

## BCH 307

### Membrane Biochemistry

#### Introduction

The outer walls of a house or car provide a strong, inflexible barrier that protects its human inhabitants from an unpredictable and harsh external world. You might expect the outer boundary of a living cell to be constructed of an equally tough and impenetrable barrier because it must also protect its delicate internal contents from a nonliving, and often inhospitable, environment. Yet cells are separated from the external world by a thin, fragile structure called the plasma membrane that is only 5 to 10 nm wide. It would require about five thousand plasma membranes stacked one on top of the other to equal the thickness of a single page of this handbook.

**Membranes form boundaries** both around the cell (the plasma membrane) and around distinct subcellular compartments (e.g. nucleus, mitochondria, lysosomes, etc.). They act as selectively permeable barriers allowing the inside environment of the cell or organelle to differ from that outside. Membranes are involved in signaling processes; they contain specific receptors for external stimuli and are involved in both chemical and electrical signal generation. All membranes contain two basic components: lipids and proteins. Some membranes also contain carbohydrate. The composition of lipid, protein and carbohydrate varies from one membrane to another. For example, the inner mitochondrial membrane has a larger amount of protein than lipid due to the presence of numerous protein complexes involved in oxidative phosphorylation and electron transfer, whereas the myelin sheath membrane of nerve cells, which serves to electrically insulate the cell, has a larger proportion of lipid.

#### A Brief History of Studies on Plasma Membrane Structure

The first insights into the chemical nature of the outer boundary layer of a cell were obtained by **Ernst Overton** of the University of Zürich during the **1890s**. Overton knew that nonpolar solutes dissolved more readily in nonpolar solvents than in polar solvents, and that polar solutes had the opposite solubility. Overton reasoned that a substance entering a cell from the medium would first have to dissolve in the outer boundary layer of that cell. To test the permeability of the outer boundary layer, Overton placed plant root hairs into hundreds of different solutions containing a diverse array of solutes. He discovered that the more lipid-soluble the solute, the more rapidly it would enter the root hair cells. He concluded that the dissolving power of the outer boundary layer of the cell matched that of **fatty oil**. The first proposal that cellular membranes might contain a lipid bilayer was made in **1925** by two Dutch scientists, **E. Gorter and F. Grendel**. These researchers extracted the lipid from human **red blood cells** and measured the amount of surface area the lipid would cover when spread over the surface of water. Since mature mammalian red blood cells lack both nuclei and cytoplasmic organelles, the plasma membrane is the only lipid-containing structure in the cell. Consequently, all of the lipids extracted from the cells can be assumed to have resided in the cells' plasma membranes. The ratio of the surface area of water covered by the extracted lipid to the surface area calculated for the red blood cells from which the lipid was extracted varied between 1.8 to 1 and 2.2 to 1. Gorter and Grendel speculated that the actual ratio was 2:1 and was 2:1 and concluded that the plasma membrane contained a

bimolecular layer of lipids, that is, a lipid bilayer. They also suggested that the polar groups of each molecular layer (or leaflet) were directed outward toward the aqueous environment. This would be the thermodynamically favored arrangement, because the polar head groups of the lipids could interact with surrounding water molecules, just as the hydrophobic fatty acyl chains would be protected from contact with the aqueous environment. Thus, the polar head groups would face the cytoplasm on one edge and the blood plasma on the other. Gorter and Grendel arrived at the conclusion that membranes contain a lipid bilayer.

In the 1920s and 1930s, cell physiologists obtained evidence that there must be more to the structure of membranes than simply a lipid bilayer. It was found, for example, that lipid solubility was not the sole determining factor as to whether or not a substance could penetrate the plasma membrane. Similarly, the surface tensions of membranes were calculated to be much lower than those of pure lipid structures. This decrease in surface tension could be explained by the presence of protein in the membrane. In **1935, Hugh Davson and James Danielli** proposed that the plasma membrane was composed of a lipid bilayer that was lined on both its inner and outer surface by a layer of globular proteins. They revised their model in the early 1950s to account for the selective permeability of the membranes they had studied. In the revised version, Davson and Danielli suggested that, in addition to the outer and inner protein layers, the lipid bilayer was also penetrated by protein-lined pores, which could provide conduits for polar solutes and ions to enter and exit the cell.

Experiments conducted in the late **1960s** led to a new concept of membrane structure, as detailed in the **fluid mosaic model** proposed in **1972** by **S. Jonathan Singer and Garth Nicolson** of the University of California, San Diego. In the fluid-mosaic model, which has served as the “**central dogma**” of membrane biology for more than three decades, the lipid bilayer remains the core of the membrane, but attention is focused on the physical state of the lipid. Unlike previous models, the bilayer of a fluid-mosaic membrane is present in a fluid state, and individual lipid molecules can move laterally within the plane of the membrane.

The structure and arrangement of membrane proteins in the fluid-mosaic model differ from those of previous models in that they occur as a “mosaic” of discontinuous particles that penetrate the lipid sheet. Most importantly, the fluid-mosaic model presents cellular membranes as dynamic structures in which the components are mobile and capable of coming together to engage in various types of transient or semi-permanent interactions.

## **An Overview of Membrane Functions**

### **1. Compartmentalization.**

Membranes are continuous, unbroken sheets and, as such, inevitably enclose compartments. The plasma membrane encloses the contents of the entire cell, whereas the nuclear and cytoplasmic membranes enclose diverse intracellular spaces. The various membrane-bounded compartments of a cell possess markedly different contents. Membrane compartmentalization allows specialized activities to proceed without external interference and enables cellular activities to be regulated independently of one another.

## **2. Scaffold for biochemical activities/Biochemical reactions centre**

Membranes not only enclose compartments but are also a distinct compartment themselves. As long as reactants are present in solution, their relative positions cannot be stabilized and their interactions are dependent on random collisions. Because of their construction, membranes provide the cell with an extensive framework or scaffolding within which components can be ordered for effective interaction.

## **3. Providing a selectively permeable barrier.**

Membranes prevent the unrestricted exchange of molecules from one side to the other. At the same time, membranes provide the means of communication between the compartments they separate. The plasma membrane, which encircles a cell, can be compared to a moat around a castle: both serve as a general barrier, yet both have gated “bridges” that promote the movement of selected elements into and out of the enclosed living space.

## **4. Transporting solutes.**

The plasma membrane contains the machinery for physically transporting substances from one side of the membrane to another, often from a region where the solute is present at low concentration into a region where that solute is present at much higher concentration. The membrane’s transport machinery allows a cell to accumulate substances, such as sugars and amino acids, that are necessary to fuel its metabolism and build its macromolecules. The plasma membrane is also able to transport specific ions, thereby establishing ionic gradients across itself. This capability is especially critical for nerve and muscle cells.

## **5. Responding to external stimuli.**

The plasma membrane plays a critical role in the response of a cell to external stimuli, a process known as signal transduction. Membranes possess receptors that combine with specific molecules (ligands) or respond to other types of stimuli such as light or mechanical tension. Different types of cells have membranes with different receptors and are, therefore, capable of recognizing and responding to different environmental stimuli. The interaction of a plasma membrane receptor with an external stimulus may cause the membrane to generate a signal that stimulates or inhibits internal activities. For example, signals generated at the plasma membrane may tell a cell to manufacture more glycogen, to prepare for cell division, to move toward a higher concentration of a particular compound, to release calcium from internal stores, or possibly to commit suicide.

## **6. Intercellular interaction.**

Situated at the outer edge of every living cell, the plasma membrane of multi cellular organisms mediates the interactions between a cell and its neighbors. The plasma membrane allows cells to recognize and signal one another, to adhere when appropriate, and to exchange materials and information. Proteins within the plasma membrane may also facilitate the interaction between extracellular materials and the intracellular cytoskeleton.

## **7. Energy transduction.**

Membranes are intimately involved in the processes by which one type of energy is converted to another type (energy transduction). The most fundamental energy transduction occurs during photosynthesis when energy in sunlight is absorbed by membrane-bound pigments, converted into chemical energy, and stored in carbohydrates. Membranes are also involved in the transfer of

chemical energy from carbohydrates and fats to ATP. In eukaryotes, the machinery for these energy conversions is contained within membranes of chloroplasts and mitochondria.

### The Chemical Composition of Membranes

Membranes are lipid–protein assemblies in which the components are held together in a thin sheet by non-covalent bonds. As noted above, the core of the membrane consists of a sheet of lipids arranged in a bimolecular layer. The lipid bilayer serves primarily as a structural backbone of the membrane and provides the barrier that prevents random movements of water-soluble materials into and out of the cell.

The proteins of the membrane, on the other hand, carry out most of the specific functions of membrane. Each type of differentiated cell contains a unique complement of membrane proteins, which contributes to the specialized activities of that cell type. The ratio of lipid to protein in a membrane varies, depending on the type of cellular membrane (plasma vs. endoplasmic reticulum vs. Golgi), the type of organism (bacterium vs. plant vs. animal), and the type of cell (cartilage vs. muscle vs. liver). For example, the inner mitochondrial membrane has a very high ratio of protein/lipid in comparison to the red blood cell plasma membrane, which is high in comparison to the membranes of the myelin sheath that form a multilayered wrapping around a nerve cell. To a large degree, these differences can be correlated with the basic functions of these membranes. The inner mitochondrial membrane contains the protein carriers of the electron-transport chain, and relative to other membranes, lipid is diminished. In contrast, the myelin sheath acts primarily as electrical insulation for the nerve cell it encloses, a function that is best carried out by a thick lipid layer of high electrical resistance with a minimal content of protein. Membranes also contain carbohydrates, which are attached to the lipids and proteins.

Table: Major components of plasma membranes in various organisms

|                                      | Components (% by weight) |              |        | Sterol type  | Other lipids                    |
|--------------------------------------|--------------------------|--------------|--------|--------------|---------------------------------|
|                                      | Protein                  | Phospholipid | Sterol |              |                                 |
| Human myelin sheath                  | 30                       | 30           | 19     | Cholesterol  | Galactolipids, plasmalogens     |
| Mouse liver                          | 45                       | 27           | 25     | Cholesterol  | —                               |
| Maize leaf                           | 47                       | 26           | 7      | Sitosterol   | Galactolipids                   |
| Yeast                                | 52                       | 7            | 4      | Ergosterol   | Triacylglycerols, steryl esters |
| <i>Paramecium</i> (ciliated protist) | 56                       | 40           | 4      | Stigmasterol | —                               |
| <i>E. coli</i>                       | 75                       | 25           | 0      | —            | —                               |

### Membrane Lipids

Membranes contain a wide diversity of lipids, all of which are amphipathic; that is, they contain both hydrophilic and hydrophobic regions. There are three main types of membrane lipids: glycerophospho lipids, sphingophospho lipids, glycolipids and cholesterol.

Table: Lipids composition of some biological membranes:

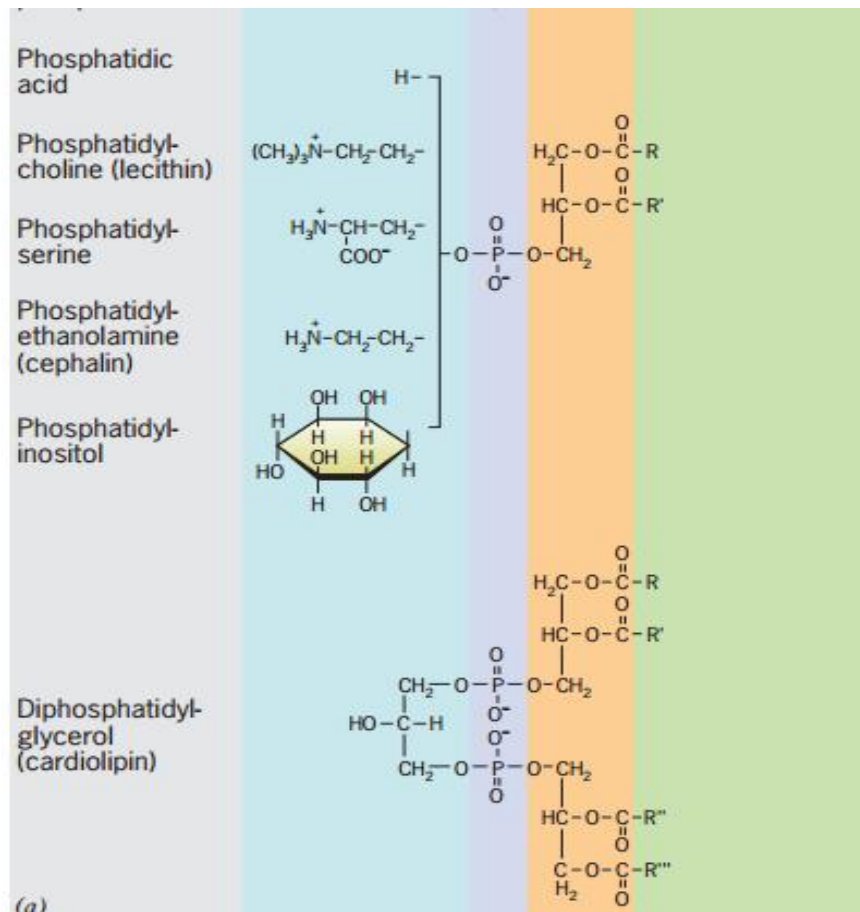
| Lipid                     | Human erythrocyte | Human myelin | Beef heart mitochondria | <i>E. coli</i> |
|---------------------------|-------------------|--------------|-------------------------|----------------|
| Phosphatidic acid         | 1.5               | 0.5          | 0                       | 0              |
| Phosphatidylcholine       | 19                | 10           | 39                      | 0              |
| Phosphatidyl-ethanolamine | 18                | 20           | 27                      | 65             |
| Phosphatidylglycerol      | 0                 | 0            | 0                       | 18             |
| Phosphatidylserine        | 8.5               | 8.5          | 0.5                     | 0              |
| Cardiolipin               | 0                 | 0            | 22.5                    | 12             |
| Sphingomyelin             | 17.5              | 8.5          | 0                       | 0              |
| Glycolipids               | 10                | 26           | 0                       | 0              |
| Cholesterol               | 25                | 26           | 3                       | 0              |

### Glycerophospho lipids

Most membrane lipids contain a phosphate group, which makes them phospholipids. Because most membrane phospholipids are built on a glycerol backbone, they are called phosphoglycerides. Unlike triglycerides, which have three fatty acids and are not amphipathic, membrane glycerides are diglycerides—only two of the hydroxyl groups of the glycerol are esterified to fatty acids; the third is esterified to a hydrophilic phosphate group.

Without any additional substitutions beyond the phosphate and the two fatty acyl chains, the molecule is called phosphatidic acid, which is virtually absent in most membranes. Instead, membrane phosphoglycerides have an additional group linked to the phosphate, most commonly either choline (forming phosphatidylcholine, PC), ethanolamine (forming phosphatidylethanolamine, PE), serine (forming phosphatidylserine, PS), or inositol (forming phosphatidylinositol, PI). Each of these groups is small and hydrophilic and, together with the negatively charged phosphate to which it is attached, forms a highly water-soluble domain at one end of the molecule, called the head group. At physiologic pH, the head groups of PS and PI have an overall negative charge, whereas those of PC and PE are neutral. In contrast, the fatty acyl chains are hydrophobic, unbranched hydrocarbons approximately 16 to 22 carbons in length. A membrane fatty acid may be fully saturated (i.e., lack double bonds), monounsaturated (i.e., possess one double bond), or polyunsaturated (i.e., possess more than one double bond). Phosphoglycerides often contain one unsaturated and one saturated fatty acyl chain.

With fatty acid chains at one end of the molecule and a polar head group at the other end, all of the phosphoglycerides exhibit a distinct amphipathic character.



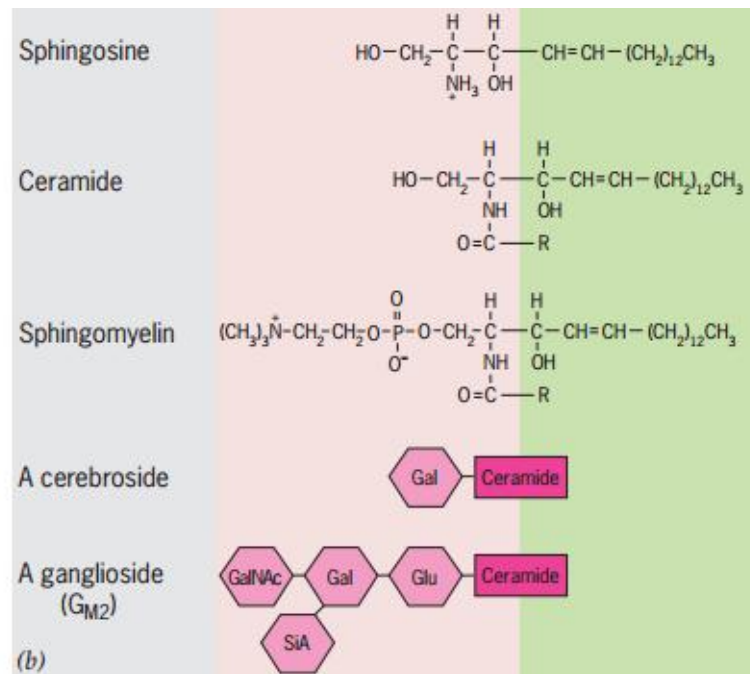
## Sphingolipids

A less abundant class of membrane lipids, called sphingolipids, are derivatives of sphingosine, an amino alcohol that contains a long hydrocarbon chain. Sphingolipids consist of sphingosine linked to a fatty acid (R) by its amino group. This molecule is a ceramide. The various sphingosine-based lipids have additional groups esterified to the terminal alcohol of the sphingosine moiety. If the substitution is phosphocholine, the molecule is sphingomyelin, which is the only phospholipid of the membrane that is not built with a glycerol backbone.

## Glycolipid

If the substitution is a carbohydrate, the molecule is a glycolipid. If the carbohydrate is a simple sugar, the glycolipid is called a cerebroside; if it is a small cluster of sugars that includes sialic acid, the glycolipid is called a ganglioside. Hundreds of different gangliosides have been identified by differences in their carbohydrate chains. Since all sphingolipids have two long, hydrophobic hydrocarbon chains at one end and a hydrophilic region at the other, they are also amphipathic and basically similar in overall structure to the phosphoglycerides.

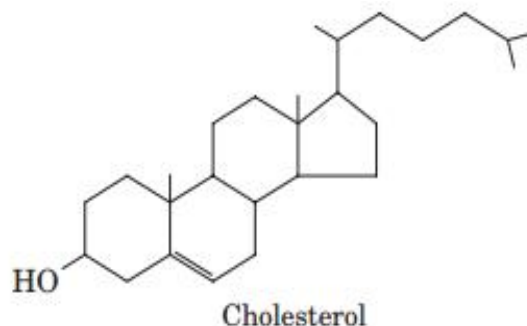
Glycolipids, with galactose are characteristically found in the plasma membranes of cells in neural tissue, and those with glucose in the plasma membranes of cells in non-neural tissues.



In humans, at least 60 different sphingolipids have been identified in cellular membranes. Many of these are especially prominent in the plasma membranes of neurons, and some are clearly recognition sites on the cell surface, but a specific function for only a few sphingolipids has been discovered thus far.

### Cholesterol

Another lipid component of certain membranes is the sterol cholesterol, which in certain animal cells may constitute up to 50 percent of the lipid molecules in the plasma membrane. Plant cells contain cholesterol-like sterols, but biologists disagree as to whether or not they completely lack cholesterol. Cholesterol molecules are oriented with their small hydrophilic hydroxyl group toward the membrane surface and the remainder of the molecule embedded in the lipid bilayer. The hydrophobic rings of a cholesterol molecule are flat and rigid, and they interfere with the movements of the fatty acid tails of the phospholipids



### The Structure and Functions of Membrane Proteins

Depending on the cell type and the particular organelle within that cell, a membrane may contain hundreds of different proteins. Each membrane protein has a defined orientation relative to the cytoplasm, so that the properties of one surface of a membrane are very different from those of

the other surface. This asymmetry is referred to as membrane “sidedness.” In the plasma membrane, for example, those parts of membrane proteins that interact with other cells or with extracellular substances are exposed to the extracellular space, whereas those parts of membrane proteins that interact with cytoplasmic molecules are exposed to the cytosol.

Membrane proteins can be grouped into three distinct classes distinguished by the intimacy of their relationship to the lipid bilayer. These are

### **1. Integral proteins that penetrate the lipid bilayer.**

Integral proteins are transmembrane proteins; that is, they pass entirely through the lipid bilayer and thus have domains that protrude from both the extracellular and cytoplasmic sides of the membrane. Some integral proteins have only one membrane-spanning segment, whereas others are multispinning.

Most integral membrane proteins function in the following capacities: as receptors that bind specific substances at the membrane surface, as channels or transporters involved in the movement of ions and solutes across the membrane, or as agents that transfer electrons during the processes of photosynthesis and respiration. Like the phospholipids of the bilayer, integral membrane proteins are also amphipathic, having both hydrophilic and hydrophobic portions. As discussed below, those portions of an integral membrane protein that reside within the lipid bilayer—the transmembrane domains—tend to have a hydrophobic character. Amino acid residues in transmembrane domains form van der Waals interactions with the fatty acyl chains of the bilayer, which seals the protein into the lipid “wall” of the membrane. As a result, the permeability barrier of the membrane is preserved. Those portions of an integral membrane protein that project into either the cytoplasm or extracellular space tend to be more like the globular proteins.

These non embedded domains tend to have hydrophilic surfaces that interact with water-soluble substances (low molecular-weight substrates, hormones, and other proteins) at the edge of the membrane.

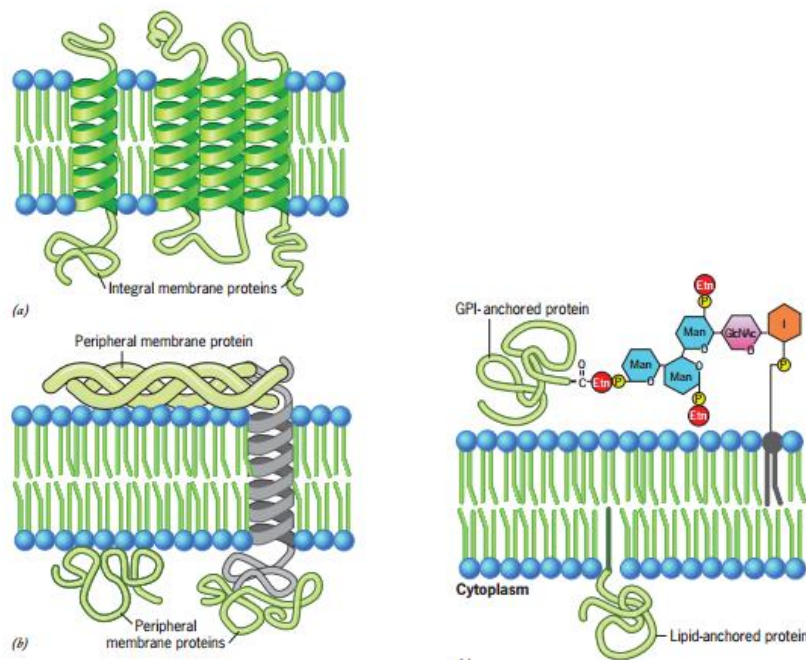
**2. Peripheral proteins** that are located entirely outside of the lipid bilayer, on either the cytoplasmic or extracellular side, yet are associated with the surface of the membrane by noncovalent bonds.

The best studied peripheral proteins are located on the internal (cytosolic) surface of the plasma membrane, where they form a fibrillar network that acts as a membrane “skeleton”. These proteins provide mechanical support for the membrane and function as an anchor for integral membrane proteins. Other peripheral proteins on the internal plasma membrane surface function as enzymes, specialized coats, or factors that transmit transmembrane signals. Peripheral proteins typically have a dynamic relationship with the membrane, being recruited to the membrane or released from the membrane depending on prevailing conditions.

**3. Lipid-anchored proteins** that are located outside the lipid bilayer, on either the extracellular or cytoplasmic surface, but are covalently linked to a lipid molecule that is situated within the bilayer. Several types of lipid-anchored membrane proteins can be distinguished. Numerous

proteins present on the external face of the plasma membrane are bound to the membrane by a small, complex oligosaccharide linked to a molecule of phosphatidylinositol that is embedded in the outer leaflet of the lipid bilayer. Peripheral membrane proteins containing this type of glycosyl-phosphatidylinositol linkage are called GPI-anchored proteins. They were discovered when it was shown that certain membrane proteins could be released by a phospholipase that specifically recognized and cleaved inositol-containing phospholipids. They act as receptors, enzymes, and cell-adhesion proteins. A rare type of anemia, paroxysmal nocturnal hemoglobinuria, results from a deficiency in GPI synthesis that makes red blood cells susceptible to lysis.

Another group of proteins present on the cytoplasmic side of the plasma membrane is anchored to the membrane by one or more long hydrocarbon chains embedded in the inner leaflet of the lipid bilayer. At least two proteins associated with the plasma membrane in this way (Src and Ras) have been implicated in the transformation of a normal cell to a malignant state.



### Membrane Structure: the Fluid Mosaic Model

Membranes are 5 to 8 nm (50 to 80 Å) thick and appear trilaminar when viewed in cross section with the electron microscope. The combined evidence from electron microscopy and studies of chemical composition, as well as physical studies of permeability and the motion of individual protein and lipid molecules within membranes, led to the development of the fluid mosaic model for the structure of biological membranes. Phospholipids form a bilayer in which the nonpolar regions of the lipid molecules in each layer face the core of the bilayer and their polar head groups face outward, interacting with the aqueous phase on either side. Proteins are embedded in this bilayer sheet, held by hydrophobic interactions between the membrane lipids and hydrophobic

domains in the proteins. Some proteins protrude from only one side of the membrane; others have domains exposed on both sides. The orientation of proteins in the bilayer is asymmetric, giving the membrane “sidedness”: the protein domains exposed on one side of the bilayer are different from those exposed on the other side, reflecting functional asymmetry. The individual lipid and protein units in a membrane form a fluid mosaic with a pattern that, unlike a mosaic of ceramic tile and mortar, is free to change constantly. The membrane mosaic is fluid because most of the interactions among its components are noncovalent, leaving individual lipid and protein molecules free to move laterally in the plane of the membrane. The integral membrane proteins can be considered as ‘icebergs’ floating in a two-dimensional lipid ‘sea’. The bilayer organization of the lipids would act both as a solvent for the amphipathic integral membrane proteins and as a permeability barrier.

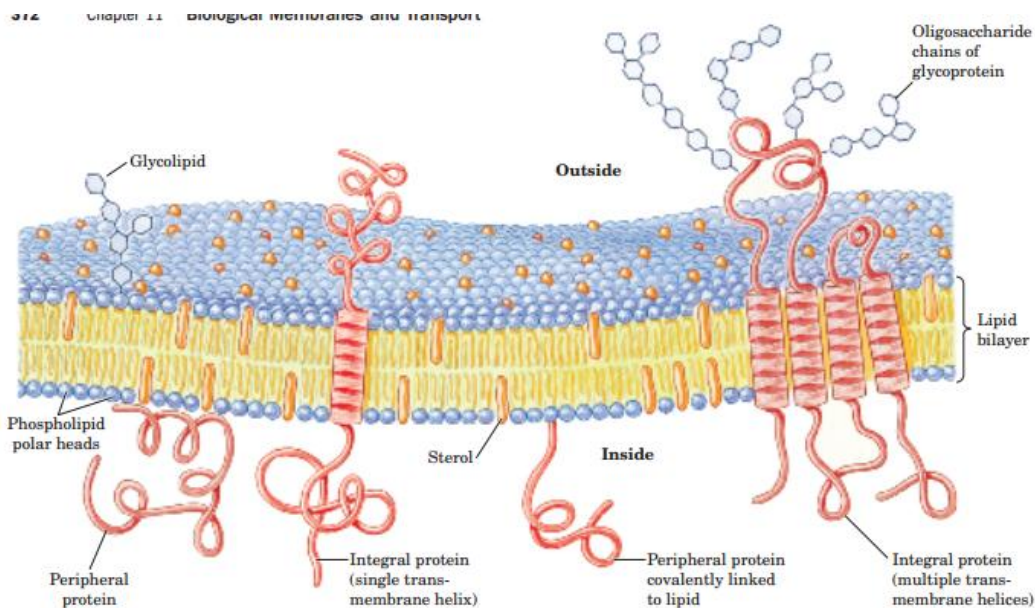


*Figure: the fluid mosaic model of membrane structure. The fatty acyl chains in the interior of the membrane form a fluid, hydrophobic region. Integral proteins float in this sea of lipid, held by hydrophobic interactions with their nonpolar amino acid side chains. Both proteins and lipids are free to move laterally in the plane of the bilayer, but movement of either from one face of the bilayer to the other is restricted. The carbohydrate moieties attached to some proteins and lipids of the plasma membrane are exposed on the extracellular surface of the membrane*

### Membrane Lipids and Membrane Fluidity

The physical state of the lipid of a membrane is described by its fluidity (or viscosity). Consider a simple artificial bilayer composed of phosphatidylcholine and phosphatidyl ethanolamine, whose fatty acids are largely unsaturated. If the temperature of the bilayer is kept relatively warm (e.g., 37°C), the lipid exists in a relatively fluid state. At this temperature, the lipid bilayer is best described as a two-dimensional liquid crystal. As in a crystal, the molecules still retain a specified orientation; in this case, the long axes of the molecules tend toward a parallel arrangement, yet individual phospholipids can rotate around their axis or move laterally within the plane of the bilayer. If the temperature is slowly lowered, a point is reached where the bilayer distinctly changes. The lipid is converted from a liquid crystalline phase to a

frozen crystalline gel in which the movement of the phospholipid fatty acid chains is greatly restricted.

The temperature at which this change occurs is called the transition temperature. The transition temperature of a particular bilayer depends on the ability of the lipid molecules to be packed together, which depends in turn on the particular lipids of which it is constructed. Saturated fatty acids have the shape of a straight, flexible rod. Cis-unsaturated fatty acids, on the other hand, have crooks in the chain at the sites of a double bond. Consequently, phospholipids with saturated chains pack together more tightly than those containing unsaturated chains. The greater the degree of unsaturation of the fatty acids of the bilayer, the lower the temperature before the bilayer gels. The introduction of one double bond in a molecule of stearic acid lowers the melting temperature almost 60°C.

Another factor that influences bilayer fluidity is fatty acid chain length. The shorter the fatty acyl chains of a phospholipid, the lower its melting temperature. The physical state of the membrane is also affected by cholesterol. Because of their orientation within the bilayer, cholesterol molecules disrupt the close packing of fatty acyl chains and interfere with their mobility. The presence of cholesterol tends to abolish sharp transition temperatures and creates a condition of intermediate fluidity. In physiologic terms, cholesterol tends to increase the durability while decreasing the permeability of a membrane.

Table: melting points of some 18 carbon fatty acids

| Fatty acid                  | cis Double bonds | M.p.(°C) |
|-----------------------------|------------------|----------|
| Stearic acid                | 0                | 70       |
| Oleic acid                  | 1                | 13       |
| Linoleic acid               | 2                | -9       |
| Linolenic acid              | 3                | -17      |
| Eicosapentanoic acid (EPA)* | 5                | -54      |

\*EPA has 20 carbons.

### The Importance of Membrane Fluidity

What effect does the physical state of the lipid bilayer have on the biological properties of the membrane? Membrane fluidity provides a perfect compromise between a rigid, ordered structure in which mobility would be absent and a completely fluid, nonviscous liquid in which the components of the membrane could not be oriented and structural organization and mechanical support would be lacking. In addition, fluidity allows for interactions to take place within the membrane. For example, membrane fluidity makes it possible for clusters of membrane proteins to assemble at particular sites within the membrane and form specialized structures, such as intercellular junctions, light-capturing photosynthetic complexes, and synapses. Because of membrane fluidity, molecules that interact can come together, carry out the necessary reaction, and move apart.

Fluidity also plays a key role in membrane assembly. Membranes arise only from preexisting membranes, and their growth is accomplished by the insertion of lipids and proteins into the fluid matrix of the membranous sheet. Many of the most basic cellular processes, including cell movement, cell growth, cell division, formation of intercellular junctions, secretion, and endocytosis, depend on the movement of membrane components and would probably not be possible if membranes were rigid, nonfluid structures.

### **Maintaining Membrane Fluidity**

The internal temperature of most organisms (other than birds and mammals) fluctuates with the temperature of the external environment. Since it is essential for many activities that the membranes of a cell remain in a fluid state, cells respond to changing conditions by altering the types of phospholipids of which they are made. Maintenance of membrane fluidity is an example of homeostasis at the cellular level and can be demonstrated in various ways. For example, if the temperature of a culture of cells is lowered, the cells respond metabolically.

The initial “emergency” response is mediated by enzymes that remodel membranes, making the cell more cold resistant. Remodeling is accomplished by (1) desaturating single bonds in fatty acyl chains to form double bonds, and (2) reshuffling the chains between different phospholipid molecules to produce ones that contain two unsaturated fatty acids, which greatly lowers the melting temperature of the bilayer. Desaturation of single bonds to form double bonds is catalyzed by enzymes called desaturases. Reshuffling is accomplished by phospholipases, which split the fatty acid from the glycerol backbone, and acyl transferases, which transfer fatty acids between phospholipids. In addition, the cell changes the types of phospholipids being synthesized in favor of ones containing more unsaturated fatty acids. As a result of the activities of these various enzymes, the physical properties of a cell’s membranes are matched to the prevailing environmental conditions.

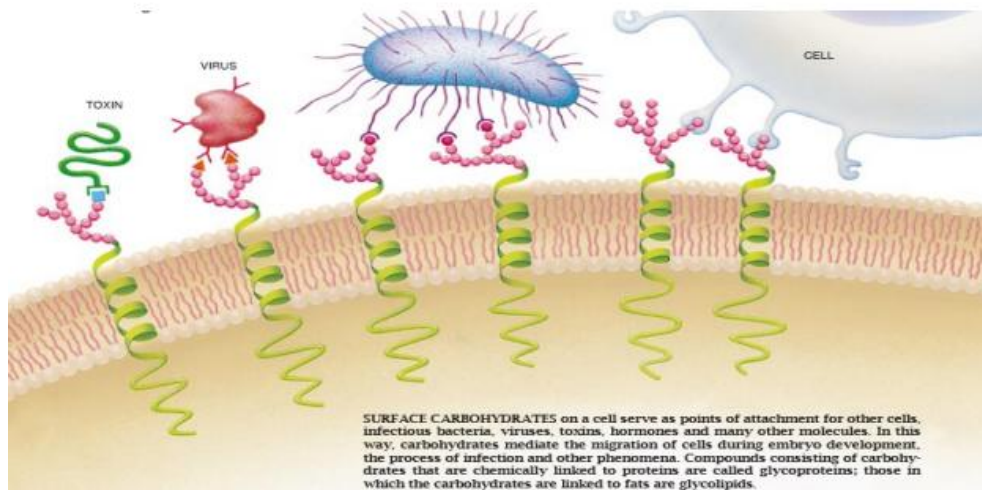
Maintenance of fluid membranes by adjustments in fatty acyl composition has been demonstrated in a variety of organisms, including hibernating mammals, pond-dwelling fish whose body temperature changes markedly from day to night, cold resistant plants, and bacteria living in hot springs. Prokaryotic cells that live at very high temperatures have plasma membranes that contain highly unusual lipids.

### **Membrane carbohydrates: cell – cell recognition and communication**

Along with membrane proteins, these carbohydrates form distinctive cellular markers, sort of like molecular ID badges, that allow cells to recognize each other. These markers are very important in the immune system, allowing immune cells to differentiate between body cells, which they shouldn’t attack, and foreign cells or tissues, which they should.

Membrane carbohydrate plays the role of a marker, identifier, a site for recognition and attachment during cell-cell recognition. Carbohydrates found on the plasma membrane of plants and animals are usually attached to proteins and lipids to form glycoproteins and glycolipids respectively. Carbohydrates promotes specific recognition and are important in Immunity

response, hostcell-Pathogen interaction, cell growth, Apoptosis, cell communication, cooperation, coordination and transporting organisms cell.



The carbohydrate moieties of certain sphingolipids define the human blood groups and therefore determine the type of blood that individuals can safely receive in blood transfusions

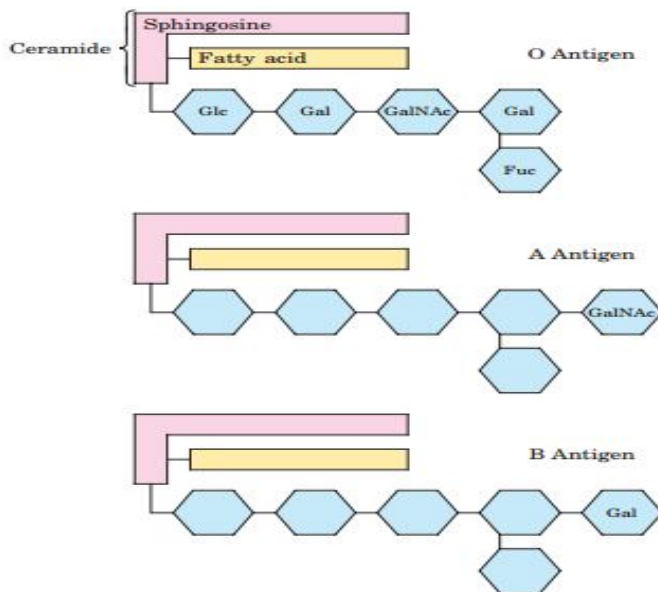


Figure: Glycolipids determines blood grouping

### MEMBRANE TRANSPORT (Small molecules)

Membrane is permeable to water, gases ( $O_2$ ,  $CO_2$ ,  $N_2$ ) and small uncharged polar molecules (e.g. urea, ethanol), but is impermeable to large uncharged polar molecules (e.g. glucose), ions ( $Na^+$ ,  $K^+$ ,  $Cl^-$ ,  $Ca^{2+}$ ) and charged polar molecules (e.g. amino acids, ATP, glucose 6-phosphate). The first group of molecules can cross the membrane unaided and without an input of energy, whereas the latter group require the presence of integral membrane transport proteins and, in some cases, an input of energy to travel through the otherwise

impermeable membrane barrier. Thus the plasma membrane and the membranes of internal organelles are selectively permeable barriers, maintaining a distinct internal environment.

### Passive transport

The passive transport of molecules across a membrane does not require an input of metabolic energy. The rate of transport (diffusion) is proportional to the concentration gradient of the molecule across the membrane. There are two types of passive transport: simple diffusion and facilitated diffusion.

### Simple diffusion

Only relatively small uncharged or hydrophobic molecules ( $\text{H}_2\text{O}$ ,  $\text{O}_2$ ,  $\text{CO}_2$ , other gases, urea and ethanol) cross the lipid bilayer by simple diffusion. No membrane proteins are involved, so there is no specificity. The molecule in aqueous solution on one side of the membrane dissolves into the lipid bilayer, crosses it, and then dissolves into the aqueous solution on the opposite side. The rate of diffusion is directly proportional to the concentration gradient of the molecule across the membrane and the process is not saturable.

### Facilitated diffusion

Unlike simple diffusion, the facilitated (or carrier-mediated) diffusion of a molecule across a biological membrane is dependent on specific integral membrane proteins, often called uniporters. The molecule binds to the protein on one side of the membrane, the protein then undergoes a conformational change, transports the molecule across the membrane and then releases it on the other side. Molecules transported across membranes in this way include hydrophilic molecules such as glucose, other sugars and amino acids. The transport proteins are specific for one particular molecule or a group of structurally similar molecules. The transport proteins are capable of being saturated, display Michaelis–Menten-type binding kinetics ( $K_m$  and  $V_{\max}$ ), and are influenced by temperature, pH and inhibitor molecules in a similar manner to enzymes.

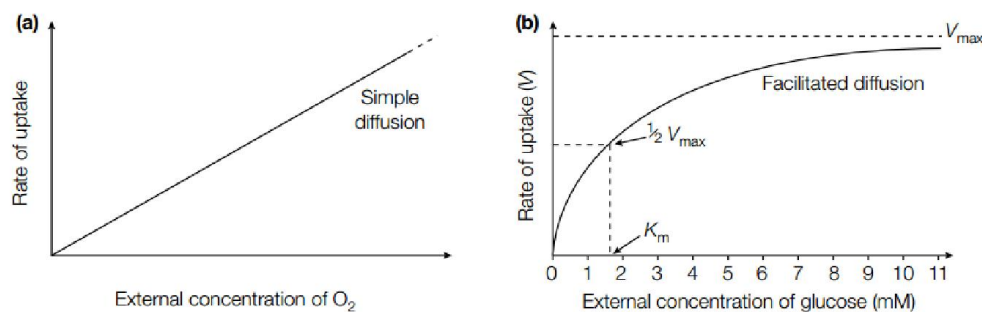


Fig. 1. Kinetics of (a) simple and (b) facilitated diffusion.

An example of facilitated diffusion is the uptake of glucose into erythrocytes by the glucose transporter. The erythrocyte glucose transporter is an integral membrane protein of mass 45 kDa that is asymmetrically orientated in the plasma membrane. This uniporter protein structure traverses the membrane with 12 helices which form a central pore through which

the glucose molecule is passed upon conformational changes in the protein. All the steps in the transport of glucose into the cell are freely reversible, the direction of movement of glucose being dictated by the relative concentrations of glucose on either side of the membrane. In order to maintain the concentration gradient across the membrane, the glucose is rapidly phosphorylated inside the cell by hexokinase to glucose 6-phosphate which is no longer a substrate for the glucose transporter. The erythrocyte glucose transporter is highly specific for D-glucose ( $K_m$  1.5 mM), the nonbiological L-isomer being transported at a barely measurable rate. D-Mannose and D-galactose, which differ from D-glucose in the configuration at one carbon atom are transported at intermediate rates. Thus the transporter has a higher affinity (lower  $K_m$ ) for glucose than for other sugars

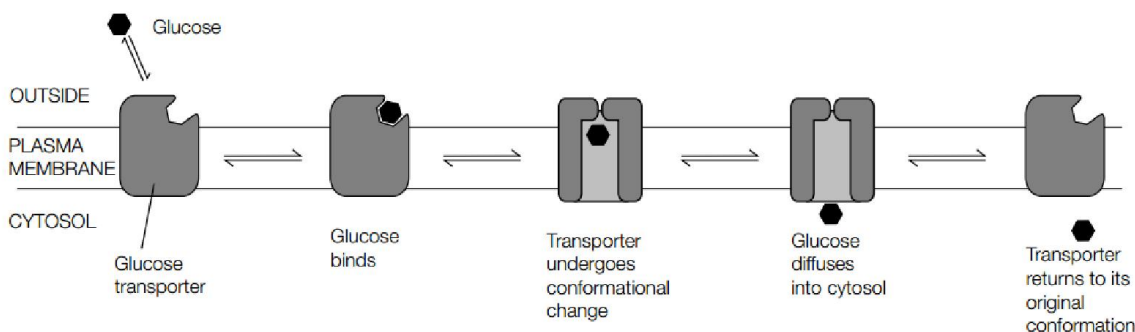


Fig. 2. Facilitated diffusion of glucose into erythrocytes.

### Active transport

The active transport of molecules requires an input of metabolic energy. This can be derived either from direct coupling to the hydrolysis of ATP or by coupling to the movement of an ion down its concentration gradient.

#### Primary (ATP-driven) active transport

In this case, the energy required for the transport of the molecule across the membrane is derived from the coupled hydrolysis of ATP, for example the movement of  $\text{Na}^+$  and  $\text{K}^+$  ions by the  $\text{Na}^+/\text{K}^+$  ATPase. All cells maintain a high internal concentration of  $\text{K}^+$  and a low internal concentration of  $\text{Na}^+$ . The resulting  $\text{Na}^+/\text{K}^+$  gradient across the plasma membrane is important for the active transport of certain molecules, and the maintenance of the membrane electrical potential. The movement across the membrane of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$  and  $\text{H}^+$ , as well as a number of other molecules, is directly coupled to the hydrolysis of ATP.

**Structure and action of the  $\text{Na}^+/\text{K}^+$  ATPase** The  $\text{Na}^+/\text{K}^+$  ATPase is an integral membrane protein consisting of 110 kDa and 55 kDa subunits. Upon hydrolysis of one molecule of ATP to ADP and  $\text{P}_i$ , the protein undergoes a conformational change and three  $\text{Na}^+$  ions are pumped out of the cell across the plasma membrane and two  $\text{K}^+$  ions are pumped in the opposite direction into the cell. Both ions are being moved up their concentration gradients across the membrane; hence the requirement for an input of energy. No transport occurs unless ATP is hydrolyzed, and no ATP is hydrolyzed if there is no  $\text{Na}^+$  and  $\text{K}^+$  to transport (i.e. it is a coupled system).

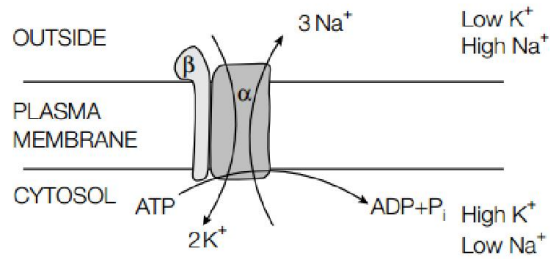


Fig. 3. The  $\text{Na}^+/\text{K}^+$ -ATPase, shown as an  $\alpha\beta$  heterodimer.

### Secondary (Ion-driven) active transport

The movement of the molecule to be transported across the membrane is coupled to the movement of an ion, usually either  $\text{Na}^+$  or  $\text{H}^+$ , down its concentration gradient. The energy for the movement of the molecule across the membrane against its concentration gradient comes from the movement of the ion down its concentration gradient. If both the molecule and the ion move in the same direction, it is termed **symport**, and the protein involved in the process is called a **symporter** (e.g.  $\text{Na}^+/\text{glucose}$  transporter); if the molecule and the ion move in the opposite direction, it is termed **antiport**, and the protein involved in the process is called an **antiporter**.

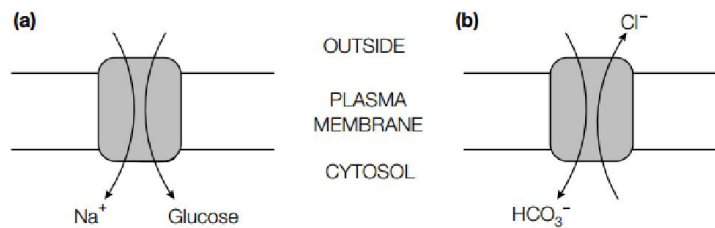


Fig. 4. Ion-driven cotransport mechanisms. (a) Symport process involving a symporter (e.g.  $\text{Na}^+/\text{glucose}$  transporter); (b) antiport process involving an antiporter (e.g. erythrocyte band 3 anion transporter).

### Glucose transport into intestinal epithelial cells

Any nutrient molecules in the lumen of the intestine have to pass through the cytosol of the epithelial cell in order to enter the blood. Glucose (or other sugars and amino acids) are transported across the apical membrane from a relatively low concentration in the lumen of the intestine to a relatively high concentration in the cytosol of the epithelial cell by a glucose/ $\text{Na}^+$  symporter protein (Fig. 5). This is a form of ion-driven active transport; the energy for the movement of glucose against its concentration gradient comes from the movement of  $\text{Na}^+$  down its concentration gradient. The blood flowing through the capillaries on the basolateral side of the epithelial cell maintains a concentration gradient of glucose across this membrane allowing the glucose to move out of the cell by facilitated diffusion through a glucose transporter (a uniporter) which is similar to the erythrocyte glucose transporter. The relatively low concentration of  $\text{Na}^+$  inside the epithelial cell is maintained by a  $\text{Na}^+/\text{K}^+$  ATPase on the basolateral membrane, an example of ATP-driven active transport (Fig. 5).

### Glucose rehydration therapy

The movement of  $\text{Na}^+$  and glucose from the lumen of the intestine across the epithelial cell to the blood sets up a difference in osmotic pressure across the cell. As a result, water flows through the cell, across the apical and basolateral membranes by simple diffusion. Hence the uptake of water requires both  $\text{Na}^+$  and glucose (or amino acids) to be present in the lumen of the intestine. The presence of water alone in the lumen of the intestine is much less effective. This is the basis of glucose rehydration therapy as a remedy for dehydration; a solution of glucose and salt ( $\text{NaCl}$ ) is administered to the patient. This is a simple, inexpensive but extremely important treatment which has saved the lives of many infants in developing countries who would have otherwise died of the effects of dehydration, usually associated with diarrhea.

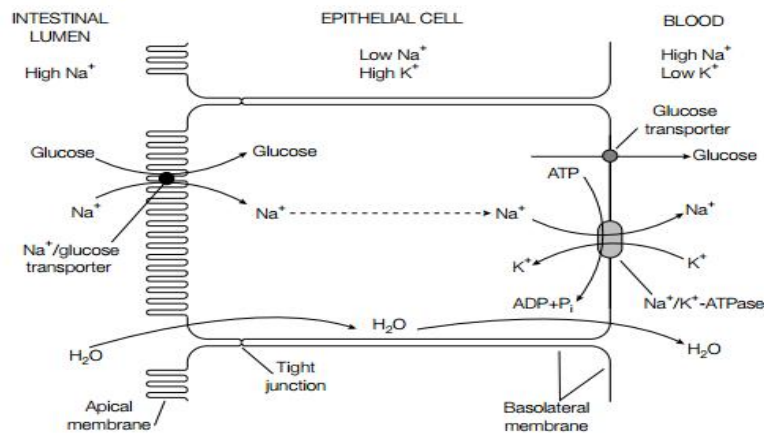


Fig. 5. Transport of glucose and water across intestinal epithelial cells.

### MEMBRANE TRANSPORT (Larger Molecules)

#### Exocytosis

A cell often needs to secrete larger molecules than can be accommodated by the transport systems discussed above. Exocytosis refers to the movement of proteins out of the cell across the plasma membrane into the extracellular space. Proteins destined to be secreted from the cell are translated on ribosomes attached to the RER. Membrane-bound vesicles containing these proteins then bud off from the RER, migrate through the cytosol and fuse with the membrane of the Golgi apparatus (Fig. 1). On transport through the endoplasmic reticulum and Golgi apparatus, various post-translational modifications to the proteins take place, such as glycosylation

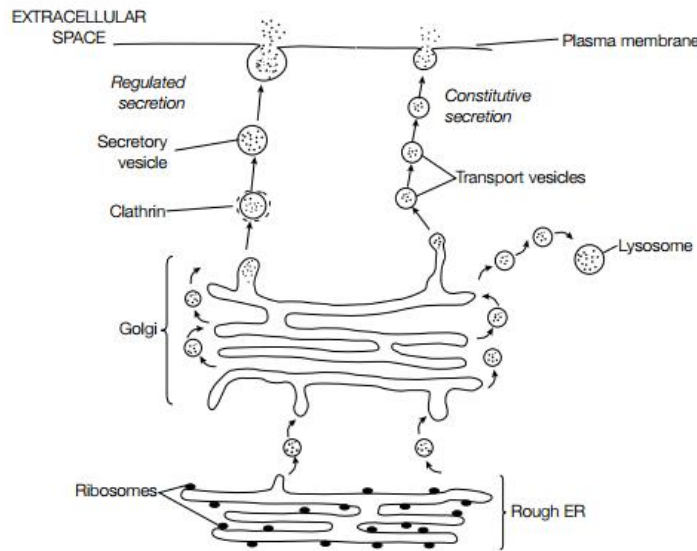


Fig. 1. Exocytosis of proteins by the constitutive and regulated secretory pathways.

The Golgi apparatus is the major sorting center of the cell where proteins and lipids are packaged into discrete vesicles and then targeted to the appropriate part of the cell. For example, vesicles containing lysosomal proteins will be targeted to the lysosomes. If the vesicles contain no specific targeting signal they will migrate through the cytosol to the plasma membrane, the so-called default or constitutive secretory pathway (Fig. 1).

In certain cells, however, an additional secretory pathway exists, the regulated secretory pathway. This pathway is found mainly in cells that are specialized for secreting products rapidly on demand in response to a particular stimulus (Fig. 1). For example, the hormone insulin and digestive enzymes are secreted by the pancreas, while neurotransmitters are secreted by nerve cells.

### Endocytosis

Endocytosis is the uptake of extracellular macromolecules across the plasma membrane into the cell. The material to be ingested is progressively enclosed by a small portion of the plasma membrane, which first invaginates and then pinches off to form an intracellular vesicle containing the ingested macromolecule. Endocytosis can be divided into three distinct types depending on the size of the ingested macromolecule and whether specific cell surface receptors are involved. These three processes are: phagocytosis, pinocytosis and receptor mediated endocytosis.

### Phagocytosis

Phagocytosis is the ingestion of large particles such as bacteria and cell debris via large endocytic vesicles called phagosomes. In order to be ingested the particle must first bind to the surface of the phagocyte, usually through specialized cell surface receptors. Once bound to the receptors, the phagocyte is stimulated to begin engulfing the particle with its plasma membrane, thereby enclosing it within a phagosome (Fig. 2a). The phagosome then fuses with a lysosome and the ingested particle is broken down. Utilizable material will be transported into the

cytosol, while indigestible substances will remain in the lysosomes, forming residual bodies. In protozoa, phagocytosis is a form of feeding, where the ingested material is broken down in the lysosomes and utilized as food. In multicellular organisms only a few specialized cells are capable of phagocytosis. Macrophages and neutrophils (white blood cells) use phagocytosis to protect the organism against infection by ingesting invading microorganisms. Macrophages are also involved in scavenging dead and damaged cells and cell debris.

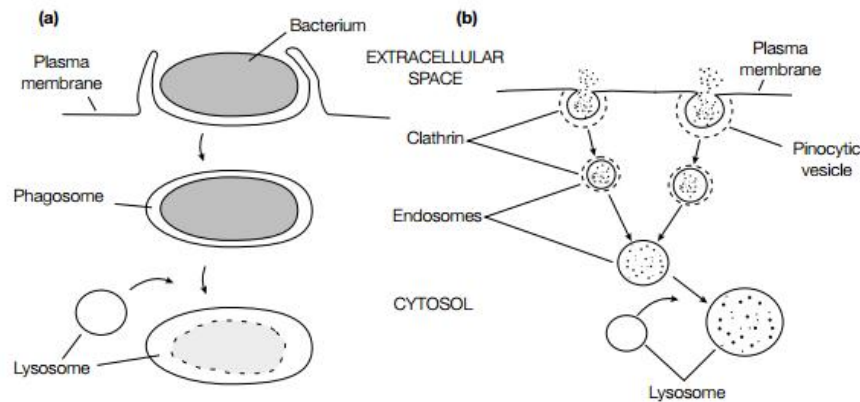


Fig. 2. (a) Phagocytosis and (b) pinocytosis.

## Pinocytosis

Unlike phagocytosis, which is a regulated form of endocytosis carried out by a small number of cell types, pinocytosis is a constitutive process that occurs continuously in all eukaryotic cells. Small areas of the plasma membrane are ingested in the form of small pinocytic vesicles that are later returned to the cell surface. As the pinocytic vesicle forms, a small amount of extracellular fluid is enclosed in the vesicle and also taken up into the cell (Fig. 2b). This nonspecific uptake of extracellular fluid is often referred to as cell-drinking or fluid-phase endocytosis. The initial endocytosis begins by the formation of a clathrin-coated pit (see below) at specialized regions of the plasma membrane (Fig. 2b). The resulting clathrin-coated endocytic vesicle (endosome) is then endocytosed, the clathrin coat dissociates and the endosome fuses with a lysosome (see below).

## Receptor-mediated endocytosis

Receptor-mediated endocytosis is the selective uptake of macromolecules from endocytosis the extracellular fluid via clathrin-coated pits and vesicles. This process, which takes place in most animal cells, involves the macromolecule binding specifically to a cell-surface receptor. Once bound to the receptor, the receptor-macromolecule complex accumulates in a clathrin-coated pit and is then endocytosed in a clathrin-coated vesicle. Receptor-mediated endocytosis provides a way of selectively concentrating particular macromolecules that are at low concentrations in the extracellular fluid, thereby increasing the efficiency of their uptake without having to take in large quantities of extracellular fluid. One of the best studied and understood receptor-mediated endocytic processes is the uptake of cholesterol by mammalian cells. Many viruses and other toxins gain entry to animal cells by receptor-mediated endocytosis. Although the cells do not purposely have cell-surface receptors that recognize the viral particle, the

virus has evolved to express a protein on its surface that mimics the correct ligand recognized by the receptor, thus allowing the virus to bind and be internalized.

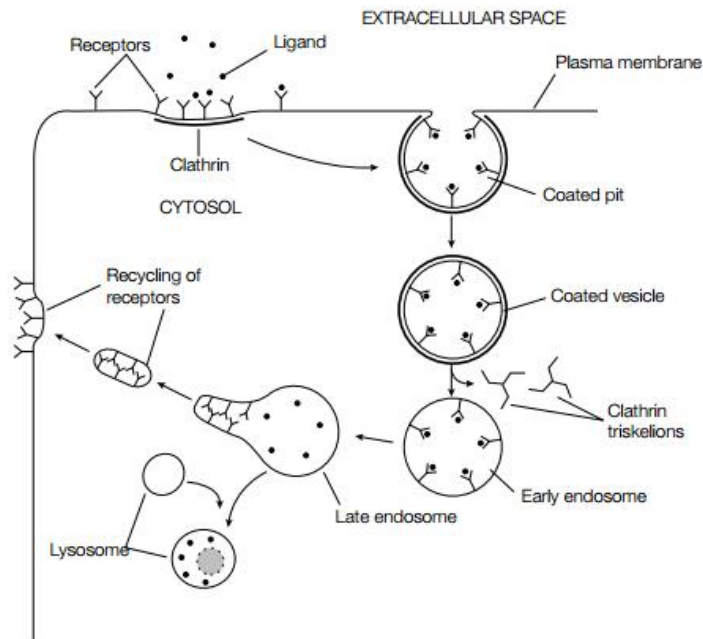


*Fig. 3. Receptor-mediated endocytosis involves clathrin-coated pits and vesicles.*

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