Passive Transport and Facilitated Diffusion 2.5

Learning Objectives

- Describe the microporous membrane as a model of passive membrane transport
- Describe the lipid bilayer model of passive membrane transport
- Define the permeability of a membrane
- Describe how the permeability depends on the microscopic character of the membrane for a porous membrane
- Describe how the permeability depends on the microscopic character of the membrane and solute for a dissolution model of passive transport
- Be able to determine the free energy change for passive transport
- Distinguish between facilitated transport and diffusional transport on the basis of saturability, specificity, rates, and competition
- Write an equation showing the rate of facilitated transport as a function of its solute concentration with zero-*trans* concentration. Identify the variables and describe their meaning
- Distinguish between an ionophore and a channel
- Describe what is meant by channel gating
- Distinguish between voltage-gated channels and ligand-gated channels

MEMBRANES POSSESS A VARIETY OF TRANSPORT MECHANISMS

As described in Chapter 2.4, membranes serve as effective barriers to the free movement of materials, thereby dividing the cell into compartments. This compartmentalization is necessary. In muscle, for example, it allows for the control of contraction by releasing Ca^{2+} ions from a store (the specialized endoplasmic reticulum of the muscle cell) into the cytoplasmic compartment. Relaxation is then brought about by removing Ca^{2+} ions from the cytosol back into the storage compartment. In another example, compartmentalization allows mitochondria to transduce the energy of oxidation of foodstuffs into the chemical energy of ATP. However, compartmentalization does not make sense if material absolutely cannot travel between the compartments. What is necessary is *selective* transport and *regulated* transport. The cell must be able to control what goes across the membranes and how fast.

There are three main mechanisms for transport:

A. Passive transport
   1. Diffusion
   2. Facilitated transport
B. Active transport
   1. Primary active transport
   2. Secondary active transport
C. Osmosis.

In this chapter, we will consider passive transport across two types of hypothetical membranes: a microporous membrane and a lipid bilayer membrane. The mechanisms of passive transport differ considerably between these two models, but the overall form of the equations is similar. In Chapter 2.6, we will consider active transport and then in Chapter 2.7, we will discuss osmosis.

A MICROPOROUS MEMBRANE IS ONE MODEL OF A PASSIVE TRANSPORT MECHANISM

Here we introduce the porous membrane as a model for biological membranes as shown in Figure 2.5.1. We consider here that a microporous membrane separates two solutions of different concentrations but the same pressure. The pores allow solute particles to pass, but the rest of the membrane that lacks pores is impermeable to the solute, and also to solvent water. We assume that the solute particles are small compared to the pores.

First we write the flux, the flow per unit area within a single pore. This is governed by Fick's Laws of Diffusion given as

$$j_s = -D\frac{\partial C(x)}{\partial x} \qquad \frac{\partial C(x)}{\partial t} = D\frac{\partial^2 C(x)}{\partial x^2} \qquad [2.5.1]$$

where j_s indicates the flux within the pore. We use the lower case "j" purposefully to distinguish it from J_s, which we will use to signify the macroscopic flux across the entire membrane. The top equation is Fick's First Law of Diffusion; the bottom equation is Fick's Second Law of Diffusion. Here we are concerned only with flux across the membrane, in one direction, and the

DOI: http://dx.doi.org/10.1016/B978-0-12-800883-6.00015-X

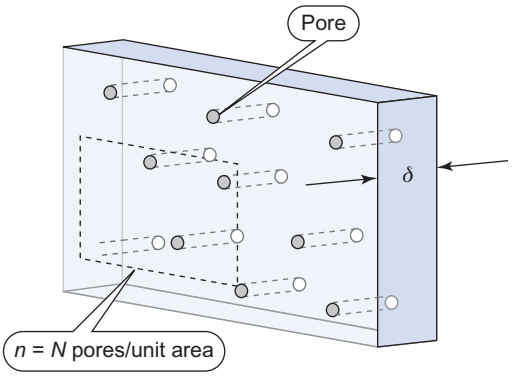

FIGURE 2.5.1 Schematic of the hypothetical microporous membrane. In this model, the membrane is a thin sheet of thickness δ. It is pierced by many cylindrical pores oriented perpendicular to the surface of the membrane. The radius of each pore is a and the number of pores, N, per unit area A is $n = N/A$. This model might not pertain to some cellular membranes, but it may describe some extracellular membranes such as the basement membrane, which supports many cells, especially epithelial cells, or it may represent the filtration membrane present in the kidney.

one-dimensional forms of Fick's laws apply to this situation. Let C_L be the concentration on the left side of the membrane and C_R be the concentration on the right. We can arrange it so that the volumes of the two baths are so large that C_L and C_R are effectively kept constant. Under these conditions, the solute flow will come to a steady state or stationary value. This means that neither the fluxes nor the concentration of solute changes with time. Fick's Second Law of Diffusion becomes

$$[2.5.2] \qquad 0 = D\frac{\partial^2 C(x)}{\partial x^2}$$

Note that this situation cannot be literally true, as solute is moving from one compartment to another, so there must be some changes in $C(x)$ with time. However, $C(x)$ can be so nearly constant that we can ignore the very slight error. The solution to this equation is that $\partial C(x)/\partial x$ is constant. This means that the concentration within the pores is linear with x. We solve this equation by two successive integrations, incorporating the boundary conditions that at $x = 0$, $C(x) = C_L$ and at $x = \delta$, $C(x) = C_R$ (see Figure 2.5.2). The concentration is written as

$$[2.5.3] \qquad C(x) = C_L + \left(\frac{C_L - C_R}{0 - \delta}\right)x$$

and the concentration gradient is

$$[2.5.4] \qquad \frac{\partial C(x)}{\partial x} = -\left(\frac{C_L - C_R}{\delta}\right) = -\frac{\Delta C}{\delta}$$

The flux in the pore is given from Eqns [2.5.1] and [2.5.4] as

$$[2.5.5] \qquad j_s = D\frac{\Delta C}{\delta}$$

The total flow of solute **per pore**, q_s, is given by the area of the pore times the flux within the pore:

$$[2.5.6] \qquad q_s = \pi a^2 j_s$$

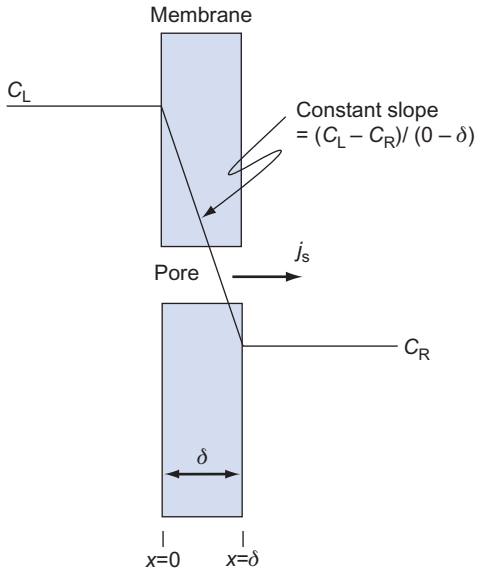

FIGURE 2.5.2 Cross-section of a microporous membrane in the vicinity of a pore. Superimposed on the cross-section is a graph of the concentration gradient. The left compartment has a higher concentration (C_L) than the right compartment with concentration C_R. Under this situation, the flux through the pore is to the right.

The total flow across an area A of the membrane containing N pores is

$$[2.5.7] \qquad Q_s = Nq_s = N\pi a^2 j_s$$

The macroscopically observed flux across the membrane is the total flow of solute (Q_s) divided by the macroscopic area of the membrane.

$$[2.5.8] \qquad \begin{aligned} J_s &= \frac{Q_s}{A} \\ &= \frac{N\pi a^2 j_s}{A} = n\pi a^2 j_s \\ &= \frac{n\pi a^2 D}{\delta}\Delta C \end{aligned}$$

According to this equation, the observed macroscopic solute flux across the membrane is linearly related to the concentration difference by a coefficient that includes the thickness of the membrane (δ), the density of pores in the membrane (n), the radius of the pores (a), and the diffusion coefficient of the solute (D). Often many of these parameters are not known with accuracy and we lump all of these terms together to write

$$[2.5.9] \qquad J_s = p\Delta C$$

where p is the **permeability** of the membrane to the solute. This **phenomenological coefficient** has the units of cm s^{-1} and includes all of the microscopic parameters of the membrane:

$$[2.5.10] \qquad p = \frac{n\,\pi a^2 D}{\delta}$$

In this model, the permeability increases when the size of the pores increases, when the number of pores per

unit area of membrane increases, when the thickness of the membrane decreases, and when the diffusion coefficient of the transported solute increases. Which of these can be regulated? Typically membranes do not regulate their thickness, nor can the diffusion coefficient be altered. **Channels** can act like pores and they can be **gated**. That is, the channels can be opened or shut. This has the effect of controlling the area through which materials can be transported and this is a common way of regulating ion transport. Another way of physiologically regulating passive transport is by controlling the number of pores (or channels) in a membrane.

The distinction between pores and channels lies in the substrate. We think of pores as holes in a substrate that will not collapse, as if we drilled a tiny hole in a thin plastic sheet. Lipid bilayers, however, will not support a watery void in their interior. Channels are proteins embedded in the membrane that line a watery pathway across the membrane and prevent its collapse by providing mechanical support on the sides of the pathway. The watery path across the membrane is made by the proteins that form the channel.

DISSOLUTION IN THE LIPID BILAYER IS ANOTHER MODEL FOR PASSIVE TRANSPORT

Consider now a markedly different model of the membrane. In this case, there are no pores, but we envision that a molecule may penetrate from the left to the right of the membrane by dissolving in the lipid bilayer core of the membrane, diffusing across the lipid, and then being extracted back into the aqueous phase on the other side of the membrane. This model of passive transport is shown schematically in Figure 2.5.3.

The dissolution of the solute in the lipid membrane is described quantitatively by a constant called the **partition coefficient** (see Chapter 2.3):

$$[2.5.11] \qquad k_s = \frac{\text{equilibrium C in the lipid phase}}{\text{equilibrium C in the water phase}}$$

If equilibrium is reached quickly at both the left and right surface of the membrane, then diffusion through the lipid phase would limit the rate of transport. Let the concentration on the left side of the membrane be C_L and the concentration on the right side of the membrane be C_R. The concentration immediately inside the membrane on the left, by Eqn [2.5.11], will be k_sC_L, and the concentration on the right inside the membrane will be k_sC_R. The steady-state flux through the lipid phase is

$$[2.5.12] \qquad J_s = D_{s,\text{lipid}}\left(\frac{k_s\,C_L - k_s\,C_R}{\delta}\right)$$

where $D_{s,\text{lipid}}$ is the diffusion coefficient of the solute in the lipid phase and δ is the thickness of the lipid phase. We write J_s here because the entire area of the membrane is available for dissolution of the solute and

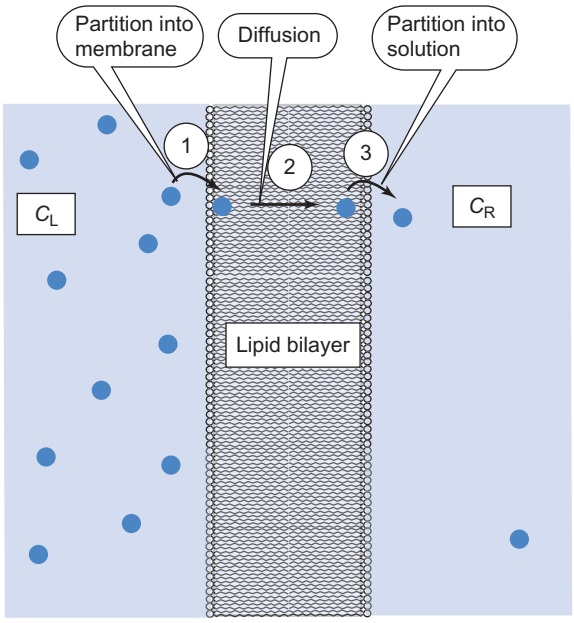

FIGURE 2.5.3 Cartoon of the lipid bilayer model of passive transport. The left and right compartments are separated by a lipid bilayer membrane. Solute, shown here as blue spheres, moves across the membrane in three well-defined steps. In step 1, the particle partitions itself into the lipid phase of the membrane. In step 2, the material diffuses across the lipid bilayer. In step 3, the material partitions itself back into the aqueous phase on the right side of the membrane. Overall transport rates are determined by the rates of steps 1, 2, and 3.

diffusion across the lipid bilayer. This equation can be rewritten as

$$[2.5.13] \qquad J_s = \frac{k_s D_{s,\text{lipid}}}{\delta}\Delta C$$

This last equation is identical in form to that derived earlier in the microporous membrane model:

$$[2.5.9] \qquad J_s = p\Delta C$$

In the case of the dissolution–diffusion–solution model, we identify the permeability as

$$[2.5.14] \qquad p = \frac{k_s D_{s,\text{lipid}}}{\delta}$$

Once again, the permeability is a single phenomenological parameter that relates the flux to the concentration difference. It incorporates all of the microscopic parameters of the membrane–solute pair into a single parameter. In this case, these microscopic parameters are the partition coefficient, the thickness of the membrane, and the diffusion coefficient of the solute in the membrane.

Equation [2.5.14] neatly sums up many experimental observations concerning the permeability of materials through biological membranes. These are briefly summarized in **Overton's rules**:

A. The permeability is proportional to the lipid solubility.
B. The permeability is inversely proportional to molecular size.

Thus we expect ethanol to permeate cell membranes quite easily because it is small and it is lipid soluble. On the other hand, glucose is larger and it is not readily lipid soluble and so it requires another mechanism to enter the cell. This mechanism is the carrier. Overton's rules derive from two main components of Eqn [2.5.14]: the dependence of p on k_s means that lipid solubility is a direct determinant of the permeability and the dependence on D_s means that size is an inverse determinant of permeability.

Lipid solubility here depends on the hydrophobicity or lipophilicity of the solute. Different chemical groups in any molecule confer hydrophilic or hydrophobic character to those parts of the molecule, as described in Chapters 2.3 and 2.4. In particular, electric charge make a solute highly hydrophilic and not lipophilic. Thus charged or ionized solutes are generally highly impermeable by this dissolution mechanism of transport. Solutes enriched in hydroxyl groups, carboxyl groups, and amino groups are generally not easily permeable through this mechanism unless they are also very small.

FACILITATED DIFFUSION USES A MEMBRANE-BOUND CARRIER

For some valuable materials, the membrane permeability is not large enough for the cell's needs and facilitated diffusion is used to carry solute across the membrane. One possible way a **carrier** could operate is shown in Figure 2.5.4. The transformations that occur to allow this transport are not known in detail. It is likely that the carrier provides something like a pore for the solute, but the pore is specifically designed to fit the solute. In this case, the solute molecule never dissolves in the lipid bilayer but is protected from it by a pocket of the protein carrier.

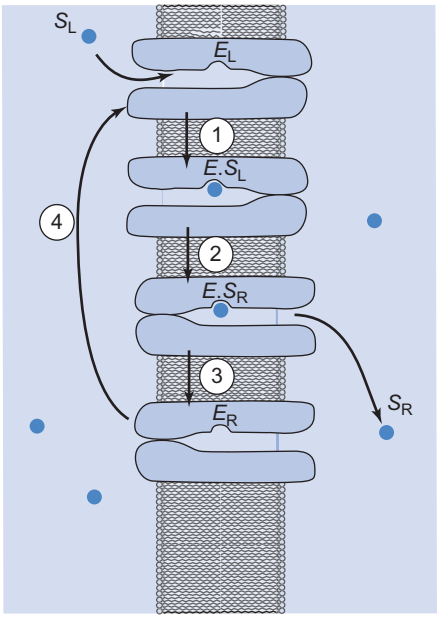

FIGURE 2.5.4 Schematic diagram of carrier-mediated passive transport. The carrier is designated as "*E*." In this scheme, an integral protein molecule in the membrane binds to a solute molecule on one side of the membrane. The carrier molecule then undergoes a transformation that has the effect of changing the side of the membrane that is accessible to the binding site. The solute molecule then dissociates from the carrier on the opposite side. The carrier then returns to its original shape.

The mechanism involved in facilitated diffusion shown in Figure 2.5.4 involves a sequence of four reactions:

$$\begin{aligned} S_L + E_L &\rightarrow E - S_L \\ E - S_L &\rightarrow E - S_R \\ E - S_R &\rightarrow E_R + S_R \\ E_R &\rightarrow E_L \\ \text{---------} & \\ \text{Sum}: S_L &\rightarrow S_R \end{aligned} \qquad [2.5.15]$$

The sum of these four reactions is the movement of solute from the left side of the membrane to the right side. The carrier concentration does not enter into the overall stoichiometry of the reaction, because its presence on both sides of the reactions cancels itself out. It acts as a catalyst for transport because it determines its rate without being altered by the process.

FACILITATED DIFFUSION *SATURATES* WITH INCREASING SOLUTE CONCENTRATIONS

Facilitated diffusion can be distinguished from a purely diffusional mechanism because facilitated diffusion is **saturable** and it is **specific**. Plots of the flux versus the concentration are not linear, as you would expect from the diffusion mechanisms as shown in Eqns [2.5.8] and [2.5.13]. The rate increases with concentration but only up to a point. This is due to the fact that there are only so many carrier molecules in the membrane. When they are all busy, there can be no further increase in the rate of transport. This is analogous to the ferrying of people across a river that is too deep for most of them to wade. Although some can wade, most must cross only by ferry. When there are not too many people, the ferries can accommodate them easily and the transport rate will increase with each increase in the number of people waiting on shore. When the crowd on the shore gets too great, however, the ferries become full and the rate of transport can be increased further only by the number of brave souls who can wade the river (diffuse across the membrane) or by increasing the number of ferries. Figure 2.5.5 shows the kinetics of a saturable transport mechanism.

These curves often closely resemble the hyperbolic plots characteristic of Michaelis–Menten enzyme kinetics and can be fit to

$$Q_{trans} = \frac{Q_{max} C}{K_m + C} \qquad [2.5.16]$$

where Q_{trans} is the flow of transported material across the membrane in moles per unit time, Q_{max} is the maximum flow, C is the concentration of the transported

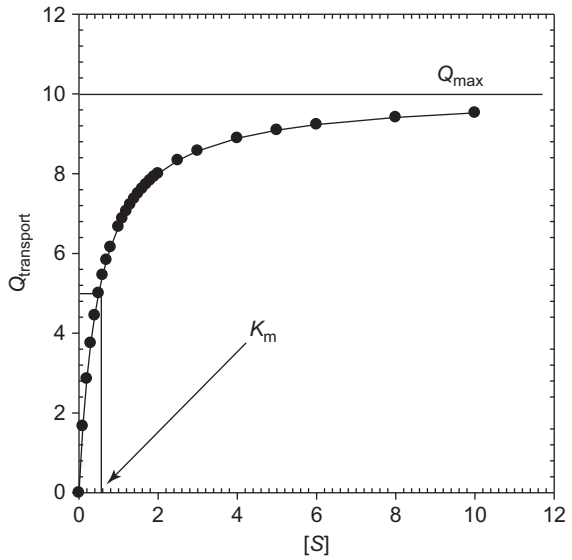

FIGURE 2.5.5 Graph of a saturable transport mechanism. The rate of transport in moles per unit time per unit area is plotted against the concentration of material on the feed side [*S*]. A maximum transport rate, Q_{max}, can be identified. The substrate concentration at half-maximal transport is used to characterize the affinity of the transport mechanism for its substrate.

solute on the feed side of the membrane, keeping the concentration on the opposite side at zero, and K_m is a constant characteristic of the carrier. The term "K_m" comes from Michaelis–Menten kinetics, and these carriers almost certainly do not have the mechanism first proposed by Michaelis and Menten. Nevertheless, the term "K_m" has come to mean "the concentration of substrate at half-maximal activity." Sometimes this is referred to as K_t, the concentration at half-maximal transport. Eqn [2.5.16] is a simplified version of the exact solution of the kinetics of the scheme shown in Figure 2.5.4.

FACILITATED DIFFUSION SHOWS *SPECIFICITY*

Another distinguishing feature of carrier-mediated facilitated diffusion is its structural **specificity**. The parts of the carriers that bind transported solute are specifically designed for that solute and not others. For example, most cell membranes in the human contain carriers for glucose. They will transport D-glucose but not its **enantiomer** (mirror image compound) L-glucose. The carrier for glucose will not transport amino acids and vice versa.

FACILITATED DIFFUSION SHOWS *COMPETITIVE INHIBITION*

The specificity of carrier-mediated facilitated diffusion also gives rise to **competitive inhibition**. Compounds that closely approximate the shape of the natural substrate may also bind to the carrier and be transported across the membrane. Since the carrier cannot carry both compounds at the same time, the transport of the natural substrate is reduced by the presence of competitive inhibitors. This competitive inhibition is closely related to that observed in enzyme kinetics. In other cases, a compound sharing some similarity with the natural substrate may bind to the carrier but not be transported. If the binding is at the transport site, such a compound might inhibit transport.

PASSIVE TRANSPORT OCCURS SPONTANEOUSLY WITHOUT INPUT OF ENERGY

The chemical reaction for the overall transport is written as

$$\text{Solute}_L \rightarrow \text{Solute}_R \qquad [2.5.17]$$

and the free energy change for the reaction is

$$\Delta G = G_R - G_L \qquad [2.5.18]$$

Substituting in with the chemical potential, we obtain

$$\Delta G = n(\Delta\mu^0 + RT \ln C_R) - n(\Delta\mu^0 + RT \ln C_L) = n\,RT \ln \frac{C_R}{C_L} \qquad [2.5.19]$$

where n is the number of moles of solute moving from left to right. Here there is no electrical work term because the charge on the molecule, z, is zero. If $C_R > C_L$, ΔG calculated from Eqn [2.5.19] will be positive. This means that the opposite process will occur. That is, solute will move from the right to the left, opposite to the direction shown in Eqn [2.5.17]. If $\Delta G = 0$, then no net movement occurs and $C_R = C_L$. If $C_R < C_L$, then ΔG calculated according to Eqn [2.5.19] will be negative and the reaction will proceed as written, with solute moving from the left to the right side of the membrane. Thus thermodynamics tells us what process can occur and with what change in free energy, but it does not give us an expression for the permeability or the rate at which the process will occur.

Diffusion through aqueous pores or through the lipid barrier of membranes or by facilitated diffusion is called passive transport because none of these mechanisms requires "outside" energy. These flows occur spontaneously. What this means is that the energy that drives them is contained within the solutions themselves. This does not mean that they occur rapidly, but only that they occur naturally without the addition of any "outside" force. The rate at which they occur depends on the mechanisms of transfer. The analysis of the mechanism gives us additional information, such as what determines and regulates the rate.

The overall ΔG for facilitated diffusion is the ΔG for the sum, which is the same in Eqns [2.5.15] and [2.5.17]. The net $\Delta G = nRT \ln C_L/C_R$. Thus the participation of the carrier, which remains unchanged by the transport reaction, does not alter the reaction *energetics* at all, whereas it does alter the reaction *kinetics*. The carrier is a catalyst. It speeds up the reaction, which in this case is transport, without entering into the stoichiometry of the reaction. This is an example of how thermodynamic

Example 2.5.1 Specificity of Transport

There are a variety of transporters for glucose that are called GLUT (for glucose transporter). The GLUT-1 transporter imports glucose into a variety of cell types. This is an integral membrane protein with a molecular weight of 45 kDa. Its K_m for glucose is 1.5 mM. It will also transport L-glucose with a K_m of 3000 mM. Glucose is typically about 100 mg% in the extracellular fluid. At what fraction of Q_{max} will glucose be transported at this concentration?

The normal plasma [glucose] is given as 100 mg%, which is 100 mg of glucose per 100 mL of plasma. This is $P_g = 100$ mg/100 mL $\times$ 1000 mL L^{-1} = 1 g L^{-1}. Since the molecular weight of glucose is 180 Da, its gram molecular weight is 180 g mol^{-1} and the normal plasma glucose concentration is

$$P_g = 1 \text{ g L}^{-1}/180 \text{ g mol}^{-1} = 0.0056 \text{ M} = 5.6 \text{ mM}$$

The rate of transport, assuming zero-*trans* glucose, is given by Eqn [2.5.16] as

$$Q_{trans} = [5.6 \text{ mM}/(1.5 \text{ mM} + 5.6 \text{ mM})]Q_{max} = \mathbf{0.789\ Q_{max}}$$

At plasma [glucose], the transporters are nearly saturated and the rate of transport could be increased mostly by affecting the number of transporters, i.e., increasing Q_{max}.

What would the L-glucose rate of transport be at the same concentration as D-glucose?

$$Q_{trans\ \text{L-glucose}} = [5.6 \text{ mM}/(3000 \text{ mM} + 5.6 \text{ mM})]Q_{max} = \mathbf{0.0019\ Q_{max}}$$

D-Glucose is transported almost 400 times more quickly than L-glucose.

Example 2.5.2 Effect of Substrate Concentration on Flux

GLUT-1 glucose transporter is the most ubiquitous form of glucose transporter. It is present in high amounts in erythrocytes and endothelial cells, the blood–brain barrier and in the proximal straight tubule of the nephron. If its K_m for glucose is 1.5 mM, how much would transport increase if plasma glucose were increased from 80 mg% to 120 mg%?

First, we convert the plasma glucose concentrations to mM. 80 mg% means 80 mg per 100 mL or 80 mg/0.1 L = 800 mg/L. The molecular weight of glucose is 180 g, so this concentration is equivalent to 0.8 g/180 g mol^{-1}/L = 4.44 mM. Similarly, 120 mg% is 6.67 mM.

If the K_m for glucose is 1.5 mM, then the transport rate at 80 mg % glucose would be

$$Q = Q_{max} \times 4.4 \text{ mM}/(1.5 \text{ mM} + 4.44 \text{ mM}) = \mathbf{0.75 Q_{max}}$$

And at 120 mg% glucose it would be

$$Q = Q_{max} \times 6.66 \text{ mM}/(1.5 \text{ mM} + 6.66 \text{ mM}) = \mathbf{0.82 Q_{max}}$$

The transport rate increases 9% when the blood glucose increases by 50%. These GLUT1 transporters are insensitive to changes in blood glucose.

GLUT2 is another glucose transporter that is present in beta cells of the islets of Langerhans, in the pancreas, and also in the kidney, intestine, and liver. Its K_m for glucose is much higher, 17 mM. How much would transport increase if plasma glucose were increased from 80 mg% to 120 mg%?

We can use the molar concentrations for glucose that we used before for the GLUT1 calculations. The transport rates are calculated at 80 mg% glucose as

$$Q = Q_{max} \times 4.4 \text{ mM}/(17 \text{ mM} + 4.44 \text{ mM}) = \mathbf{0.207 Q_{max}}$$

and at 120 mg% glucose, it would be

$$Q = Q_{max} \times 6.66 \text{ mM}/(17 \text{ mM} + 6.66 \text{ mM}) = \mathbf{0.282 Q_{max}}$$

Here the transport rate increases 36% when blood glucose increases 50%.

Thus GLUT1 and GLUT2 serve different functions. GLUT1 operates close to maximal rates relatively independently of blood glucose levels. GLUT2 increases transport almost proportionately with blood glucose, so that GLUT1 is used for basal glucose transport into metabolizing tissues, whereas GLUT2 finds use as part of the sensor apparatus for glucose concentrations in blood.

analysis of the reaction is independent of the mechanism: it tells us about the energetics without telling us anything about the reaction's path or its rate.

The saturability of carrier-mediated facilitated diffusion distinguishes it from the other passive transport mechanisms that show a linear relationship between flow and the concentration difference across the membrane. Neither mechanism can concentrate solute. Flow of material always occurs from the side with the higher concentration to the side with the lower concentration. When the concentrations on the two sides of the membrane are equal, no further flow occurs because the two solutions are in equilibrium.

IONS CAN BE PASSIVELY TRANSPORTED ACROSS MEMBRANES BY *IONOPHORES* OR BY *CHANNELS*

So far we have considered passive diffusion of nonelectrolytes. Suppose now that the diffusing species

are electrically charged. **Charged species are poorly soluble in the lipid phase**, and so they cannot merely dissolve in the lipid on one side of the membrane, diffuse across, and then enter the compartment on the opposite side of the membrane. They need either carriers or channels to get across.

IONOPHORES CARRY IONS ACROSS MEMBRANES OR FORM CHANNELS

Fungi and bacteria make a class of poison called **ionophores**. These are molecules that allow ions to cross membranes. The fungi and bacteria make these compounds to kill off competition by disrupting the permeability barrier of their competitors' membranes. These ionophores are of two types: carriers and channel formers. Figure 2.5.6 illustrates these two types of ionophores.

An example of a carrier is **A23187**. This material is commercially obtained from *Streptomyces chartreusis* and has weak antibiotic activity against gram-positive bacteria. It is particularly active for divalent cations with a specificity of $Mn^{2+} > Ca^{2+} > Mg^{2+} > Sr^{2+} > Ba^{2+} > Li^{+} > Na^{+} > K^{+}$. It is predominantly used as a carrier for Ca^{2+}. Other examples of natural molecules that act as carriers include **valinomycin** (a K^{+} ionophore) and **nigericin** (an H^{+} ionophore).

An example of a channel former is **gramicidin A**. This is an antibiotic polypeptide containing 15 amino acids that is isolated from the bacterium *Bacillus brevis*. The molecule appears to form a pore by linking two molecules of gramicidin A across the bilayer. The gramicidin pore appears to behave like a water-filled pore. Other examples of pore-forming antibiotics include **amphotericin** and **nystatin**. Amphotericin makes a channel by interacting with cholesterol in cell membranes.

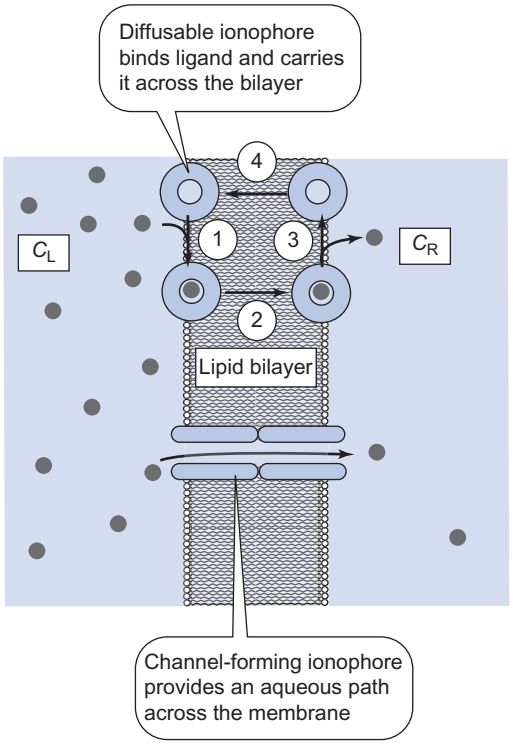

FIGURE 2.5.6 How ionophores work. Some ionophores increase the passive diffusion across a lipid bilayer by providing a hydrophilic pocket that binds a solute and sequesters it away from the hydrophobic lipid interior. These ionophores generally show specificity of transport because the pocket binds some ions better than others. For these types of ionophores, the ionophore–ligand complex is believed to diffuse across the lipid bilayer, carrying the ligand with the ionophore. Other ionophores form an aqueous channel across the lipid bilayer. These channel-forming ionophores are less specific but still show specificity due to the size and shape of the channel.

ION CHANNELS

A variety of integral membrane proteins form channels for ions. These **ion channels** exhibit some of the characteristics of carriers in that they are highly selective. These channels exhibit other characteristics such as **gating**. Gating refers to the fact that these channels act as if they have gates that are opened sometimes, allowing ions to cross the membrane, and are closed at other times, preventing ions from moving. The percent of the time the channels are opened is referred to as the open probability, p_o, and can be regulated in various ways. Some channels open when another molecule binds to the channel. These are **ligand-gated** channels. Other channels sense the local potential, probably through the presence of charged groups on the channel, and open or close depending on the potential. These are **voltage-gated** channels. A cartoon of these types of channels is shown in Figure 2.5.7.

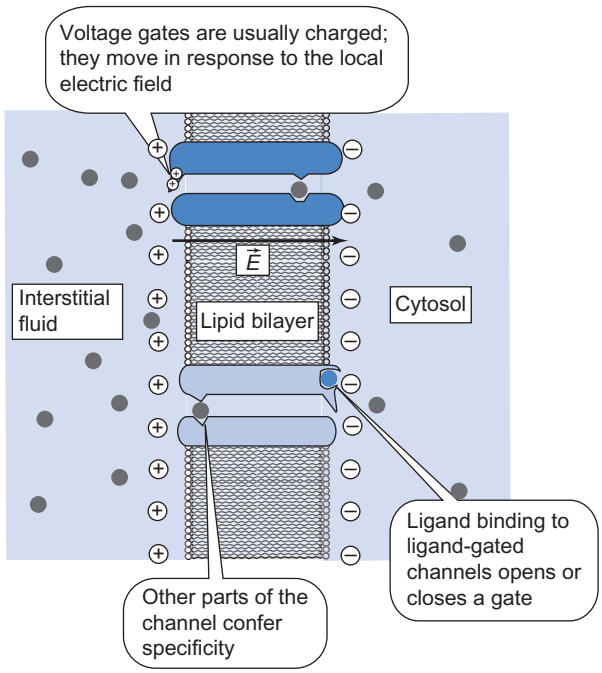

FIGURE 2.5.7 Voltage- and ligand-gated channels. Voltage-gated channels typically have a highly charged part of the protein that responds to the local electrical field produced by the separation of charge on the two sides of the membrane. Changes in this electric field alter the disposition of the gate to either open or close access to a hydrophilic pathway across the membrane. Ligand-gated channels bind a regulatory ligand that alters the shape of the channel so as to open or close its pathway.

Examples of voltage-gated and ligand-gated channels abound, and a full description of them here is premature because we have not yet studied membrane potential or action potentials. **Voltage-gated Na^+ and K^+ channels** allow ions to flow across the membrane only under specific circumstances. The flow of ions is an electric current, because charged ions are moving. The membrane itself is a tiny capacitor, as we saw in Chapter 1.3. The currents going through these channels can discharge the capacitor, changing the voltage across the membrane, or they can charge it back up again. In this way, opening of the fast Na^+ channel in neurons causes a brief, pulse-like change in the voltage across the neuronal cell membrane. The membrane potential is reestablished by a later opening of the K^+ channels. These actions produce the nerve impulse, the brief change in nerve cell membrane potential that propagates down the nerve and activates its target—either another neuron, a muscle fiber, or some secretory cell.

Other voltage-gated channels include the **T-**, **L-**, and **N-type Ca^{2+} channels**. The designation "T" signifies that this channel opens "transiently"; the "L" stands for "long-lasting"; and the N indicates that this type of channel is "neuronal." The channels generally open upon depolarization of the cell membrane and they specifically transport Ca^{2+}. The consequence is that the $[Ca^{2+}]$ inside the cell increases in the vicinity of the channel, and this Ca^{2+} binds to cellular elements to change their activity.

Ligand-gated channels may be present on the surface membrane and also on interior membranes. The endoplasmic reticulum of many cells contains a large tetrameric protein called the **IP3 receptor**. This receptor forms a channel for Ca^{2+} across the ER membrane and opens in response to IP3 (inositol trisphosphate) that is liberated from the surface membrane as part of signal transduction. Gating by IP3 causes Ca^{2+} release from the ER and the increased cytoplasmic $[Ca^{2+}]$ alters cellular activity.

Many ligand-gated channels are present on the surface membrane and respond to neurotransmitters or hormones. These channels are gated by the binding of a chemical rather than by the voltage difference across the membrane. **Acetylcholine** is the neurotransmitter involved in skeletal muscle neurotransmission. When activated, the motor neuron nerve terminal releases acetylcholine near the skeletal muscle membrane. The acetylcholine binds to **nicotinic acetylcholine receptors**, so named because of their sensitivity to nicotine, on the muscle membrane. Binding of acetylcholine opens a large conductance pathway mainly for Na^+. This causes a depolarization of the muscle membrane that propagates along the muscle surface, eventually activating muscle contraction.

WATER MOVES PASSIVELY THROUGH *AQUAPORINS*

Passive transport of water across biological membranes also occurs through water channels. These are tiny pores formed by proteins called **aquaporins**. There are a variety of aquaporins and they are present on virtually every cell membrane. AQP1 has a molecular weight of 29 kDa and forms a channel by the association of four monomers. In some membranes, the number of aquaporins is physiologically regulated so that water movement through the cell can be regulated. This is particularly important in the kidney, because the kidney has the final job of retaining water when it is scarce and excreting it when it is in excess. Although water obeys Fick's Laws of Diffusion, its movement is dominated by pressure-driven flow.

SUMMARY

Materials cross biological membranes by a variety of mechanisms including passive transport, active transport, and osmosis. Passive transport mechanisms require no input of metabolic energy. Because of this, passive transport always entails the movement of materials from regions of high concentration to regions of low concentration. The free energy change per mole in the reaction $S_L \rightarrow S_R$ is

$$\Delta\mu = RT \ln[C_R/C_L]$$

where C_R and C_L are the concentrations of S on the right- and left-hand sides of the membrane, respectively. If $C_R < C_L$, then $\Delta\mu < 0$ and the reaction proceeds from the high concentration (C_L) to the low concentration (C_R).

Passive diffusion across membranes is characterized by a linear relationship between the rate of transport and the concentration difference across the membrane:

$$J_s = p\Delta C$$

where J_s is the macroscopically observed flux and p is the permeability. This equation holds true if we envision the membrane as a microporous membrane in which diffusion occurs through tiny pores, or if we envision the solute as dissolving in the lipid bilayer and diffusing across it. The dependence of p on the microscopic characteristics of the membrane differs in these two models. For a microporous membrane

$$p = n\pi a^2 D/\delta$$

where n is the number of pores per unit area, a is the radius of the pore, D is the diffusion coefficient of the solute, and δ is the thickness of the membrane. For a solute dissolving in the lipid bilayer

$$p = KD_{lipid}/\delta$$

where K is the partition coefficient of the material in the lipid phase, D_{lipid} is the diffusion coefficient in the lipid bilayer, and δ is the thickness of the bilayer.

Some membrane proteins bind solutes and provide an alternative path across membranes. The alternative path facilitates the diffusion of the solute across the membrane. These proteins are carriers for the solutes. The kinetics of transport shows specificity, saturation,

and competition with similar solutes. The overall transport rate often obeys an equation of the form

$$Q_{trans} = Q_{max}C/[K_m + C]$$

where Q_{max} is the maximum transport rate, typically limited by the number of carriers in the membrane, and K_m is a measure of the dissociation constant of the carrier for the solute.

Passive transport mechanisms include lipid dissolution and diffusion, facilitated diffusion, ligand-gated channels, voltage-gated channels, diffusion-mediated ionophores, and pore-forming ionophores.

REVIEW QUESTIONS

1. Why is the gradient for a diffusive process linear at steady state?
2. For a microporous membrane, what effect would increasing the number of pores have on diffusive flux across a membrane? What effect would increasing the size of the pores have? What effect should result from increasing the size of the diffusing solute? Increasing the temperature?
3. For a solute dissolution model, what effect would increasing the partition coefficient have? Increasing the particle size of the diffusing solute? Increasing the temperature?
4. Why should two models as different as the microporous membrane and solute dissolution model have identical relationship between J and ΔC?
5. Under what conditions is the free energy for transfer across a membrane for a solute equal to zero?
6. How does the function relating rate of transport to concentration differ between facilitated diffusion and simple diffusion?
7. Why does simple diffusion not show specificity or competition?
8. What two general mechanisms are used to regulate the open or closed state of channels?
9. What is a channel?
10. How would you determine K_m for facilitated diffusion? Q_{max}?