UNIT
1

CELL MEMBRANES

S. J. Singer and G.L.Nicolson

The Fluid mosaic model was first proposed by S.J Singer (American Cell Biologist) and G L. Nicolson (American Biochemist) in 1972 to describe the structure of cell membrane. They published a paper entitled "The structure of cell membrane" in cell biology journal. In this model cell membrane consists of two phospholipid layers. Each phospholipid macromolecule is itself composed of a hydrophilic head and hydrophobic tail. The hydrophilic heads are attracted to water whereas the hydrophobic tails are repelled by water. This attraction and repulsion gives the stable structure to lipid bilayer.

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Learning Objectives

After studying this unit the students will be able to

- discuss the structure and composition of a biological cell membrane
- explain the biomemebrane models
- describe the transport of substances across the membrane
- examine the properties of membrane
- explain the various buffer systems and functions

INTRODUCTION

Life of a cell is entirely based on planar sandwiches called membranes that are capable of protecting and separating the cells from the world around. These membranes, generally, are impermeable to macromolecules but can facilitate movement of certain molecules across it and contribute to maintain homeostasis at a greater level .

Membranes are comprised of two layers of lipids with embedded proteins. They act as two-dimensional entity, with a polar group, facing water exterior and hydrocarbon tails facing the interior side of cell. While membranes encircling the entire cell act as barriers against external environment, membrane around each organelle creates unique interior space for specialized biochemical reactions of that organelle, finally, contributing to the life of a cell.

The current knowledge on membrane has its origin from the findings of E.coli in the year 1855 which described that a membrane consists of lipid bilayer. In the following century, biochemical experts and X-ray experts had postulated the membrane as a surface coating with proteins diffused in the place of membrane or floating or spanning, with lipid anchors.

The following content of the chapter will provide you a detailed knowledge on structure and composition of a membrane.

1.1. CHEMICAL COMPOSITION

1.1.1. Lipid

It is understood from the introductory notes that lipids can form the framework of membrane, with the anchored proteins. The lipid molecules are less than 1000 Dalton in size and consist of aliphatic/aromatic hydrocarbons. There are various types of lipids that form the bilayer structure of a membrane.

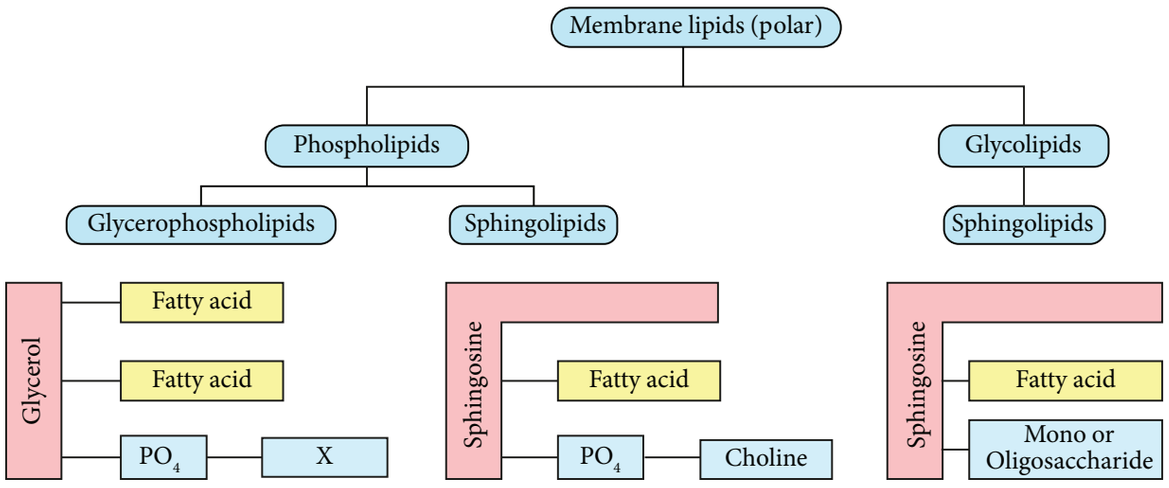


Figure 1.1 Lipid framework of a bilayered membrane

Phosphoglycerides:

Phosphoglycerides are also mentioned as glycerolphospholipids because they contain phosphate. They are the major constituents of lipid bilayer of membrane. Phosphoglycerides have three components

(i) tri-carbon (C1, C2, C3) backbone of glycerol,

(ii) 2 long chain fatty acids esterified to C1 and C2 and

(iii) phosphoric acid esterified to C3 of glycerol.

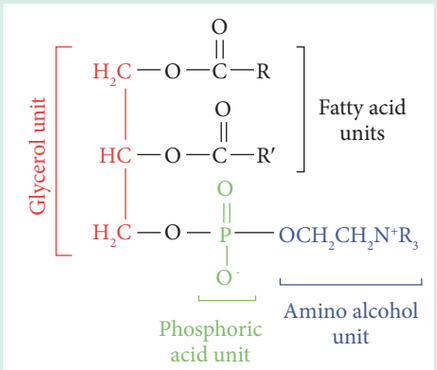
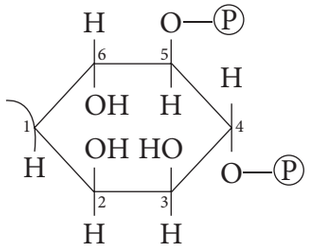
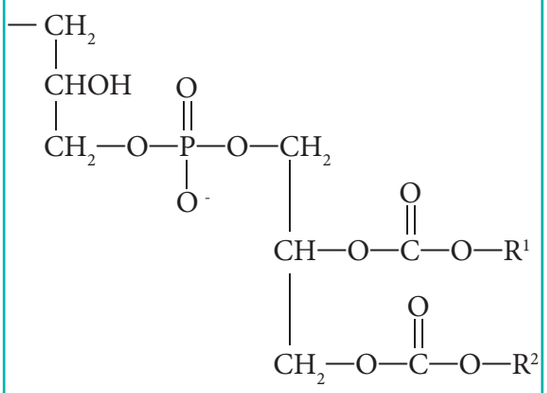


Figure 1.2 Structure of phosphoglycerides

They are amphipathic (as they have both hydrophobic (water fearing) and hydrophilic (water loving) parts. The shape of phosphoglycerides in membrane is roughly rectangular (Figure 1.2). The fatty acids which are aliphatic may have or may not have double bond or can have 2 or more double bonds.

Table 1: Common head groups found in glycerophospholipids and their basic characteristics. Source: Lafer, Eileen. "Membrane Lipids I and II: Glycerophospholipids and Sphingolipids." Lecture. San Antonio, Texas. December 6, 2011

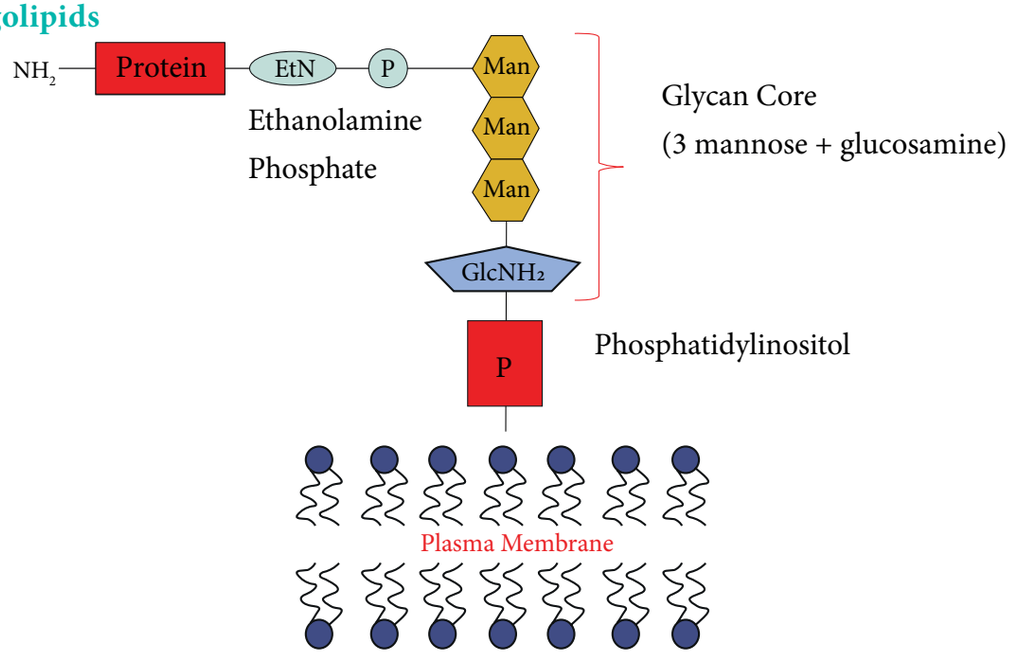
Name of glycerophos-pholipid	Name of X	Formula of X	Net charge (at. pH7)
Phosphatidic acid	-	-H	-1
Phosphatidylethanol-amine	Ethanolamine	$-CH_2-CH_2-\overset{+}{N}H_3$	0
Phosphatidylcholine	Choline	$-CH_2-CH_2-\overset{+}{N}(CH_3)_3$	0
Phosphatidylserine	Serine	$-CH_2-\underset{\displaystyle COO^-}{\underset{\displaystyle |}{CH}}-\overset{+}{N}H_3$	-1
Phosphatidylglycerol	Glycerol	$-CH_2-\underset{\displaystyle OH}{\underset{\displaystyle |}{CH}}-CH_2-OH$	-1

Phosphatidylinositol 4,5-bisphosphate	Myo-Inositol 4,5-bisphosphate		-4
Cardiolipin	Phosphatidylglycerol		-2

Glycolipids:

Glycolipids as their name indicates are sugar containing lipids. The sugar residues are always present on the external side of the membrane. There are three types of glycolipids (i) sphingolipids (ii) glycerolglycolipids in which sugars are attached to hydroxyl group on C3 of triglycerides (iii) glycosylphosphatidyl inositol (GPI). GPI anchors proteins to the outer leaflet of the plasma membrane. The C-terminal residue of a protein is attached through an amide linkage to the amino group of an ethanolamine which is connected by a sugar backbone (Mannose and Glucosamine) to the phosphaditylinositol.

Sphingolipids



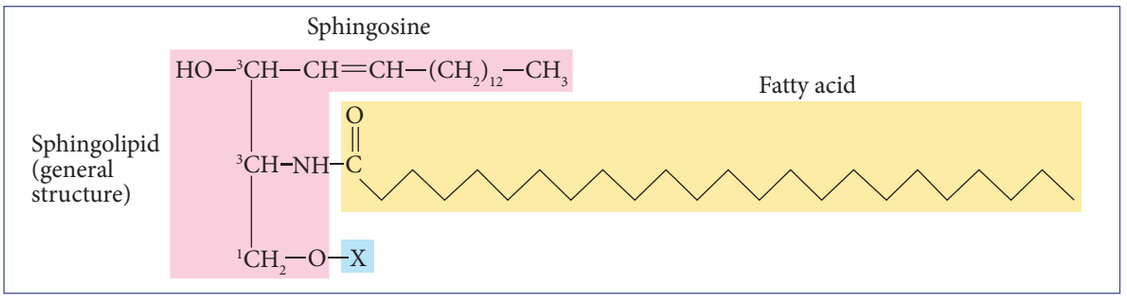


Figure 1.3. Structure of Sphingolipids.

In sphingolipids, the hydrophobic region consists of a long chain sphingoid base with generally 18 carbons, which is linked to the acyl group of a fatty acid via an amide bond. The polar head group (X) attached to C1 consists of X=H in the parent compound, ceramide.

In biological membrane, the sphingolipid components that contain a sugar molecule are referred to as glycolipids. These lipids obtain the name from sphingosine with a polar head group attached to C1 (Figure 1.3). Sphingolipids are abundantly present in membrane. There are various sphingolipids depending on (i) the fatty acid attached to C2 by an amide bond, or (ii) nature of polar head group esterified to the hydroxyl group at C1. Sphingolipids with one or more sugars are called glycophospholipids, which may be either neutral or negatively charged. In some case, a phosphate ester can link a base (choline or ethanolamine similar to phosphoglycerides) to C1 of sphingolipids and it is called sphingomyelin. Complex glycolipids like gangliosides contain oligosaccharides as their polar head group and one or more residues of sialic acid as the terminal sugar.

Sterols

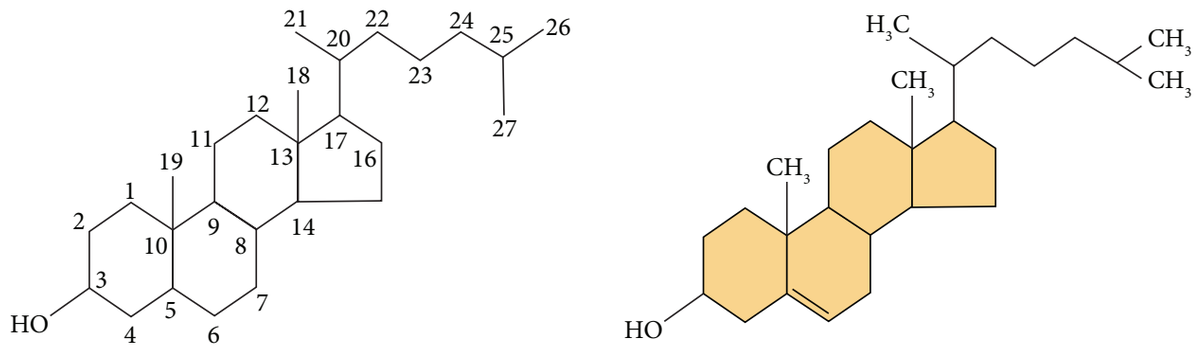


Figure 1.4: Structure of sterols

Sterols are third major lipid constituents of the lipid bilayer (Figure 1.4) cholesterol is the major type of sterol found in animal membranes while flat sterols can be found in bacteria, lower eukaryotes and plants. The cyclopentanoperhydrophenanthrene ring of cholesterol is apolar, inserting into the core of the lipid bilayer with the hydroxyl group at C3 situated at the surface. The precursor moieties of cholesterol namely isoprenol, geranyl and farnesylisoprenols act as hydrocarbon anchors for various membrane proteins.

Triglycerides

Triglycerides are composed of glycerol with fatty acids esterified to all the 'C's. They do lack polar head group but they are not present in membrane.

Lipoproteins are denoted as proteins that are chemically attached to membrane lipids, and they are present on either sides of a membrane.

Water, the universal solvent is also a component of a membrane. The cell membranes include water molecules, either bound to the polar groups visible on the polar side of the membrane molecules (structured water) or unbound (bulk water) within pores and certain ion channels crossing the membranes .

Ions are associated with membranes by simple adsorption to the two surfaces of the membrane or can just pass through ionic channels (membrane proteins) or ion pumps (membrane proteins with enzymatic character). The ions that contributes to the structure and functions of membrane are: H^+, Na^+, K^+, Cl^-, Ca^{++}, HCO_3^-

1.1.2.Proteins

Membrane proteins

Most of the functions of a membrane depend on the proteins present in it. You can recall that it was mentioned elsewhere that there are two classes of membrane proteins.

Both integral and peripheral membrane proteins which form the structure of the membrane. Integral proteins have both hydrophobic and hydrophilic interaction with hydrocarbon core and exterior of lipid cage

1.1.2.1.Integral Protein

Some of the membrane proteins are tightly embedded in the membrane and they cannot be isolated unless, the membrane is disintegrated. They are called as Integral or Intrinsic membrane proteins. They are again classified into two. (a). Transmembrane proteins, which traverse (pass through) or span the membrane. These proteins will have domains on either side of the membrane. Many cell surface receptors belong to this class. (b). Lipid anchored proteins that are present either on the cytosolic side or on the extracytosolic side. They insert themselves in the membrane by a lipid (acyl chain) attached to the N terminal end. Transmembrane proteins are of two types. Single pass transmembrane proteins that traverse the membrane only once. Multipasstransmembrane proteins that traverse the membrane more than once.

1.1.2.2.Peripheral Protein

Those proteins that are present on the surface of the membrane are called as peripheral proteins. They can be easily isolated from the membrane. eg. spectrin present in the RBC membrane.

1.2 MODELS PROPOSED FOR MEMBRANE STRUCTURE

1.2.1. Monolayer Model (1917)

If a lipid molecule containing hydrophilic group is dissolved in a highly volatile solvent and several drops of it are carefully applied to the surface of the water, the lipid spreads out to form a thin mono molecular film. Based on this, Langmuir in 1917 proposed the monolayer model of membranes. In the mono molecular structure, it is found that the hydrophilic portion of each molecule projects in to the water surface and the hydrophobic parts are directed away from the water.

1.2.2. Lipid Bilayer Model or Bimolecular lipid layer:

The model was proposed by Gorter and Grendal in 1925. Lipids from erythrocytes were extracted from erythrocytes and the amount of surface area that lipid would cover when spread over the surface of water was assessed. It was identified that the membrane contained a bimolecular layer of lipids .They found that the polar groups of each molecular layer were directed towards the outside of the bilayer.

1.2.3. Sandwich Model:

Danielli and Davidson proposed the sandwich model in 1935 which was based on the lipid bilayer model. In this model, the plasma membrane is made up of lipid bilayer, sandwiched between the two continuous layers of proteins. The lipid molecules are arranged in an ordered manner and are set at right angles to the surface. The membrane was found to be 75-100 Å thick. In this, the thickness of the protein layer was found to be 20 Å and that of the lipid layer was 35Å .

Drawbacks

This model cannot be applied to all membranes because it reveals definite proportions of lipids and proteins, which has not been true.

1.2.4.Unit membrane Model:

Unit membrane hypothesis of the structure of membrane was proposed in the year 1953 by Robertson. In unit membrane model the protein layers are said to be asymmetrical. The scientist had investigated the structure of a RBC membrane using electron microscope and found that all cellular membranes contains three layers *viz.* two outer layers of the proteins separated by a lighter middle layer of phospholipids. Thus, unit membrane model visualises cell membrane as a trilaminar structure consisting of two dark osmiophilic layers separated by a light osmiophilic layer. The lipid layer was found to be of 35 Å thickness with a dense band of proteins of 20 Å thickness existing on either side of the membrane.Mucoprotein is present on the outer surface and non mucoid protein is present on the inner surface of the RBC membrane.

This model was widely accepted because of the following features

1. The densely packed bimolecular lipid layer shows the presence of 40% lipid by weight in the membrane.
2. The model accounted for the three layered staining pattern of fixed membranes as observed in electron microscopy.
3. Phospholipids spontaneously form a bimolecular system in vitro when added to an aqueous environment and there is no requirement for work input to maintain the minimum energy conformation of the synthetic membrane.
4. The membrane is rich in hydrocarbons, so it is of high electrical resistance.
5. High permeability of natural membranes to non-polar molecules could be explained by their solubility in the non-polar lipid phase and at the same time accounted for relative impermeability to small ions which do not dissolve readily in this medium.

Drawbacks

(i) The thickness of the membrane was found to be greater in plasma membrane than in the intracellular membrane of endoplasmic reticulum or golgi complex

(ii) This model is based on the study of the myelin sheath of a nerve fibre, which is a non-typical membrane and hence cannot be used as a reference model for the cell membrane

1.2.5.Fluid Mosaic Model:

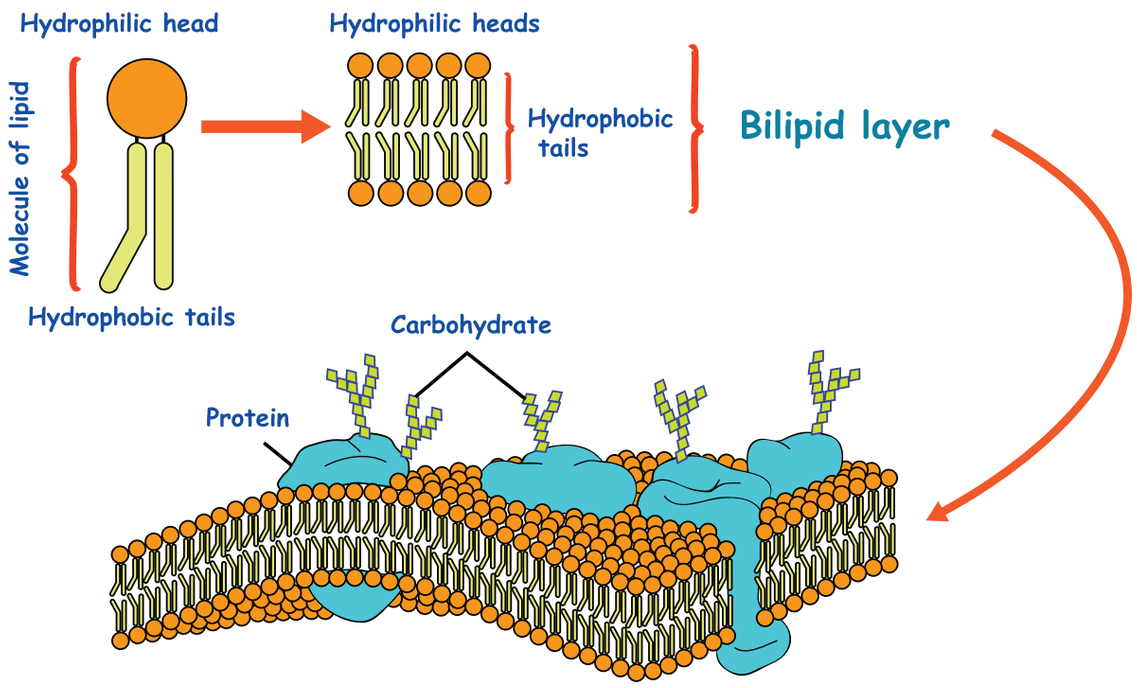


Figure 1.5: Fluid membrane bilayer

The fluid mosaic model of cell membrane was proposed in 1972 by S.J. Singer and G.L. Nicolson. The plasma membrane is a unique component of both plant and animal cells. It serves as a barrier between the cell interior and its surrounding and it allows entry

and exit of certain substances. Hence, it is referred to as a semi-permeable membrane. According to this model, the plasma membrane contains a bimolecular lipid bilayer, both the surfaces are interrupted by protein molecules. The cell membranes have been visualized as mosaics of lipids and proteins.

As mentioned earlier, the cylindrical shapes and amphipathic ratio of phosphoglycerides and sphingolipids allow the bilayer formation with planar structure. This bilayer formation is associated with increased entropy owing to the hydrophobic chain of the bilayer which in turn assists in membrane assembly.

In this model, the membrane contains various molecules such as embedded protein, carbohydrate, cholesterol, and hence it is described as a mosaic pattern. The lipids are thought to be arranged primarily in a bilayer in which peripheral and integral proteins are embedded to varying degrees. The membrane proteins are not fixed within the lipid layer but are free to move laterally. The globular proteins of the membrane are of two different types: extrinsic (peripheral proteins) and intrinsic (integral proteins).

Some proteins are attached at the polar surface of the lipid, while others either partially penetrate the bilayer or span the membrane entirely to stick out on both sides. The protein usually contains chains of sugars or oligosaccharides. Some lipids present on outer surface are glycolipids. The cell membrane is more like a fluid, rather than being a rigid or solid structure. The membrane is of fluid consistency which enables the lateral movement of lipid and protein molecules within it. The proteins of the membrane are concerned with the enzymatic activities, transport of molecules and with receptor function.

Singer and Nicolson considered the lipoprotein association to be hydrophobic and fluidity of the membrane results due to hydrophobic interaction. It should be noted that phospholipids and many intrinsic proteins are amphipathic molecules, i.e., both hydrophilic and hydrophobic groups occur within the same molecule. The fluidity of lipid is evidenced based on x-ray diffraction, differential thermal analysis and electron spin resonance (ESR) techniques.

The lipid bilayer has many dynamic properties which are as follows:

1. The internal rapid motion within each lipid molecule is possible.
2. The lipid molecule might diffuse laterally
3. The transfer of lipid molecule from one side of the bilayer to the other is possible and is referred as flip-flop movement.
4. The lipid molecules might rotate rapidly as a whole about their axis.

The fluid mosaic model of cell membrane is now widely accepted as it can be applied to membranes of all types regardless of their varying characteristics and differences in lipid:protein ratio. In fact this model can account for the molecular organisation and ultrastructure of membranes in-terms of their chemical composition.

Modern knowledge of the red cell plasma membrane and its membrane skeleton began with Marchesi and Steers's identification of spectrin in 1968. Prior to that year, almost the only thing known about membranes was that they contained a lipid bilayer. Indeed, there was a period in the 1960s when it was believed that red cell membranes contained only a single 22.5 kDa protein called "structural protein".

Three major factors can influence fluidity of a cell membrane :

1. Temperature

It will affect the mode of movement and closeness of phospholipids. During cold conditions, they are found together but when it is hot they move away farther from each other.

2. Cholesterol

These molecules are randomly or arbitrarily distributed across the lipid bilayer, so that the layer can be fluid in various environments. Cholesterol clamps phospholipids thereby controlling the unwarranted movement of molecules across.

3. Saturated and unsaturated fatty acids

Fatty acids make the tails of the phospholipid . (i) Saturated fatty acids : they are carbon chains with single bonds between them which makes them straight enabling tight packing. (ii) unsaturated fats are carbon chains with double bonds between them. These double bonds generate bends in the chain, that do not encourage tight packing. This has role in fluidity of the membrane because the twists or bends will increase the space in between the phospholipids. This will eventually leave them not to get frozen at lower temperatures. Further, molecules such as CO_2 and O_2, do need smaller spaces between the phospholipids through which they can move quicky with ease.The two possible bends that can occur are :

1. Cis-unsaturated fats, both sides of the chain stay on the same side
2. Trans-unsaturated fats, both sides of the chain are opposite from each other

1.3.MEMBRANE TRANSPORT

For the maintenance of the integrity of a cell, the internal concentration of various substances should be maintained. The movement of ions, small biomolecules and nutrients should be regulated, while waste products such as carbon dioxide (CO_2) should leave the cells. The membranes are equipped with a special feature that aid in regulation of concentration of the substances inside a cell. The lipid bilayer structure so far discussed does the major part of control transport processes. Generally, the movement of substances across the membranes are categorized based on whether or not energy is required.

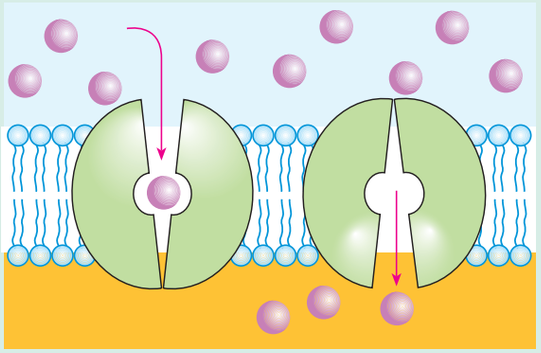
i. Passive transport - the movement of substances across the membrane without the expenditure of cellular energy.

ii. Active transport - the movement of substances across the membrane using energy from adenosine triphosphate (ATP).

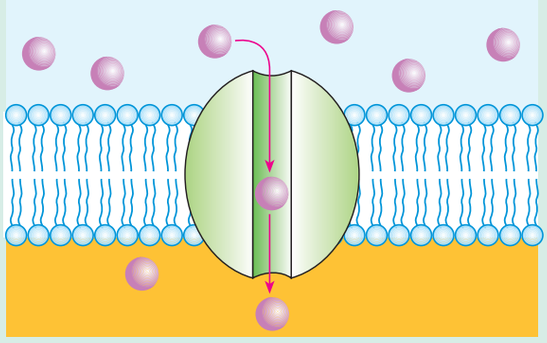
1.3.1.Passive transport

To appreciate the movements in the above mentioned passive mode, let us consider the terms viz., gradients and diffusion

A concentration gradient is the difference in concentration of a substance across a space. Molecules (or ions) will spread/diffuse from where they are more concentrated to where they are less concentrated until they are equally distributed in that space. (When molecules move in this way, they are said to move down their concentration gradient.) Three common types of passive transport include simple diffusion, osmosis, and facilitated diffusion.



Transport of matter with facilitated diffusion



Substance passage through carrier protein channel

Fig 5:. Facilitated Diffusion

1.3.2. Facilitated diffusion

Facilitated diffusion of substances crossing the cell (plasma) membrane takes place with the help of proteins such as channel proteins and carrier proteins. Channel proteins are less selective than carrier proteins, and usually mildly discriminate between their cargo based on size and charge. Carrier proteins are more selective, often only allowing one particular type of molecule only to cross.

1.3.3. Active Transport

For the transport methods described above, the cell expends no energy. Membrane proteins that aid in the passive transport of substances do so without the use of ATP. Active transport differs from diffusion in that molecules are transported away from thermodynamic equilibrium (hence, energy is required). The required energy can come from the hydrolysis of ATP, from electron movement, or from light. The energy moves a substance across a membrane, often with the help of protein carriers, and usually against its concentration gradient. One of the most common types of active transport involves proteins that serve as pumps. The word “pump” probably conjures up thoughts of using energy to pump up the tire of a bicycle or a basketball. Similarly, energy from ATP is required for these membrane proteins to transport substances—molecules or ions—across the membrane, usually against their concentration gradients (from an area of low concentration to an area of high concentration).

The maintenance of electrochemical gradients in biologic systems is so important that it consumes perhaps 30–40% of the total energy expenditure in a cell. In general, cells maintain a low intracellular Na^+ concentration and a high intracellular K^+ concentration, along with a net negative electrical potential inside. The pump that maintains these gradients is an ATPase that is activated by Na^+ and K^+ (Na^+-K^+ ATPase;). The ATPase is an integral membrane protein and requires phospholipids for activity. The ATPase has catalytic centers for both ATP and Na^+ on the cytoplasmic side of the membrane, but the K^+ binding site is located on the extracellular side of the membrane. These pumps are particularly abundant in nerve cells, which are constantly pumping out sodium ions and pulling in potassium ions to maintain an electrical gradient across their cell membranes. An electrical gradient is a difference in electrical charge across a space. In the case of nerve cells, for example, the electrical gradient exists between the inside and outside of the cell, with the inside being negatively-charged (at around -70 mV) relative to the outside. The negative electrical gradient is maintained because each Na^+/K^+ pump moves three Na^+ ions out of the cell and two K^+ ions into the cell for each ATP molecule that is used . This process is so important for nerve cells that it accounts for the majority of their ATP usage.

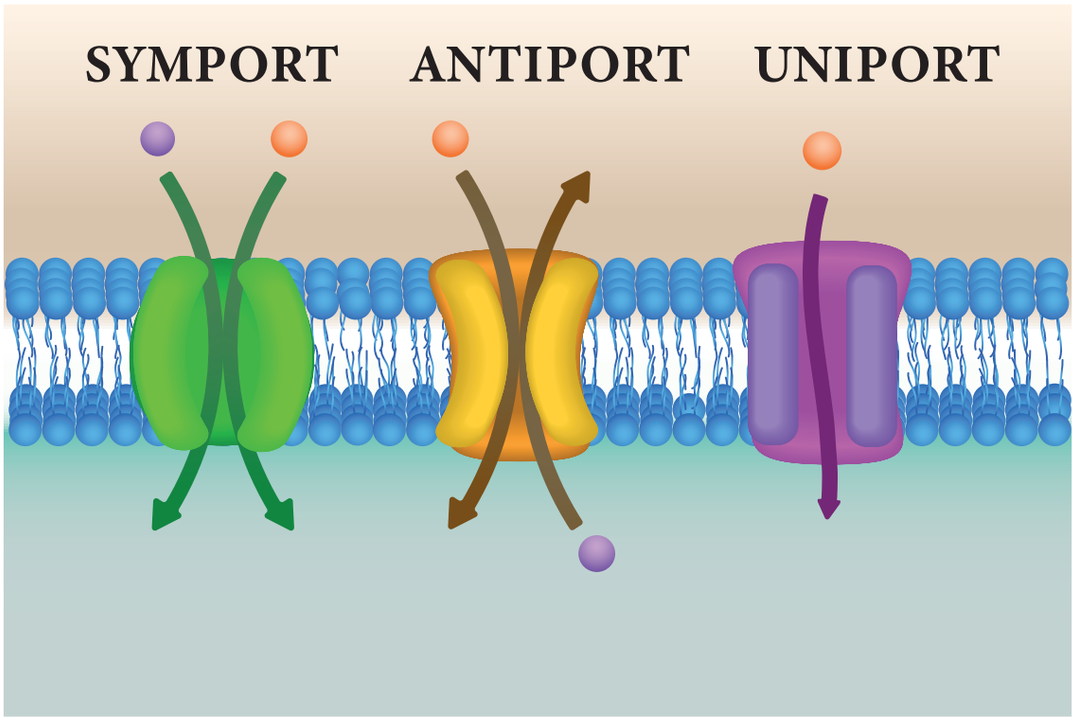


Figure 6: Active transport across membranes. Primary reaction is mediated by pumps (see text) while the porters (Uniporter, symporter and Antiporter) are referred as secondary reactions

Like pumps, carriers are found in all membranes, for exchange of molecules for metabolism or expelling out the wastes. Carriers are also known as facilitators or porters and the substrates for carriers include ions and small soluble organic molecules and sometimes, lipid soluble substances.

Carriers which transport a single substrate through a membrane down its concentration gradient are called uniporters. Many carriers transport substrates against or up concentration gradients also. In such cases, the carrier transport across the membrane is combined with the transport of one more substrate down its electrochemical gradient e.g. Glucose. Its movement from plasma to red blood cells (down the concentration gradient) and to intestines (up its concentration gradient) is along with Na^+. It is called as symporter, as both glucose and Na^+ are transferred in the same direction.

Another class of carriers are termed as antiporters. These are called anti as they transport substrates in the opposite directions to the ion gradient promoting the reaction. All of the carrier-mediated responses are reversible.

It is well understood that the membrane effectively protects the interior of the cell from the exterior. Though the membrane is generally impermeable, molecules from the outside environment are taken up via a process called endocytosis

1.3.4.Endocytosis

In the process of endocytosis, an area or a region of the membrane would surround the substance which needs to be internalised. The section of the membrane that has surrounded the material would bud into the cell forming a vesicle which retains the ingested substance.

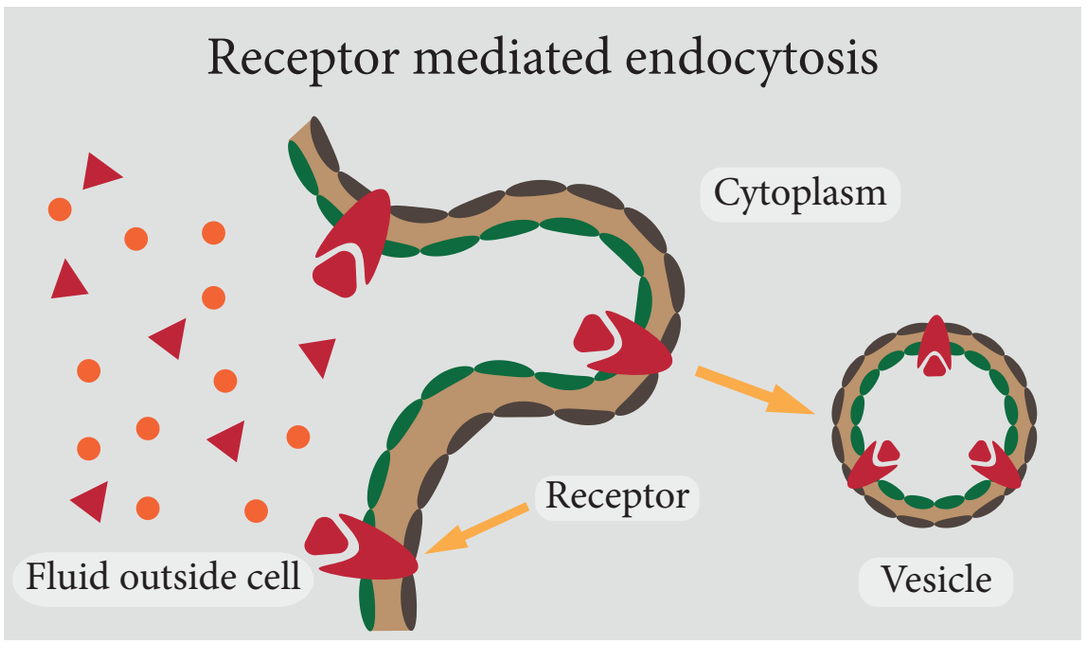


Figure7 .Receptor-mediated endocytosis. A particular substance binds to a receptor on the outer surface of the membrane and uptaken by endocytosis

Receptor-Mediated Endocytosis

Receptor-mediated endocytosis is a targeted process, unlike endocytosis in general, where receptor molecules are employed on the outer surface of the membrane to bind to a substance that need to be transported into the cell. The receptor exhibits specific binding affinity towards the substance that accomplishes the process. A protein coated vesicle called clathrin coated vesicle is attached to the cytosolic side of the membrane and this would pinch off from the membrane once the substance is transported into the coated vesicle.

Membrane uses a process called pinocytosis, a varied process of endocytosis. Pinocytosis uses smaller vesicles and occur in molecules (including liquid) whenever required by the cell from the extracellular fluid. Another varied process of pinocytosis called Potocytosis, uses a coating protein called caveolin, whose function is same as that of clathrin. In addition to caveolin, membranes contain cavities which form vacuoles and have receptors as well as lipid rafts.

In liver cell, receptor-mediated endocytosis is involved in the uptake of a type of cholesterol, called low-density lipoprotein or LDL bad cholesterol. If the receptor-mediated endocytosis is unsuccessful, bad cholesterol will not be removed and gets accumulated. This may result in familial hypercholesterolemia

1.4.VISCOSITY AND SURFACE TENSION

One of the most amazing features of biological membranes is that both the lipid and protein moieties are persistently in motion. This is the property of any molecule of a viscous liquid. The membrane acts as a two-dimensional liquid in which the protein constituents stroll like boats.

The lipid bilayer behaves as a two-dimensional liquid in the absence of covalent bonds between the lipids, whose resistance against shear deformations is characterized by the surface shear viscosity. Any relative motion between the two leaflets of a bilayer is opposed by a frictional force. Living cells actively control these forces by varying the mixture of lipids and sterols present in their membranes

Ouabain, is a plant derived toxic substance and in eastern Africa it was used as an arrow poison warfare and in hunting during ancient times. Ouabain is a cardiac glycoside and at low doses can be useful for treating arrhythmia and hypotension. It can inhibit the ATPase activity.

1.4.1.Biological importance of viscosity and surface tension

1. Blood viscosity is useful in streamlining the blood flow. Blood plasma has a normal viscosity of 15 – 20 mpoises. Alterations in the viscosity are an indication of diseased condition. Viscosity increases during macroglobulinemia, retinal hemorrhages and congestive heart failure.

2. Carbohydrate and protein solutions are highly viscous in nature. The lubricating property of the synovial fluid is achieved mainly by the viscous nature of the mucopolysaccharides present in the synovial fluid.
3. Dipalmitoyl lecithin is a surfactant that is secreted by the lung alveoli, which reduces the surface tension and prevents the collapse of lung alveoli during expiration. Certain premature infants have low levels of this surfactant leading to acute respiratory distress.

1.5. OSMOSIS

Osmosis is the net movement of water across a semi-permeable membrane from an area of lower concentration of the solute to an area of higher concentration of the solute.

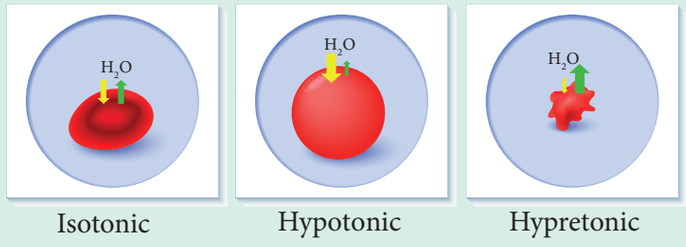


Figure 8: Osmolarity and the consequent changes in a cell

Tonicity is responsible for the movement of the water into or out of the cell. A solution's tonicity is related to its osmolarity, which is the total concentration of all solutes in the solution. A solution with low osmolarity has fewer solute particles per liter of solution, while a solution with high osmolarity has more solute particles per liter of solution. When solutions of different osmolarities are separated by a membrane permeable to water, but not to solute, water will move from the side with lower osmolarity to the side with higher osmolarity.

- If the extracellular fluid has lower osmolarity than the fluid inside the cell, it is said to be hypotonic—hypo means less than—to the cell, and the net flow of water will be into the cell.
- In the reverse case, if the extracellular fluid has a higher osmolarity than the cell's cytoplasm, it is said to be hypertonic—hyper means greater than—to the cell, and water will move out of the cell to the region of higher solute concentration.
- In an isotonic solution—iso means the same—the extracellular fluid has the same osmolarity as the cell, and there will be no net movement of water into or out of the cell.

Hypotonic, hypertonic, and isotonic are relative terms. That is, they describe how one solution compares to another in terms of osmolarity. For instance, if the fluid inside a cell has a higher osmolarity, than the surrounding fluid, the cell interior is hypertonic to the surrounding fluid, and the surrounding fluid is hypotonic to the cell interior.

1.5.1. Biological significance

1. Hemolysis and Crenation. The physiological or isotonic saline is 0.9% NaCl. When red blood cells are suspended in 0.3% NaCl (hypotonic solution), water will enter into the cells and the cell will burst releasing all its contents. This kind of lysis is called as hemolysis. The resulting membranes are called as ghosts. On the other hand, when the cells are placed in 1.5% NaCl, water comes out of the cell, leading to shrinkage of cells. The process is called as crenation.

2. The erythrocyte fragility test is based upon the osmotic diffusion property. The ability of the membrane to withstand hypotonic solution depends upon the integrity of the membrane. Certain genetic disorders like sickle cell anemia and deficiency of vitamin E makes the erythrocyte membrane more fragile.

3. Osmotic pressure of blood is largely due to its mineral ions such as sodium, potassium, chloride, calcium and protein. The osmotic pressure exerted by proteins is of considerable biological significance owing to the impermeability of the plasma membrane to the colloidal particles.

4. Absorption of water in the intestine is due to osmosis. Formation of urine in the kidneys may be attributed to osmotic pressure. The net difference in the hydrostatic pressure and osmotic pressure is responsible for the filtration of water at the arterial end of the capillary and the reabsorption of the same at the venous end. At the arterial end, the hydrostatic pressure is 22 mmHg and the osmotic pressure is 15 mm Hg. The pressure to drive out the fluid is 7 mm Hg. At the venous end, the hydrostatic pressure is 15 mm Hg and osmotic pressure is 7 mm Hg. The net absorption pressure to draw water back into the capillaries is 15 – 7 = 8 mm Hg. This is called as Starling's hypothesis.

1.6. BUFFERS

A buffer is defined as a solution which resists the change in pH that will occur on addition of small quantities of acid or base to the solution. Buffers are mixtures of weak acid and its salt or weak base and its salt. The pH of the solution is defined as the negative logarithm of hydrogen ion concentration. The pH of buffers are determined by Henderson Haselbach equation

$$pH = pKa + \log \frac{[salt]}{[acid]}$$

Regulation of blood pH

When there is a deviation of blood pH away from that of normal value, the two systems in the body work togeather for restoring the equilibrium. 1. The respiratory system modifies the rate of respiration that will in turn alter the concentration of carbon dioxide in the blood; 2. The renal system modifies the reabsorption/and or production of bicarbonate/hydrogen ions. This balance is known as “compensation”.

1.6.1 Hemoglobin Buffer system

By the process of metabolism, carbon di oxide is produced in the tissues and enters the blood. This CO_2 will be hydrated forming H_2CO_3 and this gets ionized yielding H^+ and HCO_3^-. When the oxygen tension is reduced in the tissues, oxy-hemoglobin will dissociate, thereby producing oxygen and hence reduced hemoglobin is formed.

In lungs, oxy-hemoglobin (strong acid) is formed from reduced hemoglobin releasing hydrogen ions that reacts with bicarbonate and yield carbonic acid. Owing to lower CO_2 tension in lung, the shift of equilibrium towards the production of carbon di oxide is enabled which in turn will be continuously eliminated during exhalation

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

Reduced hemoglobin will act as anion and accepts the H^+ ions to produce acid-reduced hemoglobin (HHb). By the production of weak acid, the arrival of H^+ ions are buffered thereby causing little change in pH.

On the return of blood to the lungs, owing to the formation of oxy-hemoglobin (stronger acid) as mentioned above, these H^+ ions are released . Immediately these released H^+ ions are neutralized by HCO_3^-. This is inevitable for the lungs to release CO_2.

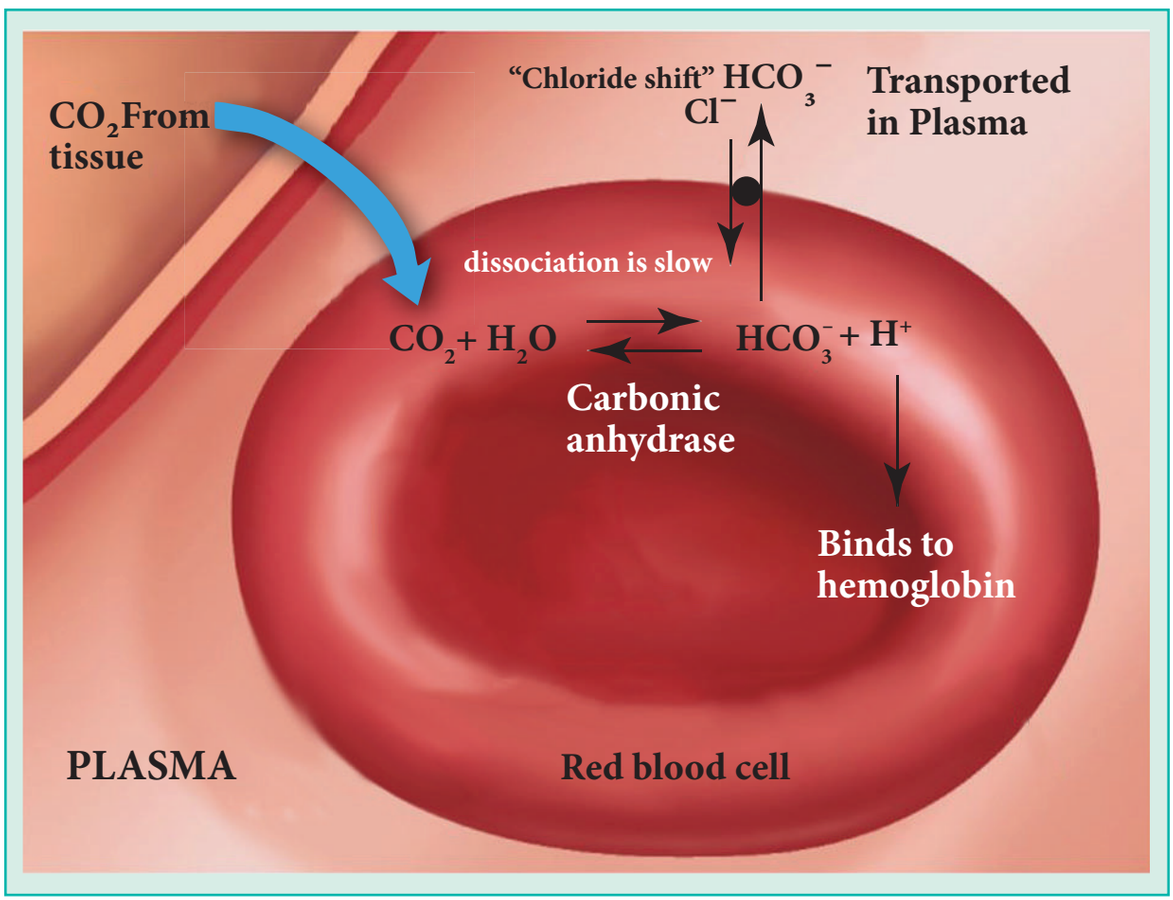


Figure 9

Chloride Shift

1. In RBCs CO_2 reacts with water forming carbonic acid in a reaction that is catalysed by carbonic anhydrase.
2. The formed carbonic acid is buffered by phosphate buffer as well as haemoglobin buffer.
3. Bicarbonate returns to plasma and gets exchanged with chloride ion that enters into (shifts into) the cell if the tension of CO_2 increases in the blood.
4. On the contrary, if the CO_2 tension is reduced, chloride will exit the cell and enters the plasma.
5. In general, red blood corpuscles are impermeable to sodium or potassium whereas permeable to hydrogen, bicarbonate and chloride ions. Potassium which is a cation is available to the plasma by anionic (chloride) exchange. This results in added CO_2 being carried by plasma as sodium bicarbonate.
6. The cycle continues as CO_2 enters and passes to the red blood cells and forms carbonic acid (partial amount of which returns to plasma) by carbonic anhydrase. The remaining carbonic acid then reacts with hemoglobin buffers yielding bicarbonate that travels to the plasma in exchange of chloride and is transported.
7. The reactions mentioned so far are reversible. In lung tissue, chloride shifts back into plasma when blood becomes arterial. This eventually releases intracellular potassium to buffer the oxy-hemoglobin. In plasma, it neutralizes sodium (Figure 9).

1.6.2. Regulation by respiratory mechanism

We have seen that the carbonic acid will dissociate into CO_2 and H_2O. If there is more of H^+ within the blood more of CO_2 elimination will be carried out by lungs. If there is more of HCO_3^-, the lungs will ensure low respiratory rate thus enhancing the retention of CO_2 so that it can be useful for forming carbonic acid that can buffer the excess of bicarbonate.

The alveolei and bronchioles of lungs do perform such functions effectively. A phospholipid molecule is secreted by a particular type of cell in the lung lining the alveoli and bronchioles. This surfactant regulates (lowers) the surface tension of the alveolar membranes thereby protecting the alveoli during exhalation and inhalation.

1.6.3. Regulation by renal mechanism

The major functions of kidneys are regulation of water and electrolyte balance. This is done by excretion of waste substances in urine. The formation of urine involves three stages (i) filtration, (ii) reabsorption, (iii) secretion. The regulatory role of renal organ is achieved by the buffering capacity of the organ. For this function membrane performs key role. Passive and active transports are involved in addition to osmosis and pinocytosis. (Figure 10).

Passive transport

- Simple diffusion
- Facilitated diffusion (glucose in basolateral membrane)

Active transport

Active reabsorption

- Primary active transport (Sodium-potassium ATPase pump)
- Secondary active transport
- Secondary active reabsorption (glucose by sodium in PT)

Active Secretion

- Primary active Secretion
- Secondary active Secretion (H^+ by sodium in PT)

Osmosis

Pinocytosis

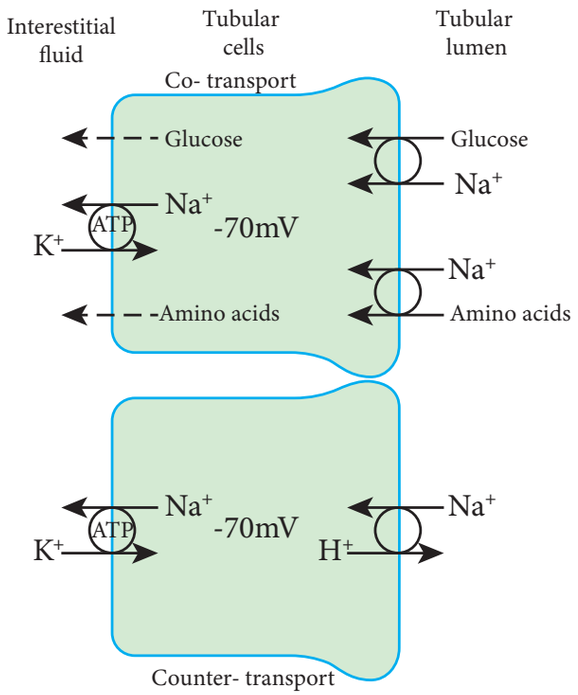


Figure 10: Movement of substances across the renal tubular membrane that aid in urine formation (by filtration, reabsorption,secretion) by kidneys.

Activity

1. Perform the experiment to know the diffusion using coloured liquid or jelly like substance and water that can help you to understand how water, oxygen and small substances are transported across the membrane (Use paper towels as membrane). Do the experiment in the presence and absence of heat i.e hot water and cold water. (Higher temperatures speed up diffusion because molecules have more kinetic energy at higher temperatures).
2. Use potato and sugar solution to understand osmosis. Calculate the difference in height of the solution inside the cut potato (well like pit engraved in potato) which you have kept in a petri dish filled with coloured water.

Summary

1. The cell membrane is an essential feature for the survival and homeostasis of a cell.
2. The membrane regulates the interaction of a cell with its external environment and it allows substances to enter or leave the cell.
3. The membrane is chiefly made up of lipids and proteins; carbohydrates make up a small percentage.

4. Lipids and proteins (integral and peripheral) maintain the structure and function of the membrane
5. Many models have been proposed for the structure of a membrane.
6. The fluid-mosaic model is the widely accepted model that best describes the structures of most of cell membranes
7. Cholesterol and the fatty acids of phospholipids control the fluidity of the membrane.
8. The proteins in the lipid bilayer perform several functions of the membrane. They act as receptors, enzymes, etc.
9. Membrane uses passive and active modes of transport.
10. The endocytotic process and variations of endocytosis allow substances to enter the cell, while exocytosis aids the exit of the substances away from the cell (these process maintain the size of membrane)
11. Membrane is bestowed with properties such as viscosity, surface tension and osmosis thus making them indispensable tool in maintenance of integrity of a cell.
12. Buffers maintain the normal physiological pH. Haemoglobin buffers are playing important role in maintaining pH of blood. The regulatory role of respiratory and renal functions are profoundly dependent on the membranes of lung and kidneys respectively

EVALUATION

I Multiple Choice Questions

1. In a membrane, carbohydrate moieties glycoproteins or glycolipids are

   A. oriented towards outside
   B. placed towards inside
   C. facing towards outside and inside
   D. randomly dispersed (Ans-A)

2. The lipid bilayer _____ in nature

   A. hydrophilic
   B. hydrophobic
   C. both
   D. the nature depends on the immediate environment (Ans-C)

3. _____membrane contains the largest amount of proteins

   A. red blood corpuscular membrane
   B. myelin sheath
   C. lysosomal membrane
   D. outer mitochondrial membrane (Ans-A)

4. The distribution of inherent proteins in the membrane is

   A. symmetrical B. asymmetrical

   C. random D. uniform (Ans-B)

5. _____statement describes the role of a cell membrane?

   A. free entry and exit of substances into or out of a cell.

   B. controlled movement of substances across the cell.

   C. Block the entry of substances into the cell.

   D. Dismisses the exit of substances from the cell. (Ans-B)

6. ----------can pass through - bilayer without support.

   A. Fat-soluble molecules

   B. Ions

   C. Both A and B

   D. None of the above

7. Channel protein adopts ---------- to transport ions

   A. Facilitated diffusion

   B. Active transport

   C. Both A and B

   D. None of the above

8. Which of the following is/are the major functions of cell membrane.

   A. compartmentalization

   B. protection from from extracellular components

   C. temperature maintenance

   D. All of the above

9. Materials are allowed to permitted through cell membrane thus maintaining a constant

   A. gradient

   B. concentration

   C. nutrients

   D. buffering

10. Fluid Mosaic Model helps to understand that the proteins are embedded in ----------fashion.

    A. eplic B. criss-cross

    C. zigzag D. mosaic

11. This sentence is true about the surface tension of a plasma membrane

    A. Surface tension of membrane is greater than pure lipid assemblies.

    B. Surface tension of membrane is lesser than pure lipid molecules.

    C. Cell membrane do not possess surface tension.

    D. Surface tension of membrane is same as that of the pure lipid.

12. One of the following is not the function of a membrane

    A. chromosomal segregation

    B. transport

    C. extracellular interaction

    D. energy transduction

13. In this process, a vesicle formed at the plasma membrane brings the substances into the cell

    A. Endocytosis

    B. Exocytosis

    C. Plasmolysis

    D. Crenation

14. Sodium and potassium are transported by a -------- protein

    A. carrier

    B. channel

    C. receptor

    D. enzyme

15. Clathrin coated pits assist in

    A. receptor-mediated endocytosis

    B. exocytosis

    C. phagocytosis

    D. diffusion

II Answer the following

1. What is a membrane?
2. What is the difference between diffusion and osmosis?
3. Give your idea on tonicity.
4. Discuss the different models of membrane structure.

5. Write about the protein composition of a membrane.
6. Write about the lipid composition of a membrane.
7. Write about the haemoglobin buffer system.
8. How kidneys are efficient in regulation of pH?
9. Give detailed note on membrane transport.
10. What are pinocytosis and potocytosis?
11. What are the three types of glycolipids in the membrane?
12. Give the mosaic model of cell membrane. List the dynamic properties and write the model is accepted.
13. What are uniporters, antiporters and symporters?
14. What do you mean by receptor-mediated endocytosis?
15. Give the biological significance of osmosis.
16. Give the biological significance of viscosity.
17. Give a note on membrane sterols
18. Give a note on sphingolipids present in the membrane.
19. What are the drawbacks of sandwich model?
20. Give a short note on active transport by membrane.

CONCEPT MAP