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Introduction to Biochemistry

The study of chemical processes within and relating to living organisms is called as **Biochemistry**. Sometimes it is also called as **biological chemistry**. The complexity of life is raised by biochemical processes. The chemical basis of biological molecules and its related process occur within living cells and between cells are focused and understand by biochemistry. This will help to study and understand tissues and organs, as well as organism structure and function. Structures, functions, and interactions of biological macromolecules, like proteins, nucleic acids, carbohydrates, and lipids are dealt by much of biochemistry. This biological macromolecule performs many of the functions associated with life and provides the structure of cells. The chemistry of the cell also depends on the reactions of smaller molecules and ions. These can be inorganic (**Example:** Water and metal ions) or organic (**Example:** The amino acids, which are used to synthesize proteins). Primarily in medicine, nutrition and agriculture the findings of biochemistry are applied. The causes and cures of diseases are investigated by biochemist in medicine. The effects of nutritional deficiencies and how to maintain health and wellness are studied in nutrition. Soil and fertilizers are investigated by biochemist in agriculture. They also try to discover ways to improve crop cultivation, crop storage, and pest control.

Cell

The structural and functional unit of life is cell. In all living organisms the basic unit of life is the cell. All organisms are made of cells or aggregates of cells. Unicellular organisms are organism composed of a single cell and multicellular organisms are organism composed of many cells. Independent existence and performing the essential functions of life are the capabilities of unicellular organisms. Live cell was first seen and described by Anton Von Leeuwenhoek and nucleus was later discovered Robert Brown. All the structural details of the cell were revealed by using electron microscope. Anything less than a complete structure of a cell does not ensure independent living.

Cells are the fundamental structural and functional unit of all living organisms or biological activity and are organized into subcellular organelles and each is assigned to specific function.

Composition of Cell

Elements: Living matter or cell is composed of many elements. Based on the quantities present in living matter elements are broadly classified into two major types.

1. **Major elements:** It constitutes about 90 % of dry body weight of human body. Carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorous (P) and sulfur (S) are the six major elements present in living matter.
2. **Minor elements:** Cell also contains several other elements in minute quantity which are functionally important. Sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), fluorine (F), chlorine (Cl), iodine (I), zinc (Zn), iron (Fe), copper (Cu), cobalt (Co), molybdenum (Mo) and selenium (Se) are minor elements present in the cells.

The most predominant and versatile element of life is carbon because it is capable to form infinite number of compounds. The reason behind is ability of carbon to form stable covalent bonds and unlimited carbon–carbon (C-C) chain. In living system about 90% of compounds invariably contain carbon.

Macromolecules or Biomolecules: Life is composed of lifeless chemical molecules. About 6,000 and 1,00,00 different types of molecules are present in bacterium and man, respectively. Among these only few are characterized till now. Proteins, nucleic acid (such as DNA and RNA), polysaccharides and lipids are the important macromolecules or biomolecules composed of amino acids, nucleotides, monosaccharides and fatty acids, respectively. Strictly speaking lipids are not polymers but fatty acids are present in majority of lipids. The building blocks and functions of important biomolecules of cells are summarized in Table 1.1.

Table 1.1 Building blocks and functions of important biomolecules of cells.

S. No	Macromolecules or Biomolecules	Repeating Unit or Building Blocks	Important Functions
1	Protein	Amino acids	Static and dynamic functions of cell and fundamental basis of structure.
2	Deoxyribonucleic acid (DNA)	Deoxyribonucleotides	Storehouse of genetic information.
3	Ribonucleic acid (RNA)	Ribonucleotides	Essentially needed for biosynthesis of protein.
4	Glycogen or Polysaccharide	Glucose or monosaccharide	Storage form of energy to meet short term demands.
5	Lipid	Fatty acid, glycerol	Structural components of membrane and storage form of energy to meet long term demands.

Structural hierarchy of organism: Elements forms building blocks and macromolecules are formed from building blocks. Supramolecular assemblies like membranes are formed from macromolecules such as proteins, nucleic acid (such as DNA and RNA), polysaccharides and lipids. This supramolecular assembly organizes into organelles, cells, tissues, organs and finally the whole organism. The structural hierarchy of organism is depicted in Figure 1.1.

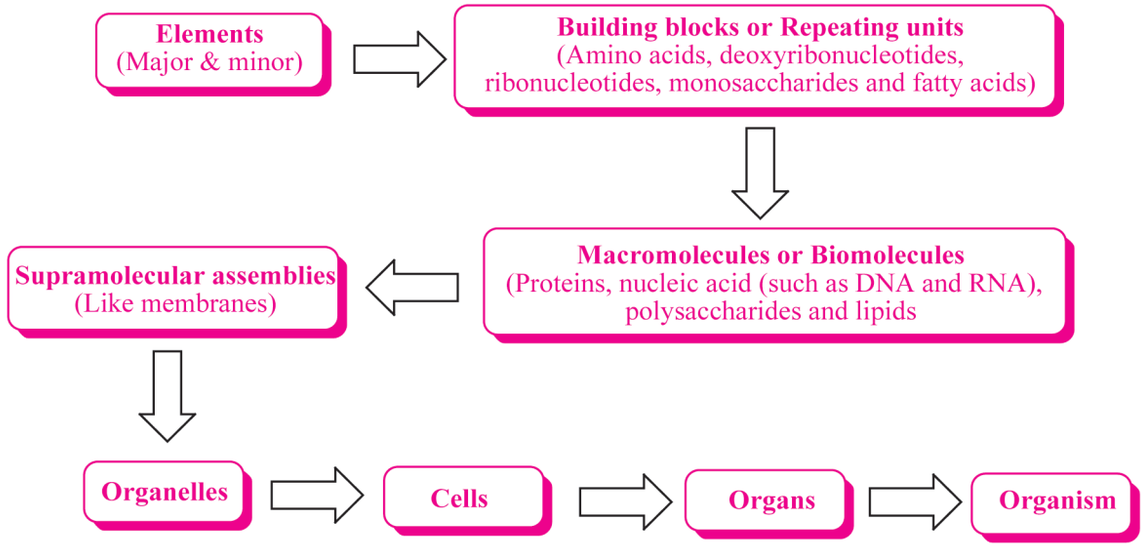


Figure 1.1 The structural hierarchy of organism.

Chemical composition of organism: The chemical composition of a normal healthy adult weighing 65 kg is summarized in Table 1.2. More than 60 % of the weight is contributed by the solvent of life i.e., water. Protein is mostly present in muscle and lipids are mostly present in adipose tissue contributes to weight followed by water. Minerals contributes about 6 % of body weight. Carbohydrate in the form of glycogen is present in low concentration and contributes less to weight.

Table 1.2 Chemical composition of a normal healthy adult weighing 65 kg.

S. No	Name of constituent	Composition (in percentage %)	Contribution to weight (in Kg)
1	Water	61.6	40
2	Protein	17.0	11
3	Lipid	13.8	9
4	Minerals	6.1	4
5	Carbohydrate	1.5	1
6	Total	100.0	65

Types of Cell

Based on the presence or absence of a membrane bound nucleus and other organelles, cells and hence organisms are broadly classified into two major types as,

1. Eukaryotic cell.
2. Prokaryotic cell.

A typical eukaryotic cell consists of a cell membrane, nucleus and cytoplasm and prokaryotic cell lacks membrane bound nucleus. Plant cells have a cell wall outside the cell membrane. **The volume of the cell is occupied by a semi-fluid matrix called cytoplasm in both prokaryotic and eukaryotic cells**.

Cells vary in their shape, size and activities/functions. **Example:** Only 0.3 µm is the length of smallest cell mycoplasmas, 3 to 5 µm is the length of bacteria and egg of an ostrich is the largest isolated single cell. 7.0 µm is the diameter of human red blood cells of multicellular organisms. Nerve cells are some of the longest cells. In addition to size, cells are also vary greatly in their shape. The shape of the cell may be columnar, polygonal, thread like, cuboid, disc-like, or even irregular. Based on the function they perform the shape of the cell may vary. Different shapes of cells are presented in Figure 1.2.

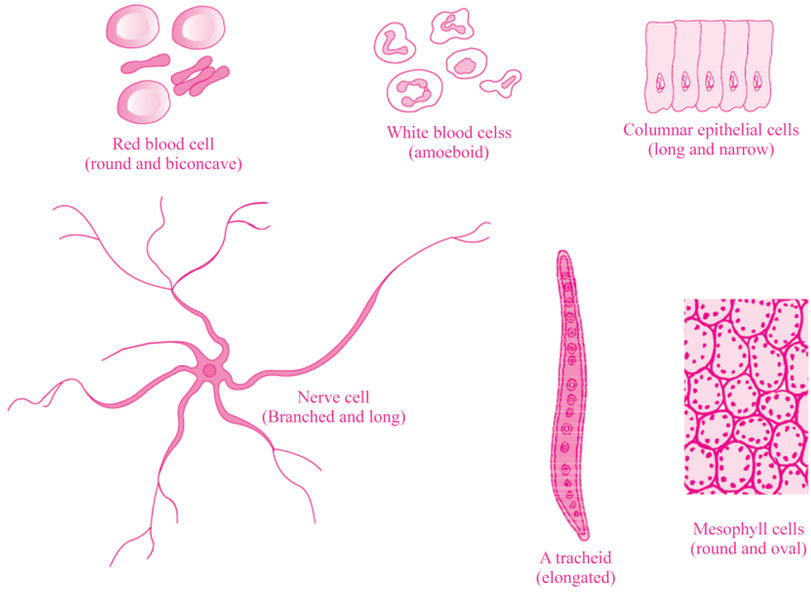


Figure 1.2 Different shapes of cells.

Prokaryotic Cells

In Greek "pro" means "before" and "karyon" means "nucleus". Prokaryotes lack nucleus and possess simple structure. In general, prokaryotic cells are smaller and multiply more rapidly as compared to

eukaryotic cells. Bacteria, blue-green algae, mycoplasma and PPLO (pleuro pneumonia like organisms) are examples for prokaryotic cells. The size and shape of prokaryotic cells vary greatly. Rod like (*bacillus*), spherical (*coccus*), comma shaped (*vibrio*) and spiral (*spirillum*) are the four basic shapes of bacteria.

A wide variety of shapes and functions are exhibited by prokaryotic cell but the fundamental organizations are similar. Except in mycoplasma, cell wall surrounds the cell membrane in all prokaryotes. Cytoplasm is the fluid matrix filling the cell and there is no well-defined nucleus. Nuclear membrane do not envelop the genetic material and is basically naked. Outside the genomic DNA, small circular DNA known as plasmid is present in many bacteria in addition to the genomic DNA (the single chromosome/ circular DNA). Unique phenotypic characters like antibiotic resistance are conferred to such bacteria by this plasmid DNA. With foreign DNA, bacterial transformation is monitored by using this plasmid DNA in higher classes. In eukaryotes nuclear membrane is present. Except for ribosomes no organelles are found in prokaryotic cells like the ones in eukaryotic cells. The characteristic of prokaryotes is mesosome which is a specialized differentiated form of cell membrane. They are essentially in the folding of cell membrane. A typical eukaryotic cell and its comparison with other organism is presented in Figure 1.3.

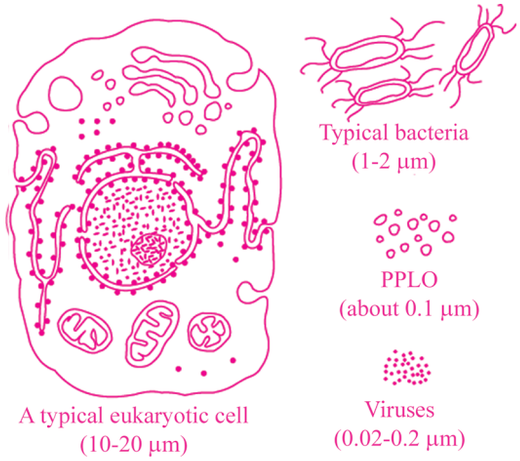


Figure 1.3 A typical eukaryotic cell and its comparison with other organism.

Cell envelope: Chemically complex cell envelope is present in many prokaryotic cells, mainly the bacterial cells. A tightly bound three layered structure is compose the cell envelope.

1. The outermost layer is glycocalyx
2. The centre layer cell wall
3. The inner layer plasma membrane.

Together these three layers perform single protective unit even though distinct functions are performed by each layer. Based on the cell envelope differences and response to Gram staining, bacteria are broadly classified into two major categories as Gram positive and Gram negative bacteria. Among different bacteria, composition and thickness of glycocalyx differs. In some bacteria the glycocalyx is slime layer (loose sheath) while in others it may be capsule (thick and tough). Apart from protection from bursting or collapsing, the cell wall prevents the bacterium by providing a strong structural support and the cell wall also determines the shape of the cell. In nature the plasma membrane is selectively permeable and interacts with the outside world. Structurally these membranes of prokaryotes are similar like eukaryotes.

In the cell extensions of plasma membrane, it forms a special membranous structure known as mesosome. Vesicles, tubules and lamellae are the different forms of extensions. Mesosomes help in cell wall formation, DNA replication and distribution to daughter cells. They also help in secretion processes, respiration, increasing the enzymatic content and surface area of the plasma membrane. Chromatophores which contain pigments are other membranous extensions into the cytoplasm which are present in some prokaryotes like cyanobacteria. Bacterial cells may be motile or non-motile. Flagella are the thin filamentous extensions from the bacterial cell wall present in motile bacterial cells. Bacteria show a range in the number and arrangement of flagella. Filament, hook and basal body are three parts of bacterial flagellum. The

longest portion of flagellum is the filament and it extends from the cell surface to the outside. In bacteria pili and fimbriae are also the surface structures besides flagella but they don't have any role in motility. The pili made up of special protein are elongated tubular structures and the fimbriae sprouting out of the cell are small bristle like fibers. It helps to attach bacteria to rocks and also to the host tissues in some bacteria.

Ribosomes: With the plasma membrane ribosomes are associated in prokaryotic cell. They are made of two subunits namely 50S and 30S units which when present together form 70S prokaryotic ribosomes and the size is about 15 to 20 nm. Ribosomes are the site of protein synthesis. Polysome or polyribosomes are the chain formed by attachment of several ribosomes into a single mRNA. The ribosomes of a polysome translate the mRNA into proteins.

Inclusion bodies: Inclusion bodies are the storage form of reserve material present in cytoplasm of prokaryotic cells. In cytoplasm they lie free and are not bound by any membrane. **Example:** Phosphate granules, cyanophycean granules and glycogen granules. Green, purple and blue green photosynthetic bacteria contain gas vacuoles.

Eukaryotic Cells

In Greek "eu" means "true" and "karyon" means "nucleus". Eukaryotes possess well defined nucleus and are more complex in structure and functions. Protists, plants, animals and fungi are examples for eukaryotic cells. Presence of membrane bound organelles with an extensive compartmentalization of cytoplasm is present in eukaryotic cells. With a nuclear envelope, an organized nucleus is present in eukaryotic cells. In addition, various complex cytoskeletal and locomotory structures are also present in eukaryotic cells. Chromosomes are the organized genetic material of eukaryotic cells.

Cell walls, large central vacuole and plastids are present in plant cells and in animal cells these are absent; hence all eukaryotic cells are not identical. In other way, centrioles are present in animal cells whereas in plant cells these are absent. Structure of plant and animal cells are presented in Figure 1.4 and Figure 1.5, respectively.

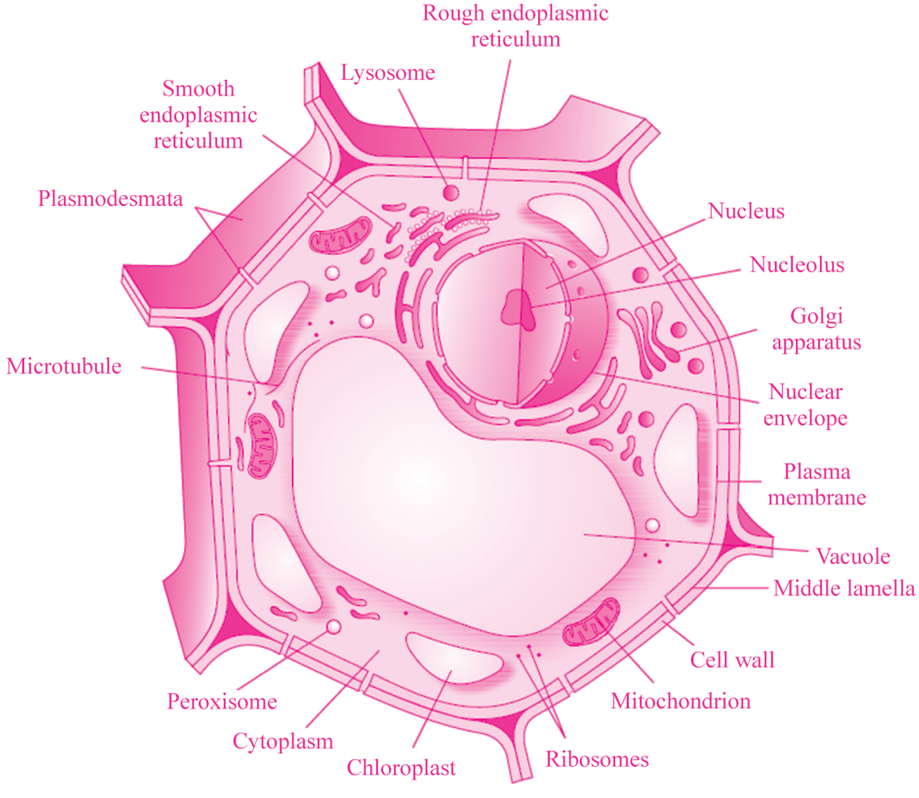


Figure 1.4 Structure of plant cell.

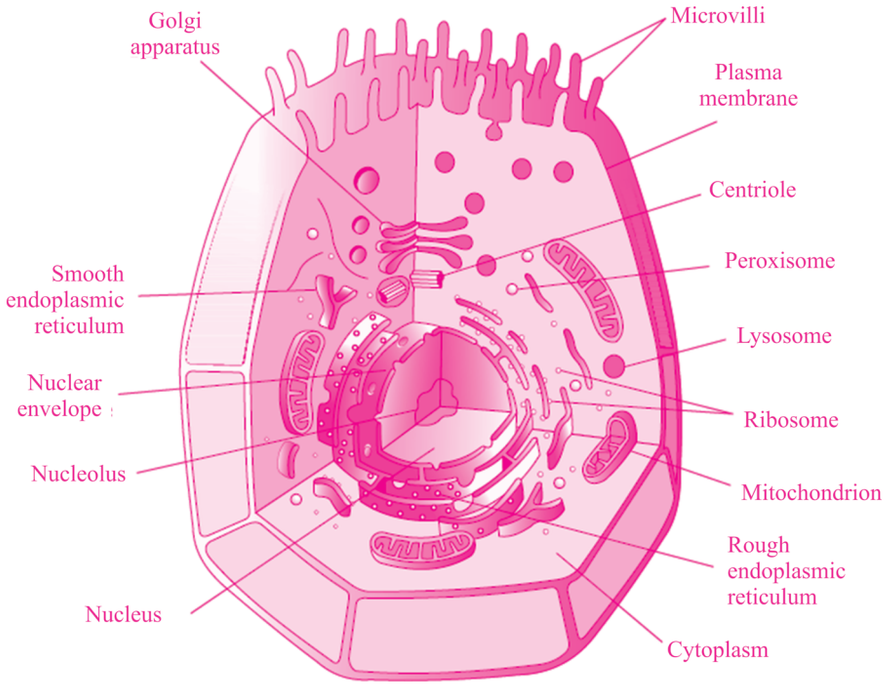


Figure 1.5 Structure of animal cell.

Human body possesses about 10^{14} cells with a size of 10 to 100 µm diameter. Plant cells possess rigid cell wall and chloroplasts which is the site of photosynthesis. Plasma membrane enveloped various subcellular organelles present in cells. Each subcellular organelles are isolated in pure form by differential centrifugation of tissue homogenate. Following are various organelles present in eukaryotic cells. They are,

1. Cell membrane
2. Nucleus
3. Mitochondria
4. Endoplasmic reticulum (ER)
5. Golgi complex
6. Lysosomes
7. Ribosomes
8. Microbodies or peroxisomes
9. Cytosol and cytoskeleton
10. Cilia and flagella
11. Centrosome and Centrioles
12. Cell wall
13. Vacuoles
14. Plastids

1. Cell Membrane:

Lipids and proteins are the major components of cell membrane. Phospholipids are the major lipids which are arranged in a bilayer. Within the membrane polar heads and hydrophobic tails of the lipids are arranged towards the outer sides and the inner part of the cell membrane, respectively. This ensures that cells are protected from the aqueous environment by the non-polar tail of saturated hydrocarbons present in cell membrane.

2. Nucleus:

It is the largest cellular organelle surrounded by the nuclear envelope, which consists of two parallel membranes with a perinuclear space between (10 to 50 nm). Between the materials present inside the nucleus and that of the cytoplasm, this perinuclear space forms a barrier. The outer membrane

usually remains continuous with the endoplasmic reticulum and also bears ribosomes on it. Fusion of the two membranes forms the nuclear envelope, at a number of places which is interrupted by minute pores known as nuclear pores having diameter of about 90 nm. Between the nucleus and the cytoplasm RNA and protein molecules are moved in both directions through these nuclear pores only. In general, per cell only one nucleus is present but frequently it is observed variations in the number of nuclei. Even some mature cells lack nucleus. For example erythrocytes of many mammals and sieve tube cells of vascular plants. The ground material present in the nucleus is commonly known as nuclear matrix or the nucleoplasm. *DNA polymerase* and *RNA polymerase* are rich in nucleoplasm. In addition, enzymes involved in glycolysis, citric acid cycle and HMP shunt pathways are also present in nucleoplasm. Nucleolus and chromatin are the component of nucleoplasm. Nucleolus is the dense body present in nucleoplasm and possess spherical structures. RNA particularly rRNA is present in nucleolus and it enters the cytosol through nuclear pores. Protein syntheses are actively carried out in the cells through larger and more numerous nucleoli.

Chromatin is a loose and indistinct network of nucleoprotein fibers present in the nucleus. Chromatin contains DNA and some basic proteins called histones, some non-histone proteins and also RNA. Nucleosomes contain DNA and histones and the assembly of nucleosomes constitutes chromatin fibers of chromosome (In Greek "chromo" means "color" and "soma" means "body"). A single human cell has approximately two meter long thread of DNA distributed among its forty six (twenty three pairs) chromosomes. Kinetochores are disc shaped structures present on the sides of the primary constriction or the centromere which is essentially present in every chromosome. Two chromatids of a chromosome are held by centromere. The chromosomes are broadly classified into four types depending on the centromere position as follows.

(a) **Metacentric chromosome:** Centromere is present in middle and forms two equal arms of the chromosome.

(b) **Sub-metacentric chromosome:** Centromere is present slightly away from the middle leads to one longer and one shorter arm of the chromosomes.

(c) **Acrocentric chromosome:** Centromere is present close to its end leads to one very long and one extremely short arm of the chromosomes.

(d) **Telocentric chromosome:** Centromere is present in terminal or sometimes a few chromosomes have non-staining secondary constrictions at a constant location leads to the appearance of a small fragment called the satellite.

3. Mitochondria:

In Greek "mitos" means "thread" and "chondros" means "granule". Under the microscope, mitochondria are not easily visible unless specifically stained. Depends on the physiological activity of the cells the number of mitochondria present in each cell may vary. In addition, considerable degree of variability is observed in terms of size and shape also. In general, it is having 0.2-1.0 μm (average 0.5 μm) diameter and 1.0-4.1 μm length with cylindrical or sausage shape. Mitochondria is composed of,

(a) **Double membrane (inner membrane and outer membrane)**: This double membrane bound structure present in each mitochondrion divides its lumen distinctly into two aqueous compartments (inner compartment and outer compartment). Cristae are the number of infoldings formed in the inner membrane towards the matrix and these cristae increases the surface area. Own specific enzymes present in the two membranes are associated with the mitochondrial function.

(b) **Matrix:** It is a dense homogeneous substance filled in the inner compartment and the outer membrane forms the continuous limiting boundary of the organelle.

Mitochondria are the sites of aerobic cellular respiration and energy metabolism. Mitochondria are considered as 'power houses' of the cell because they produce cellular energy in the form of ATP. Single circular DNA molecule, a few RNA molecules, ribosomes (70S) and the components required for protein synthesis are also present in the matrix. Fission divides the mitochondria. About one fifth of cell volume is occupied by 2,000 mitochondria. About 10 % of mitochondrial proteins are synthesized in mitochondria itself from their own independent protein synthesizing machinery.

Endomembrane System: Some of the membranous organelles are considered together as their functions are coordinated and these membranous organelles are collectively known as endomembrane system. **Organelles such as endoplasmic reticulum (ER), Golgi complex, lysosomes and vacuoles come under this endomembrane system.** Whereas organelles such as mitochondria, chloroplast and peroxisomes do not come under this endomembrane system because their functions are not coordinated with the above components.

4. **The Endoplasmic Reticulum (ER):**

Endoplasmic reticulum is a network or reticulum of tiny tubular structures scattered in the cytoplasm and it divides the intracellular space into two distinct compartments as,

(a) Luminal (inside ER) compartment and
(b) Extra luminal (cytoplasm) compartment

There are two different types of ER depending on the presence of ribosomes attached to their outer surface as follows.

(a) Rough endoplasmic reticulum (RER): This endoplasmic reticulum bearing ribosomes on their surface, hence they appeared rough. In the cells RER is frequently observed and it is involved actively in the synthesis of protein as it bears ribosomes. They are extensive and continuous with the outer membrane of the nucleus. As such microsomes do not occur in the cell. Microsomes are formed in small vesicles by disrupture of RER during the process of cell fractionation.

(b) Smooth endoplasmic reticulum (SER): Ribosomes are not present in this type of ER; hence, they appear smooth. Synthesis of lipids such as phospholipids, triacylglycerol and sterols mainly takes place in the SER of the cell. It also supplies calcium for cellular function along with metabolism of drug

5. **Golgi apparatus or Golgi bodies:**

Golgi bodies are the densely stained reticular structures present near the nucleus. Many disc-shaped, flat cisternae or sacs having diameter of 0.5 to 1.0 μm are present in Golgi apparatus. These are stacked parallel to each other. In a Golgi apparatus varied numbers of cisternae are present. Near the nucleus the Golgi cisternae are concentrically arranged with distinct the forming or convex cis face and the maturing or concave trans face. The forming and the maturing faces of the organelle are interconnected but entirely different. The principle function of the Golgi apparatus is acting as packaging materials, to be delivered either secreted outside the cell or to the intra-cellular targets. From the ER the materials in the form of vesicles to be packaged are fused with the *cis* face of the Golgi bodies and move towards the maturing face. For this reason, only Golgi bodies remain in close association with the ER. Before the number of proteins synthesized by ribosomes released from its *trans* face ER modifies in the cisternae of the Golgi bodies. Glycoproteins and glycolipids are mainly formed in the Golgi bodies because it is an important site for its formation.

6. **Lysosomes:**

In the Golgi apparatus by the process of packaging these are formed as single membrane bound vesicular structures. All types of hydrolytic enzymes such as *hydrolases* are present very rich in the isolated lysosomal vesicles. These hydrolytic enzymes are optimally active at the acidic pH and are capable of digesting biomolecules such as carbohydrates, proteins, lipids and nucleic acids. Hence, **lysosomes are regarded as digestive tract of the cell. Example:** *Lipases* digest lipids; *proteases* digest proteins; *carbohydrases* digest carbohydrates; *nucleases* digest nucleic acid.

Hydrolytic enzymes are responsible for maintaining the cellular compounds in a dynamic state through their degradation and recycling. The degraded product leaves the cell by diffusion for reutilization. Lipofuscin are the age pigments which are rich in lipids and proteins accumulate in the cells. When these hydrolytic enzymes are escaped into cytosol it results in destruction of functional biomolecules of the cells and leads to complication. **Example:** Arthritis, muscle disease and allergic disorder.

7. **Ribosomes:**

Ribosomes are the dense particles possessing granular structures composed of ribonucleic acid (RNA) and proteins. They are not surrounded by any membrane. Larger and smaller subunits are the two

subunits present in each ribosome. 60S and 40S are the two subunits of 80S ribosomes of eukaryotic cell; whereas 50S and 30S are the two subunits of 70S ribosomes of prokaryotic cell. 'S' denotes Svedberg's unit of sedimentation coefficient which is indirect measurement of density and size.

8. **Microbodies or Peroxisomes:**

These are single membrane bound minute vesicles which are spherical or oval in shape. It is present in both plant and animal cells. It contains various enzymes particularly *catalases* which protect the cells from toxic effects of hydrogen peroxide (H_2O_2) into water and carbon dioxide. It is also involved in the synthesis of plasmogens and glycolipids and oxidation of long chain fatty acids containing more than eighteen carbons. Glyoxysomes are present in plants which are a special type of peroxisomes involved in the glyoxylate pathway.

9. **Cytosol and cytoskeleton:**

Cytosol is a collective term representing cellular matrix. It is a compartment contains many enzymes, metabolites and salts in aqueous gel like medium.

Cytoskeletons are collective term representing an elaborate network of filamentous proteinaceous structures present in the cytoplasm consisting of microfilaments, microtubules and intermediate filaments. Mechanical support, motility and maintenance of the shape of the cell are the major functions of cytoskeleton.

10. **Cilia and Flagella:**

Hairs like outgrowth of the cell membrane covered with plasma membrane are known as cilia and flagella. Small structures which work like oars are cilia which cause the movement of either the surrounding fluid or the cell. When compared to cilia, flagella are longer and responsible for movement of cell. Flagella are also present in the prokaryotic bacteria but structurally differ from eukaryotic flagella. Axonemes are their core possesses a number of microtubules running parallel to the long axis. Nine doublets of radially arranged peripheral microtubules, and a pair of centrally located microtubules are present in the axoneme and this arrangement of axonemal microtubules is referred as the "9+2" array. Bridges connects the central tubules and central sheath also encloses it. Radial spoke connects to one of the tubules of each peripheral doublet; hence totally nine radial spokes are present. Linkers also interconnect the peripheral doublets. Basal bodies are the centriole-like structure from which both the cilium and flagellum are emerged.

11. **Centrosome and Centrioles:**

Centrioles are cylindrical structures present in organelle centrosome. An amorphous pericentriolar material surrounds the two centrioles of centrosome. In a centrosome both the centrioles lie perpendicular to each other in which each has an organization like the cartwheel. Nine evenly spaced peripheral fibrils of tubulin protein are present in centrioles. Each of the peripheral fibril is a triplet and the adjacent triplets are also linked. Hub is the proteinaceous substance present in the proximal region central part of the centriole. Radial spokes made of protein connects hub with tubules of the peripheral triplets. In animal cells during cell division spindle apparatus are raised from spindle fibers and the centrioles form the basal body of cilia or flagella.

12. **Cell Wall:**

In fungi and plants, cell wall forms an outer covering for the plasma membrane and the cell wall is a non-living rigid structure. The major functions of the cell wall are giving shape to the cell and protecting the cell from mechanical damage and infection. In addition, it also provides barrier to undesirable macromolecules and helps in cell-to-cell interaction. Cellulose, galactans, mannans and minerals like calcium carbonate are the components of cell wall of algae. Cellulose, hemicellulose, pectin and protein are the components of cell wall of other plants. The primary wall is the cell wall of a young plant cell, which is capable of growth. When the cell matures primary wall gradually diminishes with formation of the secondary wall on the inner (towards membrane) side of the cell. The different neighboring cells are holed or glued together by the middle lamella composed of calcium pectate. The cytoplasms of neighboring cells are connected by plasmodesmata which traversed the cell wall and middle lamellae.

13. **Vacuoles:**
    In the cytoplasm the membrane bound space found is known as vacuole. Excretory product sap, water and other not useful materials are present in vacuoles. Tonoplasts are the single membrane which bounds the vacuole. 90 % volume of the plant cell is occupied by vacuoles. In plants, compared to cytoplasm in vacuole the concentration of tonoplast is significantly higher. Hence, transport of a number of ions and other materials into the vacuole is facilitated by the tonoplast against concentration gradients. For osmoregulation and excretion, the contractile vacuole present in a*moeba* is important. Food particles engulfment forms food vacuoles in many cells as in protists.
14. **Plastids:**
    All plant cells and euglenoids contains plastids. They are large and bear some specific pigments which provide specific colors to the plants. Plastids are broadly classified into three types based on the type of pigments present in it as follows,
    1. **Chloroplasts:** Chlorophyll and carotenoid pigments are present in chloroplast. Essential light energy necessary for photosynthesis is trapped by chloroplasts. In the mesophyll cells of the leaves, majority of the chloroplasts of the green plants are found. The space limited by the inner membrane of the chloroplast is called the stroma. A number of organized flattened membranous sacs called the thylakoids, are present in the stroma. The membrane of the thylakoids encloses 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.
    2. **Chromoplasts:** Fat soluble carotenoid pigments like xanthophylls, carotene and others are present in chromoplast. Yellow, orange or red color was imparted to the plant by chromoplast.
    3. **Leucoplasts:** These are colorless plastids of varied shapes and sizes with stored nutrients. **Example:** Amyloplasts of potato store carbohydrates (starch); elaioplasts store oils and fats whereas the aleuroplasts store proteins.

Comparison between Prokaryotes and Eukaryotes

The various characteristics of prokaryotic cells are compared with eukaryotic cell in the Table 1.3.

Table 1.3 Comparison between prokaryotes and eukaryotes.

S. No	Characteristic	Prokaryotic cell	Eukaryotic cell
1	Size	1 to 10 nm (Smaller than eukaryotes).	10 to 100 nm (Larger than prokaryotes).
2	Nucleus	No well-defined nucleus present, histones are absent and DNA is found in nucleoid.	Well defined nucleus surrounded by membrane is present and DNA associated with histone is present.
3	Cell membrane	Rigid cell wall envelopes the cell.	Flexible plasma membrane envelopes the cell.
4	Subcellular organelles	Not present.	Mitochondria, nucleus, lysosomes, etc are present.
5	Cytoplasm	Cytoskeleton and organelles are absent.	Cytoskeleton and organelles are present with a network of tubules and filaments.
6	Energy metabolism	Enzymes of energy metabolism are bound to membrane as mitochondria are absent.	Enzymes of energy metabolism are present in mitochondria.
7	Cell division	Cell division takes place by fission and not by mitosis.	Cell division takes place by mitosis.

Biochemical Organization of Cell Membrane

The cell membrane is an envelope which surrounds the cell. From the external environment cells are separated and protected by plasma membrane. In addition to that cell is connected to its environment by this plasma membrane only. Moreover, plasma membrane also surrounds the subcellular organelles such as mitochondria, nucleus and lysosomes.

Composition of Membrane

From tissue to tissue the actual composition of membrane varies. In general, the followings are the three substances which are the major components of cell membrane.

1. **Lipids:** In animal cell membrane amphipathic lipids (Lipids possessing both hydrophilic and hydrophobic groups) such as glycolipids, phospholipids and cholesterol are found.
2. **Proteins:** The proteins present in the membrane are broadly classified into two major types as extrinsic protein and intrinsic protein.
3. **Carbohydrates:** Glycocalyx are the thick coating of complex polysaccharides present in many animal cell membranes. In the tissue the oligosaccharides of glycocalyx interacts with collagen of intracellular matrix (Body substance which consists of ground substance and connective tissue fibers).

Structure of Membrane

In the year 1935, Davson proposed the lipid bilayer model for membrane structure and it was modified by Danielle. For membrane structure the most acceptable model and more recent model is "**Fluid mosaic model**" proposed by Singer and Niclolson. 5 to 8 nm is the usual thickness of the biological membrane. Lipid bilayer is the essential component of a membrane. At the core of the bilayer, non-polar (hydrophobic) region of the lipids face each other and the polar (hydrophilic) region face out ward. In this lipid bilayer globular proteins are embedded irregularly. These globular proteins are broadly classified into two major categories based on their position and type of attachment.

1. **Peripheral or extrinsic membrane protein:** These proteins lie on the surface of membrane and loosely bound to the surface membrane. Hence it can be easily separated from the membrane. **Example:** Cytochrome c of mitochondria.
2. **Integral or intrinsic membrane protein:** These integral proteins are partially or totally buried in the membrane and tightly bound to the lipid bilayer. Hence it cannot be easily separated from the membrane and it can be separated using detergents or organic solvents. **Example:** Cytochrome P_{450} and receptors of hormones.

According to this, the quasi-fluid nature of lipid enables lateral movement of proteins within the overall bilayer. This ability to move within the membrane is measured as its fluidity. The fluid nature of the membrane is also important from the point of view of functions like cell growth, formation of intercellular junctions, secretion, endocytosis, cell division etc. The proteins are distributed irregularly in membrane; hence, the membrane is asymmetric in nature. Like mosaic or ceramic tile, the membranes appeared due to arrangement of lipid bilayer and protein subunits. The membrane freely changes unlike a fixed ceramic tile. That's why the structure of the membranes is considered as fluid mosaic model. The fluid mosaic model of membrane structure is presented in Figure 1.6.

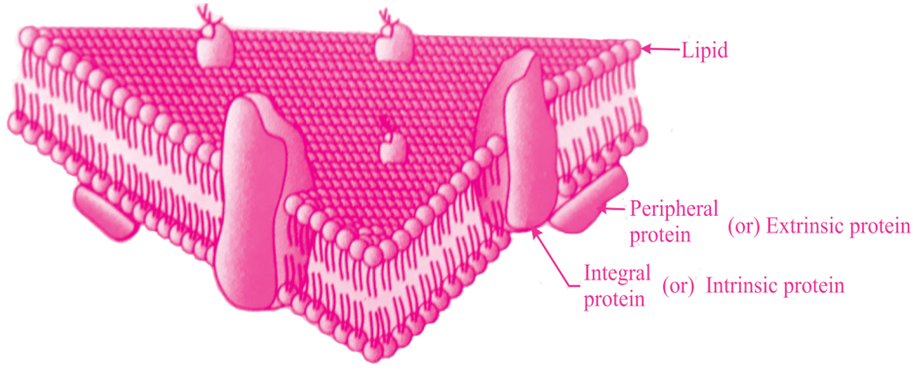


Figure 1.6 The membrane structure (Fluid mosaic model).

Transport of the Molecules Across Membranes

One of the most important functions of the plasma membrane is the transport of the molecules across it. Different types of transport of molecules across membranes are presented in Figure 1.7. The membrane is selectively permeable to some molecules present on either side of it. Depending on the size of molecules the transport processes across membranes are classified into two categories as,

1. Transport of metabolites or solutes
2. Transport of macromolecules

Transport of Metabolites or Solutes

In general, membrane forms a barrier for the free passage of compounds across the membrane.

Mechanism of transport system: Different mechanism involved in transport of metabolites or solutes is presented in Figure 1.8. Through membrane, metabolites or solutes are transported by two different mechanisms. They are,

(a) Passive transport
(b) Active transport

(a) Passive transport: It occurs along a concentration gradient (Solute moves from higher concentration to lower concentration) and this process is not dependent on the supply of metabolic energy (ATP is not necessary for passive transport). It was further subdivided into two types based on the requirements of carrier molecule as follows,

(i) Passive diffusion or Simple diffusion
(ii) Facilitated diffusion

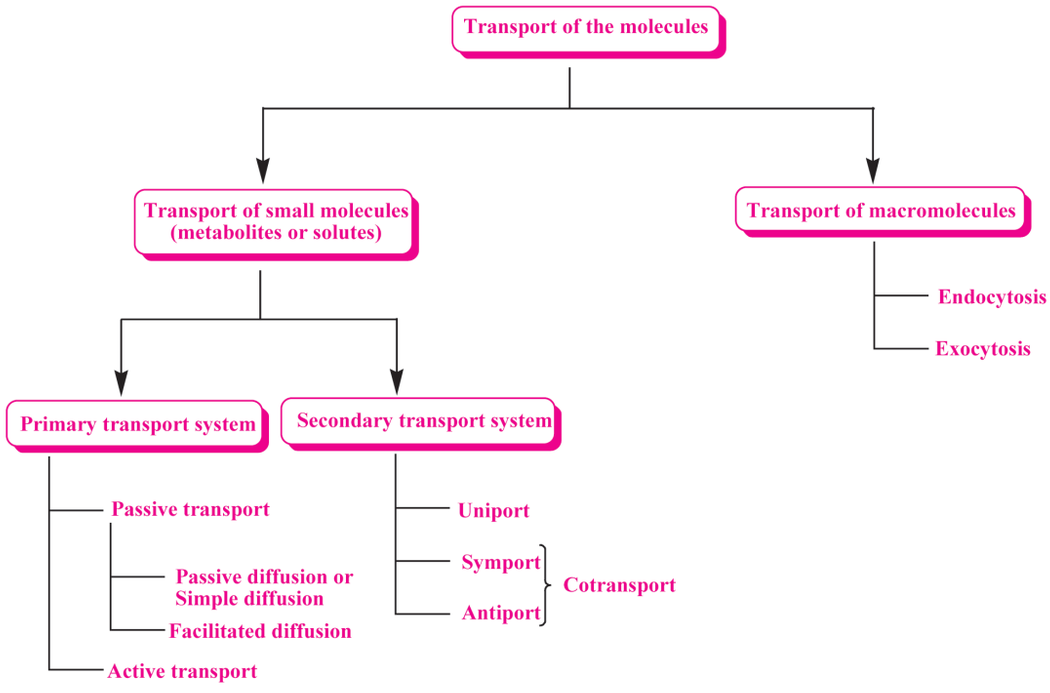


Figure 1.7 Types of transport of molecules across membranes.

(i) Passive diffusion or Simple diffusion: This process is a very simple process in which solutes move across the membrane along the concentration gradient. Presence of carrier or transport protein and supply of metabolic energy is not needed for this transport process. **Example:** Passage of water and gases.

Passive transport of water – Osmosis: The phenomenon of movement of water from dilute solution (Low osmotic pressure) to concentrated solution (High osmotic pressure) across the biological membrane is known as osmosis. In the body, movement of water occurs through osmosis which does not require any energy. Edema, diarrhea, cholera and inflammation are certain medical and health complications due to the disturbances in osmosis.

(ii) **Facilitated diffusion:** This process is also somewhat similar to passive diffusion. Like passive diffusion, solutes or metabolites move across the membrane along the concentration gradient and the supply of metabolic energy is not needed for this transport process also. The most important difference between passive and facilitated diffusion is presence of carrier or transport protein. Facilitated diffusion occurs through the mediation of carrier or transport protein; whereas passive diffusion occurs without mediation of carrier or transport protein. **Example:** Transport of active form of fatty acid i.e., acyl CoA (Carrier molecule: Carnitine); transport of glucose (Carrier molecule: Glucose transporter); transport of galactose, leucine and phenylalanine.

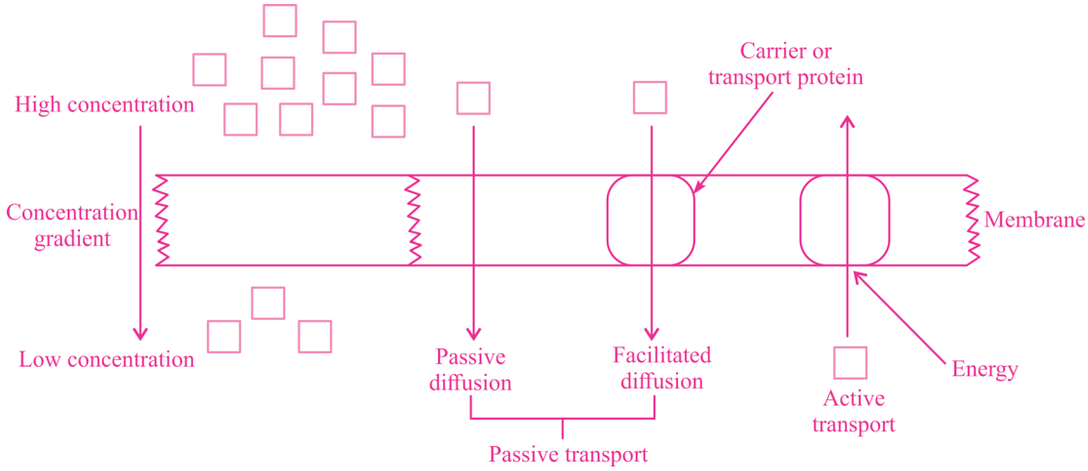


Figure 1.8 Transport of molecules across biological membrane.

Ping-Pong model: The occurrence of facilitated diffusion is explained by Ping-Pong model. Two conformations namely Ping conformation and Pong conformation exists for carrier or transport protein according to this mechanism. The carrier or transport protein exposed to the high solute concentration side in the "pong" conformation. This leads to attachment or binding of solute to specific sites of the carrier protein. Latter, carrier or transport protein undergoes conformational changes and exposed to the low solute concentration side in the "ping" conformation and releases solute molecule. Facilitated diffusion is generally regulated by hormones. This model is schematically represented in Figure 1.9. **Example:** Insulin increases transport of glucose in muscle and adipose tissue; transport of amino acid in liver and other tissue.

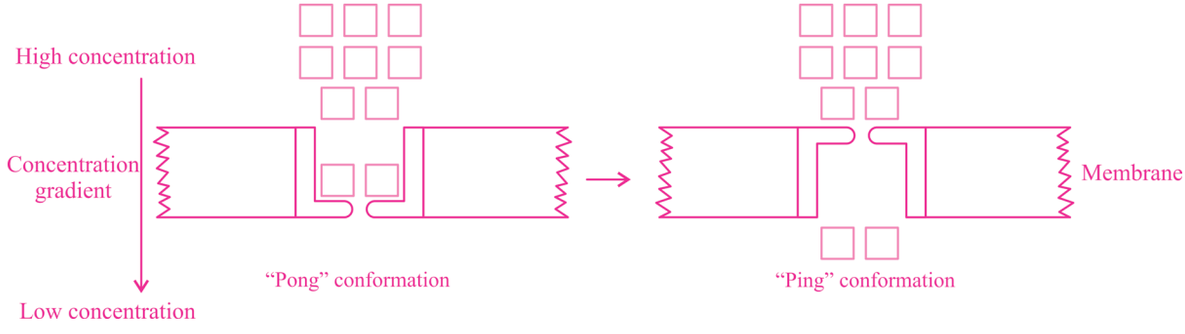


Figure 1.9 Facilitated diffusion – Ping Pong model.

(b) Active transport: It occurs against a concentration gradient (solute moves from lower concentration to higher concentration) and this process is dependent on the supply of metabolic energy (ATP is necessary for active transport). Like facilitated diffusion this process is also a carrier mediated process. Ion pumps particularly sodium-potassium (Na^+-K^+) pumps through the involvement of Na^+-K^+ *ATPase* or ion transporting *ATPase* are good examples for active transport process.

Sodium-potassium (Na^+-K^+) pump: Cells essentially need high intracellular potassium (K^+) concentration and low sodium (Na^+) concentration for their survival. Optimal glycolysis (*Pyruvate kinase* is dependent on potassium (K^+) concentration) and biosynthesis of proteins need high intracellular potassium (K^+) concentration. In addition, for nerve impulse transmission across plasma membrane sodium (Na^+) and potassium (K^+) gradients are needed. Sodium-potassium (Na^+-K^+) pump is diagrammatically represented in Figure 1.10.

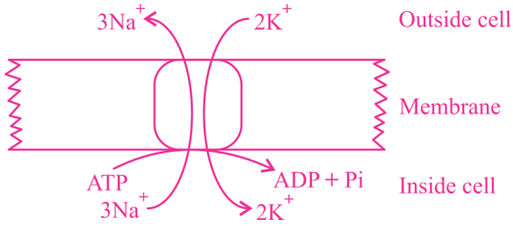


Figure 1.10 Sodium-potassium (Na^+-K^+) pump – An active transport.

In the cell, high potassium (K^+) concentration and low sodium (Na^+) concentration is maintained by the sodium-potassium (Na^+-K^+) pump. The *Na^+-K^+ ATPase* are the enzyme which is an integral plasma membrane protein having 2,50,000 molecular weight brought the above Na^+-K^+ concentration in the cells. Two α and two β subunits are present in *Na^+-K^+ ATPase*, hence it is represented as $(\alpha\beta)_2$. *Sodium-potassium (Na^+-K^+) ATPase* pumps three sodium (Na^+) to outside the cell from inside and brings two potassium (K^+) to inside the cell from outside. This requisite cytosolic sodium (Na^+) and potassium (K^+) levels are maintained using sodium-potassium (Na^+-K^+) pump by utilizing major portion of the cellular ATP (In nerve cells up to 70 %).

$$3\ Na^+\ (in) + 2\ K^+\ (out) + ATP \longrightarrow 3\ Na^+\ (out) + 2\ K^+\ (in) + ADP + Pi$$

Inhibitors of *Na^+-K^+ ATPase*: The followings are the two important inhibitors of the *Na^+-K^+ ATPase*.

1. **Ouabain:** It is a steroid derivative extracted from the seeds of an African shrub which inhibits *Na^+-K^+ ATPase*. Tribals in Africa use ouabain as poison to tip the hunting arrows (Arrow poison).
2. **Digoxin:** It is another *Na^+-K^+ ATPase* inhibitor and chemically it is steroid glycoside. In general digoxin improves cardiac contractility, hence used in the treatment of CHF (Congestive heart failure).

Types of Transport System

Based on the movement of molecules, transport systems are broadly classified into three major types as follows.

1. **Uniport system:** In this transport system, single molecules are transported through the membranes. **Example:** Transport of glucose to erythrocytes.
2. **Symport system:** In this transport system two different molecules are simultaneously transported in the same direction through the membrane. **Example:** From the gut sodium (Na^+) and glucose are transported to the intestinal mucosal cells.

3. **Antiport system:** In this transport system through the membrane two different molecules are simultaneously transported in the opposite direction. **Example:** Transport of chloride (Cl^-) and bicarbonate (HCO_3^-) in erythrocytes. Proton pump in the stomach is another example for antiport system of gastric parietal cells. In the lumen of stomach high acidic (pH ~ 1) condition is brought by the enzyme *H^+-K^+ ATPase*. For hydrolysis of one molecule of ATP, proton pump antiport two cytoplasmic protons ($2H^+$) and two extracellular potassium (K^+). The proton (H^+) combines with the chloride ion (Cl^-) secreted by the chloride ion (Cl^-) channel to form gastric HCl. Peptic ulcer is treated using omeprazole which is a drug that inhibits *H^+-K^+ ATPase* and results in decreased secretion of HCl.

   All the above-mentioned transport systems such as uniport systems, symport systems and antiport systems are considered as secondary active transport systems and are represented in Figure 1.11.

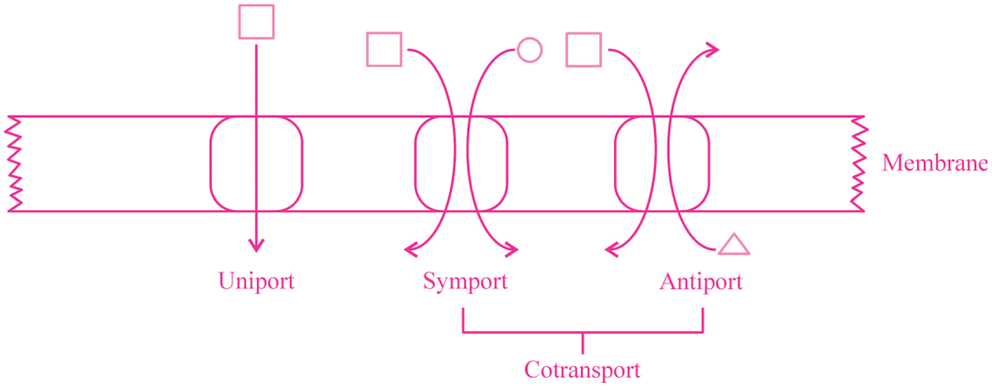


Figure 1.11 Different types of transport systems based on the movement of molecules.

Cotransport system: Through the membrane, transport of a substance coupled to the spontaneous movement of another substance is termed as cotransport system. Symport systems and antiport systems are very good examples for cotransport systems. Sodium (Na^+) cotransport system is another example. By this system amino acids and sugars are transported into the cells. Glucose (or amino acids) is passed into the cells with simultaneous movement of sodium (Na^+). Later sodium (Na^+) is pumped out from inside through *Na^+-K^+ ATPase* with the involvement of ATP.

Transport of Macromolecules

Across the membrane the transport of macromolecules such as proteins, polysaccharides and polynucleotides are also equally important. Two independent mechanisms are involved in the transport of macromolecules & are presented in Figure 1.12. They are,

1. **Endocytosis:** The processes of intake of macromolecules by the cells from the outside are termed as endocytosis. **Example:** Uptake of LDL by cells.
2. **Exocytosis:** The processes of release of macromolecules from the cells to the outside is termed as exocytosis. **Example:** Secretion of hormones such as insulin, parathyroid hormones from the cell.

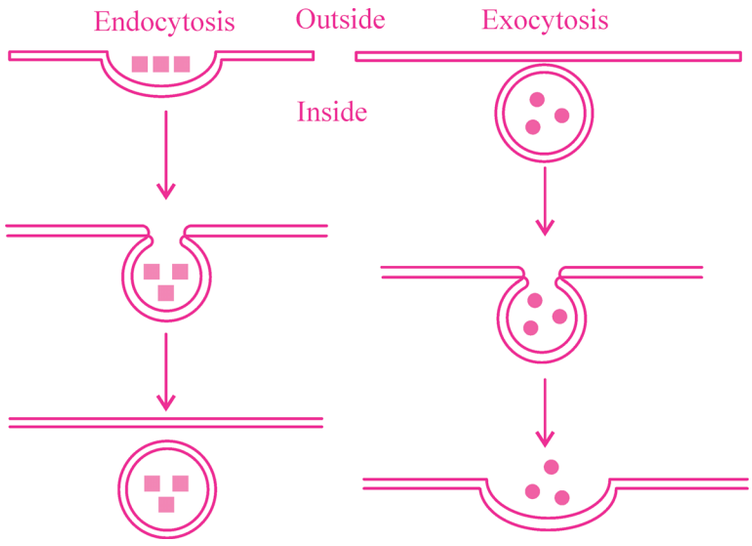


Figure 1.12 Transport of macromolecules.

Disorders of Membrane Transport System

Number of pathological conditions occur due to alterations in the membrane transport systems. The followings are some important diseases due to loss of membrane transport systems.

1. **Hartnup's disease:** In intestinal cells and renal tubules transport of neutral amino acids are decreased.
2. **Cystinuria:** It is the clinical condition in which excretion of cysteine, lysine, arginine and ornithine in urine is increased and leads to formation of renal cystine stones.
3. **Decreased glucose uptake:** Lack of specific sodium-glucose transporter decreases glucose uptake in some individuals.
4. **Decreased renal reabsorption of phosphate:** In vitamin D resistant rickets renal reabsorption of phosphate is decreased.

Bioenergetics (or) Biochemical Thermodynamics

Role of high energy compounds in biological process and basic knowledge of bioenergetics is very useful for better understanding of biological oxidation.

Study of energy changes (utilization and transfer) in biochemical reaction is termed as bioenergetics or biochemical thermodynamics. In bioenergetics mechanism of chemical reaction is not concerned but it concerned about the initial and final states of energy components of reactants. Based on energy released or consumed in biochemical reaction it was broadly classified into two types. They are,

1. **Exergonic reaction:** In this type biochemical reaction energy is released. ΔG^{o} value of this reaction is negative and the reactions will take place spontaneously. Almost all catabolic reactions are exergonic reactions. **Example:** Breakdown of ATP into ADP and inorganic phosphate liberates 7.3 Cal/mol energy.

$$ATP + H_2O \longrightarrow ADP + Pi \qquad (\Delta G^{\circ} = -7.3 \text{ Cal/mol})$$

2. **Endergonic reaction:** In this biochemical reaction energy is consumed or utilized by the reactants. It needs energy; hence, energy must be supplied. ΔG^{o} value of this reaction is positive and the reactions will not take place spontaneously. Almost all anabolic reactions, muscle contraction, nervous excitation, etc. are good examples for endergonic reactions. **Example:** Synthesis of ATP from ADP and inorganic phosphate. This reaction occurs only when 7.3 Cal/mol energy is supplied at least.

$$ADP + Pi \longrightarrow ATP + H_2O \quad (\Delta G^{O} = + 7.3 \text{ Cal/mol})$$

Terms used in Bioenergetics:

To understand the bioenergetics reactions, it is necessary to know about the following terms

1. Free energy
2. Enthalpy
3. Entropy

1. **Free energy:** It is defined as the energy actually available for utilization or to do work. The feasibility of chemical reaction is predicted valuably using changes in free energy and is represented by the symbol "ΔG". If the reaction is accompanied by decrease in free energy then the reaction can occur spontaneously.

   Standard free energy change: It is defined as the free energy change when the reactants or products are at a concentration of 1 mol/*l* at pH 7.0. This standard free energy is denoted by the symbol "ΔG°".

   The free energy change (ΔG) may be either,

   (a) Negative free energy change: In a chemical reaction if there is a loss of free energy then ΔG is represented by negative sign and the reaction is called as exergonic reaction and the reaction proceeds spontaneously. Free energy changes of almost all catabolic reactions possess negative sign only. **Example:** Breakdown of ATP into ADP and inorganic phosphate liberates 7.3 Cal/mol energy.

$$ATP + H_2O \longrightarrow ADP + Pi \qquad (\mathbf{\Delta G^{\circ} = - 7.3 \text{ Cal/mol}})$$

   (b) Positive free energy change: In a chemical reaction if free energy is supplied then the ΔG is represented by positive sign and the reaction is called as endergonic reaction and the reaction does not proceed spontaneously. Free energy changes of almost all anabolic reactions possess positive sign only. **Example:** Synthesis of ATP from ADP and inorganic phosphate and the reaction occurs only when 7.3 Cal/mol energy is supplied at least.

$$ADP + Pi \longrightarrow ATP + H_2O \quad (\Delta G^{O} = + 7.3 \text{ Cal/mol})$$

   (c) Zero free energy change: In a chemical reaction ΔG becomes zero when it is at equilibrium.

$$A \rightleftharpoons B \qquad (\mathbf{\Delta G^{\circ} = 0})$$

The free energy change (ΔG) is generally dependent on the actual concentrations of reactants and products at a constant temperature and pressure. Consider the below reaction in which reactant "A" is converted to product "B".

$$\underset{\text{(Reactant)}}{\mathbf{A}} \longrightarrow \underset{\text{(Product)}}{\mathbf{B}}$$

The following mathematical relationship can be derived when the reactant "A" is converted to product "B".

$$\Delta G = \Delta G^{o} + RT\, ln \frac{[B]}{[A]}$$

Where,

ΔG = Free energy change
ΔG° = Standard free energy change
R = 1.987 Cal / mol (Gas constant)
T = Absolute temperature in Kelvin (273 + °C)
ln = Natural logarithm
[B] = Concentration of product
[A] = Concentration of reactant

When reaction is at equilibrium then the free energy change is zero i.e., ΔG = 0. Substitute the value of ΔG in above equation.

$$0 = \Delta G^{o} + RT\, ln \frac{[B]_{eq}}{[A]_{eq}}$$

Therefore, $$\Delta G^{o} = -RT\, ln \frac{[B]_{eq}}{[A]_{eq}}$$

Where, K_{eq} = Equilibrium constant

The free energy change (ΔG) is an additive value for pathways. A series of reactions are often involved in biochemical pathways. In such reaction the free energy change (ΔG) is an additive value. Whether the particular pathway will proceed or not is crucially determined by the sum of the free energy change (ΔG). The pathway can operate when the sum of the free energy change (ΔG) is negative even though some of the individual reactions may have positive free energy change (ΔG).

2. **Enthalpy:** It is a measure of the change in the heat content of the reactants compared to products. It is denoted by the symbol "ΔH". During the thermodynamic reaction either the heat may be released or absorbed. Based on this the chemical reactions are broadly classified into two major types as,
   - **(a) Exothermic reaction:** During a chemical reaction if the heat is released then the reaction is said to be exothermic reaction. **Example:** Sodium hydroxide dissolved in water.
   - **(b) Endothermic reaction:** During a chemical reaction if the heat is absorbed then the reaction is said to be endothermic reaction. **Example:** Benedict's test, Fehling's test, etc.
3. **Entropy:** It is the change in the randomness or disorder of reactants and products. It is usually represented by "ΔS". When the reaction attains equilibrium, entropy attains a maximum. In general, temporary decrease in entropy was observed in the reactions of biological systems.

Relationship between the change of free energy, enthalpy and entropy:

The relationship between the change of free energy, enthalpy and entropy is expressed in the below mentioned equation.

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

Where,

ΔG = Free energy change

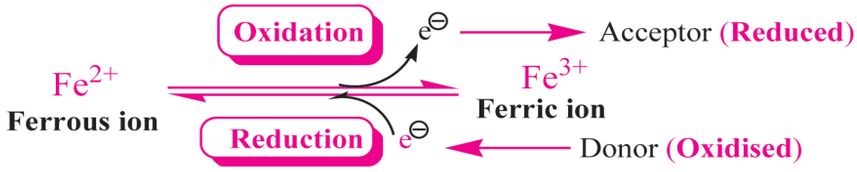
ΔH = Enthalpy

T = Absolute temperature in Kelvin (273 + °C)

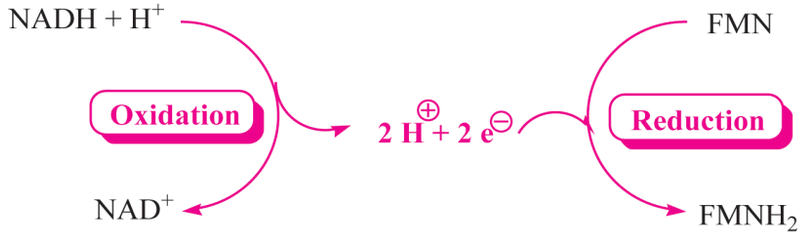
ΔS = Entropy

Biological Oxidation

Oxidation is defined as addition of oxygen (or) loss of hydrogen (or) loss of electrons and reduction is defined as loss of oxygen (or) gain of hydrogen (or) gain of electron. The electron lost in the oxidation is accepted by acceptor which is said to be reduced. Both oxidation and reduction are coupled with each other. Hence oxidation–reduction reactions are commonly known as redox reaction (If one compound is getting oxidized the other one must reduce). **Example:** Inter conversion of Fe^{2+} (ferrous ion) to Fe^{3+} (ferric ion).



If the oxidation reduction reaction takes place in biological system then it is known as biological redox reaction (or) simply biological oxidation. The general oxidation–reduction principle is applicable to biological systems also. **Example:** The oxidation of NADH + H^+ to NAD^+ is coupled with simultaneous reduction of FMN to $FMNH_2$. In this example NADH + H^+ / NAD^+ and FMN / $FMNH_2$ are called as redox pair which differ in their tendency to lose or gain electrons.



Redox Potential

Redox potential is otherwise known as oxidation–reduction potential. A quantitative measure of the tendency of a redox pair to lose or gain electrons is known as redox potential. Specific standard redox potential (E_o Volts) is assigned to each redox pair based on their tendency to lose or gain electrons at 25 °C and pH 7.0. The redox potential (E_o) is directly related to the change in the free energy ($\Delta G°$).

The specific standard redox potential (E_o) may be either positive or negative. More negative redox potential (E_o Volts) indicates greater tendency of reductant to lose electrons and a more positive redox potential (E_o Volts) indicates greater tendency of oxidant to accept electrons. Generally, the electrons flow from a redox pair with more negative redox potential (E_o Volts) to another redox pair with more positive redox potential (E_o Volts). Specific standard redox potential (E_o volts) of various redox pair system is summarized in Table 1.4.

Table 1.4 Specific standard redox potential (E_o Volts) of various redox pair system.

S. No	Redox pair	E_o in Volts
1	Succinate / α-ketoglutarate	- 0.67
2	$2H^+ / H_2$	- 0.42
3	$NADH + H^+ / NAD^+$	- 0.32
4	$NADP^+ / NADP + H^+$	- 0.32
5	$FMN / FMNH_2$ (Enzyme bound)	- 0.30
6	Lipoate (ox / red)	- 0.29
7	$FAD / FADH_2$	- 0.22
8	Pyruvate / Lactate	- 0.19
9	Fumarate / succinate	+ 0.03
10	Cytochrome b (Fe^{3+} / Fe^{2+})	+ 0.07
11	Coenzyme Q (ox / red)	+ 0.10
12	Cytochrome c_1 (Fe^{3+} / Fe^{2+})	+ 0.23
13	Cytochrome c (Fe^{3+} / Fe^{2+})	+ 0.25
14	Cytochrome a (Fe^{3+} / Fe^{2+})	+ 0.29
15	$½ O_2 / H_2O$	+ 0.82

Co-Enzyme System Involved in Biological Oxidation

Co-enzymes are defined as the non-protein, organic, low molecular weight and easily dialyzable substances associated with the functions of enzymes.

Holoenzyme (Active enzyme) ⟶ **Apoenzyme** (Protein part) + **Co-enzyme** (Non-protein part)

The followings are the various co-enzymes which are involved in biological oxidation. They are,

1. Flavin mononucleotide (FMN)
2. Flavin adenine dinucleotide (FAD)
3. Nicotinamide adenine dinucleotide (NAD^+)
4. Nicotinamide adenine dinucleotide phosphate ($NADP^+$)
5. Lipoic acid

Details of these co-enzymes along with its chemical structure is explained in enzyme topic.

Energy Rich Compounds

It is otherwise known as high energy compounds or high energy phosphates. In the biological systems certain compounds on hydrolysis yield energy. Energy rich compounds or high energy compounds are substances which possess sufficient free energy to liberate at least 7 Cal/mol at pH 7.0. Energy rich compounds are otherwise known as high energy compounds. Compared to hydrolysis of ATP into ADP and inorganic phosphates, all the high energy compounds liberate more energy when undergo hydrolysis. High energy compounds except acetyl CoA generally contains phosphate group in their structure, hence it is also known as high energy phosphates. **Example:** Phosphoenol pyruvate, carbamoyl phosphate, cAMP, 1,3-bisphosphoglycerate, phosphocreatine, acetyl phosphate, S-adenosylmethionine (SAM), pyrophosphate (PPi), acetyl CoA and ATP.

Compounds which liberate less than 7.0 Cal/mol (which is lower than hydrolysis of ATP into ADP and inorganic phosphate) are referred as low energy phosphates or low energy compounds. **Example:** ADP, glucose-1-phosphate, fructose-1-phosphate, glucose-6-phosphate and glycerol-3-phosphate. The standard free energy liberated during hydrolysis of some important compounds is summarized in Table 1.5.

Table 1.5 The standard free energy (ΔG°) liberated during hydrolysis of some important compounds.

S. No	Compounds Name	ΔG° (in Cal / mol)
High energy phosphates or High energy compounds		
1	Phosphoenol pyruvate	- 14.8
2	Carbamoyl phosphate	- 12.3
3	cAMP	- 12.0
4	1,3-Bisphosphoglycerate	- 11.8
5	Phosphocreatine	- 10.3
6	Acetyl phosphate	- 10.3
7	S-Adenosylmethionine (SAM; Sulfonium compound)	- 10.0
8	Pyrophosphate (PPi)	- 8.0
9	Acetyl CoA (Thioester)	- 7.7
10	ATP (Breakdown into ADP & inorganic phosphate)	- 7.3
Low energy phosphates or Low energy compounds		
11	ADP (Breakdown into AMP & inorganic phosphate)	- 6.6
12	Glucose-1-phosphate	- 5.0
13	Fructose-1-phosphate	- 3.8
14	Glucose-6-phosphate	- 3.3
15	Glycerol-3-phosphate	- 2.2

Classification of high energy compounds

High energy compounds are broadly classified into five major types according to type of bonds present in their structure. They are,

1. Pyrophosphate
2. Acyl phosphate
3. Enol phosphate
4. Thioester or thiol ester
5. Phosphagens or guanidino phosphate

Acid anhydride bonds particularly phospho anhydride bonds are high energy bonds which are usually present in all high energy compounds. Condensation of two acidic groups or related compounds generally produces this acid anhydride bonds. These bonds liberate free energy when it undergoes hydrolysis; hence it is known as high energy bonds. The symbol '~' is used by Lipmann to represent high energy bonds. ATP is instantly written as AMP-P-P. The various examples and bonds present in different classes of high energy compounds are summarized in Table 1.6.

Table 1.6 High energy compounds.

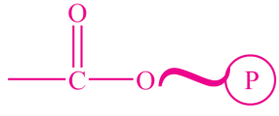
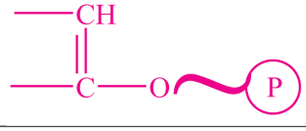
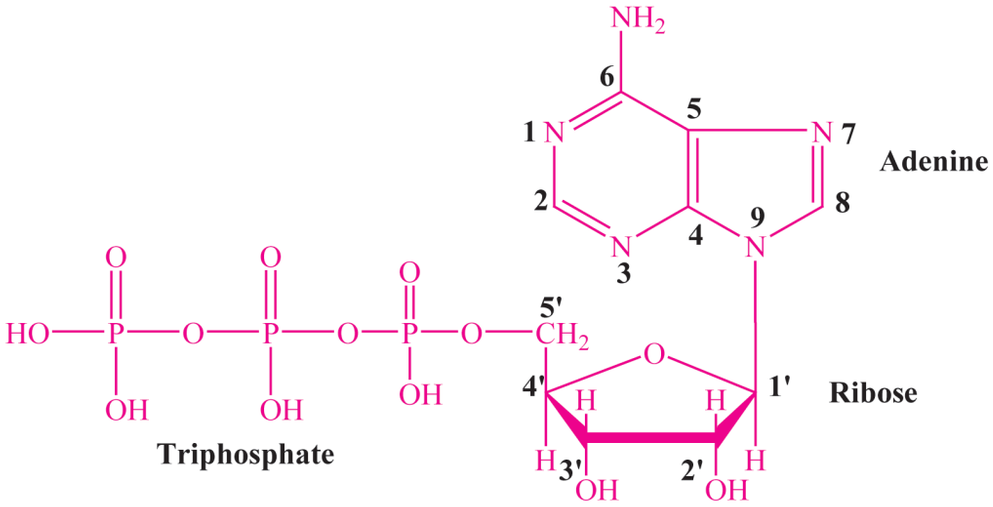
S. No	Class	Type of bond present	Example
1	Pyrophosphate		Pyrophosphate, ATP
2	Acyl phosphate		Carbamoyl phosphate, 1,3-bisphosphoglycerate, acetyl phosphate
3	Enol phosphate		Phosphoenol pyruvate

Table 1.6 *Contd...*

S. No	Class	Type of bond present	Example
4	Thioester or thiol ester		Acetyl CoA, acyl CoA
5	Phosphagens or guanidino phosphate		Phosphocreatine, phosphoarginine

Adenosine Triphosphate (ATP)



Adenosine triphosphate (ATP)

In living cells, the most important high energy molecule present is adenosine triphosphate (ATP). It is a nucleotide and composed of

1. Adenine (nitrogen base)
2. Ribose (sugar) and
3. A triphosphate groups.

In the triphosphate moiety of ATP, it contains two phospho anhydride bonds, hence ATP is a high energy compounds. ATP-ADP cycle evidences that ATP is the energy currency of the cell.

ATP-ADP Cycle

Large amount of energy (7.3 Cal/mol) is released when ATP is hydrolyzed to ADP and inorganic phosphate.

$$ATP + H_2O \longrightarrow ADP + Pi \qquad (\Delta G^\circ = -7.3\ Cal/mol)$$

Several processes in the biological systems such as muscle contraction, active transport, biosynthesis, etc. utilizes the energy liberated from the ATP when it breaks. In addition, energy rich compounds are biosynthesized from low energy compounds by reacting with high energy phosphates which is donated by ATP. Whilst, the compounds possessing higher free energy content donates high energy phosphates to ADP in order to produce ATP. ATP-ADP cycle is represented in Figure 1.13.

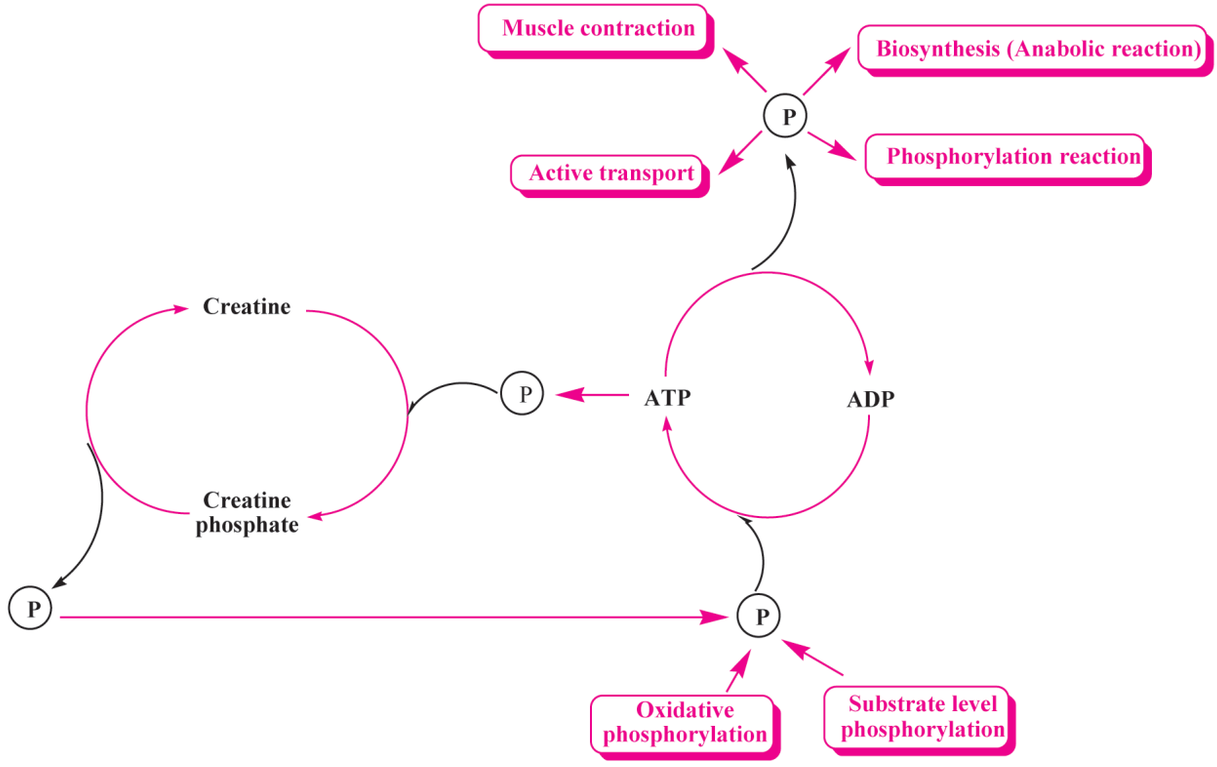


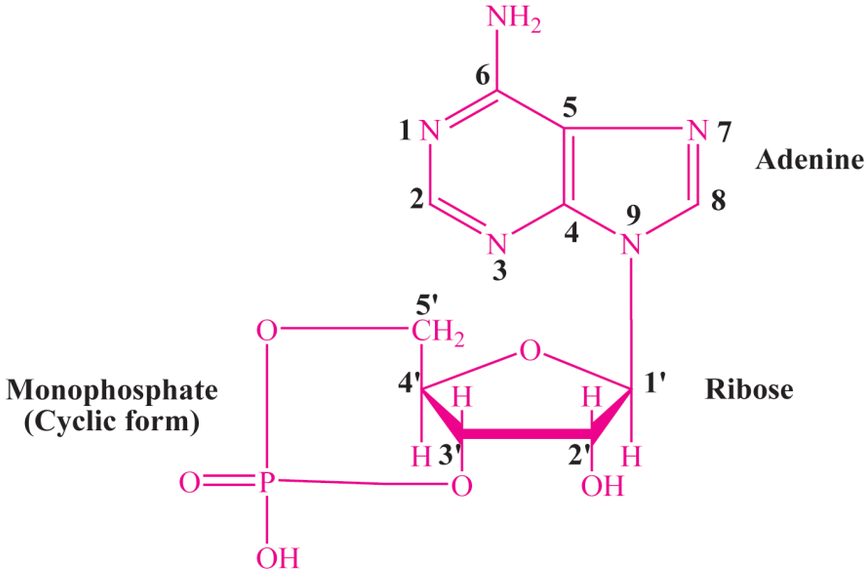
Figure 1.13 ATP-ADP cycle along with formation and breakdown of ATP (Phosphate will not exist free in biological system. It is only transferred).

Biological Significance of ATP

Biological importance of ATP is as follows

1. ATP is the short-term energy store of the cell.
2. Universally for all living things ATP is the energy currency of cell.
3. ATP is the source of phosphate moiety for phosphorylation reaction.
4. In addition, ATP can also donate pyrophosphate (PPi) and adenosine monophosphate (AMP) to other suitable acceptor for the formation of important biological compounds.
5. It easily participates in many biological reactions.
6. ATP is necessary for muscle contraction.
7. ATP is useful for nerve impulse transmission.
8. ATP is important for normal growth and development.
9. ATP is necessary for active transport.
10. ATP is also useful for homeostasis.
11. ATP is needed for all anabolic reactions.
12. ATP is helpful for intracellular signaling.
13. ATP is useful for the synthesis of nucleic acid.
14. ATP also plays a role in synthesis of proteins for the activation of amino acids.

Cyclic Adenosine Monophosphate (cAMP)

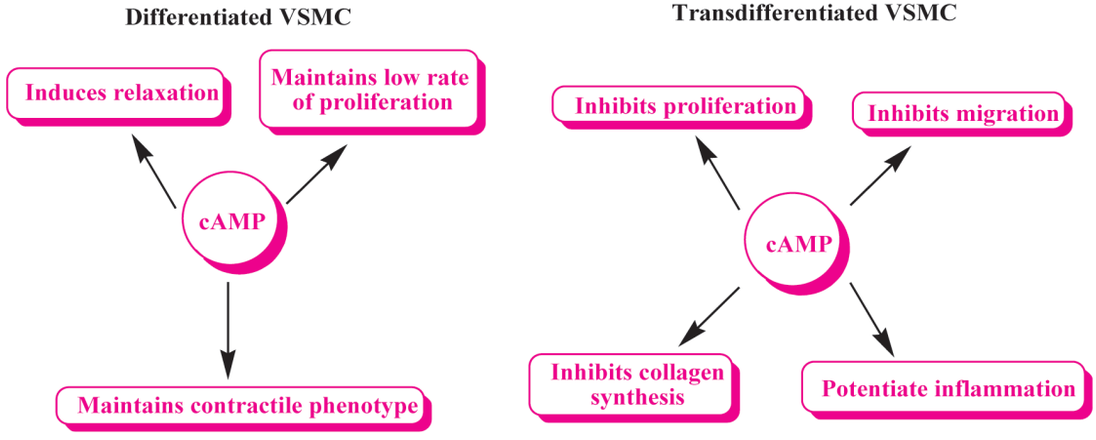


Cyclic adenosine monophosphate (*c*AMP)

In living cells, another important high energy molecule present is cyclic adenosine monophosphate (cAMP). The prefix 'cyclic' indicates the cyclic structure formed between phosphate moiety present at C-5 of ribose and hydroxyl group present at C-3 of ribose molecule.

It is a nucleotide and composed of

1. Adenine (nitrogen base)
2. Ribose (sugar) and
3. A monophosphate group.



Biological Significance of cAMP

Biological responses of cAMP are,

1. It is the intracellular secondary messenger present in many biological processes.
2. It is used for signal transduction intracellularly.
3. cAMP plays an important key regulatory role in most type of cells.
4. *Adenyl cyclase* particularly alters cAMP and this cAMP regulates the enzyme called *phosphodiesteraeses*.

5. cAMP mediates some short-term aspects of synaptic transmission. In addition, some rapid actions of certain neurotransmitter on ion channels that do not involve ligand gated channels are mediated through cAMP.
6. Along with other intracellular messenger, cAMP plays a central role in mediating other aspects of synaptic transmission.
7. Virtually all other effects of neurotransmitter on target neuron functioning both short and long term are achieved through intracellular messengers.

Synthesis of ATP

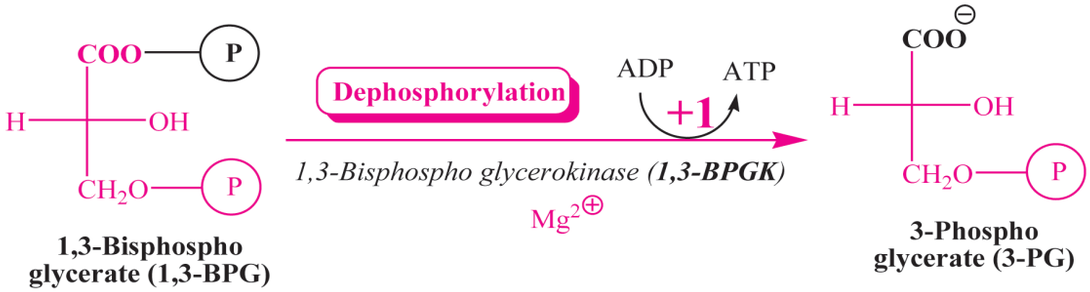
ATP is generally synthesized by phosphorylation reaction from ADP and inorganic phosphate. Generally, the phosphorylation reaction used to synthesize ATP is broadly classified into two different types.

1. Substrate level phosphorylation
2. Oxidative phosphorylation

Substrate Level Phosphorylation

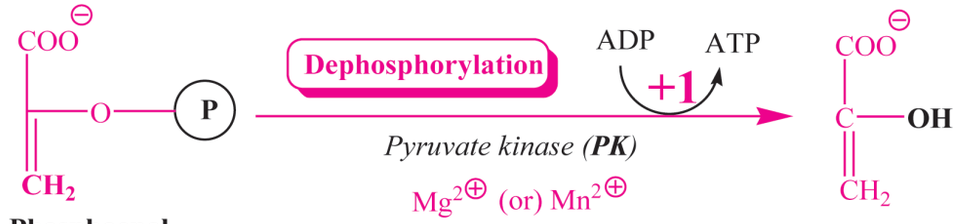
In this type of phosphorylation, ATP is directly synthesized in the metabolism during oxidation of substrate without involvement of ETC (Electron Transport Chain). To produce ATP, high energy phosphates are transferred usually from high energy compounds such as 1,3-bisphosphoglycerate, phosphoenol pyruvate (intermediates of glycolysis) and succinyl CoA (intermediates of citric acid cycle).

Example 1: 1,3-Bisphospho glycerate (1,3-BPG) produced 3-phospho glycerate (3-PG) by dephosphorylation reaction in presence of *phospho glycero kinase* (PGK) (In ATP / GTP involved reactions the enzymes acted are "*kinase*" and the substrate is "1,3-bisphospho glycerate").



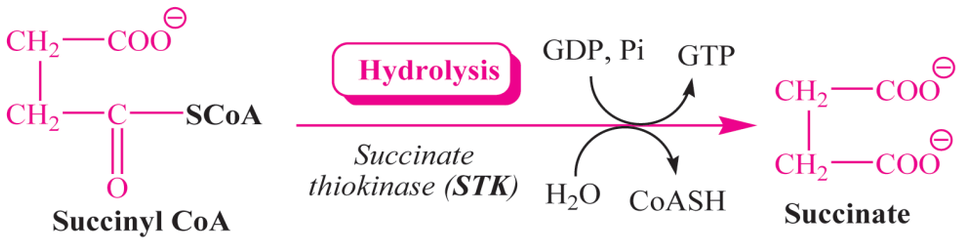
1,3-Bisphospho glycerate (1,3-BPG) **3-Phospho glycerate (3-PG)**

Example 2: Phosphoenolpyruvate undergoes dephosphorylation and produce pyruvate in enol form in presence of *pyruvate kinase* (PK) (PEP) (In ATP / GTP involved reactions, the enzymes acted are "*kinase*" and the product formed is "pyruvate").



Phosphoenol pyruvate (PEP) **Pyruvate (Enol form)**

Example 3: Succinyl CoA is hydrolyzed into succinate with loss of co-enzyme A. The reaction is catalyzed by *succinate thiokinase* (STK) enzyme (In ATP / GTP involved reactions, the enzymes acted are "*kinase*", sulphur atom of CoA is involved and the product formed is "succinate"). One GDP is converted into GTP in this step by reacting with inorganic phosphate.



Oxidative Phosphorylation

In aerobic organism, oxidative phosphorylation is the major source of ATP. Oxidative phosphorylation is defined as the process of synthesizing ATP from ADP and Pi (inorganic phosphate) with the involvement ETC (electron transport chain). In general, the transport of electrons through the ETC is linked with the release of free energy. Oxidative phosphorylation is taking place in complex–V of inner mitochondrial membrane.

Phosphorous oxygen ratio (P:O): The phosphorous oxygen ratio i.e., P:O is defined as the number of inorganic phosphate molecules utilized for ATP generation for every atom of oxygen consumed. Otherwise it represents the number of molecules of ATP synthesized per pair of electrons carried through ETC. In general, the phosphorous oxygen ratio (P:O) for mitochondrial oxidation of NADH is three.

$$NADH + H^+ + \frac{1}{2}O_2 + 3\ ADP + 3\ Pi \longrightarrow NAD^+ + 3\ ATP + H_2O$$

The phosphorous oxygen ratio (P:O) for mitochondrial oxidation of $FADH_2$ is two.

$$FADH_2 + \frac{1}{2}O_2 + 2\ ADP + 2\ Pi \longrightarrow FAD + 2\ ATP + H_2O$$

Ten and six protons are pumped across the mitochondrial membrane by NADH + H^+ and $FADH_2$, respectively. Four protons are required for the synthesis of one ATP. Hence, there is a strong evidence that the phosphorous oxygen ratio (P:O) for mitochondrial oxidation of NADH + H^+ and $FADH_2$, is 2.5 and 1.5, respectively.

Site of oxidative phosphorylation in ETC: In ETC, there are three exergonic site which results in the synthesis of 3 ATP molecules.

1. Oxidation of $FMNH_2$ by Co-enzyme Q
2. Oxidation of cytochrome b by cytochrome c_1.
3. *Cytochrome oxidase* reaction.

Each one of the above sites represents the coupling site for the synthesis of one ATP. NADH + H^+ pass through all three coupling sites that results in production of three ATP. Whereas, $FADH_2$ by passing the first coupling site and pass through only last two coupling sites results in production of two ATP.

Energetic of oxidative phosphorylation: The simplified reactions involved in the transport of electrons to redox pair $\frac{1}{2}O_2$ / H_2O (E_0: + 0.82 V) from redox pair NAD^+ / NADH + H^+ (E_0: - 0.32 V) is represented as follows,

$$\frac{1}{2}O_2 + NADH + H^+ \longrightarrow H_2O + NAD^+$$

Between these two redox pairs the redox potential difference is 1.14 V [ΔE_0 = E_0 of accepted redox pair - E_0 of donated redox pair i.e., 0.82 – (-0.32) which is 0.82 + 0.32)]. This 1.14 V redox potential equals to 52 Cal/mol of energy. In ETC, from the above electron transfers three ATPs are generated. Hence, the total energy of these three ATPs is 21.9 Cal/mol (One ATP energy is 7.3 Cal/mol. Hence, for three ATPs it is 7.3 X 3 = 21.9). From this, the energy conservation efficiency is calculated as follows,

$$\text{Energy conservation efficiency} = \frac{\text{Energy of 3 ATPS}}{\text{Total energy}} \times 100 = \frac{21.9}{52} \times 100 = 42\%$$

Thus, only 42 % of energy is trapped in the form of three ATPs when NADH + H^+ are oxidized and the remaining energy is lost as heat. This lost heat is not a waste one because it is necessary to maintain body temperature and it allows continuous generation of ATP in ETC.

Mechanism of Oxidative Phosphorylation

The mechanism involved in oxidative phosphorylation is tried to explain by several hypothesis. Out of several mechanisms proposed for oxidative phosphorylation the following three are most important.

1. Chemical coupling mechanism
2. Chemiosmotic mechanism
3. Conformational coupling mechanism

1. **Chemical coupling mechanism:** In the year 1953, Edward Slater proposed this hypothesis. According to this mechanism, in ETC during the course of electron transfer, ADP reacts with inorganic phosphate and produces a series of phosphorylated high energy intermediates which are utilized for synthesis of ATP. This reaction is believed to be analogous to the substrate level phosphorylation reactions occurring in glycolysis and TCA cycle. In addition, till date no evidence could prove this hypothesis since all attempts made to isolate any one of the phosphorylated high energy intermediates are not successful.

ADP + Pi ⟶ Several phosphorylated high energy intermediates ⟶ ATP

2. **Chemiosmotic mechanism:** This mechanism is widely accepted and it was proposed in the year 1961 by Peter Mitchell. This mechanism clearly explains the utilization of electrons transport in ETC for the production of ATP from ADP and inorganic phosphate. The energy stored in battery separated by positive and negative charges is generally used for comparison of chemiosmotic mechanism and is represented in Figure 1.14.

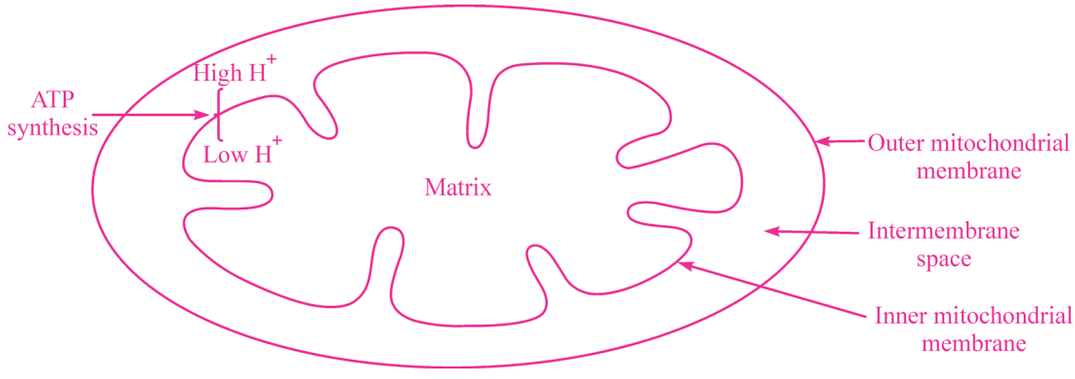


Figure 1.14 Outline of chemiosmotic mechanism for oxidative phosphorylation.

The inner mitochondrial membrane is impermeable to ions such as protons (H^+) and hydroxyl ions (OH^-). In ETC, across the coupling membrane (i.e., inner mitochondrial membrane) protons are translocated along with the transport of electrons to the inter membrane space from mitochondrial matrix. Electrochemical gradient or proton gradient takes place as a result of pumping of protons. The reason behind is accumulation of more protons (H^+) on the outer side of the inner mitochondrial membrane than the inner side. This formed electrochemical gradient or proton gradient due to flow of electrons in ETC is sufficient enough for the synthesis of ATP from ADP and inorganic phosphate.

The electrochemical gradient or proton gradient is utilized by the enzyme known as *ATP synthase* present in complex–V of inner mitochondrial membrane and produces ATP. The enzyme is also known as *ATPase* as it hydrolyzes ATP into ADP and inorganic phosphate. *ATP synthase* is a complex enzyme and are made up of two functional subunits namely F_1 and F_0. The structure of *ATP synthase* is comparable with the structure of lollipops. ATP is synthesized when the protons accumulated on the inter membrane space re-enter into the mitochondrial matrix. Chemiosmotic mechanism for oxidative phosphorylation is schematically represented in Figure 1.15.

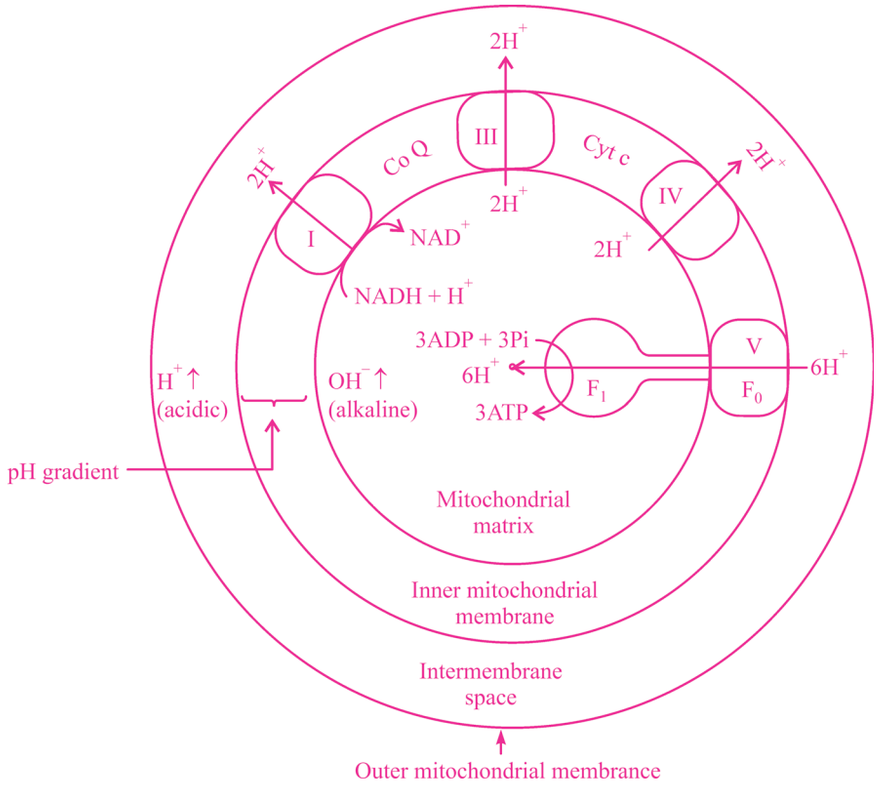


Figure 1.15 Schematic representation of chemiosmotic mechanism for oxidative phosphorylation.

Evidence for chemiosmotic mechanisms: There are several evidences available for supporting chemiosmotic mechanisms.

1. ATP synthesis takes place in inner mitochondrial membrane only.
2. Inner mitochondrial membrane is impermeable to various ions such as H^+, K^+ etc.
3. Additions of proton in inter membrane space of mitochondria results in increased ATP synthesis.
4. Any substances which increases the membrane permeability leads to decreased ATP synthesis. **Example:** 2, 4-Dinitrophenol (2,4-DNP).

3. **Conformational coupling mechanism:** According to this mechanism, inner mitochondrial membrane undergoes some conformational changes therefore ADP and Pi (inorganic phosphate) come close to each other during electron transfer in ETC which results in formation of ATP. There is an evidence for this mechanism as inner mitochondrial membrane undergoes conformational changes and it is explained by rotary motor model for ATP generation.

Rotary motor model for ATP generation: In 1964 Paul Boyer proposed that ATP is synthesized due to a conformational change in the mitochondrial membrane proteins. Now this hypothesis of Paul Boyer is considered as rotary motor or engine driving model or binding change model which is widely accepted. The enzyme *ATP synthase* is a complex one present in complex–V of inner mitochondrial membrane and it contains two major sub complexes namely F_0 and F_1. To the sub complex F_0 composed of channel protein C subunits, *F_1-ATP synthase* is attached. *F_1-ATP synthase*

consist of three subunits namely α, β and γ. Generally, γ subunit is present centrally and is surrounded by alternative α and β subunits. Three α, three β and one γ subunit is present in F_1-*ATP synthase*. The γ subunit is rotated physically in response to proton flux leads to induction of conformational changes in β_3 subunit results in release of ATP. Different conformations were adapted by the three β subunits of F_1-*ATP synthase* according to the binding change mechanism. One subunit has O (open) conformation; the second one has L (loose) conformation; and the third one has T (tight) conformation. γ subunit rotation is induced by protons through unknown mechanism. This leads to conformational changes in β subunits. In L-conformation, ADP and inorganic phosphate binds to β subunits. When this L-site of β subunits changed to T-conformation, ATP is synthesized. The O-site is changed to L-conformation which binds to ADP and inorganic phosphate. The T-site is changed to O-conformation and releases ATP. This conformational change of β subunits is repeated and three ATPs are generated for each rotation. The release of ATP from O-conformation is energy dependent whereas synthesis of ATP is not dependent on energy. This is very crucial in synthesis of ATP by rotary motor model. This enzyme *ATP synthase* acting as a proton driving motor is a good example for rotary catalysis. Hence, in the world the smallest molecular motor is *ATP synthase*. Rotary motor model for ATP generation is represented in Figure 1.16.

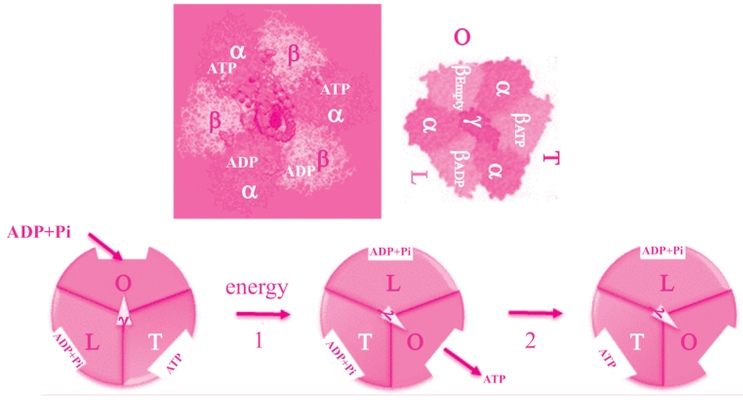


Figure 1.16 Rotary motor model for ATP generation.

Inhibitors of oxidative phosphorylation:

Oxidative phosphorylation can be inhibited by the following components.

1. Uncouplers
2. Ionophores
3. Inhibitors of *ATP synthase*
4. Inhibitors of adenine nucleotide carrier.

1. **Uncouplers:** These are the substances which uncouple or delink the transport of electrons in ETC during oxidative phosphorylation. Uncoupler oxidizes the reducing equivalents such as NADH + H^+ and $FADH_2$ without production of ATP. **Example:** 2,4-Dinitrophenol (2,4-DNP) and physiological uncouplers such as thyroxine, thermogenin and long chain fatty acids.
2. **Ionophores:** Ionophores is defined as lipophilic substances which increases the membrane permeability of various ions such as H^+, K^+, Cl^-, HCO_3^-, Na^+ etc. across the biological membrane. Even uncouplers are proton ionophores (uncouplers increase the membrane permeability of H^+ ions). **Example:** Valinomycin, gramicidin-A and nigericin are K^+ ionophores; 2,4-dinitophenol (2,4-DNP) is H^+ ionophores.
3. **Inhibitors of *ATP synthase*:** *ATP synthase* is an enzyme responsible for the synthesis of ATP. Hence, inhibition of *ATP synthase* may lead to decreased ATP synthesis. **Example:** Oligomycin
4. **Inhibitors of adenine nucleotide carrier:** ADP is an important component for the synthesis of ATP. Hence decreased supply of ADP may lead to decreased synthesis of ATP. This type of

inhibitors inhibits adenine nucleotide carrier which leads to block of adequate supply of ATP.
Example: Atractyloside

Electron Transport Chain (ETC) or Respiratory Chain or Electron Transport System (ETS)

Through a series of biochemical reactions, energy rich biomolecules such as carbohydrates (specifically glucose), amino acids and fatty acids are oxidized in body into carbon dioxide and water. During these metabolic reactions from various metabolic intermediates the reducing equivalents are transferred into co-enzyme NAD^+ and FAD to produce NADH + H^+ and $FADH_2$ respectively. Through electron transport chain (ETC) these two reduced coenzymes finally reduce oxygen to water. Free energy loss is usually associated with ETC. From ADP and inorganic phosphate, ATP is synthesized by utilizing the part of this free energy . ETC is important for regeneration of oxidized form of reducing equivalent NAD^+ and FAD. Overview of biological oxidation and ETC are represented in Figure 1.17 and 1.18, respectively.

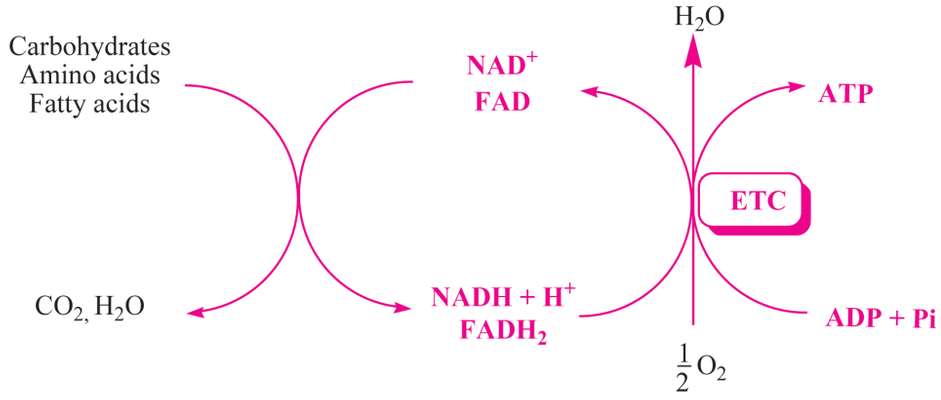


Figure 1.17 Overview of biological oxidation.

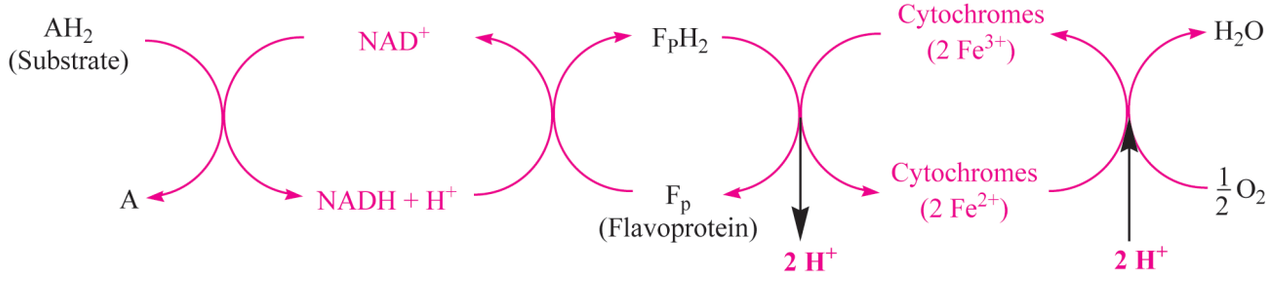


Figure 1.18 Overview of electron transport chain (ETC).

Mitochondria: Mitochondria are considered as the power house of the cell because mitochondria are the centre for metabolic oxidation reactions. The reduced coenzymes like NADH + H^+ and $FADH_2$ are oxidized to NAD^+ and FAD respectively in ETC of mitochondrion with energy liberation in the form of ATP. Five distinct parts are present in mitochondria. They are,

1. Outer mitochondrial membrane
2. Inner mitochondrial membrane
3. Inter membrane space
4. The cristae and
5. The mitochondrial matrix

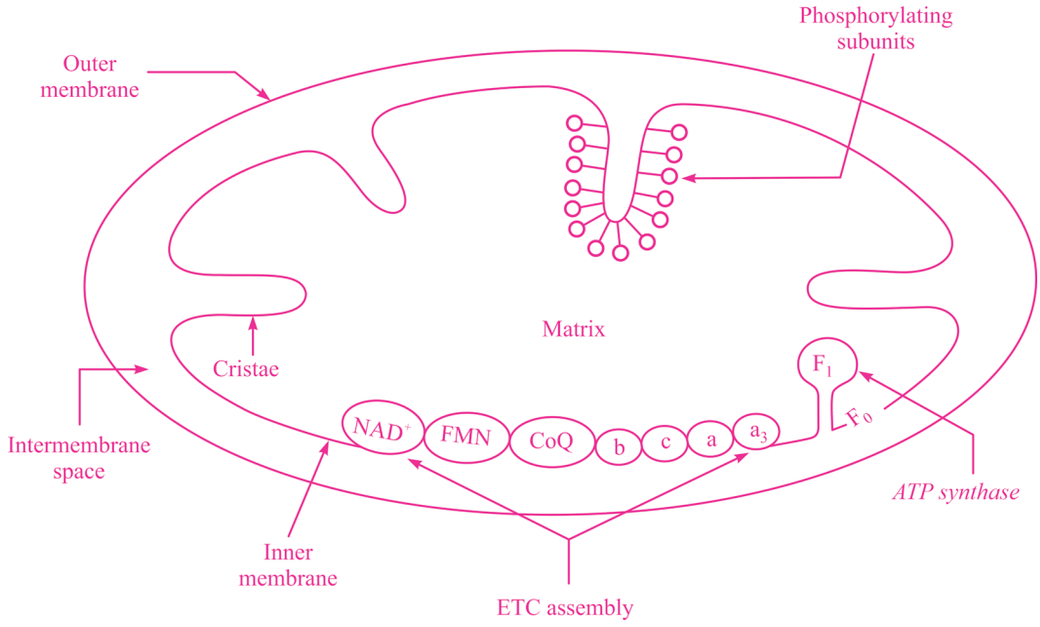


Figure 1.19 Structure of mitochondria depicting ETC.

In the inner mitochondrial membrane of the mitochondria, ETC and ATP synthesizing systems are located which is a specialized structure and rich in proteins. Inner mitochondrial membrane is impermeable to ions like sodium (Na^+), potassium (K^+), proton (H^+), etc. and small molecule like ADP, ATP, etc. The surface area of the inner mitochondrial membrane is greatly increased by forming cristae (highly folded membrane). The centre of ATP production i.e., a specialized particle phosphorylating subunits which look likes lollipops are present in the inner surface of the inner mitochondrial membrane. The mitochondrial matrix is the interior ground substance of the mitochondria. The enzymes involved in TCA cycle, oxidation of amino acids and β-oxidation of fatty acids are rich in the mitochondrial matrix. Structure of mitochondria depicting ETC is presented in Figure 1.19.

Mechanism of ETC

Five distinct enzyme or respiratory complexes are present in the inner mitochondrial membrane of the mitochondria. They are complex–I, complex–II, complex–III, complex–IV and complex–V. Electrons are carried by the first four complexes i.e., complex–I to complex–IV and the ATP is synthesized in complex–V. Certain mobile electron carriers such as, NADH, co-enzyme Q, cytochrome C and oxygen are present in ETC besides these five enzyme complexes. The electrons are transported collectively by the complex–I to complex–IV and the mobile electron carriers. Finally, the electrons react with oxygen and produced water. ETC of mitochondria utilizes the largest portion of oxygen consumed by the body. Mechanism of ETC with multiprotein complexes involved in ETC is represented schematically in Figure 1.20.

Components of ETC: There are the five major components which are distinct carriers that participate in the electron transport chain. These carriers are sequentially arranged and carry electrons from the substrate and finally combine with oxygen to produce water. The five major components of ETC are, 1) Nicotinamide nucleotides, 2) Flavoproteins, 3) Iron-sulphur proteins, 4) Co-enzyme Q and 5) Cytochromes.

1. **Nicotinamide nucleotide:** NAD^+ and $NADP^+$ are the two coenzymes of vitamin B_3 i.e., niacin. Out of these two, NAD^+ is actively involved in ETC. Generally, from the substrates (AH_2) such as glyceraldehyde-3-phosphate, pyruvate, isocitrate, α-ketoglutarate and malate, NAD^+ removes two hydrogen and reduced to NADH + H^+ in presence of *dehydrogenase* enzyme.

$$\underset{\textbf{Substrate}}{AH_2} + NAD^+ \xrightleftharpoons[\textit{Dehydrogenase}]{\textbf{Redox reaction}} \underset{\textbf{Product}}{A} + NADH + H^+$$

Whereas $NADP^+$ is reduced to NADPH + H^+ in presence of $NADP^+$ dependent *dehydrogenase* enzyme which is not a substrate for ETC. NADPH + H^+ is mainly involved in fatty acid synthesis and cholesterol synthesis

2. **Flavoproteins:** *NADH-coenzyme Q reductase* or *NADH dehydrogenase* and *succinate-coenzyme Q reductase* or *succinate dehydrogenase* are the two important flavoproteins of the ETC. Prosthetic group present in flavoprotein *NADH dehydrogenase* is FMN. The co-enzyme FMN is converted to $FMNH_2$ by accepting two electrons and protons from NADH + H^+. *NADH dehydrogenase* is closely associated with iron-sulphur (Fe-S) proteins or non-heme iron (NHI) proteins.

$$FMN + NADH + H^+ \xrightarrow[\text{NADH-coenzyme Q reductase or NADH dehydrogenase}]{\text{Reduction}} FMNH_2 + NAD^+$$

Prosthetic group present in flavoprotein *succinate dehydrogenase* is FAD. The coenzyme FAD is converted to $FADH_2$ by accepting two electrons and protons from succinate. *Succinate dehydrogenase* is also closely associated with iron-sulphur (Fe-S) proteins or non-heme iron (NHI) proteins.

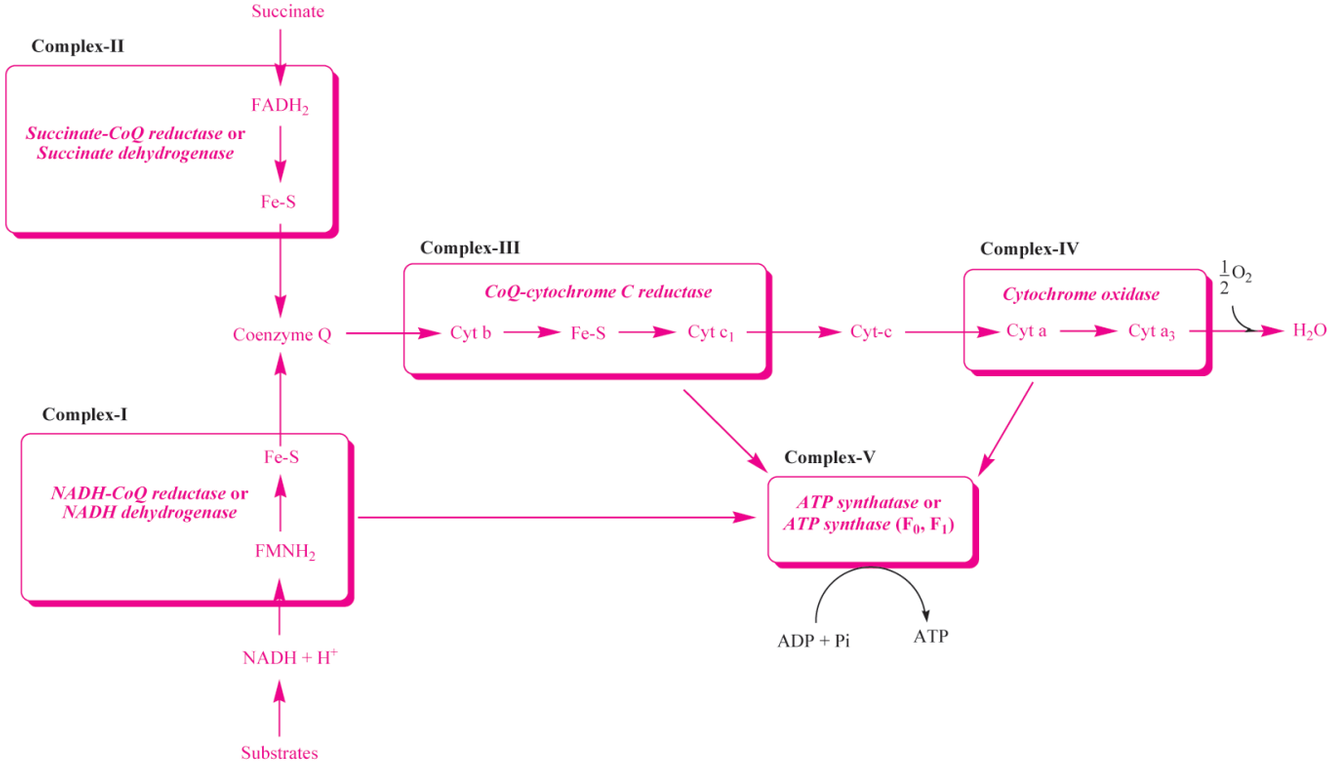
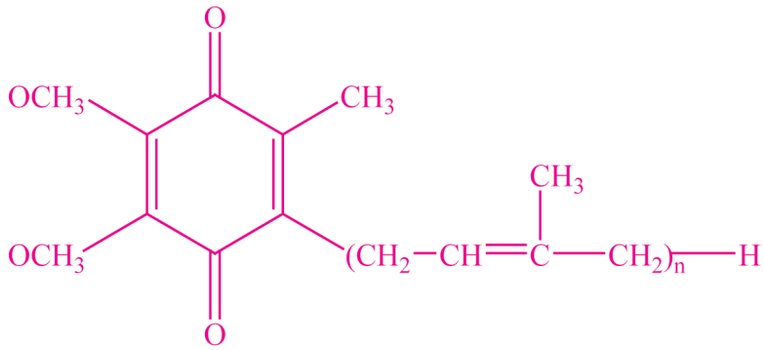


Figure 1.20 Mechanism of ETC with multiprotein complexes involved in ETC.

$$FAD + Succinate \xrightarrow[\text{Succinate-coenzyme Q reductase or Succinate dehydrogenase}]{\text{Reduction}} FADH_2 + Fumarate$$

3. **Iron-sulphur (FeS) proteins:** There are about six iron-sulphur proteins which are involved in ETC and the exact mechanism involved in iron-sulphur protein is not clearly understood. In generals iron-sulphur proteins exist in two forms such as ferric i.e., Fe^{3+} (oxidized) and ferrous i.e., Fe^{2+} (reduced) which are inter convertible. Out of several iron-sulphur proteins, one is involved in the transfer of electron form $FMNH_2$ to coenzyme Q; the other one is involved in the transfer of electron form $FADH_2$ to coenzyme Q; the next one is the transfer of electron form cytochrome b to cytochrome c_1.
4. **Co-enzyme Q:** It is ubiquitous in living system; hence, it is also known as ubiquinone. Co-enzyme Q is a quinone derivative with a variable isoprenoid side chain. Quinone ring with ten isoprenoid unit side chain is present in mammalian tissues, hence it is commonly known as co-enzyme Q_{10} or CoQ_{10}. Co-enzyme Q is a lipophilic electron carrier. It accepts electron from both $FMNH_2$ produced in ETC and $FADH_2$ produced outside ETC (**Example:** *Succinate dehydrogenase, acyl CoA dehydrogenase*, etc.) In some organism like mycobacteria, co-enzyme Q is not present, hence, in these organism vitamin K performs the similar functions of co-enzyme Q. In body co-enzyme Q is directly synthesized because in animal there is no known vitamin precursors for co-enzyme Q .



5. **Cytochromes:** In general, cytochromes are hemoprotein containing heme as prosthetic group. Porphyrin ring with iron atom is present in heme. Compared to other hemoproteins such as hemoglobin, methemoglobin, heme present in cytochromes is different. In cytochromes, the iron is present in both reduced (ferrous i.e., Fe^{2+}) and oxidized (ferric i.e., Fe^{3+}) form alternatively which is essential for the transport of electrons in ETC. In case of other hemoproteins such as hemoglobin, methemoglobin, heme is present only in ferrous (Fe^{2+}) state.

   Depending upon the types of heme present and the respective absorption spectrum, initially three cytochromes are designated as cytochrome a, cytochrome b and cytochrome c. Latter, several additional cytochromes are discovered which are designated as cytochrome c_1, cytochrome b_1 and cytochrome a_3.

   Electrons are transported from co-enzyme Q into cytochromes in the following orders b, c_1, c, a and a_3. These cytochromes act as an effective electron carrier due to the presence of reversible oxidation–reduction property of heme iron present in it.

   Heme group and 104 amino acid containing small protein is cytochrome c (Molecular weight: 13,000). With an intermediate redox potential, cytochrome c acts as a central molecule in ETC. Cytochrome c can be easily extracted because it is loosely bounded in the inner mitochondrial membrane.

   In ETC, the terminal component is *cytochrome oxidase*. Cytochromes a and a_3 are collectively known as *cytochrome oxidase*. This is the only cytochrome which can directly react with molecular oxygen atom. Additionally, *cytochrome oxidase* also contains copper besides iron. Like iron, this copper also undergoes oxidation –reduction (cupric i.e., Cu^{2+} - curprous i.e., Cu^{+}) during the transport of electrons. Finally, water is produced in ETC from the transported electrons, free protons and the molecular oxygen atom.

Inhibitors of ETC

There are many site specific ETC inhibitors. Generally, inhibitors block the electron transport by binding with one component of ETC. This may lead to the accumulation of reduced components before the inhibitor

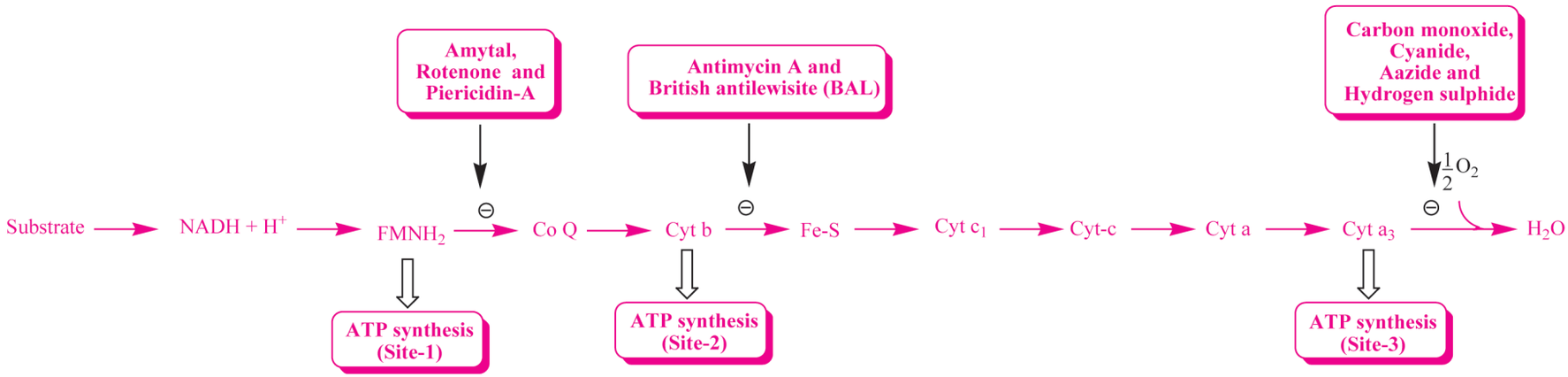


Figure 1.21 ETC with sites of ATP synthesis and inhibitors.

blockade step and oxidized components after the inhibitor blockade step. ATP synthesis i.e., phosphorylation mainly depends on ETC. Hence, ATP synthesis is also inhibited by the site specific ETC inhibitors. The inhibitors may act on three different sites of ATP synthesis as follows.

1. **Inhibitors at site-1:** The following chemical agents inhibits the synthesis of ATP in site-1 of ETC i.e., NADH and co-enzyme Q. **Example:** Amytal (barbiturate drug), rotenone (fish poison) and piericidin-A (antibiotic).
2. **Inhibitors at site-2:** The compounds inhibit the synthesis of ATP in site-2 of ETC i.e., cytochrome b and cytochrome c. **Example:** Antimycin-A (antibiotic) and British antilewisite i.e., BAL (Antidote used for war gas).
3. **Inhibition at site-3:** The compounds inhibit the synthesis of ATP in site-3 of ETC i.e., *cytochrome oxidase*. **Example:** Carbon monoxide (reacts with reduced form of cytochrome), cyanide and azide (reacts with oxidized form of cytochrome) and hydrogen sulphide (reacts with both oxidized and reduced form of cytochrome).

The most potent inhibitor of ETC is cyanide. It blocks the ETC by binding with the ferric ion of *cytochrome oxidase* which leads to death. Tissue asphyxia (mostly in CNS) is the reason behind the cyanide death. ETC with sites of ATP synthesis and inhibitors are presented in Figure 1.21.

PROBABLE QUESTIONS

PART – A: Multiple Choice Questions

1. _____ is the fundamental structural and functional unit of all living organisms.
   (a) Element (b) Macromolecule
   (c) Cell (d) Organ
2. Which of the following element is not a major element present in living matter?
   (a) Carbon (b) Sodium
   (c) Phosphorus (d) Sulphur
3. _____ are the building blocks of lipids.
   (a) Fatty acid (b) Glycerol
   (c) Both a & b (d) None of the above
4. What is the percentage of water present in a normal healthy adult weighing 65 kg?
   (a) 52.4 (b) 61.6
   (c) 70.8 (d) 82.0
5. in both prokaryotic and eukaryotic cells, the volume of the cell occupied by a semi-fluid matrix called _____.
   (a) Nucleus (b) Vacuole
   (c) Liposome (d) Cytoplasm
6. Which of the following is not a prokaryotic cell?
   (a) Fungi (b) Bacteria
   (c) PPLO (pleuro pneumonia like organisms) (d) Blue-green algae
7. _____ are the storage form of reserve material present in cytoplasm of prokaryotic cells.
   (a) Liposome (b) Mitochondria
   (c) Ribosome (d) Inclusion bodies
8. Which of the following are present in plant cells and absent in animal cells?
   (a) Cell walls (b) Large central vacuole
   (c) Plastids (d) All the above

9. In which chromosome, centromere is present in middle and forms two equal arms of the chromosome?
   (a) Metacentric chromosome (b) Sub-metacentric chromosome
   (c) Acrocentric chromosome (d) Telocentric chromosome
10. If enthalpy change for a reaction is zero, then $\Delta G°$ equals to _____
   (a) $-T\Delta S°$ (b) $T\Delta S°$
   (c) $-\Delta H°$ (d) $\ln k_{eq}$
11. $\Delta G°$ is defined as the _____
   (a) Residual energy present in the reactants at equilibrium
   (b) Residual energy present in the products at equilibrium
   (c) Difference in the residual energy of reactants and products at equilibrium
   (d) Energy required in converting one mole of reactants to one mole of products
12. For a reaction if $\Delta G°$ is positive, then _____
   (a) The products will be favored
   (b) The reactants will be favored
   (c) The concentration of the reactants and products will be equal
   (d) All of the reactant will be converted to products
13. The study of energy relationships and conversions in biological systems is called as _____
   (a) Biophysics (b) Biotechnology
   (c) Bioenergetics (d) Microbiology
14. Which of the following statement is false?
   (a) The reaction tends to go in the forward direction if ΔG is large and positive
   (b) The reaction tends to move in the backward direction if ΔG is large and negative
   (c) The system is at equilibrium if $\Delta G = 0$
   (d) The reaction tends to move in the backward direction if ΔG is large and positive
15. Anabolism and catabolism are chemically linked in the form of _____
   (a) ADP (b) ATP
   (c) Phosphodiester linkage (d) ASP
16. Which of the following statements is false about ATP hydrolysis?
   (a) It is highly exergonic (b) Activation energy is relatively high
   (c) $\Delta G^o = -30.5$ kJ/mol (d) $\Delta G^o = 30.5$ kJ/mol
17. An endergonic reaction _____
   (a) Proceeds spontaneously (b) Does not require activation energy
   (c) Releases energy (d) Requires energy
18. An exergonic reaction _____
   (a) Proceeds spontaneously (b) Does not require activation energy
   (c) Releases energy (d) Requires energy
19. Phosphoryl groups are derivatives of _____
   (a) Phosphorous acid (b) Phosphoric acid
   (c) Acetic acid (d) Citric acid
20. Water does a nucleophilic attack on phosphate monoester by producing _____
   (a) Phosphorous chloride (b) Phosphorous sulfide
   (c) Inorganic phosphate (d) Organic phosphate

21. Which is an example of chemical to osmotic energy conversion that occurs in living organisms?
    (a) ATP-driven muscle contraction
    (b) ATP-dependent photon emission in fireflies
    (c) Light-induced electron flow in chloroplasts
    (d) ATP-driven active transport across a membrane
22. Which of the following statements about redox potential is false?
    (a) $NADH/NAD^+$ redox pair has the least redox potential
    (b) Oxygen/H_2O redox pair has the highest redox potential
    (c) The components of the electron transport chain are organized in terms of their redox potential
    (d) The redox potential of a system is usually compared with the potential of the hydrogen electrode
23. Which out of the following is not a flavoprotein?
    (a) *Succinate dehydrogenase* (b) Cytochrome c
    (c) *Xanthine oxidase* (d) *NADH dehydrogenase-Q reductase*
24. Which out of the following has the highest redox potential?
    (a) NAD^+ (b) FMN
    (c) FAD (d) O_2
25. Which one out of the following is not a NAD^+ requiring enzyme?
    (a) *Lactate dehydrogenase* (b) *Pyruvate dehydrogenase* complex
    (c) *Malate dehydrogenase* (d) *Acyl CoA dehydrogenase*
26. Which of the following enzyme catalyzes the direct transfer and incorporation of O_2 into a substrate molecule is
    (a) *Reductase* (b) *Oxidase*
    (c) *Oxygenase* (d) *Peroxidase*
27. Loss of electrons can be termed as _____
    (a) Metabolism (b) Anabolism
    (c) Oxidation (d) Reduction
28. Gain of electrons can be termed as _____
    (a) Metabolism (b) Anabolism
    (c) Oxidation (d) Reduction
29. This is the major source of ATP in aerobic organism.
    (a) Substrate level phosphorylation (b) Oxidative phosphorylation
    (c) Glycolysis (d) TCA cycle
30. Name the compound with the greatest free energy
    (a) ATP (b) Phosphocreatine
    (c) Cyclic AMP (d) Phosphoenolpyruvate
31. 1,3-Bisphosphoglycerate is a example for which type of high energy compound?
    (a) Acyl phosphate (b) Enol phosphate
    (c) Pyrophosphate (d) Guanidino phosphate
32. In general, high energy compounds contain which type bond in its structure?
    (a) Peptide (b) Glycoside
    (c) Acid anhydride (d) Covalent

33. What happened to free energy changes when a reaction is at equilibrium?
    (a) Maximum (b) Minimum
    (c) Average (d) Zero
34. Standard redox potential of NAD^+/NADH pair is _____ volts.
    (a) - 0.67 (b) - 0.22
    (c) +0.29 (d) - 0.32
35. The $FMNH_2$ is oxidized by _____
    (a) Cytochrome-c (b) Coenzyme Q
    (c) Cytochrome-a (d) Cytochrome-b
36. Which complex of inner mitochondrial membrane is the site of oxidative phosporylation?
    (a) III (b) IV
    (c) V (d) VI
37. Inner mitochondrial membrane is impermeable to which of the following ions?
    (a) H^+ (b) K^+
    (c) OH^- (d) All
38. Which of the following antibiotics act as ionophores for potassium ions.
    (a) Antimycin & Valinomycin (b) Piercidin-A & Valinomycin
    (c) Nigercin & Valinomycin (d) Antimycin & Piercidin-A
39. Protein that contains a nucleic acid derivative of riboflavin is called _____
    (a) Nucleic acid (b) Amino acid
    (c) Flavoprotein (d) None
40. NADP-linked *dehydrogenase* catalyzes _____
    (a) Glucose 6-phosphate+$NADP^+ \leftrightarrow$ 6-phosphogluconate + NADPH + H^+
    (b) Lactate + $NAD^+ \leftrightarrow$ pyruvate + NADH + H^+
    (c) Pyruvate + CoA + $NAD^+ \leftrightarrow$ acetyl-CoA + CO_2 + NADH + H^+
    (d) L-Malate + $NAD^+ \leftrightarrow$ oxaloacetate + NADH + H^+
41. A lipid-soluble benzoquinone with a long isoprenoid side chain is?
    (a) Ubiquinone (b) Cytochrome-b
    (c) Cytochrome-c (d) Cytochrome-a
42. The only membrane bound enzyme in the citric acid cycle is _____
    (a) *Succinate dehydrogenase* (b) *NADH dehydrogenase*
    (c) *ATP synthase* (d) *Acyl CoA dehydrogenase*
43. In ETC, complex-I is also called _____
    (a) *NADH dehydrogenase* (b) *Succinate dehydrogenase*
    (c) Cytochrome bc1 complex (d) *Cytochrome oxidase*
44. In ETC, complex-II is also called _____
    (a) *NADH dehydrogenase* (b) *Succinate dehydrogenase*
    (c) Cytochrome bc1 complex (d) *Cytochrome oxidase*
45. In ETC, complex-III is also called _____
    (a) *NADH dehydrogenase* (b) *Succinate dehydrogenase*
    (c) Cytochrome bc1 complex (d) *Cytochrome oxidase*

46. In ETC, complex-IV is also called _____
    (a) *NADH dehydrogenase* (b) *Succinate dehydrogenase*
    (c) Cytochrome bc1 complex (d) *Cytochrome oxidase*
47. In mitochondria, hydride ions are removed from substrates by _____
    (a) NAD-linked *dehydrogenases* (b) NADP-linked *dehydrogenases*
    (c) *ATP synthase* (d) *Succinate dehydrogenases*
48. Which of the following is the prosthetic group of *NADH dehydrogenase*?
    (a) NADH (b) FAD
    (c) NADPH (d) FMN
49. If mitochondria were blocked at the site of NADH oxidation and were treated with succinate as substrate, what would the P : O ratio is?
    (a) Same as that normally produced by succinate
    (b) One more than normally produced by succinate
    (c) One less than normally produced by succinate
    (d) Zero
50. ATP synthesis by chemiosmosis is by _____
    (a) *ATP dehydrogenase* (b) *Gyrase*
    (c) *ATP synthase* (d) *Dehydrogenase*

Key for Multiple Choice Questions

1 (c)	2 (b)	3 (c)	4 (b)	5 (d)
6 (a)	7 (d)	8 (d)	9 (a)	10 (a)
11 (d)	12 (b)	13 (c)	14 (d)	15 (b)
16 (d)	17 (d)	18 (c)	19 (b)	20 (c)
21 (d)	22 (a)	23 (b)	24 (d)	25 (d)
26 (c)	27 (c)	28 (d)	29 (b)	30 (d)
31 (a)	32 (c)	33 (d)	34 (d)	35 (b)
36 (c)	37 (d)	38 (c)	39 (c)	40 (a)
41 (a)	42 (a)	43 (a)	44 (b)	45 (c)
46 (d)	47 (a)	48 (d)	49 (a)	50 (c)

PART – B: Short Answers

1. Explain the composition of cell.
2. Write a note on structural hierarchy of organism.
3. Define cell and list out different types of cell. Explain any one type of cell.
4. What are ribosomes and inclusion bodies?
5. How does an animal cell differs from plant cell.
6. Draw neat diagram of mitochondria and explain?
7. What is Golgi complex and endoplasmic reticulum?
8. Write the structural components of eukaryotic ribosomes.
9. Write a note on endomembrane system.
10. Compare prokaryotes and eukaryotes.
11. Write the characteristics of vesicular transport systems across cell membrane.
12. What is intrinsic and extrinsic protein?

13. What is free energy and free energy change?
14. Write the biochemical organization and functions of plasma membrane.
15. Write the characteristics of carrier mediated transport system.
16. Write the difference between passive transport and facilitated diffusion.
17. What is endocytosis and exocytosis?
18. Explain facilitated diffusion with the help of Ping-Pong model.
19. What is cotransport system?
20. Note on disorders of membrane transport system
21. What are the components present in the cell membranes?
22. What is energy rich compounds?
23. What are the main reasons for the ATP acts as universal energy currency molecule?
24. Write a brief account on ATP cycle.
25. Write about the biological significance of ATP & cAMP.
26. Explain redox potential & free energy constant.
27. Explain the biological significance of ATP.

PART – C: Long Answers

1. Write a detailed note on prokaryotic cells.
2. Explain eukaryotic cell with neat labelled diagram.
3. Write a detailed note on biochemical organization of cell membrane with neat labelled diagram.
4. How small molecules and macro molecules are transported in biological system. Explain with suitable diagram.
5. Write a detailed note on bioenergetics.
6. Define free energy, enthalpy and entropy. Explain the relationship between free energy, enthalpy and entropy.
7. List out various co-enzymes involved in biological oxidation and explain any two in detail with chemical structure.
8. Define and classify energy rich compounds. Explain any one compound in detail with chemical structure.
9. Explain about ETC & its mechanism.
10. Explain substrate level phosphorylation with suitable example.
11. Write an essay on electron transport chain & oxidative phosphorylation.
12. Write the biosynthesis and biological significance of ATP.
13. Write the biosynthesis and biological significance of cyclic AMP.
14. Explain the different mechanism or hypothesis proposed for oxidative phosphorylation.
15. Explain rotary motor model for ATP generation. Add a note on inhibitors of oxidative phosphorylation.
16. Outline how cyclic AMP is formed and destroyed. Comment on biological importance of cyclic AMP.
17. Difference between active and passive transport process.
18. Explain the components of respiratory chain in detail.
19. Explain the role of AMP in metabolism. Explain about active membrane transport.
20. Explain the terms: a) Passive diffusion; b) Active transport.
21. Write short notes on cyclic AMP.
22. Write briefly on: a) biological significance of ATP, b) Free energy, c) Energy rich compounds.
23. Write an account of high energy compounds metabolism.
24. Write a detailed note on inhibitors of ETC.
25. How does ATPs synthesized in biological system.