Notes on apoptosis, transport across the plasma membrane, cell fractionation, Centrifugation, cell membrane, and edema

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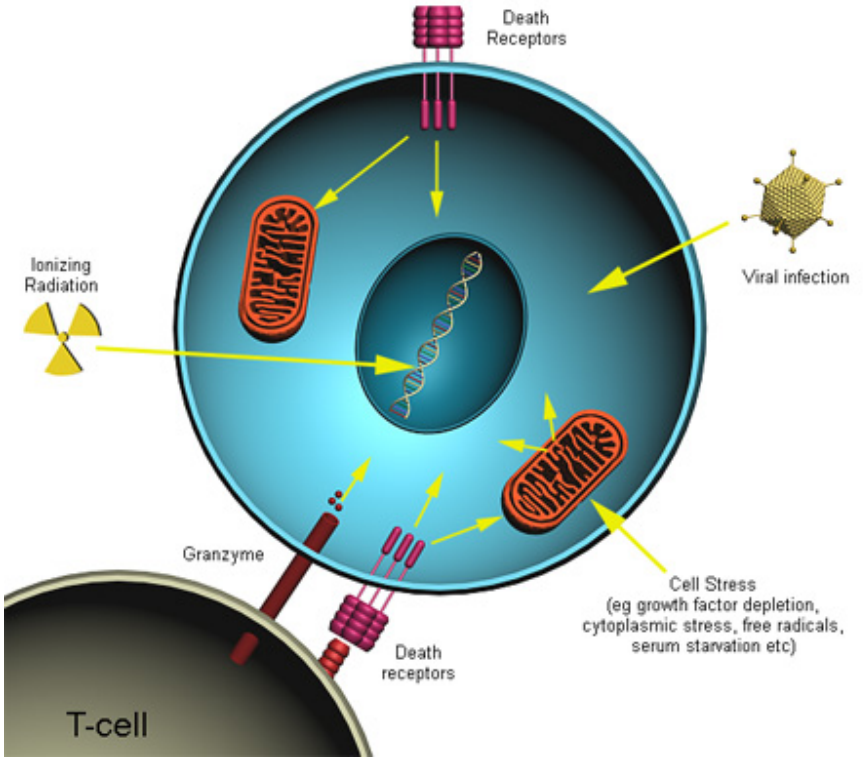
Apoptosis

Apoptosis, or programmed cell death, is a normal component of the development and health of multicellular organisms. Cells die in response to a variety of stimuli and during apoptosis they do so in a controlled, regulated fashion. This makes apoptosis distinct from another form of cell death called necrosis in which uncontrolled cell death leads to lysis of cells, inflammatory responses and, potentially, to serious health problems. Apoptosis, by contrast, is a process in which cells play an active role in their own death (which is why apoptosis is often referred to as cell suicide).

Mechanisms:

Upon receiving specific signals instructing the cells to undergo apoptosis a number of distinctive changes occur in the cell. A family of proteins known as caspases are typically activated in the early stages of apoptosis. These proteins breakdown or cleave key cellular components that are required for normal cellular function including structural proteins in the cytoskeleton and nuclear proteins such as DNA repair enzymes. The caspases can also activate other degradative enzymes such as DNases, which begin to cleave the DNA in the nucleus.

Typically, the cell begins to shrink following the cleavage of lamins and actin filaments in the cytoskeleton (A). The breakdown of chromatin in the nucleus often leads to nuclear condensation and in many cases the nuclei of apoptotic cells take on a "horse-shoe" like appearance (B). Cells continue to shrink (C), packaging themselves into a form that allows for their removal by macrophages. These phagocytic cells are responsible for clearing the apoptotic cells from tissues in a clean and tidy fashion that avoids many of the problems associated with necrotic cell death. In order to promote their phagocytosis by macrophages, apoptotic cells often ungergo plasma membrane changes that trigger the macrophage response. One such change is the translocation of phosphatidylserine from the inside of the cell to the outer surface. The end stages of apoptosis are often characterised by the appearance of membrane blebs (D) or blisters process. Small vesicles called apoptotic bodies are also sometimes observed (D, arrow).



Transport across the Plasma Membrane

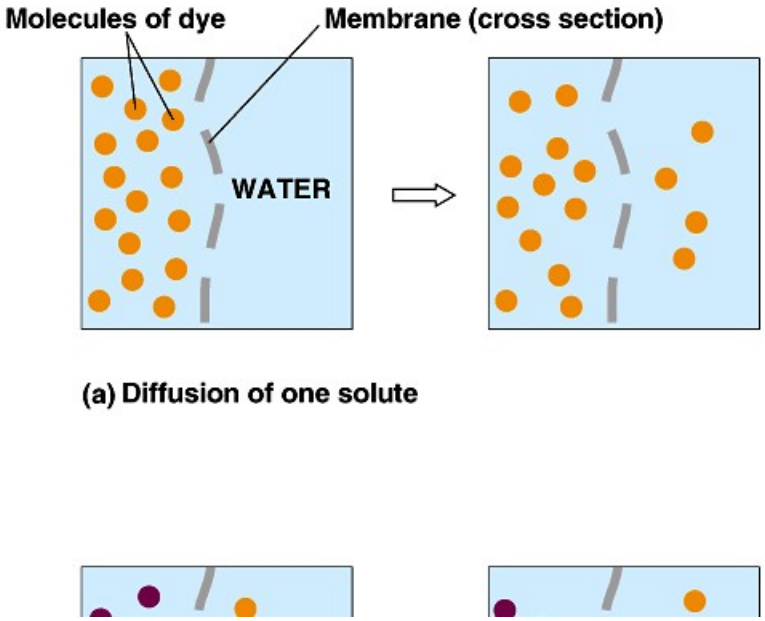
The plasma membrane does not simply form a "sack" in which to keep all the cytoplasm and other cellular organelles. The plasma membrane is a very important structure which functions to allow certain substances to enter or leave the cell. It can "pump" other substance into the cell against the concentration gradient or pump other "wastes" etc. out of the cell.

Types:

A. **Passive transport processes**- Some of the transport process happens "**passively**" without the cell needing to expend any energy to make them happen.
B. **Active transport processes**. Other transport processes require energy from the cell's reserves to "power" them. These processes are called "**active transport processes**".

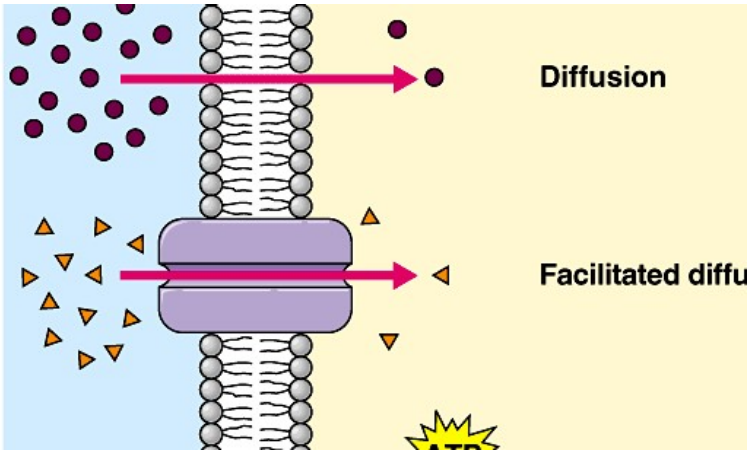
A) Passive Transport Processes-

a) **Diffusion**: is the movement of ions or molecules from regions of **higher** concentration **to** regions of **lower** concentration. (**Down** a concentration gradient)



(a) Diffusion of one solute

The plasma membrane will allow certain substances to cross it but not others. Such a membrane is referred to as "**selective permeable**". The plasma membrane's permeability depends on a large part on its makeup. Both the protein portion and the phospholipid portion of the membrane are involved in the permeability.



Osmosis: Osmosis is the movement of water from a region of high water concentration to a region of lower water concentration through a semi permeable membrane.

Facilitated Diffusion-

This is similar to simple diffusion in the sense that it is diffusion (across a membrane) from a high concentration to a lower concentration.**However**, this time the rate of diffusion is greatly accelerated by the action membrane proteins that act as carrier molecules and aid in diffusion. These "carrier proteins" are known as "**Permeases**".

a) **Facilitated Diffusion of Ions**

Facilitated diffusion of ions takes place through proteins, or assemblies of proteins, embedded in the plasma membrane. These transmembrane proteins form a water-filled channel through which the ion can pass **down** its concentration gradient. The transmembrane channels that permit facilitated diffusion can be opened or closed. They are said to be **"gated"**.

Types of gated ion channels:

- ligand-gated
- mechanically-gated
- voltage-gated
- light-gated

i) Ligand-gated ion channels.

Many ion channels open or close in response to binding a small signaling molecule or **"ligand"**. Some ion channels are gated by extracellular ligands; some by intracellular ligands. In both cases, the ligand is **not** the substance that is transported when the channel opens.

External ligands

External ligands (shown here in green) bind to a site on the extracellular side of the channel.

Examples:

- **Acetylcholine** (**ACh**). The binding of the neurotransmitter acetylcholine at certain synapses opens channels that admit Na^+ and initiate a nerve impulse or muscle contraction.
- **Gamma amino butyric acid** (**GABA**). Binding of GABA at certain synapses — designated $GABA_A$ — in the central nervous system admits Cl^- ions into the cell and inhibits the creation of a nerve impulse.

Internal ligands

Internal ligands bind to a site on the channel protein exposed to the cytosol.

Examples:

- "Second messengers", like **cyclic AMP** (**cAMP**) and **cyclic GMP** (**cGMP**), regulate channels involved in the initiation of impulses in neurons responding to odors and light respectively.

ii) Mechanically-gated ion channels

Examples:

- Sound waves bending the cilia-like projections on the hair cells of the inner ear open up ion channels leading to the creation of nerve impulses that the brain interprets as sound.
- Mechanical deformation of the cells of stretch receptors opens ion channels leading to the creation of nerve impulses.

iii) Voltage-gated ion channels

Some channels open or close in response to changes in the charge (measured in volts) across the plasma membrane.

Example: As an impulse passes down a neuron, the reduction in the voltage opens sodium channels in the adjacent portion of the membrane. This allows the influx of Na^+ into the neuron and thus the continuation of the nerve impulse.

Some 7000 sodium ions pass through each channel during the brief period (about 1 millisecond) that it remains open. This was learned by use of the patch clamp technique.

b) Facilitated Diffusion of Molecules

Some small, hydrophilic organic molecules, like sugars, can pass through cell membranes by facilitated diffusion. The process requires transmembrane proteins. In some cases, these — like ion channels — form water-filled pores that enable the molecule to pass in (or out) of the membrane following its concentration gradient.

Example: **Maltoporin**. This homotrimer in the outer membrane of E. coli forms pores that allow the disaccharide maltose and a few related molecules to diffuse into the cell.

Osmosis

- Osmosis is a special term used for the diffusion of water through cell membranes.
- Although water is a polar molecule, it is able to pass through the lipid bilayer of the plasma membrane. **Aquaporins** — transmembrane proteins that form hydrophilic channels — greatly accelerate the process, but even without these, water is still able to get through.
- Water passes by diffusion from a region of higher to a region of lower concentration.

  Example: the reabsorption of water from the kidney tubules back into the blood depends on the water following behind the active transport of Na^+.

B) Active Transport

Active transport is the movement of a substance against its concentration gradient (from low to high concentration). In all cells, this is usually concerned with accumulating high concentrations of molecules that the cell needs, such as ions, glucose and amino acids. If the process uses chemical energy, such as from adenosine triphosphate (ATP), it is termed primary active transport. Secondary active transport involves the use of an electrochemical gradient.

1. **Direct Active Transport.** Some transporters bind ATP directly and use the energy of its hydrolysis to drive active transport.

2. **Indirect Active Transport.** Other transporters use the energy already stored in the gradient of a directly-pumped **ion**.

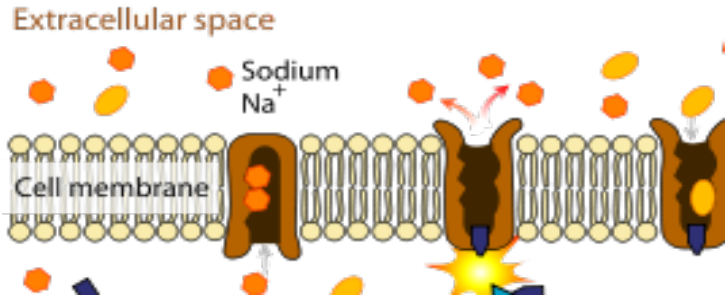
Direct Active Transport

1. The Na^+/K^+ ATPase

The cytosol of animal cells contains a concentration of potassium ions (K^+) as much as 20 times higher than that in the extracellular fluid. Conversely, the extracellular fluid contains a concentration of sodium ions (Na^+) as much as 10 times greater than that within the cell.

These concentration gradients are established by the active transport of both ions. And, in fact, the same transporter, called the Na^+/K^+ ATPase, does both jobs. It uses the energy from the hydrolysis of ATP to

- actively transport 3 Na^+ ions out of the cell
- for each 2 K^+ ions pumped into the cell.



2. The H^+/K^+ ATPase

The parietal cells of your stomach use this pump to secrete gastric juice. These cells transport protons (H^+) from a concentration of about 4 x 10^{-8} M within the cell to a concentration of about 0.15 M in the gastric juice (giving it a pH close to 1). Small wonder that parietal cells are stuffed with mitochondria and uses huge amounts of**ATP** as they carry out this three-million fold concentration of protons.

3. The Ca^{2+} ATPases

A Ca^{2+} ATPase is located in the **plasma membrane** of all eukaryotic cells. It uses the energy provided by one molecule of ATP to pump one Ca^{2+} ion out of the cell. The activity of these pumps helps to maintain the ~20,000-fold concentration gradient of Ca^{2+} between the cytosol (~ 100 nM) and the ECF (~ 20 mM).

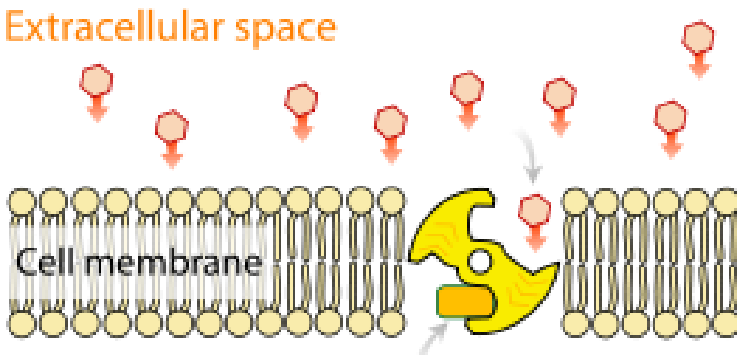
4. ABC Transporters

ABC ("**A**TP-**B**inding **C**assette") transporters are transmembrane proteins that

- expose a ligand-binding domain at one surface and a
- ATP-binding domain at the other surface.

Indirect Active Transport

Indirect active transport uses the downhill flow of an ion to pump some other molecule or ion against its gradient. The driving ion is usually sodium (Na^+) with its gradient established by the Na^+/K^+ ATPase.



1. Symport Pumps

In this type of indirect active transport, the driving ion (Na^+) and the pumped molecule pass through the membrane pump in the **same** direction.

Examples:

- **The Na^+/glucose transporter**. This transmembrane protein allows sodium ions and glucose to enter the cell together. The sodium ions flow **down** their concentration gradient while the glucose molecules are pumped **up** theirs. Later the sodium is pumped back out of the cell by the Na^+/K^+ ATPase.
- Sodium-driven symport pumps also return **neurotransmitters** to the presynaptic neuron.
- **The Na^+/iodide transporter**. This symporter pumps iodide ions into the cells of the thyroid gland and also into the cells of the mammary gland.

2.Antiport Pumps

In antiport pumps, the driving ion (again, usually sodium) diffuses through the pump in one direction providing the energy for the active transport of some other molecule or ion in the opposite direction.

Example: Ca^{2+} ions are pumped out of cells by sodium-driven antiport pumps.

Endocytosis

Endocytosis is the process by which cells take in materials. The cellular membrane folds around the desired materials outside the cell. The ingested particle becomes trapped within a pouch, vacuole or inside the cytoplasm. Often enzymes from lysosomes are then used to digest the molecules absorbed by this process.

Biologists distinguish two main types of endocyctosis: pinocytosis and phagocytosis.

- In pinocytosis, cells engulf liquid particles (in humans this process occurs in the small intestine, cells there engulf fat droplets).
- In phagocytosis, cells engulf solid particles.

Cell Fractionation

Cell fractionation is a combination of various methods used to separate a cell organelles and components.

Steps:

There are three principal steps:

1. **Disruption (homogenization)-** Homogenization is the process of breaking open the cells. Cells are broken apart by chemicals, enzymes, or sound waves.
2. **Macro Filtration-** This step may not be necessary depending on the source of the cells. Animal tissue however is likely to yield connective tissue which must be removed. Commonly, filtration is achieved either by pouring through gauze or with a suction filter and the relevant grade ceramic filter.
3. **Purification-**This is achieved by Differential centrifugation and at the end of this process, we get mitochondria, the nucleus, the chloroplast and etc.

A. Cell Disruption Method

i. **Lysis**

For easily disrupted cells such as mammalian cells grown in culture media, a mild osmosis-based method for cell disruption (lysis) is commonly used. Simply lowering the ionic strength of the media will cause the cells to swell and burst. In some cases it is also desirable to add a mild surfactant and some mild mechanical agitation or sonication to completely disassociate the cellular components. For cells that are more difficult to disrupt, such as bacteria, yeast, and algae, hypotonic shock alone generally is insufficient to open the cell.

ii. **Blenders**

For molecular separations, mechanical blenders are often used, varying in sophistication from household blenders to high speed blenders with specially designed blades and chambers (e.g. a Virtis Tissue Homogenizer). The mechanical procedures are augmented by various organic solvents (for phase separations) and/or detergents to assist the denaturation and separation of molecules (e.g. DNA from histones).

iii. **Mortars, Pestles**

The most common procedures use Ten Broeck or Dounce homogenizers, both of which are glass mortar and pestle arrangements with manufactured, controlled bore sizes. The addition of a motor driven teflon pestle creates the Potter-Elvijem homogenizer.

iv. **Bead method**

Another common laboratory-scale mechanical method for cell disruption uses small glass, ceramic or steel beads and a high level of agitation by stirring or shaking of the mix. The method, often referred to as "beadbeating", works well for all types of cellular material. It has the advantage of being able to disrupt very small sample sizes.An equal volume of beads are added to the cell or tissue suspension in a test tube and the sample is mixed on a common laboratory vortex mixer. level, beadbeating is done in closed vials, centrifuge tubes, or sealed microtiter plates. The sample and 0.1 to 3 mm diameter beads are agitated at about 2000 oscillations per minute.Cell disruption is complete in 1–3 minutes of shaking.

v. **Sonication**

Another common laboratory-scale method for cell disruption applies ultrasound (20–50 kHz) to the sample (*sonication*). High-frequency is generated electronically and the mechanical energy is transmitted to the

sample via a metal probe. The probe is placed into the cell-containing sample and the high-frequency oscillation causes a localized low pressure region resulting in cavitation and impaction, ultimately breaking open the cells.

vi. **Detergent methods**

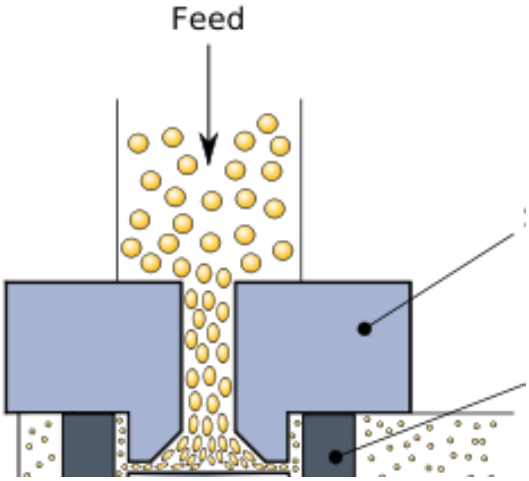
Detergent-based cell lysis is an alternative to physical disruption of cell membranes, although it is sometimes used in conjunction with homogenization and mechanical grinding. Detergents disrupt the lipid barrier surrounding cells by disrupting lipid:lipid, lipid:protein and protein:protein interactions. The ideal detergent for cell lysis depends on cell type and source and on the downstream applications following cell lysis.

vii. **The 'cell bomb'**

Another laboratory-scale system for cell disruption is rapid decompression or the "cell bomb" method. In this process, cells in question are placed under high pressure (usually nitrogen or other inert gas up to about 25,000 psi) and the pressure is rapidly released. The rapid pressure drop causes the dissolved gas to be released as bubbles that ultimately lyse the cell.

viii. **Valve-type processors**

Valve-type processors disrupt cells by forcing the media with the cells through a narrow valve under high pressure (20,000–30,000 psi or 140–210 MPa). As the fluid flows past the valve, high shear forces in the fluid pull the cells apart. By controlling the pressure and valve tension, the shear force can be regulated to optimize cell disruption. Due to the high energies involved, sample cooling is generally required, especially for samples requiring multiple passes through the system.

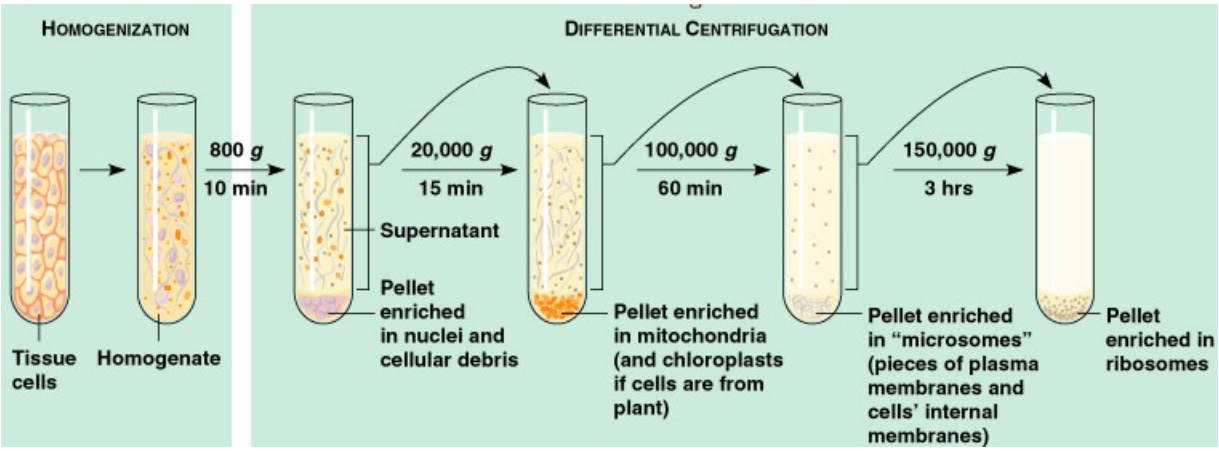


Centrifugation

The most widely used technique for fractionating cellular components is the use of centrifugal force. Procedures employing low speed instruments with greater volume capacity and refrigeration are known as "preparative" techniques. Analytical procedures, on the other hand, usually call for high speed with a corresponding lower volume capacity. A centrifuge working at speeds in excess of 20,000 RPM is an ultracentrifuge.

Organelles may be separated in a centrifuge according to a number of basic procedures. They can be part of a moving boundary, a moving zone, a classical sedimentation equilibrium, a preformed gradient isodensity, an equilibrium isodensity or separated at an interface.

The diagram below is an example of cell fractionation. The first two test tubes demonstrate the result of homogenization and the last four portray the process of differential centrifugation.



Cell membrane

The **cell membrane** or **plasma membrane** is a biological membrane that separates the interior of all cells from the outside environment. The cell membrane is selectively permeable to ions and organic molecules and controls the movement of substances in and out of cells. It basically protects the cell from outside forces. It consists of the lipid bilayer with embedded proteins.

Structure-

Fluid mosaic model

According to the fluid mosaic model of S.J. Singer and G.L. Nicolson (1972), biological membranes can be considered as a two-dimensional liquid in which all lipid and protein molecules diffuse more or less easily. Although the lipid bilayers form the basis of the membranes, the plasma membrane also contains a large quantity of proteins, which provide more structure. Examples of such structures are protein-protein complexes, pickets and fences formed by the actin-based cytoskeleton, and potentially lipid rafts.

Lipid bilayer-

Lipid bilayers form through the process of self-assembly. The cell membrane consists primarily of a thin layer of amphipathic phospholipids which spontaneously arrange so that the hydrophobic "tail" regions are isolated from the surrounding polar fluid, causing the more hydrophilic "head" regions to associate with the intracellular (cytosolic) and extracellular faces of the resulting bilayer. This forms a continuous, spherical lipid bilayer.

[Forces such as van der Waals, electrostatic, hyrdogen bonds, and noncovalent interactions, are all forces that contribute to the formation of the lipid bilayer. Overall, hydrophobic interactions are the major driving force in the formation of lipid bilayers. Lipid bilayers are generally impermeable to ions and polar molecules. The arrangement of hydrophilic heads and hydrophobic tails of the lipid bilayer prevent polar solutes (e.g. amino acids, nucleic acids, carbohydrates, proteins, and ions) from diffusing across the membrane, but generally allows for the passive diffusion of hydrophobic molecules. This affords the cell the ability to control the movement of these substances via transmembrane protein complexes such as pores, channels and gates.]

Composition

Cell membranes contain a variety of biological molecules, notably lipids and proteins.

Lipids

The cell membrane consists of three classes of amphipathic lipids:

- **phospholipids** (egphosphatidylinositol, phosphatidylethanolamine, sphingomyelin etc),
- **glycolipids,** and
- **cholesterols**.

The amount of each depends upon the type of cell, but in the majority of cases phospholipids are the most abundant.

The fatty chains in phospholipids and glycolipids usually contain an even number of carbon atoms, typically between 16 and 20. The 16- and 18-carbon fatty acids are the most common. Fatty acids may be saturated or unsaturated. The entire membrane is held together via non-covalent interaction of hydrophobic tails. In animal cells cholesterol is normally found dispersed in varying degrees throughout cell membranes, in the irregular spaces between the hydrophobic tails of the membrane lipids.

Carbohydrates

Plasma membranes also contain carbohydrates, predominantly glycoproteins, but with some glycolipids (cerebrosides and gangliosides). For the most part, no glycosylation occurs on membranes within the cell; rather generally glycosylation occurs on the extracellular surface of the plasma membrane.

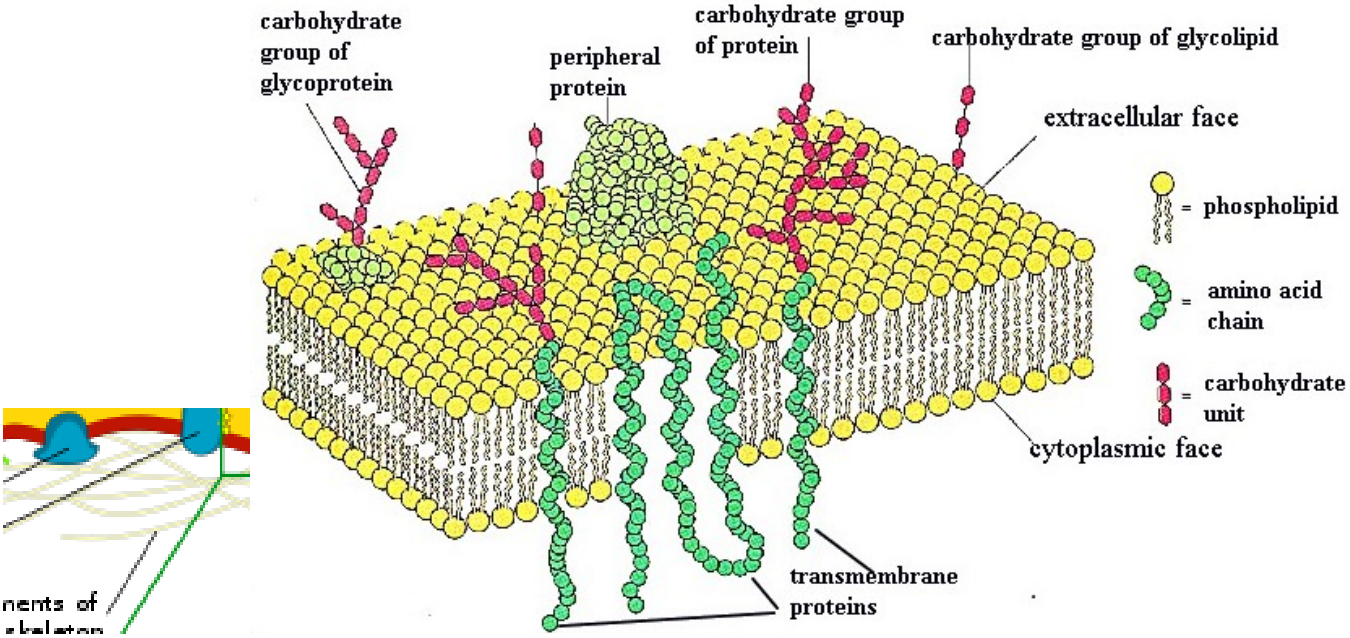
Proteins

Proteins within the membrane are key to the functioning of the overall membrane. These proteins mainly transport chemicals and information across the membrane. Every membrane has a varying degree of protein content. Proteins can be in the form of peripheral or integral.

a. **Integral proteins or transmembrane proteins**- have a hydrophilic cytosolic domain, that anchors it within the cell membrane, and a hydrophilic extracellular domain that interacts with external molecules. The hydrophobic domain consists of one, multiple, or a combination of α-helices and β sheet protein motifs. **Examples** -Ion channels, proton pumps, G protein-coupled receptor

b. **Lipid anchored proteins**- Covalently bound to single or multiple lipid molecules; hydrophobically insert into the cell membrane and anchor the protein. The protein itself is not in contact with the membrane. Eg-G proteins

c. **Peripheral proteins-** Attached to integral membrane proteins, or associated with peripheral regions of the lipid bilayer. These proteins tend to have only temporary interactions with biological membranes, and, once reacted the molecule, dissociates to carry on its work in the cytoplasm. Eg-Some enzymes,some hormones



Function

The cell membrane surrounds the cytoplasm of a cell and, in animal cells, physically separates the intracellular components from the extracellular environment. The cell membrane also plays a role in anchoring the cytoskeleton to provide shape to the cell, and in attaching to the extracellular matrix and other cells to help group cells together to form tissues.

The membrane is differentially permeable and able to regulate what enters and exits the cell, thus facilitating the transport of materials needed for survival. To do so, the membrane employs a number of transport mechanisms:

1. Diffusion : Some substances such as carbon dioxide (CO_2), oxygen (O_2), and water, can move across the plasma membrane by diffusion, which is a passive transport process.

2. Osmosis : Because the membrane acts as a barrier for certain molecules and ions, they can occur in different concentrations on the two sides of the membrane. Such a concentration difference across a semipermeable membrane can set up a osmotic flow for the solvent. Water can thus be transported across the membrane by osmosis.

3. Mediated Transport : Nutrients such as sugars and materials of growth such as amino acid must enter the cell, and the waste of metabolism must leave. Such molecules are moved across the membrane by special proteins called transport proteins or permeases.

4. Endocytosis : Endocytosis is the process in which cells absorb molecules by engulfing them. The plasma membrane creates a small deformation inward, called an invagination, in which the substance to be transported is captured. Endocytosis is a pathway for internalizing solid particles (cell eating or phagocytosis), small molecules and ions (cell drinking or pinocytosis), and macromolecules. Endocytosis requires energy and is thus a form of active transport.

5. Exocytosis : The membrane of a vesicle can be fused with the plasma membrane, extruding its contents to the surrounding medium. This is the process of exocytosis. Exocytosis occurs in various cells to remove undigested residues of substances brought in by endocytosis, to secrete substances such as hormones and enzymes, and to transport a substance completely across a cellular barrier.

EDEMA

Edema is an abnormal accumulation of fluid beneath the skin or in one or more cavities of the body that produces swelling. Generally, the amount of interstitial fluid is determined by the balance of fluid homeostasis, and increased secretion of fluid into the interstitium or impaired removal of this fluid may cause edema.

Classification:

Cutaneous edema is referred to as "pitting" when, after pressure is applied to a small area, the indentation persists for some time after the release of the pressure.

Peripheral pitting edema is the more common type, resulting from water retention. It can be caused by systemic diseases, pregnancy in some women, either directly or as a result of heart failure, or local conditions such as varicose veins, thrombophlebitis, insect bites, and dermatitis.

Non-pitting edema is observed when the indentation does not persist. It is associated with such conditions as lymphedema, lipoedema andmyxedema.

Six factors can contribute to the formation of edema:

1. increased hydrostatic pressure;
2. reduced oncotic pressure within blood vessels;
3. increased tissue oncotic pressure;
4. increased blood vessel wall permeability e.g. inflammation;
5. obstruction of fluid clearance via the lymphatic system;
6. changes in the water retaining properties of the tissues themselves. Raised hydrostatic pressure often reflects retention of water and sodium by the kidney.

Oedema can cause:

1. skin discolouration
2. fluid-filled areas of skin that temporarily hold the imprint of your finger when pressed (known as pitting oedema)
3. aching, tender limbs
4. stiff joints
5. weight gain or weight loss
6. raised blood pressure and pulse rate