Chapter 3
Membrane Potential

Ionic Gradient and Electric Potential

The specific ionic composition of the cytosol usually differs greatly from that other surrounding fluid. In virtually all cell including microbial, plant and animal cells. the cytosolic pH is kept near 7 and the cytosolic concentration of K^+ is much higher that that of sodium ion (Na^+). in particular, in both invertebrates and vertebrates the concentration of K^+ is 20 - 40 times higher in cell than in the blood, while the concentration of Na^+ is lower in cells than in the blood. The concentration of calcium ion (CA^{2+}) free in the cytosol is generally less than 1 micromolar, a 1,000 time lower than that in the blood.

The plasma membrane contains channel proteins that allow the principal cellular ions (Na^+, K^+, Ca^{2+}, and Cl^- to move through it at different rates down their concentration gradients. Ion concentration gradients and selective movements of ions through channels create a difference in electric potential between the inside and outside of cell. The magnitude of this potential - 70 millivolts (mV) with the inside of the cell negative with respect to the outside- does not seem like much until we realize that the plasma membrane is only about 3.5 nm thick. Thus the voltage gradient across the plasma membrane is 0.07 V per 3.5×10^{-7} cm, or 200,000 volts per centimeter. The plasma membrane, like all biological membranes, is an electrical device called a capacitor. A thin sheet of nonconducting material surrounded on both sides by electrically conducting material (the polar heads) that can store an electric charge across it.

The ionic gradients and electric potential across the plasma membrane drive many biological processes. Opening and closing of Na^+, K^+, and Ca^{2+} channels are essential to the conduction of an electric impulse down the axon of a nerve cell. In many animal cells, the concentration gradient of Na^+ ions and the membrane electric potential power the uptake of other molecules against their concentration gradient; amino acids frequently enter cells in this manner. In most cells, a rise in the concentration of Ca^{2+} ions in the cytosol is an important regulatory signal. In muscle cells, for instance is initiates contraction; in the exocrine ells of the pancreas, it triggers secretion of digestive enzymes.

Table 2: Ion Concentrations in Invertebrate and Vertebrate Cells

	Cell (mM)	**Blood (mM)**
Squid Axon*		
K^+	400	20
Na^+	50	440
Cl^-	40-150	560
Ca^{2+}	0.0003	10
$X^{-@}$	300-400	
Mammalian C		
K^+	139	4
Na^+	12	145
Cl^-	4	116

HCO_3^-	12	29
$X^{-@}$	138	9
Mg^{2+}	0.8	1.5
Ca^{2+}	,0.0002	1.8

K^+ Channels Generate Membrane Electric Potential

In the experimental system outlined below the distribution of K^+, Na^+, and Cl^- ions is similar to that between an animal cell and its aqueous environment. A membrane separates a 15 mM KCl/150 mM NaCl solution on the right side (representing the outside of the cell) from a 150 mM KCl/15 mM NaCl solution on the left (the inside). A Potentiometer (voltmeter) is connected to the solution on each side to measure any difference in electric potential across the membrane. If the membrane is impermeable to all ions, no ions will flow across it and there will be no electric potential difference.

Now suppose that the membrane contains Na^+ ions then tend to move down their concentration gradient from the right side to the left, leaving an excess of negative Cl^- ions compared with Na^+ ions on the right side and generating an excess of positive Na^+ ions compared with Cl^- ions on the left side. The excess Na^+ ions on the left and Cl^- on the right remain near their respective surface of the membrane. There is now a separation of charge across the membrane, which a potentiometer can measure as an electric potential, or voltage. The right side is negative with respect to the left. As more and more Na^+ ions move through channels across the membrane, the magnitude of this charge difference increases. However, continued movement of the Na^+ ions eventually is inhibited by the excess of positive charges accumulated on the left side of the membrane and by the attraction Na^+ ion to the excess negative charge built upon the right side. The system soon reaches an equilibrium point at which the two opposing factors that determine the movement of Na^+ ions. The membrane electric potential and the ion concentration gradient -balance each other out. At equilibrium, no net movement of Na^+ ions occurs across the membrane. Thus the excess negative (Cl^-) charges bound to the right surface of the membrane are separated from and attracted to the excess positive (Na^+) ones on the left. In this way, the phospholipid membrane acts as a capacitor, and stores the charge across it exactly as does a capacitor in an electric circuit.

The magnitude of the resulting sodium equilibrium potential in volts (the electric potential across a membrane permeable only to Na^+ ions) is given by the Nernst equation, which is derived from basic principles of physical chemistry:

$$\mathbf{E_{na}= (RT/ZF)\ln [Na_l]/[Na_r]}$$

where:
R (the gas constant) =1.987 cal/(degree.mol), or 8.28 joules/(degree.mol),
T (the absolute temperature) = 293 K at 20 C,
Z (valence) = +1,
F (the Faraday constant) =23,062 Cal/(mol.V), or 96,000 coulombs/(mol.V),
and $[Na_l]$ and $[Na_r]$ are the Na^+ ion concentration on the left and right sides at equilibrium.

The equation is similar to the equation used to calculate the voltage change associated with oxidation or reduction reactions, which also involve movement of electric charges. At 20^oC, the above equation reduces to

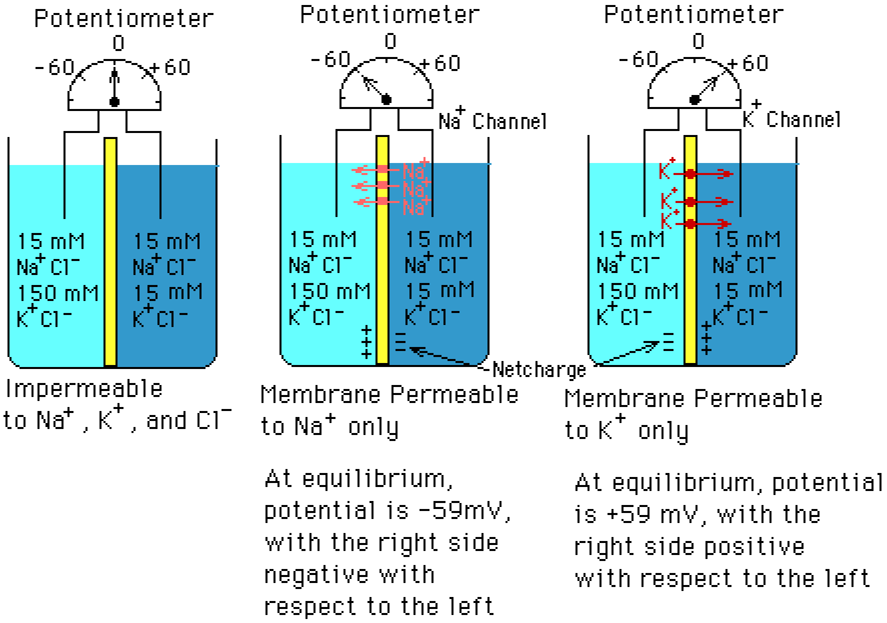
$$\mathbf{E_{na}= 0.059 \log_{10} [Na_l]/[Na_r]}$$

When $[Na_l]/[Na_r]$ =0.1 then E_{na}=0.059 V or -59 mV

If the membrane is permeable only to K^+ ion and not to Na^+ or Cl^- ions, the calculation is the same.

$$E_{na}= 0.059 \log_{10} [K_l]/[K_r]$$

The magnitude of the membrane electric potential is the same (59 mV), except that the right side is now positive with respect to the left. This is precisely the opposite polarity to that obtained with selective Na^+ permeability.



If the membrane is permeable to Na^+ and K^+ ions to the same degree, then each moves down its concentration gradient: Na^+ from the right side to the left, and K^+ from left to right. In this case, nonmembrane electric potential is expected and none is observed. An intermediate situation between these two extremes can also occur. When the membrane is permeable to both Na^+ and K^+ ions but more permeable to K^+ ions, the right side initially has a positive potential relative to the left, but the magnitude of the potential is some what less than K_k= +59 mV. Eventually due to the diffusion of Na^+ and K^+ Ions , there will be an equal concentration of both ions on both sides of the membrane and no membrane electric potential.

As noted earlier, the membrane potential across the plasma membrane of animal cells is about - 70 mV; that is the cytosolic face is negative with respect to the exoplasmic (outside) face. These membranes contain many open K^+ channels but few open Na^+ or Ca^{2+} channels. As a result the major ionic movement across the plasma membrane is that of K^+ from the inside, outward. This leaves an excess of negative charge on the inside, and an excess of positive on the outside, and is the major determinant of the inside negative membrane potential. Quantitatively, the usual potential of -70 mV is close to that of the potassium equilibrium potential calculated from the Nernst equation.

The Na^+- K^+ ATPase, ion pump moves K^+ ions into the cytosol from the extracellular medium, generating the K^+ concentration gradient. Movement of K^+ ions through K^+ channels from the cytosol outward, down their concentration gradient, generates the inside-negative membrane potential. These always-open K^+ channels, the so-called resting K^+ channels, have just been cloned and sequenced, but as yet we know little of their molecular structure. However, much is know of the structure and function of other types of K^+ channels that are found mainly in nerve cells and that open or close in response to change in membrane potential or to other signals.

Ion concentration Gradients and Electric Potential Drive the Movement of Ions Across Biological Membranes.

Two forces govern the movement of such ions as K^+, Cl^-, and Na^+ across selectively permeable membranes: the membrane electric potential and the ion concentration gradient. These forces may act in the same direction or in opposite directions. The free-energy change ΔG corresponding to the transport of 1 mole of Na^+ ions from the outside (exterior) to the inside (cytosol) of a typical mammalian cell is about -3 kcal/mol. Since ΔG is <), this reaction thermodynamically favored. About half of this ΔG value is contributed by the membrane electric potential and half is contributed by the Na^+ ions concentration gradient. It is important to understand these forces in some detail, since the inward movement of Na^+ ions is used to power the uphill movement of several ions and small molecules into or out of animal cells catalyzed by symport and antiport proteins.

The free energy change generated from Na^+ concentration gradient is

$$\Delta G_c = RT \ln([Na_{in}]/[Na_{out}])$$

At the concentrations of Na_{in} and Na_{out} shown in above Figure which are typical for many cells, $\Delta G_c = -1.45$ kcal/mol, the change in free energy for the thermodynamically favored transport of 1 mol of Na^+ ions from outside to inside the cell if there were no membrane electric potential. The free-energy change generated from the membrane electric potential is

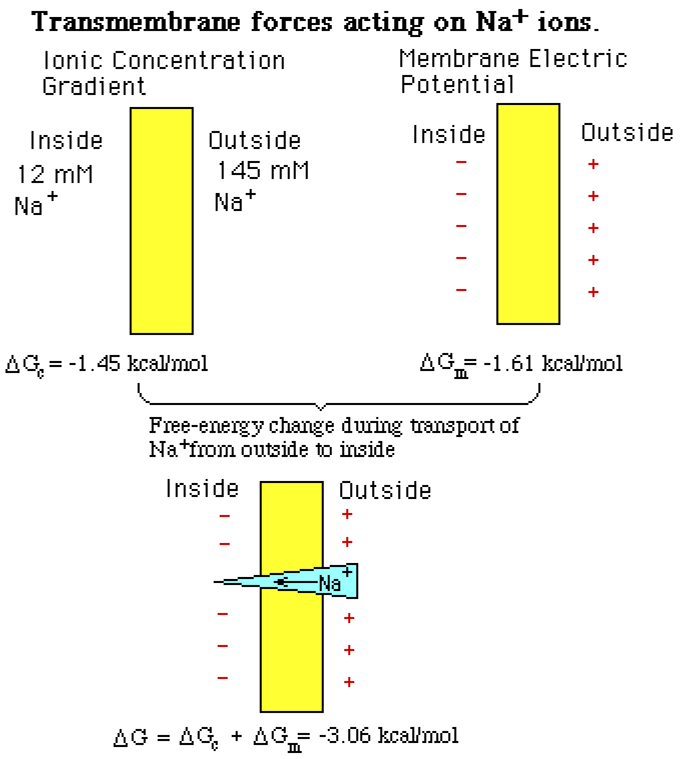
$$\Delta G_m = FE$$

Where:
F = the Faraday constant
E = the membrane electric potential.

If E =-70 mV, then ΔG_m = -1.6 kcal mol, the change in free energy for the thermodynamically favored transport of 1 mol of Na^+ ion from outside to inside the cell if there were no Na^+ concentration gradient. Given both forces acting on Na^+ ions, the total ΔG will be the sum of the two partial values.

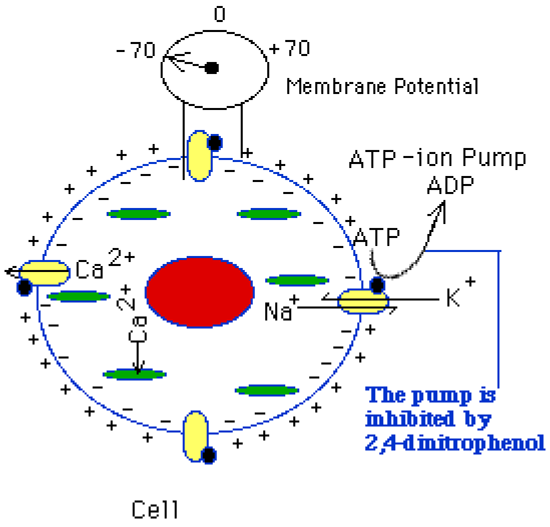
$$\Delta G = \Delta G_c + \Delta G_m = (-1.45) + (-1.61) = -3.06 \text{ kcal/mol}$$

In this typical example the Na^+ concentration gradient and the membrane electric potential contribute almost equally to total ΔG for transport of Na^+ ions



Active Ion Transport and ATP Hydrolysis

We turn now to the ATP-powered pumps that transport ions against their concentration gradients. The Na^+ -K^+ ATPase, for instance, pumps K^+ into the cell and Na^+ outwards, thus establishing the high cytosolic concentration of K^+ that s essential for generation of the cytosol-negative potential across the plasma membrane. The Ca^{2+} ATPases pump Ca^{2+} out of the cytosol into the extracellular medium or into intracellular organelles, thereby maintaining the concentration of Ca^{2+} in the cytosol much lower than that in the extracellular medium.



Early evidence for the existence of these pumps can from studies in which aerobic production of adenosine triphosphate (ATP) in a cell was inhibited by 2,4-dinitrophenol. The ion concentration inside the cell gradually approached that of the exterior environment as the ions moved through plasma membrane channels down their electric and concentration gradients. Eventually the cell died because cell require a high concentration of K^+ for protein synthesis.

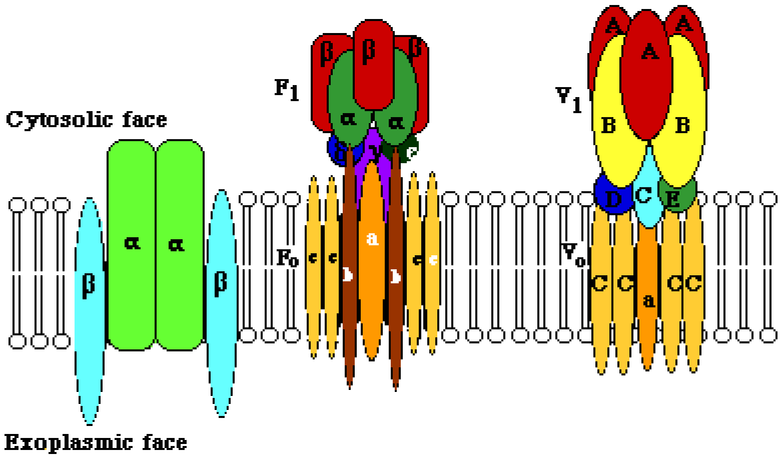
A significant fraction of available energy in every cell is required to maintain the concentration gradients of such ions as $Na^=$, $K^=$, and Ca^{2+} across the plasma and intracellular membranes. In nerve and kidney cells, for example, up to 25% of the ATP produced by the cell is used for ion transport, and in human erytrocytes, up to 50% of the energy stored in ATP molecules is used for this purpose.

Ion pumps can be grouped into three classes (P, V, and F)

There are three classes of ATP-powered ion pumps, called P, V, and F. Ion pumps in the P class, the simplest in structure, are composed of four transmembrane subunits: two alpha and two beta polypeptides. The larger □□subunit is phosphorlated during the transport process, and the transported ions are thought to move through this subunit. Included in this class are the $Na^=$-K^+ ATPase in the plasma membrane and several Ca^{2+} ATPases including one in the plasma membrane, which transports Ca^{2+} ions from the cytocol to the SR lumen.

Ion pumps of the V and F classes are similar in structure to each other but unrelated to P-class pumps. All known members of these two classes transport only protons. F-class pumps contain at least three kinds of transmembrane proteins and B-class pumps contain at least two kinds of proteins. Both classes contain at least five kinds of extrinsic polypeptides that form the cytosolic domain.

V-class ATP-powered pumps maintain the low pH of plant vacuoles and of lysosomes and other vesicles in animal cells by using the energy release by ATP hydrolysis to pump protons from the cytosolic to the exoplasmic face of the membrane up the proton electrochemical gradient.



Subunit	Molecular Weight (kD)	Subunit	Molecular Weight (kD)	Subunit	Molecular Weight (kD)
α	120	α	55	B	57
β	50	β	50	A	70
		γ	31	C	44
		δ	19	D	30
		ε	15	E	26
		a	30	a	20
		b	17	c	16
		c	8