process by which molecules are broken down and energy is produced. The body then receives the energy it needs to perform the most basic functions. Catabolism breaks down carbohydrates, amino acids, and lipids in the following stages.

- Step 1: Digestion

  First, large molecules are digested into smaller counterparts. This occurs for all molecules too large to be directly absorbed. ATP then pumps everything back into cells.
- Step 2: Energy Release Begins

  Acetyl coenzyme A further breaks down molecules, beginning the release of energy.

- Step 3: Energy Produced

  The molecules are oxidized, releasing all stored energy.

  Carbohydrates are broken down into simple sugars, protein into amino acids, amino acids into keto acids, and fat lipids into free fatty acids and glycerol.

  Anabolism is the process by which the body utilizes the energy released by catabolism to synthesize complex molecules.

  These complex molecules form cellular structures and act as the building blocks of the body. Though working in an opposite fashion to catabolism, anabolism similarly has 3 steps.

- Step 1: Precursor Production

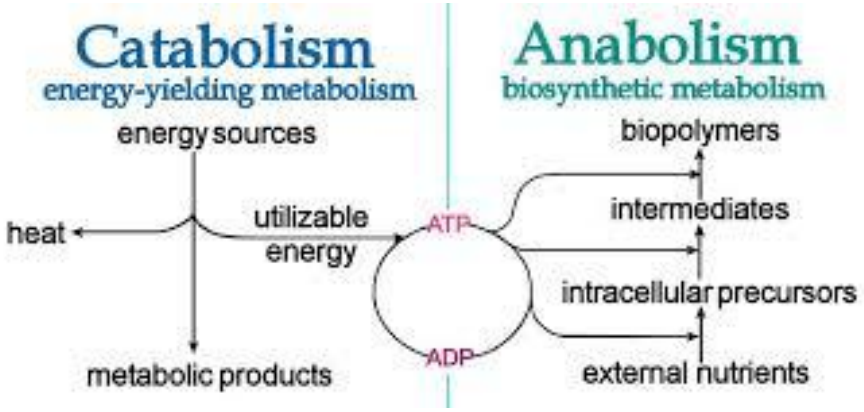
  Precursors of complex molecules are produced, such as amino acids and monosaccharide's.

- Step 2: Activation

  These precursors are activated into reactive forms by ATP.

- Step 3: Assembly

  Proteins, polysaccharides, lipids, and nucleic acids are assembled and ready for use.



Standard for energy change

The free energy change of a chemical process under standard state conditions, ΔG^o, can be determined four different ways:

- From Free Energies of Formation
- From Enthalpy Changes and Entropy Changes
- From Equilibrium Constants

- From Cell Potentials

Using Free Energies of Formation to Determine Standard State Free Energy Changes

If we know the standard free energy changes of formation, ΔG^o_f, of each species in a change we can determine the standard state free energy change, ΔG^o, for the change using the following equation:

$$\Delta G^o = \Sigma\ \Delta G^o_{f(products)} - \Sigma\ \Delta G^o_{f(reactants)}$$

Using Enthalpy Changes and Entropy Changes to Determine Standard State Free Energy Changes

If we know the enthalpy change, ΔH^o, and the entropy change, ΔS^o, for a chemical process, we can determine the standard state free energy change, ΔG^o, for the process using the following equation:

$$\Delta G^o = \Delta H^o - T\Delta S^o$$

In this equation T is the temperature on the Kelvin scale. In introductory courses we make the assumption that ΔH^o and ΔS^o, do not change as the temperature changes.

Using Equilibrium Constants to Determine Standard State Free Energy Changes

If we know the equilibrium constant, K_{eq}, for a chemical change (or if we can determine the equilibrium constant), we can calculate the standard state free energy change, ΔG^o, for the reaction using the equation:

$$\Delta G^o = - RT \ln K_{eq}$$

In this equation

- R = 8.314 J mol^{-1} K^{-1} or 0.008314 kJ mol^{-1} K^{-1}.
- T is the temperature on the Kelvin scale.
- K_{eq} is the equilibrium constant at the temperature T.

Using Cell Potentials to Determine Standard State Free Energy Changes

If we know the standard state cell potential, E^o, for an electrochemical cell (or if we can determine the standard state cell potential), we can calculate the standard state free energy change, ΔG^o, for the cell reaction using the equation:

$$\Delta G^o = -nFE^o$$

In this equation

- n is the number of moles of electrons exchanged in the cell reaction.
- F is 96.485 kJ $volt^{-1}$ $mole^{-1}$ (the "Faraday").

E^o is the cell potential under standard state conditions.

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MULTIPLE CHOICE QUES
Unit 1

Question	Opt A	Opt B
The most predominant chemical constituent of life	Water	protein
The cellular organelles regarded as the digestive tra	Nucleus	Golgi apparatus
Gases such as oxygen and carbon dioxide cross the p	secondary active transpo	passive diffusion throug
Which of the following is an example of primary acti	Cl--HCO_3- exchange	Na^+ - H^+ exchange
The sodium pump	Exchanges extracellular N	Is important for maintai
A substance can only be accumulated against its ele	Facilitated diffusion	Passage through ion cha
The movement of molecules from an area of high cc	Osmosis	Diffusion
What is the collective term for all of the chemical pr	Anabolism	catabolism
Change in color of particular reactant can be detecte	Spectrometer	calorimeter
According to the Beer-Lambert Law, on which of the	Distance that the light ha	Colour of the solution
What is the name of an instrument used to measure	Coulometer	Colourmeter
The wavelength of an absorption is 495 nm. In what	Radiowave	Infrared
Aqueous KMnO4 solutions are purple. A plot of absc	linear with a positive grac	non-linear
Ribosomes help in	Protein synthesis	Photosynthesis
Food is converted to energy in	Nucleus	Nucleolus
Extra cellular DNA is found in	Chloroplast	Endoplasmic reticulum
Fluid mosaic model was given by	Robertson	Schwann
The cellular organelles called “suicide bags” are	Lysosomes	Ribosomes
The power house of the cell is	Nucleus	Cell membrane
The Golgi complex	Synthesizes proteins	Produces ATP
Plasma membrane is made up of	Protein, lipid, carbohydra	Lipid, carbohydrate
Plant cell is mainly composed of	Cellulose	Starch
Anabolism and catabolism are types of	chemical reaction	chain reactions
Overall chemical reaction that takes place with in a	metabolism	anabolism
Study of chemical components as well as chemical p	Microbiology	Biochemistry
Which of the following is a chemical link between c	AMP	ADP
Tunnels which allow specific ions to pass through th	selectively permeable tur	permeable tunnels
Type of transport which always involves a protein is	passive transport	active transport
Mitochondrial DNA is	Circular double stranded	Circular single stranded
Which of these is part of the cell membrane?	triglycerides	phospholipids
How do fat-soluble molecules normally get into a ce	they dissolve in the fat la	they pass through prote
The phospholipids are unusual molecules because:	they have hydrophilic reg	they have hydrophobic
Which of the following statements best describes th	two layers of protein with	two layers of lipid with
The movement of chloride ions from an area where	diffusion	active transport
If a cell has a solute concentration of 0.07% which o	0.01% solute	0.1% solute

Which of the following is necessary in order for osm	a permeable membrane	a semi-permeable mem
Which of these are passive transport mechanisms?	osmosis	diffusion
In an isotonic solution there would be:	no net movement of wat	net movement of water
The sodium-potassium pump (which carries sodium	active transport	endocytosis
The process of a cell engulfing a solid object is:	phagocytosis	exocytosis
What is likely to happen to a plant cell that is placed	it becomes turgid	it becomes flaccid
When a cell bursts due to osmosis, it is in a solution	hypertonic	isotonic
Why do plant cells behave differently to animal cells	Plant cells are permeable	Plant cells do not carry
Which of these equations is correct?	ATP + inorganic phosphat	ADP + inorganic phosph
Atoms which have same number of protons but diff	isotopes	isomers
To determine mass of other compound by comparin	relative molecular mass	relative atomic mass
Sum of protons (p+) and neutrons (n0) in an atom is	atomic number	nucleon number
Chloride ion has number of protons of	17	18
Smaller particles in atom are called	atomic particles	sub-atomic particles
Number of protons and electrons in n atom is	different	same
Electrons orbit around nucleus and bears	positive charge	negative charge
Positively charged particle of atom is called	protons	neutrons
Neutrons carry	positive charge	negative charge
Total number of protons in an atom of each elemen	atomic number	atomic mass
Nucleus in an atom consists of	protons	neutrons
Molecules which contains fixed number of same typ	elements	compounds
If an atoms loses electron ion obtained is charged	positively	negatively
If 3 Na+ ions pumped out of cell and 2 K+ pumped ir	1	2
The filter color used to measure optical density of a	Blue	Yellow
The cuvette used for analysis of sample at UV range	Glass cuvette	Quartz

R EDUCATION
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STIONS

Opt C	Opt D	Answer
lipid	carbohydrate	Water
mitochondria	endoplasmic reticul	endoplasmic reticulum
specific gas transpor	primary active trans	passive diffusion through the lipid bilayer
Na^{+}-Ca^{2+} exchange	The Na^{+}, K^{+} ATPase	The Na^{+}, K^{+} ATPase
Can only be inhibite	Is an ion channel	Is important for maintaining a constant cell volume
Diffusion through a	Active transport	Active transport
Active Transport	Phagocytosis	Diffusion
metabolism	synthesis	metabolism
colorimeter	all of them	colorimeter
Solution concentrati	Extinction coefficien	Colour of the solution
Colorimeter	Calorimeter	Colorimeter
Ultraviolet-visible	Microwave	Ultraviolet-visible
an exponential curve	linear with a negativ	an exponential curve
Lipid synthesis	Respiration	Protein synthesis
Chloroplast	Mitochondria	Mitochondria
Ribosomes	Nucleus	Chloroplast
Dave Donson	Singer and Nicolson	Singer and Nicolson
Nucleolus	Golgi's bodies	Lysosomes
Mitochondria	Lysosomes	Mitochondria
Provides a pathway f	Forms glycoproteins	Forms glycoproteins
Protein, lipid	Protein	Protein, lipid, carbohydrate
Protein	Lipid	Cellulose
metabolism	complex reactions	metabolism
complex reaction	catabolism	metabolism
Fresh water biology	Chemical biology	Biochemistry
ATP	All of these	ATP
both A and B	channel proteins	channel proteins
lateral diffusion	flip flop	active transport
Linear double helix	None of these	Circular double stranded
ATP	more than one of th	phospholipids
they are absorbed b	they never get in	they dissolve in the fat layers of the membrane and enter the cell
they are triglyceride	both A and B	they have hydrophobic regions
a double layer of lipi	A single layer of pro	a double layer of lipid molecules with protein molecules suspende
osmosis	exocytosis	diffusion
1% solute	10% solute	0.01% solute

an isotonic solution	ATP	a permeable membrane
phagocytosis	both A and B	both A and B
net movement of wa	bursting of the cell	no net movement of water
exocytosis	passive transport	active transport
pinocytosis	diffusion	phagocytosis
it undergoes plasmo	it bursts	it becomes turgid
hypotonic	either A or C	hypotonic
Plant cells contain a	Plant cells have a ce	Plant cells have a cell wall
ATP + ADP --> inorga	ATP + ADP --> organ	ADP + inorganic phosphate --> ATP
spectators	allotropes	isotopes
relative molecular ra	relative atomic radiu	relative atomic mass
Avogadro's number	protonic identity	nucleon number
24	34	17
smaller particles	neutral particles	sub-atomic particles
average	constant	same
no charge	neutral charge	negative charge
electrons	charges	protons
no charge	neutral charge	no charge
molecular mass	atomic scale	atomic number
electrons	a and b	a and b
mixtures	all of them	elements
neutral	smaller	positively
3	4	1
Red	Green	Blue
Silica	All	Quartz

by diffusion

d in the layer

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DEPARTMENT OF MICROBIOLOGY
(For the candidates admitted from 2015 onwards)

Subject	**:**	**Biochemistry**	**Semester**	**:**	**I**
Subject code	**:**	**17MBU103**	**Class**	**:**	**I B.Sc Microbiology**

UNIT-II: COURSE MATERIAL

Unit-II

Monosaccharides-families, stereo isomerism, epimers, mutarotation and anomers. Forms of glucose and fructose, Haworth projection. Sugar derivatives. Disaccharides-occurrence, concept of reducing and non-reducing sugars and Haworth projections. Polysaccharides-storage and structural polysaccharides.

Suggest Readings

1. Nelson, D.L and Cox, M.M. (2008). Lehninger Principles of Biochemistry, 5th edition. W.H. Freeman and Company.

Carbohydrates

A carbohydrate is a biological molecule consisting of carbon (C), hydrogen (H) and oxygen (O) atoms, usually with a hydrogen–oxygen atom ratio of 2:1 (as in water); in other words, with the empirical formula $C_m(H_2O)_n$ (where *m* could be different from *n*). Carbohydrates are hydrates of carbon; technically they are polyhydroxy aldehydes and ketones. Carbohydrates are also known as saccharides, the word saccharide comes from Greek word sakkron which means sugar.

Functions of Carbohydrate

All animals derive the major portion of their food calories from the different types of Carbohydrates in their diets. Most of the energy for the metabolic activities of the cell in all organisms is derived from the oxidation of Carbohydrate. Important functions of Carbohydrate are that of storing food, acting as a framework in body, performs are listed below.

Carbohydrate functions as Bio Fuel

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

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

Carbohydrate functions as Primary Source of Energy

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

Carbohydrate functions as storage food

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

- Polysaccharide starch acts as storage food for plants.
- Glycogen stored in liver and muscles acts as storage food for animals.
- Insulin acts as storage food of dahlias, onion and garlic.

Thus carbohydrate performs the function of storing food.

Carbohydrate functions as framework in body

Different Carbohydrates especially Polysaccharides act as framework in living organism.

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

Thus carbohydrates function as contributing material to the cellular structure.

Carbohydrate functions as Anticoagulant

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

Carbohydrate functions as Antigen

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

Carbohydrate functions as Hormone

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

Carbohydrates provide raw material for industry

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

Other Functions

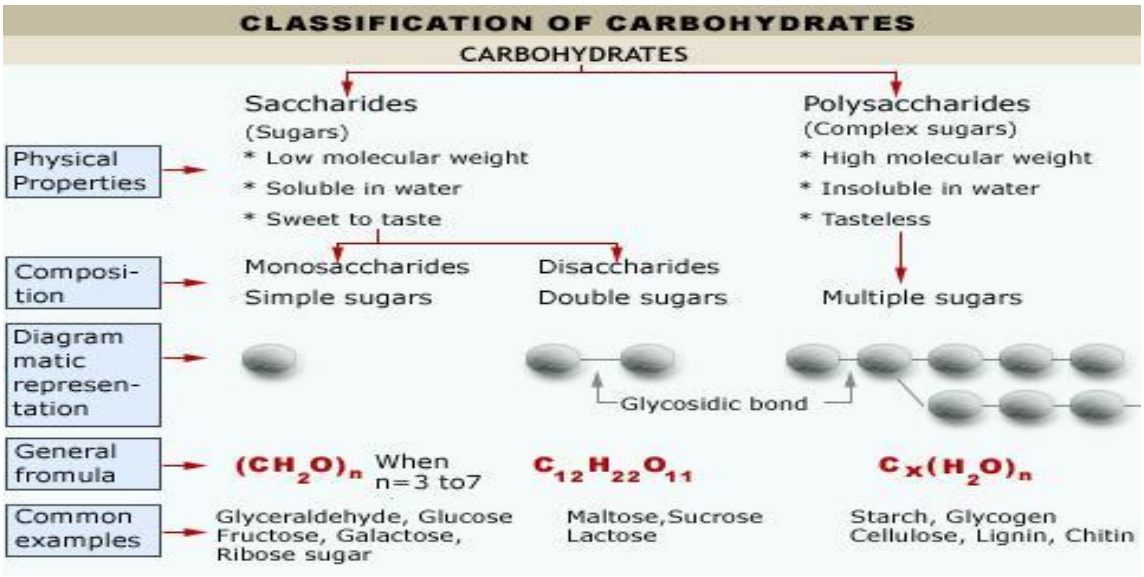
Agar is polysaccharide used in culture media, laxative and food.

Cellulose acts as roughage of food. It stimulates peristalsis movement and secretion of digestive enzymes.

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

Classes of carbohydrates

Carbohydrates are classified into three groups



Monosaccharides (From Greek, *mono*=one; *sakchron*=sugar)

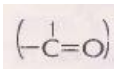
The following table shows the classification of monosaccharides based on the number of their carbon atoms, their general structure, and examples for each.

- They have the general formula $Cn(H_2O)_n$, and they cannot be further hydrolyzed.
- The monosaccharides are divided into different categories, based on the functional group and the number of carbon atoms.

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

Classification of monosaccharide with selected examples

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

Ketoses: When the functional group is a keto  group, they are referred to as ketoses

e.g. dihydroxyacetone, fructose.

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

Monosaccharides	*Occurrence*	*Biochemical importance*
Trioses		
Glyceraldehyde	Found in cells as phosphate	Glyceraldehyde 3-phosphate is an intermediate in glycolysis
Dihydroxyacetone	Found in cells as phosphate	Its 1-phosphate is an intermediate in glycolysis
Tetroses		
D-Erythrose	Widespread	Its 4-phosphate is an intermediate in carbohydrate metabolism
Pentoses		
D-Ribose	Widespread as a constituent of RNA and nucleotides	For the structure of RNA and nucleotide coenzymes (ATP, NAD^+, $NADP^+$)
D-Deoxyribose	As a constituent of DNA	For the structure of DNA
D-Ribulose	Produced during metabolism	It is an important metabolite in hexose monophosphate shunt
D-Xylose	As a constituent of glycoproteins and gums	Involved in the function of glycoproteins
L-Xylulose	As an intermediate in uronic acid pathway	Excreted in urine in essential pentosuria
D-Lyxose	Heart muscle	As a constituent of lyxoflavin of heart muscle
Hexoses		
D-Glucose	As a constituent of polysaccharides (starch, glycogen, cellulose) and disaccharides (maltose, lactose, sucrose). Also found in fruits	The 'sugar fuel' of life; excreted in urine in diabetes. Structural unit of cellulose in plants
D-Galactose	As a constituent of lactose (milk sugar)	Converted to glucose, failure leads to galactosemia
D-Mannose	Found in plant polysaccharides and animal glycoproteins	For the structure of polysaccharides
D-Fructose	Fruits and honey, as a constituent of sucrose and inulin	Its phosphates are intermediates of glycolysis
Heptoses		
D-Sedoheptulose	Found in plants	Its 7-phosphate is an intermediate in hexose monophosphate shunt, and in photosynthesis
Disaccharides	*Occurrence*	*Biochemical importance*
Sucrose	As a constituent of cane sugar and beet sugar, pineapple	Most commonly used table sugar supplying calories
Lactose	Milk sugar	Exclusive carbohydrate source to breast fed infants. Lactase deficiency (lactose intolerance) leads to diarrhea and flatulence
Maltose	Product of starch hydrolysis, occurs in germinating seeds	An important intermediate in the digestion of starch

Stereoisomerism of monosaccharides:

All the monosaccharides except dihydroxyacetone contain one or more asymmetric (chiral) carbon atoms and thus occur in optically active isomeric forms. The simplest aldose, glyceraldehyde, contains one chiral center (the middle carbon atom) and therefore has two different optical isomers, or **enantiomers**

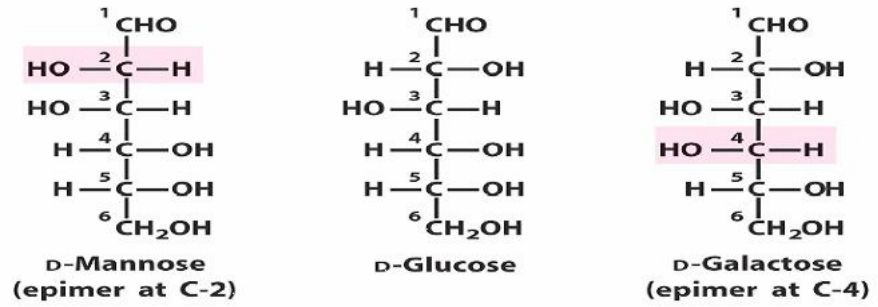
- Carbon 2 of glyceradehyde is a chiral center.
- There are thus 3 steroisomers of glyceraldehydes: D-glyceraldehyde and L-glyceraldehyde.

- By convention, sugars are written with the most oxidized carbon (i.e. aldehyde or ketone) at the top.
- The chiral center farthest from the most oxidized carbon determines if it is D or L.
- If the hydroxyl points to the left, then it is the L configuration if to the right then it is D.
- In general, only the D isomers are used biologically, but there are many exceptions to this generalization.
- Sugars can be conveniently written as Fischer projections to indicate stereochemistry.
- The most oxidized carbon is placed at the top and each carbon between it and the last carbon is a cross from which are appended the hydrogen and hydroxyl group.
- It makes a difference if the hydroxyl group is written to the or lift.
- It is important to recognize that a Fischer projection indicates the stereochemistry of each chiral center.
- One must imagine that the groups to the left and right (-H and –OH) are coming out the plane towards the viewer, while the substituent's above and below are out of the plane directed away from the viewer.
- In general, a molecule with *n* chiral centers can have 2^n stereoisomers. Glyceraldehyde has $2^1 = 2$; the aldohexoses, with four chiral centers, have $2^4 = 16$ stereoisomers.

Epimers

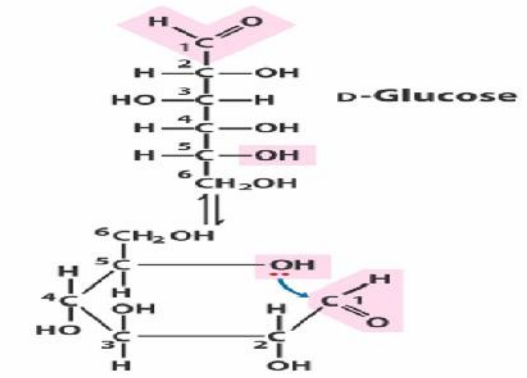
Carbohydrates that differ only in their stereochemistry at one position are called Epimers.

- Eg. Glucose and mannose (C-2)
- Glucose and galactose (C-4)



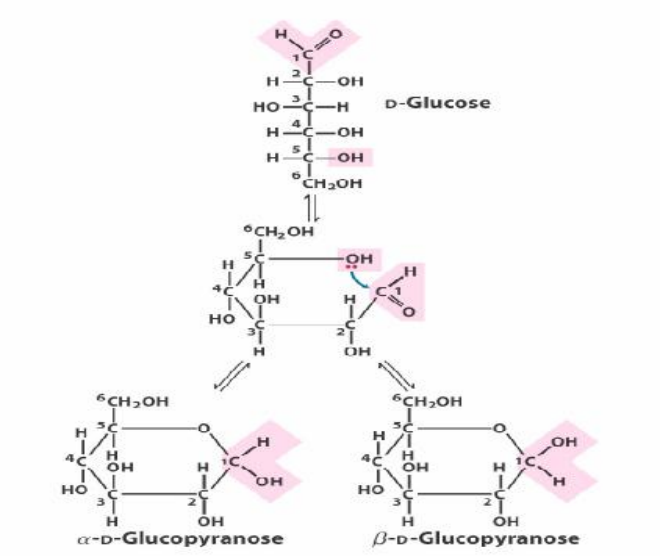
Mutarotation and anomers:

- In aqueous solution, D-glucose exists in one of 2 forms: α-D-glucose and ß-D-glucose.
- This is because Aldehydes can react with alcohols to form a hemiacetal.
- In this case, the hydroxyl oxygen attacking the molecule it is an intermolecular reaction, which results in formation of a ring.
- Rings with 6 members are the most stable, but 5-membered rings are possible.
- The oxygen that attacked the carbonyl carbon will be a member of the ring.
- The carbonyl oxygen is converted to a hydroxyl group in the process.
- The stereochemistry of this hydroxyl group is determined by the position of the carbonyl during the attack; it can be one of 2 possible configurations: α or ß.
- Six-member rings resemble pyran and are referred to as pyranosides.
- Five – member rings resemble furan and are referred to as furanosides.



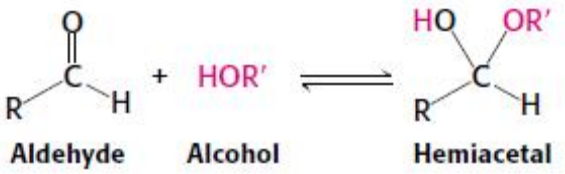
Anomers

- Isomeric forms of monosaccharides that differ only in their configuration about the hemiacetal or hemiketal carbon atom are called **anomers**. The hemiacetal (or carbonyl) carbon atom is called the **anomeric carbon**. The _ and _ anomers of D-glucose interconvert in aqueous solution by a process called **mutarotation**
- The aldehyde or ketone carbon is referred to as the anomeric carbon, as this is the chiral center that differs between 2 Anomers.
- For D-sugars the anomer has the hydroxyl group down in the Haworth projection and on the same side as the ring oxygen in the Fisher projection.

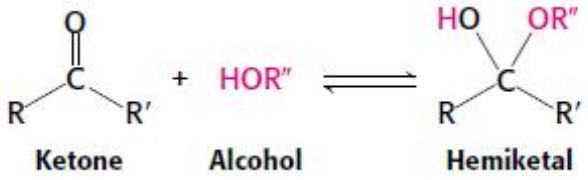


Haworth Projections

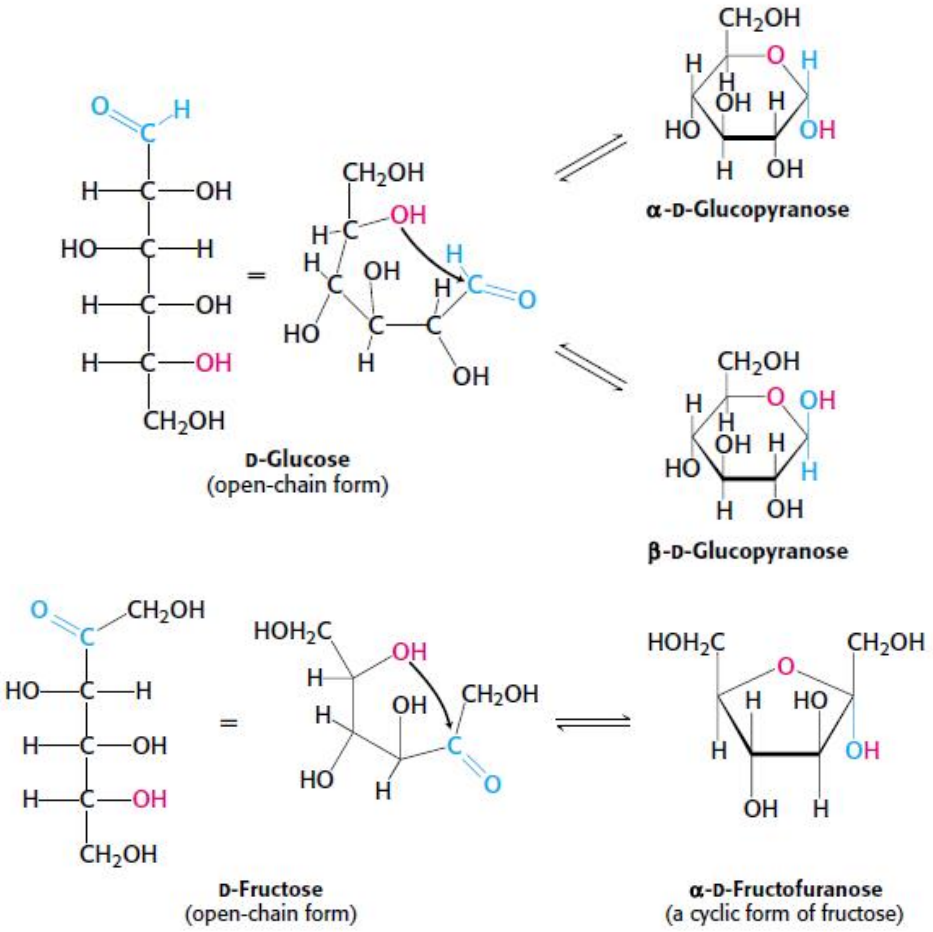
The predominant forms of ribose, glucose, fructose, and many other sugars in solution are not open chains. Rather, the open-chain forms of these sugars cyclize into rings. In general, an aldehyde can react with an alcohol to form a hemiacetal.



For an aldohexose such as glucose, the C-1 aldehyde in the open-chain form of glucose reacts with the C-5 hydroxyl group to form an intramolecular hemiacetal. The resulting cyclic hemiacetal, a six-membered ring, is called pyranose because of its similarity to pyran. Similarly, a ketone can react with an alcohol to form a hemiketal.



The C-2 keto group in the open-chain form of a ketohexose, such as fructose, can form an intramolecular hemiketal by reacting with either the C-6 hydroxyl group to form a six-membered cyclic hemiketal or the C-5 hydroxyl group to form a five-membered cyclic hemiketal. The five-membered ring is called a furanose because of its similarity to furan.



The depictions of glucopyranose and fructofuranose shown below are Haworth projections. In such projections, the carbon atoms in the ring are not explicitly shown. The approximate plane of the ring is perpendicular to the plane of the paper, with the heavy line on the ring projecting toward the reader. Like Fischer projections, Haworth projections allow easy depiction of the stereochemistry of sugars.

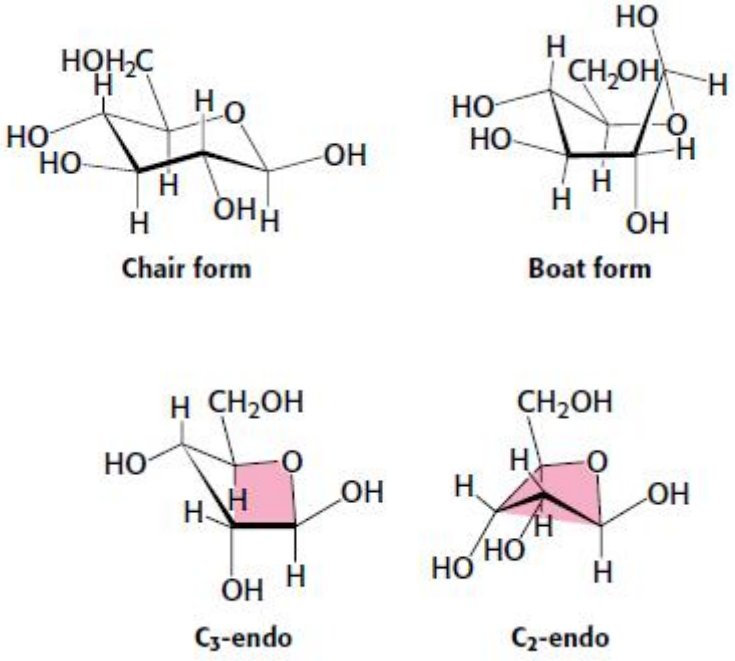
An additional asymmetric center is created when a cyclic hemiacetal is formed. In glucose, C-1, the carbonyl carbon atom in the open-chain form, becomes an asymmetric center. Thus, two ring structures can be formed: α-D-glucopyranose and β-D-glucopyranose. For D sugars drawn as Haworth projections, the designation α means that the hydroxyl group attached to C-1 is below the plane of the ring; β means that it is above the plane of the ring. The C-1 carbon atom is called the anomeric carbon atom, and α and β forms are called anomers. An equilibrium mixture of glucose contains approximately one-third α anomer, two-thirds β anomer, and <1% of the open-chain form.

The same nomenclature applies to the furanose ring form of fructose, except that α and β refer to the hydroxyl groups attached to C-2, the anomeric carbon atom. Fructose forms both pyranose and furanose rings. The pyranose form predominates in fructose free in solution, and the furanose form predominates in many fructose derivatives. Pentoses such as D-ribose and 2-deoxy-D-ribose form furanose rings, as we have seen in the structure of these units in RNA and DNA.

Chair and boat forms of glucose

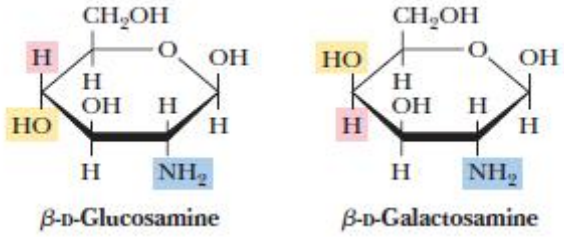
The six-membered pyranose ring is not planar, because of the tetrahedral geometry of its saturated carbon atoms. Instead, pyranose rings adopt two classes of conformations, termed chair and boat because of the resemblance to these objects. In the chair form, the substituents on the ring carbon atoms have two orientations: axial and equatorial. Axial bonds are nearly perpendicular to the average plane of the ring, whereas equatorial bonds are nearly parallel to this plane. Axial substituents sterically hinder each other if they emerge on the same side of the ring (e.g., 1,3-diaxial groups). In contrast, equatorial substituents are less crowded. The chair form of β-D-glucopyranose predominates because all axial positions are occupied by hydrogen atoms. The bulkier –OH and –CH2OH groups emerge at the less-hindered periphery. The boat form of glucose is disfavored because it is quite sterically hindered.

Furanose rings, like pyranose rings, are not planar. They can be puckered so that four atoms are nearly coplanar and the fifth is about 0.5 Å away from this plane. This conformation is called an envelope form because the structure resembles an opened envelope with the back flap raised. In the ribose moiety of most biomolecules, either C-2 or C-3 is out of the plane on the same side as C-5. These conformations are called C2- endo and C3-endo, respectively.



Sugar Derivatives

Amino sugars, including D-glucosamine and D-galactosamine, contain an amino group (instead of a hydroxyl group) at the C-2 position. They are found in many oligosaccharides and polysaccharides, including chitin, a polysaccharide in the exoskeletons of crustaceans and insects.



Glucosamine

Glucosamine ($C_6H_{13}NO_5$) is an amino sugar and a prominent precursor in the biochemical synthesis of glycosylated proteins and lipids. Glucosamine is part of the structure of the polysaccharides chitosan and chitin, which compose the exoskeletons of crustaceans and other arthropods, as well as the cell walls of fungi and many higher organisms. Glucosamine is one of the most abundant monosaccharides. It is produced commercially by the hydrolysis of crustacean exoskeletons or, less commonly, by fermentation of a grain such as corn or wheat.

Glucosamine is naturally present in the shells of shellfish, animal bones, bone marrow, and fungi. D-Glucosamine is made naturally in the form of glucosamine-6-phosphate, and is the biochemical precursor of all nitrogen-containing sugars.[30]Specifically in humans, glucosamine-6-phosphate is synthesized from fructose 6-phosphate and glutamine by glutamine fructose-6-phosphate transaminase as the first step of the hexosamine biosynthesis pathway. The end-product of this pathway is uridine diphosphate N-acetylglucosamine (UDP-GlcNAc), which is then used for making glycosaminoglycans,proteoglycans, and glycolipids.

Galactosamine

Galactosamine is a hexosamine derived from galactose with the molecular formula $C_6H_{13}NO_5$. This amino sugar is a constituent of some glycoprotein hormones such as follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Other sugar constituents of FSH and LH include glucosamine, galactose and glucose. Galactosamine is a hepatotoxic, or liver-damaging, agent that is sometimes used in animal models of liver failure.

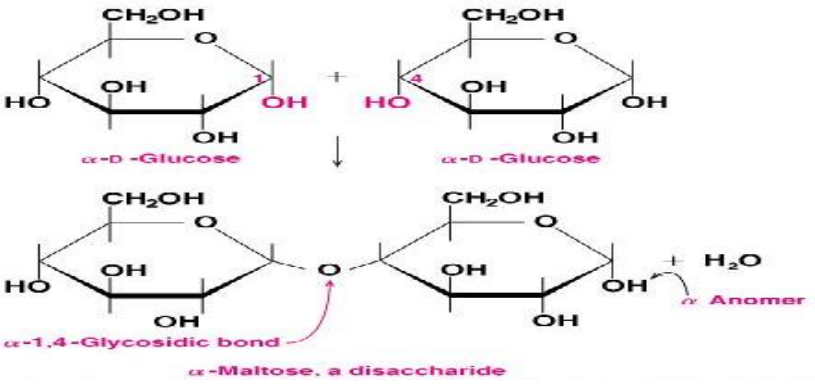
Disaccharides

- A disaccharide is formed when a hydroxyl group on one monosaccharide reacts with the anomeric carbon of another monosaccharide to form a glycosidic bond.
- Each disaccharide has a specific glycosidic linkage (depending on which hydroxyl reacts with which anomer).
- The three most common disaccharides are **maltose, lactose and sucrose.**
- When hydrolyzed using acid or an enzyme, the following monosaccharide are produced.
- The disaccharides are of two types
  1. Reducing disaccharides with free aldehyde or keto group e.g. maltose, lactose.
  2. Non-reducing disaccharides with no free aldehyde or keto group e.g. sucrose,

Maltose

Occurrence: Not occur in our body, but present in germinating cereals and malt; It is the breakdown product of starch

Structure: Maltose (malt sugar or corn sugar) is composed of two glucose molecules are joined through α-1,4 glycosidic linkage



Properties

- Because one of the glucose molecules is a hemiacetal (having a free aldehyde group) it can undergo mutarotation (Gradual change in specific rotation; Glucose if freshly prepared have sp rotation of +112°, but on standing gives a rotation of + 52.).
- It exist in α and β forms
- Since it is having a free aldehyde group, it reduce compounds and and so maltose is a reducing sugar.
- Reduce Fehling and Benedicts solution but not Barfoeds solution
- Forms osazone with phenyl hydrazine
- Maltose can be fermented by yeast to produce ethanol.
- Maltose is also used in cereals, candies and malted milk.

  Hydrolysis: Hydrolyzed by maltase present in alimentary canal; two glucose molecules are released upon hydrolysis

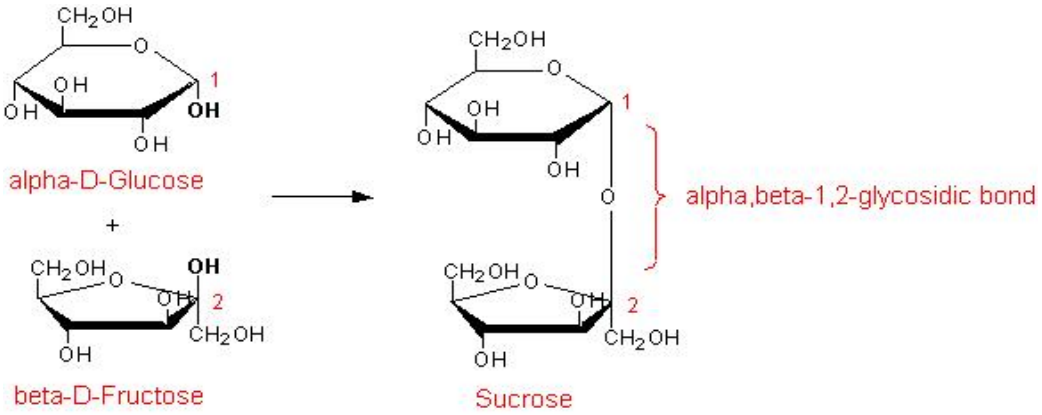
Sucrose

Occurrence

It is the sweetest of all the sugars; does not exist in our body, occur in cane sugar, pineapple, carrot root, sweet potato and honey. Sucrose is the most abundant disaccharide and is commercially produced from sugar cane and sugar beets.

Structure

Sucrose (table sugar) consists of one glucose molecule and one fructose molecule linked by an α,β-1,2-glycosidic bond.



- It is not having a free aldehyde or ketone group, so don't have mutarotation; does not exist in α and β forms. **Because the glycosidic bond in sucrose involves both** anomeric carbons, neither monosaccharide can undergo mutorotation, and so sucrose is not a reducing sugar.

Properties

- White crystalline solid powder; sparingly soluble in water
- The specific rotation of fructose is 66.5, but upon hydrolysis it is changed to -19.5. This because the hydrolyzed product, fructose, which is having more levorotary than the glucose. This reaction is called inversion and the sugar is called invert sugar.
- it does not reduce Fehling , Benedicts s and Barfoeds solution
- it cannot from crystals with phenyl hydrazine
- **Hydrolysis:**
- Hydrolyzed by sucrose present in alimentary canal; one glucose and one fructose molecules are released upon hydrolysis

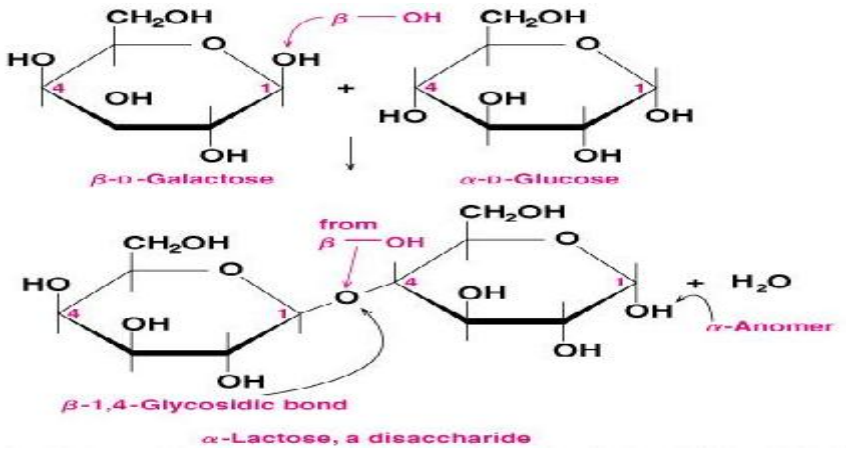
Lactose

Occurrence

- Present in human milk (9.8%) produced by mammary gland of human beings; It comes from milk products (about 4-5% of cow's milk).; also occur in urine during pregnancy.

Structure

- **Lactose** (milk sugar) consists of one glucose molecule and one galactose molecule linked by a β-1,4 glycosidic bond.



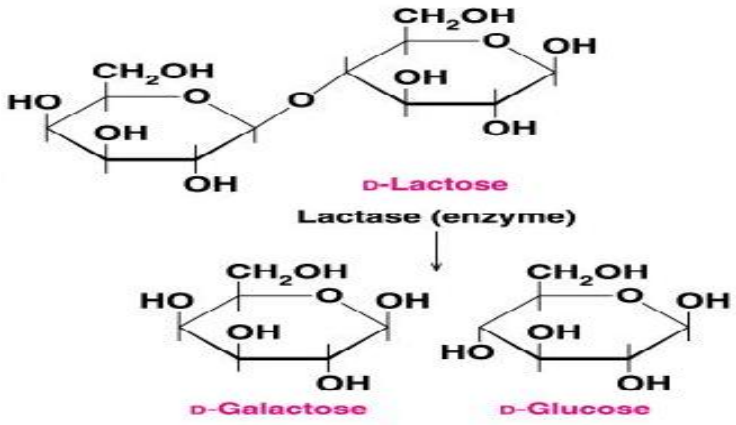
- Because the glucose is a hemiacetal, it can undergo mutorotation, and it is having a free aldehyde group, which reduce compounds and so lactose is a reducing sugar.

Properties

- White crystalline solid powder; sparingly soluble in water
- The specific rotation is + 55.2·
- Exist in α and β forms
- Reduce Fehling and Benedicts solution but not Barfoeds solution
- Forms osazone with phenyl hydrazine

Hydrolysis of Lactose

Hydrolyzed by lactase present in alimentary canal; one glucose and one galactose molecules are released upon hydrolysis.



- Some people don't produce enough lactase, the enzyme that hydrolyzes lactose, and so can't digest lactose.
- Many adults become lactose intolerant, and develop abdominal cramps, nausea and diarrhea.
- Lactase can be added to milk products (or taken as a supplement) to combat this problem

Polysaccharides

A **polysaccharide** is a polymer consisting of hundreds to thousands of monosaccharide joined together by glycosidic linkages.

They are further classified into

Homopolysaccharides

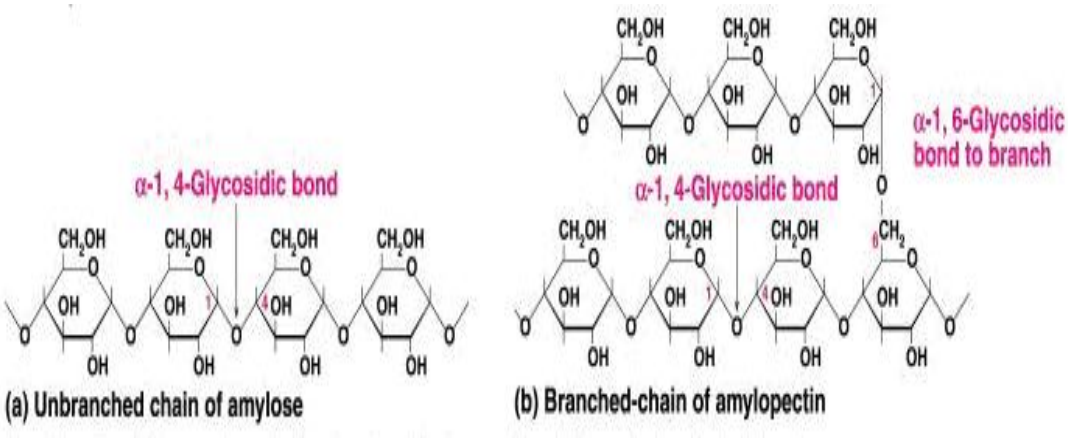
1. Storage polysaccharides: Eg-Starch (Plant); glycogen (animal)
2. Structural polysaccharides: Eg-Cellulose (Plant); Chitin (animal)

Heteropolysaccharides

1. Glycoproteins
2. Glycosaminoglycans

- Heparin/Heparin sulfate
- Chondroitin sulfate
- Keratin sulfate
- Hyaluronic acid

- Three biologically important polysaccharides are **starch, glycogen** and **cellulose** all three are polymers of D-glucose, but they differ in the type of glycosidic bond and/or the amount of branching
- Starch and glycogen are used for storage of carbohydrates
- Starch is found in plants and glycogen in animals
- The polymers take up less room than would the individual glucose molecules, so are more efficient for storage
- Cellulose is a structural material used in formation of cell walls in plants Plant Starch (Amylose and Amylopectin)



Storage polysaccharides

Starch

- Half of the carbohydrate ingested by human is starch.
- It is the source of carbohydrates and fundamental source of energy.
- Starch is the carbohydrate reserve of plants which is the most important dietary source for higher animals, including man.

Occurrence: It is the storage form of carbohydrate in plants ; It is present in cereals, potato, and legumes, root, tubers, tubers, vegetables etc fruits. It is found as granules in cytoplasm of chloroplast

Structure

- Starch is a homopolymer composed of D-glucose units held by a-glycosidic bonds.
- It is known as glucosan or glucan.

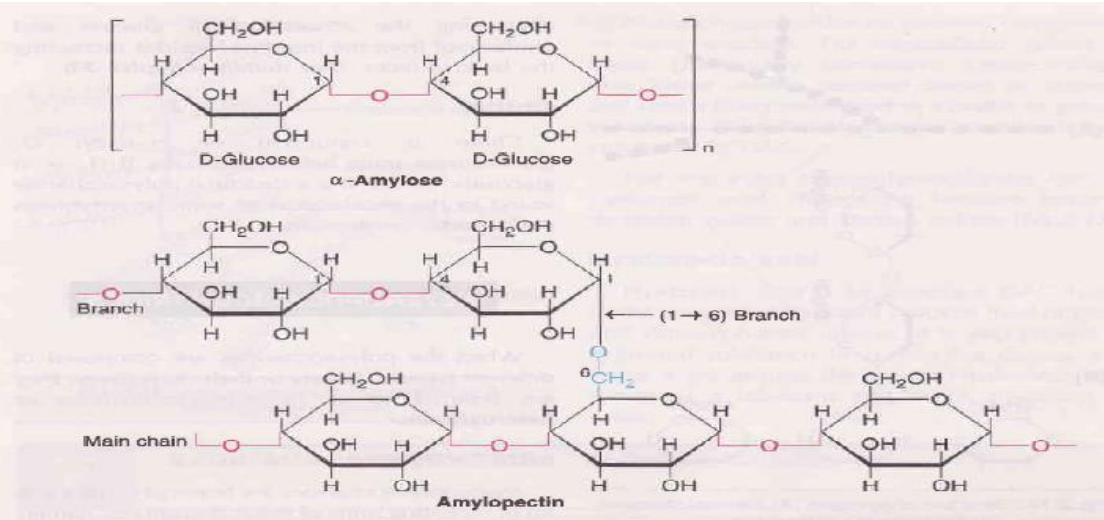
- Starch consists of two polysaccharide components-water soluble amylose (15-20%) and a water insoluble amylopectin (80-85%).

Amylose (α- amylose)

It is a long unbranched polysaccharide; made of α- D glucose joined by α (1 → 4) glycosidic linkage. In starch it constitute about 15-20%. It is in the helical form and 6 glucose unit per turn. It have nearly 300-400 glucose units; molecular weight is 1000-50,000. It form blue color with iodine.

Amylopectin (β-amylose)

Amylopectin on the other hand, is a branched polysaccharide atleast 80 branch with an interval of 24-30 glucose units(20-30 glucose units per branch).It is made of α- D glucose joined by α 1,4 glycosidic linkage and the branch is established with α 1,6 glycosidic linkage(α (1 → 6) glycosidic bonds at the branching points and α (1 → 4) linkages everywhere). In starch it constitute about80-85%. It have nearly 300-5500 glucose units; molecular weight is 5,00,000. It form blue colour with iodine.



Structure of starch (~-amylose and amylopectin)

Properties of starch

White , soft powder, tasteless; insoluble in water; specific rotation is+196.

Hydrolysis

Starch is a glucosan, because it yields only glucose molecule on hydrolysis; with water it form hydrated micelle

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

α- amylase

Amylose ------→ Maltose + glucose

α- amylase attacks the α 1,4 glycosidic linkage. It is present in saliva

α- amylase/ β- amylase

Amylo pectin ----------→ Maltose + glucose

α- amylase attacks the α 1,4 glycosidic linkage. It is present in saliva. α 1,6 glycosidic linkage is attacked by as α 1,6 glucosidase

Starch with mineral acid gives glucose. This glucose reacts with iodine and give gradual change in colour ie., -blue----------purple----------red----------none

Starch on partial hydrolysis yield dextrin which gives stiffness to cloths

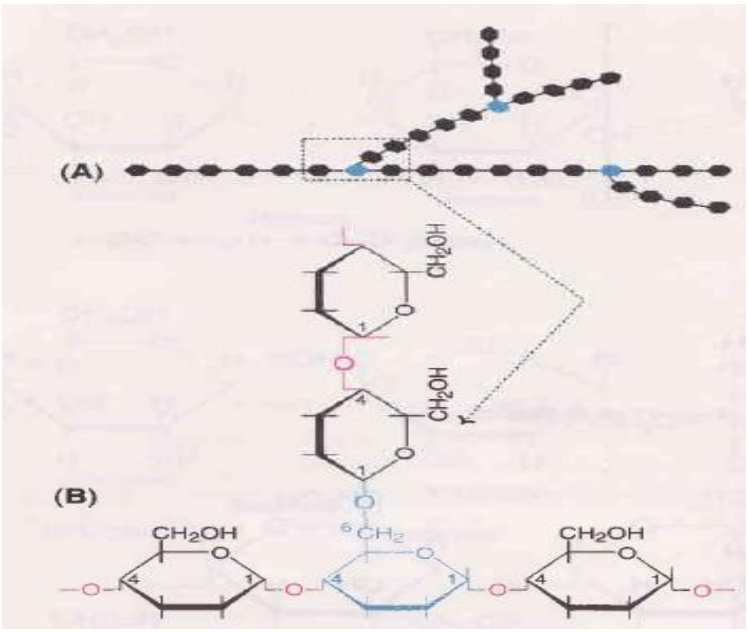
Glycogen

Glycogen is the carbohydrate reserve in animals, hence often referred to as animal starch. It is the reserve carbohydrate found in liver and muscle of animal and human beings

It is present in high concentration in liver, followed by muscle, brain etc. Liver have more glycogen (7% of its weight) than muscle. Glycogen is also found in plants that do not possess chlorophyll (e.g. yeast, fungi).

Structure

- The structure of glycogen is similar to that of amylopectin with more number of branches. It is a branched polymer of carbohydrate ; made of α-D glucose; Glucose is the repeating unit in glycogen joined together by α (1 → 4) glycosidic bonds, and α (1 → 6) glycosidic bonds at branching points, the branching is established by α 1,6 glycosidic linkage.
- The molecular weight (up to 1 x 10^8) and the number of glucose units (up to 5000-25,000) vary in glycogen depending on the source from which glycogen is obtained.



Structure of glycogen (A) General structure (B) Enlarged at a branch point

Properties

White, tasteless powder; readily soluble in water; Non reducing; give red color with iodine

Hydrolysis

On complete hydrolysis, glycogen yields glucose and maltose

Dextrin

This is formed by the partial (incomplete) hydrolysis of starch by salivary amylase; and also by dilute mineral acid and heat.

Inulin

It is a fructosan; made of repeating units of fructose. It is found in roots and tubers of dahlia and dandelions; it mainly used in assessing the kidney function.

Structural Polysaccharides

Structural polysaccharides are the polysaccharides that are found to form the structure of an organism.

Eg. Cellulose - in plants

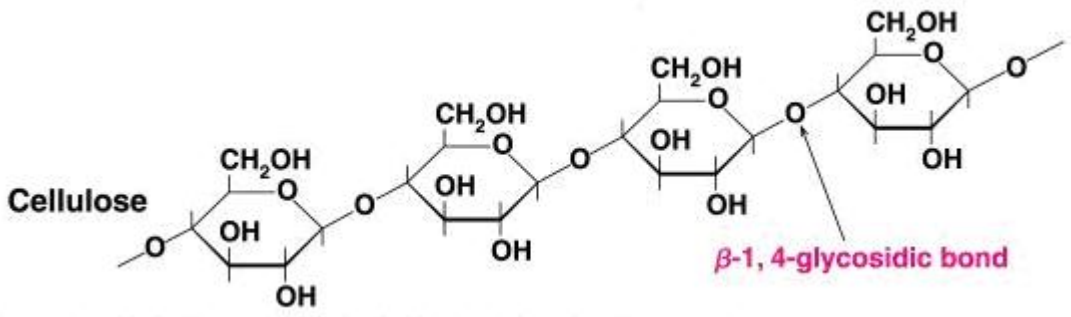
Chitin - found in outer skeleton of insects and crabs

Lignin - wood

Cellulose

It is the most abundant of all biomolecule in biosphere. 50% carbon in vegetation is contributed by cellulose. In plant, it is the main constituent of supporting tissue. It is not present in animal.

- Cellulose is a polymer made with repeated glucose units bonded together by *beta*-linkages.
- The structural components of plants are formed primarily from cellulose.
- Wood is largely cellulose and lignin, while paper and cotton are nearly pure cellulose.



Properties

- Cellulose is insoluble in water. It does not change color when mixed with iodine. On hydrolysis, it yields glucose. It is the most abundant carbohydrate in nature.
- Fibrous, tough, white solid; insoluble in ordinary solvents and water; give no color with iodine.

Hydrolysis

- Humans and many other animals lack an enzyme to break the *beta*-linkages, so they do not digest cellulose.
- Certain animals such as termites can digest cellulose, because bacteria possessing the enzyme are present in their gut.
- It is not acted upon by amylase in human intestine, so doesn't have any nutritive value. It adds bulk to the intestinal constituents and stimulates the peristaltic movement of bowel so it aids in relieving constipation.
- On complete hydrolysis by cellulase enzyme it yields α-D glucose. This enzyme is mainly present in termites, which are able to digest the wood.

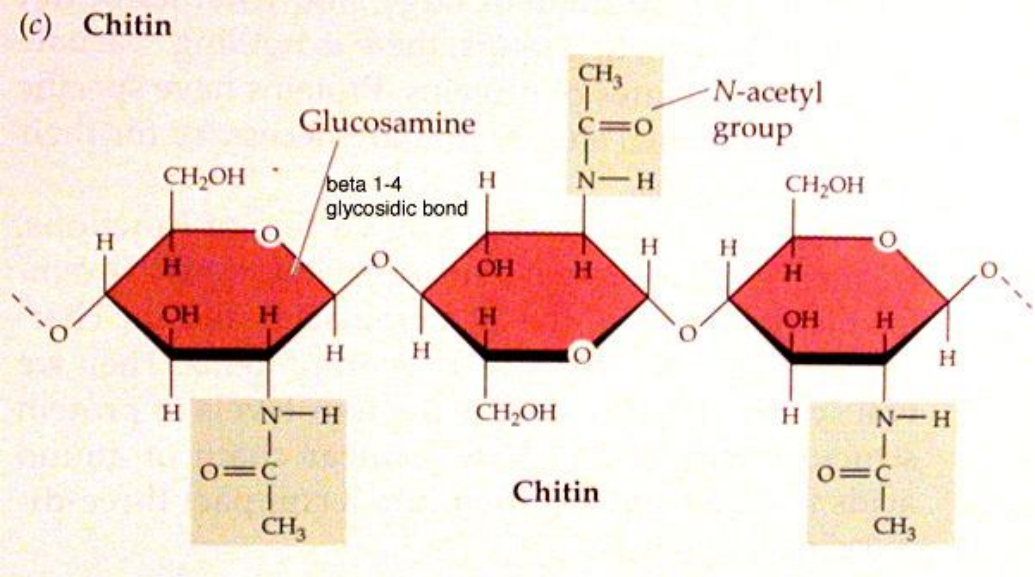
- It is also hydrolyzed by acids such as sulfuric acid, nitric acid and sodium hydroxide.

Chitin

Chitin is a polysaccharide found in the outer skeleton of insects, crabs, shrimps, and lobsters and in the internal structures of other invertebrates.

Structure

It is a long-chain polymer of a *N*-acetylglucosamine, a derivative of glucose, Joined through ß(1-4) linked units of the amino sugar N-acetyl-glucosamine.



Properties

In its unmodified form, chitin is translucent, pliable, resilient, and quite tough. but in most invertebrates it occurs largely as a component of composite materials

Application

Chitin is the main source of production of chitosan, which is used in a number of applications, such as a flocculating agent, a wound healing agent, a sizing and strengthening agent for paper, and a delivery

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DEPARTMENT OF MICROBIOLOGY

I B.SC MICROBIOLOGY – FIRST SEMESTER

17MBU105A – BIOCHEMISTRY

MULTIPLE CHOICE QUESTIONS

Unit 2

Question	Opt A	Opt B	Opt C	Opt D	Answer
Two monosaccharides are j	Peptide bond	Phosphodiester bon	Glycosidic l	Hydrogen l	Glycosidic bond
All the following are storag	Starch	Cellulose	Dextran	Glycogen	Dextran
The glycosidic linkage betw	β 1 – 4	α 1 – 2	α 1 – 4	β 1 – 2	α 1 – 4
The glycosidic linkage betw	α 1 – 4	β 1 – 4	α 1 – 6	β 1 – 6	α 1 – 6
Which of the following sug	Sucrose	Glucose	Galactose	Mannose	Sucrose
Maltose is a disaccharide o	Glucose and galact	Glucose and glucose	Glucose an	Fructose ar	Glucose and glucose
Glycosidic bond in sucrose i	α 1 – 4	β 1 – 4	α 1 – 2	β 1 – 2	β 1 – 2
Majority of the monosacch	L-type	D-type	DL-types	None of th	D-type
Example of Epimers is	Glucose & Galacto	Glucose & Ribose	Mannose &	a & c	a & c
The end product of hydroly	Soluble starch	Glucose	Dextrins	Maltose	Glucose
Cellulose fibers resemble w	ß-sheets	α-helices	ß-turns	None of th	ß-sheets
Hydrolysis of lactose yields	galactose and fruct	galactose and gluco	glucose an	fructose an	galactose and glucose
Boat and chair conformatio	in pyranose sugars	in any sugar without	in any suga	only in D-gl	in pyranose sugars
Storage polysaccharide ma	amylopectin	glycogen	cellulose	collagen	glycogen
The glycosaminoglycan whi	Dermatan sulphate	Chondroitin sulphat	Keratan sul	Heparan su	Keratan sulphate
Keratan sulphate is found i	Heart muscle	Liver	Adrenal co	Cornea	Cornea
Repeating units of hyaluror	N-acetyl glucosami	N-acetyl galactosam	N-acetyl gl	N-acetyl ga	N-acetyl glucosamine a
The approximate number o	10	20	40	80	80
In amylopectin the interval	10–20	24–30	30–40	40–50	24–30
The general formula for pol	$(C_6H_{10}O_5)_n$	$(C_6H_{12}O_5)_n$	$(C_6H_{10}O_6)$	$(C_6H_{10}O_6)$	$(C_6H_{10}O_5)_n$
α-D-glucose and β -D-gluco	Stereoisomers	Epimers	Anomers	Keto-aldo p	Anomers
The general formula of mo	$C_nH_{2n}O_n$	$C_2nH_2O_n$	$C_nH_2O_{2n}$	$C_nH_{2n}O_{2n}$	$C_nH_{2n}O_n$
The aldose sugar is	Glycerose	Ribulose	Erythrulose	Dihydoxyac	Glycerose
A triose sugar is	Glycerose	Ribose	Erythrose	Fructose	Glycerose
A pentose sugar is	Dihydroxyacetone	Ribulose	Erythrose	Glucose	Ribulose
The pentose sugar present	Lyxose	Ribose	Arabinose	Xylose	Lyxose
Polysaccharides are	Polymers	Acids	Proteins	Oils	Polymers
The number of isomers of g	2	4	8	16	16
Two sugars which differ fro	Epimers	Anomers	Optical isor	Stereoisom	Epimers
Isomers differing as a resul	Epimers	Anomers	Optical isor	Steroisome	Epimers
The most important epime	Galactose	Fructose	Arabinose	Xylose	Galactose
α-D-glucose + 112.0 → + 52	Optical isomerism	Mutarotation	Epimerisati	D and L iso	Mutarotation
Compounds having the sam	Stereoisomers	Anomers	Optical isor	Epimers	Epimers
In glucose the orientation c	D or L series	Dextro or levorotato	α and β an	Epimers	D or L series
The sugar found in milk is	Galactose	Glucose	Fructose	Lactose	Lactose
Invert sugar is	Lactose	Sucrose	Hydrolytic p	Fructose	Hydrolytic products of
Sucrose consists of	Glucose + glucose	Glucose + fructose	Glucose + g	Glucose + r	Glucose + fructose

The monosaccharide units	Maltose	Sucrose	Cellulose	Cellobiose	Maltose
Which of the following is a	Isomaltose	Maltose	Lactose	Trehalose	Trehalose
Which of the following is a	Sucrose	Trehalose	Isomaltose	Agar	Isomaltose
A dissaccharide formed by	Lactose	Maltose	Trehalose	Sucrose	Trehalose
A polysacchharide which is	Glycogen	Starch	Inulin	Dextrin	Glycogen
The homopolysaccharide u	Agar	Inulin	Pectin	Starch	Agar
The polysaccharide used in	Glycogen	Agar	Inulin	Hyaluronic	Inulin
The constituent unit of inul	Glucose	Fructose	Mannose	Galactose	Fructose
The polysaccharide found i	Pectin	Chitin	Cellulose	Chondroiti	Chitin
Which of the following is a	Dextrins	Agar	Inulin	Chitin	Agar
A positive Benedict's test is	Sucrose	Lactose	Maltose	Glucose	Sucrose
Starch is a	Polysaccharide	Monosaccharide	Disaccharic	None of th	Polysaccharide
A positive Seliwanoff's test	Glucose	Fructose	Lactose	Maltose	Fructose
Osazones are not formed w	Glucose	Fructose	Sucrose	Lactose	Sucrose
The most abundant carboh	Starch	Glycogen	Cellulose	Chitin	Cellulose
The total Glucose in the bo	10–15	20–30	40–50	60–80	20–30
Whcih of the following feat	Contain asymmetr	Are of 2 types – aldo	Tend to exi	Include glu	Tend to exist as ring st
The following examples are	Amylopectin	Heparin	Peptidogly	Hyaluronic	Amylopectin
Glucosamine is an importa	Homopolysacchari	Heteropolysacchario	Mucopolys	Dextran	Mucopolysaccharide
Glycogen is present in all b	Liver	Brain	Kidney	Stomach	Brain
Iodine test is positive for st	Mucoproteins	Agar	Glycogen	Cellulose	Glycogen
The distinguishing test betv	Bial's test	Seliwanoff's test	Barfoed's t	Hydrolysis	Barfoed's test
Cane sugar is known as	Galactose	Sucrose	Fructose	Maltose	Sucrose

ler Section 3 of UGC Act 1956)

ınd D-glucuronic acid

sucrose

ructures in solution

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DEPARTMENT OF MICROBIOLOGY
(For the candidates admitted from 2015 onwards)

Subject	**:**	**Biochemistry**	**Semester**	**:**	**I**
Subject code	**:**	**17MBU103**	**Class**	**:**	**I B.Sc Microbiology**

UNIT-III: COURSE MATERIAL

Unit-III

Classification and functions of lipids, storage lipids-structure and function of fatty acids. Triacylglycerols. Saponification. Structural lipids-structure, functions and properties of phosphoglycerides and sphingolipids.

Suggest Readings

1. Nelson, D.L and Cox, M.M. (2008). Lehninger Principles of Biochemistry, 5th edition. W.H. Freeman and Company.

Lipids

Definition

- The lipids are heterogeneous group of compounds related to fatty acids.
- They constitute a broad group of naturally occurring molecules that include fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E, and K), monoglycerides, diglycerides, triglycerides, phospholipids, and others.

Biological significance

The main biological functions of lipids include

- Fat serve as an efficient sources of energy storage,
- Serve as insulating material
- Helps in blood clotting
- Serve as structural components of cell membranes, and as important signaling molecules.
- Lipoproteins and glycolipids are important for maintaining cellular integrity.

Classification of Lipids

- They are broadly classified into simple lipids, complex lipids, derived lipids and miscellaneous lipids based on their chemical composition.

Simple lipids

Esters of fatty acids with alcohols. These are mainly of two types

Fats and oils (triacylglycerols)

- These are esters of fatty acids with glycerol.
- The difference between fat and oil is only physical.
- Thus, oil is a liquid while fat is a solid at room temperature.

Waxes

- Esters of fatty acids (usually long chain) with alcohols other than glycerol.
- These alcohols may be aliphatic or alicyclic.
- Cetyl alcohol is most commonly found in waxes.

Complex (or compound) lipids

- These are esters of fatty acids with alcohols containing additional groups such as phosphate, nitrogenous base, carbohydrate, protein etc.

- They are further divided as follows

Phospholipids

- They contain phosphoric acid and frequently a nitrogenous base.
- This is in addition to alcohol and fatty acids.

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

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

Glycolipids

- These lipids contain a fatty acid, carbohydrate and nitrogenous base.
- The alcohol is sphingosine; hence they are also called as glycosphingolipids.
- Glycerol and phosphate are absent e.g., cerebrosides, gangliosides.

Lipoproteins

- Macromolecular complexes of lipids with proteins.

Other complex lipids

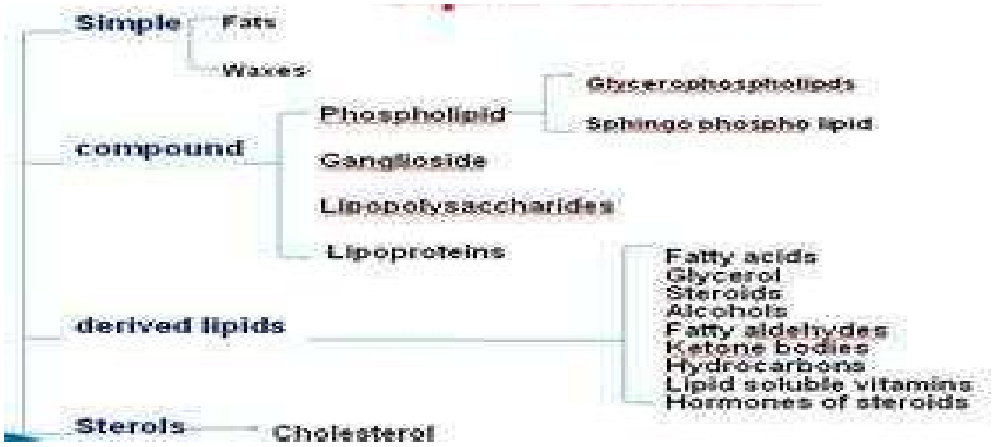
- Sulfolipids, amino lipids and lipopolysaccharides are among the other complex lipids.

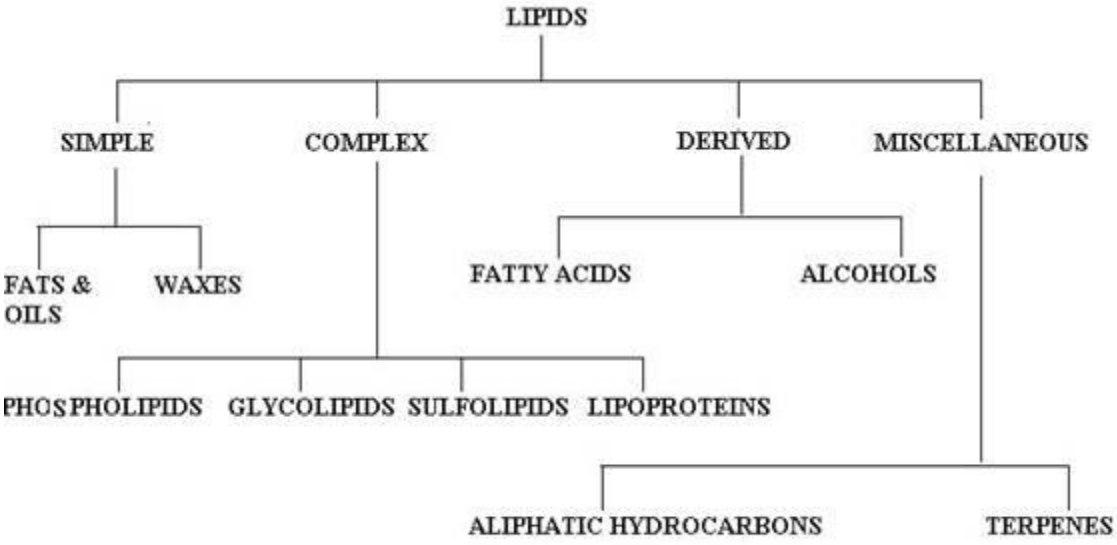
Derived lipids

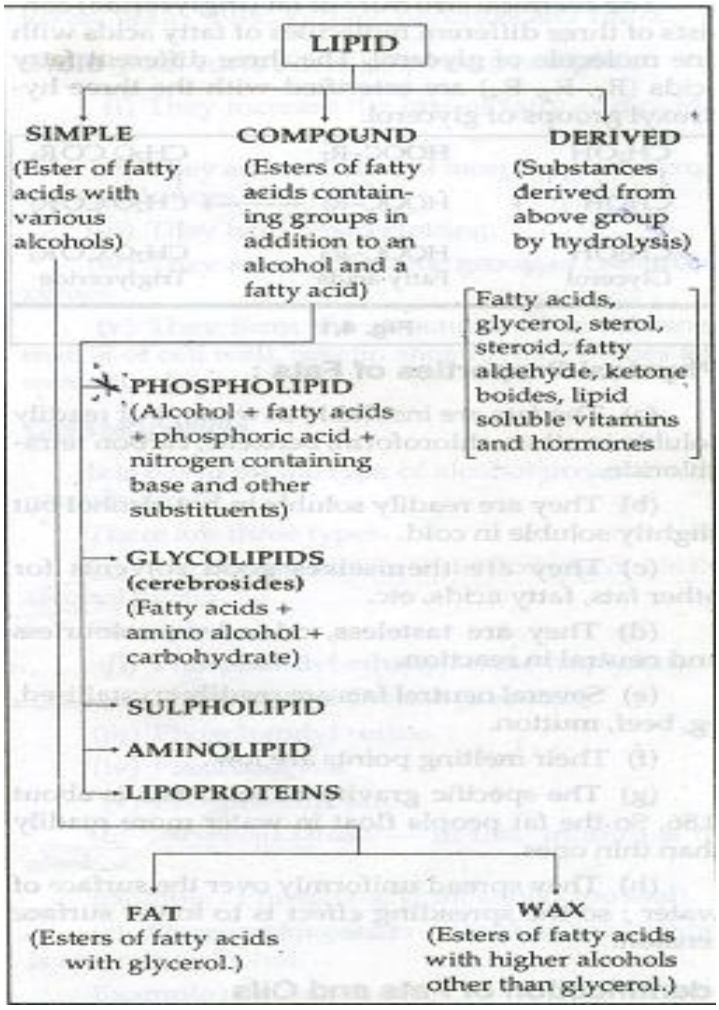
- These are the derivatives obtained on the hydrolysis of group 1 and group 2 lipids which possess the characteristics of lipids.
- These include glycerol and other alcohols, fatty acids, mono- and diacylglycerols, lipid (fat) soluble vitamins, steroid hormones, hydrocarbons and ketone bodies.

Miscellaneous lipids

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


Simple
Fats
Waxes
compound
Phospholipid
Glycerophospholipids
Sphingophospho lipid
Ganglioside
Lipopolysaccharides
Lipoproteins
derived lipids
Fatty acids
Glycerol
Steroids
Alcohols
Fatty aldehydes
Ketone bodies
Hydrocarbons
Lipid soluble vitamins
Hormones of steroids
Sterols
Cholesterol


LIPIDS
SIMPLE
COMPLEX
DERIVED
MISCELLANEOUS
FATS & OILS
WAXES
FATTY ACIDS
ALCOHOLS
PHOSPHOLIPIDS
GLYCOLIPIDS
SULFOLIPIDS
LIPOPROTEINS
ALIPHATIC HYDROCARBONS
TERPENES

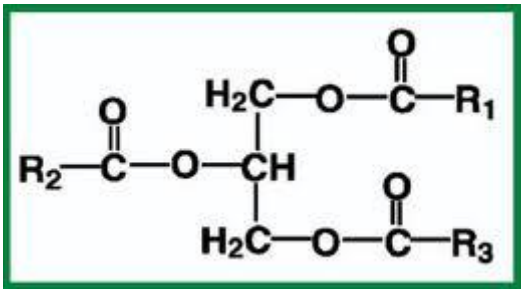


Simple Lipids

Fats

- Fat, any substance of plant or animal origin that is nonvolatile, insoluble in water, and oily or greasy to the touch. They are esters of fatty acids with glycerol.
- They are found in nature in large quantities. Fats are usually solid at ordinary temperatures, such as 25 °C (77 °F), but they begin to liquefy at somewhat higher temperatures.
- Chemically, fats are identical to animal and vegetable oils, consisting primarily of glycerides, which are esters formed by the reaction of three molecules of fatty acids with one molecule of glycerol.

  Eg-Triacylglycerol



- They are the best reserve of food material in the human body.
- They act as insulator for the loss of body heat.
- They act as a padding material for protecting internal organs.
- The chemical structure of fat (triglyceride) consists of three different molecules of fatty acids with one molecule of glycerol. The three different fatty acids (R_1, R_2, R_3) are esterified with the three hydroxyl groups of glycerol.

Physical and Chemical Properties

- Fats (and oils) may be divided into animal and vegetable fats according to source. Further, they may be classified according to their degree of unsaturation as measured by their ability to absorb iodine at the double bonds.
- This degree of unsaturation determines to a large extent the ultimate use of the fat.
- Liquid fats (i.e., vegetable and marine oils) have the highest degree of unsaturation, while solid fats (vegetable and animal fats) are highly saturated.
- Solid vegetable fats melting between 20 and 35 °C (68 and 95 °F) are found mainly in the kernels and seeds of tropical fruits.
- They have relatively low iodine values and consist of glycerides containing high percentages of such saturated acids as lauric, myristic, and palmitic.
- Fats are practically insoluble in water and, with the exception of castor oil, are insoluble in cold alcohol and only sparingly soluble in hot alcohol.
- They are soluble in ether, carbon disulfide, chloroform, carbon tetrachloride, petroleum benzin, and benzene. Fats have no distinct melting points or solidifying points because they are such complex mixtures of glycerides, each of which has a different melting point.

- Glycerides, further, have several polymorphic forms with different melting or transition points.
- Fats can be heated to between 200 and 250 °C (392 and 482 °F) without undergoing significant changes provided contact with air or oxygen is avoided.
- Above 300 °C (572 °F), fats may decompose, with the formation of acrolein (the decomposition product of glycerol), which imparts the characteristic pungent odour of burning fat.
- Hydrocarbons also may be formed at high temperatures.
- Fats are hydrolyzed readily.
- This property is used extensively in the manufacture of soaps and in the preparation of fatty acids for industrial applications.

Physical Properties of Fats

- The fats are insoluble in water, but readily soluble in ether, chloroform, benzene, carbon tetrachloride.
- They are readily soluble in hot alcohol but slightly soluble in cold.
- They are themselves good solvents for other fats, fatty acids, etc.
- They are tasteless, odourless, colorless and neutral in reaction,
- Several neutral fats are readily crystallized, eg, beef, mutton
- Their melting points are low.
- The specific gravity of solid fats is about 0.86. So the fat people float in water more readily than thin ones.
- They spread uniformly over the surface of water; so the spreading effect is to lower surface tension.

Chemical properties of fats

Hydrolysis

1. Hydrolysis of triacylglycerol takes place by lipases producing fatty acids and glycerol.
2. Phospholipases attack the ester linkage of phospholipids.

Saponification

- Boiling with an alcoholic solution of strong metallic alkali hydrolyzes triglycerides into glycerol and fatty acids are called saponification.
- The products are glycerol and the alkali salts of the fatty acids which are called soaps.
- Fats, phospholipids, glycolipids and waxes are called saponifiable lipid.
- Steroids, polyisoprenoids and higher alcohols are grouped as unsaponifiable lipids because they cannot give rise to soap.

Saponification number

- The number of milligrams of KOH is required to saponify 1 gram of fat or oil.
- The amount of alkali needed to saponify a given quantity of fat will be depended upon the number of -{:OOH group present. It is inversely proportional to the average molecular weight of the fatty acids in the fat i.e. the fats containing short chain fatty acids will have more -{:OOH groups per gram than long chain fatty acids and this will take up more alkali and hence will have higher saponification number.

Example: Butter containing a larger proportion of short chain fatty acids such as butyric and caproic acids, has relatively high saponification number 220 to 230.

Acid number

- The number of milligrams of KOH is required to neutralize the free fatty acids of 1 gram of fat.
- Significance: The acid number indicates the degree of rancidity of the given fat.

Iodine number

- This is the amount (in grams) of iodine absorbed by 100 grams of fat.
- This is the measure of the degree of unsaturation of a fat.
- 3. Significance: If the fat contains higher number of unsaturated fatty acids, it becomes essential for the protection of heart disease. These unsaturated fatty acids being combined with the cholesterol are oxidized in the liver producing bile acids, bile salts, Vitamin D, gonadotrophin hormones. They prevent atherosclerosis.

Acetyl number

- The number of milligrams of KOH required to neutralize the acetic acid obtained by saponification of 1 gram of fat after it has been acetylated.
- This is a measure of the number of hydroxy acid groups in the fat.

Polenske number

- The number of milliliters of 0.l (N) KOH required to neutralize the insoluble fatty acids from 5 grams of fat.

Reichert-Miessl number

- This is the same as the Polenske number except that the soluble fatty acids are measured by titration of the distillate obtained by steam distillation of the saponification mixture.
- Significance: It measures the amount of volatile soluble fatty acids.

Halogenation

- Chlorine, bromine and iodine atoms may be added to the double bonds of unsaturated fatty acids containing fats.

Rancidity

- Nearly all natural fats are oxidized when ex- posed to air, light, moisture, particularly, if warm, it develops an unpleasant odour and taste. The enzyme lipase which in the presence of moisture and temperature bring about hydrolysis rapidly.
- This happens so due to the formation of peroxides at the double bonds of unsaturated fatty acids.
- Vitamin E is an important natural antioxidant and prevents development of rancidity.

Soaps

- Soaps are metallic salts of fatty acids.
- Soaps are formed by adding alkalis to fatty acids.
- Soaps of unsaturated fatty acids are softer and more water soluble than those of saturated fatty acids.
- Potassium soap of an acid is more water soluble and softer than the sodium soap, calcium and magnesium soaps are far less soluble.

Compound lipids

Phospholipids

These are complex or compound lipids containing phosphoric acid, in addition to fatty acids, nitrogenous base and alcohol. Based on the type of alcohol present in the phospholipid they are classified into three types.

Glycerophosphatides - In this, glycerol is the alcohol group.

Example

- Phosphatidyl ethanolamine (cephalin).
- Phosphatidyl choline (Lecithin).
- Phosphatidyl serine.
- Plasmalogens.
- Phosphatidic acid.

Phosphoinositides - In this, inositol is the, alcohol.

Example: Phosphatidyl inositol (Lipositol).

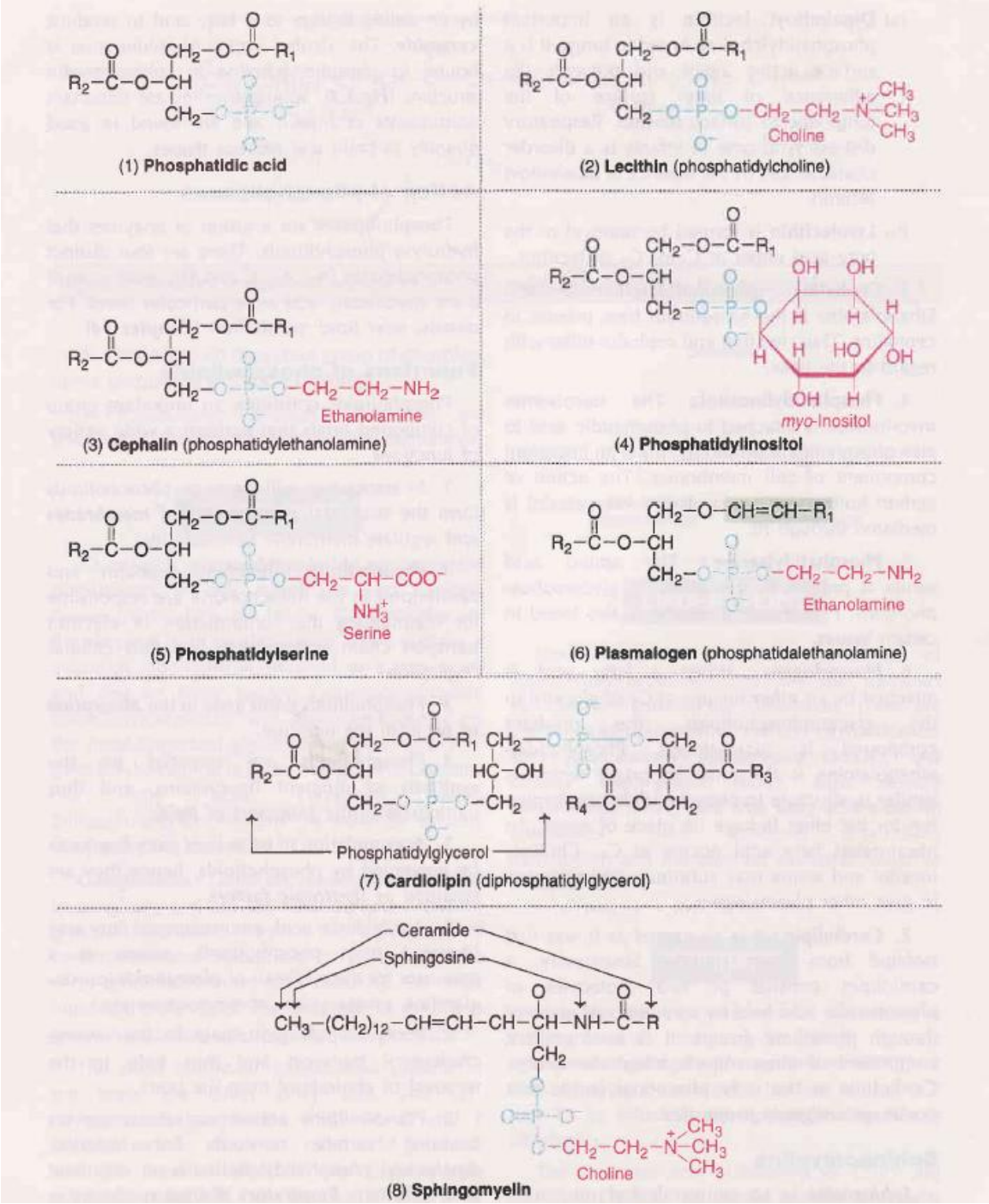
Phosphosphingosides - In this, sphingosine is an amino alcohol. .

Example: Sphingomyelin, ceramide.

Structure

- **Phosphatidic acid:** This is the simplest phospholipid. It does not occur in good concentration in the tissues.
- **Lecithins (phosphatidylcholine):** These are the most abundant group of phospholipids in the cell membranes.
- **Cephalins (phosphatidylethanolamine):** Ethanolamine is the nitrogenous base present in cephalins, thus lecithin and cephalin differ with regard to the base.
- **Phosphatidylinositol:** The steroisomer myo-inositol is attached to phosphatidic acid to give Phosphatidylinositol.
- **Phosphatidylserine:** The amino acid serine is present in this group of glycerophospholipids. Phosphatidylthreoninise also found in certain tissues.
- **Plasmalogens:** When a fatty acid is attached by an ether linkage at C1 of glycerol in the glycerophospholipids, the resultant compound is plasmalogen.

- **Cardiolipin**: It is so named as it was first isolated from heart muscle. Structurally, a cardiolipin consists of two molecules of phosphatidic acid held by an additional glycerol through phosphate groups.



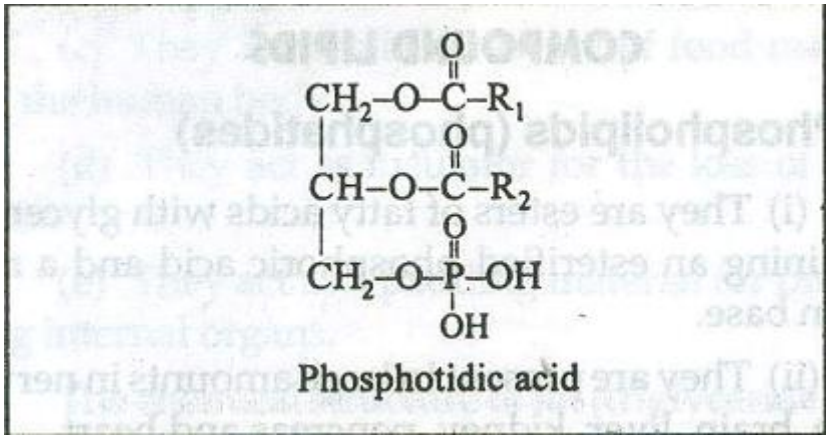
Structure of phospholipids

Phosphatidic acid and phosphat idyl glycerols

Phosphatidic acid is important as an intermediate in the synthesis of triacylglycerols and phospholipids.

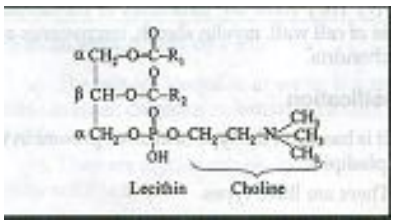
Cardiolipin

- It is formed from phosphatidyl glycerol.
- Chemically, it is diphosphatidyl glycerol.
- It is found in inner membrane of mitochondria and bacterial wall.



Lecithins (Phosphatidylcholine)

The lecithins contain glycerol and fatty acids, phosphoric acid and choline (nitrogenous base). Lecithins generally contain a saturated fatty acid at a position and an unsaturated fatty acid at ß position. They can exist in a or ß forms.



Physical Properties

- Lecithins are waxy, white substances but become brown soon when exposed to air.
- They are soluble in ordinary fat solvents except acetone.
- They decompose when heated.
- They constitute valuable agents for the emulsifications of fats and oils.

Chemical Properties of Lecithin

- When aqueous solution of lecithins is shaken with ~S04' choline is split off, forming phosphatidic acid.'
- When lecithins are boiled with alkalis or mineral acids, not only choline is split off; phosphatidic acid is further hydrolyzed to glycerophosphoric acid and 2 molecules of fatty acids.

Lecithin H_2SO4-+ Phosphatidic acid + cholin.

Phosphatidic acid -+ Glycerophosphoric acid + fatty acids (2 mol)'

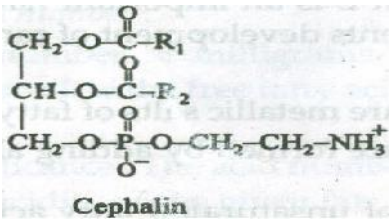
Physiological Functions of Lecithin

- It facilitates the combinations with proteins to from lipoproteins of plasma and cells.
- Acetylcholine formed from choline has an important role in the transmission of nervous impulses across synapses.
- Choline is the most important lipotropic agent as it can prevent formation of fatty liver.
- Lecithin lowers the surface tension of lung alveoli. Dipalmityllecithin is a major constituent of "lung surfactant" which prevents the adherence ql the inner surface of the alveoli of the lungs (preventing the collapse of the alveoli) by its surface tension lowering effect. The absence of this in the alveolar membrane of some premature infants causes the respiratory distress syndrome in them.
- It lowers the surface tension of water molecule and helps in the emulsification of fat.

Difference of Lecithin and Cephalin

Cadmium chloride compound of Cephalin is soluble but cadmium chloride compound of lecithin is insoluble.

Cephalins (Phosphatidyl ethanplamine)

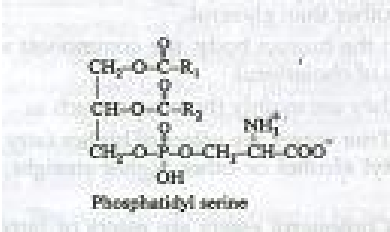


Cephalin

They always occur in the tissues in association with lecithins and are very similar in

properties. The only difference is the nitrogenous base.

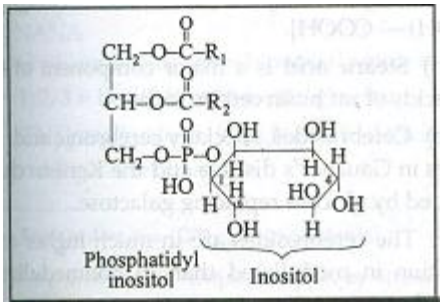
Phosphatidyl Serine



A cephaline like phospholipid is found in tissues.

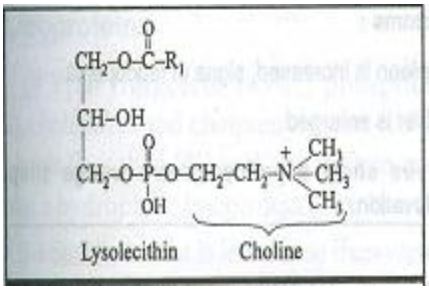
Phosphatidyl inositol (Lipositol or Phosphoinasitides)

Phosphatidyl~ inositol Inositol



- It acts as second messenger in Ca ++ de- pendent hormone action.
- Some signals must provide communication between the hormone receptor on the plasma membrane and intracellular Ca ++ reservoirs.
- They are more acidic than the other phospholipids.

Lysophospholipids:



- These are phosphoacylglycerols containing only one acyl radical in ex position eg, Lysolecithin.

Formation

(a) By the action of phospholipase A_2.

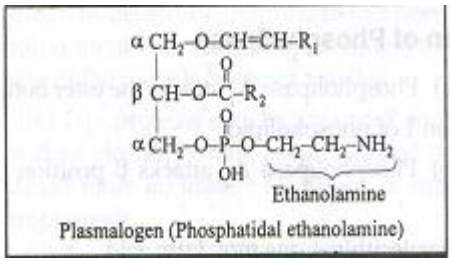
(b) By interaction of lecithin and cholesterol in presence of the enzyme lecithin

cholesterol acyl transferase, so lysolecithin and cholesterol ester are formed

Lecithin + cholesterol .t LCAT

Lysolecithin + cholesterol ester.

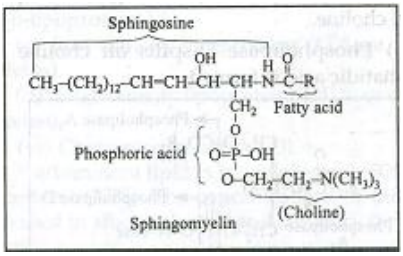
Plasmalogens



Plasmalogen (Phosphatidal ethanolamine)

- These are the contents of brain and muscle.
- Structurally, these resemble lecithins and cephalins but give a positive reaction when tested for aldehydes with Schiff's reagent (fuchsin sulfurous acid) after pretreatment of the phospholipid with mercuric chloride. Plasmalogen (phosphatidal ethanolamine)
- They possess an ether link in ex position instead of ester link. The alkyl radical is an unsaturated alcohol.

Sphingomyelins



- These are found in large quantities in brain and nerve tissue.
- The concentrations of these phospholipids are increased in Niemann-Pick disease in the liver and spleen.
- These contain sphingosine (18 carbon) (amino alcohol) fatty acid, phosphoric acid and choline. No glycerol is present.
- In sphingosine molecule -N~ group.· binds a fatty acid by an amide linkage to produce ceramide. When phosphate group is attached to ceramide it is called ceramide phosphate.

- When choline is split off from sphingomyelin, ceramide phosphate is left.

Clinical Aspect

- In Niemann-.Pick disease excess amount of sphingomyelin are deposited in brain, liver, spleen.
- It is a lipid storage disease (lipidoses) and hereditary. It is caused by the deficiency of enzyme sphingomyelinase.
- The clinical findings are:

(a) Enlarged liver and spleen.

(b) Mental retardation.

(c) Nervous system is affected.

(d) Anemia and leukocvtosis.

Action of Phospholipase

(a) Phospholipase A_1 attacks the ester bond in position **1** of phospholipid.

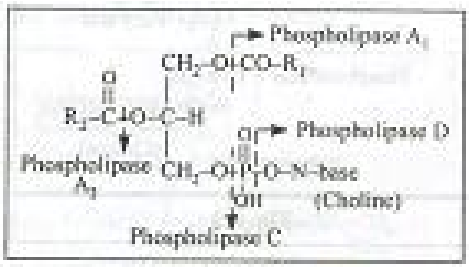
(b) Phospholipase A_2 attacks β position and form

Lysolecithin + one mol. fatty acid.

(c) Phospholipase B (lysophospholipase) attacks lysolecithin and hydrolyzes ester bond in a position and forms glyceryl phosphoryl choline + 1 mol fatty acid.

(d) Phospholipase C hydrolyzes phosphate ester bond and produces α, β diacyl glycerol + phosphoryl choline.

(e) Phospholipase D-splits off choline and phosphatidic acid is formed



Function of Phospholipids

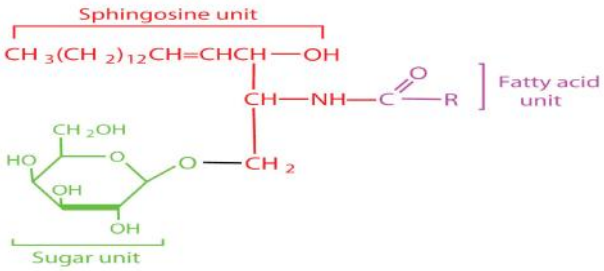
- Phospholipids constitute an important group of compound lipids that perform a wide variety of functions.

- In association with proteins, phospholipids form the structural components of membranes and regulate membrane permeability.
- Phospholipids (lecithin, cephalin and cardiolipin) in the mitochondria are responsible for maintaining the conformation of electron transport chain components, and thus cellular respiration.
- Phospholipids participate in the absorption of fat from the intestine.
- Phospholipids are essential for the synthesis of different lipoproteins, and thus participate in the transport of lipids.
- Accumulation of fat in liver (fatty liver) can be prevented by phospholipids, hence they are regarded as lipotropic factors.
- Arachidonic acid, an unsaturated fatty acid liberated from phospholipids, serves as a precursor for the synthesis of eicosanoids (prostaglandins, prostacyclinst, hromboxanes etc.,).
- Phospholipids participate in the reverse cholesterol transport and thus help in the removal of cholesterol from the body.
- Phospholipids act as surfactants (agents. lowering surface tension). For instance dipalmitoyl phosphatidylcholinies an important lung surfactant. Respiratory distress syndrome infants are associated with insufficient production of this surfactant.
- Cephalins, an important group of phospholipids participate in blood clotting.
- Phospholipids (phosphatidylinositol) are involved in signal transmission across membranes.

Glycolipids

Glycolipids are lipids with a carbohydrate attached. Their role is to provide energy and also serve as markers for cellular recognition. Eg: One type of glycolipid found in human red blood cells is involved in the ABO blood type antigens.

They contain an amino alcohol (Sphingosine) attached with an amide linkage to fatty acid and glycosidically to a carbohydrate moiety (Sugar, amino sugar, sialic acid).



Classification

They are classified into (i) Cerebrosides, (ii) Gangliosides.

Cerebrosides

- Cerebrosides contain galactose, a high molecular weight fatty acid and sphingosine.

Therefore, they may also be classified as sphingolipids.

- They are the chief constituent of myelin sheath.
- They may be differentiated by the type of fatty acid in the molecule.

These are

- Kerasin-Containing lignoceric acid [CH_3 - (CH_2)22 - COOH].
- Cerebron-Containing a hydroxylignoceric acid (cerebronic acid).
- [CH3-(C~h1 - CH(OH) - COOH].
- Nervon-Containing an unsaturated homologue of lignoceric acid called nervonic acid. [CH_3 - {C~h- CH = CH - (CH_2)13 - COOH].
- Oxynervon-Containing hydroxynervonic acid [CH_3 - (CH_2)7 - CH = CH - (CH_2)12- CH(OH) - COOH].
- Stearic acid is a major component of the fatty acids of rat brain cerebrosides.
- Cerebrosides, specially cerebronic acid, increases in Gaucher's disease and the Kerasin characterized by glucose replacing galactose.
- The cerebrosides are in much higher concentration in medullated than in nonmedullated nerve fibers.

Clinical Aspect

Gaucher's disease

- The cerebroside content of the reticuloendothelial cell (spleen) is very high.
- In cerebroside molecule, the kerasin is characterised by glucose replacing galactose.

- The disease is caused by the deficiency of enzyme glucocerebrosidase.

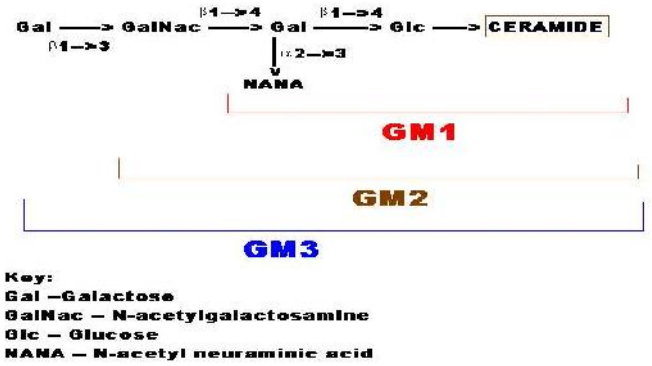
Symptoms

- Spleen is increased, signs of leucopenia.
- Liver is enlarged.
- Eyes show a yellow brown wedge shaped elevation.

Gangliosides

- These are glycolipids occurring in the brain.
- Gangliosides contain ceramide (sphingosine + fatty acids), glucose, galactose, N-acetylgalactosamine and sialic acid.
- Some gangliosides also contain dihydrosphingosine or Gangliosine in place of sphingosine.
- Most of the gangliosides contain a glucose, two molecules of galactose, one N-acetylgalactosamine and upto three molecules of sialic acid.

They are further classified into GM1, GM2, GM3. The following figure depict this.

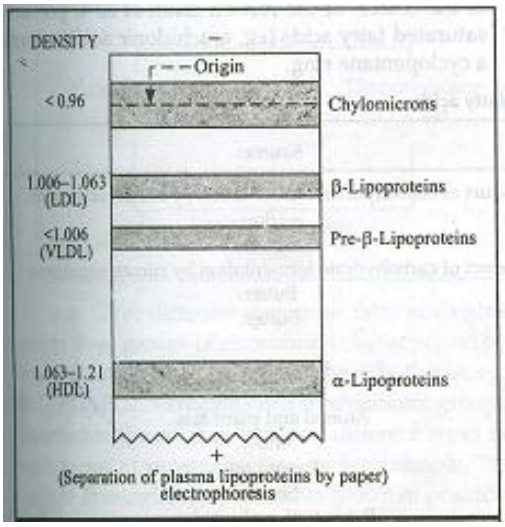


Lipo Proteins

Lipids are transported in blood as large macromolecules called lipoproteins. These are complexes with proteins. Free fatty acids are the exception, mainly binding to albumin.

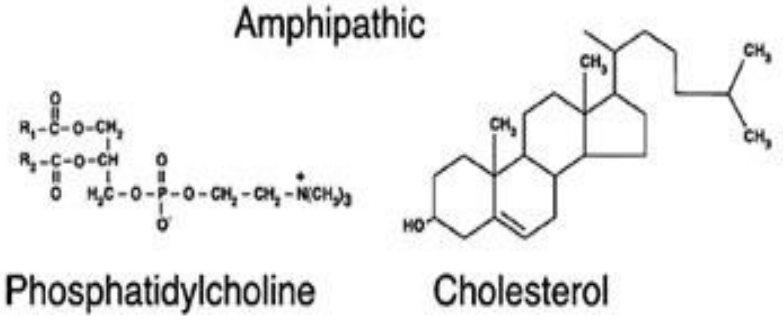
(i) Triacylglycerol (45%), phospholipids (35%), cholesterol and cholesteryl esters (15%), free fatty acids (less than 5%) and also protein combine to form a hydrophilic lipoprotein complex.

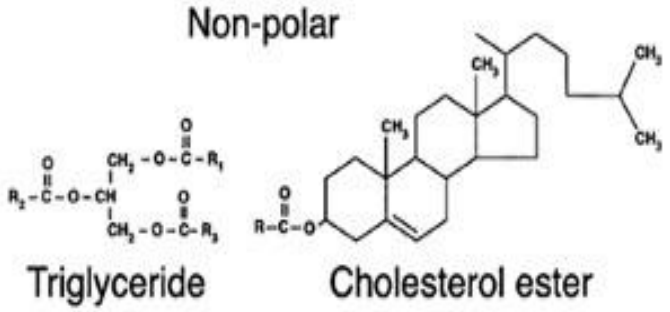
(ii) Since pure fat is less dense than Water, the proportion of lipid to protein in lipoproteins in plasma is separated by ultracentrifugation.

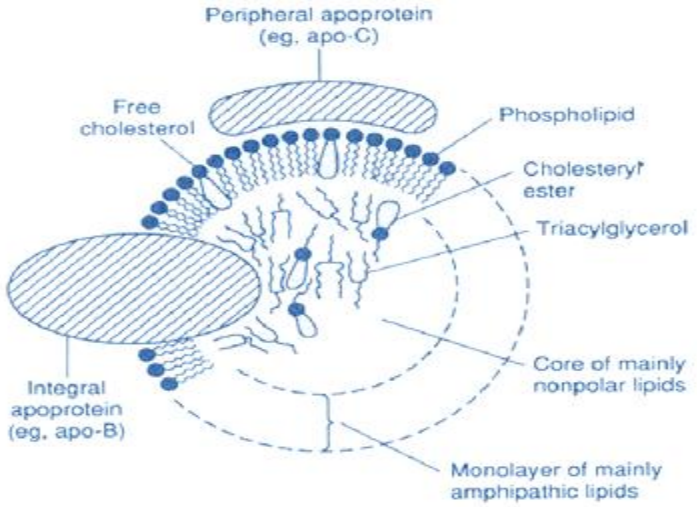


Structure of lipo protein

Hydrophobic lipids, triglycerides and phospholipids are within the lipoprotein core, with the polar portions of phospholipids and the water-soluble alcohol portion of free cholesterol projecting into the aqueous environment, causing solubilization of the lipoprotein.







Types of lipoproteins

Lipoprotein classes can be separated physico chemically, either by electrophoresis which uses surface charge or by ultracentrifugation which uses relative density. Four major groups of lipoproteins have been identified which are important physiologically and in clinical diagnosis in some metabolic disorders of fat metabolism.

(i) chylomicrons,
(ii) Very-low-density lipoprotein (VLDL),
(iii) Intermediate-density lipoprotein (IDL),
(iv) Low-density lipoprotein (LDL)
(v) High-density lipoprotein (HDL).

Predominant lipid is triacylglycerol (50%) and cholesterol (23%). The concentrations of these are increased in atherosclerosis and coronary thrombosis etc.

LDL: Predominant lipid is cholesterol (46%) and phospholipids (23%).Increase in atherosclerosis and coronary thrombosis, etc.

HDL: Predominant lipid is phospholipid (27%) and proteins (45%). The protein moiety lipoprotein is known as an apo protein which constitute nearly 60% of some HDL and 1% of chylomicrons. Many lipoproteins contain more than one type of apoprotein polypeptide.

The larger lipoproteins (such as chylomicrons and VLDL) consist of a lipid core of non-polar triacylglycerol and cholesteryl ester surrounded by more polar phospholipid,

cholesterol and apoproteins.

The table gives the properties of different lipo proteins

Lipoprotein class	Density (g/mL)	Diameter (nm)	Protein % of dry wt	Phospholipid %	Triacylglycerol % of dry wt
HDL	1.063-1.21	5 – 15	33	29	8
LDL	1.019 – 1.063	18 – 28	25	21	4
IDL	1.006-1.019	25 - 50	18	22	31
VLDL	0.95 – 1.006	30 - 80	10	18	50
chylomicrons	<0.95	100 - 500	1 - 2	7	84

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Importance

- To transport and deliver the lipids to tissues.
- To maintain structural integrity of cell surface and sub cellular particles like mitochondria and microsomes.
- The β-lipoprotein fraction increases in severe diabetes mellitus, atherosclerosis etc. Hence determination of the relative concentrations of α and β-lipoproteins and pre-β- lipoproteins are of diagnostic importance.

Aminolipids

Phosphatidyl ethanolamine and serines are aminolipids and sphingomyelins and gangliosides contain substituted amino groups.

Sulpholipids (Sulphatides)

- These have been isolated from brain and other animal tissues.
- These are sulphate derivatives of the a1actosyl residue in cerebrosides.

Derived Lipids

Fatty Acids

- In chemistry, especially biochemistry, a fatty acid is a carboxylic acid with a long aliphatic tail (chain), which is either saturated or unsaturated.

- Most naturally occurring fatty acids have a chain of an even number of carbon atoms, because they are synthesized from 2-carbon units and are straight chain derivatives (from 4 to 28.)
- These are obtained by the hydrolysis of fats. Fatty acids are usually derived from triglycerides or phospholipids.
- When they are not attached to other molecules, they are known as "free" fatty acids.
- The straight chain may be saturated (containing no double bonds) or unsaturated (containing one or more double bonds).
- Carbon atoms of fatty acids are numbered from the carboxyl carbon (carbon No.1). The carbon atom adjacent to the carboxyl carbon (Carbon No. 2) is also known as the α-carbon. Carbon atom No. 3 is the β-carbon and the end methyl carbon is known as the γ-carbon.
- Various conventions are used for indicating the number and position of the double bonds, eg, Δ^9 indicates a double bond between carbon atoms 9 and 10 of the fatty acid.

Functions

- Fatty acids are important sources of fuel because, metabolized, they yield large quantities of ATP.
- Many cell types can use either glucose or fatty acids for this purpose.
- In particular, heart and skeletal muscle prefer fatty acids.
- The brain cannot use fatty acids as a source of fuel; it relies on glucose or ketone bodies

Types

1. Straight chain.
2. Branched chain.
3. Substituted (methyl substituted -cerebronic acid)
4. Cyclic (chaulmoogric acid) used in leprosy.

Straight chain

(a) Saturated {odd (less than 10 carbon atom)} & even (greater than 10 carbon atom)}.

(b) Unsaturated (odd & even). (Straight chain even number fatty acid is common)

Saturated Fatty Acids

General formula for saturated fatty acids is CnH2n+l COOE. Other higher fatty acids occur in waxes. A few branched-chain fatty acids have also been isolated from both plant and animal sources. Prostanoids include Prostaglandins (PC), and thromboxanes (TX).

General characteristics of prostanoid

(a) All are 20 carbon compound.

(b) Trans double bond at 13 position.

(c) -OH group at 15 position.

Saturated Fatty Acids

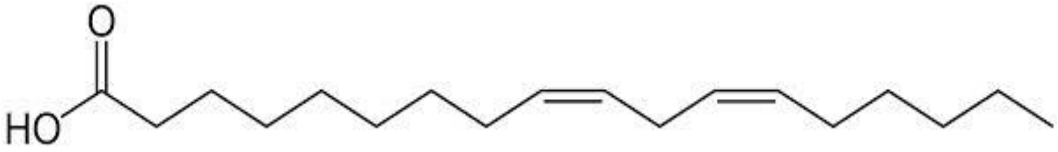
Acid	*Formula*	*Carbon atoms*	*Sources*
Acetic	CH_3COOH	2	Product of carbohydrate fermentation by rumen organisms
Propionic	C_2H_5COOH	3	— do—
Butyric	C_3H_7COOH	4	Butter.
Caproic	$C_5H_{11}COOH$	6	Product of carbohydrate fermentation,by rumen organisms
Caprylic	$C_7H_{15}COOH$	8	Butter.
Decanoic (Capric)	$C_9H_{19}COOH$	10	Butter.
Lauric	$C_{11}H_{23}COOH$	12	Coconut oils.
Myristic	$C_{13}H_{27}COOH$	14	Coconut oils.
Palmitic	$C_{15}H_{31}COOH$	16	Animal and plant fats.
Stearic	$C_{17}H_{35}COOH$	18	–do–
Arachidic	C_{19} H_{39} COOH	20	Peanut oil.
Behenic	$C_{21}H_{43}COOH$	22	Seeds.
Lignoceric	$C_{23}H_{47}COOH$	24	Peanut oil, crebrosides.

Unsaturated fatty acids

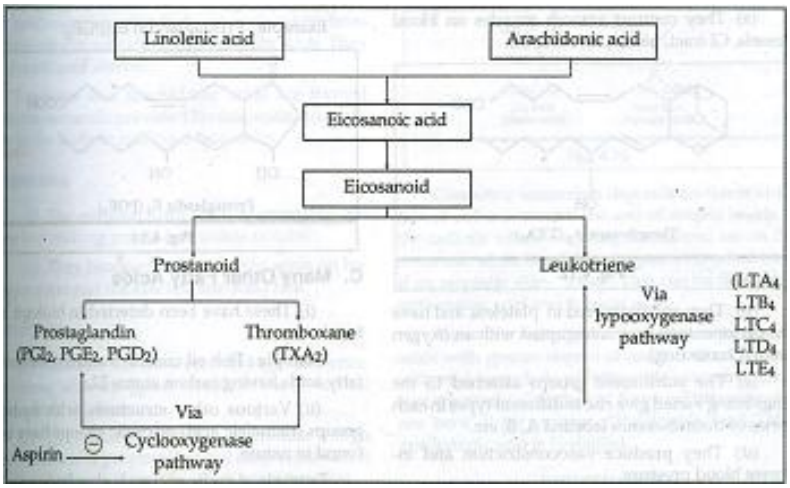
A.General formula C_nH2n-l COOH

Type of acid	*Acid*	*Formula*	*Unsaturation at carbon atoms*	*Number of double bonds*	*Sources*
Mono-unsaturated	Palmitoleic	$C_{15}H_{29}COOH$	Δ^9	1	Near all fats
"	Oleic	$C_{17}H_{33}COOH$	Δ^9	1	do
Poly-unsaturated	Linoleic	$C_{17}H_{31}COOH$	Δ^9, Δ^{12}	2	Animal and plant fat
"	Linolenic	$C_{17}H_{29}COOH$	$\Delta^9, \Delta^{12}, \Delta^{15}$	3	do
"	Arachidonic	$C_{19}H_{31}COOH$	$D^5, \Delta^8, \Delta^{11}, \Delta^{14}$	4	Peanut oil
Eicosanoids					
Prostanoids & Leukotrienes	Timnodonic	$C_{19}H_{29}COOH$	$\Delta^5, \Delta^8, \Delta^{11}, \Delta^{14}, \Delta^{17}$	5	Fish oils, eg. cod liver oil
"	Clupanodonic	$C_{21}H_{31}COOH$	$\Delta^7, \Delta^{10}, \Delta^{13}, \Delta^{16}, \Delta^{19}$	5	Fish oils, phospholipids in brain
"	Cervonic	$C_{21}H_{31}COOH$	$\Delta^4, \Delta^7, \Delta^{10}, \Delta^{13}, \Delta^{16}, \Delta^{19}$	6	Fish oils, phospholipids in brain

- Fatty acids with one double bond are monounsaturated and those with 2 or more double bonds are collectively known as polyunsaturated fatty acids (PUFA).
  Eg: Linoleic acid



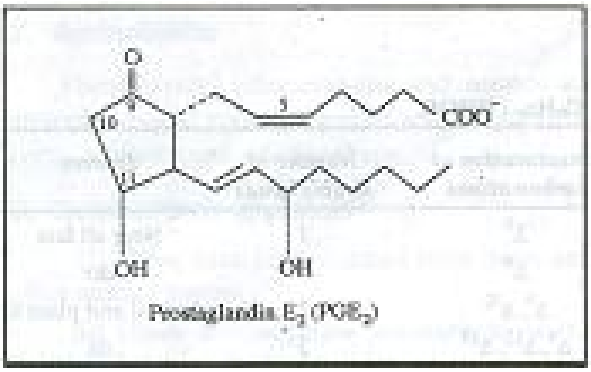
Classification



Three different eicosanoic fatty acids give rise to three groups of eicosanoids characterized by the number of double bonds in the side chains, eg, PG_1, PG_2, PG_3. Variations in the substituent groups attached to the rings give rise to different types in each series of prostaglandins, as for example, "E" type of Prostaglandin has a keto group in position9,whereas the "F" type has a hydroxyl group in this position,

Prostacyclins (PGI)

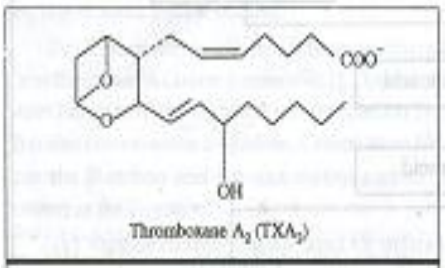
- They are formed in vascular endothelium and continually formed in heart. They are also formed in kidneys.
- They are formed from cyclic endoperoxide PGH_2 by the action of microsomal Prostacyclin synthetase.
- They inhibit platelet aggregation and gastric secretion from the pyloric mucosa.



Prostaglandin E_2 (PGE_2)

- They decrease blood pressure and protect coronary arteries.
- They increase renal blood flow and stimulate renin production.
- They are inhibited by hyperlipemia, vit. E deficiency and radiation.

Thromboxanes

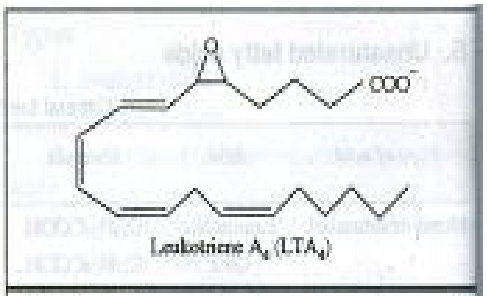


Thromboxane A_2 (TXA_2)

- They contract smooth muscles on blood vessels, GI tract, uterus, bronchioles.
- They are discovered in platelets, and have the cyclopentane ring interrupted with an oxygen atom (Oxane ring).
- The substituent groups attached to the rings being varied give rise to different types in each series of thromboxanes labelled A, B, etc.
- They produce vasoconstriction and in- crease blood pressure.
- They cause release of serotonin and calcium ion (Ca ++) from platelet granules.
- lmidazoles inhibit their synthesis.

Leukotrienes

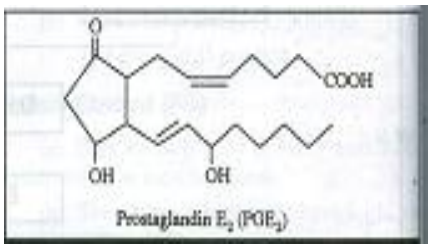
- They are the third group of eicosanoid derivatives formed via the lipoxygenase pathway rather than cyclization of the fatty acid chain.

- 

- They are first described in leukocytes.
- They are characterized by the presence of three conjugated double bonds.
- They are stimulators of mucus secretion and are responsible for vasoconstriction of bronchial muscles.
- They are inhibited by prolonged use of aspirin.
- The groups of compounds known as prostaglandins are synthesized from arachidonic acid in the body. They have pharmacologic and biochemical activity.

Prostaglandins (PG)

- They virtually exist in every mammalian tissue and act as local hormones.
- They have important physiologic anc pharmacologic activities.
- They are synthesized in vivo by cyclization of the center of the carbon chain of 2OC polyunsaturated fatty acids (eg, arachidonic acid) to fom a cyclopentane ring.
- **Example:** Prostaglandin E_2(PGE_2)



Many Other Fatty Acids

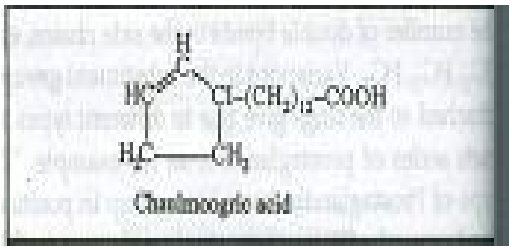
- These have been detected in biologic material.

Example: Fish oil contain 5 and. 6 unsaturated fatty acids having carbon atoms 22.

- Various other structures with hydroxy groups (ricinoleic acid) or cyclic groups have been found in nature. .

Example of cyclic groups is chaulmoogric acid which was used many years ago in the

treatment of leprosy.



Chaulmoogric acid

Essential fatty acids	Chemical name	General formula	No. of double bonds	Structure	Sources
Linoleic	9, 12-Octadecadi-enoic acid	$C_nH_{2n-3}COOH$	$2(\Delta^9,\Delta^{12})$	CH_3-$(CH_2)_4$ $(CH=CH-CH_2)_2$ $(CH_2)_6$ COOH	Corn, Peanut, Cotton seed, Soyabean oil.
Linolenic	6,9,12-Octadeca-trienoic acid	$C_nH_{2n-5}COOH$	$3(\Delta^6,\Delta^{12},\Delta^{15})$	CH_3-$(CH_2)_4$ $(CH=CH-CH_2)_3$ $(CH_2)_3$ COOH	Found frequently with linoleic acid but particularly in linseed oil.
Arachidonic	5,8,11,14-eicosatetra-enoic acid	$CnH_{2n-7}COOH$	$4(\Delta^5,\Delta^8,\Delta^{11},\Delta^{14})$	CH_3-$(CH_2)_4(CH$ $=CH-CH_2)_4$ $(CH_2)_2$ COOH	Found in small quantities with linoleic and linolenic acids but particularly in peanut oil.

Essential Fatty Acids

Burr and Burr (1930) introduced the term "Essential Fatty Acids" (EF A) on the basis that they are essential for the growth and health of young albino rats. These polyunsaturated fatty acids which are not synthesized in the body but are taken from natural sources are called essential fatty acids. They are (mentioned above): linolenic and arachidonic acids are formed from linoleic acids provided linoleic acids are available in the body in sufficient quantities.

Properties

- The essential fatty acids of vegetable oils have low melting points and iodine number.
- They become saturated fatty acids on hydrogenation and the oils become solid fats.

Functions

- The essential fatty acids in high concentration along with the lipids constitute the structural elements of the tissues.
- The lipids of gonads also contain a high concentration of polyunsaturated fatty acids which suggest the importance of reproductive function.

- They effect the prolongation of clotting time and increase the fibrinolytic activity.
- They retard atherosclerosis being esterified and emulsified with cholesterol and are incorporated into lipoproteins for transport to the liver for further oxidation.
- They cure skin lesions.
- The deficiency of these acids in the diet of babies causes eczema.

Isomerim in Unsaturated Fatty Acids

Variations in the locations of the double bond in unsaturated fatty acid chains produce isomers. Oleic acid has 15 different positional isomers.

Geometric isomerism depends on the orientation of radicals around the axis of double bonds. If the radicals which are being considered are on the same side of the bond, the compound is called "cis", if on opposite side, "trans". This can be illustrated with maleic acid and fumaric acid.

There are more geometric isomers in case of acids with greater degree of unsaturation. The un- saturated long chain of fatty acids occurring in nature are nearly all in tbe 'cis' form and the molecules are "bent" at the position of the double bond. Thus, arachidonic acid is D-shaped.

Refined and Hydrogenated Oils

Refined oil: It is prepared in the following manner:

- Free fatty acids are removed by alkali treatment
- Colouring matter is removed by activated carbon.
- Odour is '∼ov"" by superheated steam,

Essential fatty acids, or EFAs, are fatty acids that humans and other animals must ingest because the body requires them for good health but cannot synthesize them. The term "essential fatty acid" refers to fatty acids required for biological processes but does not include the fats that only act as fuel. Only two fatty acids are known to be essential for humans: alpha-linolenic acid (anomega-3 fatty acid) and linoleic acid (an omega-6 fatty acid).

Some other fatty acids are sometimes classified as "conditionally essential," meaning that they can become essential under some developmental or disease conditions;

examples include docosahexaenoic acid (an omega-3 fatty acid) and gamma-linolenic acid(an omega-6 fatty acid).

The essential fatty acids start with the short chain polyunsaturated fatty acids (SC-PUFA):

- ω-3 fatty acids:
- α-Linolenic acid or ALA (18:3n3)
- ω-6 fatty acids:
- Linoleic acid or LA (18:2n-6)

These two fatty acids cannot be synthesized by humans because humans lack the desaturase enzymes required for their production.

They form the starting point for the creation of longer and more desaturated fatty acids, which are also referred to as long-chain polyunsaturated fatty acids (LC-PUFA):

ω-3 fatty acids

eicosapentaenoic acid or EPA (20:5n-3)

docosahexaenoic acid or DHA (22:6n-3)

ω-6 fatty acids

gamma-linolenic acid or GLA (18:3n-6)

dihomo-gamma-linolenic acid or DGLA (20:3n-6)

arachidonic acid or AA (20:4n-6)

ω-9 fatty acids are not essential in humans because they can be synthesized from carbohydrates or other fatty acids.

Functions of Essential Fatty Acids

Essential fatty acids have a ton of benefits in our body. They are

- They help with cellular development and the formation of healthy cell membranes, and they have actually been shown to block tumor formation in animals, as well as block the growth of human breast cancer cells.
- Essential fatty acids assist in the development and function of the brain and nervous system.
- Helps to regulate proper thyroid and adrenal activity.
- They play a role in thinning blood, which can prevent blood clots that lead to heart attacks and stroke.

- They also possess natural anti-inflammatory qualities that can relieve symptoms of both arthritis and other autoimmune system diseases.
- Essential fatty acids regulate blood pressure, immune responses and liver function, as well as help with blood clotting and breaking down cholesterol.
- Diet low in these fatty acids has been shown to create skin problems, including eczema, dandruff, split nails and brittle hair.

Hydrogenated oils

The refined oils are hydrogenated under optimum temperature and pressure with hydrogen in the" presence of nickel catalyst. . Unsaturated fatty acids are converted into saturated fatty' acids.

Hydrogenation

Oleic acid----------Stearic acid

The liquid oil becomes solid fat and the unsaturated fatty acid content decreases. Vanaspati is hydrogenated refined groundnut oil.

Alcohols

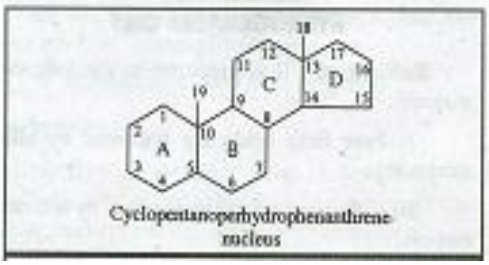
Alcohols found in lipid molecules include glycerol, cholesterol and higher alcohols (cetyl alcohol), usually found in the waxes.

The unsaturated alcohols are important pigments. Phytyl alcohol is a constituent of chlorophyll and lycophyll ($C_{40}H_{56}O_2$); a polyunsaturated dihydroxy alcohol occurs in tomatoes as a purple pigment.

Steroids

The steroids are often found in association with fat. They have a similar cyclic nucleus resembling phenanthrene (rings A, B, C) to which a cyclopentane ring (D) is attached. The parent substance is better designated as cyclopentano-perydrophen anthrene. The positions on the steroid nucleus are numbered as shown in the figure.

Methyl side chains occur typically at positions 10 and 13 (constituting C atoms 19 and 18). A side chain at position 17 is usual (as in cholesterol). *H* the compound has one or more hydroxyl groups and no carbonyl or carboxyl groups, it is a *sterol,* and the name terminates in-OL.



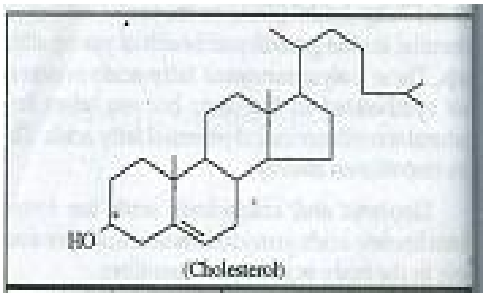
Cyclopentanope Rhydrophenanthrene. nucleus

Steroids may be divided in the following manner:

- Sterols=cholesterol, ergosterol, coprosterol.
- Bile acids-Glycocholic acid and taurocholic acid.
- Sex hormones-Testosterone, Estradiol.
- Vitamin D-Vit. 02 and 03 .
- Adrenocortical hormones-Corticosterone.
- Cardiac glycosides-Stropanthin.
- Saponins-Digitonin.

Cholesterol

It is widely distributed in all cells of the body. It occurs in animal fats but not in plant fats. Its structure is given below. The metabolism of cholesterol is discussed in the chapter of lipid metabolism



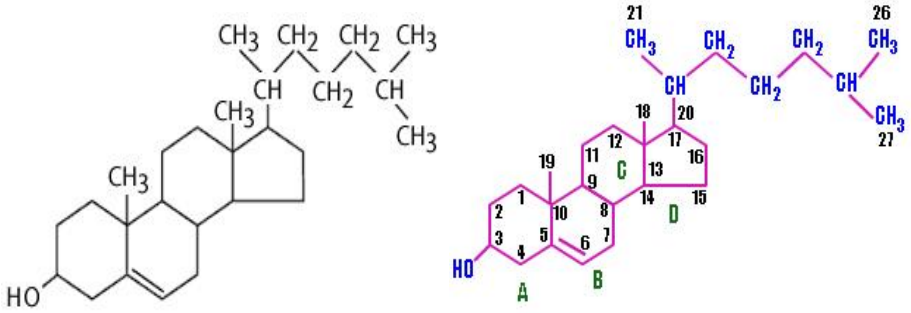
(Cholesterol)

Cholesterol and significance

Cholesterol

- Cholesterol, exclusively found in animals, is the most abundant animal sterol.
- It is widely distributed in all cells and is a major component of cell membranes and lipoproteins.
- Cholesterol (Creek: chole-bile) was first isolated from bile.

- Cholesterol literally means 'solid alcohol from bile.'



Functions of Cholesterol

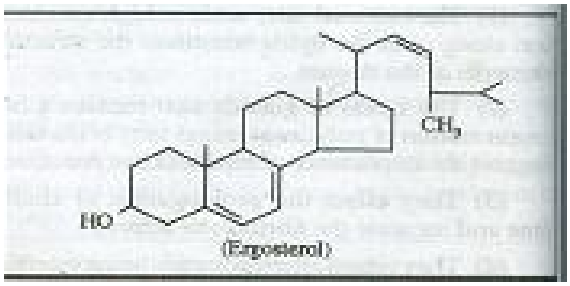
- Cholesterol is a poor conductor of heat and electricity, since it has a high dielectric constant.
- It is present in abundance in nervous tissues.
- It appears that cholesterol functions as an insulating cover for the transmission of electrical impulses in the nervous tissue.
- Cholesterol performs several other biochemical functions which include its role in membrane structure and function, in the synthesis of bile acids, hormones (sex and cortical) and vitamin D.

Erogesrol

(i) It occurs in ergot and yeast.

(ii) It is the precursor of vitamin 0.

(iii) It acquires antirachitic properties with the opening of ring B when irradiated with ultraviolet light.



Coprosterol

It occurs in feces as a result of the reduction by bacteria in the intestine of the double

bond between C_5, and C_6 of cholesterol.

Important Tests

(1) Greese spot test: A drop of oil placed over a piece of ordinary paper. A translucent spot is visible. This indicates the presence of fat.

(2) Emulsification test: 2 ml water is taken in one test tube and 2 ml of diluted bile salt solution in another test tube. Add 3 drops of the given oil to each test tube and shake vigorously. Note the stability of the emulsification formed.

(3) Saponification test: Take 10 drops of coconut oil in a test tube. Add 20 drops of 40% NaOH and 2 ml of glycerol to it. Gently boil for about 3 minutes until complete saponification occurs. If oil globules are visible, boiling must be continued. Divide the solution into 3 parts to carry the following experiments in test tube 1, 2, 3.

To test tube No. 1 add saturated solution of NaCl. Note that the soap separates out and floats to the surface (salting out process).

To test tube No.2 add a few drops of cone. HCl. An oily layer of the fatty acids rises to the surface.

To test tube No.3 add a few drops of $CaCl_2$ solution. The insoluble calcium soap is precipitated. . .

Unsaturation test

Add 10 drops of Huble's iodine reagent to 10 rnl of chloroform. The chloroform assumes a pink colour due to the free iodine. The solution is divided equally into three test tubes as (a), (b) and (c) and three types of oil are added.

Add the oil No. 1 to the test tube (a) drop by drop shaking the tube vigorously after each addition till the pink colour of the solution just disappears. The number of oil drops required are noted. The experiment is repeated by oil 2 and 3 adding to test tubes (b) and (c), respectively. The more the number of drops required to discharge the pink colour, the less is the unsaturation.

Colour Reactions to Detect Sterols

Liebermann-Burchard Reaction: A chloroform solution of a sterol when treated with acetic anhydride and sulphuric acid gives a green colour .This reaction is the basis of a colorimetric estimation of blood cholesterol.

Salkowski test: A red to purple colour appears when a chloroform solution 0; the sterol is treated with an equal volume of concentrated sulphuric acid. .

Clinical Orientation

- The high concentration of polyunsaturated fatty acids in the lipids of gonads are important in reproductive function. .
- The essential fatty acid deficiency causes swelling of mitochondrial membrane resulting. in the reduction in efficiency of oxidative phosphorylation producing increased heat.
- Docosahexenoic acid formed from dietary linolenic acids enhances the electrical response of the photoreceptors to illumination. Therefore; linolenic acid of the diet is essential for optimal vision.
- The deficiency of essential fatty acids causes skin lesions, abnormal pregnancy and lactation in adult females, fatty liver, kidney damage.
- The genetic deficiency of lecithin cholesterol acyl transferase (LCAT) .causes Norum's Disease.
- Sitosterol decreases the intestinal absorption of exogenous and endogenous cholesterol and thereby lowers the blood cholesterol level.
- The deficiency of the enzyme sphingomyelinase. Causes the large accumulations of sphingomyelins in brain, liver and spleen of children resulting in the Niemann-Pick disease with the symptoms of enlarged abdomen, liver, spleen and mental deterioration.
- Absence of dipalmityl lecithin (DPL} in premature foetus produces respiratory distress syndrome (Hyaline-membrane disease).
- The inherited Gaucher's Disease in infancy and childhood is caused by the deficiency of the enzyme glucocerebrosldase involving the large accumulations of glucocereqrosides (usually Kerasin) in the liver, spleen, bone marrow, and brain with the manifestations of weight loss, failure in growth, and progressive mental retardation.
- The autosomal recessive Tay-Sach's Disease (GM_2 Gangliosidosis) results in the accumulation of large amounts of gangliosides in the brain and nervous tissues due

to the absence of the enzyme hexosaminidase A with the association of progressive development of idiocy and blindness in infants soon after birth.

- The inherited disorder Metachromatic Leukodystrophy (MLD) happens on the salfatide,

  formed from galactocerebroside, accumulation in various tissues owing to the deficiency of the enzyme sulfatase (Aryl salfatase) with the symptoms of weakness, ataxia, defects in locomotion, paralysis, difficulties in speech in children before three years of age and psychiatric manifestation including progressive dementia in adults.

- Obesity and atherosclerosis are distinctly related to the concentrations of cholesterol and polyunsaturated fatty acids in the body.

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Question	Opt A	Opt B	Opt C
An example of a hydroxy fatty acid is	Ricinoleic acid	Crotonic acid	Butyric acid
An example of a saturated fatty acid is	Palmitic acid	Oleic acid	Linoleic acid
If the fatty acid is esterified with an alcohol c	Lipositol	Plasmalogen	Wax
A fatty acid which is not synthesized in the b	Palmitic acid	Lauric acid	Linolenic acid
Essential fatty acid:	Linoleic acid	Linolenic acid	Arachidonic acid
The fatty acid present in cerebrosides is	Lignoceric acid	Valeric acid	Caprylic acid
The number of double bonds in arachidonic a	1	2	4
In humans, a dietary essential fatty acid is	Palmitic acid	Stearic acid	Oleic acid
A lipid containing alcoholic amine residue is	Phosphatidic acid	Ganglioside	Glucocerebroside
Cephalin consists of	Glycerol, fatty acic	Glycerol, fatty aci	Glycerol, fatty acids, ph
In mammals, the major fat in adipose tissues	Phospholipid	Cholesterol	Sphingolipids
Glycosphingolipids are a combination of	Ceramide with on	Glycerol with gala	Sphingosine with galact
The importance of phospholipids as constitu	Fatty acids	Both polar and nc	Glycerol
In neutral fats, the unsaponificable matter in	Hydrocarbons	Triacylglycerol	Phospholipids
Higher alcohol present in waxes is	Benzyl	Methyl	Ethyl
Kerasin consists of	Nervonic acid	Lignoceric acid	Cervonic acid
Gangliosides are complex glycosphingolipids	Liver	Brain	Kidney
Unsaturated fatty acid found in the cod liver	Clupanodonic acid	Cervonic acid	Elaidic acid
Phospholipid acting as surfactant is	Cephalin	Phosphatidyl inos	Lecithin
An oil which contains cyclic fatty acids and or	Elaidic oil	Rapeseed oil	Lanoline
Unpleasant odours and taste in a fat (rancidi	Lead	Copper	Tocopherol
Gangliosides derived from glucosylceramide	Sialic acid	Glycerol	Diacylglycerol
'Drying oil', oxidized spontaneously by atmos	Coconut oil	Peanut oil	Rape seed oil
Deterioration of food (rancidity	Cholesterol	Vitamin E	Peroxidation of lipids
The number of ml of N/10 KOH required to n	Reichert-Meissel r	Polenske number	Acetyl number
Molecular formula of cholesterol is	C27H45OH	C29H47OH	C29H47OH
The cholesterol molecule is	Benzene derivativ	Quinoline derivat	Steroid
Salkowski test is performed to detect	Glycerol	Cholesterol	Fatty acids
Palmitic, oleic or stearic acid ester of cholest	Elaidic oil	Lanoline	Spermaceti
Dietary fats after absorption appear in the ci	HDL	VLDL	LDL
Free fatty acids are transported in the blood	Combined with alk	Combined with fa	Combined with β -lipop
Long chain fatty acids are first activated to ac	Cytosol	Microsomes	Nucleus
The enzyme acyl-CoA synthase catalyses the	AMP	ADP	ATP
Carnitine is synthesized from	Lysine and methio	Glycine and argin	Aspartate and glutamat
The enzymes of β-oxidation are found in	Mitochondria	Cytosol	Golgi apparatus
Long chain fatty acids penetrate the inner mi	Freely	As acyl-CoA deriv	As carnitine derivative
Dietary fibres are rich in	Cellulose	Glycogen	Starch

The end products of saponification:	glycerol	acid	soap
Triglycerides are	Heavier than wate	Major constituent	Non-polar
Cerebronic acid is present in	Glycerophospholip	Sphingophospholi	Galactosyl ceramide
Acylsphingosine is also known as	Sphingomyelin	Ceramide	Cerebroside
The highest phospholipids content is found ir	Chylomicrons	VLDL	LDL
The major lipid in chylomicrons is	Triglycerides	Phospholipids	Cholesterol
Number of carbon atoms in cholesterol is	17	19	27
The lipoprotein richest in cholesterol is	Chylomicrons	VLDL	LDL
The nitrogenous base in lecithin is	Ethanolamine	Choline	Serine
All the following are omega-6-fatty acids exc	Linoleic acid	α-Linolenic acid	γ-Linolenic acid
All the following have 18 carbon atoms excep	Linoleic acid	Linolenic acid	Arachidonic acid
A 20-carbon fatty acid among the following is	Linoleic acid	α -Linolenic acid	β -Linolenic acid
Predominant fatty acids in meat are	Saturated	Monounsaturated	Polyunsaturated
Cholesterol is present in all of the following e	Egg	Fish	Milk
Which of the following has the highest chole	Meat	Fish	Butter
Cholesterol is a	Animal sterol	M.F. C27 H46O	5 methyl groups
Lieberman-Burchard reaction is performed to	Cholesterol	Glycerol	Fatty acid
Fatty acids are oxidized by	α -oxidation	β -oxidation	ω -oxidation
Which of the following is not an unsaturated	Oleic acid	Stearic acid	Linaoleic acid
Calorific value of lipids per gm is	4 Kcal	8 Kcal	9 Kcal
Saponification:	Hydrolysis of fats l	Hydrolysis of glyc	Esterification
In cephalin, choline is replaced by	Serine	Ethanolamine	Betaine
A fatty acid not synthesized in man is	Oleic	Palmitic	Linoleic

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RTMENT OF MICROBIOLOGY
ROBIOLOGY – FIRST SEMESTER
BU105A – BIOCHEMISTRY
TIPLE CHOICE QUESTIONS
Unit 3

Opt D	Answer
Oleic acid	Ricinoleic acid
Erucic acid	Palmitic acid
Cephalin	Wax
Palmitoleic acid	Linolenic acid
All these	All these
Behenic acid	Lignoceric acid
6	4
Linoleic acid	Linoleic acid
Sphingomyelin	Sphingomyelin
Glycerol, fatty acids, p	Glycerol, fatty acids, phosphoric acid and ethanolamine
Triacylglycerol	Triacylglycerol
Sphingosine with phos	Ceramide with one or more sugar residues
Phosphoric acid	Both polar and nonpolar groups
Cholsesterol	Hydrocarbons
Cetyl	Cetyl
Clupanodonic acid	Lignoceric acid
Muscle	Brain
Timnodonic acid	Timnodonic acid
Phosphatidyl serine	Lecithin
Chaulmoogric oil	Chaulmoogric oil
Ergosterol	Tocopherol
Hyaluronic acid	Sialic acid
Linseed oil	Linseed oil
Phenolic compounds	Peroxidation of lipids
Non volatile fatty acid	Reichert-Meissel number
C23H41OH	C27H45OH
Straight chain acid	Steroid
Vitamin D	Cholesterol
Chaulmoogric oil	Lanoline
Chylomicron	Chylomicron
In unbound free salts	Combined with albumin
Mitochondria	Cytosol
GTP	ATP
Proline and hydroxypr	Lysine and methionine
Nucleus	Mitochondria
Requiring Na depende	As carnitine derivative
Proteoglycans	Cellulose

Both (A and (C	Both (A and (C
Hydrophilic	Non-polar
Gangliosides	Galactosyl ceramide
Sulphatide	Ceramide
HDL	HDL
Free fatty acids	Triglycerides
30	27
HDL	LDL
Betaine	Choline
Arachidonic acid	α-Linolenic acid
Stearic acid	Arachidonic acid
Arachidonic acid	Arachidonic acid
Mono and poly-unsatı	Saturated
Pulses	Pulses
Milk	Butter
All of these	All of these
Vitamin D	Cholesterol
All of these	All of these
Palmitic acid	Stearic acid
None of these	9 Kcal
Reduction	Hydrolysis of fats by alkali
Sphingosine	Ethanolamine
Stearic	Linoleic

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DEPARTMENT OF MICROBIOLOGY
(For the candidates admitted from 2015 onwards)

Subject	**:**	**Biochemistry**	**Semester**	**:**	**I**
Subject code	**:**	**17MBU103**	**Class**	**:**	**I B.Sc Microbiology**

UNIT-IV: COURSE MATERIAL

Unit-IV

Classification and functions of proteins and amino acids, structure of amino acids and concept of zwitterions. Ninhydrin reaction. Natural modifications of amino acids in proteins. Non-protein amino acids, Oligopeptides: Structure and functions of glutathione, insulin and aspartame. Primary and secondary structure of proteins-alpha helix, beta pleated sheet. Tertiary and quaternary structures of proteins. Human haemoglobin structure.

Suggest Readings

1. Nelson, D.L and Cox, M.M. (2008). Lehninger Principles of Biochemistry, 5th edition. W.H. Freeman and Company.

Proteins

Proteins are important macromolecules of the cells, formed by the polymerization of amino acids. Proteins are the mode of expression of the genetic information. They performs various biology functions in the cells, such as they act as the structural components of cells, enzymes, hormones, pigments, storage proteins and some toxins in the cells.

Classification of proteins

Proteins are classified based on the following criterions:

(1) Structure

(2) Composition

(3) Function

Structure

Based on the structure, proteins are classified into 3 groups

(a) Fibrous proteins

(b) Globular Proteins

(c) Intermediate proteins

Fibrous Proteins

- They are linear in shape
- Secondary structure is the most important functional structure of fibrous proteins
- Usually, these proteins do not have tertiary structures
- Physically fibrous proteins are very tough and strong
- They are insoluble in the water
- Long parallel polypeptide chains cross linked at regular intervals
- Fibrous proteins form long fibres or sheaths.

Functions of fibrous proteins

- Perform the structural functions in the cells

Example: Collagen, Myosin, Silk and keratin

Globular Proteins

- Globular proteins are spherical or globular in shape
- The polypeptide chain is tightly folded into spherical shapes
- Tertiary structure is the most important functional structure in globular proteins

- Physically they are soft than fibrous proteins.
- They are readily soluble in water.
- Most of the proteins in the cells belong to the category of globular proteins.

Functions

- Form enzymes, antibodies, and some hormones.

Example: Insulin, Haemoglobin, DNA polymerase and RNA polymerase.

Intermediate proteins

- Their structure is intermediate to linear and globular structures.
- They are short and more or less linear shaped proteins
- Unlike Fibrous proteins, they are soluble in water.

Functions:

- Blood clotting proteins

Example: Fibrinogen

Composition

They are broadly divided into two types

(a) Simple proteins

(b) Conjugated proteins

Simple proteins

Simple proteins composed of only amino acids

Proteins may be fibrous or globular

They possess relatively simple structural organization

Example: Collagen, Myosin, Insulin, Keratin

Conjugated proteins

- Conjugated proteins are complex proteins
- They contain one or more non-amino acid components.
- Here the protein is tightly or loosely bound to one or more non-protein parts.
- The non-protein parts of these proteins are called prosthetic groups.
- The prosthetic group may be metal ions, carbohydrates, lipids, phosphoric acids, nucleic acids and FAD.
- The prosthetic group is essential for the biological functions of these proteins.

- Conjugated proteins are usually globular in shape and are soluble in water.
- Most of the enzymes are conjugated proteins.

Based on the nature of prosthetic groups, the conjugated proteins are further classified as follows.

Phosphoprotein: Prosthetic group is phosphoric acid, Example-casein of milk, vitellin of egg yolk.

Glycoproteins: Prosthetic group is carbohydrates, Example- most of the membrane proteins, mucin (Component of saliva).

Nucleoprotein: Prosthetic group is nucleic acid, Example- proteins in chromosomes, structural proteins of ribosome.

Chromoproteins: Prosthetic group is pigment of chrome, Example: Haemoglobin, Phytochrome and Cytochrome.

Lipoproteins: Prosthetic group is lipids, Example: Membrane proteins.

Flavoproteins: Prosthetic group is FAD (Flavin Adenine Dinucleotide), Example: Proteins of Electron Transport System (ETS).

Metalloproteins: Prosthetic group is metal ions, Example: Nitrate reductase.

Functions

Structural proteins

- Form the component of the connective tissue, bone, tendons, cartilage, skin, feathers, nails, hairs and horn
- Most of them are fibrous proteins and are insoluble in water.

Example: Collagen, Keratin and Elastin

Enzymes

- They are the biological catalysts
- Enzymes reduce the activation energy of reactants and speed up the metabolic reactions in the cells.
- Most of them are globular conjugated proteins.

Hormones

- They include the proteinaceous hormones in the cells.

Example: Insulin, Glucagon

Respiratory pigments

- They are coloured proteins
- All of them are conjugated proteins and they contain pigments (chrome) as their prosthetic group.

Example: Haemoglobin, Myoglobin

Transport proteins

- They transport the materials in the cells
- They form channels in the plasma membrane
- They also form one of the components of blood and lymph in animals

Example: Serum albumin

Contractile proteins

- They are the force generators of muscles
- They can contract with the expense of energy from ATP molecules.

Example: Actin, Myosin

Storage proteins

- They act as the store of metal ions and amino acids in the cells
- Found in seeds, egg and milk
- Abundantly seen in pulses (Legume seeds).

Example: Ferritin which stores iron, casein

Toxins

- They are toxic proteins

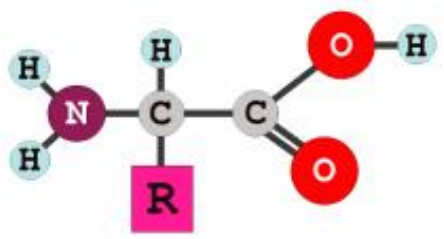
Example: Snake venom

Protein Class	Functions	Examples
Structural Proteins	They are used as bricks and mortars to construct the biological buildings and machineries	α-Keratin of fur, feathers, hairs and claws; collagens of skin, bone and cartilage
Carrier Proteins	They carry metabolites from one site to the other to make biological processes a reality	Haemoglobin carries oxygen; transferrin transports iron
Storage Proteins/ Nutrient Proteins	They serve as biological store houses to preserve nutritional proteins which act as source of essential amino acids	Casein of milk, ovalbumin and ovomucoid of egg, and glutelin of wheat, ferritin
Enzyme Proteins	They act as biological catalysts and make an otherwise slow or improbable reaction fast and feasible	Digestive enzymes trypsin and pepsin, papain from papaya and ribonucleases
Hormone Proteins	They act as biological signals; mediate and regulate physiological processes	Insulin, glucagons and adrenocorticotrophic hormone
Defense Proteins	Protect against foreign invaders like bacteria and viruses, make survival possible under hostile conditions	Antibodies, thrombin, antifreeze proteins and lysozyme in tears
Proteins as Toxins/ Poisons	They are toxic/poisonous to others but provide a defense tool to organisms they belong to	Snake venoms, diphtheria toxin, ricin in caster bean, gossypin of cotton seed

Functions of proteins

Amino acids

Amino acids as the building blocks of proteins. Amino acids is defined as a molecule containing an amine group (-NH2), carboxyl group (-COOH) and the variable group denoted as R, different among different amino acids. R groups is also called the side chain, The overall amino acid formula can be represented as : R-CH(NH2)-COOH. An average molecular weight is about 135 daltons.



Classification of Amino acids

Amino acids are broadly classified into four types

(a) Non-Polar

(b) Polar

(c) Acidic

(d) Basic

Other type of classifications

(a) Essential

(b) Non-essential

(c) Semi-essential

Non-polar Amino Acids

- The non-polar amino acids contain mostly hydrocarbon R groups that do not bear positive or negative charges.
- Non-polar (i.e., hydrophobic) amino acids play an important role in maintaining the three-dimensional structures of proteins, because they interact poorly with water.

Two types of hydrocarbon side chains are found in this group:

(a) Aromatic

(b) Aliphatic

Aromatic amino acids

- Aromatic amino acid contains aromatic ring in their structure
- Benzene is one of the simplest aromatic amino acid.

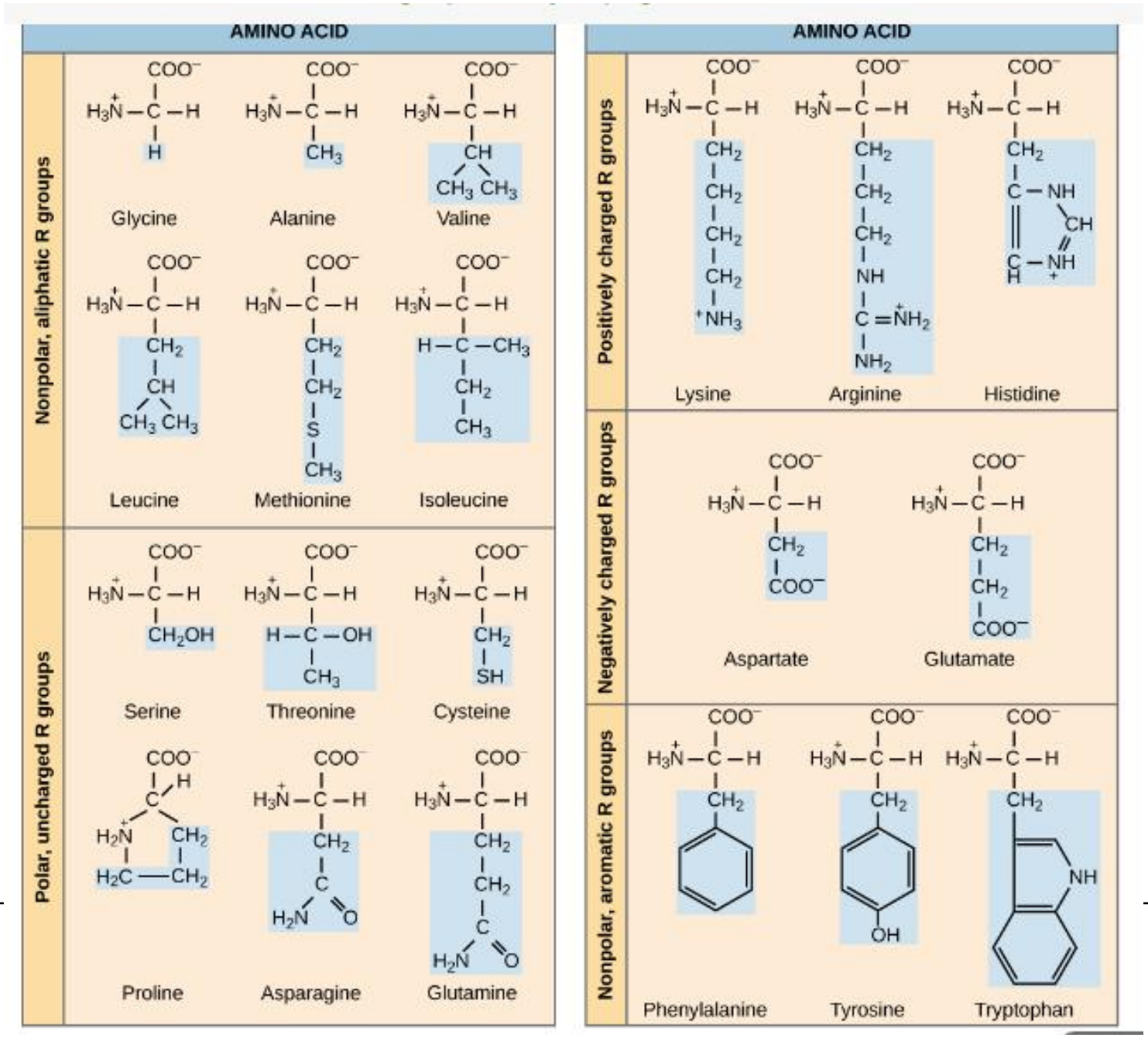
Aliphatic amino acids

- The term aliphatic refers to non-aromatic amino acids such as methane and cyclohexane.
- Phenylalanine and tryptophan contain aromatic ring structures.
- Glycine, alanine, valine, leucine, isoleucine, and proline have aliphatic R groups.

- A sulfur atom appears in the aliphatic side chains of methionine and cysteine. Methionine contains a thioether group (—S—CH3) in its side chain.

Polar Amino Acids

- Polar amino acids have functional groups capable of hydrogen bonding, they easily interact with water.
- Polar amino acids are described as hydrophilic, or "water-loving."
- Serine, threonine, tyrosine, asparagine, and glutamine belong to this category.
- Serine, threonine, and tyrosine contain a polar hydroxyl group, which enables them to participate in hydrogen bonding, an important factor in protein structure.
- The hydroxyl groups serve other functions in proteins.



Acidic Amino Acids

- Two standard amino acids have side chains with carboxylate groups.
- Because the side chains of aspartic acid and glutamic acid are negatively charged at physiological pH, they are often referred to as aspartate and glutamate.

Basic Amino acids

- Basic amino acids bear a positive charge at physiological pH.
- They can therefore form ionic bonds with acidic amino acids.
- Lysine, which has a side chain amino group, accepts a proton from water to form the conjugate acid (—NH3).

Classification based on nutritional requirements

Essential amino acids:

These amino acids cannot be synthesized in the body and have to be present essentially in the diet.

Examples: Valine, Isoleucine, Leucine, Lysine, Methionine, Threonin, Tryptophan and Phenylalanine.

Semi-essential amino acids

These amino acids can be synthesized in the body but the rate of synthesis is lesser than the requirement (e.g. during growth, repair or pregnancy)

Example: Arginine and Histidine

Non-essential amino acids

These amino acids are synthesized in the body, thus their absence in the diet does not adversely affect the growth.

Example: Glycine, Alanine and the other remaining amino acids.

Physical Properties of amino acids

- Colourless
- Crystalline
- May be sweet (Glycine, Alanine, Valine)
- Tasteless (Leucine)
- Bitter (Arginine, Isoleucine).
- Soluble in water, acids, but insoluble in organic solvents.

- High melting point (More than 200° C).

Chemical Properties of amino acids

Reaction due to NH2 group

- Reaction with acids to form salt
- Reaction with nitrous acids to liberate Nitrogen
- Reaction with CO2 to form carbamino compounds

Reaction due to COOH group

- Reaction with strong alkalies to form salt
- Reaction with alcohols to form esters

Reaction due to both NH2 and COOH group

- Amino acids condense with each other by COOH group at one amino acids with NH2 of other amino acid to form peptide bond.

Functions of amino acids

Histidine

- Found in high concentrations in hemoglobin.
- Useful in treating anemia due to relationship to hemoglobin.
- Has been used to treat rheumatoid arthritis.
- Precursor to histamine.
- Associated with allergic response and has been used to treat allergy.
- Assists in maintaining proper blood pH.

Isoleucine

- Muscle tissue uses Isoleucine as an energy source.
- Required in the formation of hemoglobin.

Leucine

- Potent stimulator of insulin.
- Helps with bone healing.
- Helps promote skin healing.
- Modulates release of Enkephalins, which are natural pain-reducers.

Lysine

- Helps form collagen, the connective tissue present in bones, ligaments, tendons, and joints.
- Assists in the absorption of calcium.
- Essential for children, as it is critical for bone formation.
- Involved in hormone production.
- Lowers serum triglyceride levels.

Methionine

- Assists in breakdown of fats.
- Precursor of the amino acids Cysteine and Taurine.
- Helps reduce blood cholesterol levels.
- Antioxidant.
- Assists in the removal of toxic wastes from the liver.
- Helps prevent disorder of hair, skin, and nails due to sulfur and anti-oxidant activity.
- Required for synthesis of RNA and DNA.
- Natural chelating agent for heavy metals, such as lead and mercury.

Phenylalanine

- Precursor to the hormone, Thyroxin.
- Enhances mood, clarity of thought, concentration, and memory.
- Suppresses appetite.
- Major part of collagen formation.
- Powerful anti-depressant.
- Used in the treatment of Parkinson's Disease.

Threonine

- Required for formation of collagen.
- Helps prevent fatty deposits in the liver.
- Aids in production of antibodies.
- Can be converted to Glycine (a neurotransmitter) in the central nervous system.
- Acts as detoxifier.

- Needed by the GI (gastrointestinal) tract for normal functioning.

Tryptophan

- Precursor to the key neurotransmitter, serotonin, which exerts a calming effect.
- Effective sleep aid, due to conversion to serotonin.
- Effective in some forms of depression.
- Treatment for migraine headaches.
- Stimulates growth hormone.
- Tryptophan must compete with 5 other amino acids to pass through the blood-brain barrier and enter the brain. Those 5 are: tyrosine, phenylalanine, leucine, isoleucine, and valine and are called Large Neutral Amino Acids (LNAA).

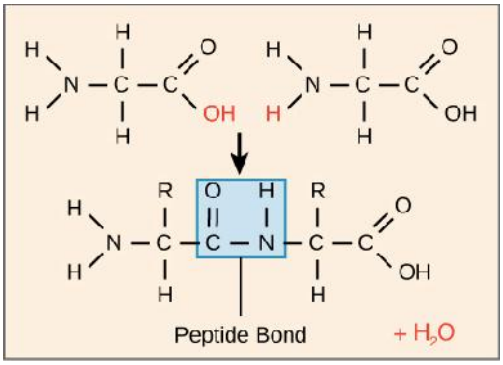
Valine

- Competes with Tyrosine and Tryptophan in crossing the blood-brain barrier.
- The higher the Valine level, the lower the brain levels of Tyrosine and Tryptophan.
- Actively absorbed and used directly by muscle as an energy source.

Peptide bonds

Each protein in your cells consists of one or more polypeptide chains. Each of these polypeptide chains is made up of amino acids, linked together in a specific order. The chemical properties and order of the amino acids are key in determining the structure and function of the polypeptide

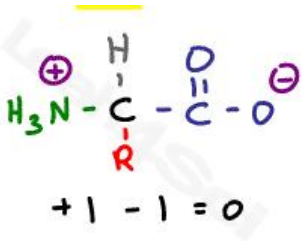
The amino acids of a polypeptide are attached to their neighbors by covalent bonds known as peptide bonds. Each bond forms in a dehydration synthesis (condensation) reaction. During protein synthesis, the carboxyl group of the amino acid at the end of the growing polypeptide chain reacts with the amino group of an incoming amino acid, releasing a molecule of water. The resulting bond between amino acids is a peptide bond.



At one end, the polypeptide has a free amino group, and this end is called the amino terminus (or N-terminus). The other end, which has a free carboxyl group, is known as the carboxyl terminus (or C-terminus).

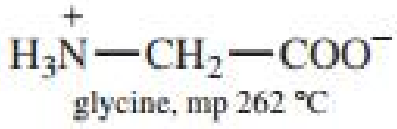
Concept of Zwitterions

Zwitterion is the dipolar form of an amino acid which occurs when H^+ ion is transferred from an acid group to an amine group.



The dipolar nature of amino acids gives them some unusual properties:

1. Amino acids have high melting points, generally over 200° C.



2. Amino acids are more soluble in water than they are in ether, dichloromethane, and other common organic solvents.
3. Amino acids have much larger dipole moments than simple amines or simple acids.
4. Amino acids are less acidic than most carboxylic acids and less basic than most amines.

Ninhydrin reaction

The reaction between alpha-amino acid and ninhydrin involved in the development of color.

alpha-amino acid + ninhydrin ---> reduced ninhydrin + alpha-amino acid + H_2O

alpha-amino acid + H_2O ---> alpha-keto acid +NH_3

alpha-keto acid + NH_3 ---> aldehyde + CO_2

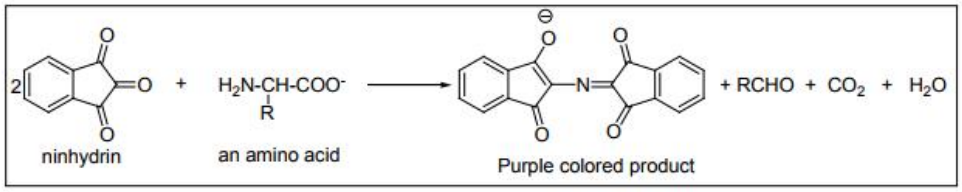
Step 1: it is an oxidative deamination reaction that removes two hydrogen from the alpha amino acid to yield an alpha-imino acid. Simultaneously, the original ninhydrin is reduced and loses an oxygen atom with the formation of a water molecule.

In Step 2: The NH group in the alpha imino acid is rapidly hydrolyzed to form an alpha keto acid with the production of an ammonia molecules.

Step 3: This alpha-keto acid further undergoes decarboxylation reaction under a heated condition to form an aldehyde that has one less carbon atom than the original amino acid. A carbon dioxide molecule is produced.

Further the overall, reaction becomes

alpha-amino acid + 2 ninhydrin ---> CO_2 + aldehyde + final complex(BlUE) + $3H_2O$



Non-Non-Protein amino acids

The amino acid which are not involved in the protein synthesis are called as non protein amino acids.

These non-protein amino acids are classified into two types

(a) Alpha

(b) Non-alpha

Alpha amino acids

- Ornithine
- Citrulline
- Thyroxine

- S-adenosylmethionine
- Homecysteine
- Ovathiol
- Azaserine

Non-Alpha amino acids

- Beta-alanine
- Beta-aminoisobutyric acid
- Gama-aminobutyric acid
- Aminolevulinic acid
- taurine

Alpha Amino acid

Ornithine

- Ornithine is precursors of polyamine
- Ornithine enters liver, mitochondria and participates in urea synthesis.

Citrulline

- Citrulline is intermediates in the biosynthesis of urea.
- L-ornithine transcarbamoylase catalyzes transfer of the carbamoyl group carbamoyl phosphate to ornithine, forming cirtulline and orthophosphate. While the reaction occurs in the mitochondrial matrix, both the formation of ornithine and the subsequent metabolism of citrulline take place in the cytosol.

Thyrosine

- Tyrosine forms norepinephrine and epinephrine and following iodination the thyroid hormones triiodothyronine and thyroxine.
- Use of measurement of blood thyroxine or thyroid stimulating hormone (TSH) in the neonatal diagnosis of congenital hypothyroisidm.
- The amino acid thyrosine is the starting point in the synthesis of the catecholamines and of the thyroid hormones tetraiodothyronine (thyroxine; T4) and triiodothyronine (T3).

Ovathiol

- Sulfur containing amino acid found in fertilized eggs, and acts as an antioxidant.

Azaserine

- Purine deficiency states, while rare in humans, generally reflect a deficiency of folic acid.
- Compounds that inhibit formation of tetrahydrofolates and therefore block purine synthesis have been used in cancer chemotherapy.
- Inhibitory compounds and the reactions they inhibit include, azaserine, diazanorleucine, 6-mercaptopurine and mycophenolic acid.

Non-alpha amino acids

Beta-Alanine and Aminoisobutyrate

- Alanine and aminoisobutyrate are formed during catabolism of the pyrimidines uracil and thymine.
- Traces of alanine also result from the hydrolysis of alanyl dipeptides by the enzyme carnosinase.
- Aminoisobutyrate also arises by transamination of methylmalonate semialdehyde, a catabolite of L-valine.
- The initial reaction of alanine catabolism is transamination to malonate semialdehyde.
- Subsequent transfer of coenzyme A from succinyl-CoA forms malonyl-CoA semialdehyde which is then oxidized to malonyl-CoA and decarboxylated to the amphibolic intermediate acetyl-CoA.

Beta-Alanyl Dipeptides

- The alanyl dipeptides carnosine and anserine (N-methylcarnosine) activate myosin ATPase chelate copper, and enhance copper uptake.
- Analyl-imidazole buffers the pH of anaerobically contracting skeletal muscle.
- Biosynthesis of carnosine is catalyzed by carnosine synthetase in a two-stage reaction that involves initial formation of an enzyme-bound acyl-adenylate of alanine and subsequenct transfer of the alanyl moiety to L-histidine.
- Hydrolysis of carnosine to alanine and L-histidine is catalyzed by carnosinase. The heritable disorder carnosinase deficiency is characterized by carnosinuria.

- Homocarnosine present in human brain at higher levels than carnosine is synthesized in brain tissue by carnosine synthetase. Serum carnosinase does not hydrolyze homocarnosine.
- Homocarnosinosis, a rare genetic disorder, is associated with progressive spastic paraplegia and mental retardation.

Gama-Aminobutyrate

- Gama-Aminobutyrate (GABA) functions in brain tissue as an inhibitory neurotransmitter by altering transmembrane potential differences.
- GABA is formed by decarboxylation of glutamate by L-glutamate decarboxylase.
- Trasamination of aminobutyrate forms succinate semialdehyde which can be reduced to hydroxybutyrate by L-lactate dehydrogenase,or be oxidized to succinate and thence via the citric and cycle to CO_2 and H_2O.
- A rare genetic disorder of GABA metabolism involves a defective GABA amino transferase, an enzyme that participate in the catabolism of GABA subsequent to its postsynpatic release in brain tissue.
- Defects in succinic semialdehyde dehydrogenase are responsible for another rare metabolic disorder of aminobutyrate catabolism characterized by 4-hydroxybutyric aciduria.

Structure and functions of Glutathione

Glutathione (GSH) is often referred to as the body's master antioxidant.

Composted of three amino acids

- Cysteine
- Glycine
- Glutamate

Glutathione can be found in virtually every cell of the human body.

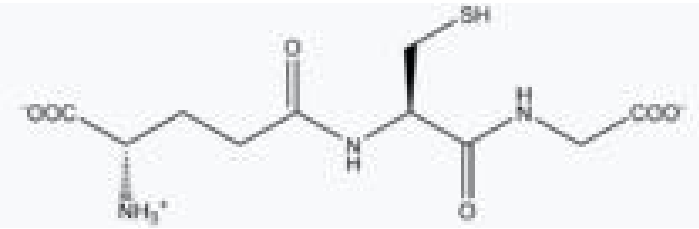
The highest concentration of glutathione is in the liver, making it critical in the body's detoxification process.

Glutathione is also an essential component to the body's natural defense system.

Viruses, bacteria, heavy metal toxicity, radiation, certain medications, and even the normal process of aging can all cause free-radical damage to healthy cells and deplete glutathione.

Glutathione depletion has been correlated with lower immune function and increased vulnerability to infection due to the liver's reduced ability to detoxify.

As the generation of free radicals exceeds the body's ability to neutralize and eliminate them, oxidative stress occurs.



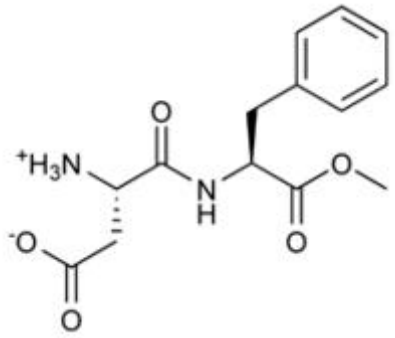
Functions of Glutathione

- It maintains levels of reduced glutaredoxin and glutathione peroxidase.
- It is one of the major endogenous antioxidants produced by the cells, participating directly in the neutralization of free radicals and reactive oxygen compounds
- Regulation of the nitric oxide cycle is critical for life, but can be problematic if unregulated
- It is used in metabolic and biochemical reactions such as DNA synthesis and repair, protein synthesis, prostaglandin synthesis, amino acid transport, and enzyme activation.
- Thus, every system in the body can be affected by the state of the glutathione system, especially the immune system, the nervous system, the gastrointestinal system, and the lungs.
- It has a vital function in iron metabolism.
- It has roles in progression of the cell cycle, including cell death.
- GSH levels regulate redox changes to nuclear proteins necessary for the initiation of cell differentiation.
- Differences in GSH levels also determine the expressed mode of cell death, being either apoptosis or cell necrosis.
- Manageably low levels result in the systematic breakage of the cell whereas excessively low levels result in rapid cell death.

Aspartame

- Aspartame is an artificial sweeter.

- Aspartame is 180 to 200 times sweeter than normal sugar.
- Aspartame is not suitable for baking because if often breaks down when heated and loses much of its wetness and at temperatures above 90̊ Fa component of it can covert to formaldehyde.



These aspartame is synthesized from two amino acids

- Aspartic acid
- Phenylalanine

Aspartame has the chemical formula of $C_{14}H_{18}N_2O_5$.

Upon ingestion, aspartame breaks down into several residual chemicals, including

- Aspartic acid
- Phenylalanine
- Methanol
- Formaldehyde

Methanol and Formaldehyde

- Approximately 10% of aspartame is broken down into methanol in the small intestine. Most of the methanol is absorbed and quickly converted into formaldehyde.
- High concentration, formaldehyde can kill cells and tissues.

Phenylalanine

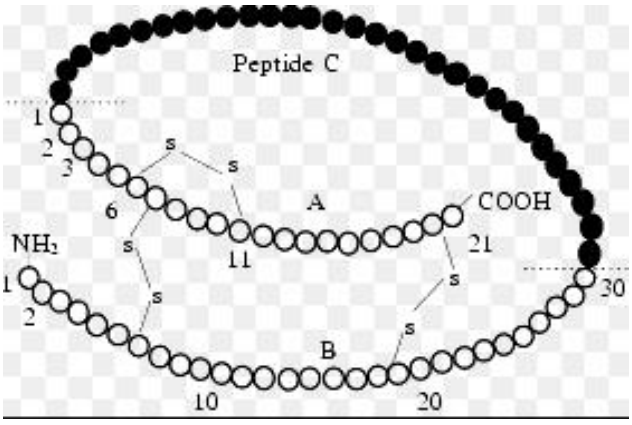
- One of the functional groups in aspartame is phenylalanine, which is unsafe for those born with phenylketonuria, a rare genetic conditions.
- Approximately 50% of aspartame is broken down into phenylalanine, which is completely safe for everyone except sufferers of phenylketonuria.

Aspartic acid

- Approximately 40 percent of aspartame is broken down into aspartic acid.
- Aspartic acid belongs to a class of chemicals that in high concentrations act as an excitotoxin, damage on brain and nerve cell.

Structure of Insulin

Insulin is a hormone secreted by the pancreas that regulates glucose levels in the blood. Without insulin, cells cannot use the energy from glucose to carry out functions within the body. Insulin was first discovered in 1921 by Frederick Grant Banting and Charles. The FDA approved insulin in 1939.



Insulin is composed of two peptide chains referred to as the A chain and B chain. A and B chains are linked together by two disulfide bonds, and an additional disulfide is formed within the A chain. In most species, the A chain consists of 21 amino acids and the B chain of 30 amino acids.

Although the amino acid sequence of insulin varies among species, certain segments of the molecule are highly conserved, including the positions of the three disulfide bonds, both ends of the A chain and the C-terminal residues of the B chain. These similarities in the amino acid sequence of insulin lead to a three dimensional conformation of insulin that is

very similar among species, and insulin from one animal is very likely biologically active in other species. Indeed, pig insulin has been widely used to treat human patients.

Insulin molecules have a tendency to form dimers in solution due to hydrogen-bonding between the C-termini of B chains. Additionally, in the presence of zinc ions, insulin dimers associate into hexamers.

Functions of insulin

- Insulin is made in the pancreas by beta cells.
- After the body takes in food, these beta cells release insulin, which enables cells in the liver, muscles and fat tissues to take up glucose and either store it as glycogen or allow blood to transfer it to organs in the body for use as an energy source.
- This process stops the use of fat as a source of energy.
- When glucose levels are elevated in the blood, insulin is produced at higher rates by the pancreas in order to maintain normal sugar concentrations in the blood.
- Without insulin, the body cannot process glucose effectively and glucose begins to build up in the blood stream instead of being transported to different cells.
- In contrast with elevated levels of glucose in the blood, when there is a deficit of glucose available to the body, alpha cells in the pancreas release glucagon, a hormone that causes the liver to convert stored glycogen into usable glucose which is then released into the bloodstream.

Some of the effects of the insulin on the metabolism include:

- Controlling cell intake of substances like glucose in many organs like muscles and adipose tissues.
- Controlling amino acid uptake, thus increasing DNA replication and protein synthesis
- Altering the activity of enzymatic cells

Other Cellular effects of insulin include:

- Increasing synthesis of glycogen. Glycogen is a type of storage for glucose and is stored in the liver. Levels of blood glucose determine whether glucose is stored as glycogen or is excreted. Low levels of glucose cause the liver to excrete glucose, while higher levels of glucose allow glucose to be stored as glycogen.

- Increasing the synthesis and esterification of fatty acids. This is caused by the insulin causing fat cells to convert blood lipids to triglycerides. Esterification is caused when the insulin causes the adipose tissue to convert fats from fatty acid esters.
- Increasing the esterification of fatty 4. Decreasing protein breakdown (proteolysis) 5. Reducing lipolysis 6. Increasing uptake of substances like amino acid and potassium 7. Relaxing wall of arteries of muscles, which vasodilation 8. Increasing secretion of HCl into the stomach.

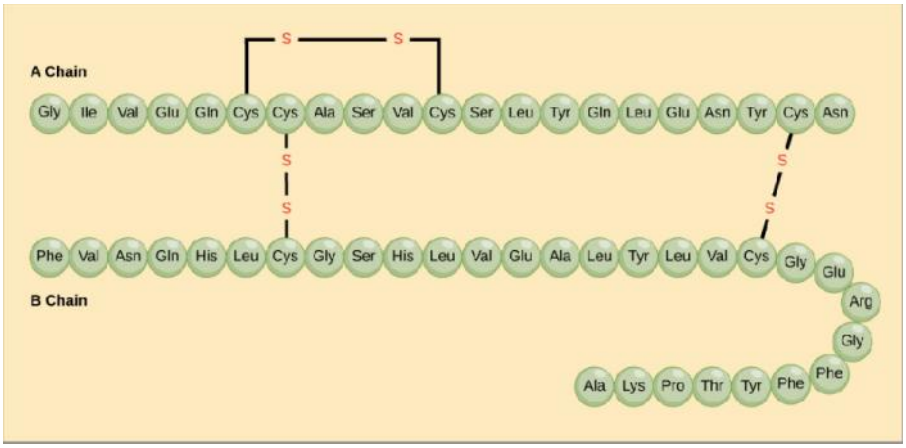
Protein structure

- Egg whites contain large amounts of proteins called albumins, and the albumins normally have a specific 3D shape, thanks to bonds formed between different amino acids in the protein. Heating causes these bonds to break and exposes hydrophobic (water-hating) amino acids usually kept on the inside of the protein. The hydrophobic amino acids, trying to get away from the water surrounding them in the egg white, will stick to one another, forming a protein network that gives the egg white structure while turning it white and opaque.
- The shape of a protein is very important to its function.
- To understand how a protein gets its final shape or conformation, we need to understand the four levels of protein structure: primary, secondary, tertiary, and quaternary.

Primary structure

- The simplest level of protein structure, **primary structure**, is simply the sequence of amino acids in a polypeptide chain.
- For example, the hormone insulin has two polypeptide chains, A and B, shown in diagram below. (The insulin molecule shown here is cow insulin, although its structure is similar to that of human insulin.)
- Each chain has its own set of amino acids, assembled in a particular order.

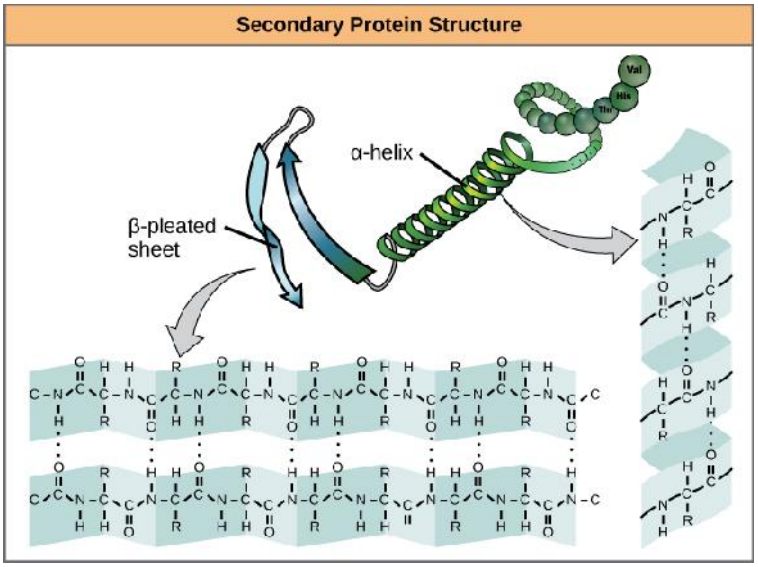
- For instance, the sequence of the A chain starts with glycine at the N-terminus and ends with asparagine at the C-terminus, and is different from the sequence of the B chain.



- Insulin consists of an A chain and a B chain. They are connected to one another by disulfide bonds (sulfur-sulfur bonds between cysteines).
- The A chain also contains an internal disulfide bond. The amino acids that make up each chain of insulin are represented as connected circles, each with the three-letter abbreviation of the amino acid's name.

Secondary structure

- The next level of protein structure, **secondary structure**, refers to local folded structures that form within a polypeptide due to interactions between atoms of the backbone. (The backbone just refers to the polypeptide chain apart from the R groups – so all we mean here is that secondary structure does not involve R group atoms).
- The most common types of secondary structures are the α helix and the β pleated sheet. Both structures are held in shape by hydrogen bonds, which form between the carbonyl O of one amino acid and the amino H of another.

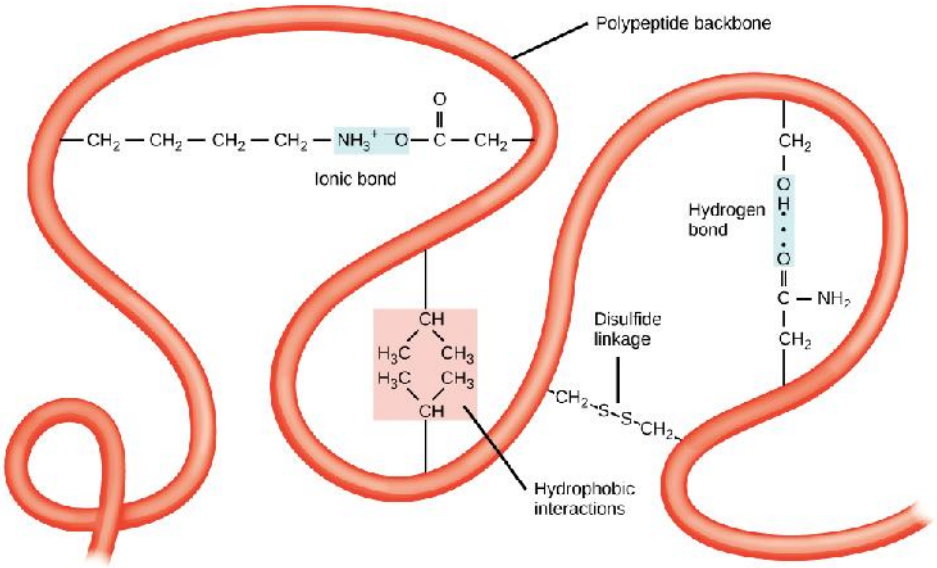


- In an **α helix**, the carbonyl (C=O) of one amino acid is hydrogen bonded to the amino H (N-H) of an amino acid that is four down the chain. (E.g., the carbonyl of amino acid 1 would form a hydrogen bond to the N-H of amino acid 5).
- This pattern of bonding pulls the polypeptide chain into a helical structure that resembles a curled ribbon, with each turn of the helix containing 3.6 amino acids.
- The R groups of the amino acids stick outward from the α helix, where they are free to interact.
- In a **β pleated sheet**, two or more segments of a polypeptide chain line up next to each other, forming a sheet-like structure held together by hydrogen bonds.
- The hydrogen bonds form between carbonyl and amino groups of backbone, while the R groups extend above and below the plane of the sheet.
- The strands of a β pleated sheet may be **parallel**, pointing in the same direction (meaning that their N- and C-termini match up), or **antiparallel**, pointing in opposite directions (meaning that the N-terminus of one strand is positioned next to the C-terminus of the other).

Tertiary structure

- The overall three-dimensional structure of a polypeptide is called its **tertiary structure**. The tertiary structure is primarily due to interactions between the R groups of the amino acids that make up the protein.

- R group interactions that contribute to tertiary structure include hydrogen bonding, ionic bonding, dipole-dipole interactions.
- For example, R groups with like charges repel one another, while those with opposite charges can form an ionic bond. Similarly, polar R groups can form hydrogen bonds and other dipole-dipole interactions. Also important to tertiary structure are **hydrophobic interactions**, in which amino acids with nonpolar, hydrophobic R groups cluster together on the inside of the protein, leaving hydrophilic amino acids on the outside to interact with surrounding water molecules.
- Finally, there's one special type of covalent bond that can contribute to tertiary structure: the disulfide bond. **Disulfide bonds**, covalent linkages between the sulfur-containing side chains of cysteines, are much stronger than the other types of bonds that contribute to tertiary structure.



Quaternary structure

- Many proteins are made up of a single polypeptide chain and have only three levels of structure. However, some proteins are made up of multiple polypeptide chains, also known as subunits. When these subunits come together, they give the protein its **quaternary structure**.

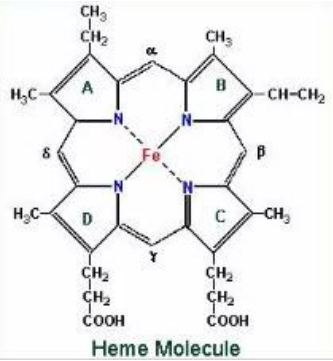
- one example of a protein with quaternary structure: hemoglobin. hemoglobin carries oxygen in the blood and is made up of four subunits, two each of the α and β types. In general, the same types of interactions that contribute to tertiary structure (mostly weak interactions, such as hydrogen bonding and London dispersion forces) also hold the subunits together to give quaternary structure.

Hemoglobin

- Hemoglobin, a chromo protein, found exclusively in red blood cells is actually a conjugated protein containing heme as prosthetic group and globin as the protein part apoprotein.
- The normal concentration of Hb in an adult varies from 14.0 to 16.0 gm%.Approximately 90 mg/kg of Hb is produced and destroyed in the body every day.
- Hb has a molecular weight of about 67,000.
- Each gram of Hb contains 3.4 mg of iron.
- Heme is present as a prosthetic group in hemoglobin as well as in myoglobin, cytochromes, peroxidases, catalases and tryptophan pyrrolases etc.
- Heme is produced by the combination of iron with a porphyrin ring.
- The heme protion is alike in all forms of hemoglobin

Structure of Heme

- Heme is a derivative of porphyrin, porphyrins are cyclic compounds formed by the fusion of 4 pyrrole rings linked by methenyl bridges.
- Since an atom of iron is present heme is called ferroprotoporphyrin.
- These rings are names as I,II,III, IV and the bridges are names as alpha, beta, gamma and delta.
- Porphyrins contain side chains attached to each of the other four pyrrole rings.
- Different porphyrins vary in nature of the side chains that are attached to each of the pyrrole rings.



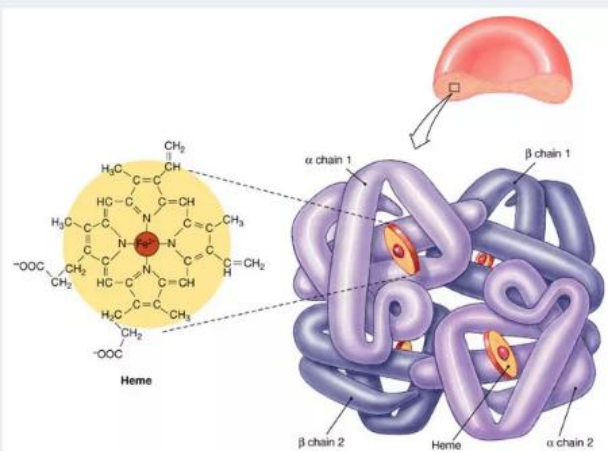
- Heme consists of one ferrous atom (Fe++) that is co-ordinated in the centre of the tetra pyrrole ring of protoporphyrin IX.
- The double bonds are resonating and therefore keep shifting in their position.
- When the ferrous atom in heme gets oxidized to ferric form. Hematin is formed, which loses the property of carrying oxygen and is brown in color, as compared to that of heme which is red in color.

Structure of Globin

1. Different hemoglobins are produced during embryonic, fetal and adult life.
2. Each consists of a tetramer of globin polypeptide chains.
3. The major adult hemoglobin HbA has the structure $\alpha_2\beta_2$.

Polypeptide chains

- Each polypeptide chain contains heme in the heme pocket. Thus one Hb molecule contains 4 Heme units.
- The subunits of hemoglobin are arranged array with a tight spherical overall appearance and each individual polypeptide is folded in such a manner to maximize polar residues being on the exposed surface and non-polar interactions being internal, making this large protein water soluble. The interior surface of the molecule lined with non-polar groups froms a hydrophobic pocket into which heme is inserted.



- The arrangement of polypeptides is held together by hydrogen bonding, hydrophobic interactions and multiple ionic interactions that take place at the contact points between subunits.
- These subunits interactions play a critical role in the binding of oxygen to hemoglobin.
- In the amino acid sequence of each polypeptide chain, certain residues appear to be critical to stability and function.
- Such residues are usually the same in α or β chains.
- The NH2 terimal valines of the beta chains are important in 2,3-BPG interactions. The C-terminal residues are important in the salt bridges.
- Each heme moiety can bind a single oxygen molecule, a molecule of hemoglobin can transport up to four oxygen molecules.
- Each heme unit holds an iron ion is such a way that the iron can interact with an oxygen molecule, forming oxyhemoglobin.
- Blood containg RBCs filled with oxyhemoglobin is bright red.
- The iron oxygen interaction is very weak; the two can easily be separated without damaging the heme unit or the oxygen molecules.
- The binding of an oxygen molecule to the iron in a heme unit is therefore completely reversible.
- A hemoglobin molecule in which the iron has separated from the oxygen molecule is called deoxyhemoglobin.

Primary structure of hemoglobin

- Normal alpha chain contains 141 AA residues in linear sequence.
- The non-alpha chains are all 146 amino acids in length; the beta chain begins with valine and histidine.
- The C-terminal residues are Tyrb145 and Hisb146.The delta chain differs from the beta chain in only 10 residues.
- The first eight residues are the C-terminal residues (127-146) are the same in the delta and beta chains. Tetramers of beta chains maybe found in a thalassemia.
- The gamma chain of fetal hemoglobin differs from the beta chain by 39 residues.
- The N-terminal residues of the gamma chain and beta chain are glycine and valine respectively, while the C-terminal residues.
- Try145 and His146 are the same as in gamma and beta chains. Appreciable quantities of free gamma are found in the red cells of some infants with a thalassemia, free gamma chains like beta chains can form homotetramers known as hemoglobin barts.

Secondary structure of hemoglobin

- About 75 percent of the amino acids in α or β chains are in a helical arrangement.
- All studied hemoglobins have a similar helical content.
- Eight helical arease lettered A to H, occur in the β chains.
- Hemoglobin nomenclature specifies that amino acids within helices are designated by the amino acid number and the helix letter, while amino acids between helices bear the number of the amino acid and the letters of the two helices. Thus residues EF3 is the third residue of the segment connecting the E and F helices, while residues F8 is the eighth residue of the F helix. Alignment according to helical designation makes homology evident; residue F8 is the proximal heme-linked histidine and the histidine on the distal side of the heme is E7.

Tertiary structure

- The tertiary folding of each globin chain forms an approximate sphere. Tertiary folding fives rise to at least 3 functionally important characteristics of the hemoglobin molecules.

- Polar or charged side chains tend to be directed to the outside surface of the subunit and conversely, non-polar structure tend to the directed inwards. The effect of this is to make the surface of the molecule hydrophilic and the interior hydrophobic
- An open toped cleft in the surface of the subunit known as haem pocket iscreated.
- This hydrophobic cleft protects the ferrous ion from oxidation.
- The amino acids which form the inter-subunit bonds responsible for maintaining the quaternary structure and thus the function of the haemoglobin molecule are brought into the correct orientation to permit these bonds to form.

Quarternary structure

T-form

- The deoxy form of hemoglobin is called the "T" form or taut or tense form. In this form the two αβ dimmers interact through a network of ionic bonds and hydrogen bonds that constrain the movement of the polypeotide chains. The T form is the low oxygen affinity form of hemoglobin.

R form

- The binding of hemoglobin causes rupture of some the ionic bonds and hydrogen bonds between the αβ dimmers. This leads to a structure called "R" or relaxed form, in which the polypeptide chains have more freedom of movement. The R form is the high affinity form of hemoglobin.

Functions of hemoglobin

Hemoglobin as oxygen carrier

- The main function of hemoglobin is to carry oxygen from the lungs to all the tissues of the body. This is due to the affinity of hemoglobin for oxygen. When hemoglobin comes in contact with oxygen, it combines with it and form oxy-hemoglobin. This is a weak bond. When blood reaches to tissues, where oxygen is deficient, the bond is broken and oxygen diffuses out to tissues.

Hemoglobin as carbon dioxide carrier

- Some of carbon dioxide is transported from tissues to lungs through hemoglobin. Although the majority of it is transported via plasma but still it carries some of CO2 to lungs.

Color of blood

- The red color of blood is due to hemoglobin. When red blood cells are separated from the blood, the red color disappears. This means that the red color of blood is due to red blood cells. Hence the name red blood cells is given to it. And as we know that hemoglobin is present inside red blood cells, therefore it gives red coloration to RBCs

Buffering action

- Hemoglobin also acts as a buffer. Buffer means to resist change in pH.Blood has 7.4 pH and it remains in the narrow range. Because, if it changes the life of the person may be endangered. Therefore, hemoglobin plays very important role in keeping the pH of blood constant.

Erythrocyte metabolism

- Hemoglobin plays an important role in the modulation of erythrocyte metabolism.

Interaction with drugs

- Not only for oxygen, but hemoglobin act a very important role the transport of various drugs to their site of action.

Physiological active catabolites

- Hemoglobin is a source of various physiological active catabolites.

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Question	Opt A	Opt B	Opt C
Proteins contain	Only L- α - amino acids	Only D-amino acids	DL-Amino a
The optically inactive amino acid is	Glycine	Serine	Threonine
At neutral pH, a mixture of amino acids in solut	Dipolar ions	Nonpolar molecules	Positive an
The true statement about solutions of amino a	All amino acids contain	All amino acids cont	Some amin
pH (isoelectric pH) of alanine is	6.02	6.6	6.8
Since the pK values for aspartic acid are 2.0, 3.9	3	3.9	5.9
Sulphur containing amino acid is	Methionine	Leucine	Valine
All the following are sulphur containing amino a	Cysteine	Cystine	Methionine
An aromatic amino acid is	Lysine	Tyrosine	Taurine
The functions of plasma albumin are	Osmosis	Transport	Immunity
Amino acid with side chain containing basic gro	2-Amino 5-guanidovale	2-Pyrrolidine carbox	2-Amino 3-
An essential amino acid in man is	Aspartate	Tyrosine	Methionine
Non essential amino acids	Are not components of	May be synthesized	Have no ro
An example of polar amino acid is	Alanine	Leucine	Arginine
The amino acid with a nonpolar side chain is	Serine	Valine	Asparagine
A ketogenic amino acid is	Valine	Cysteine	Leucine
An amino acid that does not form an α-helix is	Valine	Proline	Tyrosine
An amino acid not found in proteins is	β-Alanine	Proline	Lysine
In mammalian tissues serine can be a biosynthe	Methionine	Glycine	Tryptophar
A vasodilating compound is produced by the de	Arginine	Aspartic acid	Glutamine
Biuret reaction is specific for	–CONH-linkages	–CSNH2 group	–(NH)NH2 g
Sakaguchi's reaction is specific for	Tyrosine	Proline	Arginine
Million-Nasse's reaction is specific for the amin	Tryptophan	Tyrosine	Phenylalan
Ninhydrin with evolution of CO2 forms a blue c	Peptide bond	α -Amino acids	Serotonin
Which of the following is a dipeptide?	Anserine	Glutathione	Glucagon
Which of the following is a tripeptide?	Anserine	Oxytocin	Glutathione
Casein, the milk protein is	Nucleoprotein	Chromoprotein	Phosphopr
An example of phosphoprotein present in egg y	Ovoalbumin	Ovoglobulin	Ovovitellin
A simple protein found in the nucleoproteins of	Prolamine	Protamine	Glutelin
Histones are	Identical to protamine	Proteins rich in lysin	Proteins wi
The protein present in hair is	Keratin	Elastin	Myosin
Both α-helix and β-pleated sheet conformation	Watson and Crick	Pauling and Corey	Waugh and
Each turn of α-helix contains the amino acid res	3.6	3	4.2
Distance traveled per turn of α–helix in nm is	0.53	0.54	0.44
Along the α-helix each amino acid residue adva	0.15	0.1	0.12
The number of helices present in a collagen mo	1	2	3
In proteins the α-helix and β-pleated sheet are	Primary structure	Secondary structure	Tertiary str
The α-helix of proteins is	A pleated structure	Made periodic by di	A non-perio

Tertiary structure of a protein describes	The order of amino acic	Location of disulphic	Loop regio
In a protein molecule the disulphide bond is no	Reduction	Oxidation	Denaturati
Denaturation of proteins results in	Disruption of primary st	Breakdown of pepti	Destructio
The enzyme trypsin is specific for peptide bond	Basic amino acids	Acidic amino acids	Aromatic a
Chymotrypsin is specific for peptide bonds cont	Uncharged amino acid r	Acidic amino acids	Basic amin
The end product of protein digestion in G.I.T. IS	Dipeptide	Tripeptide	Polypeptid
At isoelectric pH, an amino acid exists as	Anion	Cation	Zwitterion
At a pH below the isoelectric point, an amino a	Cation	Anion	Zwitterion
An amino acid having a hydrophilic side chain is	Alanine	Proline	Methionine
An amino acid that does not take part in α helix	Histidine	Tyrosine	Proline
Primary structure of a protein is formed by	Hydrogen bonds	Peptide bonds	Disulphide
α-Helix is formed by	Hydrogen bonds	Hydrophobic bonds	Electrostati
Aromatic amino acids can be detected by	Sakaguchi reaction	Millon-Nasse reactic	Hopkins-Co
Two amino groups are present in	Leucine	Glutamate	Lysine
During denaturation of proteins, all of the follo	Primary structure	Secondary structure	Tertiary str
All the following are branched chain amino acic	Isoleucine	Alanine	Leucine
Millon's test is for identification of	Tyrosine	Tryptophan	Proline
Hopkins-Cole test is for identification of	Tyrosine	Tryptophan	Arginine
Collagen is very rich in	Glycine	Serine	Aspartic ac
In glutathione (a tripeptide) is present apart fro	Serine	Glycine	Leucine
2-Amino 3-OH propanoic acid is	Glycine	Alanine	Valine
All amino acids have one asymmetric carbon at	Arginine	Aspargine	Histidine

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IOLOGY – FIRST SEMESTER

05A – BIOCHEMISTRY

E CHOICE QUESTIONS

Unit 4

Opt D	Answer
Both A) an	Only L- α - amino acids
Valine	Glycine
Hydrophob	Dipolar ions
All amino a	All amino acids contain both positive and negative charges
7.2	6.02
6	3
Asparagine	Methionine
Threonine	Threonine
Arginine	Tyrosine
both A)an	Osmosis
2-Amino pr	2-Amino 5-guanidovaleric acid
Serine	Methionine
May be syr	May be synthesized in the body from essential amino acids
Valine	Arginine
Threonine	Asparagine
Threonine	Cysteine
Tryptophar	Proline
Histidine	β-Alanine
Phenylalan	Glycine
Histidine	Histidine
All of these	–CONH-linkages
Cysteine	Arginine
Arginine	Tyrosine
Histamine	α -Amino acids
β -Lipoprot	Anserine
Kallidin	Glutathione
Glycoprote	Phosphoprotein
Avidin	Ovovitellin
Globulin	Protamine
Insoluble ir	Proteins rich in lysine and arginine
Tropocolla	Keratin
Y.S.Rao	Pauling and Corey
4.5	3.6
0.48	0.54
0.2	0.15
4	3
Quaternary	Secondary structure
Stabilised b	A non-periodic structure

The ways o	The ways of protein folding
X-ray diffra	Denaturation
Irreversible	Destruction of hydrogen bonds
Next to sm	Basic amino acids
Small amin	Uncharged amino acid residues
Amino acid	Amino acid
None of th	Zwitterion
Undissocia	Cation
Serine	Serine
Tryptophar	Proline
All of these	Peptide bonds
Disulphide	Hydrogen bonds
Xanthoprot	Xanthoproteic reaction
Threonine	Lysine
Quaternary	Secondary structure
Valine	Alanine
Arginine	Tyrosine
Cysteine	Tryptophan
Glutamic a	Glycine
Phenyl alar	Glycine
Serine	Serine
Glycine	Glycine

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DEPARTMENT OF MICROBIOLOGY
(For the candidates admitted from 2015 onwards)

Subject	**:**	**Biochemistry**	**Semester**	**:**	**I**
Subject code	**:**	**17MBU103**	**Class**	**:**	**I B.Sc Microbiology**

UNIT-V: COURSE MATERIAL

Unit-V

Structure and classification of enzymes, mechanism of action of enzymes. Km equation and enzyme activity. Allosteric enzyme and its mechanism. Multienzyme complex, enzyme inhibition. Vitamins-classification and characteristics, sources and importance.

Suggest Readings

1. Nelson, D.L and Cox, M.M. (2008). Lehninger Principles of Biochemistry, 5th edition. W.H. Freeman and Company.

Enzymes

Enzymes are soluble, colloidal, organic catalyst formed by living cells that catalyze a specific biochemical reaction by lowering the activation energy and in the process they remain unchanged.

Types of Enzymes

1. Exo-enzymes

- Enzymes that function outside the cell are called so, e.g. zymase, lysozyme, digestive enzymes.

2. Endo-enzyme

- Enzymes that function inside the cell are called so, e.g. enzymes of glycolysis, Krebs cycle, protein biosynthesis etc.

3. Zymogens

- These are inactive precursors or pro-enzymes forms of exo-enzymes. They become activated prior to enzymatic action, e.g., proteases.

4. Constitute or housekeeping enzymes

- Those enzymes are always present and synthesized in cell, e.g., glycolytic enzymes.

5. Inducible enzymes

- Most enzymes are synthesized only when they are needed e.g. Nitric oxide synthase, cycloxygenase, aldehyde dehydrogenase etc.

6. Isoenzymes (isozymes)

- These are the different forms of the same enzymes which catalyze the same chemical reaction but, differ each other chemically, immunologically, and electrophoretically and in kinetic properties. For example, in maize 18 isozymes found for peroxidase. In plants aspertate kinase exist in two isozyme forms. Aspertate kinase catalyzes the amino acid biosynthesis from aspertate, LDH (Lactic acid dehydrogenase)

7. Ribozyme or RNA Enzymes

- e.g. ribonuclease-P (RNAase-P), Peptidyl transferase (23S rRNA of larger subunit of ribosome) etc.

8. Abzymes

- These are the antibodies that act as enzymes.

Structure (Chemical Nature) of Enzyme

All enzymes are generally globular proteins except some RNA enzymes like Ribonuclease-P, ribozyme and peptidyl transferase.

On the basis of number of polypeptide chains, enzymes are of 2 types

(a) Monomeric enzymes:

- Consist of one polypeptide chain (subunit), e.g., ribonuclease, lysozyme, hexokinase etc. These are functional in their 3 dimensional or tertiary structures.

(b) Oligomeric enzymes:

- Consist of more than one olgolypeptide chain. They are functional in their quaternary structure. For example, aldolase consists of 4 chains (tetrameric), Rubisco of Calvin cycle consists of 24 chains, and Enolase is a dimmer.

On the basis of chemical nature, enzymes are also 2 types

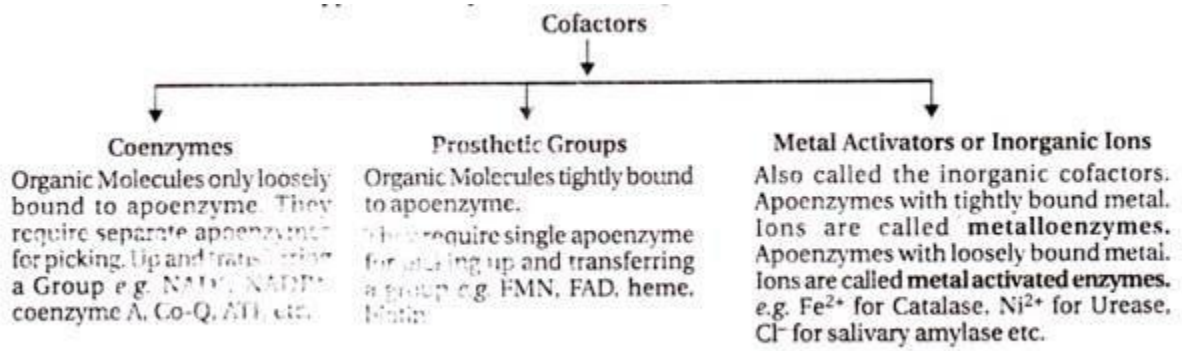
(i) Simple enzymes

- They consist of only proteins, e.g. urease, lysozyme, pepsin, trypsin etc.

(ii) Holoenzyme or Conjugate enzyme

- These enzymes consist of proteinous part called apoenzyme and non-proteinous part called co-factor.

Holoenzyme = Apoenzyme + Co-factor (active) (Proteinous part) (Non-proteinous part). The cofactors are of 3 types: Co-enzymes, Prosthetic groups and inorganic ions.



The surface of a functional enzyme contains 2 sites *i.e. catalytic site* and *allosteric sites.*

Catalytic Site or Active Site or Active Spot

- It is a small three-dimensional (3D) area on or near enzyme surface that binds the specific substrate(s) and convert into products. The unique 3D shape of a catalytic site may alter by denaturation (unfolding) through high temperature or exposure to extremes of pH. This results in the loss of catalytic activity.
- In monomeric enzymes, the catalytic site is often a cleft or crevice, but in multimeric enzymes, it resides at the interface between polypeptides. In some enzymes, the catalytic site is rigid or non- flexible to accommodate a substrate. But in most cases, the binding of substrate induces a conformational change in the catalytic site, e.g., glucose (substrate) induces a conformational change of hexokinase. An enzyme may have one or more active sites. Each active site consists of 3-12 amino acids that come together by folding of polypeptides. In a holoenzyme, the catalytic site also contains cofactor for its function.

The amino acids residues of catalytic site have 4 roles

- Provide charged R-groups to attract substrate,
- Some act as template for holding substrate,
- Some provide functional groups that perform chemical changes by lowering activation energy
- A few residues determine substrate specificity
- Enzymes : Amino Acid residues in active site
- Pepsin : Tyrosine only
- Aldolase : Glycine – Histidine – Alanine

Allosteric Sites

- These are special sites on enzyme surface other than catalytic site, which when bind with effectors or modulators alter the conformation of the catalytic site. The enzymes having allosteric sites are called allosteric enzymes. Allosteric sites are of two types: activator site and inhibitor site. An allosteric activator when binds to activator site increase the enzyme activity while an allosteric inhibitor decreases the enzyme activity by binding the inhibitor site.

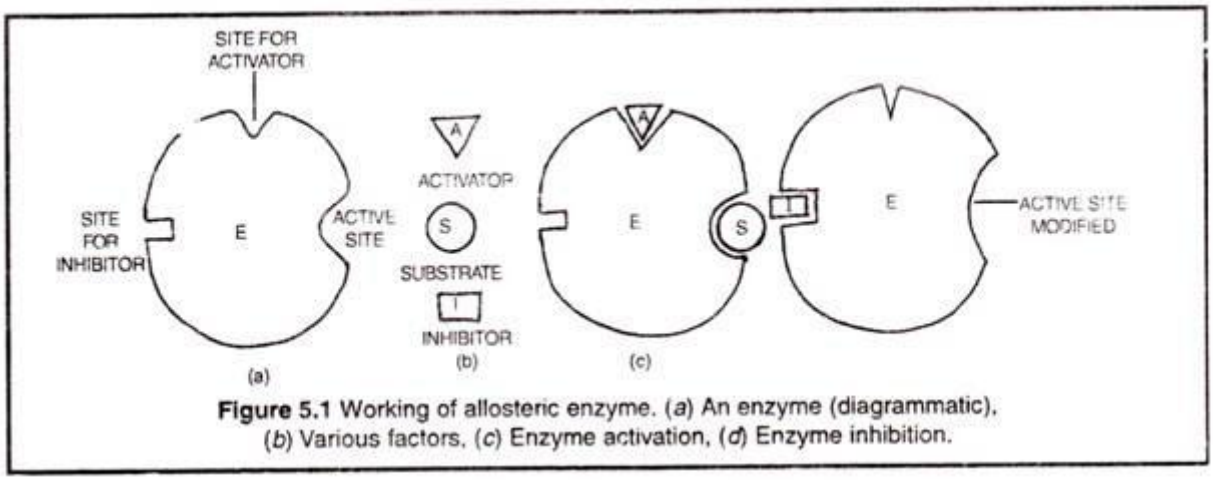


Figure 5.1 Working of allosteric enzyme. (*a*) An enzyme (diagrammatic), (*b*) Various factors, (*c*) Enzyme activation, (*d*) Enzyme inhibition.

Cofactors

Cofactors, mostly metal ions or coenzymes, are inorganic and organic chemicals that assist enzymes during the catalysis of reactions.

Coenzymes are non-protein organic molecules that are mostly derivatives of vitamins soluble in water by phosphorylation; they bind apoenzyme to proteins to produce an active holoenzyme.

Apoenzymes are enzymes that lack their necessary cofactor(s) for proper functioning; the binding of the enzyme to a coenzyme forms a holoenzyme. Holoenzymes are the active form of an apoenzyme.

Cofactors can be metals or coenzymes, and their primary function is to assist in enzyme activity.

They are able to assist in performing certain, necessary, reactions the enzyme cannot perform alone.

They are divided into coenzymes and prosthetic groups.

A holoenzyme refers to a catalytically active enzyme that consists of both apoenzyme (enzyme without its cofactor(s)) and cofactor.

There are two groups of cofactors: metals and small organic molecules called coenzymes.

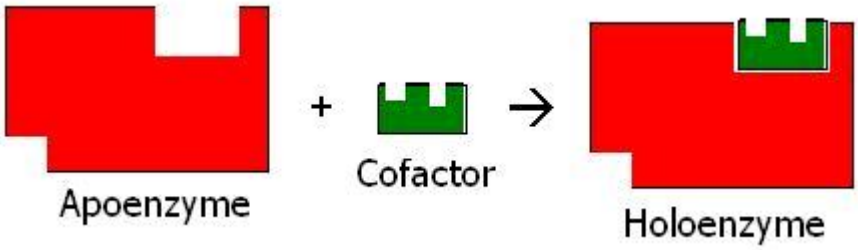
Coenzymes are small organic molecules usually obtained from vitamins.

Prosthetic groups refer to tightly bound coenzymes, while co-substrates refer to loosely bound coenzymes that are released in the same way as substrates and products.

Loosely bound coenzymes differ from substrates in that the same coenzymes may be used by different enzymes in order to bring about proper enzyme activity.

Enzymes without their necessary cofactors are called apoenzymes, which are the inactive form of an enzyme. Cofactors with an apoenzyme are called a holoenzyme, which is the active form.

General formula



Metal cofactors

Metal ions are known as the common cofactors.

In some enzymes, the function as a catalyst cannot be carried out if a metal ion is not available to be bound the active site.

In daily nutrition, this kind of cofactor plays a role as the essential trace elements such as: iron (Fe^{3+}), manganese (Mn^{2+}), cobalt (Co^{2+}), copper (Cu^{2+}), zinc (Zn^{2+}), selenium (Se^{2+}), and molybdenum (Mo^{5+}).

For example, Mg2 is used in glycolysis. In the first step of converting glucose to glucose 6-phosphate, before ATP is used to give ADP and one phosphate group, ATP is bonded to Mg2 which stabilizing the other two phosphate groups so it is easier to release only one phosphate group without resonate with other two.

In some bacteria such as genus Azotobacter and Pyrococcus furiosus, metal cofactors are also discovered to play an important role. An example of cofactors in action is the zinc-mediated function of carbonic anhydrase or the magnesium-mediated function of restriction endonuclease.

Enzyme Classification

The International Union of Biochemistry (IUB, 1961) adopted a scheme for systematic functional classification and nomenclature of enzymes.

The recommendations of IUB are as follows

- All known enzymes have been grouped into six major classes on the basis of reaction type they catalyze,
- Each class further sub-divided into subclasses and sub-subclasses,
- Each enzyme is assigned two names i.e., recommended (trivial) name and systematic name,
- Each enzyme is identified by a unique four digit classification number.
- For example, hexokinase is recommended name, its systematic name is glucose phosphotransferase and its classification number in EC 2.7.1.1. Here, "EC" stands for Enzyme commission, the first number (2) stands for the major class, the second number (7) stands for the sub class, the third number (1) indicates sub-class and the fourth number (1) denotes the serial number assigned in its sub-classes.

Table 5.2. IUB classification of enzymes

Major Class (Type of reaction catalyzed)	Common exmaples	Kind of reaction	Specific Example
1. ***Oxidoreductases*** *(Transfer of electrons)*	Oxidases Reductases Dehydrogenase	$A^{+3} + B^{+2} \rightarrow A^{+2} + B^{+3}$	Alcohol + NAD ↓ *Alcohol dehydrogenase* Aldehyde + $NADH_2$
2. ***Transferases*** *(Transfer of functional groups)*	Transaminase Transketolase Transaldolase	$A - X + B \rightarrow A + B - X$	Glucose + ATP ↓ *Glukokinase or hexokinase* Glucose-6-Phosphate + ADP
3. ***Hydrolases*** *(Hydrolysis Reactions)*	Amylases Lipases Proteases Nucleases	$A–B+H_2O \rightarrow A–OH + B–H$	Sucrose ↓ *Sucrase* Glucose + Fructose
4. **Lyases or *Desmolases*** *(Group elimination to form double bonds without hydrolysis)*	Aldolase Decarboxylase Fumarase Citrate synthase	$A - B \rightarrow A = B + X - Y$ X Y	Histidine ↓ *Hlstidine decarboxylase* Histidine + CO_2
5. ***Isomerases*** *(Transfer of Groups within a molecule*	Isomerase Mutase Epimerase	$A - B \rightarrow A - B$ Y X X Y	Glucose – 6-Phosphate ↓ *Isomerase* Fructose-6-Phosphate
6. ***Ligases or Synthetases*** *(Bond formation couples with ATP hydrolysis)*	Synthetases Carboxylases	$A + B + ATP \rightarrow$ $A - B + ADP + Pi$	Pyruvate + CO_2 + ATP ↓ *Pyruvate carboxylase* Oxaloacetate + ADP + Pi

1. Oxidoreductases catalyze a variety of oxidation-reduction reactions. Common names include dehydrogenase, oxidase, reductase and catalase.

2. Transferases catalyze transfers of groups (acetyl, methyl, phosphate, etc.). Common names include acetyltransferase, methylase, protein kinase and polymerase. The first three subclasses play major roles in the regulation of cellular processes. The polymerase is essential for the synthesis of DNA and RNA.

3. Hydrolases catalyze hydrolysis reactions where a molecule is split into two or more smaller molecules by the addition of water. Common examples are given below.

- Proteases split protein molecules. Examples: HIV protease and caspase. HIV protease is essential for HIV replication. Caspase plays a major role in apoptosis.
- Nucleases split nucleic acids (DNA and RNA). Based on the substrate type, they are divided into RNase and DNase. RNase catalyzes the hydrolysis of RNA and DNase acts on DNA. They may also be divided into exonuclease and endonuclease. The exonuclease progressively splits off single nucleotides from one end of DNA or RNA. The endonuclease splits DNA or RNA at internal sites.
- Phosphatase catalyzes dephosphorylation (removal of phosphate groups). Example: calcineurin. The immunosuppressive drugs FK506 and Cyclosporin A are the inhibitors of calcineurin.

4. Lyases catalyze the cleavage of C-C, C-O, C-S and C-N bonds by means other than hydrolysis or oxidation. Common names include decarboxylase and aldolase.

5. Isomerases catalyze atomic rearrangements within a molecule. Examples include rotamase, protein disulfide isomerase (PDI), epimerase and racemase.

6. Ligases catalyze the reaction which joins two molecules. Examples include peptide synthase, aminoacyl-tRNA synthetase, DNA ligase and RNA ligase.

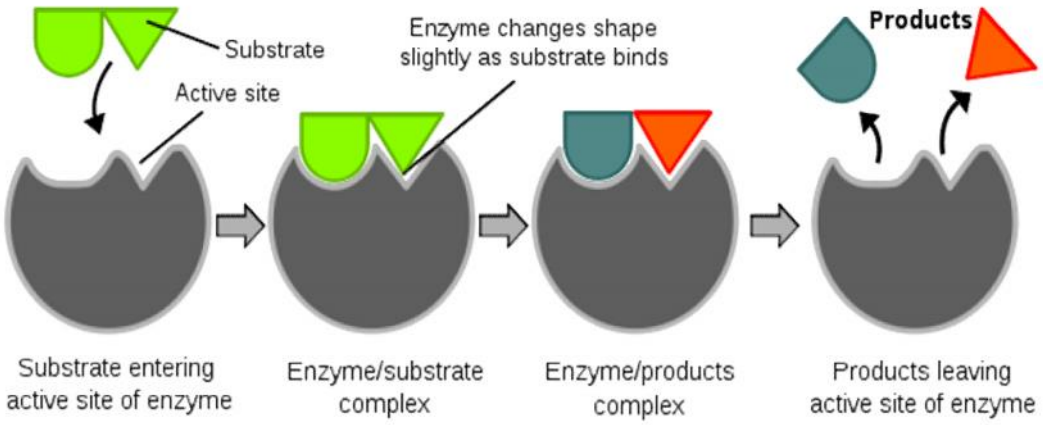
The IUBMB committee also defines subclasses and sub-subclasses. Each enzyme is assigned an EC (Enzyme Commission) number. For example, the EC number of catalase is EC1.11.1.6. The first digit indicates that the enzyme belongs to oxidoreductase (class 1). Subsequent digits represent subclasses and sub-subclasses.

Mechanism of Enzyme Action:

- Arrhenius first pointed out that, all the molecules in a given population do not have the same kinetic energy some molecules are energy poor and other are energy rich. Higher is the energy barrier the grater is the inactiveness of reaction. This energy

barrier can be overcome by the enzymes and making the molecule active with available energy level.

- To explain the velocity of enzymatic reaction Leonor Michaels and Moud Menten (1913) proposed following assumptions.
- Only a single substrate and a single product are formed in enzymatic reaction.
- The process continued essentially to its completion.
- Concentration of substrate is much greater than the enzyme in the system.
- An intermediate enzyme substrate complex is formed.
- The rate of decomposition of the substrate is proportional to the concentration of the enzyme substrate complex.
- They proposed an equation popularly accepted as Michaelis. Menten's equation, which concerned the velocity of enzymatic reaction.
- Where Km is the Michaelis constant 'S' is the substrate concentration, Vmax - maximum velocity of the reaction and V0 is the initial velocity.
- Km value is constant for all enzymes up to the half of the maximum velocity of reaction. Greater is the ES complex period the lower is the Km value.
- There are several theories has been put forwarded by different biochemists to explain the mechanism of the enzyme action.



(i) Lock and key thoery

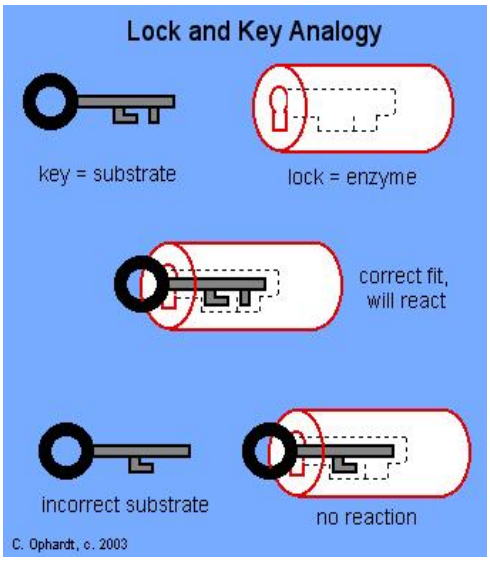
In the **lock-and-key model** of enzyme action:

- the active site has a rigid shape

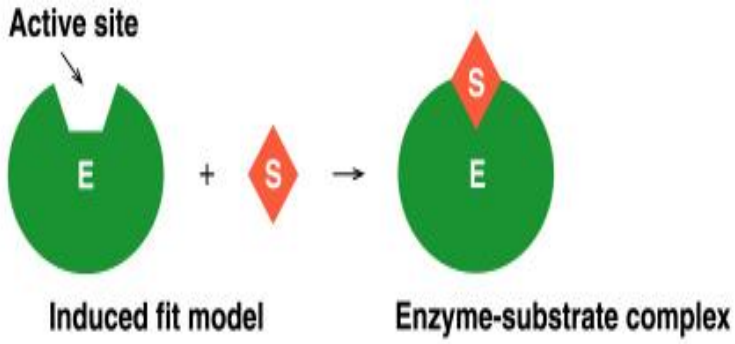
- only substrates with the matching shape can fit

- the substrate is a key that fits the lock of the active site

This is an older model, however, and does not work for all enzymes



(II) Inducted fit Theory: In the induced-fit model of enzyme action



- the active site is flexible, not rigid
- the shapes of the enzyme, active site, and substrate adjust to maximize the fit, which improves catalysis
- there is a greater range of substrate specificity
- This model is more consistent with a wider range of enzymes

Enzyme Catalyzed Reactions

- When a substrate (S) fits properly in an active site, an enzyme-substrate (ES) complex is formed:

$$E + S \leftrightarrows ES$$

- Within the active site of the ES complex, the reaction occurs to convert substrate to product (P):

  $$ES \rightarrow E + P$$

- The products are then released, allowing another substrate molecule to bind the enzyme

  - this cycle can be repeated millions (or even more) times per minute

- The overall reaction for the conversion of substrate to product can be written as follows:

  $$E + S \leftrightarrows ES \rightarrow E + P$$

Factors affecting Enzyme Activity

- The activity of an Enzyme is affected by its environmental conditions. Changing these alter the rate of reaction caused by the enzyme. In nature, organisms adjust the conditions of their enzymes to produce an Optimum rate of reaction, where necessary, or they may have enzymes which are adapted to function well in extreme conditions where they live.

Temperature

- Increasing temperature increases the Kinetic Energy that molecules possess. In a fluid, this means that there are more random collisions between molecules per unit time.
- Since enzymes catalyse reactions by randomly colliding with Substrate molecules, increasing temperature increases the rate of reaction, forming more product.
- However, increasing temperature also increases the Vibrational Energy that molecules have, specifically in this case enzyme molecules, which puts strain on the bonds that hold them together.
- As temperature increases, more bonds, especially the weaker Hydrogen and Ionic bonds, will break as a result of this strain. Breaking bonds within the enzyme will cause the Active Site to change shape.

- This change in shape means that the Active Site is less Complementary to the shape of the Substrate, so that it is less likely to catalyse the reaction. Eventually, the enzyme will become Denatured and will no longer function.
- As temperature increases, more enzymes' molecules' Active Sites' shapes will be less Complementary to the shape of their Substrate, and more enzymes will be Denatured. This will decrease the rate of reaction.
- In summary, as temperature increases, initially the rate of reaction will increase, because of increased Kinetic Energy. However, the effect of bond breaking will become greater and greater, and the rate of reaction will begin to decrease.
- The temperature at which the maximum rate of reaction occurs is called the enzyme's Optimum Temperature. This is different for different enzymes. Most enzymes in the human body have an Optimum Temperature of around 37.0 °C.

pH - Acidity and Basicity

- pH measures the Acidity and Basicity of a solution. It is a measure of the Hydrogen Ion (H+) concentration, and therefore a good indicator of the Hydroxide Ion (OH-) concentration. It ranges from pH1 to pH14. Lower pH values mean higher H+ concentrations and lower OH- concentrations.
- Acid solutions have pH values below 7, and Basic solutions (alkalis are bases) have pH values above 7. Deionised water is pH7, which is termed 'neutral'.
- H+ and OH- Ions are charged and therefore interfere with hydrogen and ionic bonds that hold together an enzyme, since they will be attracted or repelled by the charges created by the bonds. This interference causes a change in shape of the enzyme, and importantly, its Active Site.
- Different enzymes have different Optimum pH values. This is the pH value at which the bonds within them are influenced by H+ and OH- Ions in such a way that the shape of their Active Site is the most Complementary to the shape of their Substrate. At the Optimum pH, the rate of reaction is at an optimum.
- Any change in pH above or below the Optimum will quickly cause a decrease in the rate of reaction, since more of the enzyme molecules will have Active

Sites whose shapes are not (or at least are less) Complementary to the shape of their Substrate.

- Small changes in pH above or below the Optimum do not cause a permanent change to the enzyme, since the bonds can be reformed. However, extreme changes in pH can cause enzymes to Denature and permanently lose their function.
- Enzymes in different locations have different Optimum pH values since their environmental conditions may be different. For example, the enzyme Pepsin functions best at around pH2 and is found in the stomach, which contains Hydrochloric Acid (pH2).

Concentration

- Changing the Enzyme and Substrate concentrations affect the rate of reaction of an enzyme-catalysed reaction. Controlling these factors in a cell is one way that an organism regulates its enzyme activity and so its Metabolism.
- Changing the concentration of a substance only affects the rate of reaction if it is the limiting factor: that is, it the factor that is stopping a reaction from preceding at a higher rate.
- If it is the limiting factor, increasing concentration will increase the rate of reaction up to a point, after which any increase will not affect the rate of reaction. This is because it will no longer be the limiting factorand another factor will be limiting the maximum rate of reaction.
- As a reaction proceeds, the rate of reaction will decrease, since the Substrate will get used up. The highest rate of reaction, known as the Initial Reaction Rate is the maximum reaction rate for an enzyme in an experimental situation.

Substrate Concentration

- Increasing Substrate Concentration increases the rate of reaction. This is because more substrate molecules will be colliding with enzyme molecules, so more product will be formed.
- However, after a certain concentration, any increase will have no effecton the rate of reaction, since Substrate Concentration will no longer be the limiting

factor. The enzymes will effectively become saturated, and will be working at their maximum possible rate.

Enzyme Concentration

- Increasing Enzyme Concentration will increase the rate of reaction, as more enzymes will be colliding with substrate molecules.
- However, this too will only have an effect up to a certain concentration, where the Enzyme Concentration is no longer the limiting factor.

Allosteric enzymes

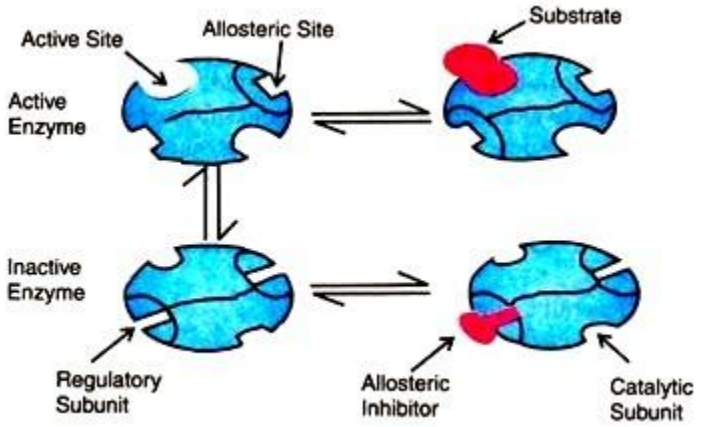
Allosteric enzymes are enzymes that change their conformation upon binding of an effector. An allosteric enzyme is an oligomer whose biological activity is affected by *altering* the conformation(s) of its quaternary structure. Allosteric enzymes tend to have several subunits. These subunits are referred to as protomers. In a given conformational state, these enzymes can bind substrate (S), inhibitor (l), and activator (A).

Whereas enzymes with single active sites display normal Michaelis-Menten kinetics, allosteric enzymes have multiple active sites and show cooperative binding. As a result, allosteric enzymes display a sigoidal dependence on the concentration of their substrates, allowing them to greatly vary catalytic output in response to small changes in effector concentration. Effector molecules, which may be the substrate itself (homotropic effectors) or some other small molecule (heterotropic effector), may cause the enzyme to become more active or less active. The binding sites for heterotropic effectors, called allosteric sites, are separate from the active site.

Properties of Allosteric Enzymes:

- Allosteric or Regulatory enzymes have multiple subunits (Quaternary Structure) and multiple active sites. Allosteric enzymes have active and inactive shapes differing in 3D structure. Allosteric enzymes often have multiple inhibitor or activator binding sites involved in switching between active and inactive shapes.
- Allosteric enzymes have characteristic “S”-shaped curve for reaction rate vs. substrate concentration. Because the substrate binding is “Cooperative.” And the binding of first substrate at first active site stimulates active shapes, and promotes binding of second substrate.

- A modulator is a metabolite, when bound to the allosteric site of an enzyme, alters its kinetic characteristics. The modulators for allosteric enzyme may be either stimulatory or inhibitory. A stimulator is often the substrate itself. The regulatory enzymes for which substrate and modulator are identical are called homo-tropic.
- When the modulator has a structure different then the substrate, the enzyme is called heterotropic. Some enzymes have more then one modulators. The allosteric enzymes also have one or more regulatory or allosteric sites for binding the modulator. Enzymes with several modulators generally have different specific binding sites for each.

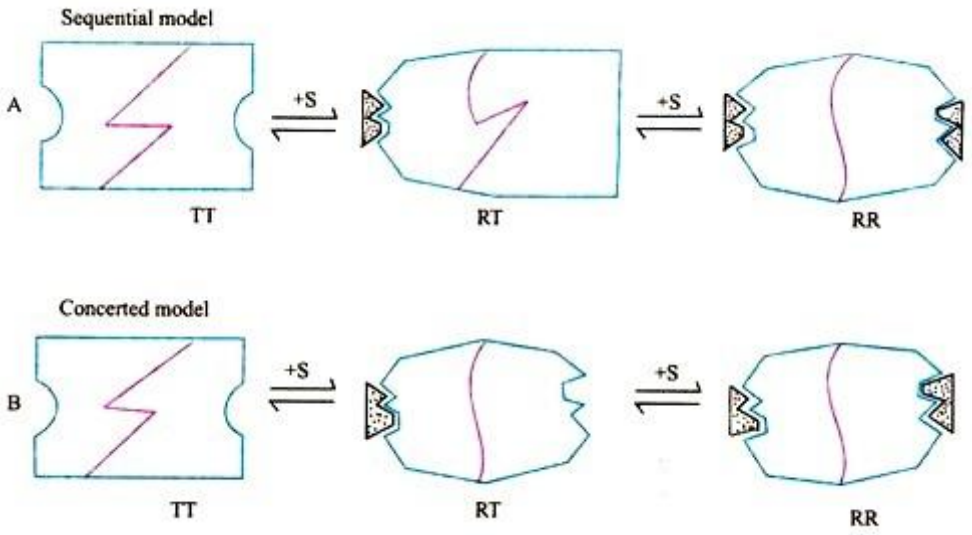


Mechanism of Action of Allosteric Enzymes:

Two general models for the inter-conversion of inactive and active forms of allosteric enzymes have been proposed:

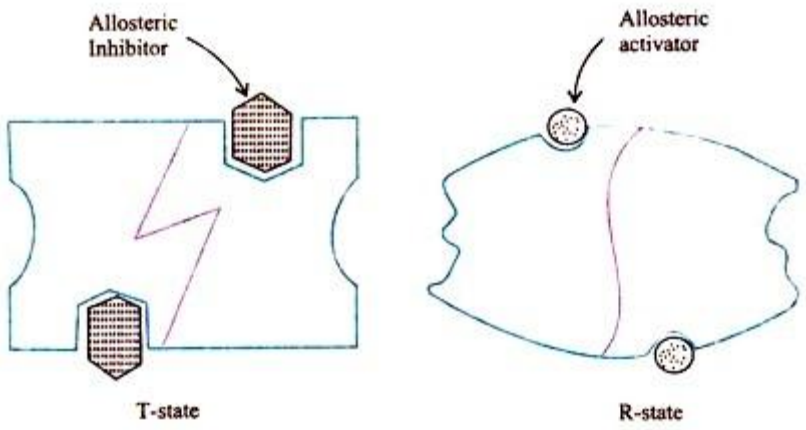
Simple sequential model:

- This model was proposed by Koshland Jr. in the year 1966. According to this theory, the aliosteric enzyme can exist in only two conformational changes individually. Consider an aliosteric enzyme consisting of two identical subunits, each containing an active site.
- The T (tense) form has low affinity and the R (relaxed) form has high affinity for substrate. In this model, the binding of substrate to one of the subunits induces a T → R transition in that subunit but not in the other subunits.



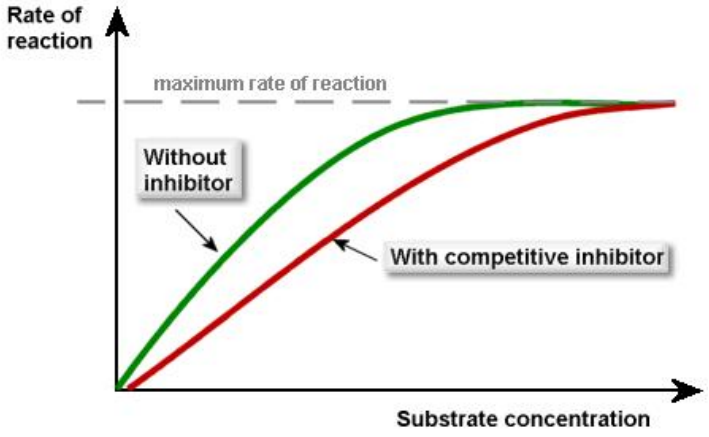
Concerted or Symmetry Model

- This model was proposed by Jacques Monod and his colleagues in 1965. According to them, an allosteric enzyme can exist in still two conformations, active and relaxed or inactive form.
- All subunits are either in the active form or all are in inactive form. Every substrate molecule that binds with enzyme increases the probability of transition from the inactive to the active site. The effect of allosteric activators and inhibitors can be explained quite easily by this model.
- An allosteric inhibitor binds preferably to the T form whereas an allosteric activator binds to the R form (Fig. 12.17B). An allosteric inhibitor shifts The R → T conformational equilibrium towards T. Whereas an allosteric activator shifts it toward R.
- The result is that an allosteric activator increases the binding to substrate of the enzyme, whereas an allosteric inhibitor decreases substrate binding. Symmetry is conserved in this model but not in the sequential model.

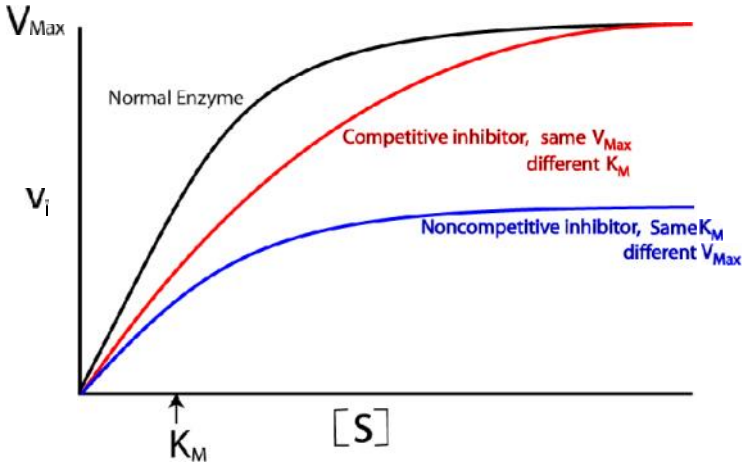


Enzyme Inhibitors

- Enzyme Inhibitors reduce the rate of an enzyme catalyzed reaction by interfering with the enzyme in some way. This effect may be permanent or temporary.
- Competitive Enzyme Inhibitors work by preventing the formation of Enzyme-Substrate Complexes because they have a similar shape to the substrate molecule.
- This means that they fit into the Active Site, but remain unreacted since they have a different structure to the substrate. Therefore less substrate molecules can bind to the enzymes so the reaction rate is decreased.
- Competitive Inhibition is usually temporary, and the Inhibitor eventually leaves the enzyme. This means that the level of inhibition depends on the relative concentrations of substrate and Inhibitor, since they are competing for places in enzyme Active Sites.



- Non-competitive Enzyme Inhibitors work not by preventing the formation of Enzyme-Substrate Complexes, but by preventing the formation of Enzyme-Product Complexes. So they prevent the substrate from reacting to form product.
- Usually, Non-competitive Inhibitors bind to a site other than the Active Site, called an Allosteric Site. Doing so distorts the 3D Tertiary structure of the enzyme, such that it can no longer catalyse a reaction.
- Since they do not compete with substrate molecules, Non-competitive Inhibitors are not affected by substrate concentration.

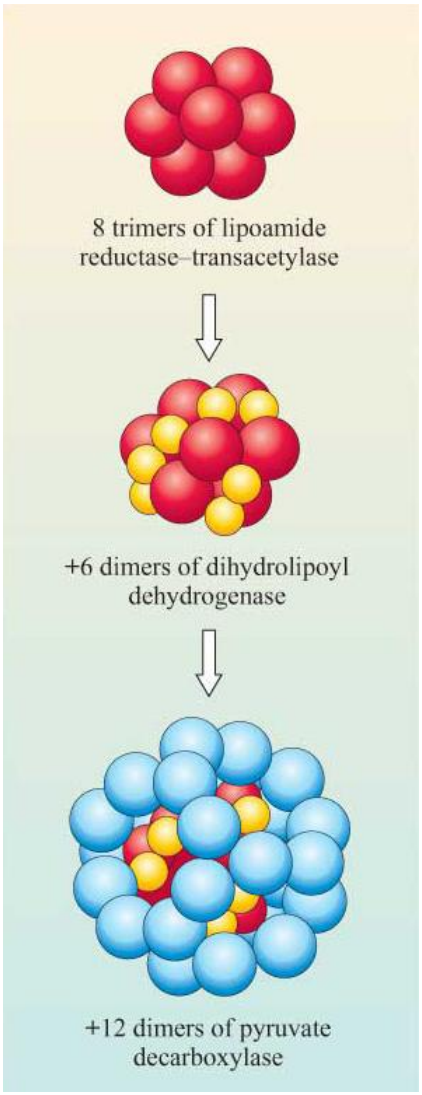


- Many Non-competitive Inhibitors are irreversible and permanent, and effectively denature the enzymes which they inhibit. However, there are a lot of non-permanent and reversible Non-competitive Inhibitors which are vital in controlling Metabolic functions in organisms.
- Enzyme Inhibitors by organisms are used in controlling metabolic reactions. This allows product to be produced in very specific amounts.

Multienzyme complexes

In free solution, the rate of an enzyme-catalysed reaction depends on the concentration of the enzyme and the concentration of its substrate. For an enzyme operating at suboptimal concentrations, the reaction is said to be *diffusion-limited*, since it depends on the random collision of the enzyme and substrate. If we consider a metabolic pathway, the product of one reaction is the substrate for the next enzyme in the pathway. Direct transfer of a metabolite from one enzyme to another would avoid dilution of the metabolite in the bulk aqueous environment and would increase the rate of reaction.

In the cell, enzymes of a particular pathway are frequently organised spatially so that such *metabolic channelling* can occur. Some enzymes are associated with other enzymes involved in a particular pathway to form **multienzyme complexes**. For the enzymes in such complexes, the diffusion of the substrate is not rate-limiting. Pyruvate dehydrogenase is a complex of three different enzymes that collectively catalyse the oxidation of pyruvate. In fact, in eukaryotic cells, most enzymes do not diffuse freely in the cytosol but are effectively concentrated in particular parts of the cell along with other enzymes or proteins involved in related processes. Concentration of enzymes in this way can be achieved by specific protein–protein interactions.



Pyruvate dehydrogenase is a multienzyme complex comprising multiples of three different enzymes: eight of lipoamide reductase–transacetylase (a trimer), six of dihydrolipoyl

dehydrogenase (a dimer) and 12 of pyruvate decarboxylase (a dimer), giving a total of 60 polypeptide chains per complex.

Michaelis-Menten Equation.

First derivation, we start with kinetic mechanism.

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

E is enzyme, S is substrate, ES is the enzyme-substrate complex, and P is product. This equation includes the assumption that during the early stages of the reaction, so little product is formed that the reverse reaction (product combining with enzyme and re-forming substrate) can be ignored). Another assumption is that the concentration of substrate is much greater than that of total enzyme ([S]>>[Et]), so it can essentially be treated as a constant.

From general chemistry we can equate that rate of this process (k3[ES]) to the change in product concentration as a function of time (d[P]/dt),or equivalently, we can designate the rate with an italicized v (v) as follows.

$$\frac{d[P]}{dt} = v = k_3[ES]$$

Because the concentration of the enzyme-substrate complex ([ES]) cannot be measured experimentally, we need an alternative expression for this term. Because the enzyme that we add to the reaction will either be unbound (E) or bound (ES) we can express the fraction of bound enzyme as follows.

$$\frac{[ES]}{[E_t]} = \frac{[ES]}{[ES]+[E]}$$

If we multiply the numerator and denominator of the right-hand side of the above equation. We are in effect, multiplying by one and we do not change the value of this expression. When we do this we obtain.

$$[ES] = \frac{[E_t]}{1 + \frac{[E]}{[ES]}}$$

We have almost achieved our goal of isolating [ES], Next we need to come up with an alternative expression for the ration [E]/[ES]. We do this by recalling that a major assumption in enzyme kinetics is the steady state assumption. Basically, it says the rate of change of [ES] as a function of time is zero: d[ES]/dt=0. Another way to express the steady state assumption is that the rate of formation of ES equals the rate of breakdown of ES.

$$k_1[\mathrm{E}][\mathrm{S}] = k_2[\mathrm{ES}] + k_3[\mathrm{ES}] = (k_2 + k_3)[\mathrm{ES}]$$

The left hand side of the equation expresses the rate of formation of ES and the right hand side expresses the two ways that ES can breakdown.

We can rearrange the equation to isolate the ration [E]/[ES].

$$\frac{[\mathrm{E}]}{[\mathrm{ES}]} = \frac{(k_2 + k_3)}{k_1[\mathrm{S}]}$$

We now define a new constant, the Michaelis constant (Km)

$$K_m = \frac{(k_2 + k_3)}{k_1}$$

If we substitute Km back into equation we obtain

$$\frac{[\mathrm{E}]}{[\mathrm{ES}]} = \frac{K_m}{[\mathrm{S}]}$$

We now substitute the ration Km/[S] from equation in place of the ratio [E]/[ES] and we obtain

$$[\mathrm{ES}] = \frac{[\mathrm{E}_t]}{1 + \frac{K_m}{[\mathrm{S}]}}$$

If we multiply the numerator and denominator of the right hand side of equation by [S], we are in effect, multiplying by one and we do not change the value of this expression.

When we do this we obtain

$$[\mathrm{ES}] = \frac{[\mathrm{E}_t][\mathrm{S}]}{[\mathrm{S}] + K_m} = \frac{[\mathrm{E}_t][\mathrm{S}]}{K_m + [\mathrm{S}]}$$

Now we have achieved our goal of isolating [ES] and we can substitute this alternative expression of [ES] into equation. We obtain

$$v = \frac{k_3[E_t][S]}{K_m + [S]}$$

Next, we imagine what happens to equation [S]>>Km as follow

$$v \approx \frac{k_3[E_t][S]}{[S]} = k_3[E_t] = k_{cat}[E_t]$$

The constant K_{cat} in the right hand most term of equation is used to signify that k_3 is considered the catalytic constant. Under such conditions, when [S] is said to saturating, the enzyme in functioning as fast as it can and we define k_3[Et] (or kcat[Et]) to be equal to V_{max} the maximum velocity that can be obtained. Therefore the equation can be rewritten into the familiar form of Michaelis-Menten equation.

$$v = \frac{V_{max}[S]}{K_m + [S]}$$

Vitamin

- Vitamins are organic compounds required by the body in small amounts for metabolism, to protect health, and for proper growth in children. Vitamins also assist in the formation of hormones, blood cells, nervous-system chemicals, and genetic material. They generally act as catalysts, combining with proteins to create metabolically active enzymes that in turn produce hundreds of important chemical reactions throughout the body. Without vitamins, many of these reactions would slow down or cease.

Chemical Composition

Vitamins are organic compounds of different chemical nature. These are alcohols, aldehyde, organic acids, their derivatives or nucleotide derivatives.

Classification of Vitamins

Vitamins are classified according to their ability to be absorbed in fat or water.

Fat Soluble Vitamins: These are oily and hydrophobic compounds. These are stored in the liver and are not excreted out of the body. Bile salts and fats are required for their

absorption. Vitamin A, D, E and K are fat soluble vitamins. Because these vitamins can be stored, their excessive intake may have toxic effect and can result in **Hypervitami nosis**.

Water Soluble Vitamins: Vitamin B complex and vitamin C are water soluble. They are compounds of carbon, hydrogen, oxygen and nitrogen. They are not stored in the body therefore they required daily in small amount.

Fat Soluble Vitamin

Vitamin A

Vitamin A (Retinol)

Vitamin A is a pale yellow primary alcohol derived from carotene. It includes Retinol (alcoholic form), Retinal (Aldehyde form) and Retinoic acid (acidic form).

Source

• In animal form, vitamin A is found in milk, butter, cheese, egg yolk, liver, and fish-liver oil.

• In plant source it obtained from vegetables as carrots, broccoli, squash, spinach, kale, and sweet potatoes.

Physiological Significance

• All three forms of vitamin A are necessary for proper growth, reproduction, vision, differentiation and maintenance of epithelial cells.

• Vitamin A accelerates normal formation of bone and teeth.

• Retinoic acid is needed for glycoprotein synthesis.

Deficiency of Vitamin A

• An early deficiency symptom is night blindness (difficulty in adapting to darkness).

• Other symptoms are excessive skin dryness

• Lack of mucous membrane secretion, causing weakness to resist bacterial attack

• Dryness of the eyes due to a malfunctioning of the tear glands.

Hypervitaminosis of Vitamin A: Excess vitamin A can interfere with growth, stop menstruation, damage red blood corpuscles, and cause skin rashes, headaches, nausea, and jaundice.

Vitamin D (Calciferol or Antirachitic Vitamin)

Source

• Vitamin D is obtained from egg yolk, cod liver oil and liver oil from other fishes.

• It is also manufactured in the body when sterols, which are commonly found in many foods, migrate to the skin and become irradiated.

Physiological Significance

• This vitamin is necessary for normal bone formation and for retention of calcium and phosphorus in the body.

• It also protects the teeth and bones against the effects of low calcium intake by making more effective use of calcium and phosphorus.

• It decreases pH in the lower intestine.

Deficiency

• Vitamin D deficiency produces **rickets** in children and **Osteomal aci a** in adult.

• Rickets is characterized by abnormities of the rib cage and skull and by bowlegs, due to failure of the body to absorb calcium and phosphorus.

• Osteomalacia is characterized by softness of pelvic girdle, ribs and femoral bones.

Hypervitaminosis of Vitamin D

• Because vitamin D is fat-soluble and stored in the body, excessive consumption can cause vitamin poisoning, kidney damage, lethargy, and loss of appetite.

Vitamin E (Tocopherol or Fertility Vitamin)

Source

• It is found in vegetable oils, wheat germ, liver, and leafy green vegetables.

• They are also present in little amount in meat, milk and eggs.

Physiological Significance

• Vitamin E acts as antioxidants. They play some role in forming red blood cells and muscle and other tissues and in pre venting the oxidation of vitamin A and fats.

• It is also associated with cell maturation and differentiation.

Deficiency

• Deficiency of vitamin E causes sterility in both male and females.

• It causes muscular dystrophy.

• In children it causes haemolysis, creatinuria.

Vitamin K (Phylloquinone or Anti hemorragic Vitamin or Coagulation Vitamin)

Vitamin K is a complex unsaturated hydrocarbon found in two forms Vitamin K (Phylloquinone) and Vitamin K (Menaquinone).

Source

• The richest sources of vitamin K are alfalfa, fish livers, leafy green vegetables, egg yolks, soybean oil and liver.

• It is also produced by bacteria in human intestine therefore no dietary supplement is needed.

Physiological Significance

• This vitamin is necessary mainly for the coagulation of blood.

• It aids in forming prothrombin, an enzyme needed to produce fibrin for blood clotting.

• Acts as an inducer for the synthesis of RNA.

• It is also required for the absorption of fat.

Deficiency

• Digestive disturbances may lead to defective absorption of vitamin K and hence to mild disorders in blood clotting.

Hypervitaminosis of Vitamin K

• Administration of large doses of vitamin K produces haemolytic anemia and jaundice in infants because of breakdown of RBCs.

Water Soluble Vitamin

Known also as vitamin B complex, these are fragile, water-soluble substances, several of which are particularly important to carbohydrate metabolism. They include

Vitamin B1 (Thiamine),

Vitamin B2 (Riboflavin),

Vitamin B3 (Niacin or Nicotinic Acid),

Vitamin B6 (Pyridoxine),

Vitamin B12 (Cobalamin) etc.

Vitamin B1

(Thiamine)

Vitamin B1 (Thiamine Chloride)

Thiamine, or vitamin B. a colorless, crystalline substance. It is readily soluble in water and slightly in ethyl alcohol

Source

• Vitamin B1is abundantly found in germinating seeds, un-milled cereals, beans, orange juice, tomato, egg, meat, fish, organ meats (liver, heart, and kidney), leafy green vegetables, nuts, and legumes.

Physiological Significance

• Act s as a catalyst in carbohydrate metabolism, enabling pyruvic acid to be absorbed and carbohydrates to release their energy.

• Thiamine also plays a role in the synthesis of nerve-regulating substances.

Deficiency

• Deficiency in thiamine causes beriberi, which is characterized by muscular weakness, swelling of the heart, and leg cramps.

6. Vitamin B2 (Riboflavin)

Source

• The best sources of riboflavin are liver, milk, meat, dark green vegetables, whole grain and enriched cereals, pasta, bread, and mushrooms.

Physiological Significance

• It is essential for carbohydrate metabolism. Enzyme containing riboflavin is called **Fl avoproteins**.

• It acts as coenzyme for enzyme catalyzing oxidation-reduction reaction.

Deficiency

• Its deficiency causes **Glossitis** (inflammation of tongue).

• Lack of thiamine causes skin lesions, especially around the nose and lips, and sensitivity to light.

Vitamin B3

Source

• The best sources of niacin are liver, poultry, meat, canned tuna and salmon, whole grain and enriched cereals, dried beans and peas, and nuts.

• The body also makes niacin from the amino acid tryptophan.

Physiological Significance

• Nicotinic acid is essential for the normal functioning of skin, intestinal tract and the nervous system.

• Vitamin B3 works as a coenzyme in the release of energy from nutrients.

Deficiency

• A deficiency of niacin causes **pellagra**, the first symptom of which is a sunburnlike eruption that breaks out where the skin is exposed to sunlight.

• Later symptoms are a red and s wollen tongue, diarrhea, mental confusion, irritability, and, when the central nervous system is affected, depression and mental disturbances.

Pantothenic Acid or Vitamin B5

Source

• Its main sources are liver, milk, meat, eggs, wheat germ, wheat bran, potatoes, sweet potatoes, tomatoes, cabbage, cauliflower and broccoli. Fruit and other vegetables also have pantothenic acid.

Physiological Significance

• Pantothenic acid is essential for growth of infants and children,

• It plays a major role in the metabolism of proteins, carbohydrates, and fats.

Deficiency

• Its deficiency causes nausea, vomiting, gastrointestinal disorders, improper growth and fatty liver.

Vitamin B6 (Pyridoxine):

Source

• The best sources of pyridoxine are whole (but not enriched) grains, cereals, bread, liver, avocadoes, spinach, green beans, and bananas.

• It is also found in milk, eggs, fish, chicken, beaf, pork and li ver.

Physiological Significance

• Pyridoxine, or vitamin B, is necessary for the absorption and metabolism of amino acids.

• It also plays roles in the use of fats in the body and in the formation of red blood cells.

Deficiency

• Pyridoxine deficiency is characterized by skin disorders, cracks at the mouth corners, smooth tongue, convulsions, dizziness, nausea, anemia, and kidney stones.

Vitamin B7 (Biotin)

Biotin is also known as “anti-egg white injury factor” or as H-factor.

Source:

• Biotin occurs in combined state as biocytin. It is found in yeast, liver, kidney, milk and molasses.

Physiological Significance:

• Biotin serves as prosthetic group for many enzymes which catalyze fixation of CO into organic molecules.

• It helps in synthesis of fatty acids.

Deficiency:

• Its deficiency caused the destruction of intestinal bacteria.

• It leads to nausea and muscular pain.

Vitamin B9 or M or Bc (Folic Aci d)

Source

• Folic acid is found in yeast, liver and kidney.

• Fish meat and green leafy vegetables, milk and fruits also provide folic acid.

Physiological Significance

• Folic acid acts as a coenzyme and help in synthesis of purines and thymine during DNA synthesis.

• It helps in formation and maturation of red blood cells.

Deficiency

• Folic acid deficiency gives rise to **megaloblasticanemia**.

• The patient suffers from retarded growth, weakness, infertility, inadequate lactation in females and gastrointestinal disorders.

Vitamin B12 (Cynocobalamin)

Vitamin B12 or **Cobalamin**, or **Anti -Pernicious Anaemic Factor (APA)**, one of the most recently isolated vitamins.

Source

• Cobalamin is obtained only from animal sources—liver, kidneys, meat, fish, eggs, and milk. Vegetarians are advised to take vitamin B supplements.

Physiological Significance

• It is necessary in minute amounts for the formation of nucleoproteins, proteins, and red blood cells.

• It is necessary for the functioning of the nervous system.

• It stimulates the appetite of the subject.

Deficiency

• Due to its deficiency **Pernicious Anemia** results which is characterized by symptoms of ineffective production of red blood cells, faulty myelin (nerve sheath) synthesis, and loss of epithelium (membrane lining) of the intestinal tract.

Lipoic Acid

• Lipoic acid is a sulphur containing fatty acid. It is widely distributed in natural foods. Lipoic acid functions as a coenzyme inoxidative decarboxylati on of pyruvic acid and a-ketoglutaric acid. Its deficiency disorders have not been recorded.

Inositol

Source

• Yeast, meat, milk, nuts, fruits, vegetables and grains contains Inositol.

Physiological Significance

• It increases peristalsis of small intestine, increase the rate of contraction of heart muscles.

Deficiency

• Deficiency symptoms include retarded growth, failure of lactation, loss of hair over the body (alopecia) etc.

Choline

Source

• Choline is found in liver, egg yolk, meat, cereals, rice, milk, fruits and vegetables.

Physiological Significance

• Acetyl choline is a chemical mediator of parasympathetic activities and other activities of nervous system.

• It prevents accumulation of fat in the liver.

Deficiency

• Its deficiency causes fatty liver, slipped tendon diseases etc.

Vitamin C (Ascorbic Acid or Antiscorbutic Vitamin)

Source

• Sources of vitamin C include citrus fruits, fresh strawberries, cantaloupe, pineapple, and guava.

• Good vegetable sources are Broccoli, Brussels sprouts, Tomatoes, Spinach, Kale, Green Peppers, Cabbage, and Turnips.

Physiological Significance

• Vitamin C is important in the formation and maintenance of collagen, the protein that supports many

body structures and plays a major role in the formation of bones and teeth.

• It also enhances the absorption of iron from foods of vegetable origin.

• The connective tissue fibrils and collagen are synthesized with the help of vitamin C.

• It play important role in wound repair.

• It protects body against stress.

Deficiency

• This well -known Scurvy is the classic manifestation of severe ascorbic acid deficiency. Its symptoms are loss of the cementing action of collagen and include hemorrhages which lead to loosening of teeth and cellular changes in the long bones of children.

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Question	Opt A	Opt B	Opt C
A Holoenzyme is	Functional unit	Apo enzyme	Coenzyme
Example of an extracellular enzyme is	Lactate dehydrogenase	Cytochrome oxidas	Pancreatic lipase
Enzymes, which are produced in inactive fo	Papain	Lysozymes	Apoenzymes
An example of ligases is	Succinate thiokinase	Alanine racemase	Fumarase
An example of lyases is	Glutamine synthetase	Fumarase	Cholinesterase
Activation or inactivation of certain key reg	Tyrosine	Phenylalanine	Lysine
The enzyme which can add water to a carb	Hydratase	Hydroxylase	Hydrolase
Fischer's 'lock and key' model of the enzym	The active site is compl	The active site is co	Substrates change confo
From the Lineweaver-Burk plot of Michaeli	1/V	V	1/S
A sigmoidal plot of substrate concentration	Michaelis-Menten kine	Co-operative bindir	Competitive inhibition
The kinetic effect of purely competitive inh	Increases Km without a	Decreases Km with	Increases Vmax without
An inducer is absent in the type of enzyme:	Allosteric enzyme	Constitutive enzym	Co-operative enzyme
In reversible non-competitive enzyme activ	Vmax is increased	Km is increased	Km is decreased
In competitive enzyme activity inhibition	The structure of inhibit	Inhibitor decreases	Km remains unaffective
In enzyme kinetics Vmax reflects	The amount of an activ	Substrate concentra	Half the substrate conce
In enzyme kinetics Km implies	The substrate concentr	The dissocation cor	Concentration of enzym
In a competitive enzyme activity inhibition	Apparent Km is decreas	Apparent Km is incr	Vmax is increased
In non competitive enzyme activity inhibiti	Increases Km	Decreases Km	Does not effect Km
The pH optima of most of the enzymes is	Between 2 and 4	Between 5 and 9	Between 8 and 12
Coenzymes are	Heat stable, dialyzable,	Soluble, colloidal, p	Structural analogue of e
Factors affecting enzyme activity:	Concentration	pH	Temperature
The normal serum GOT activity ranges from	3.0–15.0 IU/L	4.0–17.0 IU/L	4.0–60.0 IU/L
The normal GPT activity ranges from	60.0–250.0 IU/L	4.0–17.0 IU/L	3.0–15.0 IU/L
The normal serum acid phosphatase activit	5.0–13.0 KA units/100 r	1.0–5.0 KA units/10	13.0–18.0 KA units/100
The normal serum alkaline phosphatase act	1.0–5.0 KA units/100 m	5.0–13.0 KA units/10	0.8–2.3 KA units/100 ml
The isoenzymes LDH5 is elevated in	Myocardial infarction	Peptic ulcer	Liver disease
LDH1 and LDH2 are elevated in	Myocardial infarction	Liver disease	Kidney disease
The pH optima for salivary analyse is	6.6–6.8	2.0–7.5	7.9
The pH optima for pancreatic analyse is	4	7.1	7.9
The substrate for amylase is	Cane sugar	Starch	Lactose
Vitamin A or retinal is a	Steroid	Polyisoprenoid com	Benzoquinone derivative
β-Carotene, precursor of vitamin A, is oxida	β-Carotene dioxygenas	Oxygenase	Hydroxylase
Preformed Vitamin A is supplied by	Milk, fat and liver	All yellow vegetable	All yellow fruits
Fat soluble vitamins are	Soluble in alcohol	one or more Proper	Stored in liver
The normal serum concentration of vitamir	5–10	15–60	100–150
One manifestation of vitamin A deficiency i	Painful joints	Night blindness	Loss of hair
Deficiency of Vitamin A causes	Xeropthalmia	Hypoprothrombine	Megaloblastic anemia
An important function of vitamin A is	To act as coenzyme for	To play an integral r	To prevent hemorrhages

Retinal is a component of	Iodopsin	Rhodopsin	Cardiolipin
Richest source of Vitamin D is	Fish liver oils	Margarine	Egg yolk
Deficiency of vitamin D causes	Ricket and osteomalaci	Tuberculosis of bon	Hypthyroidism
Vitamin K2 was originally isolated from	Soyabean	Wheat gram	Alfa Alfa
Vitamin synthesized by bacterial in the inte	A	C	D
The most important natural antioxidant is	Vitamin D	Vitamin E	Vitamin B12
Creatinuria is caused due to the deficiency	A	K	E
The daily requirement of riboflavin for adul	0–1.0	1.2–1.7	2.0–3.5
The precursor of CoA is	Riboflavin	Pyridoxamine	Thiamin
FAD is a coenzyme for	Succinate dehydrogena	Glycerol-3-phospha	Sphingosine reductase
Niacin contains a	Sulphydryl group	Carboxyl group	Amide group
Vitamin B12 is	Not stored in the body	Stored in bone mar	Stored in liver
Daily requirement of vitamin C in adults is a	100 mg	25 mg	70 mg
Precursor of Vitamin A is	α-Carotene	β-Carotene	γ-Carotene
Conversion of β-carotene into retinal requi	β-Carotene dioxygenas	Bile salts	Molecular oxygen
Provitamins A include	Retinal	Retionic acid	Carotenes
Provitamin D3 is	Cholecalciferol	Ergosterol	7-Dehydrocholesterol
Ergosterol is found in	Animals	Plants	Bacteria
A water soluble form of vitamin K is	Phylloquinone	Farnoquinone	Menadione
The non-protein part of rhodopsin is	Retinal	Retinol	Carotene
Antisterility vitamin is	Vitamin B1	Vitamin B2	Vitamin E
The sulphur-containing vitamins among the	Thiamine	Riboflavin	Niacin

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ARTMENT OF MICROBIOLOGY
CROBIOLOGY – FIRST SEMESTER
MBU105A – BIOCHEMISTRY
LTIPLE CHOICE QUESTIONS

Unit 5

Opt D	Answer
All of these	All of these
Hexokinase	Pancreatic lipase
Proenzymes	Proenzymes
Aldolase	Succinate thiokinase
Amylase	Fumarase
Serine	Serine
Esterase	Hydratase
The active site is flexible	The active site is complementary in shape to that of substance
S	1/S
Non-competitive inhibitic	Co-operative binding
Decreases Vmax without	Increases Km without affecting Vmax
Isoenzymic enzyme	Constitutive enzyme
Concentration of active e	Concentration of active enzyme is reduced
Inhibitor decreases Vmax	The structure of inhibitor generally resembles that of the substrate
Enzyme substrate compl	The amount of an active enzyme
Half of the substrate conc	The substrate concentration that gives one half Vmax
Vmax is decreased	Apparent Km is increased
Increases Km	Does not effect Km
Above 12	Between 5 and 9
Different forms of enzym	Heat stable, dialyzable, non protein organic molecules
All of these	All of these
0.9–4.0 IU/L	4.0–17.0 IU/L
0.1–14.0 IU/L	3.0–15.0 IU/L
0.2–0.8 KA units/100 ml	1.0–5.0 KA units/100 ml
13.0–21.0 KA units/100 r	5.0–13.0 KA units/100 ml
Infectious diseases	Liver disease
Brain disease	Myocardial infarction
8.6	6.6–6.8
8.6	8.6
Ribose	Starch
6-Hydroxychromane	Polyisoprenoid compound containing a cyclohexenyl ring
Transferase	β-Carotene dioxygenase
Leafy green vegetables	Milk, fat and liver
All these	All these
0–5	15–60
Thickening of long bones	Night blindness
Pernicious anemia	Xeropthalmia
To maintain the integrity	To maintain the integrity of epithelial tissue

Glycoproteins	Rhodopsin
Butter	Fish liver oils
Skin cancer	Ricket and osteomalacia
Putrid fish meal	Putrid fish meal
K	K
Vitamin K	Vitamin E
D	E
4.0–8.0	1.2–1.7
Pantothenate	Pantothenate
All of these	All of these
All of these	Carboxyl group
Stored in RE cells	Stored in liver
100 mg	70 mg
All of these	All of these
All of these	All of these
All of these	Carotenes
Ergocaliferol	7-Dehydrocholesterol
All of these	Plants
None of these	Menadione
Repsin	Retinal
Vitamin K	Vitamin E
Pyridoxine	Thiamine

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DEPARTMENT OF MICROBIOLOGY
(For the candidates admitted from 2015 onwards)

Subject	**:**	**Biochemistry**	**Semester**	**:**	**I**
Subject code	**:**	**17MBU103**	**Class**	**:**	**I B.Sc Microbiology**

Maximum: 50 marks

PART-A (20 X 1 = 20 Marks)
Answer all Questions

1 Water

2 Endoplasmic reticulum

3 Passive diffusion through the lipid bilayer

4 Na^+, K^+ ATPase

5 Metabolism

6 An exponential curve

7 Singer and Nicolson

8 Diffusion

9 Colorimeter

10 Protein synthesis

11 Glycosidic bond

12 α 1-4

13 D-type

14 a & c

15 Glucose and Glucose

16 β 1-2

17 Galactose and glucose

18 In pyranose sugars

19 $(C_6H_{10}O_5)n$

20 Anomers

Part-B (3 x 2 = 6 Marks)
Answer all Questions

21 Differentiate anabolism and catabolism.

Anabolism	Catabolism
Metabolic process that builds molecules the body needs.	Metabolic process that breaks down large molecules into smaller molecules
Requires energy	Releases energy
Estrogen, testosterone, insulin, growth hormone	Adrenaline, cortisol, glycagon, cytokines
Anabolic exercises which are often anaerobic in nature, generally build muscle mass	Catabolic exercises are usually aerobic and good at burning fat and calories

22 Define epimers.

Either of two stereoisomers that differ in the arrangement of groups on a single asymmetric carbon atom (such as the first chiral center of a sugar's carbon chain).

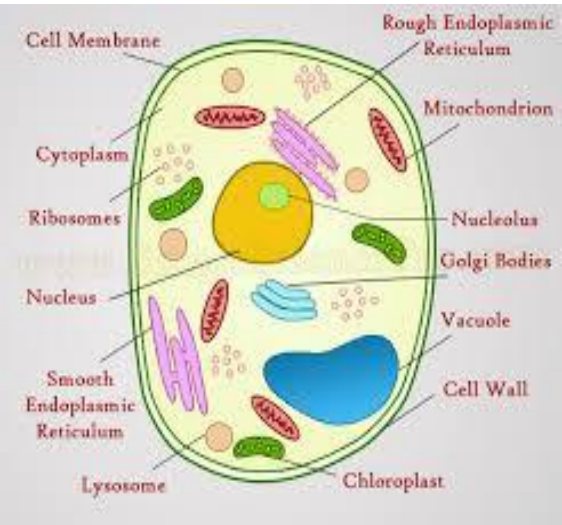
23. Define anomers.

An anomer is a cyclic saccharide and also an epimer, where the difference in the configuration specifically occurs at the hemiacetal or acetal carbon. This carbon is called the anomeric carbon and it is derived from the carbonyl carbon (aldehyde or ketone functional group) of the open-chain form of the carbohydrate molecule. Anomerization is the process of conversion of one anomer to the other. The two

anomers are distinguished by naming them alpha (α) or beta (β).

Part-C (3 x 8 = 24 Marks)
Answer all Questions

24. a) Write in detail the structure of an animal cell and its organelles.



Animal Cell Structure

The Animal cells are smaller than the plant cells which vary in their sizes and are irregular in shape. It comprises of the following parts:

Cell Membrane: A thin semipermeable membrane layer of protein and fats surrounding the cell. It helps in owning the cell together and permits entry and exits of nutrients into the cell.

Nuclear Membrane: It is the double membrane that surrounds the nucleus.

Nucleus: A celestial body containing several organelles including the nucleolus. It contains DNA and other cell's hereditary information.

Centrosome: It is a small organelle found near to the nucleus which has a thick center and radiating tubules. The centrosomes are where microtubules are produced.

Lysosome (Cell Vesicles): They are round organelle surrounded by a membrane comprising of digestive enzymes which help in digestion, excretion and in cell renewal process.

Cytoplasm: A jelly-like double membrane organelles found outside the cell nucleus in which the organelles are located.

Golgi Body: A flat smooth layered, sac-like organelle which is located near the nucleus and involved in manufacturing, storing, packing and transporting the particles throughout the cell.

Mitochondrion: They are spherical to rod-shaped organelles with a double membrane. They are the powerhouse of a cell as they play an important role in releasing energy.

Ribosome: They are small organelles made up of RNA-rich cytoplasmic granules and they are the sites of protein synthesis.

Vacuole: A membrane-bound organelles present inside a cell involved in maintaining shape and storing water, food, wastes, etc.

Nucleopore: They are tiny holes present in the nuclear membrane which are involved in the movement of nucleic acids and proteins within the cell.

24, b) Describe the principle and instrumentation of a colorimeter.

A colorimeter is a device used to test the concentration of a solution by measuring its absorbance of a specific wavelength of light.

Principle

The colorimeter is based on Beer-Lambert's law, according to which the absorption of light transmitted through the medium is directly proportional to the medium concentration.

Instrumentation

The instrument use for colorimetric is colorimeter. This apparatus will comprise of the following parts:

1. Light source

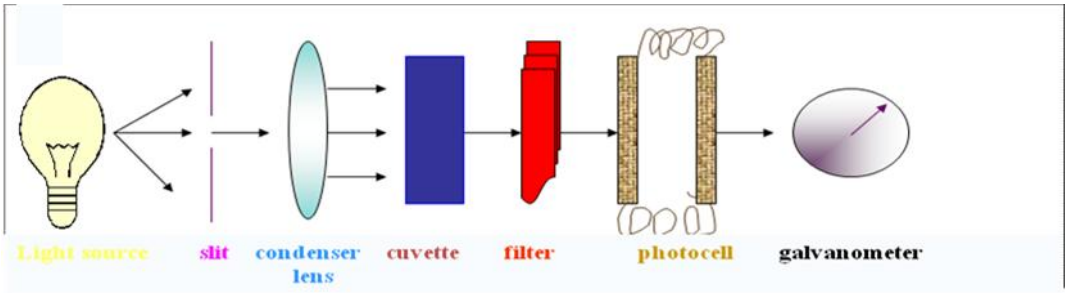
2. Filter (the device that selects the desired wavelength to obtain monochromatic light)
3. Cuvette chamber (the transmitted light passes through compartment wherein the solution containing the colored solution are kept in cuvette, made of glass or disposable plastic)
4. Detector (this is a photosensitive element that converts light into electrical signals)
5. Galvanometer (measures electrical signal quantitatively)

White light from a tungsten lamp passes through a slit, then a condenser lens, to give a parallel beam which falls on the solution under investigation contained in an absorption cell or cuvette. The cell is made of glass with the sides facing the beam cut parallel to each other.

Beyond the absorption cell is the filter, which is selected to allow maximum transmission of the color absorbed. If a blue solution is under examination, then red is absorbed and a red filter is selected.

The light then falls on to a photocell which generates an electrical current in direct proportion to the intensity of light falling on it.

This small electrical signal is increased by the amplifier which passes to a galvanometer of digital readout to give absorbance reading directly.

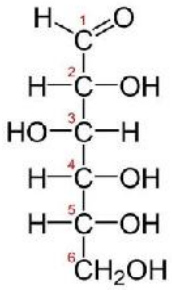


25. a) Write a note on monosaccharides and its forms.

A monosaccharide is the most basic form of carbohydrates. Monosaccharides can by combined through glycosidic bonds to form larger carbohydrates, known as oligosaccharides or polysaccharides.

Structure of Monosacchardies

All monosaccharides have the same general formula of $(CH_2O)_n$, which designates a central carbon molecule bonded to two hydrogens and one oxygen. The oxygen will also bond to a hydrogen, creating a hydroxyl group. Because carbon can form 4 bonds, several of these carbon molecules can bond together. One of the carbons in the chain will form a double bond with an oxygen, which is called a carbonyl group. If this carbonyl occurs at the end of the chain, the monosaccharide is in the *aldose* family. If the carboxyl group is in the middle of the chain, the monosaccharide is in the *ketose* family.



Glucose is one of the most common monosaccharides in nature, used by nearly every form of life. This simple monosaccharide is composed of 6 carbons, each labeled in the image. The first carbon is the carbonyl group. Because it is at the end of the molecule, glucose is in the aldose family. Typically, monosaccharides with more than 5 carbons exist as rings in solutions of water. The hydroxyl group on the fifth carbon will react with the first carbon. The hydroxyl group gives up its hydrogen atom when it forms a bond with the first carbon. The double bonded oxygen on the first carbon bonds with a new hydrogen when the second bond with the carbon is broken. This forms a fully connected and stable ring of carbons.

Example of Monosaccharides

Glucose

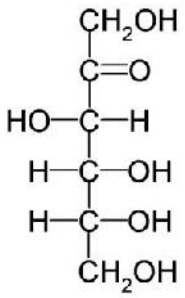
Glucose is an important monosaccharide in that it provides both energy and structure to many organism. Glucose molecules can be broken down in glycolysis, providing energy and precursors for cellular respiration. If a cell does not need any more energy at the moment, glucose can be stored by combining it with other monosaccharides. Plants store these long chains as starch, which can be disassembled and used as energy

later. Animals store chains of glucose in the polysaccharide glyocogen, which can store a lot of energy.

Glucose can also be connected in long strings of monosaccharides to form polysaccharides that resemble fibers. Plants typically produce this as cellulose. Cellulose is one of the most abundant molecules on the planet, and if we could weigh all of it at once it would weigh millions of tons. Each plant uses cellulose to surround each cell, creating rigid cell walls that help the plants stand tall and remain turgid. Without the ability of monosaccharides to combine into these long chains, plants would be flat and squishy.

Fructose

Although almost identical to glucose, fructose is a slightly different molecule. The formula ($(CH_2O)_6$) is the same, but the structure is much different.

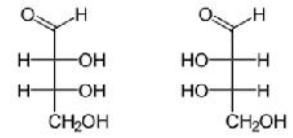


Notice that instead of the carbonyl group being at the end of the molecule, as in glucose, it is the second carbon down. This makes fructose a ketose, instead of an aldose. Like glucose, fructose still has 6 carbons, each with a hydroxyl group attached. However, because the double bonded oxygen in fructose exists in a different place, a slightly different shaped ring is formed. In nature, this makes a big difference in how the sugar is processed. Most reactions in cells are catalyzed by specific enzymes. Different shaped monosaccharides each need a specific enzyme to be broken down.

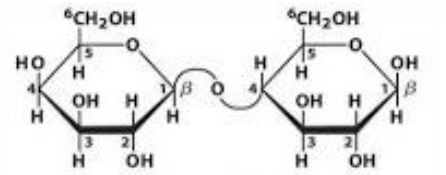
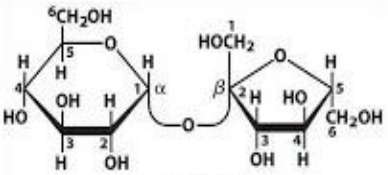
Fructose, because it is a monosaccharide, can be combined with other monosaccharides to form oligosaccharides. A very common disaccharide made by plants is sucrose. Sucrose is one fructose molecule connected to a glucose molecule through a glycosidic bond.

D- and L-Forms

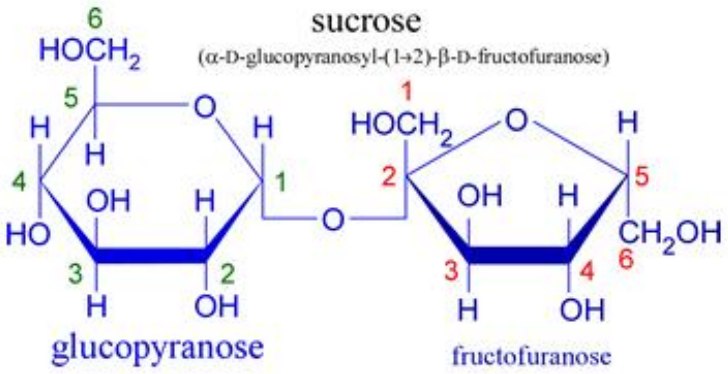
Monosaccharides can appear in either D- (dextro) or L- (levo) form, which are the mirror images of each other. Most naturally occurring monosaccharides are in the D-form and most synthetically produced are in the L-form. D- and L- forms have different properties.



25. b) Differentiate reducing and non-reducing sugars. Add a note on the Haworth projection of sucrose.

Reducing sugars	Non-reducing sugars
Such sugar bear a free aldehyde (-CHO) or ketonic (-CO) group	These sugars do not have such groups
Reducing sugars have the capacity to reduce cupric ions of Benedict's or Fehling solution to cuprous ions	Non- reducing sugar fail to reduce the cupric ions of Benedict's solution to cuprous ions.
Examples: Maltose, Lactose, Melibiose, Cellobiose, Gentiobiose  Lactose (β form) β-D-galactopyranosyl-(1→4)-β-D-glucopyranose Gal(β1→4)Glc Lactose	Example: Sucrose, Trehalose  Sucrose α-D-glucopyranosyl β-D-fructofuranoside Glc(α1↔2β)Fru Sucrose

A Haworth projection is used to represent the cyclic structure of monossacharides in a simple 3D perspective. SInce sucrose is formed from one glucose and one fructose molecule, its Haworth projection will show an α-D-glucopyranosyl ring and a β-D-fructofuranose ring connected through an α−β(1→2) glycosidic bond.



Now, a reducing sugar is a sugar that either has an aldehyde functional group attached, or is capable of forming one by way of isomerism. What basically takes place is a redox reaction in which the aldehyde is oxidized and another compound is reduced.

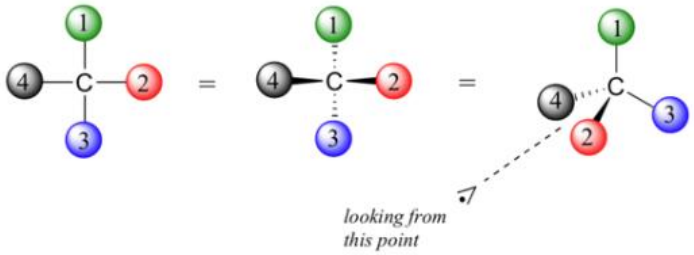
Another criterion that needs to be met in order to have a reducing sugar is that the anomeric carbon must be free to open up the ring structure and allow for the redox reaction to take place. A sugar must exist as the linear form in solution in order to be a reducing sugar.

In sucrose's case, both anomeric carbons, i.e. the one belonging to glucose (marked **1**in green in the above picture) and the one belonging to fructose (marked **2** in red), cannot open their respective rings because they are tied up in forming the glycosidic bond that keeps the sucrose molecule together.

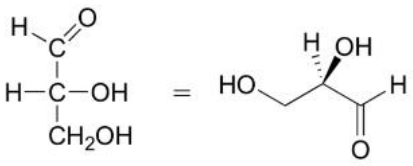
As a result, sucrose cannot react with Benedict's reagent (basically a Cu2+ solution) to reduce the metal cations and form a Cu2O precipitate. Hence, sucrose is a non-reducing sugar**.**

26. a) Draw the Fischer and Haworth projections of glucose and fructose.

Fisher projections show sugars in their open chain form. In a Fischer projection, the carbon atoms of a sugar molecule are connected vertically by solid lines, while carbon-oxygen and carbon-hydrogen bonds are shown horizontally. Stereochemical information is conveyed by a simple rule: vertical bonds point into the plane of the page, while horizontal bonds point out of the page.

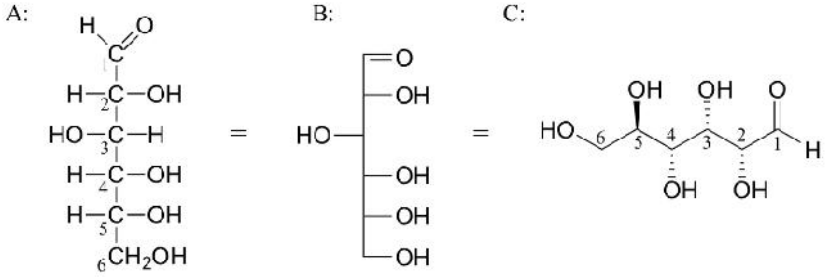


Below are two different representations of (R)-glyceraldehyde, the smallest sugar molecule (also called D-glyceraldehyde in the stereochemical nomenclature used for sugars).

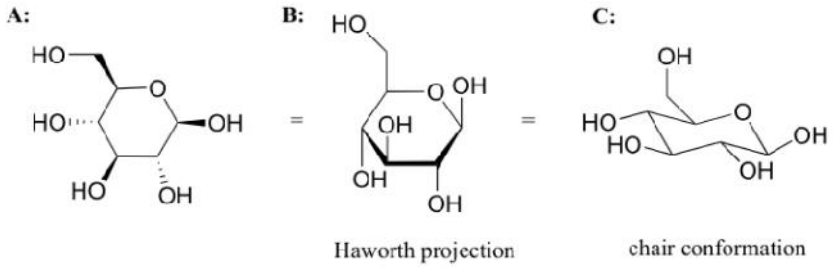


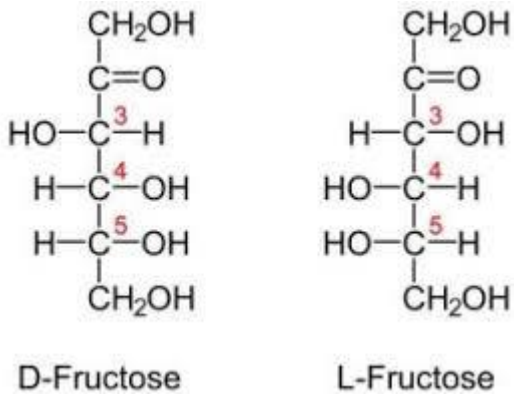
(R)-glyceraldehyde
(D-glyceraldehyde)

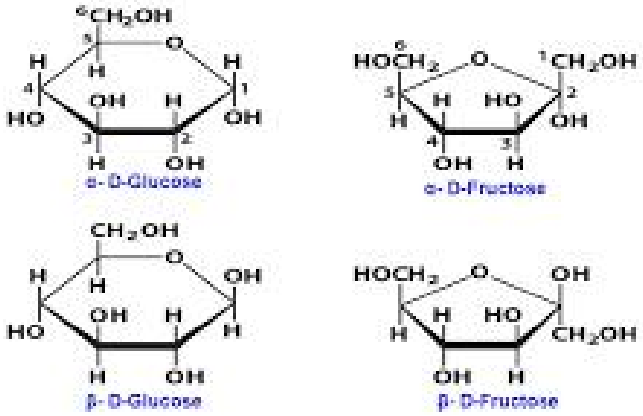
Below are three representations of the open chain form of D-glucose: in the conventional Fischer projection (A), in the "line structure" variation of the Fischer projection in which carbons and hydrogens are not shown (B), and finally in the 'zigzag' style (C) that is preferred by organic chemists.



While Fischer projections are used for sugars in their open-chain form, Haworth projections are often used to depict sugars in their cyclic forms. The beta diastereomer of the cyclic form of glucose is shown below in three different depictions, with the Haworth projection in the middle.







26. b) Write a note on disaccharides with appropriate examples.

Disaccharides

- A disaccharide is formed when a hydroxyl group on one monosaccharide reacts with the anomeric carbon of another monosaccharide to form a glycosidic bond.
- Each disaccharide has a specific glycosidic linkage (depending on which hydroxyl reacts with which anomer).
- The three most common disaccharides are **maltose, lactose and sucrose.**

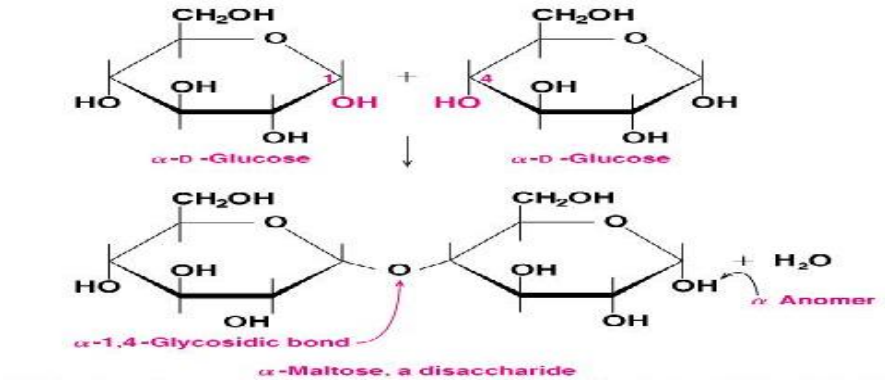
- When hydrolyzed using acid or an enzyme, the following monosaccharide are produced.
- The disaccharides are of two types

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

Maltose

Occurrence: Not occur in our body, but present in germinating cereals and malt; It is the breakdown product of starch

Structure: Maltose (malt sugar or corn sugar) is composed of two glucose molecules are joined through α-1,4 glycosidic linkage



Properties

- Because one of the glucose molecules is a hemiacetal (having a free aldehyde group) it can undergo mutorotation (Gradual change in specific rotation; Glucose if freshly prepared have sp rotation of +112°, but on standing gives a rotation of + 52.).
- It exist in α and β forms
- Since it is having a free aldehyde group, it reduce compounds and and so maltose is a reducing sugar.
- Reduce Fehling and Benedicts solution but not Barfoeds solution
- Forms osazone with phenyl hydrazine
- Maltose can be fermented by yeast to produce ethanol.
- Maltose is also used in cereals, candies and malted milk.

  Hydrolysis: Hydrolysed by maltase present in alimentary canal; two glucose molecules are released upon hydrolysis

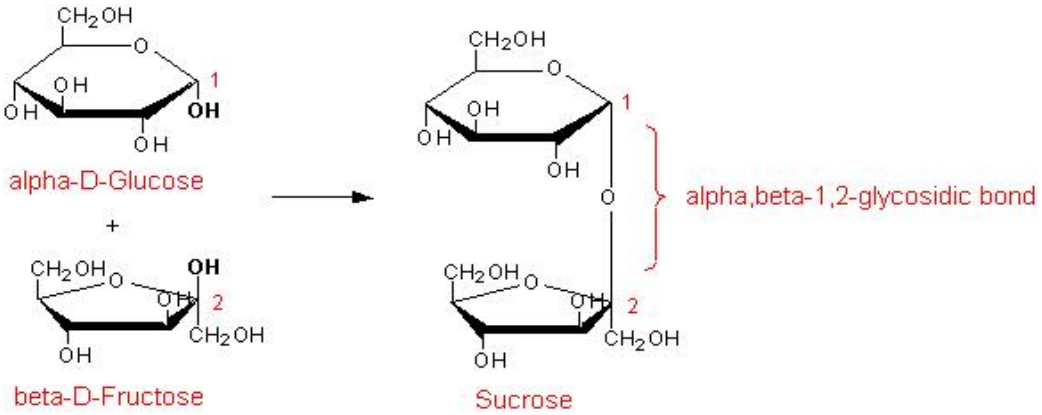
Sucrose

Occurrence

It is the sweetest of all the sugars; doesnot exist in our body, occur in cane sugar, pineapple, carrot root, sweet potato and honey. Sucrose is the most abundant disaccharide and is commercially produced from sugar cane and sugar beets.

Structure

Sucrose (table sugar) consists of one glucose molecule and one fructose molecule linked by an α,β-1,2-glycosidic bond.



- It is not having a free aldehyde or ketone group, so don't have mutarotation; does not exist in α and β forms. **Because the glycosidic bond in sucrose involves both** anomeric carbons, neither monosaccharide can undergo mutorotation, and so sucrose is not a reducing sugar.

Properties

- White crystalline solid powder; sparingly soluble in water
- The specific rotation of fructose is 66.5. but upon hydrolysis it is changed to -19.5. This because the hydrolysed product, fructose, which is having more levo rotary than the glucose . This reaction is **called inversion and the sugar is called invert sugar.**
- it does not reduce Fehling , Benedicts s and Barfoeds solution
- it cannot from crystals with phenyl hydrazine
- **Hydrolysis:**

- Hydrolysed by sucrase present in alimentary canal; one glucose and one fructose molecules are released upon hydrolysis

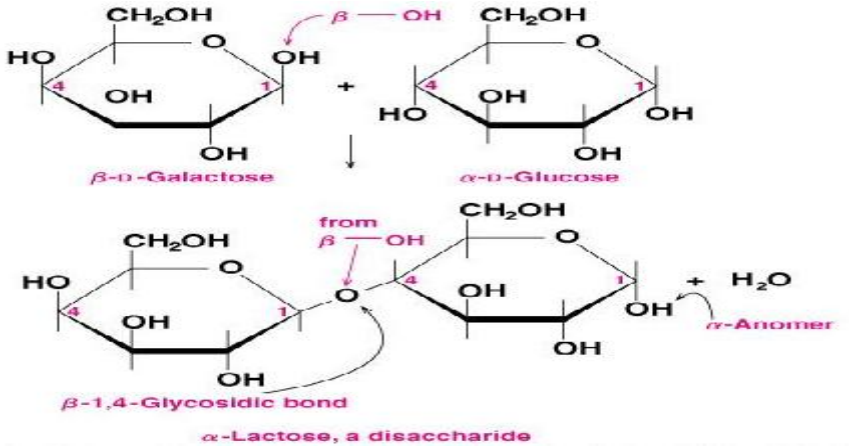
Lactose

Occurrence

- Present in human milk (9.8%) produced by mammary gland of human beings; It comes from milk products (about 4-5% of cow's milk).; also occur in urine during pregnancy.

Structure

- **Lactose** (milk sugar) consists of one glucose molecule and one galactose molecule linked by a β-1,4 glycosidic bond.



- Because the glucose is a hemiacetal, it can undergo mutorotation, and it is having a free aldehyde group, which reduce compounds and so lactose is a reducing sugar.

Properties

- White crystalline solid powder; sparingly soluble in water
- the specific rotation is + 55.2.
- Exist in α and β forms
- Reduce Fehling and Benedicts solution but not Barfoeds solution
- Forms osazone with phenyl hydrazine

Reg. No. _____

[16MBU103]

KARPAGAM UNIVERSITY

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed University *established Under Section 3 of UGC Act 1956*)

DEPARTMENT OF BIOCHEMISTRY

SECOND INTERNAL EXAMINATIONS - AUGUST 2017

I B.Sc., MICROBIOLOGY - First Semester

17MBU103 - BIOCHEMISTRY

DATE: **MAXIMUM: 50 MARKS**

TIME: 2 HOURS

Part-A (20 X 1 = 20 marks)

Answer All Questions

Choose the correct option from the following:

1. All the following are storage polysaccharides except

   a. Starch b. Cellulose c. Dextran d. Glycogen

2. Storage polysaccharide made by animals is

   a. amylopectin b. glycogen c. cellulose d. collagen

3. The end product of hydrolysis of "Starch" by amylase is …

   a. Soluble starch b. Glucose c. Dextrins d. Maltose

4. Keratan sulphate is found in abundance in

   a. Heart muscle b. Liver c. Adrenal cortex d. Cornea

5. Repeating units of hyaluronic acid are

   a. N-acetyl glucosamine and D-glucuronic acid
   b. N-acetyl galactosamine and D-glucuronic acid
   c. N-acetyl glucosamine and galactose
   d. N-acetyl galactosamine and L- iduronic acid

6. A fatty acid which is not synthesized in the body and has to be supplied in the diet is

   a. Palmitic acid b. Lauric acid c. Linolenic acid d. Palmitoleic acid

7. The fatty acid present in cerebrosides is

   a. Lignoceric acid b. Valeric acid c. Caprylic acid d. Behenic acid

8. The end products of saponification:

   a. glycerol b. acid c. soap d. Both a & c

9. Glycosphingolipids are a combination of

   a. Ceramide with one or more sugar residues b. Glycerol with galactose
   c. Sphingosine with galactose d. Sphingosine with phosphoric acid

10. Cephalin consists of

    a. Glycerol, fatty acids, phosphoric acid and choline
    b. Glycerol, fatty acids, phosphoric acid and ethanolamine
    c. Glycerol, fatty acids, phosphoric acid and inositol
    d. Glycerol, fatty acids, phosphoric acid and Serine

11. Molecular formula of cholesterol is

    a. $C_{27}H_{45}OH$ b. $C_{29}H_{47}OH$ c. $C_{29}H_{47}OH$ d. $C_{23}H_{41}OH$

12. An example of a saturated fatty acid is

    a. Palmitic acid b. Oleic acid c. Linoleic acid d. Erucic acid

13. Dietary fibres are rich in

    a. Cellulose b. Glycogen c. Starch d. Proteoglycans

14. All the following have 18 carbon atoms except

    a. Linoleic acid b. Linolenic acid c. Arachidonic acid d. Stearic acid

15. Gangliosides derived from glucosylceramide contain in addition one or more molecules of

    a. Sialic acid b. Glycerol c. Diacylglycerol d. Hyaluronic acid

16. The importance of phospholipids as constituent of cell membrane is because they possess

    a. Fatty acids b. Both polar and nonpolar groups c. Glycerol d. Phosphoric acid

17. Cephalin consists of

    a. Glycerol, fatty acids, phosphoric acid and choline
    b. Glycerol, fatty acids, phosphoric acid and ethanolamine
    c. Glycerol, fatty acids, phosphoric acid and inositol
    d. Glycerol, fatty acids, phosphoric acid and Serine

18. The number of double bonds in arachidonic acid is

    a. 1 b. 2 c. 4 d. 6

19. Which of the following is omega-3 polyunsaturated fatty acid?

    a. Linoleic acid b. α-Linolenic acid c. γ-Linolenic acid d. Arachidonic acid

20. The glycosaminoglycan which does not contain uronic acid is

a. Dermatan sulphate b. Chondroitin sulphate

c. Keratan sulphate d. Heparan sulphate

Part-B (3 x 2 = 6 Marks)
Answer All Questions

21. What is saponification?

22. Draw the structure of cholesterol.

23. Differentiate between starch and glycogen in terms of its glycosidic linkage.

Part-C (3 x 8 = 24 Marks)
Answer All Questions

24. a). Explain in detail about the structural polysaccharides.

(or)

b). Explain in detail about the storage polysaccharides.

25. a). Describe the structure, functions and properties of phosphoglycerides.

(or)

b). Describe the structure of phosphatidylethanolamine and phosphatidylcholine.

26. a). Explain in detail the various function of lipids.

(or)

b). Explain in detail the structure, functions and properties of tricayl glycerols.

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DEPARTMENT OF MICROBIOLOGY
(For the candidates admitted from 2015 onwards)

Subject	**:**	**Biochemistry**	**Semester**	**:**	**I**
Subject code	**:**	**17MBU103**	**Class**	**:**	**I B.Sc Microbiology**

Maximum: 50 marks

PART-A (20 X 1 = 20 Marks)
Answer all Questions

1 Dextran

2 Glycogen

3 Glucose

4 Cornea

5 N-acetyl glucosamine and D-glucuronic acid

6 Linolenic acid

7 Lignoceric acid

8 Both a & b

9 Ceramide with one or more sugar residue

10 Glycerol, fatty acid, phosphoric acid and ethanolamine

11 $C_{27}H_{45}OH$

12 Palmitic acid

13 Cellulose

14 Arachidonic acid

15 Sialic acid

16 Both polar and non-polar groups

17 Glycerol, fatty acids, phosphoric acid and ethanolamine

18 4

19 α-Linolenic acid

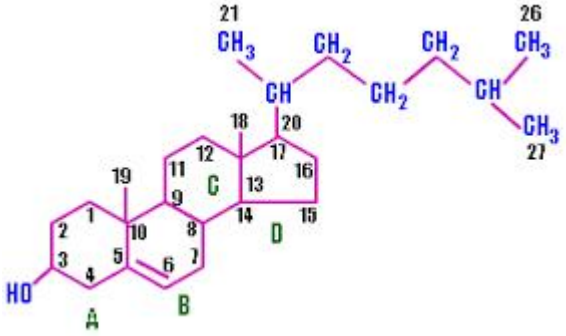
20 Keratin sulphate

Part-B (3 x 2 = 6 Marks)
Answer all Questions

21 What is saponification.

Saponification is a process by which triglycerides are reacted with sodium or potassium hydroxide (lye) to produce glycerol and a fatty acid salt, called 'soap'. The triglycerides are most often animal fats or vegetable oils. When sodium hydroxide is used, a hard soap is produced. Using potassium hydroxide results in a soft soap.

22 Draw the structure of cholesterol.



23. Differentiate between starch and glycogen in terms of its glycosidic linkage.

- Starch and glycogen are made from alpha-glucose. This is an isomer of glucose in which the hydroxyl (-OH) group attached to carbon number 1 is below the plane of the ring.
- Starch is itself composed of two types of polymer:amylose and amylopectin. In amylose, the glucose monomers are linked by 1,4 glycosidic bonds.
- In amylopectin there are two types of glycosidic bonds: 1,4 and 1,6.

- Glycogen is similar in structure to amylopectin, but branches more frequently.

Part-C (3 x 8 = 24 Marks)
Answer all Questions

24. a) Explain in detail about the structural polysaccharides.

Structural Polysaccharides

Structural polysaccharides are the polysaccharides that are found to form the structure of an organism.

Eg. Cellulose - in plants

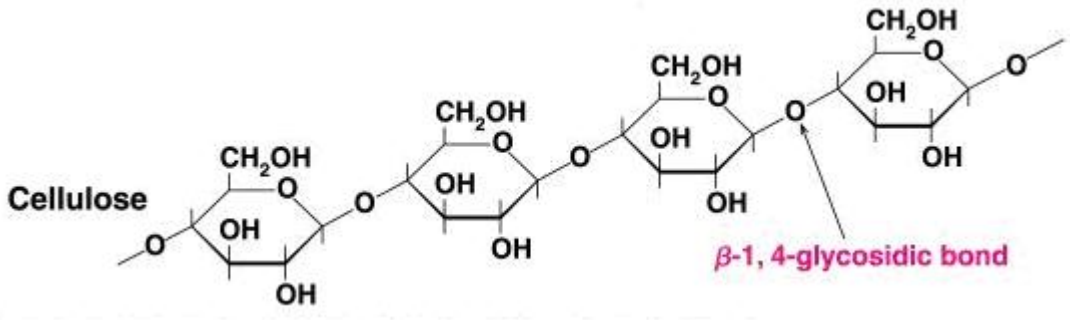
Chitin - found in outer skeleton of insects and crabs

Lignin - wood

Cellulose

It is the most abundant of all biomolecule in biosphere. 50% carbon in vegetation is contributed by cellulose.In plant , it is the main constituent of supporting tissue.it is niot present in animal.

- Cellulose is a polymer made with repeated glucose units bonded together by *beta*-linkages.
- The structural components of plants are formed primarily from cellulose.
- Wood is largely cellulose and lignin, while paper and cotton are nearly pure cellulose.



Properties

- Cellulose is insoluble in water. It does not change color when mixed with iodine. On hydrolysis, it yields glucose. It is the most abundant carbohydrate in nature.

- Fibrous, tough, white solid ; insoluble in ordinary solvents and water; give no colour with iodine.

Hydrolysis

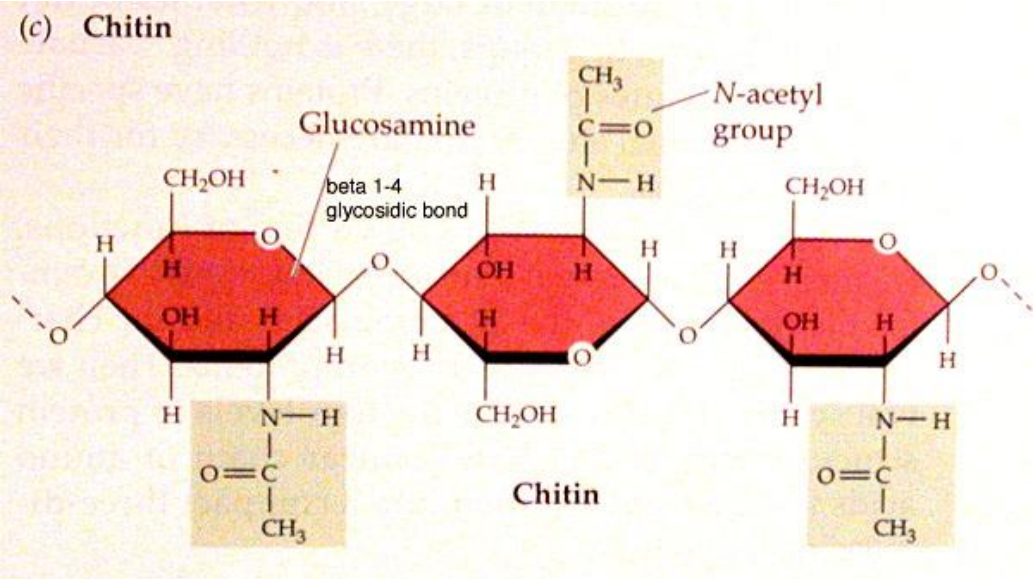
- Humans and many other animals lack an enzyme to break the *beta*-linkages, so they do not digest cellulose.
- Certain animals such as termites can digest cellulose, because bacteria possessing the enzyme are present in their gut.
- It is not acted upon by amylase in human intestine , so doesn't have any nutritive value. It add bulk to the intestinal constituents and stimulate the peristaltic movement of bowel so it aids in relieving constipation.
- On complete hydrolysis by cellulase enzyme it yield α-D glucose. This enzyme is mainly present in termites, which are able to digest the wood.
- It is also hydrolysed by acids such as sulfuric acid, nitric acid and sodium hrdroxide.

Chitin

Chitin is a polysaccharide found in the outer skeleton of insects, crabs, shrimps, and lobsters and in the internal structures of other invertebrates.

Structure

It is a long-chain polymer of a *N*-acetylglucosamine, a derivative of glucose, Joined through ß(1-4) linked units of the amino sugar N-acetyl-glucosamine.



Properties

In its unmodified form, chitin is translucent, pliable, resilient, and quite tough. but in most invertebrates it occurs largely as a component of composite materials

Application

Chitin is the main source of production of chitosan, which is used in a number of applications, such as a flocculating agent, a wound healing agent, a sizing and strengthening agent for paper, and a delivery

24. b). Explain in detail about the storage polysaccharides.

Starch

- Half of the carbohydrate ingested by human is starch.
- It is the source of carbohydrates and fundamental source of energy.
- Starch is the carbohydrate reserve of plants which is the most important dietary source for higher animals, including man.

Occurrence: It is the storage form of carbohydrate in plants ; It is present in cereals, potato, and legumes, root, tubers, tubers, vegetables etc fruits. It is found as granules in cytoplasm of chloroplast

Structure

- Starch is a homopolymer composed of D-glucose units held by a-glycosidic bonds.
- It is known as glucosan or glucan.
- Starch consists of two polysaccharide components-water soluble amylose (15-20%) and a water insoluble amylopectin (80-85%).

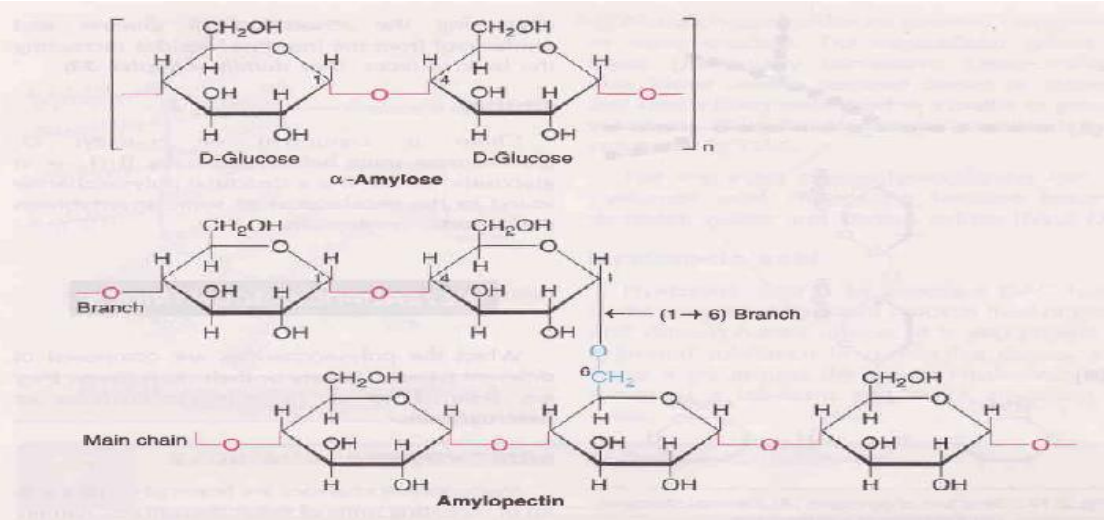
Amylose (α- amylose)

It is a long unbranched polysaccharide; made of α- D glucose joined by α (1 → 4) glycosidic linkage. In starch it constitute about 15-20%. It is in the helical form and 6 glucose unit per turn. It have nearly 300-400 glucose units; molecular weight is 1000-50,000. It form blue colour with iodine.

Amylopectin (β- amylose)

Amylopectin on the other hand, is a branched polysaccharide atleast 80 branch with an interval of 24-30 glucose units(20-30 glucose units per branch).It is made of α- D

glucose joined by α 1,4 glycosidic linkage and the branch is established with α 1,6 glycosidic linkage(α (1 → 6) glycosidic bonds at the branching points and α (1 → 4) linkages everywhere). In starch it constitute about80-85%. It have nearly 300-5500 glucose units; molecular weight is 5,00,000. It form blue colour with iodine.



Structure of starch (~-amylose and amylopectin)

Properties of starch

White , soft powder, tasteless; insoluble in water; specific rotation is+196.

Hydrolysis

Starch is a glucosan, because it yields only glucose molecule on hydrolysis;with water it form hydrated micelle

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

α- amylase

Amylose ------→ Maltose+glucose

α- amylase attacks the α 1,4 glycosidic linkage.It is present in saliva

α- amylase/ β- amylase

Amylo pectin ----------→ Maltose+glucose

α- amylase attacks the α 1,4 glycosidic linkage.It is present in saliva:α 1,6 glycosidic linkage is attacked by as α 1,6 glucosidase

Starch with mineral acid gives glucose. This glucose reacts with iodine and give gradual change in colour ie., -blue_____purple_____red_____none

Starch on partial hydrolysis yield dextrin which gives stiffness to cloths

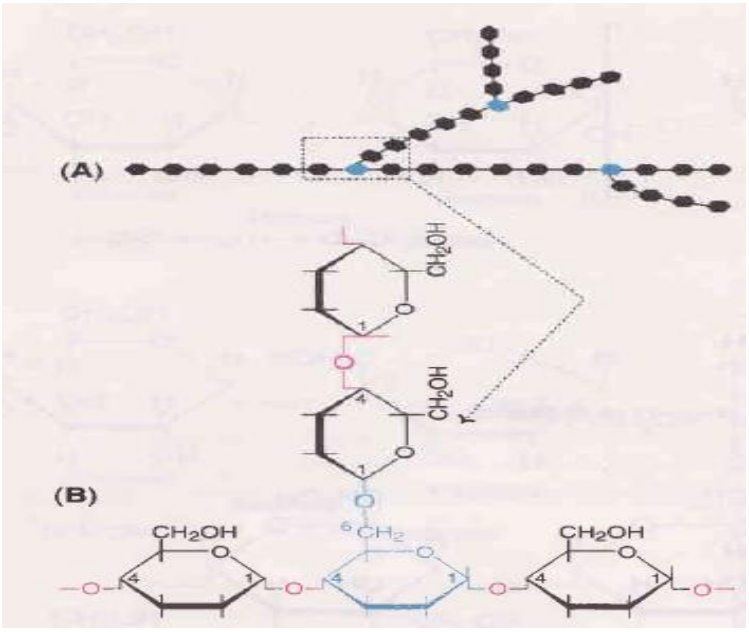
Glycogen

Glycogen is the carbohydrate reserve in animals, hence often referred to as animal starch. It is the reserve carbohydrate found in liver and muscle of animal and human beings

It is present in high concentration in liver, followed by muscle, brain etc. Liver have more glycogen (7% of its weight) than muscle. Glycogen is also found in plants that do not possess chlorophyll (e.g. yeast, fungi).

Structure

- The structure of glycogen is similar to that of amylopectin with more number of branches. It is a branched polymer of carbohydrate ; made of α-D glucose; Glucose is the repeating unit in glycogen joined together by α (1 → 4) glycosidic bonds, and α (1 → 6) glycosidic bonds at branching points, the branching is established by α 1,6 glycosidic linkage.
- The molecular weight (up to 1 x 10^8) and the number of glucose units (up to 5000-25,000) vary in glycogen depending on the source from which glycogen is obtained.



Structure of glycogen (A) General structure (B) Enlarged at a branch point

Properties

White, tasteless powder; readily soluble in water; Non reducing; give red colour with iodine

Hydrolysis

On complete hydrolysis, glycogen yields glucose and maltose

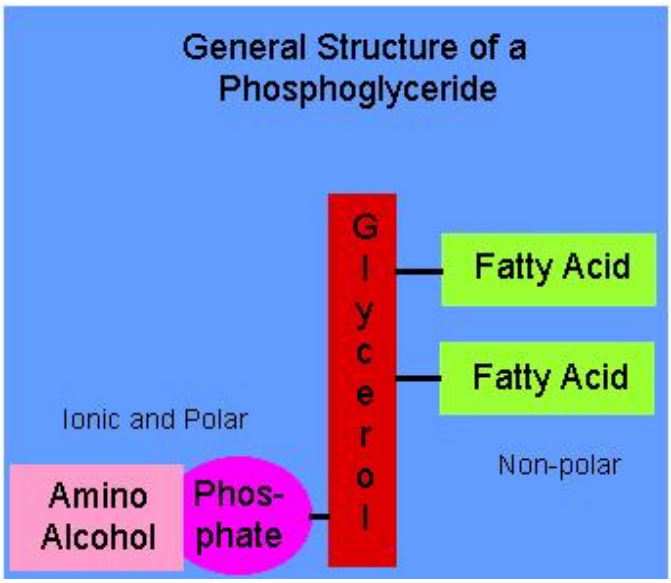
Dextrin

This is formed by the partial (incomplete) hydrolysis of starch by salivary amylase; and also by dilute mineral acid and heat.

Inulin

It is a fructosan; made of repeating units of fructose.it is found in roots and tubers of dahlia and dandelions; it mainly used in assessing the kidney function.

25. a). Describe the structure, functions and properties of phosphoglycerides.



Phospholipids are similar to the triglycerides with a couple of exceptions. Phospholglycerides are esters of only **two fatty acids**, phosphoric acid and a tri-functional alcohol - glycerol (IUPAC name is 1,2,3-propantriol). The fatty acids are attached to the glycerol at the 1 and 2 positions on glycerol through ester bonds. There may be a variety of fatty acids, both saturated and unsatured, in the phospholipids.

The third oxygen on glycerol is bonded to phosphoric acid through a **phosphate ester** bond (oxygen-phosphorus double bond oxygen). In addition, there is usually a

complex amino alcohol also attached to the phosphate through a second phosphate ester bond. The complex amino alcohols include choline, ethanolamine, and the amino acid-serine.

The properties of a phospholipid are characterized by the properties of the fatty acid chain and the phosphate/amino alcohol. The long hydrocarbon chains of the fatty acids are of course non-polar. The phosphate group has negatively charged oxygen and positively charged nitrogen to make this group ionic. In addition there are other oxygen of the ester groups, which make on whole end of the molecule strongly ionic and polar.

Phospholipids are major components in the lipid bilayers of cell membranes.

There are two common phospholipids:

Lecithin contains the amino alcohol, choline.

Cephalins contain the amino alcohols serine or ethanolamine.

Lecithin

Lecithin is probably the most common phospholipid. It is found in egg yolks, wheat germ, and soybeans. Lecithin is extracted from soy beans for use as an emulsifying agent in foods. Lecithin is an emulsifier because it has both polar and non-polar properties, which enable it to cause the mixing of other fats and oils with water components.

Lecithin is also a major component in the lipid bilayers of cell membranes.

Lecithin contains the ammonium salt of choline joined to the phosphate by an ester linkage. The nitrogen has a positive charge, just as in the ammonium ion. In choline, the nitrogen has the positive charge and has four methyl groups attached.

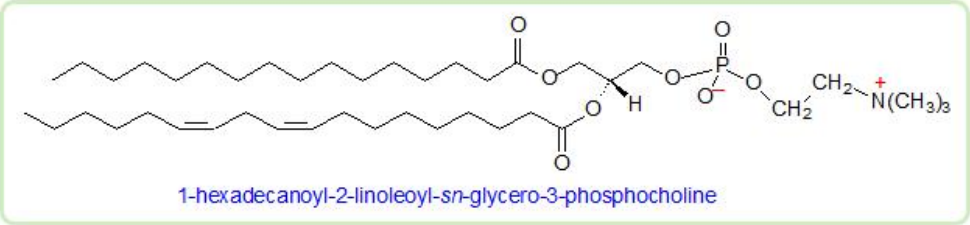
Cephalins

Cephalins are phosphoglycerides that contain ehtanolamine or the amino acid serine attached to the phosphate group through phosphate ester bonds. A variety of fatty acids make up the rest of the molecule.

Cephalins are found in most cell membranes, particularly in brain tissues. They also iimportant in the blood clotting process as they are found in blood platelets.

25. a). Describe the structure of phosphatidylethanolamine and phosphatidylcholine.

Phosphatidylcholine (once given the trivial name 'lecithin') is usually the most abundant phospholipid in animals and plants, often amounting to almost 50% of the total complex lipids, and as such it is obviously a key building block of membrane bilayers. In particular, it makes up a very high proportion of the outer leaflet of the plasma membrane. Phosphatidylcholine is also the principal phospholipid circulating in plasma, where it is an integral component of the **lipoproteins**, especially the HDL. On the other hand, it is less often found in bacterial membranes, perhaps 10% of species, but there is none in the 'model' organisms *Escherichia coli* and *Bacillus subtilis*.



1-hexadecanoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine

Phosphatidylcholine is a neutral or zwitterionic phospholipid over a pH range from strongly acid to strongly alkaline. In animal tissues, some of its membrane functions appear to be shared with the structurally related sphingolipid, sphingomyelin, although the latter has many unique properties of its own.

In animal tissues, phosphatidylcholine tends to exist in mainly in the diacyl form, but small proportions (in comparison to phosphatidylethanolamine and phosphatidylserine) of alkylacyl and alkenylacyl forms may also be present. Data for the compositions of these various forms from bovine heart muscle are listed in our web pages on **ether lipids**. As a generalization, animal phosphatidylcholine tends to contain lower proportions of arachidonic and docosahexaenoic acids and more of the C_{18} unsaturated fatty acids than the other zwitterionic phospholipid, phosphatidylethanolamine. Saturated fatty acids are most abundant in position *sn*-1, while polyunsaturated components are concentrated in position *sn*-2. Indeed, C_{20} and C_{22} polyenoic acids are exclusively in position *sn*-2, yet in brain and retina the unusual very-long-chain polyunsaturated fatty acids (C_{30} to C_{38}) of the *n*-6 and *n*-3 families occur in position *sn*-1. Dietary factors obviously influence fatty acid compositions, but in comparing animal species, it would be expected that the structure of the phosphatidylcholine in the same metabolically active tissue would

be somewhat similar in terms of the relative distributions of fatty acids between the two positions

As a lecithin, phosphatidylethanolamine consists of a combination of glycerol esterified with two fatty acids and phosphoric acid. Whereas the phosphate group is combined with choline in phosphatidylcholine, it is combined with the ethanolamine in phosphatidylethanolamine. The two fatty acids may be the same, or different, and are usually in the 1,2 positions (though they can be in the 1,3 positions).

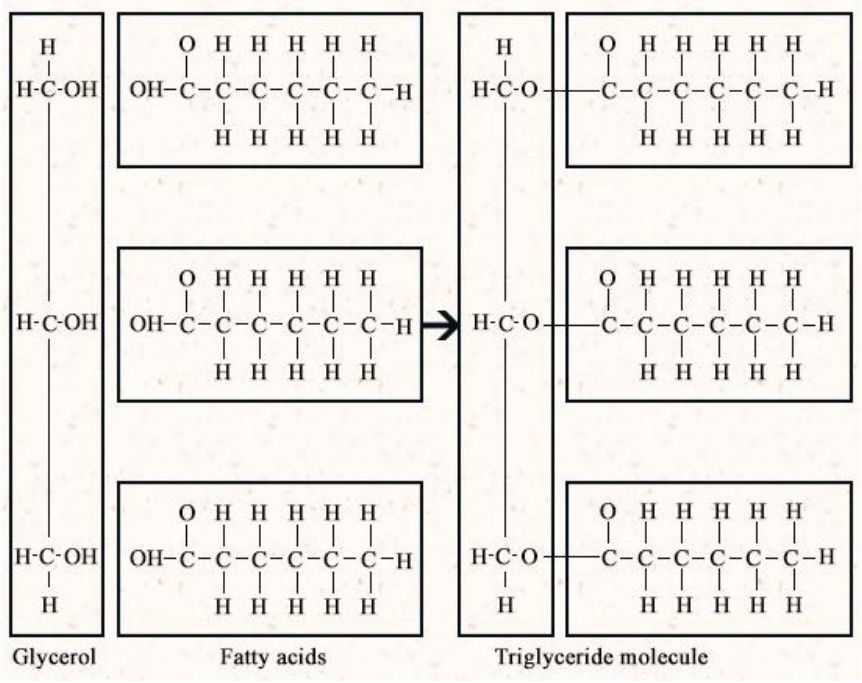
26. a). Explain in detail the various function of lipids.

- They are stored in adipose tissue (triglycerides) and are one of the major energy source. Lipids are the best energy source for humans.
- Some lipids are essential nutrients like fat-soluble vitamins A, (necessary for vision) and D (necessary for calcium metabolism), present in some fats and oils of animal origin, vitamin E (prevention of autoxidation of unsaturated lipids), present in vegetable oils, and vitamin K (normal clotting of blood) present in green leaves, essential fatty acids, in particular linoleic and α-linolenic acids, founders of the family of omega-6 and omega-3 fatty acids respectively.
- During growth they are utilized as "bricks" for construction of biological membranes (phospholipids, cholesterol and glycolipids together with proteins), so contributing to construction of that barrier that separates intracellular environment from extracellular one and, inside cell, circumscribes organelles like mitochondria, Golgi apparatus or nucleus, and whose integrity is the basis of life itself; moreover they are also important for maintenance, physiochemical properties and repairing of cell membranes themselves.
- Many hormones are lipids: steroid hormones, like estrogens, androgens and cortisol, are formed from cholesterol (essential also during embryogenesis), prostaglandins, prostacyclin, leukotrienes, thromboxanes, and other compounds (all eicosanids) from omega-3 and omega-6 polyunsaturated fatty acids with 20 carbon atoms.
- On plasmatic cell membranes they can act as receptors, antigens and membrane anchors for proteins and can modify the structure, and therefore the functionality, of membrane enzymes.

- Many lipids, like diacylglycerol, ceramides, sphingosine and platelet-activating factor act as regulators of intracellular processes.
- There are fat deposits not accessed during a fast, classified as structural fat, the function of which is to hold organs and nerves in the right position protecting them against traumatic injuries and shock; fat pads on the palms and buttocks protect the bones from mechanical pressure.
- A subcutaneous layer of fat is present in humans: it insulates the body reducing the loss of body heat and contributing to maintain body temperature.
- On epidermis they are involved in maintaining water barrier.
- They are electrical insulator of axon of neurons that are covered over and over again by plasmatic membranes of Swann cells, in peripheral nervous system, and of oligodendrocytes in central nervous system; these plasmatic membranes have a lipid content greater than that of the other cells. This lipoprotein coating is called myelin sheath.
- On digestive tract they facilitate the digestive process depressing gastric secretion, slowing gastric emptying and stimulating biliary and pancreatic flow.
- Bile salts (by-products of cholesterol) are natural detergents synthesized in the liver and secreted into bile. They solubilize phospholipids and cholesterol in the bile, permitting the secretion of cholesterol into the intestine (the excretion of both cholesterol and bile salts is the major way by which cholesterol is removed from the body). Bile salts also aid in the digestion and absorption of fat and soluble-fat vitamins in gut.
- In many animals, some lipids are secreted into external environment and act as pheromones that attract or repel other organisms.
- They affect the texture and flavor of food and so its palatability.
- Food manufacturers use fat for its textural properties, e.g. in baked goods fat increase the tenderness of the product.

26. b). Explain in detail the structure, function and properties of triacyl glycerols.

Triacylglycerols (triglycerides) are lipids, a type of fat, and their level in the blood is considered to be a measure of an individual's heart health. The dietary fat is synthesized in the liver, and moreover, can be obtained from food, particularly that which is derived from animal-based sources. Monounsaturated, polyunsaturated, and saturated fats that we get from our diet are also considered as triglycerides.



A triglyceride molecule is made up of 3 molecules of fatty acids that are connected to a glycerol molecule. While a glycerol molecule is made up of 3 carbon molecules with an OH bond on each, the fatty acid molecule is made up of a long chain of carbon and hydrogen (hydrocarbon) atoms with a carboxyl (-COOH) group at one end.

Functions

- Protects internal organs
- Provides heat insulation
- Acts as an energy source
- Promotes nutrient absorption

Properties

- Hydrolysis
- Saponification
- Rancidity

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DEPARTMENT OF MICROBIOLOGY
(For the candidates admitted from 2015 onwards)

Subject	**:**	**Biochemistry**	**Semester**	**:**	**I**
Subject code	**:**	**17MBU103**	**Class**	**:**	**I B.Sc Microbiology**

Maximum: 50 marks

PART-A (20 X 1 = 20 Marks)
Answer all Questions

1 Only L-α-amino acids

2 Methionine

3 β-alanine

4 α-amino acids

5 Pauling and Corey

6 Zwitterion

7 Denaturation

8 Amino acid

9 3.6

10 3

11 All of these

12 Succinate thiokinase

13 Concentration of active enzyme is reduced

14 The amount of an active enzyme

15 All of these

16 Heat, stable, dialyzable, non protein organic molecules

17 All of these

18 1.2-1.7

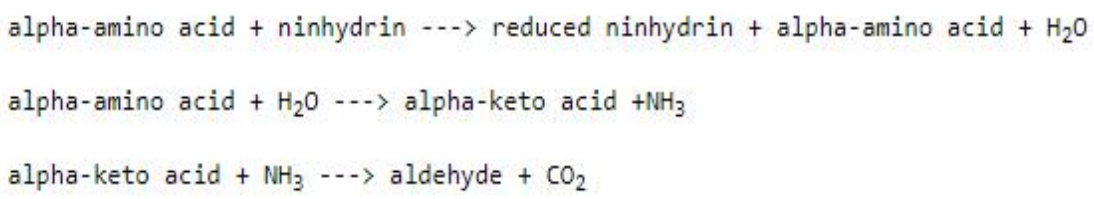
19 Stored in liver

20 Putrid fish meal

Part-B (3 x 2 = 6 Marks)
Answer all Questions

21 What is the importance of Ninhydrin reaction.

Ninhydrin is also used in amino acid analysis of proteins. Most of the amino acids, except proline, are hydrolyzed and react with ninhydrin.



22 Describe enzyme activity.

Enzyme activity = moles of product/time.

Specific activity = enzyme activity/amount of enzyme.

23. What are the main functions of proteins.

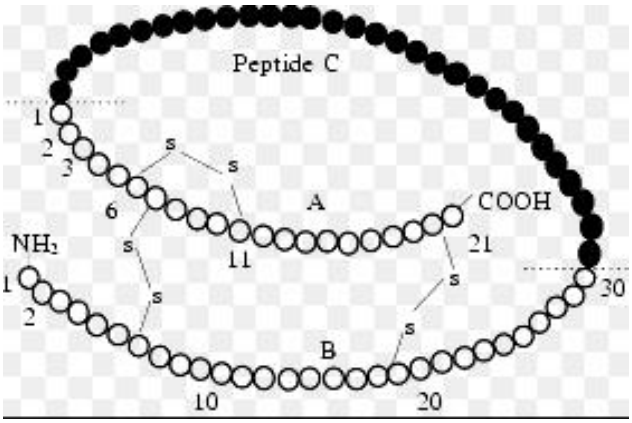
- Structural functions
- Driving Biochemical reactions
- Hormonal regulation
- Regulation of cell division
- Immune system actions

Part-C (3 x 8 = 24 Marks)
Answer all Questions

24. a). Describe the structure and functions of insulin.

Structure of Insulin

Insulin is a hormone secreted by the pancreas that regulates glucose levels in the blood. Without insulin, cells cannot use the energy from glucose to carry out functions within the body. Insulin was first discovered in 1921 by Frederick Grant Banting and Charles. The FDA approved insulin in 1939.



Insulin is composed of two peptide chains referred to as the A chain and B chain. A and B chains are linked together by two disulfide bonds, and an additional disulfide is formed within the A chain. In most species, the A chain consists of 21 amino acids and the B chain of 30 amino acids.

Although the amino acid sequence of insulin varies among species, certain segments of the molecule are highly conserved, including the positions of the three disulfide bonds, both ends of the A chain and the C-terminal residues of the B chain. These similarities in the amino acid sequence of insulin lead to a three dimensional conformation of insulin that is very similar among species, and insulin from one animal is very likely biologically active in other species. Indeed, pig insulin has been widely used to treat human patients.

Insulin molecules have a tendency to form dimers in solution due to hydrogen-bonding between the C-termini of B chains. Additionally, in the presence of zinc ions, insulin dimers associate into hexamers.

Functions of insulin

- Insulin is made in the pancreas by beta cells.
- After the body takes in food, these beta cells release insulin, which enables cells in the liver, muscles and fat tissues to take up glucose and either store it as glycogen or allow blood to transfer it to organs in the body for use as an energy source.
- This process stops the use of fat as a source of energy.
- When glucose levels are elevated in the blood, insulin is produced at higher rates by the pancreas in order to maintain normal sugar concentrations in the blood.
- Without insulin, the body cannot process glucose effectively and glucose begins to build up in the blood stream instead of being transported to different cells.
- In contrast with elevated levels of glucose in the blood, when there is a deficit of glucose available to the body, alpha cells in the pancreas release glucagon, a hormone that causes the liver to convert stored glycogen into usable glucose which is then released into the bloodstream.

Some of the effects of the insulin on the metabolism include:

- Controlling cell intake of substances like glucose in many organs like muscles and adipose tissues.
- Controlling amino acid uptake, thus increasing DNA replication and protein synthesis
- Altering the activity of enzymatic cells

Other Cellular effects of insulin include:

- Increasing synthesis of glycogen. Glycogen is a type of storage for glucose and is stored in the liver. Levels of blood glucose determine whether glucose is stored as glycogen or is excreted. Low levels of glucose cause the liver to excrete glucose, while higher levels of glucose allow glucose to be stored as glycogen.
- Increasing the synthesis and esterification of fatty acids. This is caused by the insulin causing fat cells to convert blood lipids to triglycerides. Esterification is caused when the insulin causes the adipose tissue to convert fats from fatty acid esters.

- Increasing the esterification of fatty 4. Decreasing protein breakdown (proteolysis) 5. Reducing lipolysis 6. Increasing uptake of substances like amino acid and potassium 7. Relaxing wall of arteries of muscles, which vasodilation 8. Increasing secretion of HCl into the stomach.

24. b). Describe the Michaelis-Menten Equation.

First derivation, we start with kinetic mechanism.

E is enzyme, S is substrate, ES is the enzyme-substrate complex, and P is product. This equation includes the assumption that during the early stages of the reaction, so little product is formed that the reverse reaction (product combining with enzyme and re-forming substrate) can be ignored). Another assumption is that the concentration of substrate is much greater than that of total enzyme ([S]>>[Et]), so it can essentially be treated as a constant.

From general chemistry we can equate that rate of this process (k3[ES]) to the change in product concentration as a function of time (d[P]/dt),or equivalently, we can designate the rate with an italicized v (v) as follows.

$$\frac{d[\mathrm{P}]}{dt} = v = k_3[\mathrm{ES}]$$

Because the concentration of the enzyme-substrate complex ([ES]) cannot be measured experimentally, we need an alternative expression for this term. Because the enzyme that we add to the reaction will either be unbound (E) or bound (ES) we can express the fraction of bound enzyme as follows.

$$\frac{[\mathrm{ES}]}{[\mathrm{E_t}]} = \frac{[\mathrm{ES}]}{[\mathrm{ES}]+[\mathrm{E}]}$$

If we multiply the numerator and denominator of the right-hand side of the above equation. We are in effect, multiplying by one and we do not change the value of this expression. When we do this we obtain.

$$[ES] = \frac{[E_t]}{1 + \frac{[E]}{[ES]}}$$

We have almost achieved our goal of isolating [ES], Next we need to come up with an alternative expression for the ration [E]/[ES]. We do this by recalling that a major assumption in enzyme kinetics is the steady state assumption. Basically, it says the rate of change of [ES] as a function of time is zero: d[ES]/dt=0. Another way to express the steady state assumption is that the rate of formation of ES equals the rate of breakdown of ES.

$$k_1[E][S] = k_2[ES] + k_3[ES] = (k_2 + k_3)[ES]$$

The left hand side of the equation expresses the rate of formation of ES and the right hand side expresses the two ways that ES can breakdown.

We can rearrange the equation to isolate the ration [E]/[ES].

$$\frac{[E]}{[ES]} = \frac{(k_2 + k_3)}{k_1[S]}$$

We now define a new constant, the Michaelis constant (Km)

$$K_m = \frac{(k_2 + k_3)}{k_1}$$

If we substitute Km back into equation we obtain

$$\frac{[E]}{[ES]} = \frac{K_m}{[S]}$$

We now substitute the ration Km/[S] from equation in place of the ratio [E]/[ES] and we obtain

$$[ES] = \frac{[E_t]}{1 + \frac{K_m}{[S]}}$$

If we multiply the numerator and denominator of the right hand side of equation by [S], we are in effect, multiplying by one and we do not change the value of this expression.

When we do this we obtain

$$[ES] = \frac{[E_t][S]}{[S] + K_m} = \frac{[E_t][S]}{K_m + [S]}$$

Now we have achieved our goal of isolating [ES] and we can substitute this alternative expression of [ES] into equation. We obtain

$$v = \frac{k_3[E_t][S]}{K_m + [S]}$$

Next, we imagine what happens to equation [S]>>Km as follow

$$v \approx \frac{k_3[E_t][S]}{[S]} = k_3[E_t] = k_{cat}[E_t]$$

The constant K_{cat} in the right hand most term of equation is used to signify that k_3 is considered the catalytic constant. Under such conditions, when [S] is said to saturating, the enzyme in functioning as fast as it can and we define k_3[Et] (or kcat[Et]) to be equal to V_{max} the maximum velocity that can be obtained. Therefore the equation can be rewritten into the familiar form of Michaelis-Menten equation.

$$v = \frac{V_{max}[S]}{K_m + [S]}$$

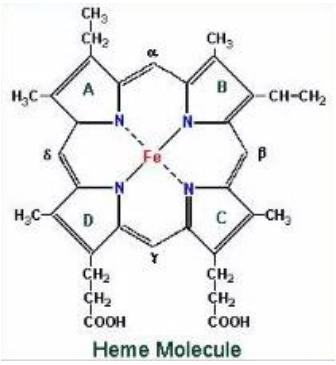
25. a). Explain in detail the structure and functions of haemoglobin.

- Hemoglobin, a chromo protein, found exclusively in red blood cells is actually a conjugated protein containing heme as prosthetic group and globin as the protein part apoprotein.
- The normal concentration of Hb in an adult varies from 14.0 to 16.0 gm%.Approximately 90 mg/kg of Hb is produced and destroyed in the body every day.
- Hb has a molecular weight of about 67,000.
- Each gram of Hb contains 3.4 mg of iron.
- Heme is present as a prosthetic group in hemoglobin as well as in myoglobin, cytochromes, peroxidases, catalases and tryptophan pyrrolases etc.

- Heme is produced by the combination of iron with a porphyrin ring.
- The heme protion is alike in all forms of hemoglobin

Structure of Heme

- Heme is a derivative of porphyrin, porphyrins are cyclic compounds formed by the fusion of 4 pyrrole rings linked by methenyl bridges.
- Since an atom of iron is present heme is called ferroprotoporphyrin.
- These rings are names as I,II,III, IV and the bridges are names as alpha, beta, gamma and delta.
- Porphyrins contain side chains attached to each of the other four pyrrole rings.
- Different porphyrins vary in nature of the side chains that are attached to each of the pyrrole rings.



Heme Molecule

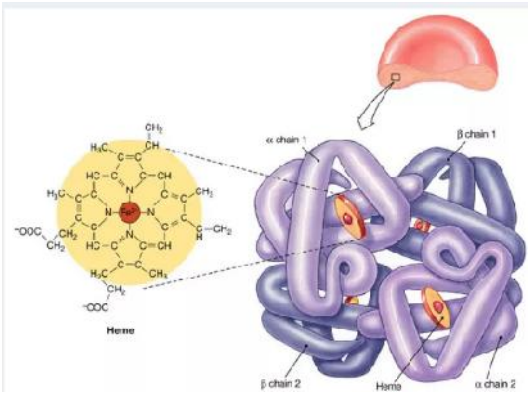
- Heme consists of one ferrous atom (Fe++) that is co-ordinated in the centre of the tetra pyrrole ring of protoporphyrin IX.
- The double bonds are resonating and therefore keep shifting in their position.
- When the ferrous atom in heme gets oxidized to ferric form. Hematin is formed, which loses the property of carrying oxygen and is brown in color, as compared to that of heme which is red in color.

Structure of Globin

1. Different hemoglobins are produced during embryonic, fetal and adult life.
2. Each consists of a tetramer of globin polypeptide chains.
3. Themajor adult hemoglobin HbA has the structure $\alpha_2\beta_2$.

Polypeptide chains

- Each polypeptide chain contains heme in the heme pocket. Thus one Hb molecule contains 4 Heme units.
- The subunits of hemoglobin are arranged array with a tight spherical overall appearance and each individual polypeptide is folded in such a manner to maximize polar residues being on the exposed surface and non-polar interactions being internal, making this large protein water soluble. The interior surface of the molecule lined with non-polar groups froms a hydrophobic pocket into which heme is inserted.



- The arrangement of polypeptides is held together by hydrogen bonding, hydrophobic interactions and multiple ionic interactions that take place at the contact points between subunits.
- These subunits interactions play a critical role in the binding of oxygen to hemoglobin.
- In the amino acid sequence of each polypeptide chain, certain residues appear to be critical to stability and function.
- Such residues are usually the same in α or β chains.
- The NH2 terimal valines of the beta chains are important in 2,3-BPG interactions. The C-terminal residues are important in the salt bridges.
- Each heme moiety can bind a single oxygen molecule, a molecule of hemoglobin can transport up to four oxygen molecules.

- Each heme unit holds an iron ion is such a way that the iron can interact with an oxygen molecule, forming oxyhemoglobin.
- Blood containg RBCs filled with oxyhemoglobin is bright red.
- The iron oxygen interaction is very weak; the two can easily be separated without damaging the heme unit or the oxygen molecules.
- The binding of an oxygen molecule to the iron in a heme unit is therefore completely reversible.
- A hemoglobin molecule in which the iron has separated from the oxygen molecule is called deoxyhemoglobin.

Primary structure of hemoglobin

- Normal alpha chain contains 141 AA residues in linear sequence.
- The non-alpha chains are all 146 amino acids in length; the beta chain begins with valine and histidine.
- The C-terminal residues are Tyrb145 and Hisb146.The delta chain differs from the beta chain in only 10 residues.
- The first eight residues are the C-terminal residues (127-146) are the same in the delta and beta chains. Tetramers of beta chains maybe found in a thalassemia.
- The gamma chain of fetal hemoglobin differs from the beta chain by 39 residues.
- The N-terminal residues of the gamma chain and beta chain are glycine and valine respectively, while the C-terminal residues.
- Try145 and His146 are the same as in gamma and beta chains. Appreciable quantities of free gamma are found in the red cells of some infants with a thalassemia, free gamma chains like beta chains can form homotetramers known as hemoglobin barts.

Secondary structure of hemoglobin

- About 75 percent of the amino acids in α or β chains are in a helical arrangement.
- All studied hemoglobins have a similar helical content.
- Eight helical arease lettered A to H, occur in the β chains.
- Hemoglobin nomenclature specifies that amino acids within helices are designated by the amino acid number and the helix letter, while amino acids between helices

bear the number of the amino acid and the letters of the two helices. Thus residues EF3 is the third residue of the segment connecting the E and F helices, while residues F8 is the eighth residue of the F helix. Alignment according to helical designation makes homology evident; residue F8 is the proximal heme-linked histidine and the histidine on the distal side of the heme is E7.

Tertiary structure

- The tertiary folding of each globin chain forms an approximate sphere. Tertiary folding fives rise to at least 3 functionally important characteristics of the hemoglobin molecules.
- Polar or charged side chains tend to be directed to the outside surface of the subunit and conversely, non-polar structure tend to the directed inwards. The effect of this is to make the surface of the molecule hydrophilic and the interior hydrophobic
- An open toped cleft in the surface of the subunit known as haem pocket iscreated.
- This hydrophobic cleft protects the ferrous ion from oxidation.
- The amino acids which form the inter-subunit bonds responsible for maintaining the quaternary structure and thus the function of the haemoglobin molecule are brought into the correct orientation to permit these bonds to form.

Quarternary structure

T-form

- The deoxy form of hemoglobin is called the "T" form or taut or tense form. In this form the two αβ dimmers interact through a network of ionic bonds and hydrogen bonds that constrain the movement of the polypeotide chains. The T form is the low oxygen affinity form of hemoglobin.

R form

- The binding of hemoglobin causes rupture of some the ionic bonds and hydrogen bonds between the αβ dimmers. This leads to a structure called "R" or relaxed form, in which the polypeptide chains have more freedom of movement. The R form is the high affinity form of hemoglobin.

Functions of hemoglobin

Hemoglobin as oxygen carrier

- The main function of hemoglobin is to carry oxygen from the lungs to all the tissues of the body. This is due to the affinity of hemoglobin for oxygen. When hemoglobin comes in contact with oxygen, it combines with it and form oxy-hemoglobin. This is a weak bond. When blood reaches to tissues, where oxygen is deficient, the bond is broken and oxygen diffuses out to tissues.

Hemoglobin as carbon dioxide carrier

- Some of carbon dioxide is transported from tissues to lungs through hemoglobin. Although the majority of it is transported via plasma but still it carries some of CO2 to lungs.

Color of blood

- The red color of blood is due to hemoglobin. When red blood cells are separated from the blood, the red color disappears. This means that the red color of blood is due to red blood cells. Hence the name red blood cells is given to it. And as we know that hemoglobin is present inside red blood cells, therefore it gives red coloration to RBCs

Buffering action

- Hemoglobin also acts as a buffer. Buffer means to resist change in pH.Blood has 7.4 pH and it remains in the narrow range. Because, if it changes the life of the person may be endangered. Therefore, hemoglobin plays very important role in keeping the pH of blood constant.

Erythrocyte metabolism

- Hemoglobin plays an important role in the modulation of erythrocyte metabolism.

Interaction with drugs

- Not only for oxygen, but hemoglobin act a very important role the transport of various drugs to their site of action.

Physiological active catabolites

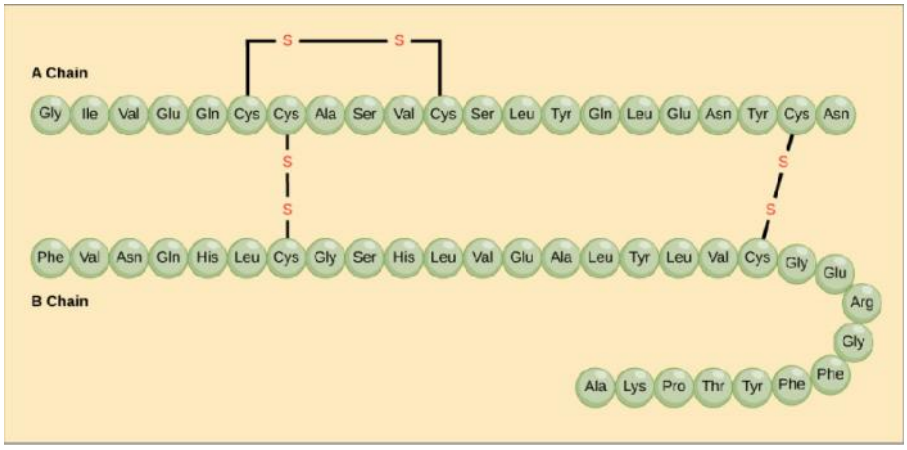
- Hemoglobin is a source of various physiological active catabolites.

25. b). Describe the different structures of proteins.

- Egg whites contain large amounts of proteins called albumins, and the albumins normally have a specific 3D shape, thanks to bonds formed between different amino acids in the protein. Heating causes these bonds to break and exposes hydrophobic (water-hating) amino acids usually kept on the inside of the protein. The hydrophobic amino acids, trying to get away from the water surrounding them in the egg white, will stick to one another, forming a protein network that gives the egg white structure while turning it white and opaque.
- The shape of a protein is very important to its function.
- To understand how a protein gets its final shape or conformation, we need to understand the four levels of protein structure: primary, secondary, tertiary, and quaternary.

Primary structure

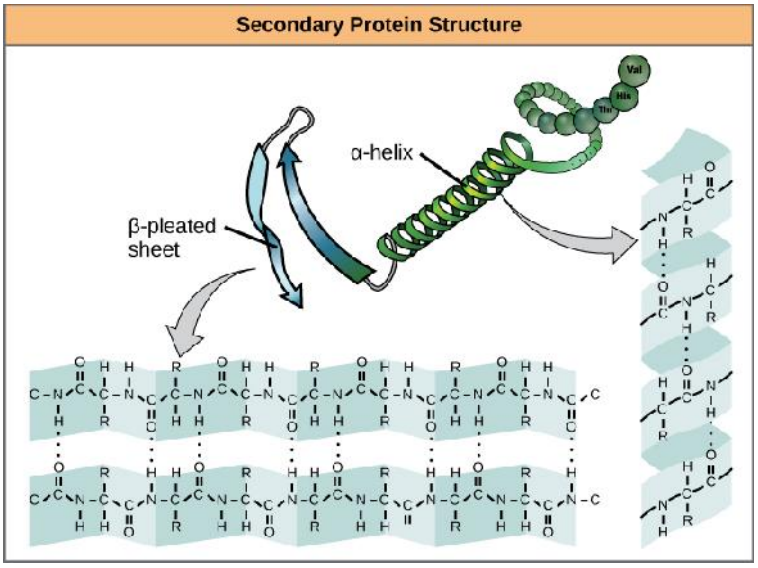
- The simplest level of protein structure, **primary structure**, is simply the sequence of amino acids in a polypeptide chain.
- For example, the hormone insulin has two polypeptide chains, A and B, shown in diagram below. (The insulin molecule shown here is cow insulin, although its structure is similar to that of human insulin.)
- Each chain has its own set of amino acids, assembled in a particular order.
- For instance, the sequence of the A chain starts with glycine at the N-terminus and ends with asparagine at the C-terminus, and is different from the sequence of the B chain.



- Insulin consists of an A chain and a B chain. They are connected to one another by disulfide bonds (sulfur-sulfur bonds between cysteines).
- The A chain also contains an internal disulfide bond. The amino acids that make up each chain of insulin are represented as connected circles, each with the three-letter abbreviation of the amino acid's name.

Secondary structure

- The next level of protein structure, **secondary structure**, refers to local folded structures that form within a polypeptide due to interactions between atoms of the backbone. (The backbone just refers to the polypeptide chain apart from the R groups – so all we mean here is that secondary structure does not involve R group atoms).
- The most common types of secondary structures are the α helix and the β pleated sheet. Both structures are held in shape by hydrogen bonds, which form between the carbonyl O of one amino acid and the amino H of another.

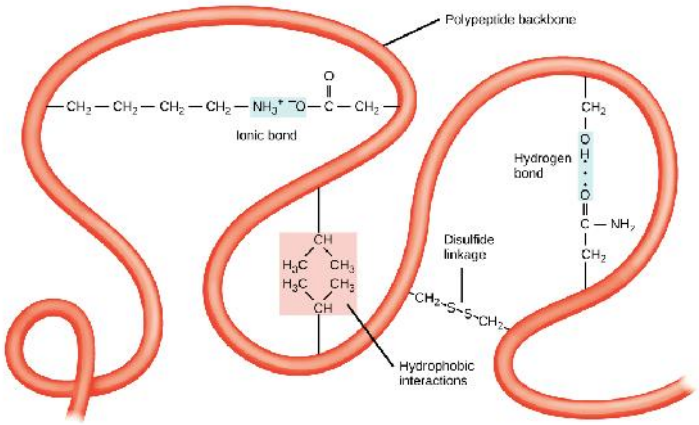


- In an **α helix**, the carbonyl (C=O) of one amino acid is hydrogen bonded to the amino H (N-H) of an amino acid that is four down the chain. (E.g., the carbonyl of amino acid 1 would form a hydrogen bond to the N-H of amino acid 5).
- This pattern of bonding pulls the polypeptide chain into a helical structure that resembles a curled ribbon, with each turn of the helix containing 3.6 amino acids.

- The R groups of the amino acids stick outward from the α helix, where they are free to interact.
- In a **β pleated sheet**, two or more segments of a polypeptide chain line up next to each other, forming a sheet-like structure held together by hydrogen bonds.
- The hydrogen bonds form between carbonyl and amino groups of backbone, while the R groups extend above and below the plane of the sheet.
- The strands of a β pleated sheet may be **parallel**, pointing in the same direction (meaning that their N- and C-termini match up), or **antiparallel**, pointing in opposite directions (meaning that the N-terminus of one strand is positioned next to the C-terminus of the other).

Tertiary structure

- The overall three-dimensional structure of a polypeptide is called its **tertiary structure**. The tertiary structure is primarily due to interactions between the R groups of the amino acids that make up the protein.
- R group interactions that contribute to tertiary structure include hydrogen bonding, ionic bonding, dipole-dipole interactions.
- For example, R groups with like charges repel one another, while those with opposite charges can form an ionic bond. Similarly, polar R groups can form hydrogen bonds and other dipole-dipole interactions. Also important to tertiary structure are **hydrophobic interactions**, in which amino acids with nonpolar, hydrophobic R groups cluster together on the inside of the protein, leaving hydrophilic amino acids on the outside to interact with surrounding water molecules.
- Finally, there's one special type of covalent bond that can contribute to tertiary structure: the disulfide bond. **Disulfide bonds**, covalent linkages between the sulfur-containing side chains of cysteines, are much stronger than the other types of bonds that contribute to tertiary structure.



Quaternary structure

- Many proteins are made up of a single polypeptide chain and have only three levels of structure. However, some proteins are made up of multiple polypeptide chains, also known as subunits. When these subunits come together, they give the protein its **quaternary structure**.
- one example of a protein with quaternary structure: hemoglobin. hemoglobin carries oxygen in the blood and is made up of four subunits, two each of the α and β types. In general, the same types of interactions that contribute to tertiary structure (mostly weak interactions, such as hydrogen bonding and London dispersion forces) also hold the subunits together to give quaternary structure.

26. a). Explain the classification and functions of various vitamins with suitable examples.

Classification of Vitamins

Vitamins are classified according to their ability to be absorbed in fat or water.

Fat Soluble Vitamins: These are oily and hydrophobic compounds. These are stored in the liver and are not excreted out of the body. Bile salts and fats are required for their absorption. Vitamin A, D, E and K are fat soluble vitamins. Because these vitamins can be stored, their excessive intake may have toxic effect and can result in **Hypervitaminosis**.

Water Soluble Vitamins: Vitamin B complex and vitamin C are water soluble. They are compounds of carbon, hydrogen, oxygen and nitrogen. They are not stored in the body therefore they required daily in small amount.

Fat Soluble Vitamin

Vitamin A

Vitamin A (Retinol)

Vitamin A is a pale yellow primary alcohol derived from carotene. It includes Retinol (alcoholic form), Retinal (Aldehyde form) and Retinoic acid (acidic form).

Source

• In animal form, vitamin A is found in milk, butter, cheese, egg yolk, liver, and fish-liver oil.

• In plant source it obtained from vegetables as carrots, broccoli, squash, spinach, kale, and sweet potatoes.

Physiological Significance

• All three forms of vitamin A are necessary for proper growth, reproduction, vision, differentiation and maintenance of epithelial cells.

• Vitamin A accelerates normal formation of bone and teeth.

• Retinoic acid is needed for glycoprotein synthesis.

Deficiency of Vitamin A

• An early deficiency symptom is night blindness (difficulty in adapting to darkness).

• Other symptoms are excessive skin dryness

• Lack of mucous membrane secretion, causing weakness to resist bacterial attack

• Dryness of the eyes due to a malfunctioning of the tear glands.

Hypervitaminosis of Vitamin A: Excess vitamin A can interfere with growth, stop menstruation, damage red blood corpuscles, and cause skin rashes, headaches, nausea, and jaundice.

Vitamin D (Calciferol or Antirachitic Vitamin)

Source

• Vitamin D is obtained from egg yolk, cod liver oil and liver oil from other fishes.

• It is also manufactured in the body when sterols, which are commonly found in many foods, migrate to the skin and become irradiated.

Physiological Significance

• This vitamin is necessary for normal bone formation and for retention of calcium and phosphorus in the body.

• It also protects the teeth and bones against the effects of low calcium intake by making more effective use of calcium and phosphorus.

• It decreases pH in the lower intestine.

Deficiency

• Vitamin D deficiency produces **rickets** in children and **Osteomal aci a** in adult.

• Rickets is characterized by abnormities of the rib cage and skull and by bowlegs, due to failure of the body to absorb calcium and phosphorus.

• Osteomalacia is characterized by softness of pelvic girdle, ribs and femoral bones.

Hypervitaminosis of Vitamin D

• Because vitamin D is fat-soluble and stored in the body, excessive consumption can cause vitamin poisoning, kidney damage, lethargy, and loss of appetite.

Vitamin E (Tocopherol or Fertility Vitamin)

Source

• It is found in vegetable oils, wheat germ, liver, and leafy green vegetables.

• They are also present in little amount in meat, milk and eggs.

Physiological Significance

• Vitamin E acts as antioxidants. They play some role in forming red blood cells and muscle and other tissues and in pre venting the oxidation of vitamin A and fats.

• It is also associated with cell maturation and differentiation.

Deficiency

• Deficiency of vitamin E causes sterility in both male and females.

• It causes muscular dystrophy.

• In children it causes haemolysis, creatinuria.

Vitamin K (Phylloquinone or Anti hemorragic Vitamin or Coagulation Vitamin)

Vitamin K is a complex unsaturated hydrocarbon found in two forms Vitamin K (Phylloquinone) and Vitamin K (Menaquinone).

Source

• The richest sources of vitamin K are alfalfa, fish livers, leafy green vegetables, egg yolks, soybean oil and liver.

• It is also produced by bacteria in human intestine therefore no dietary supplement is needed.

Physiological Significance

• This vitamin is necessary mainly for the coagulation of blood.

• It aids in forming prothrombin, an enzyme needed to produce fibrin for blood clotting.

• Acts as an inducer for the synthesis of RNA.

• It is also required for the absorption of fat.

Deficiency

• Digestive disturbances may lead to defective absorption of vitamin K and hence to mild disorders in blood clotting.

Hypervitaminosis of Vitamin K

• Administration of large doses of vitamin K produces haemolytic anemia and jaundice in infants because of breakdown of RBCs.

Water Soluble Vitamin

Known also as vitamin B complex, these are fragile, water-soluble substances, several of which are particularly important to carbohydrate metabolism. They include

Vitamin B1 (Thiamine),

Vitamin B2 (Riboflavin),

Vitamin B3 (Niacin or Nicotinic Acid),

Vitamin B6 (Pyridoxine),

Vitamin B12 (Cobalamin) etc.

Vitamin B1

(Thiamine)

Vitamin B1 (Thiamine Chloride)

Thiamine, or vitamin B. a colorless, crystalline substance. It is readily soluble in water and slightly in ethyl alcohol

Source

• Vitamin B1is abundantly found in germinating seeds, un-milled cereals, beans, orange juice, tomato, egg, meat, fish, organ meats (liver, heart, and kidney), leafy green vegetables, nuts, and legumes.

Physiological Significance

• Act s as a catalyst in carbohydrate metabolism, enabling pyruvic acid to be absorbed and carbohydrates to release their energy.

• Thiamine also plays a role in the synthesis of nerve-regulating substances.

Deficiency

• Deficiency in thiamine causes beriberi, which is characterized by muscular weakness, swelling of the heart, and leg cramps.

6. Vitamin B2 (Riboflavin)

Source

• The best sources of riboflavin are liver, milk, meat, dark green vegetables, whole grain and enriched cereals, pasta, bread, and mushrooms.

Physiological Significance

• It is essential for carbohydrate metabolism. Enzyme containing riboflavin is called **Fl avoproteins**.

• It acts as coenzyme for enzyme catalyzing oxidation-reduction reaction.

Deficiency

• Its deficiency causes **Glossitis** (inflammation of tongue).

• Lack of thiamine causes skin lesions, especially around the nose and lips, and sensitivity to light.

Vitamin B3

Source

• The best sources of niacin are liver, poultry, meat, canned tuna and salmon, whole grain and enriched cereals, dried beans and peas, and nuts.

• The body also makes niacin from the amino acid tryptophan.

Physiological Significance

• Nicotinic acid is essential for the normal functioning of skin, intestinal tract and the nervous system.

• Vitamin B3 works as a coenzyme in the release of energy from nutrients.

Deficiency

• A deficiency of niacin causes **pellagra**, the first symptom of which is a sunburnlike eruption that breaks out where the skin is exposed to sunlight.

• Later symptoms are a red and s wollen tongue, diarrhea, mental confusion, irritability, and, when the central nervous system is affected, depression and mental disturbances.

Pantothenic Acid or Vitamin B5

Source

• Its main sources are liver, milk, meat, eggs, wheat germ, wheat bran, potatoes, sweet potatoes, tomatoes, cabbage, cauliflower and broccoli. Fruit and other vegetables also have pantothenic acid.

Physiological Significance

• Pantothenic acid is essential for growth of infants and children,

• It plays a major role in the metabolism of proteins, carbohydrates, and fats.

Deficiency

• Its deficiency causes nausea, vomiting, gastrointestinal disorders, improper growth and fatty liver.

Vitamin B6 (Pyridoxine):

Source

• The best sources of pyridoxine are whole (but not enriched) grains, cereals, bread, liver, avocadoes, spinach, green beans, and bananas.

• It is also found in milk, eggs, fish, chicken, beaf, pork and li ver.

Physiological Significance

• Pyridoxine, or vitamin B, is necessary for the absorption and metabolism of amino acids.

• It also plays roles in the use of fats in the body and in the formation of red blood cells.

Deficiency

• Pyridoxine deficiency is characterized by skin disorders, cracks at the mouth corners, smooth tongue, convulsions, dizziness, nausea, anemia, and kidney stones.

Vitamin B7 (Biotin)

Biotin is also known as “anti-egg white injury factor” or as H-factor.

Source:

• Biotin occurs in combined state as biocytin. It is found in yeast, liver, kidney, milk and molasses.

Physiological Significance:

• Biotin serves as prosthetic group for many enzymes which catalyze fixation of CO into organic molecules.

• It helps in synthesis of fatty acids.

Deficiency:

• Its deficiency caused the destruction of intestinal bacteria.

• It leads to nausea and muscular pain.

Vitamin B9 or M or Bc (Folic Aci d)

Source

• Folic acid is found in yeast, liver and kidney.

• Fish meat and green leafy vegetables, milk and fruits also provide folic acid.

Physiological Significance

• Folic acid acts as a coenzyme and help in synthesis of purines and thymine during DNA synthesis.

• It helps in formation and maturation of red blood cells.

Deficiency

• Folic acid deficiency gives rise to **megaloblasticanemia**.

• The patient suffers from retarded growth, weakness, infertility, inadequate lactation in females and gastrointestinal disorders.

Vitamin B12 (Cynocobalamin)

Vitamin B12 or **Cobalamin**, or **Anti -Pernicious Anaemic Factor (APA)**, one of the most recently isolated vitamins.

Source

• Cobalamin is obtained only from animal sources—liver, kidneys, meat, fish, eggs, and milk. Vegetarians are advised to take vitamin B supplements.

Physiological Significance

• It is necessary in minute amounts for the formation of nucleoproteins, proteins, and red blood cells.

• It is necessary for the functioning of the nervous system.

• It stimulates the appetite of the subject.

Deficiency

• Due to its deficiency **Pernicious Anemia** results which is characterized by symptoms of ineffective production of red blood cells, faulty myelin (nerve sheath) synthesis, and loss of epithelium (membrane lining) of the intestinal tract.

Lipoic Acid

• Lipoic acid is a sulphur containing fatty acid. It is widely distributed in natural foods. Lipoic acid functions as a coenzyme inoxidative decarboxylati on of pyruvic acid and a-ketoglutaric acid. Its deficiency disorders have not been recorded.

Inositol

Source

• Yeast, meat, milk, nuts, fruits, vegetables and grains contains Inositol.

Physiological Significance

• It increases peristalsis of small intestine, increase the rate of contraction of heart muscles.

Deficiency

• Deficiency symptoms include retarded growth, failure of lactation, loss of hair over the body (alopecia) etc.

Choline

Source

• Choline is found in liver, egg yolk, meat, cereals, rice, milk, fruits and vegetables.

Physiological Significance

• Acetyl choline is a chemical mediator of parasympathetic activities and other activities of nervous system.

• It prevents accumulation of fat in the liver.

Deficiency

• Its deficiency causes fatty liver, slipped tendon diseases etc.

Vitamin C (Ascorbic Acid or Antiscorbutic Vitamin)

Source

• Sources of vitamin C include citrus fruits, fresh strawberries, cantaloupe, pineapple, and guava.

• Good vegetable sources are Broccoli, Brussels sprouts, Tomatoes, Spinach, Kale, Green Peppers, Cabbage, and Turnips.

Physiological Significance

• Vitamin C is important in the formation and maintenance of collagen, the protein that supports many body structures and plays a major role in the formation of bones and teeth.

• It also enhances the absorption of iron from foods of vegetable origin.

• The connective tissue fibrils and collagen are synthesized with the help of vitamin C.

• It play important role in wound repair.

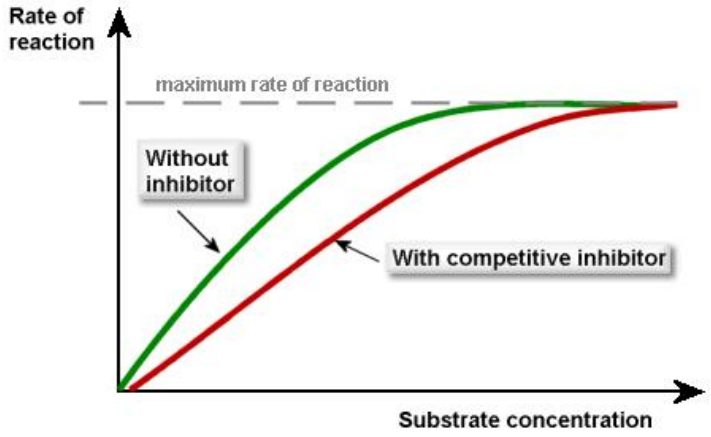
• It protects body against stress.

Deficiency

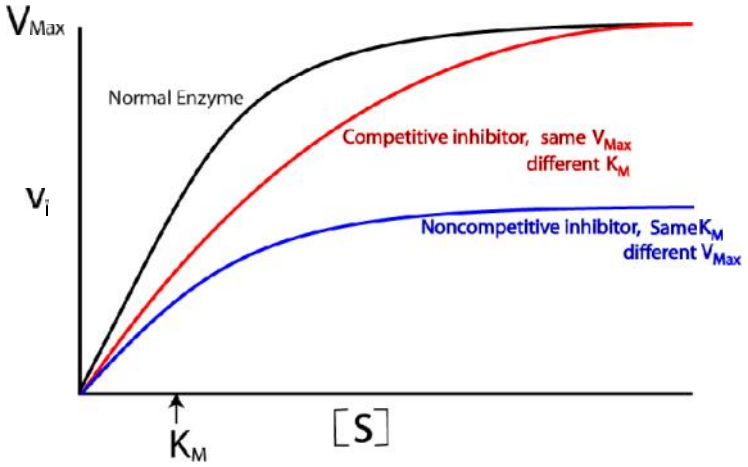
• This well -known Scurvy is the classic manifestation of severe ascorbic acid deficiency. Its symptoms are loss of the cementing action of collagen and include hemorrhages which lead to loosening of teeth and cellular changes in the long bones of children.

26.b). Write in detail on the competitive and non-competitive enzyme inhibition.

- Enzyme Inhibitors reduce the rate of an enzyme catalyzed reaction by interfering with the enzyme in some way. This effect may be permanent or temporary.
- Competitive Enzyme Inhibitors work by preventing the formation of Enzyme-Substrate Complexes because they have a similar shape to the substrate molecule.
- This means that they fit into the Active Site, but remain unreacted since they have a different structure to the substrate. Therefore less substrate molecules can bind to the enzymes so the reaction rate is decreased.
- Competitive Inhibition is usually temporary, and the Inhibitor eventually leaves the enzyme. This means that the level of inhibition depends on the relative concentrations of substrate and Inhibitor, since they are competing for places in enzyme Active Sites.



- Non-competitive Enzyme Inhibitors work not by preventing the formation of Enzyme-Substrate Complexes, but by preventing the formation of Enzyme-Product Complexes. So they prevent the substrate from reacting to form product.
- Usually, Non-competitive Inhibitors bind to a site other than the Active Site, called an Allosteric Site. Doing so distorts the 3D Tertiary structure of the enzyme, such that it can no longer catalyse a reaction.
- Since they do not compete with substrate molecules, Non-competitive Inhibitors are not affected by substrate concentration.



- Many Non-competitive Inhibitors are irreversible and permanent, and effectively denature the enzymes which they inhibit. However, there are a lot of non-permanent and reversible Non-competitive Inhibitors which are vital in controlling Metabolic functions in organisms.
- Enzyme Inhibitors by organisms are used in controlling metabolic reactions. This allows product to be produced in very specific amounts.

Reg. No.....

[12BTU101]

KARPAGAM UNIVERSITY

(Under Section 3 of UGC Act 1956)
COIMBATORE – 641 021
(For the candidates admitted from 2012 onwards)

B.Sc. DEGREE EXAMINATION, NOVEMBER 2014

First Semester

BIOTECHNOLOGY

BIOCHEMISTRY

Time: 3 hours

Maximum : 100 marks

PART – A (15 x 2 = 30 Marks)
Answer ALL the Questions

1. Define atom
2. Define covalent bonds
3. Define acids.
4. How are carbohydrates classified?
5. What are amino acids?
6. What is a peptide bond?
7. Write any four functions of triglycerides
8. Define glycolipids.
9. What are the types of RNA?
10. Define enzymes
11. What are co-enzymes?
12. Write in brief about the Michaelis-Menten equation.
13. Illustrate glycolysis.
14. Show the TCA cycle.
15. List the types of. Vitamins

PART B (5 X 14= 70 Marks)
Answer ALL the Questions

16. a. Explain in detail about the va der Waals equation.

Or

b. Define acids and bases. Write in detail about the chemical characteristics of acids and bases.

17. a. Give a detailed account of the structural characteristics of carbohydrates.

Or

b. Write in detail about the structural configuration of proteins.

18. a. Give a detailed account of lipids.

Or

b. Describe the basic structure of DNA.

19. a. Give a detailed account on enzyme catalysis

Or

b. Describe enzyme inhibition.

20. a. Write in detail about the process of photophosphorylation.

Or

b. Describe in detail about the structure and functions of hormones.

Reg. No.....

[12BTU101]

KARPAGAM UNIVERSITY

(Under Section 3 of UGC Act 1956)
COIMBATORE – 641 021
(For the candidates admitted from 2012 onwards)

B.Sc. DEGREE EXAMINATION, APRIL 2013

First Semester

BIOTECHNOLOGY

BIOCHEMISTRY

ime: 3 hours Maximum : 100 marks

PART – A (15 x 2 = 30 Marks)
Answer ALL the Questions

. Define covalent bond.
. Write about the buffers.
. Define acids and bases.
. What are polysaccharides?
. Give the projection formulas for any three positively charged amino acids
. Define α-helix.
. Give the structure and systemic name of Arachidic and Lignoceric acids.
. Define Lipoproteins.
. What are the major pyrimidine bases?
0. Give the basic principle of enzyme activity
1. Define K_{eq} of an enzyme.
2. Define steady state kinetics.
3. Differentiate catabolic and anabolic pathways.
4. Define entropy.
5. Describe the structure of insulin.

PART B (5 X 14= 70 Marks)
Answer ALL the Questions

16. a. Explain in detail about the convalent and non-covalent interactions among molecules.

Or

b. Write in detail about the pH and pKa

17. a. Give a detailed account of the structure of monosaccharides with suitable examples.

Or

b. Write in detail about the structure of amino acids and peptides.

18. a. Give a detailed account of lipoproteins.

Or

b. Describe the types of RNA.

19. a. Give a detailed account on the factors affecting enzyme catalysis.

Or

b. Describe the mechanism of enzyme regulation.

20. a. Write in detail about the TCA cycle.

Or

b. Describe in detail the electron transport chain.

Reg. No.....

[17MBU103]

KARPAGAM UNIVERSITY

Karpagam Academy of Higher Education
(Established Under Section 3 of UGC Act 1956)
COIMBATORE – 641 021
(For the candidates admitted from 2017 onwards)

B.Sc., DEGREE EXAMINATION, NOVEMBER 2017

First Semester

MICROBIOLOGY

BIOCHEMISTRY

Time: 3 hours Maximum : 60 marks

PART – A (20 x 1 = 20 Marks) (30 Minutes)
(Question Nos. 1 to 20 Online Examinations)

PART B (5 x 2 = 10 Marks) (2 ½ Hours)
Answer ALL the Questions

21. Structure of ribose and deoxyribose.
22. Define mutarotation.
23. Write the reaction of a protein with ninhydrin.
24. What are Zwitterions?
25. What is apoenzyme?

PART C (5 x 6 = 30 Marks)
Answer ALL the Questions

26. a. Write a note on the transport of molecules across the membrane.

Or

b. Describe the principle and instrumentation of a calorimeter.

27. a. Describe the Haworth projection formulae and chair and boat forms of glucose.

Or

b. Explain in detail about the storage polysaccharides.

28. a. Describe the structure, functions and properties of phosphoglycerides.

Or

b. Explain in detail the structure, functions and properties of tricayl glycerols.

29. a. Explain in detail the structure and functions of haemoglobin.

Or

b. Describe the tertiary structure of proteins.

30. a. Describe the Michaelis - Menten equation.

Or

b. Write in detail on the competitive and non-competitive enzyme inhibitors.
