GOVERNMENT OF TAMILNADU

HIGHER SECONDARY FIRST YEAR

BIO-CHEMISTRY

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Department of School Education

Untouchability is Inhuman and a Crime

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Government of Tamil Nadu

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Key features …

	Scope of BIO-Chemistry	Awareness about higher education avenues in the field of Bio-Chemistry	
	Learning objectives	Describe the specific competency / performance capability acquired by the learner	
	Do you know	Additional information provided to relate the content to day-to-day life / development in the field	
	Example Problems	Model problems worked out for clear-cut comprehension by the learners	
	Q.R code	Quick access to concepts, videos, animations and tutorials	
	ICT	opens up resources for learning; enables the learners to access, extend transform ideas / informations	
	Summary	A glance on the substance of the unit	
	Concept map	Inter relating the concepts for enabling learners to visualize the essence of the unit	
	Evaluation	To assess the level of understanding through multiple choice question,numerical problems etc…	
	Books for Reference	List of relevant books for further reading	
	Glossary	Important terms are enlisted with equivalent Tamil words	

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The courses offered, top colleges and competitive exams conducted for higher education after 12th in Chemistry field were summarized below.



Courses Bachelor Degree	Institution	Selection Mode	Careers in Chemistry
• B.Sc Biochemistry • B.ScBiotechnology • B.Sc Chemistry • B.ScBioinformatics • B.Sc Microbiology • B.Sc Zoology • B.ScBotony	• Arts and Science Colleges	• Counseling based on 12th Marks in Science Subjects	• Biochemistry Research and Development • Food products and processing systems • Animal systems • Plant systems • Environmental study systems • Agribusiness systems • High School teaching faculty • Quality control section
Allied Health Science Courses			
• B.Sc Nursing • B.Sc Medical Lab Technology • B.ScAnaesthetic Technology • B.ScClinical oplometry • B.ScMedical Microbiology • B.ScRadiotherapy Technology • B.Sc Physician Assistant • B.Pharm	• Medical Colleges and Paramedical Institutes	• Counseling based on 12th Marks in Science Subjects	• Diagnostic services • Support services • Health informatics • Therapeutic services • Preventive care
Diploma Paramedical Courses			
• Dialysis Technician • X-ray Technician • Medical Lab Technician • Operation theatre Technician • Radiation Technician • Anesthetic Technician • Medical Record Technician	• Paramedical Institutes	• Counseling based on 12th Marks in Science Subjects	• Human supportive services • Diagnostic services • Prognostic services • Therapeutic services • Preventive care
Masters Degree			

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Courses Bachelor Degree	Institution	Selection Mode	Careers in Chemistry
Master Degree Courses			
• M.Sc Biochemistry • M.Sc Animal Biochemistry • M.Sc Plant Biochemistry • M.Sc Medical Biochemistry • M.ScLifesciences • M.Sc Molecular Biology • M.Sc Biotechnology • M.Sc Microbiology • M.Sc Genetics • M.ScZology • M.Sc Bioinformatics • M.ScNanoscience and Technology • M.Sc Medical Microbiology • M.Sc Applied Microbiology • M.Sc Forensic Science • M.Sc Biophysics	• University of Madras • Pondicherry University • Madurai Kamarajar University • Bharathidasan University • Bharathiar University • ManonmaniamSundaranar University • Periyar University • Tamilnadu Agricultural University • Annamalai University • SASTRA University • VIT University • Arts and Science Colleges • Medical Colleges	• Based on the percentage scored indegree and Separate Entranceexams will be conducted by the • concern institute	• Biomedical agent • Research fellow • Quality controller • Forensic scientist • Research and Development scientist • Patent attorney • Health, Safety and Environmental Managers • Pharmaceutical Analyst • Lab in-charges • Project Assistants • Higher secondary teachers
Engineering Degree Courses			
• M.Tech Biotechnology	• Anna University and Private Engineering Colleges	• Common Entrance Examination Test - TANCET	• Biomedical Industry • Industrial Scale Production • Pharma industry • Health, Safety and Environmental Managers
M.Phil			
• M.Phil Biochemistry • M.PhilLifesciences • M.Phil Genetics • M.Phil Microbiology • M.Phil Biotechnology • M.Phil Endocrinology • M.PhilNanoscience and Technology • M.Phil Bioinformatics • M.Phil Biophysics	• Universities • Arts and Science Colleges	• Entrance test or direct interview depend upon specific university and college.	• Research and Development • Teaching faculty in colleges • Industrial related jobs

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Courses Bachelor Degree	Institution	Selection Mode	Careers in Chemistry
Ph.D Courses			
• Ph.D Biochemistry • Ph.DLifesciences • Ph.D Genetics • Ph.D Microbiology • Ph.D Biotechnology • Ph.D Endocrinology • Ph.DNanoscience and Technology • Ph.D Bioinformatics • Ph.D Biophysics • Ph.D Immunology	• University of Madras • Pondicherry University • Madurai Kamarajar University • Bharathidasan University • Bharathiar University • ManonmaniamSundaranar University • Periyar University • Tamilnadu Agricultural University • Annamalai University • SASTRA University • VIT University • Indian Institute of Sciences • Indian Institute of Technology • Tata Institute of Fundamental Research (TIFR) • Bhabha Atomic Research Centre (BARC) • National Institute of Immunology • AIMS	• Separate Entrance Examination will be conducted by concern institute or universitie.	• Scientists posts in Various industries • Scientist posts in government organizations • Post-doctoral fellow • Teaching faculty in Universities • Top position in Research and Development wing

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Entrance Exams and Pattern

JEE-Main Paper I -3 hrs Paper II-3 hrs www.jeemain.nic.in	• Indian Institutes of Information Technology (IIITs) • Indian Institute of Engineering Science and Technology (IIEST) • National Institutes of Technology (NITs) • Indian Institute of Technology (IITs) • Government Funded Technical Institutes (GFTIs)	OMR based and Online based (choice of candidate)	Notification: 1st week of Dec Exam Date: April Negative marking: 4/-1 Total Marks:360	Physics Chemistry Mathematics Total Questions	30 30 30 90
JEE-Advanced Paper I -3 hrs Paper II-3 hrs Should qualify JEE main (paper-I) www.jeeadv.ac.in	• Indian Institutes of Information Technology (IIITs) • Indian Institute of Engineering Science and Technology (IIEST) • National Institutes of Technology (NITs) • Indian Institute of Technology (IITs) • Government Funded Technical Institutes (GFTIs)	Online	Notification: last week of April Exam Date: May second week Negative marking: 4/-1 Total Marks: 306 (Variable each year)	Physics Chemistry Mathematics Total Questions	18 18 18 54
BITSAT http://www.bitsadmission.com Time: 3 hrs	• Birla Institute of Technology and Science	Online	Notification: 2nd week of Dec Exam Date: 2nd week of May Negative marking: 3/-1 Total Marks: 450	Physics Chemistry Mathematics English Proficiency Logical Reasoning Total Questions	40 40 45 15 10 150
CUCET https://cucetexam.in Time: 2 hrs	• All Central Universities	Online	Notification: 2nd week of Feb Exam Date: April Negative marking: 1/0.25 Total Marks:175	English General Awareness Mathematical Aptitude Analytical Skill Science Total Questions	25 25 25 25 75 175
JAM http://jam.iitb.ac.in/ Time: 3 hrs	• All IITs	Online	Notification: September Exam Date: February Negative marking: 1/0.33 2/0.66 Total Marks:100	Chemistry Total Questions	60 60

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CSIR-UGC Net http://csirhrdg.res.in Time: 3 hrs	• Ph.D. admission in all Universities and Colleges	OMR	Sessions: June From out : March Exam Date: June Session: December From out : September Exam Date: December	General Science, Quantitative Reasoning & Analysis and Research Aptitude Chemistry Total Questions	60 15 75
GATE http://gate.iitg.ac.in/ Time: 3 hrs	• Ph.D. admission in all IITs	OMR	From out : September Exam Date: February Negative marking: 1/0.33 2/0.66 Total Marks:100	General Aptitude Chemistry Total Questions	10 55 65
TANCET https://www.annauniv.edu Time: 2 hrs	• Anna University Entrance exam for technology courses	Pen and Paper based exam	From out : January Exam Date: March Negative marking: 1/0.33 Total Marks:100	Engineering Mathematics Basic Engineering and Sciences Chemistry Total Questions	20 20 60 100
University of Madras http://www.unom.ac.in Time: 3 hrs	• M.Sc. Entrance exam	Pen and Paper based exam	From out : 2nd week of Feb Exam Date: April Negative marking: Nil Total Marks:100	Analytical Chemistry Inorganic Chemistry Organic Chemistry Physical Chemistry Total Questions	25 25 25 25 100
Anna University https://www.annauniv.edu Time: 1 hr	• M.Sc. Entrance exam	Online	From out : April Exam Date : May Negative marking : 1/0.25 Total Marks : 100	Chemistry Total Questions	100 100
Annamalai University http://www.annamalaiuniversity.ac.in	• M.Sc. Admission	Based on percentage scored in degree and Personal Interview.			
	• B.Sc. Admission	Counseling based on 12th Marks in Science Subjects			
Bharathiar University www. b-u.ac.in Time: 1.30 hrs	• M.Sc. Entrance exam	Pen and Paper based exam	From out : May Exam Date : Roughly July Total Marks : 60	Chemistry Total Questions	60 60
Bharathidasan University http://www.bdu.ac.in/	• M.Sc. Entrance exam	Pen and Paper based exam	From out : last week of May Exam Date : second week of June		
Madurai Kamaraj University mkuniversity.ac.in	• M.Sc. Entrance exam	Online	From out : March Exam Date : May/June		

(Note: All these dates are tentative and they are subjected to change every year)

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Assessment

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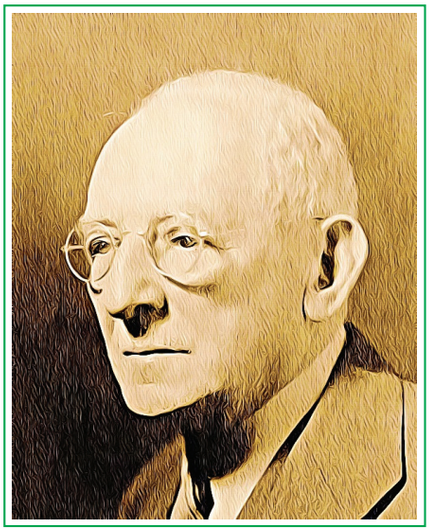
Unit 1 BASIC CONCEPTS OF BIO CHEMISTRY AND CELL BIOLOGY



Carl Alexander Neuberg

Carl Alexander Neuberg, often referred to as the father of modern biochemistry is a German-Jewish pioneer in biochemistry. He gained international recognition through his elucidation of the biochemical reactions of alcoholic fermentation in which he discovered a number of different enzymes such as carboxylase and of intermediates such as fructose-6- phosphate. This understanding had become crucial as to how metabolic pathways would be investigated by later researchers.

Learning Objectives

After studying this unit, the student will be able to

- Describe the cell
- Classify cells into different types
- Differentiate between prokaryotic and eukaryotic cells
- Explain the shapes and sizes of different cells
- Describe the functions of different cellular organelles
- Calculate pH from hydrogen ion concentration
- Apply Henderson-Hasselbalch equation to calculate the pH of a buffer
- Describe the intracellular and extracellular fluids
- State the components of blood and lymph
- Identify the pH of different body fluids
- Explain the role of buffers in regulation of pH
- Describe the importance of lungs and kidneys for acid-base regulation
- Measure the pH of different solutions using pH paper and pH meter

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Introduction

A cell, in biology, is a membrane-bound unit that contains the fundamental molecules of life. The cells of all living organisms create an elegant molecular order within them and pass a pattern of that order on to descent organisms. This creation and duplication of order, out of often disordered surroundings, is unique to a living cell, and the complexity is governed by continual expenditure of energy. A cell is responsible for the everlasting process of abstracting/obtaining energy from their surroundings- either from sunlight, as plants do, or from foodstuffs, as animals do. This is achieved by the ordered structural organization and functions of subcomponents of each cell which will be dealt in detail.

1.1 The unit of biological organisation: The Cell



In 1838, two scientists Theodor Schwann and Matthias Schleiden, were chatting after-dinner about their observations on cells. Schleiden was describing the plant cells with nuclei which made Schwann to recall the similarity of these plant cells to the animal cells which he had studied. The two scientists went immediately to Schwann's lab and examined the slides. Later Schwann published a book on animal and plant cells (Schwann 1839) but did not acknowledge anybody (including Schleiden 1838). He concluded his cell paper as :

i) The cell is the unit of structure, physiology, and organization in living things.

ii) The cell retains a dual existence as a distinct entity and a building block in the construction of organisms.

iii) Cells form by free-cell formation, similar to the formation of crystals (spontaneous generation).

Be it a complex living organism or a non-living matter, they are comprised of several basal units. If you are a living organism, you are based on the single block, the cell. If you are non-living matter like a house, you are based on the single block, the brick. Thus, simply, a cell is a basal building unit of an organism. One of the first major discoveries in biology was made by Robert Hooke (1665). He showed that the partition of the cork (plant tissue) into tiny compartments, and termed them as 'cellulae', or cells. In 1838 Schleiden, a German Botanist and his co-worker Theodor Schwann, have shown that organisms are made up of cells surrounded by a thin layer. However, they failed to correctly explain the generation of cells. Later, in 1857, Rudolph Virchow, a pathologist demonstrated that cells can be formed. only by the division of pre-existing cells. Yet, the question of first cell remains unanswered. Single cells may be adapted to survive in many different types of environments, extending between extremes of cold or heat, living in aerobic or anaerobic conditions, or even surrounded by methane gas. Some single cells can live within other organisms.

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The cells of many of the organisms are similar in size. Almost all cells maintain uniformity in size (1-2 μm in diameter) and the bigger ones may be just 5-10 times larger. Why is such uniformity in cell size maintained? The surface/volume ratio for any object of any shape depends on its size. The complex biochemistry that takes place inside the cell requires a significant volume and exchange with the external environment. If the size is too large, then there will not be enough surface for the exchange of substances with its environment and the process will not occur. Thus, the size relates to the biochemical process i.e metabolism. An unicellular bacterial cell has smaller size than that of the cells of higher organisms as their metabolism is simple while a virion(viral particle) is smaller than bacteria as they depend on their host for their survival. Yet, there are unusual sizes such as very small bacteria which are 0.2 μm in length, while cells of nervous systems of vertebrates are about a metre long.

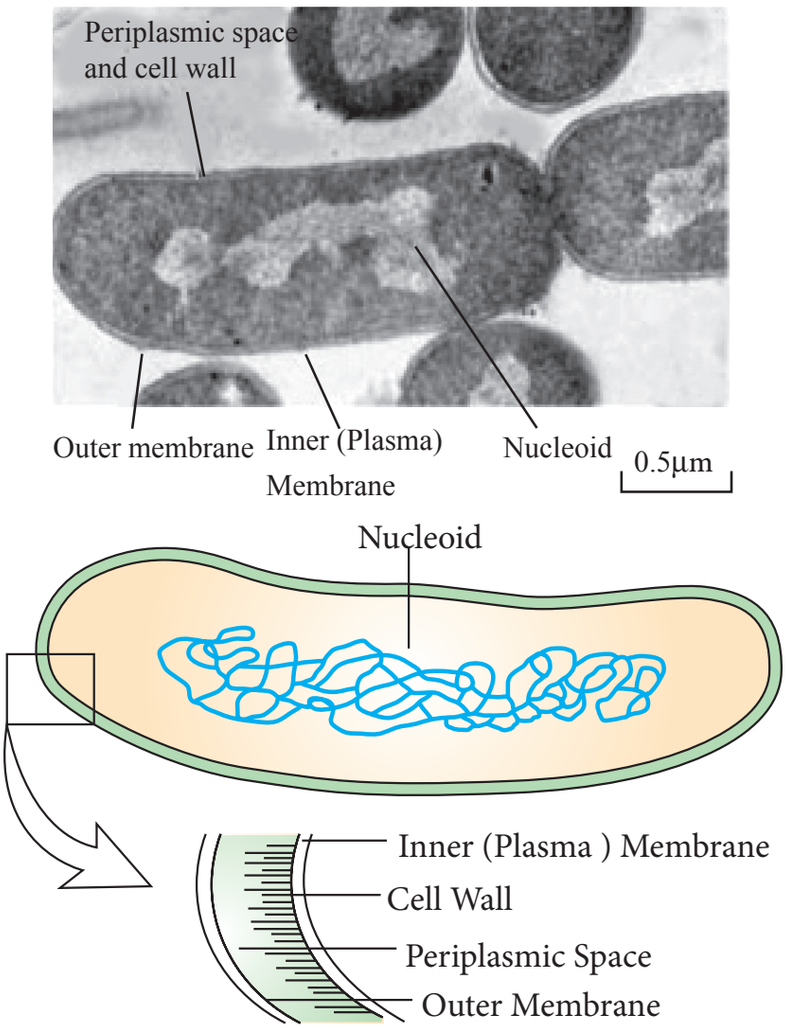

Figure 1.1 Structural organization of the prokaryotic cell

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1.2. Two major classes of cells: prokaryotic and eukaryotic

Prokaryote and eukaryote comes from the Greek word where 'Pro' means 'before' and 'Eu' means 'true'. The term "Karyon" typically means that a rounded or oval mass of protoplasm within the cytoplasm of a plant or animal cell; it is surrounded by a nuclear envelope, which encloses euchromatin, heterochromatin, and one or more nucleoli and undergoes mitosis during cell division.

The prokaryotes are unicellular, in general eubacteria (true bacteria) and archaebacteria (ancient bacteria). The cell encloses a semi-liquid component with a few suspended structures called ribosomes. Within the membrane is the cytoplasm, which contains the cytosol-a semi-liquid concentrated solution or suspension with the structures suspended within it. In prokaryotes, this semi-liquid cytoplasm is not divided into compartments, and carries the genetic information. Freely existing DNA molecules and ribosomes which are involved in protein synthesis are found in the cytosol. A prokaryotic cell can have flagella for swimming purpose and the cell may have pili that help the organism to get attached to other cells or surfaces.

The eukaryotes are ten times larger than the prokaryotic cells but are endowed with the unique feature of 'compartmentalization'. The specialized functions of a eukaryotic cellsare carried out in organelle-the membrane-surrounded structures lying within the surrounding cytoplasm. Major organelles common to most eukaryotic cells are the mitochondria, which specialize in oxidative metabolism; the endoplasmic reticulum, a folded membrane structure rich in ribosomes; the Golgi complex, membrane-bound chambers that function in secretion and the intracellular shuttling of new proteins; and the nucleus. The nucleus of a eukaryotic cell contains the cell's genetic information, encoded in DNA that is packaged into chromosomes. A portion of this DNA is subpackaged into a dense region within the nucleus called the nucleolus. Surrounding the nucleus is a nuclear envelope, pierced by pores through which the nucleus and cytoplasm communicate.



An organelle can be specific for the type of cell

In plants, inside each cell, there is a central vacuole, which can hold water, and under favourable conditions, the water enters the cell by osmosis (osmotic flow of water from area of low solute concentration outside the cell into the cell's vacuole, which has a higher solute concentration) and fills up the vacuole, creating a pressure called turgor pressure. This turgor pressure pushes the plasma membrane against the cell wall of plant, thereby stiffening the cell. This is mainly what makes non-woody parts of plants stiff and vertical.

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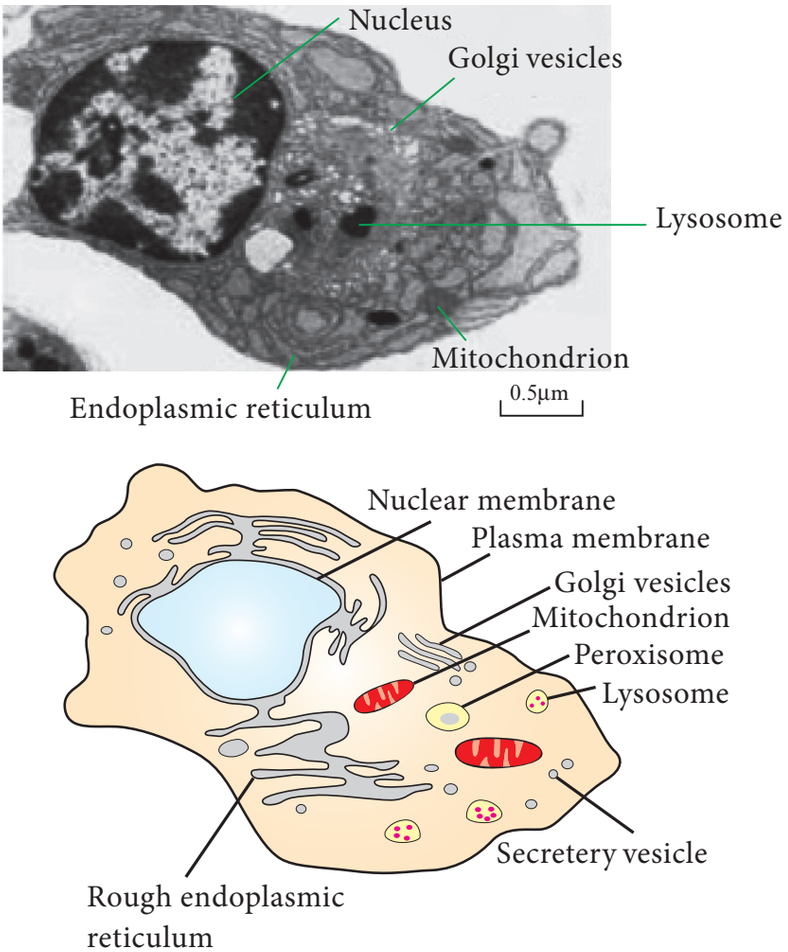

Figure 1.2 Structural organization of the eukaryotic cell

Table 1.1 Differences between prokaryotic and eukaryotic cells

Feature	Prokaryotic cell	Eukaryotic cell
Size	Usually 0.2–2 µm	Usually 5–100 µm
Nucleus	Absent	Present
Number of chromosomes	one (It is not true chromosome, but is a plasmid/extrachromosomal DNA)	More than one
True membrane bound organelles	Absent	Present
Examples	Bacteria and Archaea bacteria	Animals and Plants
Genetic recombination	Partial, unidirectional transfers	Meiosis and fusion of gametes
Lysosomes and peroxisomes	Absent	Present

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Microtubules	Absent but has homologues	Present
Endoplasmic reticulum	Absent	Present
Mitochondria	Absent	Present
Cytoskeleton	May be absent	Present
DNA.	Multiple proteins fold and condense DNA which is organized into conformations like supercoiled or wound around tetramers of proteins called HU proteins.	Eukaryotes wrap their DNA around proteins called histones.
Ribosome	Smaller	Larger
Golgi apparatus.	Absent	Present
Flagella	Submicroscopic in size and composed of only one fiber	Microscopic in size; membrane bound; usually arranged as nine doublets surrounding two singlets.
Cell wall	Usually chemically complex	Usually present in plant cells and fungi (chemically simpler)

1.3.Shape and structure of cell

Cell is a simple sphere, well-defined structure, with extended process, for example neuron or epithelial cells that have distinct apical and basolateral surfaces carrying out distinct functions. The abilities of cells to take such different forms depends on the following:

- Separation of a membrane called plasma membrane from the external environment which control import and export
- Cellular components that are constructed by food sources
- Genetic information and gene expression

1.3.1.Cell and solute levels

For unicellular organisms, homeostasis is necessary because the exterior environment may be subjected to significant fluctuations. For multicellular organisms, it enables individual cells to maintain internal environments that are distinct from that of the extracellular fluid.

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Table 1.2 Differences between prokaryotic and eukaryotic chromosomes

S.No	Prokaryotic chromosomes	Eukaryotic Chromosomes
1.	Many prokaryotes contain a single circular chromosome	Eukaryotes contain multiple linear chromosomes.
2.	Prokaryotic chromosomes are condensed in the nuceluoid via DNA supercoiling and the binding of various architectural proteins	Eukaryotic chromosomes are condensed via histones in a membrane bound nucleus
3.	Because prokaryotic DNA can interact with the cytoplasm transcription and translation occur simultaneously.	In eukaryotes, transcription occurs in the nucleus, and translation occurs in the cytoplasm.
4	Most prokaryotes contain only one copy of each gene (they are haploid) Nonessential prokaryotic genes are commonly encoded on extrachromosomal plasmids.	Most eukaryotes contain two copies of each gene (they are diploid)extrachromosomal plasmids are not commonly present in eukaryotes.
5	Prokaryotic genomes are compact and contain little repetitive DNA	Eukaryotes contain large amounts of noncoding and repetitive DNA.

The cell responds to this situation by controlling the movement of ions and water across the plasma membrane. Its ability to maintain a constant internal environment is called homeostasis. This is an important function of all cells, whether they are part of unicellular or multicellular organisms. One major role of homeostasis in animal cells is to cope with osmotic pressure by balancing the ionic composition so as to avoid the accumulation of water. To maintain homeostasis, ions and water may need to be moved into or out of a cell in a regulated manner.

The cell membrane regulates movement of water and ions. The difference in permeability to water and to ionic solutes has an important consequence in allowing osmotic pressure to develop across the membrane in response to differences in concentrations of dissolved substances on either side. Typically, the interior of the cell has higher potassium but lower sodium and calcium concentrations than that in the exterior. Sodium or potassium ions can diffuse less than the rate of water diffusion. So, there is a difference in ion concentrations on either side of the membrane. Water moves across to equalize the concentration of solutes on either side. If a cell had no mechanism to control solute levels, it would shrink or expand in response to osmotic pressure, whenever the concentration of solutes outside was greater than inside or *vice versa.*

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1.4. Subcellular organelles

An eukaryotic cell does not have a homogeneous internal environment but is divided into two major compartments, cytoplasm and nucleus and subsequently into individual compartments, each of which is surrounded by a membrane, addressed as organelles.



An organelle can be specific for the type of cell

Certain organelles like lysosomes are present in animal cells but not in plant cells. Plant cells have chloroplasts, the sites of photosynthesis and usually a large, water filled vacuole. While most of the animal cells are surrounded by a plasma membrane only, plant cells often have a rigid cell wall on the outer membrane. Plants do not have centrioles. certain cells have basal bodies, which act as anchors.

1.4.1.Cell Membrane

All plants, animal cells, prokaryotic cells, and fungal cells are bounded by a cell membrane, which is sometimes known as plasma membrane.

Chemical composition of Cell membrane: Cellular membranes including plasma membranes and internal membranes consist of mainly lipid, protein and water. Lipids constitute about 40 percent of the membrane composition. Lipids are complex mixtures of cholesterol and fatty acid esters, mainly in the form of glycerides and phospholipids. Glycerol is a three-carbon molecule that forms the backbone of membrane lipids. Within an individual glycerophospholipid, fatty acids are attached to the first and second carbons, and the phosphate group is attached to the third carbon of the glycerol backbone.

Lipid bilayer encircles a cell and is amphipathic with one end as a hydrophilic 'head' and the other end as a hydrophobic 'tail'. Each 'leaf' of the lipid bilayer has one side consisting of an array of the hydrophilic heads, while the other side consists of the hydrophobic tails. An aqueous environment causes the hydrophobic tails to aggregate, so that the hydrophobic sides of each leaf come together to form a non-ionic centre, like an oil drop in water. The hydrophilic end of the two leaves face into the ionic milieu on either side of the lipid bilayer. The lipid bilayer has the important property of fluidity which allows it to fuse with other membranes, generate new membranes by fission, and provide solvent for proteins that can reside within the layer and move around within it. It can permit water but will not permit ions, small charged molecules, and all large molecules.

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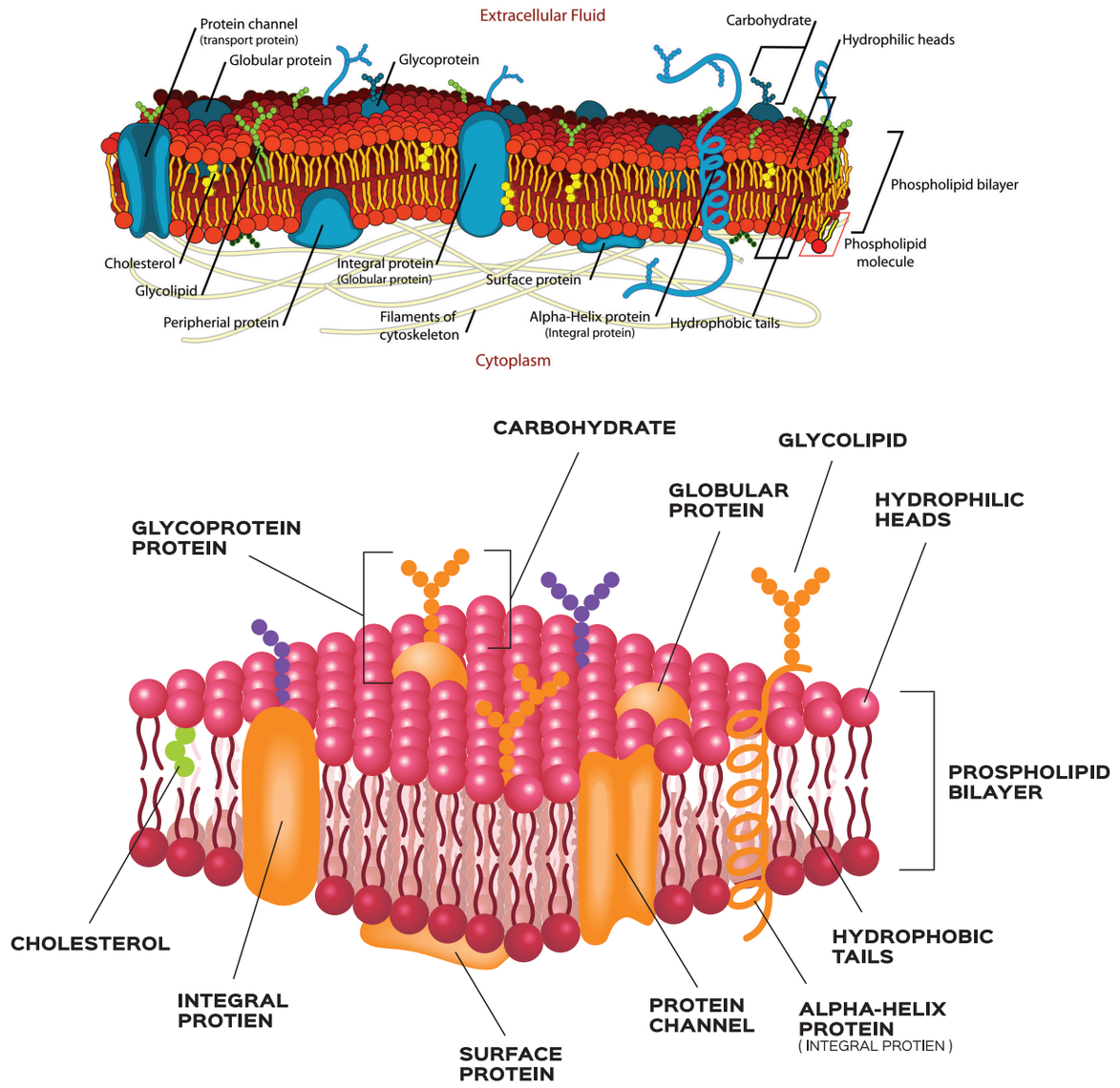

Figure 1.3 A schematic diagram of plasma membrane

The plasma membrane separates the contents of the cell from the external environment. In unicellular organism, the 'external environment' is the exterior world; for a multicellular organism, it is both the exterior world outside the organism as well as the interior world created by other cells. For this process of division of a pre-existing cell, a cell must carry within it the information for reproducing all its components. The form of this information is a single type of genetic material, DNA, which codes for all the proteins of the cell.

Functions of cell membrane

- It serves to keep all the component parts of the cell together in one place.
- It regulates the continuous movement of substances into and out of the cell.

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- It can serve as a base of attachment for the cytoskeleton in some organisms and the cell wall in others. Thus, the cell membrane also serves to maintain its shape.
- It can regulate the cell growth through the balance of endocytosis and exocytosis.
- It can maintain the concentration of water, inorganic ions and organic molecules between the cell and the environment.
- The plasma membrane also receives signals and coordinates molecular interactions at the surface such as cell to cell recognition, adhesion and communication.

1.4.2.Cell wall

The cell wall is a non-living rigid structure that forms an outer covering for the plasma membrane of fungi and plants. Cell wall not only gives shape to the cell and protects the cell from mechanical damage and infection, it also helps in cell-to-cell interaction and provides a barrier to undesirable macromolecules.

Bacterial cell wall:

Bacteria have a cell wall, which is a rigid, carbohydrate-containing structure that surrounds the bacterial cell. However, the genus Mycoplasma, do not have cell wall. The cell wall provides the bacteria with several benefits including protection of the bacterium from damage by encircling it with a tough, rigid structure. This structure is also porous. Small molecules are able to freely pass through the cell wall to the membrane, but large molecules are excluded. By performing this function, the cell wall acts as a coarse filter. The primary function of the cell wall, however, is to maintain the cell shape and prevent bursting due to osmotic pressure (called lysis).

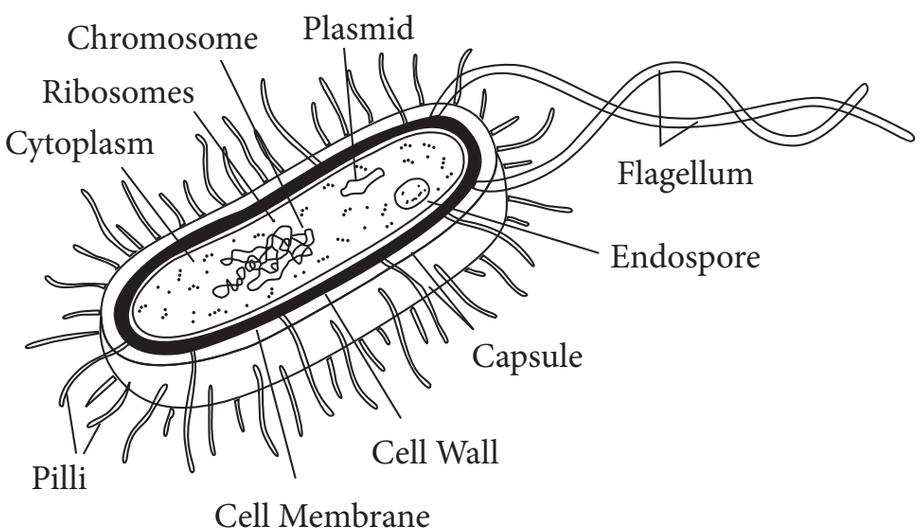

Figure 1.4 A schematic diagram of Bacteria

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Most of the bacterial cells contain cell wall which is, partially composed of a macromolecule called peptidoglycans, a mixture of amino sugars and short peptides. Human cells do not need peptidoglycans and do not make them. Certain antibiotics act by targeting such cell walls and peptidoglycans. Penicillin, one of the first antibiotics widely used to prevent the final cross-linking step, or transpeptidation as a result of which the fragile cell wall would burst, killing the bacterium.

Plant cell wall

Algae have cell wall, made of cellulose, galactans, mannans and minerals like calcium carbonate, while in other plants it consists of cellulose, hemicellulose, pectins and proteins. The cell wall of a young plant cell, the primary wall is capable of growth, which gradually diminishes as the cell matures and the secondary wall is formed on the inner (towards membrane) side of the cell. The middle lamella is a layer mainly of calcium pectate which holds or glues the different neighbouring cells together. The cell wall and middle lamellae may be traversed by plasmodesmata which connect the cytoplasm of neighbouring cells.

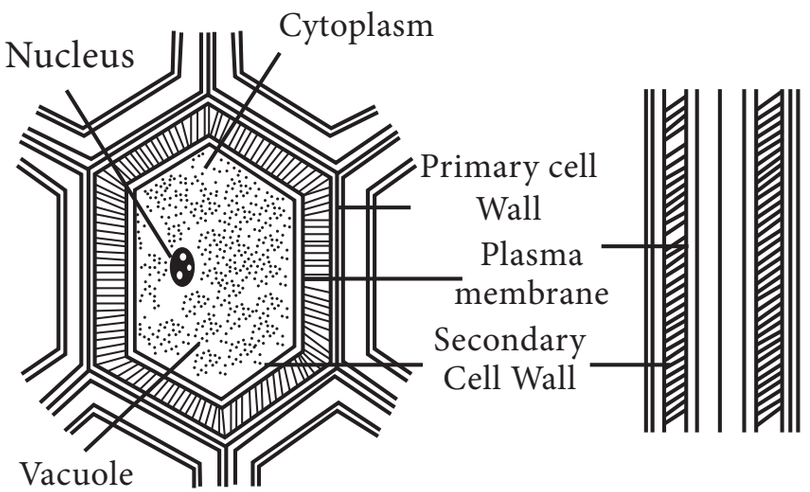

Figure 1.5.A Schematic representation of a typical plant cell wall

The main functions of the cell wall are:

- Cell wall provides structural and mechanical support.
- Cell wall determines and maintains the shape of the plant cell and governs plant architecture

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- Cell wall resists internal turgor pressure of cell.
- Cell wall regulates growth rate and diffusion of materials.
- Cell wall functions as stores of carbohydrates.
- Cell wall protects against pathogens, dehydration, and other environmental factors.

1.4.3.Nucleus

The largest organelle in the cell is nucleus which is enveloped by bound double layered nuclear membrane preserving the genetic material called chromatin. Nucleus occupies 1%-2% and 10% in yeast and animal cells respectively. The genetic material forms a mass called chromatin that is concentrated in one part of the nucleus. The outer and inner membranes are separated by lumen. The outer membrane of the nuclear envelope is continuous with the endoplasmice reticulum (ER) membrane, and the lumen of the nuclear envelope is continuous with the lumen of the ER. The inner nuclear membrane is usually supported by a network of filaments called the nuclear lamina, located in the nucleus and anchored to the inner membrane. The nucleus contains subcompartments with specialized functions and the major subcompartment in the nucleus is the nucleolus.

The pores of nuclear membranes are large enough to be completely permeable to smaller molecules, so there is no difference in the aqueous environment of the nucleus and the cytoplasm. The nucleus is considered to be the core of the cell which regulates all metabolic events.

DO YOU KNOW?

The diversity of eukaryotic organisms with their nuclei extends from single cells whose existences resemble those of free-living bacteria to complex multicellular organisms with many different types of component cells. In the nucleus, the concentration of DNA is equivalent to a gel of high viscosity. In the other compartments, proteins are concentrated at a high density.

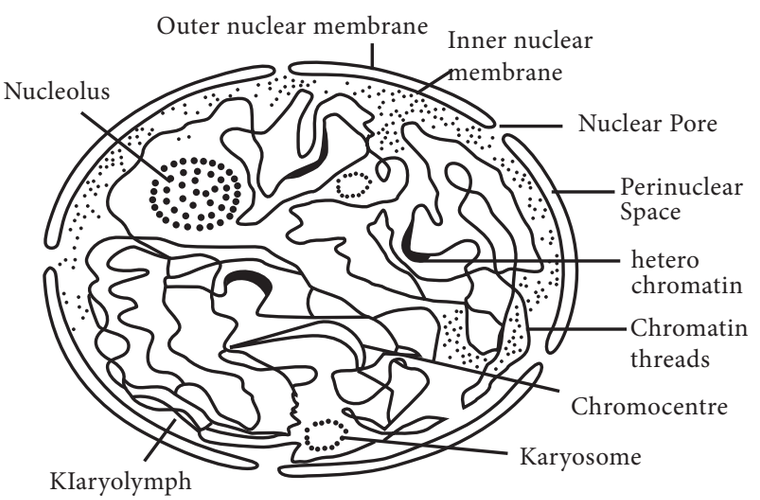

Figure 1.6 A diagrammatic representation of the ultrastructure of nucleus

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Nuclear envelope: The nucleus is separated from the cytoplasm by a double membrane, the nuclear envelope and the two membranes separated from each other by a perinuclear space of varying width. There are little holes in the nuclear envelope called nuclear pores which help the substances to move into or out of the nucleus. DNA occupies most of the space inside a nucleus. DNA is the genetic material and provides the instructions essential for building proteins. Proteins are responsible for helping with most activities in a cell. Inside the nucleus is a round body called nucleolus, which is present in a eukaryotic cell. The nucleolus is devoid of an encircling membrane. The nucleolus produces the ribosomal subunits from proteins and ribosomal RNA, also known as rRNA. It then sends the subunits out to the rest of the cell where they combine into complete ribosomes. Ribosomes make proteins; therefore, the nucleolus plays a vital role in making proteins in the cell.

1.4.4.Mitochondria- the power house of the cell

A cell has a compartment for energy production. It obtains energy from the food supplied by its environment. This energy then has to be converted into some form that can be distributed throughout the cell. The common solution is to store energy in the form of a common molecule that can be used whenever and wherever it is needed in the cell. The term 'mitochondrion' is derived from the Greek word 'mitos' which means 'thread' and 'chondrion' which means 'granule'. Mitochondria is a membrane bound cellular structure and is found in most of the eukaryotic aerobic cells. Mitochondria may assume different shapes ranging from granular to filamentous depending upon the functional state of the cell. They are spherical in yeast cells, elliptical in kidney cells, elongated in liver cells and filamentous in fibroblasts. The size of the mitochondria ranges from 0.5 to 1.0 μm in diameter.

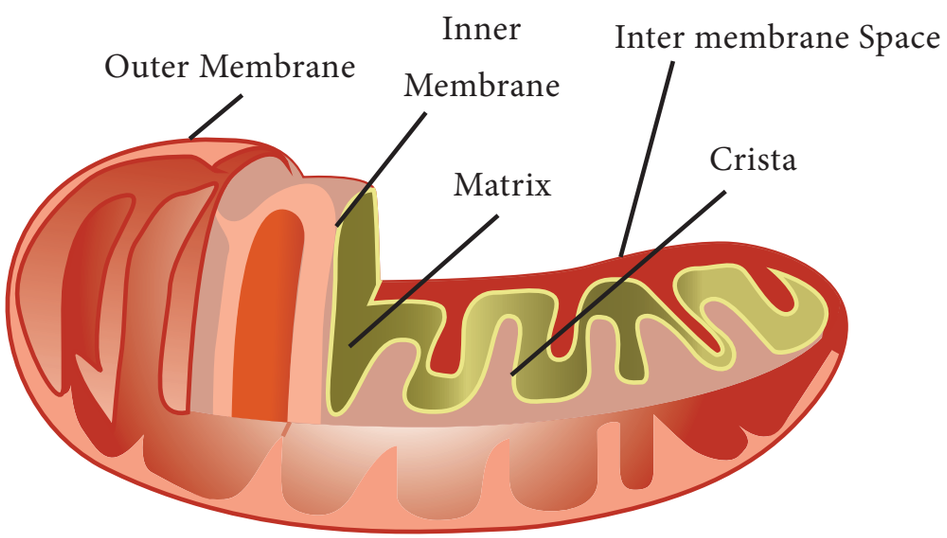

Figure 1.7 A schematic diagram of a mitochondrion

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The mitochondria consist of a smooth outer membrane, which has a large number of special proteins known as the porins, separated by a space from an inner membrane. The inner membrane is thrown into folds or invagination called cristae which extend into matrix, the mitochondrial lumen. Both the membranes are separated by a clear inter membrane space. The cristae are irregularly shaped like villous and finger like projections. The membranes are made up of phospholipids and proteins.

Functions of Mitochondria

- The mitochondria can help the living cell to convert energy supplied by the environment into ATP, the common molecule, required for chemical reactions. ATP can be generated in two pathways: in the cytosol, and in mitochondrion. First pathway exists in the cytosol of an eukaryotic cell (or within a bacterial cell) where glycolysis degrades glucose to lactate and releases two molecules of ATP.
- Second pathway is the main source of energy production as ATP (called oxidative phosphorylation and involves the electron transport chain). Pyruvate generated from glycolysis enters the matrix (lumen) of the mitochondrion, where it is degraded and combined with coenzyme A to form acetyl CoA. The acetyl part of the acetyl CoA is then degraded to carbon dioxide by the citric acid cycle, releasing hydrogen atoms. The hydrogen atoms are used to reduce the carrier NAD^+ to NADH, and then oxidation of NADH releases a proton and an electron.
- Mitochondria help the cells to maintain proper concentration of calcium ions within the compartments of the cell.
- Mitochondria also help in erythropoiesis and biosynthesis of hormones like testosterone and estrogen.
- The mitochondria of liver cells have enzymes that detoxify ammonia.
- The mitochondria also play an important role in the process of apoptosis or programmed cell death. Abnormal death of cells due to the dysfunction of mitochondria can affect the function of an organ.
- The mitochondria are involved in other cellular activities like signalling, cellular differentiation and cell senescence. They also regulate the control of cell cycle and cell growth.
- Unlike the outer membrane, the inner membrane is strictly permeable, it is permeable only to oxygen, ATP and it also helps in regulating transfer of metabolites across the membrane.
- The matrix of the mitochondria is a complex mixture of proteins and enzymes. These enzymes are important for the synthesis of ATP molecules, mitochondrial ribosomes, tRNAs and mitochondrial DNA.
- Mitochondria also affect human health. Mitochondrial disorders and cardiac dysfunction also play an important role in the aging process.

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1.4.5.Endoplasmic reticulum (ER):

Eukaryotic cells contain several interrelated membrane-bound compartments, collectively termed as 'endomembrane system' or ER.It is a continuous membrane, which is present in both plant cells, animal cells and absent in prokaryotic cells.There is a series of convoluted membrane sheets which are contiguous with the outer membrane of the nuclear envelope. This series of membrane delimited compartments in a typical eukaryotic cell are related and interact with one another by fission and fusion of their membranes. The space, which is present in the endoplasmic reticulum, is called as the lumen.

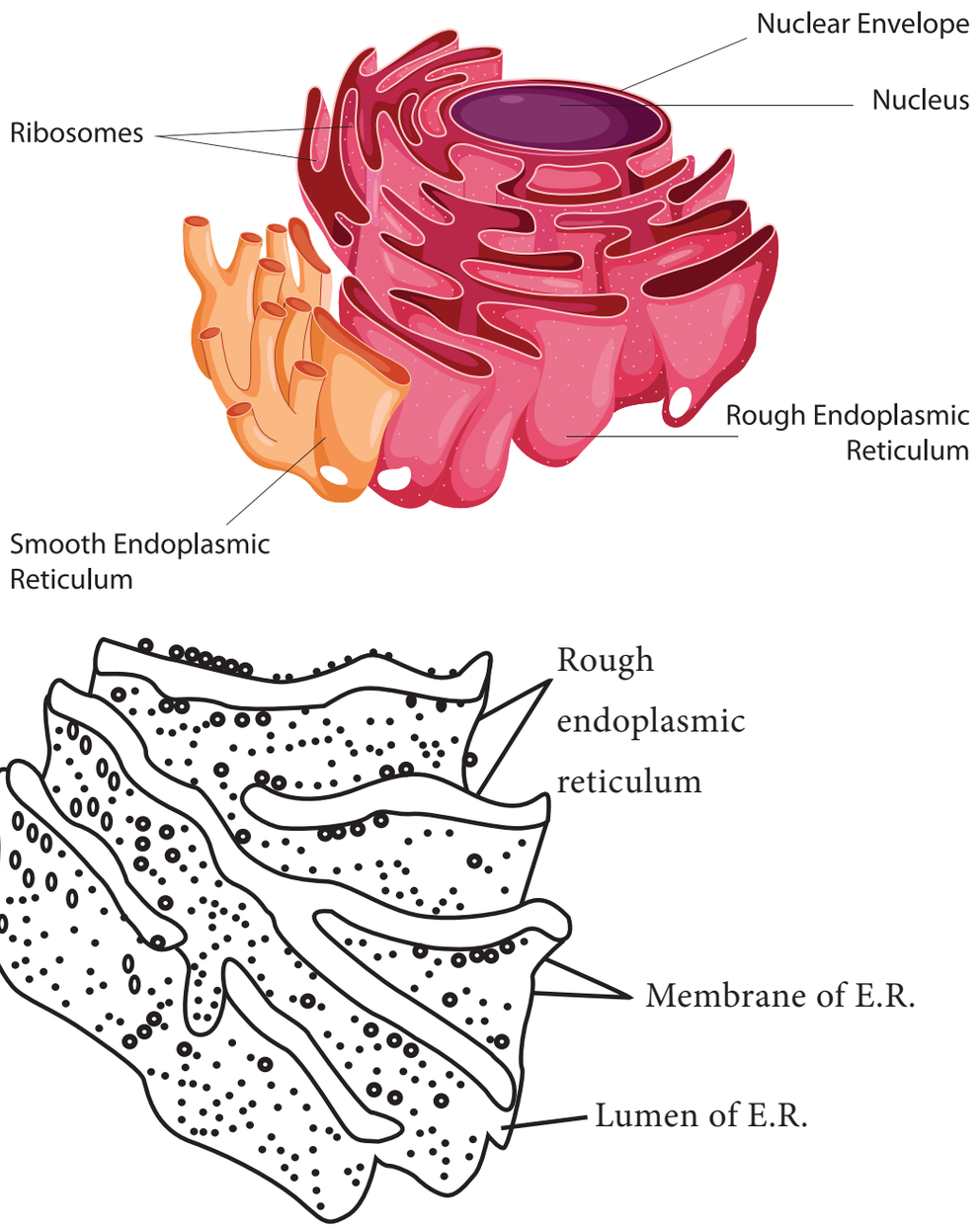

Figure 1.8 A schematic diagram of Endoplasmic Reticulum

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There are three morphological patterns in ER.

1. Granular or Rough endoplasmic reticulum
2. Smooth endoplasmic Reticulum
3. Lamellar and Vesicular endoplasmic reticulum

The rough endoplasmic reticulum contains ribosome attached to the cytoplasmic side of the membrane and it forms a lace like system. The smooth Endoplasmic reticulum lacks the attached ribosome and it forms tubular structures.

The major functions of Endoplasmic reticulum are:

- They play a vital role in the formation of the skeletal framework
- They provide the increased surface area for cellular reactions
- They help in the formation of nuclear membrane during cell division
- They play a vital role in the synthesis of proteins, lipids, glycogen and other steroids like cholesterol, progesterone, testosterone, etc.
- They are responsible for the secretion, synthesis, modification and transportation of proteins and other carbohydrates to another organelle, which includes lysosomes, Golgi apparatus, plasma membrane, etc.

1.4.6.Golgi Apparatus

Camillo Golgi (1898) had made the first report on the densely stained reticular structures near the nucleus. Hence these were later named Golgi bodies, attributed to him. They consist of many flat, disc-shaped sacs or cisternae of 0.5µm to 1.0µm diameter. These are stacked parallel to each other. Varied numbers of cisternae are present in a Golgi complex. The Golgi cisternae are concentrically arranged near the nucleus with distinct convex *cis* or the forming face and concave *trans* or the maturing face.

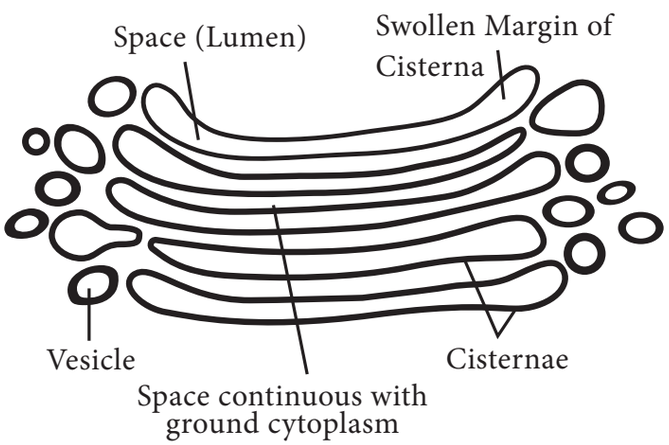

Figure 1.9.A schematic diagram of Golgi apparatus

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The *cis* and the *trans* faces of the organelle are entirely different, but interconnected. The golgi apparatus principally performs the function of packaging materials, to be delivered either to the intra-cellular targets or secreted outside the cell. Materials to be packaged in the form of vesicles from the ER fuse with the *cis* face of the golgi apparatus and move towards the maturing face. This explains, why the golgi apparatus remains in close association with the endoplasmic reticulum. A number of proteins synthesized by ribosomes on the endoplasmic reticulum are modified in the cisternae of the golgi apparatus before they are released from its *trans* face. Golgi apparatus is the important site of formation of glycoproteins and glycolipids.

Functions of Golgi apparatus

- Golgi apparatus helps in protein sorting from one compartment to another by the secretory pathway.
- Covalent modifications of proteins involving the addition of small sugar molecules occur in the ER and Golgi apparatus.

1.4.7.Ribosomes:

Ribosomes are the granular structures first observed under the electron microscope as dense particles by George Palade (1953). In the word ribosome, the pharse 'ribo' is derived from ribonucleic acid and 'somes' from the Greek word 'soma' which means 'body'. Ribosomes are tiny particles about 200 Å. They are composed of ribonucleic acid (RNA) and proteins. Ribosomes are not considered as organelles because of the lack of a membrane around them. However, when they are producing certain proteins they can become bound to the endoplasmic reticulum membrane. Free floating ribosomes are also present. Ribosomes are composed of both RNA and proteins. About 37 - 62% of ribosomes are made up of RNA and the rest is proteins. There are two types of ribosomes based on their sedimentation properties. Prokaryotes possess 70 S ribosomes and Eukaryotes possess 80 S ribosomes. The subunits of ribosomes are named owning to their sedimentation rate measured as special Svedberg Unit ('S'). The ribosomes share a core structure which is similar to all ribosomes despite differences in their size. The ribosomes are made up of two subunits - a small and a large subunit. The small subunit reads the mRNA while the large subunit joins the amino acids to form a chain of polypeptides.

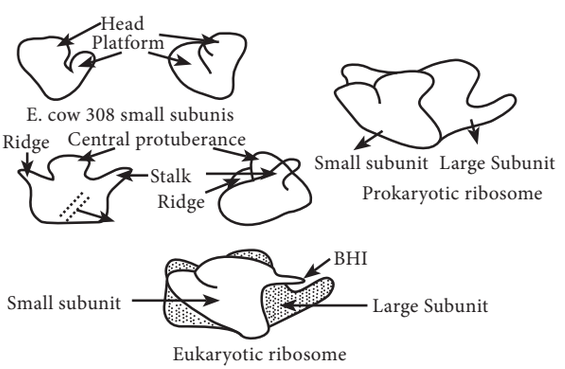

Figure 1.10 A schematic diagram of larger and smaller subunits of a ribosome

Functions of ribosomes:

- The bound and the free ribosomes are similar in structure and are involved in protein synthesis.

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- The location of the ribosomes in a cell is a determining factor of the type of protein produced. If the ribosomes are free floating throughout the cell, the proteins that are used within the cell are produced. When ribosomes are attached to endoplasmic reticulum (referred as rough endoplasmic reticulum or rough ER), the proteins that are used inside the cell or outside the cell are produced.
- The catalytic activity of the ribosome is carried out by the RNA.

1.4.8.Lysosomes

These are membrane bound vesicular structures formed by the process of packaging in the golgi apparatus. The isolated lysosomal vesicles have been found to be very rich in hydrolytic enzymes, called hydrolases such as lipases, proteases, carbohydrases, which are optimally active at the acidic pH. These enzymes are capable of digesting carbohydrates, proteins, lipids and nucleic acids.

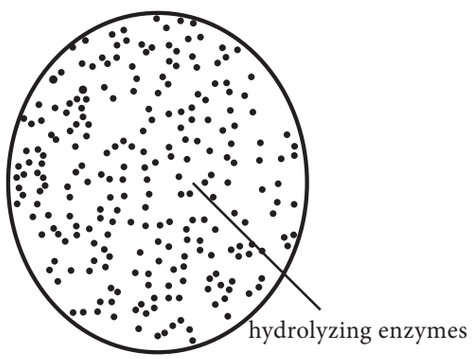

Figure 1.11 A schematic diagram of lysosome

1.4.9.Peroxisome:

Peroxisomes are microbodies that are abundantly present in mammalian liver and kidney, and also in plant cells. It depends on the type of eukaryotic cell. The matrix of Peroxisomes is rich in enzymes but a few enzymes are located in the membrane. The common enzymes present in the matrix of peroxisomes are catalases and peroxidases which metabolize a number of substrates. Enzymes present in the membrane of peroxisomes are cytochrome b5 and NADH cytochrome b5 reductase.

Functions of peroxisomes

- A major function of the peroxisome, in yeast and plant cells are to breakdown the fatty acid molecules, in a process called beta-oxidation. Peroxisomes are involved in lipid biosynthesis
- Peroxisomes contain enzymes required for the synthesis of plasmalogens
- Peroxisomes in seeds are responsible for the conversion of stored fatty acids to carbohydrates, which is critical in providing energy and raw materials for growth of the germinating plant.

1.4.10.Cytoplasm:

The ground substance that fills the interior of the cell is called cytosol or cytoplasm. It is a jelly-like substance and it is made up of eighty percent

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water and is usually clear. It appears as a transparent and colourless fluid. The cytoplasm serves as a molecular soup. It is in the cytoplasm where all the cellular organelles are suspended and are bound together by a lipid bilayer membrane. The cytoskeleton present in the cytoplasm gives the cell its shape. Cytoplasm also constitutes numerous salts and is a very good conductor of electricity.

Various metabolic activities occur in the cytoplasm. Metabolic pathways like glycolysis and cellular processes like cell division take place in the cytoplasm.

- Cytoplasm shows differential staining properties, the areas stained with the basic dyes are the basophilic areas of the cytoplasm and are termed as ergatoplasm for this material.
- It is a heterogenous mixture of opaque granules and organic compounds which gives it its colloidal nature.
- The cytoplasm conatains dissolved nutrients and it aids to dissolve waste products.
- It helps movement of the cellular materials around the cell through a process called cytoplasmic streaming.
- The peripheral zone of cytoplasm is jelly-like and is known as the plasmogel. The surrounding area of the nuclear zone is thin and liquefied in nature and is known as the plasmosol.
- The physical nature of cytoplasm is colloidal. It has a high percentage of water and particles of various shapes and sizes are suspended in it.
- It also contains proteins, of which 20-25 percent are soluble proteins including enzymes.
- Also, certain amount of carbohydrates, RNAs, inorganic salts and lipid substances are found.
- The plasmogel part of the cytoplasm is capable of absorbing water and removing it, according to the cell's need.
- The stomatal guard cells present in the leaves exhibit this property.
- An organized system of fibres can be observed by specific staining techniques.

1.4.11. Plastids

Plastids are found in all plant cells and in euglenoides. These are easily observed under the microscope as they are large. They bear some specific pigments, thus imparting specific colours to the plants. Based on the type of pigments

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plastids can be classified into different types:

Protoplastids, Amyloplastids, Leucoplastids, Etioplasts, Chloro-amyloplasts and Chromoplasts.

- Protoplasts contain brown carotenoids, chlorophyll a and chlorophyll c pigments
- Amyloplasts synthesizes starch and stores them as granules in the stroma. Some types of plastids contain enzymes for the synthesis of certain small compounds.
- The leucoplasts are the colourless plastids of varied shapes and sizes.
- Rhodoplasts contain chlorophyll a and chlorophyll d along with phycobilin and phycoerythrin pigments.
- Chloroplasts-occur in green plants are characterised by the presence of Chlorophyll a and Chlorophyll b.
- Chromoplasts synthesize and store pigments called carotenoids, which are red, orange, or yellow molecules that give some flowers and fruits their colour.

1.4.12.Chloroplasts

Chloroplasts are members of a group of plant organelles collectively known as plastids. These are associated with photosynthesis. Majority of the chloroplasts of the green plants are found in the mesophyll cells of the leaves. These are lens-shaped, oval, spherical, discoid or even ribbon-like organelles having variable length (5-10 μm) and width (2-4 μm). Their number varies from 1 per cell of the *Chlamydomonas*, a green alga to 20-40 per cell in the mesophyll. Of the two, the inner chloroplast membrane is relatively less permeable. The space limited by the inner membrane of the chloroplast is called the stroma. A number of organised flattened membranous sacs called the thylakoids, are present in the stroma. Thylakoids are arranged in stacks like the piles of coins called grana (singular: granum) or the intergranal thylakoids. In addition, there are flat membranous tubules called the stroma lamellae connecting the thylakoids of the different grana. The membrane of the thylakoids enclose a space called a lumen. The stroma of the chloroplast contains enzymes required for the synthesis of carbohydrates and proteins. It also contains small, double-stranded circular DNA molecules and ribosomes. Chlorophyll pigments are present in the thylakoids.

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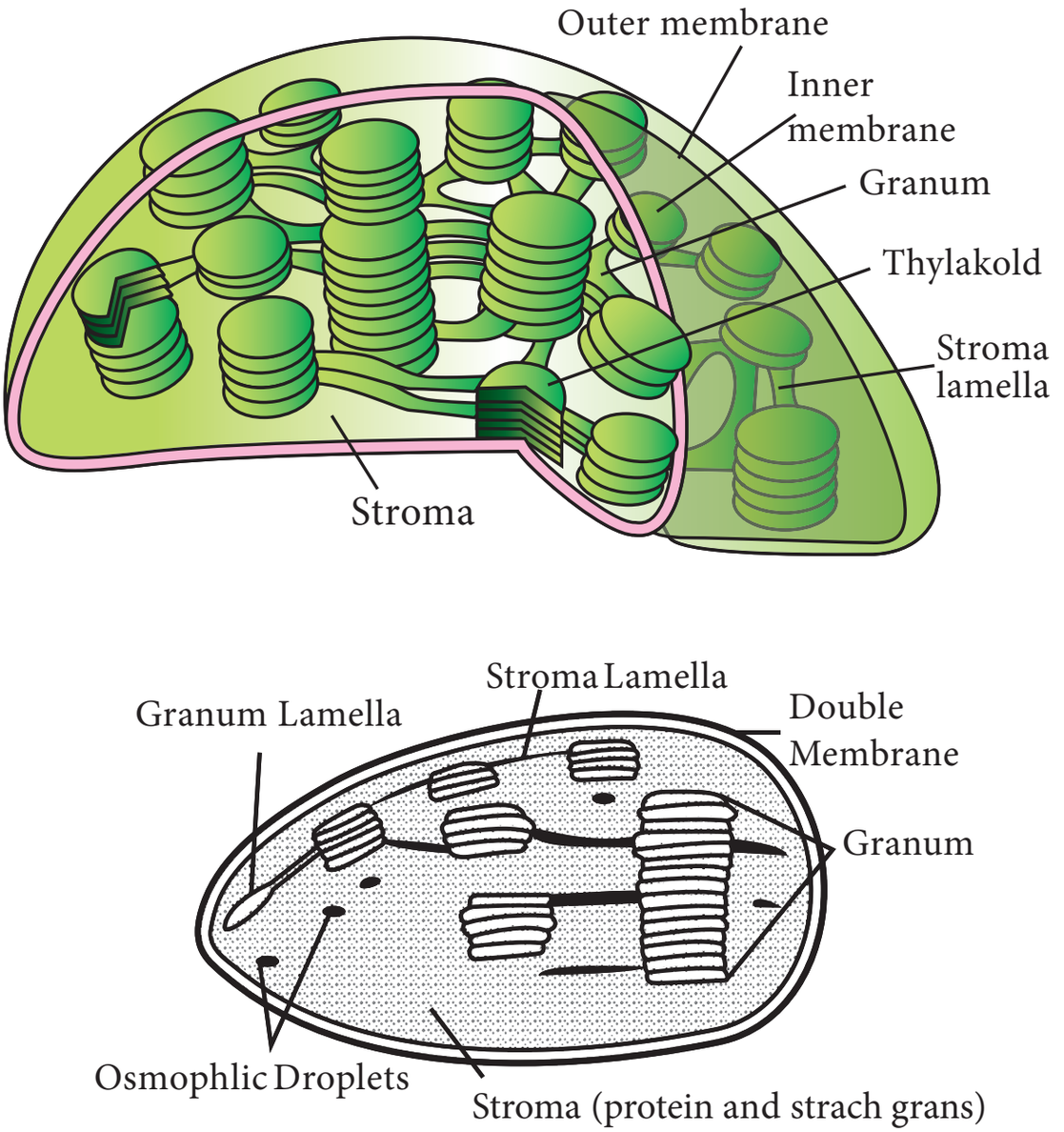

Figure 1.12 A schematic diagram of chloroplast

The thylakoids in chloroplasts contain chlorophyll and carotenoid pigments which are responsible for trapping light energy essential for photosynthesis. Chloroplasts develop in the parts of a plant, such as leaves, in which light gathering and photosynthesis will occur. Plants that are grown in the dark do not develop chloroplasts but instead develop a different type of plastid in their leaves. Chloroplasts develop into chromoplasts when tomatoes ripen from green to red and when green leaves of deciduous trees turn red orange or yellow.

Functions of chloroplast

- Chloroplasts function as the food producers of the cell and every green plant in the planet is working to convert the solar energy into sugars.

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- They are responsible for breaking down the nutrients and sugars that the cell receives and convert that into energy.
- It enables a plant to make ATP from a system in which the electrons are provided by chlorophyll that have been activated by light.

1.4.13.Vacuole:

The vacuole is the membrane-bound space found in the cytoplasm. Plant cells possess a well-developed vacuolar system, which becomes more prominent in maturing cells. It is also present in the cells of animals, fungi and bacteria but they are smaller in size. In plant cells the vacuoles can occupy up to 90 percent of the volume of the cell. Vacuoles contain water, sap, excretory product and other materials not useful for the cell. The vacuole is bound by a single membrane called tonoplast. In plants, the tonoplast facilitates the transport of a number of ions and other materials against concentration gradients into the vacuole, hence, their concentration is significantly higher in the vacuole than in the cytoplasm. In Amoeba, the contractile vacuole is important for excretion. In many cells, as in protists, food vacuoles are formed by engulfing the food particles.

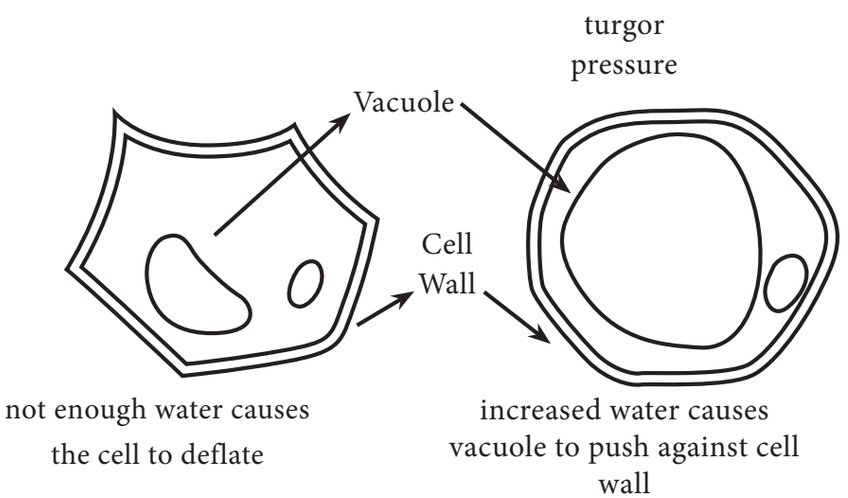

Figure 1.13 A schematic diagram of vacuole



In plants, inside each cell, there is a central vacuole, which can hold water, and under favourable conditions, the water enters the cell by osmosis (osmotic flow of water from area of low solute concentration outside the cell into the cell's vacuole, which has a higher solute concentration) and fills up the vacuole, creating a pressure called turgor pressure. This turgor pressure pushes the plasma membrane against the cell wall of plant, thereby stiffening the cell. This is mainly what makes non-woody parts of plants stiff and vertical.

In plant cells, the vacuoles accumulate a high concentration of sugars and other soluble compounds. Water enters the vacuole to dilute these sugars, generating hydrostatic pressure that is counterbalanced by the rigid wall. In this way the cells of the plant become stiff or turgid, in the same way that when an inner tube is inflated inside a bicycle tyre the combination

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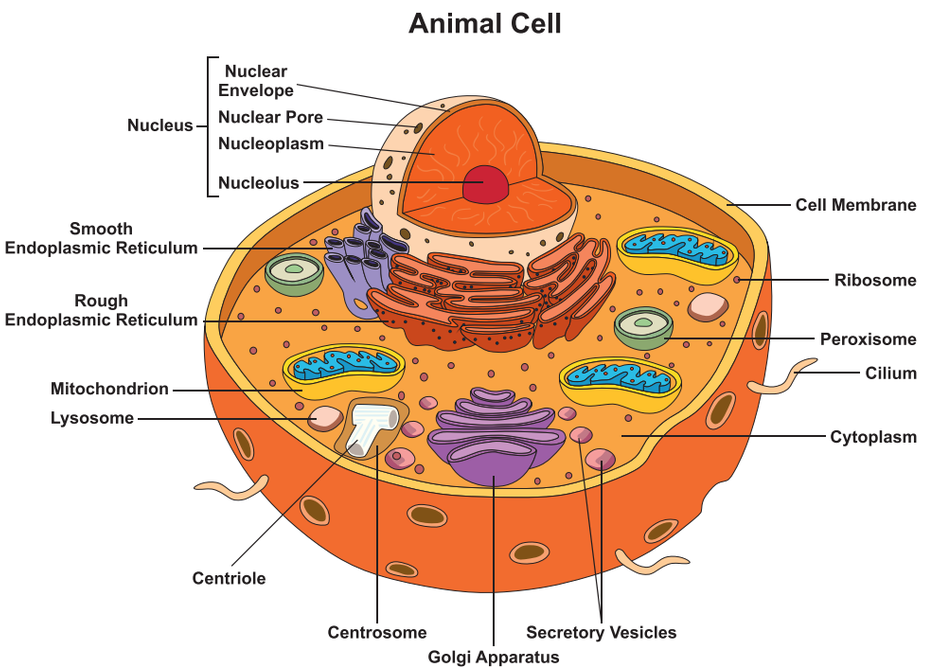
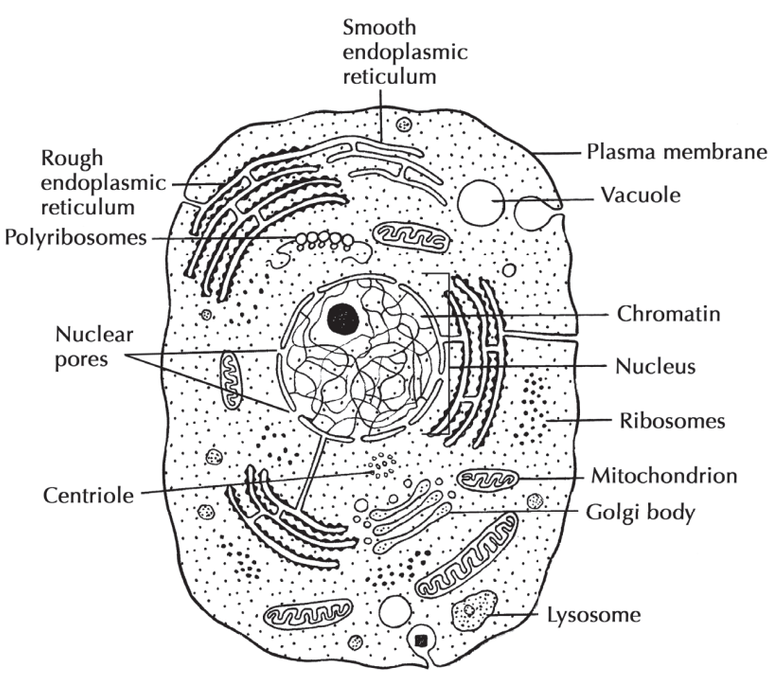
becomes stiff. Vacuoles are generally pigmented. The beautiful colors of petals and fruits are due to presence of compounds such as the purple anthocyanins in the vacuole.

Functions of vacuole:

- Vacuoles aid in storing salts, nutrients, pigments, minerals, proteins, facilitating the growth of the plant and playing a vital structural role for the plant
- It serves in other functions such as protection, storage organelles for metabolites, growth and disposal of toxic excretory substances.

1.4.14.Distinguishing features of Plant and Animal Cells





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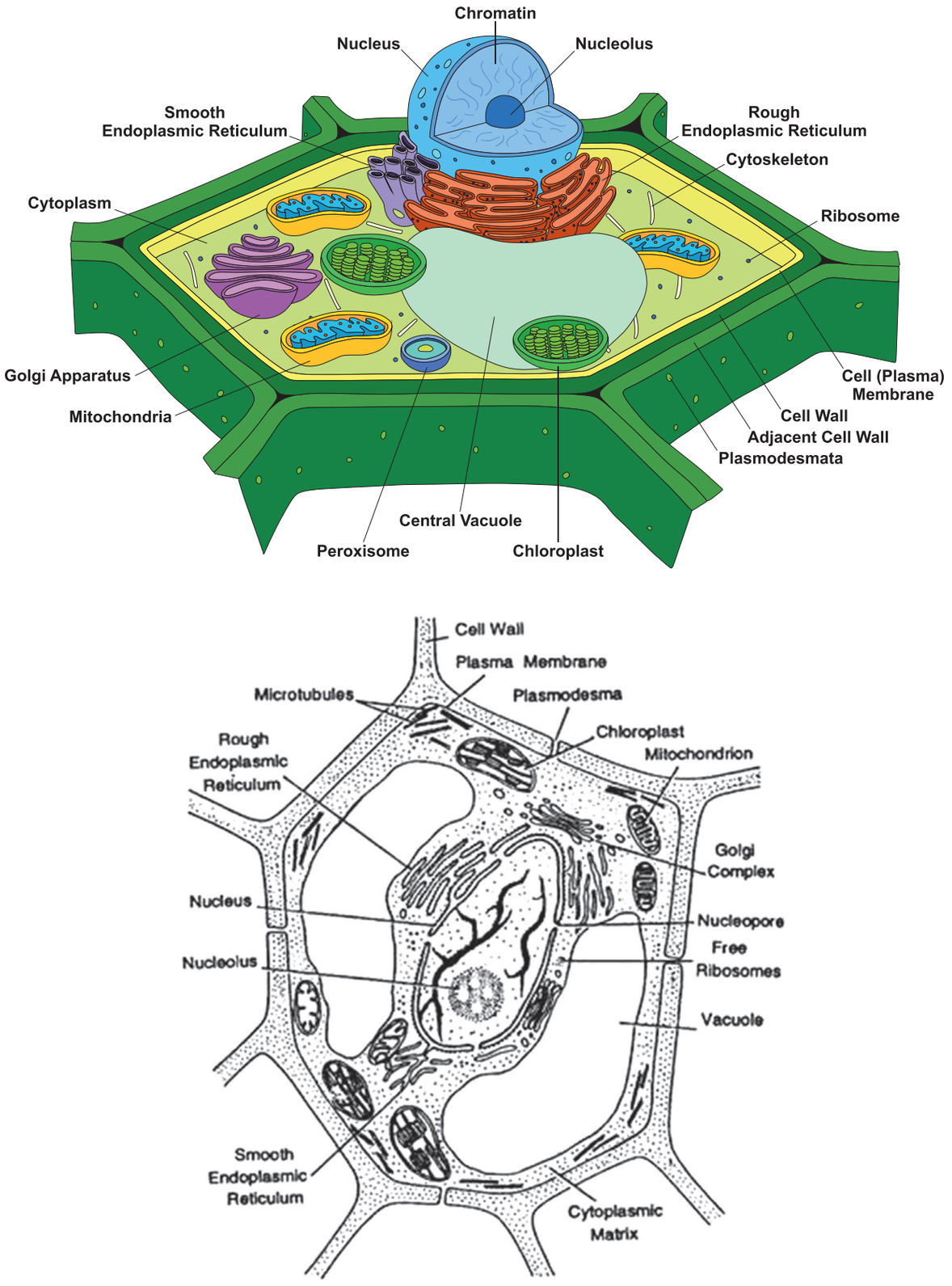

Figure1.14 Schematic representation of plant and animal cell

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Features	Plant Cell	Animal Cell
Size and Shape	Plant cells are generally rectangular in shape.	The animal cells vary in their shapes
Cell wall	Present	Absent
Plasma membrane	Present	Present
Endoplasmic reticulum	Present	Present
Nucleus	Present and lies on one side of the cell.	Present and lies in center of the cell
Mitochondria	Present and are fewer.	Present and are numerous.
Lysosomes	Present but are very rare	Present
Centrosomes	Absent	Present
Golgi apparatus	Present	Present
Cytoplasm	Present	Present
Ribosome	Present	Present
Plastids	Present with chloroplast in them.	Plastids are absent
Essential nutrients	The plant cell can synthesize amino acids, vitamins and coenzymes which are required by them	The animal cell cannot synthesize most of the amino acids, vitamins, and coenzymes which are required by them
Vacuoles	Usually large and few central vacuoles	Usually small and numerous central vacuoles
Cilia	Absent	Certain animal cells have cilia
Ribosomes	Present	Present

1.5. Acids and bases

An acid is defined as a substance that gives off protons while a base is a substance that accepts protons as per the theory of Lowry and Bronsted. Thus, an acid is a proton (H^+) donor and a base is a proton (H^+) acceptor.

The general equation that represents the dissociation of an acid is as follows:

$$\underset{\text{Acid}}{HA} \Longleftrightarrow \underset{\text{Proton}}{H^+} + \underset{\text{Conjugate base}}{A^-}$$

An acid dissociates to form proton and its conjugate base. On the other hand, the conjugate base combines with proton to form acid. The difference between an acid and its conjugate base is the presence or absence of a proton. In general, a strong acid has a weak conjugate base while a weak acid has a strong conjugate base. For instance, strong acid HCl has weak conjugate base Cl^-, weak acid HCN has a strong conjugate base CN^-.

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A few examples of acids and their corresponding conjugate bases are as follows.

Acids		Protons		Conjugate Bases
H_2O	$\rightarrow$	H^+	+	OH^-
HCl	$\rightarrow$	H^+	+	Cl^-
H_2CO_3	$\rightarrow$	H^+	+	HCO_3^-
CH_3COOH	$\rightarrow$	H^+	+	CH_3COO^-
NH_4^+	$\rightarrow$	H^+	+	NH_3



Some metallic hydroxides such as NaOH and KOH are commonly referred to as alkalies. They dissociate to form metallic ion and OH^- ion. The latter, being a base, accepts H^+ ions.

The substances which can function both as acids and bases are referred to as ampholytes. Water is the best example for an ampholyte. Similarly, amino acids are also ampholytes.

Acids and bases in biological systems

In general, acids are produced in the body as the end products of many metabolic reactions. These include the volatile acids like carbonic acid (most predominant, about 20,000 mEq/day) or non-volatile acids (about 80 mEq/day) such as lactic acid, sulphuric acid, phosphoric acid etc. Carbonic acid is formed from the metabolic product CO_2; lactic acid is produced in anaerobic metabolism; sulphuric acid is generated from proteins (sulfur containing amino acids); phosphoric acid is derived from organic phosphates (e.g. phospholipids). All these acids add up H^+ ions to the blood.

The formation of bases in the body under normal circumstances is negligible. Some amount of bicarbonate is generated from carbondioxide. The ammonia produced from amino acids is converted to urea.



A diet rich in animal proteins (non-vegetarian diet) results in more acid production by the body that ultimately leads to the excretion of urine which is profoundly acidic. A vegetarian diet has a tendency for a net production of bases. This is due to the fact that vegetarian diet produces salts of organic acids such as sodium lactate which can utilize H^+ ions produced in the body. For this reason, a vegetarian diet has an alkalizing effect on the body. This is reflected by the excretion of neutral or slightly alkaline urine.

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1.5.1 Hydrogen ion concentration and pH

The acidic or basic nature of a solution is measured by H^+ ion concentration. The conventional units such as moles/l or g/l are not commonly used to express H^+ ion concentration. Sorenson (1909) introduced the term pH to express H^+ ion concentration. pH is defined as the negative logarithm of H^+ ion concentration.

$$pH = - \log [H^+]$$



pH is an abbreviation for "power of hydrogen" where "p" represents the German word 'potenz' for power, and H is the element symbol for hydrogen. In short, pH stands for "potential of hydrogen". It was described by Soren Peder Lauritz Sorensen in 1909.

pH Scale

The pH is a narrow scale, ranging from 0 to 14 which corresponds to 1 M solution to 10^{-14} M solution of $[H^+]$. Pure water has an equal concentration of H^+ and OH^- ions i.e. 10^{-7} M each. Thus, pure water has a pH 7 which is neutral. Solutions with pH less than 7 are said to be acidic while those with pH greater than 7 are alkaline. lt must be remembered that the term acidic or alkaline are not absolute but only relative. Thus, a solution with pH 3.0 is more acidic when compared with a solution of pH 4.5. A rise in H^+ concentration decreases pH while a fall in H^+ concentration increases pH. The reverse is true for OH^- concentration. The pH of a solution containing 1N $[H^+]$ is 0 while that containing 1N $[OH^-]$ is 14.

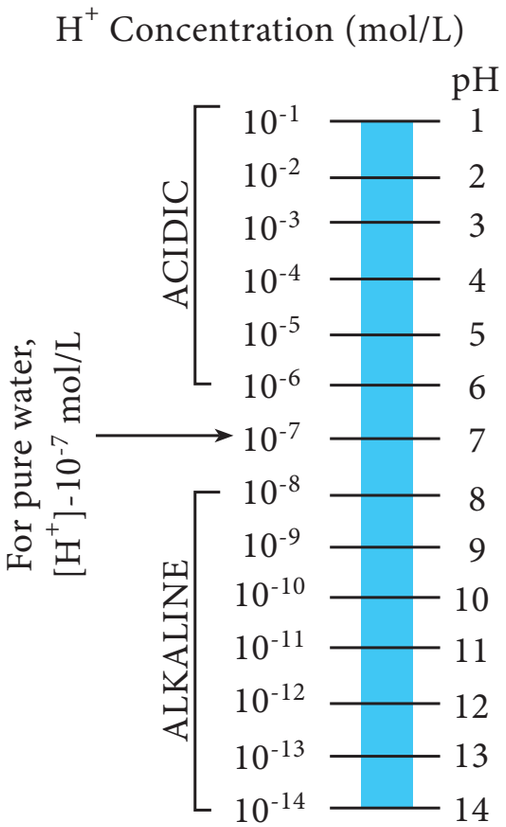

Figure 1. pH scale

Evaluate 1

Calculate the H^+ ion concentration if pH = 8.5

1.5.2 Buffers

A buffer is an aqueous solution consisting of a mixture of a weak acid and its conjugate base or a weak base and its conjugate acid. A buffer resists changes in pH when a small amount of strong acid or base is added and hence it maintains the pH of a solution. Many organisms thrive only in a relatively small pH range so they utilize buffer systems to maintain a constant pH. Buffers are more effective within 1.0 pH unit range of its pK_a value.

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Examples of buffers

1. Acetic acid and sodium acetate mixture
2. Ammonium hydroxide and ammonium chloride mixture
3. Potassium dihydrogen phosphate and dipotassium hydrogen phosphate mixture.
4. Sodium carbonate and sodium bicarbonate mixture

Buffer action

Buffer solutions achieve their resistance to pH change because of the presence of an equilibrium between the acid HA and its conjugate base A^-.

$$HA \rightleftharpoons H^+ + A^-$$

When some strong acid is added to an equilibrium mixture of the weak acid and its conjugate base, the equilibrium is shifted to the left, in accordance with Le Chatelier's principle. Because of this, the hydrogen ion concentration increases by less than the amount expected for the quantity of strong acid added. Similarly, if strong alkali is added to the mixture the hydrogen ion concentration decreases by less than the amount expected for the quantity of alkali added.

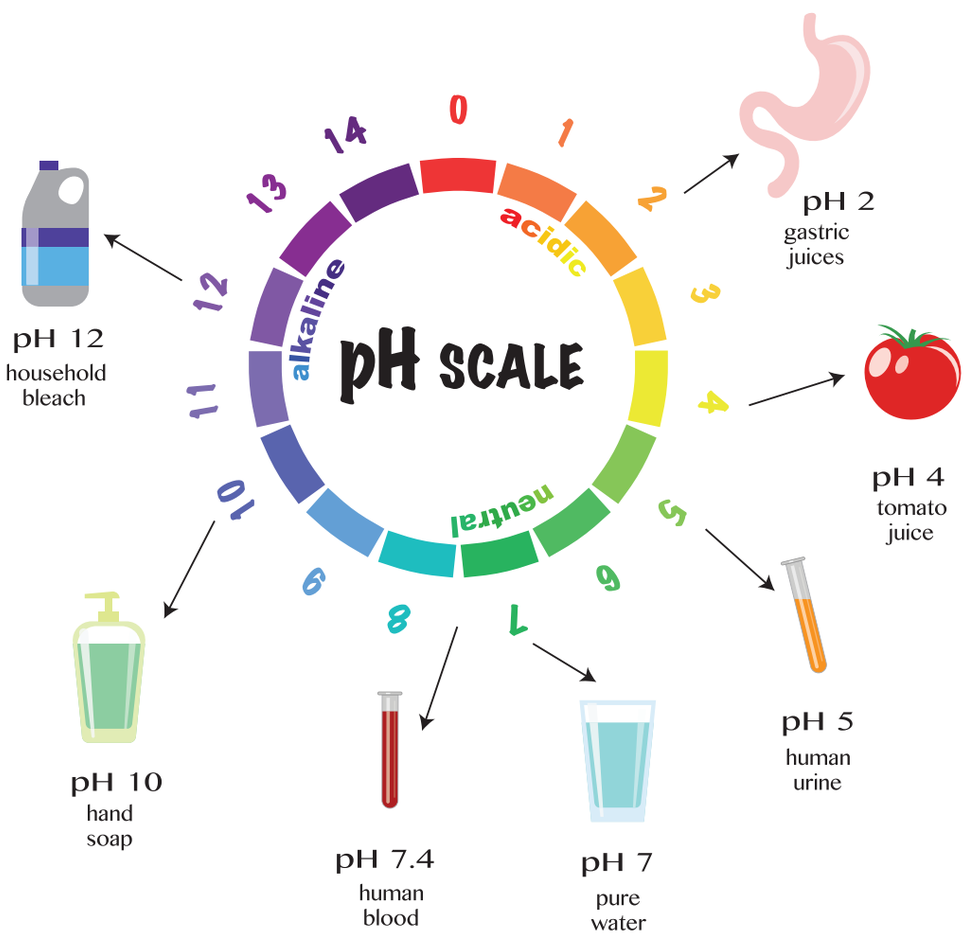

Figure 1.15 – pH paper and its colour change with respect to different samples.

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The pH paper contains a mixture of indicators which gives different colours across the entire pH range. It is used to measure pH of the sample.

Key Concept

The quantity of strong acid or base that is added to change the pH of one liter of buffer solution by one pH unit is known as **buffer capacity**.

1.5.3 Henderson–Hasselbalch equation

An equation showing the relationship between a buffer's pH and the relative amounts of the buffer's weak acid and its conjugate base is called as Henderson-Hasselbalch equation. Consider the dissociation of a weak acid (HA). At equilibrium,

$$HA \rightleftharpoons H^+ + A^-$$

The dissociation constant (K_a) is,

$$K_a = \frac{[H^+]\,[A^-]}{[HA]} \qquad \text{.....(1.1)}$$

Rearrange the equation 1.1 for hydrogen ion concentration

$$[H^+] = K_a \frac{[HA]}{[A^-]} \qquad \text{.....(1.2)}$$

Taking the logarithm to base 10, on both side of the equation 1.2,

$$\log_{10}[H^+] = \log_{10} K_a + \log_{10} \frac{[HA]}{[A^-]} \qquad \text{.....(1.3)}$$

By substituting pH and pK_a values to the equation 1.3

$$-\,pH = -\,p\,K_a + \log_{10} \frac{[HA]}{[A^-]} \qquad \text{.....(1.4)}$$

Multiplying the equation 1.4 by (–1)

$$pH = p\,K_a - \log_{10} \frac{[HA]}{[A^-]} \qquad \text{.....(1.5)}$$

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Reciprocating the log term of equation 1.5

$$pH = pK_a + \log_{10} \frac{[A^-]}{[HA]} \quad \text{.....(1.6)}$$

This form of the ionization constant equation is called the Henderson-Hasselbalch equation (equation 1.6). It is useful for calculating the pH of a weak acid solution containing its conjugate base (salt). The other forms of the Henderson-Hasselbalch equation of weak acid with its conjugate base are as follows:

$$pH = pK_a + \log \frac{[\text{conjugate base}]}{[\text{Acid}]} \quad \text{.....(1.7)}$$

or

$$pH = pK_a + \log \frac{[\text{proton aceptor}]}{[\text{proton donor}]}$$

When the concentrations of weak acid and its conjugate base or weak base and its conjugate acid are equal, the pH of the solution equals the pK_a of the buffer. This is evident from the Henderson-Hasselbalch equation.

If the pK_a of bicarbonate buffer is 6.1 and the pH of blood is 7.4, then the ratio of bicarbonate to carbonic acid ($[HCO_3^-]$ / $[H_2CO_3]$) in blood is calculated by applying the Henderson-Hasselbalch equation.

$$pH = pK_a + \log \frac{[HCO_3^-]}{[H_2CO_3]} \quad \text{..... (1.8)}$$

Substitute the pH and pK_a values into the Henderson–Hasselbalch equation.

$$7.4 = 6.1 + \log \frac{[HCO_3^-]}{[H_2CO_3]} \quad \text{.....(1.9)}$$

Rearrange the equation 1.9

$$\log \frac{[HCO_3^-]}{[H_2CO_3]} = 1.3 \quad \text{.....(1.10)}$$

Changing log term to the other side of the equation 1.10

$$\frac{[HCO_3^-]}{[H_2CO_3]} = \text{antilog } 1.3 \quad \text{.....(1.11)}$$

or

$$\frac{[HCO_3^-]}{[H_2CO_3]} = 20 \quad \text{.....(1.12)}$$

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Evaluate 2:

Calculate the pH of a 2 L solution containing 10 mL of 5 M acetic acid and 10 mL of 1 M sodium acetate. The pK_a for acetic acid is 4.76.

Evaluate 3:

The pK_a of the phosphate buffer system ($[H_2PO_4^-]/[HPO_4^{-2}]$) is 6.8. What are the relative concentrations of $[H_2PO_4^-]$ and $[HPO_4^{2-}]$ in a urine sample that has a pH of 4.8.

1.5.4 Uses of Buffers

- Buffer solutions are necessary to maintain the pH range of biological fluids. A buffer of carbonic acid ($H_2CO_3^-$) and bicarbonate (HCO_3^-) is present in blood plasma, to maintain a pH between 7.35 and 7.45.
- Enzymes are functional only under narrow pH range.
- In Industries, buffer solutions are used in fermentation processes and in setting the correct conditions for dyes used in coloring fabrics.
- They are also used for the calibration of pH meters.
- Buffers are mainly used in clinical and research laboratories. Example., phosphate buffered saline (PBS) at pH 7.4.

Determination of pH : pH meter

A pH meter is used to measure the pH of a solution. It consists of a voltmeter attached to a pH-responsive electrode and a reference electrode. The difference in electrical potential produced by hydrogen ions between pH electrode and a reference electrode is measured. A pH meter is utilized in different laboratories and industries to measure pH.

1.5.5 pH and Buffer system in Body fluids

Body fluid

All parts of the body require nutrients and the metabolic wastes produced in them need to be removed from the body. Hence, there is a need to transport various substances like digested food materials, hormones, catabolites, enzymes, various gases from one part of the body to another. These movements are achieved through body fluids. In addition to this, body fluids also provide the medium for the occurrence of metabolic reactions. Water is major component of body fluids. Water is present within and around the cells of the body, and within all the blood vessels. The total body water (TBW) is approximately 60% of body weight.

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Traditional Two Electrode pH Sensor

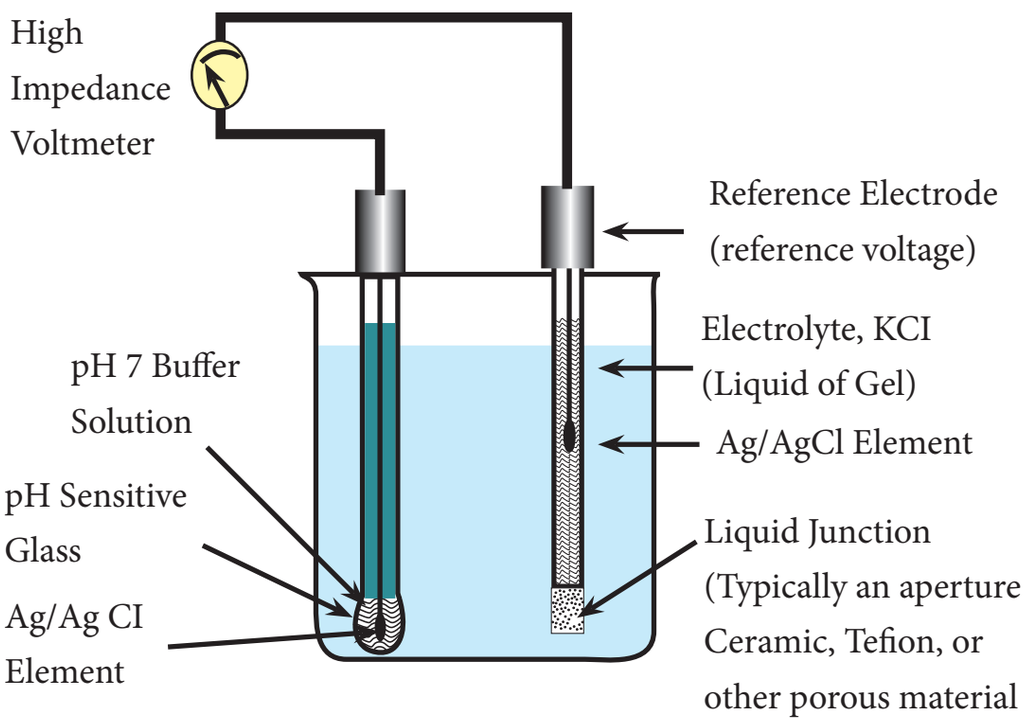

Figure 1.16 Image of simple pH meter and its schematic components.

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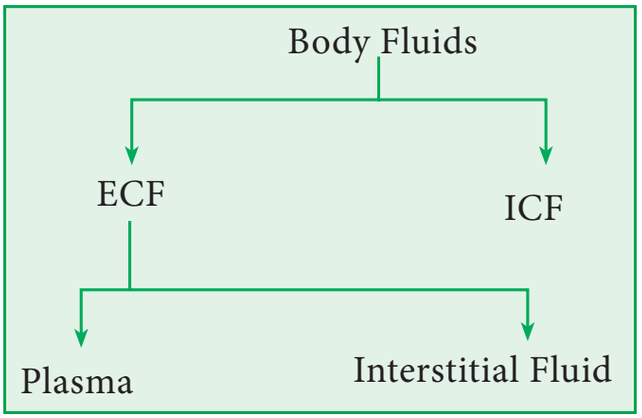

Flowchart – Classification of Body fluids

Body fluids are watery solution of dissolved substances such as oxygen, nutrients and wastes etc. Depending upon their location (compartment), they are of two types, namely, intracellular fluid (ICF) and extracellular fluid (ECF). Intracellular fluid is the fluid present within all the cells of the body. Intracellular fluid is two thirds of TBW i.e. 40% of body weight. The major cations of ICF are K^+ and Mg^{2+}. The major anions are proteins and organic phosphates.

Collectively, the fluid present in the blood and in the spaces surrounding cells is called extracellular fluid (ECF), that is, all the fluid that is outside of cells. The ECF is one thirds of TBW i.e. 20% of body weight. The major cation is Na^+. The major anions are Cl^- and HCO_3^-. The ECF is comprised of plasma (1/4th of ECF) and interstitial fluid (3/4th of ECF).

The interstitial fluid (tissue fluid) lies around and between cells. Its composition is the same as that of plasma except that it lacks larger proteins. Thus, interstitial fluid is an ultrafiltrate of plasma. Cerebrospinal fluid and lymph are examples for interstitial fluid.

Cerebrospinal fluid (CSF)

The cavities of the brain (ventricles), the spinal cord and subarachnoid region is filled with CSF. The total volume of CSF is 100 – 150 ml. It is a clear, transparent and colorless fluid. It has similar pH as that of blood (7.20 to 7.40 i.e. slightly alkaline). It protects the brain and spinal cord from shocks and maintains a uniform pressure on the nervous structures. It acts as a reservoir to regulate the contents of the cranium. To a limited extent, it acts as a medium for nutrient exchange in the nervous system.

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Lymph

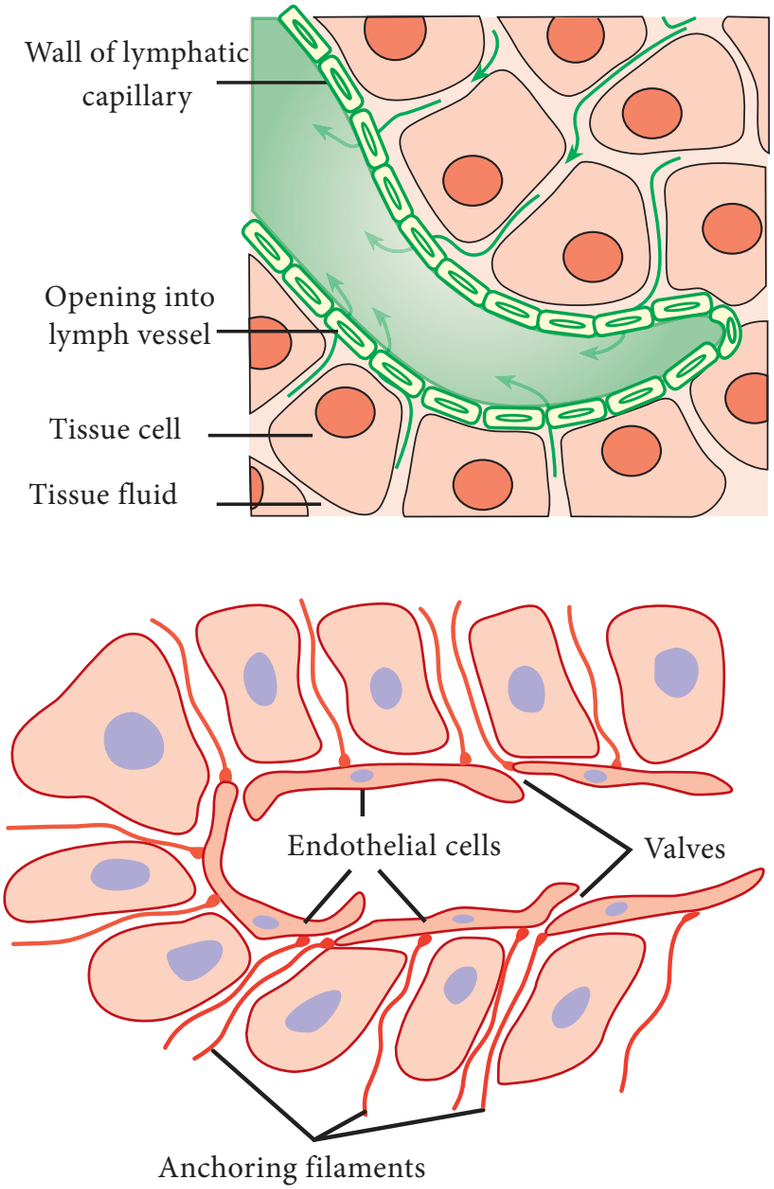

Figure 1.17 Lymph node

Formation and flow of lymph and interstitial fluid

The fluid present in the lymphatic system is called lymph. Lymph is a colorless fluid. It is composed of fluid matrix, plasma and leucocytes. It bathes tissues and organs in its protective covering. There are no erythrocytes in lymph and it has lower protein content than blood. Its pH is as same as that of blood (7.35 to 7.40 i.e. slightly alkaline).

When the blood passes through the capillaries in tissues, some water, along with many small water-soluble substances, move out into the spaces between the cells. Larger

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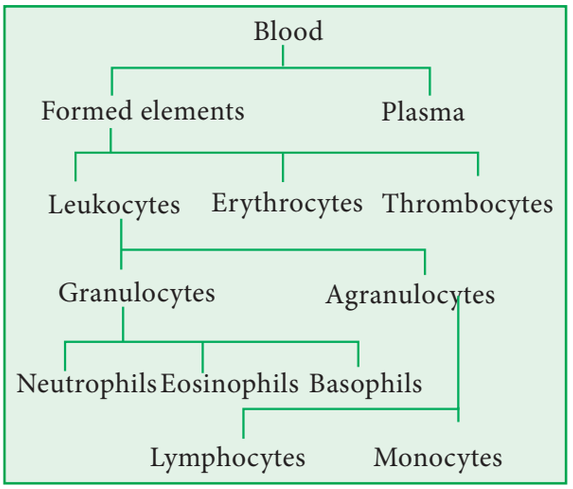
proteins and most of the formed elements are left in the blood vessels. This fluid is called the interstitial fluid or tissue fluid. Exchange of nutrients, gases, etc. between the blood and the cells always occur through this fluid. There is an elaborate network of vessels called the lymphatic system. The lymphatic system collects this lymph fluid and drains it back to the major veins such as thoracic duct and subclavian vein.

Fats are absorbed through lymph in the lacteals present in the intestinal villi. Lymph drains fluid from extracellular or intercellular spaces into blood. It is used to maintain a balance between blood and interstitial fluid.

Blood

Blood functions as a vehicle for mass transport of materials between cells and the environment or between the cells themselves for maintaining homeostasis. Blood consists of cellular portion called as formed elements (cells) that are suspended and carried in a fluid portion called plasma. The total blood volume in an adult is about 5 litres. The normal pH range of the blood is 7.35 – 7.40.

When a blood sample is centrifuged, the heavier formed elements are packed at the bottom of the centrifuge tube, leaving plasma at the top. The formed elements constitute 45% of total blood volume and the plasma accounts for the remaining 55%.



The formed elements include erythrocytes, leukocytes and platelets (thrombocytes). A cubic millimeter of adult blood normally contains 4.9 million to 5.5 million erythrocytes in males and 4.4 million to 5.0 million erythrocytes in females. The number of leukocytes in an adult human being is 5000 to 9000 leukocytes per cubic millimeter. Leukocytes include granulocytes (neutrophils, eosinophils, basophils) and agranulocytes (lymphocytes and monocytes). The normal platelet count in the blood is between 150000 and 300000 cells per cubic millimeter.

Plasma is a pale-yellow coloured liquid consisting of water and dissolved solutes. The solutes include ions like Na^+ as well as organic molecules such as metabolites, hormones, enzymes, albumins, globulins, fibrinogen and other proteins.

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Blood performs the following functions:

1. Blood transports oxygen from lungs to the tissues and carbon dioxide from tissues to the lungs.
2. It transports absorbed nutrients from digestive tract to all the body tissues.
3. It transports metabolic waste materials to kidney, lungs, skin and intestine for their removal.
4. It transports various minerals, vitamins and hormones.
5. It regulates water balance.
6. It maintains acid-base balance in the body.
7. It provides defense against various infections through leukocytes and antibodies.

DO YOU KNOW?

Blood doping is a method followed to temporarily increase the oxygen carrying capacity of blood in an attempt to gain a competitive advantage by an athlete. In this method, blood is removed from the athlete, promptly reinfusing plasma but freezing the erythrocytes for reinfusion one to seven days before the competitive event. Blood doping is illegal in collegiate athletics and Olympics for ethical and medical reasons.

DO YOU KNOW?

The sales of synthetic erythropoietin (Epogen, Procrit) provides more than $1 billion dollars annually. This hormone is often used to boost erythrocyte production in patients with suppressed erythropoietic activity, such as those with kidney failure or those undergoing chemotherapy for cancer.

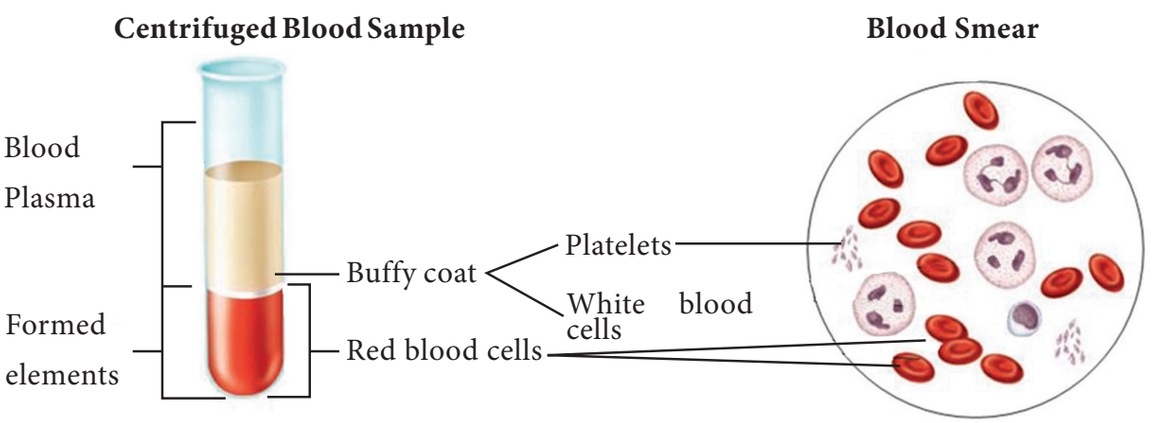

Figure 1. 18 Blood constituents; After centrifugation, blood cells settle at the bottom of the centrifuge tube leaving the plasma at the top of the tube. Leukocytes and thrombocytes form a thin light colored buffy coat at the interface between packed erythrocyte and the plasma.

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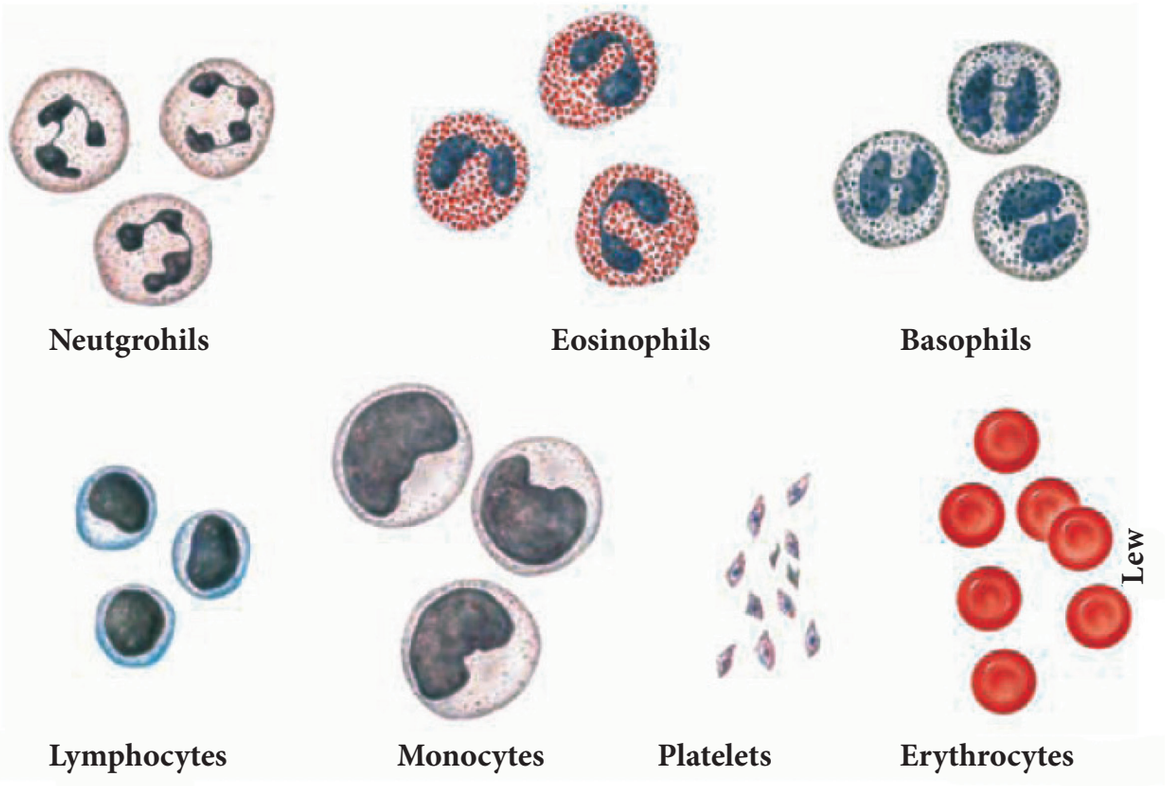

Figure 1.19 Blood cells : Leukocytes and thrombocytes (platelets) of blood are represented after staining process and erythrocytes represented without staining process.

The pH of important biological fluids is presented in the following table (Table 1.3).

Table 1.3 - pH range of the biological fluids

Sl. No	Biological Fluid	pH
1	Blood	7.35 - 7.40
2	Tears	7.20 - 7.40
3	Saliva	6.40 - 7.00
4	Gastric juice	1.50 - 3.00
5	Pancreatic juice	7.50 - 8.00
6	Interstitial fluid	7.20 - 7.40
7	Intracellular fluid	6.50 - 6.90
8	Urine	5.00 - 7.50
9	Cerebrospinal fluid	7.20 - 7.40

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Table 1.4 - Buffer systems of the body fluids

Sl. No	Body Fluid	Buffer system
1	Blood	Bicarbonate, Protein and Hemoglobin buffer system
2	Interstitial fluid	Bicarbonate buffer system
3	Intracellular fluid	Protein and Phosphate buffer system

Various Buffers of Blood

Blood contains four buffers namely

- Bicarbonate buffer system
- Phosphate buffer system
- Protein buffer system
- Hemoglobin buffer system



The buffering capacity of blood for handling carbon dioxide is estimated to be distributed among various buffer systems as follows: Haemoglobin 62%, Phosphate 22%, Plasma protein 11% and Bicarbonate buffer system 5%.

Bicarbonate buffer system

The bicarbonate buffer system consists of carbonic acid and bicarbonate ions. The pK_a of the bicarbonate buffer system is 6.1. It is the most important buffer system of blood plasma. The carbon dioxide released during fuel metabolism reacts with water by the action of the enzyme carbonic anhydrase to form carbonic acid [H_2CO_3]. Carbonic acid is a weak acid that partially dissociates into bicarbonate ion [HCO_3^-] and H^+ ion.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

As base is added and H^+ removed, carbonic acid dissociates into hydrogen ion and bicarbonate ions, and dissolved CO_2 reacts with water to replenish the carbonic acid levels. When CO_2 levels are increased, it forms more amount of carbonic acid which in turn dissociates into hydrogen ion and bicarbonate ions. Thus bicarbonate buffer functions as buffer system in blood.

Phosphate buffer system

The dihydrogen phosphate [$H_2PO_4^-$] ions and monohydrogen phosphate [HPO_4^{2-}] ions contribute to the phosphate buffer system. The pK_a of a phosphate buffer system is 6.8. Phosphoric acid dissociates into H^+ ions and dihydrogen phosphate [$H_2PO_4^-$] ions with pK_a of 2.15. Dihydrogen phosphate [$H_2PO_4^-$] ion dissociates into H^+ ions and monohydrogen phosphate [HPO_4^{2-}] ions with pK_a of 7.2 whereas monohydrogen phosphate ions dissociates into hydrogen ion and phosphate PO_4^{3-} anions with pK_a of 12.4. From the dissociation constant values, it is clearly understood that phosphate acts as an effective buffer in blood (pH = 7.4).

$$H_3PO_4 \rightleftharpoons H^+ + H_2PO_4^- \rightleftharpoons H^+ + HPO_4^{2-} \rightleftharpoons H^+ + PO_4^{3-}$$

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But, phosphate concentration is very low in blood, thus, phosphate buffer, plays a major role as an intracellular buffer in red blood cell and other types of cells where their concentrations are higher than in blood and interstitial fluid. The sodium salts of phosphoric acid also act as buffer system.

Protein buffer system

Plasma proteins are responsible for protein buffer system. The buffering capacity of proteins depends upon the pK_a of ionisable group of aminoacid side chains. Histidine residue plays a vital role as buffering agent because its imidazole group pK_a value is 6.7 and it is the more effective contributor for protein buffer system. The plasma proteins are responsible for the 2% buffering capacity of plasma. At blood pH 7.4, proteins exist as anions (Pr^-) serving as conjugate base. After accepting H^+ ions it is converted into weak acid (HPr). Thus, buffering action of proteins is due to the following dissociation reaction:

$$HPr \rightleftharpoons Pr^- + H^+$$
$$\text{Protein-H} \rightleftharpoons \text{Protein}^- + H^+$$

Hemoglobin buffer systems

Hemoglobin present in erythrocytes also plays an important role as buffering agent. It mainly buffers the acids produced during gaseous transport between lungs and tissues.

$$HHb \rightleftharpoons Hb^- + H^+$$

At tissue levels, H^+ ions released from carbonic acid bind with haemoglobin and help in the transport of CO_2 as HCO_3^-. In lungs, as haemoglobin combines with oxygen, it releases H^+ ions, which in turn bind with HCO_3^- to form carbonic acid. Carbonic acid then dissociates into CO_2 and water. Then, CO_2 is exhaled. Thus haemoglobin acts as a buffer system.

1.5.6 Acid-Base Balance

The normal pH of biological fluid is maintained in a narrow range. For example, the pH of the blood is maintained between 7.35-7.40, i.e. slightly alkaline. The changes in pH range will affect metabolic functions e.g. denaturation of proteins, enzyme activity etc. Thus, maintenance of pH is vital for normal physiological and biochemical functions of the body. The change of pH is due to the change of acid-base concentrations in the cell and biological fluids. Hence, the control and maintenance of acid-base balance is essential for the maintenance of pH.

Regulation of Acid-Base balance

The acid-base balance in the body is maintained by buffer system along with the functions of lungs and kidney.

Role of Lung

The first line of defense maintenance of pH is the control of extracellular concentrations of CO_2 and bicarbonate ions by the lungs. An increase in ventilation removes CO_2 from extracellular fluid which, in turn reduces

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hydrogen ion concentration. Conversely, decreased ventilation increases CO_2, thus increasing hydrogen ion concentration in the extracellular fluid. Both bicarbonate buffer system and hemoglobin buffer system of erythrocytes are important for the maintenance of pH by lungs.

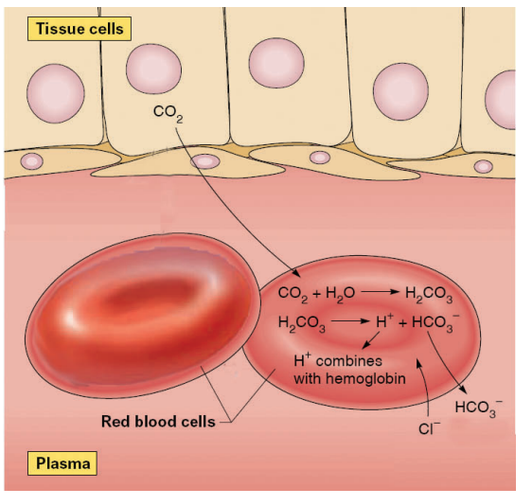

Figure 1.20 – The dissolved carbon dioxide diffuses into erythrocytes from the tissues. It is then converted into carbonic acid by the enzyme carbonic anhydrase. Carbonic acid then dissociates into hydrogen ion and bicarbonate ion. The hydrogen ion then combines with haemoglobin as HHb and the bicarbonate ion diffuses out into plasma. To maintain electrical neutrality, chloride ions diffuse into erythrocytes.



BOHR EFFECT

The unloading of oxygen is increased by the binding of H^+ (released from carbonic acid) to oxyhemoglobin. This is the Bohr effect, and results in increased conversion of oxyhemoglobin to deoxyhemoglobin.



CHLORIDE SHIFT

The exchange of chloride ions for bicarbonate ions as blood passes through the systemic capillaries is called as the chloride shift.

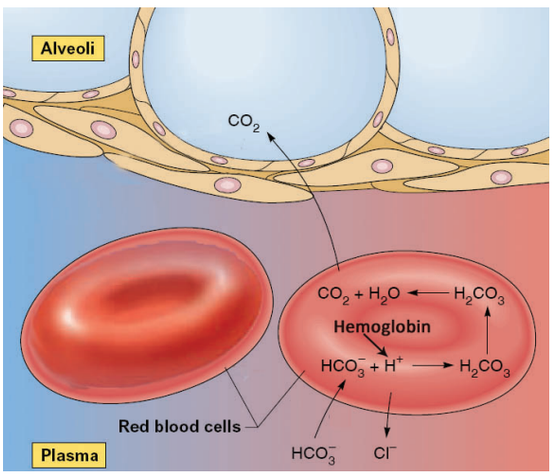

Figure 1.21 – The bicarbonate ions diffuse from plasma, combine with hydrogen ions released from hemoglobin, to form carbonic acid. The carbonic acid is then converted into carbon dioxide and water by the enzyme carbonic anhydrase. The carbon dioxide is then eliminated through exhaled air.

When blood passes through capillaries of systemic circulation, carbonic acid is formed from diffused carbon dioxide and water by the action of the enzyme carbonic anhydrase within the erythrocytes. High carbonic acid concentration favors the dissociation of carbonic acid into bicarbonate and hydrogen ions within erythrocytes. The released H^+ ions bind with deoxy haemoglobin whereas bicarbonate ions diffuse out of erythrocytes into plasma. Thus haemoglobin reduces H^+ ions within erythrocytes in tissues.

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When blood reaches pulmonary capillaries, deoxyhemoglobin is converted into oxyhemoglobin. The hydrogen ions are released from hemoglobin because oxyhemoglobin has a weaker affinity for H^+ ions. Due to this, bicarbonate ions diffuse into erythrocytes which, in turn combine with H^+ ions and form carbonic acid. At low partial pressure of carbon dioxide, carbonic anhydrase converts carbonic acid into carbon dioxide and water within erythrocytes. Thus, bicarbonate ions reduce H^+ ions in lungs.

Role of Kidney

The kidney maintains pH by excreting either acidic or basic urine. Excretion of acidic urine increases pH whereas basic urine excretion decreases pH in extracellular fluid. Large amount of H^+ ions are secreted into the tubular lumen by tubular epithelial cells. If they are removed in urine, they will increase the pH of extracellular fluid. Large amount of bicarbonate ions are also continuously secreted in the renal tubules. If they are excreted in urine, then they reduce pH by retaining H^+ ions. If more hydrogen ions are removed than bicarbonate ions, then there will be a net loss of acid, whereas, if more bicarbonate ions are filtered than hydrogen ions are secreted, then there will be a net loss of base. These functions are achieved by mainly three components, namely, bicarbonate buffer system, phosphate buffer system and ammonia. The role of these components are as follows:

Bicarbonate buffer system in kidney

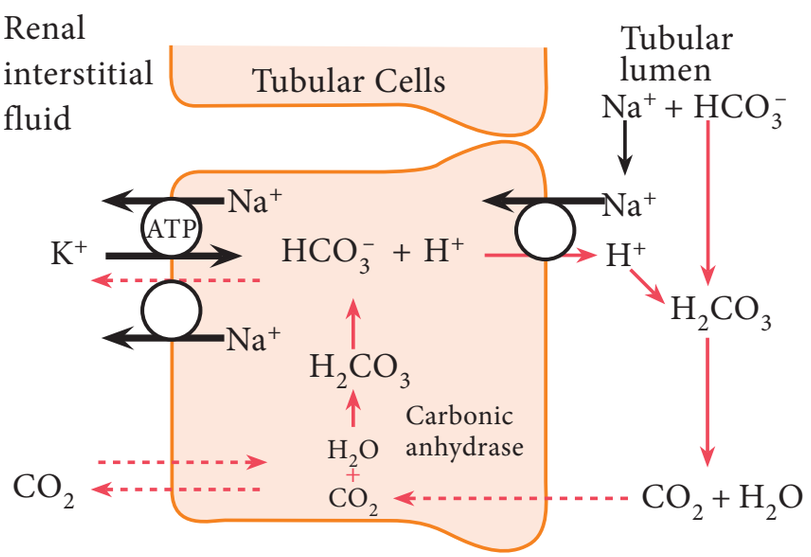

Figure 1.22 Role of bicarbonate ions in kidney tubule

The bicarbonate ions, freely filtered through glomerulus, combine with hydrogen ions and form carbonic acid which, in turn, dissociates into carbon dioxide and water. The carbon dioxide then diffuses into tubular cells where it again combines with water to form carbonic acid in the presence of carbonic anhydrase. Thus, bicarbonate and hydrogen ions are reabsorbed and retained. This pattern of H^+ ion secretion occurs in proximal convoluted tubule, ascending loop of Henle and early part of distal convoluted tubule.

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Phosphate buffer system in kidney

After the absorption of available bicarbonate ions in tubular filtrate, remaining hydrogen ions interact with HPO_4^{2-} or $NaHPO_4^-$ and form $H_2PO_4^-$ or NaH_2PO_4 which can be excreted in urine. Thus, hydrogen ions are removed from extracellular fluid.

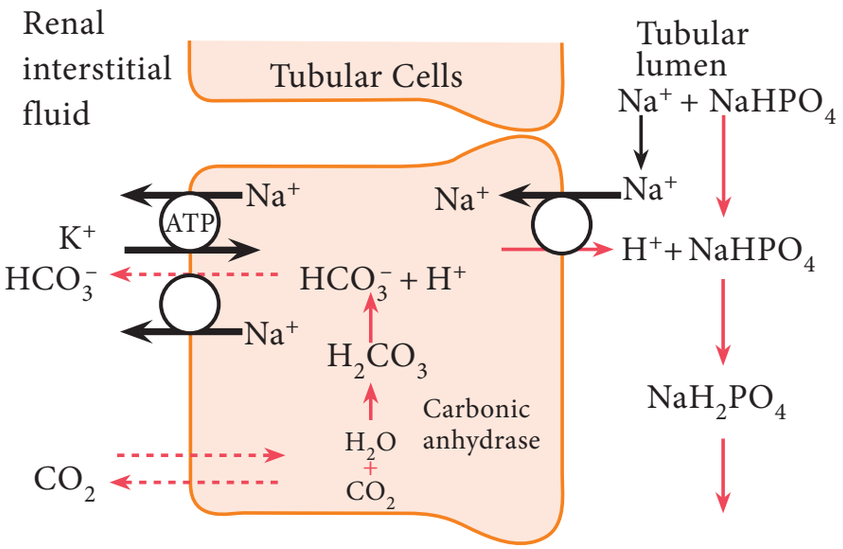

Figure 1.23 Role of phosphate ions in kidney tubule

Ammonium buffer system in kidney

Ammonia produced from glutamine in tubular epithelial cells, can freely diffuse into tubular lumen. This ammonia then combines with hydrogen ions to form ammonium ions which can be easily excreted in urine. Thus, hydrogen ions are removed from extracellular fluid.

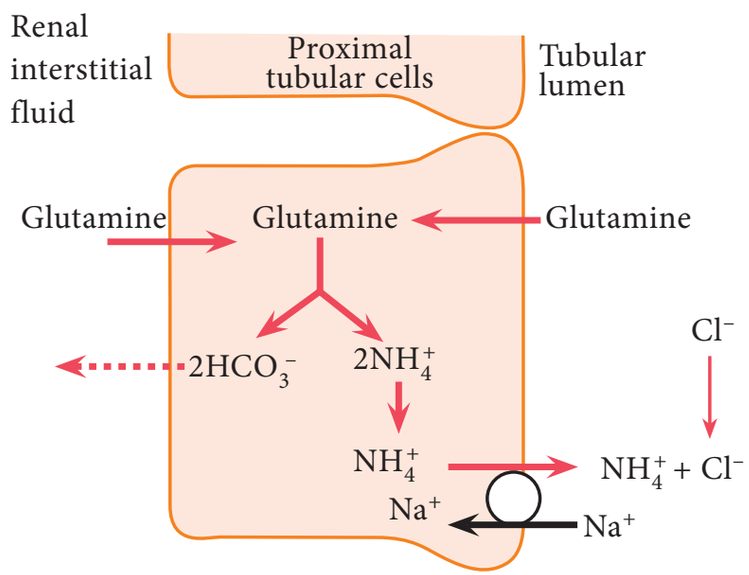

Figure 1.24 Role of ammonium in kidney tubule

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Key Concept

Increased level of carbonic acid or decreased level of bicarbonate ion results in acidosis. Decreased level of carbonic acid or increased level of bicarbonate ion results in alkalosis.



The term basicity means the number of replaceable hydrogen atoms present in one molecule of an acid. The term acidity means the number of replaceable hydroxyl groups present in one molecule of a base.



Dilution of mineral acid with water should be done with care by mixing acid slowly by the sides of the container with constant stirring. Addition of water to mineral acid should be avoided.

Evaluate 4:

- Take Saliva, Urine and Pure water
- Dip pH paper into these solutions
- Observe the changes

Sl. No.	Sample	Color of the pH paper	Approximate pH	Nature of the substance (Acidic / Basic)
1.	Saliva			
2.	Urine			
3.	Pure water			

Evaluate 5:

- Take Soap solution, 1M NaOH, lemon juice and 1M HCl.
- Dip pH meter into these solutions
- Note the pH value of these solutions

Sl. No.	Sample	pH Value
1.	Soap solution	
2.	1M NaOH	
3.	Lemon juice	
4.	1M HCl	

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Answer to evaluate 1:

Calculate $[H^+]$ from a known pH. Find $[H^+]$ if pH = 8.5

$[H^+] = 10^{-pH}$

$[H^+] = 10^{-8.5}$

$[H^+] = 3.2 \times 10^{-9}$ M

Answer to Evaluate 2:

Calculate the pH of a 2 L solution containing 10 mL of 5 M acetic acid and 10 mL of 1 M sodium acetate. The pK_a for acetic acid is 4.76.

Answer:

First, calculate the concentrations of the acid and conjugate base, expressing all concentrations in units of moles per liter (Molar Concentration).

Acetic acid: (0.01 L)(5 M)/(2 L) = 0.025 M

Sodium acetate: (0.01 L) (1M)/ (2 L)= 0.005 M

Substitute the concentrations of the acid and conjugate base into the Henderson–Hasselbalch equation.

$pH = pK_a + \log ([acetate] / [acetic acid])$

$pH = 4.76 + \log (0.005 / 0.025)$

$pH = 4.76 - 0.70$

pH = 4 .06

Answer to Evaluate 3:

The pK_a of the phosphate buffer system ($[H_2PO_4^-]/[HPO_4^{2-}]$) is 6.8. What are the relative concentrations of $[H_2PO_4^-]$ and $[HPO_4^{2-}]$ in a urine sample that has a pH of 4.8.

$$pH = pK_a + \log \frac{H_2PO_4^-}{[HPO_4^{2-}]} \qquad 1.13$$

Substitute the pH and pK_a values into the Henderson–Hasselbalch equation.

$$4.8 = 6.8 + \log \frac{H_2PO_4^-}{[HPO_4^{2-}]} \qquad 1.14$$

Rearrange the equation 1.14

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$$\log \frac{H_2PO_4^-}{[HPO_4^{2-}]} = -2.0$$

Converting log term in the above equation

$$\frac{H_2PO_4^-}{[HPO_4^{2-}]} = 10^{-2}$$

or

$$\frac{H_2PO_4^-}{[HPO_4^{2-}]} = \frac{1}{100}$$

Rearranging equation

$$[HPO_4^{2-}] = 100\ [H_2PO_4^-]$$

The concentration of $[H_2PO_4^{2-}]$ is 100 times of concentration of $[HPO_4^-]$ to get pH of 4.8 as per Henderson–Hasselbalch equation.

EVALUATION

I. Part A

1) Which one of the following is a membrane less organelle?

a. nucleus b. nucleolus c. endoplasmic reticulum

d. mitochondria.

2) The nature of the lipid bilayer is

a. hydrophilic b. hydrophobic c. amphipathic

d. lipophilic

3) The cell wall is made up of

a. glucose b. cellulose c. sucrose d. fructose

4) The suicidal bag of the cell is

a. mitochondria b. nucleus c. lysosome

d. endoplasmic reticulum

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5) Peroxisomes are involved in the biosynthesis of which of the following?

a. protein b. lipid c. carbohydrate d. nucleic acid

6) The site of the light-dependent photosynthesis reactions in chloroplasts is

a. inner membrane b. outer membrane

c. matrix d. thylakoid membrane

7) Which of the following is synthesised and processed by Endoplasmic reticulum?

a. protein b. vitamin c. nucleic acid d. lipid

8) The organelle which exchange energy with environment is

a. mitochondria b. endoplasmic reticulum

c. nucleus d. peroxisomes

9) Cell homeostasis is maintained by

a. nucleus b. cell membrane c. mitochondria d. lysosome

10) The physical nature of cytoplasm is

a. colloidal b. solid c. liquid d. vacuum

11). Which is the most common acid produced in the human body?

a. HCl b. H_2SO_4 c. H_2CO_3 d. HNO_3

12. The range of pH scale is

a. 1 to 14 b. 0 to 14 c. 0 to 7 d. 1 to 7

13. A buffer pair (HA/A^-) has a pK_a of 7.4. At a blood pH of 7.4, the concentration of HA is

a. 1/100 that of $[A^-]$

b. 1/10 that of $[A^-]$

c. equal to that of $[A^-]$

d. 10 times that of $[A^-]$

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14. What will be the ratio of ($[HPO_4^{2-}]/[H_2PO_4^-]$) of phosphate buffer at pH 7.4 and pK_a 6.8 in blood.

a. 2 b. 3 c. 4 d. 5

15. Most of the water in the human body is present in

a. intracellular fluid b. total extracellular fluid

c. plasma d. interstitial fluid

16. Which type of formed elements are high in number in blood?

a. leucocytes b. erythrocytes c. thrombocytes

d. lymphocytes

17. What is the nature of normal urine?

a. strongly acidic b. strongly basic c. slightly alkaline

d. slightly acidic

18. Identify the most important buffer system in blood plasma from the following:

a. bicarbonate buffer b. phosphate buffer c. protein buffer

d. hemoglobin buffer

19. Which form of hemoglobin have more affinity towards hydrogen ions?

a. oxyhaemoglobin b. deoxyhaemoglobin c. cyanohaemoglobin

d.methyhaemoglobin

20. Excretion of acidic urine _____ pH in extracellular fluid.

a. decreases b. increases c. does not alter

d. both a and b

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II. Part B

1. What is the function of Nuclear pore?
2. Name the types of Endoplasmic reticulum.
3. What is a Lysosome? Give its function.
4. What are the functions of Peroxisome?
5. Write a short note on chloroplast.
6. Is ribosome an organelle? How do they differ in prokaryotes and eukaryotes?
7. Justify the necessity of vacuoles in plant cell.
8. Write about the types of plastids.
9. Draw and label the parts of nucleus.
10. Brief the bilipid layered structure of plasma membrane.
11. Define: acid and base
12. Define pH
13. What is a buffer?
14. Distinguish the prokaryotic and eukaryotic cell.
15. With the help of a diagram, brief the features of cell wall of plant cell.
16. Brief the functions of mitochondria of the cell.
17. Sketch and label both plant and animal cells.
18. Brief about cytoplasm and its function.
19. List out any three uses of buffers.
20. How do you classify body fluids?
21. List out any three functions of blood.
22. State and Derive Henderson-Hasselbalch equation.

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23. Describe on the different types of buffer system in blood.

24. How does kidney maintain the pH of extracellular fluid?

25. Describe in detail about the organelles of cell like Nucleus, Endoplasmic reticulum, Mitochondria and chloroplast with neat diagram.

Activities:

1. Identify the different organelle microscopically, in both plant and animal cell models with the help of teacher.

2. Make both the models of prokaryotic and eukaryotic cells.

Reference Books

1. Staurt Ira Fox, 2011. Human Physiology, 12th edition, McGraw-Hill Publication (ISBN 978–0–07–337811–4).

2. Guyton and Hall, 2016. Text book of Medical Physiology, 13th edition, Elsevier Publications. (ISBN 978-1-4557-7005-2).

3. Gary D Christian, 2004. Analytical Chemistry, 6th edition, John Wiley and Sons Inc. (ISBN 0-471-21472-8)

Web links:

1. Online Chemistry Lab - http://onlinelabs.in/chemistry

2. Online resources for teaching and learning chemistry - http://chemcollective.org/vlabs

3. Buffers - http://www.dnatube.com/video/372/Maintenance-of-pH-Buffer

4. Online Labs - http://www.olabs.edu.in/

SUMMARY

Cell is the basic structural and functional unit of all living organism. Two Major classes of cells are Prokaryotic and eukaryotic cells. Homeostasis is an important function of all cells. It is the ability to maintain constant internal environment by controlling the movement of ions and water across the plasma membrane. All plants, animal cell, prokaryotic cells and fungal cells are bounded by a cell membrane known as plasma membrane. Cell wall is a non-living rigid structure that forms an outer covering for the plasma membrane of fungi and plants. Nucleus is the largest organelle in the cell which is

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enveloped by bound double layered nuclear membrane called chromatin. Cell has a store convert for compartment for energy production. This is called mitochondria- the power houses of the cell. Endoplasmic reticulum or ER are the several interrelated membrane-bound compartments in eukaryotic cells.

Ribosomes are the granular structures composed of ribonucleic acid and proteins. Cytosol or cytoplasm is the ground substance that fills the interior of the cell. Plastids are found in all plant cells and in euglenoides. The ability to use light for sugar synthesis from water and carbondioxide is a special feature of plant cells. This process is termed as Photosyntheis is carried out in organelles called Chloroplasts. Vacuoles is the membrane bound space found in the Cytoplasm. According to Lowry Bronsted theory. Acid is a proton-donor and a base is a proton acceptor. The acidic or basic nature of a solution is measured by H^+ ion Concentration

$pH = -\log[H^+]$

Henderson-Hasselbalch equation $pH = pK_a + \log \frac{[\text{Conjugatebase}]}{[\text{Acid}]}$

A pH meter used to measure pH of asolution.

Body fluids are water solution of dissolved substances. Two types are intra cellular fluids and extracellular fluids. The colourless fluid present in the lymphatic system is called lymph. Blood is a fluid that circulates in a closed system of blood vessels in multi cellular and highly complex vertebrate animals.

Various buffer system of the blood are.

a. Bicarbonate buffer system, b. Phosphate buffer system, c. Protein buffer system and d. Hemoglobin buffer system

The body's acid base balance is maintained by buffer systems that operates in living system (lungs and kidneys).

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CONCEPT MAP

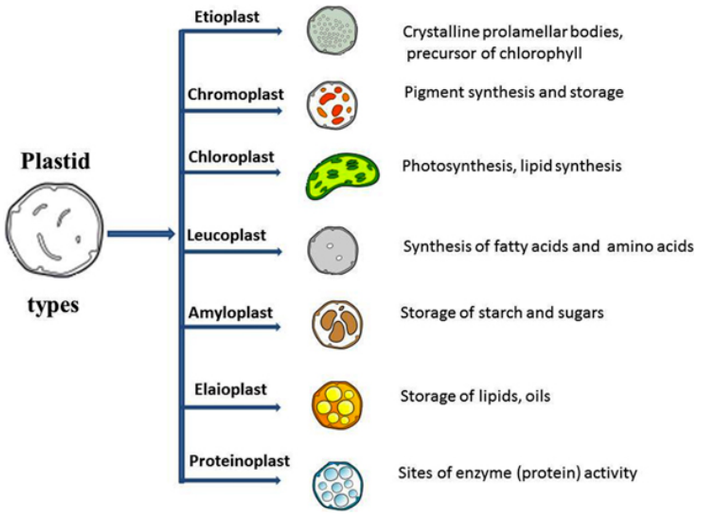
Cell

Types

1. Prokaryotic cells
2. Eukaryotic cells

Sub cellular Organelles

1. Cell membrane
2. Cell wall
3. Nucleus
4. Mitochondria
5. Endoplasmic Reticulum (ER)
6. Golgi apparatus
7. Ribosomes
8. Lysosomes
9. Peroxisomes
10. Cytoplasm
11. Plastids
12. Chloroplasts
13. Vacuole



Acids and Bases

pH and Buffer systems → Henderson Hassel Balch equation

Biological fluids

Regulation of acid base balance

Lungs
1. Bicarbonate buffer
2. Hemoglobin buffer

Kidneys
1. Phosphate buffer
2. Ammonium buffer
3. Bicarbonate buffer

Types

ECF

Plasma

Blood
1. Bicarbonate buffer
2. Protein buffer
3. Hemoglobin buffer

Interstitial Fluids
1. Lymph
2. CSF

ICF

Intra cellular fluids
1. Protein buffer
2. Phosphate buffer

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ICT Corner

Buffers and pH

By using this tool you can simulate the preparation of a buffer and measure its pH values

Please go to the URL
http://pages.uoregon.edu/tgreenbo/pHbuffer20.html
(or)
Scan the QR code on the right side

I1LRC

Step – 1

Open the Browser and type the URL given (or) Scan the QR Code. You can see a webpage as shown in the figure.

Step – 2

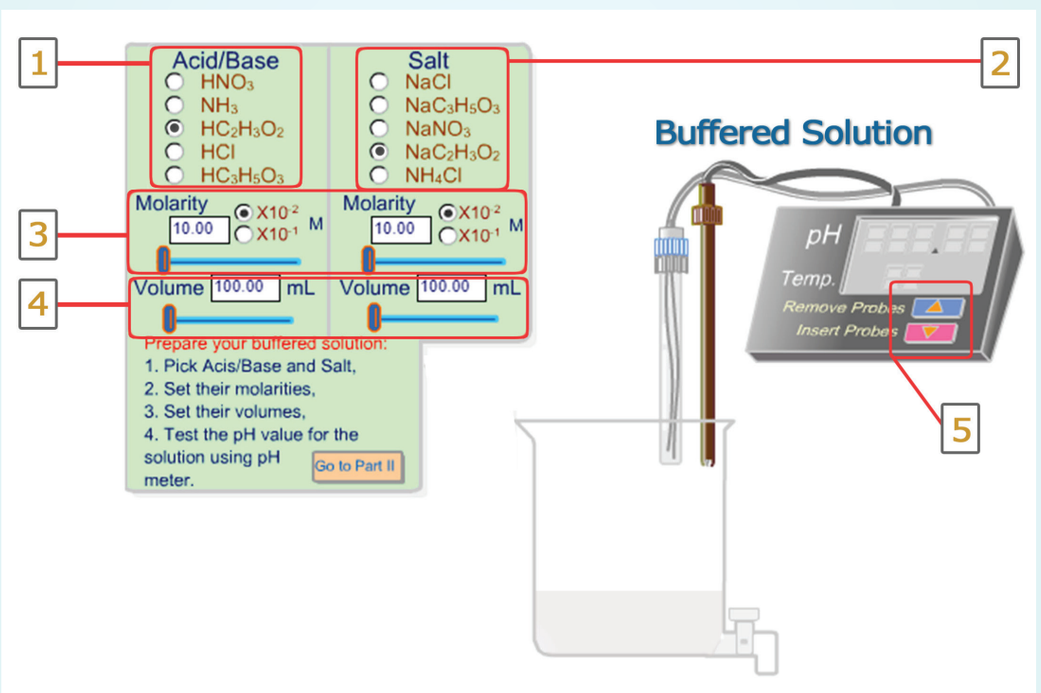
Now you can select a combination of an acid/base (Box 1) and its corresponding salt (Box 2) from the given choices and also select the desired concentrations (Box 3) and volume (Box 4) of these for the buffer.

Step – 3

In order to measure the pH of the made-up buffer click the 'Insert Probe' (Box 5) on the pH meter. Now the pH meter shows the pH. After measuring you need to remove the probe by clicking 'Remove Probe" (Box 5) to make any changes in the composition.

Step – 4

Now you can vary the concentration and volume of the components and see how the pH changes.



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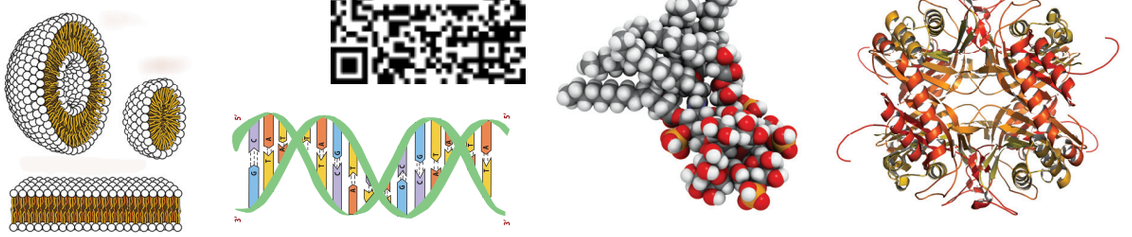
Unit 2 BIOMOLECULES





Friedrich Wöhler

Friedrich Wöhler was a German chemist best known for his synthesis of urea from ammonium cyanate in 1828 (Wohler synthesis), which was the first synthesis of an organic chemical compound from an inorganic one; it contributed to the foundation of modern organic chemistry.

Learning Objectives

After studying this unit, students will be able to

- Classify the different biomolecules.
- Describe the importance of carbohydrates.
- Distinguish monosaccharides, oligo saccharides and polysaccharides.
- Classify various types of proteins.
- Explain the functions of proteins.
- Classify various types of lipids.
- Explain the biological functions of lipids.
- Describe the biological functions of nucleic acids.
- Differentiate between DNA and RNA.

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Introduction:

All the major components of the cells are made up of four types of macromolecules namely carbohydrates, proteins, lipids and nucleic acids. These molecules are called biomolecules. In this unit, we will see the overview of these biomolecules. Detailed information about these molecules are discussed in the subsequent chapters

2.1 Carbohydrates

Carbohydrates, otherwise known as saccharides (derived from the Greek Sakcharon– sugar; many simple sugars taste sweet) are one of the abundant molecules in earth. They are essential to maintain life in both plants and animals. They are synthesised in plants by photosynthesis.

$$x\,CO_2 + y\,H_2O \xrightarrow{sunlight} C_x(H_2O)_y + xO_2$$

The compounds that we come across in our daily life such as table sugar, wood, cotton, starch, and honey etc... are all carbohydrates.

2.1.1 Importance

Carbohydrates are widely distributed in both plant and animal tissues. They occur as food reserves in the storage organs of plants and animals. They are the important source of energy which is required for the various metabolic activities of living organisms.

They provide raw material for many important industries including textiles, artificial silks, paper, films, plastics, lacquers, confectionary, drugs, fermentation and explosives.

2.1.2 Definition

Carbohydrates are defined as poly-hydroxy aldehydes or ketones. They contain hydrogen and oxygen in the same ratio as in water (2:1)

The names of most of carbohydrates are characterised by the ending '-ose'. For example glucose, fructose, sucrose, cellulose, etc.

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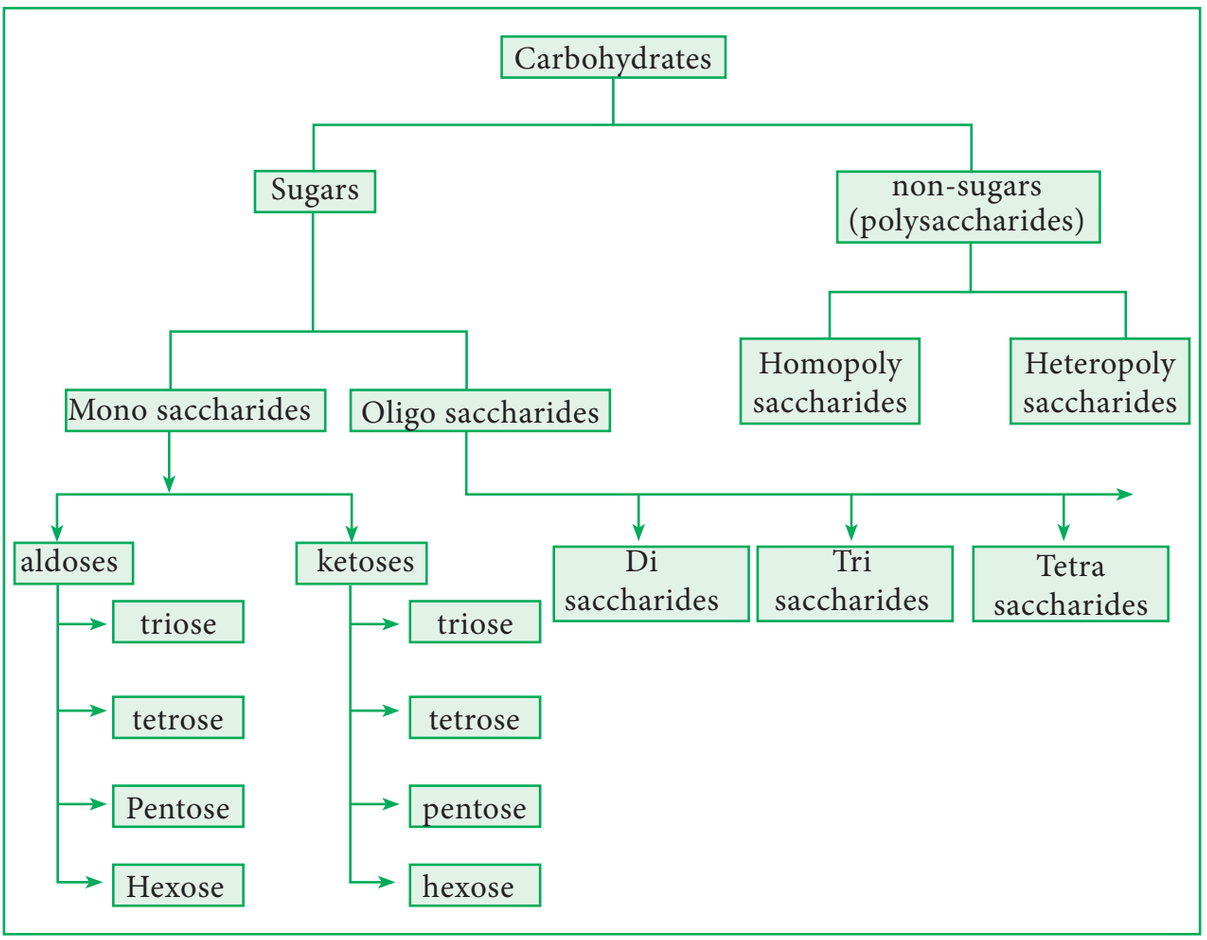
2.1.3 Classification

Carbohydrates are generally classified as Sugars and Non-sugars.



Sugars

Sugars are sweet, crystalline and soluble in water. They are classified further as below.

2.1.4 Monosaccharides

Mono saccharides have a general formula $C_n(H_2O)_n$. Based on the number of carbon atoms they are further classified into trioses, tetroses, pentoses, hexoses etc. They can also be classified as aldoses and ketoses based on the functional group present in them. They cannot be hydrolysed into simpler units. This can be further classified as aldoses and ketoses based on the functional group present in C1 position.

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Aldoses:

Aldoses contain aldehyde group (-CHO) as a functional group along with two or more hydroxyl groups. Examples: glyceraldehyde, ribose, glucose galactose

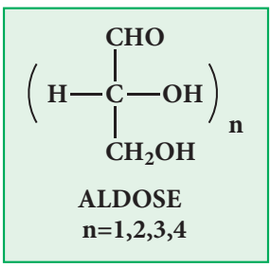

Figure 2.1 General structure of aldoses

Ketoses:

Ketoses contain keto group (>C=O) as a functional group along with two or more hydroxyl groups.

Examples: dihydroxy acetone, ribulose, fructose.

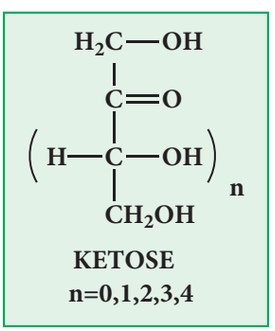

Figure 2.2 General structure of ketoses

2.1.5 Oligosaccharides

Oligosaccharides are sugars that yield 2 to 10 monosaccharide molecules on hydrolysis. This can be further classified as di, tri, tetra saccharides etc. based on the number of monosaccharide units present. In these molecules monosaccharide units are interlinked by glyosidic bridges

Disaccharides

Disaccharides have a general formula C_n $(H_2O)_{n-1}$Example: sucrose, lactose and maltose. In these molecules monosaccharide units are inter linked by a glyosidic bridges.

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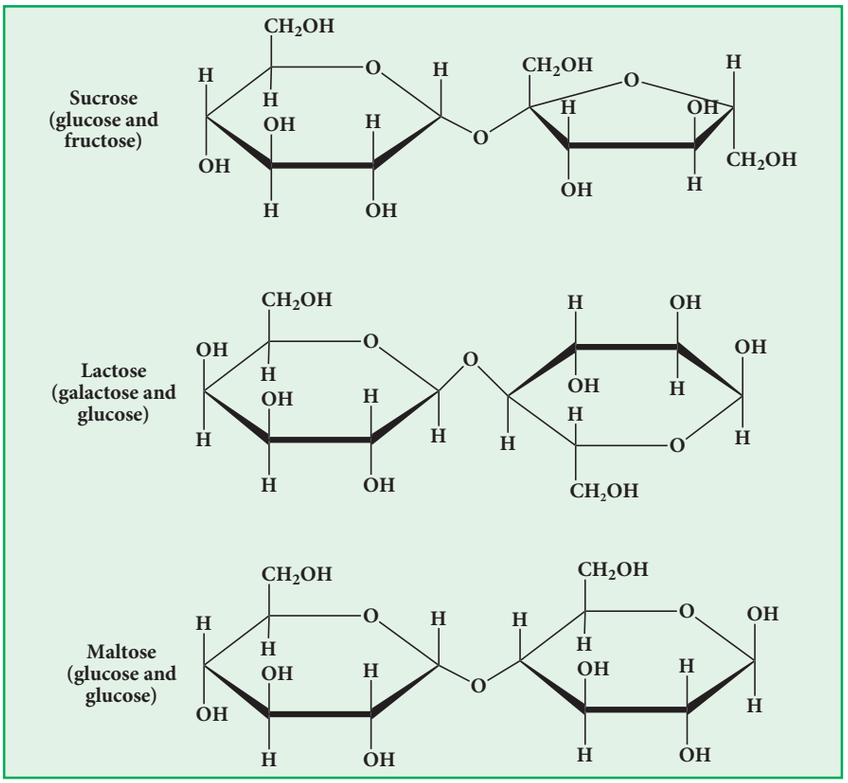

Figure 2.3 Structure of common disaccharides

Hydrolysis of disaccharides in presence of mineral acid yields corresponding monosaccharides. For example hydrolysis of sucrose gives glucose and fructose. Similarly maltose gives two molecules of glucose.

$$C_{12}H_{22}O_{11} + H_2O \xrightarrow{H^+} C_6H_{12}O_6 + C_6H_{12}O_6$$

Sucrose — Glucose — Fructose

$$C_{12}H_{22}O_{11} + H_2O \xrightarrow{H^+} C_6H_{12}O_6 + C_6H_{12}O_6$$

Maltose — Glucose — Glucose

$$C_{12}H_{22}O_{11} + H_2O \xrightarrow{H^+} C_6H_{12}O_6 + C_6H_{12}O_6$$

Lactose — Glucose — Galactose

Figure 2.4 Hydrolysis of disaccharides

Trisaccharides

This will give three monosaccharide units on hydrolysis. These have a general formula as $C_n(H_2O)_{n-2}$ Example: raffinose.

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2.1.6 Polysaccharides (Non Sugars)

Polysaccharides are the carbohydrates that yield more than ten monosaccharide units upon hydrolysis.They are further classified into homopoly saccharides and heteropoly saccharides based on the monomeric units. Example: starch, cellulose, inulin

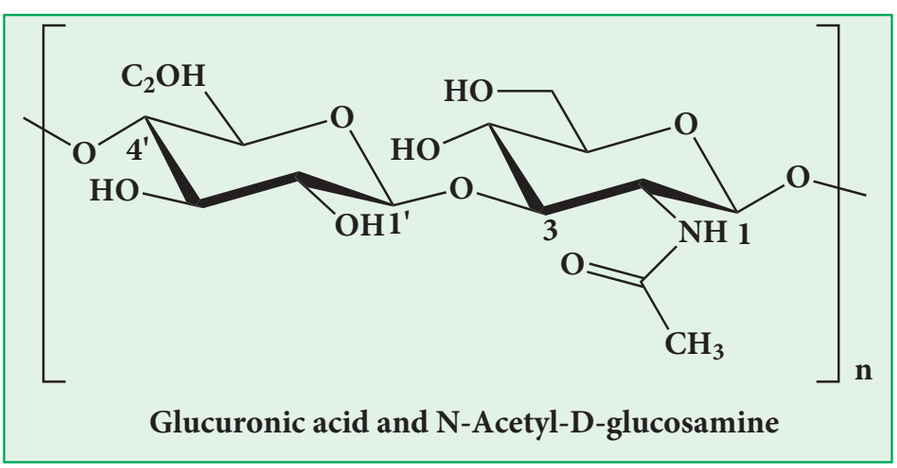
Homopolysaccharides

A homopolysaccharide yields the same type of monosaccharide units on hydrolysis. For example starch a homopolysaccharide yields only glucose upon hydrolysis. Similarly glycogen and cellulose also yield glucose on hydrolysis.

Heteropolysaccharides

A heteropolysaccharide yields more than one type of monosaccharide upon hydrolysis. Eg. Hyaluronic acid, heparin, keratan sulphate and chondroitin sulphate

These are present in extra cellular matrix and therefore they are called as mucopolysaccharides.



Glucuronic acid and N-Acetyl-D-glucosamine

Figure 2.5 Structure of hyaluronic acid

Hyaluronic acid is made up of glucuronic acid and N-acetyl glucosamine.

Starch:

Starch is the major form of stored carbohydrate in plants. Starch is composed of a mixture of two substances namely amylose, a linear polysaccharide and amylopectin, a branched polysaccharide. The detailed study of starch will be discussed in the unit 5.

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Cellulose:

Cellulose is an unbranched polymer of β-D-glucose. Because of the absence of side chains, molecules stay together to form rigid structures in plants. Wood is largely made up of cellulose and cotton is almost pure cellulose.

Cellulose may be modified in the laboratory by treating it with nitric acid to produce nitrocellulose or gun cotton which is an explosive component of smokeless powder. Partially nitrated cellulose, known as pyroxylin, is used in the manufacture of collodion, plastics, lacquers, and nail polish.

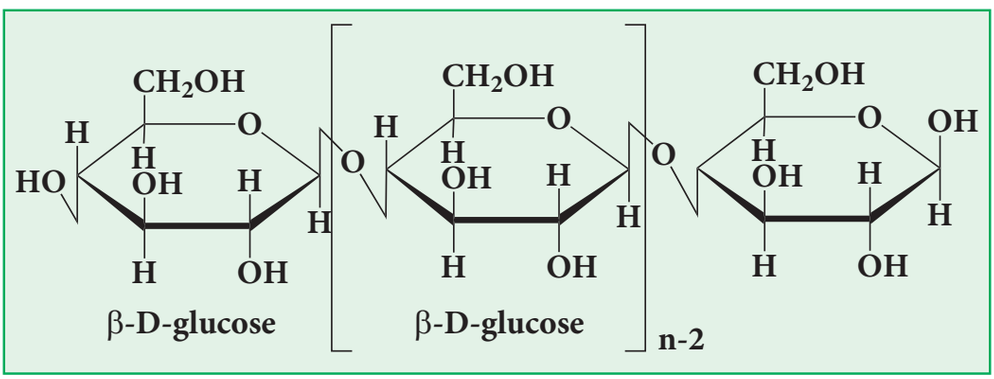

Figure 2.6 Structure of cellulose

Glycogen:

Glycogen is a storage form of glucose which is mainly present in liver and muscle. Glycogen is also known as animal starch. It is a multi branched polysaccharide of glucose. The polysaccharide structure represents the main storage form of glucose in the body.

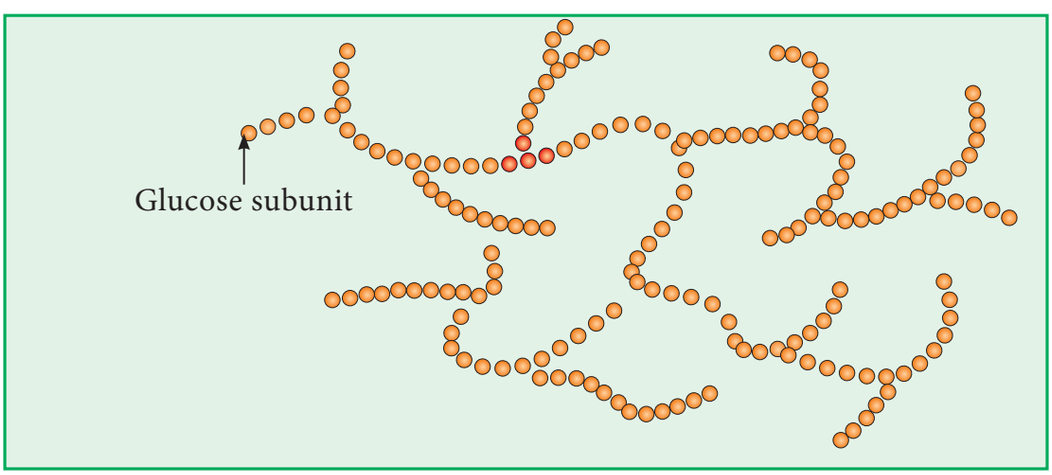

Figure 2.7 Glycogen

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2.2 Proteins

Proteins are the fundamental biological components of skin, hair, muscles, connective tissues, enzymes etc...

2.2.1 Definition

Proteins are defined as the biopolymers composed of α-amino acids linked by peptide bonds. They are also known as polypeptides. Proteins are the major constituents of all living organisms. They contain carbon, hydrogen, nitrogen, oxygen and sulphur.

2.2.2 Classification

Proteins are classified into three main groups.

a. Simple protein:

The protein that yields only α-amino acids on hydrolysis is called a simple protein. Examples: albumin and globulin

b. Conjugated protein:

The protein that yields α-amino acids and a non-protein part upon hydrolysis is called a conjugated protein. The non-protein part is called as the prosthetic group.

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

i Nucleoprotein:

Protein associated with nucleic acids is called as nucleo protein.

Example: Histone(in DNA).

ii. Phosphoprotein:

Protein containing phosphoric acid is called as phosphoprotein.

Example: casein (in milk).

iii. Glycoprotein:

Protein containing carbohydrate moiety is called as glycoprotein.

Example: mucin (in saliva)

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iv. Chromoprotein:

Protein containing heterocyclic compounds like porphyrins is called as chromo protein.

Example: myoglobin (in muscle).

v. Lipoprotein:

Protein conjugated with lipids is called lipoprotein.

Examples: chylomicron (in small intestine)

vi. Metalloprotein:

Protein containing metal ion is called as metalloprotein.

Examples: ceruloplasmin (in blood).

c. Derived protein:

Protein that is derived from simple or conjugated proteins by the action of acids, alkalies or enzyme is called as derived protein. These are partially hydrolysed proteins.

Example: peptones

2.2.3 Functional diversity of Proteins.

Repair and Maintenance:

Protein is termed as the building block of the body. Protein is vital in the maintenance of body tissue, including development and repair.

Hormones:

Some proteins acts as a hormones. Example: Insulin, a small protein, which regulates blood sugar level.

Enzymes:

Most of the enzymes are proteins, they act as biocatalysts in chemical reactions taking place in the body.

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Transportation:

Protein is a major component in transportation of certain molecules. For example, haemoglobin is a protein that transports oxygen throughout the body.

Storage:

Proteins are used to store certain molecules. Ferritin is a protein which stores iron in the liver.

Antibodies:

All antibodies are proteins. Antibodies neutralize infection, illness and diseases.

2.3 Lipids

2.3.1 Definition

Chemically, Lipids can be defined as esters of fatty acids with alcohol. They are insoluble in water and soluble in organic solvents such as alcohol, ether, benzene and chloroform.

2.3.2 Classification

Based on the chemical nature, lipids are classified as

Simple lipids

These are esters of fatty acids with glycerol or long chain alcohols. They are further classified as follows.

(a) Fats: Fats are glyceric esters of fatty acids. Lipids in animals are called as fats while the lipids in plants are called as oils. Example: Triacylglycerol.

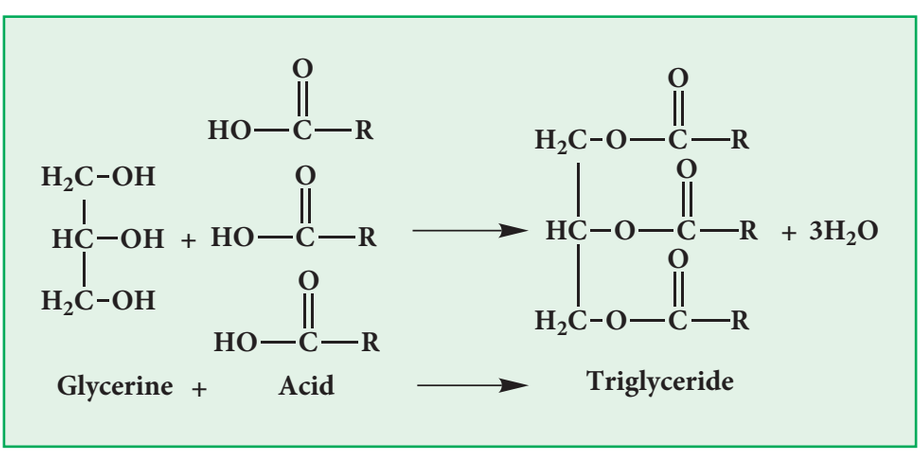

Figure 2.8 Formation of triacylglyceride

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(b) Waxes: Waxes are esters of fatty acids with long chain monohydric alcohols.

Examples: Cerylmyristate (bees wax).



Figure 2.9 Bee's wax

Compound lipids

Compound lipids are esters of fatty acids with alcohol, and they contain extra groups. Depending upon the extra group present they are subdivided as follows:

a. Phospholipids (Phosphatides)

A glyceric ester containing phosphate and nitrogen base or an alcohol are called phospholipids. These lipids are present in large amounts in nerve tissue, brain, liver, kidney, pancreas and heart. Phospholipids are further classified into three types based on the type of group connected to phosphatidyl group.

i. Glycerophosphatides:

In these phospholipids, a nitrogen base is connected with phosphatidyl group.

Examples: Lecithin, Cephalin

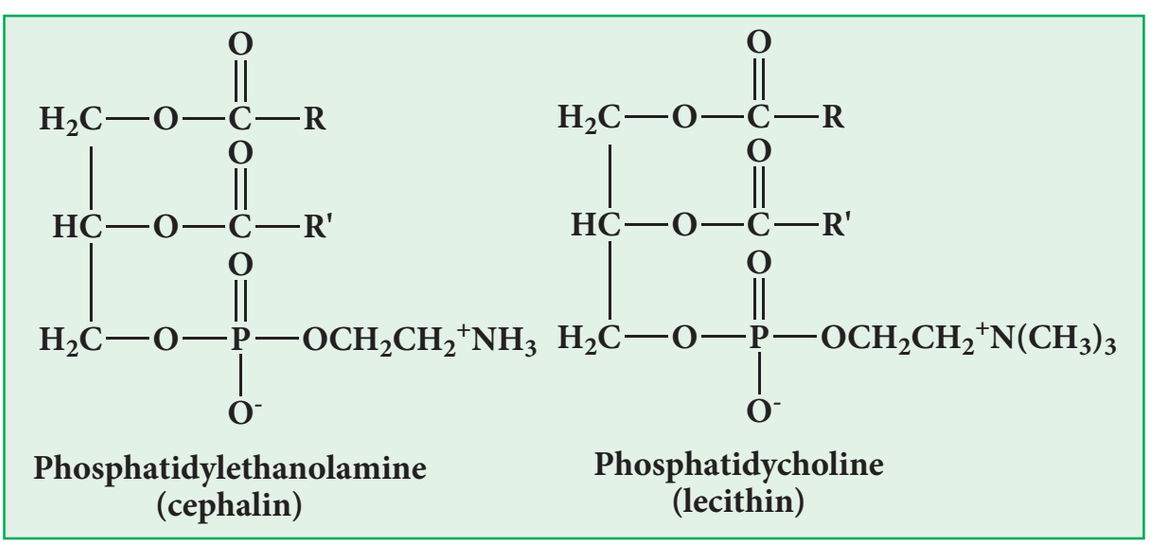

Figure 2.10 Structure of lecithin, cephalin

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ii) Phosphoinositides:

In these phospholipids, inositol is connected with phosphatidyl group. Example: Phosphatidylinositol (lipositol)

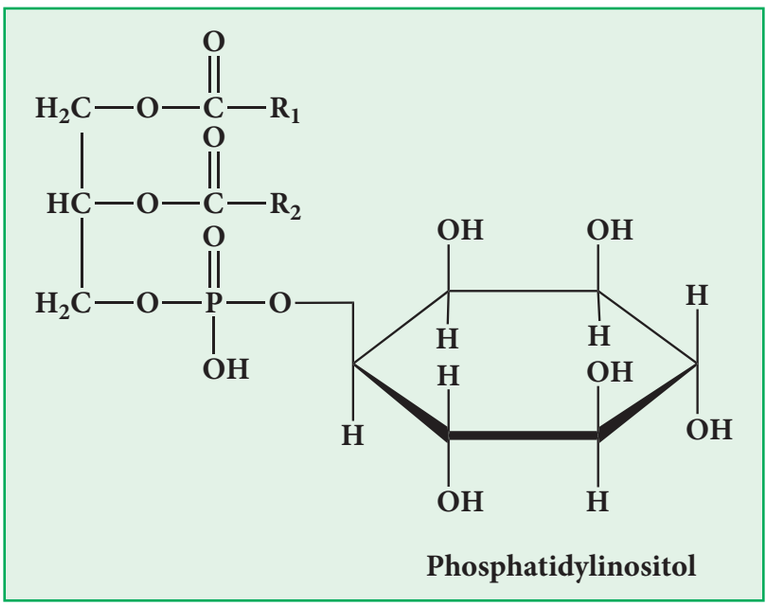

Figure 2.11 Structure of phosphatidylinositol

iii) Phosphosphingoside:

A group of phospholipids containing 1-phosphocholine combined with a ceramide (sphingosine + fatty acid). Example: Sphingomyelin

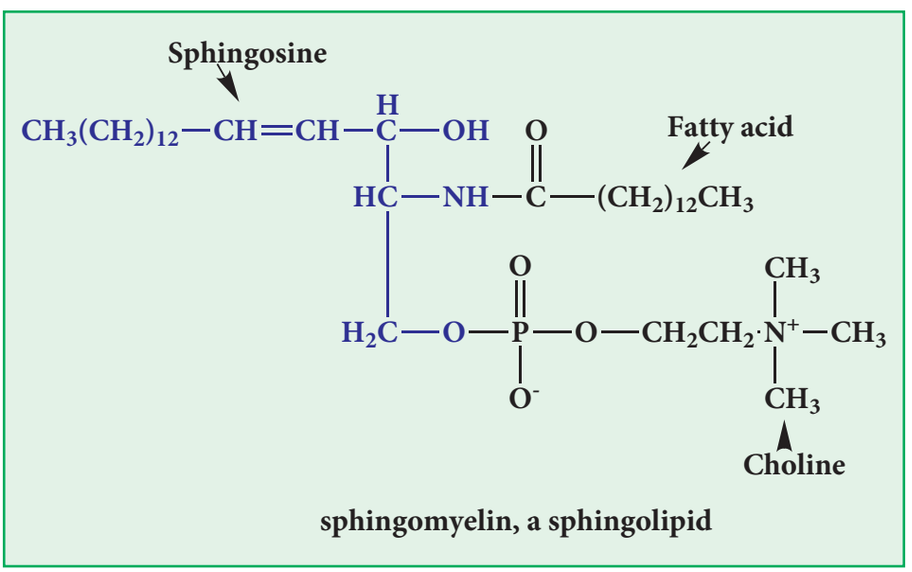

Figure 2.12 Structure of phosphosphingosides

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b. Glycolipids

The lipids which contain a carbohydrate moiety linked with ceramide is called a glycolipid.

Examples : Cerebroside and gangliosides.

c. Lipoproteins

A complex of proteins, lipid and cholesterol is called as a lipoprotein. The protein moiety in the lipoprotein is known as apoprotein.

Examples:

- Chylomicron
- Very low density lipoprotein (VLDL)
- Low density lipoprotein (LDL)
- High density lipoprotein (HDL)

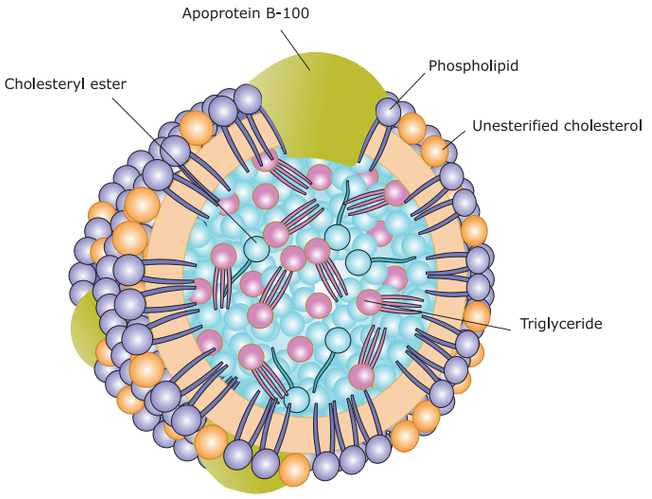

Figure 2.13 Structure of lipoprotein (LDL)

Derived lipids:

Lipids that are derived from the hydrolysis of simple and compound lipids are called derived lipids.

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Examples: diacylglycerol, fatty acids, glycerol and cholesterol.

2.3.3 Functions of lipids:

Lipids perform several biological functions such as,

- Triglycerides serve as energy reserve of the body.
- Lipids are important components of cell membranes which regulates membrane permeability.
- Phospholipids, provide fluidity and flexibility to the cell membranes.
- Lipids act as signalling molecules.
- Fat layer provides insulation from cold.
- Lipoproteins transports lipids throughout the body.

2.4 Nucleic acids

Nucleic acids are biopolymers, essential to all known forms of life.

2.4.1 Definition

Nucleic acids are the polymers of nucleotides. Nucleotides are made of three components:

1. 5-carbon sugar
2. Nitrogenous base
3. Phosphate groups

2.4.2 Structure of nucleic acids:

Sugar unit:

If the sugar unit present in the nucleic acid is a ribose, then the polymer is called ribonucleic acid (RNA) and if the sugar is deoxyribose, then the polymer is deoxyribonucleic acid (DNA).

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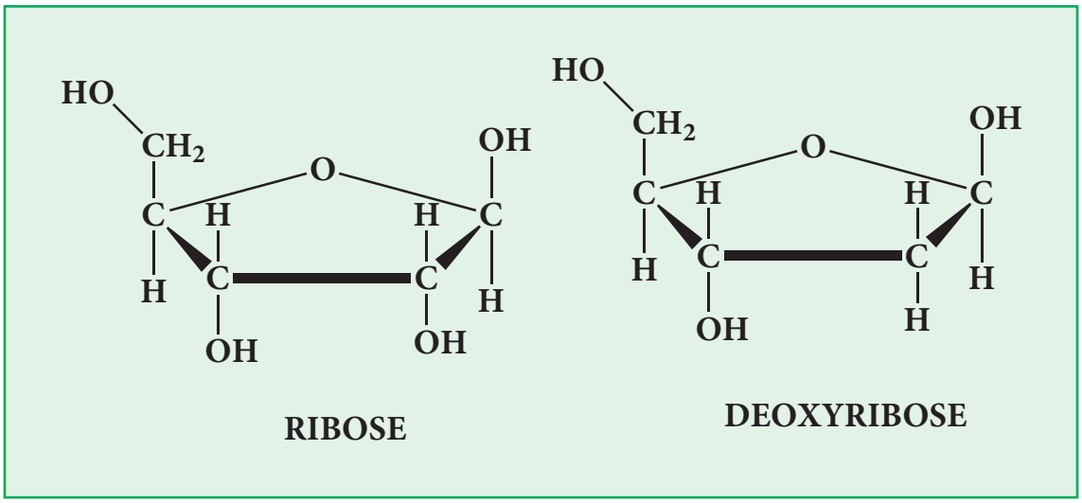

Figure 2.14 Structure of ribose and deoxy ribose

Nitrogen base:

Nucleic acids contain purine and pyrimidine bases. They are Adenine (A), Guanine (G), Cytosine(C), Thymine (T) and uracil(U).

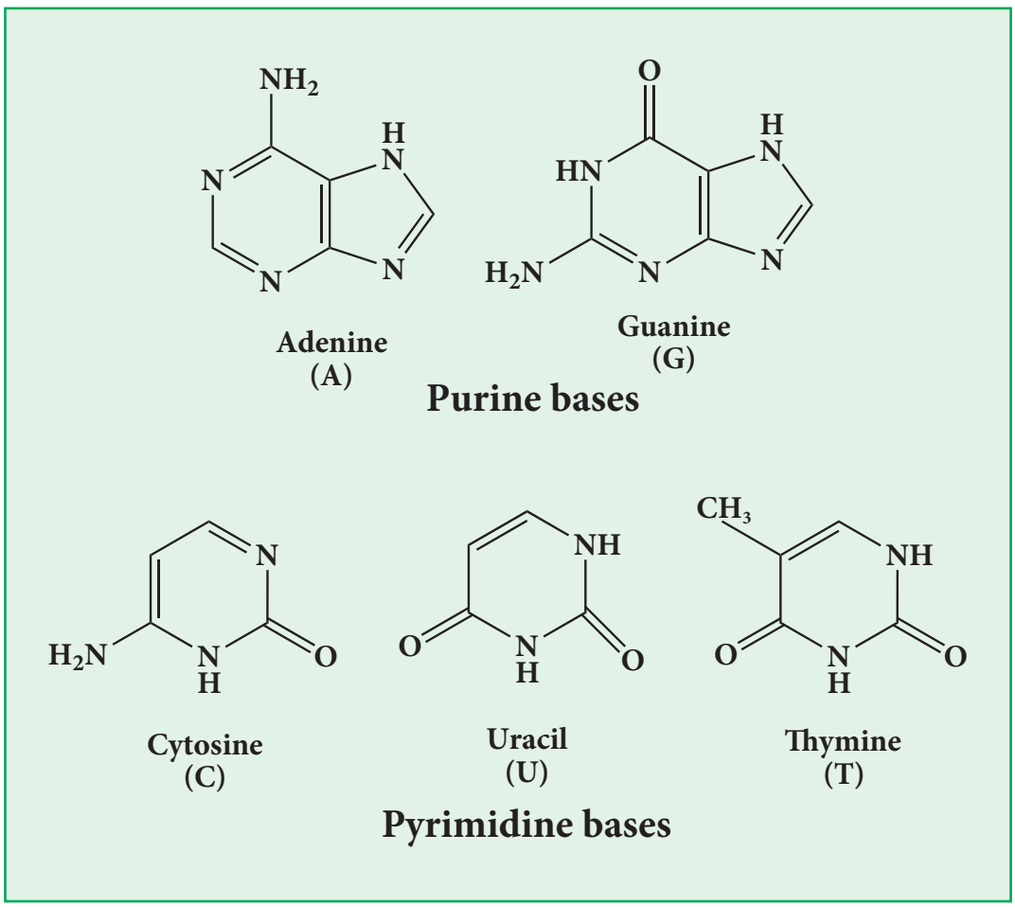

Figure 2.15 Structure of purine and pyrimidine bases

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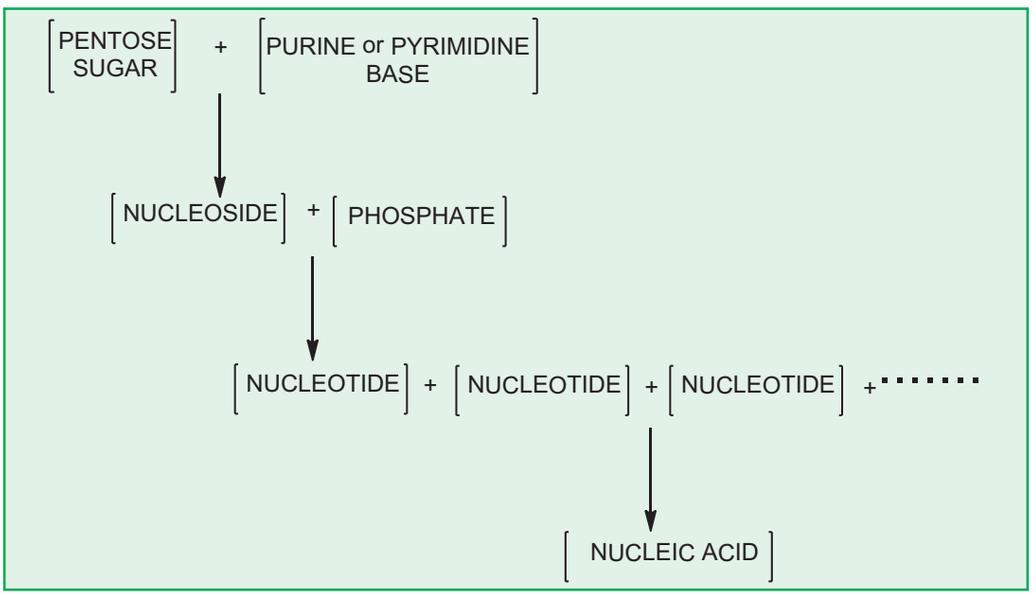
Phosphate group:

In nucleic acids, sugar unit and nitrogenous base can combine to form a nucleoside, these nucleosides combine with a phosphate to form a nucleotide, which in turn polymerises to form nucleic acids.



2.4.3 Classification

Nucleic acids are classified into two types based on the ribose sugar.

a. Deoxyribonucleic acids (DNA)

The most important constituent of chromosome. DNA is a polymer of nucleotides containing 2-deoxyribose sugar and nitrogenous bases like Adenine (A), Guanine (G), Thymine (T) and Cytosine (C).

b.Ribonucleic acids (RNA)

RNA is a polymer of nucleotides containing ribose sugar and nitrogenous bases like Adenine (A), Guanine (G), Uracil (U), and Cytosine (C).

There are three main classes of RNA molecules, they are

i. Messenger RNA (mRNA). ii. Transfer RNA (tRNA) iii. Ribosomal RNA (rRNA)

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2.4.4 Functions of DNA and RNA:

- The main function of nucleic acids is to store and transfer genetic information.
- DNA controls the synthesis of RNA in the cell.
- DNA transmits the genetic information to mRNA for the synthesis a specific protein.
- RNA directs synthesis of proteins.
- mRNA takes genetic message from DNA.
- tRNA transfers activated amino acid, to the site of protein synthesis.
- rRNA are mostly present in the ribosomes, and responsible for stability of mRNA.

EVALUATION



I. Choose the correct answer:

1. Glucose is a

   a. Monosaccharide b.Disaccharide c. Oligosaccharide

   d. Polysaccharide

2. An example of a heteropolysaccharide is

   a. Hyaluronic acid b. Cellulose c. Mannose

   d. Starch

3. Chylomicron belongs to the group of

   a. Metalloprotein b. Chromoprotein c. Lipoprotein

   d. Nucleoprotein

4. Long chain alcohols are present in

   a. Waxes b. Fats c. Oils

   d. Phospholipids

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5. A Phosphoprotein present in milk is

a. histone b. casein c. mucin d. insulin

6. The metal present in Ceruloplasmin is

a. Fe b. Ca c. Cu d. Mg

7. The differential base present in DNA and RNA is

a. Adenine b. Guanine c. Cytosine d. Uracil

8. Which of the following is ketohexose

a. Glucose b. Fructose c. Ribose d. Galactose

9. Sucrose is a

a. Disaccharide b. Monosaccharide c. Trisaccharide d. Tetra saccharide

10. Lactose is made up

a. Glucose & mannose b. Fructose & glucose

c. Ribose & ribulose d. Glucose & galactose

11. Which of the following is known as animal starch

a. Glycogen b. Amylose

c. Cellulose d. Amylopectin

12. rRNA is present in

a. Nucleus b. Plasma membrane

c. Ribosome d. Nuclear membrane

13. Phosphatidyl choline is

a. Cephalin b. Lecithin

c. Ceramide d. Myristate

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14. Which of the following nitrogenous base is not found in DNA

a. Adenine b. Thymine

c. Guanine d. Uracil

15. Sugar present in RNA is

a. Ribulose b. 2-Deoxy ribose

c. Ribose d. Glucose

16. Peptide bond is present in

a. Carbohydrates b. Proteins

c. Lipids d. Nucleic acids

17. Partially hydrolyzed proteins are

a. Peptides b. Poly peptides

c. Peptones d. Simple proteins

II. Give answer for the followings:

1. Write down the hydrolysis reaction of sucrose?
2. Give note on glycogen.
3. What are the basic differences between starch and cellulose?
4. Write note on chromoprotein.
5. What are glycolipids?
6. Give a short note on derived lipids.
7. What are nucleosides?
8. What are three types of RNA?
9. What are hetero polysaccharides? Give an example.
10. What are homo polysaccharides?
11. Give the structure of glucose.
12. Write note on phosphoprotein.
13. Write the equation for triacyl glyceride formation?

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14. Explain the lipoproteins.

15. Give structure for lecithin and cephalin?

16. Give the classification of carbohydrates with examples.

17. Explain the functional diversity of proteins

18. Explain the biological functions of lipids.

19. Give an account on classification of lipids.

20. Explain the functions of DNA and RNA.

SUMMARY

Biomolecules are molecules that are present in all living organisms. It includes the macromolecules carbohydrates, proteins, lipids and nucleic acids. These are the four major classes of biomolecules

Carbohydrates

Carbohydrates are essential to maintain the life in both plants and animals. They are synthesized in plants by photosynthesis. Carbohydrates are good source of energy. Monosaccharides are simple sugars. They have a free aldehyde or keto group. Disaccharides are made up of two monosaccharides connected by a glycosidic bond. Polysaccharides are the carbohydrates that yield more than ten monosaccharides units upon hydrolysis. They are further classified into homo polysaccharides and hetero polysaccharides based on the monomeric units. They are non-sugars and complex carbohydrates.

Proteins

Proteins are polymers of α-amino acids. These α-amino acids are joined together by the peptide bond which is formed in between the carboxyl group and amino group of successive amino acids. Proteins are formed from 20 different amino acids.

Proteins are classified into three types namely simple proteins, conjugated proteins and derived proteins. Simple protein yields only α-amino acids on hydrolysis. Conjugated protein yields α-amino acids and a non-protein part upon hydrolysis. The non-protein part is called the prosthetic group. Derived protein is formed by the action of acids, alkalis or enzymes on simple or conjugated proteins. These are partially hydrolyzed proteins.

Lipids

Lipids are generally esters of fatty acids and are building blocks of biological membranes. Lipid molecules hold a large amount of energy and are energy storage

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molecules. Based on the chemical nature, lipids are classified as simple lipids, compound lipids and derived lipids. Simple lipids are esters of fatty acids with glycerol or long chain alcohols. Compound lipids are esters of fatty acids with alcohol and they contain extra group. Derived lipids are formed by the hydrolysis of simple and compound lipids.

Nucleic Acids

Nucleic acids are made of polymer of nucleotides. Nucleotides consists of nitrogenous base, a pentose sugar and a phosphate group. Polymerized nucleotides form DNA and RNA which are genetic material.

DNA: DNA is a polymer of nucleotides containing 2-deoxyribose sugar and nitrogen bases like Adenine (A), Guanine (G), Thymine (T) and Cytosine(C).

RNA: RNA is a polymer of nucleotides containing ribose sugar and nitrogen bases like Adenine (A), Guanine (G), Uracil (U), and Cytosine(C).

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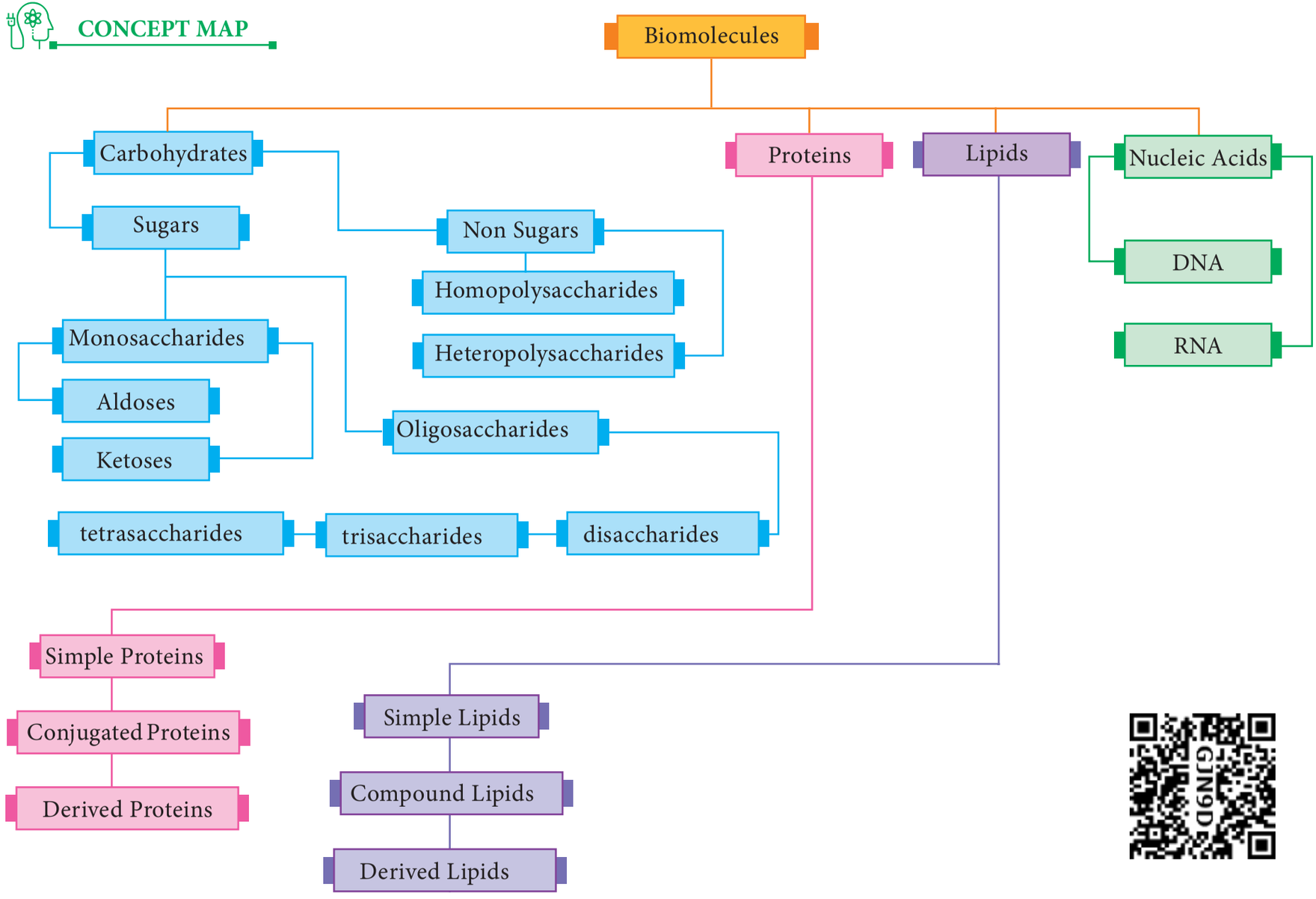
CONCEPT MAP



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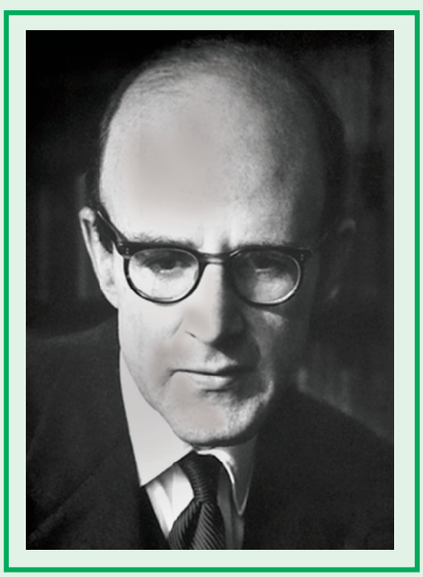
Unit 3 PROTEINS



Max Perutz

An Austria born British molecular biologist, who first discovered the three dimensional structure of the protein haemoglobin. He was awarded nobel prize for his work in 1962.

Scientific career Fields

Molecular biology
Crystallography

Awards

Nobel Prize for Chemistry (1962)
Wilhelm Exner Medal (1967)
Sir Hans Krebs Medal (1968)
Royal Medal (1971)
Copley Medal (1979)

Learning Objectives

After studying this unit the students will be able to

- Summarize the different physiological and cellular roles of proteins.
- Identify the different dietary sources of proteins
- Describe the relation between amino acids and proteins
- Describe the structure and properties of amino acids
- Identify the pK_a and pI values of amino acids when provided with a titration curve
- Explain the formation and properties of a peptide bond
- Describe the different hierarchical levels of the three dimensional structure of a protein
- Provide structure function relationships for hemoglobin and collagen
- Describe the process protein denaturation
- Relate different diseases with protein deficiency, malfunction and misfolding.

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Introduction

Proteins are the most abundant and diverse organic molecules present in living organisms. The physiological process that takes place in an organism is mediated or assisted by proteins. The name protein has its origin from the Greek word *Proteos*, which could be translated to 'primary' or 'holding first place'.

A single cell contains thousands of proteins, each with a unique structure and function. Though their structures and functions vary, all proteins are made up of a fundamental building block called amino acids. The proteins are made of one or more chains of covalently linked amino acids through peptide bonds.

3.1 Dietary sources of proteins

Proteins can be obtained from both animal and plant sources. The animal sources include milk, meat, fish, liver, egg etc. The plant sources of proteins are pulses, nuts and cereals (Fig 3.1). Proteins taken in our routine diet are broken down to amino acids (building block) during the process of digestion in stomach. These amino acids are absorbed by the body and used as building blocks to synthesize new proteins once again which depends on the specific physiological requirement of a living organism.



Figure 3.1 Dietary sources of proteins

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3.2 Amino acids

There are more than 300 amino acids found in nature. However, only 20 of them are present in proteins. Each amino acid has three distinct groups

a. A primary amino group (Except for the amino acid proline, which has a secondary amino or imino group)

b. A carboxylic acid group and

c. A distinct side chain or R group.

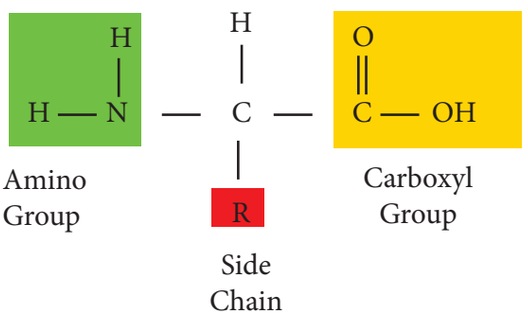

Figure 3.2 Chemical structure of an amino acid

The chemical nature of the side chain in each amino acid determines the property of that amino acid and the role it plays in a protein structure. Considering this important role of the side chain, amino acids are classified into different groups based on their side chain.

3.2.1 Amino acids with non polar side chains

These amino acids have non polar groups as their side chains and are shown in Table 3.1. These non polar side chains are similar to lipids and tend to clump together in an aqueous environment by means of a special type of interaction called hydrophobic interaction. Hence, these amino acids are also called hydrophobic amino acids. These side chain groups (are less likely to lose or gain protons) form hydrogen bond or ionic bonds.

3.2.2 Amino acids with uncharged polar side chains

The amino acids serine, threonine, tyrosine, cysteine, asparagine and glutamine have a net zero charge at neutral pH. However at alkaline pH the side chain of cysteine and tyrosine can lose a proton. The polar hydroxyl group (-OH) present in the side chains of serine, threonine and tyrosine can form a hydrogen bond. Similarly, the cysteine side chain can form disulphide bonds. The details of hydrogen bond and disulphide bonds along with their role in protein structure and stability will be explained in a later section of this unit.

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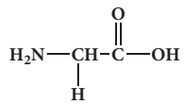
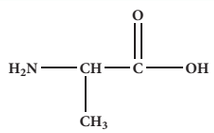
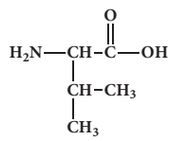
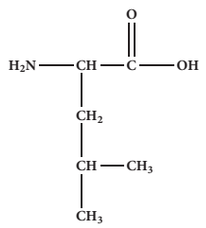
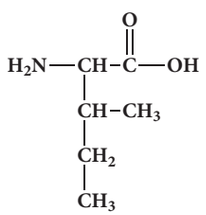
3.2.3 Amino acids with basic side chains

At physiologic pH the amino acids arginine and lysine are positively charged due to the ionization of their side chains. The side chain of the amino acid histidine acts as a weak base and in proteins histidine is either neutral or basic depending on the chemical environment.

Table 3.1 Properties of Amino acids

S. NO	Amino Acid name	Three letter code	One letter code	Structure of Amino acid	Type
1.	Glycine	GLY	G	 Glycine (Gly)	Non-polar
2.	Alanine	ALA	A	 Alanine (Ala)	Non-polar, Hydrophobic
3.	Valine	VAL	V	 Valine (Val)	Non-polar, Hydrophobic
4.	Leucine	LEU	L	 Leucine (Leu)	Non-polar, Hydrophobic
5.	Isoleucine	ILE	I	 Isoleucine (Ile)	Non-polar, Hydrophobic

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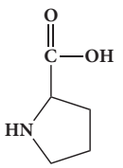
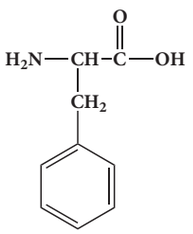
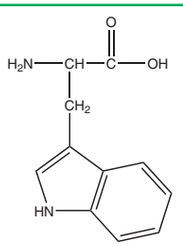
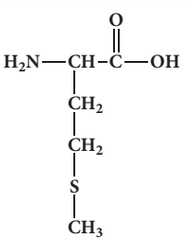
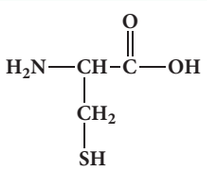
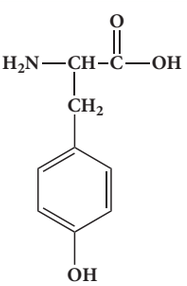
6.	Proline	PRO	P	 Proline (Pro)	Non-polar, Imino acid
7.	Phenylal-anine	PHE	F	 Phenylalanine (Phe)	Non-polar, Aromatic
8.	Trypto-phan	TRP	W	 Tryptophan(Trp)	Non-polar, Aromatic
9.	Methi-onine	MET	M	 Methionine (Met)	Sulfur containing
10.	Cysteine	CYS	C	 Cysteine (Cys)	Sulfur containing
11.	Tyrosine	TYR	Y	 Tyrosine (Tyr)	Polar, Aromatic

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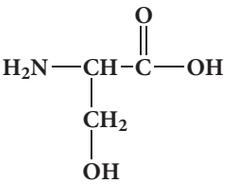
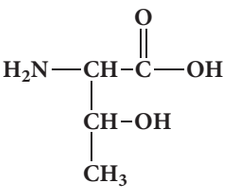
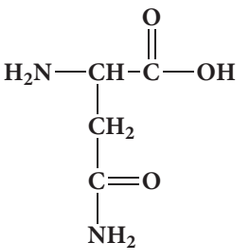
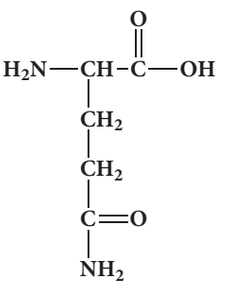
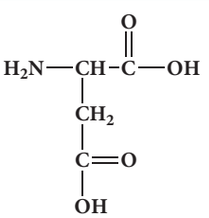
12.	Serine	SER	S	 **Serine (Ser)**	Polar, -OH containing
13.	Thre-onine	THR	T	 **Threonine (Thr)**	Polar, -OH containing
14.	Asparag-ine	ASN	N	 **Asparagine (Asn)**	Polar, Amide containing
15.	Gluta-mine	GLN	Q	 **Glutamine (Gln)**	Polar, Amide containing
16.	Aspartic Acid	ASP	D	 **Aspartic acid (Asp)**	Polar, Acidic

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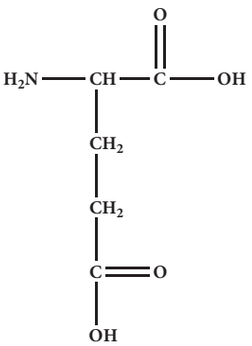
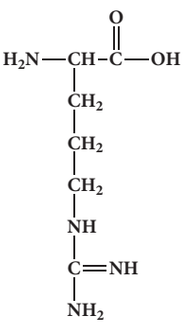
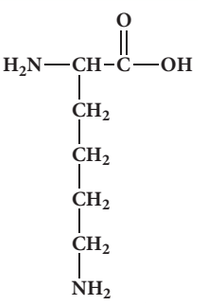
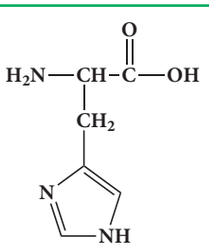
17.	Glutamic Acid	GLU	E	 Glutamic acid (Glu)	Polar, Acidic
18.	Arginine	ARG	R	 Arginine (Arg)	Polar, Basic
19.	Lysine	LYS	K	 Lysine (Lys)	Polar, Basic
20.	Histidine	HIS	H	 Histidine (His)	Polar, Basic

3.2.4 Stereo isomerism in aminoacids

All amino acids except glycine have at least one asymmetric carbon atom and hence they exist as stereo isomers and are normally represented as D and L isomers. Proteins are always made up of L amino acids. However D amino acids can be found in antibiotics, bacterial cell walls etc.

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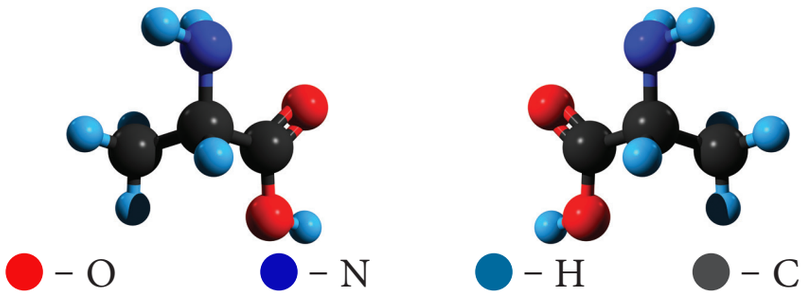

Figure 3.3 D and L Alanine

3.2.5 Acid – base properties of amino acids

Amino acids have both carboxylic acid and amino groups. Similarly, the side chains of acidic and basic amino acids contain additional ionisable groups. Recall that acids are proton donors, where as bases are proton acceptors and the quantitative relationship between pH of a solution and the concentration of the weak acid and its conjugate base is given by Henderson-Hasselbalch equation.

The dissociation of the acidic and basic groups can be explained with alanine as an example. The three different ionic forms of alanine in acidic, neutral and basic solutions is explained in Figure 3.4

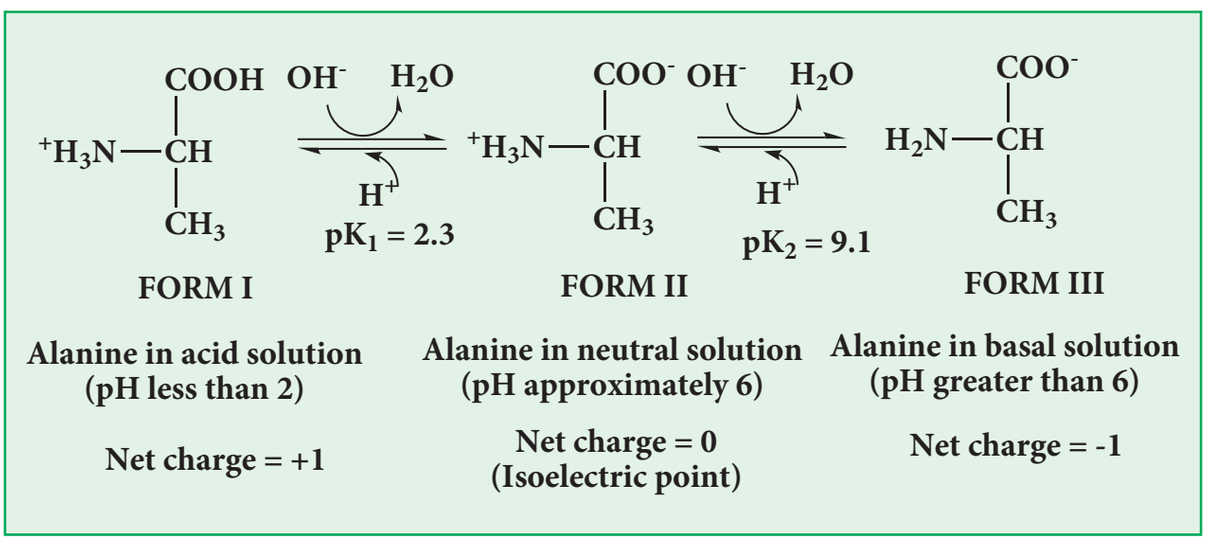

Figure 3.4 ionic forms of alanine

Note that at acidic pH both the amino and carboxylic acid groups are protonated (Form I) and as the pH increases the –COOH group dissociates to become Form II. At basic pH the $-NH_3^+$ group can dissociate to become Form III.

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The conversion among the three different forms is heavily influenced by pH. In acidic solution the amino acid behaves like a protonated derivative and moves towards cathode under an electric field. In an alkaline medium the same amino acid behaves like an anion and moves towards anode.

At a particular pH, the net charge of an amino acid becomes neutral (Form II) and such ionic forms are known as zwitter ions and the corresponding pH is known as its iso electric point (pI). The titration curve of alanine is given in figure 3.5 Note that since there are two ionisable groups in alanine, it has two pKa values denoted as pKa_1 which is equal to 2.3 and pKa_2 which is equal to 9.1. The iso electric point is denoted as pI and for alanine it is 5.7.

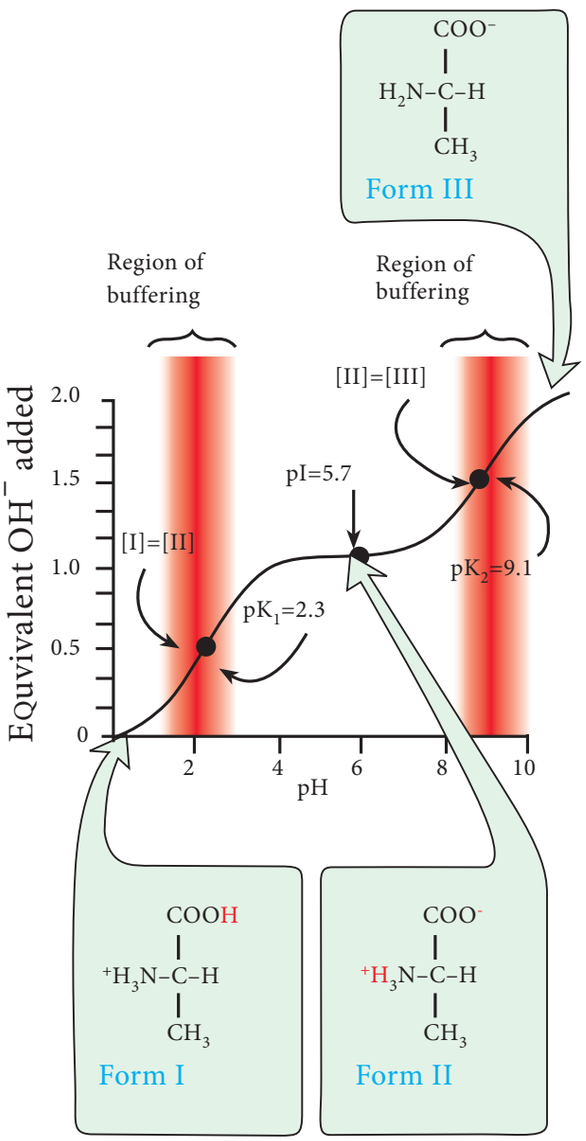

Figure 3.5: The titration curve of alanine

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3.2.6 Reaction with ninhydrin

Ninhydrin oxidatively decarboxylates an amino acid to CO_2 and an aldehyde. The reduced ninhydrin subsequently reacts with another molecule of ninhydrin to form a purple complex which absorbs light at 570 nm

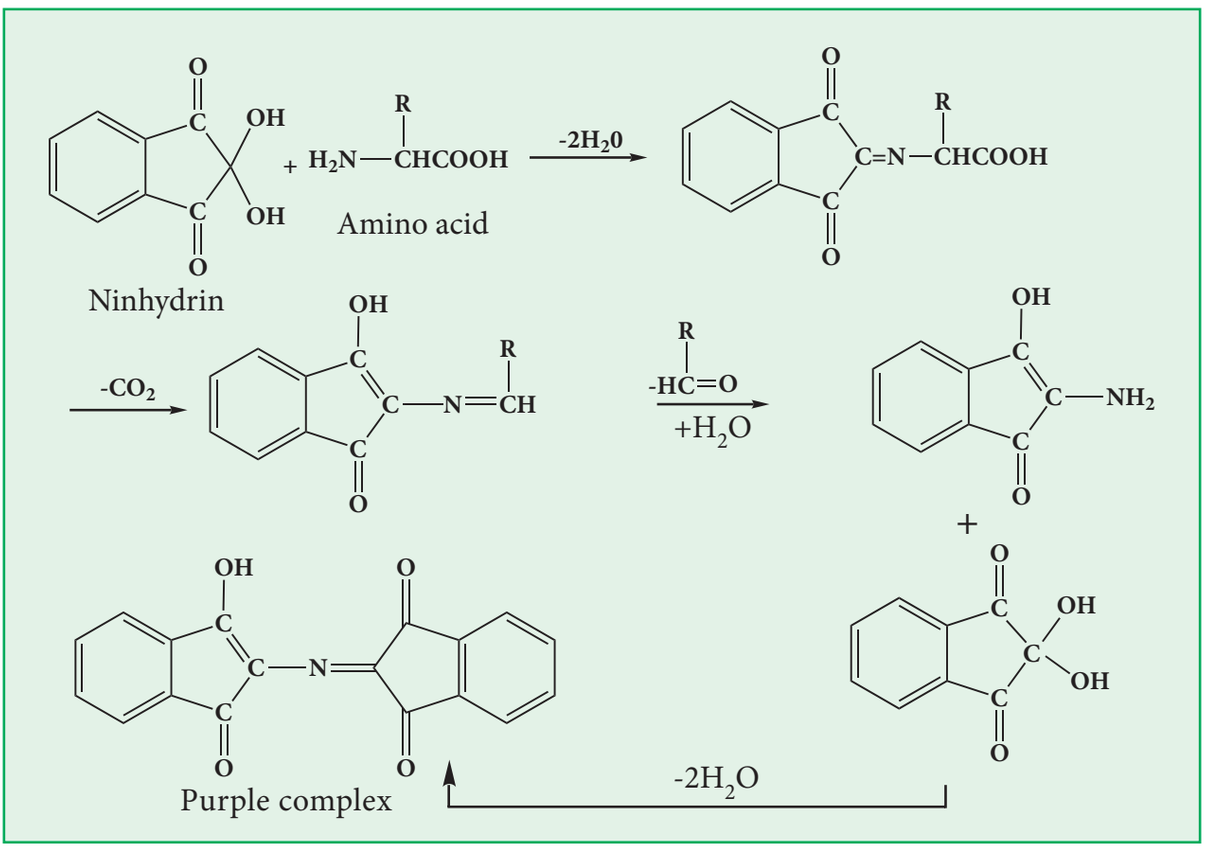

Figure 3.6 Reaction with ninhydrin

3.2.7 Essential amino acids

Certain amino acids cannot be synthesised by our body. Such amino acids must be compulsorily included in our diet for a healthy life. Such amino acids are called essential amino acids. For humans 10 amino acids are essential, that includes arginine, methionine, histidine, phenylalanine, valine,leucine, isoleucine, lysine, threonine and tryptophan (MATT VILL PHLY).

3.3 Proteins and their structure

Proteins are made up of the 20 different amino acids. These amino acids are joined together by a covalent linkage commonly known as a peptide bond. The linear sequence of these linked amino acids is specific for a protein. The amino acid sequence contains necessary information for that protein to fold into a unique three dimensional structure

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and correspondingly a unique function. The structure of proteins can be best understood by considering them in four hierarchical levels as described in figure 3.7

3.3.1 The primary structure of proteins

The amino acid sequence of a protein is known as its primary structure. Knowing the primary structure for a protein is important because even small changes (due to mutations) in the primary structure can lead to improper folding and hence impairment or complete loss of function.

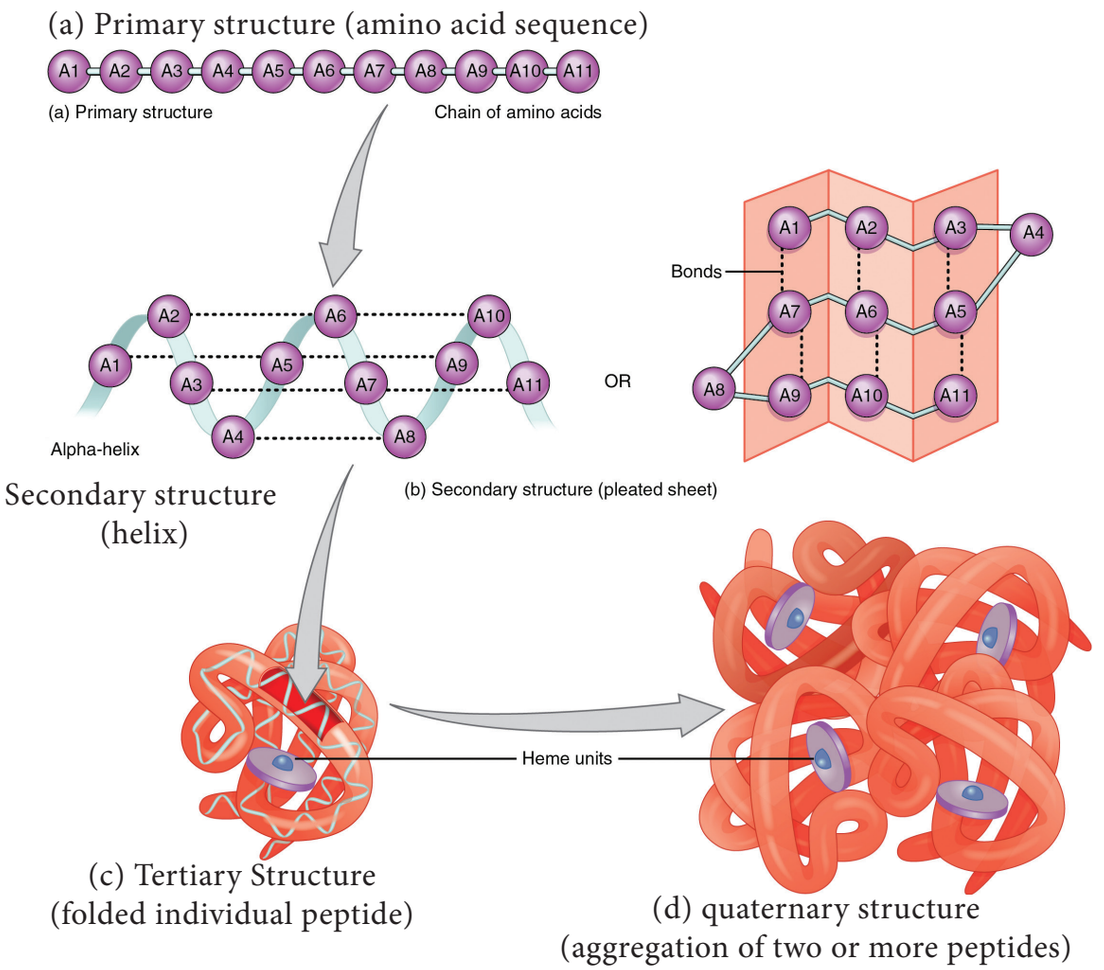

Figure 3.7 Structural organization of proteins

Peptide bonds

The amino acids in protein are covalently linked together to form peptide bonds. Peptide bonds are amide linkages between the α carboxyl group of one amino acid and the α amino group of another amino acid. For example, serine and alanine can form a peptide called serylalanine as described in figure 3.8. Since two amino acids are joined together, this molecule is known as a dipeptide. If many amino acids are joined together in the same way to form a single chain, such a chain is known as a polypeptide. The atoms excluding

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the side chains of amino acids in a polypeptide are known together as the back bone or main chain of the polypeptide.

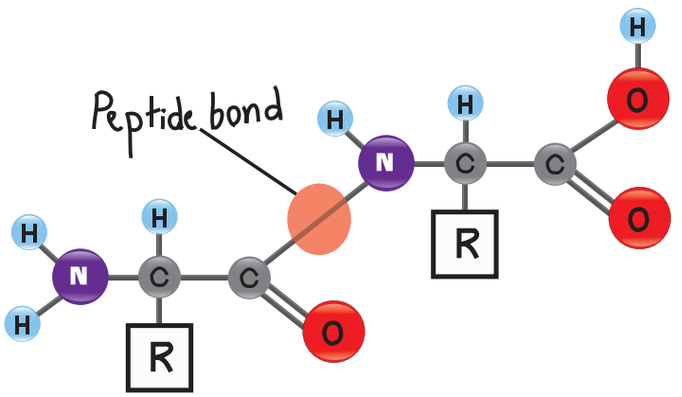

Figure 3.8 Chemical structure of a dipeptide and a peptide bond

Peptide bonds have some important properties, which are

i. Peptide bonds are generally in *trans* conformation. However in rare conditions peptide bonds formed by proline can adopt a *cis* conformation.

ii. Peptide bonds have a partial double bond character, which gives them a planar nature and hence cannot be rotated.

iii. Since peptide bonds are amide linkages the –C=O and –NH groups cannot donate or accept protons and are uncharged. The net charge of a polypeptide can come only from the N terminus amino group, C terminus carboxyl group and the side chains of the amino acids.

iv. Despite not being ionisable, the –C=O and –NH groups of peptide bonds are polar and can involve in the formation of hydrogen bonds. This property is important for the formation of secondary structures of proteins.

3.3.2 Secondary structure of proteins

The back bone of a polypeptide forms regular structural arrangements by making hydrogen bonds with its neighbouring amino acids. As a rule, these hydrogen bonds are always between the main chain –NH group and –C=O group. There are three main types of secondary structures present in proteins namely α Helix, β sheet and β turn.

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Hydrogen Bonds

Hydrogen bonds are weak electrostatic interactions between an electro negative atom and a hydrogen which is covalently linked to another electro negative atom.

α Helix

*I*t is a spiral (helical) structure of a tightly packed and coiled main chain of a polypeptide with the side chain groups of amino acids protruding outside. The helical structure is achieved by the formation of hydrogen bonds between the –C=O of an nth amino acid with the –NH group of $n+4^{th}$ amino acid. Each turn of an α helix contains 3.6 amino acids.The α helices are mostly right handed but there are rare instances where left handed α helices are also present in proteins. The amino acid Proline can produce a kink in an α helix as its secondary amino group is not geometrically compatible inside an α helix.

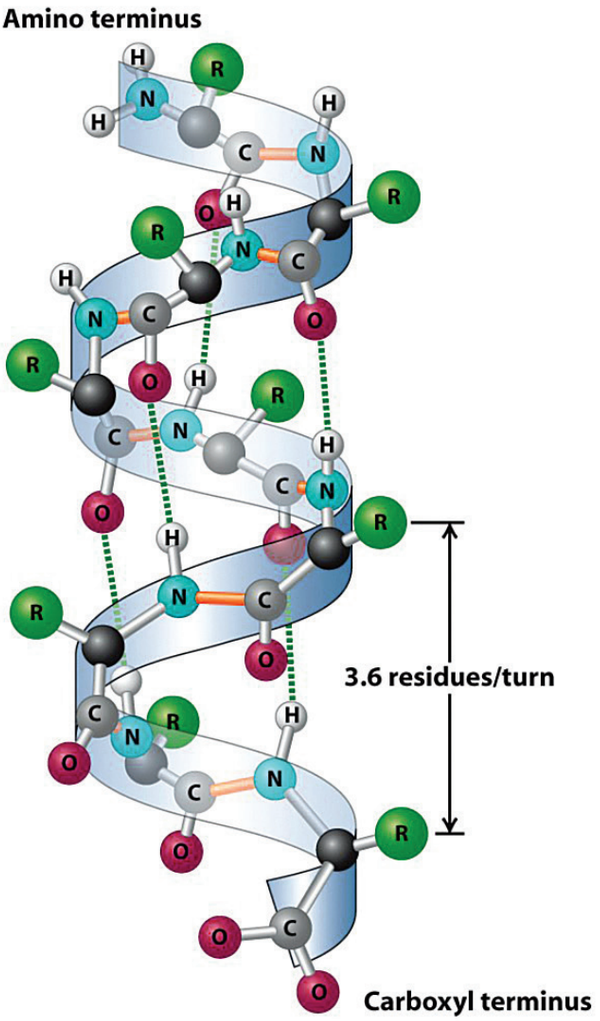

Figure 3.9 Structure of an α helix

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β pleated sheets

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 strands of a β pleated sheet may be parallel where the N- and C- termini of the strands match up or antiparallel where the N-terminus of one strand is positioned next to the C-terminus of the other.

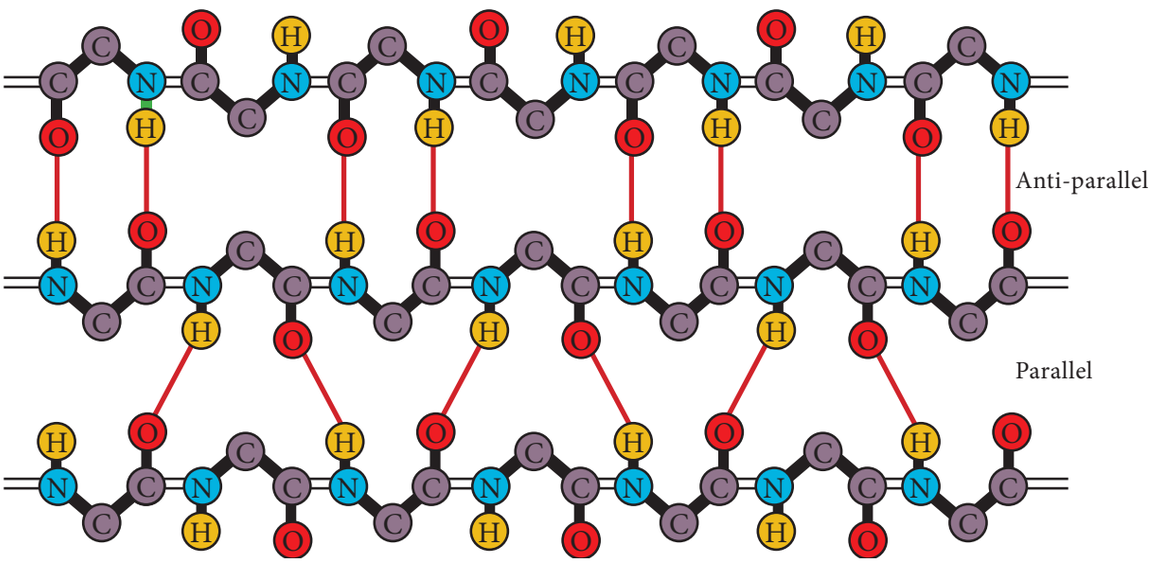

Figure 3.10 : structures of parallel and anti parallel β sheets

Table 3.2 comparison between α helix and β pleated sheet

α Helix	β Sheet
1. The interacting residues are always from a continuous stretch of polypeptide chain.	1. The interacting residues are not from a continuous stretch of a polypeptide chain.
2. Forms a tightly coiled structure	2. Forms a fully extended structure
3. The hydrogen bonds are parallel to direction of the polypeptide backbone	3. The hydrogen bonds are perpendicular to the direction of the polypeptide back bone
4. Can exist as a right handed or left handed helix	4. Can exist as parallel or anti parallel sheets
5. The amino acids Methionine, alanine, leucine, glutamic acid and lysine have higher probabilities of occurrence in α helix.	5. The amino acids isoleucine,valine,threonine, phenylalanine and tyrosine have higher probabilities of occurrence in a β sheet.

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β turns

These are secondary structural elements with four amino acids that can reverse (turn) the direction of a polypeptide and thus help the polypeptide to form a globular shape. They are mostly found on the surface of proteins. The amino acids proline and glycine are more frequently found in β turns. They are also mostly found to connect two different α helices or β strands to form super secondary structure motifs such as helix-turn helix, beta meander, beta barrel etc.

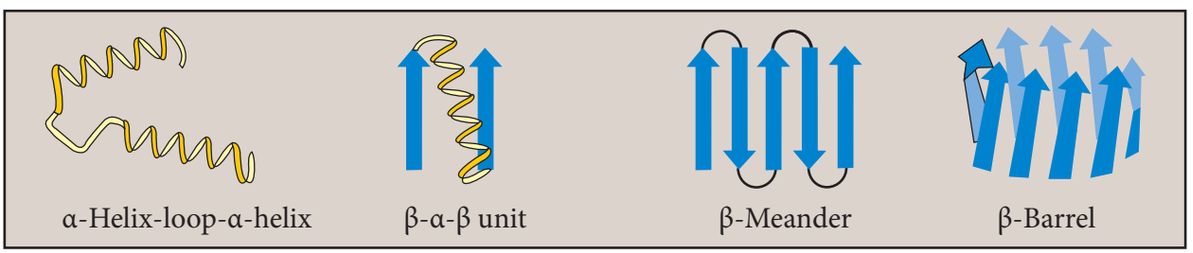

Figure 3.11 The secondary structure elements connected through β turns

3.3.3 Tertiary structure

The polypeptide folds in such a way that the secondary structure elements are packed compactly to form an overall three-dimensional structure called its **tertiary structure**. The tertiary structure is stabilized mainly by the interactions between the R groups (side chains) of the amino acids.

The interactions that contribute to tertiary structure are hydrogen bonds, ionic interactions, dipole-dipole interactions and Vander Waals Forces. The above mentioned interactions are also known as non bonded interactions.

The side chains with like charges such as Lys and Arg repel one another, while those with opposite charges such as Lys and Asp can form an ionic interaction. Similarly, polar R groups can form hydrogen bonds and other dipole-dipole interactions.

The amino acids with non polar, hydrophobic R groups cluster together on the inside of the protein through hydrophobic interactions. This cluster is also known as the hydrophobic core and it is an important feature of globular proteins. Similarly, the hydrophilic amino acids, (i.e.) the amino acids with side chains containing charged groups are present on the surface of globular proteins to interact with surrounding water molecules.

The sulphur containing side chains of two cysteine residues can form a covalent bond known as a **disulfide bond**. The disulfide bonds help to bring together two different

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parts of the same polypeptide or two different polypeptides together and are the only covalent interactions involved in the formation of tertiary structure.

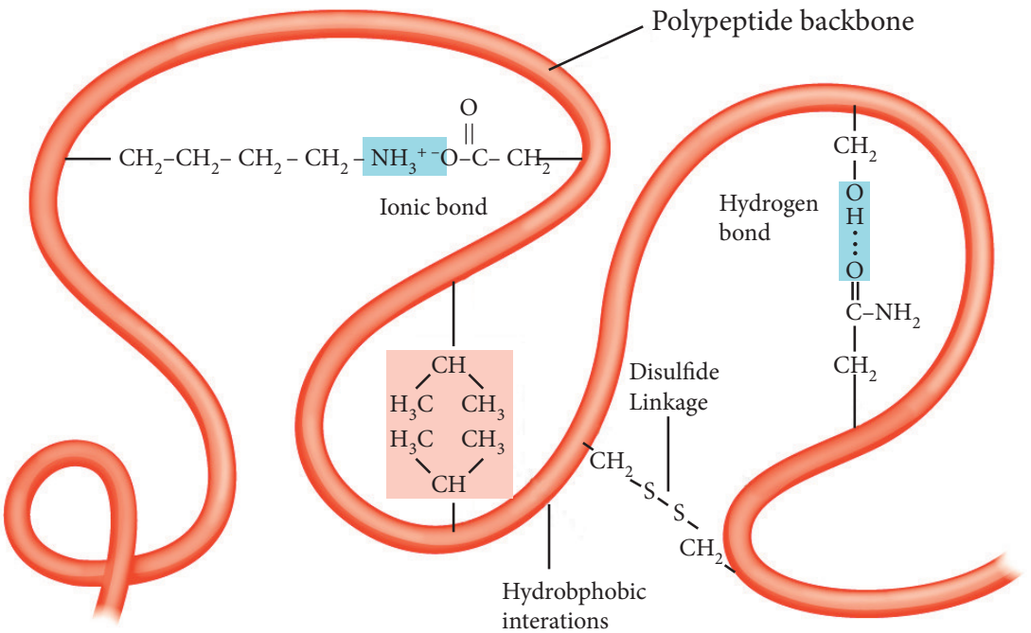

Figure 3.12: molecular interactions in a tertiary protein structure

3.3.4 Quaternary structure of proteins

Proteins that are made up of a single polypeptide chain have only three levels of structure. Some proteins are made up of more than one polypeptide chain. In such cases the tertiary structures formed by each of those polypeptide chains come together to form a quaternary structure. These individual polypeptide chains are also known as subunits. Hemoglobin, a protein which carries oxygen in blood is made up of four subunits. Similarly, DNA polymerase, an enzyme which synthesizes new strands of DNA is composed of ten subunits. The same types of interactions that contribute to tertiary structure are also involved in stabilization of the quaternary structure.

3.4 Properties of proteins

a. Generally proteins are colourless and tasteless. However, there are exceptions for example Hemoglobin is red in colour.

b. The solubility of proteins is influenced by pH. Proteins are least soluble at their iso electric point.

c. All protein solutions are optically active. The magnitude of optical activity depends on temperature, the wave length of light used and the concentration of protein.

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d. Since proteins are macro molecules, their sizes are quantitatively expressed in terms of their molecular weights with kilo Daltons as their unit (kDa). For example the human serum albumin has a molecular weight of 66 kDa.

e. Because of their giant size, proteins exhibit colloidal properties such as low diffusion and Tyndall effect.

f. Proteins undergo hydrolysis upon treatment with concentrated mineral acids like HCl and yield constituent amino acids as their hydrochlorides. Similarly, proteolytic enzymes like trypsin and chymotrypsin hydrolyse proteins.

g. When proteins are treated with alkaline copper sulphate solution (Biuret reagent) they form a violet coloured complex called Biuret complex. This reaction can be used as a qualitative and quantitative test for proteins.

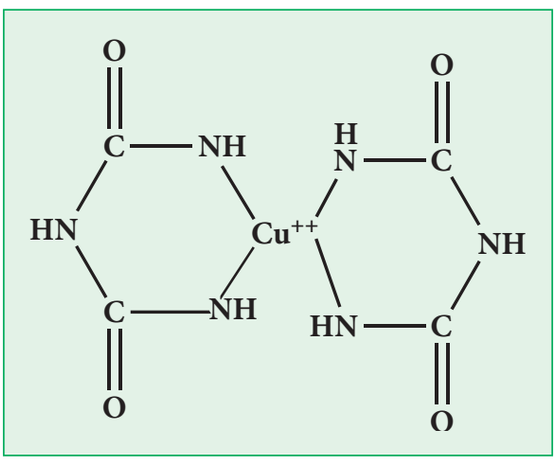

Figure 3.13 Biuret complex

3.5 Haemoglobin – an example for globular protein

Haemoglobin is found in red blood cells and is involved in the transport of oxygen from lungs to tissues. It is a tetramer containing four polypeptide chains – 2α chains and 2 β chains. Each of these chains contains a prosthetic group called heme. Heme is a protoporphyrin ring complexed with Fe^{2+}. This Fe^{2+} ion can form six bonds, four with the nitrogen atoms of the porphyrin ring, one with a histidine of hemoglobin and the other with oxygen. Thus every haemoglobin can carry four O_2 molecules.

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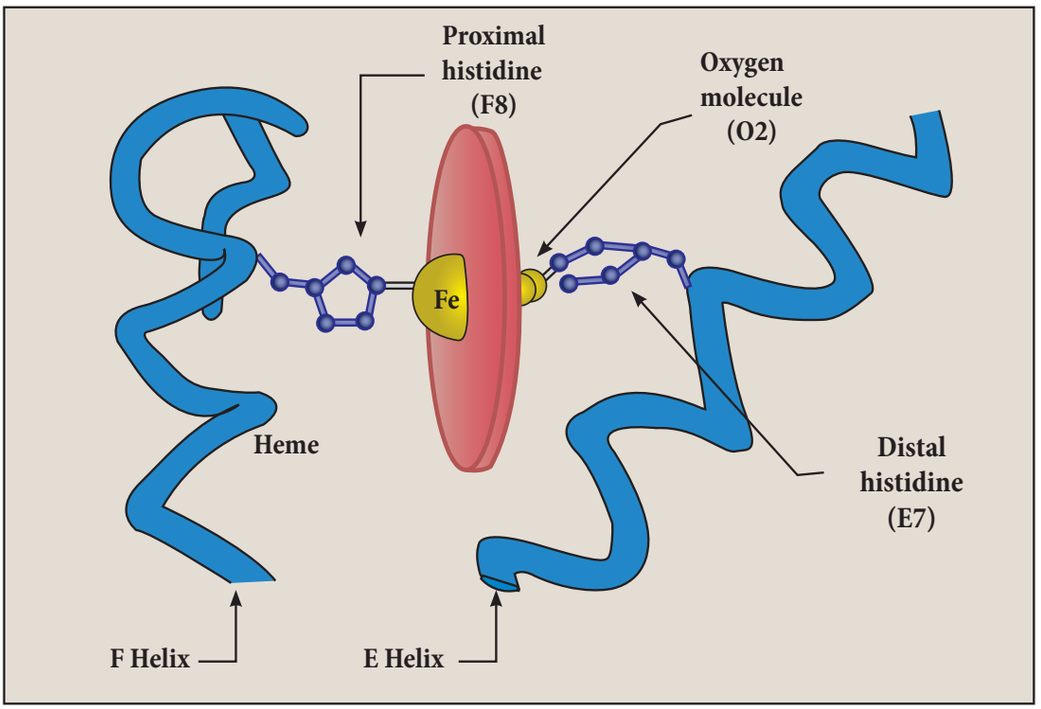

Figure 3.14 Binding of oxygen to heme in hemoglobin

Haemoglobin is an alpha helical protein, meaning it does not contain β sheets as its secondary structural elements. The haemoglobin tetramer structure could be considered as a dimerized dimer of (αβ)1 and (αβ)2. The α and β chain in each dimer are held together strongly by hydrophobic interactions. The interactions between (αβ)1 and (αβ)2 are comparatively weaker hydrogen bonds and ionic interactions. This allows the dimers to move with respect to each other forming two different conformational states: a relaxed 'R' conformation and a taut 'T' conformation. The binding and release of oxygen switches the hemoglobin between these two states.

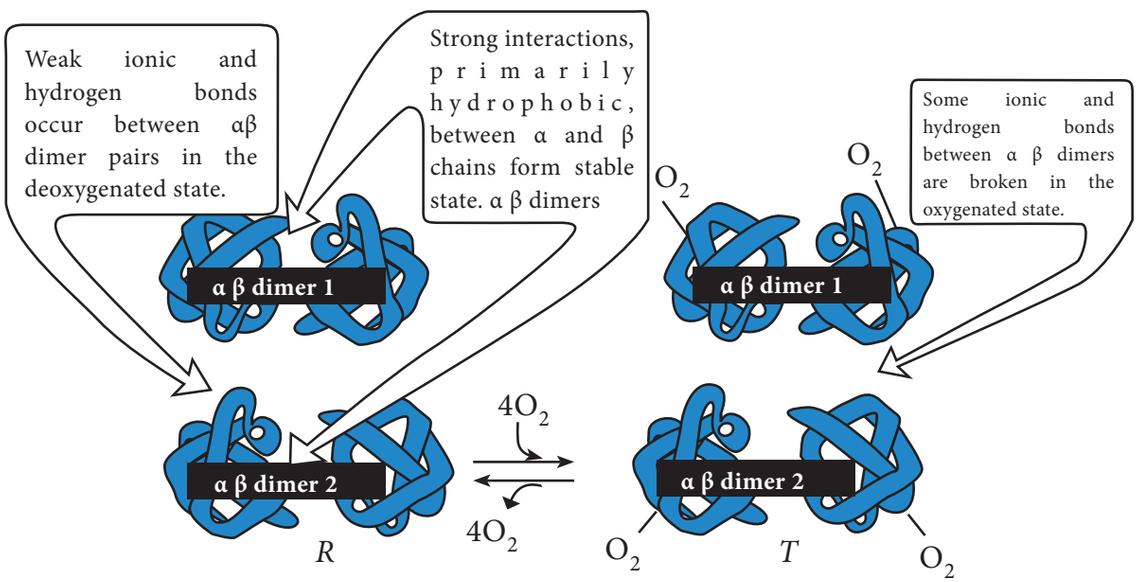

Figure 3.15 R and T conformations of haemoglobin

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Haemoglobinopathies are a set of diseases caused by synthesis of structurally abnormal haemoglobins, insufficient amount of haemoglobins or both. Sickle cell anemia, thalasemia, porphyria etc are examples of haemoglobinopathies.

3.6 Collagen - an example for fibrous protein

Collagen is the most abundant protein found in humans. Unlike globular structures discussed above, collagen forms a long coiled fibrous structure. Each collagen molecule consists of three polypeptide chains which forms an elongated triple helical structure as described in figure 3.16

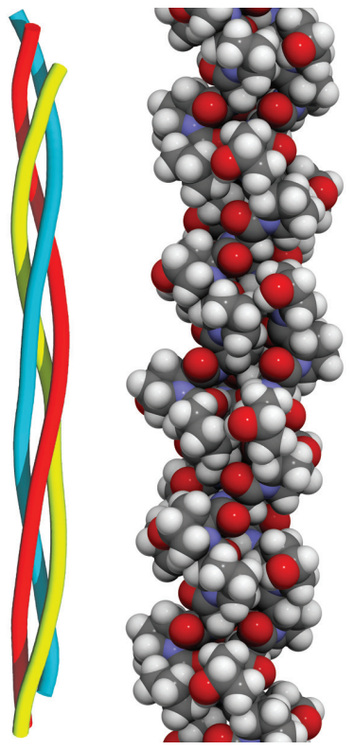

Figure 3.16 Structure of Collagen

The amino acid sequence of these polypeptide chains are always repeating units of Gly-X-Y. Where X is often proline and Y is either hydroxyproline or hydroxylysine. The hydroxyl group of hydroxylysine can also be glycosylated with glucose or galactose.

There are various types of collagen which can be broadly categorized into three groups.

a. The fibril forming collagens present in skin, bone, cartilages, tendons and blood vessels etc provide tensile strength to the corresponding tissues.

b. The network forming collagens form network like structures beneath the membranes providing them mechanical strength.

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c. The fibril associated collagens connect two fibril forming collagens or a fibril forming collagen with other components of extra cellular matrix.

More than 1000 disease causing mutations have been identified both directly in collagen genes or in the genes of enzymes involved in the synthesis of collagen. The diseases associated with collagen malfunction are known as collagenopathies. Elhers-Danlos syndrome (EDS) is a prominent collagenopathy which is due to inherited mutations in collagen processing enzymes. Stretchy skin, loose joints and vascular problems are associated with EDS.



Figure 3.17 Stretchy skin in EDS syndrome

Osteogenesis Imperfecta (O.I) is another prominent collagenopathy characterized by brittle bones, hunch back, twisted spine and retarded wound healing. The mutations in glycine residues of collagen and thereby an improper triple helical structure leads to this disease.

3.7 Denaturation and Protein folding

Each protein has a unique three dimensional structure. Upon changes in various factors like temperature, pH, ionic strength or exposure to certain chemicals like urea lead to disruption of its three-dimensional structure and turn back into an unstructured string of amino acids. When a protein loses its higher-order structure, but not its primary structure, it is said to be denatured. Denatured proteins are not functional.

For some proteins, denaturation can be reversed. Since the primary structure of the polypeptide is still intact it may be able to re-fold into its original structure, if it is returned to its normal environment. Many proteins do not fold by themselves, but instead get assistance from other proteins like chaperons.

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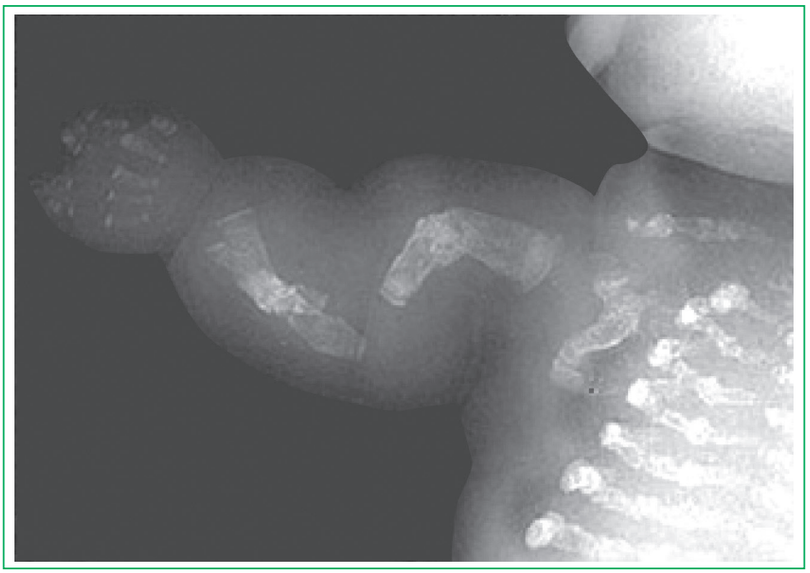

Figure 3.18 X-ray image of lethal O.I of a fetus showing fractures

The process by which a polypeptide chain acquires its 3-dimensional structure is known as protein folding. It is a complex process and the exact mechanism by which a protein folds to its three dimensional structure has not been understood so far. Quite often many proteins misfold. Some proteins when misfolded form fibrillar structure of β pleated sheets. This misfolding can be spontaneous or could be because of mutations. These misfolded proteins aggregate in neurons and can lead to amyloid disease such as Alzheimer's disease, which is a **neuro degenerative disease.**

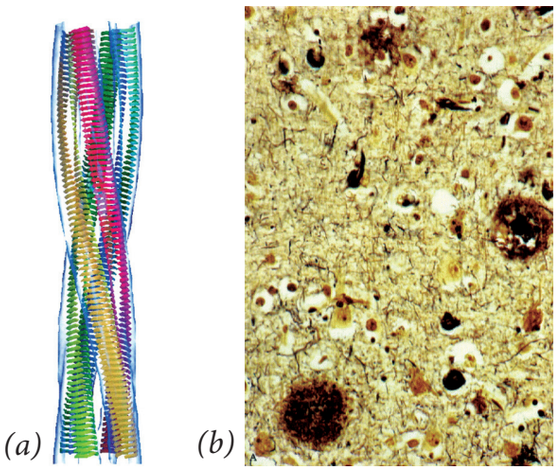

Figure 3.19 (a) Model of an amyloid fibril and (b) Amyloid plaques in temporal cortex

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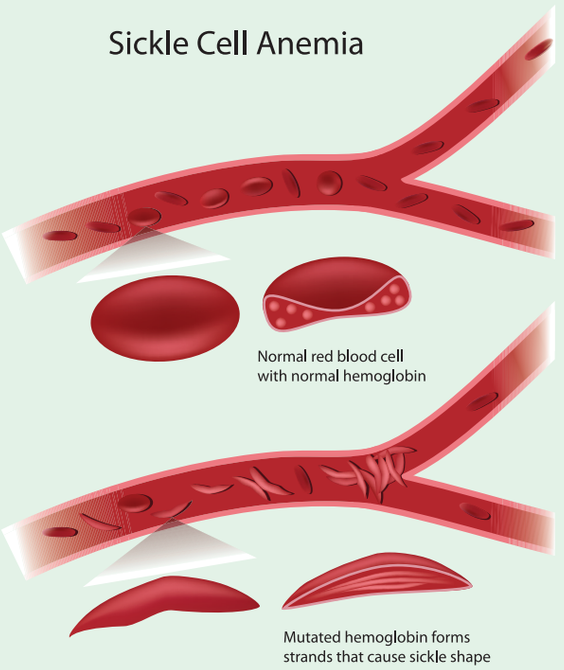
What causes Sickle Cell Anemia?



It is an inherited disease where the body does not produce healthy red blood cells. This is a genetic defect due to a mutation in the β-globin gene of hemoglobin which results in the substitution of a valine in the sixth position, instead of a glutamic acid. This mutation leads to abnormal tertiary structure of haemoglobin which in turn leads to lead to sickle shaped red blood cells. The altered shape of the red blood cells make them rigid and get stuck in the blood vessels and capillaries and thereby slows the blood flow. It is an autosomal recessive disease, meaning that the defective gene must be passed by both the father and mother to the off spring. There is no cure for this disease, but treatments can be given to reduce the symptoms like pain, swelling of limbs, fever etc..

Ramachandran Plots



G.N Ramachandran was born in the town of Ernakulam, Kerala, India to a Tamil speaking family. He completed his B.Sc. honours in Physics from St. Joseph's College, Tiruchirapalli in 1939. He joined the Indian Institute of Science, Bangalore in 1942 in the Electrical Engineering Department. He did his Ph.D. in Physics under the supervision of Nobel Laureate Sir C.V. Raman. In 1942 he received Master's Degree in Physics from Madras University. In 1954, he identified and published the Triple helical structure of Collagen using X-ray diffraction. He pioneered the field of protein structure validation through the study of available crystal structures of peptides. From his studies, in 1962, he developed the Ramachandran Plot which is used even today for stereochemical validation of protein structures. In 1970, he founded the Molecular Biophysics Unit at the Indian Institute of Science which was later known as the Centre of Advanced Study in Biophysics. He has provided a major contribution to the research field of Structural Biology from India for which we must feel proud.

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EVALUATION

I. Choose the correct answer

1. How many amino acids are found in nature?

   a) 20 b) 100 c) 300 d) 25

2. Which basic amino acid is neutral or basic based on chemical environment?

   a) Lysine b) Arginine c) Tryptophan d) Histidine

3. At Isoelectric point (pI), the ionic form of amino acid is called as

   a) Anion b) Zwitter ion c) Cation d) None of the above

4. Which amino acids cannot be synthesized by our body?

   a) Non-essential amino acids b) Polar amino acids

   c) Essential amino acids d) Aromatic amino acids

5. To which hierarchical level of protein structure does α helix belong?

   a) Primary structure b) Secondary structure

   c) Tertiary structure d) Quarternary structure

II. Fill in the blanks

1. Planarity of the peptide bond is due to ---------- character
2. Each turn of an α helix contains ---------- amino acids
3. Beta barrel is a ----------
4. The covalent bond formed between two cysteine residues is called ----------
5. The unit for protein size is ----------

Answer the following in brief

1. What are essential amino acids ?
2. What are the four hierarchical levels of protein structure?
3. Write the reaction of proteins with Ninhydrin.

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4. What are the three different types of Collagen?

5. What are the non-bonded interactions in tertiary structure of a protein?

Answer the following in detail

1. Explain the structure of Globular proteins with an example.

2. Write about the hierarchical levels of protein structure.

3. Distinguish between α helix and β pleated sheets.

4. What is a peptide bond and what are its important properties?

5. Write any five physical and chemical properties of proteins.

SUMMARY

Proteins are important class of macromolecules which are the work horses of the cell. They perform important functions like catalysis of metabolic reactions, defence against foreign bodies, structural support for the cell, transport of other molecules etc. Proteins are polymers of twenty different amino acids. There are 20 amino acids which can be constituents of proteins. Based on the properties of their side chain chemical groups, amino acids can be classified into hydrophobic, polar, acidic and basic amino acids. The polymers of these 20 amino acids are known as polypeptide chains. One or more polypeptide chains can form a protein molecule. These polypeptide chains can fold to obtain a specific three dimensional structure and obtain specific function. Proteins structures can be described in a hierarchical order known as primary, secondary, tertiary and quaternary structures. Proper folding of a polypeptide chain to a proper three dimensional structure is important for a protein to achieve its biological function. So misfolding of proteins often leads to several diseases.

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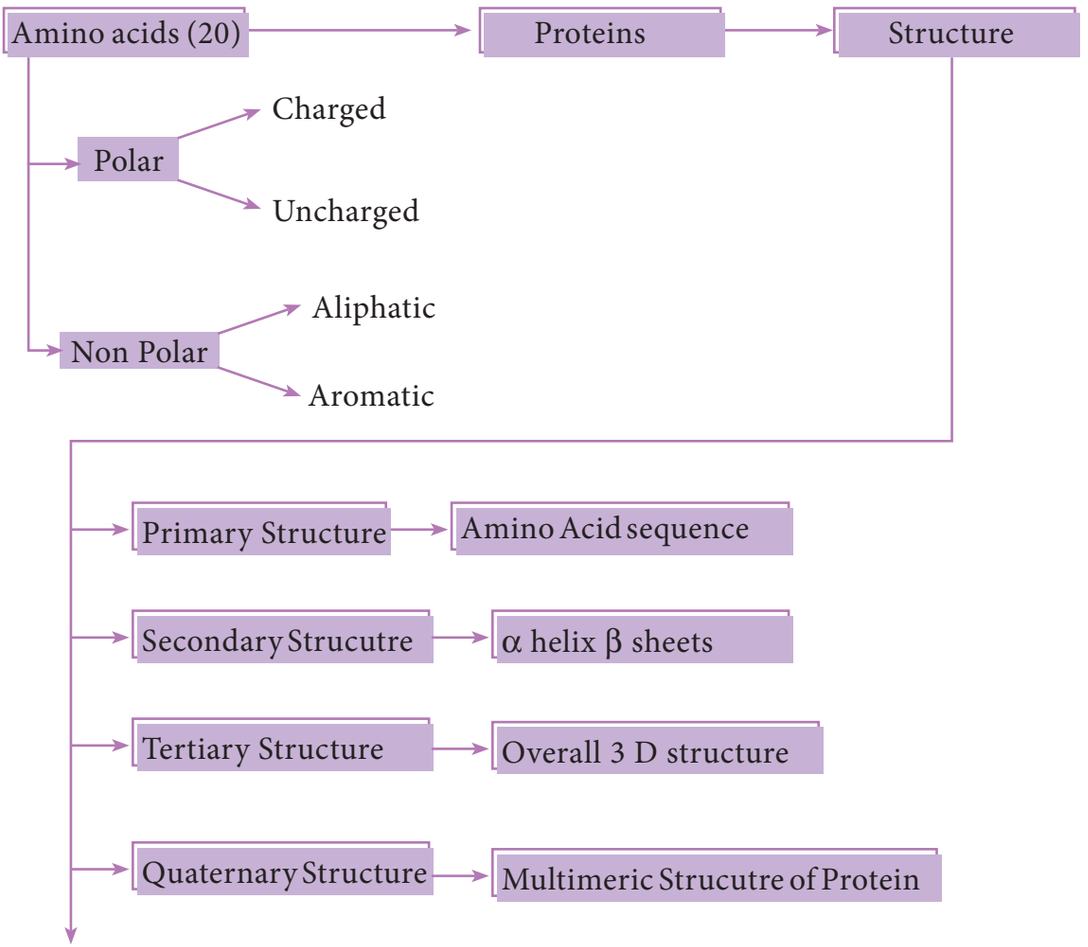
CONCEPT MAP



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Unit 4 ENZYMES



Anselme Payen

Anselme Payen was a French chemist who first isolated the enzyme diastase in 1833 and showed that it can catalyze the conversion of starch to maltose. However only in 1926 enzymes were shown to be purely made of proteins by James B Sumner, who crystallized the enzyme urease to confirm its purity.

F6WT1

Learning Objectives

After studying this unit the students will be able to:

- Classify enzymes based on their function and assign EC numbers.
- Describe the factors influencing enzyme activity.
- Explain the different types of enzyme inhibitors.
- Explain isoenzymes.
- List the uses of enzymes in various fields such as medicine, industries, scientific research etc...

Introduction

Enzymes are a type of protein present in all living things. Enzymes are produced in animal, bacteria, fungi, yeast and plants. Enzymes act as biological catalysts, increasing the rate of chemical reactions without undergoing any permanent change to themselves. Enzymes are specific in their catalytic activity. They are colloidal in nature and thermo-labile in character.

Enzymes are used to speed up biochemical reactions. The reactions can be the transformation of a chemical compound or a protein. For example, in our stomach, enzymes break down food

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molecules into simpler molecules. For example, amylase coverts starch into maltose and pepsin breaks proteins into smaller peptides.

4.1. Nature and Properties of Enzymes:

1. Enzymes are proteins.
2. Enzymes are complex macromolecules with high molecular weight.
3. Enzymes act as catalysts for biochemical reactions and are called biocatalysts.
4. They help in the breakdown of larger molecules into smaller molecules (catabolism) and in the synthesis of larger molecules (anabolism).
5. Enzymes possess high degree of specificity.
6. Most of the enzymes have high turnover number.
7. Some enzymes possess only one polypeptide chain. They are known as monomeric enzymes. (Ribonuclease, Trypsin etc.)
8. Some enzymes possess more than one polypeptide chain. They are known as oligomeric enzymes. (Lactate dehydrogenase (LDH)).
9. The activity of an enzyme increases with increase in substrate concentration and ultimately reaches a steady maximum velocity.
10. Some enzymes can possess multiple functions and when there are more number of polypeptide chains are known as multi-enzyme complexes. Example: Fatty acid synthase.
11. Each enzyme shows high activity at a particular pH and temperature called its optimum pH and temperature, respectively.

Note

Turnover number of an enzyme is defined as the maximum number of substrate molecules which can be converted to products per molecule of enzyme per unit time.

4.2 Nomenclature and Classification of Enzymes:

In early days the suffix – *ase* was added to the substrate for naming the enzymes.

Example : *Lipase* acts on lipids.

These names are known as trivial names. They do not convey complete information about the enzymatic reaction.

The International Union of Biochemistry and Molecular Biology (IUBMB) has assigned a systematic nomenclature for enzymes. The systematic name has two parts.

- The first part represents the substrate. In enzyme catalyzed reactions the reactants are known as substrates.

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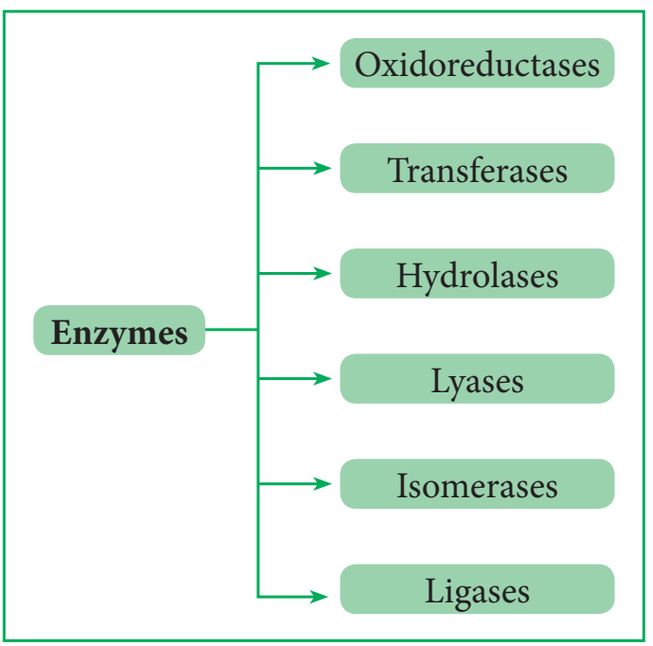
- The second part, ending in –*ase*, indicates the type of reaction catalysed.

Each enzyme is assigned a four-digit code number called Enzyme Commission (EC) number.

- The first digit represents the major class to which the enzyme belongs.
- The second digit denotes the subclass.
- The third digit denotes the sub-sub class of the enzyme within the major class.
- The fourth digit represents the serial number of the enzyme within the sub-sub class.

Example: Hexokinase (EC 2.7.1.1) and Glutamine synthetase (EC 6.3.1.2)

According to International Union of Biochemistry and Molecular Biology, the enzymes are classified into six major classes on the basis of the reaction they catalyse. The six major classes of enzymes are:



1) Oxidoreductases:

These are enzymes which catalyze the oxidation – reduction reactions between two substrates.

Examples: a) Dehydrogenase (Alcohol dehydrogenase)

b) Oxidase (Cytochrome oxidase)

c) Peroxidase (Glutathione peroxidase)

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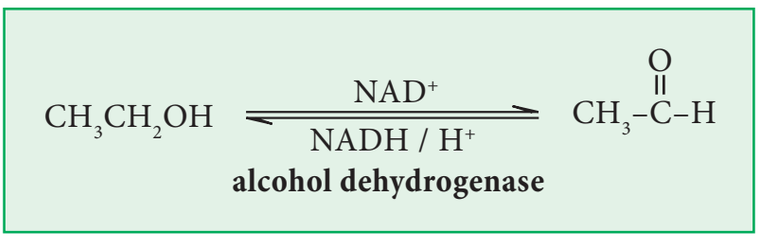
Alcohol dehydrogenase (EC 1.1.1.1) :

This enzyme oxidizes ethanol into acetaldehyde. It requires the coenzyme NAD^+ (Niacinamide Adenine Dinucleotide) which gets reduced to NADH.



2) Transferases:

These are enzymes which catalyze the transfer of certain groups such as phosphate, amino or acetyl groups from one substrate to another.

Examples:

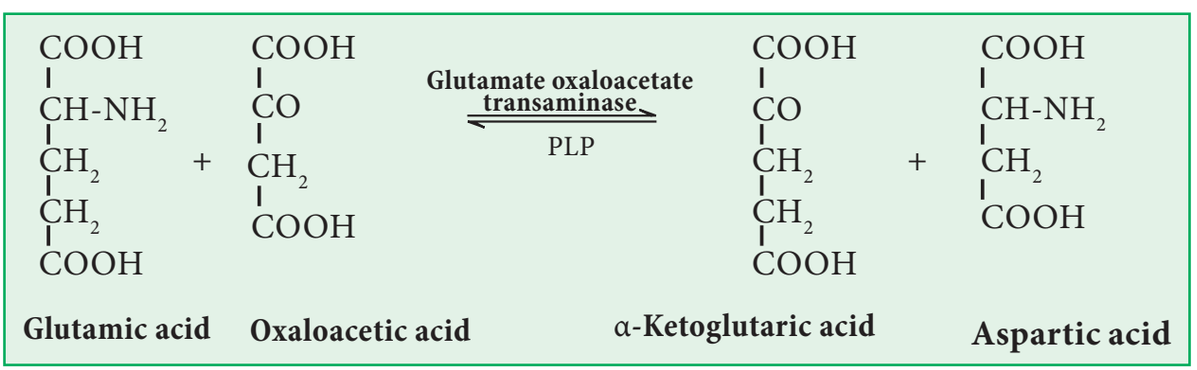
a) Transaminase (Transfers an amino group Example Aspartate amino transferase)

b) Transacylase (Transfers an acyle group Example Malonyl transacylase)

c) Phosphorylase (Transfers a phosphate group Example Glycogen phosphorylase)

Transaminases:

They catalyse the transfer of amino group from an amino acid to a keto acid. Example: Glutamate oxaloacetate transaminase (GOT) or Aspartate transaminase (AST; EC 2.6.1.1). This enzyme catalyses the transfer of an amino group from glutamic acid to oxaloacetic acid. It requires pyridoxal phosphate (PLP) as coenzyme for its activity.



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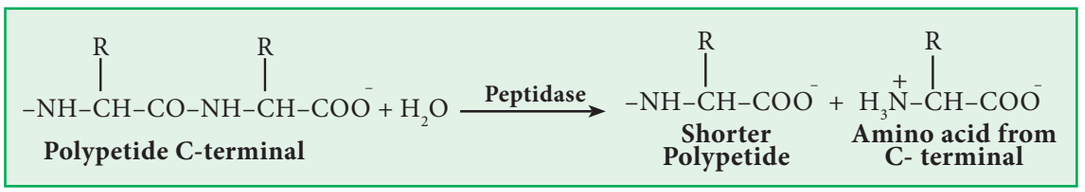
3) Hydrolases:

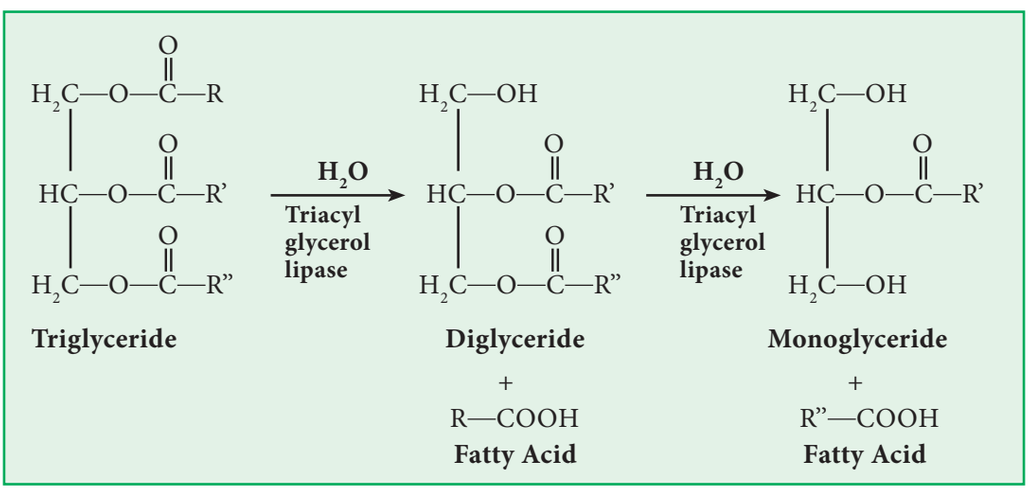
These are enzymes which catalyze the hydrolysis of substrates. They bring about the hydrolysis by adding water.

Example : a) Lipase b) Urease c) Glycosidase.



Lipase (EC 3.1.1.3):

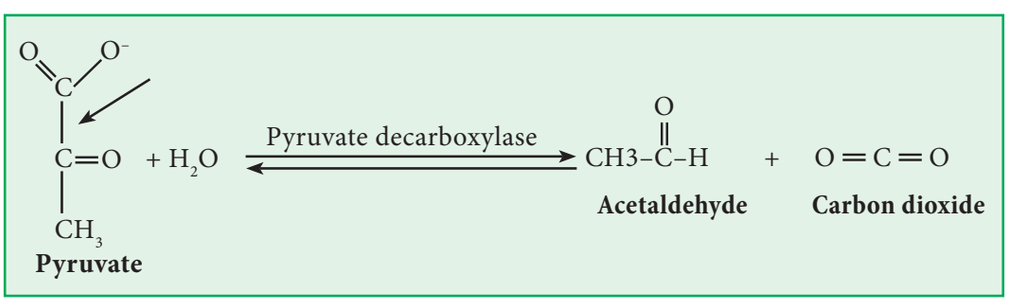
These are enzymes which hydrolyze the ester linkage. For example triacyl glycerol lipase (EC 3.1.1.3) splits the ester linkage between glycerol and fatty acid.



4) Lyases:

These enzymes catalyze the addition or elimination of groups like H_2O, CO_2, and NH_3 etc. Example: Aldolase, decarboxylase

a) Pyruvate carboxylase (EC 4.1.1.1):



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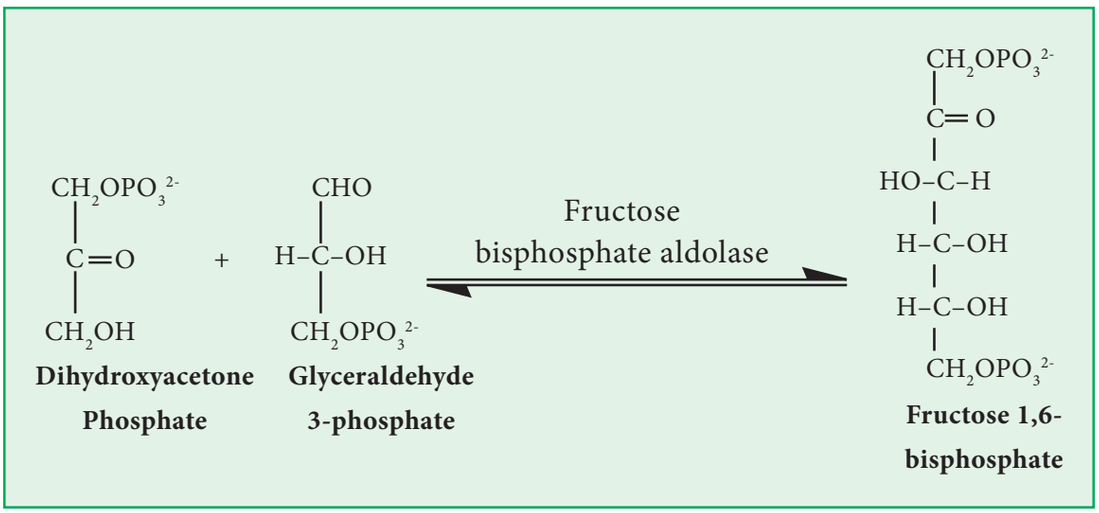
b) Fructose bisphosphate aldolase (EC 4.1.2.13):

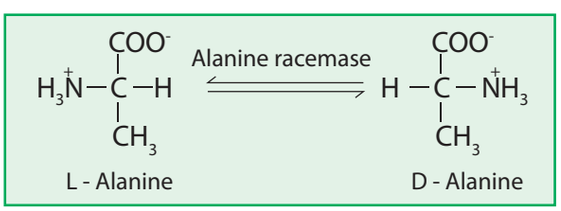
It catalyzes the reversible conversion of fructose-1,6-bisphosphate to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate by aldol cleavage of the C3–C4 bond.



5) Isomerases:

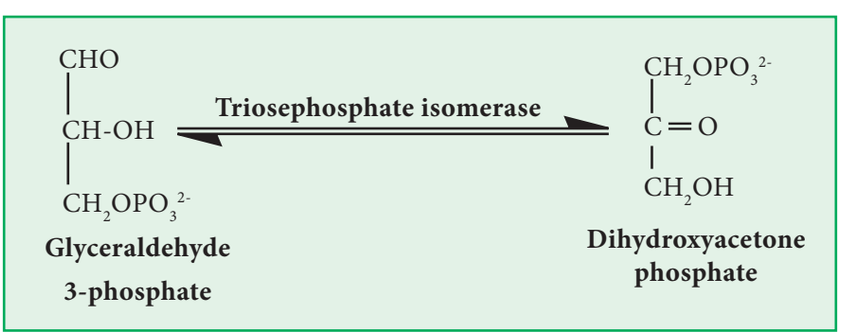
These enzymes catalyze the inter-conversion of isomers such as optical, geometrical or positional isomers.

Example : a) Alanine racemase (EC 5.1.1.1)



b) Triosephosphate isomerase (EC 5.3.1.1):

This enzyme catalyzes the isomerization of glyceraldehyde-3-phosphate into dihydroxy acetone phosphate.



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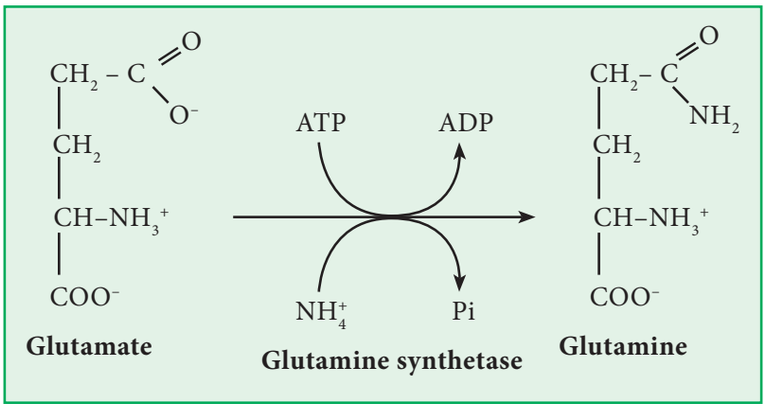
6) Ligases:

These enzymes catalyze the synthetic reactions. They link two substrates together with the utilization of ATP or GTP.

Example : Glutamine synthetase.

Glutamine synthetase (EC 6.3.1.2):

This is a ligase which catalyzes the synthesis of glutamine from glutamate and NH_3.



4.3 Coenzyme

1. Some of the enzymes are simple proteins. Examples: amylase, trypsin. Many enzymes require one or more non-protein components called cofactors. If the cofactor is an organic molecule, it is known as coenzyme. The cofactor may also be a metal ion.

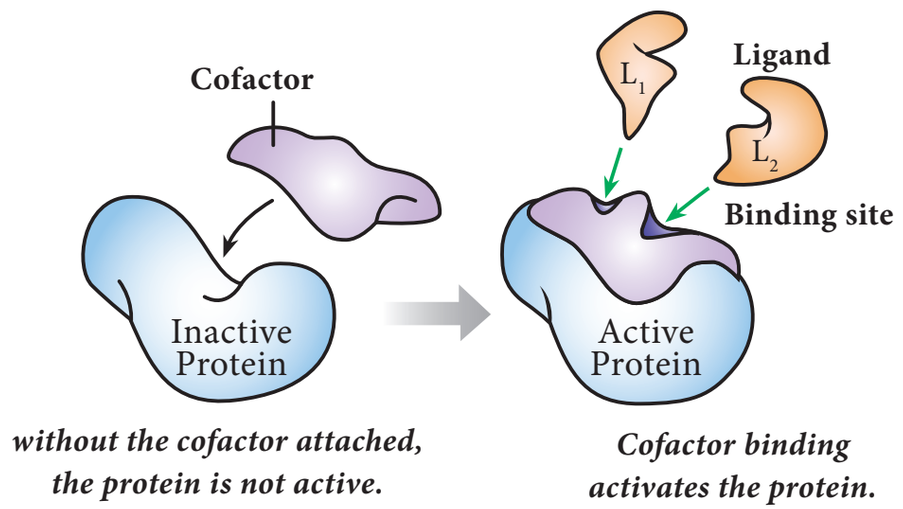


Figure 4.1 Cofactor binding

2. Coenzymes may be defined as non-protein, low molecular weight organic compounds required for the activity of enzymes. Example: Thiamine pyrophosphate (TPP).

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3. The protein part of the enzyme is known as 'apoenzyme'. The entire enzyme system consisting of the apoenzyme and coenzyme (or) prosthetic group is known as **holoenzyme**.

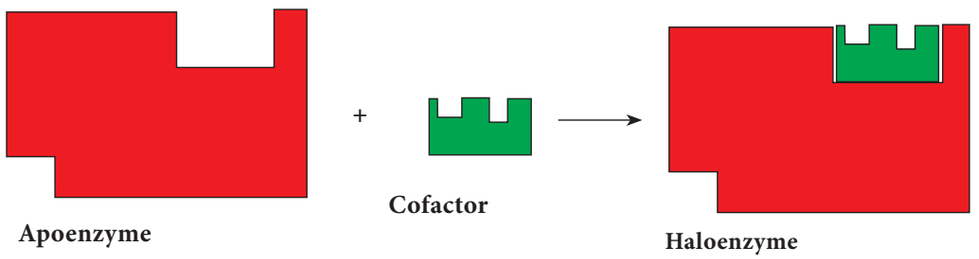

Figure 4.2 Apoenzymes and haloenzyme

4. Most of the coenzymes are linked to their apoenzymes by noncovalent forces. Example**:** The coenzyme ATP is attached to its apoenzyme hexokinase through weak non-bonding interactions.

5. Some of the coenzymes are tightly bound to their apoenzymes through covalent bonds. These are termed as prosthetic groups. Example: The prosthetic group biotin is attached to its apoenzyme carboxylase through a covalent bond.

6. The coenzymes undergo alterations during enzymatic reaction. Hence the coenzymes are regarded as second substrates (or) cosubstrates.

7. Many coenzymes are the derivatives of water soluble B-complex vitamins. **Examples:** Niacin.

8. The Coenzymes can also be organic substances other than vitamins. **Example :** ATP (adenosine triphosphate), CDP (Cytidine diphosphate)

9. Nucleotides and their derivatives can act as coenzymes.

   Example**:** NAD, FMN, FAD, coenzyme-A etc.

10. The specificity of an enzyme is mostly dependent on the apoenzyme and not on the coenzyme. For example, NAD^+ functions as coenzyme for several enzymes like alcohol dehydrogenase and lactate dehydrogenase.

11. Coenzymes function as group transfer agents (Table 4.1).

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Table 4.1 : Vitamin related Coenzymes

Coenzyme	Derived from Vitamin	Atom (or) group transferred (function)	Dependent enzyme
Thiamine pyrophosphate (TPP)	Thiamine (B_1)	Aldehyde	Transketolase
Flavin mono nucleotide (FMN)	Riboflavin (B_2)	Hydrogen and electrons	L-amino acid oxidase
Flavin adenine dinucleotide (FAD)	Riboflavin (B_2)	Hydrogen and electrons	D-amino acid oxidase
Nicotinamide adenine dinucleotide (NAD^+) (or) Diphospho pyridine nucleotide (DPN)	Niacin (B_3)	Hydrogen and electrons	Lactate dehydrogenase
Pyridoxal phosphate (PLP)	Pyridoxine (B_6)	Amino	Alanine transminase
Biotin	Biotin (B_7) or (H)	CO_2	Pyruvate carboxylase
Coenzyme A	Pantothenic acid (B_5)	Acyl	Thiokinase
Tetrahydrofolate	Folic acid	one carbon unit	formyl transferase

4.4 Factors influencing Enzyme activity

The important factors that influence an enzyme catalyzed reaction are; pH, Temperature, Substrate concentration, Enzyme concentration, Activators and inhibitors.

4.4 .1 Effect of pH :

1. Change in hydrogen ion concentration influences the enzyme activity. When the velocity is plotted against pH, a bell shaped curve is obtained.

2. The pH at which the enzymatic reaction has maximum velocity is known as **optimum pH.** Most of the enzymes possess optimum pH between 5 and 9. However there are exceptions like pepsin, alkaline phosphatase etc.

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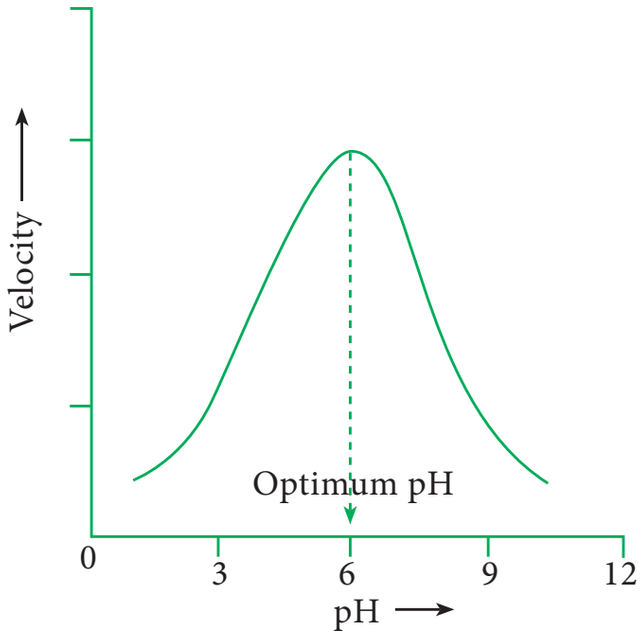

Figure 4.3 Effect of pH

Note

Denaturation: Nonproteolytic change in structure of a native enzyme that causes it to lose some or all of its unique or specific characteristics.

3. The optimum pH of some of the common enzymes are as follows:

Enzyme	Optimum pH
Pepsin	1-2
Alkaline phosphatase	10-11
Acid phosphatase	4-5

4. Enzymes possess only low activity or even become inactive at extreme pH values. This is due to the following reasons;

a) The hydrogen ion concentration affects the ionic charges on the active site of the enzyme.

b) Thus the extreme pH values lower the effective concentration of active form of enzyme and substrate. Therefore, the reaction velocity will be lowered.

c) Denaturation of enzyme occurs at extreme pH values.

4.4.2. Effect of temperature on enzyme acitivity:

1. Velocity of an enzyme reaction increases with increase in temperature upto a maximum and then declines.

2. When the velocity is plotted against temperature we obtain a plot as shown in figure 3.4.

3. The temperature at which the enzymatic reaction has maximum velocity is known as **optimum temperature.**

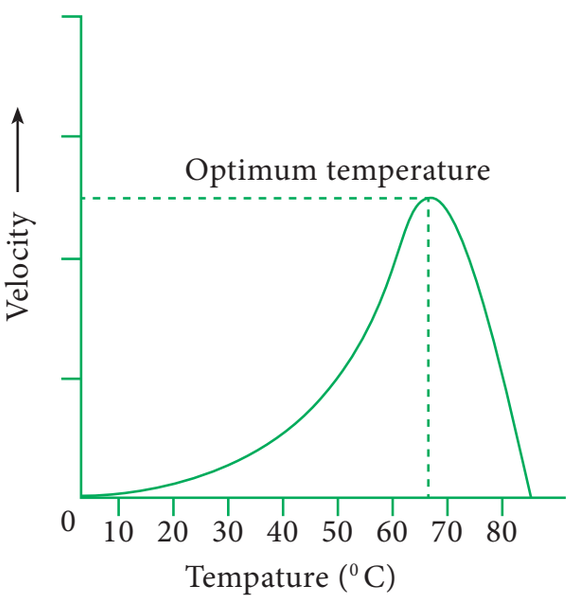

Figure 4.4 Effect of temperature

4. The optimum temperatures of some of the common enzymes are as follows:

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Enzyme	Optimum temperature (^{0}C)
Plant urease	60
Human enzymes	37

But enzymes like venom phosphokinase, muscle adenylate kinase are active even at 100^{0}C.

5. In general, at high temperatures the enzymes undergo denaturation and this leads to the rapid loss of catalytic activity.

6. Temperature co-efficient or Q_{10} is defined as the increase in velocity of the enzymatic reaction when the temperature is increased by 10^0 C. For most of the enzymes the value of Q_{10} is "2" in the temperature range 0^0 C to 40^0 C.

4.4.3 Concentration of substrate:

Formation of an enzyme-substrate complex (ES complex) is the first step in enzyme catalysis. Increase in the substrate concentration gradually increases the velocity of the enzymatic reaction up to a particular value.

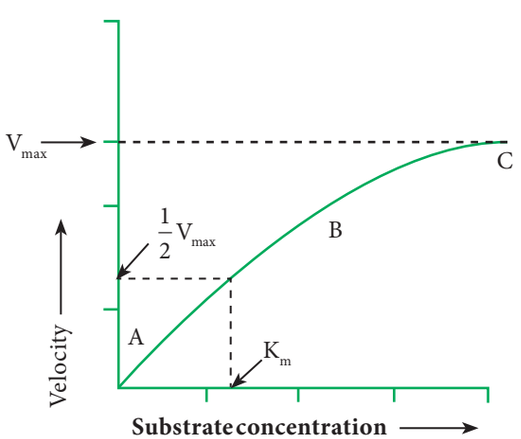

Figure 4.5 Effect of substrate concentration

A hyperbolic curve is obtained when velocity is plotted against the substrate concentration. This graph shows three distinct phases.

i) In the first phase (A), the velocity of the reaction is directly proportional to the substrate concentration.

ii) In the second phase (B), the substrate concentration is not directly proportional to the enzyme activity.

iii) In the third phase (C), the velocity remains constant and does not change with increase in the substrate concentration. (plateau)

4.4.4 Concentration of enzyme

At a constant substrate concentration, the velocity of an enzyme catalyzed reaction increases proportionately with the increase in the concentration of the enzyme. This property is utilized in determining the level of serum enzymes for the diagnosis of diseases. On plotting the velocity of the enzymatic reaction with the enzyme concentration, a straight line is obtained.

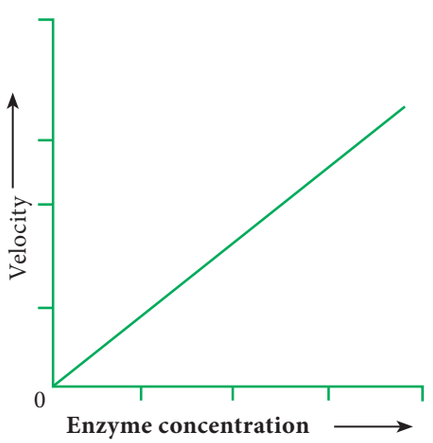

Figure 4.6 Effect of enzyme concentration

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4.4.5 Activators

Activators are the inorganic ions (cations or anions) or compounds that enhances the activity of the enzymes.

Enzyme	Activator
Phenol oxidase	Cu^{2+}
Amylase	Cl^{-}

Metal ions can be bound to the enzyme permanently or can be used just for activation. When metal ions used only for the activation of enzyme, they are called metal activated enzymes.

Examples: ATPase (Mg^{2+}, Ca^{2+}) and Enolase (Mg^{2+}).

If metal ions are bound with enzymes using chemical bond, they are called metalloenzymes.

Examples: Alcohol dehydrogenase-Zn^{2+} and Carbonic anhydrase-Zn^{2+},

Time, radiations and co-enzymes are also the other factors which affect the velocity of an enzyme reaction.

4.5 Inhibitors

An inhibitor is defined as a substance which binds with the enzyme and brings about a decrease in catalytic activity of that enzyme. For example, anti-oxidants are added as inhibitors to food to retard its spoilage on exposure to air (oxygen) and inhibition could be either reversible or irreversible.

Allosteric activators and inhibitors:

This type of inhibition takes place due to the presence of allosteric site (Greek allo = 'other'; stereos = 'space' or 'site') on the surface of the enzyme, away from the active site. The final end-product fits in the allosteric site and in some way brings about a change in shape of the enzyme so that the active site of the enzyme becomes unfit for making a complex with its substrate. Allosteric inhibition may be reversible. In many metabolic reactions, when the concentration of the final end product (usually acts as an allosteric inhibitor) in the cell falls, the activity of the enzyme is restored. Similary an enzyme can also be activated by an activator that binds to an allosteric site. This activator is called as an allosteric activator.

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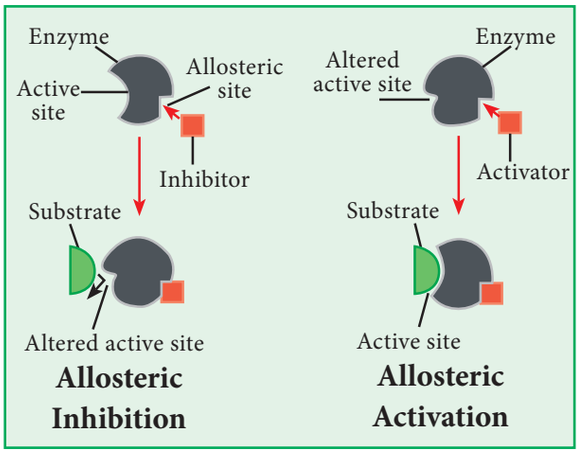

Figure 4.7 Allosteric inhibition

4.5.1 Types of Inhibition

i) Competitive Inhibition

Competitive inhibition is usually reversible. A competitive inhibitor usually closely resembles the substrate and is regarded as a substrate analogue. The inhibitor competes with substrate and binds at the active site of the enzyme but does not undergo any catalysis. As long as the competitive inhibitor is bound to the active site, the enzyme will not be available for the substrate to bind. This type of inhibition can be reversed by increasing the concentration of substrate.

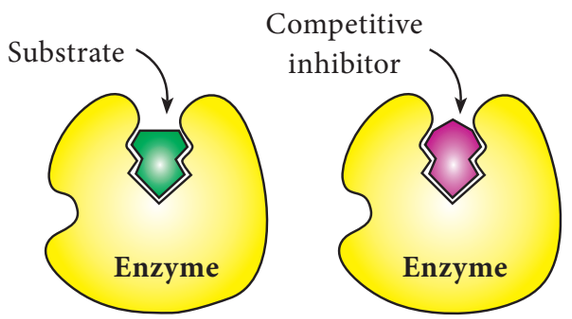

Figure 4.8 Competitive inhibition

Example: 1) Enzyme - Xanthine oxidase; Substrate - Hypoxanthine; Inhibitor – Allopurinol.

Significance of the inhibitor: Used in the control of Gout to reduce excess production of uric acid from hypoxanthine.

Example: 2) Enzyme - Succinate dehydrogenase; Substrate - Succinate; Inhibitor - Malonate

ii) Noncompetitive inhibition:

Usually a noncompetitive inhibitor binds either to free enzyme or to ES complex at a site other than the active site on the enzyme surface. This results in the change of conformation of the enzyme as well as its active site, which makes the substrate unable to bind to the enzyme effectively. This type of inhibitor has no structural resemblance with the substrate like competitive inhibitors.

Non-competitive inhibitors do not interfere with the enzyme-substrate binding. But catalysis is prevented, possibly due to the distortion of enzyme conformation.

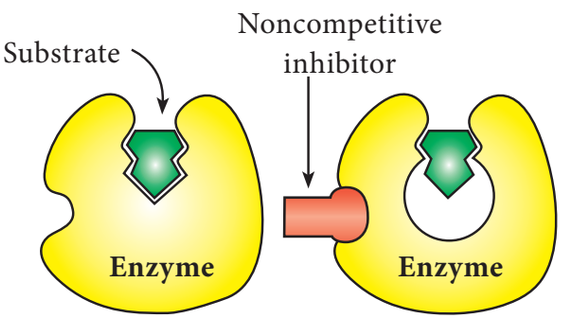

Figure 4.9 Noncompetitive inhibition

iii) Uncompetitive inhibition:

Uncompetitive inhibitors binds only to the ES complex. However, the binding of the inhibitor affects the binding of the substrate. By adding the inhibitor usually follows an allosteric effect where it binds to a different site on the enzyme than the substrate. This binding to an allosteric site changes the conformation of the enzyme so that the

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affinity of the substrate for the active site is reduced.

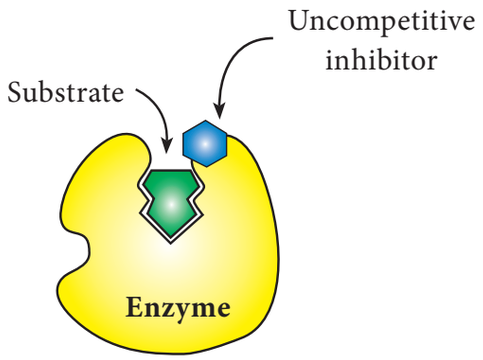

Figure 4.10 Uncompetitive inhibition

Table 4.2: Comparison between Competitive and Noncompetitive inhibition

Sl. No.	Competitive inhibition	Noncompetitive inhibition
1.	Inhibitor resembles the substrate	Inhibitor has no structural resemblance with the substrate.
2.	Inhibitor binds at the active site.	Inhibitor binds at a site other than active site
3.	Enzyme binds either with substrate or inhibitor	Enzyme binds with both substrate and inhibitor
4.	Reversible	Reversible
5.	Can be overcome by increasing substrate concentration.	Cannot be overcome by increasing substrate concentration

4.6 Industrial applications of enzymes

Enzymes are widely used in food, pharmaceutical and chemical industries. Bacterial enzymes are used for the fermentation of food items. For example,

i. Making of curd from milk by *Lactobacillus acidophilus*.

ii. Producing yoghurt and cheese from milk by *Streptococcus thermophilus*.

iii. Fermenting rice and blackgram by *Leucanostoc mesenteroides* for preparing delicious idlies.

iv. In washing powders enzymes are incorporated to remove stains from clothes.

v. Fructose syrup is produced by the isomerization of glucose by glucose isomerase.

vi. Penicillin acylase is used to convert penicillin to semisynthetic penicillins.

vii. Glucose and galactose are produced from the discarded portion during cheese making by lactase enzyme

viii. Enzymes are used for desizing fabrics. This kind of enzymatic desizing does not weaken the fabrics.

ix. In the manufacture of leather, the hide is made free from hair. This is done by employing pancreatic enzymes.

x. Pepsin is used to digest gelatin in the process of recovering silver from photographic films.

4.7 Medical applications of enzymes

i. Streptokinase or Urokinase is sometimes used to lyse intravascular blood clots.

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ii. Gastrointestinal tract enzymes (pepsin, trypsin and lipase) are given to patients suffering from indigestion.

iii. Enzyme asparaginase is used as an anticancer drug.

iv. Enzymes are used to diagnose various diseases such as AIDS.

v. Immobilized enzymes like glucose oxidase (GOD) and peroxidase (POD) are used for the estimation of blood glucose.

Table 4.3 Some important therapeutic enzymes

Enzyme	Reaction	Therapeutic use
Asparaginase	L-Asparagine + H_2O → L-aspartate + NH_3	Leukaemia
Collagenase	Collagen hydrolysis	Skin ulcers
Glutaminase	L-Glutamine + H_2O → L-glutamate + NH_3	Leukaemia
Hyaluronidase	Hyaluronate hydrolysis	Heart attack
Lysozyme	Bacterial cell wall hydrolysis	Antibiotic
Ribonuclease	RNA hydrolysis	Antiviral
β-Lactamase	Penicillin → penicilloate	Penicillin allergy
Streptokinase	Plasminogen → plasmin	Blood clots
Trypsin	Protein hydrolysis	Inflammation
Uricase	Urate + O_2 → allantoin	Gout
Urokinase	Plasminogen → plasmin	Blood clots

Serum enzymes are used as markers to detec cellular damage which in turn helps in the diagnosis of diseases.

Table 4.4 Serum enzymes

Serum enzyme	Disease
Amylase	Acute pancreatitis
GPT or ALT	Liver diseases (hepatitis), jaundice, cirrhosis of liver
GOT or AST	Heart attack
Alkaline phosphatase	Rickets, obstructive jaundice, bone cancer, hyperparathyroidism.
Acid phosphatase	Cancer of prostate gland

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Serum enzyme	Disease
Lactate dehydrogenase (LDH)	Heart attack, liver diseases, leukemia, pernicious anemia
Creatine Kinase (CK)	Myocardial infarction (early marker), hypothyroidism, alcoholism.
Aldolase	Muscular dystrophy, liver diseases
5´-Nucleotidase	Hepatitis, obstructive jaundice, tumour
γ-Glutamyltranspeptidase (γ-GT)	Alcoholism, infective hepatitis, obstructive jaundice.

EVALUATION

I. Choose the correct answer

1. The catalytic activity of an enzyme is restricted to its small portion called

   (a) active site (b) passive site

   (c) allosteric site (d) all Choices are correct

2. An active enzyme made of polypeptide chain and a co-factor is

   (a) coenzyme (b) substrate

   (c) apoenzyme (d) holoenzyme

3. In human body the optimum temperature for enzymatic activities is

   (a) 37^0C (b) 40^0C

   (c) 25^0C (d) 30^0C

4. Enzymes are sensitive to

   (a) changes in pH (b) changes in temperature

   (c) both a and b (d) none of these

5. Enzyme B requires Zn^{2+} in order to catalyze the conversion of substrate X. The zinc is best identified as a(n):

   (a) coenzyme (b) activator

   (c) substrate (d) product

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II. Fill in the blanks

6. Glutamine synthetase is an example for the class of enzymes called ----------
7. The inhibitor that resembles the substrate is called ----------
8. ---------- is used to digest gelatin in the process of recovering silver from photographic films
9. ---------- and ---------- are the enzymes used therapeutically to treat blood clots
10. ---------- is the serum enzyme that acts as an early marker for myocardial infarction.

III. True or False.

11. Enzyme substrate complex is a permanent stable complex.
12. Malonate is the competitive inhibitor of succinate dehydrogenase.
13. An enzyme substrate complex is formed in all the enzymatic reactions.
14. The degree of competitive inhibition cannot be decreased by increasing the concentration of the substrate.
15. An uncompetitive inhibitor has affinity towards ES complex.

IV. Give short answers for the following:

16. What are enzymes? Why are enzymes important for living organisms?
17. Is there a difference between the initial and the final energy levels in catalyzed and non-catalyzed reactions?
18. Give any two examples for an enzymatic reaction.
19. Justify the need for a systematic nomenclature for enzymes.
20. Relate the reaction pH with enzyme activity.

V. Answer the following:

21. What are enzyme cofactors? Describe the relationship between vitamins and enzyme cofactors?
22. With appropriate examples describe the different classes of enzymes and their nomenclature system.

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23. Describe the different types of enzyme inhibition mechanisms.

24. Compare competitive and non-competitive inhibition.

25. Write about the various industrial applications of enzymes.

26. Describe the different medical applications of enzymes.

27. Write in detail about the main factors that alter the speed of enzymatic reactions.

SUMMARY

Enzymes are proteins that work as biological catalysts. They catalyse the biochemical reactions that occur in living organisms. They are highly specific for a given reaction and accelerate the reaction rates to many folds. They help breakdown the food molecules to harvest energy from them and also to synthesise the necessary macromolecules for the development of living organisms. Enzymes have their maximum activity at a particular pH and temperature which are called the optimum pH and temperature for that enzyme. The concentration of the enzyme and the substrate concentration influence the activity of a particular enzyme. Some enzymes do require small organic molecules or metal ions in order to carry out their functions, these molecules are called co enzymes/cofactors.

Enzymes are classified into six classes based on their functions viz. oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases. The International Union of Biochemistry and Molecular Biology (IUBMB) has assigned a 4 digit number called Enzyme Commission (EC) number.

The activity of an enzyme can be inhibited by small molecules which are known as inhibitors. Based on the type of inhibition they can be classified as competitive, uncompetitive and noncompetitive inhibitors.

Enzymes have many applications in industries as well as in medicine.

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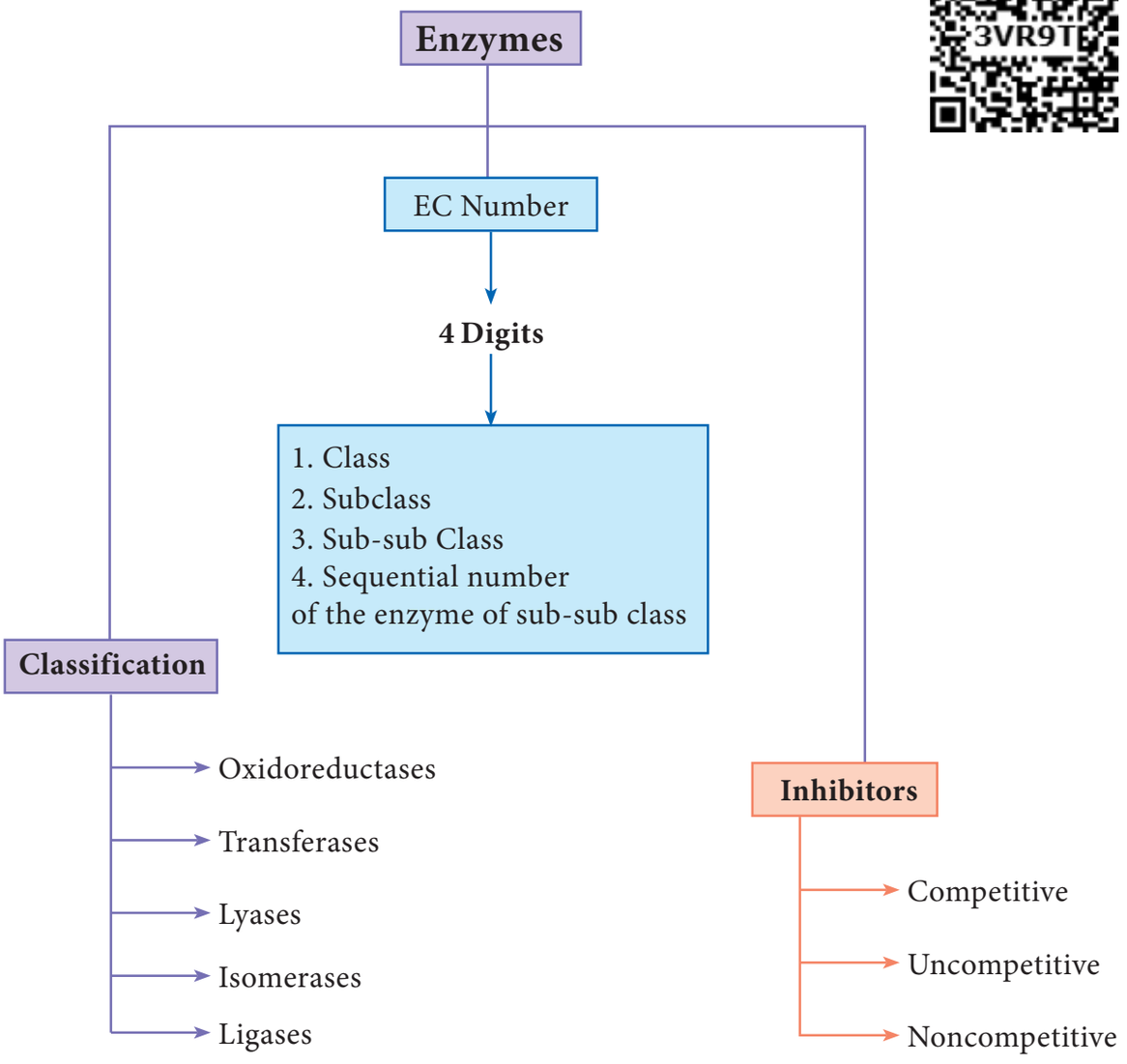
CONCEPT MAP



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Unit 5 CARBOHYDRATES



Gerty Cori

Gerty Cori along with her husband Carl Cori made important discoveries in Carbohydrate biochemistry. They were particularly interested in glucose metabolism and its hormonal regulation. Their proposed cycle of reactions is now known as Cori's cycle. They were awarded the Nobel prize in 1947 for their discoveries. Gerty Cori was the first American woman to receive a Nobel prize.

The US government has also released an US Postal Service stamp in Honour of Gerty Cori. The Cori craters in the moon and venus were also named after them.

Learning Objectives

After reading this Chapter, you will be able to:

- Explain the functions of carbohydrates
- Draw the chemical structure of glucose, galactose, fructose, maltose, lactose and sucrose.
- Write the physical and chemical properties of carbohydrates
- Draw the Haworth projection of glucose, galactose and fructose
- Describe the structural arrangement of the Homo polysaccharides (Starch & Glycogen) and Hetero polysaccharides (Heparin and hyaluronic acid)

Introduction

Carbohydrates are widely distributed in plants and animals. Plants produce carbohydrates from carbon dioxide and water by photosynthesis. The important carbohydrate found in plants is starch. Animals mainly depend upon plant sources for their carbohydrates. The carbohydrate stored in animals is glycogen.

Chemically, carbohydrates are defined as polyhydric aldehyde or ketone or compounds which produce them on hydrolysis.

Example: Glucose, fructose, starch, cellulose, glycogen etc.

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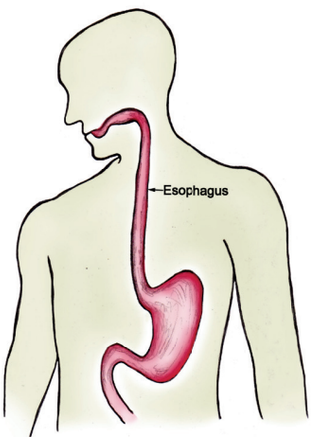
5.1 A Primary Source of Energy

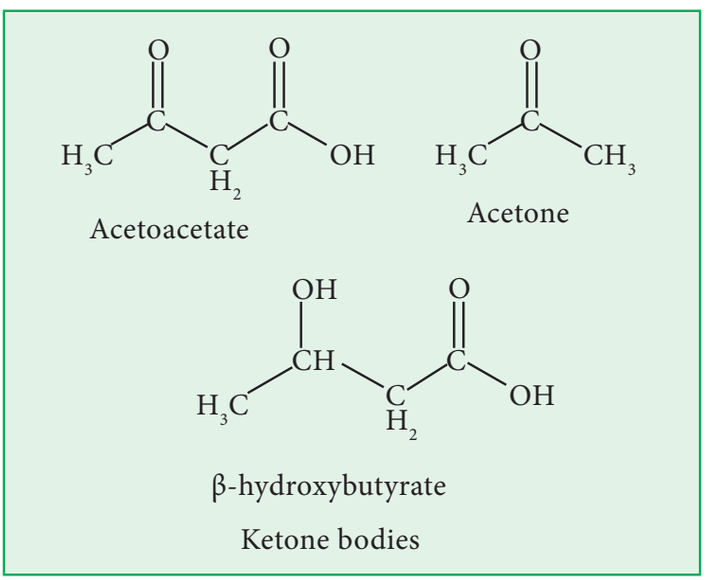
Digestion of carbohydrates starts in the mouth. Enzymes in saliva begin to break down carbohydrates. The Carbohydrates travel through the esophagus, stomach and enter the small intestine.



In the small intestine, carbohydrates get further broken down into single carbohydrate units called monosaccharides. These single molecules get absorbed across the intestine wall and are sent through the blood stream. Carbohydrate in the blood is in the form of a monosaccharide called glucose. The more carbohydrate eaten at one time, the more glucose will be released into the blood after digestion.

It is important to note, that fats and proteins can also be burnt to provide energy but fats are only burned if there is non-availability of carbohydrates. When fat is burnt in absence of carbohydrates, toxic compounds called ketone bodies are produced.

Accumulation of these ketone bodies over a long period causes a condition called 'Ketosis" In this condition, blood is unable to carry oxygen properly and this can be fatal. Thus, "one of the important functions of carbohydrate is to help burn fat properly".



i. As a source of energy:

The main function of carbohydrate is to supply energy for the body processes. A greater part of the energy in the diet (more than 50-80%) is supplied by carbohydrates. Some of the carbohydrates are immediately utilized by the tissues and the remaining are

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stored as glycogen in the liver and muscles and some are stored as adipose tissues for future energy needs.

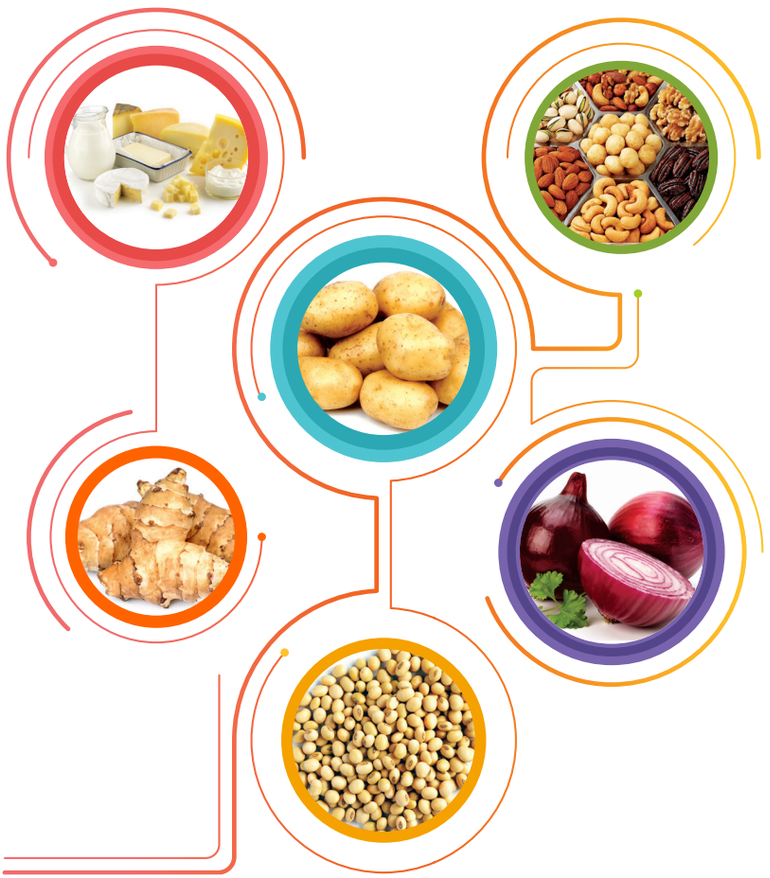

Figure 5.1 Foods rich in carbohydrate

ii. Protein-sparing action:

Carbohydrates are mainly utilized by the body for fulfilling the major part of the energy needs, thus sparing protein for tissue building and repairing. The first physiological demand of the body is the need for energy, which must be satisfied before the nutrients are used for other functions. So, this function of carbohydrates is to spare protein for body building and repair of tissues.

iii. Essential for Fat Oxidation:

Even though fat yields twice as much as energy as carbohydrate per unit weight, carbohydrate is essential for the oxidation of fats. The common expression that 'fat burns in the fire of carbohydrates' is used to emphasize, that in absence of carbohydrates, fats cannot be oxidised by the body to yield energy. A breakdown product of carbohydrate is essential for the oxidation of acetate, which is the breakdown product of fats.

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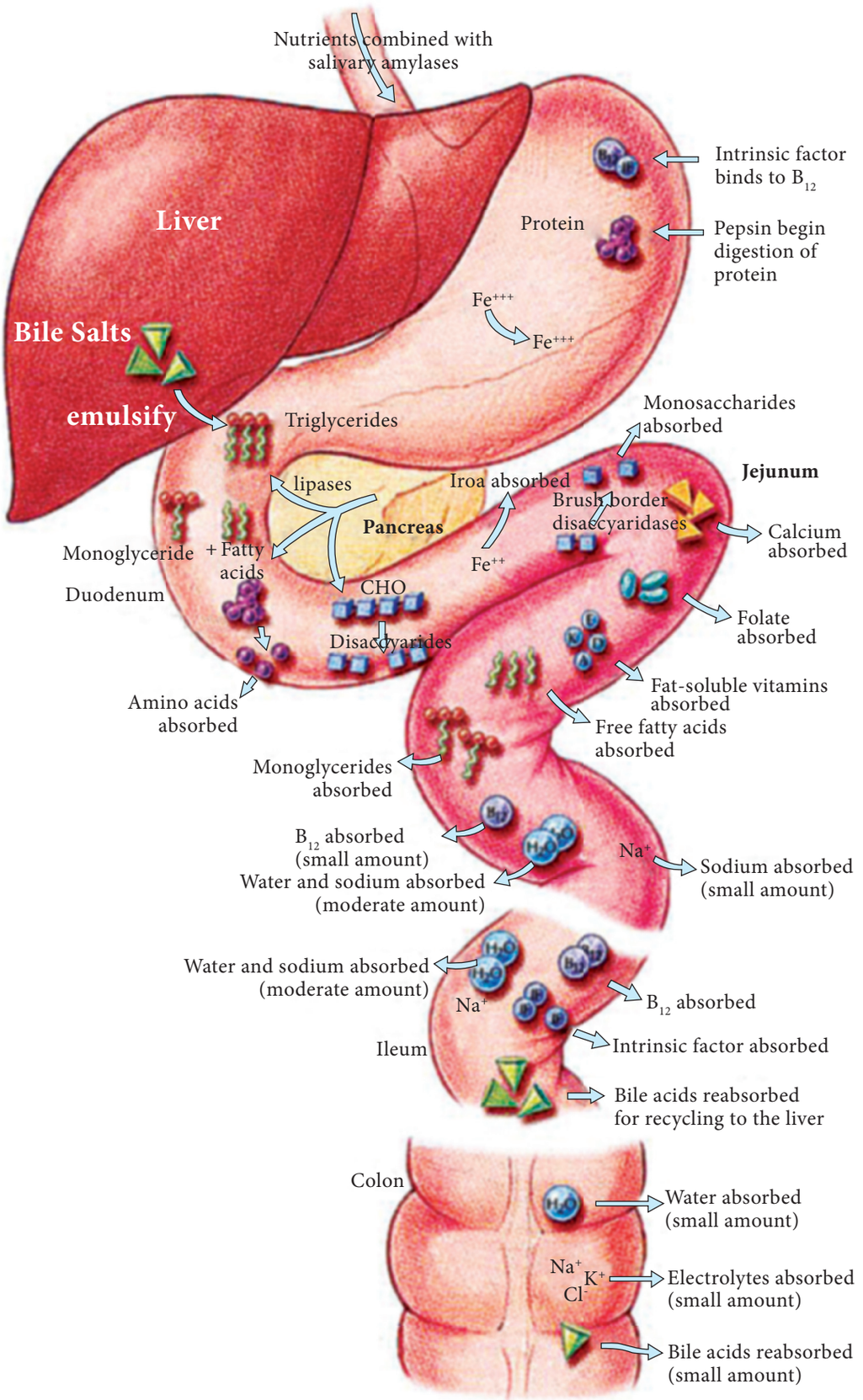

Figure 5.2 Digestion and absorption of nutrients in the gastrointestinal tract

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iv. Role in gastro-intestinal function:

Carbohydrates play an important role in the gastro-intestinal functions of mammals. The digestive system changes carbohydrates into glucose, also known as blood sugar. Some glucose is used for energy and the rest is stored in the liver and muscles for later use. When blood sugar rises, pancreas pumps out more and more insulin, a hormone that tells cells to absorb glucose for energy or storage. As cells absorb glucose, blood sugar levels begin to fall, which signals the pancreas to start making glucagon, a hormone that tells the liver to release stored glucose.



Anticoagulants, commonly referred to as blood thinners, are chemical substances that prevent or reduce coagulation of blood, prolonging the clotting time.

v. Carbohydrate functions as Antigen:

Many antigens are glycoproteins (which contain oligosaccharides) in nature and give immunological properties to the blood.

vi. Carbohydrate functions as Hormone:

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

vii. Carbohydrates provide raw material for industry:

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

viii. Other Functions

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

Cellulose acts as roughage of food. It stimulates peristaltic movement and in the secretion of digestive enzymes.

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

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5.2 Classification

Carbohydrates are often referred to as saccharides. They are classified into three main groups

i) Monosaccharides ii) Oligosaccharides iii) Polysaccharides.

5.2.1 Monosaccharides:

Monosaccharides are the simplest group of carbohydrates which cannot be hydrolysed further. They are often referred to as "simple sugars". They have the general formula $C_n(H_2O)_n$. They can be further subdivided on the basis of the number of carbon atoms (triose, tetrose, pentose etc.) and on the basis of the functional group (aldose, ketose) as given in Table – 5.1.

Table – 5.1 Classification of monosaccharides

Number of 'C' atoms	Name of the monosaccharide	Aldose	Ketose
3	Triose	Glyceraldehyde (Glycerose)	Dihydroxyacetone
4	Tetrose	Erythrose	Erythrulose
5	Pentose	Ribose	Ribulose
6	Hexose	Glucose	Fructose
7	Heptose	Glucoheptose	Sedoheptulose

5.2.2 Oligosaccharides :

Oligosaccharides are the carbohydrates which yield two to ten monosaccharide units on hydrolysis. They are further classified into di, tri, tetra (or) penta saccharides, respectively, containing 2, 3, 4 (or) 5 monosaccharide units.

Example:

a) Maltose (disaccharide) (Glucose + Glucose)

b) Sucrose (disaccharide) (Glucose + Fructose)

c) Lactose (disaccharide) (Glucose + Galactose)

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d) Isomaltose (disaccharide) (Glucose + Glucose)

e) Raffinose (Trisaccharide) (Fructose + Glucose + Galactose)

f) Stachyose (tetrasaccharide) (Galactose + Galactose + Glucose + Fructose)

g) Verbascose (pentasaccharide) (Galactose + Galactose + Galactose + Glucose + Fructose)

5.2.3 Polysaccharides

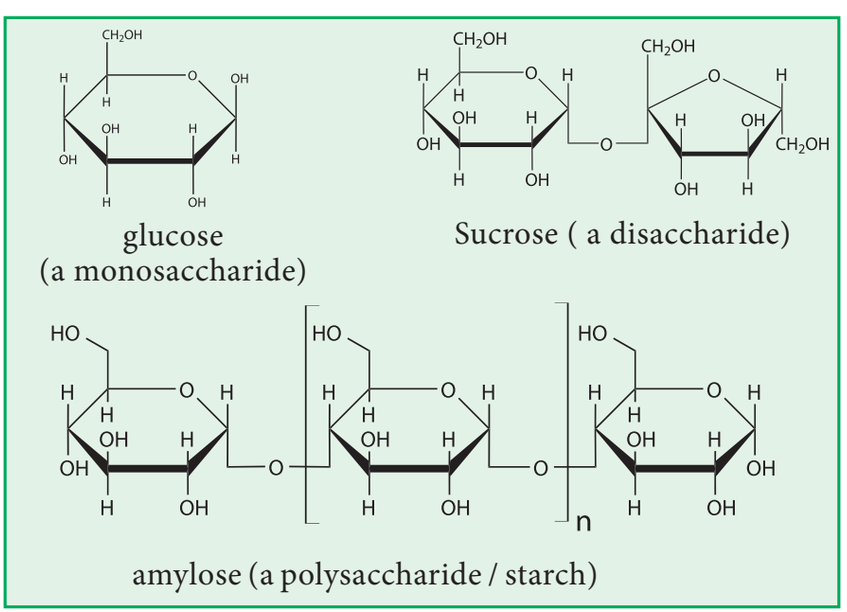
Polysaccharides are the carbohydrates which yield more than ten molecules of monosaccharides on hydrolysis. They are usually tasteless (non-sugars). They have the general formula $(C_6H_{10}O_5)_n$.

a) The polysaccharides composed of a single type of monosaccharide are called homoglycans (or) homopolysaccharides. They have the general formula $(C_6H_{10}O_5)_n$.

Example : Starch, cellulose, glycogen, insulin.

b) Polysaccharides composed of two (or) more different types of monosaccharides are called heteroglycans (or) heteropolysaccharides.

Example : Agar, heparin, pectins, gum arabic, hyaluronic acid etc.



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5.3 Structure of Glucose, Fructose and Galactose

5.3.1 Glucose:

Glucose has the molecular formula ($C_6H_{12}O_6$) and has a six member ring. Glucose may be represented by the following open chain structure. But in solution it exists only as a six membered ring structure called pyranose. Glucose is known as grape sugar.

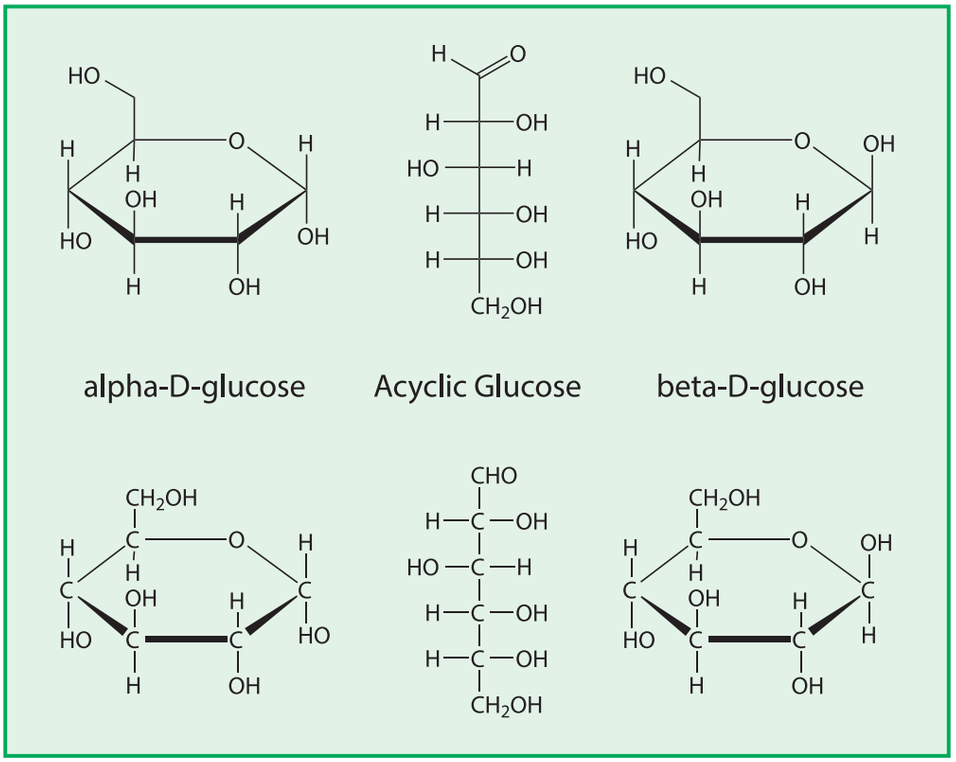

Figure 5.3 Structures of Glucose



Glucose is eaten, absorbed into the blood stream, and makes its way to the liver where it is broken down to supply energy to the entire body. This breaking down process requires insulin.

5.3.2 Fructose

Fructose has the molecular formula ($C_6H_{12}O_6$). Fructose exists mostly as a five membered ring structure called "furanose form". Fructose is known as fruit sugar as its source in the diet is fruits and vegetables. Honey is also a good source.

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Fructose is more soluble than other sugars and hard to crystallize because it is more hygroscopic and holds onto water stronger than the others. This means that fructose can be used to extend the shelf life of baked products more than other sugars.

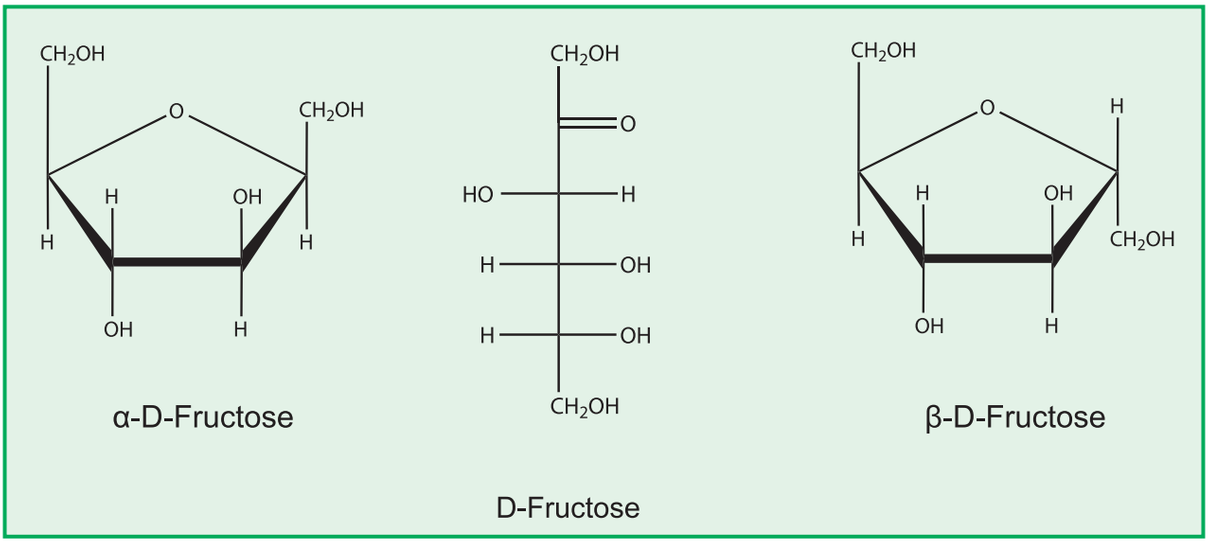

Figure 5.4 Structures of Fructose

5.3.3 Galactose

Galactose is a monosaccharide and has the same chemical formula as glucose, i.e., $C_6H_{12}O_6$. It is similar to glucose in its structure, differing only in the position of one hydroxyl group. This difference, however, gives galactose different chemical and biochemical properties to glucose. In solution, it forms 5- and 6-membered rings but also exists in linear form. Small amounts of lactose and galactose can appear in nondairy foods.

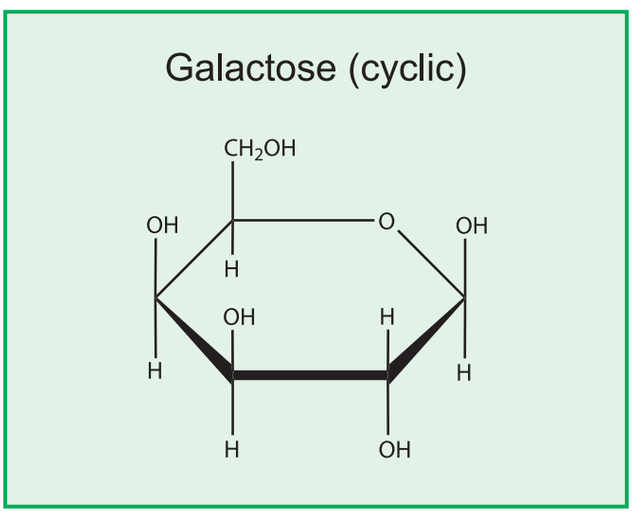

Figure 5.5 Pyranose structure of galactose

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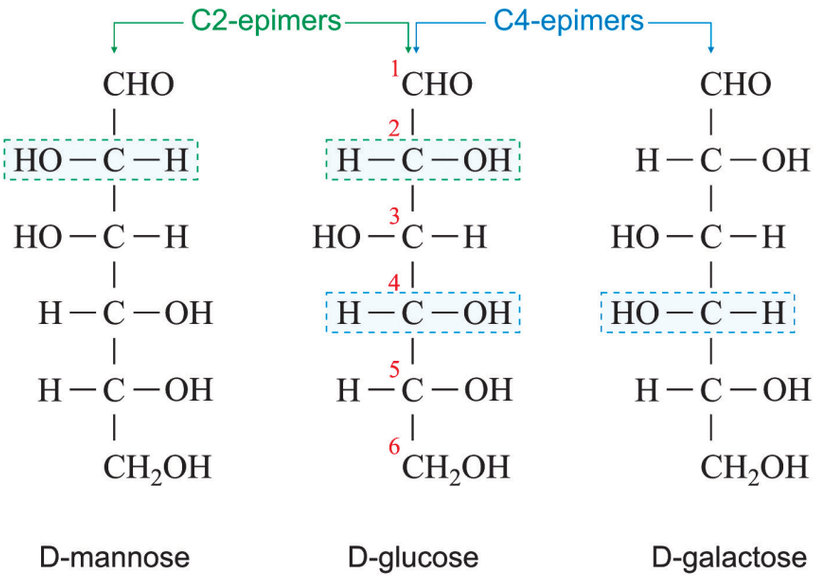
Epimers

D-sugars differing in configuration at a single asymmetric center are known as epimers. Thus D-glucose and D-galactose are epimers at C4 ; D- glucose and D-mannose are epimers at C2.



Anomers:

Sugars differing in configuration at the C1 asymmetric center are known as anomers. The C1–carbon is called as anomeric carbon. Thus α-D and β-D forms of glucose are anomers.

Mutarotation:

Mutarotation was discovered by the chemist Dubrunfaut in 1814. Mutorotation is the change in optical rotation due to change in equilibrium between two anomers.

When D-glucose is crystallized at room temperature and a fresh solution is prepared, its specific rotation of polarized light is +112°; but after 12-18 hours it changes to +52.5°. If initial crystallization is taking place at 98° C and then solubilized, the specific rotation is found to be +19°, which also changes to +52.5° within few hours. This change in rotation with time is called mutarotation.

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5.4 Properties of Glucose, Fructose and Galactose:

5.4.1 Glucose:

- Glucose can be a solid or liquid.
- Melting Point of glucose is 146˚C
- Molar mass of glucose is 180.16 g/mol
- Density of glucose is 1.54 g/cm^3
- Glucose is soluble in water and acetic acid

5.4.2 Fructose:

Fructose has a higher solubility than other sugars; therefore, it is harder for fructose to crystallize from an aqueous solution.

- Fructose is white powder
- Melting point of fructose is 103°C
- Molar mass of fructose is 180.16 g/mol
- Density of fructose is 1.69 g/cm^3.
- Fructose is soluble in water

5.4.3 Galactose:

- Galactose is White powder.
- Melting point of galactose is 167 °C
- Molar mass of galactose is 180.16 g/mol
- Soluble in water.

Note

Galactosemia is a rare genetic metabolic disorder that affects an individual's ability to metabolize the sugar galactose properly.

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Chemical Properties:

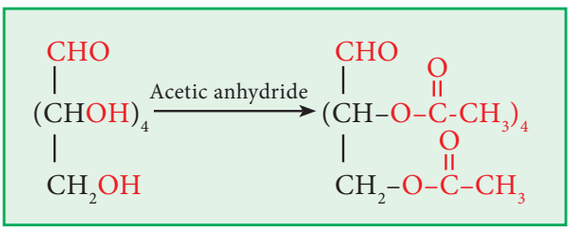
Reactions of Glucose, Fructose and Galactose:

i. Acidic character:

Both glucose and fructose behave as weak acids and form salts with $Ca(OH)_2$ (lime water).

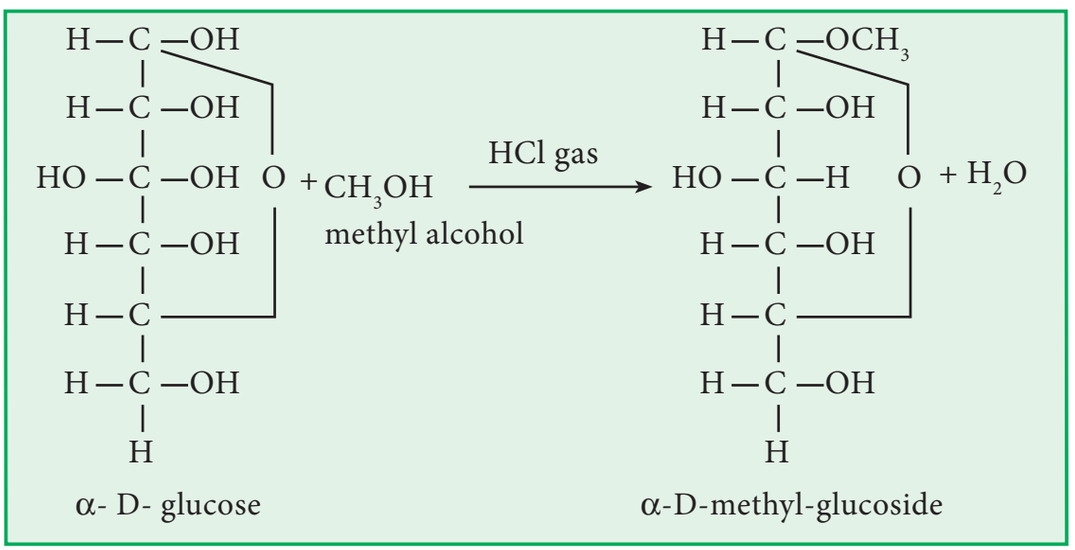
ii. Ester formation:

Glucose and Fructose form penta acetyl derivative when treated with acetic anhydride.



iii. Ether formation:

Glucose and fructose react with methanol in the presence of dry HCl gas to give ethers known as methyl glucoside and methyl fructoside, respectively.



iv. Reduction:

i. Sodium amalgum reduces glucose into sorbitol and fructose into a mixture of sorbitol and mannitol. Similarly, it reduces fructose into a mixture of sorbitol and mannitol.

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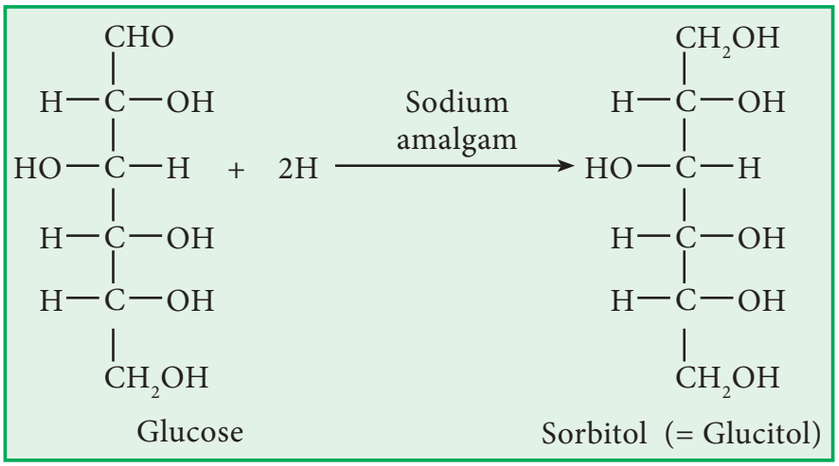
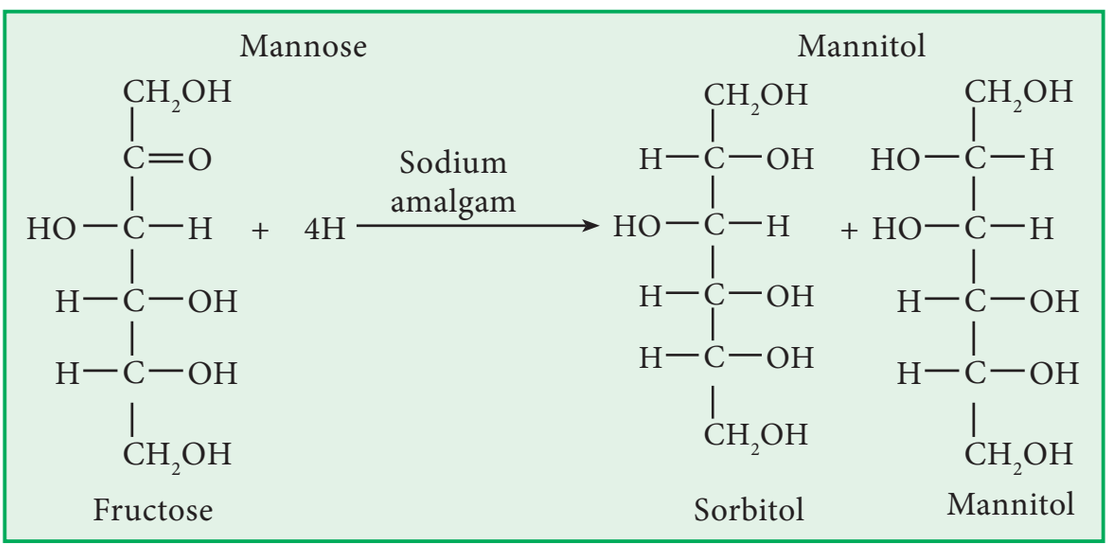
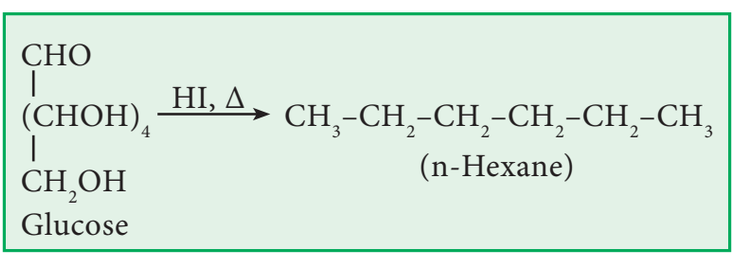




a. Both are reduced to n-hexane by HI / red 'P'.



b. Galactose on reduction with Na/Hg, gives dulcitol (and with HI/red P, n-hexane will be obtained).

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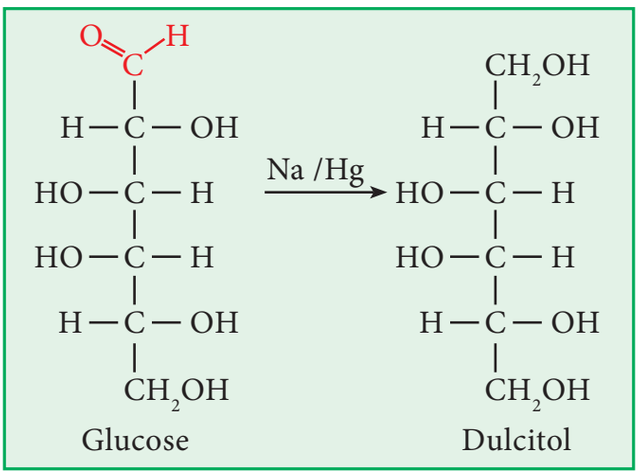
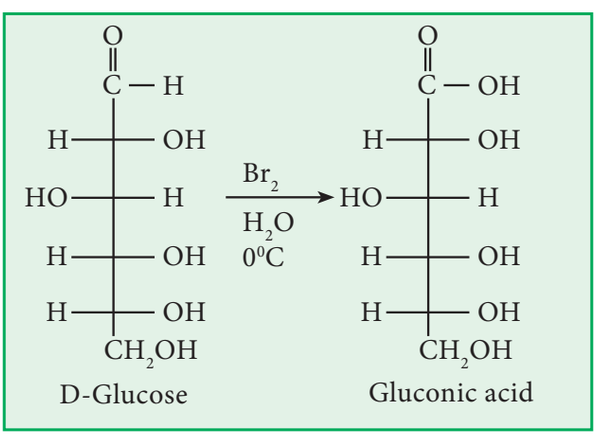


v. Oxidation:

a. Glucose is oxidized by mild oxidizing agents like bromine water into gluconic acid. Strong oxidizing agents like conc. HNO_3 oxidize glucose into gluconic acid.



b. Fructose is not oxidised by mild oxidising agents. But strong oxidising agents like conc. HNO_3 split fructose into a mixture of trihydroxy glutaric, tartaric and glycollic acids.

c. Galactose on oxidation with mild oxidizing agent such as bromine water, gives galactonic acid. On oxidation with strong oxidizing agents like HNO_3, it gives galactaric (or) mucic acid. This acid is insoluble in water and hence this reaction is used as a test for galactose. On oxidation with O_2/Pt-C (as in glucose, after protecting the –CHO group into ispropylidene group) it gives galacturonic acid.

vi. Action with Tollen's reagent:

Both glucose and fructose reduce Tollen's reagent into silver mirror.

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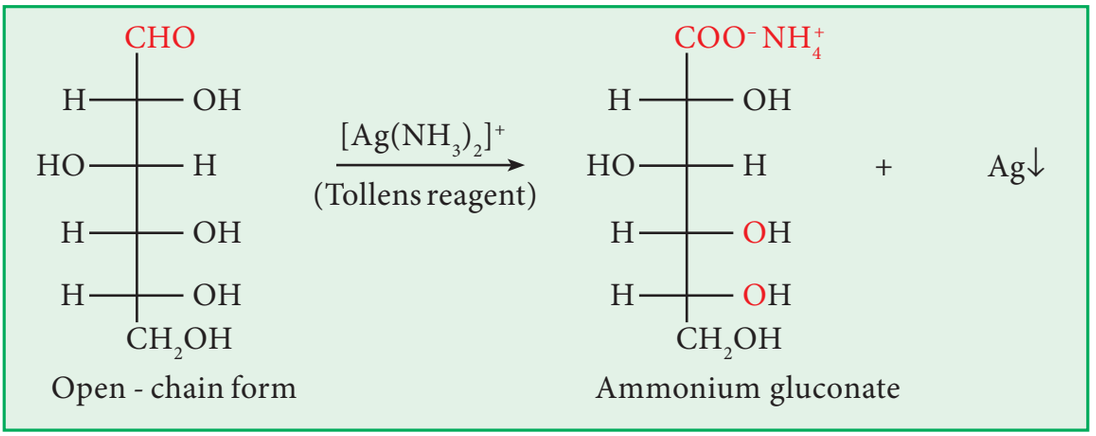
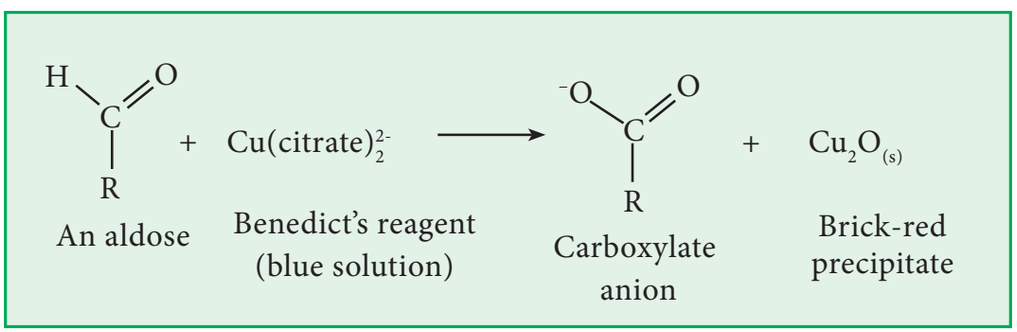


vii. Action with Fehling's solution:

Both glucose and fructose reduce Fehling's solution into red cuprous oxide.

viii. Action with Barfoed's and Benedict's reagents:

Both glucose and fructose reduce Barfoed's and Benedict's reagents into red cuprous oxide as in the case of Fehling's solution.



$$\text{Glucose} + CuO \rightarrow \text{Gluconic acid} + Cu_2O \downarrow$$

$$\text{Fructose} + CuO \rightarrow \text{Tartaric acid} + \text{glycollic acid} + Cu_2O \downarrow$$

Since both glucose and fructose reduce all these four reagents (Tollen's, Fehling's, Benedict's and Barfoed's reagent), these sugars are known as reducing sugars.

ix. Action with hydroxylamine:

a. Both glucose and fructose form oximes with hydroxylamine.

b. With NH_2OH, galactose forms galactose oxime.

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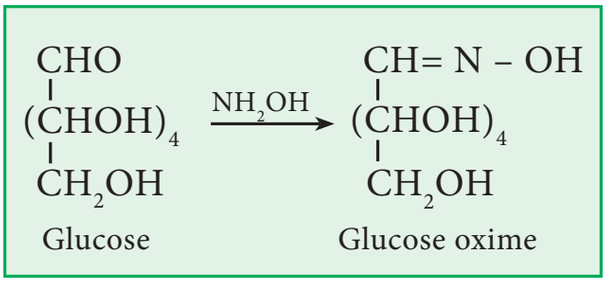
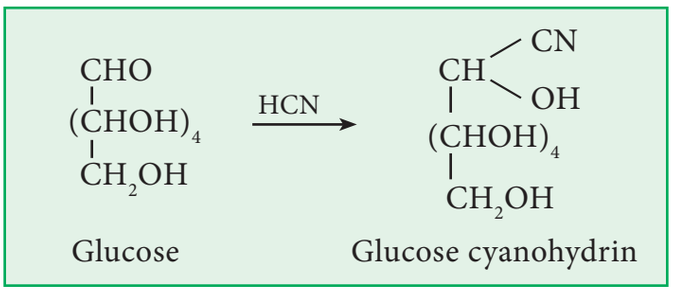


x. Action with HCN (Kiliani synthesis):

Both glucose and fructose form cyanohydrins with HCN.



Note

Tollen's Reagent:

Ammonical Silver Nitrate : $[Ag(NH_3)_2]NO_3$

Fehling's Reagent:

A- Copper(II) Sulfate

B- aqueous potassium sodium tartrate

Benedict's Reagent:

Mixture of sodium carbonate, sodium citrate and copper (II) sulfate

xi. Action with Conc. HCl:

Both glucose and fructose when heated with conc. HCl give laevulic acid.

xii. Action with alkalies:

When warmed with conc. alkali, sugars first turn yellow, then brown and finally resinify. But in the presence of dilute. alkali glucose and fructose give a mixture of D-glucose, D-mannose and D-fructose. This is known as Lobry de Bruyn - van Ekenstein rearrangement. This occurs through enediol formation.

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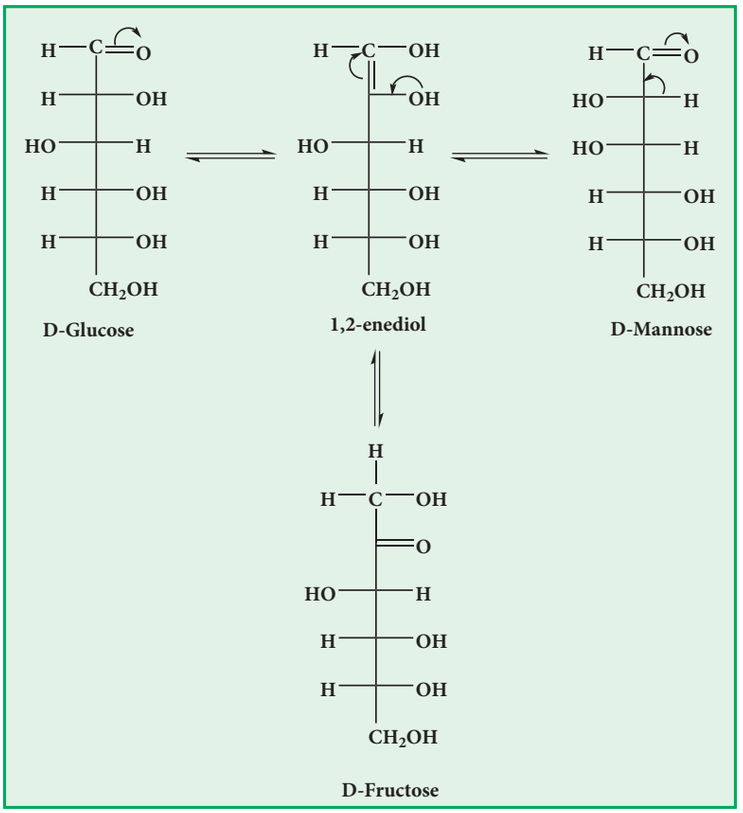
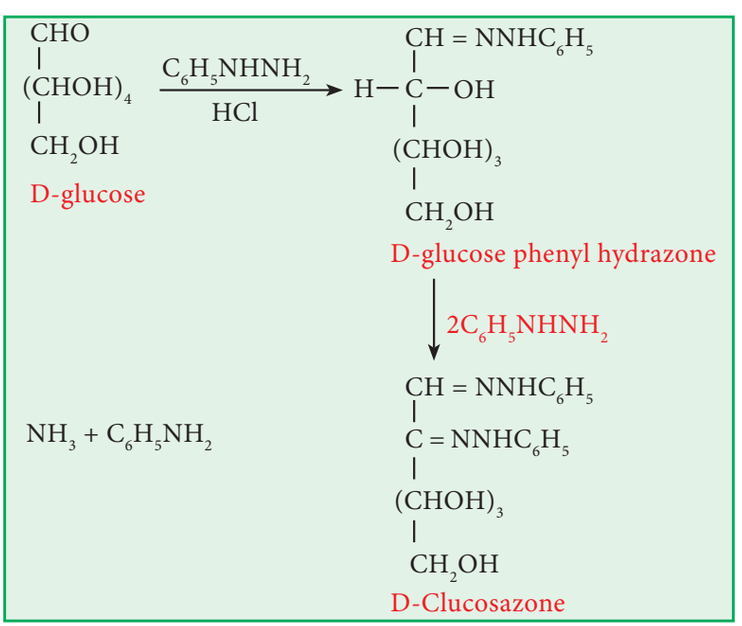


xiii. Osazone formation:

Both glucose and fructose react with excess of phenyl hydrazine to give the same type of osazone. Glucose and fructose have structural difference with respect to only first two carbon atoms, which are involved in osazone formation. The configurations in the rest of the carbon atoms are similar in both glucose and fructose. Hence they form similar osazone crystals.



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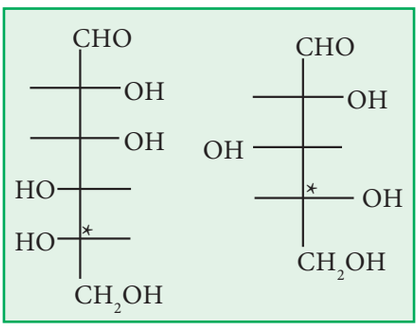
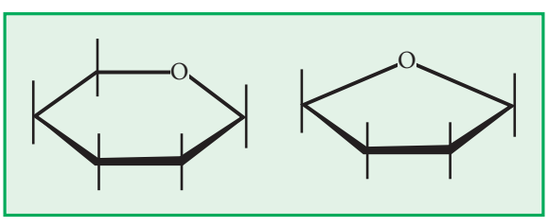
5.5 Haworth's Projection

A Haworth Projection is a common way of writing a structural formula to represent the cyclic structure of monosaccharides. It was named in the remembrance of the chemist Sir Norman Haworth. Let's consider two sugars, one an aldohexose, the other an aldopentose. Fischer projections are shown below. Recall the carbon with the star (*) next to it is the one that determines if the sugar is a D -sugar or an L-sugar.

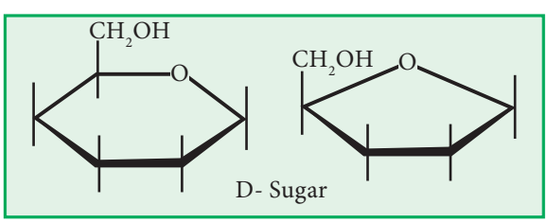


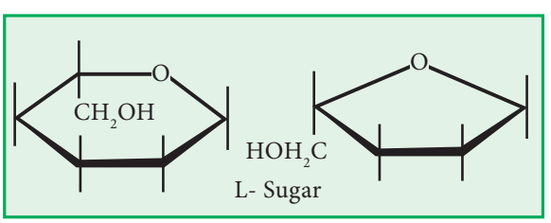
The Following are the rules.

1. Draw the basic structure for the sugar.

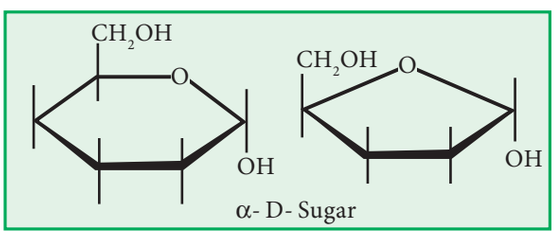


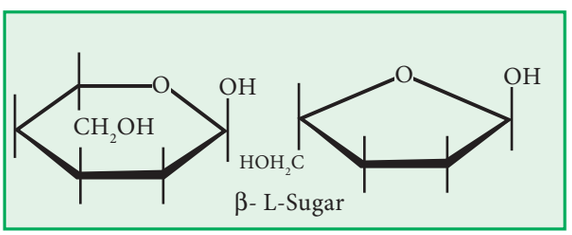
2. If the sugar is a D-sugar, place a $-CH_2OH$ above the ring on the carbon to the left of the oxygen, for an L-sugar place it below the ring.





3. For an α-sugar place an –OH below the ring on the carbon to the right of the ring oxygen, for a β-sugar place the - OH above the ring.





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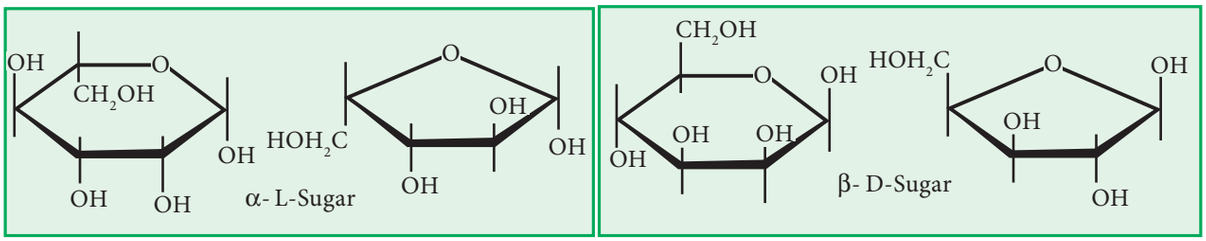
4. Finally, –OH groups on the right go below the ring and those on the left above, using the–CH_2OH group as the reference point for both projections.



5.6 Disaccharides

The carbohydrates which on hydrolysis give two monosaccharide units are known as disaccharides.

Example: Sucrose, maltose, lactose etc.,

5.6.1 Maltose:

Properties of Maltose:

a. Maltose or malt sugar is formed as an intermediate product in the acid hydrolysis of starch.

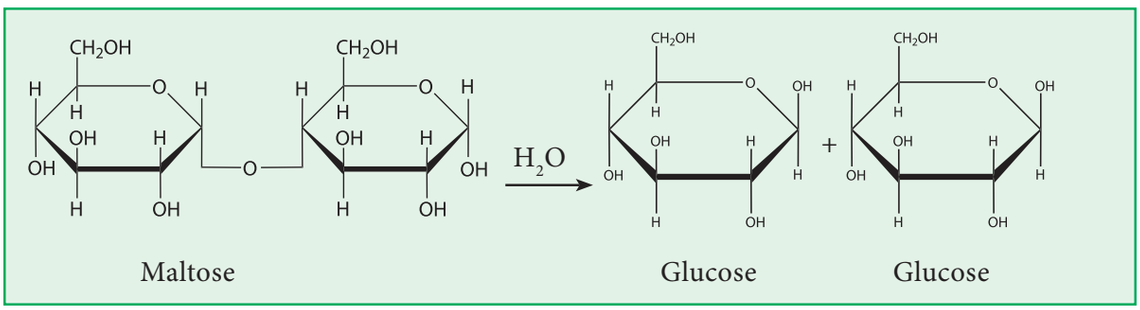
b. It is also produced during the course of digestion of starch by pancreatic amyalse.

c. It is a reducing disaccharide.

d. Maltose is composed of two α-D-glucose units held together by α(1-4) glycosidic linkage.

e. It is hydrolyzed by dilute acids (or) the enzyme maltase into two α - D - glucose units.

f. Maltose is readily fermented by yeast.



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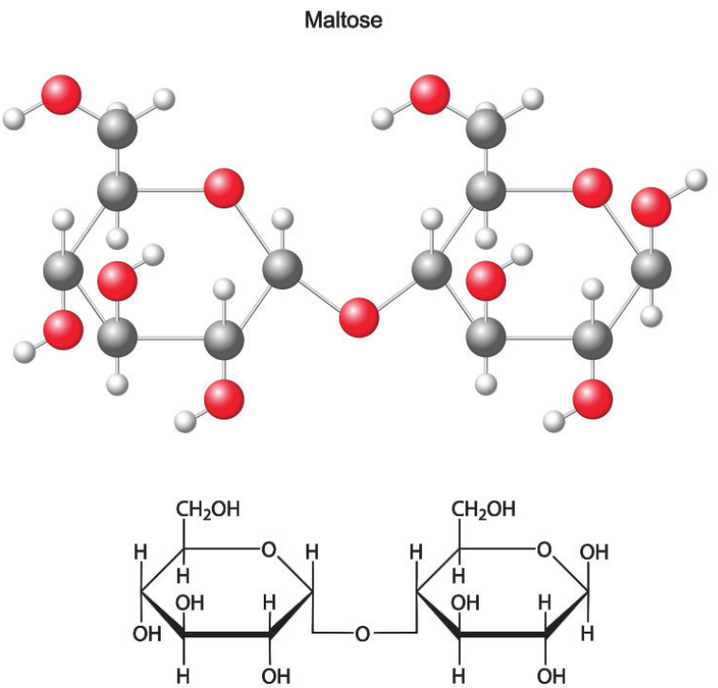
Structure of Maltose



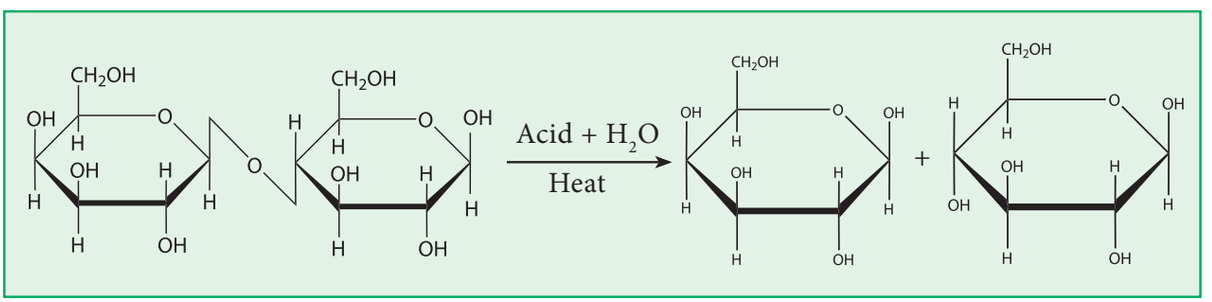
Maltose (α-D-glucopyranosyl-(1→4) α-D-glucopyranose)

5.6.2. Lactose :

a. Lactose is formed by the mammary glands. It is milk sugar.

b. It is a reducing sugar, forms osazone.

c. It is hydrolysed by acids and the enzyme lactase (β-D galactosidase) into one molecule of α- D- galactose and one molecule of α - D - glucose.



d. It is fermented by yeast.

e. In lactose, the galactose and glucose units are held together by α(1-4) linkage.

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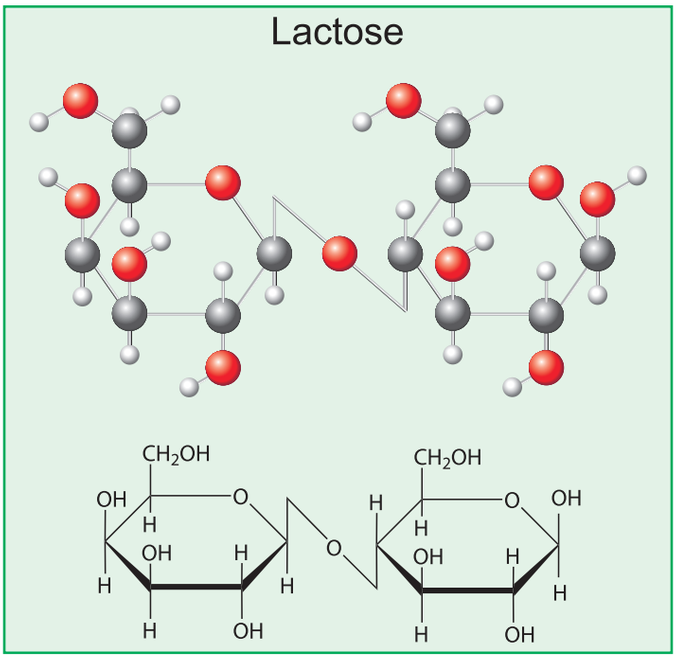
Structure of Lactose:



β-D-galactopyranosyl-(1→4) β-D-glucopyranose

(β- Lactose)

5.6.3 Sucrose:

Properties of sucrose:

a) When heated to 200°C it loses water to form a brown mass called caramel. On strong heating, it gives pure carbon with a burnt smell.

b) Concentrated sulphuric acid dehydrates sucrose into carbon. This on further oxidation by H_2SO_4 gives CO_2

$$C_{12}H_{22}O_{11} + H_2SO_4 \rightarrow C + H_2O + SO_2$$

c) When boiled with HCl, sucrose gives laevulic acid.

d) Concentrated nitric acid oxidises cane sugar (sucrose) to oxalic acid.

$$C_{12}H_{22}O_{11} + 9O_2 \rightarrow 6(COOH)_2 + 5H_2O$$

e) Sucrose is fermented by invertase into glucose and fructose which are converted to ethanol by zymase. Both these enzymes are available in yeast.

f) Sucrose on acetylation gives octa-acetyl derivative.

g) Sucrose on methylation gives octa-o-methyl derivative.

h) Sucrose does not react with HCN, NH_2OH, phenyl hydrazine, Tollen's reagent and Fehling's solution.

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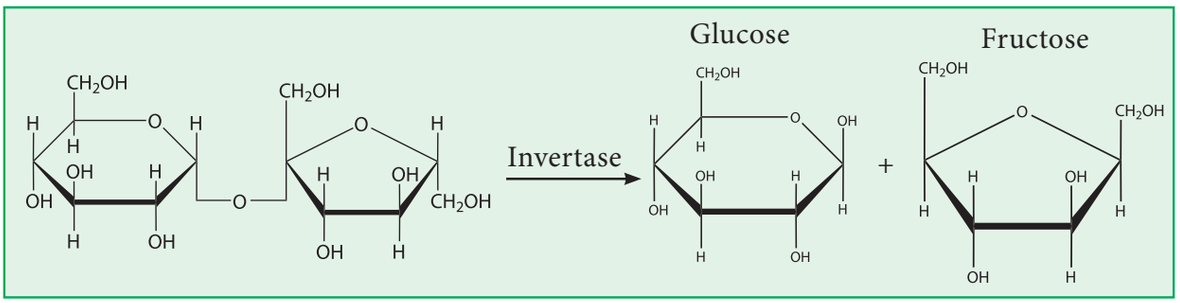
i) Controlled reduction of sucrose gives a mixture of sorbitol and mannitol.

j) It reacts with lime water $Ca(OH)_2$ to give calcium sucrate.

Hydrolysis of sucrose:

Sucrose is hydrolysed by dilute acids or enzymes like sucrase or invertase into an equimolar mixture of glucose and fructose.

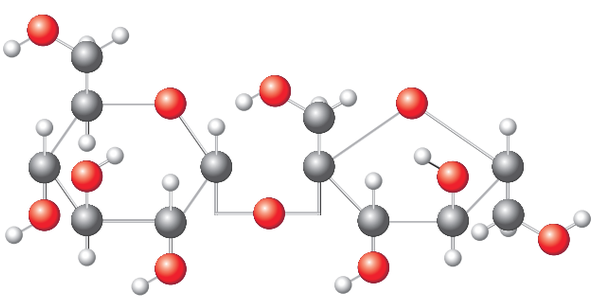


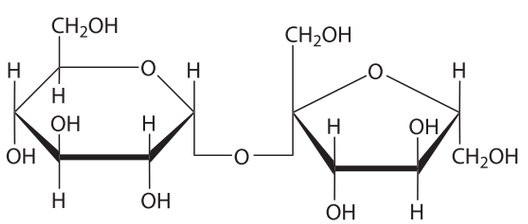
Sucrose is dextrorotatory. But the hydrolysed product is laevorotatory. Since the direction of rotation is reversed, this phenomenon is known as inversion of cane sugar. The mixture of sugars formed on hydrolysis is known as invert sugar.

According to Hudson, sucrose is first split into α-D(+) glucopyranose and β-D(+) fructofuranose, both are dextro rotatory. However, the less stable β-D(+) fructofuranose then sets up an equilibirum with its more stable isomer, α-D(–) fructopyranose which is strongly laevorotatory. Thus, the invert sugar gives a specific rotation of –28.2°.

Structure of Sucrose:

Sucrose (saccharose)





α-D-glucopyranosyl-β-D-fructofuranoside

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Table 5.2 Differences between glucose, fructose and galactose

S. No	Character	Glucose	Fructose	Galactose
1.	Optical rotation	Dexotro	Laevo	Dextro
2.	Solubility in water	Soluble in water	Soluble in water	Slightly soluble
3.	Reducing nature	Reducing sugar	Reducing sugar	Reducing sugar
4.	Action with Bromine water	Oxidised to gluconicacid	Not oxidised	Oxidised to galactonic acid
5.	Action with HNO_3	Oxidised to glucaric acid	Oxidised to a mixture of glycolic, tartaric and trihydroxy glutaric acid	Oxidised to mucic acid
6.	Action with Na/Hg	Reduced to sorbitol	Reduced to a mixture of sorbitol and mannitol	Reduced to dulcitol
7.	Osazone formation	Yellow coloured needle shaped crystals are formed within 10 minutes	Yellow coloured needle shaped crystals are formed within 5 minutes	Yellow coloured broad stick shaped crystals are formed after 20 minutes
8.	Mutarotation	Exhibits	Exhibits	Exhibits
9.	Nature	aldohexose	ketohexose	Aldohexose

5.3 Differences between sucrose, lactose and maltose

S. No	Character	Sucrose	Lactose	Maltose
1.	Commercial Name	Cane sugar	Milk sugar	Maltsugar
2.	Composition	(Glucose + Fructose)	(Galactose + Glucose)	(Glucose + Glucose)
3.	Glycosidic linkage	α(1-2)	α(1-4)	α(1-4)
4.	Reducing Nature	Non reducing Sugar	Reducing Sugar	Reducing Sugar
5.	Hydrolysing Enzyme	Invertase	Lactase	Maltase
6.	Osazone formation	Does not formed	Yellow colour osazone shaped crystals are formed	Yellow colour sun-flower shaped crystals are formed after 40 minutes

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5.7 POLYSACCHARIDES

Carbohydrates which contain more than 10 monosaccharide units are known as polysaccharides. Example : Starch, cellulose, glycogen, inulin etc.

5.7.1 HOMOPOLYSACCHARIDES

Starch:

a) Source:

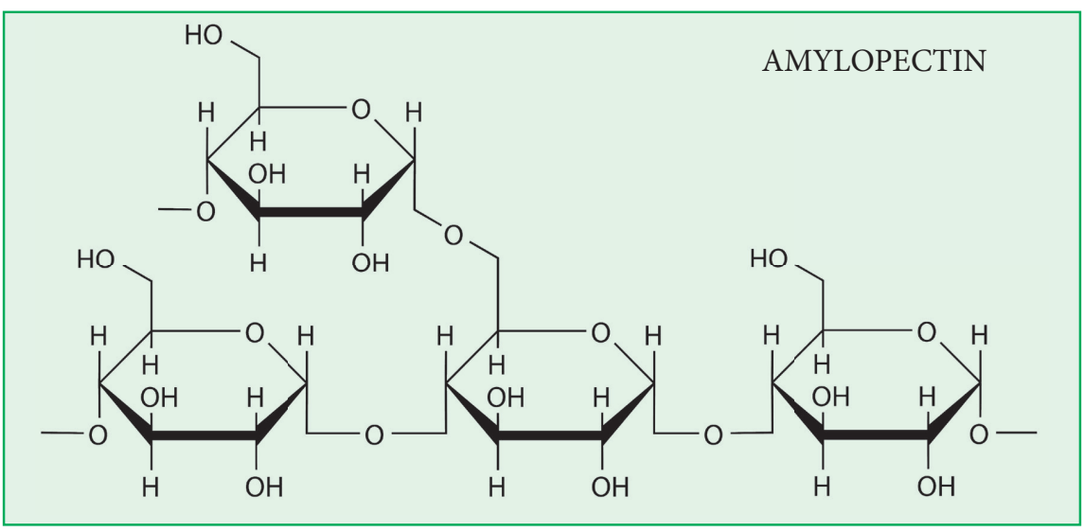
Plant materials such as roots, tubers, stem, vegetables, fruits and cereals are the main sources of starch.

b) Structure:

Starch is the nutritional reservoir in plants. Starch is a homopolysaccharide and consists of only α-D-glucose. The two chief constituents of starch are (i) Amylose (15-20%) and (ii) Amylopectin (80 - 85%).

Amylose forms the inner portion of the starch grain and is soluble in water. It is a linear, non-branched polymer of glucose. The glucose residues are united by α(1-4) linkage. The molecular weight of amylose is 60,000.

Amylopectin forms the outer covering of the starch grain and is insoluble in water. It is a highly branched polymer of glucose. The glucose residues are united by α(1- 4) linkages in the chains and by α (1 - 6) at the branch points. Its molecular weight is 2,00,000. It is like glycogen except for its lower degree of branching.



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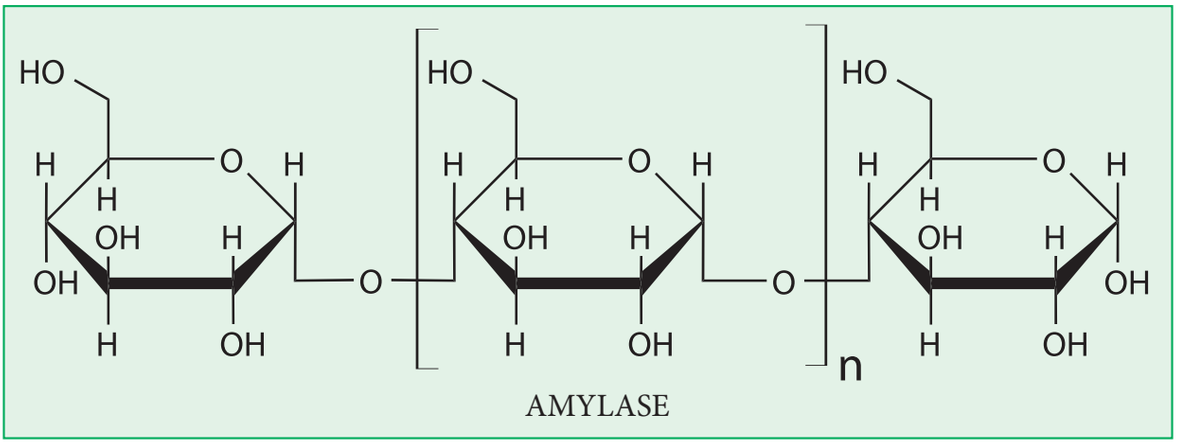


c) Hydrolysis of starch :

Starch is hydrolysed both by acids and enzymes. Both amylose and amylopectin are rapidly hydrolysed by α-amylase which is secreted by salivary glands and pancreas. α-Amylase acts upon starch and finally hydrolyses it into maltose molecules.

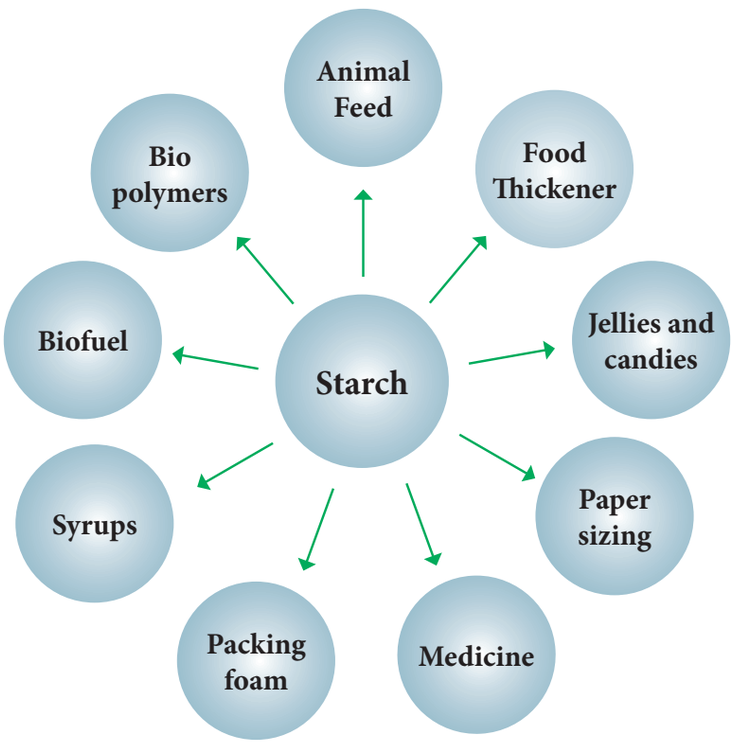
d) Uses : Starch is used

(i) as a food material. (ii) for the manufacture of glucose and alcohol.

(iii) in paper industry. (iv) in textile industry. (v) in printing.

(vi) to prepare starch acetate, nitrostarch etc. (vii) for making adhesives.

(viii) as an indicator.



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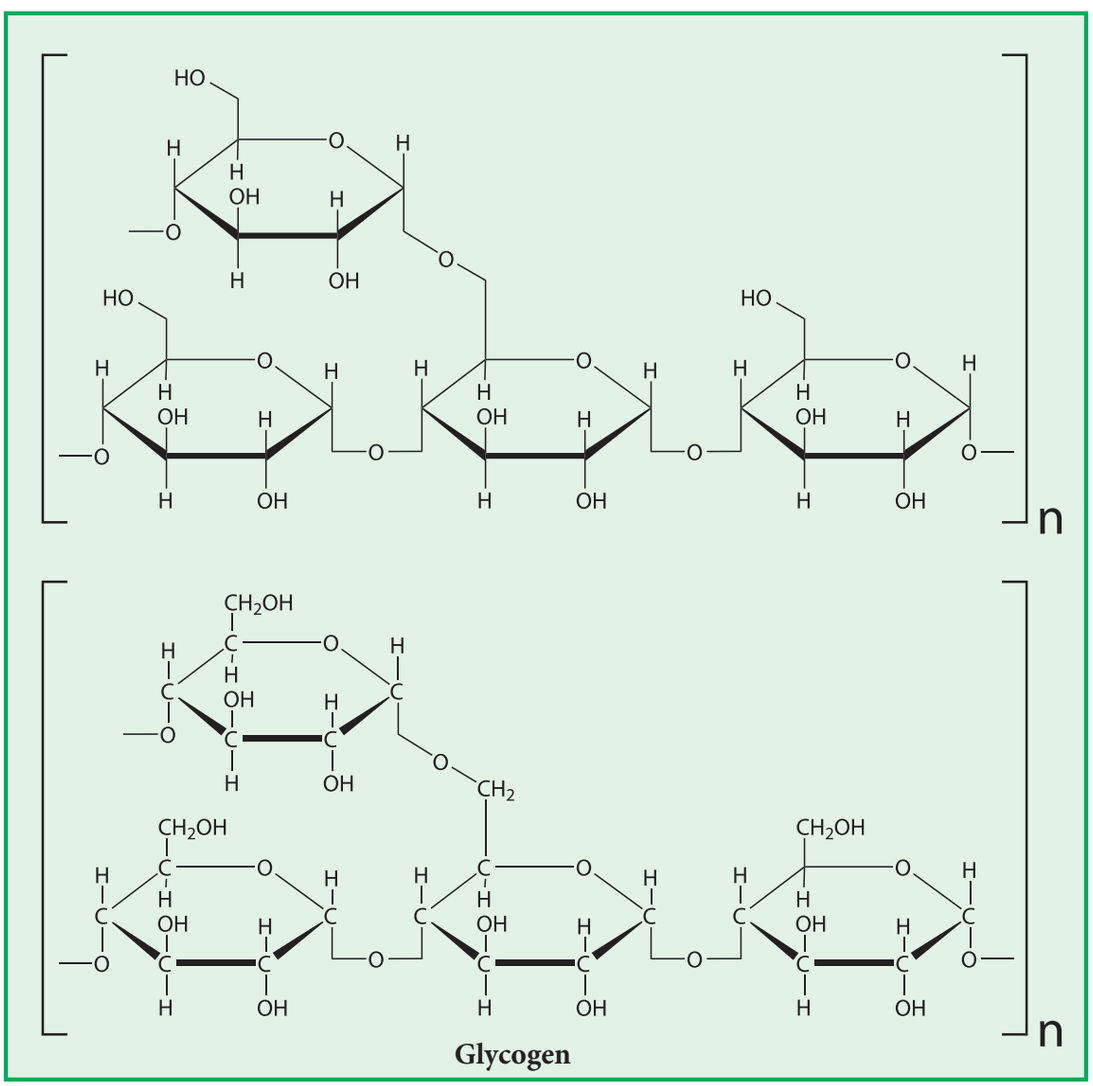
Glycogen :

a) Source :

Glycogen is the carbohydrate reserve in animals; hence often referred as animal starch. It is present in high concentrations in liver, muscle and brain.

b) Structure :

Glycogen is a very large, branched polymer of glucose residues. The structure of glycogen is similar to that of amylopectin with more number of branches. The glucose units in glycogen are linked by α(1-4) glycosidic bonds and α-(1-6) glycosidic bonds at branching points. Branching occurs about once in 10 units. The molecular weight (upto 1×10^8) and the number of glucose units (upto 25,000) vary in glycogen depending upon the source.



Glycogen

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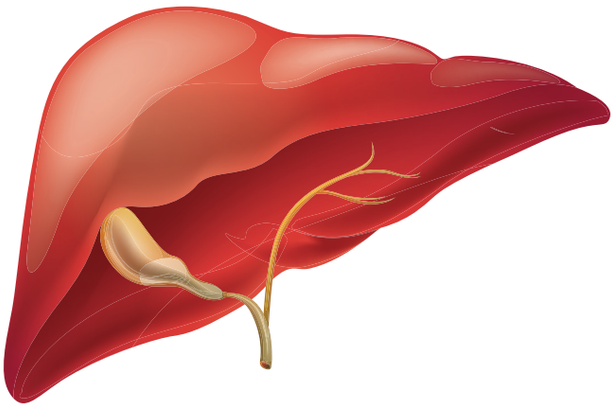
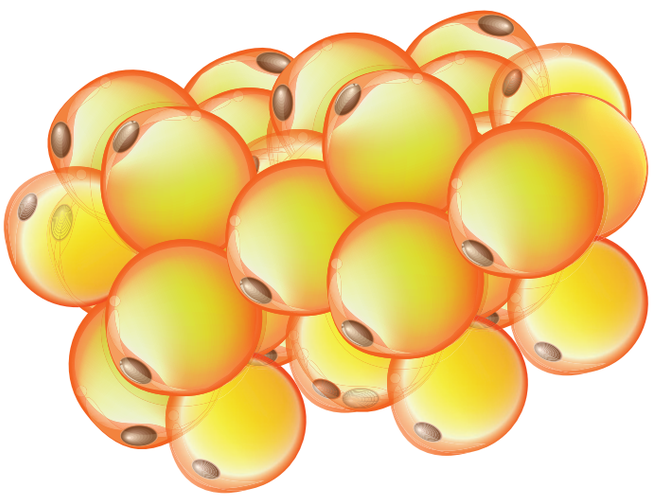
c) Uses :

(i) Excess carbohydrate in the body are deposited as glycogen.

(ii) Animal glycogen is used as food.



Liver glycogen ~300 kcal



Adipose tissue (fat)*10000kcal

5.7.2 HETEROPOLYSACCHARIDES (HETEROGLYCANS)

Glycosaminoglycans :

a. Glycosaminoglycans are otherwise known as mucopolysaccharides.

b. These are heteroglycans made up of repeating unit of aminosugars and uronic acids.

c. Because of the presence of charged groups (carboxyl group, sulphate group, acetylated amino group), they attract water molecules and so they produce viscous solutions.

d. Some of the mucopolysaccharides are found in combination with protein to form

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mucoproteins (or) mucoids (or) proteoglycans. They contain 95% carbohydrate and 5% protein.

e. Examples : i) hyaluronic acid ii) heparin iii) chondroitin sulpahte iv) keratan sulphate v) dermatan sulphate.

(i) Heparin:

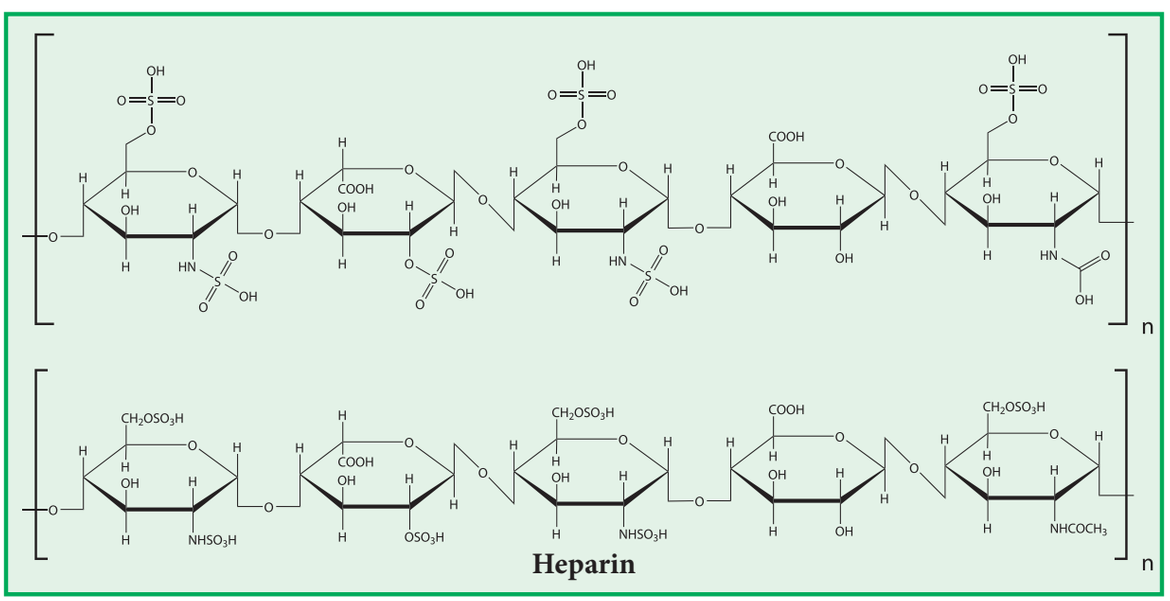
a. It is a mucopolysaccharide present in liver, lung, spleen, kidney, and blood.

b. It is a blood anticoagulant.

c. Heparin is composed of alternating units of N-sulpho-glucosamine-6 sulphate and L-iduronate-2-sulphate.

d. These two molecules are held together by α(1-4) glycosidic bond.

e. Its molecular weight is 20,000.



Heparin

ii) Hyaluronic acid :

a. It is a mucopolysaccharide present in syanovial fluid, vitreous humor of eyes, cartilage tissues, loose connective tissues and in bacteria.

b. It consists of repeated units of α-glucuronic acid and N-acetylglucosamine.

c. These two molecules are held together by α(1-3) glycosidic bond.

d. It is an unbranched chain polymer.

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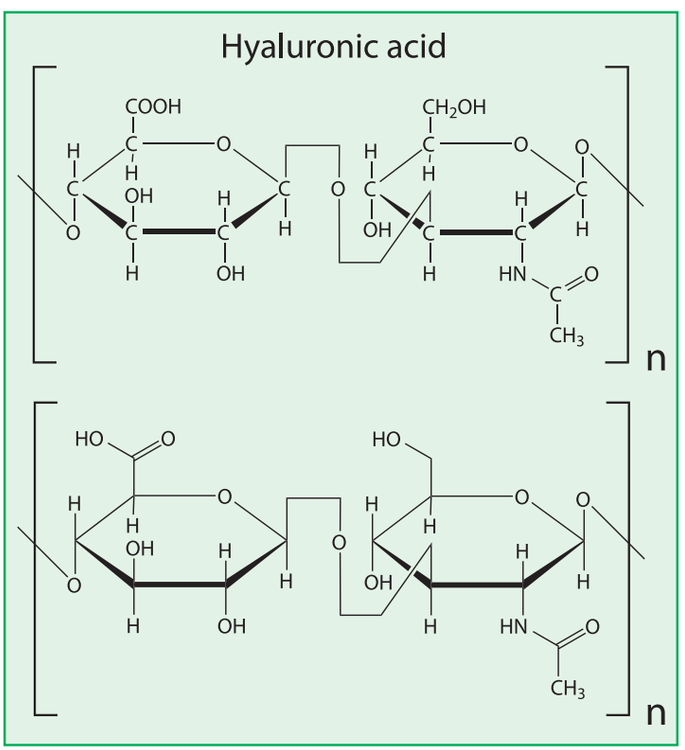
e. Its solutions are viscous and hence acts as lubricant and shock absorbent in joints.

f. In tissues, it acts as a barrier and permits the metabolites to pass through but not the bacteria and other infectious agents.

g. Hyaluronic acid contains about 250-25,000 disaccharide units, held by α-(1-4) glycosidic bonds with a molecular weight upto 4 million.

h. The α(1-4) linkage in hyaluronic acid is cleaved by the enzyme hyaluronidase. This enzyme is present in high concentration in testes, seminal fluid and certain snake venom.



EVALUATION

I. Choose the correct answer from the given four alternatives

1. The functional groups present in carbohydrates are

a) Alcohol and Carboxyl groups

b) Aldehyde and Ketone groups

c) Hydroxyl groups and Hydrogen groups

d) Carboxyl groups and others

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2. which of the following has reducing property?

a) Glucose b) Fructose

c) Starch d) Sucrose

3. The end product of hydrolysis of "Starch" by amylase is ……

a) Soluble starch b) Glucose

c) Dextrins d) Maltose

4. Fructose and Glucose can be distinguished by ……

a) Seliwanoff's reagent b) Benedict's reagent

c) Fehling's reagent d) Barfoed's reagent

II. Fill up the blanks

1. Carbohydrate consists of ....., ... and ..... molecule

2. Hydrogen Oxygen atom ratio in Carbohydrate is .....

3. Molecules having only one actual sugar group is called as .....

4. Sugars having aldehyde group are called as .....

5. Sugars with keto group are called as .....

6. Compounds having same structural formula but differing in spatial configuration are known as .....

7. The change in rotation of plane of plane polarised light with time is called .....

8. Equimolecular mixture of glucose and fructose thus formed is called ……

9. Types of Polysaccharides are ..... and .....

10. Starch is composed of amylose and ……

III. Give short answers for the following

1. What is the structural difference between glycogen and starch?

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2. What is glycogenolysis?

3. Give the structure of sucrose.

4. What is epimerisation?

5. Mention the reaction between fructose and sodium amalgam.

6. What is a racemic mixture?

IV. Answer the following:

1. What are the organic chemical groups that compose carbohydrates? How are carbohydrates classified according to the presence of those groups?

2. What are the functions of carbohydrates?

3. What is the difference between monosaccharides and disaccharides? What are some examples of them?

4. Explain the property of stereoisomerism in monosaccharides with two examples.

5. What are the reaction sequences of glucosazone formation?

6. Mention the difference between amylose and amylopectin.

7. What is the molecular formula for glucose? How can its structural formula be described?

8. What are the main biological functions of polysaccharides?

9. Explain the muta rotation of glucose.

SUMMARY

- Carbohydrates are the most abundant biomolecules, also considered as a primary source of energy.
- Structurally they can be classified as aldose (polyhydroxy aldehyde) and ketose (polyhydroxy ketones).
- Depending upon the number of sugar moieties carbohydrates are classified as monosaccharides, oligosaccharides and polysaccharides.
- Aldoses and ketoses are isomers of monosaccharides .

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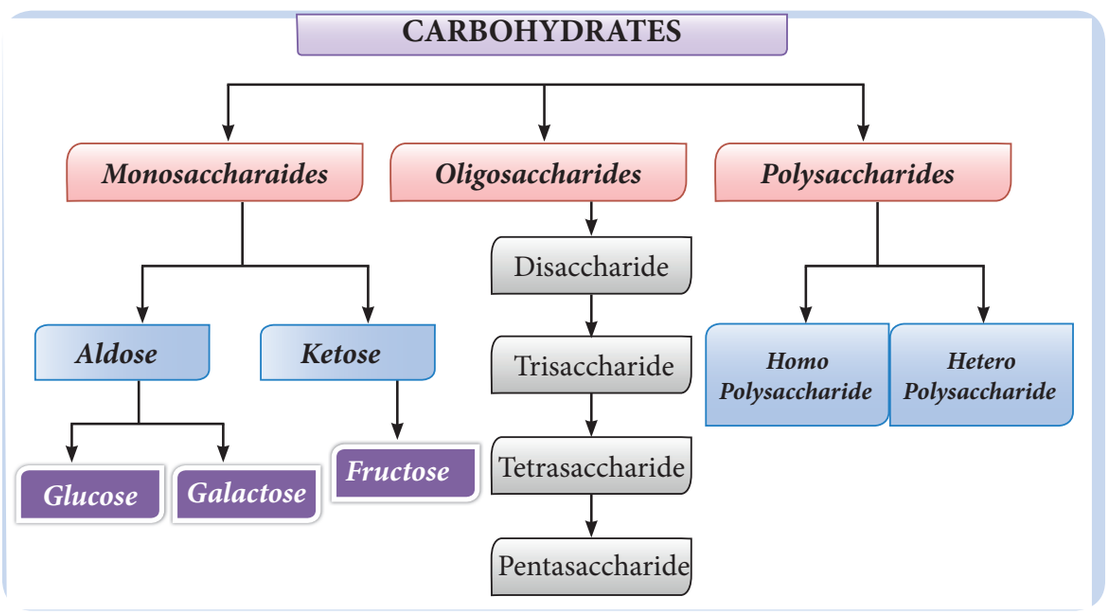
- Carbohydrates have the tendency to undergo oxidation and reduction in the presence of suitable reagents.
- The aldehyde and keto group of a monosaccharide can react with any one hydroxy group present in the same molecule to form cyclic hemiacetals or hemiketals.
- Sugars differing in configuration at the C1 asymmetric center are known as “anomers” and at C4 are called as “epimers”.
- Polysaccharides are classified as homo polysaccharides and heteropolysaccharides which have same sugar moiety and different sugar moiety, respectively.

CONCEPT MAP



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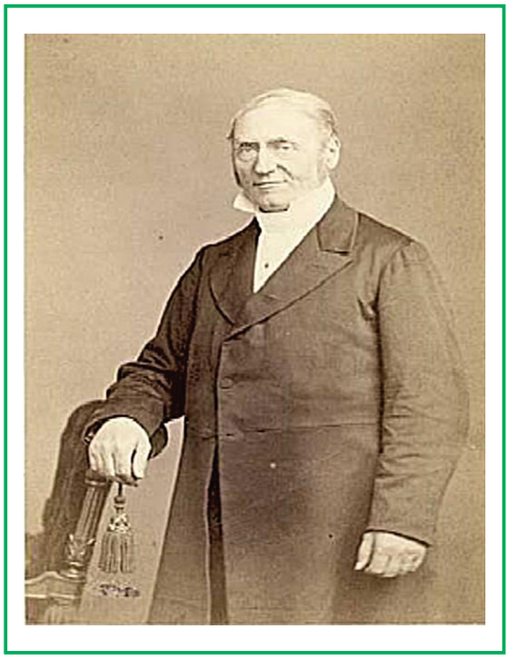
Unit 6 LIPIDS



Theodere Gobley

Theodere Gobley was a French biochemist who was a pioneer in studying the chemical components of human brain. He was the discoverer of phospholipids and lecithin. He was the first to isolate phospholipids from brain tissues and egg yolk in 1860. Later he also showed that lipids are present in other tissues and also in body fluids like blood and bile.

Learning Objectives

After studying this unit, students will be able to

- Describe the structure of lipids
- Classify lipids based on their properties
- Describe the functions of lipids.
- Elaborate the biological importance of different classes of lipids.
- Explain the clinical conditions associated with abnormal lipid levels in humans.

Introduction

Lipids are naturally occurring organic compounds that are insoluble in water but soluble in non-polar solvents like ether, chloroform or benzene. Examples include fats, oils, waxes, sterols and fat soluble vitamins.

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Unsaturated Fat (Olive oil) *Saturated Fat (Vanaspathi)*



Waxes

Figure 6.1 Examples for Lipids

6.1.1 Classification of lipids:

Simple lipids:

Simple lipids are the esters of fatty acids. Example: triglycerides and waxes.

Compound lipids:

Compound lipids are esters of fatty acids with additional groups such as phosphates. Example: phosphoglycerides and phosphoinositides

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Derived lipids:

The hydrolysis products of simple and compound lipids are called derived lipids. For example hydrolysis of triglycerides yields glycerol and fatty acid.

Similarly steroids, fatty aldehydes, ketones, alcohols, fat-soluble vitamins can also be derived from the hydrolysis of simple/compounds lipids.

6.1.2 Biological Functions of lipids:

- Lipids are important components of the cell membrane and are necessary for the structural integrity of the cell.
- They serve as energy reserve of the body.
- They act as a protective coating in aquatic organisms.
- Lipids serve as insulating material on the surface of animals living in extreme cold regions.
- They serve as component of cell recognition, species specificity and tissue immunity.
- They help in the absorption and transport of fat soluble vitamins.



Figure 6.2 Lipid insulation in polar bears

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Note

Lipids yield ~9 kcal of energy per gram, while carbohydrates and proteins yield only ~4 kcal of energy per gram.

6.2. Fatty acids

Fatty acids are the building blocks of hydrolysable lipids.

6.2.1 Classification of Fatty acids:

A simple fatty acid consists of a long linear hydrocarbon chain that may be saturated as in palmitic acid or it may have one or more double bonds as in linoleic acid. Few fatty acids like Arachidonic acid and Docosahexaenoic acid (DHA) contain more than three double bonds. Each fatty acid differs from the other, primarily in chain length, number and position of their double bonds. They are often symbolized by the number of carbon atoms in the hydrocarbon chain and number and position of the double bonds. Palmitic acid (Figure 6.3) is symbolized as 16:0 and oleic acid (Fig.6.3) as 18:1(Δ^9), Δ indicates the position of the double bond.

There are many kinds of fatty acids, isolated from various plant and animal lipids. Fatty acids with odd number of carbon atoms are significantly present in many marine organisms.

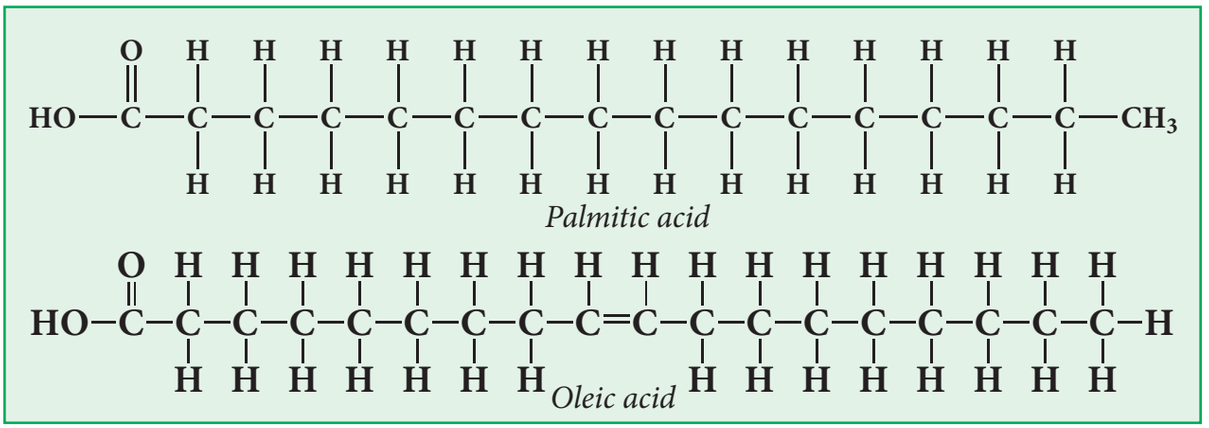

Figure 6.3 Structure of palmitic acid (saturated) and oleic acid (unsaturated)

Saturated Fatty acid

The general formula of saturated fatty acid is $C_nH_{2n+1}COOH$. If all carbon-carbon bonds in the hydrocarbon chain of the fatty acid are single covalent bonds, then the

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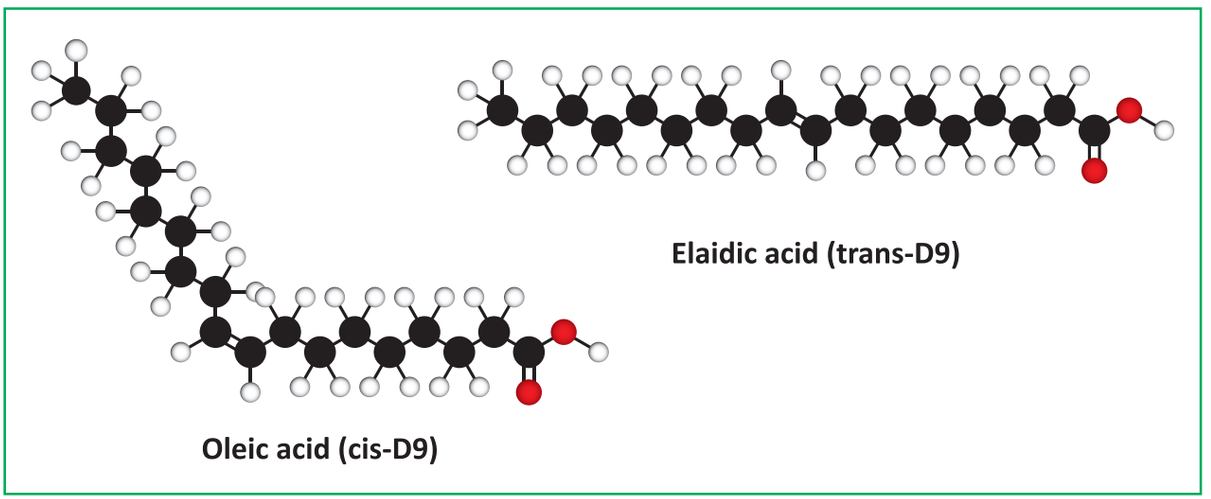
fattyacid is said to be a saturated fatty acid.



Unsaturated Fatty Acid

Figure 6.4: Stereo-chemical representation of unsaturated fatty acid

Unsaturated Fatty Acid

When one or more carbon-carbon double bonds are present, the fatty acid is called unsaturated. Depending on the number of double bonds present in the hydrocarbon chain, the fatty acid can be classified as:

- Monounsaturated fatty acid (MUFA) and
- Polyunsaturated fatty acid (PUFA)

Monounsaturated Fatty Acid:

A monounsaturated fatty acid contains one carbon-carbon double bond. Higher quantity monounsaturated fatty acids are present in olive oil, canola oil (mustard oil), peanut oil and sesame oil. The general formula is $C_nH_{2n-1}COOH$.

Polyunsaturated Fatty Acid:

Fatty acids with more than one double bond are polyunsaturated fatty acids. Foods with high amounts of polyunsaturated fats include: Walnuts, Sunflower seeds, Flax seeds or flax oil, Fish (such as salmon, mackerel, herring, albacore tuna, and trout), Corn oil and Soybean oil.

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Structure and Physical state

The vegetable oil is a liquid at room temperature. However, its hydrogenated form (Vanaspathi) is a solid. Do you know why?

The vegetable oil contains mostly unsaturated fatty acids while the Vanaspathi, the hydrogenated vegetable oil consists mainly saturated fatty acids. Saturated fatty acids are fairly linear and pack together closely through hydrophobic interactions. Close packing of saturated fats promotes stability and causes saturated fats to form solids at room temperature. As unsaturated fatty acids (especially with cis-configuration) form kinked structures as shown in the picture, close packing of unsaturated fats is prevented. Hence, these are primarily liquids at room temperature.

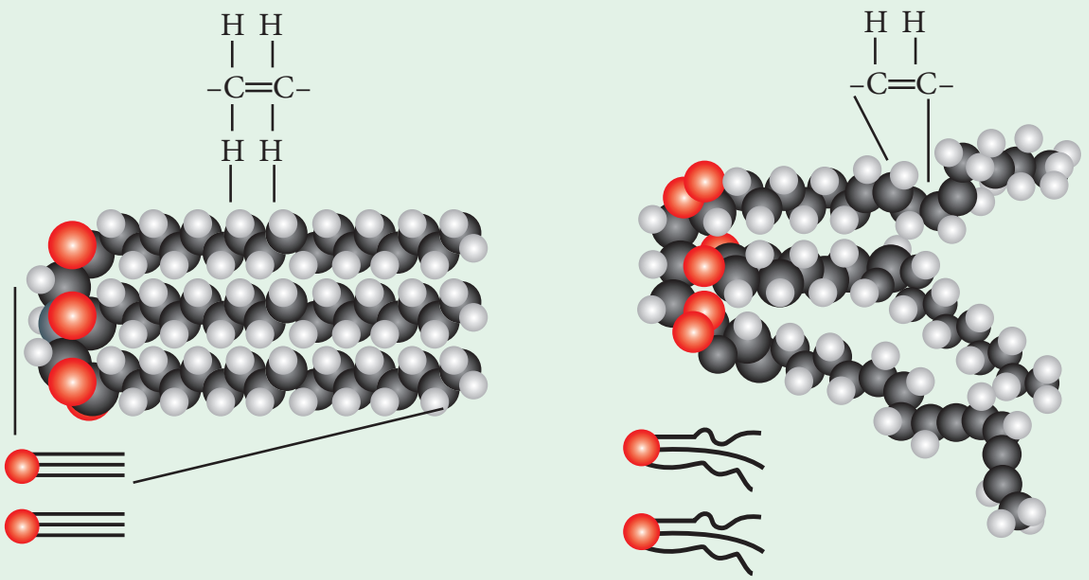

Table 6.1 & 6.2 Show the symbol, structure and common name of saturated, unsaturated fatty acid, essential fatty acid and the unsaturated trans fatty acid, elaidic acid.

Table 6.1 Saturated fatty acids

Symbol	Structure	Systemic name	Common name
12:0	$CH_3(CH_2)_{10}COOH$	*n*-Dodecanoic acid	Lauric acid
16:0	$CH_3(CH_2)_{14}COOH$	*n*-Hexadecanoic acid	Palmitic acid
18:0	$CH_3(CH_2)_{16}COOH$	*n*-Octadecanoic acid	Stearic acid

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Table 6.2: Unsaturated fatty acids

Symbol	Structure	Common name
16:1 (Δ^9)	$CH_3(CH_2)_5\ CH{=}CH(CH_2)_7\ COOH$	Palmitoleic acid
18:1 (Δ^9)	$CH_3(CH_2)_7\ CH{=}CH(CH_2)_7\ COOH$	Oleic acid
18:1 (Δ^9) (trans)	$CH_3(CH_2)_7\ CH{=}CH(CH_2)_7\ COOH$ (trans)	Elaidic acid
Essential fatty acids		
18:2 ($\Delta^{9,12}$)	$CH_3(CH_2)_4\ CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$	Linoleic acid
18:3 ($\Delta^{9,12,15}$)	$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$	Linolenic acid
20:4 ($\Delta^{5,8,11,14}$)	$CH_3(CH_2)_4\ (CH{=}CHCH_2)_3CH{=}CH(CH_2)_3COOH$	Arachidonic acid

Essential fatty acid (EFA)

Fatty acids that must be obtained from the diet as they cannot be synthesized by our body are essential fatty acids (Figure 6.5). EFA are polyunsaturated fatty acids. They are precursors of prostaglandins, a family of physiologically potent lipids.

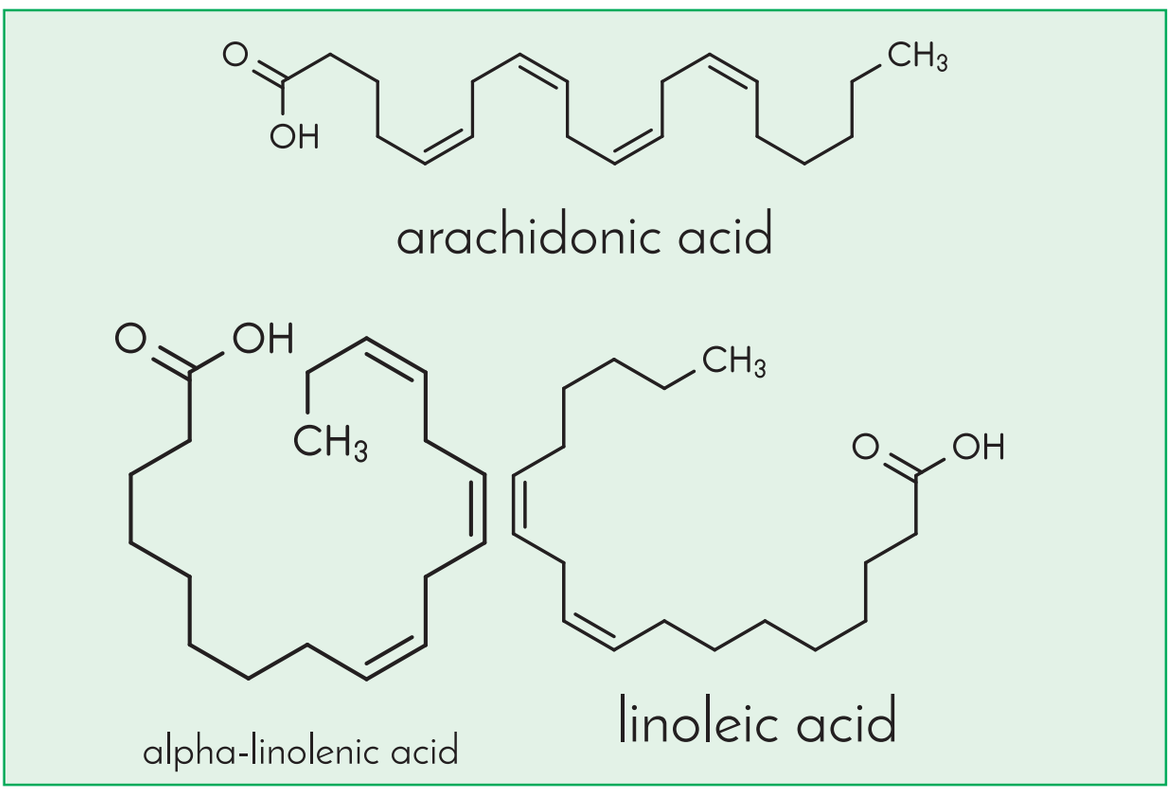

Figure 6.5 Essential fatty acids

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6.3. Triacylglycerols or Triglycerides

Triacylglycerols are simple lipids synthesized by esterification of glycerol with three molecules fatty acids. It is the storage form of fat, stored in adipose tissue. They are hydrolyzed in the gut by lipases to free fatty acids and monoglycerides.

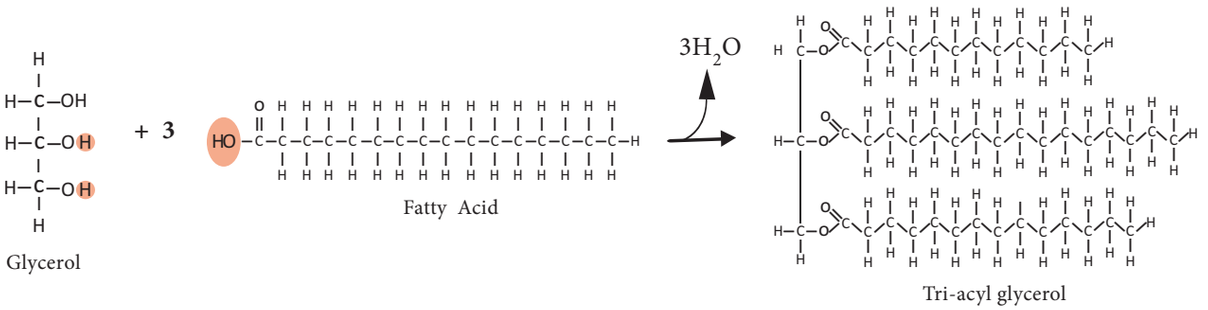

Figure 6.6 Synthesis of triglycerides

If the three hydroxyl groups of glycerol molecule are esterified with the same type of fatty acid then the lipid is called simple triacyl glycerol eg: tripalmitin. If esterification is with different fatty acids, it is called as mixed glycerides eg: dioleopalmitin.

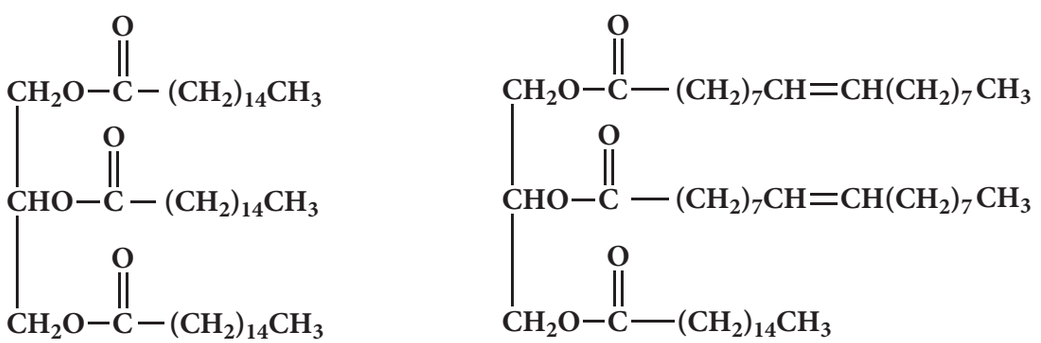

Figure 6.7 Simple and mixed triglycerides

6.3.1. Properties

Physical properties

- They are non-polar, hydrophobic, insoluble in water and soluble in organic solvents.
- Specific gravity is less than water. Therefore fats and oil float on water.

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- They serve as solvent for other fats. Example: Fat soluble vitamins A, D, E and K
- The saturated fatty acids have higher melting points than unsaturated fatty acids of corresponding length.

Common Name	Melting Point
Palmitic acid (Saturated fatty acid)	63° C
Oleic acid (Unsaturated fatty acid)	13° C

Note

The trans-double bond isomer of oleic acid, known as elaidic acid, has a linear shape and a melting point of 45° C (32° C higher than its cis isomer).

Chemical properties

i. Hydrolysis:

Triglycerides (fats) can be hydrolyzed to produce glycerol and fatty acids in the presence of acid and heat or with a suitable lipase enzyme under biological conditions.

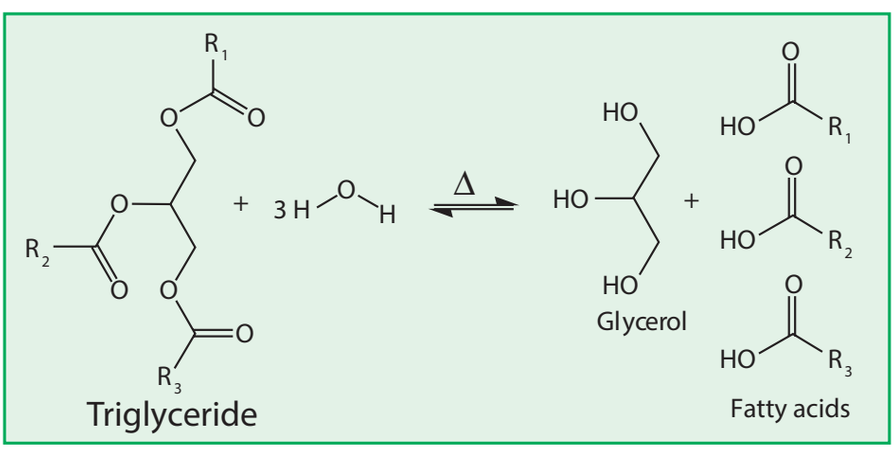

Figure 6.8 Hydrolysis of triglycerides

ii. Hydrogenation:

Hydrogenation is a process of adding hydrogen atoms to unsaturated fats until they become saturated. Hydrogenation of fat is a process used in industries, food manufacturers, to synthesize modified plant fats called hydrogenated fats that share similar texture and taste characteristics with saturated animal fats.

Note

During the processing, many fatty acids saturate and then spontaneously convert back to a unsaturated state, but in a trans-isomer form.

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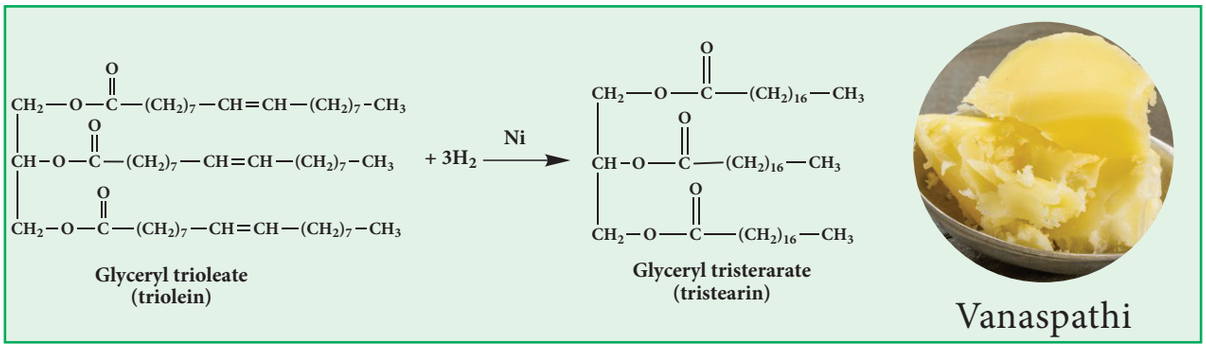

Figure 6.9 Hydrogenation of unsaturated fatty acids

iii. Saponification:

The process of hydrolysis of fat by aqueous alkali (NaOH or KOH) to yield glycerol and the salt of fatty acid (soap) is called saponification or alkaline hydrolysis of esters. Soaps are sodium or potassium salts of long chain fatty acids.

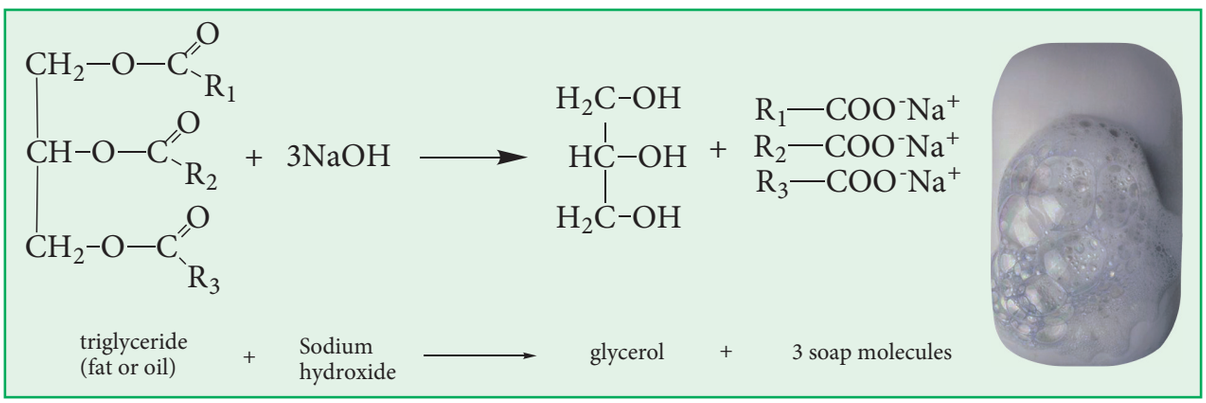

Figure 6.10 Saponification reaction of fatty acids

iv. Halogenation:

Unsaturated fatty acids have the ability to bind halogens like Cl_2, Br_2 and I_2 to their double bonds. It is a very important property which determines the degree of unsaturation of the fat or oil that determines its biological value.

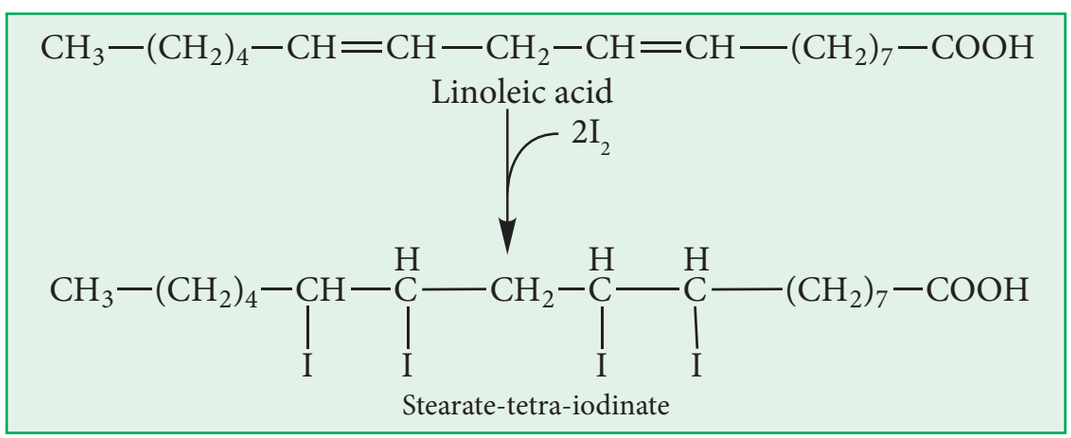

Figure 6.11 Halogenation reaction of fatty acids

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v. Rancidity:

Rancidity is a term generally used to denote unpleasant odours and flavours in foods resulting from deterioration in the fat or oil portion of a food. The triacylglycerols in fats with low molecular mass carboxylic acids undergo oxidation very quikly when exposed to air, moisture and light or hydrolysed in the presence of bacterial lipases.



How does the food industry reduces rancidity?

- Packing foods either with naturally occurring antioxidants such as Vitamin C and vitamin E or with synthetic oxidation inhibitors such as Butylated hydroxyanisole (BHA) and Butylated hydroxytoluene (BHT).
- Vacuum packaging to avoid oxygen.
- Adding inert gas such as nitrogen to the bag to replace the oxygen.
- Packing food protected from light.
- Refrigeration will reduce the rate of most reactions that take place in rancidity.

6.4. Quantitation of fat

There are certain chemical parameters used for the characterization of fats:

i. Acid number:

Acid value (or "neutralization number" or "acid number") is the number of milligram of potassium hydroxide (KOH) that is required to neutralize one gram of fat. Thus, acid number is an indication of the quantity of free fatty acid present in the fat. The fat which has been processed and stored properly has a very low acid number.

ii. Saponification number:

The saponification number is the milligrams of KOH required to saponify 1 g of fat. Thus it provides the information about the average chain length of the fatty acids in the fat. The saponification number varies inversely with the chain length of the fatty acid. The shorter the average chain length of the fatty acid, the higher is the saponification number.

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iii. Iodine number:

Iodine number is expressed as the number of grams of iodine absorbed by 100 g of fat. Not only iodine, but other halogens will also bind at double bonds. Hence bromine is often used instead of iodine because it is more reactive. The iodine number is a measure of the degree of unsaturation of the fatty acid in the fat.

Eg: the iodine numbers of linseed oil (PUFA), olive oil (MUFA), and coconut oil (saturated fatty acid) are approximately 175–201, 77–91, and 8–9.5, respectively.

iv. Polenske number:

The Polenske value indicates the level of volatile fatty acid in the fat. It is equal to the number of milliliters of 0.1N alkali (KOH) solution necessary to neutralize the water insoluble fatty acids. The value is named after the chemist who developed it, Eduard Polenske.

v. Riechert Meissl number

The Reichert value (Reichert-Meissl-Wollny number) measures the quantity of short chain fatty acid in the fat molecule. It is the number of milliliters of 0.1 N KOH solution necessary to neutralize the soluble, volatile fatty acids derived from 5 g of fat. The value is named after the chemists who developed it, Emil Reichert and EmerichMeissl.

vi. Acetyl number

Acetyl number is the measure of the free hydroxyl groups in a substance (as a fat or oil). It is the number of milligrams of potassium hydroxide required for neutralization of the acetic acid formed by the saponification of 1gm of fat after it has been acetylated.

6.4. Phospholipids

Phospholipids are compound lipids. They are amphipathic molecules, meaning they have a hydrophobic tail and a hydrophilic head group. Phospholipids are major components of the plasma membrane, which serves as a barrier between the cell and its surroundings. In a membrane, phospholipids are arranged in a structure called a lipid bilayer, with their phosphate heads facing the water and their tails pointing towards the interior of the membrane.

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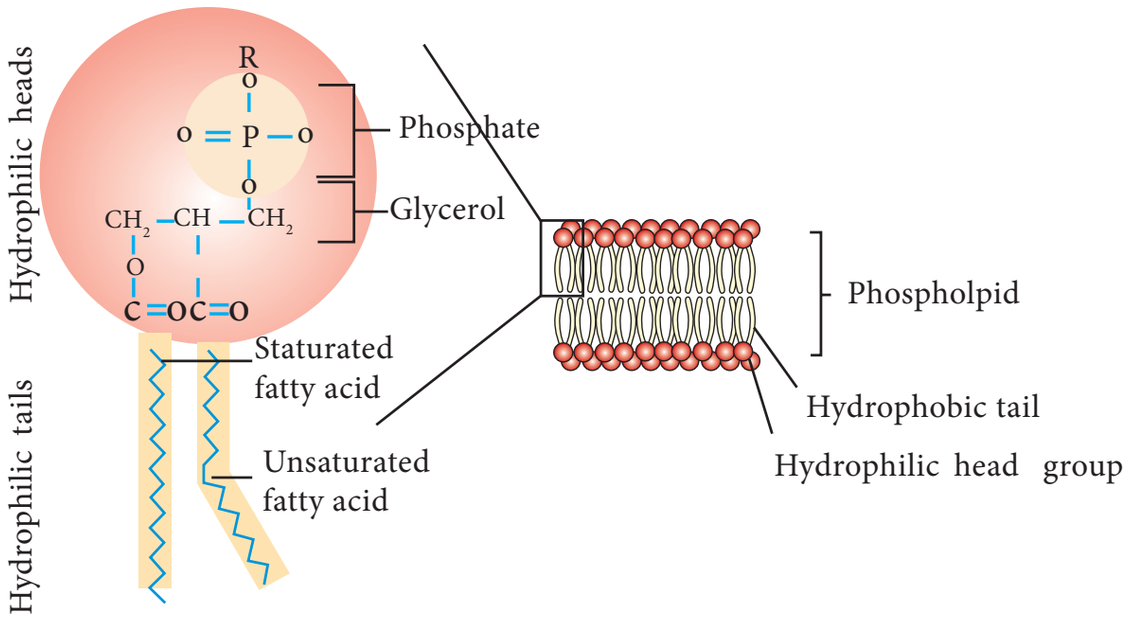

Figure 6.12 Structure of phospholipid

6.4.1 Classification:

Phospholipids are typically composed of fatty acid chains attached to a backbone of glycerol. Instead of having three fatty acid tails, phospholipids generally have two, and the hydroxyl group attached to the third carbon of the glycerol backbone is esterified by a modified phosphate group.

According to their alcohol content phospholipids are classified into two major types:

- Glycerophospholipids
- Sphingophospholipids

i. Glycero phospholipids

Glycerophospholipids are abundantly present in heart, brain, kidney, egg yolk and soyabean.

In glycerophospholipids the alcohol is glycerol, to which two fatty acids, one phosphate group and an alcohol (inositol), alcohol amine (ethanol amine, serine) or a nitrogenous base (choline) are attached. It is also known as phosphoglyceride.

There are different types of phosphoglycerides based on the attachment to the phosphate group, that confers different properties and role of the different glycerophospholipids.

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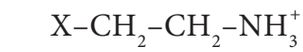
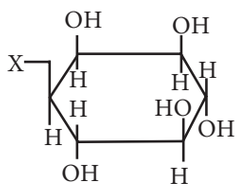
The important phosphoglycerides are:

- Phosphatidylcholine (lecithin): Choline as nitrogenous base.
- Phosphatidylserine (cephalin): Serine as alcohol amine
- Phosphatidylethanolamine: Ethanol amine an alcohol amine.
- Phosphatidylinositol: Inositol a hexahydric alcohol.

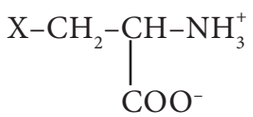
Phosphatidylethanolamine



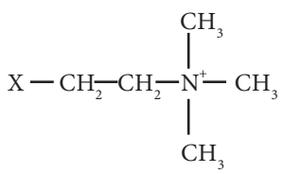
Phosphatidylinositol

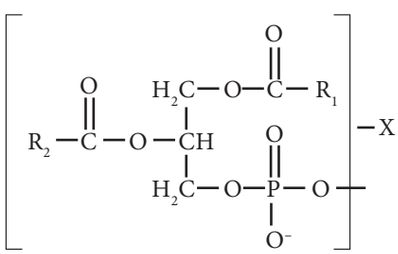


Cephalin(Phosphatidylserine)



Lecithin(Phosphatidycholine)





Phosphatidic acid

Figure 6.13 Structure of important phosphoglycerides

ii Sphingophospholipids

They contain a sphingosine back bone, fatty acid, phosphate and base. They are abundantly present in brain and nerve tissues. These compounds play important role in signal transmission and cell recognition.

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Sphingosine + Fatty acid = Ceramide Ceramide+base=sphingophospholidpid

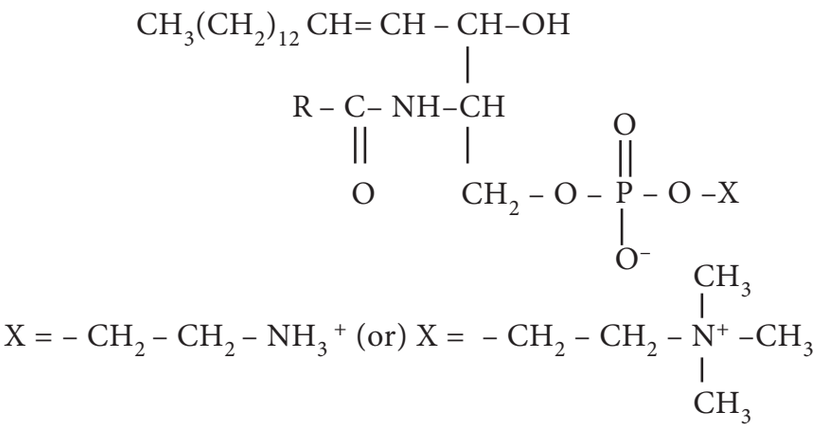

Figure 6.14 Structure of Sphingomyelin

Sphingomyelins have a phosphocholine or phosphoethanolamine molecule with an ester linkage to the 1-hydroxy group of a ceramide.

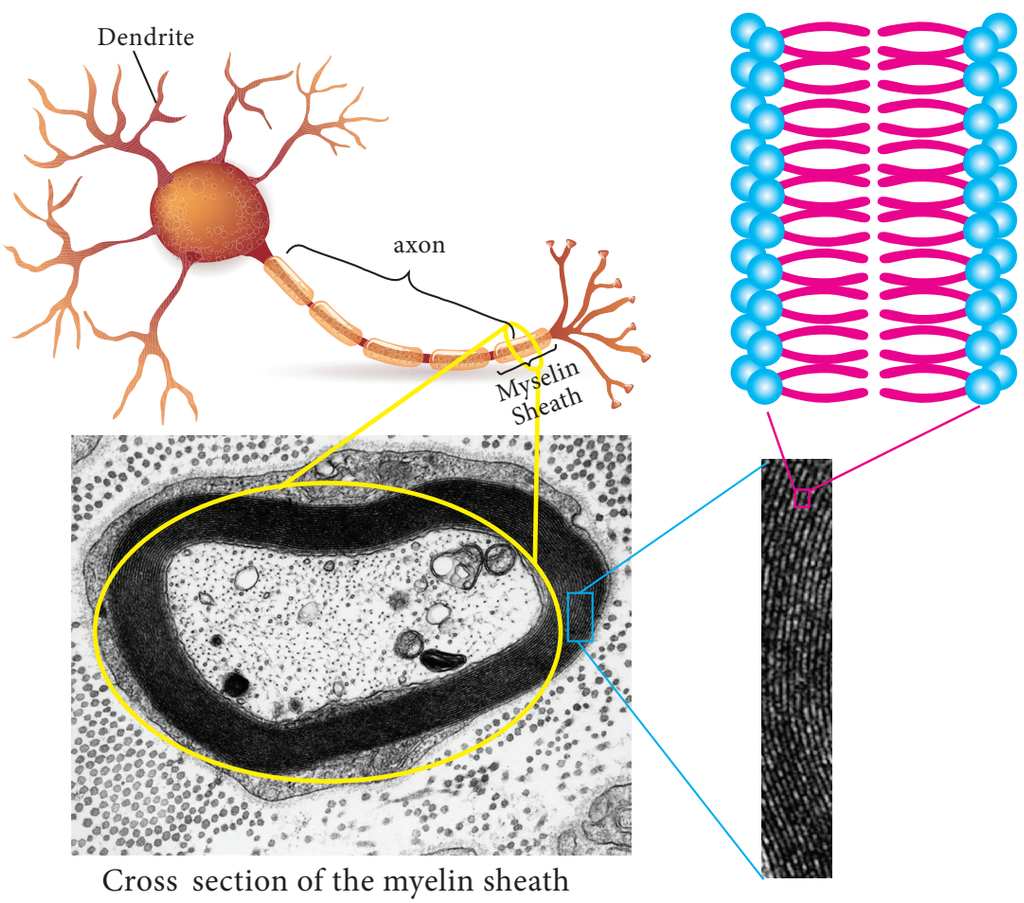

Figure 6.15 Cross section of the myelin sheath

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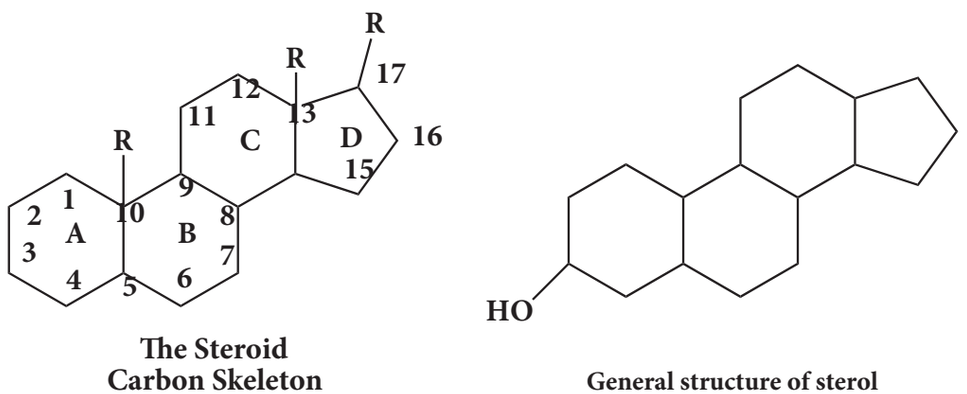
6.5 Derived Lipids:

6.5.1. Steroids:

Steroids are derived lipids. They may be recognized by their cyclo pentane perhydro phenatherene ring-tetracyclic skeleton. It consists of three six-membered ring (A,B,C) and one five-membered ring(D), as shown in the diagram.



The Steroid Carbon Skeleton

General structure of sterol

Figure 6.16 Carbon skeleton of steroids and sterols

6.5. 2 Sterols

Sterols, also known as steroid alcohols, are a subgroup of steroids with a hydroxyl group at the 3-position of the A-ring. They are amphipathic lipids as the hydroxyl group on the A ring is polar and the rest of the aliphatic chain is non-polar.

Types:

- Phytosterols (Example: Stigmasterol)
- Zoosterols (Example: Cholesterol)

Sterols of plants are called phytosterols and sterols of animals are called zoosterols. The most important zoosterol is cholesterol; phytosterols include campesterol, sitosterol and stigmasterol. Ergosterol is a sterol present in the cell membrane of fungi, where it serves a role similar to cholesterol in animal cells.

Note

Phytosterols as a nutritional supplement:

It have been shown in clinical trials to block cholesterol absorption sites in the human intestine, thus helping to reduce cholesterol absorption in humans. At present, the American Heart Association has recommended that supplemental plant sterols be taken only by those diagnosed with elevated cholesterol, as phytosterols can interfer with cholesterol absorption.

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i. Cholesterol

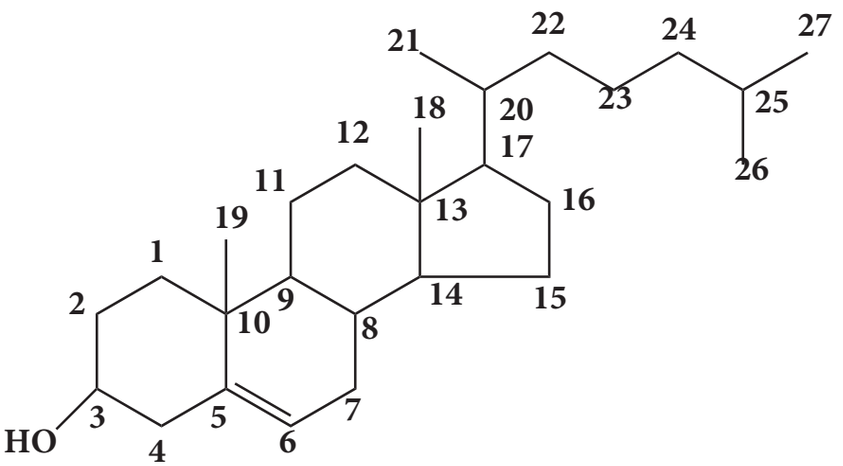
Cholesterol is widely distributed in all cells and is a major component of cell membrane and lipoproteins.

ii. Structure

Cholesterol is a C_{27} compound with molecular formula $C_{27}H_{46}O$. With a hydroxyl group at C_3 and a double bond between C_5 and C_6. An aliphatic side chain is attached at C_{17} and 5 methyl groups.

Note

Normal value of total blood cholesterol level is <200 mg/dL



Cholesterol

Figure 6.17 Structure of cholesterol

Properties of Cholesterol

1. Cholesterol exists as crystals that are white, shiny and rhombic in nature.
2. It is tasteless and odourless
3. It has a high melting point of 150°C
4. It is insoluble in water and soluble in fat solvents
5. It is a poor conductor of heat and electricity and serves as an insulator. In brain, where it is present abundantly acts as an insulator against nerve impulse which are electric in nature.

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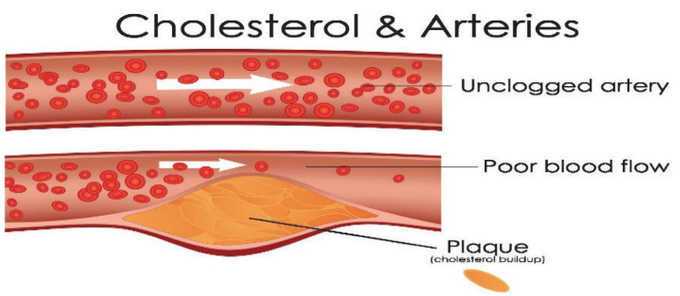
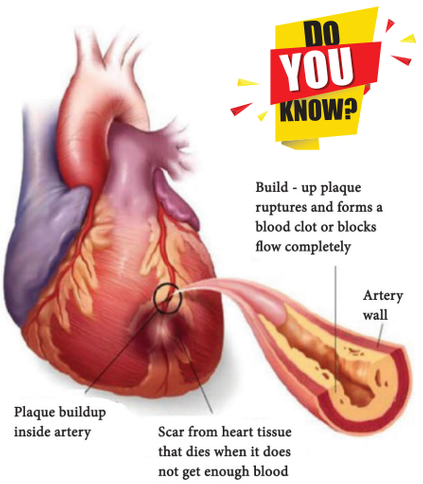
6. Cholesterol when oxidized under suitable conditions, undergoes rapid oxidation to form a ketone called cholestenone.

7. The hydroxyl group of cholesterol readily forms ester with fatty acids like stearic acid.

Atherosclerosis is a hardening of an artery. Meaning *arteria*-'artery', *sklerosis*–'hardening'. It is asymptomatic for decades until enough narrowing or closure of an artery, due to clots, occurs. And impedes blood flow to different organs enough to induce symptoms.

Typically, atherosclerosis begins at young age, as a thin layer of white-yellowish streaks with the inner layers of the artery walls and progresses from there.





Importance of Cholesterol:

Cholesterol is vital to cell membrane structure, and functions as a precursor to fat-soluble vitamins and steroid hormones

- It is also a key regulator of membrane fluidity in animals.
- It forms lipid rafts when it complexes with certain phospholipids, which makes the membranes less fluid and less subject to phase transitions. This also increases the permeability of the cell membrane to hydrogen and sodium ions.
- It also assists the liver in the manufacture of bile acids, which is essential for digestion and absorption of fat-soluble vitamins such as vitamin A, D, E and K.
- It helps in maintenance of our body temperature and protects our internal organs.
- In pharmaceutical industries, cholesterol is used in the manufacture of steroid hormones and vitamin D.

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Note

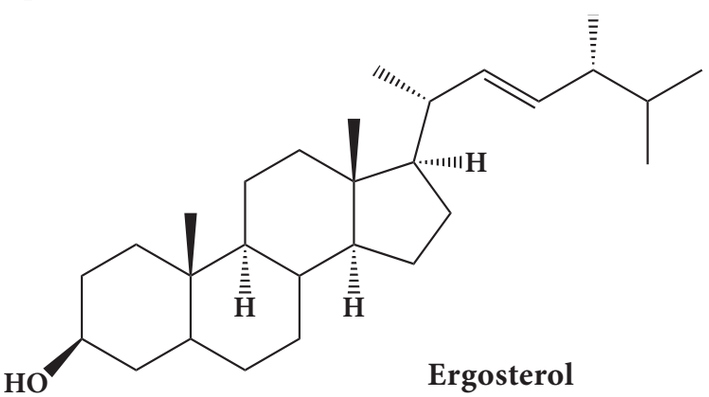
Cholesterol, is not found in plant cell and it is abundantly found in animal cell membrane and it helps stiffen the membrane

DO YOU KNOW?

Obesity is excess body fat that has accumulated to the extent that may have a negative effect on health. People are generally considered obese when their body mass index (BMI), is in the range 25-30kg/m^2. BMI is calculated by dividing a person's weight by the square of the person's eight. It is most commonly caused by a combination of excessive food intake, lack of physical acitivity, and genetic susceptibility. Over weight is associated with various diseases like cardiovascular diseases, diabetes mellitus type 2, obstructive sleep apnea, osteoarthritis and asthma

ii. Ergosterol

Ergosterol is a sterol found in cell membranes of fungi and protozoa, serving many of the same functions that cholesterol serves in animal cells. Ergosterol is a pro-vitamin form of vitamin D_2; exposure to ultraviolet (UV) light causes a chemical reaction that produces vitamin D_2.



Ergosterol

Figure 6.18 Structure of Ergosterol

YOU KNOW?

As fungi and protozoa cannot survive without ergosterol, the enzymes that synthesis ergosterol have become important targets for developing drugs against them.

iii. Biological function:

- Ergosterol is a biological precursor of vitamin D2 (ergocalciferol). In mushroom cultivation, after harvest, mushrooms are irradiated to increase their Vitamin D content.

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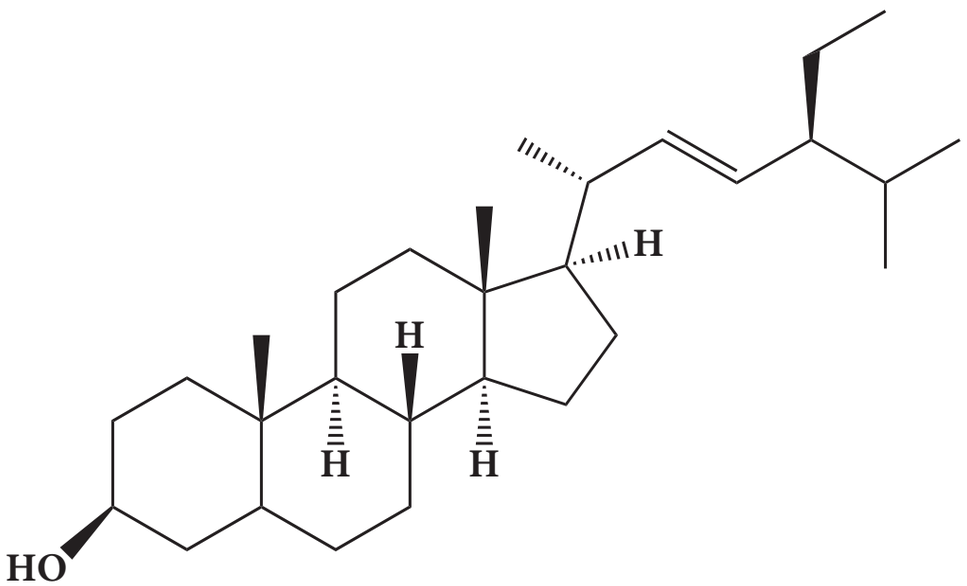
- Fungi are also grown industrially for extraction of ergosterol and synthesis of Vitamin D.

iv. Stigmasterol

Stigmasterol also known as Wulzen anti-stiffness factor, is a plant sterol. Stigmasterol is an unsaturated phytosterol occurring in a number of medicinal herbs. Stigmasterol is also found in various vegetables, legumes, nuts, seeds, and unpasteurized milk.



Stigmasterol

Figure 6.19 Structure of Stigamasterol

Applications:

- Stigmasterol is used as a precursor in the manufacture of semisynthetic progesterone, a valuable human hormone that plays an important physiological role in the regulatory and tissue rebuilding mechanisms.

- Acts as an intermediate in the biosynthesis of androgens, estrogens, and corticoids.

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EVALUATION



I. Choose the correct Answer

1. Which of the following is a characteristic of lipids?
   a) They are not soluble in water
   b) They are either fats or oil
   c) They are polar molecules
   d) They are composed of nitrogenous chains

2. What are the components of triglyceride molecules
   a) One glycerol and three fatty acids
   b) One cholesterol and two fatty acids
   c) One glycerol and one cholesterol
   d) One glycerol and two fatty acids

3. Why is butter solid at room temperature, while vegetable oil is a liquid?
   a) Butter is saturated, where vegetable oil is unsaturated
   b) Butter is polar molecule, where vegetable oil is non-polar molecule
   c) Butter is non-polar molecule, where vegetable oil is polar molecule
   d) Butter is unsaturated, where vegetable oil is saturated

4. What happens when hydrogen reacts with vegetable oil (Vanaspati)
   a) The hydrogenated vegetable oil will contain fewer trans fats
   b) The hydrogenated vegetable oil will become solid at room temperature
   c) The hydrogenated vegetable oil will become polarized
   d) The hydrogenated vegetable oil will become saturated.

5. Fatty acids are building blocks of
   a) Stigma sterol b) Tri-acyl glycerol c) Cholesterol d) Ergosterol

6. Ergosterol is a precursor for _____
   a) Vitamin A b) Vitamin E c) Vitamin C d) Vitamin D

7. _____ is a fat soluble vitamin
   a) Vitamin A b) Vitamin B12 c) Vitamin C d) Vitamin B7

8. Hydrolyses of fat with _____yield soap
   a) Acid b) Alkali c) Ketone d) Ether

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9. Polenske number indicates the _____fatty acids in the fat.

a) Volatile b) Non- volatile c) Saturated d) Unsaturated

10. Sphingo phospholipids are highly present in _____ tissue

a) Heart b) Kidney c) Brain d) Liver

11. Dioleopalmitin is a _____

a) Simply glyceride b) Mixed glyceride

c) Free fatty acid d) Steroid

12. General formula for saturated fatty acid is _____

a) $C_nH_{2n+1}COOH$ b) $C_nH_{2n-1}COOH$

c) $C_nH_{2n+1}OH$ d) $C_nH_{2n-1}OH$

13. Oleic acid is _____fatty acid

a) Saturated fatty acid b) Unsaturated fatty acid

c) PUFA d) None of these

14. Nitrogenous base present in lecithin is _____

a) Choline b) Serine

c) Ethanol amine d) Inositol

15. Sphingomyelin is a _____

a) Derived lipid b) Simple lipid

c) Triacylglycerol d) Phospholipid

II. Answer the following the question: (2 Marks)

1. What are lipids soluble in?

2. What are lipids made up of?

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3. What does bile do to fat?

4. What is iodine number?

5. What is acid number?

6. Write a short note on ergosterol?

7. Write about the melting point of different fats? Give examples.

8. Describe sphingophospholipid?

9. Draw the structure of phospholipid in lipid bilayer?

10. What are essential fatty acids? Why?

III. Answer the following the question: (3 Marks)

1. Write a note on fatty acid and their classification?

2. Briefly explain saponification/ soap preparation?

3. Give the importance of phospholipids?

4. List the properties of cholesterol?

5. Explain hydrogenation of fat?

IV. Answer the following in detail (10 Marks)

1. Write a detailed note on structure, properties and importance of cholesterol?

2. Explain in detail about phospholipids and their classification?

3. Write in detail the chemical properties of tri-acyl glycerol?

Simple experiment:

- Carry out the experiment, making careful observations. This is done to understand the key terms such as mixture, emulsion, emulsifier, hydrophilic and hydrophobic.

  Mix, water and oil in a beaker and divide the mixture into seven equal portion in seven different containers. add small amount of detergent (in second container), sugar (in thrid container), flour (in fourth container), mustard (in fifth container), egg white (in sixth container) and egg yolk (in seventh container)

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- observe the changes and draw conclusions about the properties of the lipids used in the above Experiment.

Key Answer

Water and oil	Water mixes into oil making a cloudy mixture with a thicker consistency. After 5-10 minutes the oil and water separate into two distinct layers with water on the bottom layer and oil on the top.
Adding detergent	Produces a cloudy mixture with a thin consistency and layer of foam on top. After 5–10 minutes this produces a liquid bottom layer and some foam above that indicating an emulsifier.
Adding sugar	Mixes into a thicker paste consistency then separates after 5–10 minutes into two separate layers.
Adding flour	Mixes into a milky substance with some oil globules visible in the liquid. After 5–10 minutes the solid does not dissolve but settles to the bottom, with two distinct layers of liquid and then oil as the top layer.
Adding mustard	This mixes in well producing a yellow liquid with powder particles visible on the walls of the bottle. After 5–10 minutes nothing has changed and the solution remains mixed well. This indicates to the student that the mustard is an emulsifier.
Adding egg white	Mixes into a cloudy gelatinous substance with no visible oil. After 5–10 minutes there are two (less distinct) layers. The bottom layer is a darker cream than the top with the top layer whiter and slightly foamy.
Adding egg yolk	This mixes in completely producing a cloudy yellow liquid that is still the same after 5–10 minutes, indicating an emulsifier.

SUMMARY

Lipids are a wide class of organic compounds present in living systems. They are a class of compounds soluble in non polar solvents. They have varied functions such as being energy reservoirs, acting as hormones, structural components of cell membranes etc., They also find wide applications in food industries, cosmetics, drug delivery systems etc. Lipids can be classified into simple lipids, compound lipids and derived lipids. Simple lipids are triglycerides and waxes. Triglycerides are esters of fatty acids with glycerol where as waxes are esters of fatty acids with other mono hydroxyl alcohols. Compound lipids are lipids with other chemical groups like phosphates in the case of phospholipids, sugars in the case of glycolipids and proteins in the case of lipoproteins. Derived lipids are the products of the hydrolysis of the first two classes of lipids such as fatty acids and cholesterol. Lipids have important properties like saponification, hydrogenation, rancidity etc which can be used to characterize a given lipid molecule.

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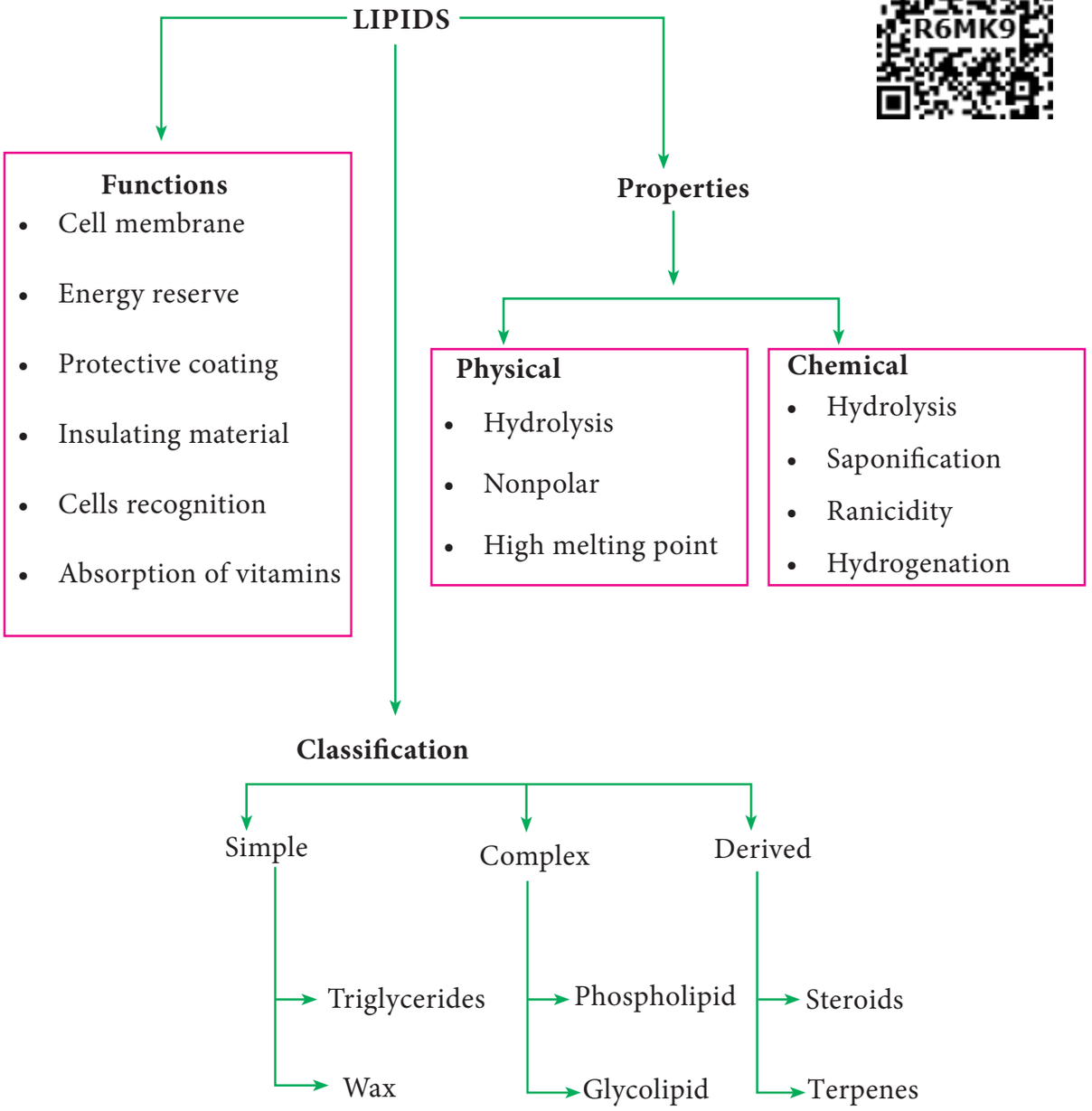
CONCEPT MAP



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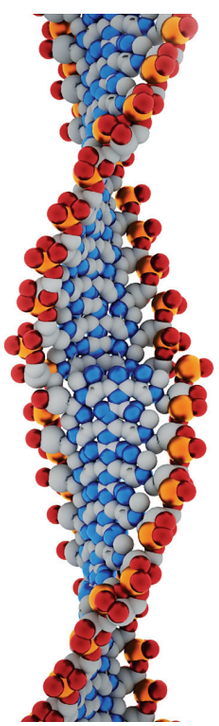
Unit 7 NUCLEIC ACIDS



The Swiss scientist Friedrich Miescher who discovered DNA in 1869 and also suggested that they may play a role in heredity.



Learning objectives

After studying this unit the students will be able to

- Explain the role played by nucleic acids in an organism
- Relate DNA with inheritance of traits from ancestors
- Describe the structural differences between bases, nucleosides and nucleotides.
- Explain the Chargaff's rule
- Elaborate the structure of DNA and RNA
- Differentiate the DNA from RNA

Introduction

We might have heard people saying, "It is in your genes?", "like mother, like daughter". Many physical traits like skin colour, curly or straight hair etc... are inherited from our parents or grandparents. We also inherit talent in special fields such as art, music, etc from then. How are these characters inherited? This is through the agents of inheritance called DNA.

Nucleic acids were first discovered by Friedrich Miescher in 1869 and were named as nuclein as they were found

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in the nuclei of leukocytes (pus cells) from discarded surgical bandages. The functions of nucleic acids were left undiscovered until early 1900.

7.1 Significance of Nucleic acids

- Nucleic acids are the molecular repositories (store houses / reserve bank) of genetic inheritance, i.e they have the ability to store and transmit information from one generation to the next.
- Ultimately, every macromolecule (proteins and RNAs) of the cell is the product of information that has been stored in the nucleotide sequences of the genes.
- Certain nucleotides like ribozymes have catalytic activities.
- Certain purine and pyrimidine analogs are used for treating cancer and AIDS.

7.2 Composition

Nucleic acids are very long, thread-like polymers, made up of a linear array of monomers called nucleotides held together by phosphodiester bridges. Nucleotides have three characteristic components (i) Base (ii) Sugar (iii) Phosphate group.

7.2.1 Common Bases of Nucleic acids

The bases of nucleic acids are heterocyclic, containing aromatic ring in their structures. They may be monocyclic pyrimidines or bicyclic purines.

i. Pyrimidine bases

- Pyrimidines are six-membered heterocyclic aromatic rings containing two nitrogen atoms (Figure 7.1) Pyrimidine ring is numbered in the clock-wise fashion. The common naturally occurring pyrimidines are cytosine, uracil, and thymine (5-methyluracil) (Figure 7.2). Cytosine and thymine are the pyrimidines typically found in DNA, whereas cytosine and uracil are common in RNA.

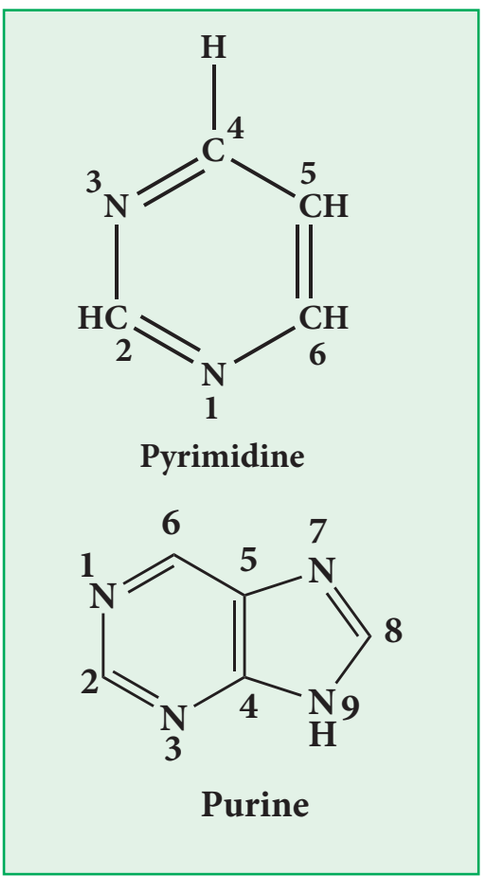

Figure 7.1. Structure of Purine and Pyrimidine

Properties of Pyrimidine bases

1. Pyrimidines are basic in nature. They are less soluble in water.

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2. They absorb UV light at 260 nm. This property is used to detect and estimate DNA and RNA in biological solutions.

3. They are capable of forming hydrogen bonds with purine nucleotides in nucleic acids.

4. They exhibit keto–enol tautomerism.

ii. Purine bases

Purine is a bicyclic ring formed by fusion of pyrimidine ring with imidazole ring. Purine ring is numbered in the anti-clockwise fashion. Adenine (6-amino purine) and guanine (2-amino-6-oxy purine), the two common purines, are found in both DNA and RNA (Figure 7.2). Other naturally occurring purine derivatives include hypoxanthine, xanthine, and uric acid. Hypoxanthine and xanthine are found only rarely as constituents of RNA, while they are intermediates (compounds formed in the synthetic pathway) in the synthesis of nucleic acids Uric acid is the catabolic end product of nucleic acids.

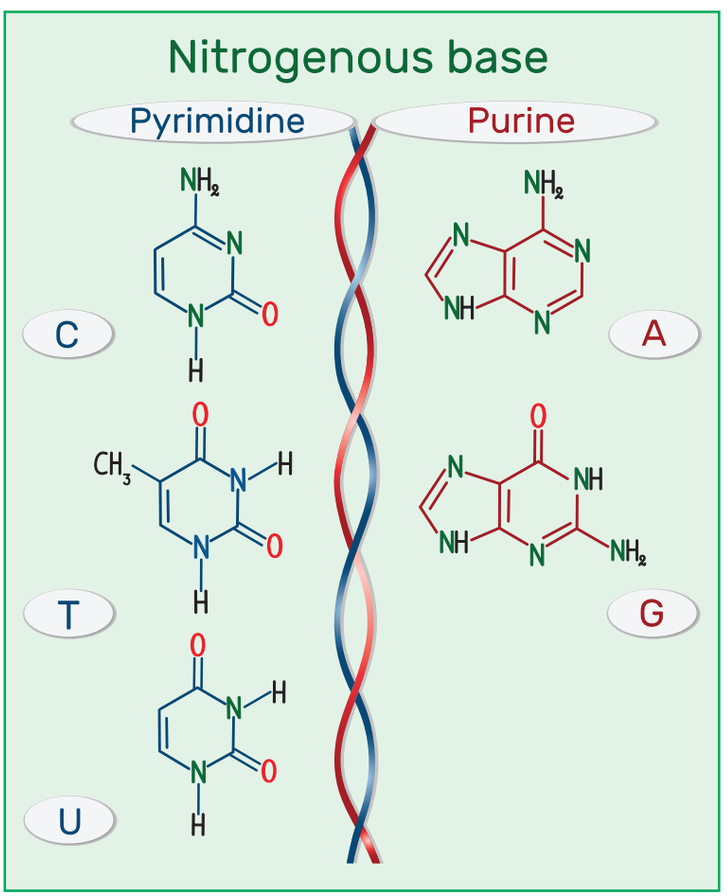

Figure 7.2. Structure of Purine and Pyrimidine bases

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Properties of Purine bases

1. Purines are basic in nature. They are sparingly soluble in water.
2. They also absorb UV light at 260 nm. This property is used to detect and estimate DNA and RNA in biological solutions.
3. They are capable of forming hydrogen bonds with pyrimidine nucleotides in nucleic acids.
4. They exhibit keto –enol tautomerism.

7.2.2 Sugars

There are two types of sugars present in nucleic acids. They are ribose and deoxyribose (Fig.7.3). Based on the sugar moiety present in the nucleic acids, they are classified as Deoxy ribonucleic acid (DNA) and Ribonucleic acid (RNA). DNA is present in the nucleus, mitochondria and chloroplasts. RNA is present in the nucleus, nucleolus, ribosome and cytoplasm. Ribose and deoxy ribose have differences in their properties. Deoxy ribose is less reactive in nature, when compared to ribose.

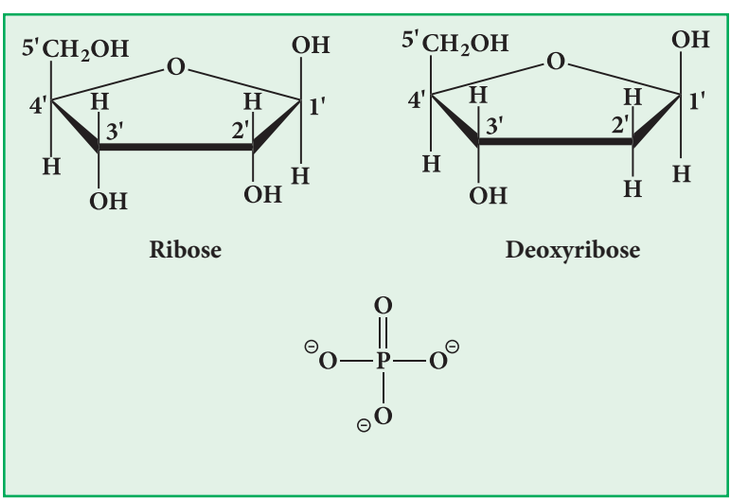

Figure 7.3 Structure of ribose, deoxy ribose and phosphate

7.2.3 Phosphate

Phosphoric acid forms phospho-diester linkage between nucleosides. Based on the number of phosphate group present in the nucleotide, they are classified as monophosphates, diphosphates and triphosphates.

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7.3 Nucleosides

A nucleoside is composed of purine or pyrimidine base and a pentose sugar. In the case of purine nucleoside, the sugar is attached to N-9 of the purine ring, whereas in pyrimidine nucleoside, the sugar is attached to the N-1 of the pyrimidine ring. This type of linkage is N-glycosidic linkage(Fig. 7.4).

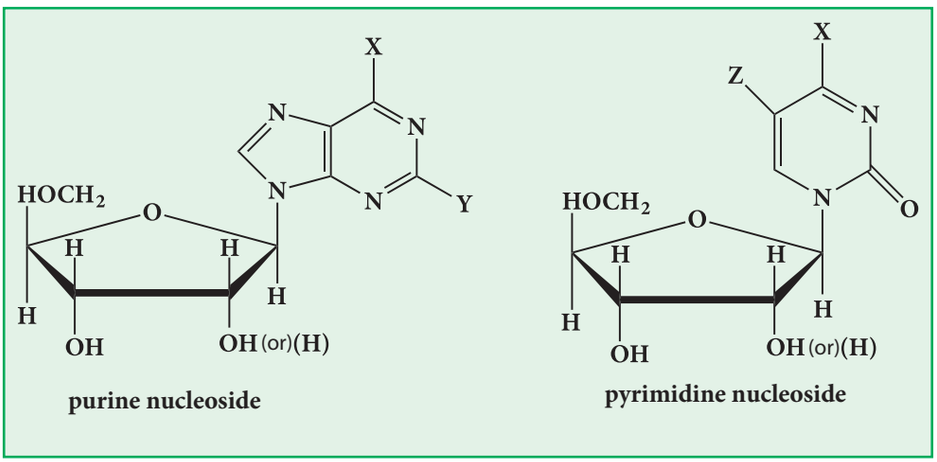

Figure 7.4 Structure of a nucleoside

7.4 Nucleotides

Nucleotides are phosphorylated forms of nucleosides. Phosphorylation (addition of phosphate group) occurs generally in the 5′OH group of the ribose or deoxy ribose sugar. A nucleotide with one phosphate group is called as monophosphate of the respective nucleoside. For example, the monophosphate of adenosine is called as Adenosine Mono Phosphate. If two phosphate groups are attached to the sugar moiety at the 5′OH group, it is called as diphosphate. eg. Cytidine Diphosphate. A nucleotide containing three phosphate groups is called as triphosphate eg. Adenosine Triphosphate (Fig. 7.5). The corresponding nucleosides and nucleotides are listed in Table 7.11 (Bases, their nucleosides and nucleotides).

Functions

- Nucleotides are the energy currency of the cells (ATP).
- They actively take part in metabolism as hydrogen donors, phosphate group donors and methyl group donors.
- They form the structural components of some co-enzymes (NAD and FAD).

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Table 7.1 Bases, their nucleosides and nucleotides

Name of the base	Sugar	Nucleoside	No. of Phosphate Groups	Nucleotide
Adenine	Ribose	Adenosine	1	AMP
			2	ADP
			3	ATP
	Deoxyribose	Deoxy Adenosine	1	dAMP
			2	dADP
			3	dATP
Guanine	Ribose	Guanosine	1	GMP
			2	GDP
			3	GTP
	Deoxyribose	Deoxy Guanosine	1	dGMP
			2	dGDP
			3	dGTP
Cytosine	Ribose	Cytidine	1	CMP
			2	CDP
			3	CTP
	Deoxyribose	Deoxy Cytidine	1	dCMP
			2	dCDP
			3	dCTP
Uracil	Ribose	Uridine	1	UMP
			2	UDP
			3	UTP
	Deoxyribose	Deoxy Uridine	1	dUMP
			2	dUDP
			3	dUTP
Thymine	Ribose	Thymidine	1	TMP
			2	TDP
			3	TTP
	Deoxyribose	Deoxy Thymidine	1	dTMP
			2	dTDP
			3	dTTP

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- Nucleotides like cAMP (cyclic AMP) and cGMP (cyclic GMP) act as second messengers involved in hormonal signaling pathways.

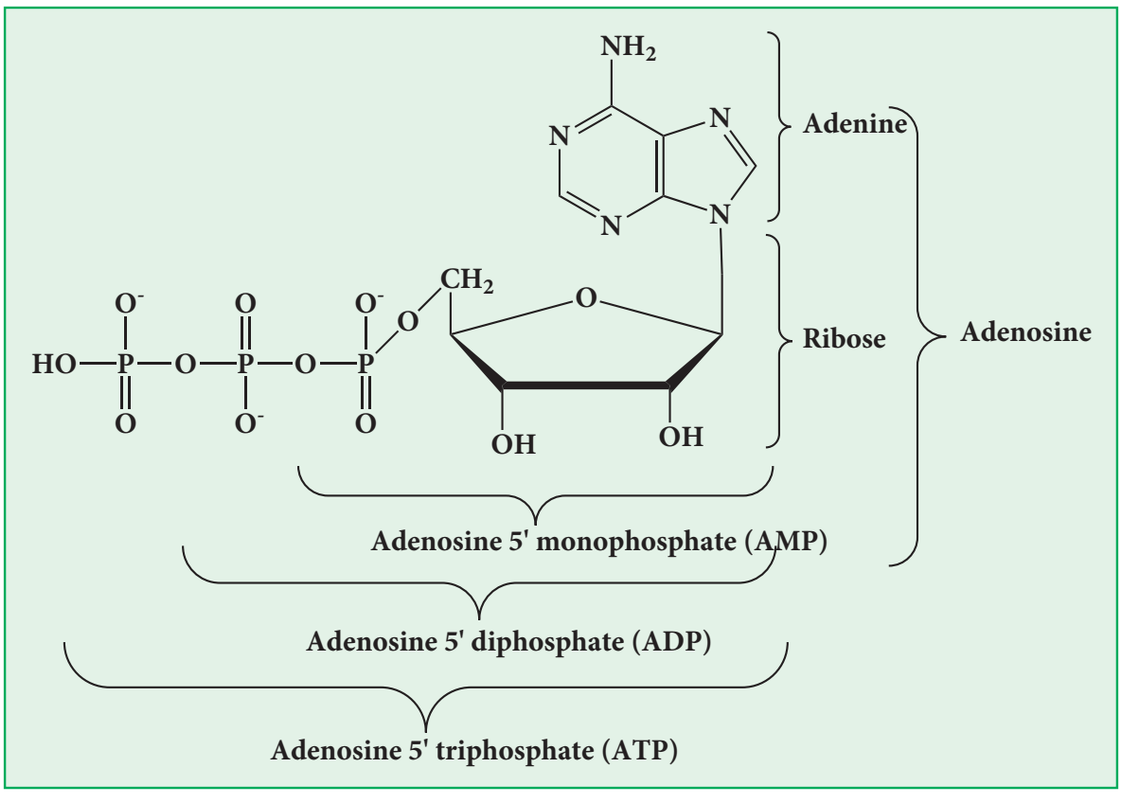

Figure 7.5 Structure of a nucleotide



Nucleosides generally do not have physiological role. However, adenosine is an exemption with biological activity. It acts as a local hormone and is involved in many different biological effects like blood vessel dilation. Supraventricular tachycardia, a heart condition characterized by rapid heart beat is treated with intravenous injections of adenosine. Adenosine promotes sleepiness. Caffeine, a chemical found in tea and coffee brings wakefulness by blocking the binding of adenosine to its receptors.

Oligonucleotides are polymers which yield two to ten residues of mononucleotides on hydrolysis. Two nucleotides join together to form dinucleotides. Example for biologically important dinucleotides are NAD and FAD, which act as co-enzymes.

Polynucleotides are polymers that yield more than ten nucleotides on hydrolysis. Polynucleotides have directions. If the first nucleotide of the sequence has 5′ triphosphate free and 3′OH group bonded to the next nucleotide, then the direction of the polynucleotide is 5′to 3′. If the first nucleotide in the sequence has 3′OH group free, then it is said to be from 3′to 5′direction.

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7.5 Structure of DNA

In 1953, J.D. Watson and F.H.C. Crick proposed a precise three dimensional model of DNA structure based on model building studies, base composition and X-ray diffraction studies carried out by Maurice Wilkins and Rosalind Franklin. This model is popularly known as the DNA double helix (Figure7.6).



The Nobel prize in Physiology or Medicine(1962) was awarded jointly to Francis Harry Crompton Crick, James Dewey Watson and Maurice Hugh Fredrick Wilkins "for discoveries concerning the molecular structure of nucleic acids



Francis Harry Compton Crick



James Dewey Watson



Maurice Hugh Fredrick Wilkins

7.5.1 Different forms of DNA

There are three forms of DNA – A, B and Z DNA. The characteristics of each form of DNA is listed in the table given below.

Table 7.2 Properties of major foms of DNA

Particulars	A DNA	B DNA	Z DNA
Helix	Right handed	Right handed	Left handed
Base pairs per turn	~11	~10.5	~12
Helical Diameter (nm)	2.6	2.0	1.8
Helical length (nm)	2.6	3.4	3.7
Shape	Broadest	Intermediate	Narrowest
Major Grove	Wide, deep	Narrow, deep	Flat
Minor Grove	Narrow, shallow	Broad, shallow	Narrow, deep

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DO YOU KNOW?

Synthetic analogs of purines, pyrimidines, nucleosides and nucleotides or sugar moiety have numerous application in clinical medicine. Oncologists employ 5- fluoro - or 5 iodouracil, and 8-azaguanine for treatment of cancer. The purine analog allopurinol is used in treatment of hyperuricemia and gout. Azathioprine is employed during organ transplantation to suppress immunologic rejection.

7.5.2 Salient features of the structure of DNA

The B form DNA, also known as the Watson- Crick DNA is the most stable and prevalent form of DNA. The important structural features of B- DNA are :

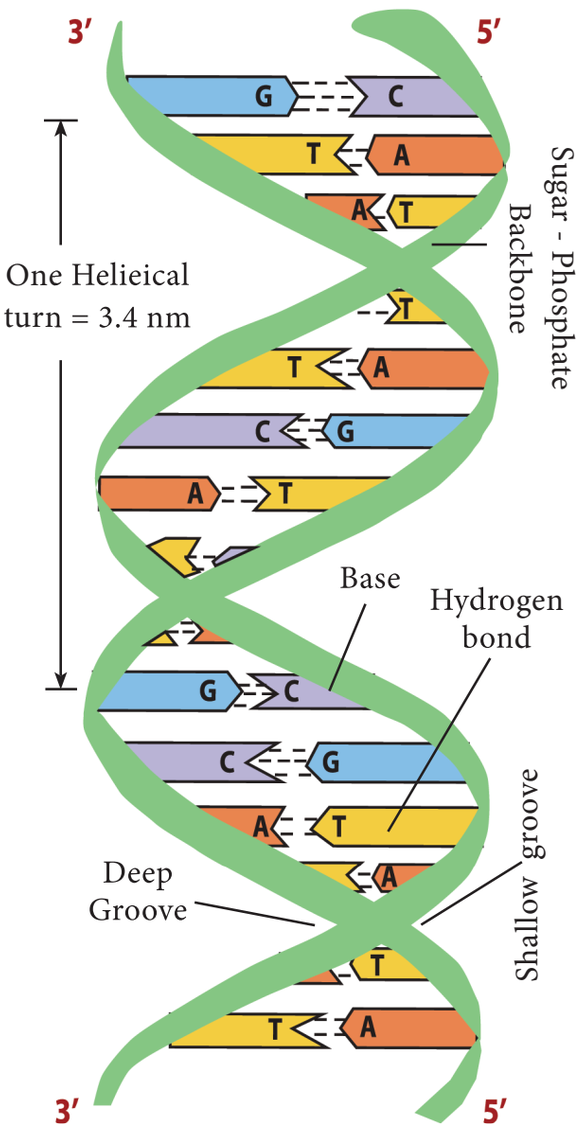
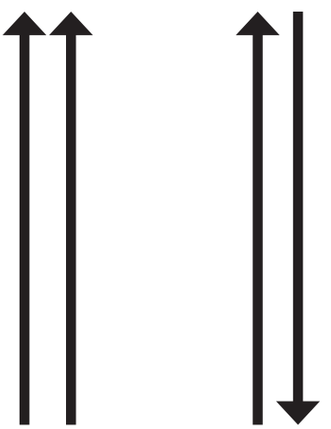


Figure. 7.6 Sructure of DNA

1. There are two polynucleotide chains in the DNA spirally twisted around each other to form a right handed double helix.



Parallel **Anti-parallel**

2. The sugar-phosphate backbones remain on the outside, while the core of the helix contains the purine and pyrimidine bases.

3. The diameter of DNA is 2 nm or 20 A°. The length of a complete turn of helix is 3.4 nm or 34 A° i.e. there are ~10.5bp per turn.

4. The DNA helix has a shallow groove called minor groove (-1.2nm) and a deep groove called major groove (-2.2nm). Proteins interact with DNA through the minor and major grooves without disrupting the DNA strands.

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5. Each polynucleotide chain is made up of four different bases. The purine bases present in DNA are adenine and guanine and the pyrimidine bases present are thymine and cytosine. The sequence of purine and pyrimidine carry the genetic information whereas the sugar and phosphate groups perform the structural role.

6. Each polynucleotide chain has direction or polarity. Further, each polynucleotide chain has 5′phosphorylated and 3′hydroxyl ends.

7. The two strands run in opposite direction (i.e.) they are antiparallel.

8. The two strands are held together by hydrogen bonds (base pairing) between the purine and pyrimidine bases of the opposite strands.

Watson and Crick deduced the rules of base pairing (Fig. 7.7). They are:

- The purine adenine (A) always pairs with the pyrimidine thymine (T).
- The purine guanine (G) always pairs with the pyrimidine cytosine (C).

Base pairing is achieved through hydrogen bonding.

Therefore, if adenine appears in one strand, thymine is found in the opposite strand and vice versa. When guanine is found in one strand, cytosine is present in the opposite strand and vice versa. So, Base sequence of one strand is complementary to the opposite strand. For example, If the sequence 5'ATGGACC3′is present in one strand, the complementary strand will be having the sequence, 3'TACCTGG5'.

There are two hydrogen bonds between A and T and three hydrogen bonds between G and C.

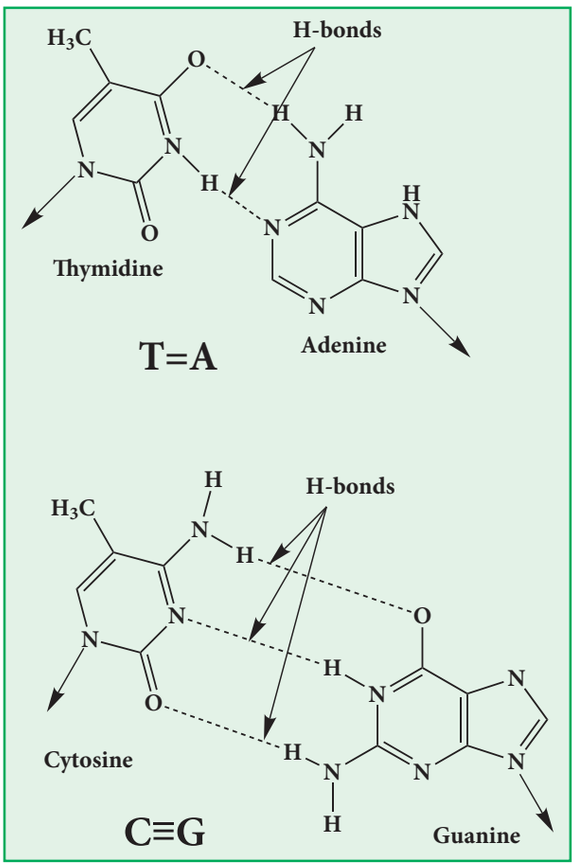

Figure. 7.7 Nucleotide base pairing

9. Base composition of DNA obeys Chargaff's rule.

Chargaff's rule

Erwin Chargaff, a scientist

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analyzed the chemical composition of DNA isolated from different species and found that irrespective of the source, the molar concentration of Adenine always equals Thymine and the molar concentration of Guanine always equals Cytosine. A=T and G=C. Therefore, A + T = G + C and the ratio of A+T /G+C = 1.0,which means that the total number of purine bases = the total number of pyrimidine bases.

7.6 Denaturation of DNA

At high temperatures (95^{0}C), the double helical structure of DNA melts due to disruption of base pairing that results in two single strands. This is called as Denaturation of DNA. The temperature at which it does so is called as Melting temperature (Tm). AT rich regions melt faster than GC rich regions. Therefore, Tm is dependent upon the composition of DNA. During denaturation, the absorption of DNA at 260nm increases. This property of DNA is called as hyperchromicity. If the temperature is brought down, the single strands rejoin to form double stranded regions. This is called as annealing or renaturating of DNA (Fig. 7.8). This property of DNA is exploited in Polymerase Chain Reaction.

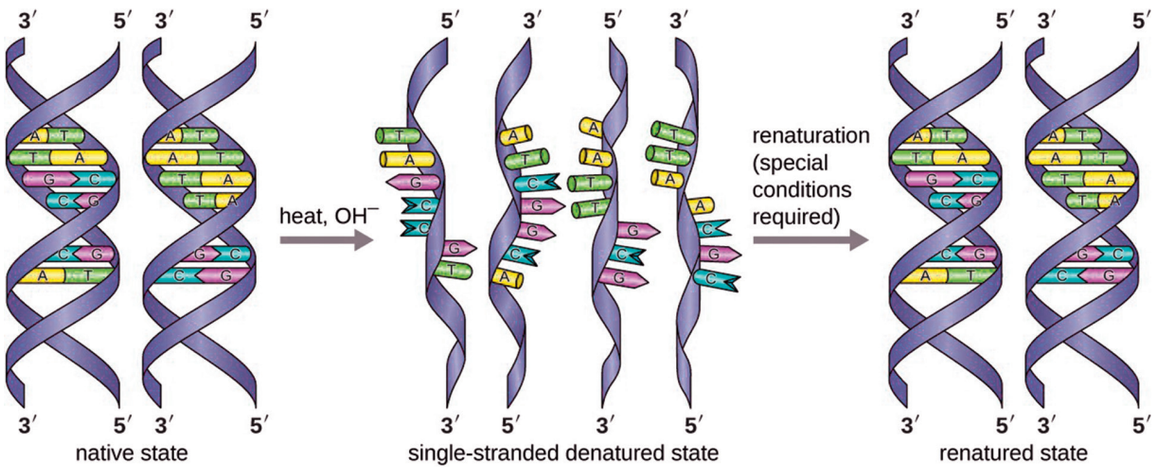

Figure. 7.8 Denaturation and renaturation of DNA

7.7 Griffith's experiment to identify the genetic material

Frederick Griffith conducted experiments in 1928 with *Streptococcus pneumoniae*, which was the milestone for discovery of DNA as the genetic material. There are two different strains of *Streptococcus pneumoniae*, one with smooth shiny colonies called the S strain, while the other with rough colonies (R). The difference is due to the presence of mucous coat in S strain bacteria, whereas the R strain bacteria lacked them. Because of the presence of the mucous coat, S strain is lethal, while R strain is not lethal.

Griffith injected both S and R strains to two different groups of mice separately. The group which was infected with the S strain developed pneumonia and died while that infected with the R strain stayed alive.

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Next, Griffith heat-killed the S strain bacteria and injected into mice, but the mice stayed alive, which suggests that heat killed S- strain are avirulent (inefficient to cause the disease). Then, he mixed the heat-killed S and live R strains and injected into the mice. The mice died. In addition, he found living S strain bacteria in dead mice (Fig. 7.9). This suggests that some transforming principle is present in the S form that has mediated the transformation of R form to S form. Later, the experiments conducted by Avery, Macleod and Mc Carty in1944 showed that the transforming principle is DNA, which carries the genetic information from one generation to another.

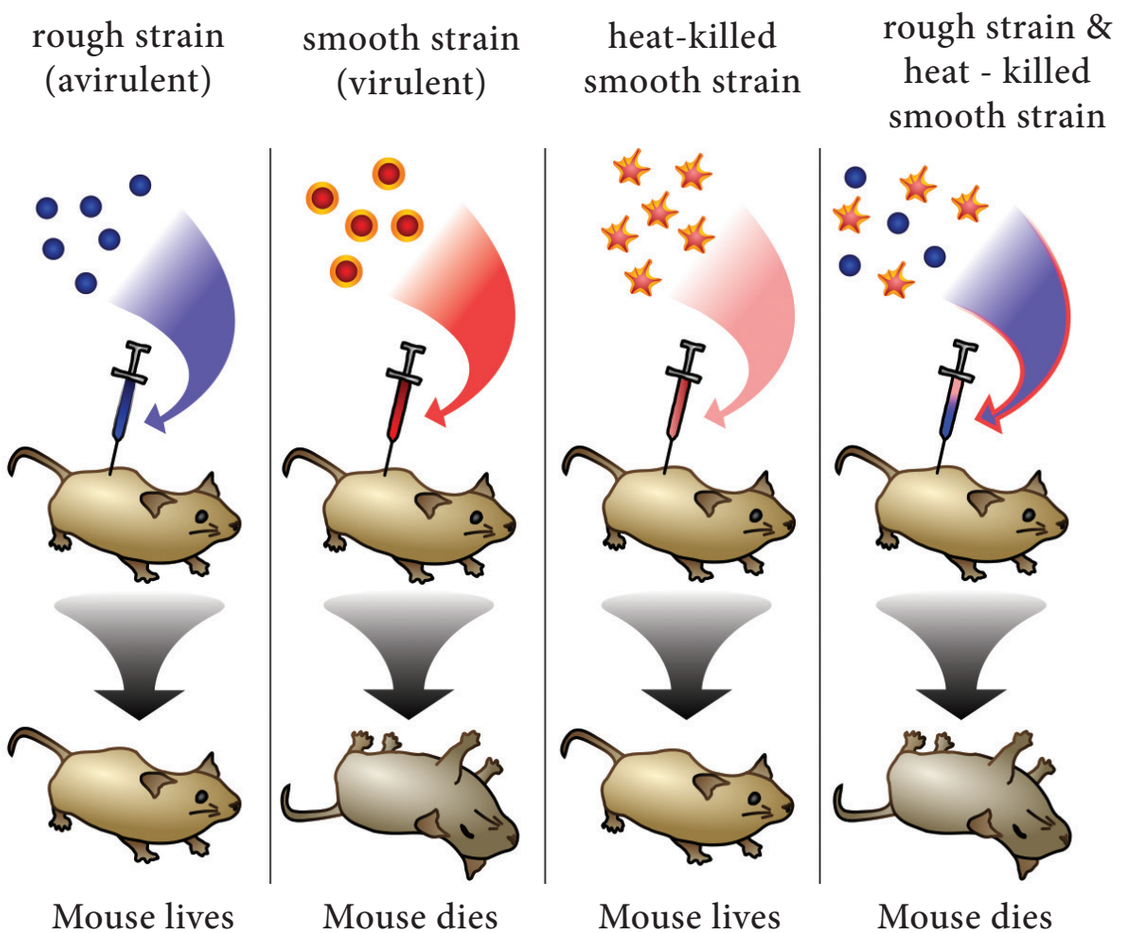

Figure. 7.9 Griffith's Experiment to identify genetic material

Functions of DNA

1. DNA is the genetic material of all living organisms except for RNA viruses like HIV. It is the greatest super chip and enormous data can be stored in high density.
2. DNA inside the zygote contains all the information required for developing into an individual organism.
3. DNA inherited from their parents is responsible for the characteristic features of an individual, i.e. shape of the eyes, ears, nose, colour of the skin, height, longevity, ability

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to withstand stress, certain congenital diseases like diabetes and hypertension.

4. DNA is the source of information for the synthesis of all cellular proteins. The segment of DNA that contains information for a protein is known as gene.

7.8 Ribonucleic acids (RNA)

RNAs, the second most abundant form of nucleic acids inside the cell are single-stranded and composed of nucleotides with ribose as their sugar. The major role of RNA is in protein synthesis as it aids in transfer of information from nucleus to cytosol, decoding, and synthesis of proteins. RNA nucleotides contain three components.

- Nitrogenous Base
- Ribose Sugar
- Phosphate Group

The nitrogenous bases include adenine (A), guanine (G), cytosine (C) and uracil (U).

Eventhough RNA is single-stranded, it has the ability to form three-dimensional structures like hairpin loops by base pairing within the strand. Adenine pairs with uracil (A=U) and guanine pairs with cytosine (G≡C). Chargaff's rule is not followed by RNA.

7.8.1 Types of RNA

There are three major types of RNAs in all prokaryotic and eukaryotic cells. They are (1). Messenger RNA (mRNA). (2). Transfer RNA (tRNA) (3). Ribosomal RNA (rRNA). They differ from each other by size, shape, formation and stability.

i. Messenger RNA

They are named so because they carry the information from nucleus to cytosol. It accounts for 1-5% of cellular RNA. They do not have specific secondary structure like helices. They are single stranded linear molecules. They consist of 1000-10,000 nucleotides. They have a free or phosphorylated 3′and 5′end. They have different life span ranging from few minutes to days.

Prokaryotic mRNA is different from eukaryotic mRNA. Prokaryotic mRNAs are polycistronic, that is they code for many proteins. While eukaryotic mRNAs are monocistronic and contain exons (expressed sequences) and introns (intervening sequences). Eukaryotic mRNAs are capped at 5′end by methylated guanosine triphosphate. Capping protects mRNA from nuclease attack. At 3′end, a polymer of adenylate (polyA) is found as tail. Poly A tail protects mRNA from nuclease attack.

Intrastrand base pairing among complementary bases allows folding of the linear molecule. As a result, haripin or loop like secondary structure is formed.

Functions

1. mRNA is a direct carrier of genetic information from the nucleus to cytoplasm.
2. It contains information required for the synthesis of protein molecules.

ii. Transfer RNA

It accounts for 10-15% of total cell RNA. Usually they consist of 50-100 nucleotides. They are single stranded

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molecules.They contain unusual bases such as methylated adenine, thymine, dihydrouracil and pseudouridine. These unusual bases are unique to tRNA. Intra-chain base pairing is observed with many base pairs, while some bases are not involved in base pairing, resulting in loops and arms formation in tRNA. These foldings in the primary structure generates a clover-leaf secondary structure (Figure 7.10).

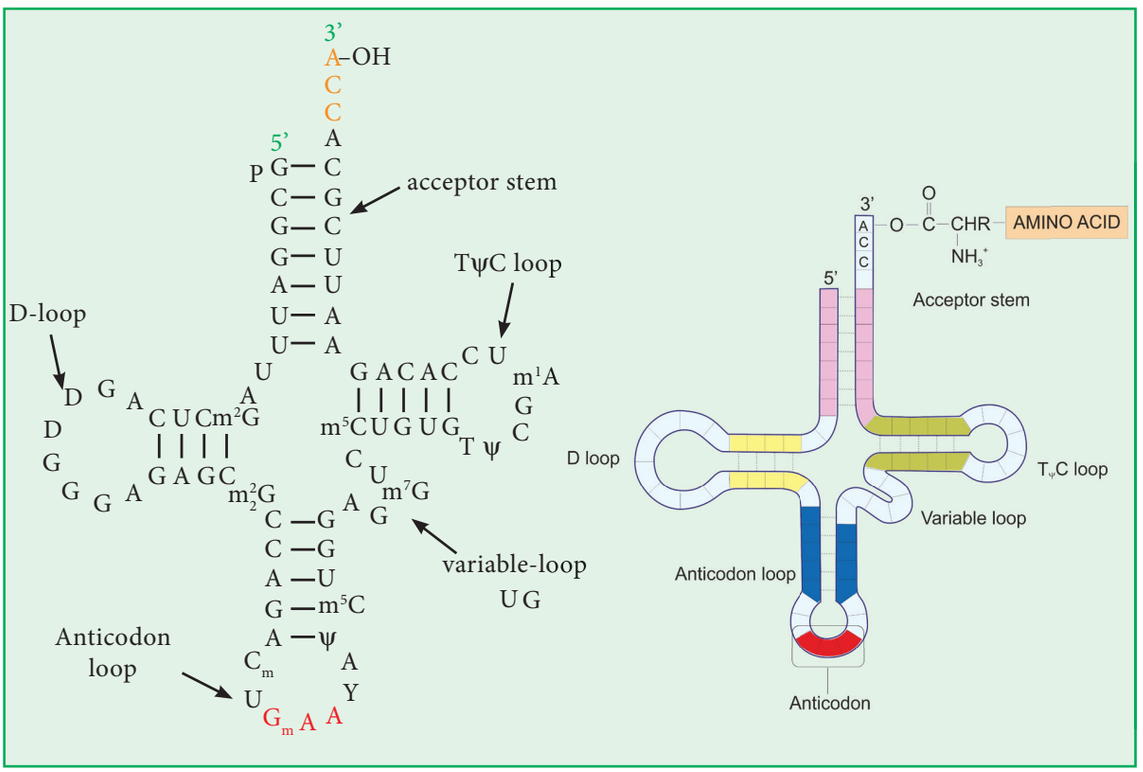

Figure 7.10 Structure of tRNA

The important features of the clover-leaf structure are,

1. An acceptor arm with base sequence "CCA" is present at the 3′end. The hydroxyl group of adenosine moiety of tRNA is responsible for getting amino acylated.
2. An anticodon arm which recognises codon on mRNA is present.
3. A TψC loop which contain an unusual base pseudouracil.
4. A D - arm which contain many dihydrouracil residues.

Functions

1. It is the carrier of amino acids to the site of protein synthesis.
2. There is at least one t-RNA molecule to each of 20 amino acids required for protein synthesis.

iii. Ribosomal RNA

Ribosomal RNA accounts for 80% of the total cellular RNA. It is present in ribosomes in combination with proteins. It is known as ribonucleoprotein. The length

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of rRNA ranges from100-600 nucleotides. rRNA molecules have a secondary structure. Intra-strand base pairing between complementary bases often generate double helical segments or loops.

Functions

1. They are required for the formation of ribosomes.
2. They are involved in the initiation of protein synthesis.



Apart from these RNAs, there are also a heterogenous group of RNAs present in the nucleus called as hnRNA and small non-coding RNAs of approximately 22 nucleotide length called as micro RNA or miRNAs.

7.9 Differences between DNA and RNA

S. No.	DNA	RNA
1	Sugar moiety is Deoxy ribose	Sugar moiety is Ribose
2	The bases present are Adenine, Thymine, Guanine and Cytosine. Uracil is not present.	The bases present are Adenine, Uracil, Guanine and Cytosine. Thymine is rarely present.
3	Double stranded molecules	Single stranded molecules
4	Obeys Chargaff's rule	Does not obey Chargaff's rule
5	Bases are not modified	Bases are modified
6	It is stable and not hydrolysed easily by alkalis	It is unstable and hydrolysed easily by alkalis
7	DNA content is constant in all the cells except during cell division	Varies from cell to cell
8	The life time of DNA is comparatively high.	RNA is short lived.
9	No natural DNA is catalytic	RNA can be catalytic
10	Present in the nucleus, mitochondria and chloroplast	Present in the nucleus, mitochondria, nucleolus, ribosomes and cytosol.

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EVALUATION



I Choose the correct answer

1. The genetic material in higher organisms is

   a. mRNA b. rRNA

   c. DNA d. Protein

2. _____ ____ ring is numbered in the clock wise direction.

   a. Pyrimidine b. Purine

   c. Thiamine d. All the above

3. Which nucleotide sequence has a high Tm?

   a. AAATTT b. GGGCCC

   c. AAGTTC d. GGATTC

4. Purine and Pyrimidine bases absorb at _____ nm.

   a. 260 b. 280

   c. 300 d. 650

5. Which of the following contains base, sugar and phosphate in its structure?

   a. Adenine b. Adenosine

   c. AMP d. Deoxy adenosine

6. _____ acts as second messenger in hormonal signaling pathways.

   a. AMP b. ADP

   c. cAMP d. ATP

7. Which of the following form of DNA has 11 base pairs per turn?

   a. A DNA b. B DNA

   c. C DNA d. Z DNA

8. Which is the stable form of DNA?

   a. A DNA b. B DNA

   c. C DNA d. Z DNA

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9. The diameter of B-DNA is _____nm.

a. 2 b. 20

c. 10 d. 34

10. If guanine is present in one strand, the complementary strand will be having _____.

a. Adenine b. Guanine

c. Cytosine d. Thymine

11. Chargaff's rule is obeyed by

a. DNA b. tRNA

c. mRNA d. rRNA

12. Forming of double stranded DNA on bringing back the temperature of Denatured DNA is called as

a. Rejoining b. Denaturation

c. Melting d. Annealing

13. _____'s experiment identified DNA as the genetic material.

a. Meischer b. Griffith

c. Avery, Mc Carty and Macloed d. Watson and Crick

14. _____ has a clover leaf structure.

a. DNA b. tRNA

c. mRNA d. rRNA

15. _____ is the carrier of amino acids to the site of protein synthesis.

a. DNA b. tRNA

c. mRNA d. rRNA

16. The differential base present in DNA and RNA is ……..

a) Adenine b) Guanine

c) Cytosine d) Uracil

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II Give short answers (Two marks)

1. Who discovered nucleic acid? When and how?
2. Write down the composition of nucleic acids.
3. Name the common bases present in nucleic acids.
4. What is a nucleotide? Give examples.
5. Write shortly on the sugars present in the nucleic acid.
6. Give the nucleosides and nucleotides of adenine.
7. What is a dinucleotide? Give examples.
8. Write about the directionality of nucleotides.
9. State Chargaff's rules.
10. Give the rules of base pairing.
11. What is the complementary strand for 5'GTAATTGC3'?
12. What are the major types of RNA?

III Give short answers (Three marks)

1. What are bases? How are they classified? Give example for each group.
2. List the properties of pyrimidine bases.
3. Write short notes on purine bases.
4. What are Oligo nucleotides Give examples?
5. Enumerate the functions of nucleotides.
6. Outline the differences between A, B and Z DNA.
7. List down the functions of DNA.
8. Write about the characteristic features of mRNA.
9. What are ribosomal RNAs? Give an account on them.

IV Answer the following questions in elaborate manner (Five marks)

1. List the Significance and functions of nucleic acid.
2. Detail on the bases, their types and properties.
3. Write an essay on the composition of nucleic acids.

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4. Draw a neat labelled diagram and explain the salient features of Watson-Crick Structure of DNA.

5. Outline the experiment carried out by Griffith to identify the genetic material.

6. With a neat illustration, explain the structure of t-RNA.

7. Give the differences between DNA and RNA.

Activity

The students may be asked to prepare a three dimensional structure of DNA or to prepare a chart explaining the structure of DNA.

SUMMARY

Nucleic acids are the molecular repositories of genetic inheritance. Every macro molecules of the cell is the product of the information that has been stored in the nucleotide sequences of genes. Nucleic acids are long threadlike polymers made up of monomers called nucleotides held together by phospho diester bridges. Nucleotides have three components namely base, sugar and a phosphate group.

The bases are either monocyclic pyrimidines (cytosine, uracil and thymine) or the bicyclic purines (adenine and guanine). There are two types of sugars are present in nucleic acid. They are ribose and deoxyribose. When the sugar is ribose it is called ribonucleic acid (RNA) and if the sugar is deoxy ribose it is called Deoxyribonucleic acid (DNA). The phosphate groups are used to connect the nucleotides with each other. Depending upon the number of phosphate groups they can be classified as monophosphates, diphosphates and triphosphates.

DNA is the genetic material of all living organisms except RNA viruses like HIV. The B form DNA, also known as the Watson-crick DNA, is the most stable and prevalent form of DNA. The two antiparellel strandes of DNA are complementary to each others. The diemeter of DNA is 2nm and helical length is 3.4nm. It has ~10.5basepairs per turn of the helix. It has three different forms namely A-DNA, B-DNA and Z-DNA. There are three major types of RNA are known. They are messenger RNA (mRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA).

They differ from each other by size, shape, formation and stability. The mRNA is a direct carrier of genetic information from nucleic acid to cytoplasm. tRNA is the carrier of amino acids to the site of protein synthesis. rRNA are required or the formation of ribosomes.

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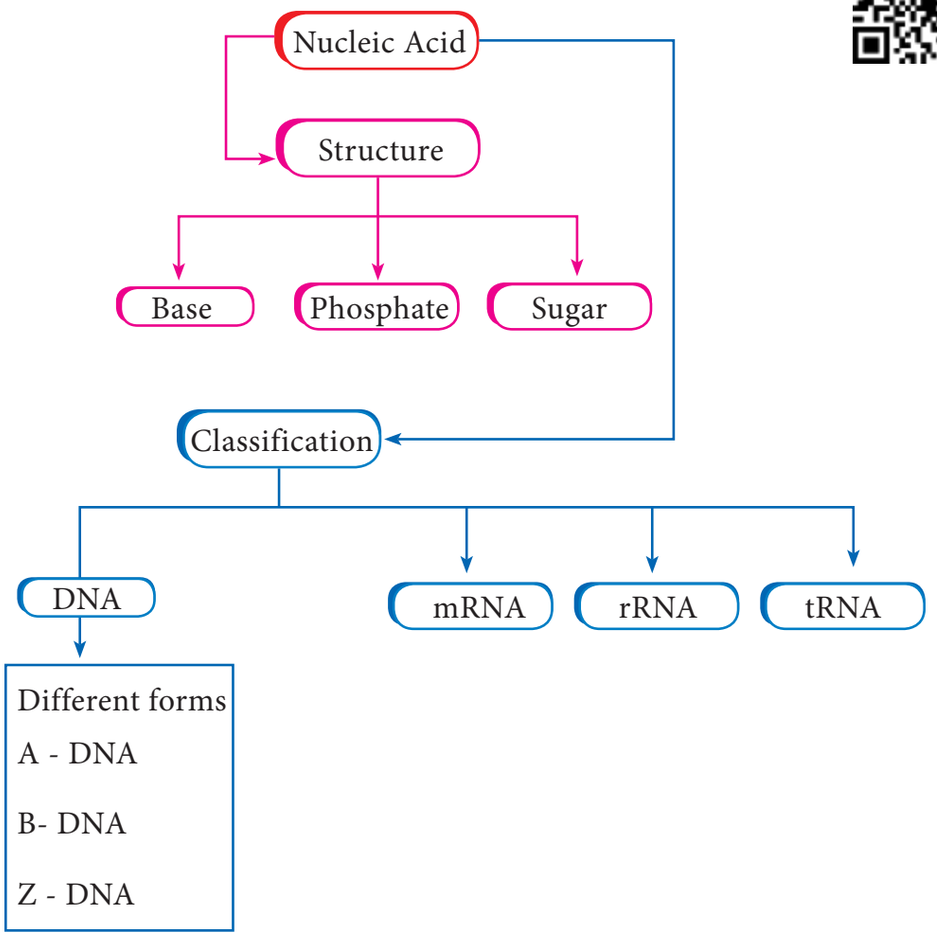
CONCEPT MAP



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Unit 8 Vitamins



Christiaan Eijkman

Christiaan Eijkman was Dutch physician who showed that the disease Beriberi was actually a nutritional defect. Interestingly, Eijkman discovery was accidental where he noted that chicken fed with polished rice developed Beriberi like symptoms, and feeding them back with unpolished rice made the chicken normal. This eventually led to the identification of the vitamin thiamine (B1). He was awarded Nobel prize along with Sir Fredrick Hopkins for the discovery of vitamins in 1929

Learning Objectives

After studying this unit the students will be able to:

- Distinguish fat and water soluble vitamins
- List the natural sources & dietary requirement of vitamins
- Describe the structure, function and the mode of storage of vitamins in our body
- Explain the different diseases associated with vitamin deficiencies
- Relate vitamins and co-enzyme functions.

Introduction

Vitamins are low molecular weight organic compounds indispensable for the normal activity of the organisms. Their absence causes deficiency symptoms. Most of the vitamins or their derivatives are coenzymes which are essential for many important enzymatic reactions in the cells.

Vitamins are required in small quantities for a variety of biochemical functions in our body. Most of them cannot be synthesised by the body and must be supplied through the diet. Unlike

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carbobydrates and lipids, they are not metabolised for providing energy. Normally, a well balanced diet will supply all the necessary vitamins in sufficient quantities.

Classification:

Vitamins are broadly classified into two groups based on their solubility.

i. Fat soluble vitamins

Vitamins A, D, E and K

ii. Water soluble vitamins

Vitamins B complex (B_1, B_2, B_3, B_6 B_{12}, Biotin, Folic acid, Pantothenic acid) and C

8.1 Fat soluble vitamins

The members of this group are vitamin A, vitamin D, vitamin E and vitamin K. All these vitamins are not soluble in water but are readily soluble in fat. These vitamins are present in the liver and fatty tissues. These vitamins can be stored in human body and hence getting too much of these vitamins can also cause problems.

8.1.1 Vitamin A (Retinol):

Vitamin A or Retinol is a poly isoprenoid compound containing a cyclohexenyl ring (Fig. 8.1) . It is found only in foods of animal origin. It is present in almost all species of fish, birds and mammals. The precursor of vitamin A, carotenoid is found in foods of plant origin. The body has the ability to convert carotenoid compound present in the diet into vitamin A. For example beta carotene found in vegetables such as carrot is converted into vitamin A by the symmetrical cleavege by the enzyme β-carotene -15,15'-dioxygenase.

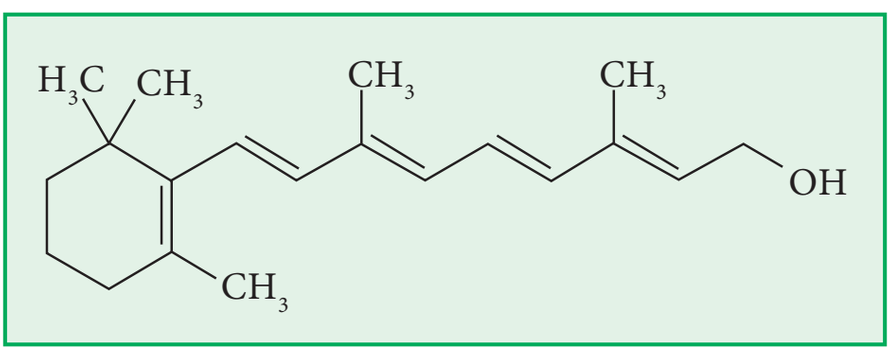

Figure 8.1 Structure of vitamin A

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Table 8.1 Comparison of fat and and water soluble vitamins

S. No.	Characteristics	Fat soluble vitamins	Water soluble vitamins
1	Solubility	Fat soluble	Water soluble
2	Absorption	Bile salts are required	Simple intestinal absorption
3	Transportation	Transported by carrier protein	Travels freely in the body without requiring carrier protein (except Vitamin B_{12})
4	Storage	Stored in liver and fatty tissues	Not stored (except vitamin B_{12})
5	Excretion	Usually the surplus vitamins are stored	Surplus vitamins are detected in kidney and removed in urine
6	Accumulation	Usually hypervitaminosis occurs	Usually hypervitaminosis deosn't occur (except in high dosage and slow release of some B vitamins)
7	Deficiency compensation	Required in periodic doses (weeks or months)	Required in frequent doses (1 - 3 days)

Sources:

Liver, oil, butter, milk, egg-yolk, tomato, carrot, green yellow vegetables, spinach and fruits such as mango and papaya (Figure 8.2).

Vitamin-A

Meat : 25000 IU / 100 g

Spinach (Cooked): 1200 IU / 100 g

Beta Carotene

Carrots (Cooked) : 2500 IU / 100 g

Drumstick leaves: 7000-8000 microgram / 100g

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Figure 8.2 Sources of vitamin A

Functions of vitamin A:

- Vitamin A plays a significant role in the visual cycle (as a component of rhodopsin).
- Retinoic acid plays a key role in glycoprotein synthesis.
- It is essential for the normal structure and functions of epithelial tissues.
- Retionic acid inhibits the enzyme collagenase and thus prevents the breakdown of collagen.
- Retinoic acid is essential for sulfation of the mucopolysaccharides.
- It promotes fertility.
- It is needed for the formation of bone and teeth.
- β-carotene is an antioxidant and plays a role in trapping peroxy radicals in tissues.

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Absorption and storage

Vitamin A and carotene are absorbed from the small intestine into the lymphatic system. The maximum absorption is reached after 3 to 5 hours of food consumption. The rate of absorption of vitamin A is more rapid than that of carotene. In the adult healthy human, only 3% of β-carotene is converted into vitamin A. About 95% of the vitamin A stored in our body is found in the liver and small amount is present in the lungs, adipose tissue and kidneys.

DO YOU KNOW?

Cooking and mashing the vegetables, is it good or bad for β-carotene absorption?

We increase the absorption of β-carotene by steaming, juicing or mashing vegetables. We have to chew well the raw vegetables and fruits in order to maximise the absorption. This causes the cell membranes to rupture and releases more carotene for absorption.

Also the raw vegetables and fruits contain β-carotene in all-trans configuration and cooked vegetables and fruits contain cis-configuration of β-carotene which are more stable and more bioavailable. Therefore, it is best to cook vegetables and fruits in order to maixmise the β-carotene absorption.

Deficiency:

The earliest sign of vitamin A deficiency is concerned with vision. Initially, there is a loss of sensitivity to green light, followed by impairment to adapt to dim light. This condition leads to night blindness. On prolonged or severe deficiency, ulceration of cornea occurs and this condition is known as xerophthalmia or keratomalacia (Figure 8.3).



Figure 8.3 keratomalacia.

8.1.2 Vitamin D:

Vitamin D is a sterol compound. It is represented by a group of steroids occuring chiefly in animals, plants and yeast. There are two distinct forms of Vitamin D, namely Vitamin D_2 and Vitamin D_3. Vitamin D_3 is more active than D_2.

Vitamin D_2:

Vitamin D_2 is also known as ergocalciferol. It is produced by the exposure of ergosterol to UV radiation. Ergosterol occurs in plants, milk and yeast.

Vitamin D_3:

Vitamin D_3 is also known as cholecalciferol. It is the natural form of the vitamin ingested from food. It can also be formed by the exposure of 7-dehydrocholesterol present in the skin to sunlight (UV radiation).

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Transformation from inactive provitamin D to the active form is accomplished by exposure to sunlight (UV rays). Cholecalciferol is converted to calcifediol (25-hydroxy cholecalciferol) in the liver. Similarly, Ergocalciferol is converted to 25-hydroxyergocalciferol. These calicifediols are further hydroxylated to form calcitriols (1,25-dihydroxy cholecalciferol & 1,25-dihydroxy ergocalciferol), the biologically active form of vitamin D. The calcitriol circulates as a hormone in the blood.

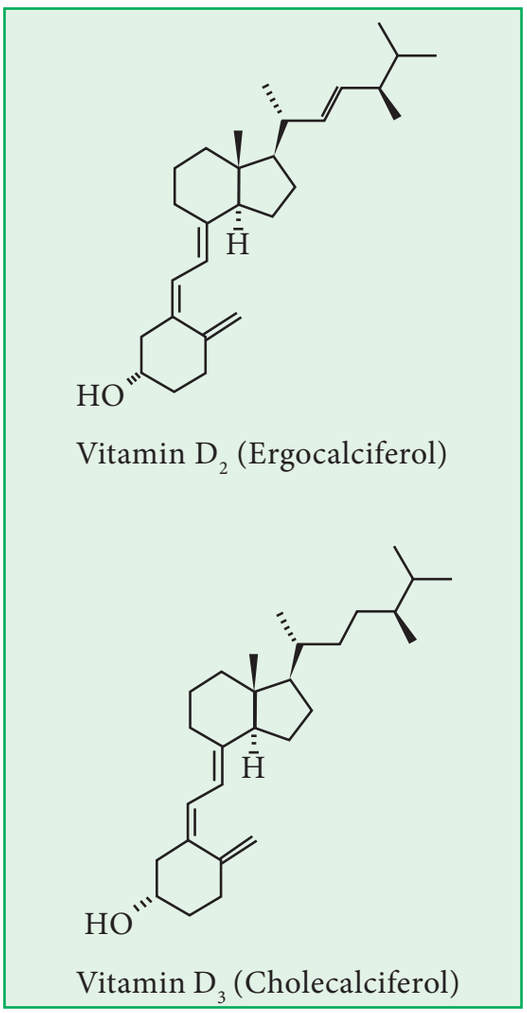

Figure 8.4 Structure of vitamin D

Sources:

Fish liver oil is the richest source of vitamin D. Milk, butter and egg yolk also contain considerable quantity of vitamin D (Fig. 8.5).

Egg yolks: 3- 10 microgram/g

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Functions:

- Vitamin D is required for normal growth in mammals. This is probably related to absorption and utilization of calcium and phosphorous.
- It helps in the normal development of bone and teeth.

Absorption and storage

- Vitamin D absorption occurs from small intestine. Fat and bile are essential for its absorption. Vitamin D enters into the general circulation via lymph and stored mainly in liver and kidneys.

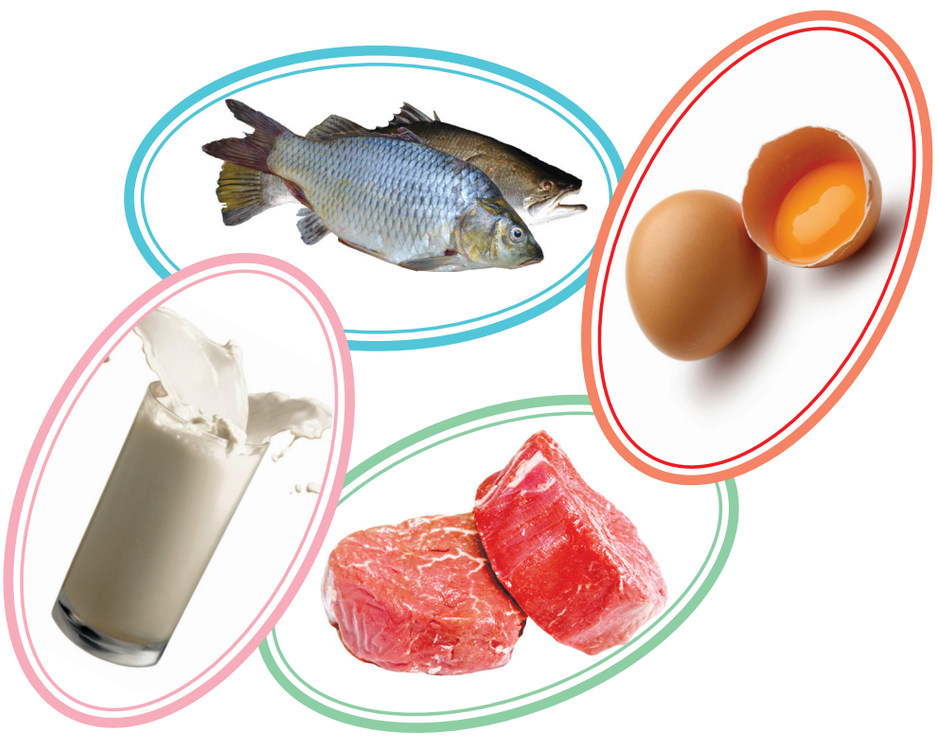

Figure 8.5 Sources of vitamin D

Deficiency:

Deficiency of this vitamin causes rickets in growing children and osteomalacia in adults (Fig. 8.6). It is due to softening of bones resulting from lack of calcium and phosphate.

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Figure 8.6 Rickets and Osteomalacia

Toxicity:

Vitamin D toxicity can occur if one consumes over 10,000 IU per day (1000 IU per day in children) for several months. This causes hypervitaminosis D, which increases the blood calcium levels. This results in bone loss and kidney stones. Long-term overconsumption of vitamin D can cause calcification of organs such as the heart, blood vessels and the kidneys.

8.1.3 Vitamin E:

Vitamin E comprises of a group of isoprenoid substituted compounds called tocopherols (Fig. 8.7). It also includes tocotrienols. The most common form of vitamin E is alpha tocopherol which is present in large quantities in human body. The most abundant form of vitamin E in diet is gamma tocopherol.

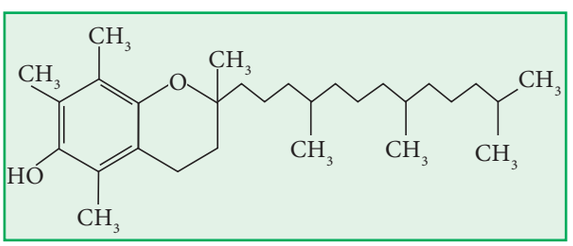

Figure. 8.7 Structure of vitamin E (alpha tocopherol)

Sources:

Cotton seed oil, sun flower oil, wheat germ oil and leafy vegetables are the rich natural sources (Figure. 8.8).

Groundnut oil: 261mg/100g

Wheat germ oil: 150 mg/100g

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Figure 8.8 Sources of vitamin E

Functions:

- **Antioxidant property**:

Vitamin E is involved in removal of free radicals and prevents their effects on unsaturated lipids of membrane and thus helps maintain the integrity of cell membrane.

- It protects the red blood cells against hemolysis.
- It plays a role in normal functioning of muscles.
- Vitamin E is essential for reproductive processes.
- It plays an important protective role during ageing of cells.
- Vitamin E is essential for biosynthesis of coenzyme Q.

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Absorption and storage:

Vitamin E, like other fat soluble vitamins, is absorbed along with fat in the small intestines. It is stored in the liver, muscle and body fat.

Deficiency:

Vitamin E deficiency causes the following conditions in animals.

- Reproductive failure
- Muscular dystrophy
- Combined deficiency of vitamin E and selenium causes hepatic necrosis.

8.1.4 Vitamin K:

Vitamins belonging to the K group are poly isoprenoid substituted naphthoquinones. It is known as the anti-hemorrhagic vitamin.

Three compounds which have the biological activity of vitamin K are:

- Phylloquinone,which is found in green leafy vegetables.
- Menaquinones, which are a family of closely related compounds synthesized by the intestinal bacteria, with different lengths of the side chain.
- Menadione, a synthetic compound which can be metabolized to yield phylloquinone.

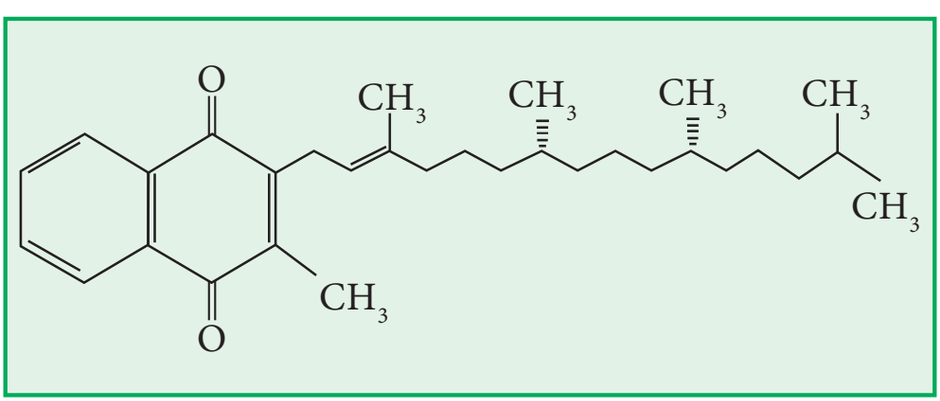

Figure 8.9 Structure of vitamin K

Sources:

Green vegetables, soybean oils, tomatoes, spinach and cabbage are chief plant sources (Fig. 8.10).

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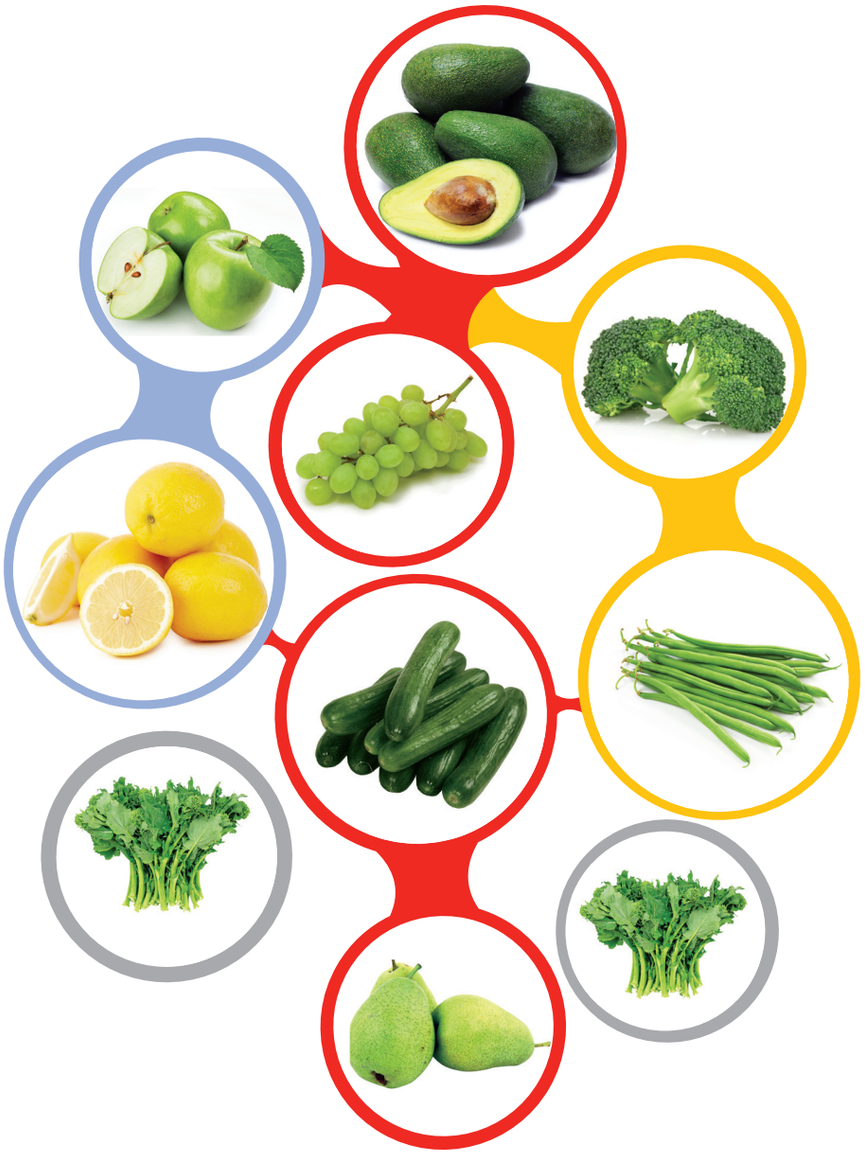

Figure 8.10 Sources of vitamin K

Functions:

- Vitamin K is essential for the synthesis of prothrombin, a substance necessary for blood clotting. That is why it is called as an anti-haemorrhagic vitamin.
- Vitamin K is needed to carboxylate specific glutamate residues of calcium binding proteins of bones, spleen and kidneys. This enhances the capacity of these proteins to deposit calcium in the tissues concerned.
- It plays a key role in the respiratory chain mechanism and oxidative phosphorylation.

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Absorption and storage

- Absorption occurs in intestine. Being fat-soluble, its absorption is enhanced by sufficient amount of bile salts. mainly in the jejunum by the way of lymphatus. Liver stores appreciable amounts of vitamin K.

Deficiency:

- Deficiency of vitamin K is very rare, as most of the daily foods contain this vitamin. In addition, intestinal flora of micro organisms synthesize adequate quantity of vitamin K.
- The deficiency of vitamin K leads to a lowering of prothrombin level and increased clotting time of blood. This may lead to hemorrhagic conditions (Figure. 8.11).



Figure 8.11 Hemorrhagic condition

8.2 Water soluble vitamins:

The members of this group are B-complex vitamins and vitamin C . They are readily soluble in water and can be transported freely in the blood and the watery fluids between the cells. However, vitamin B_{12} needs a binding protein for the transport. Excess of these vitamins are eliminated via kidneys. Unlike other members of this family the vitamin B_{12} is eliminated through bile. As these are readily soluble in water these vitamins are easily lost during cooking.

8.2.1 B-complex vitamins

The vitamin B has a group of vitamins (B_1, B_2, B_3, B_5, B_6, B_{12}, biotin and folate). All these vitamins needs to be converted into the corresponding coenzymes which is their active form. They are useful in the synthesis of many neurotransmitters such as acetylcholine.

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i. Vitamin B_1 (Thiamine)

Vitamin B_1 which is also called as thiamine contains pyrimidine and thiazole ring. Thiamine pyrophosphate (TPP) is known as active thiamine. Thiamine pyrophosphotransferase (ATP dependent enzyme) present in brain and liver is responsible for conversion of thiamine to thiamine pyrophosphate.

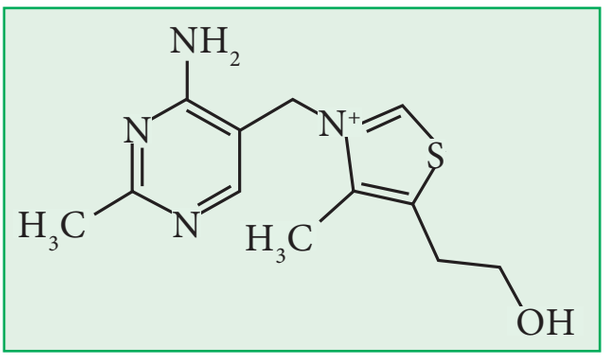

Figure 8.12 Thiamine

Sources:

Liver, pork, meat, and rice are rich sources. Other good sources are beans and nuts (Figure 8.13).

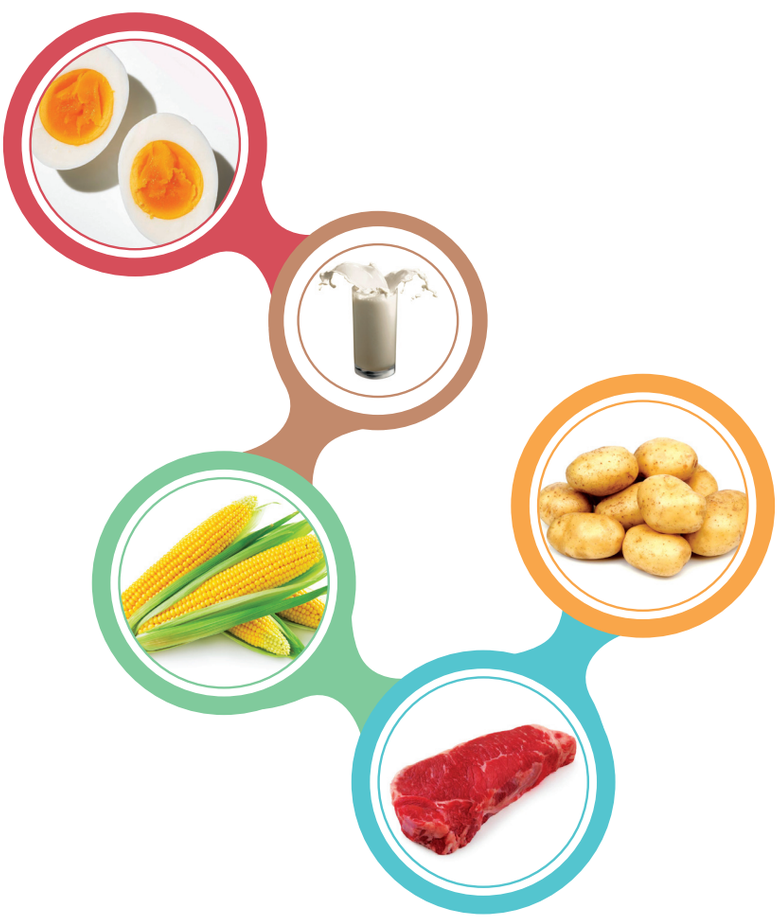

Figure. 8.13 Sources of vitamin B1

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Functions:

- Thiamine act as a coenzyme in the form of thiamine pyrophosphate (TPP) in many enzymatic reactions. These are involved mainly in the breakdown of glucose to yield energy.
- Thiamine pyrophosphate (TPP) also acts as a coenzyme for transketolase reactions in the phosphogluconate oxidative pathway of carbohydrate metabolism. This reaction is essential for ribose formation, a constituent of DNA and RNA.
- Vitamin B_1 is also required in tryptophan metabolism for the activity of the enzyme tryptophan pyrolase.
- The adequate level of thiamine provides healthy nerves, a good mental outlook, a normal appetite and digestion.

Absorption and storage:

Free thiamine is readily absorbed by the small intestine. Thiamine is not stored in the human body. The excess thiamine is excreted in urine and also degraded by the enzyme thiaminase.

Deficiency:

The symptoms of thiamine deficiency occur because the tissue cells are unable to receive sufficient energy from glucose. Therefore, they cannot carry out their normal functions. Early symptoms of thiamine deficiency include fatigue, irritability, depression and numbness of the leg and poor tone of the gastrointestinal tract together with constipation.

Beriberi is the disease caused by thiamine deficiency. Beriberi is characterized by oedema in the legs. Usually, beriberi is caused by carbohydrate rich and low thiamine diets such as polished rice.



Figure 8.14 Deficiency due to vitamin B1

ii. Vitamin B_2 (Riboflavin)

Vitamin B_2 which is also known as riboflavin consists of a heterocyclic isoalloxazine ring attached to ribitol (a pentose alcohol). It is a yellow coloured compound.

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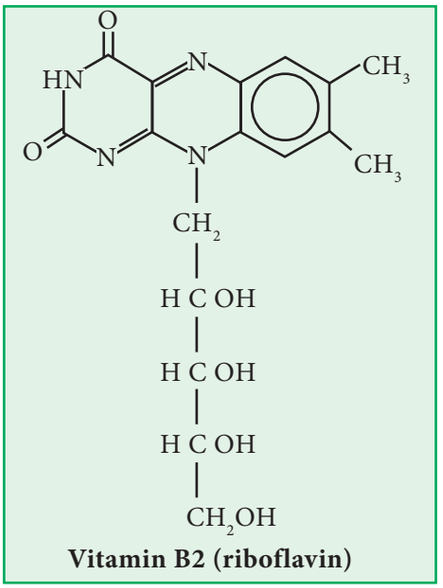

Figure 8.15 Structure of vitamin B_2

Sources:

It is widely distributed in plants. Soy beans, green vegetables are good sources of this vitamin. High concentration occurs in yeasts, milk and egg (Figure 8.16).



Figure 8.16 Sources of vitamin B_2

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Functions:

Riboflavin is a component of two important coenzymes, namely flavin mono nucleotide (FMN) and flavin adenine dinucleotide (FAD). They play key roles in various enzymatic reactions.

FMN and FAD are synthesized as follows.

$$\text{Riboflavin} + \text{ATP} \rightarrow \text{FMN} + \text{ADP}$$

$$\text{FMN} + \text{ATP} \rightarrow \text{FAD} + \text{PP}_i$$

FMN and FAD combine with different apoenzymes to form a large number of redox enzymes.

Eg. FMN is associated with the enzyme cytochrome c reductase.

FAD is found in xanthine oxidase and acyl CoA dehydrogenase

Riboflavin is essential for a healthy skin and for good vision in bright light.

Absorption and storage

The vitamin is phosphorylated in the intestinal mucosa during absorption. It is absorbed by the small intestine through the portal vein and is distributed to all tissues. The major part is excreted in urine and a small part is metabolized in the body.

Deficiency:

Riboflavin deficiency leads to cheilosis. It is characterised by the development of fissures developing in the lips and at the corners of the mouth.

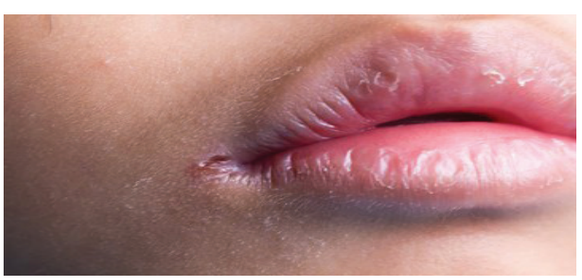

Figure 8.17 Deficiency due to vitamin B_2

Riboflavin is extremely sensitive to strong light. Due to sensitivity, riboflavin deficiency may occur in new born infants with hyper bilirubinemia who are treated by phototherapy.

iii. Vitamin B3 (Niacin)

Vitamin B_3 which is also called as niacin or nicotinic acid is pyridine 3-carboxylic acid. It occurs in tissues as nicotinamide (Figure 8.18).

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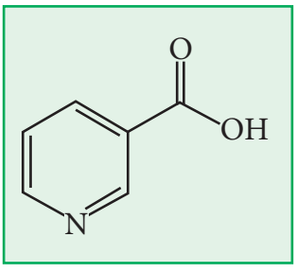

Figure 8.18 Structure of vitamin B_3

Sources:

This vitamin is widely distributed in cereals, dark green leafy vegetables. Liver and kidney are rich sources of this vitamin.

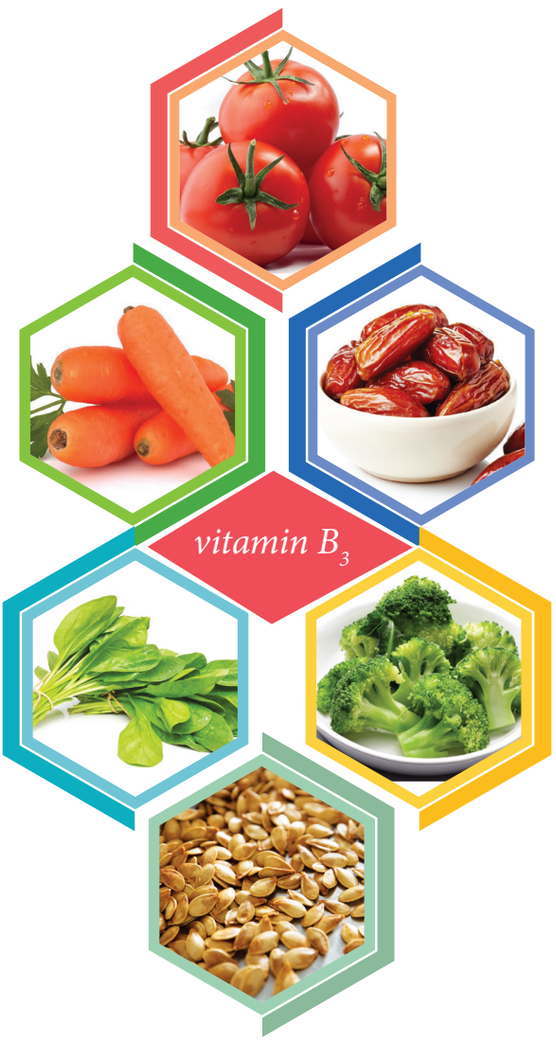

Figure 8.19 Sources of vitamin B_3

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Functions:

- Niacin is found in the form of its amide which is an important constituent of the coenzymes NAD^+ and $NADP^+$ and they take part in redox reactions which are associated with many dehydrogenases. For example NAD^+ is the coenzyme for lacate dehydrogenase. $NADP^+$ is the coenzyme for glutathione reductase.
- It promotes the formation of fat from carbohydrates.
- Niacin is essential for healthy skin, normal functions of the gastro intestinal tract and maintenance of the nervous system.

Absorption and storage

Nicotinic acid and nicotinamide are absorbed by the intestine through the portal vein into the general circulation. Excess nicotinic acid is not stored in the body.

Deficiency:

- Nicotinic acid deficiency causes a disease called pellagra (Figure 8.20).
- Dermatitis-skin exposed to the sun, soreness of the mouth and swelling of the tongue.
- Diarrhoea.
- Dementia-mental changes including depression and confusion.



Figure 8.20 Deficiency due to Vitamin B_3 (Pellagra)

iv. Vitamin B_6 (Pyridoxine)

Vitamin B_6 or Pyridoxine is also called as adermin. Vitamin B_6 consists of three closely related pyridine derivatives.

- Pyridoxine
- Pyridoxal
- Pyridoxal amine

The metabolically active form of vitamin B_6 is pyridoxal phosphate. It is formed from pyridoxal.

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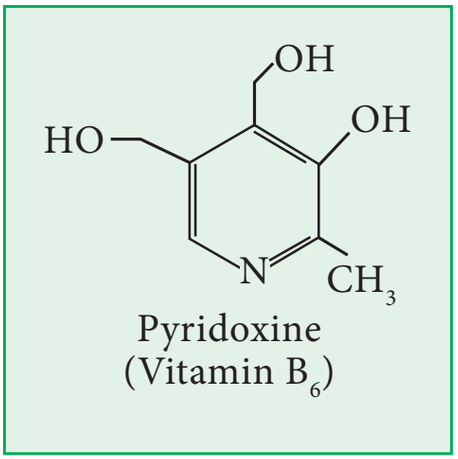

Figure 8.21 Structure of vitamin B_6

Sources:

Rich sources of this vitamin are yeast, whole grain, cereals and egg-yolk. Moderate amounts are present in organ meats like liver and kidney.

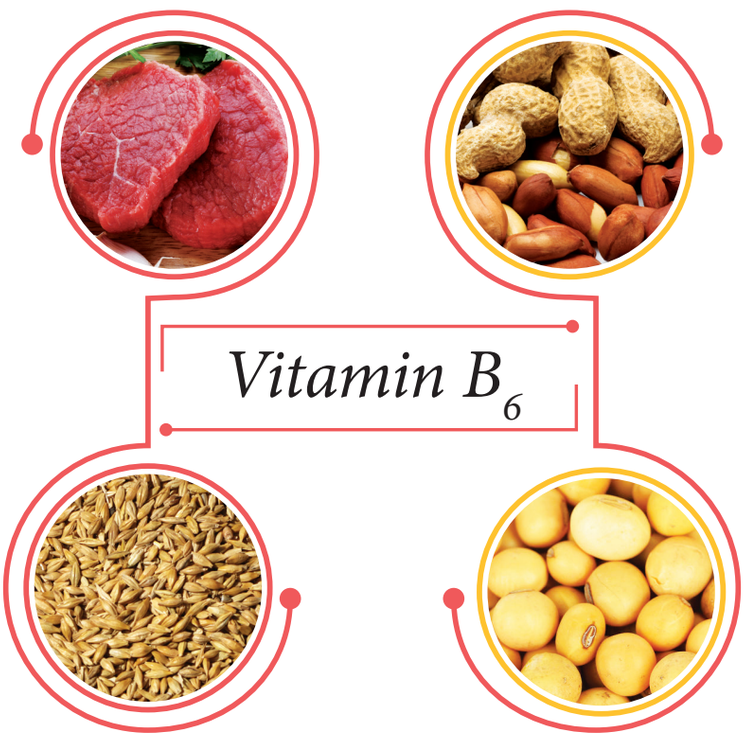

Figure 8.22 Sources of vitamin B_6

Functions:

- Pyridoxal phosphate acts as coenzyme for several enzymes of amino acid metabolism.

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Example:

Glutamate + Oxaloacetate $\xrightarrow{\text{AST}}$ α-Ketoglutarate + Aspartate

- It is involved in the formation of heme in hemoglobin.
- Pyridoxal phosphate acts as a coenzyme for the decarboxylase in decarboxylation reactions. Amino acids are decarboxylated to form their corresponding amines.

Histidine $\xrightarrow[B_6 \text{ - phosphate}]{\text{Decarboxylase}}$ Histamine

- Vitamin B_6 is involved in the synthesis of coenzyme A from pantothenic acid.
- It is also involved in the production of antibodies.
- Pyridoxal phosphate plays a key role in glycogenolysis.

Absorption and storage

Pyridoxine is readily absorbed by the small intestine. The excess amount if ingested is not stored in the body, and is excreted in urine.

Deficiency:

Deficiency of vitamin B6 is extremely rare. In infants its deficiency causes irritabilities, insomnia, muscular weakness and convulsion. The cause of the convulsions are severe impairment of the activity of the enzyme glutanate ecarboxylase, which is dependant on pyridoxal phosphate. The product of Glutamate decarboxcylase is γ-aminobutyric acid (GABA), which is a reguletory neurotransmitter in the central nervous system.

v. Vitamin B_{12}:

Vitamin B_{12} has complex ring structure, similar to porphyrin ring which has a cobalt ion (Co^{3+}) at its center (Figure. 8.23). The active coenzyme forms of vitamin B_{12} are methylcobalamine and deoxyadenolsyl cobalamine. The six co-ordination valencies of cobalt ion (Co^{3+}) are satisfied by the four nitrogens of reduced tetrapyrrole and the fifth by the nitrogen of 5, 6 dimethyl benzimidazole moiety and the sixth one either by CN^- (cyanocobalamine) or H_2O (aquacobalamine) or OH^- (Hydroxycobalamine) or CH_3 (methylcobalamine)

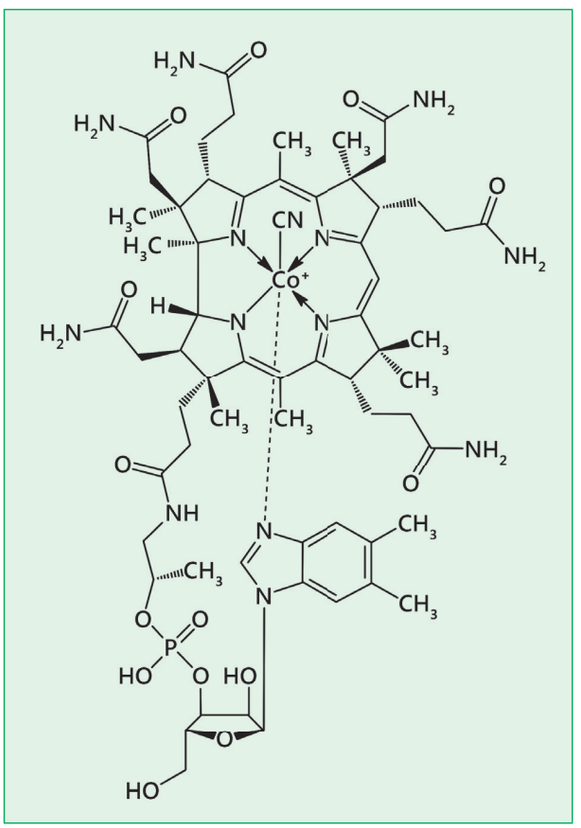

Figure 8.23 Structure of Vitamin B_{12}

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Sources:

Vitamin B_{12} is found in animal but not in vegetable foods and is unique in that it is the only known vitamin that holds an ion of metal (cobalt) in its molecule. Eggs and meat supply ample amounts of this vitamin.

Functions:

- Many microorganisms require vitamin B_{12} for growth.
- Vitamin B_{12} is required as coenzyme for the conversion of L-methylmalonyl CoA to Succinyl CoA by the enzyme methylmelonyl CoA mutase. This reaction is essential for in the metabolisom of some branched-chain amino acids and odd-chain fatty acids
- Vitamin B_{12} is required for the maturation of red blood cells in the bone marrow and for the synthesis of proteins.

Absorption and Storage

Vitamin B_{12} is absorbed from ileum. For the absorption of vitamin B_{12} from the intestines, a factor called "Intrinsic Factor" (IF) secreted by the stomach is essential. Vitamin B_{12} is stored in fair amounts in the liver.

Deficiency:

When absorption is prevented by lack of intrinsic factor, it leads to the condition called pernicious anemia. It is characterized by a drastic decrease in red blood cell count and leads to formation of macrocytic red blood cells.

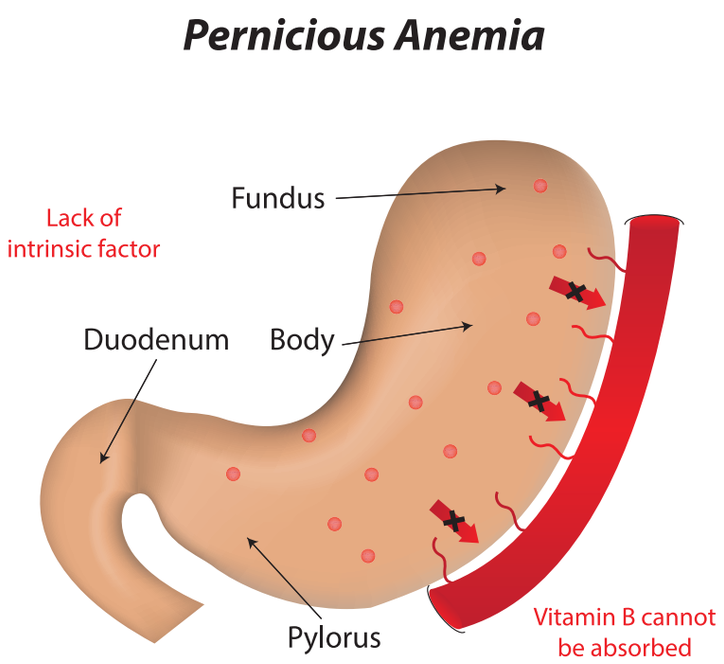

Figure 8.24 Pernicious Anemia

Vitamin B_{12} deficiency causes an increased concentration of methyl malonic acid, which competes with malonyl CoA and impairs fatty acid synthesis.

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vi. Biotin (Vitamin B_7):

Biotin is a heterocyclic monocarboxylic acid. It is a Sulphur-containing water soluble vitamin.

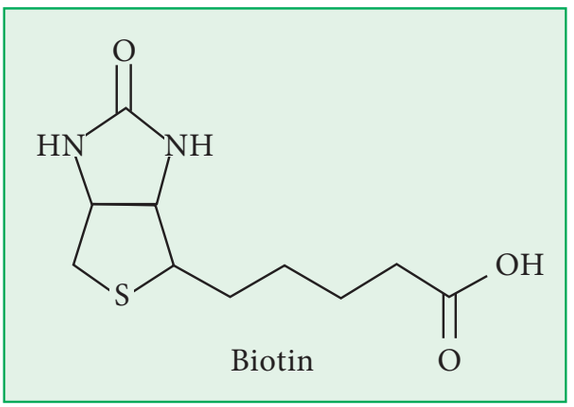

Figure 8.25 Structure of biotin

Sources:

Foods rich in biotin are liver, kidney milk and egg-yolk. Vegetables, grains are good sources.

Functions:

- It is essential for the synthesis of lipids.
- Biotin functions as the coenzyme for the enzyme called carboxylases, which catalyzes carboxylation reactions. Eg. Acetyl CoA carboxylase converts acetyl CoA to malonyl - CoA, which is required for fatty acid synthesis.

CO_2 + biotin- enzyme + ATP → Carboxy biotin - enzyme + ADP + Pi

Carboxy biotin enzyme + acetyl CoA $\xrightarrow{\text{Acetyl CoA carboxylase}}$ malonyl - CoA + biotin- enzyme

- It helps in the conversion of pyruvic acid to oxaloacetic acid. The enzyme that catalyzes this reaction is pyruvate carboxylase.
- It helps to maintain the skin and the nervous system in good condition.
- It involves in deamination of certain amino acids like aspartate, serine and threonine.

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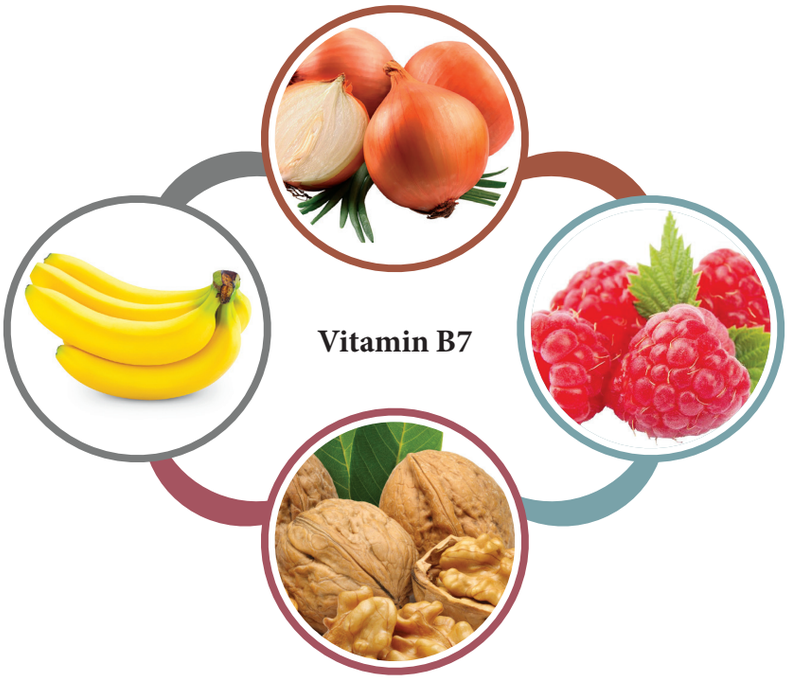

Figure. 8.26 Sources of biotin

Deficiency:

Egg white contains a protein called avidin (egg white injury factor) which binds very tightly with biotin, preventing its absorption and thereby inducing biotin deficiency. The symptoms include depression, hair loss and muscle pain.

vii. Folic acid (Vitamin B9):

Folic acid otherwise known as vitamin B9, folavin or folate is essential for cell division. Its active coenzyme is tetrahydrofolate (THF). The naturally occuring folate is susceptible to high heat and UV light and also be subject to oxidation.

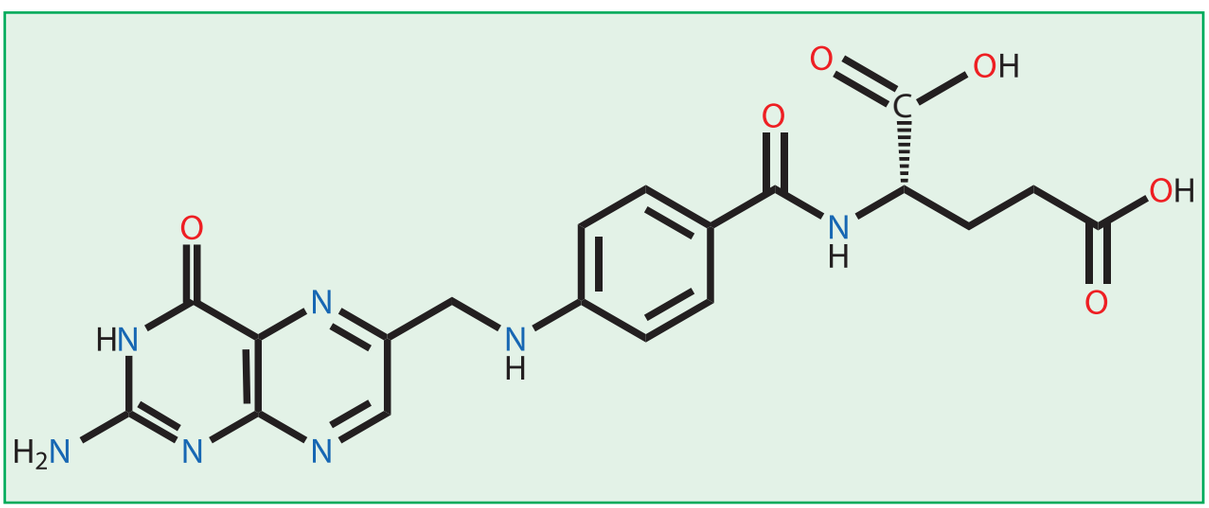

Fig. 8.27 Structure of folic acid

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Sources

Folate naturally occurs in a wide variety of foods, including dark green leaf vegetables, fruits, nuts, soybeans, chickpeas, dairy products, poultry and meat, eggs, seafood, grains. Vegetables such as avocado, beetroot, spinach, liver, yeast, asparagus, kale, and brussels and sprouts are among the foods with the highest levels of folate.

Function

- Folic acid is essential for cell division and growth
- It is important for preventing birth defects.
- It is used in the synthesis of amino acid methionine.
- Tetrahydrofolate (THF) is needed to transfer one carbon units in the biosynthetic reactions.

Absorption and storage

Folates are absorbed in the small intestine. The body can store a small amount in liver. The excess is removed from liver via bile.

Deficiency

Folate deficiency signs include anemia (megaloblastic anemia) and dysfunction of the gastrointestinal tract. The anemia is caused by the abnormal blood cell division resulting in fewer and larger red blood cells.

Lack of folate in the diet can cause neural tube defects in an embryo of a pregnant woman. These can cause fatal birth defects.

viii. Pantothenic acid (Vitamin B5):

Pantothenic acid is found in every living cell including plant, animal and microbes. It is a part of coenzyme A which is an essential coenzyme involved in the catabolism of carbohydrates, proteins and fat.

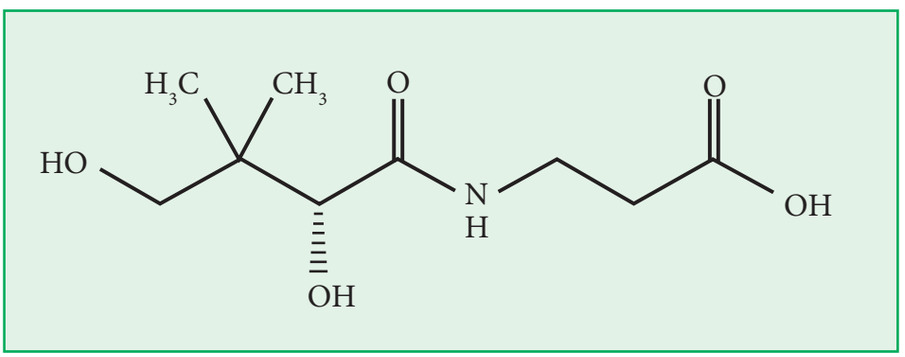

Fig. 8.28 Structure of pantothenic acid

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Sources

Pantothenic acid is present in dried mushrooms, avocado, dried egg yolks and sunflower seeds in high amounts. Outer layer of whole grains contains the vitamin, but milling removes much of the pantothenic acid.

Function

- Pantothenic acid forms a part of coenzyme A which is an essential coenzyme for metabolising carbohydrates, fat and proteins.
- It is necessary to synthesise vitamin D, steroid hormones and red blood cells
- It also helps to boost the immunity

Absorption and storage

Free pantothenic acid is absorbed into intestinal cells via a saturable, sodium-dependent active transport system. However, in foods, most pantothenic acid is in the form of CoA or bound to acyl carrier protein (ACP). Since the intestinal cells can only absorb this vitamin in free pantathenic acid, it is converted into free panthothenic acid in intestine.

Deficiency

Pantothenic acid deficiency is very rare and seen only in cases of severe malnutrition. Pantothenic acid is found in many common foods and average diets are thought to have an adequate amount of it.

8.3 Vitamin C

Vitamin C is called as ascorbic acid. Ascorbic acid is an enediol-lactone of an acid with configuration similar to that of the sugar L-glucose.

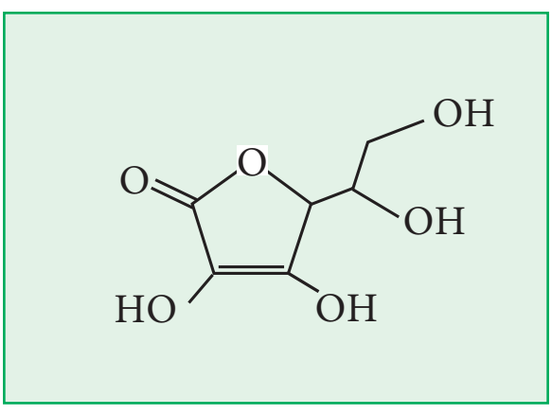

Fig. 8.29 Structure of vitamin C

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Sources:

Citrus fruits like orange, lemon are especially rich in vitamin C. Water melons, tomatoes, grape and leafy vegetables are also good sources.

Amla – 600-700mg/100g

Guava- 200-300mg/100g

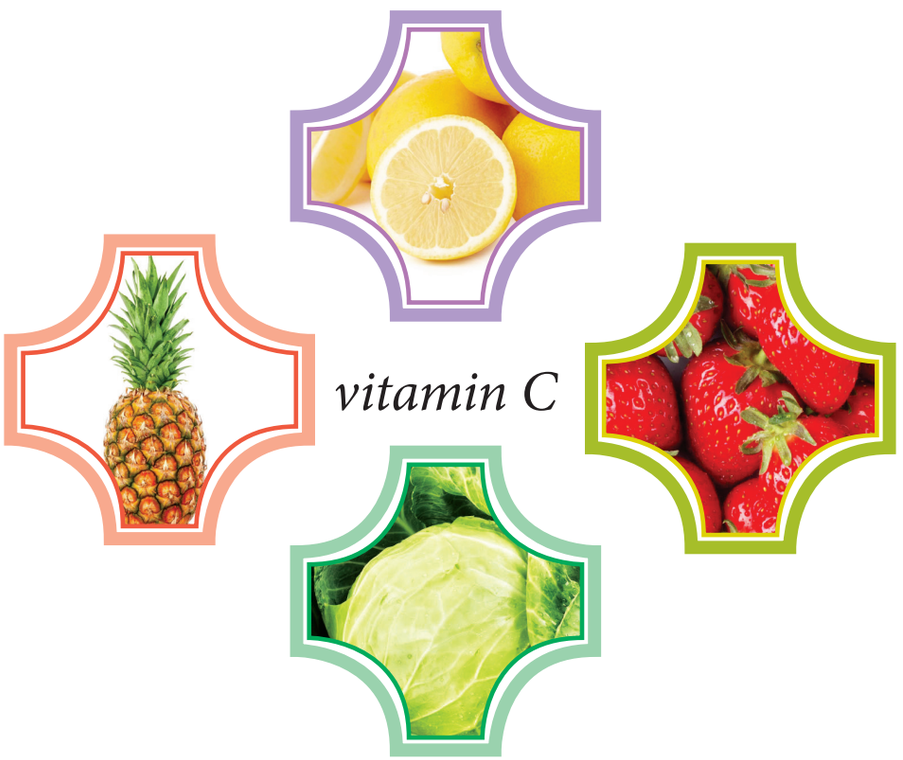

Fig. 8.30 Sources of vitamin C

Functions:

Vitamin C is involved in cellular oxidation-reduction reactions inside the cell as hydrogen carrier.

Ascorbic acid (Reduced form) ⟶ **Dehydro ascorbic acid (Oxidized form)**

- Vitamin C is essential for building collagen, the connective tissue protein which cements the cells and tissues together.
- It regulates carbohydrate metabolism.

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- Vitamin C is required as coenzyme for the enzyme dopamine hydroxylase which catalyzes the conversion of dopamine to nor-epinephrine.
- It is involved in the maturation of red blood cells.
- The absorption of iron is significantly enhanced by the presence of vitamin C.
- It has a general antioxidant role , especially in the regeneration of oxidized vitamin E in membranes.

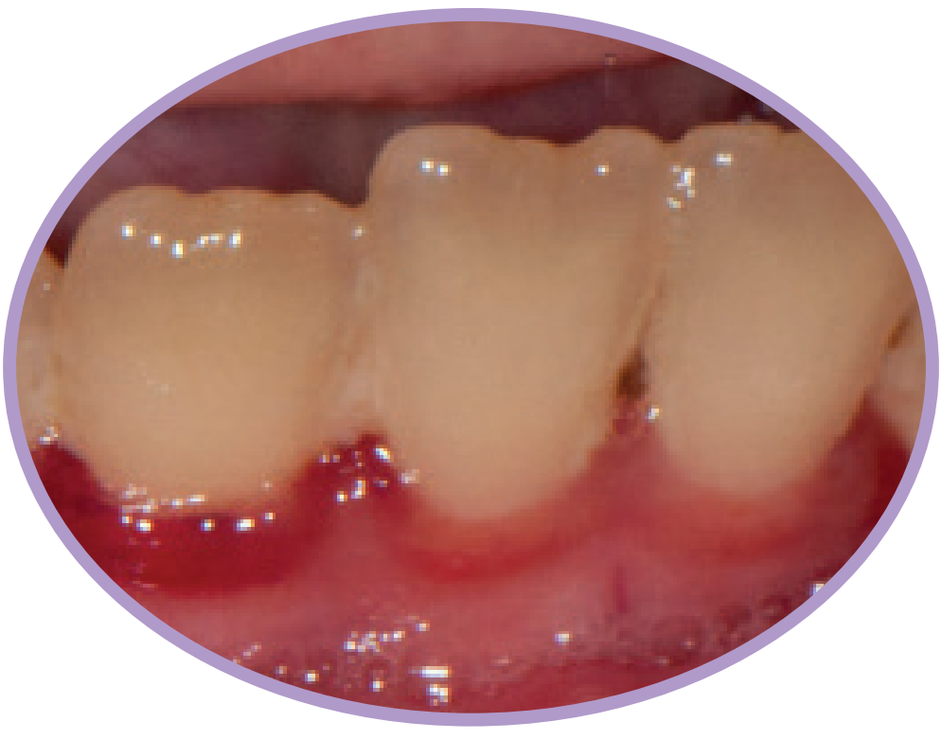

Fig. 8.31 Deficiency due to vitamin C

Absorption and storage

Ascorbic acid is rapidly absorbed from the intestines and passed on through the portal vein to the general circulation. Vitamin C is found in highest concentrations in the adrenals, the pituitary and retina than in circulation. Excessive intake of the vitamin do not increase beyond the optimal levels.

Deficiency:

Scurvy is the classical syndrome of vitamin C deficiency. It is related to defective collagen synthesis which is indicated by fragile skin, muscle weakness, bleeding of the gums, loose teeth and delayed wound healing.

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Table 8.1. Recommended Daily Allowances(RDA) of fat and water soluble vitamins for Adults

VITAMINS	RDA
Vitamin A	5000 IU
Vitamin D	10 mg
Vitamin E	25-30 mg
Vitamin K	2 mg
Thiamine (B_1)	1.5 - 2.0 mg
Riboflavin (B_2)	1.6 - 2.0 mg
Niacin (B_3)	17 - 20 mg
Pyridoxine (B_6)	2 - 3 mg
Folic acid (B_9)	500 µg
Cobalamin (B_{12})	3 µg
Pantothenic acid (B_5)	5 - 12 mg
Biotin (B_7)	25 - 50 µg
Vitamin C	75 mg

EVALUATION

I. Choose the best answer:

1. Night blindness is caused by the deficiency of

   a. Vitamin A b. Vitamin D c. Vitamin E d. Vitamin K

2. Which of the following vitamin is essential for biosynthesis of co-enzyme Q?

   a. Vitamin A b. Vitamin D c. Vitamin E d. Vitamin C

3. Anti hemorrhagic vitamin is

   a. Vitamin K b. Vitamin E c. Vitamin D d. Vitamin C

4. FAD is found in

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a. Cytochrome C reductase b. Xanthine oxidase

c. Acyl CoA dehydrogenase d. All of these

5. Cobalt is present in

a. Vitamin B1 b. Vitamin B_2 c. Vitamin B_6 d. Vitamin B_{12}

7. A deficiency of vitamin B_{12} causes

a. Scurvy b. Rickets c. Pernicious anemia d. Beriberi

8. Rickets is due to deficiency of

a. Vitamin D b . Vitamin A c. Vitamin C d. Vitamin B1

9. Pyridoxal phosphate is a coenzyme for the reaction, except

a. Transamination b. Deamination c. Decarboxylation d. Oxidation – reduction

10. Beriberi is caused by deficiency of

a. Thiamine b. Thymine c. Threonine d. Tyrosine

11. Pellagra occurs due to deficiency of

a. Biotin b. Niacin c. Pantothenic acid d. Folic acid

12. All the following vitamins have antioxidant property, except

a. Beta carotene b. Ascorbic acid c. Tocopherol d. Cholecalciferol

13. Increased prothrombin time is observed in the deficiency of

a. Vitamin K b. Vitamin B2 c. Vitamin A d. Vitamin B12

14. Thiamine pyrophosphate is required for the following enzymatic activity

a. Hexo kinase b. Transketolase

c.Transaldolase d. Glucose 6-phosphatase

15. Which of the following vitamin is required for collagen synthesis?

a. Ascorbic acid b. Nicotinic acid

c. Pantothenic acid d. Folic acid

16. Biologically active form of vitamin D is

a. 1, 25 dihydroxycholecalciferol b.24, 25 dihydroxycholecalciferol

c. 25 hydroxycholecalciferol d. 1, 24 dihydroxycholecalciferol

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II. Give short answer for the following:

1. How will you classify vitamins?
2. Name the fat soluble vitamins.
3. What are the different forms of vitamin B6?
4. Name one important enzyme in which $NADP^+$ acts as coenzyme.
5. What is the biologically active form of thiamine?
6. What are the effects of vitamin B12 deficiency?
7. What are the types of vitamin D?
8. Name the naturally occurring sources of vitamin K?
9. Write the chemical name of vitamin E.
10. State the sources of thiamine.

III. Give short answer for the following:

1. Give the functions of vitamin D
2. Explain the antioxidant property of vitamin E
3. Write a note on deficiency of vitamin B1
4. Give an example of metabolic reactions where TPP acts as coenzyme
5. State the biochemical functions of vitamin B3.
6. What is the biologically active form of riboflavin?
7. Write short notes on niacin deficiency.
8. What are the deficiency manifestations of vitamin D?

IV. Answer the followings

1. What are the functions of vitamin A?
2. Give an account on functions and deficiency symptoms of Vitamin K
3. Explain the functions of thiamine.
4. Write the various biological functions of pyridoxine.
5. Explain the functions and deficiency of vitamin C.

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SUMMARY

Vitamins are low molecular weight organic compounds that are essential for many functions of living organisms. They can be classified as water soluble and fat-soluble vitamins based on their solubility. The fat-soluble vitamins that include A, D, E and K gets readily dissolved in fat and are insoluble in water. These vitamins are present in liver and fatty tissues and the excess can be stored in human body. The water-soluble vitamins include C and B complex vitamins (B1, B2, B3, B6, B12, Biotin, Folic acid and Pantothenic acid).

Vitamin A (Retinol) plays an essential role in the visual cycle. Its deficiency causes keratomalacia. Green leaves, meat and carrots are good sources for this vitamin.

Vitamin D which exists in two forms D_2 and D_3 is essential for absorption of calcium and phosphorous and for normal growth. Its deficiency causes rickets in children and osteomalacia in adults. Fish liver oil is rich in vitamin D.

Vitamin E is an antioxidant and its deficiency cause reproductive failure and muscular dystrophy. Groundnut oil and wheat germ oil are good sources of this vitamin.

Vitamin K is essential for the biosynthesis of prothrombin a necessary substance for blood clotting. Its deficiency causes hemorrhagic condition. Green vegetables, soybean oil and spinach are good sources for vitamin K

The B-complex vitamins work together and are essential for overall growth of organism. It helps in harvesting energy from carbohydrates, fat and protein and some other important functions.

Vitamin C is involved in cellular oxidation-reduction reactions as a hydrogen carrier. Its deficiency causes scurvy syndrome. Citrus fruits like lemon and orange are rich in vitamin C.

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CONCEPT MAP



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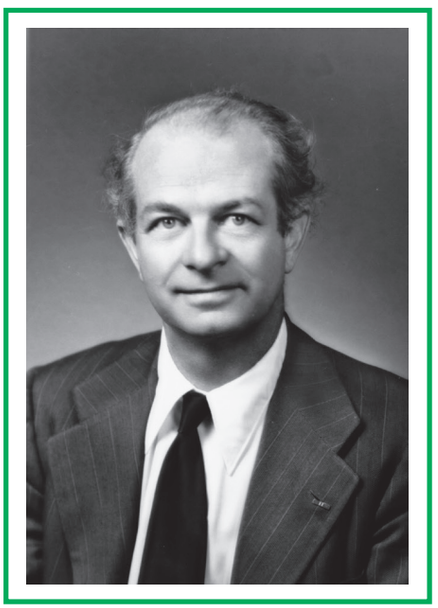
Unit 9 MINERALS



Linus Pauling

Linus Carl Pauling was an American chemist, biochemist, peace activist, author and educator. He was recognised as one of the 20 greatest scientists of all time. In addition to his contribution to chemistry and he also worked with many biologists. He demonstrated that the hemoglobin molecule changes structure when it gains or loses an oxygen atom. He also uncovered that the sickle cell anemia is caused by an abnormal protein. His success with sickle cell anemia led Pauling to speculate that a number of other diseases, including mental illnesses such as schizophrenia, might result from flawed genetics.

Learning Objectives

After studying this Unit, students will be able to

- Distinguish between the micro and macro nutrients.
- Know the various sources of micro and macro nutrients.
- Appreciate the biological functions of minerals.
- Understand the deficiency causes of various minerals.

Introduction

Minerals are inorganic elements that are present in body fluids and tissues. They are supplied to the body through diet, for example sodium from table salt and sulfur from proteins. They are required for a variety of biochemical and physiological functions such as enzyme action, nerve impulse transmission and muscle contraction. Each mineral is required in a specific amount ranging from micrograms to grams per day.

Minerals constitute about eight percent of total body mass of a human being. Unlike carbohydrates, fats and proteins, minerals donot furnish energy but they influence the rate of biochemical reactions through enzymes. Unlike vitamins, minerals are not destroyed during cooking process. However, some loss may occur due to their solubility in water.

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9.1 Classification:

The minerals required in human nutrition can be classified into two main groups.

Macro elements

Macro elements like sodium and potassium are required in large amount (>100mg/day) and present in large quantities in the body.

Micro elements

Micro elements like iron and iodine are required in small quantities (<100mg/day) and are present in small amount in tissues and body fluids.

9.2 Macro elements:

Some of the macro elements are calcium, phosphorus, sodium, potassium, chloride, magnesium and sulphur.

9.2.1Calcium

Calcium (Ca) is the most abundant inorganic mineral in the body. About 99% of it exists in the bone and teeth and 1% in soft tissues and extracellular fluid.
Functions:

- Calcium plays a role in blood coagulation by producing substances for thromboplastic activity of blood.
- Calcium is essential for bone and teeth formation.
- Calcium ions are necessary for nerve transmission and muscle contraction.
- Normal excitability of heart is calcium dependent.
- Calcium is involved in the process of mitosis.
- It also acts as cofactor for certain enzymes like succinate dehydrogenase.
- Calcium plays a role as secondary messenger in hormone action.
- Calcium is involved in the process of membrane fusion in endocytosis and exocytosis.
- It involves in maintenance of plasma membrane potential.
- Calcium binds trypsin near its active site to prevent autodigestion of trypsin.

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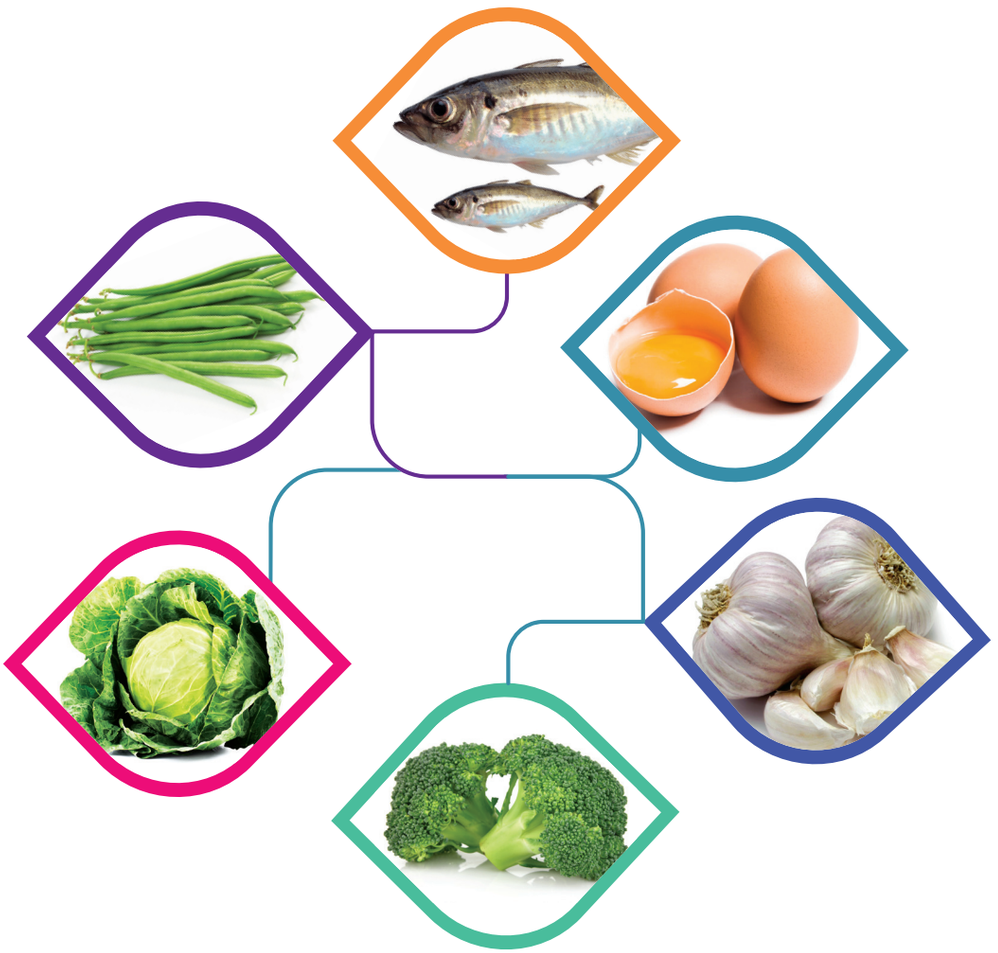

Figure 9.1 Sources of calcium

Dairy products remain one of the most important dietary sources of calcium. Other sources include egg-yolk, beans and cabbage.

Absorption:

Calcium is taken in the diet as calcium salts of phosphate, carbonate, tartrate and oxalate. Calcium is actively absorbed in the upper small intestine.

Factors affecting calcium absorption:

Many factors are said to influence the absorption of the calcium in the intestine. They are:

1. **pH:** An acidic pH favours calcium absorption.

2. **Protein:** A high protein diet favours the absorption of calcium.

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3. **Vitamin D:** Vitamin D promotes absorption of calcium.

4. **Sex hormones:** Sex hormones seem to have an effect on calcium and phosphorus balance.

5. **Lactic acid:** Lactic acid produced by microbial fermentation of sugars in the gut,increases the solubility of calcium salts, and increases their absorption.

6. **Fatty acids:** When the absorption of fats is impaired, calcium salts of fatty acids are formed to a greater extent retarding the absorption of calcium.

7. **Oxalates:** Oxalic acid from vegetables like cabbage, and spinach forms insoluble calcium oxalates, thus lowering the calcium absorption.

8. **Phosphates:** Excess of phosphate in diet lowers the calcium absorption. The ratio of calcium and phosphorus in the diet should be 1:1 for optimal absorption of calcium.

9. **Phytic acid:** Phytic acid present in cereals decreases absorption of calcium.

Excretion:

The excretion of calcium is partly through the kidneys but mostly via small intestine. Small amount of calcium may also be lost in sweat.

Deficiency:

Calcium deficiency in body causes the following disorders.

Osteoporosis

Osteoporosis is characterized by demineralization of bones (bone resorption) resulting in progressive loss of bone density.

Tetany

Tetany is a condition characterized by neuromuscular irritability and convulsion, which is associated with a state of hypocalcaemia.

Rickets

Rickets is more directly related to vitamin D deficiency, but calcium and phosphorus metabolism are also involved.

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Figure 9.2 Rickets

9.2.2. Phosphorus

Phosphorus is the principal anion of the cell. It is widely present as phosphates in proteins, nucleic acids and other various cellular components.Phosphorus and calcium are related in sources and metabolism.

Functions

1. Phosphorus is essential for formation of bones and teeth.
2. High-energy phosphate compounds like ATP (Adenosine Tri Phosphate) and Creatine Phosphate play a role in storage and transport of energy.
3. Phosphorylation and dephosphorylation reactions modify the activity of many enzymes.
4. Phospholipids, the important constituent lipid of cell membranes and nervous tissue contain phosphorus.
5. Acid-base balance is maintained by phosphate buffer in the kidneys.
6. Several co-enzymes such as $NADP^+$ and TPP involved in enzymatic reactions contain phosphate.

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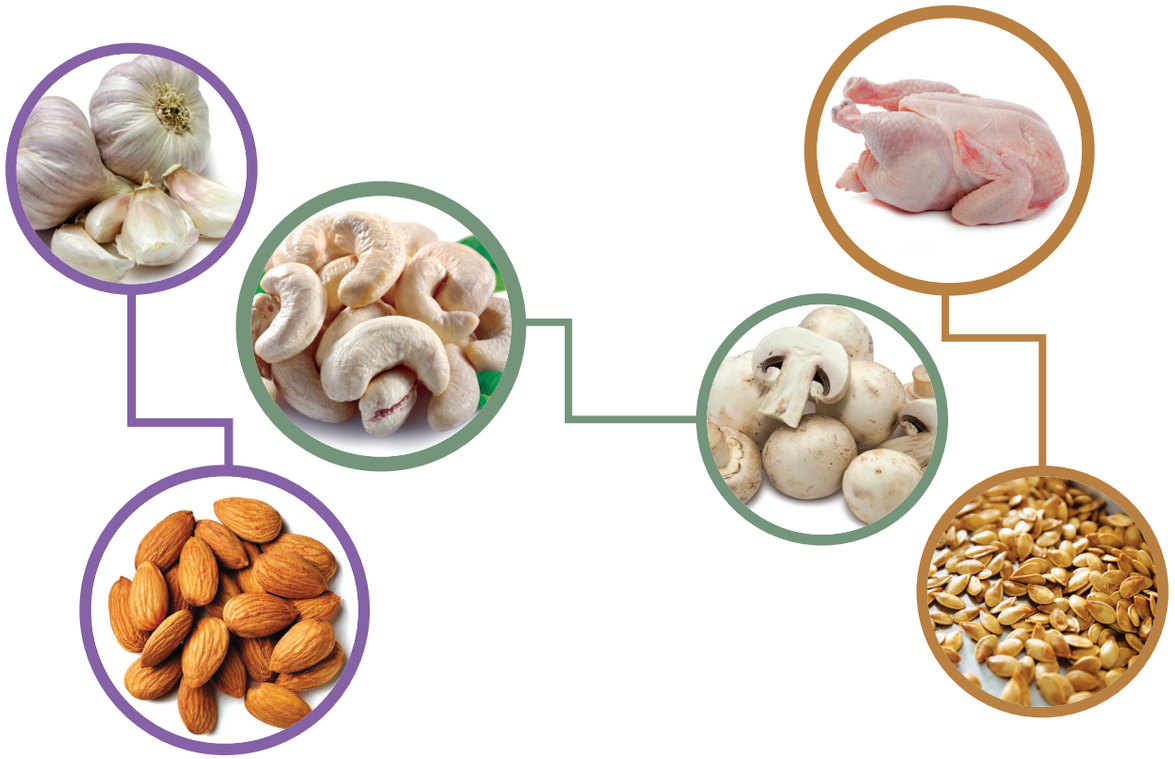

Figure 9.3 Sources of phosphorous

Foods rich in calcium are also rich in phosphorus. Animal sources include fish, meat, egg, milk, liver and kidneys. Plant sources of phosphorus are nuts, beans, green vegetables and fruits.

Absorption and Excretion:

Both calcium and phosphorus are absorbed from small intestine. Moderate amounts of fatty acid favor absorption of phosphorus. High calcium content in diet decreases the absorption of phosphorus.

Phosphates are mainly excreted by the kidneys as sodium dihydrogen phosphate through urine.

Deficiency:

Deficiency states for phosphorus are rare. Rickets, osteomalacia and osteoporosis are important dietary deficiency disorders of calcium and phosphorus. Low level of blood phosphorus is characterized by defective bone and teeth formation.

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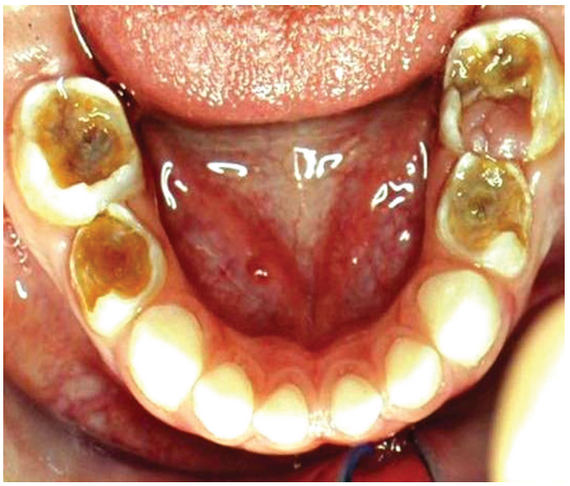

Figure 9.4 Defective teeth formation

9.2.3. Sodium

Sodium is the predominant cation of the extra cellular fluid. It is taken in the diet as sodium chloride.

Functions:

1. Sodium ion is mainly associated with chloride and bicarbonate in the regulation of acid-base equilibrium.

2. It maintains osmotic pressure of the body fluids and thus protects the body against excessive fluid loss.

3. Sodium ions are involved in the transmission of nerve impulses.

4. It plays an important role in the absorption of glucose and nutrients from small intestine by active transport.

5. Sodium ions are also involved in heart function.

Sources:



Figure 9.5 sources of sodium

Sodium is widely distributed in animal sources than plants. However, major source is common salt used in cooking. Cauliflowers, carrot and milk are also good sources of sodium.

Absorption and excretion:

Sodium is completely absorbed from the gastro intestinal tract by active transport. Normal diet contains about 5-10 grams of sodium as sodium chloride. The same amount of sodium is excreted daily through urine and sweat.

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Deficiency:

Mineralocorticosteroids secreted by adrenal glands, regulate the metabolism of sodium.Decreased level of plasma sodium(hyponatremia) may be due to lack of aldosterone and loss from gastro-intestinal tract associated with conditions like diarrhoea.

9.2.4. Potassium

Potassium is the main intracellular cation, about 98% of potassium is present inside the cell.

Functions:

Many functions of potassium and sodium are carried out in co-ordination with each other.

1. Potassium maintains the intracellular osmotic pressure, water balance and acid-base balance.

2. Potassium, along with sodium influences neuromuscular activity of cardiac and skeletal muscles.

3. The glycolytic enzyme, pyruvate kinase requires potassium as cofactor.

4. It maintains the alkalinity of the bile and blood.

5. Protein synthesis is dependent on potassium levels inside the cells.

Sources:

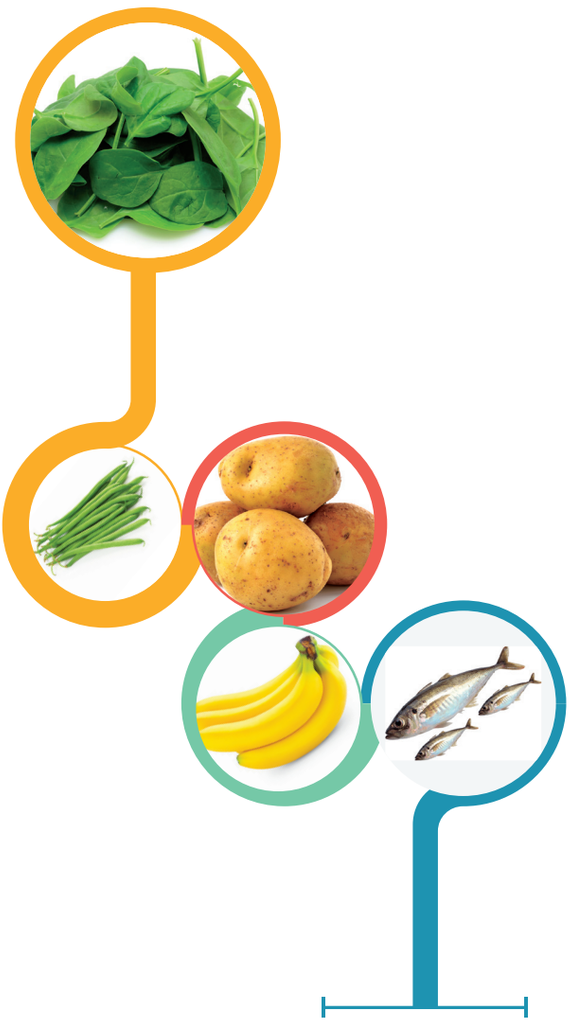

Figure 9.6 Sources of potassium

Animal sources of potassium include meat, fish, egg and milk. Vegetables like onion, carrot, fruits like apple, dates, banana, tender coconut water and grapes contain potassium.

Absorption and excretion:

Potassium is absorbed readily by passive diffusion from gastro intestinal tract and it is mainly excreted in the urine.

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Deficiency



Figure 9.7: Potassium deficiency symptoms

Deficiency of potassium leads to depression in cardiac and nervous system. Severe vomiting, diarrhoea, loss of appetite, fasting or starvation over a long period of time may lead to deficiency of potassium. It also occurs during renal failure and shock. Fatigue, growth retardation, muscular weakness, heart and respiratory dysfunction are common signs of potassium deficiency.

9.2.5. Chlorine

Chlorine is taken in diet as sodium chloride. Chloride is the major anion in the extracellular fluid.

Functions:

1. Chloride plays a vital role in acid-base balance by way of chloride shift and maintains blood pH.

2. Chlorides also contribute to formation of gastric HCl.

3. It also helps to maintain plasma osmotic pressure.

4. Chloride ion is important as an activator of salivary amylase.

5. Chloride mainly acts as a counter ion against cations in maintaining the electrical neutrality in body fluids.

Sources

Table salt (Sodium chloride) is the main source of chloride.

Absorption and excretion:

Chloride is completely absorbed from the gastro intestinal tract. Chloride is mainly eliminated in the urine and also in the sweat.

Deficiency

Deficiency states of chloride are rare. Most often, sodium and chloride are associated with each other in their functions. Any clinical condition like dehydration, affecting sodium concentration has an identical effect on chloride concentration.

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9.2.6. Magnesium

Magnesium is the second most abundant intracellular cation after potassium. Adult human body contains about 25g of magnesium. Bones contain nearly 70% of the body magnesium contributing as magnesium phosphate, which is about 1.5% of bone matter.

Functions

1. Magnesium, along with sodium, potassium and calcium controls the neuro muscular irritability.
2. Magnesium is involved in the synthesis of proteins and nucleic acids.
3. Magnesium is essential for the activity of peptidases and ribonucleases.
4. It is an activator of many glycolytic enzymes, particularly in muscle.

   Example : pyruvate kinase and enolase.
5. It plays an important role in oxidative phosphorylation.

Sources



Figure 9.8 Sources of Magnesium

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Magnesium is widely distributed in vegetables and also found in all animal tissues. As magnesium is an essential part of chlorophyll, green vegetables are important sources.

Absorption and excretion

Greater part of the daily ingested dietary magnesium is absorbed from the small intestine. The major quantity of magnesium is excreted in the feces and remaining is excreted through urine.

Factors affecting absorption

1. Excess of calcium decreases the absorption of magnesium.

2. Vitamin D, parathormone and growth hormone increases the absorption of magnesium.

Deficiency

The decreased level of serum magnesium causes depression, tetany and muscular weakness.



Figure 9.9 Tetany

9.2.7. Sulphur

Sulphur is present in our body as sulphur containing amino acids such as methionine and cysteine and also as sulphate. B-complex vitamins like Thiamine and biotin contain sulphur.

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Functions

1. S-Adenosylmethionine(SAM) acts as a methyl group donor for certain enzymes.
2. Sulphur containing amino acids are responsible for structural maintenance of proteins like insulin and keratin.
3. It is present in compounds like acetyl CoA and succinyl CoA which are needed for various reactions.
4. Glutathione is a valuable sulphur containing Tripeptide needed for the detoxification of H_2O_2.
5. Sulphur is present in heteropolysaccharides like heparin, a natural anti-coagulant.

Sources



Figure 9.10 sources of sulphur

Sulphur intake is mainly in the form of cysteine and methionine present in protein. Adequate protein in diet fulfills sulphur requirement.

Absorption and excretion:

Inorganic sulphate (SO_4^{2-}) is absorbed as such from the intestine into the portal circulation. Sulphur is excreted in the urine.

Deficiency

Specific sulphur deficiency state has not been reported in human beings.

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9.3 Micro elements

The micro elements which are essential for normal body functions are Iron(Fe), Copper(Cu), Iodine(I), Fluorine(F), Zinc(Zn), Cobalt(Co), Manganese(Mn), Chromium(Cr), Molybdenum(Mo) and Selenium(Se).

9.3.1. Iron

Iron is one of the most essential micro elements in the body. It plays a vital role in many oxidation-reduction reactions. Hemoglobin and cytochromes contain Iron in them.

Functions

1. Iron is involved in the transport of oxygen by hemoglobin.
2. Iron is essential for synthesis of cytochromes, a component of electron transport chain.
3. Myoglobin is an iron containing protein similar to hemoglobin and is present in muscle tissue.
4. Succinate dehydrogenase requires iron as a co-factor.
5. Iron improves immune status.

Sources



Figure 9.11 sources of Iron

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Meat, fish, liver are rich sources of iron. Cereals, nuts, spinach, dates are good plant sources of iron.

Absorption and excretion

Normally, about 5 to 10% of dietary iron is absorbed by the active transport process. Most absorption occurs in the duodenum. Infants and children absorb a higher percentage of iron from food than adults. Iron deficient children absorb twice as much as that of normal children. Excess dietary iron is stored as ferritin. Only lesser amounts are excreted in the urine, feces and sweat.

Factors affecting iron absorption

1. Impaired iron absorption takes place in patients who have total removal of stomach or a removal of the considerable amount of the intestine.

2. A diet high in phosphate causes decreased absorption due to the formation of insoluble ferric phosphate.

3. Copper deficiency reduces iron absorption, as copper helps in transport of iron.

4. Phytic acid and oxalic acid interfere with iron absorption.

5. Vitamin C increases iron absorption.

Deficiency

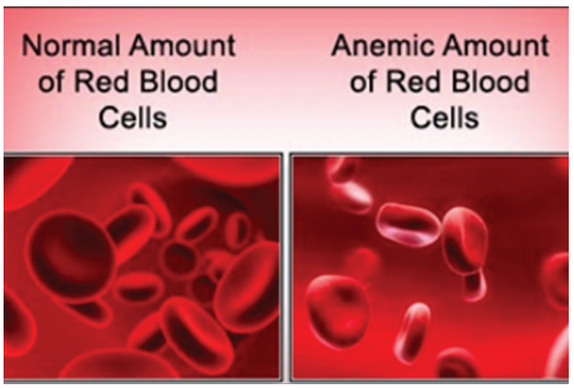

Figure 9.12 Iron deficiency anemia

Deficiency of iron causes iron deficiency anemia. The deficiency may be due to dietary or lack of iron absorption due to gastro intestinal tract diseases like diarrhoea.

9.3.2. Copper

Copper is an essential micro element. Copper content in an adult human is about 100 mg. Copper in whole blood is almost evenly distributed between cell and plasma. The highest concentrations are found in liver and kidney.

Functions

1. Copper forms integral part of certain enzymes like cytochrome oxidase and catalase.

2. Ceruloplasmin a copper containing protein is required for iron absorption.

3. Superoxide dismutase (SOD) contains copper ion, SOD converts super oxide radicals to hydrogen peroxide.

4. It is also present in cytochrome C, which is involved in electron transport in mitochondria.

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5. Copper is also needed for bone formation as well as for the maintenance of myelin within the nervous system.

6. Copper helps to form insoluble elastin fibres by cross-linking soluble pro-elastin.

Sources



Figure 9.13 Sources of Copper

Copper is present in many foods and the best sources are meat, nuts, legumes and cereals.

Absorption and excretion:

Absorption of copper into the blood stream occurs via the villi of the small intestine. About 30 percent of the dietary copper is absorbed in the duodenum.Only 10 - 60 µg of copper is excreted in normal urine in 24 hours.

Deficiency

1. A diet deficient in copper causes loss of weight.

2. Copper deficiency produces microcytic hypochromic anemia.

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3. Elastin formation is impaired in copper deficiency.

4. Copper deficiency turns hair grey, which however, can be controlled by administration of copper.

5. Deficiency of copper is sometimes associated with decrease in taste sensitivity, which is restored by oral administration of copper.



Figure 9.14 Grey hair (Copper deficiency)

9.3.3. Iodine

The adult human body contains about 50 mg of organically bound iodine. Nearly half of this is present in muscles. Most of the body iodine is present in the thyroid gland, but all cells contain traces of iodine.

Functions

The thyroid gland is chiefly concerned with the uptake of iodine for the synthesis of the thyroid hormones, tetra Iodothyronine(Thyroxin T4) and tri Iodothyronine(T3) which influence a large number of metabolic functions. These iodine containing hormones regulate energy metabolism, synthesis of proteins and cholesterol and also in the conversion of carotene to vitamin A.

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Sources

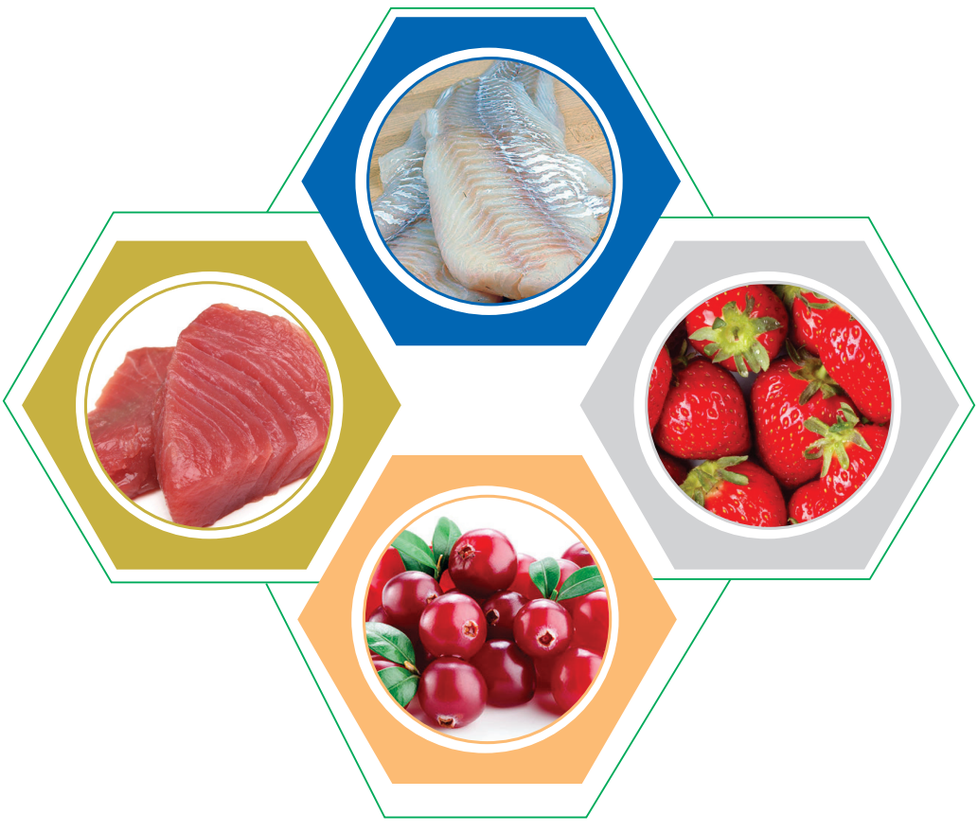

Figure 9.15 Sources of Iodine

Sea water is rich source ofIodine. Vegetables grown in Iodine-rich soil will naturally be the good sources of iodine. Animal sources of iodine include milk, sea fish and crabs.

Absorption and excretion

Absorption is through the villi of the small intestine into the blood stream and 90 % of the iodine of the thyroid gland is in organic combination and stored in the follicular colloids as thyroglobulin.

Inorganic Iodine is mostly excreted by the kidney. Milk of lactating women also contains some iodine.

Deficiency

A deficiency of iodine leads to a decreased production of thyroxine, and in turn a lowered rate of energy metabolism. In an attempt to produce more thyroid hormones the thyroid gland enlarges. This condition is called simple or endemic goiter.

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Figure 9.16 goiter

9.3.4. Fluorine

Fluorine exists in the body as compounds called fluorides.It is used as an anti-coagulant during collection of blood for the determination of blood sugar.

Functions

1. Fluorine is required in traces for the development of bones and teeth.
2. It is necessary for the prevention of dental caries.
3. It is used in combination with vitamin D for the treatment of osteoporosis.

Sources

The body receives fluorine mainly from drinking water, in which concentration varies with soil content. Some sea fish and tea also contain small amounts of fluorine.

Absorption and excretion:

Absorption of fluoride is via the small intestine into the blood stream. Most of the fluorides that are not retained by the bones and teeth are excreted rapidly into the urine. It is also excreted through sweat and intestine.

Deficiency

The absence of fluorine in the diet causes dental caries.

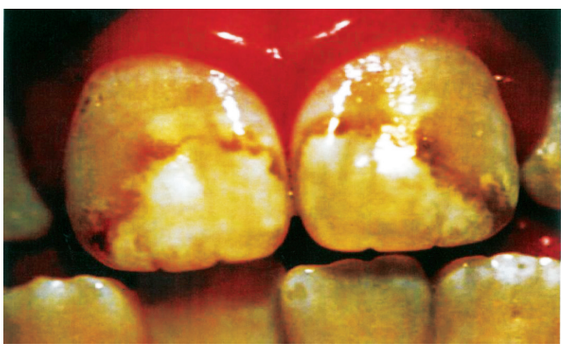

Figure 9.17 Dental Fluorosis

9.3.5. Zinc

Adult human body contains approximately 1 to 2 grams of zinc. Like iron, zinc is absorbed according to the body needs. Prostate gland is very rich in zinc.

Functions

1. Zinc forms an integral part of several metallo- enzymes in the body.
2. Zinc is essential for growth and reproduction.
3. It has role in maintaining the plasma concentration of vitamin A.
4. Zinc is essential for the storage of insulin in the β-islet cells.
5. Zinc is necessary for wound healing.

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Sources



Figure 9.18 Sources of Zinc

Fish, meat, liver, egg and certain sea foods are high in zinc. Vegetable sources are cereals, pulses, yeast and wheat germ. Milk including breast milk also is a good source of zinc. The colostrum is an especially rich source.

Absorption and excretion:

Zinc present in animal foods is well absorbed in the small intestine, especially from the duodenum. Zinc present in plant foods are poorly absorbed due to the presence of phytic acid which interferes with its absorption. Zinc is mostly excreted in urine.

Deficiency

1. Zinc deficiency causes poor growth and hypogonadism during adolescence.
2. In zinc deficiency, there is poor wound healing.
3. Zinc deficiency may result in impairment of sensitivity to taste.

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4. Deficiency of zinc may interfere with storage and secretion of insulin.

5. Zinc deficiency causes alopecia (hair loss)



Figure 9.19 alopecia

9.3.6.Cobalt

Cobalt is a component of vitamin B12. Elemental cobalt of the diet can be converted to Cobalamine by the intestinal bacteria. Cobalt occurs in small amount in all tissues, higher concentrations occur in liver and kidneys.

Functions

Most of the cobalt is present in vitamin B12, which is necessary for maturation of red blood cells.

Cobalt is an activator of the enzyme Phosphoglucomutase and glycyl - glycine peptidase.

Sources

It is largely available in food. Normal average diet contains about 5 to 8 micro grams of cobalt. One µg of vitamin B12 contains ~0.0225- 0.045 µg of cobalt.

Absorption and excretion

Cobalt is absorbed from the small intestine. About 65% of ingested cobalt is excreted in the urine and the remaining in the feces.

Deficiency

Cobalt deficiency is rare in human beings.

9.3.7. Manganese

Manganese is an essential trace element. It is present as Mn^{2+} ion and found in high concentrations in the mitochondria.

Functions

1. Manganese acts as a cofactor for number of enzymes including arginase, hexokinase and isocitrate dehydrogenase.
2. Manganese is essential for normal bone structure, reproduction and functioning of the central nervous system.
3. Manganese also functions with vitamin K in the formation of prothrombin.
4. It inhibits lipid peroxidation.
5. Manganese also participates in glycoprotein and proteoglycan synthesis.
6. It helps in Porphyrin synthesis.
7. Manganese is involved in fatty acid and cholesterol synthesis.

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Sources



Figure 9.20 Sources of Manganese

Manganese is universally distributed in plant and animal tissues, nuts, cereals and vegetables. The average diet can provide approximately 3 to 4 mg of manganese.Tea is exceptionally rich in manganese.

Absorption and excretion

Manganese is readily absorbed in the small intestine. Normally 3 to 4 % of Manganese in diet is absorbed. Large quantity of Manganese is excreted mostly in the feces. Only very small quantities of manganese are excreted in the urine.

Factors affecting absorption:

Manganese absorption is inhibited by iron.

Deficiency

A deficiency of Mn reduces appreciably the synthesis of oligosaccharides.

The deficiency of Manganese leads to impaired growth and skeletal abnormalities.

9.3.8. Chromium

Chromium is widely distributed throughout the body. The adult human body contains only 6 mg of chromium.

Functions

Chromium accelerates the utilization of glucose.

It reduces serum cholesterol level.

Chromium is also said to be important in the metabolism of plasma lipoproteins.

Sources

Chromium is highly available in average diets. Significant amount of chromium is obtained in the diet by cooking foods in steel containers.

Absorption and excretion:

It is absorbed in the small intestine. Chromium is mainly excreted in urine.

Deficiency

Chromium deficiency is characterized by impaired growth, weight loss and disturbances in glucose, lipid and protein metabolism.

9.3.9. Molybdenum

Molybdenum occurs in traces in the human body.Molybdenum occurs in some hemoflavo proteins.

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Functions

1. Molybdenum is required for the function of metallo-enzyme xanthine oxidase.
2. Presence of small amount of molybdenum helps in the utilization of copper.

Sources



Figure 9.21 Sources of Molybdenum

Molybdenum is available in normal diets. Liver and kidney are good animal sources. Whole grains and legumes are vegetable sources.

Absorption and excretion

About 50 - 70 % of the ingested Molybdenum is readily absorbed in the small intestine. About half of the absorbed molybdenum is excreted in urine.

Deficiency

Molybdenum deficiency is rare in human beings.

9.3.10. Selenium

Selenium is an essential trace element for all species including humans.

Functions

1. Selenium is essential for normal growth and fertility.
2. Selenium is a component of the enzyme which reduces oxidized glutathione.
3. It is involved in immune mechanism and synthesis of ATP.
4. Vitamin E and Selenium prevent peroxidative damage to cellular and sub-cellular organelles and chiefly the membrane.
5. Selenium may be a cancer protective agent.
6. Supplements of selenium probably protect against toxic effects of heavy metals like mercury and silver.

Sources

Selenium is largely present in different foods. Selenium is present in foods of plant origin grown in selenium rich soils. Any normal diet can meet the daily requirement of selenium.

Absorption and excretion

The principal dietary forms of selenium,selenocysteine and selenomethionine are absorbed from gastrointestinal tract. Selenium homeostasis is achieved by removal of excess selenium in urine.

Deficiency

Selenium deficiency is very rarely seen in human beings. Deficiency of selenium leads to muscular dystrophy and heart diseases.

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Table9.1.Recommended Daily Allowances (RDA) of macro and micro elements for Adults

S.N	MINERALS	RDA
1	Calcium	0.8 g
2	Phosphorus	1.0 g
3	Sodium	1-5 g
4	Potassium	4 g
5	Chlorine	2-5g
6	Magnesium	300mg
7	Iron	10-15 mg
8	Copper	2.5 mg
9	Iodine	100 – 150 µg
10	Fluorine	1.5-4 mg
11	Zinc	15 mg
12	Manganese	5 mg
13	Chromium	50 – 200 µg
14	Molybdenum	0.5 mg
15	Selenium	50 – 200 µg

Table 9.2. List of some enzymes requiring or containing mineral as cofactors (or) activators

S.No.	Enzyme	Cofactor
1	Tyrosinase	Copper
2	DNA Polymerase	Zinc
3	Cytochrome Oxidase	Iron
4	Hexokinase	Magnesium
5	Glutathione peroxidase	Selenium
6	Arginase	Manganese
7	Xanthine oxidase	Molybdenum
8	Dipeptidase	Cobalt
9	Pyruvate kinase	Potassium
10	Urease	Nickel

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EVALUATION

I. Choose the best answer:

1. Which one of the following is involved in bone formation?

   a. Calcium b. Selenium

   c. Iron d. Cobalt

2. Adrenocortico - steroids regulates the metabolism of

   a. Iodine b. Sodium

   c. Copper d.Chromium

3. Co-factor for pyruvate kinase is

   a. Potassium b. Iron

   c. Phosphorus d. Copper

4. Iron is needed for biosynthesis of

   a. Hemoglobin b. Myoglobin

   c. Cytochrome d. All of these

5. Goiter is caused by the deficiency of

   a. Iron b. Iodine

   c. Magnesium d.Cobalt

6. Which one of the following is necessary for the prevention of dental caries?

   a.Fluorine b.Chlorine

   c. Sodium d. Potassium

7. Cobalt is an activator of

   a. Phosphoglucomutase b. Hexokinase

   c. Pyruvate kinase d. Aldolase

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8. Iodine is required for the formation of

a. Insulin b. Vitamin B12

c. Thyroxine d. Calcitonin

9. Intestinal absorption of iron is enhanced by

a. Phytic acid b. Ascorbic acid

c. Oxalic acid d. Alkaline pH

10. Which of the following mineral is associated with parathyroid hormone?

a. Calcium b. Magnesium

c. Phosphorus d. Sodium

11. Molybdenum is a constituent of all of the following, except

a. Xanthine oxidase b. Aldehyde oxidase

c. Sulphite oxidase d. Cytochrome oxidase

12. Carbonic anhydrase contains the mineral

a. Copper b. Iodine

c. Zinc d. Iron

13. Glutathione peroxidase contains

a. Calcium b. Iron

c. Selenium d. Chromium

14. In wound healing, the following trace element is involved

a. Iron b. Copper

c. Zinc d. Selenium

15. The mineral which improves vitamin E effect

a. Chromium b. Iron

c. Iodine d. Selenium

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16. Transferrin is involved in

a. Hormone metabolism

b. Diagnosis of Wilson's disease

c. Transport of Iron

d. Transport of bilirubin.

II. Give short answer for the following

1. Name the macro elements
2. What are the most common sources of sodium?
3. What is the site of absorption of calcium?
4. Name the dietary sources of phosphorus.
5. Write a note on iron deficiency.
6. How is copper absorbed?
7. Name an enzyme which requires copper for activity.
8. What are sources of magnesium?
9. Give the factors affecting absorption of magnesium.
10. Mention the metabolic functions of T4 and T3.
11. Explain the deficiency symptoms of zinc.
12. Write a note on deficiency symptoms of manganese.
13. Briefly explain the toxicity of selenium.

III. Answer the following in one or two sentences

1. State the functions of potassium in the body.
2. Give the deficiency states of calcium.
3. State some important functions of magnesium.
4. List the factors which influence the absorption of iron.
5. Mention the functions of molybdenum.
6. Write the biological functions of sulphur.
7. What are functions of chromium?

Answer the following in detail

1. Explain the factors affecting absorption of calcium.
2. Give an account on functions, factors affecting absorption and deficiency state of iron.
3. Mention the functions and deficiency symptoms of copper.
4. Write down the biological functions and deficiency symptoms of selenium.
5. Give the functions of fluorine and zinc.

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SUMMARY

Minerals are inorganic substances that play major roles in many metabolic functions. They are divided among two classes, macro elements and micro elements. In general, most of these elements are components of enzymes, which are catalysts of chemical reactions in the body. Additionally, minerals regulate and control the normal function of human and animal tissues, muscles, and organs.

Macro elements including sodium, potassium, calcium, magnesium, and phosphorus in large amounts. In specific, sodium, chloride and potassium play a vital role in maintaining proper pH balance of blood and water. Phosphorous & calcium acts as a major structural component of bones and teeth. These minerals are typically available in dairy products, green vegetables, nut, beans, milk, egg, meat, fish and table salt. Deficiency of macro elements like sodium, calcium, calcium, magnesium and potassium causes diarrhoea, dehydration, osteoporosis, tetany and muscle weakness respectively.

Micro elements also called trace elements are needed in small amounts but important for the proper functioning of the body. Some of the essential trace minerals include iron, zinc, copper, selenium, chromium and iodine. Iron carries oxygen throughout the body in the blood. Zinc helps in growth, reproduction and wound healing. Selenium maintains the fertility. Chromium accelerates the utilization of glucose and reduces serum cholesterol. Iodine responsible for the synthesis of thyroid hormones and regulates the energy metabolism. Drinking water, vegetables, whole grains, legumes, cereals, nuts, seafood, liver, kidney, meat, & fish are the rich sources of micro elements. Iodine deficiency leads to simple goiter and iron deficiency causes anaemia and diarrhoea. Shortage of micro elements leads to loss of weight, dental caries, skeletal abnormalities, weight loss and disturbance in metabolism are the other effects.

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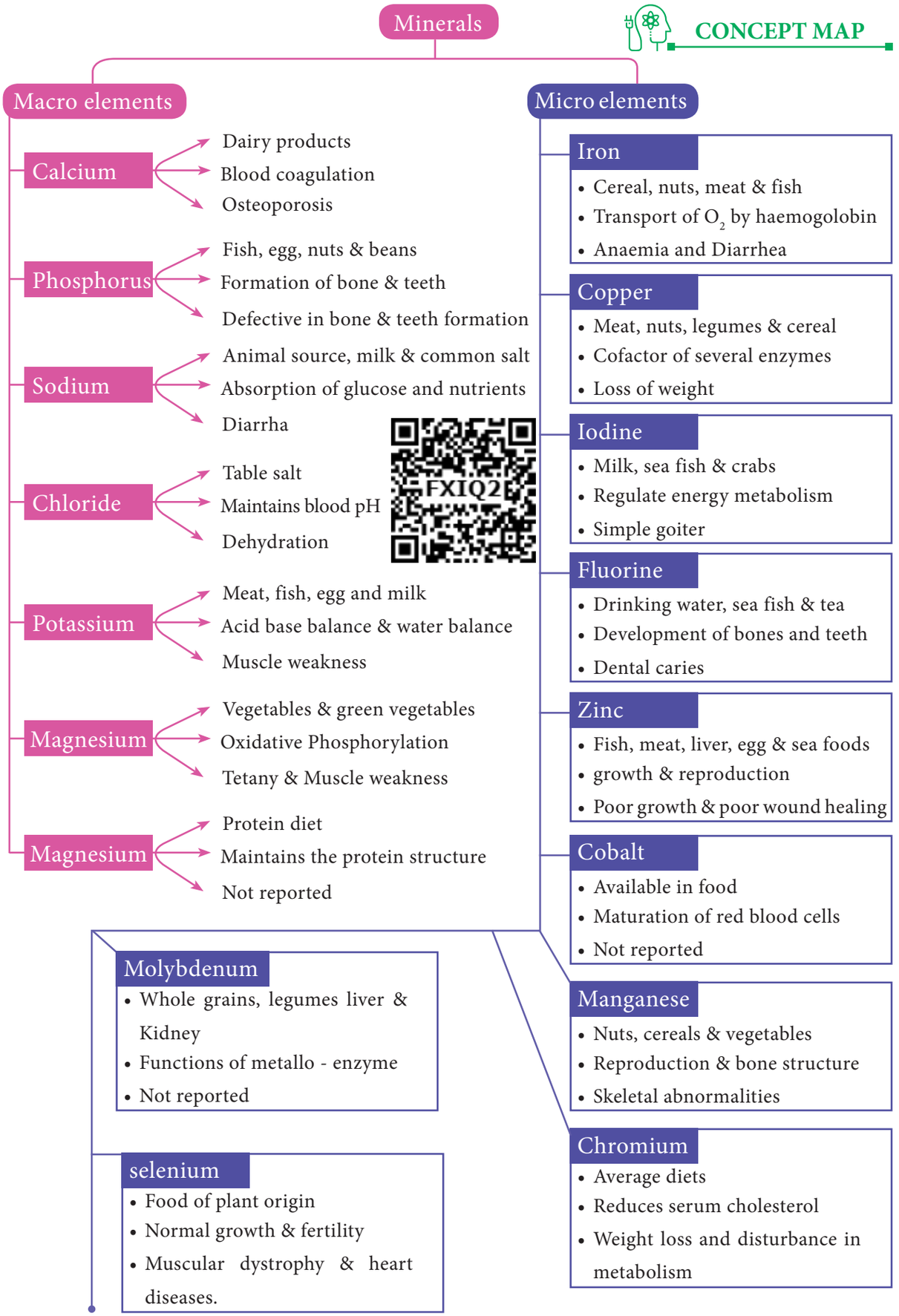
CONCEPT MAP



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Unit 10 BIOCHEMICAL TECHNIQUES



Mikhail Semyonovich Tsvet

Mikhail Semyonovich Tsvet was a Russian botanist coined the word chromatography. He invented the column chromatography procedure in 1906 to separate the plant pigments chlorophyll and carotenoids from their natural sources. He used the liquid adsorbant chromatography method to make this extraction. The adsorbant he used was calcium corbonate and the elutant was petroleum ether. He was also the person to name this technique as chromatography.

U0M6V

Learning Objectives

After studying this unit, the student will be able to

- Describe the principles and types of chromatographic techniques
- Describe the principle and types of electrophoresis
- Explain the principles and applications of centrifugation
- Describe the principles and applications of spectrophotometry
- Select a suitable biochemical technique for a given analytical problem.

Introduction

Biochemical techniques was used to evaluate the composition and properties of biological samples. It has applications in different areas of biological sciences. The information gained from such analysis provide indication of the substances that are present (qualitative) or may specify the exact amount of the substances (quantitative) present in the sample.

Qualitative methods are used to identify the substances while the quantitative methods can determine the amount of substances present in the sample

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In recent years, many methods have been developed for a widespread of analysis and are marketed as assay kits. The kits used as diagnostic tools range from some simple colorimetric assays to more sophisticated procedures involving antibodies or nucleic acids. The kits include all necessary standards and can be used in manual procedures or using an automated instrument. In biochemical and research laboratories, a biochemist should be able to efficiently handle techniques such as chromatography, centrifugation, electrophoresis and spectrophotometry as they are the major techniques used in biochemistry.

10.1 Chromatography

Mikhail Tsvet, a Russian Botanist invented chromatography in the year 1906, which is derived from the Greek word meaning colour (chroma) and writing (graphein). It is an important technique of identifying, separating and purifying different properties of chemical components. Two main approaches of chromatography are analytical and preparative. Analytical methods uses small amount of sample to identify or characterise them. Preparative methods use larger quantities of samples and are mainly used for isolation process.

10.2 Principle of chromatography

Chromatography is defined as the method of separation of different substances based on their partition coefficients in two immiscible phases. Chromatography involves a stationary and a mobile phase. The sample to be analysed, when placed in the stationary phase (solid, bonded coating) will gradually move along in the same direction as the mobile phase (liquid or gas). A component that is soluble in the stationery phase takes a longer time to travel as compared to the one that is insoluble. Therefore, the differences in their mobility can be ascribed to the interaction of the sample with the mobile and stationary phase. For a compound distributing itself between equal volumes of two immiscible solvents A & B, the distribution or partition co-efficient (Kd) is given as,

$$K_d = \frac{\text{Concentration of a component in the moving phase}}{\text{Concentration of that component in the stationery phase}}$$

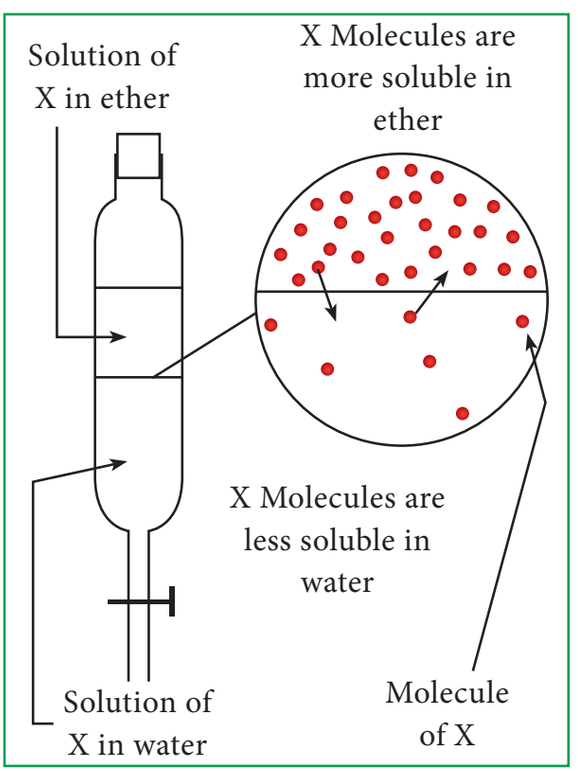

Fig.10.1 : Principle of Chromatography

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As shown in Fig.10.1, substances present in X will tend to cross the boundary between the two liquid layers, to form a dynamic equilibrium.

10.3 Types of chromatography

There are several types of chromatography which are classified based on the interaction of the sample with the mobile and stationary phase. In general, the different chromatographic techniques fall under two major categories: Partition chromatography and adsorption chromatography. Partition chromatography involves partition between two liquids. The stationary phase is a liquid and is held in an inert supporting liquid. In adsorption chromatography, the stationary phase is a finely divided adsorbent such as silica gel and the mobile phase can be a gas or more commonly a liquid. The different forms of chromatography are represented in Fig 10.2

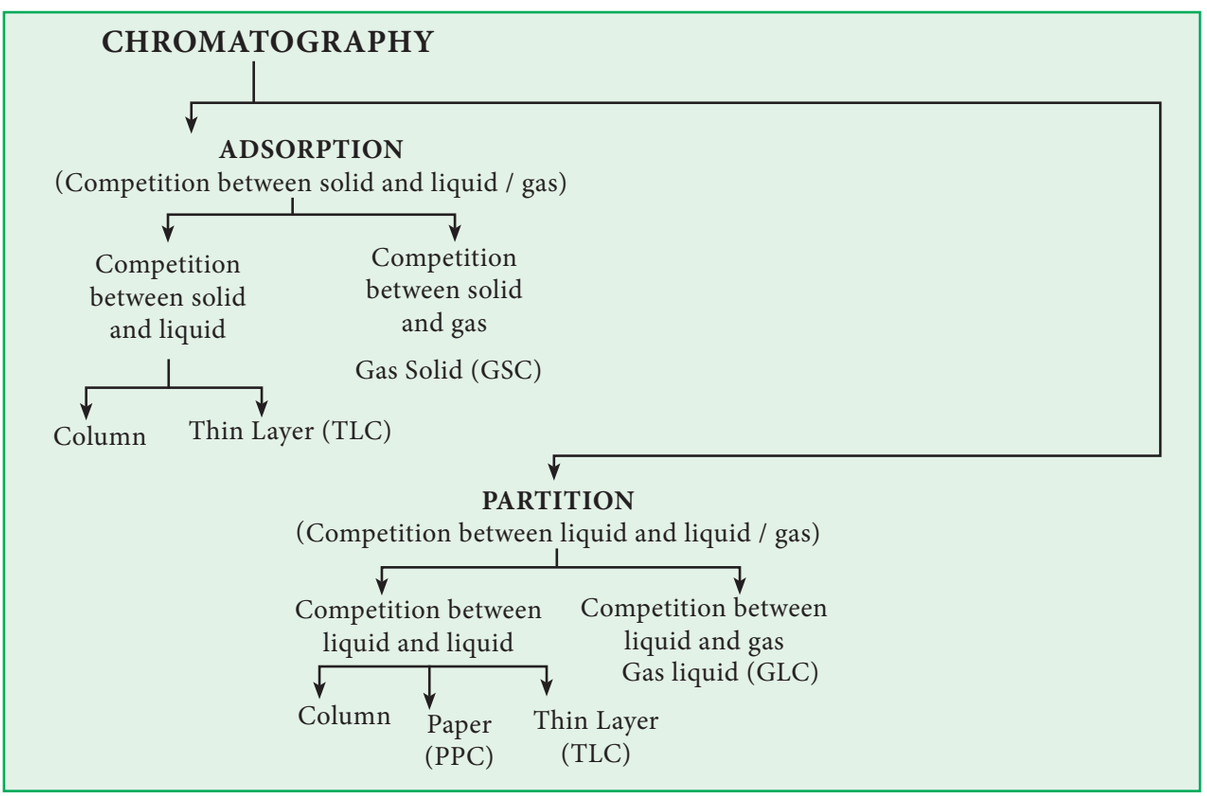

Figure.10.2. Types of Chromatography

10.3.1 Column chromatography

In column chromatography, a mixture of substances, in solution is applied to a column filled with a permeable solid matrix immersed in a solvent. As shown in Fig.10.3, sample, for example, a mixture of protein is applied to the column matrix (packed into a glass or metal surface). After equilibration, the proteins are eluted out using a specific

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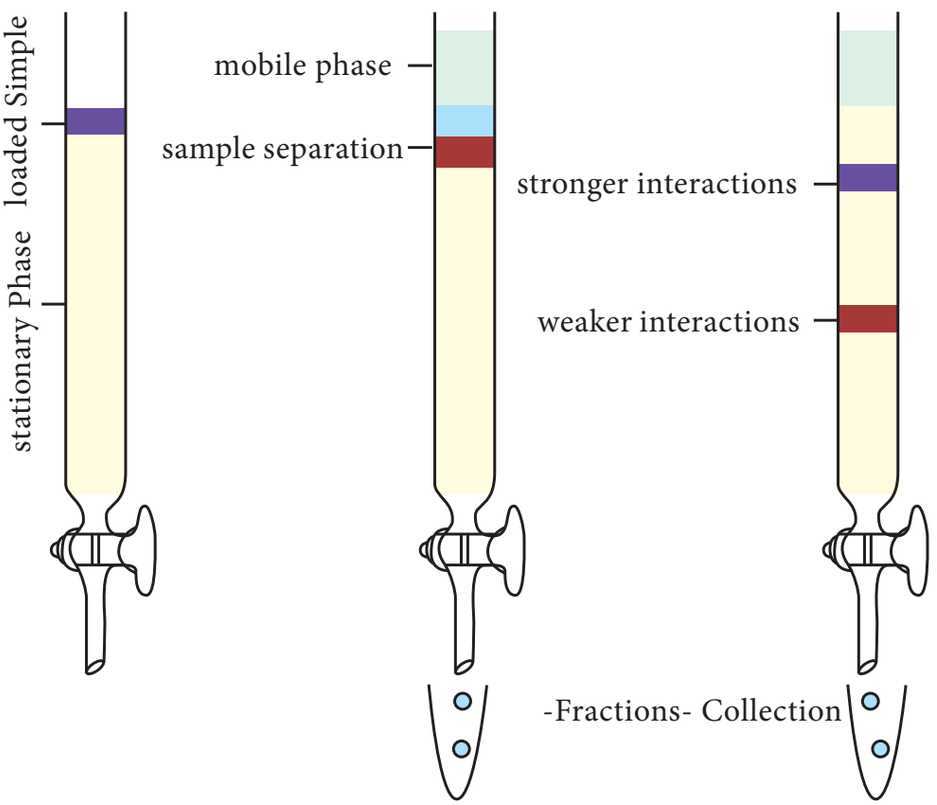
mobile phase. Since different proteins are retarded to different extents by the interaction with the matrix, they can be collected separately. According to the choice of the matrix, proteins can be separated based on the charge, hydrophobicity, size or ability to bind to a characteristic group.





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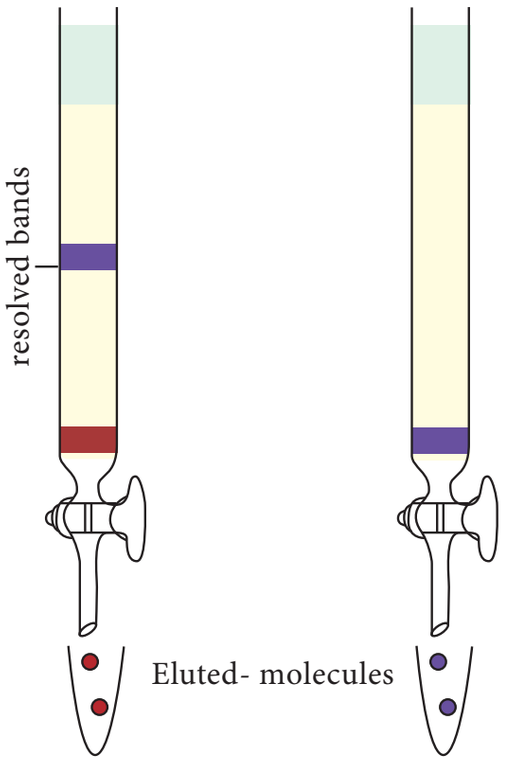

Fig.10.3 Column chromatography

10.3.2 Paper chromatography

Paper chromatography was discovered by the Russian investigators Izmailov and Schraiber. The filter paper strips, made up of cellulose are used to support the stationary phase (water) while the mobile organic phase moves on the suspended paper strip in a chamber. In this technique, the solution containing the mixture of compounds is spotted at a point above 3cm from the end of a Whatmann filter paper (Fig.10.4). After spotting the sample, the paper is dried and equilibrated into a glass jar which contains the desired solvent, for example butanol: acetic acid: water in the ratio of 4:1:5, which acts as a mobile phase. It should be ensured that the point of application remains well above the level of the solvent in the jar. The paper is suspended in such a manner so that it hangs freely without touching the sides of the container. The solvent ascends on the paper and is called as ascending chromatography. The factor governing the separation of compounds in the sample depends on the partition between two immiscible phases.

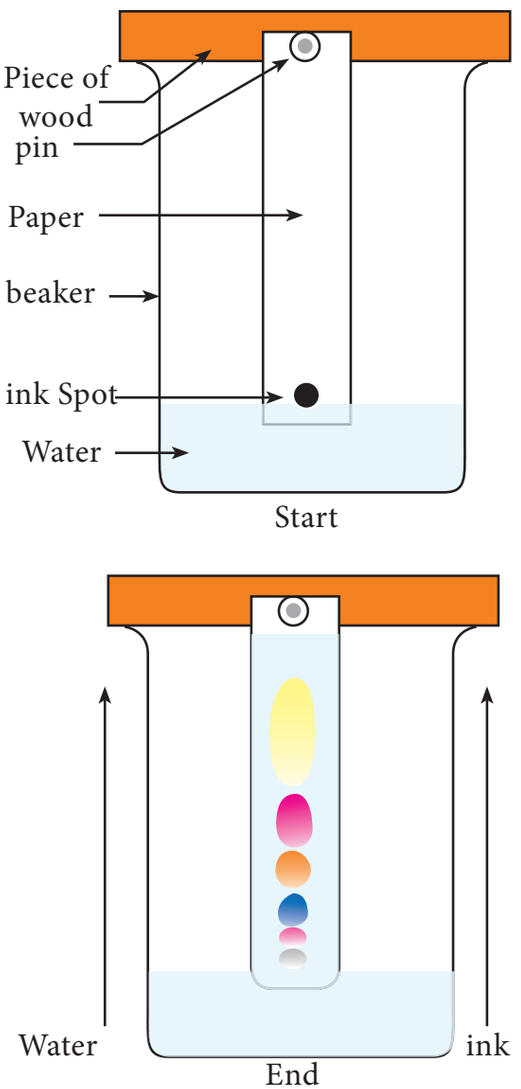

Fig.10.4 : Paper chromatography

When the solvent reaches the top of the paper, the paper is removed and the distance to which the solvent travelled is marked. In order to locate the compounds, the paper strip is dried and sprayed with

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a specific developer. For example, to identify amino acids the strip is sprayed with 0.5% ninhydrin dissolved in acetone to visualize the colored spots indicating a particular amino acid. The ratio of the distance travelled by a component (i.e. amino acid) to that travelled by the solvent front, both measured from the marked point of application of the mixture, is called the resolution front or retardation factor (Rf) value for that component. Thus,

$$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent front}}$$

10.3.3 Adsorption chromatography

Adsorption is the process through which some substances physically bind to the surface of a solid substance like charcoal. Mobile phase is either a liquid (liquid – solid chromatography) or gas (gas-solid chromatography). Adsorption chromatography is based on the interaction between the solute molecules and active sites on the stationery phase. If stationary phase is much polar than the mobile phase, high polar compounds in the sample will bind tightly to the stationary phase while less polar compounds will bind less to the stationary phase. Therefore, the less lightly bound compound will be eluted out by the mobile phase earlier than the one that binds tightly.

10.3.4 Thin layer chromatography

Principle: Thin layer chromatography (TLC) is adsorption chromatography performed on adsorbent materials supported on glassplates. This technique combines the advantages of paper chromatography with those of column chromatography. In TLC, the stationery phase (alumina powder or silica gel with calcium sulphate as binder) is coated on a glass, plastic or foil plates. The thin layer is air dried at room temperature and activated in a hot air oven at 100 - 250°C. The mixture to be separated is spotted on the plate at one end and kept vertical in a container with mobile phase. After a few minutes, the components are separated through the thin layer and dried. The spots are detected by spraying the plates with specific staining reagents.

TLC is superior to paper Chromatography for the following reasons:

1. Concentrated sulfuric acid spray followed by heating may be used to develop the chromatogram by charring, which makes the spots of organic compounds visible.

2. The time taken to separate a mixture of amino acids by paper chromatography can be reduced by TLC.

3. The choice of adsorbents to allow separation of components is restricted in paper chromatography. In TLC, a wide range of adsorbents can be used.

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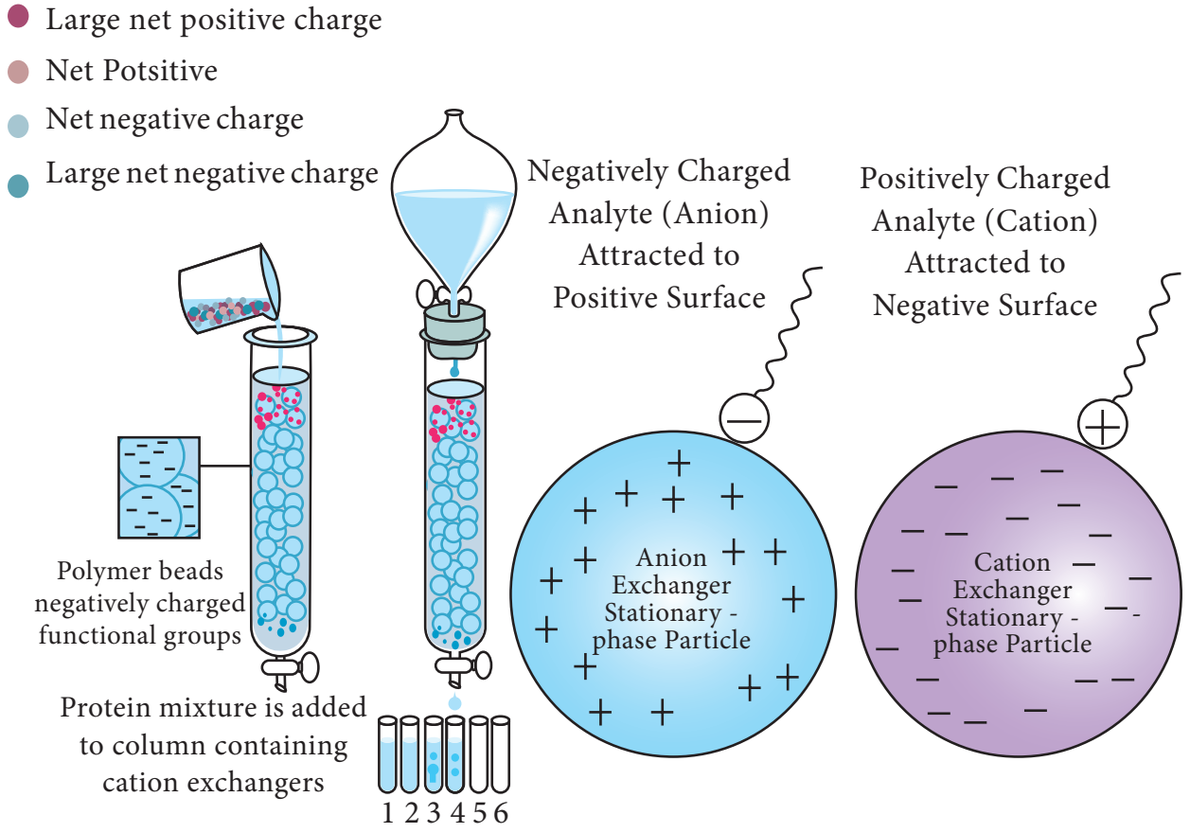
10.3.5 Ion-exchange chromatography

Principle : Ion exchange chromatography was developed by D'Alelio in 1942 using synthetic ion exchange medium based on polystyrene resin. Ion exchange chromatography is a displacement technique in which the ions in either the sample or the buffer displace the existing mobile ions associated with the fixed resin ions. Ion-exchange resins are insoluble, porous matrix containing numbers of ionic groups capable of binding ions of an opposite charge from the surrounding solution. As described in Fig.10.5, proteins move through the column depending upon the net charge at the pH being used.



Protein move through the column at rates determined by their net charge at the pH being used. With cation exchangers, proteins with a more negative net charge move faster and elute earlier.

Fig.10.5 Ion exchange chromatography

When the proteins are attached to the column material, increasing salt concentration or buffers with different pH are passed through the column. Proteins will be washed of the column at different times depending on their molecular structure, net charge and side group. Anion exchangers contain immobilized cationic groups that bind to anions and vice-versa. Examples of anion exchangers are Diethylaminoethyl (DEAE), aminoethyl (AE). Cationic exchangers include carboxymethyl (CM) cellulose, sulphopropyl (SP).

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10.3.6 Molecular sieve chromatography

Principle : Gel permeation or molecular size exclusion chromatography is based on the separation of molecules depending on the molecular size and shape. It employs column which contains pores of varying diameter. When the sample is added to the column, molecules of larger size travel faster than the smaller molecules due to permeation into the gel pores. Therefore, larger molecules are eluted first from the column, followed by molecules of smaller size (Fig. 10.6). The gel materials used in gel permeation chromatography are shown in Table.10.1.

Table.10.1.

Name	Type	Fraction range (k Da)
Sephadex G25	Dextran	1-5
Sephadex G100	Dextran	4-150
Sephadex G200	Dextran	5-600
Bio-Gel P30	Polyacrylamide	2.4-40
Bio - Gel P300	Polycrylamide	60-400
Sepharose 6B	Agarose	10-4,000
Sepharose 4B	Agarose	60-20,000

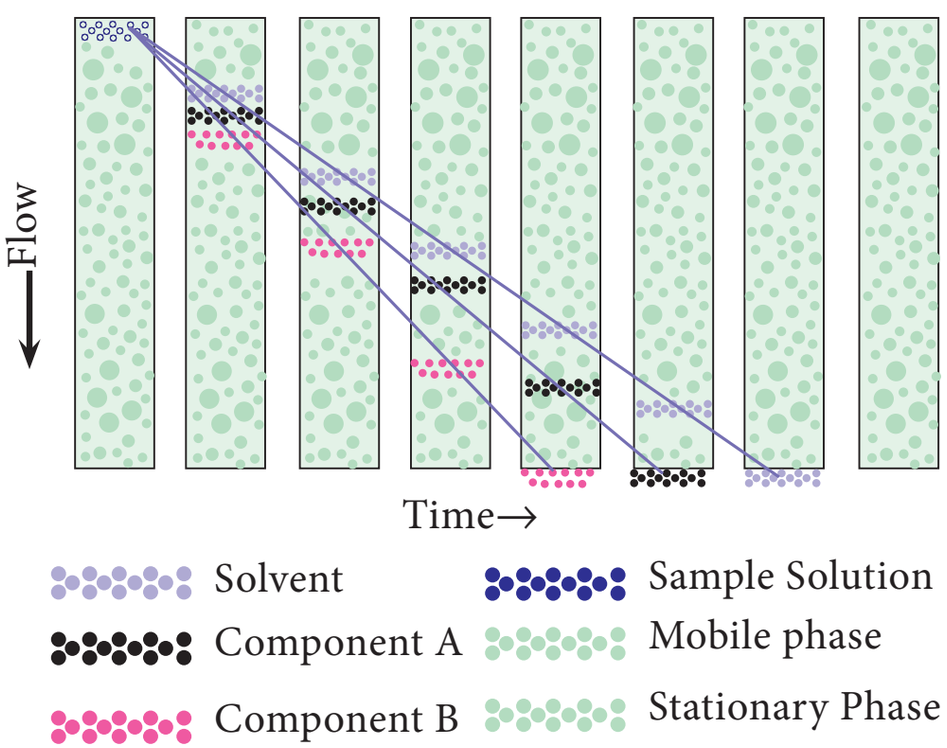

Figure 10.6 : Molecular Sieve Chromatography

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10.3.7 Affinity chromatography

Affinity chromatography is an efficient technique, where separation of a biomolecule can be achieved through highly specific interactions. The interaction is based on the chemical nature of the molecule present in the sample.

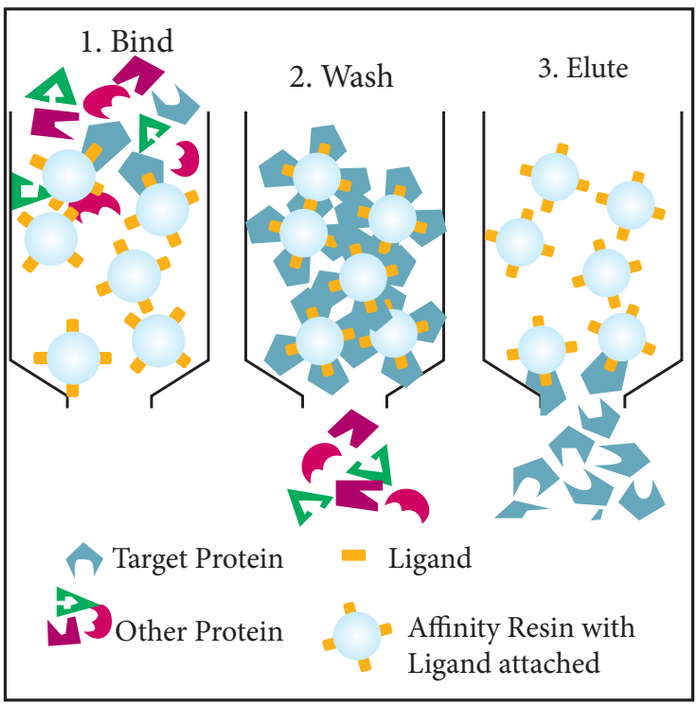

Figure 10.7 Affinity chromatography

When a complex mixture containing the specific compound to be purified is added to the immobilized ligand, only the specific compound will bind reversibly to the ligand leaving the other constituents. The target compound (protein) can be suitably recovered by displacement from the ligand using selective buffer with altered pH (Figure.10.7).

10.3.8 High performance liquid chromatography

HPLC (High Performance Liquid Chromatography) is a highly improved form of liquid column chromatography used to separate the components of a mixture. Columns used in the HPLC system are generally made in stainless steel to withstand the high pressures. Sample administration is through an automated injection system. Mobile phase (mixture of polar or non-polar liquid components) are contained in a glass reservoir. Eluents used for HPLC must be purified. Pumping systems for delivery of the eluent are one of the most important features of HPLC systems. A detector is used to retrieve the data and its analysis in the form of chromatogram (Fig.10.8). Applications of HPLC include isolation and purification of biologically active compounds, purification of chemical compounds, and developing processes for synthesizing chemical compounds.

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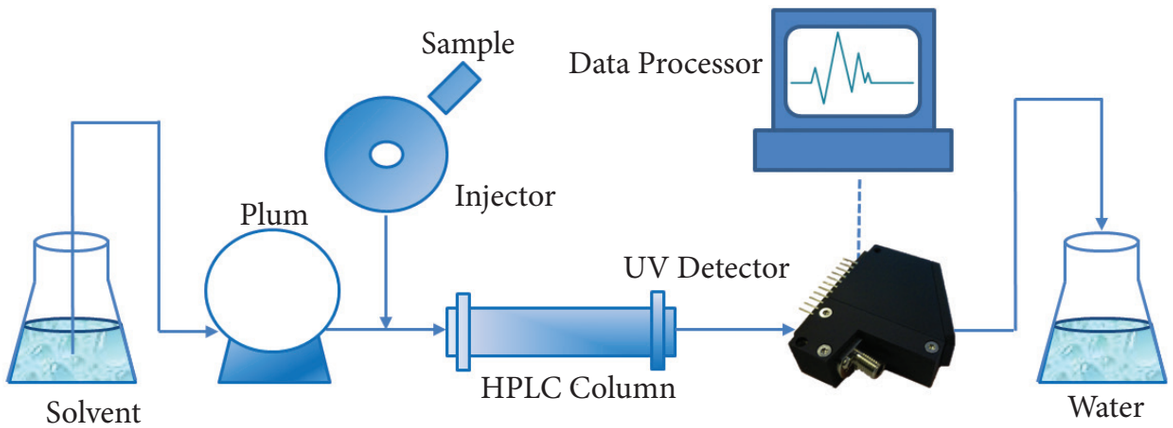

Figure 10.8 : High performance liquid chromatography

10.4. Electrophoresis

In 1948, a Swedish Physical Biochemist, Arne Tiselius was awarded the Nobel prize in chemistry for the discovery of proteins in blood serum and for studying the properties of proteins through electrophoresis. Till now, electrophoresis continues to be an important technique to identify and characterize biological macromolecules. Amino acids, peptides, proteins and nucleic acids possess ionisable groups and can be made to exist in solution as cations or anions. When a mixture of these components is subjected to an electric field, they migrate differently and can be separated.

Principle

Electrophoresis is defined as the migration of charged particles, under the influence of an electric field at a definite pH. In a mixture of proteins, each protein with its electrical charge will move differently in an electric field. This electrophoretic mobility depends on the pH of the medium, strength of the field, net charge of the molecule and size/shape of the molecule. Electrophoresis is used for the analysis of large molecules (proteins and nucleic acids) and simpler charged molecules (peptide, simpler ions).

Types of electrophoresis

The following are different types or electrophoresis.

1. Paper electrophoresis
2. Cellulose acetate electrophoresis
3. Capillary electrophoresis
4. Gel electrophoresis

Agarose gel electrophoresis, Polyacrylamide Gel Electrophoresis (SDS PAGE, Native PAGE and two- dimensional electrophoresis).

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10.4.1 Paper electrophoresis

Paper electrophoresis is an inexpensive method and requires only micro-quantities of protein. The apparatus consists of two troughs to accommodate a buffer through which an electric current is applied (Fig.10.9). Paper is a popular support medium as it is easy to handle, less expensive and is readily available. Paper contains 98% of cellulose. Paper electrophoresis has potential limitations. The greatest problem is the thickness and large pore size of the paper. The separation of proteins by paper electrophoresis takes longer time which limits its use.

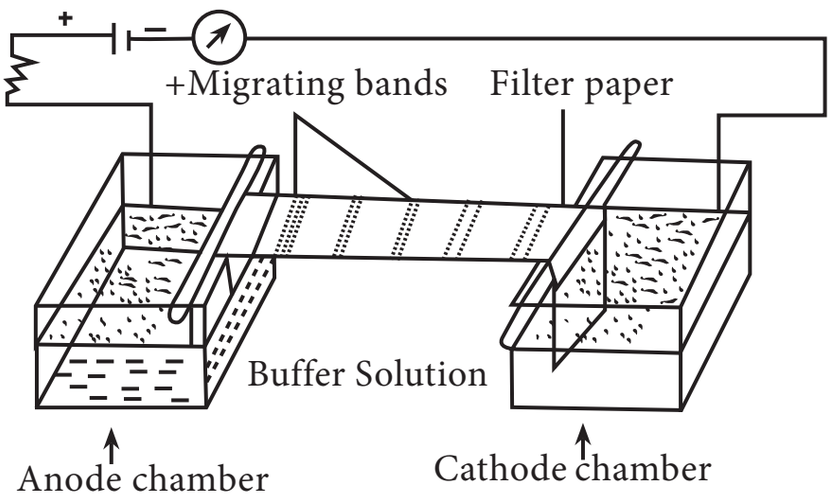

Figure 10.9 Paper electrophoresis

10.4.2 Gel Electrophoresis

i. Polyacrylamide Gel Electrophoresis

Polyacrylamide gel is prepared from acrylamide and bis-acrylamide in a suitable buffer. Polymerization of Acrylamide and bisacrylamide is achieved by a free radical reaction promoted by N,N,N',N'tetramethylethylenediamine (TEMED). This free radical process is initiated by Ammonium per sulfate (APS) used in gel. Acrylamide and bisacrylamide monomers are weak neurotoxin whereas, the polymerised polyacrylamide is non-toxic. While handling acrylamide solutions, care should be exercised and spectacles, gloves and mask should be worn.

ii. Sodium dodecyl Sulphate (SDS) polyacrylamide gel electrophoresis

Sodium dodecyl Sulphate Polyacrylamide Gel Electrophoresis(SDS-PAGE) is an electrophoretic technique very commonly used in Biochemistry, Molecular biology and forensic science. This technique was first described by Laemmli in the year 1970 and till now dominates in scientific research.

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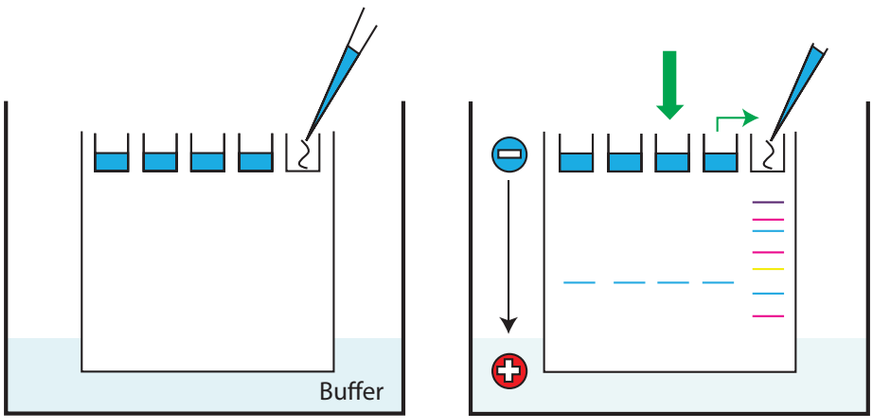
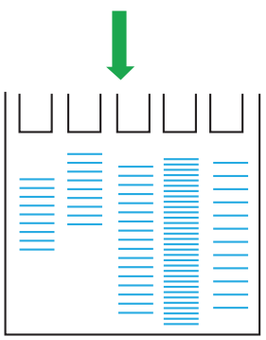
Electrophoresis apparatus: The electrophoretic apparatus consists of a reservoir tank to fill running buffer, transparent insulating cover, gel plates, spacers and gel comb to form wells. Platinum electrodes provide even current with the help of a regulated power pack. The gel is packed in-between two glass plates with the help of spacers. Clear wells are obtained using comb. Samples are layered in the little slots cut in the top of the gel slab using gel comb. Buffer is cautiously layered over the samples, and a voltage is applied to the gel using power pack for a period of usually 1-3 h. The proteins migrate in the gel depending upon their electrophoretic mobility, which is dependent on the size.





Protein samples and marker loaded in vertical SDS - PAGE system

Direction of migration of samples in vertical SDS - PAGE System



SDS - PAGE gel after Coomassie blue staining

Fig.10.10 SDS-PAGE

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Protein samples to be run on SDS-PAGE are added to sample solubilizing buffer containing beta mercaptoethanol (disrupt disulphide bridges), SDS, glycerol (to make the solution denser and enable proteins to sink in the gel) and bromophenol blue (tracking dye).

SDS -PAGE contain resolving gel, used for separation of proteins and stacking gels for concentrating the proteins prior to entry into resolving gel. Sodium dodecyl sulphate (SDS) is an anionic detergent, which binds to proteins, and provides a constant negative charge per unit mass. Protein-SDS complexes will therefore move towards the anode during electrophoresis and their mobilities are inversely proportional to the log of their molecular weights. Since the SDS impart proteins have the same charge per unit length, all proteins travel with the same mobility. However, as the mixture of proteins pass through the resolving gel, the proteins separate, owing to the molecular sieving properties of the gel. The smaller proteins move fast as they can pass through the pores of the gel. But larger proteins move slowly since they are retarded by frictional resistance due to sieving effect of the gels. When the dye reaches the bottom of the gel, the current is turned off. After electrophoresis, the gel is carefully removed from the glass plate, immersed in buffer and stained with appropriate stain solution (Fig.10.10)

Protein staining: Proteins can be detected using Coomassie Brilliant Blue G250 (CBB) solution. CBB dye stains protein with a detection limit of 40µg. For proteins of less quantity, another sensitive detection known as silver staining (1-5ng detection limit), can be performed.

Applications : SDS–PAGE is used to determine the molecular weight of proteins. To achieve this, a standard mixture of proteins of various molecular weight (molecular weight ladder) was added for direct comparison of migration distance. The molecular weights around 15-200kDa can be analyzed in this manner.

iii. Two -Dimensional gel electrophoresis

Two dimensional gel electrophoresis was introduced by O'Farell in the year 1975. It is a combination of two techniques, iso electric focusing and SDS-PAGE. Iso-electric focusing is an electrophoretic technique where proteins are separated based on their iso-electric point (pI). pI is the pH at which the amino acid does not migrate in an electric field (zwitterion form). When a gradient of pH is applied to a gel, and electric field applied to it, one end becomes more positive than the other. Relatively at all pH other than its iso electric point, proteins have a charge (positive or negative) and will be pulled to the opposite side of the gel. In two dimensional electrophoresis, proteins are separated based on isoelectric point and molecular mass. To accomplish this, proteins are first separated by isoelectric focusing where they are separated by their respective isoelectric point. Second dimension of separation is achieved through SDS-PAGE, where proteins are separated

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according to their molecular weight. Each spot on the resulting 2D gel correspond to single protein species present in the sample (Fig.10.11)

Applications : SDS–PAGE can be used to determine the molecular weight of proteins. To achieve this, a standard mixture of proteins of various molecular weight (molecular weight ladder) was added for direct comparison of migration distance. The molecular weights around 15-200kDa can be analyzed in this manner. Another application of SDS PAGE is to check the purity of a protein sample. Presence of a single band denote the protein sample is pure.

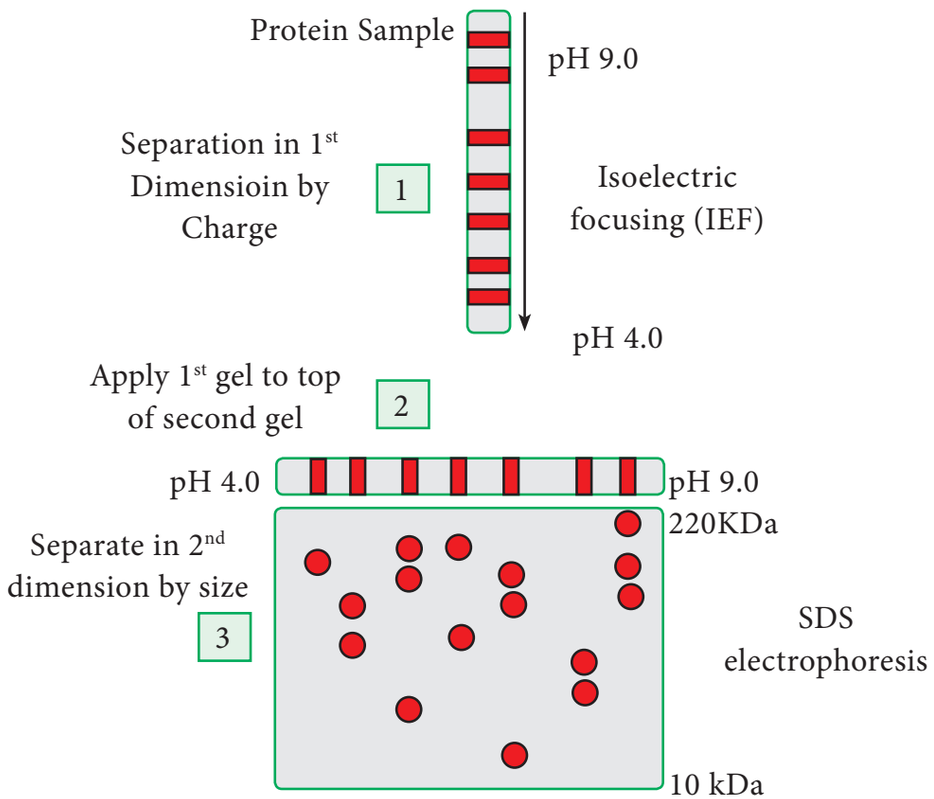

Fig.10.11 Two dimensional gel electrophoresis

iv Agarose gel electrophoresis

Agarose is one of the several components that can be separated from agar. The major source of agar is certain species of sea weed. Agarose is a linear polymer made up of alternating units of galactose and 3,6- anhydrogalactose. Agarose gels are completely transparent when cooled to room temperature.

In Agarose gel electrophoresis, DNA or RNA molecules can be separated based on their size. This is achieved by the movement of negatively charged nucleic acid molecules through an agarose matrix in a horizontal electrophoresis. Molecules with smaller size

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move faster and migrate farther as compared to longer ones. The distance between DNA or RNA bands of a given length is determined by the percentage of agarose in the gel.

10.5 Centrifugation techniques

10.5.1 Principle

Centrifugation is a separation technique based on the properties of the particle in an applied centrifugal field. Particles which differ in size, shape and density sediment at different rates in a medium held in a tube. The rate of sedimentation depends upon the applied centrifugal field, density and radius of the particle as well as the density and viscosity of the suspending medium. The rate of sedimentation can be expressed as rpm (revolutions per minute) or g (gravitational force). The rate of sedimentation is dependent upon the centrifugal field (G) which is determined by the square of the angular velocity of the rotor (ω^2) and radial distance (r) from the axis of rotation, expressed in the equation$G = \omega^2 r$

The instrument used to hold the sample and generate the centrifugal force is called the centrifuge. This is accomplished through the use of various rotors of specific diameter. A rotor can hold the sample and spin along its own axis at different speeds to generate centrifugal force.

10.5.2 Types of Rotors:

Rotors are important components in a centrifuge and there are several types of rotors. The schematic representation of rotors used in centrifuge is shown in Fig.10.12.

1. **Fixed angle rotor:** In fixed angle rotor, particles move radially outward under the influence of a centrifugal field. The tubes are held at the angle 20 to 45 degrees. Particles have only a short distance to travel, to form a pellet at the bottom of the tube.

2. **Swinging bucket rotor:** The swinging bucket rotor, starts off in a vertical position and during acceleration of the rotor swing out to a horizontal position. During centrifugation, the solution in the tube, is aligned perpendicular to the axis of rotation and parallel to the applied centrifugal field, the tube returning to its original position as the rotor slows down.

3. **Vertical rotor:** Vertical rotor presents the shortest possible path for a particle. In this rotor, the pellet is deposited along the entire length of the tube.

4. **Zonal rotor:** The zonal rotors may be of the batch or continuous flow type. They are designed to minimize the wall effect that is encountered in swinging-bucket and fixed

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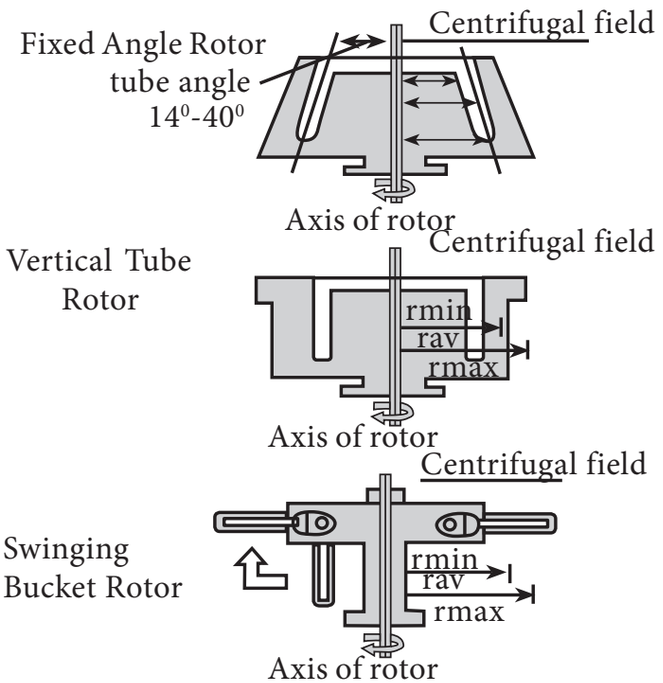
angle rotors, and to increase sample size.



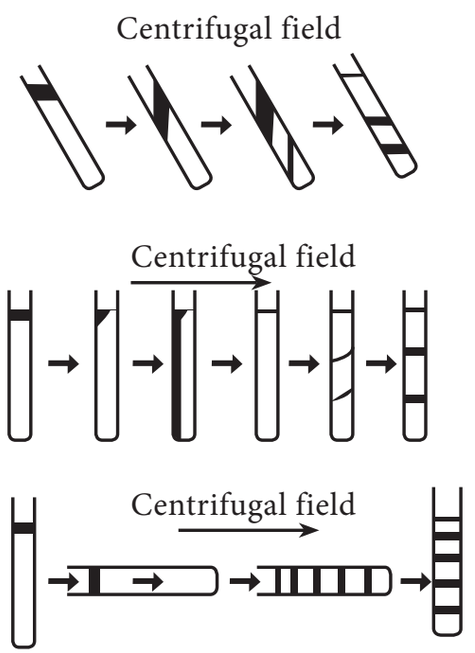


Fig.10.12 Types of rotors

10.5.3 Types of Centrifugation:

Two main centrifugation techniques are in general use

a. Preparative centrifugation: This technique is used for separation, isolation and purification of whole cells, plasma membrane, ribosomes, nucleic acids, and many subcellular organelles

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b. Analytical centrifugation: This technique is used to study the characteristic feature of a pure macromolecule. Specially designed rotors and detector systems are needed to continuously monitor the process of sedimentation of the material in the centrifugal field.

i. Differential centrifugation:

Differential centrifugation is based on the difference in the sedimentation rate of the particles of different size and density. It is used for the isolation of sub-cellular organelles. The centrifugal force is proportional to the radius of the centrifugal head and to the square of angular velocity. Therefore, it is possible to use small rotor heads at very high speeds.

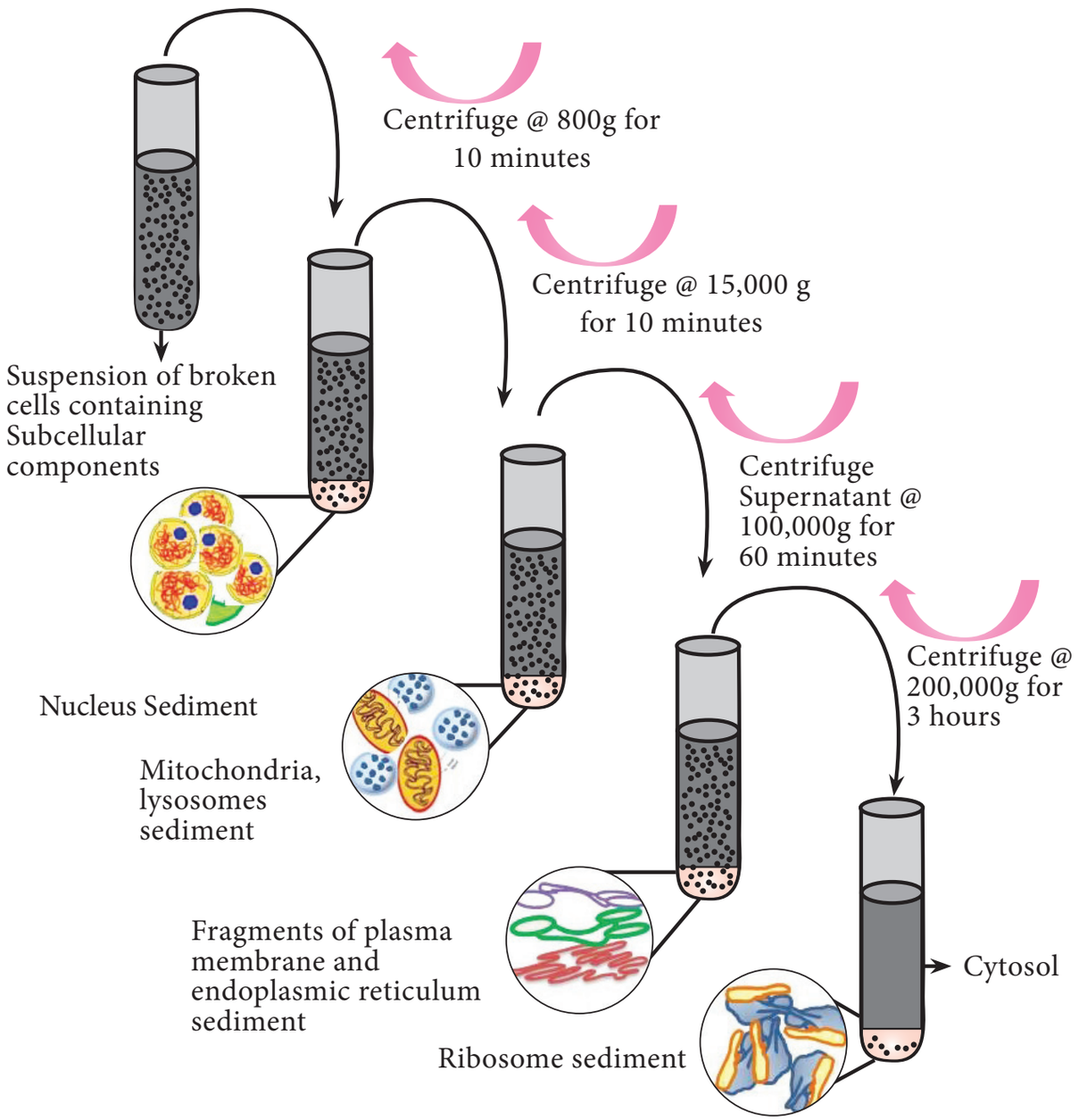

Figure 10.13 Differential centrifugation

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The rotor heads are run in a vacuum. The tube containing the homogenate is held at an angle to the axis. This technique is commonly used for collecting cells or producing subcellular fractions from a tissue homogenate. For example, homogenizing a rat liver at relatively low speed will pellet the larger dense nuclei and centrifugation at higher speed will sediment particles to further lower size (Fig.10.13)

ii. Density gradient centrifugation

This technique was developed by Scientist Brakke in the year 1960, where he proposed three ways through which density gradients may be used.

a. Rate zonal density gradient centrifugation:

This type of centrifugation involves, layering of sample solution on the top of the gradient, where the density continuously increases towards the bottom of the tube. It is used to measure the rate of sedimentation for separation of particles with different sedimentation co-efficient.

b. Isopycnic density gradient centrifugation:

Isopycnic (Equal) density gradient centrifugation depends on the buoyant density of the particle and not on the size and shape of the particle. This centrifugation is used for the separation of similar size particles which differ in their densities

c. Equilibrium iso-density centrifugation:

In equilibrium iso-density centrifugation, the sample is mixed with gradient medium to give a solution of uniform density. The gradient is self-forming during centrifugation. Salts of heavy metals like caesium, sucrose, and silica are used as the gradient material. Comparison of rate zonal density gradient centrifugation and isopycnic gradient centrifugation is represented in Fig.10.14

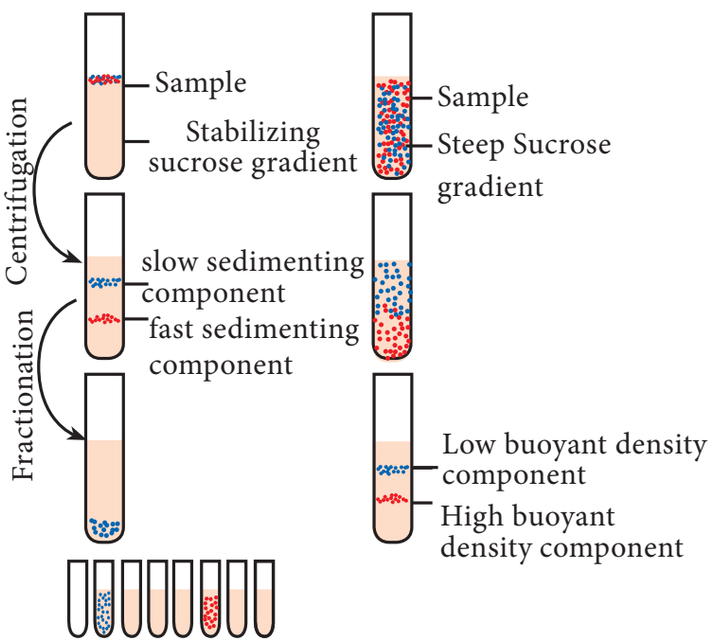

Figure 10.14 Equilibrium iso-density centrifugation

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10.5.4 Analytical Ultra centrifugation:

Analytical ultracentrifugation, developed by Svedberg, is a versatile method for the separation of small particles such as particles of 10 microns in size. It can operate safely at about rotor speeds upto 60,000 – 10,00,000 rpm. Protein molecules, under the influence of high centrifugal force can be made to sediment toward one end of a centrifuge tube. The rate of sedimentation for a protein under a certain centrifugal force depends on the density, shape and size of the molecule. It is used to determine the sedimentation co-efficient and molecular weight of the macromolecules in solution.

10.6 Spectrophotometry

The use of spectroscopic methods to evaluate bio-molecules continues to be an important area in Biochemistry. Spectroscopy is the study of the absorption and emission of radiation by matter. Using spectroscopic methods, it is possible to analyze the colour developed by the bio-molecule exhibited by the substances that absorb radiation from the visible region of the spectrum. Measurements of the intensity and wavelength of the radiation that is either absorbed or emitted provide the basis for sensitive method of detection and quantification. The study of functions of the body in both health and diseased states require the analysis of blood, serum and body fluids. The applications of spectrometry has produced more diagnostic kits to quantify the constituents present in blood, tissue, urine and other biological material.

We might have noticed that the colours of two solutions of the same substance, one a deep and another lighter colour. When the colour of the solution deepens, it is an indication that it is more concentrated. This forms the basis of spectrophotometry, where the intensity of the colour is a direct measure of the amount of the material present in the solution. Light is a form of electromagnetic radiation. When it falls on a solution one can expect three changes. a). the light can be reflected by the substance b). the light can be absorbed by the substance c). only certain wavelengths can be absorbed and the remaining transmitted. The absorbance of transmitted light is of prime importance in spectrophotometry. The observed colour is due to the absorption of a certain wavelength of light in the visible spectrum and transmittance of the other wavelength.

Photometric instruments are designed to measure the intensity of radiation. Instruments prefixed with the term 'spectro' are capable of splitting radiation into a spectrum

10.6.1 Principle of Beer-Lambert's law

The absorption of light by a solution is shown in Fig.10.15. When a beam of monochromatic light (I_o) is allowed to pass through a homogenous light absorbing

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medium, the intensity of light coming out (I) decreases exponentially, with increase in the concentration and pathlength of the medium through which it is passed. The fraction of such incident light absorbed by a given solution at a particular wave length is related to the thickness of the absorbing layer i.e., the path length and concentration of the absorbing species. This relation may be combined using Beer-Lambert's law to determine the concentration of analyte in a solution using spectrophotometry.

logIo/I = εcl, where Io and I are the intensities of incident and transmitted light, respectively. l, is the path length, c is the concentration of the absorbing species and ε is known as the molar extinction co-efficient. The expression logIo/I is absorbance.

10.6.2 Photo electric colorimeter

Principle:A beam of light with a precise wavelength is passed through the sample through a mono-chromator and lens, which navigate the coloured light to the output device which measures the extent of colour as compared to a standard. A microprocessor then calculates the absorbance or percent transmittance. If the concentration of the solution is greater, more light will be absorbed, which can be identified by measuring the difference between the amount of light at its origin and that after passing through the solution.

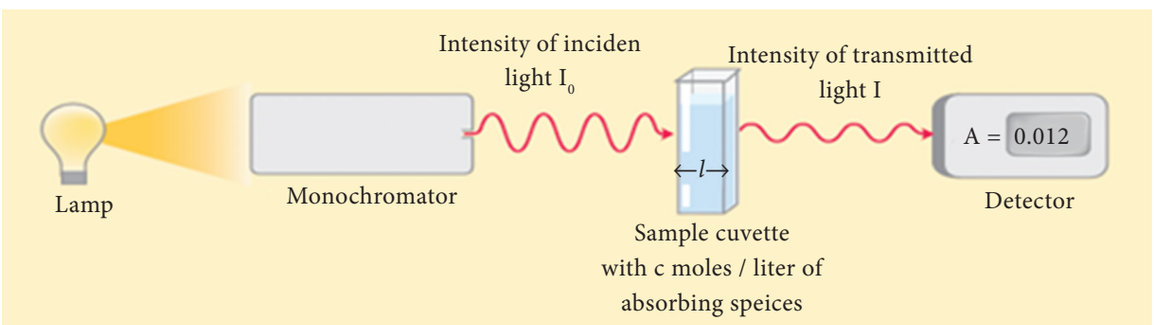

Figure 10.15 Spectrophotometry

Components of a Colorimeter :

A schematic representation of the working of the colorimeter is shown in Fig.10.16

The essential parts of a colorimeter are: a) light source, which is from a tungsten lamp and the light passes through a slit b) an aperture which can be adjusted accordingly c) a filter - where the light from the lamp source converts polychromatic light to a monochromatic light d). photocell or tube which converts the light energy to electrical energy e) a detector measures the light which was transmitted by the sample f) cuvette – a glass tube to place the solution.

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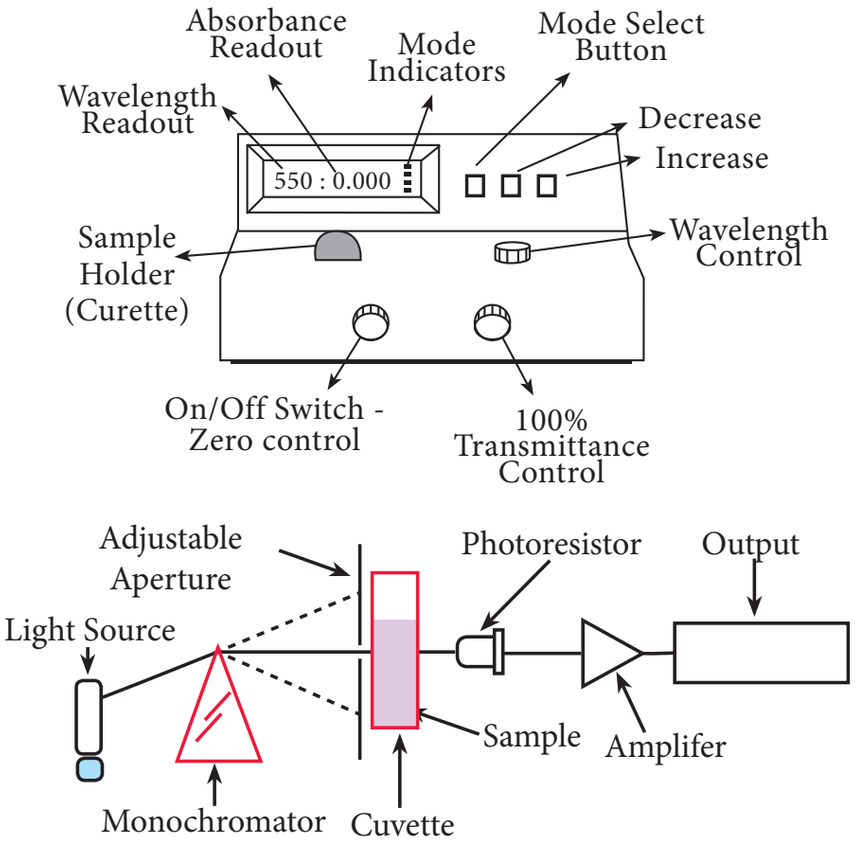

Figure 10.16 Colorimeter

In a colorimeter, beam of light is passed through an optical filter that transmits only a particular band of wavelength. The variation in the monochromatic light transmitted through a colorless sample (blank) and the amount of monochromatic light transmitted through a test sample is a measurement of the amount of monochromatic light absorbed by the sample. The monochromatic light absorbed is directly proportional to the concentration of the sample producing the colour and the path length through the sample.

Applications

A well known application of the colorimeter is to determine the concentration of the solute in a solution. To monitor the growth of a bacterial culture, colorimeter is used. As the culture grows, the medium becomes cloudier and absorbs more light which can be quantified. Colorimeters are used for testing water quality and screening of chemicals.

10.6.3 Colorimetric analysis

General steps in carrying out a photometric analysis is explained using blood glucose estimation procedure as an example.

a. Separation of the substance from the complex mixture. For example, estimation of blood glucose requires the precipitation of lipids and proteins using de-proteinising agents which otherwise interfere with the colour reaction of glucose.

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b. Qualitative conversion to a coloured or light absorbing substance. For example, after de-proteinization of the sample, the supernatant conataining glucose is made to react with orthotoluidine reagent to give a greenish blue coloured complex.

c. Measurement of light absorption of sample. For example, the colour intensities of the complex can be measured.

d. Calculation of the concentration of the substance. Comparing the molecular extinction with that of a relative standard solution of known concentration.

$$\text{Concentration of unknown} = \frac{\text{Absorbance of unknown}}{\text{Absorbance of known (Standard)}} \times \text{Concentration of known}$$

10.6.4 UV absorption spectrophotometry

Absorption spectrophotometry utilizes the phenomena of absorption of light by the sample being analysed. Different materials absorb different wavelengths based on the molecular and chemical nature of the compound. Absorption spectra can use any form of waves. The common type of waves used in absorption spectra include infrared, gamma, atomic, X-ray and visible light. The common wavelength region used in the absorption spectra is .

Applications of spectrophotometry

- Spectrophotometry is used in diverse area in the field of biological science.
- To identify classes of compounds in pure and biological samples.
- For quantitative analysis of proteins, lipids and nucleic acid etc.
- To assay the enzyme activity and their kinetics.
- Elucidation of the structure of organic compounds.
- Measurement of growth kinetics.

10.7 Biosensor

A biosensor is an analytical device which converts a biological response into an electrical signal. The term 'biosensor' is often used to cover sensor devices used in order to determine the concentration of substances and other parameters of biological interest even where they do not utilise a biological system directly. The analyte is coupled to various process including enzyme, antibody and later transduced to obtain a measurable signal. Examples include glucometer and several other diagnostic applications

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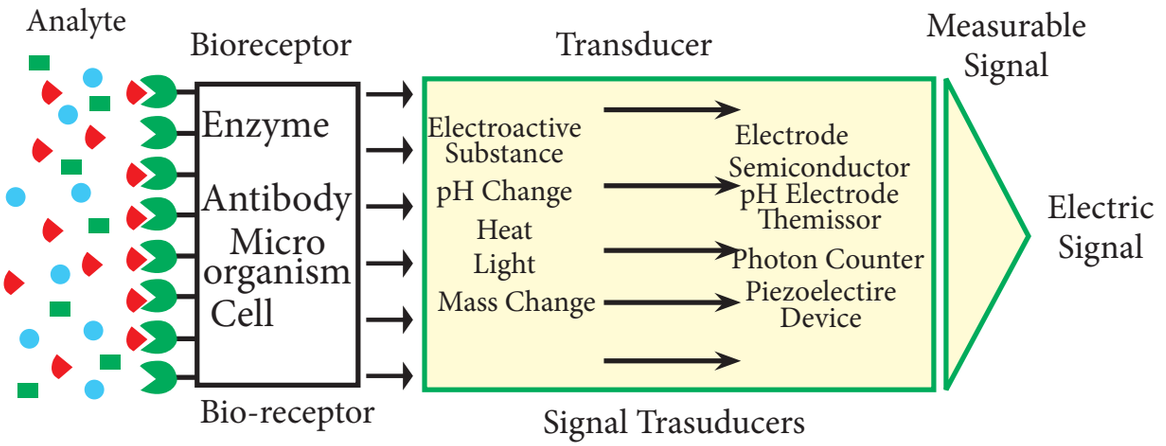

Figure 10.17 Bio - Sensors

EVALUATION

I. Choose the correct answer

1. Chromatography is based on the principle of

   a) Conductivity

   b) Distribution coefficient

   c) Counter balance of charges

   d) Centrifugal force

2. This separation technique of charged molecule under the influence of electric current is described as

   a) Paper chromatography

   b) Ion exchange chromatography

   c) Spectrophotometry

   d) Electrophoresis

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3. Separation of proteins by gel electrophoresis is based on

   1. Relative size of proteins

   2. Mobilities or migration rates in a charged electrical field

   3. Charged species

   4. Hydrophobicity of proteins

4. The purpose of adding SDS in PAGE is to

   a) solubilize the protein

   b) stabilize the complex protein

   c) impart uniform charge on protein

   d) to reduce the heat of buffer

5. In gel filtration chromatography, separation is achieved based on

   a) Size and net charge

   b) Size and shape

   c) Size and specific affinity

   d) Shape and net charge

6. A gradient commonly used in differential centrifugation is

   a. Sucrose b. Maltose c. polyacrylamide d. agar

II Give one word answer

1. Give any one material to prepare thin layer chromatogram

2. What is the speed range of ultra centrifuge?

3. A chromatographic technique involves two phases. What are they?

4. The detection limit of CBB-250 stain is_____.

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III. Answer the following (3 marks)

1. Define Beer Lamberts law

2. What are the limitations of paper chromatography

3. What are the applications of spectrophotometry

4. Comment on ion exchange resins

5. Write a note on 2D gel electrophoresis

IV Answer the following (5 marks)

1. Describe the various types of rotors used in centrifugation

2. Write the principle involved in SDS-PAGE

3. What is the basis of affinity chromatography

4. Describe the method of isolation of subcellular organelles

5. Outline the components of a biosensor

SUMMARY

Analytical techniques are a very important component of biochemistry. Its application ranges from clinical diagnosis to fundamental research. For example, the clinical test to estimate the blood glucose concentration is a combination of techniques such as centrifugation, spectroscopy etc. Similarly these methods are routinely used every day in every biochemical research laboratory. So it is important for a biochemist to have a good knowledge in these methods. This chapter explains the details of the important biochemical methods such as centrifugation, chromatography, electrophoresis and spectroscopy.

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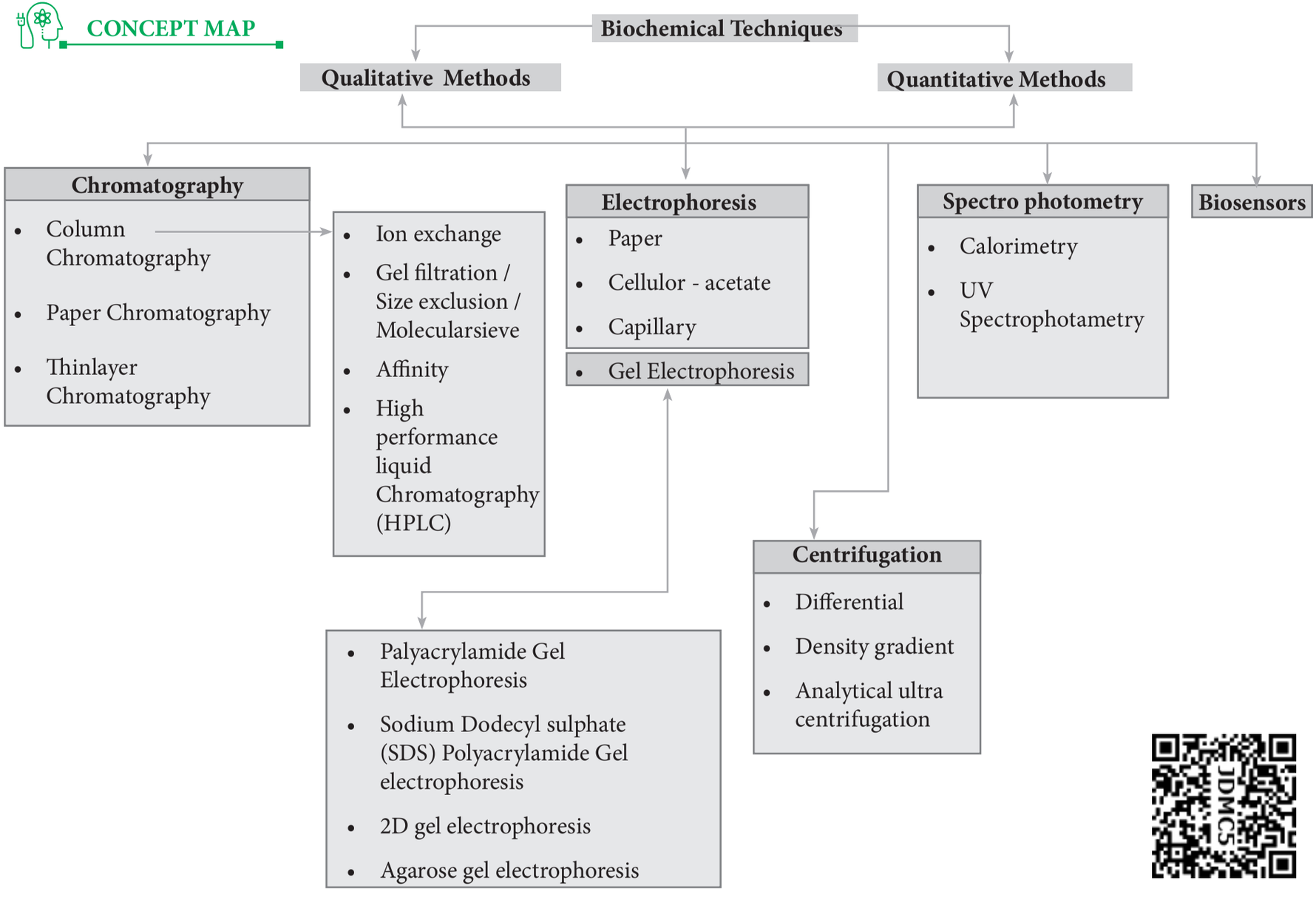
CONCEPT MAP



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ICT Corner

Paper Chromatography

By using this tool you will learn to use a paper chromatography to detect the presence of colouring agents E102 and E131.

Please go to the URL
http://www.physics-chemistry-interactive-flash-animation.com/matter_change_state_measurement_mass_volume/chromatography_high_school.htm
(or)
Scan the QR code on the right side

Step – 1

Open the Browser and type the URL given (or) Scan the QR Code. You can see a webpage which displays the term "Chromatography" in the middle and arrow with word "Enter" is present. Now click the arrow.

Step – 2

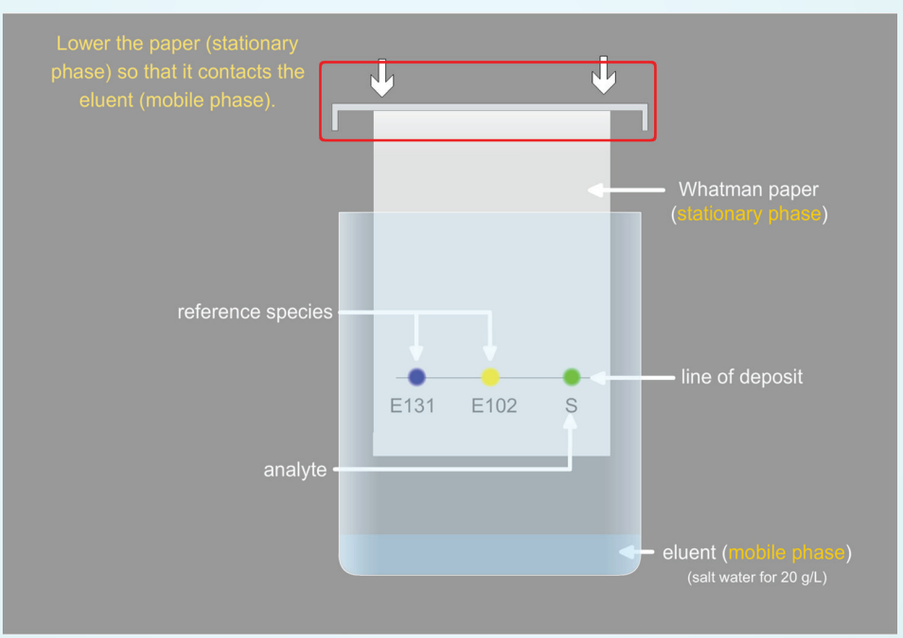
Now the page will explain the experimental setup and conditions and arrow at the bottom with the word "**Chromatography**". After reading the text click the arrow again.

Step – 3

Now you can see a page as shown in the figure. Now lower the paper so that it contacts the eluent by clicking the region marked by the red box and move it downwards. Now the experiment starts and you can see the result in few seconds.

Step – 4

After the simulation you can see few evaluation questions which you can try to answer.



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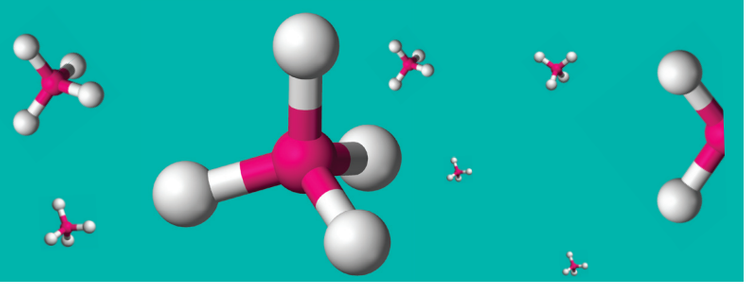
GLOSSARY

A-Z



1	activator	இயக்குவிப்பான்
2	agar	கடற்பாசி கூழ்
3	alopecia	வழுக்கை
4	anti–coagulant	உறைவெதிர்ப்பி
5	anti–stiffness factor	எதிர்விறைப்புக் காரணி
6	biocatalyst	உயிரூக்கிகள்
7	blood vessel	இரத்த குழல்கள்
8	cartilage	குருத்தெலும்பு
9	cereals	தானியங்கள்
10	cerebrospinal fluid	மூளை தண்டுவட திரவம்
11	chloroplast	பசுங்கணிகம்
12	chylomicron	நுண்கோள கொழுப்புக் குமிழ்
13	collagenopathy	கொல்லாஜன் கோளாறு
14	colorimetry	நிறவளவியல்
15	colostrum	சீம்பால்
16	convulsion	வலிப்பு
17	demineralization	தாது உப்பு நீக்கம்
18	dental caries	பற்சொத்தை
19	diarrhoea	வயிற்றுப் போக்கு
20	duodenum	சிறுகுடலின் முன்பகுதி
21	elutant	கரைத்திரவம்
22	encephalitis	மூளை வீக்கம்
23	endemic goitre	வட்டாரக் கழலை
24	endoplasmic reticulum	எண்டோபிளாச வலைப் பின்னல்
25	erythrocyte	இரத்த சிவப்பு அணுக்கள்
26	excretion	கழிவு நீக்கம்

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27	fasting	நோன்பு
28	feces	மலம்
29	fibril	நுண்ணிழை
30	gastro intestinal tract	இரைப்பைக் குடல்
31	goitre	முன்கழுத்துக் கழலை
32	gun cotton	வெடிபஞ்சு
33	haemolysis	இரத்தமழிதல்
34	hepatic necrosis	கல்லீரல் அழற்சி
35	hunch back	கூன் முதுகு
36	hypocalcaemia	இரத்தத்தில் கால்சியம் பற்றாக்குறை
37	hypogonadism	இனப்பெருக்க இயக்க குறைபாடு
38	ileum	சிறுகுடலின் கீழ்பகுதி
39	kidney	சிறுநீரகம்
40	lacquer	மெருகெண்ணெய்
41	liver	கல்லீரல்
42	membrane	செல் சவ்வு
43	meninges	மூளையுறைகள்
44	meningitis	மூளைக்காய்ச்சல்
45	muscular dystrophy	தசைநார் தேய்வு
46	mutation	திடீர்மாற்றம்
47	nerve impulses	நரம்பு தூண்டுதல்கள்
48	nuts	கொட்டைகள்
49	obesity	உடல் பெருத்தல்
50	obstructive sleep apnea	தூக்கத்தில் மூச்சுத்திணறல்
51	organelle	உள்ளுறுப்புகள்
52	osteoarthritis	கீழ்வாதம்
53	Osteogenesis imperfecta (O.I)	சீரற்ற எலும்புருவாக்கம்
54	osteomalacia	எலும்பு மெலிவு
55	phytosterols	தாவர ஸ்டெரால்கள்
56	plastics	நெகிழிகள்
57	proteolytic enzymes	புரத சிதைவு நொதி
58	pulses	பருப்பு வகைகள்

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59	reproductive failure	மலட்டுத் தன்மை
60	resorption	அழித்தல்
61	serum	இரத்த திரவம்
62	sheath	உறை
63	sickle cell anaemia	கதிர் அரிவாள் இரத்த அணுச்சோகை
64	skeletal muscles	எலும்பு தசை
65	starvation	பட்டினி
66	tendon	தசைநாண்
67	thoracic duct	வயிற்றுக் குழல்
68	vascular bundles	கடத்துதிசுக் கற்றை
69	vesicle	சிறு கொப்புளம்
70	villi	குடலுறிஞ்சி
71	whole grains	முழுதானியங்கள்
72	xerophthalmia	கருவிழிநைவு

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