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

Peripheral membrane proteins

These proteins have the following characteristics:

- They are embedded on any one side of the membrane and are also called as **extrinsic proteins,** e.g. cytochrome c which is present in the outer surface of the inner mitochondrial membrane.
- They are immersed in the membrane only partially and are weakly bound to the hydrophilic region of the specific integral proteins.
- They may have various modes of attachment, e.g. some bind to integral proteins such as an antigen. On the other hand, some peripheral proteins such as cytochrome b_5 have sequences of hydrophobic amino acids at one end of the peptide chain which serve as an anchor in the membrane lipid.
- They can be released by relatively mild procedures that leave the membrane intact, such as by treatment with a salt solution of high ionic strength or by extremes of pH.

CARBOHYDRATES

Some of the membrane proteins and lipids bear short chains of carbohydrates (**oligosaccharides**), covalently attached either to a protein as **glycoprotein** or to a lipid as **glycolipid**.

Carbohydrate content of biological membranes may vary between 3-10%. Oligosaccharide chains are normally located on the outer surface of the membrane or on the terminal side of the endoplasmic reticulum.

STRUCTURE OF A BIOLOGICAL MEMBRANE

As described above, some of the proteins span the lipid bilayer whereas others are immersed only partially. This is called as the **fluid mosaic model** because the membrane is **fluid** in consistency, consisting of a **mosaic** of proteins and lipids which are **free to drift** about in the plane of the membrane (Fig. 3.6).

It has following characteristics:

- It has a sheet-like structure forming a closed boundary and has a thickness of about 7-10 nm.
- It forms non-covalent, asymmetric assemblies of amphiphilic lipids and proteins.
- It is electrically polarized (inside negative with respect to outside).

Membrane fluidity

Membrane fluidity refers to the flexible nature of the membrane, i.e. it can shrink or expand. Membrane fluidity is regulated by several factors:

1. Presence of unsaturated fatty acids – Since *cis*-double bonds cause fatty acyl chains to bend (i.e. form kink), the membrane is less tightly packed and therefore more fluid in nature. Further, higher the unsaturation more is the fluidity.
2. Increased Ca^{2+} decreases membrane fluidity.
3. Cholesterol content of the biomembrane affects its fluidity.

FUNCTIONS OF A BIOLOGICAL MEMBRANE

Biological membranes perform several functions:

1. **Compartmentalization:** It separates two different microenvironments, e.g. the cell membrane separates the intracellular compartment from the extracellular matrix or extracellular fluid.

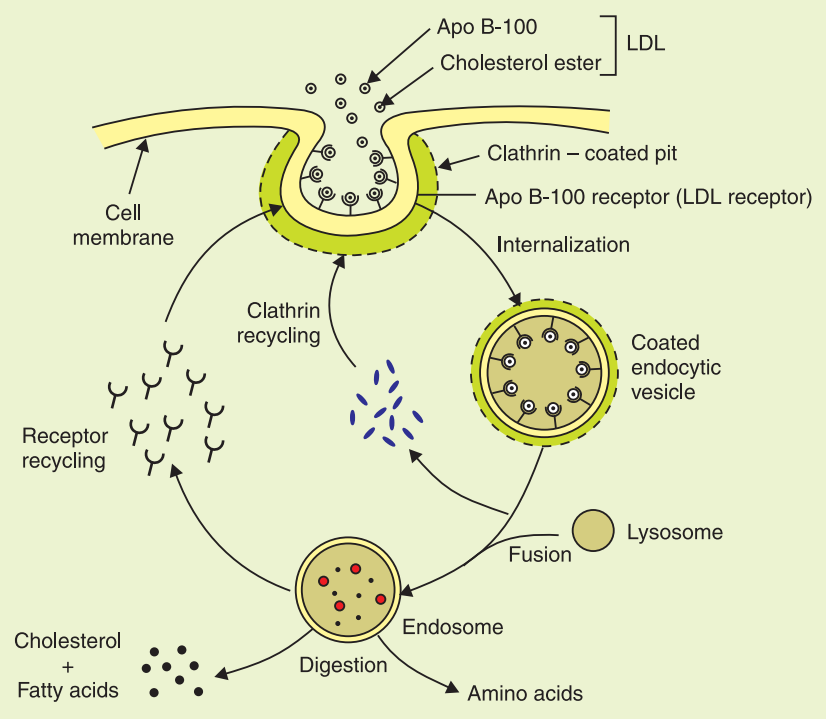


Fig. 4.11. Receptor-mediated pinocytosis.

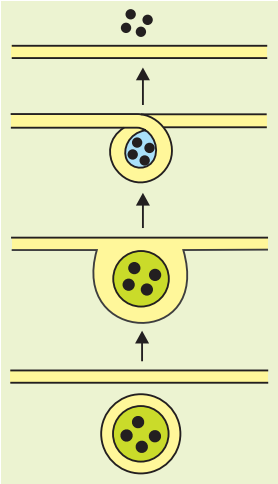


Fig. 4.12. Exocytosis.

concentrations equalizes as a result of **diffusion**. Diffusion occurs when substances move from areas of high concentration to low concentration down a concentration gradient. However, if there is an impermeable solute in one of the solutions such as A^-, concentration of the solution does not equalize. Concentration of the solution with impermeable solute remains high even at equilibrium (Fig. 4.13).

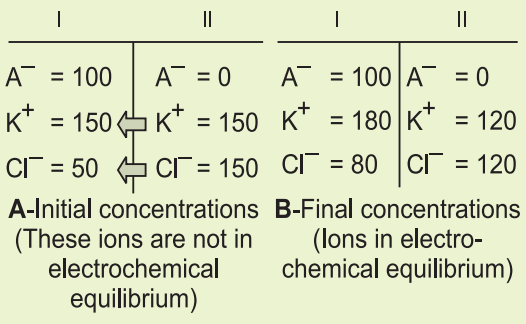


Fig. 4.13. Donnan membrane equilibrium.

Clinical usefulness of the Donnan Membrane Equilibrium

The Donnan effect can be applied to living cells since their cell membranes are selectively permeable, which means that they allow some

direction. Accordingly, hypoventilation takes place, blood pCO_2 rises, and these reactions are pushed to the right, which in turn **results in a high carbonic acid concentration in the blood** and a less alkaline shift in blood pH. Hypoventilation, however, is limited because it causes retention of CO_2, which further stimulates ventilation.

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

Buffering of acid-base disturbances by the respiratory response although is rapid but is relatively coarse. It brings blood pH close to normal but can not eliminate a fixed acid or base from the body. So, it can not restore the pH to normal.

Regulation by the Kidney

Renal response to an acid-base disturbance though is slow but more accurate. The kidney regulates acid-base balance by controlling bicarbonate reabsorption and secreting acid. **Both of these processes depend upon the formation of H^+ and HCO_3^- from CO_2 and H_2O with in the tubule cells.**

Secretion of H^+ ions: Normally, H^+ formed in the reaction is actively secreted into the tubular fluid, in exchange for Na^+. Within the tubule cell, Na^+ as well as HCO_3^- is pumped out of the cell into the interstitial fluid. Sodium uptake by the tubular cell is partly passive, flowing down the electrochemical gradient and partly active via Na^+–H^+ antiport system (Fig. 6.1).

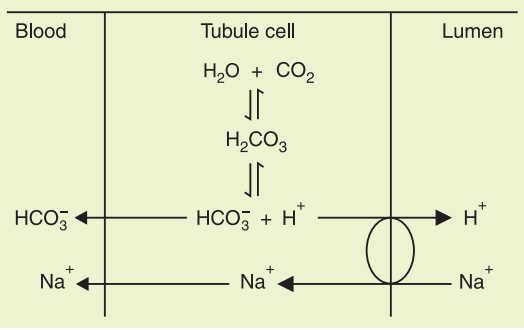


Fig. 6.1. Secretion of H^+.

Mechanisms of the removal of the secreted H^+ ions

H^+ ions that are secreted into the tubular fluid can be removed by any of the three mechanisms, suggesting that three processes are involved in the acidification of urine.

1. By reabsorption of the filtered bicarbonate,
2. By excretion of titratable acids, and
3. Excretion of ammonium ions.

All the three mechanisms involve H^+ secretion and are associated with the addition of bicarbonate to the peritubular capillary blood by the kidney tubules.

Reabsorption of the Filtered Bicarbonate

H^+ ions that are secreted into the tubules (in exchange for Na^+) combine with bicarbonate that has been filtered by the glomeruli. This, in turn, leads to the formation of carbonic acid in the tubular fluid. Dissociation of this acid to CO_2 and water is catalyzed in the lumen by carbonic anhydrase. The CO_2 rapidly diffuses through the cell membrane. **The secretion of H^+, thus, reabsorbs the filtered HCO_3^-, indirectly** (Fig. 6.2)**.**

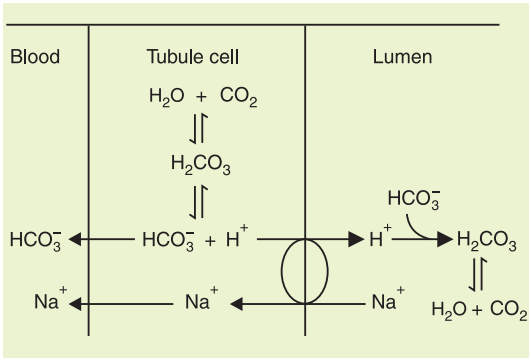


Fig. 6.2. By the reabsorption of the filtered bicarbonate.

Excretion of Titratable Acid

As reabsorption of sodium bicarbonate proceeds, the tubular fluid becomes depleted of HCO_3^- and the pH drops. Following this, **H^+ ions are taken up by the phosphate buffer**. At this stage,

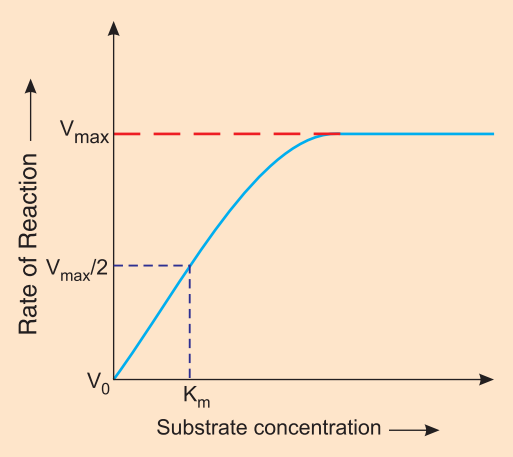


Fig. 7.7. Effect of substrate concentration on enzyme activity.

Michaelis and Menten derived a relationship between substrate concentration and reaction velocity, and described an equation called **Michaelis-Menten equation**, which is as follows:

$$V_0 = \frac{V_{max}\,[S]}{K_m + [S]}$$

where V_0 is the initial reaction velocity, V_{max} maximum velocity of the reaction, [S] concentration of the substrate, and $\mathbf{K_m}$ a constant, which is referred to as **Michaelis constant**.

K_m is defined as the substrate concentration at which the reaction velocity is half of the maximum velocity, i.e. $K_m = V_{max/2}$.

Effect of Enzyme Concentration

Reaction velocity is also affected by the concentration of the enzyme. Reaction velocity is proportional to the concentration of the enzyme present in the reaction mixture. With a two-fold increase in the concentration of the enzyme, the enzyme will combine with double the amount of the substrate, and the amount of the product so formed will also be two-folds. This relationship, however, holds true up to certain limit, i.e. only for the lower concentration of the enzyme but with the adequate concentration of the substrate (Fig. 7.8).

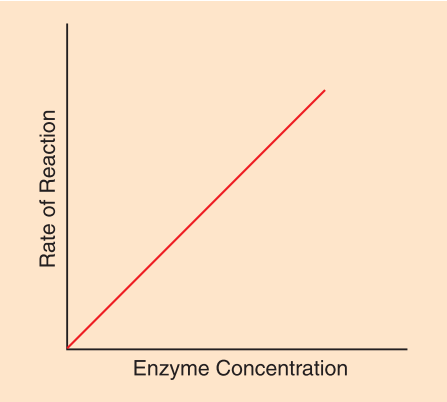


Fig. 7.8. Effect of enzyme concentration on enzyme activity,

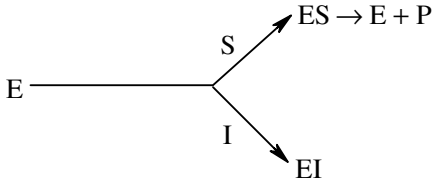
ENZYME INHIBITION

Phenomenon of decrease in the rate of the enzymatic reaction brought about by the addition of some substances is called enzyme inhibition. An inhibitor may affect enzyme activity either competitively or noncompetitively.

COMPETITIVE INHIBITION

A competitive **inhibitor competes with the substrate** for binding at the active sites of the enzyme. **Chemical structure** of such an inhibitor closely **resembles the substrate,** e.g. malonate, which inhibits the action of succinate dehydrogenase, has structure similar to succinate.

The inhibitor forms the complex with the enzyme, which is called as the **enzyme-inhibitor complex** (EI) and inhibits the action of the enzyme.



Binding of enzyme with the competitive inhibitor and the substrate depends upon the relative concentrations of the two and is usually a reversible process. By increasing the concentration of the substrate, degree of inhibition can