CHAPTER 1

# Cell and Subcellular Components

Competency Achievement: **The student should be able to:**

**BI1.1** Describe the molecular and functional organization of a cell and its subcellular components

**PY1.1** Describe the structure and functions of a mammalian cell

## MOLECULAR ORGANIZATION OF CELL

Simple precursors, or raw materials, such as $CO_2$, $H_2O$, $NH_3$, $N_2$ and $NO_3^-$, and their derivatives like pyruvate, citrate, succinate, 3-phosphoglycerate, etc. (commonly referred to as metabolites) are required to synthesize basic structural molecules known as building blocks. These include:

- Monosaccharides (Chapter 3),
- Fatty acids and glycerol (Chapter 4),
- Amino acids (Chapter 5), and
- Nucleotides (Chapter 7),

These building blocks, either through polymerization or other chemical means, form macromolecules such as polysaccharides, lipids, proteins and nucleic acids. Interaction among various macromolecules results in supramolecular complexes, e.g. multienzyme complexes, cytoskeleton, ribosomes, etc. An orderly assembly of such complexes forms various membrane-bound organelles or nonmembranous entities (subcellular fractions), all of which are defined within a physical boundary, to form a cell (Fig. 1.1).

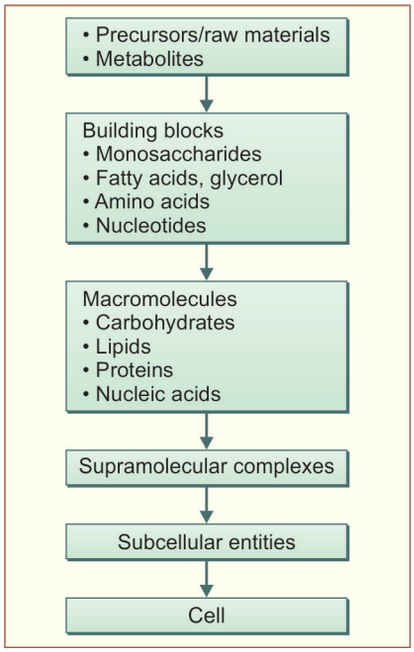


**Fig. 1.1:** Hierarchy of molecular organization

## THE CELL

The cell is a structural and functional unit of life. All animals and plants are made up of a large number of such units, in a manner to the utilization of bricks in the construction of a building. Living cells are divided into two groups, i.e. the prokaryotic cells and the eukaryotic cells. As their name suggests (*pro* = prior to; *karyot* = nucleus; *eu* = true), the fundamental difference between them is the absence or presence of a true nucleus.

### Prokaryotic Cells

The simplest form of the cell is a prokaryotic cell. Prokaryotes, e.g. bacteria, are unicellular and have one of the three basic shapes, viz. spheroidal (cocci), rod-like (bacilli) and helically-coiled (spirella).

A prokaryotic cell is small (1 to 10 nm), relatively simple in structure and has only a single membrane called cell membrane which is usually surrounded by

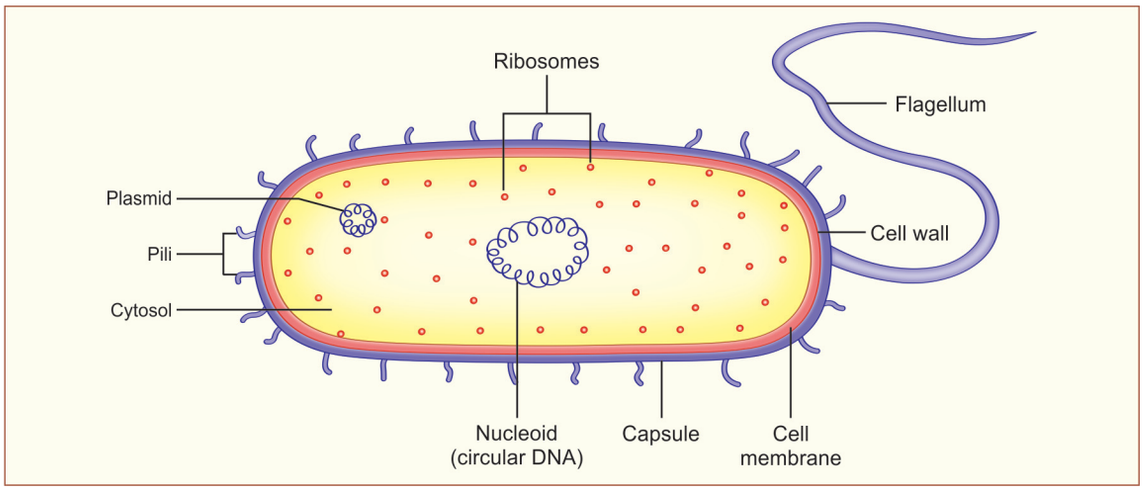


**Fig. 1.2:** A prokaryotic cell

a rigid cell wall of characteristic structure. There may or may not be a surrounding capsule. Besides, there is a single chromosome comprised of a molecule of double helical DNA which is densely coiled to form a nuclear zone. Reproduction is by asexual division. The best characterized prokaryotic cell is *Escherichia coli*. Some prokaryotes possess pili and flagella for adhesion and movement, respectively (Fig. 1.2).

## Eukaryotic Cells

Animals, plant, fungi and protozoa are called eukaryotes which may be unicellular as well as multicellular. Eukaryotic cells are one to ten thousand times larger and are more complex in structure than the prokaryotic cells. They may vary from one tissue to another with respect to its function, e.g. the liver parenchymal cell, adipose cell, nerve cell, renal tubular cell, white blood cell, etc.

Generally, the eukaryotic cell has a well-defined membrane-bound nucleus containing several chromosomes. Their chromosomes undergo replication of DNA during mitosis and get separated into daughter chromosomes, i.e. these cells reproduce by cell division.

A typical eukaryotic cell contains various organelles such as a **nucleus, endoplasmic reticulum, Golgi apparatus, mitochondria,** etc. (Fig. 1.3).

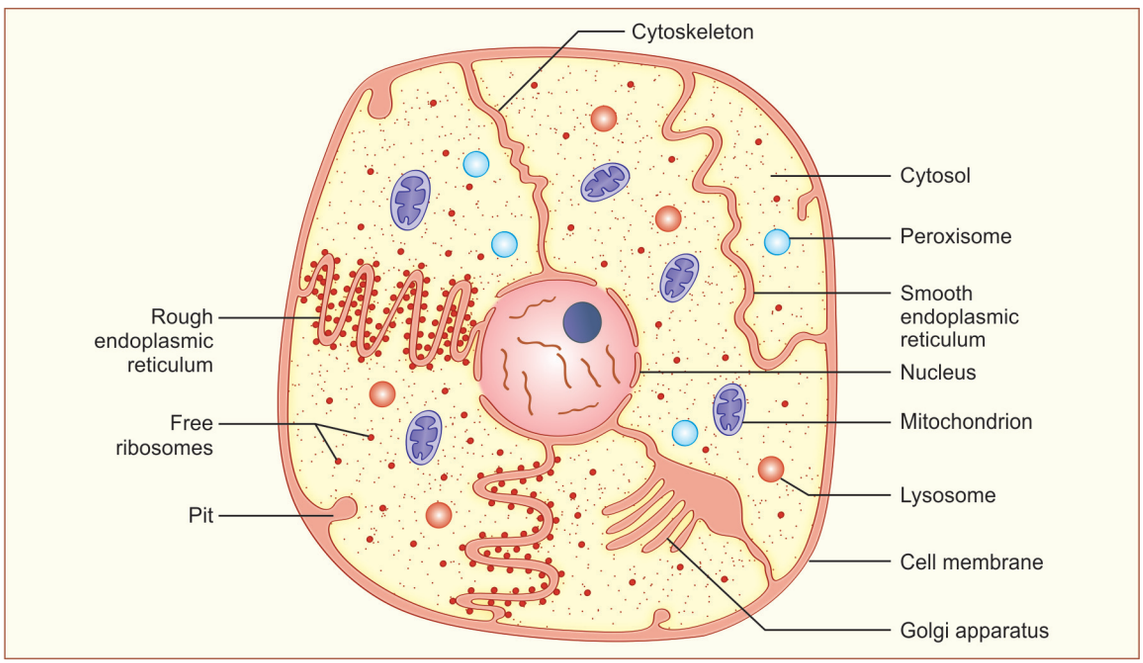


**Fig. 1.3:** The eukaryotic cell

Major differences between prokaryotic and eukaryotic cell structure are given in Table 1.1.

**Table 1.1** Major differences between prokaryotic and eukaryotic cell structure

| Parameters | Prokaryotic cells | Eukaryotic cells |
|---|---|---|
| Cell size | Small | Large |
| Overall organization | Simple | Complex |
| Boundary | Cell membrane and cell wall (sometimes surrounded by a capsule) | Cell membrane |
| Subcellular entities | Few | Many |
| Nucleus | Single DNA double helices in a poorly defined region called nucleoid | Well-defined nucleus with a membrane and multiple DNA double helices organized into chromatin |
| Reproduction | Asexual | Sexual |
| Examples | Bacteria, blue-green algae | Plant and animal cells |

## MAMMALIAN CELL AND SUBCELLULAR COMPONENTS

**Mammalian cells** live and grow, within their normal environment, in the body in contact with other cells and extracellular matrix. They are supplied with energy, in the form of carbohydrates and with nutritional components by the circulating blood. A subcellular component or **organelle** is defined as a subcellular membranous entity, which can be isolated by high speed centrifugation. The nonmembranous entities, such as ribosomes, cytoskeleton and cytosol, are designated as **subcellular fractions**.

### Fractionation of Subcellular Components

Functions of different subcellular components can be studied after their fractionation (separation and isolation) from the cell, by various steps. These include:

1. *Preparation of tissue homogenate:* The cells are disrupted under mild conditions, e.g. the tissue may be grinded in a pestle and mortar, or is homogenized by using a **homogenizer.**

   In a homogenizer, a manually operated, or motor driven, pestle is rotated within a glass tube containing a small piece of the tissue in some suitable solution (an isotonic medium such as 0.25 M sucrose solution, adjusted to pH 7.4) between 0 and 4°C to avoid loss of biological activities. Mechanical force disrupts cells and causes liberation of the cellular components into the medium. This is called **tissue homogenate**. Besides homogenization, use of a deter-gent or osmotic shock can also disrupt plasma membrane.

2. *Differential centrifugation:* Due to the differences in their size and density, various cellular components can be separated by centrifugation at variable speeds and timings:

   i. ***Low speed centrifugation:*** The homogenate is first centrifuged at low speed (500 × g for 10 min) to separate nuclear fraction, which contains nuclei and the unruptured cells in the pellet.

   ii. ***Intermediate speed centrifugation:*** The **supernatant** is, thereafter, again centrifuged at an intermediate speed (10,000 × g for 20 min) to get mitochondrial fraction, which contains mitochondria, lysosomes and peroxisomes in the pellet.

   iii. ***High speed centrifugation (ultracentrifugation):*** High speed centrifugation (100,000 × g for 60 min) of the leftover supernatant gives the microsomal fraction. The microsomal fraction contains free ribosomes, fragments of the endoplasmic reticulum and the plasma membrane in the pellet.

   The supernatant, after separation of the microsomal fraction, is a clear solution which corresponds to the cell sap or cytosol and contains free ribosomes and soluble molecules. It is, thus, possible to isolate each subcellular component, from most of the organs and cells, in a relatively pure form, by various modifications of this general procedure, i.e. differential centrifugation (Fig. 1.4).

3. *Identification of subcellular entities:* Each entity can be assessed by the measurement of some suitable marker (an enzyme or chemical constituent of the particular fraction) either by the use of an electron microscope or by biochemical estimation. Various intracellular organelles/subcellular fractions, their functions and markers are given in Table 1.2.

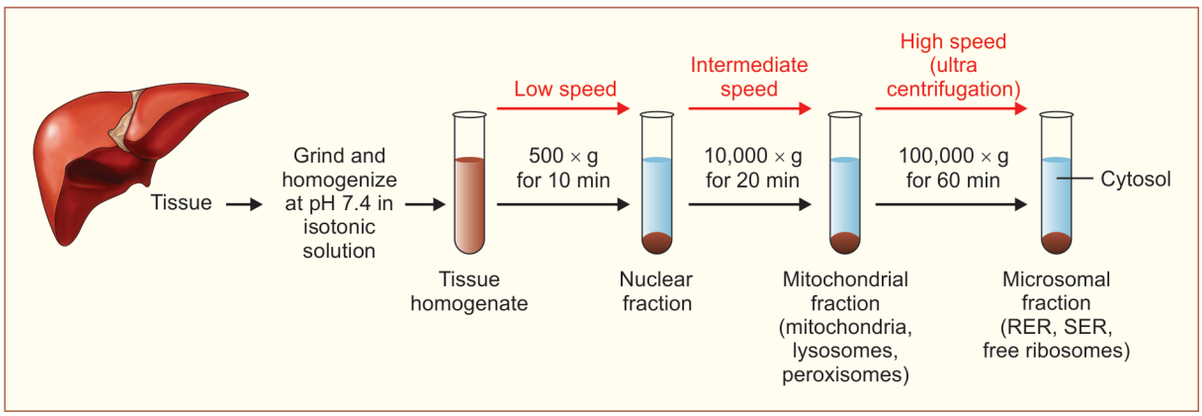


**Fig. 1.4:** Separation of subcellular fractions by differential centrifugation

**Table 1.2**: Subcellular entities, their functions and biochemical markers

| Subcellular entity | Function | Biochemical marker |
|---|---|---|
| Cell membrane | Transport across cell membrane, bears receptor and enzymes, cell-cell recognition | $Na^+/K^+$-ATPase |
| Nucleus | DNA replication, cell division, control of RNA and protein synthesis | DNA polymerase |
| Endoplasmic reticulum | Protein synthesis, lipid metabolism, xenobiotic metabolism | Glucose-6-phosphatase |
| Golgi apparatus | Post-translational modification of proteins and their sorting | Galactosyltransferase |
| Mitochondria | ATP synthesis, fatty acid oxidation, Krebs cycle, cytosolic $Ca^{2+}$ regulation | Succinate dehydrogenase, glutamate dehydrogenase |
| Lysosomes | Intracellular digestion and detoxification | Acid phosphatase |
| Peroxisomes | Fatty acid oxidation, detoxification of $H_2O_2$ | Catalase, uric acid oxidase |
| Cytoplasm | Glycolysis, fatty acid synthesis, bears the cytoskeleton | Lactate dehydrogenase |

## Structure and Functions of Subcellular Components

### *The Nucleus*

The nucleus is the largest component of the cell. It contains DNA, which is organized into separate chromosomes, and is surrounded by a membrane, called nuclear membrane.

Nuclear membrane, or nucleolemma, consists of two layers which are separated by an intermembrane space termed perinuclear space (cisterns). The outer membrane though is continuous with the endoplasmic reticulum but the two layers of the nuclear membrane are fused together at several places producing nuclear pores for the exchange of materials between the nucleus and the cytoplasm. The nucleus is filled with nucleoplasm which has a discrete body, called nucleolus, and a thread-like structure, called chromatin (Fig. 1.5).

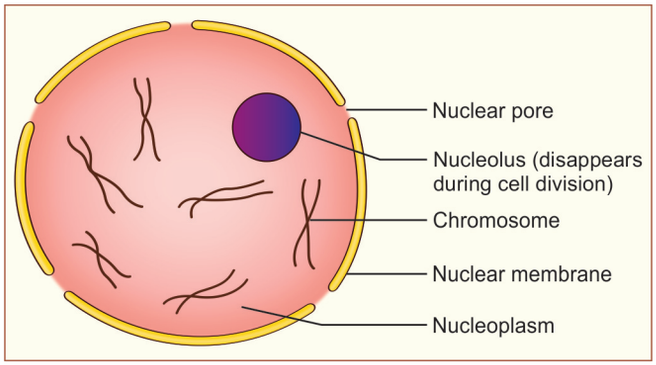


**Fig. 1.5:** The nucleus

***Nucleolus:*** The number of nucleoli may vary from one cell type to another. The genes for three of the four ribosomal RNA molecules are located in the nucleolus. Nucleoli are rich in RNA and disappear during cell division.

***Chromatin:*** It contains most of the cellular DNA in association with basic proteins termed histones. At the time of cell division, the chromatin is organized into small thread-like structures called **chromosomes**. Human somatic cell contains 23 pairs of chromosomes.

***Important functions of nucleus include:***

- Control of cell-division (DNA replication).
- Protein synthesis (by controlling the synthesis of RNA).

  The regulation of DNA synthesis and other functions of the nucleus are severely disturbed in some pathological conditions, such as cancer.

  **DNA polymerase** is a marker of the nucleus.

### *The Endoplasmic Reticulum*

Endoplasmic reticulum is a system of membranes (lipid bilayer structures) with a network of vesicular spaces. This network is present throughout the cytoplasmic matrix and grows by its own synthesis. These membranes run parallel to each other creating channels which are called cisternae. The interior of the endoplasmic reticulum thus is well connected with perinuclear spaces and through pores on the cell surface, with the extracellular space. Cisternae have a role in the exchange of materials between the cell and the extracellular fluid.

***The surface of the endoplasmic reticulum may or may not bear ribosomes. Accordingly, endoplasmic reticulum is of two types:***

- ***Rough endoplasmic reticulum (RER):*** It is also called the granular type of endoplasmic reticulum since it has small granules attached to it. These granules are termed ribosomes.
- ***Smooth endoplasmic reticulum (SER):*** It is also called as the agranular type of endoplasmic reticulum since it consists of the membranous structure only and does not contain ribosomes on its outer surface. The SER has enzymes for the biosynthesis of lipids and glycoproteins. Further, SER are very important in hepatocytes where these are primarily concerned with oxidative metabolism and for the detoxification of many drugs and other toxic organic molecules.

  **Glucose-6-phosphatase** is a marker enzyme for the endoplasmic reticulum.

### *The Ribosomes*

Ribosomes consist of ribonucleoprotein particles of two sizes, i.e. 50S and 30S in prokaryotes or 60S and 40S in eukaryotes. Because of their high RNA content, ribosomes are the site of protein synthesis. Ribosomes on the RER are associated with the synthesis of proteins for export from the cell. Free ribosomes, on the other hand, are present in the cytoplasm and synthesize proteins for use within the cell.

**RNA** is used as a marker for the ribosomes.

### *The Golgi Apparatus*

The Golgi apparatus is a smooth membrane system with vacuoles. It is rich in lipids and is considered to be the site where secretions from other organelles are brought and assembled. The newly synthesized proteins are also transferred from RER and stored in the Golgi apparatus temporarily. Some of the synthesized proteins also undergo post-translational modifications within the Golgi apparatus and thereafter are transported to different destinations. The Golgi apparatus is thus especially active in cells which produce proteins for export. They form secretory granules for the proteins after their synthesis on the ribosomes.

**Galactosyltransferase** is a marker enzyme for the Golgi apparatus.

Endoplasmic reticulum with Golgi apparatus has a role in the formation of other cellular organelles, such as lysosomes and peroxisomes.

### *The Mitochondria*

The mitochondria are the major organelle of eukaryotic cell lacking any direct structural relationship with other organelles and contain their own DNA (Fig. 1.6).

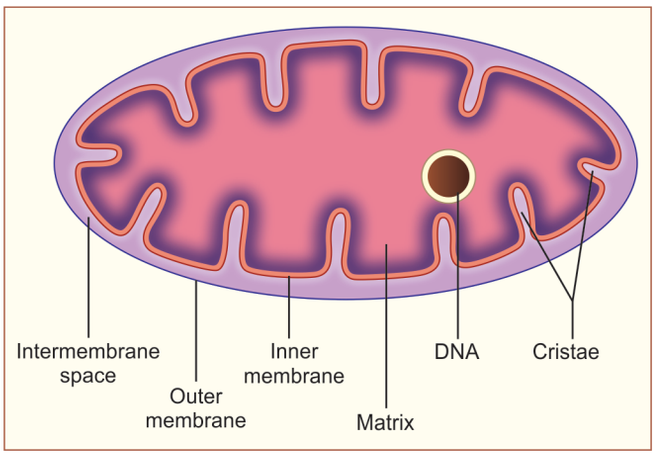


**Fig. 1.6:** A mitochondrion

A mitochondrion produces energy in the form of ATP for the cellular functions and is thus called a **power house** of the cell. Thus, depending upon energy requirement of the cell, mitochondria may vary in size, shape and number, from cell to cell. Besides producing energy, mitochondria also help to control the level of

calcium in the cytoplasm. Most of the cells contain several hundred mitochondria.

*Mitochondrial membrane:* It is a double-layered structure where the two layers are separated from each other by 50–100Å intermembrane space. Several enzymes especially those involved in the nucleotide metabolism are located here.

- The **outer membrane** of the mitochondria has a smooth structure. It is composed of both lipids and proteins and is freely permeable to most of the small molecules. Several enzymes involved in lipid metabolism such as the enzymes for fatty acid elongation, glycerol phosphate acyltransferase and phospholipase A are associated with the outer membrane of the mitochondria.
- The **inner membrane** of the mitochondria has a denser structure. It has more proteins than lipids. The inner membrane has extensive irregular folding called cristae. Cytochromes, the enzymes of electron transport chain and flavoproteins are localized within the inner membrane of the mitochondria.

*Matrix:* The intra-mitochondrial space (enclosed within the inner membrane) is called the mitochondrial matrix. This chamber contains enzymes for β-oxidation of fatty acids, for citric acid cycle and glutamate dehydrogenase. In addition, mitochondria also have DNA, referred to as mtDNA.

**Succinate dehydrogenase** and **glutamate dehydrogenase** are marker enzymes for mitochondria.

*Mitochondrial DNA:* Mitochondria are the only cellular organelles that contain their own chromosomal DNA (mtDNA), which is maternally inherited. Human mtDNA is a small double-stranded circular molecule (about 16,000 base pairs), encoding 13 polypeptides that are integrated into the inner mitochondrial membrane along with other polypeptides encoded by nuclear genes. In addition, it encodes 2 rRNAs and 22 tRNAs that are used in protein synthesis within the organelle. mtDNA differs from nuclear DNA in several aspects.

### *Differences between nuclear DNA and mitochondrial DNA*

A comparison between nuclear DNA and mtDNA is given in Table 1.3.

In contrast to nuclear DNA, the mtDNA is exposed to high levels of mutagenic free radicals and is not protected by the usual DNA repair mechanisms. This results in mutations in mtDNA that affect mitochondrial structure and function, leading to various muscular and neurological disorders. The mtDNA is more vulnerable to damage because:

- Histones (DNA binding proteins) are absent.
- Being located in the mitochondrial matrix, it is exposed to free radicals generated in the respiratory chain.
- Lack of introns.
- A few DNA repair mechanisms.

*Mutations in mtDNA are responsible for several disorders* associated with the process of oxidative phosphorylation, aging and degenerative diseases such as:

1. Leber's hereditary optic neuropathy.
2. Mitochondrial encephalopathy.
3. Chronic progressive external ophthalmoplegia.
4. Mitochondrial encephalopathy, lactic acidosis and stroke-like episodes (MELAS).

### *The Lysosomes*

The lysosomes are small sac-like organelles surrounded by a membrane. These are intermediary in size between the mitochondria and the microsomes. Lysosomes are rich in all types of hydrolases, such as for

**Table 1.3:** Comparison between nuclear DNA and mitochondrial DNA (mtDNA)

| Parameters | Nuclear DNA | mtDNA |
|---|---|---|
| Location | Nucleoplasm | Mitochondrial matrix |
| Inheritance | Both paternal and maternal | Maternal |
| Shape | Linear | Circular |
| Base pairs | $3 \times 10^9$ | $16 \times 10^3$ |
| Introns | Present | Absent |
| Histones | Present | Absent |
| Encoded proteins | Used within the cell as well as exported | Used within mitochondria |
| Repair mechanisms | Well developed | Poorly developed |
| Mutation rate | Low | High |

carbohydrates, lipids, proteins and nucleic acids since digestion of these macromolecules takes place within the lysosomes. Release of lysosomal enzymes as well as absence of lysosomal enzymes is of clinical importance (Chemistry to Clinics 1.1 and 1.2).

**Chemistry to Clinics 1.1: Suicide Bags**

Various hydrolases are enclosed in the lysosomal membrane in such a way that they are not able to result in lysis of their own cells. However, a disruption of the lysosomal membrane leads to the release of lysosomal enzymes. This in turn causes digestion of cellular components leading to their release from the cell. This process is called autolysis. Due to this specific role of lysosomes these are also called suicide bags. Lysosomal enzymes play key roles in phagocytosis, acute and chronic inflammation such as arthritis.

**Chemistry to Clinics 1.2: Lysosomal Storage Disorders**

Absence of the specific lysosomal enzyme is seen in a number of genetic disorders. This in turn results in accumulation of various cellular components which cannot be digested (hydrolysed) due to inherited deficiency of the lysosomal enzyme. Lysosomes of the affected individuals become enlarged with the undigested material and thus interfere in normal cellular processes. Various such lysosomal disorders are known:

- *Lysosomal acid lipase deficiency:* It results in Wolman's disease in infants, and cholesterol ester storage disease in adults. There is accumulation of triacylglycerol and cholesterol esters in tissues, particularly in the liver.
- *Maltase deficiency:* It results in glycogen storage disease type II.
- *Alpha-L-iduronidase deficiency:* It results in Hurler's syndrome with accumulation of glycosaminoglycans.

**Acid phosphatase** is a marker enzyme of the lysosomes.

***Age-related pigments:*** Any foreign particle which enters the cell is completely destroyed by the lysosomal enzymes. The lysosomes are normally active after their fusion with a vacuole. These vacuoles may contain ingested foreign particles, such as dead bacteria and result in their lysis (the process is called heterophagy). Further, in these vacuoles, the destruction of redundant/damaged cellular components such as mitochondria also occurs (the process is called autophagy). If not destroyed, indigestible material gets accumulated in the vesicles which are referred to as residual bodies that may be removed by exocytosis. The residual bodies containing a high concentration of lipids are designated as lipofuscin. Since these residual bodies accumulate in cells of the older individuals, these are also called age-related pigments.

### *The Peroxisomes*

Certain oxidative enzymes, e.g. uric acid oxidase, D-amino acid oxidase and catalase are associated with special organelles called peroxisomes or microbodies. The major function of the peroxisomes is in hepatocytes where these are involved in the oxidation of fatty acids by a modified α-oxidation pathway. Besides, peroxisomes also represent a very primitive cellular organelle which provides protection to the cell from the toxicity of hydrogen peroxide ($H_2O_2$).

**Catalase** and **uric acid oxidase** are marker enzymes of the peroxisomes.

Absence of functional peroxisomes results in **Zellweger syndrome**, a rare autosomal recessive disease characterized by abnormalities of several organs due to the decreased levels of plasmalogens.

### *The Cytosol*

The cytosol or cell sap is a structureless material filling the cell (aqueous matrix) in which all the cellular organelles float. It is a colloidal solution of proteins containing nearly 70% water. Besides proteins, the cytosolic fraction also contains various enzymes for glycolysis, gluconeogenesis and HMP shunt, and a variety of organic as well as inorganic substances such as glucose, potassium and magnesium.

The cytosol is in contact with all the cellular organelles and is an important vehicle for the transport of metabolites from one organelle to the other.

The cytosol of all eukaryotic cells also contains a network of fibers, collectively called the **cytoskeleton**, which includes microtubules, intermediate filaments and microfilaments.

Lactate dehydrogenase is a marker enzyme of the cytosol fraction of the cell.

### *The Plasma Membrane*

Plasma membrane or cell membrane is the outer membrane of the cell. It is in contact with the extracellular matrix. In addition to the cell membrane, eukaryotic cells also contain internal membrane systems (nuclear membrane, mitochondrial membrane, lysosomal membrane, etc.) which form specialized compartments within the cell. Cell membrane and other membranes are collectively referred to as **biological membranes** that determine which substances are to enter or exit from the enclosed region.

$Na^+$, $K^+$-ATPase is a marker for plasma membrane.

## COMPOSITION OF A BIOLOGICAL MEMBRANE

Biological membranes are composed of lipids, proteins and carbohydrates. Different membranes within the cell and between cells, however, have different composition (Fig. 1.7).

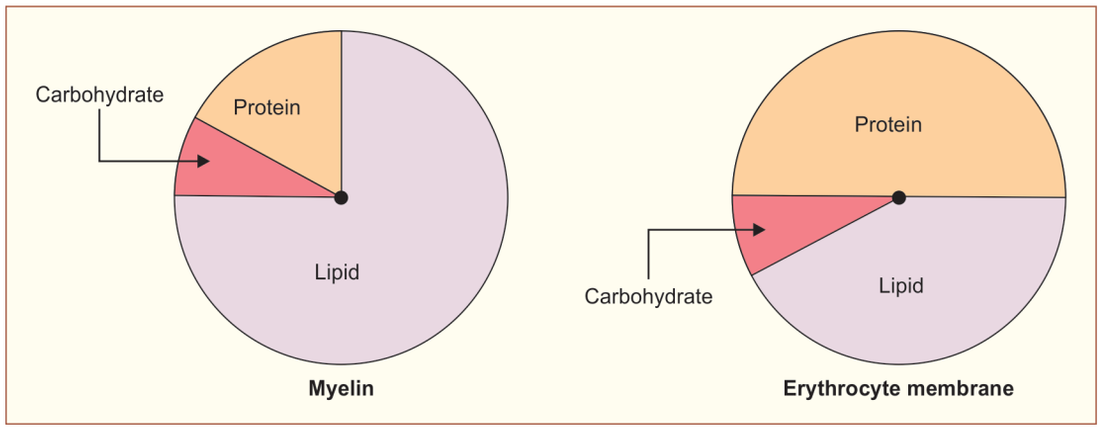


**Fig. 1.7:** Composition of biological membranes

## Lipids

Lipids form >50% of the total membrane constituents. Membrane lipids comprise both hydrophobic as well as hydrophilic regions, and thus are termed **amphipathic** molecules. Fatty acids in lipids have a polar head group and a nonpolar tail (Fig. 1.8).

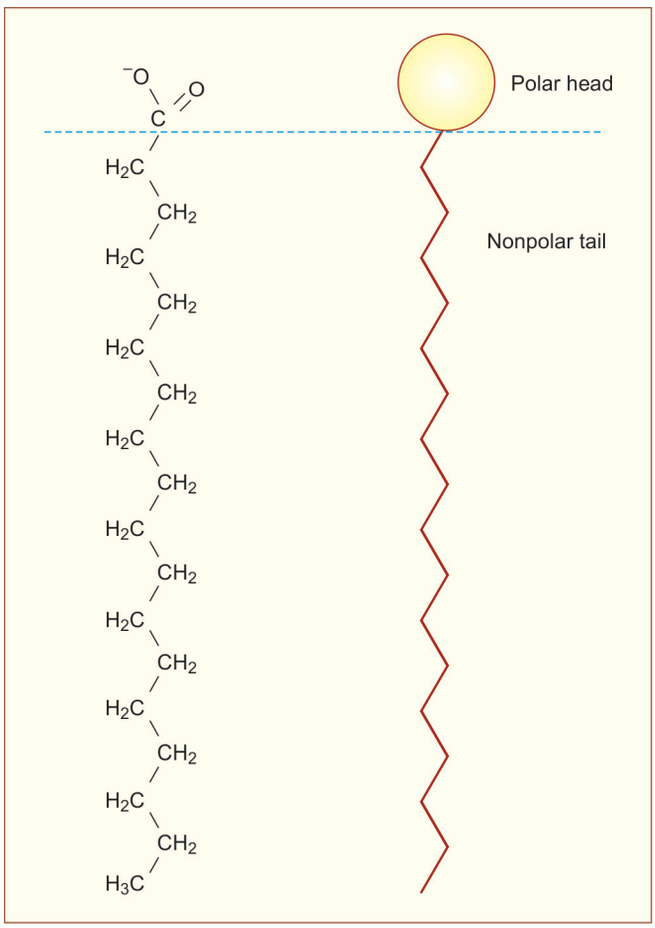


**Fig. 1.8:** A fatty acid

Fatty acids present in the tail are both saturated as well as unsaturated.

**Phospholipids** form the major proportion of the lipid component of cell membranes (Fig. 1.9).

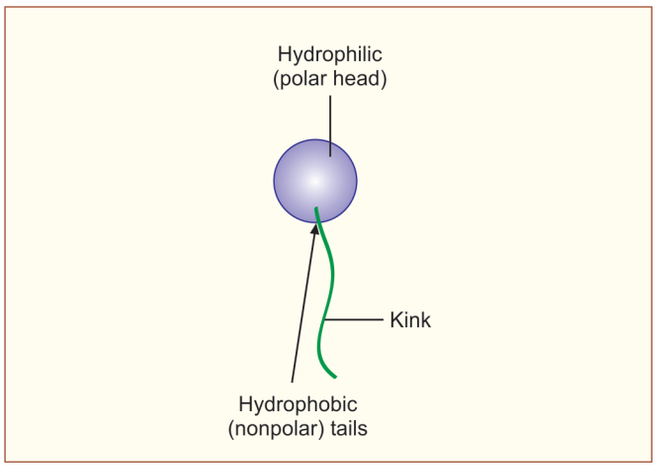


**Fig. 1.9:** A phospholipid

Besides, **free/unesterified cholesterol** is also present.

There are also qualitative differences between the classes of lipids and the individual lipids in different membranes within the cell as well as between the cells, e.g. plasma membrane has higher concentration of cholesterol and sphingolipids while intracellular membranes primarily contain glycerophospholipids with little sphingolipids or cholesterol.

In an aqueous solution, amphiphilic molecules form structurally ordered aggregates, such as micelles and bilayers, which form the structural basis of biological membranes.

*Micelles:* A micelle is a spheroidal aggregate where a large number of amphiphilic molecules, e.g. soaps and detergents, are arranged in such a way that their hydro-

philic groups (at the globular surface) interact with the aqueous solvent while the hydrophobic groups are associated at the centre, i.e. away from the solvent (Fig. 1.10).

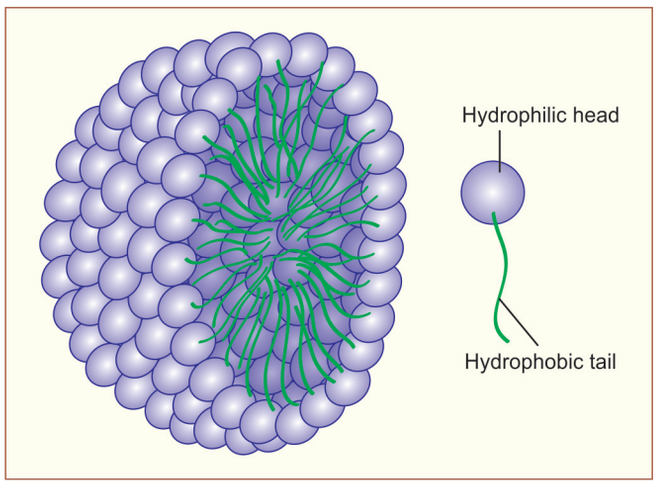


**Fig. 1.10:** A micelle

Micelles are formed when the cross-section of the hydrophilic head group exceeds that of the hydrophobic tails. This molecular arrangement eliminates unfavourable contact between water and hydrophobic tails of amphiphiles.

***Lipid bilayers:*** Bilayers are formed when the cross-section of the hydrophilic head group of amphiphilic lipids equals that of the hydrophobic tails. Lipid bilayers are the key structures in biological membranes. A lipid bilayer exists as a sheet, i.e. an expanded planer-aggregate, in which hydrophobic regions of phospholipids are protected from the aqueous environment while the hydrophilic regions are immersed in water (Fig. 1.11).

These are extremely stable structures which are held together by noncovalent interactions of the hydrocarbon chains and ionic interactions of the charged head groups with water. Lipophilic molecules such as cholesterol intercalate between fatty acyl residues of phospholipids and have minimal contact with the aqueous phase.

**Liposomes** are a special type of vesicles surrounded by lipid bilayer and are of clinical significance (Chemistry to Clinics 1.3).

**Chemistry to Clinics 1.3: Liposomes**

A lipid bilayer when closes on itself and forms a spherical vesicle which separates the external environment from the internal compartment is called a liposome.

***Preparation:*** A variety of techniques have been devised for the synthesis of liposomes in a laboratory. These can be prepared by using synthetic phospholipids or lipids extracted from natural membranes suspended in an aqueous solvent and subjected to very high frequency ultrasonic waves.

***Types:*** Depending on the preparation technique employed, liposomes may either be bound by a single lipid bilayer (**unilamellar liposomes**) or may consist of more than one concentric lipid layers alternating with aqueous layers (**multilamellar liposomes**; Fig. 1.12).

### Properties

1. Closed, self-sealing, solvent-filled vesicles formed from a suspension of phospholipids (glycerophospholipids or sphingomyelins) with or without cholesterol.
2. Usually have a diameter of 20–200 nm, and in a given preparation, are uniform in size.
3. Nontoxic.
4. Biodegradable.

### Significance

1. The outer structures of liposomes are similar to a biological membrane. Thus, liposomes serve as models of biological membranes.

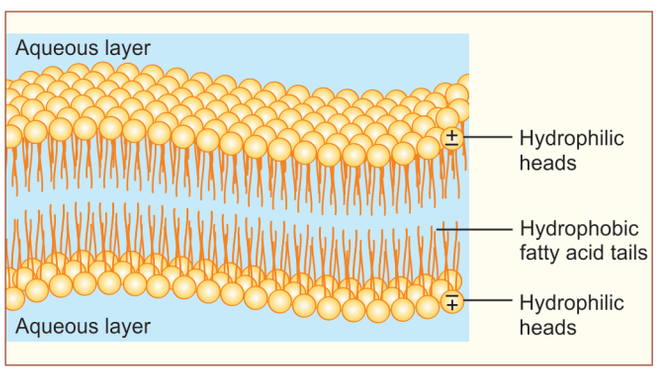


**Fig. 1.11:** A lipid bilayer

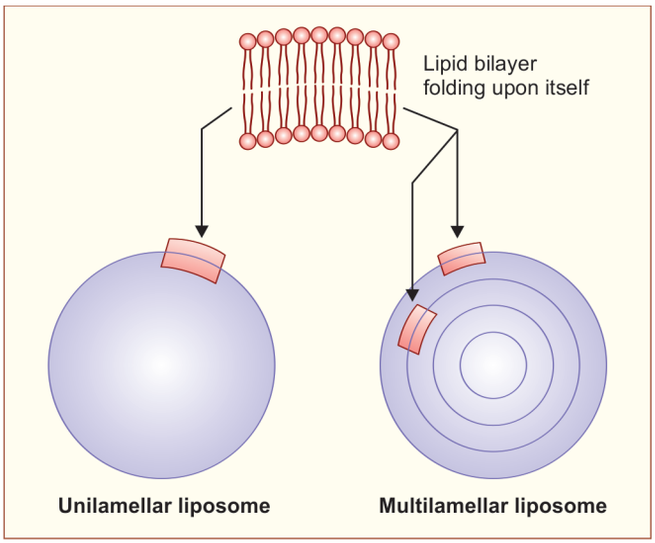


**Fig. 1.12:** Liposomes

2. When administered intravenously, liposomes are absorbed by many cells through fusion with the plasma membrane. This has a profound clinical application because the technique can be used to deliver those compounds to a patient which cannot be absorbed when administered orally. Thus, they are used as vehicles for drug delivery, e.g. liposomal. Amphotericin B is commercially available for the treatment of systemic fungal infections in immunocompromised patients. Liposomes have also been prepared with enzymes and DNA.
3. A major problem associated with liposomes is their nonspecific uptake by the reticuloendothelial system following intravenous administration. This can be circumvented by a two-fold strategy: Firstly, by coating them with specific ligands so that they are targeted to the tissue bearing the cognate receptors, and secondly, by incorporating polyethylene glycol in the bilayer which avoids uptake by the reticuloendothelial system. Such liposomes act as 'homing devices' for the encapsulated molecules including drug(s). It also minimizes the side effects of the drug(s).

## Proteins

Proteins form another major portion of the membrane and play several roles such as the structural components, transport systems, enzymes and recognition sites for hormones. Protein concentration varies from about 20% in the myelin sheath to about 80% in the inner membrane of the mitochondria.

Membrane proteins are classified by their mode of interaction with the membrane, as integral membrane proteins, peripheral membrane proteins and lipid-linked proteins (Fig. 1.13).

**Integral membrane proteins:** These proteins have the following characteristics:

- Span the cell membrane from one side to the other.
- Also called **transmembrane proteins** or **intrinsic proteins**.

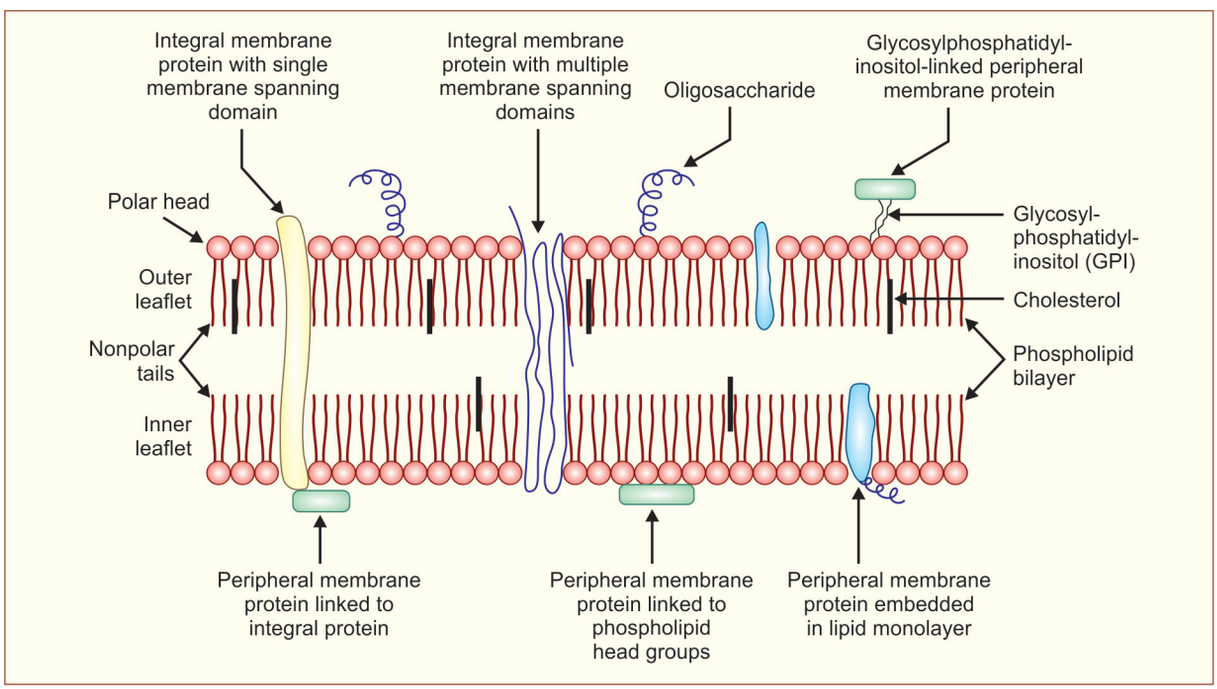


**Fig. 1.13:** Fluid-mosaic model of biological membrane

- Amphiphilic molecules tightly bound to the membrane by hydrophobic interactions.
- Some proteins contain α-helical structure consisting primarily of hydrophobic amino acids. This, in turn, forms a transmembrane sequence which has three domains, i.e. a sequence exterior to the cell containing the $-NH_2$ terminal end, a transmembrane sequence and a sequence extending into the cell with the –COOH terminal end.
- Can be separated from the membrane only by drastic treatment with certain agents that disrupt membranes, such as organic solvents or detergents.
- *Example:* **Glycophorin A**, which spans the erythrocyte membrane.

***Peripheral membrane proteins:*** These proteins have the following characteristics:

- Proteins which are embedded on any one side of the membrane.
- Also called **extrinsic proteins**.
- Immersed in the membrane only partially and are weakly bound to the hydrophilic region of the specific integral proteins.
- May have various modes of attachment: Some bind to integral proteins, such as an antigen. On the other hand, some peripheral proteins such as cytochrome b5, have sequences of hydrophobic amino acids at one end of the peptide chain which serve as an anchor in the membrane lipid.
- Can be released by relatively mild procedures that leave the membrane intact, such as by treatment with a salt solution of high ionic strength or by extremes of pH.
- *Example:* **Cytochrome *c***, in the outer surface of the inner mitochondrial membrane.

***Lipid-linked proteins:*** These proteins have the following characteristics:

- Membrane-associated proteins containing covalently attached lipids.
- Lipids anchor proteins to the membrane and thus mediate protein–protein interaction or modify structure and activity of the protein to which these are attached.
- Removal of the lipid fraction from these proteolipids leads to denaturation of the membrane proteins and loss of their biological functions.
- *Example:* **Lipophilin** presents in the myelin.

### Carbohydrates

Some of the membrane proteins and lipids bear short chains of carbohydrates (oligosaccharides), covalently attached either to a protein **(glycoprotein)** or to a lipid **(glycolipid)**. Carbohydrate content of biological membranes varies between 3 and 10%. Oligosaccharide chains are normally located on the outer surface of the membrane or on the terminal side of the endoplasmic reticulum.

## STRUCTURE OF A BIOLOGICAL MEMBRANE

As described above, the **lipid bilayer** forms the structural basis of a biological membrane. Some proteins span the lipid bilayer, whereas others are only immersed partially. This is called the **fluid-mosaic model** because the membrane is **fluid** in consistency, consisting of a **mosaic** of proteins and lipids which are **free to drift** about in the plane of the membrane (Fig. 1.13).

The fluid-mosaic model of a biological membrane has following characteristics:

- Sheet-like structure forming a closed boundary; thickness ≈ 7–10 nm.
- Noncovalent, asymmetric assemblies of amphiphilic lipids and proteins.
- Electrically polarized (inside negative with respect to outside).
- Membrane fluidity is regulated by:
  i. The presence of unsaturated fatty acids. Since *cis*-double bonds cause fatty acyl chains to bend (i.e. form kink), the membrane thus becomes less tightly packed and therefore is more fluid in nature (Figs 1.9 and 1.13).
  ii. *The degree of unsaturation of fatty acids:* Higher the unsaturation more is the fluidity.
  iii. $Ca^{2+}$ (increased $Ca^{2+}$ decreases membrane fluidity).
  iv. Cholesterol content of biomembranes.

At high temperature, cholesterol prevents lateral movement of phospholipid hydrocarbon tails thereby preventing an abnormal rise in membrane fluidity. At low temperature, it prevents the close packaging of the same hydrocarbon tails thereby preventing an abnormal fall in membrane fluidity. Hence, it is aptly said that **cholesterol 'modulates' membrane fluidity**. Abnormally high membrane cholesterol is observed in **spur cells** (Fig. 1.14).

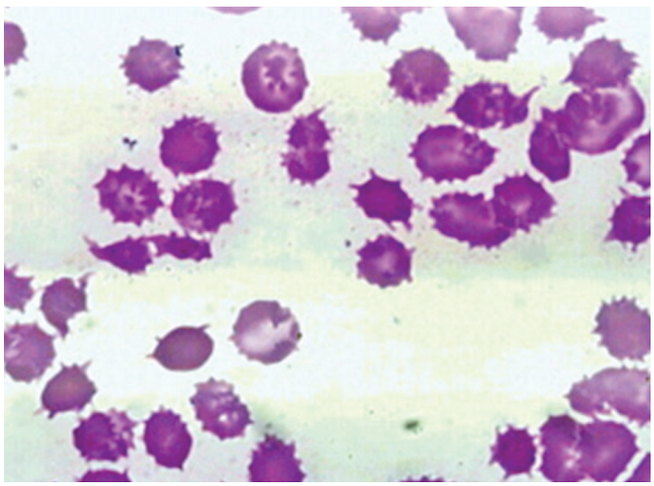

Fig. 1.14: Spur cells or acanthocytes: Red blood cells with multiple irregularly distributed, thorn-like spicules often with drumstick ends, projecting from the cell membrane. These biomembranes have an abnormally high cholesterol content derived from cholesterol-rich plasma lipoproteins

## FUNCTIONS OF A BIOLOGICAL MEMBRANE

1. ***Compartmentalization:*** It separates two different microenvironments, e.g. the cell membrane separates the intracellular compartment from the extracellular matrix or extracellular fluid.
2. ***Cell shape:*** Plasma membrane proteins have a structural role, i.e. maintain shape of the cell and define its boundaries.
3. ***Cell movement:*** Specific arrangement of plasma membrane proteins is critical in controlling the movements of some cells, e.g. movement of neutrophils from the intravascular to the extravascular compartment requires a change in cell shape as well as movement, known as diapedesis.
4. ***Enzymes:*** Many of the membrane proteins are enzymes and are located either within or on the cell membrane. The inner mitochondrial membrane is essential for localization and correct orientation of the respiratory enzymes within it for their maximum efficiency. The membrane of the endoplasmic reticulum plays an important role in localizing lipophilic substrates for their oxidation by the enzymes present therein.
5. ***Receptor molecules:*** Membrane proteins act as recognition sites, such as hormone receptors for insulin or glucagon or immunoreceptors present on B lymphocytes for the recognition and response to foreign antigens, etc.
6. ***Signal transduction:*** Various membrane proteins help in the transmission of signals, such as transmission of nerve impulses.
7. ***Translocation of substances:*** Membrane proteins regulate translocation of molecules, such as amino acids, glucose and various ions.

## MEMBRANE TRANSLOCATION

***'Translocation' versus 'transport':*** As a general rule, the term 'translocation' should be used to describe any type of transmembrane movement irrespective of the size of the substance to be moved. Further, it may or may not require a 'helper', located in the membrane itself. However, when such a helper or 'transporter' is required, the process should be called 'transport'.

## MEMBRANE TRANSLOCATION SYSTEMS

The translocation may or may not require the formation of a membrane-bound vesicle; accordingly, the translocation may be **vesicular or nonvesicular** (Fig. 1.15).

### Functional Mechanisms of Translocation

Translocation of a substance across the cell membrane can be described, in a functional sense, according to the number of molecules transported and the direction of their movement (Fig. 1.16):

***Uniport:*** The process which allows the movement of one type of molecules in only one direction, e.g. glucose uptake in erythrocytes.

***Cotransport:*** The process where transfer of one solute depends upon the simultaneous or sequential transfer of the other. It includes:

- ***Symport:*** Two types of molecules when move in the same direction, it is called symport, e.g. the $Na^+$ glucose transporter 1 (SGLT1) or the $Na^+$ amino acid transporter in the cells lining the small intestine and proximal renal tubules.
- ***Antiport:*** Two types of molecules when move in the opposite direction, it is called antiport, e.g. the $Na^+ = K^+$ antiport, the $Na^+ = Ca^{2+}$ transporter and the $Cl^- = HCO_3^-$ antiport.

## NONVESICULAR TRANSLOCATION SYSTEMS

These are of two types, i.e. the nonmediated translocation system and the mediated translocation system (Fig. 1.15).

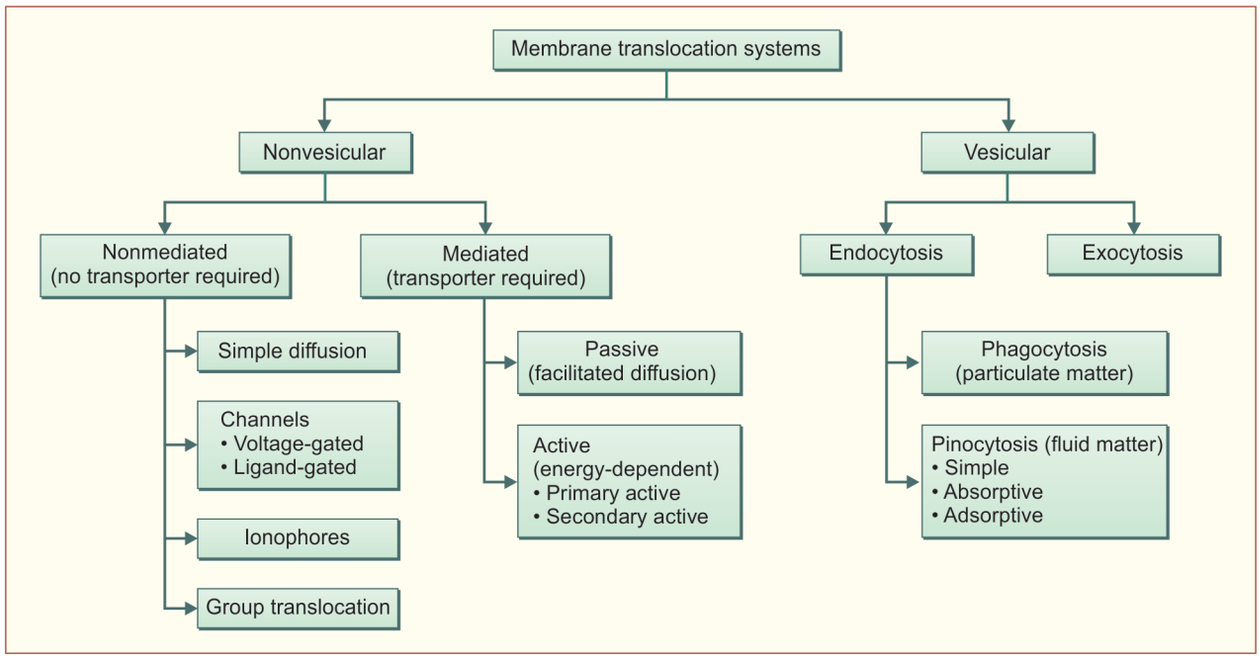


**Fig. 1.15:** Membrane translocation systems

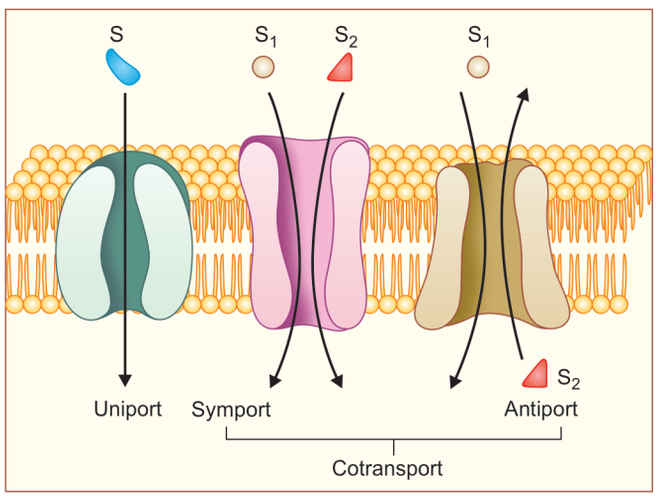


**Fig. 1.16:** Transport of substances with respect to the number of molecules and the direction of movement

## NONMEDIATED TRANSLOCATION

- Some solutes ($O_2$, $N_2$, $CO_2$, NO, urea, etc.) move across the cell membrane by diffusing down an electrochemical gradient and do not require metabolic energy. This, passive nonmediated translocation is called **simple diffusion**.
- It is limited by the thermal agitation of the specific molecule, by the concentration gradient across the membrane, and by its solubility in the hydrophobic core of the membrane bilayer. Electrolytes, since are poorly soluble in lipids, acquire a shell of water by electrostatic interaction. The size of the shell is directly proportional to the charge density of the electrolytes. For example, electrolytes with a large charge density have a larger shell of hydration and thus a slower diffusion rate.
- The direction of flow is always **from a higher to a lower concentration** and the net movement of a molecule from one side to the other continues until concentration on each side is at chemical equilibrium.
- Diffusion of a substance may also occur through membrane channels or pores.

### Channels

- Pore-like structures **formed by integral membrane proteins**.
- Permit rapid movement of specific ions or molecules from one side of membrane to the other (Fig. 1.17).
- The permeability of a channel depends upon the size, the extent of hydration, and the charge density on the ion.
- Specific channels exist for $Na^+$, $K^+$, $Ca^{2+}$, $Cl^-$, etc. These channels are **very selective** and permit the passage of only one type of ions.
- Channels are opened transiently, i.e. they are gated. The channel opening and closing involves a change in the voltage/membrane potential (**voltage-gated channel**) or the binding of a chemical agent (**ligand-gated channel**; Table 1.4).

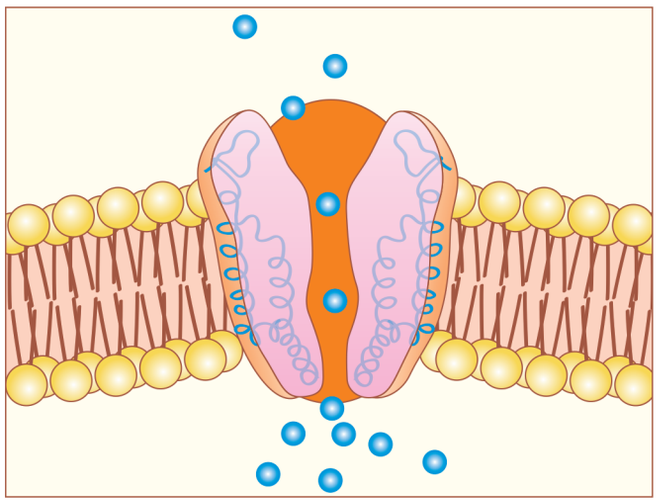

Fig. 1.17: Ion channel

**Table 1.4:** Types of ion channels

| Cannel | Responsive factor |
|---|---|
| Voltage-gated | Change in membrane potential, e.g. $Na^+$, $K^+$ and $Ca^{2+}$ channels in the heart |
| Ligand-gated | • A specific extracellular molecule, such as acetylcholine for the acetylcholine receptor channel of the neuromuscular junction.<br>• A specific intracellular molecule, e.g. cAMP for $Ca^{2+}$ channels in myocytes. |

- Sometimes, one ion can regulate the activity of the other ion, e.g. a decrease in $Ca^{2+}$ ion concentration in the extracellular fluid increases membrane permeability and diffusion of $Na^+$. This, in turn, depolarizes the membrane and triggers nerve discharge.
- A number of pharmacologic agents that modulate these channels are used therapeutically.
- Mutations in genes encoding polypeptide constituents of ion channel may lead to certain diseases termed **channelopathies**, e.g. myasthenia gravis and cystic fibrosis (Chemistry to Clinics 1.4).

***Movement of water across biological membranes—aquaporins:*** Water can move rapidly in and out of cells, but the partition coefficient of water into lipids is low; therefore, the permeability of the membrane lipid bilayer for water is also low. Specific membrane proteins that function as water channels explain the rapid movement of water across the plasma membrane. These water channels are small nearly 30 kDa, integral membrane proteins known as **aquaporins** (**AQP**). Many different forms have been discovered so far; at least six forms are expressed in cells in the kidneys and seven forms in the gastrointestinal tract, tissues in which water movement across plasma membranes is particularly rapid (Chemistry to Clinics 1.5).

**Chemisty to Clinics 1.4: Channelopathies**

1. *Myasthenia gravis:* It is an acquired autoimmune disease characterized by muscle weakness due to decreased neuromuscular signal transmission. Autoantibodies against acetylcholine receptors accelerate their turnover and reduce their number. Acetylcholinesterase inhibitor drugs are given to enhance the stay of acetylcholine at the neuromuscular junction. Ultimately, the patients require the removal of the culprit antibodies from the plasma at regular intervals, a process called 'plasmapheresis'.
2. *Cystic fibrosis:* It though is a multiorgan disease but its gene product is a cystic fibrosis transmembrane conductance regulator (CFTR), which is a cAMP-dependent $Cl^-$ channel. Patients have reduced membrane permeability which impairs fluid and electrolyte secretion and leads to luminal dehydration.

**Chemistry to Clinics 1.5: Nephrogenic Diabetes Insipidus**

Nephrogenic diabetes insipidus is renamed as arginine vasopressin resistance (AVP-R) and is, primarily, due to pathology of the kidney. This is in contrast to central or neurogenic diabetes insipidus, which is caused by insufficient levels of vasopressin (also called antidiuretic hormone, ADH). Nephrogenic diabetes insipidus caused by an improper response of the kidney to vasopressin, leading to a decrease in the ability of the kidney to concentrate the urine by removing free water. Rarely, a mutation in the "aquaporin 2" gene impede the normal functionality of the kidney water channel, which results in the kidney being unable to reabsorb water. This mutation is often inherited in an autosomal recessive manner.

## Ionophores

Besides channels, the other transmembrane route for the nonmediated translocation is through ionophores, whose features include:

- These are small organic molecules such as antibiotics which are synthesized by some bacteria and function as shuttles.
- Ionophores **increase permeability of the membrane to a particular ion** by binding the ion, diffusing it through the membrane and releasing it on the other side. To ensure the net transport, uncomplexed ionophores return to the original side of the membrane and are ready to repeat the process.
- Because of their ability to complex specific ions and facilitate their transport, ionophores contain hydrophilic centres for ion-binding and are surrounded by peripheral hydrophobic regions. This

allows the molecules to dissolve effectively in the membrane and diffuse through it. The net diffusion of a substance thus depends upon:

1. Its concentration gradient across the membrane.
2. The electric potential across the membrane.
3. The permeability coefficient of the substance for the membrane.
4. The hydrostatic pressure gradient across the membrane, and
5. Temperature.

- Each ionophore, e.g. valinomycin or nigericin has definite ion specificity. **Valinomycin** translocates $K^+$ by an electronegative import mechanism. **Nigericin** is an electrically neutral antiporter which translocates $K^+$ in exchange for $H^+$ across the membrane.

### Group Translocation

***Principle:*** Group translocation involves chemical modification of the transported substrate. The **γ-glutamyl cycle (Meister cycle)** transports **amino acids** across the plasma membrane. During the translocation, the substrate (amino acid) undergoes alteration and is released into the cell as a different molecule.

***Mechanism:*** The pathway involves the membrane-bound enzyme γ-glutamyl transpeptidase (γ-GT). The amino acid transported is the substrate to which the γ-glutamyl residue of glutathione (GSH), a tripeptide, is transferred. This leads to the formation of a dipeptide containing γ-glutamyl residue of GSH with the transported amino acid. The dipeptide, γ-glutamyl amino acid, is transported into the cell and the complex is then hydrolyzed by a separate cytosolic enzyme to liberate the free amino acid. Glutamate is released as 5-oxoproline while cysteinylglycine is cleaved to its component amino acids (Fig. 1.18).

### Salient Features

- Group translocation is especially active in renal epithelial cells, liver and enterocytes.
- All amino acids except proline can be transported by group translocation.
- Rapid process.
- High capacity.
- ***Energetically expensive:*** The energy for the transport comes from the hydrolysis of a peptide bond in GSH. For the system to continue, GSH must be resynthesized which requires 3 molecules of ATP. Hence, for each amino acid translocated across the membrane, 3 ATPs are used.

## CARRIER-MEDIATED TRANSPORT

Molecules that cannot freely diffuse through the membrane by themselves, need to be transported in association with specific carrier molecules/transport proteins.

### Salient Features of a Transport Protein

- Carrier molecules (variously designated as carriers, permeases, porters, **translocases or transporters**), behave much like enzymes.
- Have a high degree of **structural stereospecificity** for the substances to be transported.
- Demonstrate **saturation kinetics**, i.e. when binding sites on all the transport proteins are occupied, the system is saturated and the rate of transport reaches a plateau.

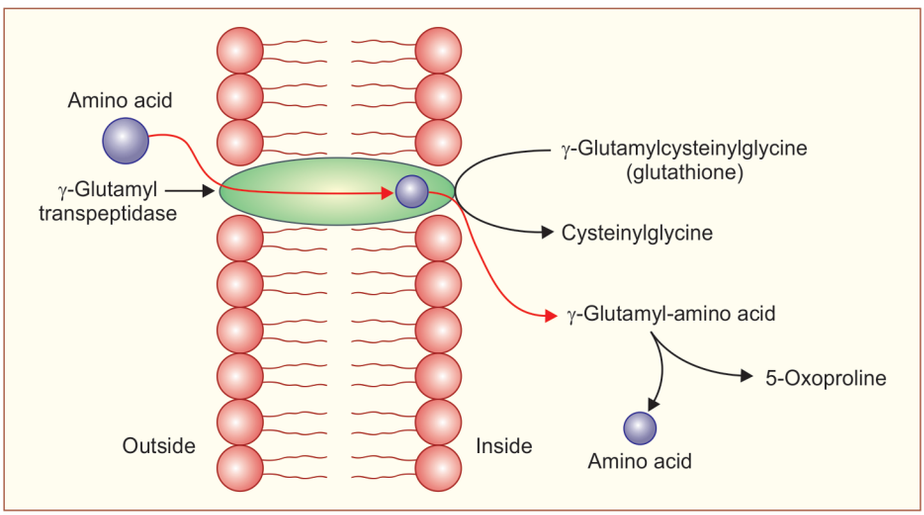


**Fig. 1.18:** Group translocation (γ-glutamyl cycle)

- Can be **inhibited** by both competitive and non-competitive inhibitors. The inhibition can prevent transport by blocking the binding sites or by interacting with the transport protein and altering its conformation so that it becomes non-functional.
- All cell membranes contain highly specific transporters for the movement of ions ($Na^+$, $K^+$, $Cl^-$, $HCO_3^-$, etc.) as well as organic compounds (such as amino acids and sugars).
- The transport of a solute molecule mediated by a transport protein has four aspects, explained by a **'ping-pong' mechanism** (Fig. 1.19):
  1. ***Recognition:*** Transport proteins have receptor sites to which the solute attaches. The transporter thus recognizes an appropriate solute from the aqueous environment for its translocation across the membrane.
  2. ***Translocation:*** After binding of the solute with the transporter protein, there occurs a conformational change in the transporter protein which translocates (moves) the solute molecule to the opposite side of the membrane.
  3. ***Release:*** A change in the conformation of the transporter protein decreases affinity of the solute and release it to the new environment.
  4. ***Recovery:*** After release of the solute, the transport protein reverts to its original conformation to accept another solute molecule, i.e. the transporter is recovered in its original conformation.
- Carrier-mediated transport includes (Fig. 1.15):
  1. **Passive transport** (passive mediated transport) or **facilitated diffusion**.
  2. **Active transport**.

***Polarized epithelial cell:*** Before discussing the details of carrier-mediated transport, it is useful to understand the concept of polarized epithelial cells lining the **small intestine** and the **renal tubules** (Fig. 1.20).

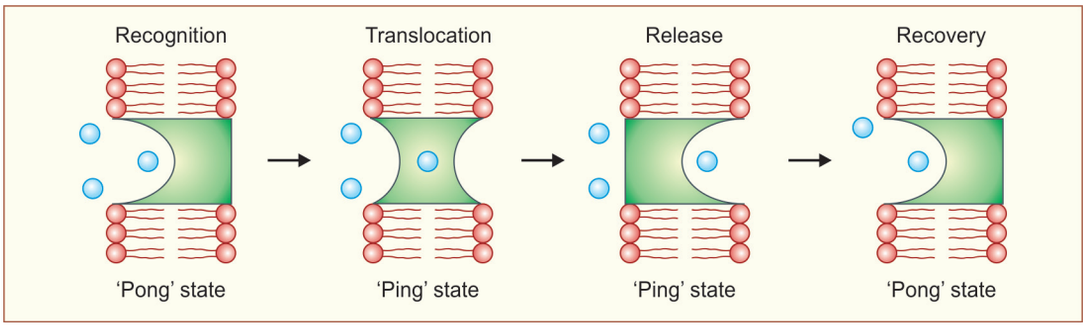


**Fig. 1.19:** 'Ping-pong' model for mediated membrane translocation

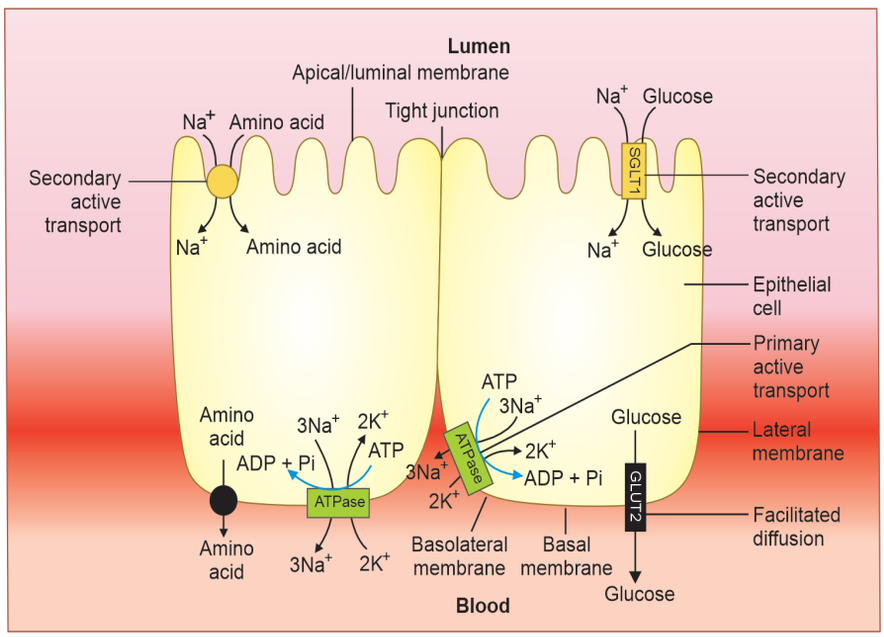


**Fig. 1.20:** Transport of glucose in epithelial cell

Epithelial cells occur in layers or sheets that allow the directional movement of solutes not only across the plasma membrane but also from one side of the cell layer to the other. Such regulated movement is achieved because the plasma membranes of epithelial cells have **two distinct regions** with different morphologies and different transport systems. These regions are the **apical membrane** facing the lumen, and the **basolateral membrane** facing the blood supply. The polarized organization of the cells is maintained by the presence of **tight junctions** at the areas of contact between adjacent cells. Tight junctions prevent proteins on the apical membrane from migrating to the basolateral membrane and vice versa. Thus, the entry and exit steps for solutes can be localized to opposite sides of the cell. This is the key to transcellular transport across epithelial cells.

## PASSIVE MEDIATED TRANSPORT/ FACILITATED DIFFUSION

- Passive mediated transport, also referred to as facilitated diffusion, leads to the translocation of solutes through membrane transport proteins **without the expenditure of metabolic energy**.
- The process can operate either unidirectionally or bidirectionally and the net flux across the membrane occur down a concentration gradient, i.e. the molecules flow from a higher to the lower concentration.
- 'Ping-pong' mechanism explains facilitated diffusion of molecules across the biological membrane with the help of a transport protein which exists in two principal conformations (Fig. 1.19).
- Several hormones (such as insulin, glucocorticoids, growth hormone, etc.) regulate facilitated diffusion by changing the number of transport proteins available.
- Examples of the transport proteins which mediate facilitated diffusion include:
  1. ***Glucose transporters (GLUT):*** A group of transport proteins present in the plasma membrane of mammalian cells for the transport of D-glucose, e.g. GLUT1, GLUT2, etc. These are referred to as glucose permeases. They translocate several D-sugars by a uniport mechanism.
  2. ***$Cl^-/HCO_3^-$ exchanger:*** It is an anion exchanger protein for the antiport movement of $Cl^-$ and $HCO^{3-}$ in RBC membrane.
  3. ***ATP-ADP translocase:*** An antiport for the exchange of anions ($ADP^{3-}$ and $ATP^{4-}$) between the cytosol and the mitochondrial matrix, located in the inner mitochondrial membrane.

## ACTIVE TRANSPORT

In active transport, the transport protein moves a specific molecule against the concentration gradient, i.e. from a lower concentration to the higher concentration. It is a process that requires energy which, in most cases, is coupled to the hydrolysis of ATP (Fig. 1.21).

The active transport can be grouped as:

- **Primary active transport,** and
- **Secondary active transport.**

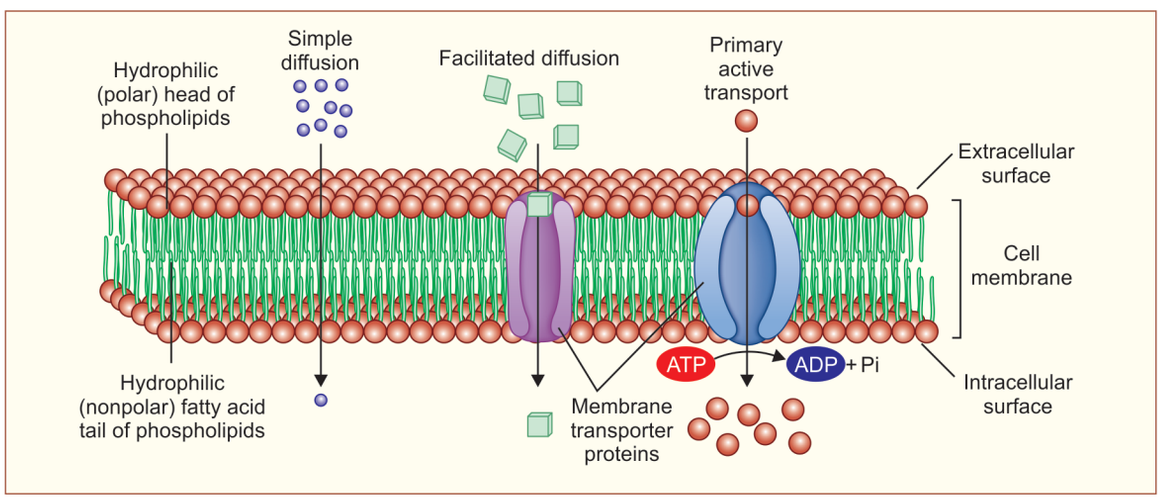


**Fig. 1.21:** Transport systems

## Primary Active Transport

This transport system has the same characteristics as the passive transport system but it is an **endergonic process**. Examples of such transporters include membrane-bound ATPases that translocate cations. They are further classified as:

- ***P type transporters:*** The transporter protein is phosphorylated and dephosphorylated during the transport activity.
- ***V type transporters:*** These are present in the membrane of the lysosomes, the Golgi vesicles and the secretory vesicles. These are responsible for acidification of the interior of these vesicles.
- ***F type transporters:*** These are present in mitochondria and are involved in ATP synthesis.

## Some of the Primary Active Transport Systems

Primary active transport systems are important in the maintenance of electrochemical gradient in biological systems and consume nearly one-third of the total energy expenditure of a cell. Examples of primary active transport system include $Na^+/K^+$-ATPase, $Ca^{2+}$-ATPase, $H^+/K^+$-ATPase and neurotransmitter transporters:

1. **$Na^+/K^+$-ATPase:** The $Na^+/K^+$-ATPase ($Na^+$-$K^+$ pump) is an antiport that moves three $Na^+$ ions out of the cell in exchange of two $K^+$ ions into the cell, with the concomitant hydrolysis of intracellular ATP (Fig. 1.22).

   The pump is an integral part of the membrane and has binding sites for both ATP and $Na^+$ on the cytoplasmic side of the membrane, and $K^+$ binding site on the extracellular side of the membrane. Drugs that inhibit the $Na^+$=$K^+$ pump are of clinical importance (Chemistry to Clinics 1.6).

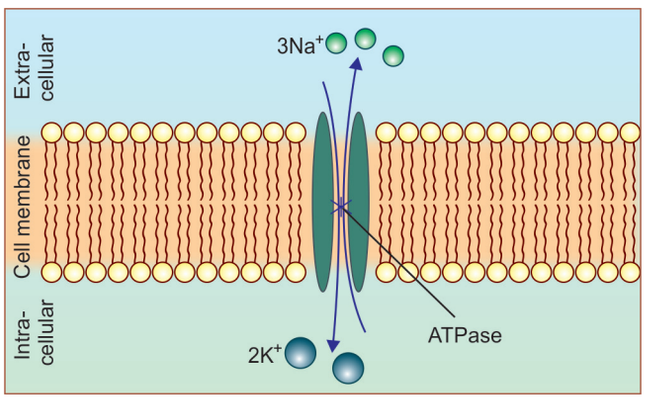


**Fig. 1.22:** $Na^+$-$K^+$ pump

**Chemistry to Clinics 1.6: Heart Failure**

The cell membrane of cardiomyocytes (cells of the myocardium) contain many transport pumps. Two of them are, $Na^+/K^+$-ATPase and $Na^+/Ca^{2+}$ exchanger. The $Na^+/K^+$-ATPase serves its usual function of maintaining low intracellular $Na^+$ concentrations. The $Na^+/Ca^{2+}$ exchanger relies on this $Na^+$ gradient to extrude $Ca^{2+}$ out of the cells. Cardiac glycosides (digitalis) such as digoxin and ouabain abolish this gradient by inhibiting the $Na^+/K^+$-ATPase. High intracellular $Na^+$ concentration slows the extrusion of $Ca^{2+}$ by the $Na^+/Ca^{2+}$ exchanger. Increased availability of $Ca^{2+}$ results in increased force of contraction that is clinically useful in the management of cardiac failure (Fig. 1.23).

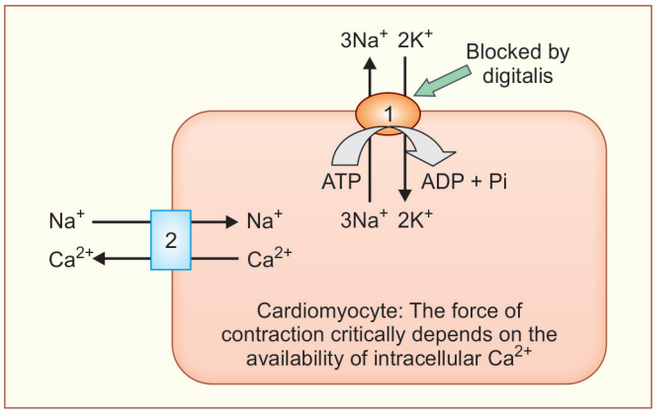


**Fig. 1.23:** Role of digitalis in heart failure: Normally, the $Na^+$-$K^+$-ATPase in the cardiomyocyte membrane moves $Na^+$ out of the cell in exchange for $K^+$ (1). The low cytosolic $Na^+$ maintains the $Na^+$ gradient for the functioning of the $Na^+$-$Ca^{2+}$ exchanger so that low cytosolic $Ca^{2+}$ levels are maintained nothing to be done here. In a failing heart, the aim of treatment is to make the myocardium contract with greater force (2). This is achieved with digitalis, which, by inhibiting $Na^+$, $K^+$-ATPase, abolishes the $Na^+$-gradient. Consequently, $Ca^{2+}$ cannot exit the cell and attains sufficiently high intracellular levels to mediate excitation-contraction coupling and generate greater contractile force. As more blood is pumped out, the symptoms of heart failure are alleviated

2. ***$Ca^{2+}$-ATPase:*** $Ca^{2+}$ is an important intracellular messenger referred to as a second messenger. It regulates various cellular processes such as muscle contraction, release of neurotransmitters and glycogen breakdown. It is also an important activator of oxidative metabolism. In order to maintain low cytosolic $Ca^{2+}$ concentration, it is actively transported out of the cell across the plasma membrane, the endoplasmic reticulum or the sarcoplasmic reticulum. The $Ca^{2+}$-ATPase ($Ca^{2+}$ pump) actively pumps two $Ca^{2+}$ out of the cytosol at the expense of ATP hydrolysis. The mechanism of $Ca^{2+}$-ATPase resembles that of the $Na^+/K^+$-ATPase. In eukaryotes, the $Ca^{2+}$ transporter is regulated by the cytosolic $Ca^{2+}$ level through a calcium binding protein termed calmodulin.

3. ***$H^+/K^+$-ATPase:*** Parietal cells in the gastric mucosa secrete HCl. Intracellular hydration of $CO_2$ by carbonic anhydrase forms carbonic acid ($H_2CO_3$) which splits into $H^+$ and $HCO_3^-$. The secretion of $H^+$ involves the $H^+/K^+$-ATPase, also called the proton pump, in the luminal membrane. This is an antiport and as $H^+$ is pumped out, the $K^+$ which enters the cell is subsequently externalized to the lumen. The chloride channels secrete $Cl^-$ into the lumen so that the overall secreted product is HCl. Inhibition of $H^+/K^+$-ATPase is of clinical importance (Chemistry to Clinics 1.7).

**Chemistry to Clinics 1.7: Peptic Ulcer**

Excess production of HCl along with a failure of mucosal defense mechanisms, can damage the gastric mucosa and may lead to peptic ulcer. The $H^+/K^+$-ATPase of the gastric mucosa is activated by histamine stimulation of the cell surface receptor (type 2 histamine receptors). Compounds, such as cimetidine and its analogs (type 2 antihistamine drugs) block the process by competing with histamine for its binding to the receptor and, in turn, reduce HCl production. These drugs are therefore widely used to alleviate the painful and otherwise fatal symptoms of peptic ulcer. However, proton pump inhibitors such as omeprazole are better because they are selective inhibitors of $H^+/K^+$-ATPase and are therefore more powerful than the antihistamines. It is now recognized that many ulcers are caused by infection with the bacteria *Helicobacter pylori* and can better be cured by the use of antibiotics besides a reduction in acidity.

4. ***Neurotransmitter transporters:*** Regulating the temporal availability of synaptically released neurotransmitter is critical to normal neuronal function. The removal of neurotransmitter from the synaptic cleft by active transport back into the presynaptic terminal or perisynaptic astrocytes (in the central nervous system) is the major way in which synaptic signaling is terminated at most of the synapses. Many disease processes in the central and peripheral nervous systems are due to disorders of synaptic signaling, and may involve altered function of neurotransmitter transporters (Chemistry to Clinics 1.8).

**Chemistry to Clinics 1.8: Neurotransmitter Transport Disorders**

Dopamine, serotonin, glutamate, norepinephrine, etc. are some of the well-known neurotransmitters. Each has its own reuptake mechanism mediated by specific membrane transporters which may be affected in several diseases:

- *Defect in the dopamine transporter:* Alzheimer's disease, attention deficit hyperactivity disorder, obesity, Parkinson's disease, schizophrenia and substance abuse.
- *Defect in the serotonin transporter:* Autism, gastric motility disorders, mood disorders and obsessive-compulsive disorder.
- *Defect in the glutamate transporter:* Amyotrophic lateral sclerosis, epilepsy, schizophrenia, and stroke.
- *Defect in the norepinephrine transporter:* Mood disorders, severe orthostatic hypotension and substance abuse.

Knowing the tertiary and quaternary structure of the transporters is important for developing selective drugs to target them.

## Secondary Active Transport

Secondary active transport is also called **ion gradient-driven active transport**. In this process, free energy of the electrochemical gradient, generated by an ion-pumping ATPase, drives the transport of another substance, such as a sugar or an amino acid, against its concentration gradient. $Na^+$ glucose transport system and the amino acid transport system are the examples of secondary active transport system.

1. ***$Na^+$ glucose transport system:*** An example is the absorption of glucose in the proximal renal tubules and the small intestine (Fig. 1.20).

   Glucose enters the intestinal epithelial cells by secondary active transport using the electrogenic $Na^+$ glucose transporter 1 (**SGLT1**) in the apical membrane. This increases the intracellular glucose concentration above the blood glucose concentration, and the glucose molecules move passively out of the cell and into the blood via an equilibrating carrier mechanism (**GLUT2**) in the basolateral membrane. The intestinal GLUT2, like the erythrocyte GLUT1, is a sodium-independent transporter that moves glucose down its concentration gradient. However, unlike GLUT1, the GLUT2 transporter can accept other sugars, such as galactose and fructose for absorption. The **$Na^+/K^+$-ATPase** that is located in the basolateral membrane pumps out the $Na^+$ (that enter the cell with glucose via SGLT1). In short, the successful uptake of glucose and sodium (symport) is ***'secondarily'*** dependent on the $Na^+$ gradient maintained by the primary active transport by Na $Na^+/K^+$-ATPase (Fig. 1.20). The **polarized** organization of the epithelial cells and the integrated functions of the plasma membrane transporters form the basis by which cells accomplish transcellular movement of both glucose and sodium ions, and is also exploited clinically in oral rehydration therapy (Chemistry to Clinics 1.9).

**Chemistry to Clinics 1.9: Oral Rehydration Solution**

The administration of oral rehydration solution (ORS) has dramatically reduced the mortality resulting from cholera and other diseases that involve extreme losses of water/solutes from the gastrointestinal tract. The main ingredients of ORS are glucose, NaCl or $NaHCO_3$, KCl and water. The glucose and $Na^+$ are reabsorbed by the sodium-glucose transporter 1 (SGLT1) in the apical membrane of enterocytes, i.e. epithelial cells lining the lumen of the small intestine. Transfer of solutes on the basolateral aspect of the enterocytes increases the osmolarity compared with the luminal osmolarity thereby favoring the osmotic absorption of water. In this manner, the absorption of glucose accompanied by the obligatory increase in absorption of NaCl and water, help to compensate for the diarrheal losses of water/solutes, and treat dehydration.

A comparison between passive mediated transport (facilitated diffusion) and active transport is shown in Table 1.5.

**Table 1.5:** Comparison between facilitated diffusion and active transport

| Parameter | Facilitated diffusion | Active transport |
|---|---|---|
| Specific binding site | Present | Present |
| Saturation kinetics | Yes | Yes |
| Inhibition by structural analogs | Yes | Yes |
| Direction of operation | Uni- or bidirectional | Unidirectional |
| Mode of operation | Along electrical/ chemical gradient | Against electrical/ chemical gradient |
| Energy dependent | No | Yes |

2. ***Amino acid transport systems:*** Amino acids are transported by luminal epithelial cells of the intestine by the Na-dependent pathway, similar to the $Na^+$-dependent glucose transport system. At least seven different brush-border specific transport systems have been identified for the uptake of different classes of L-amino acids and the dipeptides in the luminal membrane. These include the amino acid transporter for:
   - Neutral amino acids with short or polar side chain such as serine, threonine and alanine.
   - Neutral amino acids with aromatic or hydrophobic side chain, e.g. phenylalanine, tyrosine, tryptophan, methionine, valine, leucine and isoleucine.
   - Imino acids, e.g. proline and hydroxyproline.
   - β-amino acids, such as β-alanine and taurine.
   - Basic amino acids, e.g. lysine and arginine as well as cystine.
   - Acidic amino acids, such as aspartate and glutamate, and
   - Dipeptides.

Each one of these transport systems is specific for a group of closely related amino acids and operates as $Na^+$ symport system. Several pathological conditions due to a defect in the membrane transport system for specific amino acids are known (Chemistry to Clinics 1.10).

**Chemistry to Clinics 1.10: Amino Acid Transport Disorders**

- *Hartnup's disease* is due to a decrease in the transport of neutral amino acids including tryptophan in the epithelial cells of the intestine and in the renal tubules. It is characterized by pellagra-like features due to niacin deficiency consequent to tryptophan deficiency.
- In *cystinuria* renal reabsorption of cystine and basic amino acids (arginine and lysine) is abnormal. This in turn may result in the formation of cystine stones in the kidney.

## VESICULAR TRANSLOCATION

Cells translocate certain macromolecules across the plasma membrane by mechanisms which involve vesicle formation with or from the plasma membrane and are referred to as **endocytosis** and **exocytosis** (Fig. 1.15).

All these processes require energy (ATP), $Ca^{2+}$ and the cytoskeletal system for proper functioning.

### Endocytosis

Endocytosis is a mechanism for the uptake of large molecules such as polysaccharides, proteins or polynucleotides into the cytoplasm. In this process, a region of the plasma membrane invaginates, enclosing a small volume of the extracellular fluid and its contents within a bud, and generates endocytotic vesicles. The vesicle then pinches off, as fusion of the plasma membrane seals the neck of the vesicle at the original site of invagination. The resulting small vesicle is called an **endosome**. It moves into the interior of the cell and delivers its contents to some other organelle, bound by a single membrane, e.g. a lysosome, by fusion of the two membranes. This 'hybrid vesicle' is called a **secondary lysosome**. Due to the presence of hydrolytic enzymes, the macromolecular contents are digested to their monomers, such as amino acids, simple sugars or nucleotides, which then diffuse out of the vesicle in the cytoplasm.

There are two general types of processes referred to as endocytosis, i.e. phagocytosis and pinocytosis.

- ***Phagocytosis (or cell eating):*** It occurs only in specialized cells like macrophages and granulocytes for the ingestion of large particles, such as bacteria, viruses, etc.

- ***Pinocytosis:***
  - ***Simple pinocytosis*** (or ***cell drinking***) leads to cellular uptake of fluid and its contents as a result of invagination of the plasma membrane (Fig. 1.24).

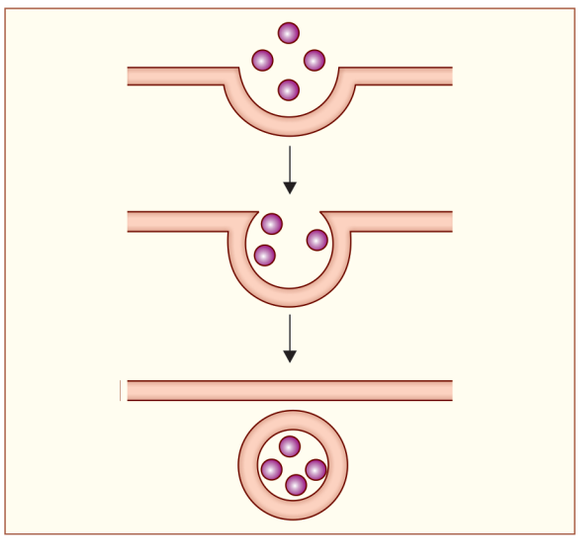

**Fig. 1.24:** Endocytosis (simple pinocytosis)

  - ***Absorptive pinocytosis*** or ***receptor-mediated pinocytosis*** is a very selective type of pinocytosis that occurs in coated pits, lined with the protein **clathrin**, resulting in the formation of the clathrin-coated vesicles. The high affinity receptors permit selective concentration of the ligand from the medium, e.g. low density lipoproteins (LDL), transferrin, etc. and the receptors are subsequently internalized by means of the coated pits containing the receptors. The coated vesicle may fuse with lysosomes, the contents are digested and clathrin is recycled back to the membrane (Fig. 1.25).
  - Sometimes, in case of some hormones, clathrin is not required for receptor-mediated pinocytosis. The internalized vesicle fuses with another organelle such as Golgi complex, i.e. no secondary lysosomes are formed. The process is known as ***adsorptive pinocytosis***.

## Exocytosis

Exocytosis is the reverse of endocytosis. It involves contact of two inside surface monolayers from the cytosolic side and release of macromolecules to the exterior of a cell. A secretory vesicle in the cytoplasm,

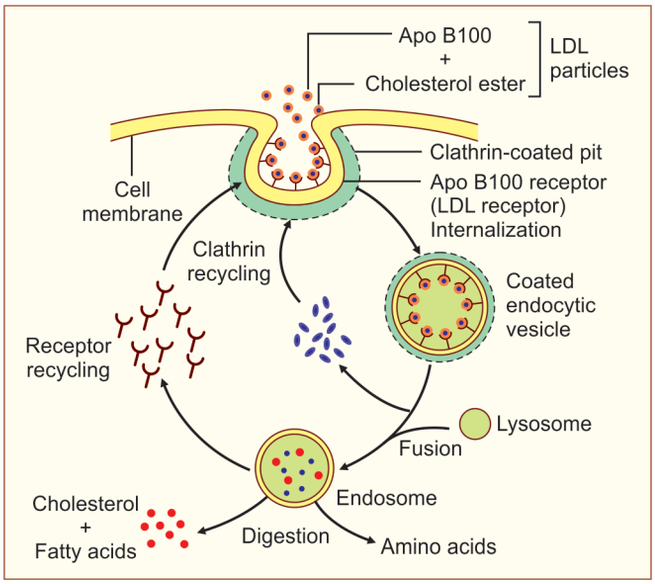


**Fig. 1.25:** Receptor-mediated pinocytosis

originating in the Golgi complex or the endoplasmic reticulum, moves to the inner surface of the plasma membrane and fuses with it, releasing the vesicular contents outside the membrane (Fig. 1.26).

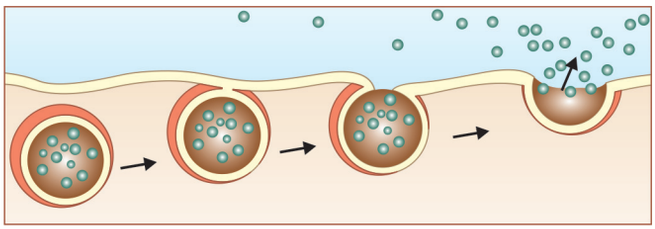

**Fig. 1.26:** Exocytosis

**The secreted/exocytosed molecules may have either of three possible fates:**

1. They become a part of the cell membrane surface, e.g. antigens.
2. They become a part of the extracellular matrix, e.g. collagens.
3. They enter the blood and carried to distant sites, e.g. hormones like insulin.

In some diseases characterized by uncontrolled cell division, vesicles may be thrown out to the cell exterior, and contain molecules actually meant for intracellular use only. The process is not a true exocytosis and such vesicles are not true secretory vesicles. They are called **exosomes** (Chemistry to Clinics 1.11).

**Chemistry to Clinics 1.11: Exosomes**

All cells contain the machinery for the targeted destruction of intracellular macromolecules when they are no longer needed. For example, 'proteasomes' are meant for targeted destruction of proteins and 'exosomes' are meant for targeted degradation of RNA. Exosomes contain RNAse-P activity for RNA cleavage and all the RNA as substrates, marked for degradation. In cells undergoing rapid division, exosomes may be thrown out into the surrounding. Hence, the term 'exosomes' also refers to small microvesicles surrounded by lipid bilayer, shed by tumors into the blood. The exosomes contain the complete set of messenger RNAs (mRNAs) and micro-RNAs (miRNAs), collectively known as the transcriptome. The exosomes can be isolated from the blood and the transcriptome subjected to reverse transcription polymerase chain reaction for detecting DNA mutations. Such blood-based detection of mutations is very valuable in diagnosing tumors where there may be constraints in obtaining a tissue biopsy, as in case of cancers of lungs, pancreas and ovaries.

## MEMBRANE ASSOCIATED PHENOMENON: GIBBS-DONNAN EQUILIBRIUM

Consider two solutions separated by a semipermeable membrane as shown in Fig. 1.27.

Both the solutions I and II contain diffusible ions such as $Na^+$ and $Cl^-$; in addition, solution I contains impermeable/nondiffusible anions designated as nondiffusible ions ($ND^-$). According to Gibbs and Donnan, the $ND^-$ create an osmotic gradient and influence the distribution of the permeable ions across the membrane so that **at equilibrium**:

1. The product of diffusible ions in solutions I and II are equal ($36 \times 16 = 24 \times 24 = 576$).
2. Both the solutions remain electrically neutral.
3. The total number of every ion remains the same as that before equilibrium; however, the total ion concentration is higher on the side containing the $ND^-$.

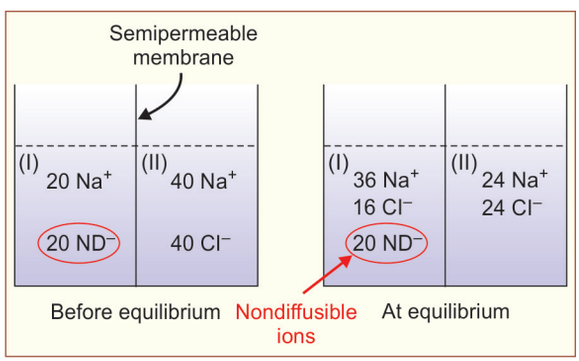


**Fig. 1.27:** Gibbs-Donnan membrane equilibrium

Gibbs-Donnan equilibrium has **important biological applications:**

- Responsible for the **differential solute** (glucose, urea, electrolytes, etc.) distribution between plasma and other biological fluids such as intracellular fluid, interstitial fluid, gastric juice, cerebrospinal fluid, etc.
- Explains the **osmotic gradient and pH gradient** between plasma and other biological fluids.
- Explains the **'chloride-shift'** in RBC.
- Finds its clinical application in **dialysis** (Chemistry to Clinics 1.12).

**Chemistry to Clinics 1.12: Dialysis**

Dialysis (Greek '*dialusis*' = 'dissolution'; '*dia*' = 'through'; '*lysis*' = 'loosening'), an artificial means to purify blood, works on the principle of Gibbs-Donnan membrane equilibrium. It is a useful maneuver to partly compensate for the loss of renal function in patients of renal failure. The blood (in case of **hemodialysis**) from a peripheral artery of the patient is allowed to flow on one side of a semipermeable membrane while an artificially prepared fluid of known solute concentration, called the **dialysate**, flows by the other side but in opposite direction (Fig. 1.28).

Proteins present in the blood are the large $ND^-$. The solute concentration in the dialysate is so designed as to maximize diffusion of water and solutes across the membrane. The net effect is the reduction of toxic molecules to low levels in the blood while improving the levels of the essential ones. The 'purified' blood is returned through a peripheral vein.

The basic procedure is the same in case of **peritoneal dialysis**. Here, the dialysate is run through a tube into the peritoneal cavity and the peritoneal membrane itself serves as the semipermeable membrane. The dialysate is retained for some time and eventually drained out through the tube, removing excess water and waste products.

## STEM CELLS

***Cell potency:*** Stem cells are the **master cells** and potential building blocks of the body because they can change into and thus create all other tissues, organs and systems in the body (Fig. 1.29).

This 'changing' is known as differentiation. Thus, 'multipotent' stem cells become 'committed' stem cells once they follow a particular line of differentiation (Table 1.6).

***Availability:*** Stem cells are found in the bone marrow and peripheral blood of adults and the umbilical cord blood of newborn infants. The cord blood cells are preferred because the baby's blood cells have not yet developed their typical set of antigens and because

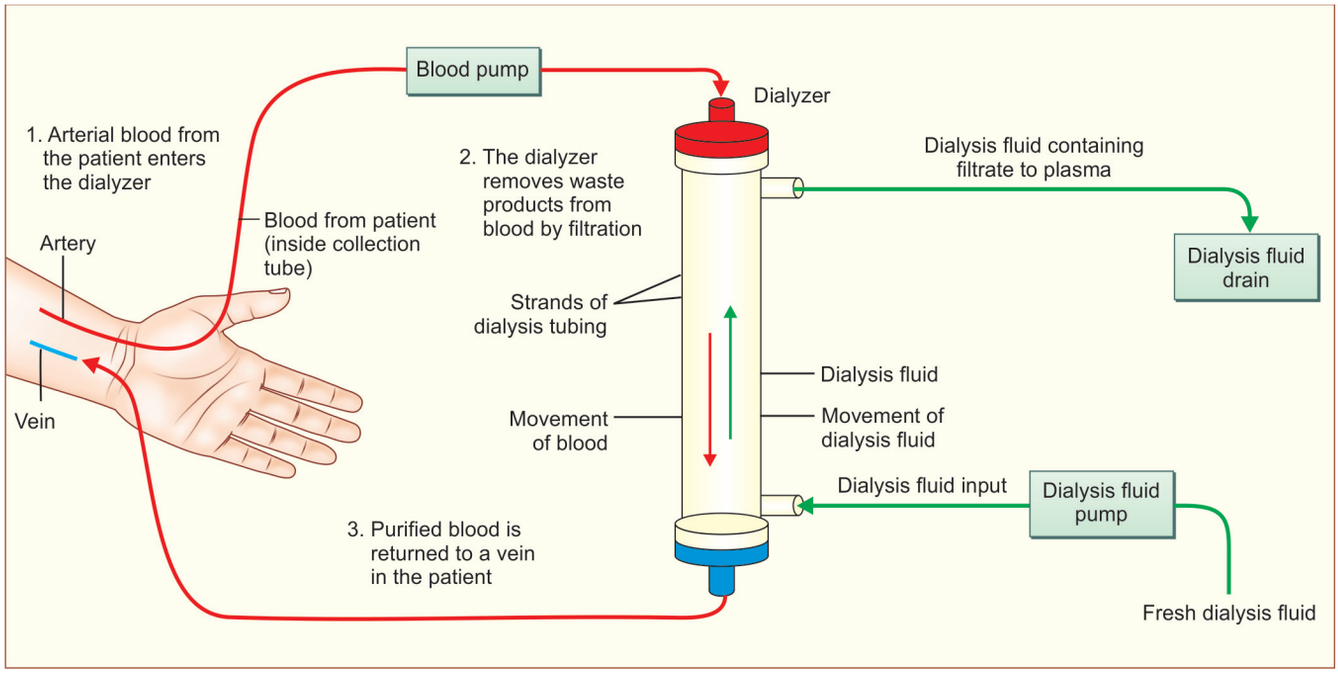


**Fig. 1.28:** Hemodialysis

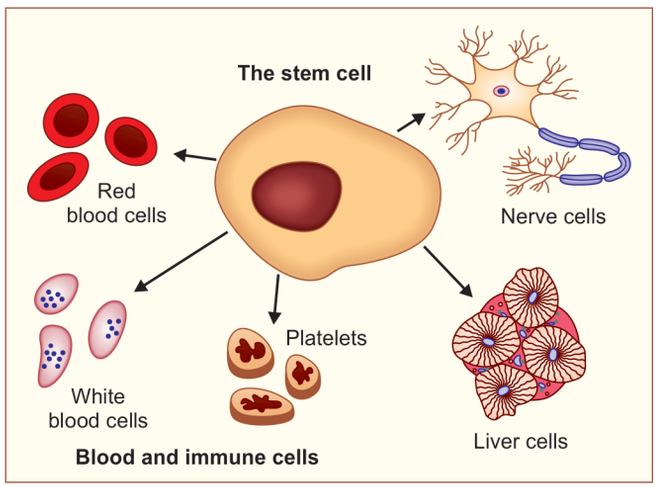


**Fig. 1.29:** Examples of cells derived from stem cells

umbilical cord blood lacks well-developed immune cells that usually cause graft-versus-host disease.

In addition to cells with blood-forming potential, few mesenchymal stem cells are also found in bone marrow. These cells can give rise to a large number of tissue cells, such as bone, cartilage, fat and connective tissue cells. However, such adult stem cells may be obtained from any tissue, are capable of self-renewal and are multipotent with the potential to form all cell types in the concerned tissue. Thus, **adult stem cells** are 'undifferentiated cells' in a 'differentiated tissue'.

**Table 1.6:** Stem cell potency

| Stem cells | Potency of differentiation |
|---|---|
| Totipotent stem cells | The zygote and cells derived from it during the first 4 days of conception give rise to all cell types and even whole organism |
| Pluripotent stem cells | Cells of the inner cell mass after 4 days of conception give rise to all cell types except placenta, but cannot form the whole organism |
| Multipotent stem cells | Form several cell types restricted to a particular tissue/organ, e.g. hemopoietic stem cells in the bone marrow (forming RBC, WBC and platelets) and neuronal stem cells (forming various cells of the nervous system) |
| Unipotent/committed stem cells | Form a specific cell type, e.g. erythroid progenitor cells and myeloid progenitor cells |

*Stem cell therapy:* The possibilities for stem cell therapy are unlimited, once we understand how to control their differentiation into all types of tissue cells:

- The major clinical use of stem cells to date has been to restore a patient's hemopoietic system that has been completely disrupted by radiation or chemo-

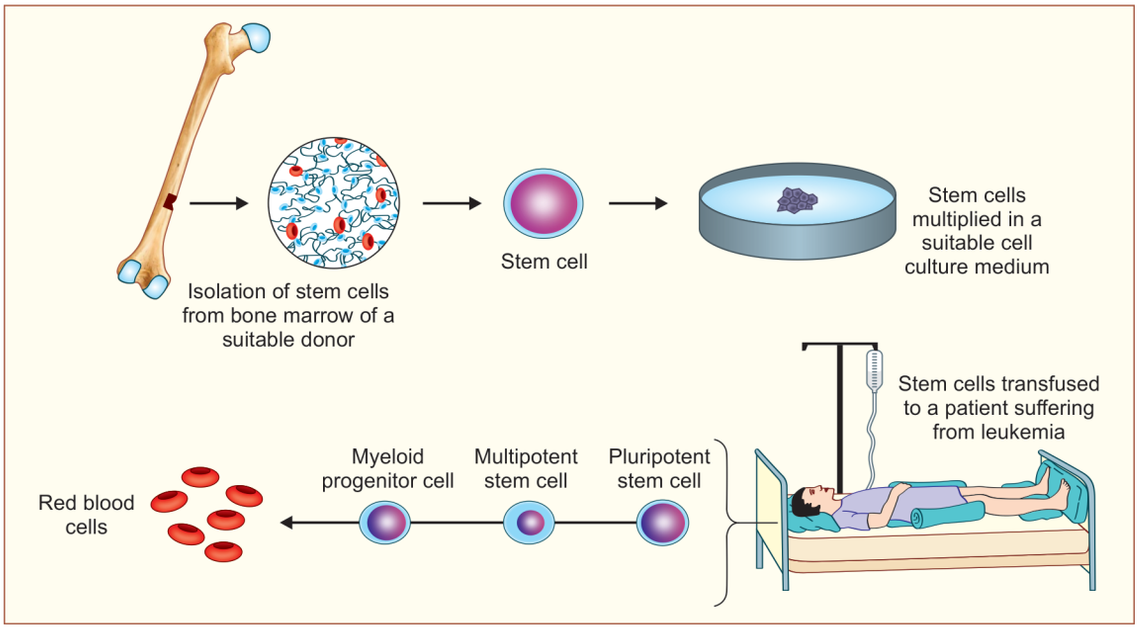


**Fig. 1.30:** A patient received treatment for leukemia (tumor of white blood cells) due to which his red blood cell count dropped resulting in severe anemia. By selecting a suitable donor and using **stem cell-based hematotherapy,** the red blood cells are restored

therapy for treatment of leukemia and solid tumors (Chemistry to Clinics 1.13).

- Based on the same principle, patients with AIDS could be supported by periodic infusions of T cell precursors.
- Infusions of neutrophil progenitors would help patients with cancer to recover from aggressive cancer therapy.
- Patients with certain anemias would benefit from infusions of red cell progenitors.

### Chemistry to Clinics 1.13: Stem Cell-based Hematotherapy

Traditionally, in bone marrow transplantation, the entire hematopoietic system of the recipient, including the most primitive multipotent stem cells, is ablated and restored with cells from the donor. However, using stem cell-based 'hematotherapy', only the specific committed stem cells are transplanted that develop along specific lineages into the mature cells that are nonfunctional in a patient with a particular disease. The healthy portion of the patient's hemopoietic system is thus kept intact (Fig. 1.30).

- Platelet progenitors may be used in patients with inborn or acquired thrombocytopenia.
- Treatment of some of the most common degenerative diseases such as Alzheimer's disease, Parkinson's disease, muscular dystrophy, spinal cord injury, stroke, and various liver and cardiac diseases.

*Role of molecular biology:* The clinical potential of stem cell therapy can be enhanced by combining traditional stem cell harvesting methods with modern molecular biology methods. Therapeutic cloning might enable the creation of embryonic stem cells that are genetically identical to the patients' cells. Embryonic stem cells are highly desirable to work with because they have the capacity to become any mature cell (totipotency). Existing stem cells can be genetically manipulated by replacing a defective or missing gene.

*Demerits of stem cell therapy:* One limitation for the use of stem cells lies in the problem of avoiding tumor growth because stem cells are naturally programmed to divide. Additionally, the benefits of stem cell therapy must be carefully balanced with ethical and social concerns.

## Some Important Questions

**1. Describe structure, functions, isolation and biochemical markers of various subcellular entities of a eukaryotic cell.**

**2. Discuss composition of a biological membrane.**

**3. Biomedical importance of stem cells.**

**4. Explain:**

i. Mitochondria as a powerhouse of the cell.
ii. Functions of the rough endoplasmic reticulum.
iii. Differences between nuclear and mitochondrial DNA.
iv. Molecular organization of a cell.
v. Differences between primary and secondary active transport.

**5. Describe:**

i. Fluid-mosaic model of cell membrane
ii. Important functions of a biological membrane
iii. Ion channels
iv. Carrier-mediated transport
v. Amino acid transport systems
vi. Transport disorders

**6. Write notes on:**

i. The nucleus
ii. Ribosomes
iii. The Golgi apparatus
iv. Matrix
v. Micelles
vi. Lipid bilayer
vii. Liposomes
viii. Integral membrane proteins
ix. Peripheral proteins
x. Nonmediated transport
xi. Channelopathies
xii. Nephrogenic diabetes insipidus
xiii. Endocytosis
xiv. Suicide bags
xv. Age pigments
xvi. Lysosomal disorders
xvii. Ionophores
xviii. Facilitated diffusion
xix. Group translocation
xx. Exocytosis
xxi. Pinocytosis
xxii. Gibbs-Donnan equilibrium

## Multiple Choice Questions

**1. The following is not a true organelle:**

A. Ribosome B. Mitochondrion
C. Lysosome D. Nucleus

**2. Galactosyltransferase is the biomarker for the following subcellular entity:**

A. Cell membrane
B. Cytoplasm
C. Golgi apparatus
D. Endoplasmic reticulum

**3. Protein synthesis occurs in:**

A. Ribosomes only
B. Mitochondria only
C. Ribosomes and mitochondria
D. Smooth endoplasmic reticulum and mitochondria

**4. Protein sorting occurs in:**

A. Golgi apparatus
B. Endoplasmic reticulum
C. Cell membrane
D. Mitochondria

**5. Mitochondrial DNA is:**

A. Paternally inherited and more prone to mutations than nuclear DNA
B. Paternally inherited and less prone to mutations than nuclear DNA
C. Maternally inherited and more prone to mutations than nuclear DNA
D. Maternally inherited and less prone to mutations than nuclear DNA

**6. Zellweger syndrome is due to absence or loss of functions of:**

A. Lysosomes
B. Peroxisomes
C. Ribosomes
D. Endoplasmic reticulum

**7. The ideal source for obtaining stem cells is:**

A. Adult peripheral blood
B. Adult bone marrow
C. Neonatal peripheral blood
D. Umbilical cord blood

**8. In the myelin sheath, lipid:protein ratio is nearly:**

A. 4:1 B. 1:4
C. 1:1 D. 3:2

**9. In the renal collecting tubules, expression of aquaporin 2 (AQP2) is increased by:**

A. ADH B. ANP
C. Aldosterone D. Angiotensin II

**10. Valinomycin and nigericin translocate:**

A. $K^+$ B. $Na^+$
C. $Ca^{2+}$ D. $Cl^-$