
**THE BIOCHEMICAL SOCIETY**

# BIOLOGICAL MEMBRANES

BIOCHEMISTRY
ACROSS THE SCHOOL CURRICULUM
GUIDANCE NOTES FOR ADVANCED BIOLOGY No. 8

# Biological Membranes

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# How membranes are organized

## We cannot live without them

To stay alive, all living things need membranes. Membranes are barriers which give cells their outer boundaries (plasma membranes) and their inner compartments (organelles). Being selectively permeable, membranes control the movement of substances into and out of cells, regulating the composition of the fluid within individual cells. Membranes control the flow of information between cells either by recognizing signal molecules received from other cells, or by sending chemical or electrical signals to other cells. Finally, membranes are involved in the capture and release of energy — photosynthesis and oxidative phosphorylation take place on membranes. Biological membranes are, therefore, more than just an inert barrier or covering: they play an active part in the life of the cell.

## Three membrane components

Biological membranes are made of three major components: lipids, proteins and sugars. All membranes have a common general structure (Figure 1), in which two-layered sheets of lipid molecules have proteins embedded in them. The structure is highly fluid and most of the lipid and protein molecules can move about in the plane of the membrane. The lipid and protein molecules are held together mainly by non-covalent interactions. Sugars are attached by covalent bonds to some of the lipid and protein molecules. They are found on one side of the

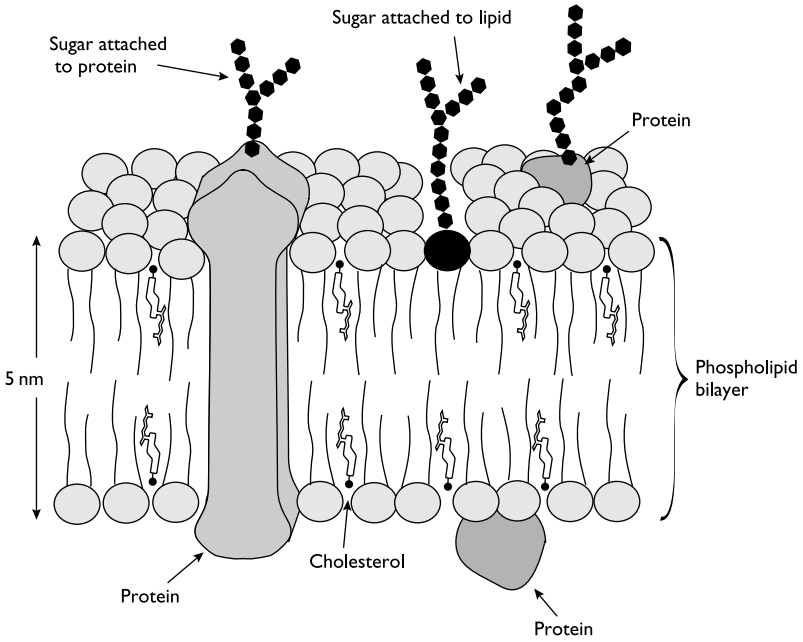



**Figure 1.** Diagrammatic representation of a biological membrane.

membrane only: for example, on the outer surface of the plasma membrane.

## Three types of lipid

Lipids are biologically important substances that are insoluble in water but soluble in organic solvents such as propanone (acetone), ethanol, trichloromethane (chloroform), ethoxyethane (diethyl ether) and light petroleum (b.p. 40–60 °C). There are three major types of lipid found in biological membranes: phospholipids, glycolipids and cholesterol. They each play different roles in the membrane.

### Phospholipids contain phosphate

The most common type of phospholipid consists of glycerol (propan-1,2,3-triol) linked to two fatty acid chains, phosphate and choline (Figure 2). The fatty acid chains usually contain between 14 and 24 carbon atoms. One chain is usually unsaturated, containing from one to four *cis* double bonds. Each double bond puts a bend in the fatty acid chain.

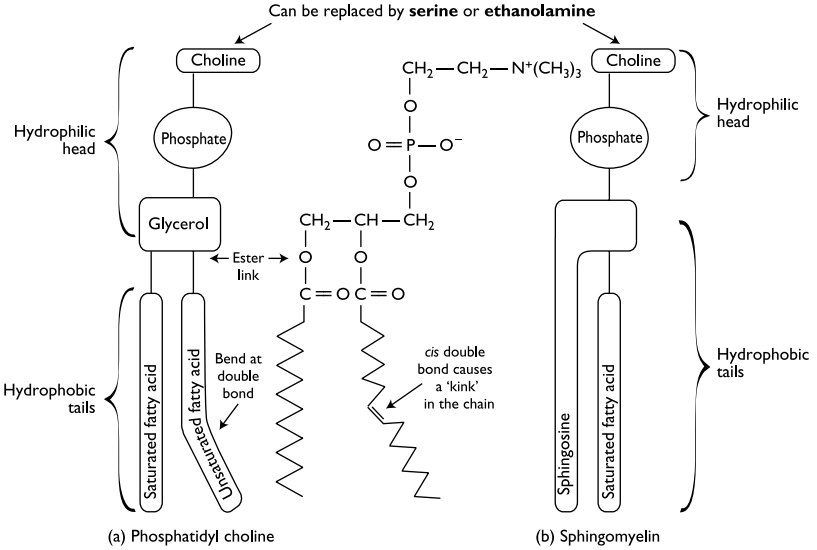



**Figure 2.** (a) Phosphatidyl choline, a glycerophospholipid; (b) sphingomyelin, a sphingophospholipid.

Because they contain glycerol, lipids of this type are called glycerophospholipids. The three major glycerophospholipids contain choline, or serine, or ethanolamine attached to the phosphate. Serine is $HOCH_2CH(COO^-)NH_3^+$. Ethanolamine is $HOCH_2CH_2NH_3^+$. Do you see from Figure 2 how each molecule can replace choline to form part of a phospholipid? Another type of phospholipid contains sphingosine instead of glycerol. The most common example, sphingomyelin, contains choline attached to the phosphate.

## Glycolipids contain sugars

In common with phospholipids, glycolipid molecules contain either glycerol or sphingosine linked to fatty acid chains (Figure 3). They differ from phospholipids in that glycolipids have a sugar, such as glucose or galactose, instead of the phosphate-containing head. Glycolipids in animal membranes almost always contain sphingosine, whereas those in bacterial and plant membranes principally contain glycerol. In all cases, glycolipids are found on the outer surface of the plasma membrane with their sugars exposed at the cell surface.

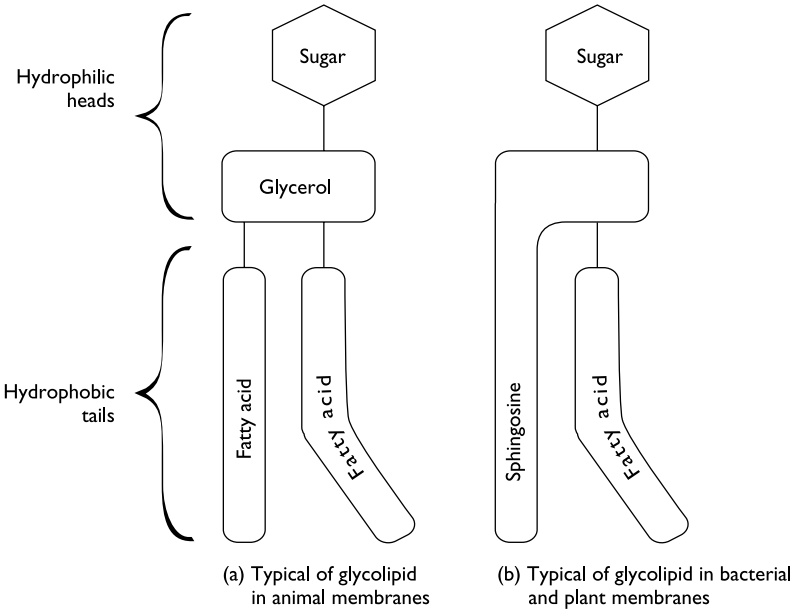



**Figure 3.** Glycolipid structures.

Cholesterol: in a class of its own

The third type of membrane lipid is cholesterol, a molecule that is structurally quite different from the phospholipids and glycolipids. Cholesterol contains a four-ring steroid structure together with a short hydrocarbon side-chain and a hydroxy group (Figure 4). Cholesterol is found in some mammalian membranes, and also in one type of micro-organism — the mycoplasmas. (Mycoplasmas are small bacteria which have a plasma membrane but no cell wall. They may be the simplest lifeforms capable of independent growth and metabolism.) It is not usually found in most bacterial membranes, nor in any plant membranes.

## Two-faced membrane lipids

A common feature of membrane lipids is that they are amphipathic. This means that they have a hydrophilic (water-loving, or polar) region and a hydrophobic (water-fearing, or non-polar) region. [For more information about solubility in water, see BASC booklet *Essential Chemistry for Biochemistry*, pp. 10–12.]

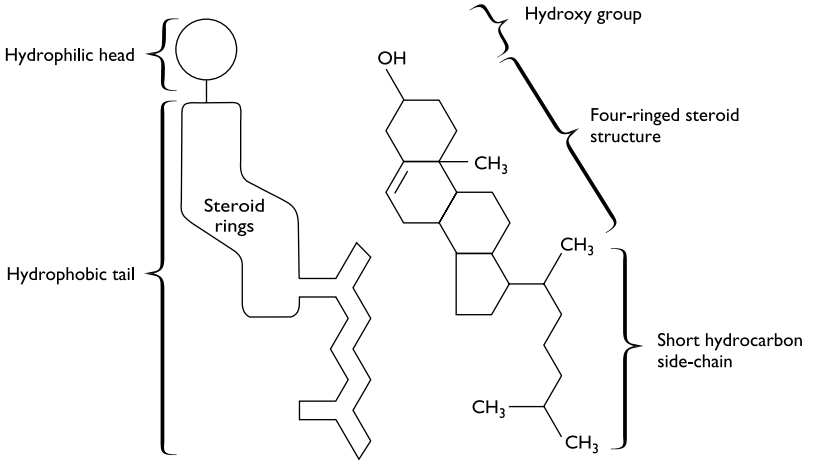
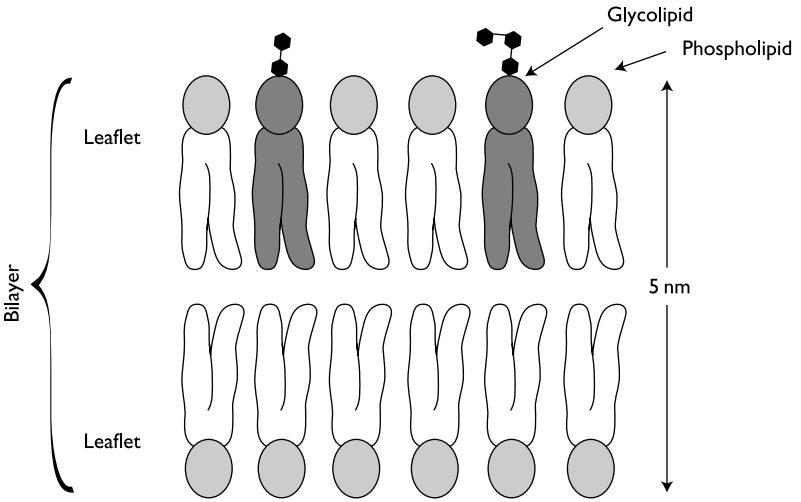



**Figure 4.** Cholesterol.

Phospholipids and glycolipids each have a hydrophilic head and two hydrophobic tails. If submerged in water, these molecules will spontaneously associate to form bilayers, with their hydrophobic tails sandwiched between the hydrophilic heads (Figure 5).

**Figure 5.** A membrane bilayer.



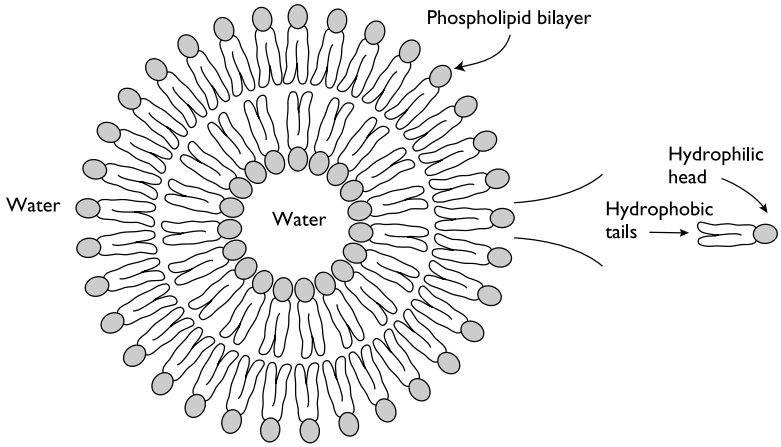



**Figure 6.** Section through a liposome.

Bilayers form because the hydrocarbon tails have a strong tendency to stay away from water, and are 'squeezed together' by water molecules. Such bilayers will close on themselves to form sealed compartments, called liposomes, to eliminate the edges where the tails would be in contact with water (Figure 6). Liposomes are useful model membranes for research, and may also be used to deliver drugs to particular organs of the body. Liposomes are absorbed by many cells by fusion with the cell plasma membrane. If methods can be developed for targeting liposomes to particular tissues, drugs could be carried in liposomes to these tissues.

Cholesterol, too, is amphipathic due to its hydrophobic rings and side-chain, and its hydrophilic hydroxy group. It can be incorporated into phospholipid bilayers (Figure 7), but cannot form a bilayer on its own.

## Membrane proteins

Many of the specific functions of membranes are carried out by proteins. Accordingly, the amount and types of protein vary considerably from membrane to membrane. The more active a membrane is in metabolism, the more protein it contains.

There are several different ways in which proteins are associated with lipid bilayers to form functional membranes (Figure 8).

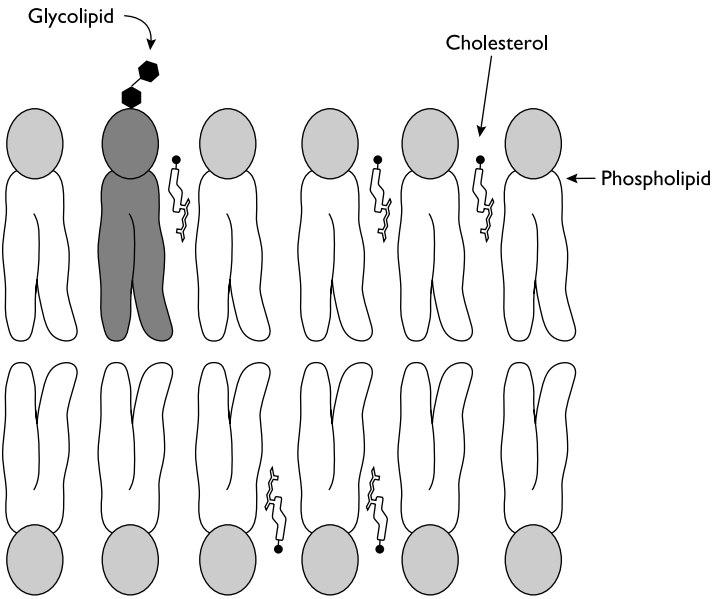
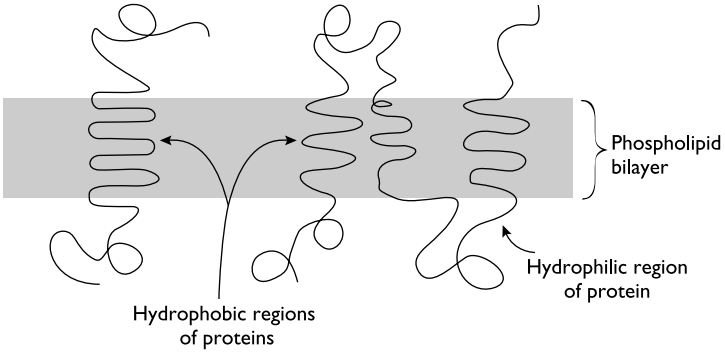



**Figure 7.** Cholesterol incorporated into a membrane bilayer.

(i) Many membrane proteins extend across the lipid bilayer. Such transmembrane proteins have hydrophobic regions that are embedded within the bilayer and interact with the hydrophobic tails of the phospholipids. These regions are often helical, forming rigid 'tubes' studded with hydrophobic amino acid side-chains. There may be one or

**Figure 8.** Transmembrane proteins embedded in the bilayer.

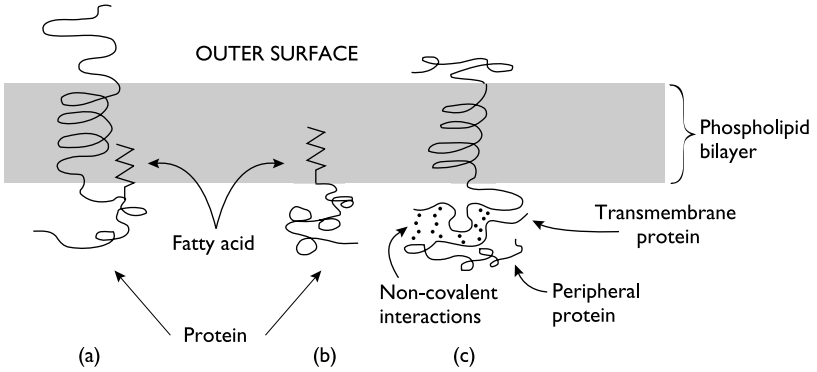


more such regions in a transmembrane protein. The hydrophilic regions of these proteins extend from both sides of the membrane. Some transmembrane proteins may be covalently linked to a fatty acid chain which is inserted into the phospholipid bilayer (Figure 9a).

(ii) Some intracellular proteins do not span the membrane but are covalently attached to the inner surface, by either a fatty acid chain or a phospholipid (Figure 9b). Such proteins are sometimes termed anchored proteins; they are firmly attached to the membrane and can only be removed by treatments (e.g. using detergents or organic solvents) which disrupt the membrane. The proteins are, therefore, called integral membrane proteins. Examples are the enzyme cholinesterase which is found in synapses, and the G-proteins involved in sending messages across membranes.

(iii) Many proteins are weakly bound to one or other surface of the membrane by non-covalent interactions with other membrane proteins (Figure 9c). They can be removed by mild treatments (such as altering the pH or ionic strength) which leave the membrane intact. Such proteins are called peripheral membrane proteins. An example is cytochrome *c* of the inner mitochondrial membrane.

**Figure 9.** (a) Transmembrane protein attached to bilayer by fatty acid chain; (b) integral membrane protein (e.g. cholinesterase) attached by fatty acid chain; (c) peripheral membrane protein (e.g. cytochrome *c*) attached to a transmembrane protein.



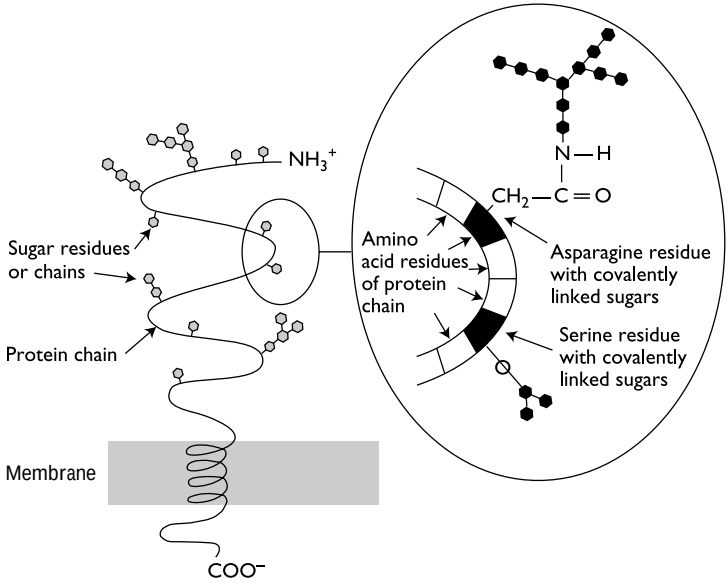



**Figure 10.** Diagrammatic representation of a glycoprotein embedded in a membrane bilayer (e.g. glycophorin A, a glycoprotein found in red cell membranes).

### Glycoproteins contain sugars

Most of the proteins of the plasma membrane that are exposed to the cell surface have covalently linked sugars (Figure 10). The sugars may be linked either to the $-CONH_2$ side-chain of the amino acid asparagine, or to the $-OH$ in the side-chains of serine or threonine. The sugars are present as short, branched chains containing from about 4 to 12 residues.

## Two-dimensional fluids

Lipid bilayers are two-dimensional fluids, since membrane lipids and many proteins are able to move past each other along the membrane (Figure 11). For phospholipids, this lateral diffusion is very rapid: the diffusion coefficient (the area over which a molecule moves in one second) is about 1 $\mu m^2 \cdot s^{-1}$. This means that a phospholipid molecule diffuses about 2 $\mu$m in 1 s. Thus it can move from one end of a bacterial cell to the other in 1 s, or around the perimeter of a human red blood cell in 12 s. Individual lipid molecules also rotate very rapidly along their head-to-tail axes, and the flexible tails can 'wave about' at their ends.

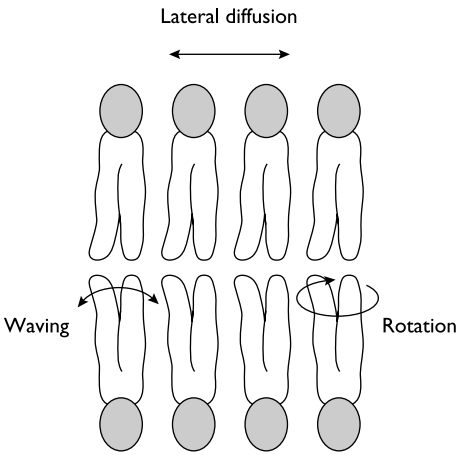



**Figure 11.** Movements of phospholipids in bilayers.

Membrane proteins are more variable in their mobility — some are almost as mobile as the phospholipids. Rhodopsin, a protein which absorbs light in the retina, is fairly mobile and has a diffusion coefficient of 0.4 $\mu m^2 \cdot s^{-1}$; the protein also rotates. Fibronectin, a protein which binds cells to each other and to other surfaces, is about 40000 times slower, with a diffusion coefficient of about 100 $nm^2 \cdot s^{-1}$.

## Two separate layers

In contrast with the rapid lateral diffusion, lipid molecules rarely move from the monolayer that they are in to the opposite one, and often the lipid composition of the two layers is quite different. The transfer of a phospholipid molecule from one layer to the other — known as transverse diffusion or flip-flop — is rare because the polar, hydrophilic head would have to penetrate the non-polar, hydrophobic hydrocarbon core of the bilayer (Figure 12). Measurements of flip-flop times of labelled phospholipids in artificial vesicles show that on average a phospholipid molecule flip-flops only once in several hours.

However, in membranes of the endoplasmic reticulum, where phospholipids are synthesized, there is a rapid flip-flop of particular lipids across the bilayer. This is achieved by proteins called phospholipid translocators (or flippases).

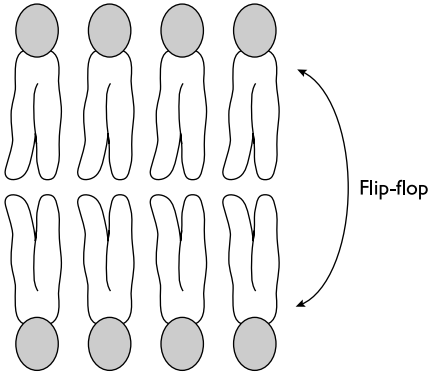



**Figure 12.** Flip-flop movements of phospholipids within bilayers are very rare.

Proteins, being larger than phospholipids and having more extensive polar regions, are even less likely to flip-flop. Indeed, flip-flop of protein molecules has so far not been observed.

## Membrane fluidity

The fluidity of a lipid bilayer is affected by temperature, fatty acid composition and cholesterol content. At low temperatures, the hydrocarbon tails of bilayer lipids can pack closely together to form an ordered arrangement (or gel state) which is fairly rigid. As temperature is increased, the lipid molecules vibrate more rapidly, causing the bilayer to 'melt' into a more disordered arrangement (or liquid state) which is more fluid. The same sort of change can be seen in butter, which is solid when cool but liquid when warmed. The temperature at which the lipid bilayer melts is called the transition temperature; for most biological membranes this is in range 10–40 °C.

Bilayer fluidity also depends on the lipid composition: the transition temperature is lower (i.e. the bilayer is more fluid) if the lipid tails are short or have double bonds. Short chains will interact less with one another than will long chains, hence a lower temperature is needed to melt the bilayer containing them. Double bonds put bends in the hydrocarbon tails, making it more difficult for the phospholipids to pack together, and bilayer fluidity is thus increased. This is why olive oil is liquid at room temperature, whereas lard (beef fat) is solid: 80% of the

olive oil acyl chains contain one or more double bonds, compared with only 46% of the beef fat acyl chains.

Bilayer fluidity is influenced by cholesterol. Eukaryotic animal membranes contain approximately one cholesterol molecule for every two phospholipid molecules. Cholesterol fits in between the bilayer phospholipids with its hydroxy group close to the phospholipid heads, and its hydrophobic rings and side-chain buried within the fatty acid chains of the membrane interior. The rigid steroid ring interacts with the neighbouring regions of the lipid tails and stiffens them, making the membrane less fluid.

## Membrane carbohydrate

Sugar residues attached to either proteins or lipids usually make up less than 10% of the membrane weight. Because they can give rise to a wide variety of structures in relatively short chains, they give individual cell types distinguishing features. Therefore, they may be involved in cell recognition. For example, the surfaces of red blood cells have carbohydrates arranged in branched chains; differences in the arrangements give rise to different blood-group antigens (i.e. A, B and O). Cell-surface differences are also responsible for the specificity of action of cells with hormones, drugs, viruses or bacteria. The cause of cell-surface differences is believed to be related to characteristic surface-carbohydrate components.

## Membrane structure summarized

The fluid mosaic model of membrane structure, proposed by S.J. Singer and G.L. Nicolson in 1972, depicts the membrane as a seething mass of lipids and proteins in which movements of molecules take place in two dimensions. On one surface, chains of sugar molecules stick out and wave about. Biological membranes are thus lipid–protein–sugar ‘sheets’, in which the permeability barrier and structural integrity are provided by the lipids; specific functions are carried out by the proteins; and the distinctive ‘appearance’ is provided by the sugars.

# 2

# How membrane structure was proved

Some of the experimental evidence in support of the fluid mosaic model.

## The lipid bilayer

The first experiments to show that membranes were bilayers were done by E. Gorter and F. Grendel in 1925. The membrane lipids from red blood cells were extracted into propanone (acetone) then floated on the surface of water. They were found to form a continuous macromolecular layer which occupied an area of about twice the surface area of the original red blood cells. Because the only membrane in red blood cells is the plasma membrane (there are no organelles), it was concluded that the membrane was organized in a continuous bilayer.

Later experiments have confirmed the bilayer structure of biological membranes. X-ray diffraction studies show that the interiors of membranes are low in electron density, whereas the outside edges are high in electron density. This is consistent with a bilayer structure which has hydrocarbon tails in the interior and polar heads on the outer surface. Freeze-fracture electron microscopy, in which membranes are frozen and split into their separate monolayers, is another confirmation of the bilayer structure. X-ray diffraction measurements and electron microscope studies show that many natural membranes are approximately 5 nm thick.

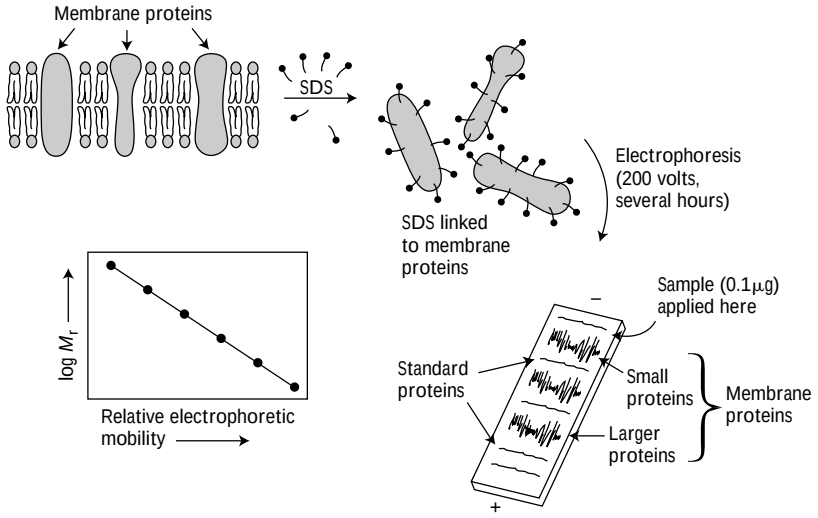
## Membrane proteins

Membrane proteins can be visualized, and their molecular mass measured, by SDS/polyacrylamide-gel electrophoresis (Figure 13).

SDS (sodium dodecyl sulphate) is an amphipathic molecule with a negatively charged hydrophilic head and a hydrophobic tail. It disrupts membrane structure and binds to the hydrophobic regions of membrane proteins to give them an overall negative charge. Electrophoresis is carried out on a slab of polyacrylamide gel, and the proteins are visualized by staining. The gel acts as a sieve so that small proteins move furthest through the gel, while large ones are retarded and stay near the point of application. The distance moved is inversely proportional to the logarithm of the molecular mass.

The first evidence for the existence of integral membrane proteins was obtained from freeze-fracture techniques. Membranes are rapidly frozen in liquid nitrogen (to –196 °C) before being fractured with a cold microtome knife. The bilayer splits into its two monolayers which, after suitable treatment, can be seen under the electron microscope. The inner faces are studded with globular particles 5.0–8.5 nm in diameter, which are membrane proteins. (We know that they are proteins because they are not present if the membrane is treated with proteolytic enzymes before freeze fracture.)

**Figure 13**. Analysis of membrane proteins by SDS/polyacrylamide-gel electrophoresis.



## Bilayer mobility

Cell fusion shows that membrane proteins are mobile, so the bilayer is fluid. L. Frye and M. Edidin (1970) labelled proteins in human and mouse cells with red and green fluorescent dyes respectively. The cells were fused and viewed under a light microscope. The red and green labels were originally localized within regions of the original cell membranes, but after 40 min they were distributed randomly over the entire surface of the hybrid cell, thus showing that integral proteins diffuse freely in the lipid bilayer.

Fluorescence photobleaching recovery shows that proteins and lipids in membranes are mobile. A cell-surface component (protein or lipid) is labelled with a fluorescent dye. A small area (say 3 $\mu m^2$) of cell surface is viewed under a fluorescence microscope, and the fluorescent molecules in this region are destroyed with a very narrow beam of laser light. The fluorescence of the observed region is monitored, whereupon it is found that it will recover its fluorescence as time passes. Owing to the fluidity of the membrane, bleached molecules leave the region and fluorescent molecules enter it. The diffusion coefficients of lipids and proteins can be measured by this method.

A third method of measuring the mobility of components in bilayers is to use artificial bilayers of known composition. They can be in the form of either spherical vesicles (liposomes), or planar sheets (black membranes) formed across the hole in a partition between two solutions. Use of spin-labelled phospholipids — which have heads bearing a group that contains an unpaired electron whose spin generates a detectable signal — has shown that bilayer lipids move rapidly, but flip-flop extremely slowly. Artificial bilayers can also be used to study membrane permeability.

## Surface sugars

Use of carbohydrate-binding proteins called lectins show that carbohydrates are located on only the outer surface of plasma membranes. Lectins recognize specific sequences of sugars, and they bind to membrane-surface glycolipids and glycoproteins. The lectins can be treated with ferritin so that they can be seen under the electron microscope.

# 3

# How membranes are made

## New membranes from old

Membranes grow by the expansion of pre-existing membranes. In prokaryotes, membrane components are synthesized on the inner surface of the plasma membrane; in eukaryotes they are synthesized on the 'outer' (cytosolic) face of the endoplasmic reticulum (ER). The flow of membranes in eukaryotic cells is from ER to Golgi complex to plasma membrane to cell exterior — unless special signals direct the flow to other organelles, to vesicles for secretion under hormonal or nervous control, or even back to the ER (Figure 14).

## Membrane lipids

Phospholipid molecules are assembled step-by-step (Figure 15). First, two fatty acids, which are activated, and embedded by their hydrocarbon chains in the ER membrane, are attached one by one to cytosolic glycerol phosphate. The resulting amphipathic molecule (diacylglycerol phosphate) is embedded in the ER membrane by its two hydrocarbon tails. Next, the phosphate is replaced by the polar head-group (for example, phosphate-choline) to form a membrane phospholipid. The enzymes that catalyse these steps are integral proteins which are embedded in the ER membrane so that their active sites face the cytosol.

These reactions occur in the outer monolayer of the ER membrane; however, flippase enzymes, present in the ER membrane of eukaryotes and in the plasma membrane of prokaryotes, transfer some of the newly formed phospholipids to the opposite monolayer (Figure 16). Flippases do not bind all phospholipids equally well, thus the two membrane monolayers end up having different distributions of phospholipid. For

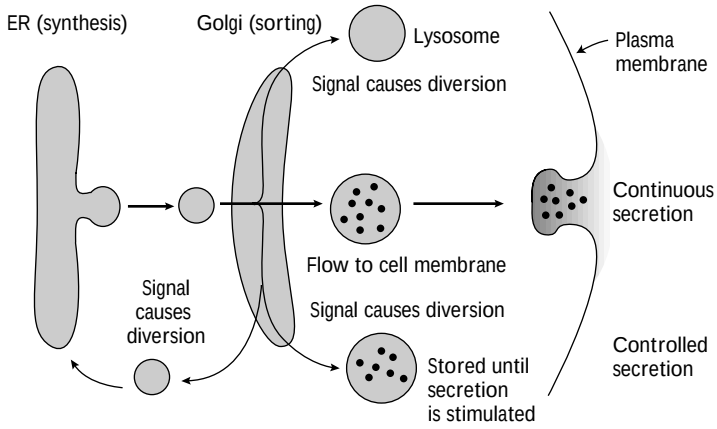
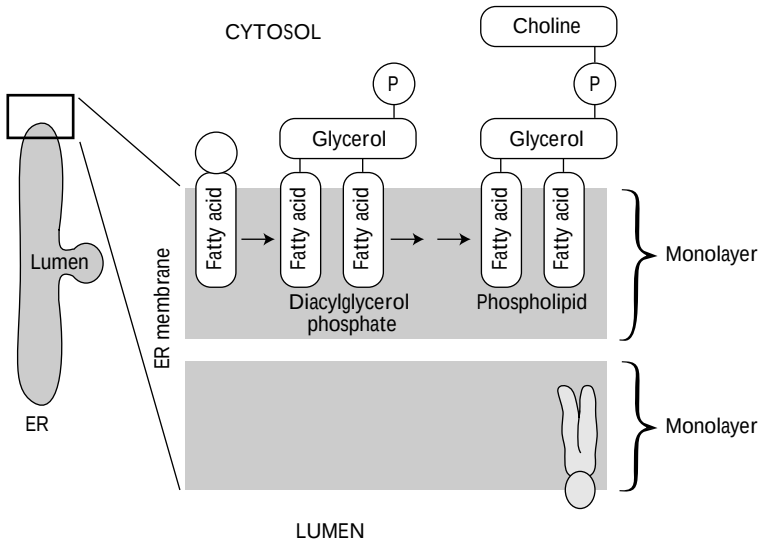



**Figure 14.** Flow of membranes in eukaryotic cells.

example, the inner ER monolayer, which produces the outer monolayer of the plasma membrane, becomes enriched with the phospholipids that contain choline.

The final stage in membrane phospholipid synthesis is the transfer of phospholipids from the ER membrane to other cellular membranes.

**Figure 15.** Formation of phospholipids.



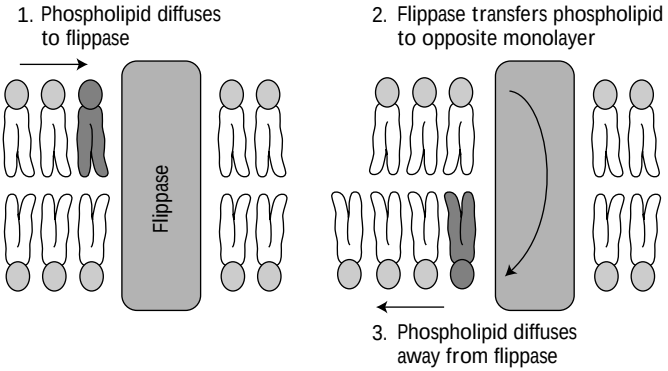
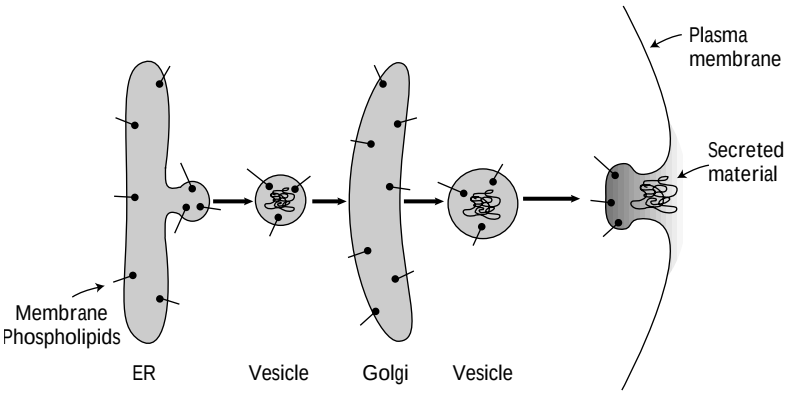



**Figure 16.** Action of flippases.

Membrane vesicles bud off from the ER and fuse with membranes of the Golgi complex. Other vesicles bud off from the Golgi complex and fuse with membranes of other organelles (such as lysosomes) or with the plasma membrane. The membranes of transport vesicles contain phospholipid, glycolipid and cholesterol, as well as membrane proteins. Materials contained within these vesicles are either taken up by the organelles with which they fuse, or secreted from the cell if fusion occurs with the plasma membrane (Figure 17).

Organelles, such as mitochondria, acquire proteins from the ER by a different mechanism. Water-soluble carrier proteins called phospho-

**Figure 17.** Transfer of phospholipids from the ER membrane to other cellular membranes.



lipid-exchange proteins (or phospholipid-transfer proteins) remove phospholipids from the ER membrane and deposit them in the membranes of the appropriate organelles.

The ER also synthesizes cholesterol and the sphingosine–fatty acid compound that forms the basis of sphingolipids. In the Golgi complex, phosphate-choline is added to form the phospholipid sphingomyelin, or sugars are added to form glycolipids. The glycolipids remain on the inner bilayer of the Golgi complex and are taken to the plasma membrane by transport vesicles. These vesicles may be the same ones that take secretory proteins and integral membrane proteins to the plasma membrane.

## Membrane proteins

Synthesis of membrane proteins begins on ribosomes in the cytosol, but the first stretch of protein synthesized (the amino terminus) contains a signal that directs the ribosome to the ER. This signal is a sequence of 15–20 amino acids, of which about half are hydrophobic. The signal is recognized by a signal recognition particle (SRP), a complex of protein with RNA which brings the growing protein chain and attached ribosome to the ER and binds it to an SRP receptor in the ER membrane. The SRP is released and the growing protein chain is directed into a protein-conducting channel which penetrates the ER membrane so that the protein, as it is synthesized, emerges in the ER lumen.

The signal sequence is sometimes called a start-transfer signal because it directs the protein to pass through the ER membrane. Once inside the lumen, this signal sequence is removed by a membrane-bound peptidase: a soluble protein is formed which will eventually be secreted from the cell (e.g. digestive enzymes secreted into the gut). Proteins destined to become integral transmembrane proteins contain one or more stop-transfer signals, each of which is a sequence of about 20 hydrophobic amino acids within the protein chain. Such signals arrest the passage of the growing protein chain through the ER membrane, so the protein remains embedded in the membrane. Several such start-transfer and stop-transfer sequences will result in the synthesis of transmembrane proteins that pass back and forth across the ER membrane.

Sugars are attached to membrane proteins during their synthesis in the ER. Chains of sugars are built up on a sugar donor (called dolichol phosphate) which is embedded in the ER membrane, then transferred to the growing protein chain as it emerges into the ER lumen.

Transport vesicles carry proteins in their membranes from the ER to the Golgi complex where they are further modified (for example, by the addition of further sugars), sorted and sent to their final destination. Unless directed otherwise, proteins will be taken by secretory vesicles to the plasma membrane. Therefore, proteins that are synthesized in cells contain signals that determine their ultimate destinations.

# 4

# How small molecules cross membranes

## To-ing and fro-ing

Throughout the life of a cell, materials continually pass in and out. Cells take in essential nutrients and excrete poisonous wastes. They also move ions in or out in a controlled way so that the ionic composition of the cell interior is different from that of the outside.

## Selectively permeable bilayers

Not all molecules can cross membranes equally well: membranes are selectively permeable. This is because the major structural component of biological membranes is the phospholipid bilayer, with its charged heads and hydrophobic core. Small hydrophobic molecules can readily cross phospholipid bilayers (whether these bilayers are artificial or in biological membranes), by dissolving in the hydrophobic core. This process is known as simple diffusion. The smaller the molecule and the more fat-soluble it is, the faster it will penetrate the membrane. Thus small non-polar molecules such as $O_2$ and $N_2$, and uncharged polar molecules such as $CO_2$, ethanol or urea, can rapidly cross lipid bilayers; they cross a 10 nm bilayer in seconds.

The rate of diffusion is described by Fick's law. This states that the rate of diffusion (moles per second) across a membrane, is directly proportional to the difference in solute concentration ($C_o$-$C_i$) on each side of the membrane, to the area ($A$) of the membrane and to the permeability coefficient (P), which is itself inversely proportional to membrane thickness ($d$). Therefore, the rate of diffusion is increased by increasing

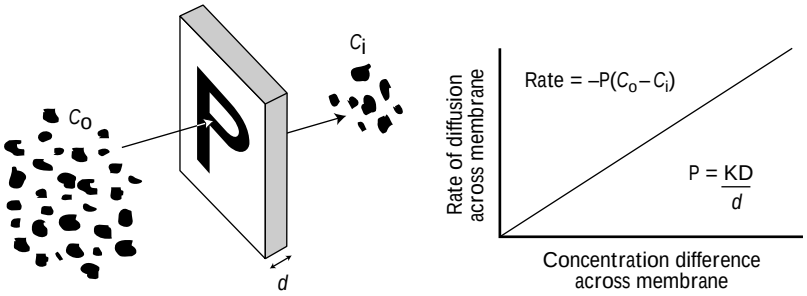



**Figure 18.** Passive diffusion of an uncharged molecule across a membrane. Abbreviations used: $C_o$, concentration outside (mol·cm$^{-3}$); $C_i$, concentration inside (mol·cm$^{-3}$); P, permeability coefficient (cm·s$^{-1}$); K, partition coefficient (ratio of the solubilities of the diffusing material in lipid and water; no units); D, diffusion coefficient (cm$^2$·s$^{-1}$); *d*, membrane thickness (cm). The negative sign means that the flow is downhill.

the concentration difference, increasing the surface area and decreasing the distance over which it occurs (Figure 18).

Large, uncharged polar molecules such as glucose might take hours to pass through a 10 nm bilayer. Charged molecules, e.g. ions such as $Na^+$ and $Cl^-$, are much less likely to get across, since they are impeded by the hydrophobic core. They may move through the fleeting gaps that occur between the rapidly moving bilayer lipid molecules, but such crossings are very slow, taking weeks to penetrate a 10 nm bilayer. Special transport mechanisms are needed to get these molecules across membranes.

## Water: small and swift

Water is unusual in that, although it is polar, it can cross lipid bilayers rapidly, passing through a 10 nm bilayer in about one millisecond. Water also moves more rapidly through biological membranes than do substances that are dissolved in water. For example, water molecules move through membranes about $10^5$ times faster than do glucose molecules, and $10^{10}$ times faster than do $Na^+$ and $K^+$ ions. You can get an idea of just how fast water can pass across membranes into cells by watching how quickly red cells burst when put into water, or by noticing how quickly the leaves of a wilting plant regain their ‘stiffness’ when placed in a vase of water.

Several reasons have been suggested for this rapid movement of water. (i) The water molecule is very small, so its size helps it to cross the membrane. (ii) Its concentration (55.5 $mol \cdot dm^{-3}$) is very high, therefore its abundance helps it to cross. (iii) Its dipolar nature may help it to cross the charged lipid head region of the bilayer. Whatever the reason, water does dissolve to a very slight extent in the hydrophobic core of the membrane; there is about one water molecule for every 20–40 lipid molecules. This means that any change in water concentration on one side of the bilayer will result in a rapid flow of water across the membrane.

## Downhill and uphill

Molecules in solution move of their own accord from a region of high concentration to a region of low concentration. We say they move 'downhill', where the hill is the concentration gradient. Energy can be obained from the downhill flow; the amount (known as the free energy change, $\Delta G$) is proportional to the concentration difference. For example, a thousand-fold difference will produce a greater flow than a ten-fold difference. Conversely, energy is needed to reverse the downhill flow — to make molecules move 'uphill', from a low concentration to a high concentration, against their concentration gradient. [For more information on $\Delta G$, see BASC booklets *Metabolism*, p. 6, and *Photosynthesis*, pp. 3–4.]

The above also applies to charged molecules (i.e. to ions). But there is also an additional factor involved. Many biological membranes are electrically positive on one side and negative on the other, there being a potential difference (voltage gradient) across them. This arises from differences in the distribution of positive and negative ions on the two sides of the membrane, and cells use energy to maintain this membrane potential.

The movement of ions will be affected by this membrane potential. The inside of many cells is electrically negative compared with the outside, so the entry of positive ions is favoured, whereas that of negative ions is opposed. For a positive ion moving into the cell, the strength of the inward attraction will depend on the concentration gradient. But it will also depend on the number of charges on the ion (Z): an ion with two positive charges will be attracted more strongly

than an ion with only one positive charge. The movement of ions will also depend on the size of the potential difference across the membrane ($\Delta\Psi$): the more negative the inside of the cell, the greater will be the attraction experienced by an entering positive ion. The hill for charged molecules is, therefore, a combination of concentration gradient and electrical gradient — the **electrochemical gradient**.

## Getting things across cell membranes

Ions, sugars and amino acids cannot diffuse across phospholipid bilayers fast enough to meet the cells' needs. They are transported by integral membrane proteins called transport proteins, and the process is sometimes called **mediated transport**. There are two types of transport protein: **channels** and **carriers**.

### Channels

Channel proteins form water-filled pores across the bilayer, through which inorganic ions move in single file. Ions flow downhill, down their electrochemical gradient, at a rate of about $10^8$ ions per second. Channels are generally built from four, five or six protein subunits, which are assembled to form a pore (Figure 19). The amino acid side-chains which line the pore will determine its **selectivity**: pores that admit positive ions are lined with negative side-chains, whereas pores that admit negative ions are lined with positive side-chains. The size of ion admitted is determined by the diameter of the narrowest part of the pore — the sodium channel, for example, has a diameter of 0.5 nm so will exclude ions that are wider than this. Dissolved ions are surrounded by a 'shell' of water molecules (the hydration sphere) which must be removed before the ions can pass through the channel.

The shape of the protein subunits, and therefore whether the channel is closed or open, is affected by the binding of signal molecules to the subunits, or even by a change in membrane potential. Channels are said to be **gated**, either ligand-gated (if they are opened by the binding of a signal molecule) or voltage-gated (if they are opened by a change in membrane potential).

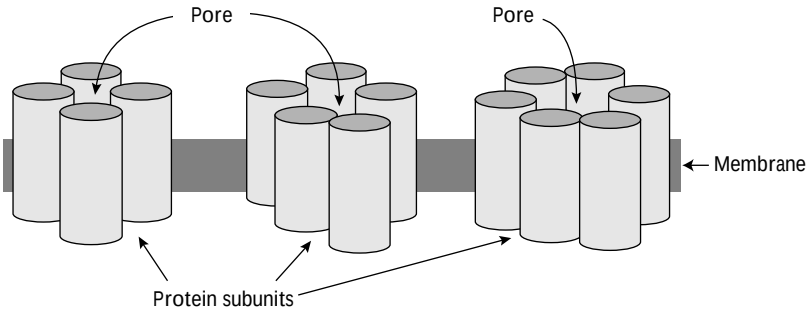
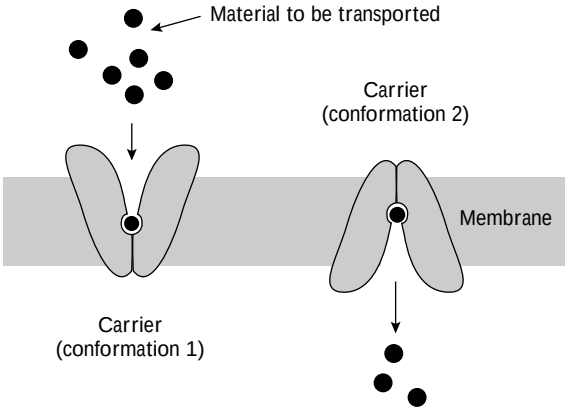



**Figure 19.** Diagrammatic representation of membrane channels.

## Carriers

Carrier or transporter proteins bind specific molecules or ions and transfer them across the membrane. Each carrier usually transports one type of molecule (eg. sugars, amino acids, ions) and often only one particular molecule of its class (e.g. the glucose transporter transports glucose but not other sugars). The binding of the substance to be transported causes its carrier to change shape, therefore the bound substance is exposed first on one side of the membrane and then on the other. Also, the affinity of the binding site for the bound substance decreases, so the transported molecule is released on the opposite side of the membrane

**Figure 20.** Carriers change their conformation (shape) to transport materials across membranes.



(Figure 20). The maximal transport rate for carriers is about $10^4$ molecules per second: they are much slower than channels.

Some carrier proteins transport only one solute across the membrane. These are called uniports, and an example is the glucose transporter in red blood cell membranes. Others transport two solutes at the same time so they are called co-transporters. If the two solutes are transported in the same direction, it is called symport; if they are transported in opposite directions, this is known as antiport. An example of symport is the co-transport of amino acids and $Na^+$ into the cells of the gut. An example of antiport is the $Na^+/K^+$ pump which pumps $Na^+$ out of cells and $K^+$ into cells (Figure 21).

## Passive and active transport

All channel proteins, and many carrier proteins, transfer molecules or ions across the membrane downhill. This process is called passive transport because no input of energy is needed to bring it about (Figure 22). It is also called facilitated diffusion because the normal process of diffusion is helped or facilitated by the membrane proteins. Facilitated diffusion differs from simple diffusion in that it is selective and saturable (i.e. when all the carriers have bound a solute molecule, the rate of diffusion is not increased by increasing the concentration of solute).

**Figure 21.** Types of carrier-mediated transport.

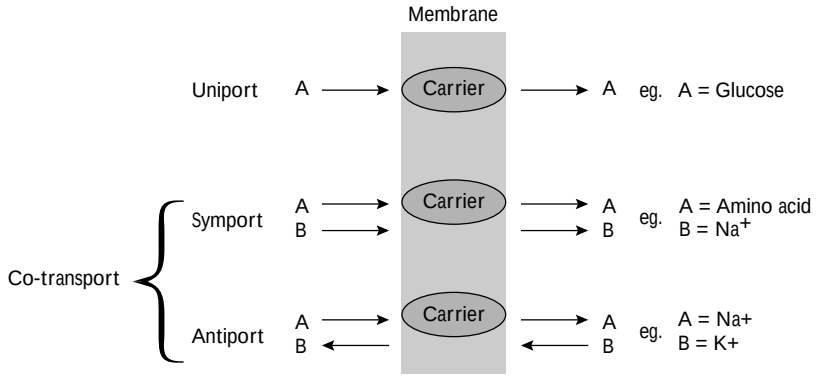


Figure 18 gives a graph of diffusion rate against concentration difference for simple diffusion. Sketch the corresponding graph for carrier-mediated (facilitated) diffusion.

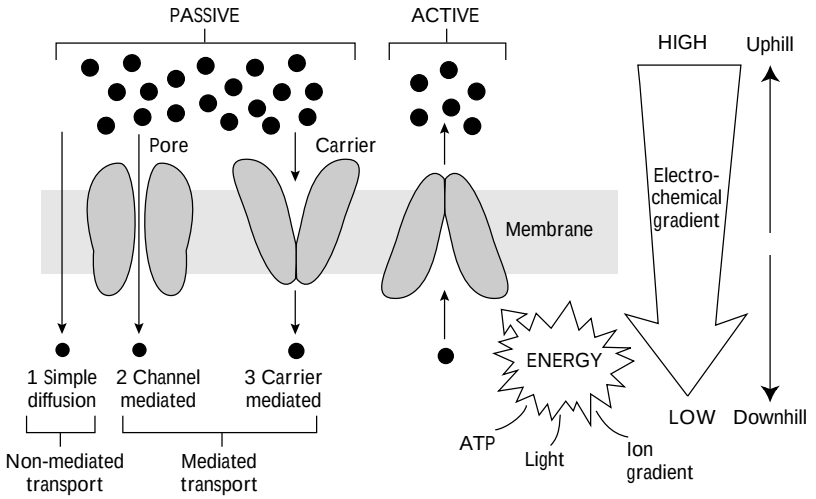
Cells also have transport proteins that transfer solutes across the membrane uphill, against their electrochemical gradient. This process is called active transport because an input of energy is needed to bring it about (Figure 22). It is always done by carrier proteins, not by channels.

The energy to drive active transport may come from a number of sources. The most common source is the hydrolysis of ATP. Others include light energy and the energy stored in ion gradients. The original ion gradient is said to arise from a primary active transport process which uses a direct source of energy, such as ATP or light. The transport that depends on the ion gradient (which in turn has to be generated by primary active transport) is referred to as secondary active transport.

### Transport driven by ATP

All animal cells actively pump $Na^+$ ions out and pump $K^+$ ions in. These two transport processes are carried out by the enzyme $Na^+/K^+$-exchanging ATPase, also called the sodium pump. The sodium pump is an integral protein of the plasma membrane. It is a tetramer of two types

**Figure 22.** Passive and active transport.



of subunit, one large and one small. The large subunit contains the ATP-binding site and is involved in ion transport. The small subunit has sugars on its extracellular surface, but its function is not known (Figure 23). The mechanism by which ATP drives ion transport is complex, but a simplified view follows.

(i) Binding of $Na^+$ ions on the cytosolic side triggers the phosphorylation of the large subunit by ATP.

(ii) Phosphorylation changes the shape of the subunit so that the $Na^+$-binding site faces the outside and $Na^+$ ions are released.

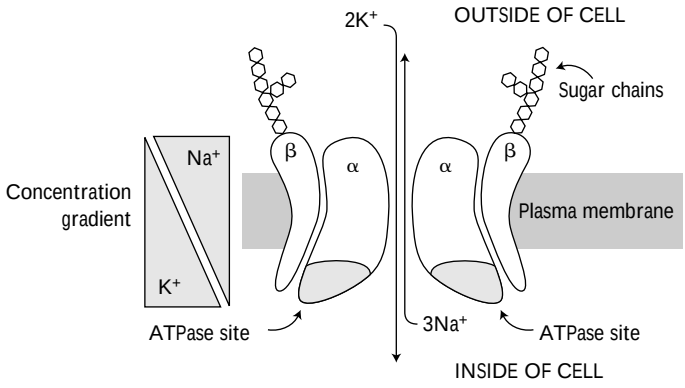
(iii) $K^+$ ions then bind and trigger the dephosphorylation of the subunit.

(iv) Dephosphorylation causes the subunit to change back to its original shape so that the binding site faces the inside.

(v) $K^+$ ions are released and ATP can be bound again, ready to repeat the cycle.

Ion pumps that work in a similar way are found in the cells lining the stomach, and in the sarcoplasmic reticulum of muscle cells. The gastric enzyme $H^+/K^+$-exchanging ATPase uses ATP hydrolysis to pump $H^+$ ions out of the cells and into the stomach interior, in exchange for $K^+$ ions. The muscle enzyme $Ca^{2+}$-transporting ATPase pumps $Ca^{2+}$

**Figure 23.** Diagrammatic representation of the sodium pump ($Na^+/K^+$-exchanging ATPase).



ions from the cytosol into the sarcoplasmic reticulum during relaxation of muscle cells.

### Transport driven by light

A purple bacterium called *Halobacterium halobium* lives in high-salt environments and contains, in its plasma membrane, a pump that uses light energy to pump $H^+$ ions out of the cell. The resulting proton gradient is then used to synthesize ATP. The pump consists of an integral membrane protein with a covalently attached molecule of retinal, hence the purple colour. Retinal is sensitive to light; the absorption of light will cause its straight hydrocarbon tail to bend (as a *trans* double bond is isomerized to *cis*). As a result, an attached proton-bearing group is moved into a position where it can pass on its proton to an acceptor group, which results ultimately in the transfer of a proton from inside the cell to outside. The retinal then returns to its straight-chain form, ready for the light-driven transport of another proton.

### Transport driven by ion gradients

Active transport can be driven by gradients of $H^+$ or $Na^+$ ions which, in turn, have been generated by another active transport system. This is called secondary active transport: the transfer of ions downhill drives the transport of other molecules uphill. An example is the transport of amino acids or glucose uphill into intestinal cells driven by the co-transport of sodium in the same direction, but downhill. The sodium is later extruded by an ATP-driven pump.

The intestinal co-transport of glucose and sodium is exploited in the rehydration of patients with cholera. Cholera toxin causes diarrhoea that will kill the patient if body fluids and salts are not replaced quickly. In oral rehydration therapy, patients are given a fluid which contains (among other substances) $Na^+$ and glucose; the presence of glucose allows uptake of sodium by the intestinal $Na^+$/glucose co-transport system.

# 5

# How large particles cross membranes

## Giving out and taking in

Small molecules, such as sugars, amino acids and inorganic ions, are both actively and passively transported. But cells also transport across their membranes macromolecules such as proteins, and even particles several micrometres in size such as bacterial cells. Cells secrete macromolecules by exocytosis and take in macromolecules by endocytosis. In exocytosis the macromolecules are contained within vesicles which fuse with the plasma membrane to release their contents to the outside. In endocytosis the reverse takes place: the substance (macromolecule or particle) to be taken in by the cell is gradually enclosed by a region of plasma membrane until a vesicle containing the substance is formed (Figure 24).

In both exocytosis and endocytosis, the macromolecules involved are contained within vesicles so that they do not mix with other components of the cytosol. The vesicles fuse only with specific membranes, causing a controlled transfer of molecules into and out of the cell. The process is similar to the transfer of newly made macromolecules from the ER via the Golgi body to other cell organelles. Both exocytosis and endocytosis depend on membrane fusion, which is itself a consequence of membrane fluidity — the ability of membrane components to move about.

## Exocytosis

Exocytosis may be constitutive or regulated. Constitutive exocytosis goes on all the time (it is part of the cell's constitution). In eukaryotic

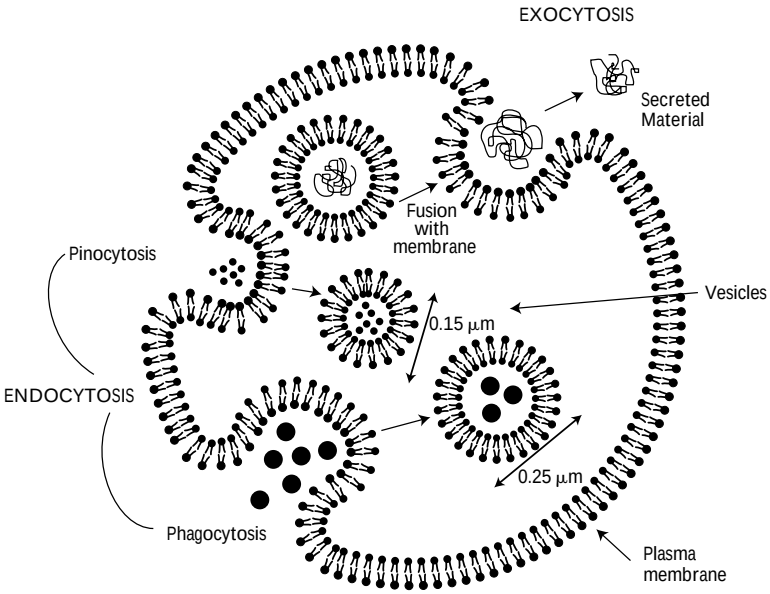



**Figure 24**. Exocytosis and endocytosis.

cells, transport vesicles derived from the ER continually carry newly made membrane components, such as lipids and proteins, from the Golgi body to the plasma membrane. In addition, cells secrete various types of molecules constantly. For example, fibroblasts secrete the large protein procollagen, which forms the collagen of connective tissue. Constitutive secretion occurs in all cells.

Regulated secretion occurs only when needed. The proteins or small molecules to be secreted are stored in secretory vesicles, which fuse with the plasma membrane in response to a signal from outside the cell. Regulated secretion operates only in cells which secrete their products on demand. The cells of our gut, for example, secrete digestive enzymes — large proteins such as pepsin, amylase and lipase. Endocrine glands such as the pancreas secrete the peptide hormones insulin and glucagon. The signal for secretion is often a hormone which binds to a receptor in the plasma membrane.

During exocytosis, the vesicle membrane becomes incorporated into the plasma membrane. The plasma membrane of a pancreatic cell has an area of about 30 $\mu m^2$. When the cell is secreting enzymes, a large amount

of vesicle membrane is incorporated into the plasma membrane; the membrane proteins and lipids are later recovered by endocytosis and incorporated into new secretory vesicles, so the surface area remains relatively unchanged.

## Endocytosis

There are two types of endocytosis, which differ in the size of the vesicles formed. In pinocytosis (or cell drinking), fluid or small particles are taken into small vesicles about 150 nm in diameter. In phagocytosis (or cell eating), large particles such as micro-organisms and cell debris are taken into large vesicles (or vacuoles) about 250 nm in diameter. Most cells carry out pinocytosis; only specialized phagocytic cells carry out phagocytosis.

Many of the particles taken up by either process end up in lysosomes. Phagocytic vesicles (phagosomes) fuse directly with lysosomes. Pinocytic vesicles are initially transferred to endosomes, from where they are taken to lysosomes, unless retrieved specifically. The breakdown products of lysosomal enzyme action are transported across the lysosomal membrane into the cytosol for reuse. Many of the membrane constituents of endosomes are recycled to the plasma membrane by exocytosis.

## Receptor-mediated endocytosis

Specific macromolecules are taken into cells by receptor-mediated endocytosis (Figure 25). The method is used, for example, on hormones bound to plasma membrane receptors. There, receptor–hormone complexes cluster in special regions of the plasma membrane called coated pits. These are dents in the membrane which have on their cytosolic side a coating of a protein called clathrin.

Endocytosis begins with the invagination of the coated pit containing a cluster of receptors to be engulfed. The clathrin forms a lattice around it, which causes the formation of a coated vesicle about 80 nm in diameter. The vesicle rapidly loses its clathrin coat, which returns to the plasma membrane to form a new coated pit. The ‘bare’ vesicle fuses with an endosome whose acidic interior (maintained by ATP-driven proton pumps) causes most protein–receptor complexes to

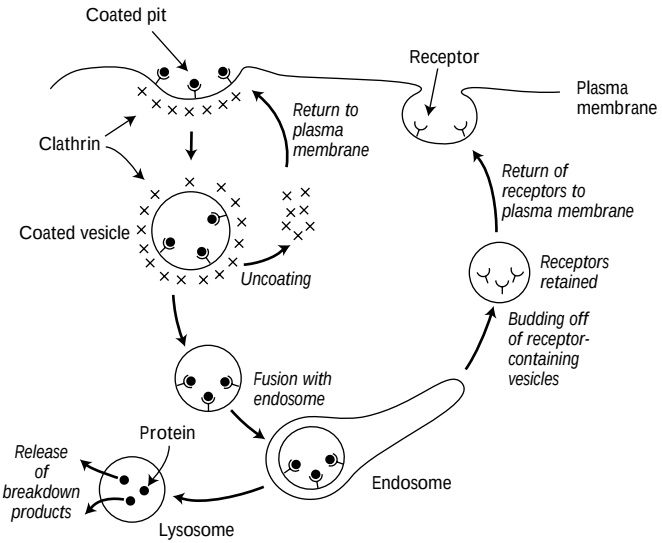



**Figure 25.** Receptor-mediated endocytosis.

dissociate. This sorting enables the proteins and the receptors to be directed to different destinations.

Vesicles containing the receptors bud off from the endosomes and are returned to the plasma membrane. Proteins are taken to the lysosomes, where they are broken down and released into the cytosol.

An important example of receptor-mediated endocytosis in animals is the uptake of the cholesterol needed for the manufacture of new membranes or steroid hormones. Most cholesterol is carried in the blood, complexed with protein and phospholipids in spherical particles called low-density lipoproteins (LDLs). When the cells need cholesterol, they make LDL receptors which cluster in coated pits on the plasma membrane and are, therefore, continually being taken into the cell and returned. Any bound LDL will be taken in with them; however, whereas the receptor is recycled, the LDL will be taken to lysosomes for degradation. The cholesterol is released into the cytosol for reuse.

Many viruses and toxins also enter cells by receptor-mediated endocytosis.

## Membrane fusion: key to exocytosis and endocytosis

Both exocytosis and endocytosis involve the fusion of two separate membrane bilayers. At some point, two membranes that were originally separate become continuous with each other, allowing some mixing of the components of the two original membranes. Fusion does not occur spontaneously and is thought to involve a number of events. First, the two layers come close to one another, a process called bilayer adherence. For close approach to occur, surface water must be removed from the bilayer surfaces. Next, the bilayers are destabilized at the point of fusion, so the lipids of the two bilayers can mix: this is called bilayer joining. Finally, the two new bilayers separate from the point of fusion.

It is probable that membrane fusion is catalysed by special proteins; such fusigenic proteins have been found in viruses, in which they catalyse the fusion of virus and endosome membranes.

The mechanism of membrane fusion is still not fully understood. However, it is an important process that occurs not only in exocytosis and endocytosis, but also in cell division, cell fusion, and within cells, as transport vesicles bud from one organelle and fuse with another.

# 6

# How messages cross membranes

## Messages as well as materials

Membranes are freely permeable to lipids, so fat-soluble materials can easily cross them. They are impermeable to charged molecules, thus charge gradients can exist across them. Membranes have proteins embedded in them that help certain molecules to cross them. They are also fluid and can allow large molecules to cross by wrapping around them to form vesicles. All these features are relevant to the transport of materials across membranes. But these same features are also pertinent to the transport of messages across membranes.

## Fat-soluble messengers

Certain hormones (chemical messengers produced by one cell to affect another cell) are fat-soluble. Examples are the steroids (cortisol, androgens and oestrogens), vitamins A and D, and thyroxine. Cortisol, for example, is secreted by the adrenal cortex in response to — among other things — prolonged low blood glucose. It travels in the blood to other organs, such as the liver, bearing the message, "Make more glucose!".

Being fat-soluble, cortisol molecules diffuse across the plasma membrane of liver cells and bind to specific receptor proteins in the cytosol. The binding of cortisol changes the shape of the receptor, and the receptor–cortisol combination then causes the synthesis of more glucose (Figure 26).

## Water-soluble messengers

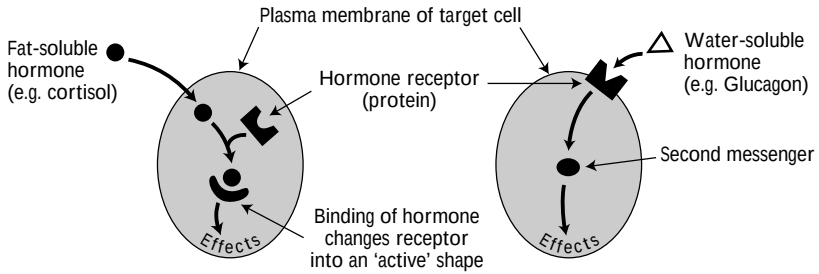
Many hormones (e.g. adrenaline, glucagon and insulin) are soluble in water and therefore cannot cross the plasma membrane of target cells. Their receptors, which are also proteins, are found embedded in the plasma membrane, where they move about the cell surface until they bind their messenger molecule (Figure 26).

Hormones are not the only messengers that bind to receptors on the cell surface. Odorants (smell molecules) and tastants (taste molecules) also bind to receptors on the surface of their sense cells. Even light interacts with a receptor molecule, called rhodopsin.

Cell-surface receptors are usually integral proteins. Indeed, many of them have similar structural features: the light-receptor rhodopsin, odorant receptors and adrenaline receptors (called β-adrenergic receptors) are similar in that their peptide chain contains seven α-helical regions embedded in the membrane (Figure 27). On their outer surface is a site which recognizes the messenger, whereas the region facing the cytosol bears a site which recognizes a GTP-binding protein (see below).

In all of the above examples, interaction of the messenger with the receptor protein changes the shape of the receptor so that it can in turn interact with proteins that are bound to the inner surface of the plasma membrane. These proteins are known as GTP-binding proteins (or G-proteins) because in their active form they have GTP bound to them. G-proteins contain three subunits and are anchored to the inner monolayer of the plasma membrane by two fatty acid chains (Figure 28). The G-protein that interacts with the light-receptor is called transducin.

**Figure 26.** Mode of action of fat- and water-soluble hormones.



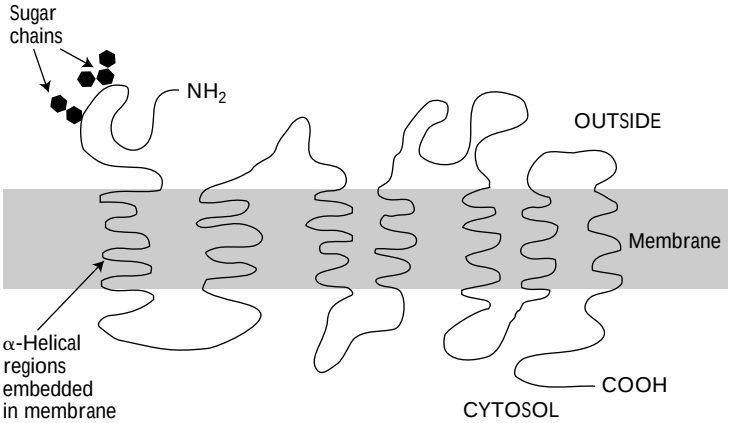



**Figure 27.** Structural features of cell-surface receptors.

## Second messengers

What happens next depends upon the individual message system. In hormone communication (including the β-adrenergic receptors), the G-protein activates an enzyme which produces a second messenger molecule. Because an enzyme is involved, many second messenger molecules are produced for each hormone molecule that binds to its receptor, and the original hormone signal is amplified.

For many hormones the enzyme is adenylate cyclase, an integral membrane protein which brings about the production of the second messenger cyclic adenosine monophosphate (cAMP) from ATP. The second messenger triggers a series of enzyme-catalysed reactions which, among other effects, may bring about phosphorylation of ion channels in the membrane, resulting in their opening.

In olfaction (the detection of smells), cAMP binds to ligand-gated ion channels and causes them to open. Less is known about gustation (taste detection). However, for sweet-tasting molecules, cAMP causes $K^+$ channels in the taste cell membranes to close. In photoreception (the detection of light), the G-protein brings about the closing of ligand-gated cation channels in the rod-cell plasma membrane.

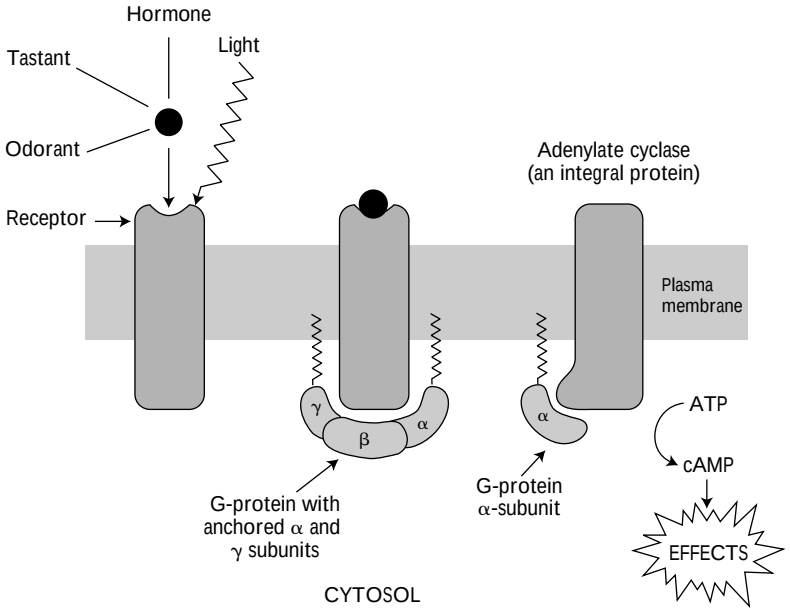



**Figure 28.** Vision, taste, smell and some hormones work through G-proteins.

## Nerve impulses

The result of the opening or closing of ligand-gated ion channels is a change in membrane potential and the generation of a nerve impulse to carry information to the brain. Nerve impulses arise because biological membranes are impermeable to ions. The ATP-driven sodium pump (the $Na^+/K^+$-exchanging ATPase) generates an ion gradient across the plasma membranes of nerve cells, so that normally the inner surface is –70 mV compared with the outer surface.

As a result of the stimuli produced by, for example, taste or smell molecules, the ion gradient is reduced by leakage of ions across the membrane. A small region of the membrane becomes depolarized; this is the origin of the nerve impulse (or action potential, as it is called) which can travel along the axon of the nerve cell.

## What happens at the synapse

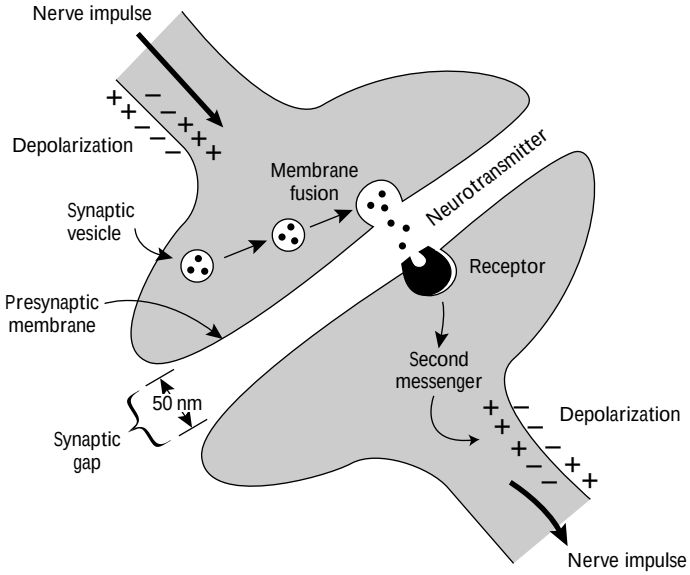
When the nerve impulse reaches the end of the axon, it finds a gap called a synapse between it and the next nerve cell (or the muscle cell in the case of a nerve that stimulates a muscle). Another property of membranes — their fluidity — is used to transmit the message across the synapse (Figure 29).

When the nerve impulse reaches the presynaptic membrane, it brings about the fusion of hundreds of synaptic vesicles with the presynaptic membrane. Each vesicle contains neurotransmitter molecules which are released into the synaptic gap by exocytosis. The molecules diffuse across the 50 nm gap to the next membrane (in nerve or muscle), bind to receptors there and generate a new nerve impulse which travels on.

## We cannot live without them

The mechanism of signal transduction (molecular message sending) in hormone action, vision, taste and smell are basically similar — and depend for their workings on the molecular properties of membranes. Membranes: we cannot live without them!

**Figure 29.** Transmission of messages across the synapse.



# 7

# How you can study membranes

## An experimental membrane

A useful experimental material for use in schools is beetroot. Beetroot cells contain a pigment that is released upon rupture of the plasma membrane. Washed, thin slices of beetroot tissue can be used in experiments to test the effects of various factors on membrane stability. Alternatively, small-scale experiments can be carried out using rhubarb cells on microscope slides. Rhubarb cells are obtained by peeling off the red epidermis of rhubarb stalk; use $0.5\ cm \times 0.5\ cm$ squares for the experiments. Under the microscope, the cells can be seen to contain a red pigment, and its behaviour on different experimental treatments can be studied.

The hazards involved in the experiments can be controlled by good laboratory practice.

(i) Do not pipette by mouth.
(ii) Wear gloves when handling tissues, detergents or enzymes.
(iii) Rinse any spillages in water.

## Experiments

1. Place a washed square slice of beetroot in a boiling tube containing $5\ cm^3$ water. Measure the release of red pigment with time, either by direct observation or by means of a colorimeter — a device that measures electronically the colour density (or absorbance) of solutions.

2. Using the above experimental set-up, investigate the effects of different conditions on the release of pigment from beetroot cells. Conditions that could be tried include: (i) temperature; (ii) prior freezing then thawing of the tissue; (iii) pH; (iv) sodium chloride concentration; (v) sucrose concentration; (vi) presence of salts of different metals (e.g. $Na^+$, $K^+$, $Ca^{2+}$, $Cu^{2+}$, $Mg^{2+}$, $Fe^{2+}$, $Al^{3+}$); (vii) presence of detergent (use washing-up liquid); (viii) presence of a proteolytic enzyme, such as trypsin. [A 'biological' (i.e. enzyme-containing) washing powder might also be tried. How might you decide whether any effects were due to enzyme or detergent?]

# 8

# How you can find out more about membranes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