Chapter 4
Substance Transport Across Membranes

4.1 Brief Overview

The plasma membrane controls the traffic in both directions between a cell and its environment of small molecules, macromolecules, supra-molecular complexes or larger particles. The types of substance transport across the cell membrane may be classified, by taking into account the size of the particles, into two categories: (a) *macrotransport* of relatively large quantities of solution, molecular complexes, or even other cells, and (b) *microtransport* of ions, small molecules and macromolecules.

Macro-transport refers to three types of processes (Fig. 4.1): *endocytosis*, whereby various particles are internalized by the cells, *exocytosis*, in which macroparticles are externalized by the cell, and *transcytosis*, which consists of an endocytosis process followed by exocytosis of the same particle. During endocytosis and exocytosis, the target particles are wrapped in membranous material which is produced and/or destroyed by the cell as necessary.

A particular type of endocytotic process is *phagocytosis*, whereby *immunocompetent* cells (i.e., macrophages, involved in an organism's defense against viruses and microbes), are able to engulf and destroy viruses and microbes invading the organism, as well as dead cells and cellular remnants.

Figure 4.2 illustrates two different instances of exocytosis: *constitutive* or *unregulated secretion* and *regulated secretion*. In the first case the particles (proteins) are sorted and packed into vesicles by the Golgi aparatus and then delivered to the plasma membrane, which they will eventually cross by following the mechanism of membrane fusion sketched in Fig. 4.2. The *regulated* or *receptor-mediated secretion* is triggered by a signal (such as a hormone or a neurotransmitter) received by a receptor on the plasma membrane. Therefore, in this case, the proteins are only secreted as needed. More detailed information about this fascinating topic can be found in biology textbooks (Alberts et al., 2002, Lodish et al., 2004).

Microtransport across membrane is performed also in a variety of modalities. By taking into account the energy involved during the process, one can distinguish: *passive transport*, and *active transport*. The *passive transport* is always directed

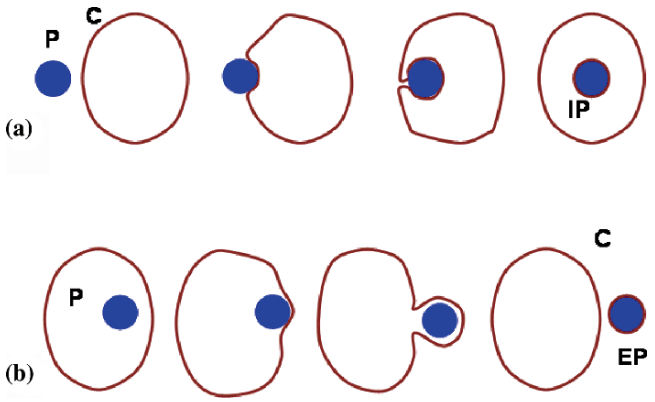

Fig. 4.1 Schematic representation of two modalities of biological macrotransport across membrane: (**a**) endocytosis; (**b**) exocytosis. The process involving both (**a**) and (**b**) is called transcytosis. Significance of the symbols: C – cell; P – particle; IP – internalized particle; EP – externalized particle.

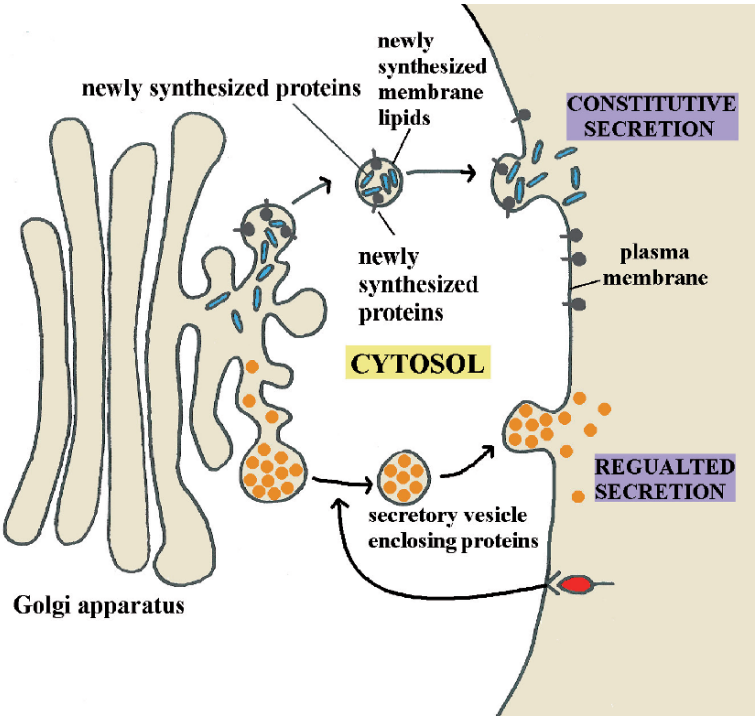

Fig. 4.2 Constitutive (unregulated) and receptor-mediated (regulated) exocytosis.

from higher concentrations of molecules to lower concentrations, that is, in the direction of the concentration gradient. This transport is due to diffusion of the particles and thus occurs spontaneously, without energy consumption (i.e., it is *exergonic*, with $\Delta G < 0$, and with entropy increase, $\Delta S_{ex} > 0$). Passive transport can be classified into: *simple diffusion*, which also occurs in non-biological systems, and *facilitated transport*, which is *specific* to biological systems.

In *active transport* the substance is always transferred *from lower to higher concentrations of molecules*, that is, in the opposite direction of the concentration gradients, and involves free energy consumption (i.e., the process is *endergonic*, with $\Delta G > 0$, and antientropic, $\Delta S_{en} < 0$).

4.2 Diffusion in Biological Systems

In biological systems, the *simple* (*passive*) *diffusion* may take place in bulk liquid phases as well as across membranes. As we have emphasized on several occasions, the interior of a cell is a highly *inhomogeneous* and *anisotropic milieu*. Therefore, the process of simple diffusion of cytosolic particles undergoes successive discontinuities as the particles encounter successive membrane interfaces, which give the whole diffusion process a complex character of chained serial processes. There is indication that diffusion processes in the cytosol may not follow classical pattern that diffusion in homogeneous media does, as we will discuss in the next chapter. At this stage in our discussion, however, we only consider the general properties of the medium as averages over large volumes, in particular when talking about transport between two reservoirs of substance. In the case of cellular membranes, the microtransport is performed across the *lipid matrix*, constituted by the lipid bilayer, through *short-lived pores* or through the cores of specialized proteins called *ionic channels* or *carriers*.

In the following, we shall assume, for simplicity, that the dimensions of the cellular compartments are much greater than the dimensions of the diffusing particles (rigorously, greater than *the mean free path* of the particles). Although rather crude, this approximation permits the use of well-known classical laws to mathematically describe the simple diffusion, as we shall see momentarily. One can regard the simple diffusion as a result of huge numbers of chaotic collisions (due to thermal agitation) both with other particles and with the solvent molecules.

4.2.1 Fick's Laws of Diffusion

In order to put the description of diffusion on a quantitative basis, we shall first review some indispensable concepts such as *flux, flux density*, and *gradient*.

The *flux*, J_Y, of a physical quantity, Y, across a surface area, A, is given by the *first derivative of Y with respect to time*:

$$J_Y = \frac{dY}{dt} \tag{4.1}$$

For instance, the flux of mass through a specified surface area is the mass flow, $J_m = dm/dt$. The flux divided by the *surface area* (i.e., flux through a unit surface area),

$$j_Y = \frac{1}{A}\frac{dY}{dt} \tag{4.2}$$

is called *flux density*.

By using the definition of the gradient of a conjugate scalar quantity, X_Y, as the vector quantity

$$\textbf{grad}\, X_Y \equiv \nabla X_Y = \frac{\partial X_Y}{\partial x}\vec{i} + \frac{\partial X_Y}{\partial y}\vec{j} + \frac{\partial X_Y}{\partial x}\vec{k} \tag{4.3}$$

where $\vec{i}$, $\vec{j}$, $\vec{k}$ are unit vectors associated with the spatial coordinates of the Cartesian reference frame, and ∇ is the del operator (represented by the symbol "nabla"), we can introduce a *vectorial equation* describing the transport of the quantity, Y:

$$\vec{j}_Y = \frac{\vec{J}_Y}{A} = -C_Y \nabla X_Y \tag{4.4}$$

where C_Y is a specific coefficient describing the transport of Y. This is *Fick's first law* for transport across surfaces, which basically states that the flux of the quantity Y is directly proportional to the gradient of the conjugated quantity X_Y.

By setting $Y = m$ (mass), $X_Y = \rho$ (density), and $C_Y = D$ (translational diffusion coefficient), we obtain Fick's *first law of simple diffusion*:

$$\vec{J}_m = -AD\nabla\rho \tag{4.5}$$

with $[J_m]_{\mathrm{SI}} = \mathrm{kg/s}$.

In the case of one-dimensional diffusion of neutral particles across membranes, Fick's first law is written in the following scalar form:

$$J_m = -AD\frac{d\rho}{dx} \tag{4.6}$$

We emphasize that it is more common in the fields of biophysics and biochemistry to use instead of mass, m, the *quantity of substance*, ν, expressed in mol, and instead of *density*, ρ, the *molar concentration*, c_M, expressed in mol/l. In this case, an analog of (4.5) is obtained as:

$$\vec{J}_\nu = -AD\nabla c_M \tag{4.7}$$

where $J_\nu = d\nu/dt$, and $[J_\nu]_{\mathrm{SI}} = \mathrm{mol/s}$.

The diffusion coefficient, D, depends on the external medium temperature, T, and dynamic viscosity coefficient, η, as well as on the particle geometry. In the case of a spherical particle of radius, r, the diffusion coefficient is given by Einstein's formula:

$$D = \frac{k_B T}{6\pi\eta r} \tag{4.8}$$

where k_B is the Boltzmann constant.

At a first glance, equation (4.8) seems to predict a linear dependence of the diffusion coefficient on temperature. In fact, the temperature dependence of D is more intricate, because the viscosity coefficient itself depends on temperature, being inversely proportional to T. Equation (4.8) also predicts that the bigger the particles the slower the diffusion. For instance, in the case of ions and small molecules, $D \approx 10^{-9}\,\mathrm{m^2 s^{-1}}$, while in the case of macromolecules, $D \approx 10^{-11}\,\mathrm{m^2 s^{-1}}$.

The temporal variation of the particle concentration is directly proportional to the spatial variation of the concentration gradient, according to *Fick's second law* of diffusion:

$$\frac{\partial c_M}{\partial t} = -\nabla\left(-D\nabla c_M\right) \tag{4.9}$$

which is easily obtained from equation (4.7) and the *continuity equation* (conservation of mass).

If D is the same in all space directions, Fick's second law becomes:

$$\frac{\partial c_M}{\partial t} = D\nabla^2 c_M \equiv D\Delta c_M \tag{4.10}$$

where Δ is the Laplacean. In one dimension, the second law of diffusion takes the form:

$$\frac{\partial c_M}{\partial t} = D\frac{\partial^2 c_M}{\partial x^2} \tag{4.10′}$$

which suggests a straightforward physical interpretation: the rate of concentration change at a given point is directly proportional to the second derivative, or the "curvature" of the concentration. This means that, if the local curvature of concentration is large (i.e., the concentration varies strongly in space), then the concentration changes rapidly with time. The diffusion equation (4.10′) reflects the mathematical formulation of the "natural tendency for the wrinkles in a distribution to disappear. More succinctly: Nature abhors a wrinkle" (Atkins and de Paula, 2002). However, we note that living bodies also generate and exploit all kinds of wrinkles (e.g., concentration gradients, electrical potential gradients). It is therefore the interplay between the mechanisms of creating curvatures and the above mechanisms of actually removing them that distinguishes inanimate from animate objects.

4.2.2 Simple Diffusion Through Membranes

If the molecules present in the cytosol or in the outer medium are soluble in the cell membrane, they can diffuse from one side to another across the membrane. For instance, lipid-soluble molecules (e.g., fatty acids, alcohols, etc.) can diffuse through biological membranes, while lipid-insoluble charged (e.g., amino acids) and uncharged molecules (e.g., glucose) cannot diffuse easily. We shall consider a homogenous but anisotropic membrane of thickness, d_m (Fig. 4.3) separating two homogenous solutions of the same solute, but with different solute concentrations: $c_{M1} > c_{M2}$. In order to characterize mathematically the *simple diffusion through*

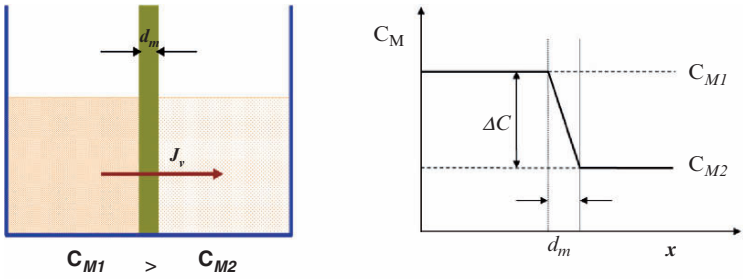

Fig. 4.3 Simple diffusion through a membrane of thickness d_m. Note that the concentration falls linearly across the membrane, with the slope $\delta c/\delta x = \delta c/d_m$. See text for the significance of the symbols.

membrane (also called *permeation*), we write Fick's first law, for a short, but finite time interval, δt, as follows:

$$J_v = \frac{\delta v}{\delta t} = -AD\frac{\delta c_M}{\delta x} = -AD\frac{c_{M2} - c_{M1}}{d_m} \tag{4.11}$$

where we have substituted the membrane thickness, d_m, with δx. This equation indicates that the flux through the membrane is directly proportional to the solute concentration difference. It assumes also that the concentrations in the media on each side of the membrane are independent of time. This is usually a good approximation in the case of biological cells, where the diffusion through the membrane is much slower than diffusion within each of the two media, which means that the membrane is the only major barrier to the motion of solute molecules. (There is another implicit approximation made in deriving equation (4.11), which will be discussed in the next section.)

By replacing the ratio D/d_m with a parameter, P, called *permeability coefficient*, equation (4.11) becomes:

$$J_v = \frac{\delta v}{\delta t} = -AP(c_{M2} - c_{M1}) \tag{4.12}$$

If there are no molecules in the second compartment (or if they are rapidly consumed as a result of some biochemical reaction), equation (4.12) gives:

$$J_v = A_P c_{M1} \propto c_{M1} \tag{4.13}$$

which suggests that the flux of simple (passive) diffusion is directly proportional to the concentration of the particle transported and, therefore, does not present saturation.

Observation: In reality, significant discontinuities in the concentrations are to be expected in biological membranes due to the absorption of the diffusing substances at the membrane surfaces, the change of ion concentrations in the electric double layers associated to membrane surfaces, and variations in the diffusing particles mobilities inside the membrane caused by dielectric inhomogeneities (Glaser, 2001).

Membranes of different cells present different values of the permeability coefficients. For instance, in the case of *erythrocyte membrane*, P is $3 \times 10^{-6}\,\mathrm{m\,s^{-1}}$ for water, and $2 \times 10^{-9}\,\mathrm{m\,s^{-1}}$ for glucose. In general the permeability of the lipid bilayer of the cell membranes spans about 10 orders of magnitude, ranging from $10^{-14}\,\mathrm{m\,s^{-1}}$, for Na^+, to $\sim 5 \times 10^{-5}\,\mathrm{m\,s^{-1}}$ for water molecules (Stryer, 1988; Alberts et al., 2002).

4.2.3 Determination of Membrane Permeability from Membrane Potential Energy Profile

The method used above for deriving the expression for the flux of substance passing through the membrane [equation (4.12)] has the distinct advantage of simplicity. However, as already seen, that derivation relied on the validity of Fick's first law, and on the assumption that the mean free path of diffusing molecules is smaller than the spatial dimensions of the media, which cannot be *a priori* tested for the case of very thin membranes. Also, as Davson and Danielli (1970) have pointed out, the major difficulty with applying Fick's laws to media separated by biological membranes is the assumption that the diffusing particles are much larger than the membrane molecules which resist the motion of the solute. Actually, most passively diffusing molecules in biological membranes are comparable in size to the phospholipids that make up the lipid bilayer. In this section, we introduce a method for deriving a formula equivalent to equation (4.12) along the lines presented by Danielli (see Davson and Danielli, 1970).

A solute particle which diffuses freely within an aqueous medium (such as the cytosol of a cell) encounters two types of resistance when it crosses a membrane interface that separates the first medium from another with lower concentration of solute: the potential energy barriers at the interfaces between the two media and the membrane polar layer (μ_a), and the energy barrier at crossing the polar layer of the membrane into the "bulk" of the membrane hydrophobic layer (μ_i). There are also smaller energy barriers associated with the particle jumping from one site to another after entering the membrane (μ_m).

Figure 4.4 shows two potential energy profiles that correspond to solute molecules with different polarities (relative to water). Depending on the nature of the molecule, the first and last barriers (i.e., those located at the two interfaces of the membrane) may or may not be the highest of all the barriers that the particle will meet. The concentrations across the membrane will therefore vary accordingly.

As regards the particular motion of the diffusing molecules, in general, each molecule can oscillate about a mean position between barriers or, if it possesses enough kinetic energy, can jump over the barrier to reach the next minimum of potential.

The concentrations of solute in the internal and external media are denoted by c_1 and c_2, respectively, while the concentrations at the internal minima of potential energy by $c'_1, c'_2, \ldots, c'_n$. We start from the medium 1 and write the rate of transfer

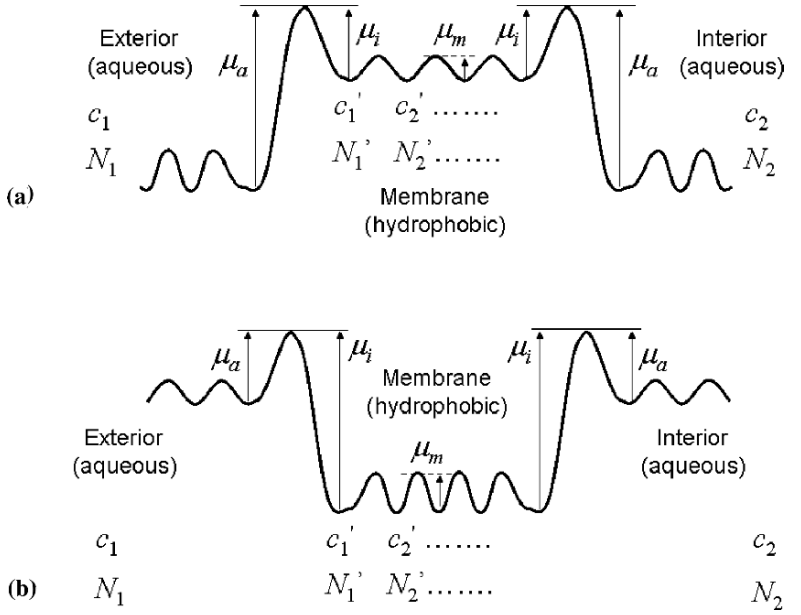

Fig. 4.4 Potential energy profiles of (**a**) a polar solute molecule (i.e., less soluble in the membrane hydrophobic layer) and (**b**) a solute molecule that is less polar than water (i.e., more soluble in membrane).

of the number of molecules, N_1, from the left to the right side of the membrane,

$$\frac{dN_1}{dt} = ac_1 \tag{4.14}$$

and the rate of transfer of molecules, N'_1, in the reverse direction,

$$\frac{dN'_1}{dt} = -bc'_1 \tag{4.15}$$

where a and b are two rate constants. Similarly, we can write the forward and reverse rates of transfer of solute at the interface with medium 2 as:

$$\frac{dN'_n}{dt} = bc'_n \tag{4.16}$$

$$\frac{dN_2}{dt} = -ac_2 \tag{4.17}$$

When a steady flow from left to right is reached, the flux of substance dS/dt across the membrane can be written as:

$$J = \frac{dS}{dt} = \frac{dN_1}{dt} + \frac{dN'_1}{dt} = ac_1 - bc'_1, \tag{4.18}$$

and:

$$J = \frac{dS}{dt} = \frac{dN'_n}{dt} + \frac{dN_2}{dt} = bc'_n - ac_2 \tag{4.19}$$

By taking the semisum of (4.18) with (4.19), we obtain:

$$J = \frac{dS}{dt} = \frac{a}{2}(c_1 - c_2) + \frac{b}{2}\left(c'_n - c'_1\right) \tag{4.20}$$

A similar treatment for the steady flow within the membrane will give:

$$J = \frac{dS}{dt} = e\left(c'_1 - c'_2\right) \tag{4.21}$$

$$J = \frac{dS}{dt} = e\left(c'_2 - c'_3\right), \tag{4.22}$$

$$\vdots$$

$$J = \frac{dS}{dt} = e\left(c'_{n-1} - c'_n\right) \tag{4.23}$$

where n is the number of (identical) barriers in the membrane and e is the rate constant inside the membrane.

By summing up equations (4.21) through (4.23) and dividing by n, we obtain:

$$J = \frac{dS}{dt} = \frac{e}{n}\left(c'_1 - c'_n\right) \tag{4.24}$$

Finally, by imposing the condition that the right-hand side of equation (4.20) equals the right-hand side of equation (4.24), an expression for $c'_1 - c'_n$ is obtained, which is substituted into equation (4.24) to obtain:

$$\frac{dS}{dt} = -PA(c_1 - c_2) \tag{4.25}$$

where

$$P = \frac{ea}{A(2e + bn)} \tag{4.26}$$

represents the permeability coefficient of the membrane.

As seen, equation (4.25) is of the same type as equation (4.12). In either case, the membrane permeability can be determined experimentally. It can be therefore concluded that both, Fick's laws as well as the above general considerations of potential energy barriers and rate of change in the number of molecules (or moles) between energy barrier maxima give the same results for the flux of substance across the membrane as a result of free diffusion.

Quiz 1. Plot the concentration profile across the membrane and from compartment 1 to compartment 2, corresponding to both cases shown in Fig. 4.4.

4.3 Osmosis and Osmotic Pressure

A particular case of *simple diffusion* is *osmosis*, which takes place when two solutions with different concentrations of solutes are separated by a *selective membrane*, that is, a membrane that permits the diffusion of solvent molecules but not of the solute (Fig. 4.5). Since we are exclusively interested in describing the process of osmosis in living matter, we shall refer here only to water as a solvent.

The fact that the solute concentration is lower in one compartment than in the other one can be reinterpreted as the "*concentration of water*" is lower in the compartment on the left-hand-side. Due to thermal agitation, the water molecules will spontaneously (i.e., with $\Delta G < 0$) diffuse from the compartment with more "concentrated" water towards the one with a lower water "concentration," where the level of the solution will increase as a result (Fig. 4.5). This process of solvent passive diffusion through a selective membrane is called *direct osmosis*. The direct osmosis is an *entropy-generating process* $(\Delta S > 0)$, having the tendency to equalize the solute (or solvent) concentrations among the two compartments.

Observation: One may indeed speak of the solvent (i.e., water) concentration. For instance, at $t = 4\,^{\circ}\mathrm{C}$, one mol $(\nu = 1)$ of pure water has a mass, $m = 18\,\mathrm{g}$, occupying a volume, $V = 18\,\mathrm{cm}^3$. The molar concentration of the pure water, $C_M = \nu/V$, is $C_M^{H_2O} \approx 55.55\,\mathrm{M}$.

We can write the expressions for the chemical potentials of water "dissolved" in the two compartments, also by taking pressure into account:

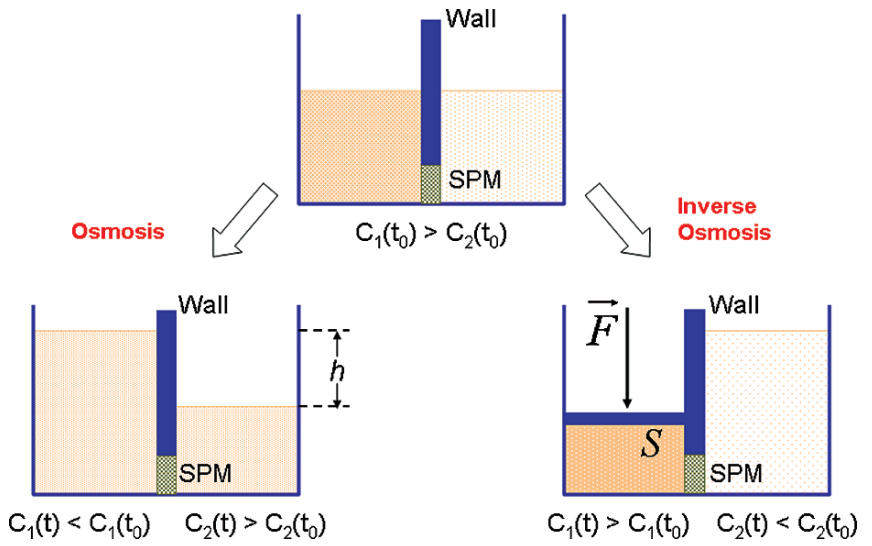

Fig. 4.5 Direct and inverse osmosis through a selective permeable membrane (SPM) separating two solutions with different initial solute concentrations, $C_1(t_0) > C_2(t_0)$.

$$\mu_1 = \mu_0 + RT \ln X_1^w + v^w(p_a + \rho^w g h_1) \tag{4.27}$$

$$\mu_2 = \mu_0 + RT \ln X_2^w + v^w(p_a + \rho^w g h_2) \tag{4.28}$$

where R is the ideal gas constant, T is the absolute temperature, ${X^1}_w$ and ${X^2}_w$ are the concentrations of water in mole fraction units, μ_0 is the standard chemical potential of water *dissolved* in pure water (i.e., the chemical potential of the pure solute in the same state of aggregation as the solvent), v^w is the partial molar volume of water, p_a is the atmospheric pressure, and $\rho^w g h_i (\mathrm{i} = 1,\ 2)$ is the hydrostatic pressure (where the density has been assumed equal between the two compartments).

In a hypothetical situation of imponderability (i.e., $g = 0$), the equality between the chemical potentials given by equations (4.27) and (4.28) gives at equilibrium:

$$X_1^w = X_2^w \tag{4.29}$$

However, in the presence of *gravity*, equal concentrations of solute between the two compartments cannot be achieved, since a *hydrostatic pressure* would counterbalance the process of water ascension in the compartment on the left-hand side, as given by:

$$X_1^w = X_2^w e^{v^w \rho^w g h / RT} \tag{4.30}$$

The hydrostatic pressure in this equation, $p_h = \rho^w g h$, is a measure of the "suction pressure," known as *osmotic pressure*, π, and which is exerted by the solution containing larger amount of solute onto the one containing less.

If one applies *external pressure*, $p_e = F/S$, on the more concentrated solution (Fig. 4.5) some of the water molecules will be forced to traverse the membrane towards the left (because the solute cannot pass through the membrane), reaching the first compartment. As a result, the concentrated solution will become more concentrated, while the dilute one will become even more diluted. At equilibrium, the following equation can be easily derived by including the external pressure term in equation (4.30):

$$X_1^w = X_2^w e^{v^w(-p_e + \rho^w g h)/RT} \tag{4.31}$$

This energy-consuming process ($\Delta G > 0$), called *reverse osmosis*, can be encountered in, e.g., some special glands of marine animals, where the salted water of seas and oceans is purified to be used as drinking water. This reverse-osmosis process is also used by humans to obtain *desalinated* water from salted sea water.

4.3.1 van't Hoff Laws

As has been shown by van't Hoff, there exists an interesting and productive analogy between *chemical solutions* and *gases* (van't Hoff, 1887; also reprinted in Kepner, 1979). Osmotic pressure of *ideal* (dilute) solutions is described by *van't Hoff laws*

which are equivalent to the laws of the ideal gas; in the case of very concentrated solutions and protein solutions (the correspondent of real gases), deviations from the van't Hoff laws occur, similar to the case of nonideal gases.

Let us consider a volume, V, of solution obtained by dissolving a mass, m_s, of solute with molecular mass, μ. The percentile concentration is $C_S = \frac{m_s}{V}$ at the absolute temperature, T. The osmotic pressure, π, of the solution satisfies a relation, formally identical with the *general equation* of state of the ideal gas:

$$\pi V = \frac{m_s}{\mu} RT \tag{4.32}$$

Using the definition of C_s it follows that:

$$\pi = \frac{C_S}{\mu} RT \tag{4.33a}$$

or, because $m_s/\mu = \nu$ and $\nu/V = C_M$ (molar concentration), one can write an equivalent relation:

$$\pi = C_M RT \tag{4.33b}$$

This last expression includes van't Hoff's laws for ideal solutions, which state that *the osmotic pressure*, π, of an *ideal solution* is: (a) directly proportional to the percentile solute concentration, C_S or molar concentration, C_M; (b) directly proportional to the absolute temperature, T, of the solute (an expected result, because the average thermal agitation energy of the molecules is proportional to the absolute temperature).

Osmotic pressure is an important parameter for biological fluids (cytosol, interstitial liquid, blood plasma, etc.). All these liquids must be compatible from the point of view of their osmotic pressures. The osmotic pressure of biological fluids is equal to the osmotic pressure of a *standard solution*, called *physiological saline solution*: an aqueous solution containing 0.145 M NaCl (or, approximately 9% NaCl) at pH=7 and room temperature.

All solutions having *the same osmotic pressure* as the *physiological saline* solution are called *isotonic* solutions. Other solutions are either *hypotonic*, when their osmotic pressure *is lower* than that of an isotonic solution, or *hypertonic*, in the opposite case.

When the cells are suspended in a *hypotonic aqueous solution*, the water molecules will enter the cells, due to osmosis (see above), increasing the cellular volume and provoking thus cellular *swelling* (Fig. 4.6). If the process is not stopped, the swelling will be followed by *cytolysis*, that is, by membrane rupture. A particular case of cytolysis is *hemolysis* (i.e., the lysis of erythrocytes). By using this phenomenon of hemolysis of erythrocytes suspended in water, for example, one can obtain *hemoglobin*.

If, on the contrary, the cells are suspended in *hypertonic solutions*, they will begin to *shrink* (Fig. 4.6), and, if not removed from the hypotonic solution, *plasmolysis* will occur, that is, the cytosol will be completely lost. In the case of plant cells or bacteria, the cell membranes can detach from the rigid cell walls, as a result of severe hypertonic treatment.

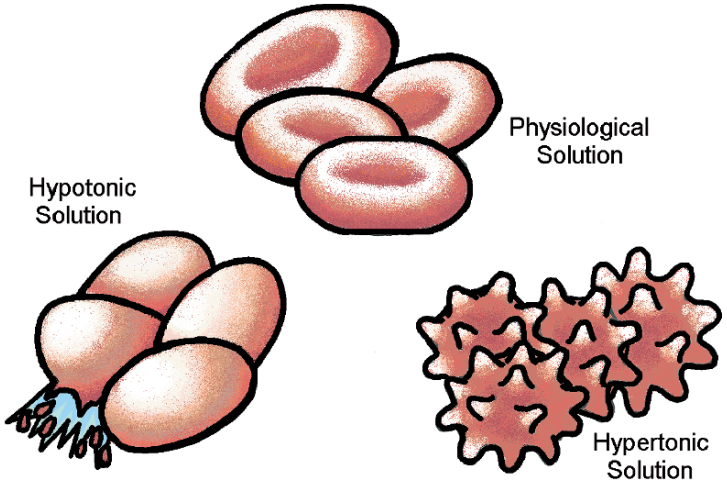

Fig. 4.6 The effect of hypotonic and hypertonic solutions on red blood cells.

Observation: In the case of electrolyte solutions, it is necessary to use in equation (4.33b) a corrected concentration, C^*_{MS} of the solute, which takes into account the *dissociation degree*, α, of the solute molecules, defined as the ratio between the dissociated molecules, N_D, and the total number, N, of initial solute molecules ($\alpha = N_D/N$). Due to the dissociation of the solute molecules, the number of osmotically active particles increases, and the osmotic pressure increases as a result. Considering the most frequent case of molecular dissociation in only two fragments, one can easily find that $C^*_{MS} = (1+\alpha)C_M$. In the case of complete dissociation ($\alpha = 1$, as in the case of NaCl), it results that $C^*_{MS} = 2\ C_M$. Therefore, the osmotic pressure of the *physiological saline solution* ($C_M = 0.145\,\text{M};\ \text{pH} = 7;\ T = 298.15\,\text{K}$), $\pi^* = 2\pi$, is equivalent with the osmotic pressure of a solution of an undissociated solute (e.g., glucose solution) that is two times more concentrated.

Quiz 2. Show that $C^*_{MS} = (1+\alpha)C_M$.

Quiz 3. Compute the osmotic pressure of the standard solution of physiological saline solution at room temperature, $t = 25\,^\circ\text{C}$.

4.3.2 Deviations from van't Hoff Laws

The *analogy* between solutions and gases *holds* even in the case of *real solutions and real gases*. For instance, one can adopt the *virial equation of state* from the theory of *real gases*, in order to describe the behavior of real solutions of macromolecules (e.g., proteins); in fact, there are several known virial equations of state, one of which is the following (Atkins and de Paula, 2002):

$$\frac{pV_M}{RT} = 1 + \frac{B}{V_M} + \frac{C}{V_M^2} + \ldots \tag{4.34}$$

where p is the gas pressure, V_M, the molar volume, R the gas constant, T, the absolute temperature, B and C constants. By using only the first terms of the right-hand side of equation (4.34), and using the notation $V_M = V/\nu$ (where $\nu = m_s/\mu$, and m_s is the solute mass) the virial equation of state for real macromolecular solutions is:

$$\pi V \approx \nu RT + \frac{\nu^2 RTB}{V} \tag{4.35}$$

Dividing both terms of equation (4.35) by V, and taking into account that $m_S/V = C_s$ (i.e., mass percentile concentration), one can write:

$$\pi \approx RT\left(\frac{C_s}{\mu}\right) + RTB\left(\frac{C_s}{\mu}\right)^2 \tag{4.36}$$

This last expression for the osmotic pressure of the real solutions can be used to estimate experimentally the *molecular mass* of a macromolecule by simply measuring the osmotic pressure (using an *osmometer*) of a series of solutions with different solute concentrations.

Quiz 4. Using $\pi/C \approx RT/\mu + aC$ (where $RTB/\mu^2 = constant = a$), show graphically how the molar mass, μ, of a macromolecule, can be determined by measuring the osmotic pressure of a series of solutions with different macromolecular concentrations (assume that the temperature is equal to the room temperature of 298.15 K).

4.3.3 Osmotic Pressure of Biological Liquids

The osmotic pressure of biological fluids (such as cytosol, blood, interstitial liquid, cerebrospinal liquid, synovial liquid, etc.) can be calculated according to "Dalton's law," i.e., π is equal to the sum of the *partial pressures* of the different components (ions, i, small molecules, m, and macromolecules, M):

$$\pi = \sum_{k=1}^{N_i} \pi_{ik} + \sum_{k=1}^{N_m} \pi_{mk} + \sum_{k=1}^{N_M} \pi_{Mk} \tag{4.37}$$

where N_i, N_m, N_M represent the number of ionic (Na^+, K^+, Cl^-, HCO_3^-, etc.), micro-molecular and macromolecular species in solution. The contribution of macromolecules is generally much less than that of ions and micro-molecules. In the case of the blood plasma, for example, the total osmotic pressure of about 7.6 bar comes mostly from ions and micro molecules (representing about 1% of its mass), while the macromolecules (representing 9% of the plasma mass) are exerting an osmotic pressure of only 0.037 bar ($\approx$28 mmHg).

The small pressure exerted by the macromolecules is called *colloid osmotic pressure*. The *colloid osmotic pressure* of the *interstitial liquid*, π_{IL} (i.e., the liquid bathing the tissue cells) is smaller than the plasma colloid osmotic pressure π_P.

Any tissue is irrigated by a complex network of capillary vessels through which blood flows, having practically the same colloid osmotic pressure as plasma. The wall of the blood capillaries (called *endothelial wall*) is impermeable to proteins but is permeable to water molecules, ions, and micro molecules. Therefore there is a difference between the colloid osmotic pressures across the capillary wall, called the *effective colloid osmotic pressure*, π_{ECO}, given by the relation:

$$\pi_{ECO} = \pi_P - \pi_{IL} \approx 22\,\text{mm Hg} > 0 \tag{4.38}$$

It is this "positive" effective colloid osmotic pressure that drives water molecules out of the interstitial space, diffusing through the capillary walls in order to equalize the global osmotic pressures of the two liquids.

4.3.4 The Cellular "Osmotic Pressure Menace"

The cell membrane is permeable to water molecules, ions and small molecules, but impermeable to macromolecules. It is interesting to note that the cell membrane is more permeable to water than to smaller ions, due to the existence of the specific *water channels*, called *aquaporins*, that prevent even protons from passing through (Lodish et al., 2004). Although the cell membrane is essential for proper functioning of the cell, it exposes the cell to a permanent danger: due to the selective transfer of water inside the cells, imposed by the difference in *the colloid osmotic pressure*, the cell is under a permanent threat to *swell* and to finally *burst*. This imminent "osmotic pressure menace" is fortunately actively avoided using an energy-consuming process of ion expulsion from cells, in order to maintain isotonicity of the cytosol. We shall see later that this active transport of ions is produced by certain molecular machines called *ionic pumps*, which use energy liberated from ATP hydrolysis.

Observation: In plant cells as well as in bacteria, fungi, and algae, this danger of cellular burst, although present, is counteracted by a special cell morphology: their plasma membrane is surrounded by *rigid walls*, so that, even when suspended in *hypotonic solutions*, these cells maintain their volume, due to the mechanical pressure exerted by their walls. This mechanical pressure that pushes the plasma membrane towards the rigid wall is called *turgor pressure*. Moreover, plant cells are endowed with vacuoles which contain concentrated electrolyte and are able to "absorb" water from the cytosol through osmosis.

One can also speak of "osmotic stress" induced by addition of inert osmolyte (e.g., polyethylene glycol) to any aqueous system, which generates compacting forces on membranes and other interesting biochemical and biophysical perturbations, as shown by Cohen and Highsmith (1997) (see also: http://*aqueous.labs.brocku.ca:osfile.html* and http://www. *mgsl.dcrt.nih.gov/docs/osmdata/osmdata.html*).

4.4 Facilitated Transport

As discussed in section 4.2, small essential ions (e.g., Na^+, K^+ and Cl^-) and small molecules (e.g., glucose) are not easily dissolved into the lipid bilayer and therefore present very small permeability coefficients. However, in reality they do permeate the membrane very efficiently, which suggests that an alternative modality of permeation is at work. Indeed, the flow of small ions and molecules into or out of the cells is *facilitated* by the existence of a special type of *transporters*. This process of transport through the membrane is called *facilitated transport* or *facilitated diffusion* and occurs spontaneously *down* the particles concentration gradients. Similar to the simple (passive) diffusion, facilitated diffusion is an exergonic process and a generator of entropy (i.e., $\Delta G < 0,\ \Delta S > 0$). Unlike the simple diffusion, however, facilitated diffusion is aided by specific *channel proteins* or by specific *carrier proteins* inserted in the cell membrane, and it is only encountered in biological systems.

4.4.1 Channel-Mediated Transport

Protein channels allow selective passage of a specific ion or molecule (e.g., Na^+, K^+, Cl^-, Ca^{2+}, H_2O, etc.), and are essentially *two-state structures* whose opening or closing are activated *chemically, electrically*, *mechanically*, or by *light*. There are *endogenous* ion channels, which are secreted by the cells in whose membranes they reside, as well as *exogenous* channels, which are formed in the plasma membrane by insertion of molecules secreted by other cells. A common example is the secretion of *gramicidin A* channel by the bacteria *Bacillus brevis* (Fig. 4.7). The gramicidin channel is a dimer formed by association across the membrane of two β*-helix* polypeptide strands (i.e., parallel β-strands, folded into a large helix). These exogenous channels are non-gated and permanently open; they allow passage of Na ions at a very high rate of $10^7\,Na^+/s$. Because of this, gramicidin channel inserted into the membranes of the target cells kills those cells by "erasing" their electrochemical gradients, which are vital for proper cell functioning. Therefore, gramicidin acts like an antibiotic, constituting a *chemical weapon* against the nutrient competitors of *Bacillus brevis*. The antibiotic activity of pore-forming molecules, such as gramicidin A, is exploited in pharmacology; several other pore-forming molecules (e.g., *nistatin, amfotericin B*) are used as *antifungal drugs*.

4.4.2 Carrier-Mediated Transport

Certain small molecules including sugars (glucose, fructose, mannose) and amino acids cannot diffuse through the cell membranes, because they are insoluble in the lipid matrix of the membrane, and can not be translocated through ion channels

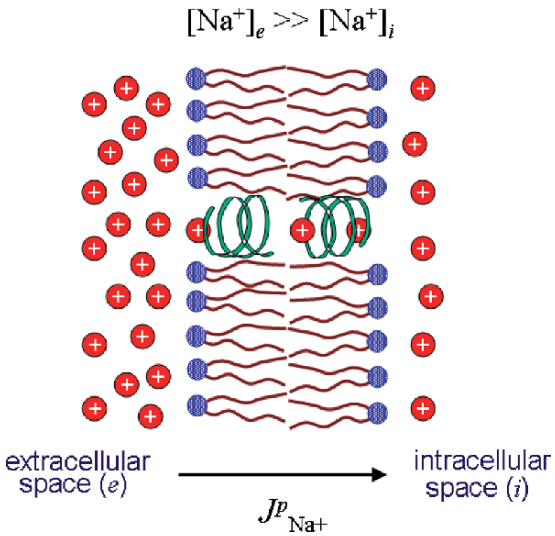

Fig. 4.7 The pore formed by two gramicidin molecules inserted into the membrane bilayer of the aggressed fungi cell. J^p_{Na} = passive flux of Na^+. $\oplus$ – sodium ions; Blue filled circles – polar heads of lipid molecules. Only Na ions are depicted, for the sake of simplicity.

either. These important molecules can still enter the cell through the plasma membrane via some specific carriers, called *uniporter* carriers. The process is driven by concentration gradients across the membrane, caused by consumption of the transported molecules in various metabolic reactions.

Observation: Although highly specific, carriers cannot function against concentration gradients.

As in the case of channels, there are *endogenous* as well as *exogenous carriers*. The latter type is secreted by certain bacteria and inserted into the membrane of a host cell. An example is *valinomycin*, an antibiotic secreted by *Streptomyces fulvissimus*. Due to its appreciable hydrophobicity, this carrier is able to insert itself into the membrane of target cells, inducing uncontrolled K^+ leakage from the cells. Therefore valinomycin, like gramicidin A, represents another natural *chemical weapon* in the struggle of cellular species for survival.

Unlike ionic channels, carriers undergo cyclic *conformational changes*, as they bind molecules from one side of the membrane and release them to the other side. This process of repeated binding–release of small molecules is time-consuming, which confers carrier-facilitated diffusion (10^2–10^4 ions/s) a diffusion rate which is several orders of magnitude lower than the channel-facilitated diffusion (10^7–10^8 ions/s).

A well-studied example of endogenous carrier is the *glucose carrier* of the erythrocyte (or red blood cell), which is called GLUT1 (Lodish et al., 2004), and has two distinct conformational states: one associated with the binding of glucose on the extracellular side of the membrane and another when the glucose is released on

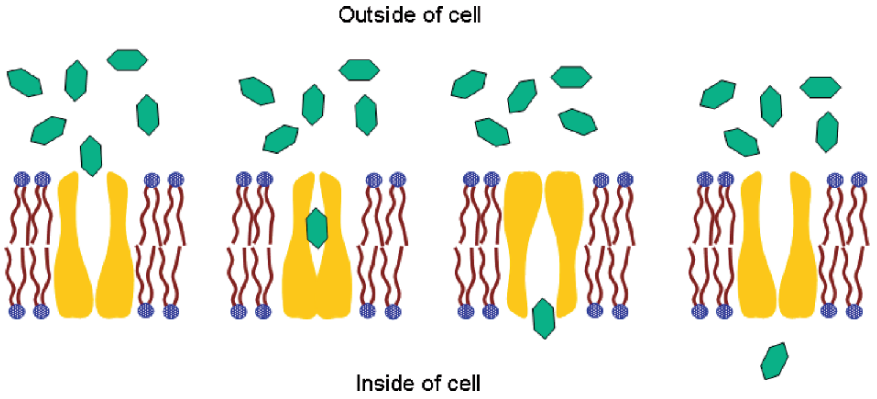

Fig. 4.8 Schematic representation of the mode of operation of a glucose carrier embedded in a phospholipid matrix. The hexagonal shapes stand for sugar molecules.

the intracellular side (Fig. 4.8). Although glucose is continuously transported across the membrane into the erythrocyte, it is not accumulated inside the cell because it is rapidly transformed into *glucose 6-phosphate* by the glucose metabolism. Therefore the GLUT1 carrier functions ceaselessly. It is interesting to note that there are two other competitors for GLUT1 binding site: D-galactose and D-mannose, which, however, bind to GLUT 1 with lesser affinities compared to glucose.

By analogy to enzymatic reactions, the process of facilitated diffusion of a sugar, S, mediated by a carrier, C, is described by:

$$S + C \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} SC \xrightarrow{k_{dis}} S \tag{4.39}$$

where k_1 and k_{-1} are reaction rate coefficients for the external side of the membrane, and k_{dis} represents the rate constant of complex dissociation at the intracellular face of the membrane.

From the reaction (4.39) one can derive the rate of SC complex formation,

$$J_{C\to SC} = \frac{d[SC]}{dt} = k_1[S][C] \tag{4.40}$$

as well as the rate of complex dissociation,

$$J_{SC\to C} = \frac{d[SC]}{dt} = (k_{-1} + k_{dis})[SC] \tag{4.41}$$

where $[X]$ = molar concentration (measured in $\mathrm{mol/dm^3} = \mathrm{M}$) of the X species.

In a steady-state, $J_{C\to SC} = J_{SC\to C}$ and one can write:

$$k_1[S][C] = (k_{-1} + k_{dis})[SC] \tag{4.42}$$

or

$$\frac{[S][C]}{[SC]} = \frac{k_{-1} + k_{dis}}{k_1} = K_M \tag{4.43}$$

where K_M represents the *Michaelis constant*.

The concentration, $[C]$, of the *free carrier* molecules is given by the obvious relation:

$$[C] = [C]_T - [SC] \tag{4.44}$$

where $[C]_T$ represents the total concentration of the carrier molecules. Substituting $[C]$ from (4.44) into (4.43), and after rearranging, one obtains:

$$[SC] = [C]_T \frac{[S]}{[S] + K_M} \tag{4.45}$$

From (4.45) it follows immediately that the rate of S release on the intracellular side of the membrane into the cell, or the *flux of facilitated diffusion* is given by:

$$J_{FD} \equiv \frac{d[S]}{dt} = k_{dis}[SC] = k_{dis}[C]_T \frac{[S]}{[S] + K_M} (\text{mol/s}) \tag{4.46}$$

From the last expression it follows that: (i) if $[S] = 0$, then $J_{FD} = 0$; (ii) if $[S] \ll K_M$, then, J_{FD} is directly proportional to $[S]$; (iii) if $[S] \to \infty$, that is, when sugar is in excess, then $J_{FD} \to k_{dis}[C]_T = J_{FD}^{Max}$, in this case all the carriers being saturated with sugar molecules; (iv) finally, if $[S] = K_M$, then $J_{FD} = J_{FD}^{Max}/2$. Therefore, K_M represents that sugar concentration for which the flux of the facilitated diffusion is *half of the maximum flux*.

The flux dependence on the concentration of solute (sugar, in this case) is given comparatively in Fig. 4.9 for carrier-facilitated diffusion and simple diffusion.

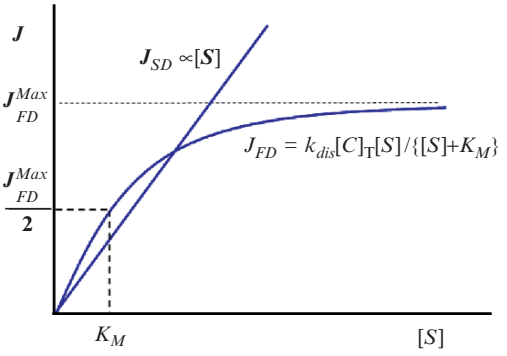

Fig. 4.9 Qualitative dependence of the simple diffusion flux (J_{SD}) and the facilitated diffusion flux (J_{FD}) on the concentration $[S]$ of the transported molecules.

4.4.3 Main Characteristics of Facilitated Transport

The two types of facilitated transport described above present the following features:

1. They are both *performed passively*, without energy consumption and driven by a concentration and/or electrical gradient
2. The *rate* of facilitated diffusion is *much higher* than the simple diffusion through lipid bilayers for a given substance
3. Both processes are *highly specific*, in the sense that a certain type of molecule can be translocated only by a given type of channel or carrier
4. Their rate of transport presents *saturation* as the small molecule concentration is increased, due the limited number of channels and/or carriers, within the cell membrane
5. Both types present *competition*, that is, the presence of other types of molecules capable to bind to the channel or carrier decreases the transport rate of the molecule of interest
6. They may be blocked by *inhibitors* (for instance, the Na^+ channels of the axon membranes can be blocked by the toxins *tetrodotoxin*, TTX, and *saxitoxin*, STX)

Observation: Under certain conditions, the saturation plateau can be increased or decreased by inserting transporters into or eliminating them from the membranes, as it is done for instance in the treatment of certain diseases.

4.5 Active Ion Transport

Living cells maintain a huge ion concentration disparity between their intracellular and extracellular faces of the membrane. As a general though not absolute rule, the concentration of Na^+, Cl^- and Ca^{2+} is much higher *outside* the cells, while the K^+ concentration is much higher *inside* the cells. Passive diffusion processes discussed above have the tendency to equalize this strongly antientropic ion distribution. However, with some minor fluctuations, this asymmetry is actively maintained by the cell, this being a *sine qua non* condition for cellular viability.

There are specific biological mechanisms by which ions are transported against their electrical and chemical gradients (collectively termed *electrochemical gradients*), thereby ensuring ionic concentration asymmetry between both sides of the cell membrane; these mechanisms are called *active transport*. This type of solute transport (Fig. 4.10) against concentration gradients is performed with consumption of cellular energy (i.e., $\Delta G > 0$ for the solute) and generation of entropy ($\Delta S < 0$ for the solute). Most cells are using up to one third of their ATP reserve in order to maintain active transport, while *excitable cells* such as neurons, which will be discussed in chapter 7, consume even more – approximately *three quarters* of their ATP reserve. This kind of active transport is usually termed *primary* active transport; there is also a type of transport called *secondary* active transport, which is driven by concentration gradients of a solute that has been created by primary active transport.

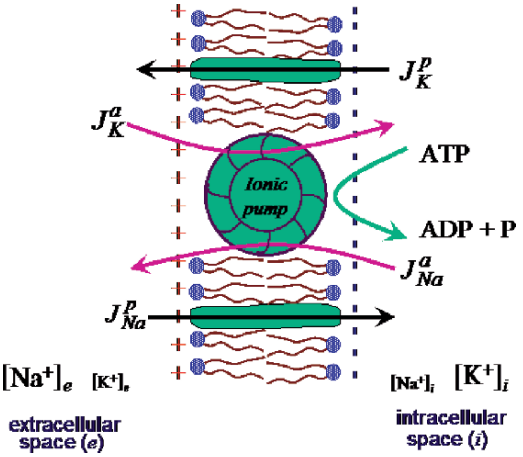

Fig. 4.10 Schematic representation of a membrane ionic pump and its function. Significance of symbols: J_{Na}^a, J_K^a, J_{Na}^p, and J_K^p, active (superscript a) and passive (superscript p) fluxes of Na^+ and K^+ through protein specific channels, depicted in green; $[Na^+]$ and $[K^+]$, ion concentrations (the different font sizes signify that the concentration is comparatively large or small). Purple filled circles are the polar heads of the membrane lipid bilayers. Electric polarization of the membrane is produced by net positive (outside the cell) and negative (inside the cell) charges.

The main characteristics of the primary active transport can be summarized as follows:

1. It is a highly endergonic process ($\Delta G > 0$)
2. It is absent from dead cells
3. It depends on temperature to a much higher degree than it does simple passive diffusion

> **Observation:** In order to characterize the influence of the temperature on the rate of a process (physical, chemical, biological), occasionally one uses the so-called *temperature quotient*, Q_{10}, given by:
>
> $$Q_{10} = J_{t+10}/J_t \tag{4.47}$$
>
> where J_t is the rate of the process at a temperature, t, while J_{t+10} is its rate at $t + 10\,^\circ\mathrm{C}$. Interestingly, for most physical and chemical processes, this quotient varies over the interval [2, 3], while for biological processes, $Q_{10} > 3$.

4. It may be indirectly diminished by certain *metabolic inhibitors* (such as cyanides, azides, dinitrophenols) and by hypoxia (i.e., reduced oxygen supply), which impede ATP synthesis
5. It may be blocked by specific inhibitors including *cardiotonic steroids* (e.g., ouabain and digitoxigenin) which have a high affinity for ionic pumps (Berg et al., 2002)

> **Observation:** It has been known for a very long time that the cardiotonic steroids ouabain and digitoxigenin (Stryer, 1988) exert pharmacological effects on the heart by increasing the contraction force of the cardiac muscle. *Cardiotonic steroids* are used clinically in the treatment of congestive heart failure.

Although the specific biological process responsible for actively "pumping" Na^+ into and K^+ out of the erythrocyte ghosts was first observed long time ago (Gardos cited by Stein, 1990), detailed knowledge of the "machines" (called later *ionic pumps*) performing such functions, in all the living cells, is still being accumulated.

The study of structure and mechanisms of action of ionic pumps continues to be an active area of research to these days, as will be discussed in more details in chapter 7.

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