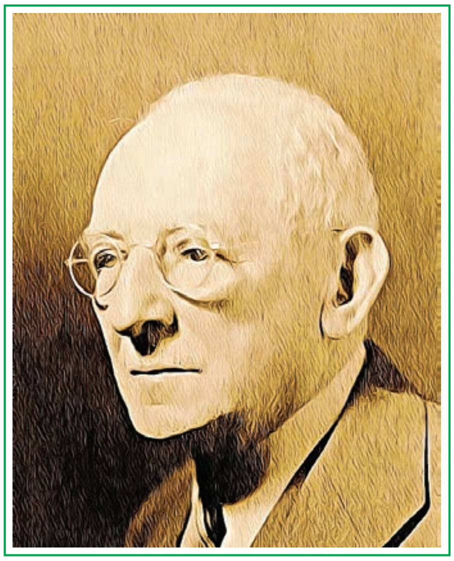
Unit 1

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

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

DO YOU KNOW?

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.

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.

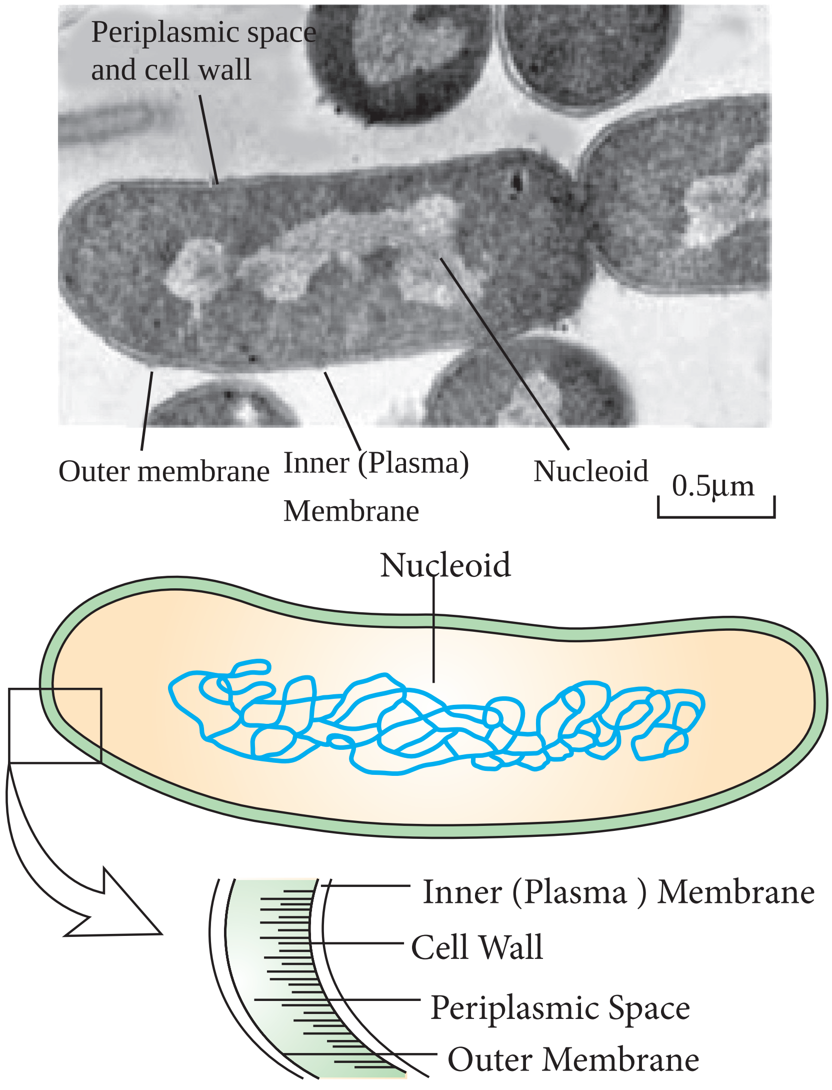


Figure 1.1 Structural organization of the prokaryotic cell

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.

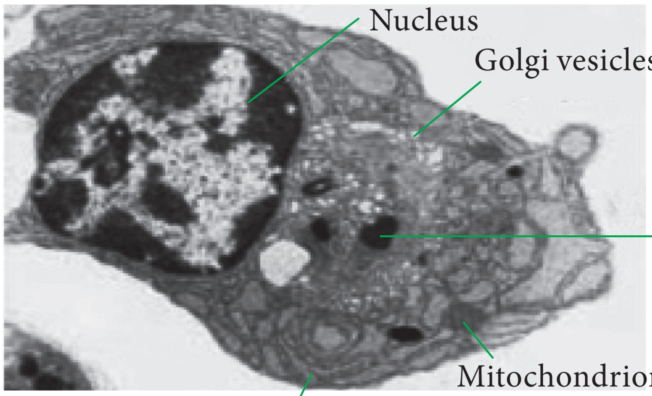
The prokaryotes are unicellular, in general eubacteria (true bacteria) and archaebacteria (ancient bacteria). The cell encloses a semi-liquid component with a few suspended structures called ribosomes. Within the membrane is the cytoplasm, which contains the cytosol-a semi-liquid concentrated solution 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.

DO YOU KNOW?

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.



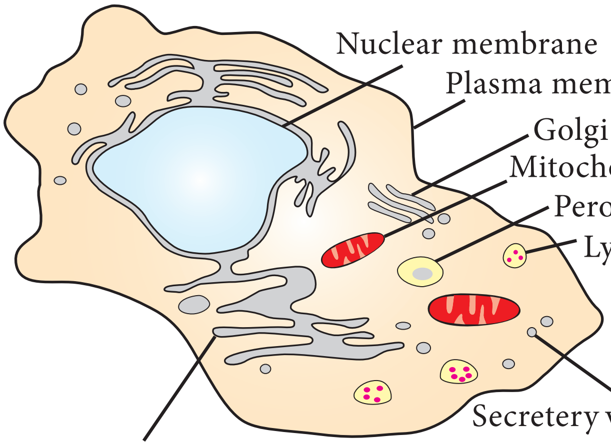


Figure 1.2 Structural organization of the eukaryotic cell

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

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.

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.*

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.

DO YOU KNOW?

An organelle can be specific for the type of cell

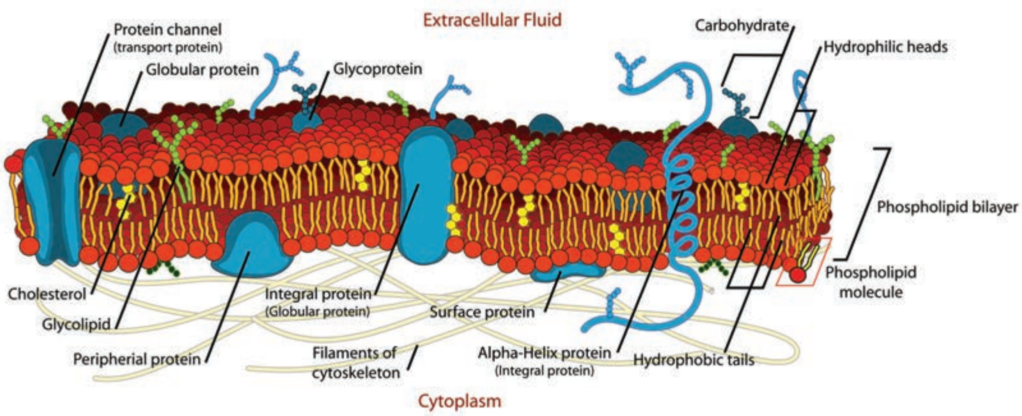
Certain organelles like lysosomes are present in animal cells but not in plant cells. Plant cells have chloroplasts, the sites of photosynthesis and usually a large, water filled vacuole. While most of the animal cells are surrounded by a plasma membrane only, plant cells often have a rigid cell wall 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.



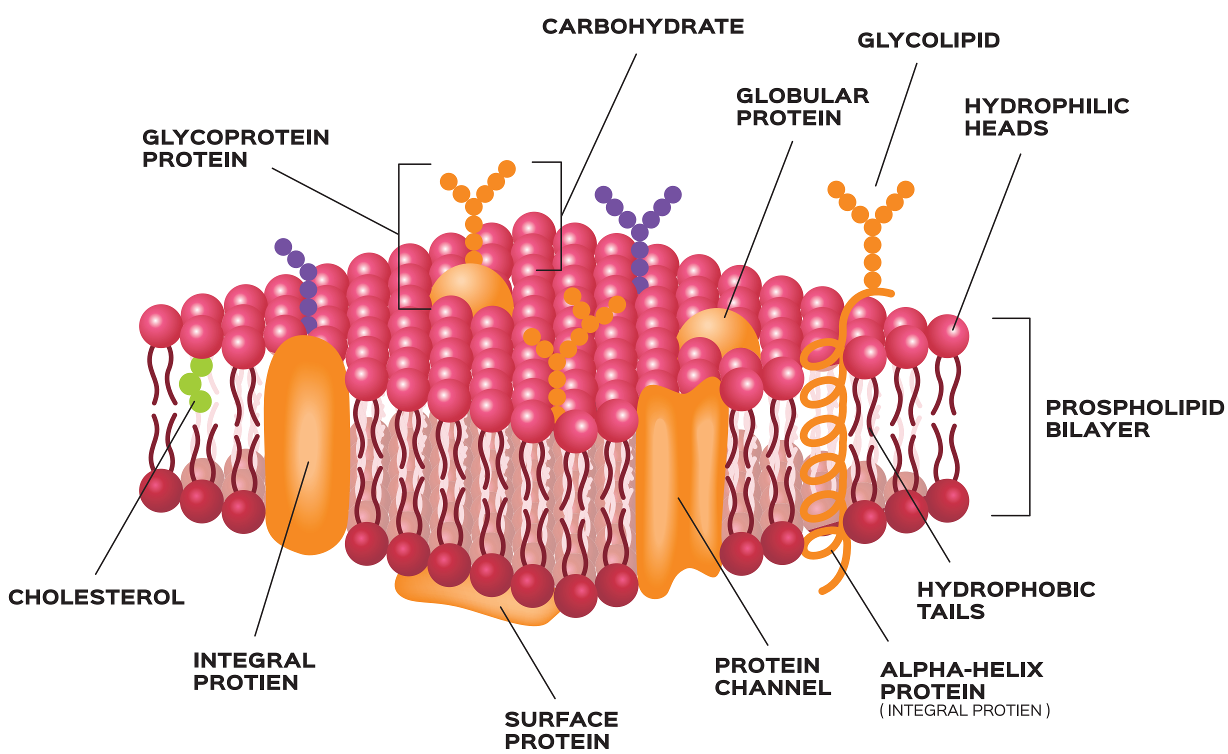


Figure 1.3 A schematic diagram of plasma membrane

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.

- 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).

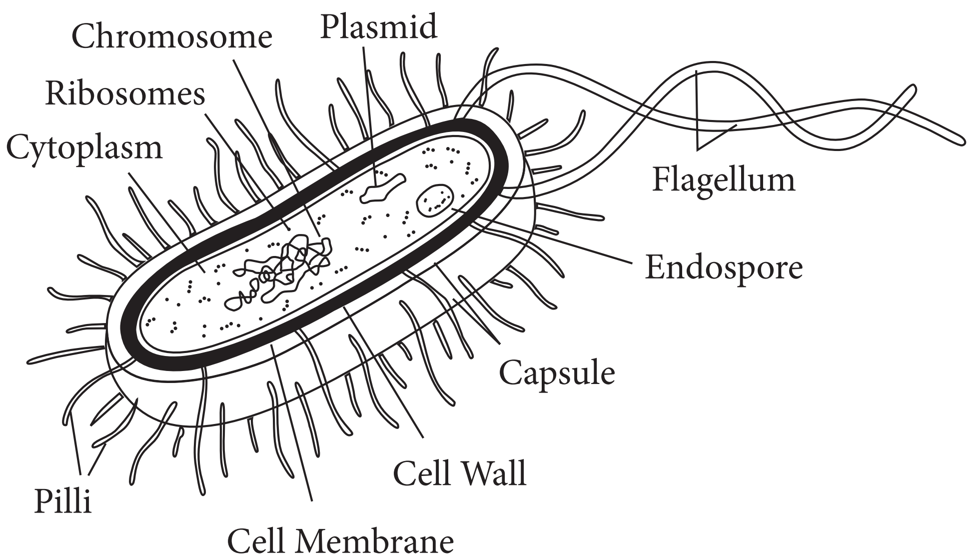


Figure 1.4 A schematic diagram of Bacteria

DO YOU KNOW?

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.

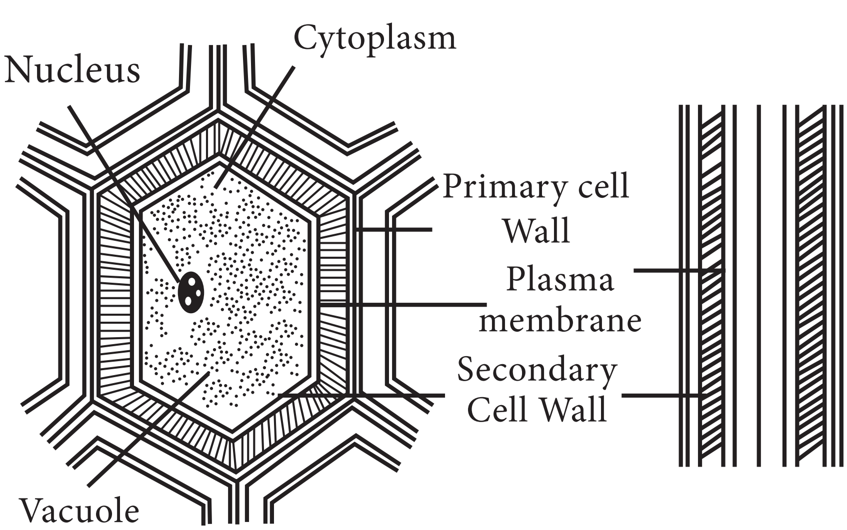


Figure 1.5.A Schematic representation of a typical plant cell wall

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

- 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.

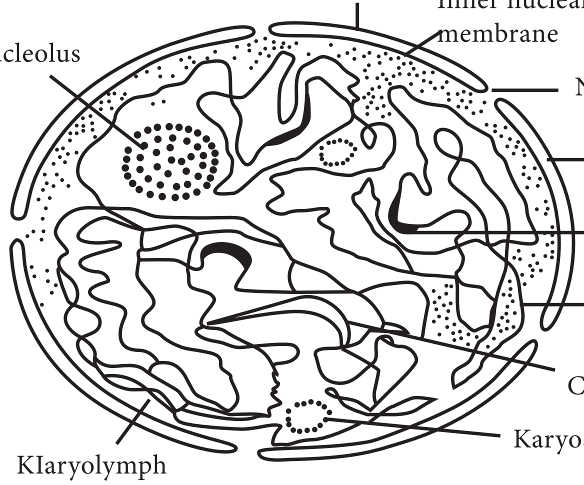


Figure 1.6 A diagrammatic representation of the ultrastructure of nucleus

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.

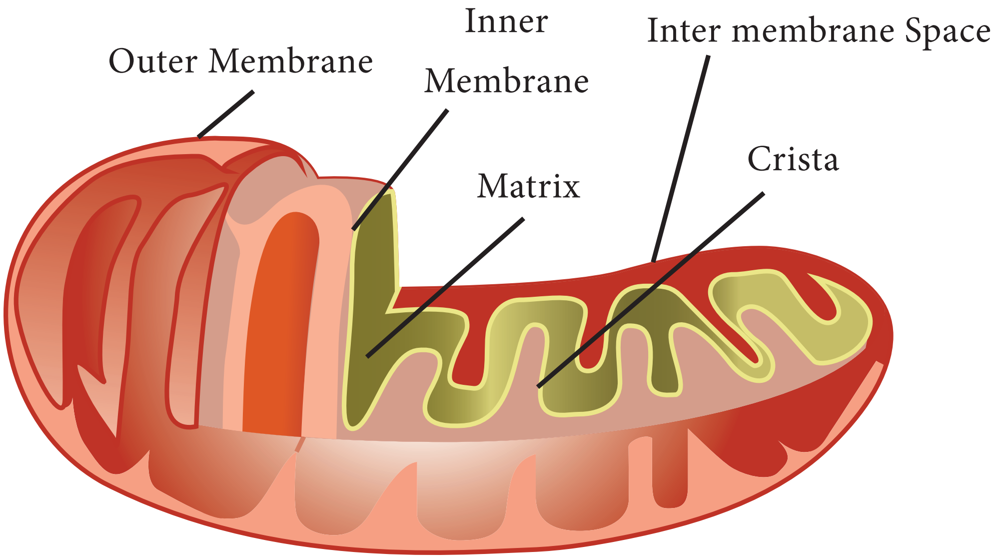


Figure 1.7 A schematic diagram of a mitochondrion

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.

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.

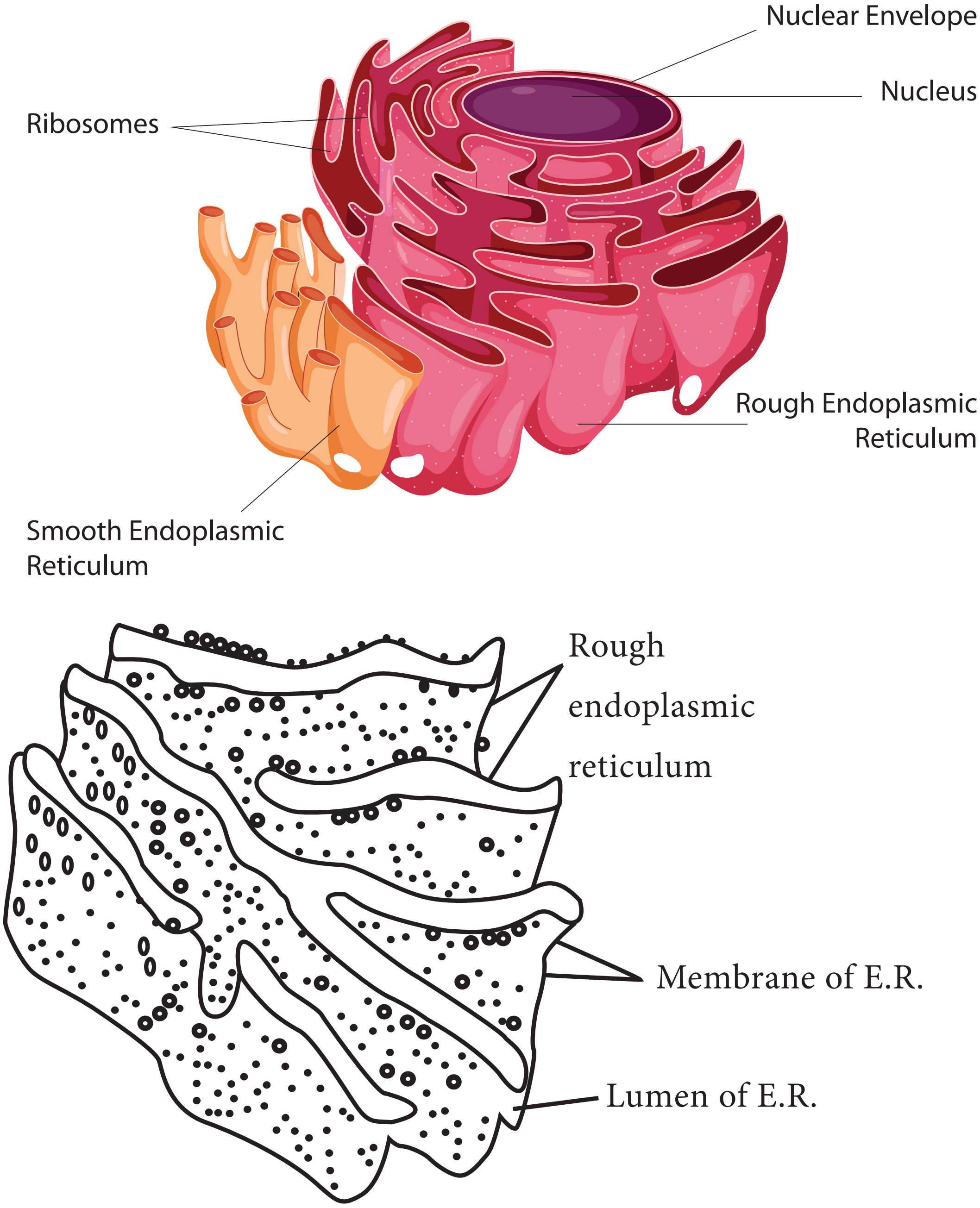


Figure 1.8 A schematic diagram of Endoplasmic Reticulum

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.

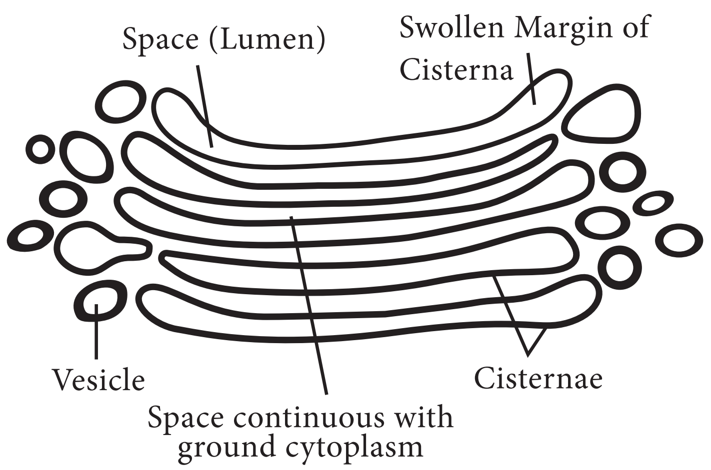


Figure 1.9.A schematic diagram of Golgi apparatus

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.

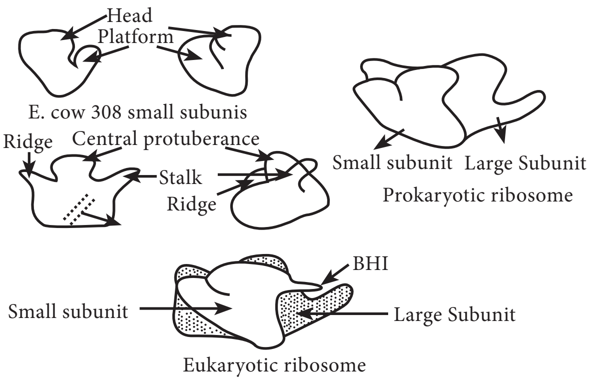


Figure 1.10 A schematic diagram of larger and smaller subunits of a ribosome

Functions of ribosomes:

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

- 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.

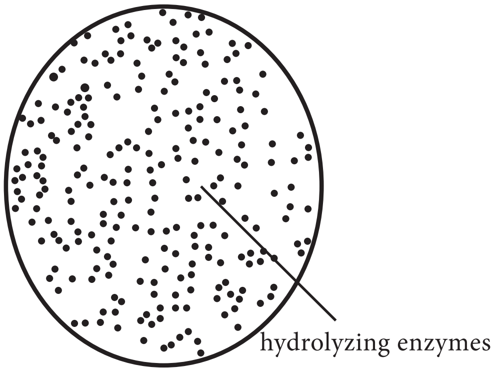


Figure 1.11 A schematic diagram of lysosome

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 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 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.

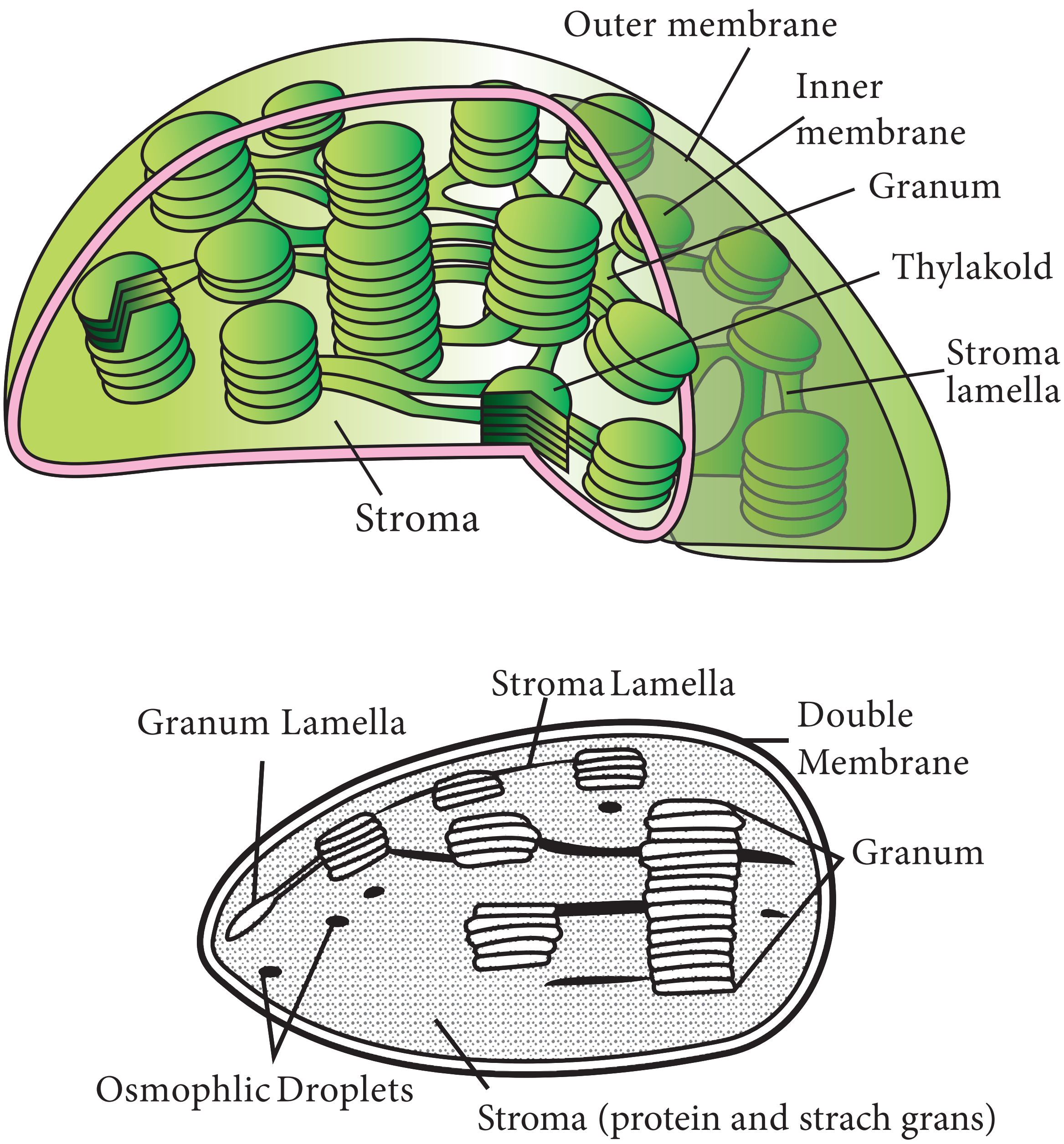


Figure 1.12 A schematic diagram of chloroplast

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.

- 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.

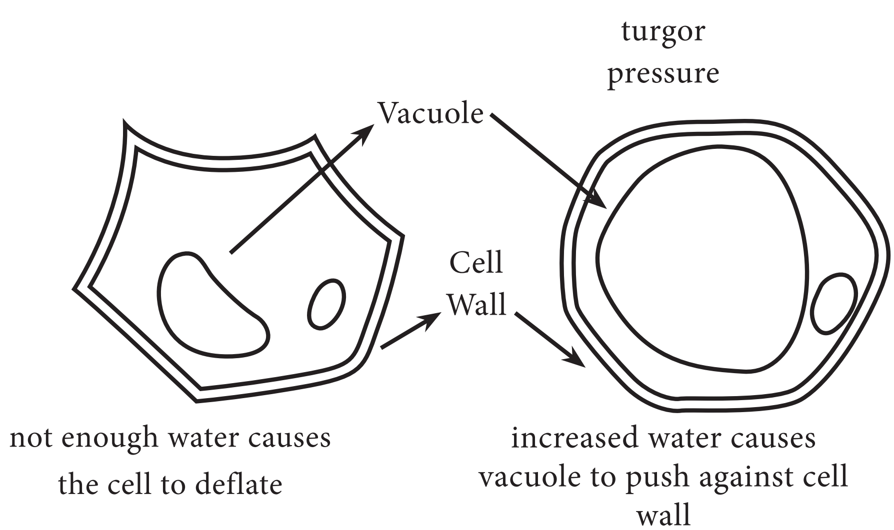


Figure 1.13 A schematic diagram of vacuole

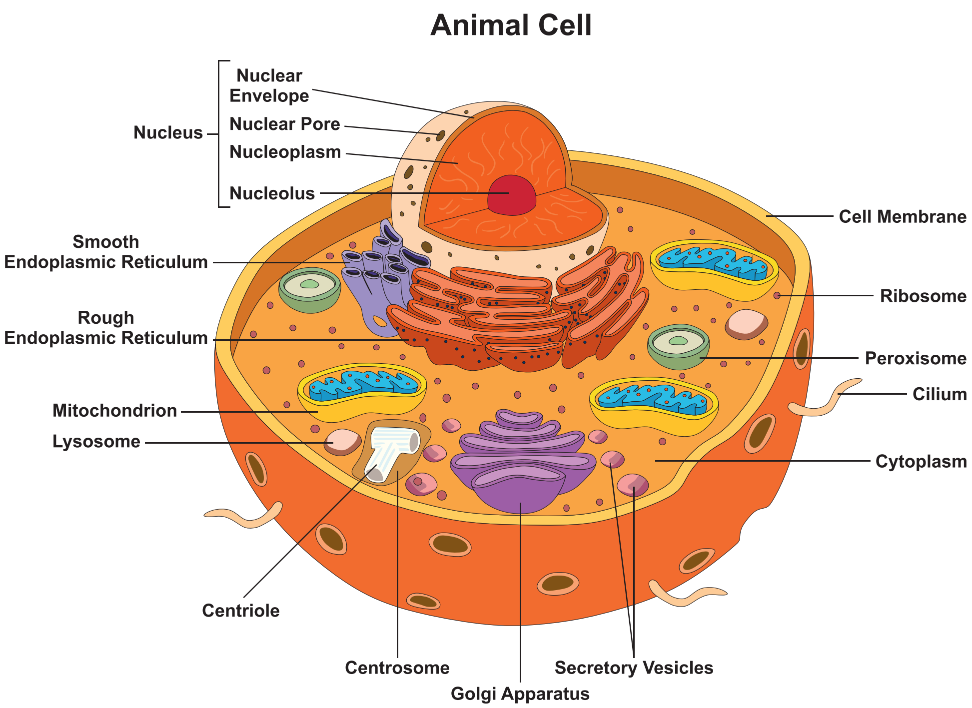
> **DO YOU KNOW?** In plants, inside each cell, there is a central vacuole, which can hold water, and under favourable conditions, the water enters the cell by osmosis (osmotic flow of water from area of low solute concentration outside the cell into the cell's vacuole, which has a higher solute concentration) 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.

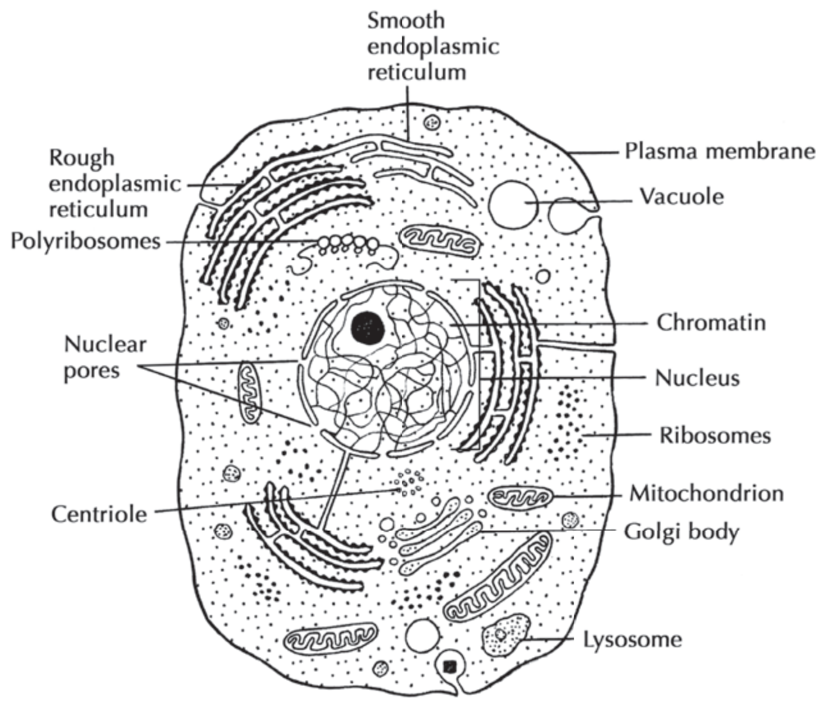
In plant cells, the vacuoles accumulate a high concentration of sugars and other soluble compounds. Water enters the vacuole to dilute these sugars, generating hydrostatic pressure that is counterbalanced by the rigid wall. In this way the cells of the plant become stiff or turgid, in the same way that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

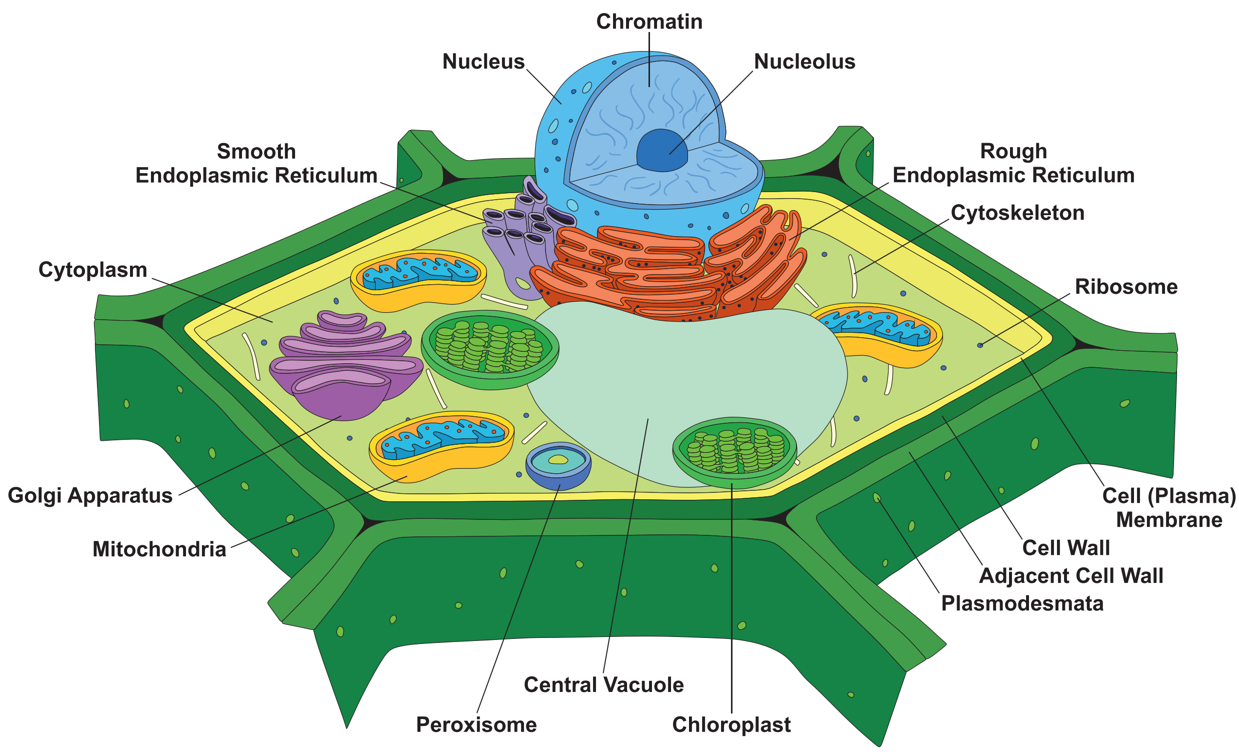
- Vacuoles aid in storing salts, nutrients, pigments, minerals, proteins, facilitating the growth of the plant and playing a vital structural role for the plant
- It serves in other functions such as protection, storage organelles for metabolites, growth and disposal of toxic excretory substances.

1.4.14.Distinguishing features of Plant and Animal Cells





Plant Cell



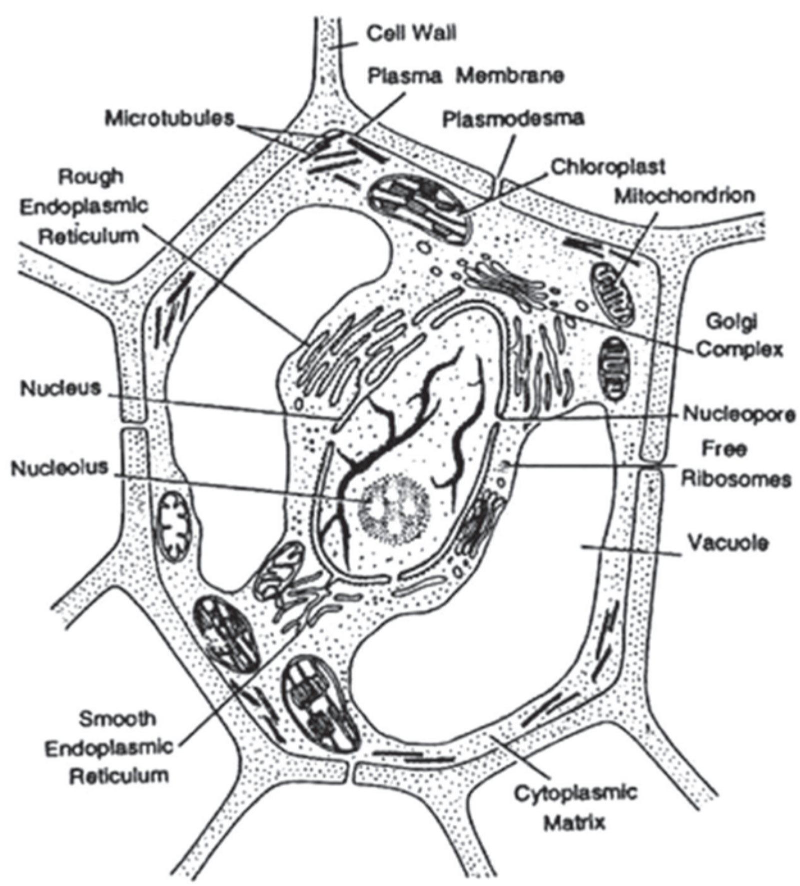


Figure1.14 Schematic representation of plant and animal cell

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:

HA	$\Longleftrightarrow$	H^+	+	A^-
Acid		Proton		Conjugate base

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

DO YOU KNOW?

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.

DO YOU KNOW?

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.

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.

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

> **DO YOU KNOW?** 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. It 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.

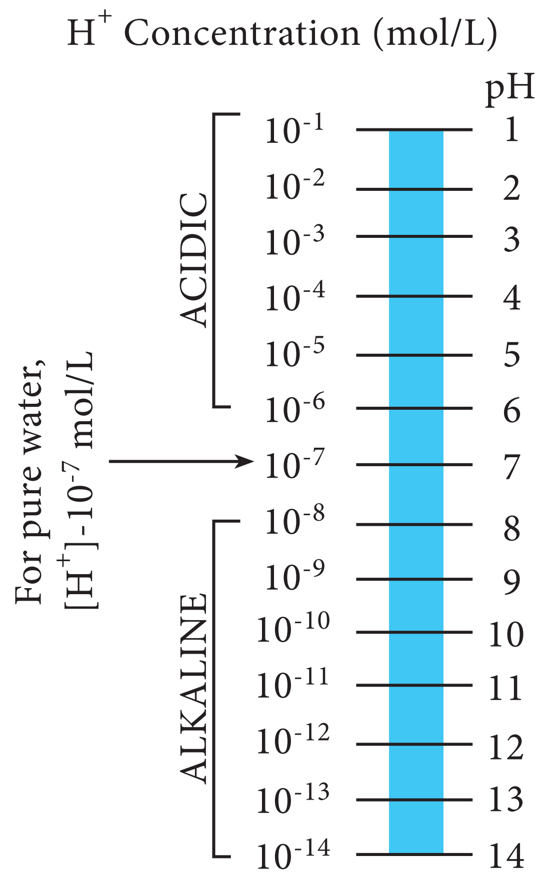


Figure 1. pH scale

> **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.

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.

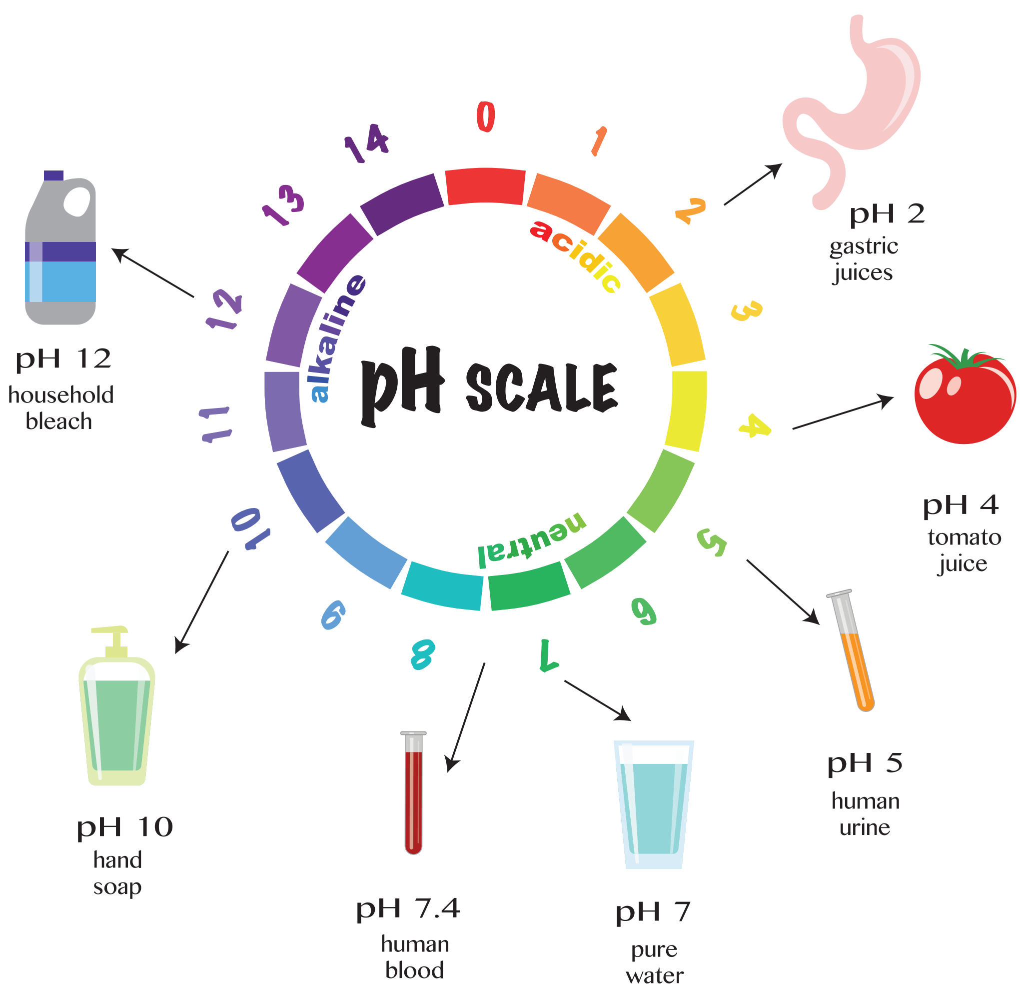


Figure 1.15 – pH paper and its colour change with respect to different samples.

DO YOU KNOW? 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,

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

Rearrange the equation 1.1 for hydrogen ion concentration

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

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

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

By substituting pH and pK_a values to the equation 1.3

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

Multiplying the equation 1.4 by (–1)

$$pH = pK_a - \log_{10} \frac{[HA]}{[A^-]} \quad \text{..........................(1.5)}$$

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

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

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.

Traditional Two Electrode pH Sensor

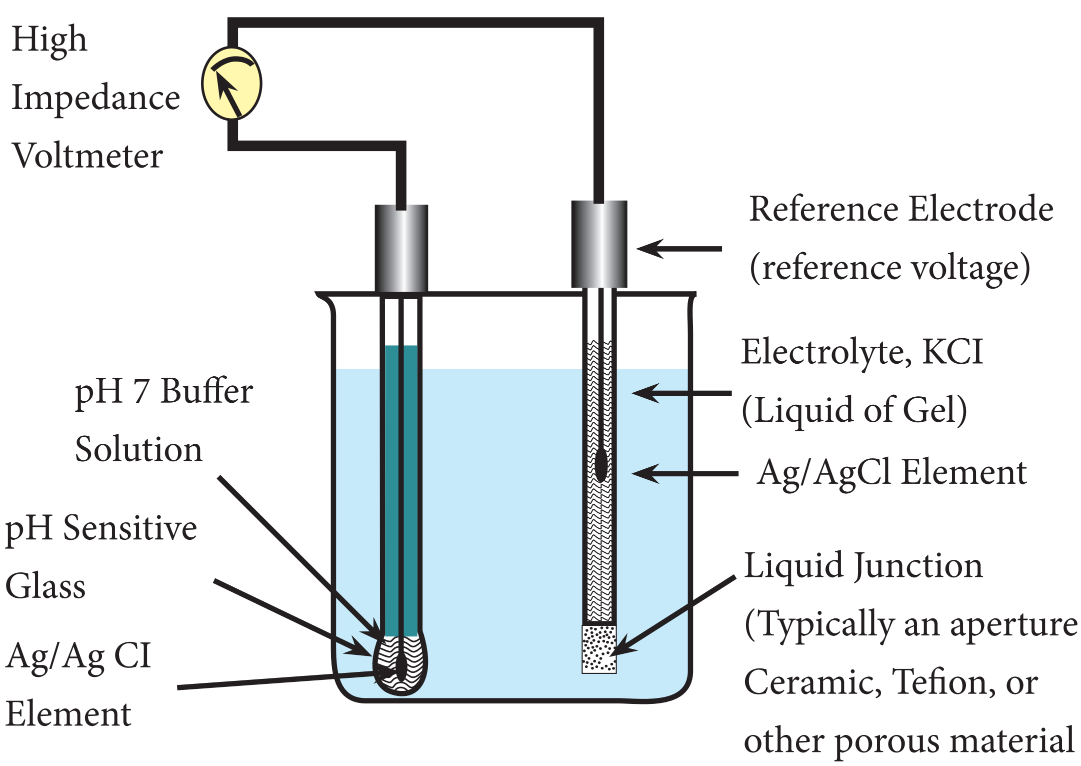
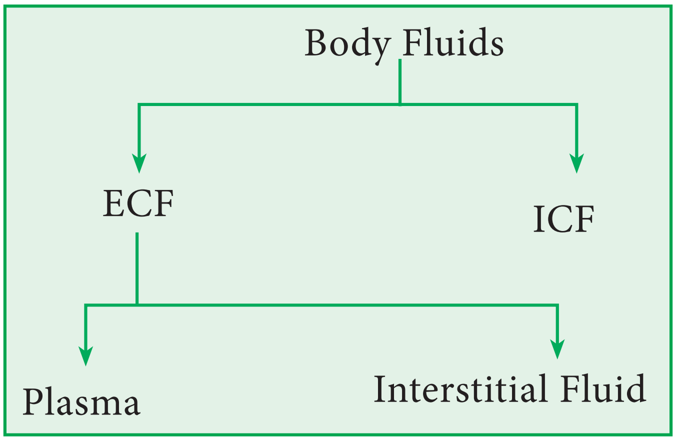


Figure 1.16 Image of simple pH meter and its schematic components.



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.

Lymph

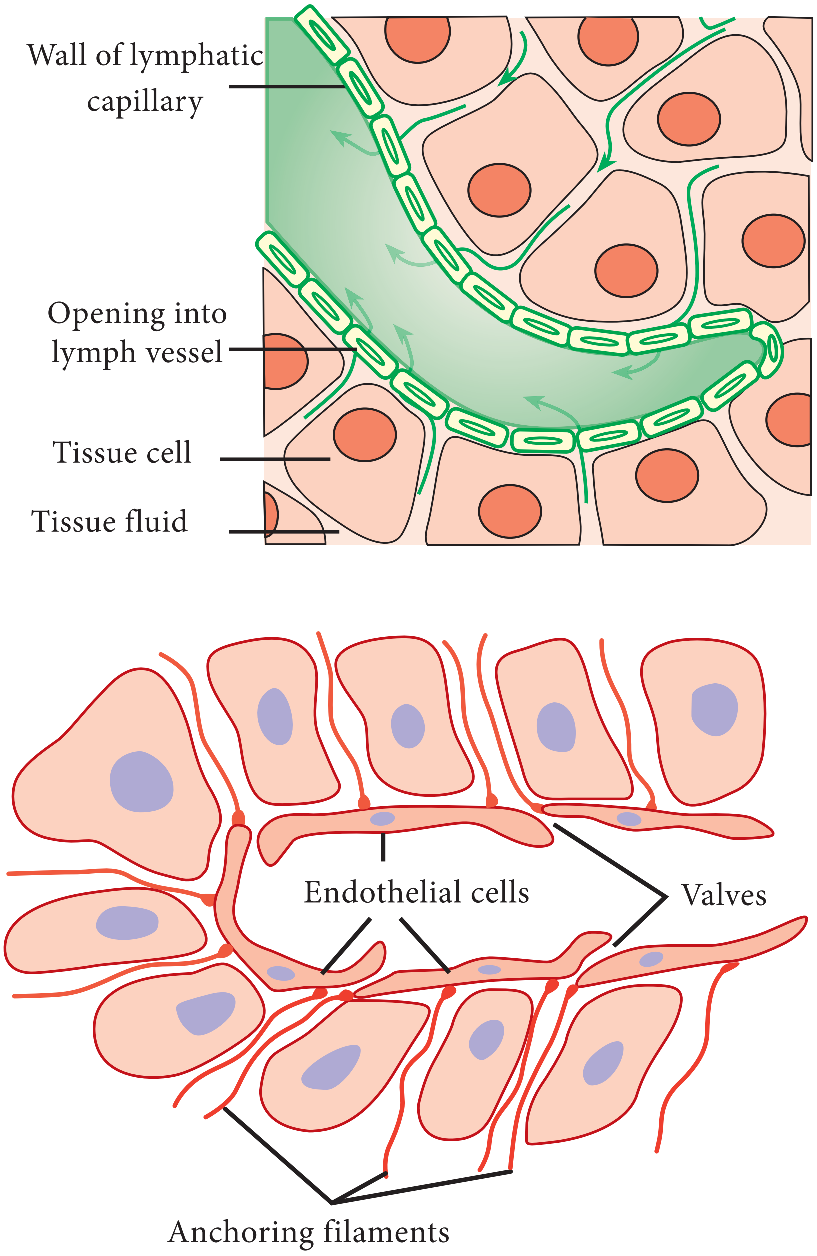


Figure 1.17 Lymph node

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).

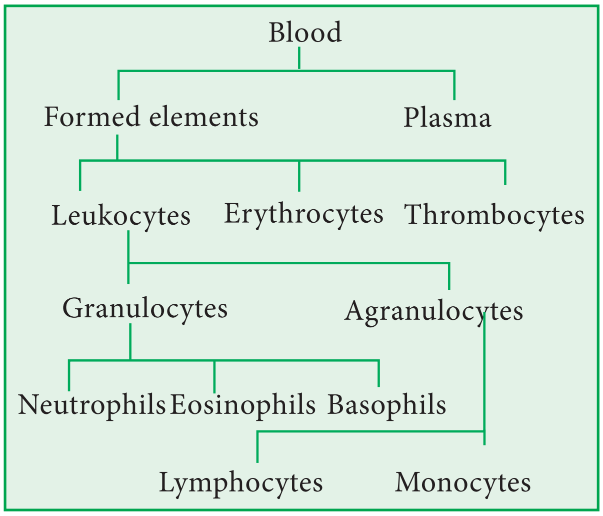
When the blood passes through the capillaries in tissues, some water, along with many small water-soluble substances, move out into the spaces between the cells. Larger proteins and most of the formed elements are left in the blood vessels. This fluid is called the interstitial fluid or tissue fluid. 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%.

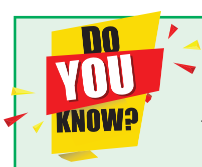


The formed elements include erythrocytes, leukocytes and platelets (thrombocytes). A cubic millimeter of adult blood normally contains 4.9 million to 5.5 million erythrocytes in males and 4.4 million to 5.0 million erythrocytes in females. The number of leukocytes in an adult human being is 5000 to 9000 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.

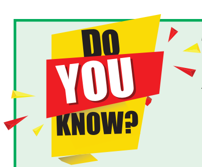
Plasma is a pale-yellow coloured liquid consisting of water and dissolved solutes. The solutes include ions like Na^+ as well as organic molecules such as metabolites, hormones, enzymes, albumins, globulins, fibrinogen and other proteins.

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.



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.



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.

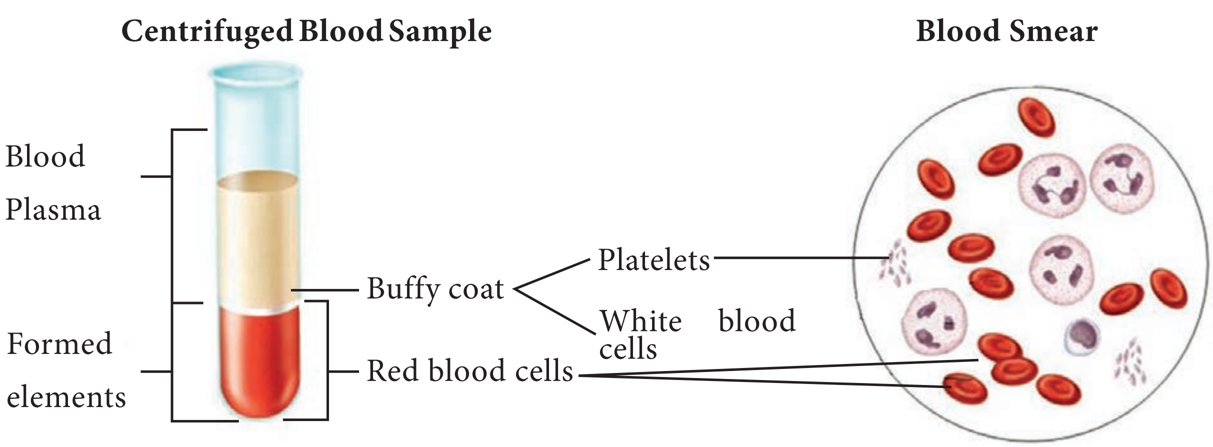


Figure 1. 18 Blood constituents; After centrifugation, blood cells settle at the bottom of the centrifuge tube leaving the plasma at the top of the tube. Leukocytes and thrombocytes form a thin light colored buffy coat at the interface between packed erythrocyte and the plasma.

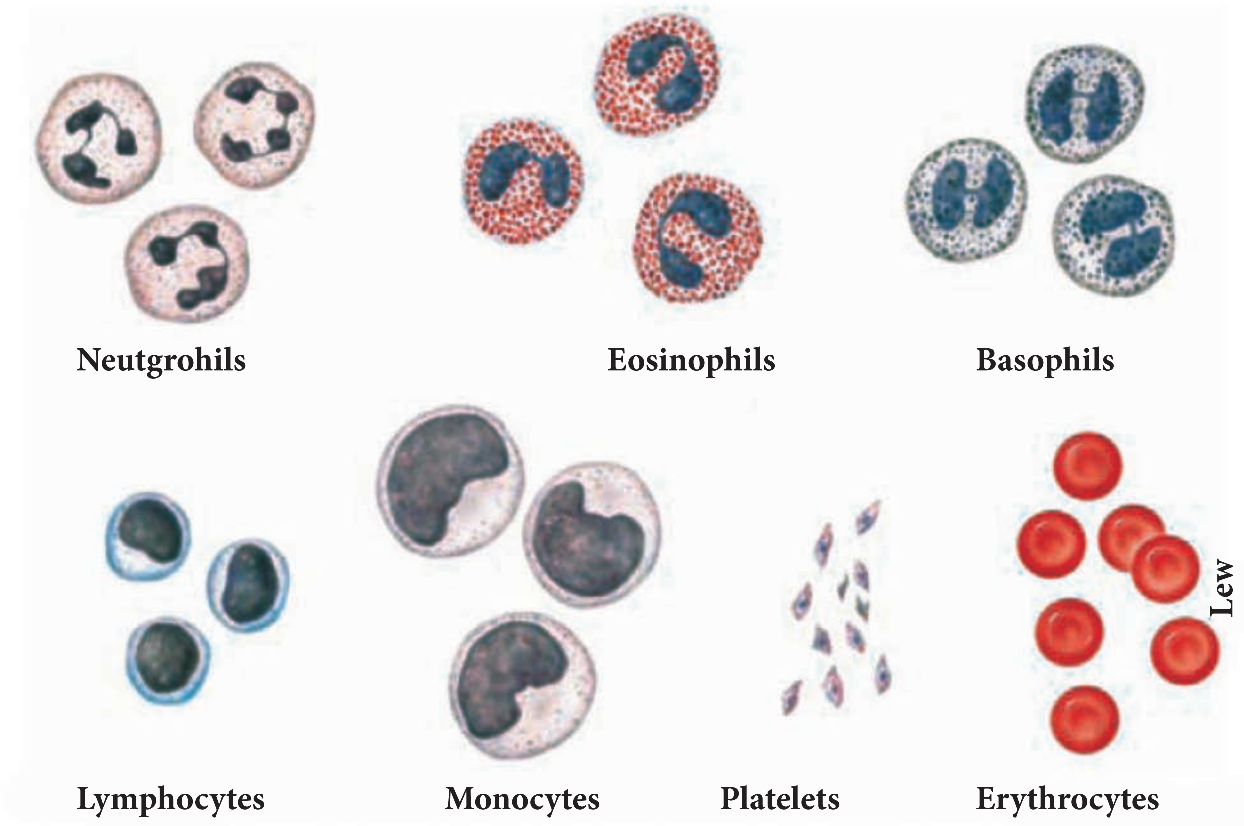


Figure 1.19 Blood cells : Leukocytes and thrombocytes (platelets) of blood are represented after staining process and erythrocytes represented without staining process.

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

DO YOU KNOW? 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).

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

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:

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

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 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.

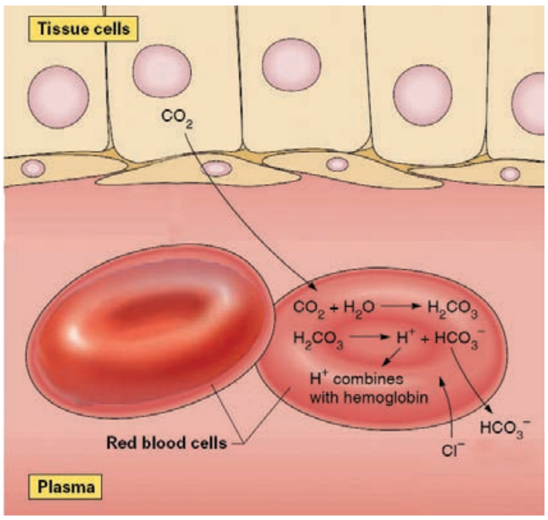

Figure 1.20 – The dissolved carbon dioxide diffuses into erythrocytes from the tissues. It is then converted into carbonic acid by the enzyme carbonic anhydrase. Carbonic acid then dissociates into hydrogen ion and bicarbonate ion. The hydrogen ion then combines with haemoglobin as HHb and the bicarbonate 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.

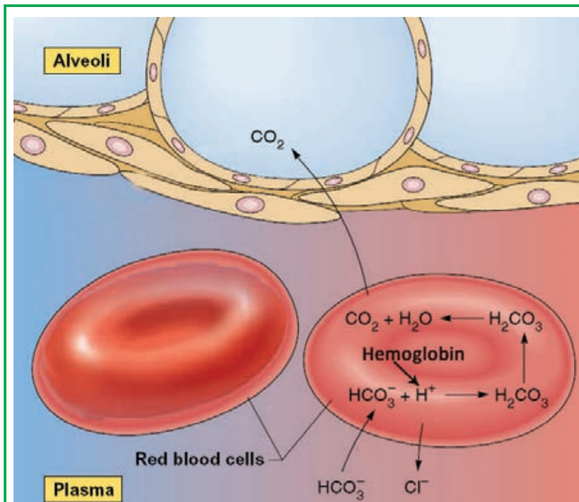

Figure 1.21 – The bicarbonate ions diffuse from plasma, combine with hydrogen ions released from hemoglobin, to form carbonic acid. The carbonic acid is then converted into carbon dioxide and water by the enzyme carbonic anhydrase. The carbon dioxide is then eliminated through exhaled air.

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.

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

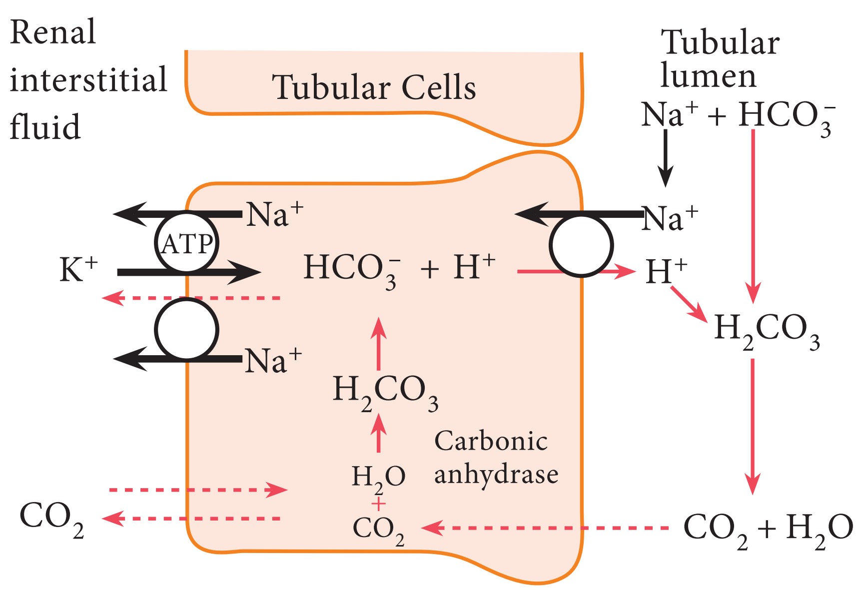

Figure 1.22 Role of bicarbonate ions in kidney tubule

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.