CHAPTER 2

Cellular Homeostasis

2.1 The Cell Membrane

The cell membrane provides a boundary separating the internal workings of the cell from its external environment. More importantly, it is selectively permeable, permitting the free passage of some materials and restricting the passage of others, thus regulating the passage of materials into and out of the cell. It consists of a double layer (a *bilayer*) of phospholipid molecules about 7.5 nm (=75 Å) thick (Fig. 2.1). The term *lipid* is used to specify a category of water-insolubie, energy rich macromolecules, typical of fats, waxes, and oils. Irregularly dispersed throughout the phospholipid bilayer are aggregates of globular proteins, which are apparently free to move within the layer, giving the membrane a fluid-like appearance. The membrane also contains water-filled pores with diameters of about 0.8 nm, as well as protein-lined pores, called *channels*, which allow passage of specific molecules. Both the intracellular and extracellular environments consist of, among many other things, a dilute aqueous solution of dissolved salts, primarily NaCl and KCl, which dissociate into Na^+, K^+, and Cl^- ions. The cell membrane acts as a barrier to the free flow of these ions and maintains concentration differences of these ions. In addition, the cell membrane acts as a barrier to the flow of water.

Molecules can be transported across the cell membrane by passive or active processes. An active process is one that requires the expenditure of energy, while a passive process results solely from the inherent, random movement of molecules. There are three passive transport mechanisms to transport molecules through the cell membrane. *Osmosis* is the process by which water is transported through the cell membrane. Simple diffusion accounts for the passage of small molecules through pores and of lipid-

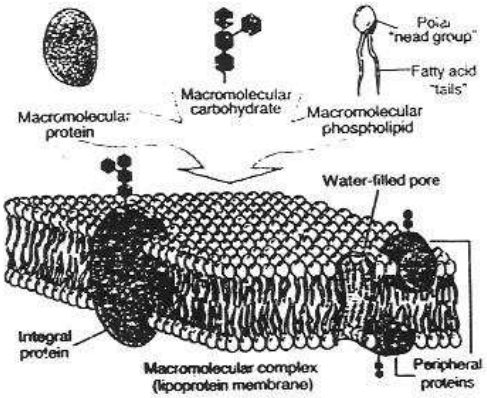

Figure 2.1 Schematic diagram of the cell membrane. (Davis et al., 1985, Fig. 3-1, p. 41.)

soluble molecules through the bilipid layer. For example, water, urea (a nitrogenous waste product of metabolism), and hydrated chloride ions diffuse through membrane pores. Oxygen and carbon dioxide diffuse through the membrane readily because they are soluble in lipids. Sodium and potassium ions pass through ion-specific channels, driven by diffusion and electrical forces. Some other mechanism must account for the transport of larger sugar molecules such as galactose, glucose, and sucrose, as they are too large to pass through membrane pores (Fig. 2.2). *Carrier-mediated diffusion* occurs when a molecule is bound to a carrier molecule that moves readily through the membrane. For example, the transport of glucose and amino acids across the cell membrane is believed to be by a carrier-mediated process.

Concentration differences are set up and maintained by active mechanisms that use energy to pump ions against their concentration gradient. One of the most important of these pumps is the Na^+–K^+ pump, which uses the energy stored in ATP molecules to pump Na^+ out of the cell and K^+ in. Another pump, the Ca^{2+} ATPase, pumps Ca^{2+} out of the cell or into the endoplasmic reticulum. There are also a variety of exchange pumps that use the energy inherent in the concentration gradient of one ion type to pump another ion type against its concentration gradient. For example, the Na^+–Ca^{2+} exchanger removes Ca^{2+} from the cell at the expense of Na^+ entry, and similarly for the Na^+–H^+ exchanger. Typical values for intracellular and extracellular ionic concentrations are given in Table 2.1.

Differences in ionic concentrations create a potential difference across the cell membrane that drives ionic currents. Water is also absorbed into the cell because of concentration differences of these ions and also because of other large molecules contained in the cell, whose presence provides an osmotic pressure for the absorption of water. It is the balance of these forces that regulates both the cell volume and the membrane potential.

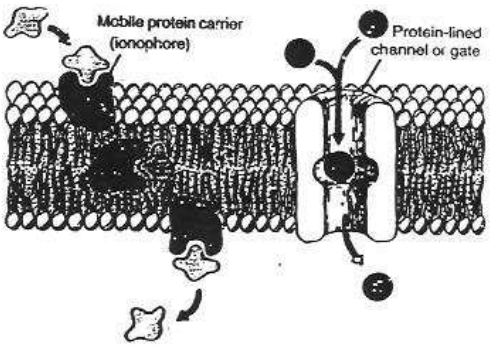

Figure 2.2 Schematic diagram of the cell membrane containing a protein carrier and a protein-lined ionic channel. (Davis et al., 1985, Fig. 3-7, p. 45.)

Table 2.1 Typical values for intracellular and extracellular ionic concentrations, from three different cell types. Concentrations are given in units of mM, and potentials are in units of mV. Extracellular concentrations for the squid giant axon are for seawater, while those for frog muscle and red blood cells are for plasma. Later in this chapter we discuss Nernst potentials and resting potentials. (Adapted from Mountcastle, 1974, Table 1-1.)

	Squid Giant Axon	Frog Sartorius Muscle	Human Red Blood Cell
Intracellular concentrations			
Na^+	50	13	19
K^+	397	138	136
Cl^-	40	3	78
Mg^{2+}	80	14	5.5
Extracellular concentrations			
Na^+	437	110	155
K^+	20	2.5	5
Cl^-	556	90	112
Mg^{2+}	53	1	2.2
Nernst potentials			
V_{Na}	+56	+55	+55
V_K	−77	−101	−86
V_{Cl}	−68	−66	−9
Resting potentials	−65	−99	−6 to −10

2.6 The Membrane Potential

The principal function of the active transport processes described above is to regulate the intracellular ionic composition of the cell. For example, the operation of the Na^+–K^+ pump results in high intracellular K^+ concentrations and low intracellular Na^+ concentrations. As we will see, this is necessary for a cell's survival, as without such regulation, cells could not control their volume. However, before we consider models for cell volume regulation, we consider the effects of ionic separation. It is a consequence of the control of cell volume by ionic transport that the cell develops a potential difference across its membrane.

2.6.1 The Nernst Equilibrium Potential

One of the most important equations in electrophysiology is the Nernst equation, which describes how a difference in ionic concentration between two phases can result in a potential difference between the phases. We do not derive the Nernst equation from first principles, but give a nonrigorous derivation in Section 2.6.2. Derivations of the Nernst equation using the theory of chemical equilibrium thermodynamics can be found in standard physical chemistry textbooks (for example, Levine, 1978; Denbigh, 1981).

Suppose we have two reservoirs containing the same ion S, but at different concentrations, as shown schematically in Fig. 2.10. The reservoirs are separated by a semipermeable membrane. The solutions on each side of the membrane are assumed to be electrically neutral (at least initially), and thus each ion S is balanced by another ion, S′, with opposite sign. For example, S could be Na^+, while S′ could be Cl^-. Because we ultimately wish to apply the Nernst equation to cellular membranes, we call the left of the membrane the inside and the right the outside.

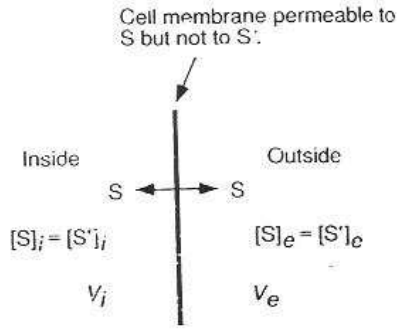

Figure 2.10 Schematic diagram of a membrane separating two solutions with different ionic concentrations.

If the membrane is permeable to S but not to S', the concentration difference across the membrane results in a flow of S from one side to another, say, from left to right. However, because S' cannot diffuse through the membrane, the diffusion of S causes a buildup of charge across the membrane. This charge imbalance, in turn, sets up an electric field that opposes the further diffusion of S through the membrane. Equilibrium is reached when the electric field exactly balances the diffusion of S. Note that at steady state there will be more S ions on one side than on the other, and thus neither side of the membrane is exactly electrically neutral. However, although the diffusion of S causes an electric potential to develop, it is important to realize that only a small amount of S moves across the membrane. To a good approximation the concentrations of S on either side of the membrane remain unchanged, the solutions on either side of the membrane remain electrically neutral, and the small excess charge accumulates near the interface.

At equilibrium the potential difference, V_S, across the membrane is given by the *Nernst potential*,

$$V_S = \frac{RT}{zF} \ln\left(\frac{[S]_e}{[S]_i}\right) = \frac{kT}{zq} \ln\left(\frac{[S]_e}{[S]_i}\right), \tag{2.54}$$

where subscripts i and e denote internal and external concentrations respectively. R is the universal gas constant, T is the absolute temperature, F is Faraday's constant, k is Boltzmann's constant, q is the charge on a proton, and z is the charge on the ion S. Values of these constants, and their units, are given in the Appendix. One particularly important relationship is

$$k = \frac{R}{N_A}, \tag{2.55}$$

where N_A is Avogadro's number. Because of this, the Nernst equation can be written in the two equivalent forms shown above. Throughout this book we follow the standard

convention and define the potential difference across the cell membrane as

$$V = V_i - V_e, \tag{2.56}$$

i.e., the intracellular minus the extracellular potential. When $V = V_S$, there is no net current of S between the phases, as the diffusion of S is exactly balanced by the electric potential difference.

Typical concentrations (in this case, for squid axon) are 397, 50, and 40 mM for potassium, sodium, and chloride, respectively, in the intracellular space, and 20, 437, and 556 mM in the extracellular space. With these concentrations, the Nernst potentials for squid nerve axon are $V_{Na} = 56$ mV, $V_K = -77$ mV, $V_{Cl} = -68$ mV (using $RT/F = 25.8$ mV at 27°C. See Table 2.1).

The Nernst equation is independent of how the ions move through the membrane and is dependent only on the concentration difference. In this sense, it is a "universal" law. Any equation that expresses the transmembrane current of S in terms of the membrane potential, no matter what its form, must have a reversal potential of V_S; i.e., the current must be zero at the Nernst potential $V = V_S$. However, although this is true when only a single ion species crosses the membrane, the situation is considerably more complicated when more than one type of ion can cross the membrane. In this case, the membrane potential that generates zero total current does not necessarily have no net current for each individual ion. For example, a current of S in one direction might be balanced by a current of S′ in the same direction. Hence, when multiple ion types can diffuse through the membrane, the phases are not, in general, at equilibrium, even when there is no total current. Therefore, the arguments of chemical equilibrium used to derive the Nernst equation cannot be used, and there is no universal expression for the reversal potential in the multiple ion case. In this case, the reversal potential depends on the model used to describe the individual transmembrane ionic flows (see Chapter 3).

2.6.2 Electrodiffusion: The Goldman–Hodgkin–Katz Equations

In general, the flow of ions through the membrane is driven by concentration gradients and also by the electric field. The contribution to the flow from the electric field is given by *Planck's equation*

$$\mathbf{J} = -u\frac{z}{|z|}c\nabla\phi, \tag{2.57}$$

where u is the *mobility* of the ion, defined as the velocity of the ion under a constant unit electric field; z is the valence of the ion, so that $z/|z|$ is the sign of the force on the ion; c is the concentration of S; and ϕ is the electrical potential, so that $-\nabla\phi$ is the electrical field.

There is a relationship, determined by Einstein, between the ionic mobility u and Fick's diffusion constant:

$$D = \frac{uRT}{|z|F}. \tag{2.58}$$

When the effects of concentration gradients and electrical gradients are combined, we obtain the *Nernst–Planck equation*

$$\mathbf{J} = -D\left(\nabla c + \frac{zF}{RT}c\nabla\phi\right). \tag{2.59}$$

If the flow of ions and the electric field are transverse to the membrane, we can view (2.59) as the one-dimensional relation

$$J = -D\left(\frac{dc}{dx} + \frac{zF}{RT}c\frac{d\phi}{dx}\right). \tag{2.60}$$

The Nernst equation

The Nernst equation can be derived from the Nernst–Planck electrodiffusion equation (2.60). When the flux J is zero, we find

$$-D\left(\frac{dc}{dx} + \frac{zF}{RT}c\frac{d\phi}{dx}\right) = 0, \tag{2.61}$$

so that

$$\frac{1}{c}\frac{dc}{dx} + \frac{zF}{RT}\frac{d\phi}{dx} = 0. \tag{2.62}$$

Now suppose that the cell membrane extends from $x = 0$ (the inside) to $x = L$ (the outside), and let subscripts i and e denote internal and external quantities respectively. Then, integrating from $x = 0$ to $x = L$ we get

$$\ln(c)\Big|_{c_i}^{c_e} = \frac{zF}{RT}(\phi_i - \phi_e). \tag{2.63}$$

and thus the potential difference across the membrane, $V = \phi_i - \phi_e$, is given by

$$V = \frac{RT}{zF}\ln\left(\frac{c_e}{c_i}\right). \tag{2.64}$$

which is the Nernst equation.

This derivation of the Nernst equation relies on the Nernst–Planck electrodiffusion equation, and so is not a derivation from first principles. The derivation from first principles can be given, but it is beyond the scope of this text. The interested reader is referred to Levine (1978) or Denbigh (1981) for the details.

The constant field approximation

In general, the electric potential ϕ is determined by the local charge density, and so J must be found by solving a coupled system of equations (this is discussed in detail in Chapter 3). However, a useful result is obtained by assuming that the electric field in the membrane is constant, and thus decoupled from the effects of charges moving through the membrane. Suppose we have two reservoirs separated by a semipermeable membrane of thickness L, such that the potential difference across the membrane is V. On the left of the membrane (the inside) $[S] = c_i$, and on the right (the outside)

$[S] = c_e$. If the electric field is constant through the membrane, we have $\partial\phi/\partial x = -V/L$, where $V = \phi(0) - \phi(L)$ is the membrane potential.

At steady state and with no production of ions, the flux must be constant. In this case, the Nernst–Planck equation (2.59) is an ordinary differential equation for the concentration c,

$$\frac{dc}{dx} - \frac{zFV}{RTL}c + \frac{J}{D} = 0, \tag{2.65}$$

whose solution is

$$\exp\left(\frac{-zVFx}{RTL}\right)c(x) = -\frac{JRTL}{DzVF}\left[\exp\left(\frac{-zVFx}{RTL}\right) - 1\right] + c_i. \tag{2.66}$$

where we have used the left boundary condition $c(0) = c_i$. To satisfy the boundary condition $c(L) = c_e$, it must be that

$$J = \frac{D}{L}\frac{zFV}{RT}\frac{c_i - c_e\exp\left(\frac{-zVF}{RT}\right)}{1 - \exp\left(\frac{-zVF}{RT}\right)}, \tag{2.67}$$

where J is the flux density with units (typically) of moles per area per unit time. This flux density becomes an electrical current density (current per unit area) when multiplied by zF, the number of charges carried per mole, and thus

$$I_S = P_S\frac{z^2F^2}{RT}V\frac{c_i - c_e\exp\left(\frac{-zFV}{RT}\right)}{1 - \exp\left(\frac{-zFV}{RT}\right)}, \tag{2.68}$$

where $P_S = D/L$ is the permeability of the membrane to S. This is the famous Goldman–Hodgkin–Katz (GHK) current equation. It plays an important role in models of cellular electrical activity.

This flow is zero if the diffusively driven flow and the electrically driven flow are in balance, which occurs, provided that $z \neq 0$, if

$$V = V_S = \frac{RT}{zF}\ln\left(\frac{c_e}{c_i}\right), \tag{2.69}$$

which is, as expected, the Nernst potential.

If there are several ions that are separated by the same membrane, then the flow of each of these is governed separately by its own current–voltage relationship. In general there is no potential at which these currents are all zero. However, the potential at which the net electrical current is zero is called the Goldman–Hodgkin–Katz potential. For a collection of ions all with valence $z = \pm 1$, we can calculate the GHK potential directly. For zero net electrical current, it must be that

$$0 = \sum_{z=1} P_j\frac{c_i^j - c_e^j\exp\left(\frac{-VF}{RT}\right)}{1 - \exp\left(\frac{-VF}{RT}\right)} + \sum_{z=-1} P_j\frac{c_i^j - c_e^j\exp\left(\frac{VF}{RT}\right)}{1 - \exp\left(\frac{VF}{RT}\right)}. \tag{2.70}$$

where $P_j = D_j/L$. This expression can be solved for V, to get

$$V = -\frac{RT}{F} \ln \left(\frac{\sum_{z=-1} P_j c_e^j + \sum_{z=1} P_j c_i^j}{\sum_{z=-1} P_j c_i^j + \sum_{z=1} P_j c_e^j} \right). \tag{2.71}$$

For example, if the membrane separates sodium ($Na^+, z = 1$), potassium ($K^+, z = 1$), and chloride ($Cl^-, z = -1$) ions, then the GHK potential is

$$V_r = -\frac{RT}{F} \ln \left(\frac{P_{Na}[Na^+]_i + P_K[K^+]_i + P_{Cl}[Cl^-]_e}{P_{Na}[Na^+]_e + P_K[K^+]_e + P_{Cl}[Cl^-]_i} \right). \tag{2.72}$$

It is important to emphasize that neither the GHK potential nor the GHK current equation are universal expressions like the Nernst equation. Both depend on the assumption of a constant electric field, and other models give different expressions for the transmembrane current and reversal potential. In Chapter 3 we present a detailed discussion of other models of ionic current and compare them to the GHK equations. However, the importance of the GHK equations is so great, and their use so widespread, that their separate presentation here is justified.

2.6.3 Electrical Circuit Model of the Cell Membrane

Since the cell membrane separates charge, it can be viewed as a capacitor. The capacitance of any insulator is defined as the ratio of the charge across the capacitor to the voltage potential necessary to hold that charge, and is denoted by

$$C_m = \frac{Q}{V}. \tag{2.73}$$

From standard electrostatics (Coulomb's law), one can derive the fact that for two parallel conducting plates separated by an insulator of thickness d, the capacitance is

$$C_m = \frac{k\epsilon_0}{d}, \tag{2.74}$$

where k is the dielectric constant for the insulator and ϵ_0 is the permittivity of free space. The capacitance of cell membrane is typically found to be 1.0 μF/cm^2. Using that $\epsilon_0 = (10^{-9}/(36\pi))$F/m, we calculate that the dielectric constant for cell membrane is about 8.5, compared to $k = 3$ for oil.

A simple electrical circuit model of the cell membrane is shown in Fig. 2.11. It is assumed that the membrane acts like a capacitor in parallel with a resistor (although not necessarily ohmic). Since the current is defined by dQ/dt, it follows from (2.73) that the capacitive current is $C_m dV/dt$, provided that C_m is constant. Since there can be no net buildup of charge on either side of the membrane, the sum of the ionic and capacitive currents must be zero, and so

$$C_m \frac{dV}{dt} + I_{ion} = 0, \tag{2.75}$$

where $V = V_i - V_e$.

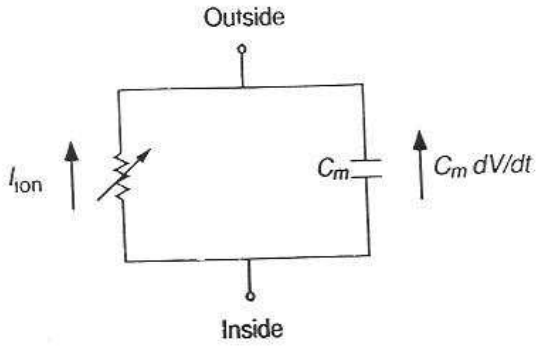

Figure 2.11 Electrical circuit model of the cell membrane.

We will meet this equation many times in this book, as it is the basis for much of theoretical electrophysiology. A significant challenge is to determine the form of I_{ion}. We have already derived one possible choice, the GHK current equation (2.68), and others will be discussed in Chapter 3.

Another common model describes I_{ion} as a linear function of the membrane potential. In Chapter 3 we will see how a linear I–V curve can be derived from more realistic models; however, because it is used so widely, we present a brief, heuristic, derivation here. Consider the movement of an ion S across a membrane. We assume that the potential drop across the membrane has two components. First, the potential drop due to concentration differences is given by the Nernst equation

$$V_S = \frac{RT}{zF} \ln\left(\frac{[S]_o}{[S]_i}\right), \tag{2.76}$$

and, second, the potential drop due to an electrical current is rI_S (if the channel is ohmic), where r is the channel resistance and I_S is the transmembrane current (positive outward) of S. Summing these two contributions we find

$$V = rI_S + V_S, \tag{2.77}$$

and solving for the current, we get the current–voltage relationship

$$I_S = g(V - V_S), \tag{2.78}$$

where $g = 1/r$ is the *membrane conductance*. The current I_S and conductance g are usually specified per unit area of membrane, being the product of the single channel conductance times the number of channels per unit area of membrane.