Cell Permeability

Changes in the extracellular osmotic pressure will create a situation in which a cell will attempt to attain equilibrium by either gaining or losing water until there is no osmotic gradient across the plasma membrane. The Boyle-Van't Hoff equation describes the volume change that must occur to achieve equilibrium, but ignors the kinetics. If the cell volume is measured as a function of time, then it can be seen that equilibrium is only achieved after an amount of time has elapsed.

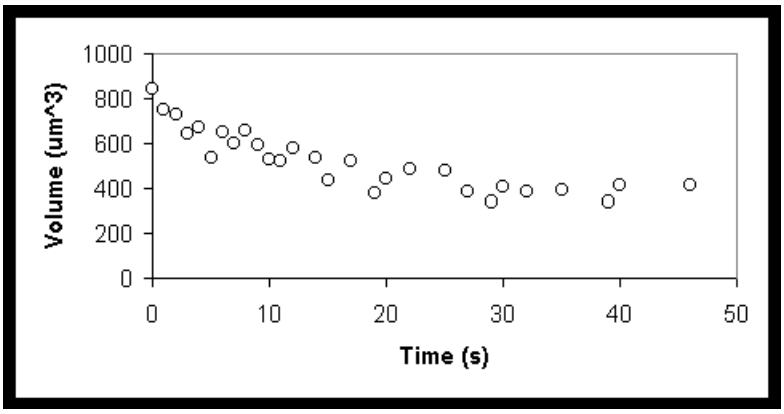

Fig. 5.2.1
Chinese Hamster Fibroblasts exposed to a concentrated saline solution.

Membrane Structure

The kinetics of water movement out of the cell are determined by the physical structure of the membrane. The detailed structure of biological membranes is extremely complicated, however, to first order we can understand permeability from a fairly simple model of membrane structure.

The first component of the membrane is a lipid bilayer. Most membrane lipids are esters of glycerol in which two of the alcohol groups are replaced by long chain fatty acids and the third is replaced by a phosphate head group.

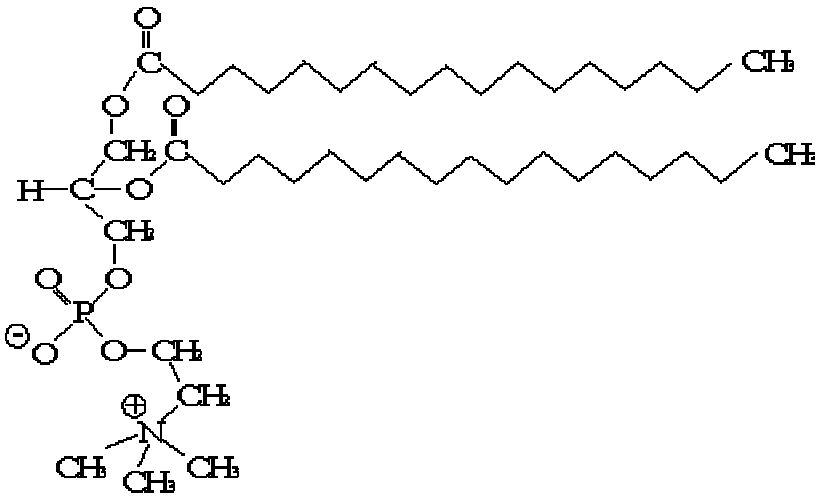

Fig. 5.2.2
The structure of phosphatidylcholine (lecithin) is shown here.

The fatty acid chains are non polar and thus hydrophobic whereas the phosphate head group is hydrophilic, making the molecule amphipathic. When these lipids are placed in water, they spontaneously form a bilayer arrangement in which the hydrophilic head groups are in contact with the water and the hydrophobic tails are separated from the aqueous phase.

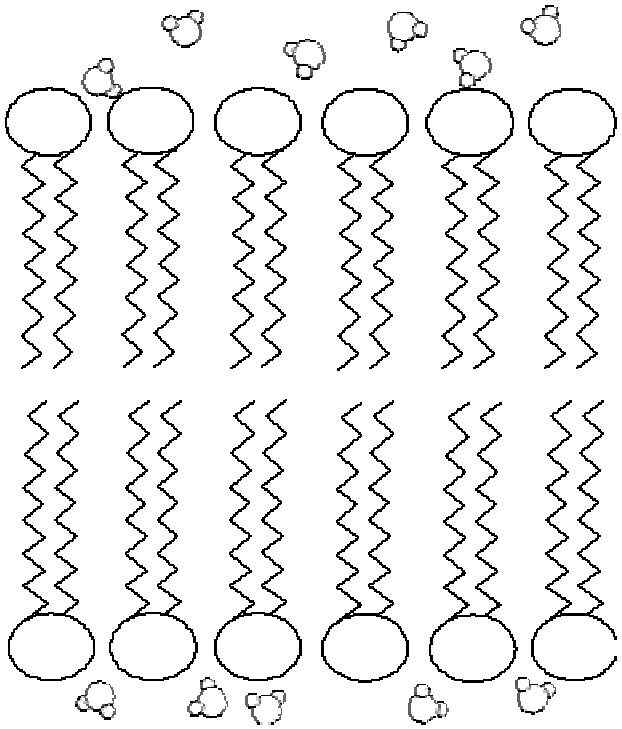

Fig. 5.2.3
A lipid bilayer in an aqueous environment.

At physiological temperatures, the lipid bilayer remains fluid but is stabilized due to the high energy required to mix the hydrophobic region with water. The most stable configuration for these bilayers is a water-filled sphere, which is indeed the structure that they form spontaneously. These liposomes also act osmotically, with the permeability properties being due to the composition of lipids that are used in their construction. Although the solubility of water in the hydrophobic region is low, it is still high enough for water to cross the bilayer in a solubility-diffusion limited manner. Using lipids found in living cells, it is possible to construct liposomes with permeability properties that encompass the entire spectrum seen between various living cell types.

The second major component of cell membranes is protein. Proteins are embedded in the plasma membrane for structural reasons, as sensors to gather information about the extracellular environment, as pathways for the transport of material between the intra and extracellular spaces, and for many other purposes (including some that we have not yet imagined). The regions within the primary structure of proteins that span the lipid bilayer are generally composed of hydrophobic amino acids. They, too, are fluid, being able to diffuse freely within the plane of the bilayer as well as rotationally. This is the fluid-mosaic model of membrane structure.

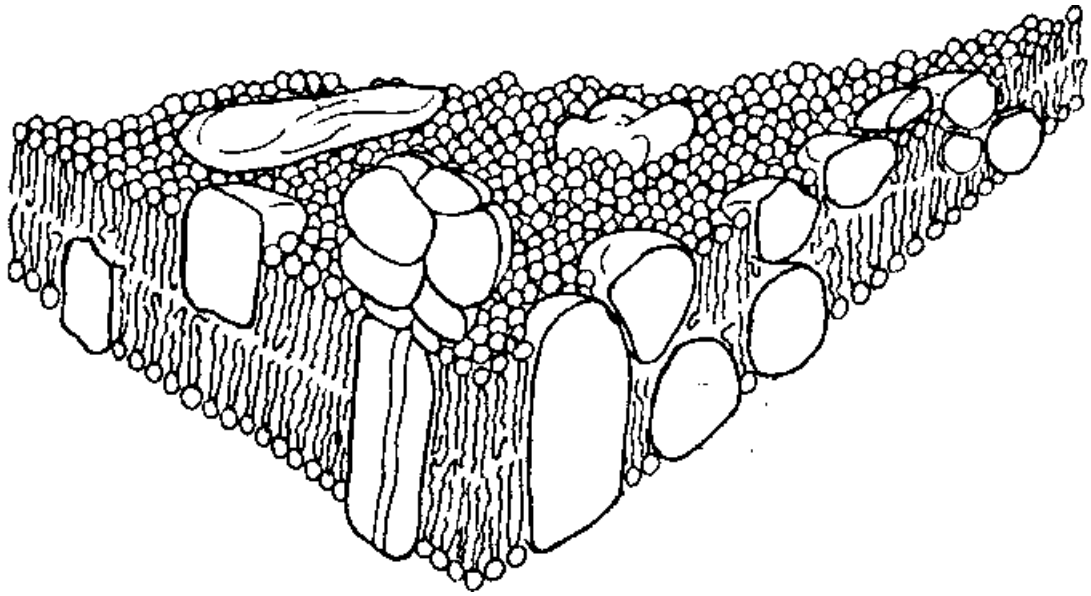

Fig. 5.2.4
The fluid-mosaic model of membrane structure

The protein component is also able to form aqueous pores through the bilayer by forming a cylinder with a hydrophobic outer surface and a hydrophilic inner surface. Water is able to move through these pores although most such pores are for other molecules and are in low enough concentrations to not dominate the permeability characteristics of the membrane. There are some cell types, however, that have specific water pore proteins that exist solely to increase the cell's water permeability; the family of aquaporin proteins.

Both diffusion through the lipid bilayer and movement through aqueous pores will be subject to a temperature dependence (the former due to the mobility of lipids and the latter due to the stabilization of the tertiary structure of the protein) that acts in addition to the temperature effects of diffusion itself. Although this information can partly be used to discern the dominant routes for water movement across a membrane, the structures are too complex and too poorly understood at present to be completely described. Most models assume an Arrhenius temperature dependence for water permeability (a relation that assumes that some activation energy must be overcome for the (energetically favorable) process to occur).

The most problematic obstacle for modeling membrane permeability comes from considerations of the size of the membrane. All biological cells have vast reserves of membrane stored as intracellular vesicles. These vesicles can fuse with the plasma membrane to increase its area, and regions of the membrane can be pinched off from the plasma membrane to decrease its area. In addition, almost all plasma membranes contain thousands of microvilli--thin extensions that protrude outward from the cell surface like hair. The two common assumptions are to either treat the membrane as the surface of a sphere (so that the membrane changes area as the 2/3 power of volume) or to assume a constant surface area (usually the area of a sphere with the isotonic cell volume). Neither is entirely accurate, but if permeability parameters are measured using one assumption, then the model will give accurate results if the same assumption is used. Using a different assumption in the model, however, can lead to enormous errors, since the area has such a significant role in the permeability equations.

Semi-Permeable Membranes

Now we wish to consider the time-dependent movement of water across cell membranes. To begin with, we'll just consider a lipid bilayer in which water is poorly soluble in the hydrocarbon tails. Water molecules will still be able to enter the bilayer interior, due to their thermal motion. We saw before that hydrophobic interactions were not actually repulsive forces, but simply less attractive. Thus a water molecule which enters a bilayer will interact with the hydrocarbons and may either cross the bilayer or leave the way it came (the process is simply a random walk).

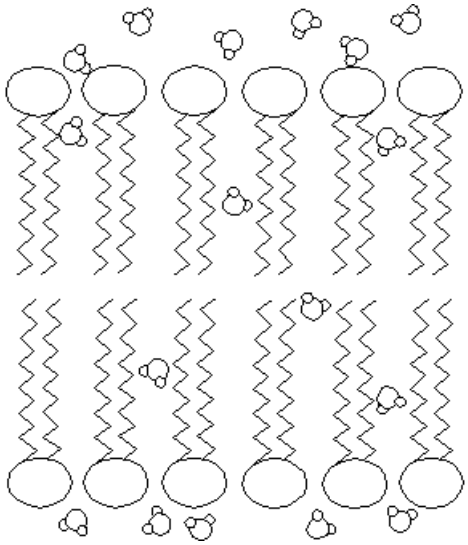

Fig. 5.2.5

So we can apply Fick's laws to this situation to see what the flux of water is across the bilayer. Using the equation

$$J = -D_w \frac{1}{\delta}\left[C(x+\delta) - C(x)\right] \qquad (5.2.1)$$

where δ is the thickness of the membrane and D_w is the diffusion constant for water inside the membrane, we can simplify this to

$$J = -D_w \frac{1}{\delta}\Delta C \qquad (5.2.2)$$

where ΔC is the concentration gradient across the membrane (we don't usually talk about the concentration of a solvent, but it's simply the number of molecules per unit volume). Since we want to know the flux over the area of the membrane, we have to multiply the right side by A

$$J = -D_w \frac{A}{\delta}\Delta C \qquad (5.2.3)$$

In the case of pure water, nothing happens, but if we put a solute which is extremely insoluble in the hydrocarbon phase of the bilayer on one side, then we create a gradient in the mole fraction

of water on either side. We can replace the concentration of water with the concentration of solute, and replace all the constants with a single new constant

$$J = L_p A \Delta C i \qquad (5.2.4)$$

where L_p is called the hydraulic conductivity and C_s is the solute concentration. We can define a quantity called the osmotic pressure of a solution as the concentration of solutes over the gas constant times the absolute temperature

$$\pi = \frac{Ci}{RT} \qquad (5.2.5)$$

This is a bit of an unusual property of a solution, and only manifests itself when two solutions are separated by a barrier that has different permeabilities to the solute and solvent. The flux equation can then be written as

$$J = L_p ART \Delta \pi \qquad (5.2.6)$$

For a cell with an intracellular and extracellular compartments, and writing flux in differential form

$$\frac{dV}{dt} = L_p ART\left(\pi_i - \pi_e\right) \qquad (5.2.7)$$

This is the standard equation for osmotic swelling and shrinkage of cells. In the case of a membrane with pores, the situation is analogous as the solute, rather than being insoluble in the lipid phase of the bilayer, is too large to fit within the pores. Thus the solute is not able to transfer momentum to the solvent within the pores, so a pressure difference develops across the width of the pores and solvent movement occurs. Making assumptions about the pore size, geometry, and the interactions that occur between the solvent and pore walls allows the derivation of a more physically meaningful permeability constant. With biological membranes, however, the situation is too complex to justify many of these assumptions, so the phenomenological permeability constant, L_p is used for convenience.

The hydraulic conductivity is a property that is unique to a particular cell type. For example, red blood cells have a much higher L_p than white blood cells, so they can reach osmotic equilibrium much faster. One of the techniques for separating white blood cells from red cells, uses this fact. Whole blood is exposed to a hypotonic solution (one which has a lower concentration of solutes than the cells normally see) and then returned to isotonic before the white blood cells have had a chance to swell past their elastic limit but after all the red cells have lysed in this manner.

Since freezing and thawing introduce such large changes in the osmotic environment of cells, L_p is probably the single most important parameter in determining a given cell type's response to low temperature exposure.

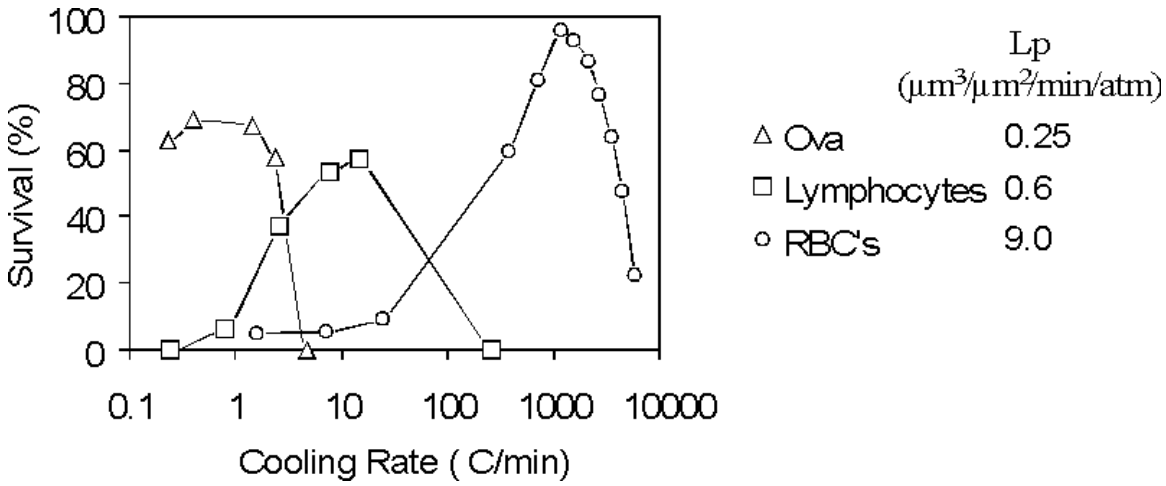

Fig. 5.2.6
Survival of three different cell types frozen at various cooling rates in 1M DMSO. In general, the higher the value of L_p, the higher the optimal cooling rate for the cell. [Data from Mazur, Freezing of Living Cells: Mechanisms and Implications. *Am J Physiol* **247**: C125-42. 1984.]

Coupled Transport of Solvent and Solute:

If we have a situation in which we have a concentration difference due to a solute that cannot permeate the membrane as well as a solute that can permeate the membrane, then we need to account for the flow of this penetrating solute as well. First, there will be a flow due to the concentration gradient of the solute across the membrane (since we're not looking at the water concentration due to the presence of solute, the direction is opposite to that used for the impermeant solute above; i.e. external concentration - internal concentration).

$$\frac{dS^{[]}}{dt} = P_s A\left({}_e C_s - {}_i C_s\right) \qquad (5.2.8)$$

Where *Ps* is the solute permeability of the membrane, *eCs* is the concentration of penetrating solute outside the cell, and *iCs* is the concentration inside the cell.

There will also be solute movement across the membrane due to solvent drag (any solute molecules inside a pore will be subject to movement through the pore due to the flow of solvent).

$$\frac{dS^{Jw}}{dt} = \overline{C_s}\frac{dV}{dt} \qquad (5.2.9)$$

Where is the average concentration of penetrating solute within the membrane: (*eCs* + *iCs*)/2

Adding these gives us our total solute flow:

$$\frac{dS}{dt} = \frac{dS^{[]}}{dt} + \frac{dS^{Jw}}{dt} = P_s A\left({}_e C_s - {}_i C_s\right) + \overline{C_s}\frac{dV}{dt} \qquad (5.2.10)$$

In the case where the size of the solute molecules is close to the size of the pores, there will be a hindrance factor due to geometrical and flow considerations. First, there will be an "effective" pore size due to the fact that the solute cannot be considered as a point.

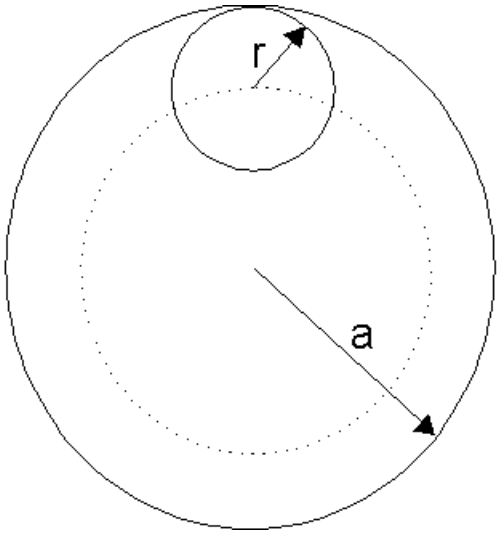

Fig. 5.2.7

The effective pore area is now

$$\pi a^2\left(1-\frac{r}{a}\right)^2 \qquad (5.2.11)$$

There will also be a "drag" effect due to interaction between the solute and the pore wall as the solute travels through the pore. Both of these effects are accounted for in a single reflection coefficient, σ (on the interval 0-1), that represents the fraction of molecules allowed through the pore. A freely permeable membrane will have $\sigma = 0$ and a membrane that is completely impermeable to the solute will have $\sigma = 1$. This has an effect on the flow of solute due to solvent drag, so the total solute flow can be rewritten as:

$$\frac{dS}{dt} = P_s A\left({}_eC_s - {}_iC_s\right) + (1-\sigma)\overline{C_s}\,\frac{dV}{dt} \qquad (5.2.12)$$

The penetrating solute will also affect the osmotic pressure of the solutions inside and outside of the cell, so the water flow equation must also be rewritten. The concentrations of penetrating solute across the pore look like:

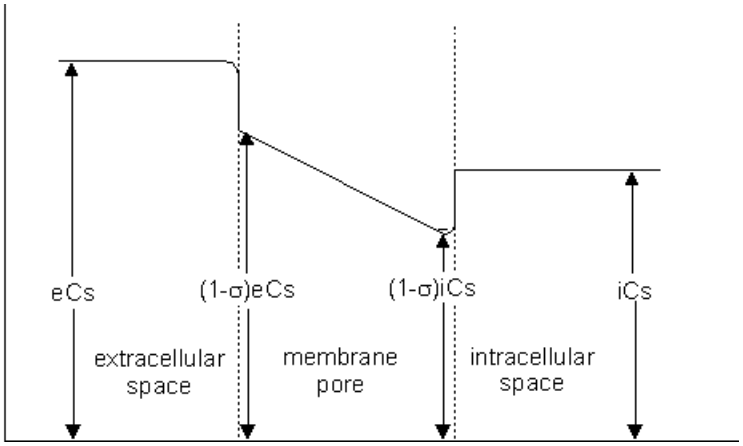

Fig. 5.2.8

So the concentration just inside the pore on the external side of the membrane is given by:

$$ {}_eC_s - (1-\sigma)_cC_s = \sigma_cC_s \qquad (5.2.13) $$

Similarly, the concentration just inside the pore on the internal side of the membrane is given by:

$$ {}_iC_s - (1-\sigma)_iC_s = \sigma_iC_s \qquad (5.2.14) $$

So the volume flow will be given by:

$$ \frac{dV}{dt} = L_pA[({}_iC_i - {}_eC_i) + \sigma({}_iC_s - {}_eC_s)] \qquad (5.2.15) $$

Where *iCi* is the concentration of impermeant solute inside the cell and *eCi* is the concentration in the external compartment.

These two equations are called the "coupled transport equations", or are often referred to as the Kedem-Katchalsky equations. They are actually simpler than the generalized equations of Kedem and Katchalsky, as hydrostatic pressure gradients are ignored. In the most general form, they can be derived from irreversible thermodynamics, using the Onsager reciprocity relation to reduce the parameters from 4 to 3. This approach is completely unsatisfying to those who like at least a smidgen of physical relevance. The 3 parameters, *Lp*, *Ps*, and σ, completely determine the permeability characteristics of a membrane, and are always determined for biological membranes (which are way too complex to allow the derivation of these parameters) by fitting experimental data to the transport equations.

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