2.6 Active Transport: Pumps and Exchangers

Learning Objectives

- Write the equation for the electrochemical potential for an ion
- Give the approximate concentration of the major ions inside and outside of a heart cell
- Be able to calculate the free energy change for ion movement across the cell membrane under given conditions of membrane potential and ion concentrations
- Be able to calculate the free energy change for ion transport when coupled to other processes
- Distinguish between active and passive transport
- Distinguish between primary and secondary active transport
- Give an example of a primary and secondary active transport mechanism
- Distinguish between P-type, V-type, F-type, and ABC-type active transporters
- Define the terms: symport, cotransporter, antiport, and exchanger

THE ELECTROCHEMICAL POTENTIAL DIFFERENCE MEASURES THE ENERGETICS OF ION PERMEATION

Here we consider the transport of four ions: Na^+, K^+, Cl^-, and Ca^{2+}, across the surface membrane of heart cells at rest. These ions have different concentrations inside and outside of the cell, and are also subjected to electrical forces because there is a potential difference across the cell membrane and thus an electric field within the membrane. The cardiomyocytes maintain a resting **membrane potential**. We will learn later how this is established, but for now it is enough to know that there is a separation of charge in the outside and inside compartments. At rest, there is an accumulation of negative charges inside the cell and positive charges outside. This separation of charges gives rise to the membrane potential, which is always taken as the difference between the electrical potential inside and outside the membrane:

$$\Delta\psi = \psi_i - \psi_o \quad [2.6.1]$$

The membrane potential is sometimes identified with the variables ψ, V_m, or E_m. The situation is described in Figure 2.6.1.

Let us first take the case of Na^+. The concentration of Na^+ outside the cell is about 145 mM and inside the cell it is 12 mM. The higher concentration outside of the cell favors a net Na^+ flow from outside to inside, driven by diffusion. Further, the negative potential inside the cell also favors Na^+ movement into the cell. We write the free energy change per mole for the movement of Na^+ from out to in as

$$\Delta\mu = \mu_i - \mu_o \quad [2.6.2]$$

Recall here that the free energy change is the free energy of the final state (inside the cell, in this case) minus the free energy of the initial state (outside the cell in this case). When we are dealing with the free energy per mole, we write μ. This is an intensive property which is defined by the conditions and not by the extent of the cell or its membrane or of the amount of material being transported. The free energy itself is an extensive property that depends on how much material is being transported. We can insert the definition of electrochemical potential that we justified earlier (see Eqn [1.7.14]):

$$\mu_x = \mu_x^0 + RT \ln C_x + z_x \mathfrak{F} \psi_x \quad [2.6.3]$$

where the subscript x denotes substance x to avoid confusion with the subscript i or o which denotes the inside or outside of the cell, respectively. Substituting in for the conditions of the inside and outside of the cell, we find

$$\begin{aligned}\Delta\mu &= \mu_i - \mu_o \\ &= \mu^0_{Na_i} + RT \ln[Na^+]_i + \mathfrak{F}\psi_i - \mu^0_{Na_o} - RT \ln[Na^+]_o - \mathfrak{F}\psi_o \\ &= RT \ln \frac{[Na^+]_i}{[Na^+]_o} + \mathfrak{F}(\psi_i - \psi_o)\end{aligned} \quad [2.6.4]$$

The standard free energy per mole (μ^0) cancels out because it is independent of condition. The μ^0 is the part of the electrochemical potential that incorporates the chemical energy involved in the formation of bonds. Since in this case there is no chemical transformation (the Na^+ ion is not chemically transformed in any way; it is simply transported from one side of the membrane to the other), $\Delta\mu^0 = 0$. Also, the z in the formula for electrochemical potential is the integer charge, which is +1 for the Na^+ ion. Remember here that ln is the natural logarithm, not logarithm base 10.

The calculation of $\Delta\mu$ for the conditions of the cell shows that $\Delta\mu < 0$ (see Example 2.6.1). This means that the reaction as written is spontaneous: it will occur in the direction written **passively**, without additional forces. Our analysis of the energy does not tell us

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anything about how fast the process will occur, because the thermodynamic analysis is independent of the mechanism, and the mechanism is what determines the rate. What we can say, however, is that a channel for Na^+ that opens in this membrane will cause a rapid inflow of Na^+ from the extracellular fluid into the cell. Net flow would not occur in the opposite direction. What we can also say is that if the membrane has any non-zero permeability to Na^+, the flux will be from the extracellular fluid (the outside) into the cell.

Now let us consider what happens with Ca^{2+}. In a completely analogous way, we write the change in free energy for Ca^{2+} entry into the cell as

$$\begin{aligned}\Delta\mu &= \mu_i - \mu_o \\ &= RT\ln[Ca^{2+}]_i + 2\mathfrak{F}\psi_i - RT\ln[Ca^{2+}]_o - \mathfrak{F}\psi_o \\ &= RT\ln\frac{[Ca^{2+}]_i}{[Ca^{2+}]_o} + 2\mathfrak{F}(\psi_i - \psi_o)\end{aligned}$$

[2.6.5]

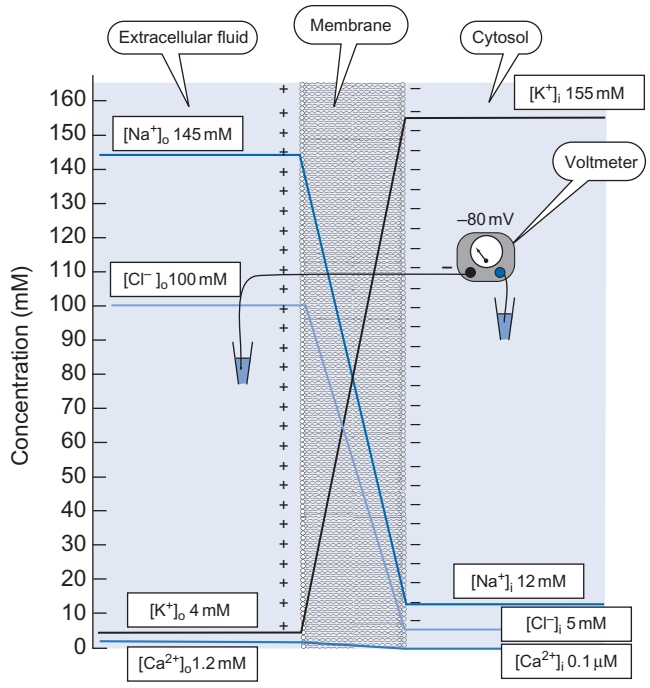

FIGURE 2.6.1 Concentrations of Na^+, K^+, and Ca^{2+} and the resting membrane potential across the resting cardiac muscle cell membrane. The subscript "o" refers to the "outside" of the cell; "i" denotes the "inside" compartment.

Here the 2 in the equation arises because Ca^{2+} has two positive charges per ion. The two charges correspond to z_x in Eqn [2.6.3]. The electrical force on a Ca^{2+} ion is twice the force on an Na^+ ion in the same electric field and thus movement produces twice the energy.

Note that this process (see Example 2.6.2) has a negative $\Delta\mu$, so it also occurs spontaneously. In this case, the free energy change is much more negative. This is due to the fact that the concentration gradient for Ca^{2+} contains more energy and the electrical energy gain is twice as great because each Ca^{2+} ion has twice the charge of an Na^+ ion. A channel for Ca^{2+} on the cell membrane would let Ca^{2+} into the cell under these conditions.

Now let us calculate the free energy change for K^+ entry into the cell. The formula is:

$$\begin{aligned}\Delta\mu &= \mu_i - \mu_o \\ &= RT\ln[K^+]_i + \mathfrak{F}\psi_i - RT\ln[K^+]_o - \mathfrak{F}\psi_o \\ &= RT\ln\frac{[K^+]_i}{[K^+]_o} + \mathfrak{F}(\psi_i - \psi_o)\end{aligned}$$

[2.6.6]

In this case, $\Delta\mu$ is positive (see Example 2.6.3). This means that K^+ under these conditions does not passively enter the cell. Rather, the spontaneous process is K^+ *exit* from the cell. If a channel specific for K^+ was to open in the membrane, K^+ would leave the cell.

In this last example, let us calculate the free energy associated with Cl^- entry into the cell. Again we write the difference in the electrochemical potentials:

$$\begin{aligned}\Delta\mu &= \mu_i - \mu_o \\ &= \mu^0_{Cl_i} + RT\ln[Cl^-]_i + (-1)\mathfrak{F}\psi_i - \mu^0_{Cl_o} + RT\ln[Cl^-]_o \\ &\quad - (-1)\mathfrak{F}\psi_o \\ &= RT\ln\frac{[Cl^-]_i}{[Cl^-]_o} - \mathfrak{F}(\psi_i - \psi_o)\end{aligned}$$

[2.6.7]

Note that the valence on the Cl^- ion is negative and it is entered that way in the equation. According to the calculation (see Example 2.6.4), at rest $[Cl^-]$ is distributed at equilibrium across the cell membrane. That is, there is no free energy change for Cl^- transport across the resting muscle membrane.

These calculations show how the electrochemical potential calculates the energetics of transport, and further

EXAMPLE 2.6.1 Free Energy of Na^+ Transport

For the conditions shown in Figure 2.6.1, calculate the free energy of transport of Na^+ from outside to inside the cardiomyocyte.

Inserting the values for the concentrations into Eqn [2.6.4] and using $R = 8.314\ J\ mol^{-1}\ K^{-1}$ ($=1.987\ cal\ mol^{-1}\ K^{-1}$; $1\ J = 0.239\ cal$) and $T = 310\ K$, and remembering that the faraday ($\mathfrak{F}$) is $9.649 \times 10^4\ C\ mol^{-1}$, we get

$$\begin{aligned}\Delta\mu &= 8.314\ J\ mol^{-1}\ K^{-1} \times 310\ K \ln[(12 \times 10^{-3}\ M)/(145 \times 10^{-3}\ M)] \\ &\quad + 9.649 \times 10^4\ C\ mol^{-1} \times (-0.080\ V) \\ &= -6.42\ kJ\ mol^{-1} - 7.72\ kJ\ mol^{-1} \\ &= \mathbf{-14.14\ kJ\ mol^{-1} = -3.38\ kcal\ mol^{-1}}\end{aligned}$$

EXAMPLE 2.6.2 Free Energy of Ca^{2+} Transport

For the conditions shown in Figure 2.6.1, calculate the free energy of transport of Ca^{2+} from outside to inside the cardiomyocyte.

Inserting the values for the concentrations and membrane potential into Eqn [2.6.5], we get:

$$\begin{aligned}\Delta\mu &= 8.314\ \text{J mol}^{-1}\ \text{K}^{-1} \times 310\ \text{K} \ln[(0.1\times10^{-6}\ \text{M})/(1.2\times10^{-3}\ \text{M})] \\ &\quad + 2 \times 9.649 \times 10^{4}\ \text{C mol}^{-1} \times (-0.080\ \text{V}) \\ &= -24.2\ \text{kJ mol}^{-1} - 15.4\ \text{kJ mol}^{-1} \\ &= \mathbf{-39.6\ kJ\ mol^{-1}} = \mathbf{-9.46\ kcal\ mol^{-1}}\end{aligned}$$

EXAMPLE 2.6.3 Free Energy of K^+ Transport

For the conditions shown in Figure 2.6.1, calculate the free energy of transport of K^+ from outside to inside the cardiomyocyte.

We can insert the values for $[K^+]_i = 155 \times 10^{-3}$ M and $[K^+]_o = 4 \times 10^{-3}$ M into Eqn [2.6.6] to calculate $\Delta\mu$:

$$\begin{aligned}\Delta\mu &= 8.314\ \text{J mol}^{-1}\ \text{K}^{-1} \times 310\ \text{K} \ln[(155 \times 10^{-3}\ \text{M})/(4 \times 10^{-3}\ \text{M})] \\ &\quad + 9.649 \times 10^{4}\ \text{C mol}^{-1} \times (-0.080\ \text{V}) \\ &= -9.43\ \text{kJ mol}^{-1} - 7.72\ \text{kJ mol}^{-1} \\ &= \mathbf{+1.71\ kJ\ mol^{-1}} = \mathbf{+0.41\ kcal\ mol^{-1}}\end{aligned}$$

EXAMPLE 2.6.4 Free Energy of Cl^- Transport

For the conditions shown in Figure 2.6.1, calculate the free energy of transport of Cl^- from outside to inside the cardiomyocyte.

We can insert the values for $[Cl^-]_i = 5 \times 10^{-3}$ M and $[Cl^-]_o = 100 \times 10^{-3}$ M into Eqn [2.6.7] to calculate $\Delta\mu$:

$$\begin{aligned}\Delta\mu &= 8.314\ \text{J mol}^{-1}\ \text{K}^{-1} \times 310\ \text{K} \ln[(5 \times 10^{-3}\ \text{M})/(100 \times 10^{-3}\ \text{M})] \\ &\quad - 9.649 \times 10^{4}\ \text{C mol}^{-1} \times (-0.080\ \text{V}) \\ &= -7.72\ \text{kJ mol}^{-1} + 7.72\ \text{kJ mol}^{-1} \\ &= \mathbf{0\ kJ\ mol^{-1}} = \mathbf{0\ kcal\ mol^{-1}}\end{aligned}$$

show that both the electrical and diffusive forces enter into the equations that determine the direction of ion flow. Things get a bit more complicated and interesting when ion flow is coupled to other processes.

ACTIVE TRANSPORT MECHANISMS LINK METABOLIC ENERGY TO TRANSPORT OF MATERIALS

All of the ion movements we have discussed so far involve movement "down" the electrochemical gradient: the free energy was higher for the initial condition than for the final condition, and the free energy change $\Delta\mu = \mu_{final} - \mu_{initial}$ is negative ($\Delta\mu < 0$). Passive diffusion through pores or the lipid bilayer, carriers, and channels are all passive. This does not mean that energy is not involved. What it means is that the energy does not derive from metabolism. The energy comes from the solutions themselves. However, cells also concentrate some materials by moving them from a region of low electrochemical potential to a region of higher electrochemical potential. This movement has $\Delta\mu > 0$. It can occur spontaneously only when the positive $\Delta\mu$ for transport is coupled to another process with a more negative $\Delta\mu$. Typically this process is ATP hydrolysis.

Primary active transport moves materials against an electrochemical gradient by the direct involvement of ATP hydrolysis. Examples of molecules that are involved in active transport include the **ion pumps**: Na, K-ATPase, Ca-ATPase, and H-ATPase.

Secondary active transport moves materials against an electrochemical gradient by the indirect involvement of ATP hydrolysis. ATP is used to establish an electrochemical gradient for something, usually Na^+, and the energy stored in the electrochemical gradient for Na^+ is then used to pump material "uphill." Examples of secondary active transport are the **Na–glucose cotransport** in the intestinal epithelium and renal proximal tubule and the **Na–Ca exchange** in the heart surface membrane.

NA,K-ATPASE IS AN EXAMPLE OF PRIMARY ACTIVE TRANSPORT

The analysis of free energy changes on ion movement that we performed earlier in this chapter indicated that

EXAMPLE 2.6.5 Calculate the Free Energy for Operation of Na,K-ATPase

For the conditions of the cell, calculate the free energy for the Na,K-ATPase.

We have already calculated that $\Delta\mu$ for ATP hydrolysis is -57.1 kJ mol^{-1} (see Chapter 1.7).

We have calculated $\Delta\mu$ for Na_o^+ going into the cell: $\Delta\mu_{Na_o \rightarrow Na_i} = -14.14$ kJ mol^{-1}. This is the opposite process of what the pump does. Thus $\Delta\mu$ for Na^+ *exit* is $\Delta\mu_{Na_i \rightarrow Na_o} = +14.14$ kJ mol^{-1}.

We also calculated $\Delta\mu$ for K^+ entry as $\Delta\mu_{K_o \rightarrow K_i} = +1.71$ kJ mol^{-1}.

Inserting the values for $\Delta\mu$, we get

$$\Delta\mu_{Na,K\text{-ATPase}} = -57.1 \text{ kJ mol}^{-1} + 3 \times 14.14 \text{ kJ mol}^{-1} + 2 \times 1.71 \text{ kJ mol}^{-1} = \mathbf{-11.26 \text{ kJ mol}^{-1}}$$

the distribution of Na^+, K^+, and Ca^{2+} is far from equilibrium. This implies that the cell actively maintains these concentrations away from equilibrium, or else the equilibrium distribution would eventually occur. The mechanism responsible for maintaining the resting concentrations of Na^+ and K^+ is the Na–K pump. This pump moves three Na^+ ions out of the cell at the same time that it transports two K^+ ions into the cell. The movement of these ions is coupled to the hydrolysis of ATP. The whole process is written as

$$[2.6.8] \quad ATP + 3Na_i^+ + 2K_o^+ \Rightarrow ADP + Pi + 3Na_o^+ + 2K_i^+$$

The $\Delta\mu$ for this entire process is equal to the sum of the $\Delta\mu$ for three separate processes:

$$[2.6.9a] \quad ATP \Rightarrow ADP + Pi$$

$$[2.6.9b] \quad 3Na_i^+ \Rightarrow 3Na_o^+$$

$$[2.6.9c] \quad 2K_o^+ \Rightarrow 2K_i^+$$

The overall free energy for this coupled process is written as

$$\Delta\mu_{Na,K\text{-ATPase}} = \Delta\mu_{ATP \Rightarrow ADP+Pi} + 3\Delta\mu_{Na_i \Rightarrow Na_o} + 2\Delta\mu_{K_o \Rightarrow K_i} \quad [2.6.10]$$

Recall that $\Delta\mu$ is the free energy per mole. In this case, it is the free energy *per mole of completed reaction* with the **stoichiometry** given by the overall reaction in Eqn [2.6.8].

$\Delta\mu_{Na,K\text{-ATPase}} = -11.26$ kJ mol^{-1} (see Example 2.6.5) means that the net change in free energy per mole of reaction, not per mole of Na^+ or K^+ or ATP, is -11.26 kJ. This is excess free energy of ATP hydrolysis beyond that required to transport Na^+ and K^+. ATP hydrolysis has a total of 57.1 kJ of energy per mole of ATP that can be harnessed to do work, and the Na,K-ATPase uses 45.84 kJ of energy per mole of reaction to do electrochemical work, under cell conditions. According to this result, there is enough energy in ATP hydrolysis to drive the Na,K-ATPase reaction. These calculations hold for the resting cell under the conditions we have investigated. The free energy for the Na,K-ATPase reaction changes with changes in cellular $[Na^+]$, $[K^+]$, $\Delta\psi$, [ATP], [ADP], or [Pi]. Changes in [ATP], [ADP], or [Pi] alter the energy available to the Na,K-ATPase to do work. Changes in $[Na^+]$, $[K^+]$, or $\Delta\psi$ alter the energy necessary for transport.

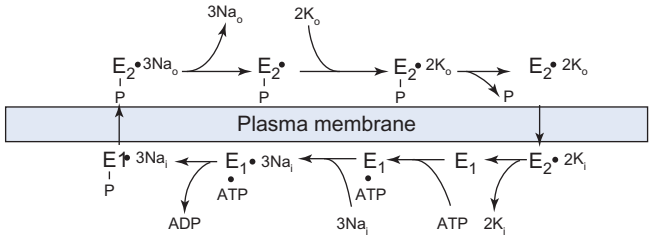

FIGURE 2.6.2 Modified post-Albers scheme for the reaction mechanism of Na,K-ATPase. Follow the reaction scheme in the direction of the arrows and you will see that the net reaction is the hydrolysis of ATP and the transport of three Na^+ ions from in to out and two K^+ ions from out to in. The processes are coupled in the reaction mechanism of the pump. The pump cannot hydrolyze ATP without binding Na^+ and K^+ in sequence. Neither can Na^+ be transported without K^+ transport and ATP hydrolysis. The coupling is made possible in part by the phosphorylation of the enzyme at an aspartic acid residue. This is shown by E–P in the diagram. The formation of a phosphoenzyme is common to the P-type active transport pumps.

The negative $\Delta\mu$ that we have calculated for the Na,K-ATPase indicates that the Na,K-ATPase reaction will occur spontaneously, but it will not tell us at what rate. The rate is a consequence of the mechanism of the pump.

NA,K-ATPASE FORMS A PHOSPHORYLATED INTERMEDIATE

The mechanism of ion pumps is generally complicated. It is useful to think of the enzyme as being characterized by a limited number of conformations to which we can give identifying labels. The reaction mechanism is then viewed as the sequential steps that occur among these conformations to achieve ATP hydrolysis and ion transport. Transformations between these conformations are determined by **rate constants**. Each step has two rate constants, one for the forward and the other for the reverse reaction. A simplified scheme for Na,K-ATPase is shown in Figure 2.6.2.

THE NA,K-ATPASE IS *ELECTROGENIC*

The overall reaction of the Na,K-ATPase shown in Eqn [2.6.8] indicates the stoichiometry of 3 Na^+ being transported out of the cell and 2 K^+ ions being transported into the cell. Thus the numbers of charges moving in

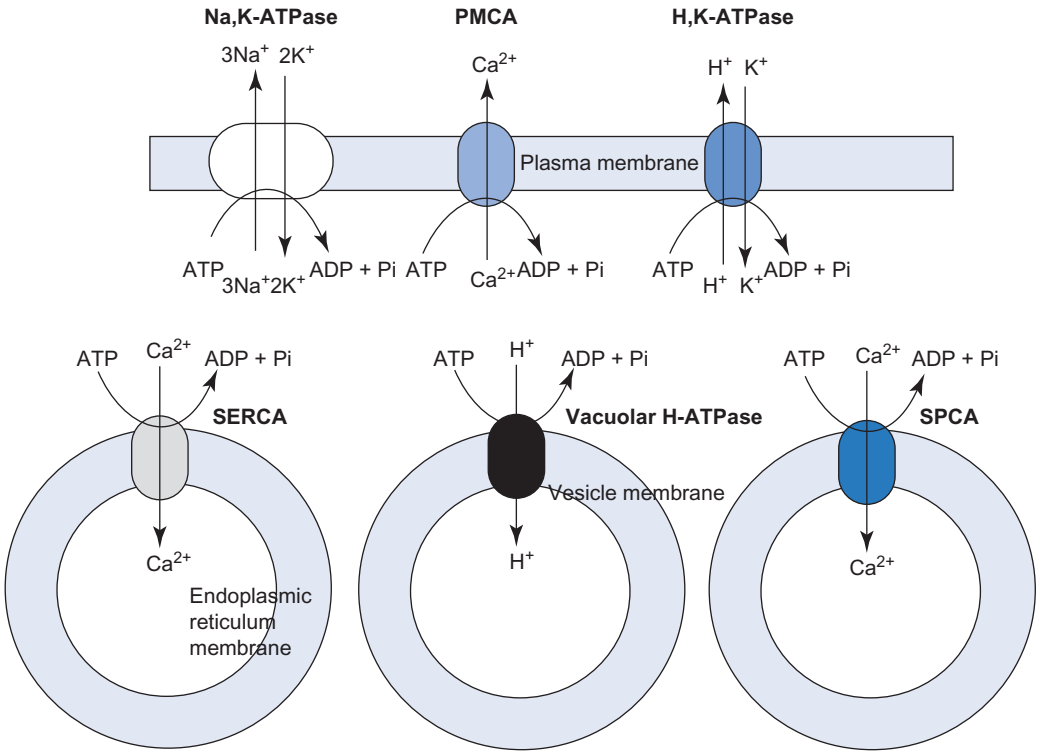

FIGURE 2.6.3 Some types of primary active transport mechanisms. Some are located on the plasma membrane of specific cell types; others, such as the smooth endoplasmic reticulum Ca-ATPase (SERCA), are located on subcellular organelles. All of the primary active transporters hydrolyze ATP. PMCA is the plasma membrane Ca-ATPase; SERCA is the smooth endoplasmic reticulum Ca-ATPase; SPCA is the secretory pathway Ca-ATPase.

the two directions are not equal, and each turnover of the pump corresponds to the movement of one net positive charge out of the cell. Thus the operation of the pump involves a transmembrane current and a net separation of charge. For this reason, the pump is called *electrogenic*—it generates an electric current and produces an electrical potential. As we will see later, its contribution to the resting membrane potential is small, but it is not zero.

THERE ARE MANY DIFFERENT PRIMARY ACTIVE TRANSPORT PUMPS

There are a variety of primary active transport pumps encoded by the human genome. These can be classified into four major groups.

P-type ATPases: These all form phosphorylated intermediates in the pump mechanism, like that shown for the Na,K-ATPase in Figure 2.6.2. Among these are **gastric H^+-ATPase** that is responsible for acidification of the stomach contents; **Na^+,K^+-ATPase** that is responsible for maintaining ionic gradients in most cells; **PMCA** (for **p**lasma **m**embrane **c**alcium ATPase) responsible for pumping Ca^{2+} out of cells; the **SERCA** family of pumps, where SERCA stands for **s**mooth **e**ndoplasmic **r**eticulum **C**a^{2+}ATPase, which is responsible for removing Ca^{2+} from the cytosol of a variety of cell types and placing it in storage in internal sacs within cells; and **SPCA**, the **s**ecretory **p**athway **C**a-ATPase, which pumps Ca^{2+} and other ions, such as Mn^{2+} and Zn^{2+}, into the Golgi to bind to secretory proteins. The SPCA is distinguishable functionally from the SERCA pumps because the SERCA pumps are inhibited by thapsigargin at low concentrations whereas SPCA is not. Figure 2.6.3 shows some of these primary active transporters.

V-type ATPases: Membranes of lysosomes and secretory vesicles contain a vacuolar-type H^+-ATPase that pumps H^+ ions from the cytoplasm into the vesicles. This V-type H^+-ATPase differs from the gastric H^+-ATPase in that it does not require K^+. The structure and mechanism of V-type ATPases differs from the P-type active transporters.

F-type ATPases: These are more commonly referred to as ATP-synthetases, because they usually work in the reverse mode to make ATP rather than hydrolyze it for the purpose of transport. The main example in the human is the F_0F_1ATPase of the inner mitochondrial membrane, which is discussed in Chapter 2.10. It uses the electrochemical gradient of H^+ to make ATP, but it can also hydrolyze ATP.

ABC transporters: The ABC here stands for "ATP-**b**inding **c**assette." This is a large family of proteins that engage in the primary active transport of a wide variety of solutes.

THE NA–CA EXCHANGER AS AN EXAMPLE OF SECONDARY ACTIVE TRANSPORT

According to our earlier calculation, $\Delta\mu$ for Ca^{2+} entry into the heart muscle cell was -39.6 kJ mol^{-1}. If left to

itself Ca^{2+} would slowly leak into the cell and disturb the resting $[Ca^{2+}]_i$. Heart cells have at least two mechanisms for pumping the Ca^{2+} back out. One of these, mentioned above, is a Ca-ATPase that directly couples ATP hydrolysis to the outward transport of a single Ca^{2+} ion. This is the PMCA Ca^{2+} pump. A second mechanism is called the **Na–Ca exchanger**. Its **stoichiometry**, or relative mole numbers, is 3Na:1Ca. The overall reaction is

$$[2.6.11] \qquad 3Na_o^+ + Ca_i^{2+} \Rightarrow 3Na_i^+ + Ca_o^{2+}$$

The $\Delta\mu$ for this entire process is equal to the sum of the $\Delta\mu$ for two separate processes:

$$[2.6.12] \qquad \begin{aligned} 3Na_o^+ &\Rightarrow 3Na_i^+ \\ Ca_i^{2+} &\Rightarrow Ca_o^{2+} \end{aligned}$$

So we write

$$[2.6.13] \qquad \Delta\mu_{Na,Ca\ exchange} = 3\Delta\mu_{Na_o \Rightarrow Na_i} + \Delta\mu_{Ca_i \Rightarrow Ca_o}$$

According to this result (see Example 2.6.6), there is enough energy in the Na^+ electrochemical gradient to drive Ca^{2+} out of the cell. However, it requires coupling the entry of three Na^+ ions for each Ca^{2+} ion that exits the cell. If the Na^+–Ca^{2+} exchange was to couple the movement of two Na^+ to each Ca^{2+}, there would be insufficient energy to drive Ca^{2+} efflux, and the exchanger would actually work in reverse mode, with the Ca^{2+} gradient driving the efflux of Na^{2+}.

The Ca^{2+} efflux that occurs through the Na–Ca exchanger is "uphill," meaning that it requires energy. Therefore, it is an active transport. The energy, however, does not come from ATP hydrolysis directly. It comes from the energy stored in the electrochemical gradient of Na^+. This energy, in turn, comes from the operation of the Na,K-ATPase that establishes and maintains the Na^+ and K^+ gradients across the cell membrane. Therefore, the Na–Ca exchange is an example of **secondary active transport**. It requires energy from a source outside of the solutes themselves. The energy is supplied by the Na^+ gradient. The Na^+ gradient is established using ATP hydrolysis.

SECONDARY ACTIVE TRANSPORT MECHANISMS ARE SYMPORTS OR ANTIPORTS

The Na–Ca exchanger, NCX, described above is an example of an **antiport**. It has this description because the two materials being transported go in opposite directions. Such a device is also called an **exchanger** or a **counter-transporter**. There are a variety of antiport secondary active transport mechanisms, as summarized in Figure 2.6.4. Thus far we have largely discussed cationic transporters, those that transport the cations. Cations are ions that migrate toward the cathode, the negatively charged electrode, and so cations are positively charged ions. Devices also transport anions or negatively charged ions. An important example of these is the Cl^-–HCO_3^- exchanger. In the red blood cell membrane this exchanger is the AE1 protein, which comprises a large fraction of the integral proteins of the erythrocyte membrane. This anion transporter exchanges Cl^- for HCO_3^- in the ratio of 1:1. This

EXAMPLE 2.6.6 Calculate the Free Energy for Operation of the Na–Ca Exchanger

We have already calculated $\Delta\mu$ for Na^+ entry as: $\Delta\mu_{Na_o \to Na_i} = -14.14$ kJ mol^{-1}

We have also calculated $\Delta\mu$ for Ca^{2+} entry as $\Delta\mu_{Ca_o \to Ca_i} = -39.6$ kJ mol^{-1}.

But in this case we are dealing with Ca^{2+}*exit*, which has $\Delta\mu = +39.6$ kJ mol^{-1}. The overall $\Delta\mu$ for the Na,Ca exchanger is thus

$$\Delta\mu_{NCX} = 3 \times (-14.14 \text{ kJ mol}^{-1}) + 39.6 \text{ kJ mol}^{-1} = \mathbf{-2.8\ kJ\ mol^{-1}}$$

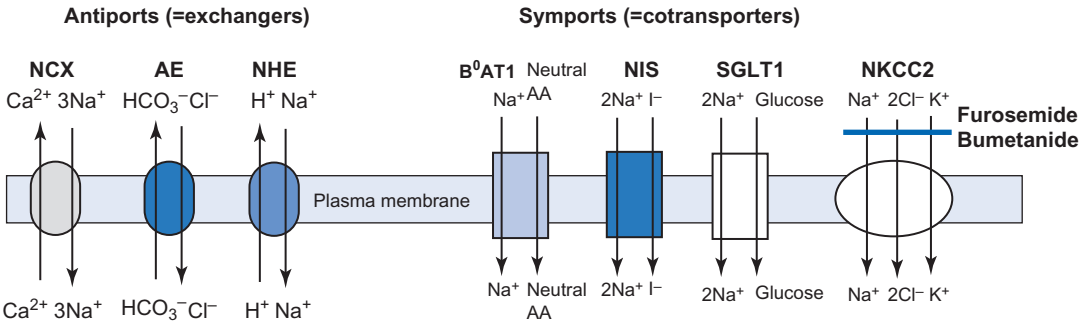

FIGURE 2.6.4 Examples of secondary active transport. NCX means "Na calcium exchanger"; AE means "anion exchanger"; NHE means "Na H exchanger"; B^0 is a specific name of a type of Na–amino acid transporter, of which there are several types; NIS means "Na iodine symport"; SGLT means "sodium glucose linked transporter." Many of these transporters exist in multiple forms or isoforms. NKCC2 means "Na, K two chloride cotransporter".

exchanger is important, as you will see later, in helping the erythrocytes to carry waste CO_2 (see Chapter 6.4).

Other secondary active transport mechanisms transport two materials in the same direction and therefore are categorized as **symports**, also called **cotransporters**. Examples include the Na–glucose transporter in the intestine and kidney membranes that transports glucose in the lumen of these organs into the absorptive cells lining the lumen. These cells also contain a number of Na–amino acid transporters that do the same thing as the glucose transporter: they transport amino acids from the lumen into the cells. The amino acids and glucose transported in this way are then transported into the blood by another mechanism, usually by facilitated diffusion. Some examples of symports are shown in Figure 2.6.4.

Secondary active transporters and facilitated diffusion proteins are classified in the family of solute carriers (SLC). There is a wide variety of these proteins, over 300 of them, and Figure 2.6.4 shows just a sampling of them. Appendix 2.6.A2 describes the nomenclature of these transport proteins.

SUMMARY

The movement of ions across cell membranes involves a change in free energy that depends on the concentration of the ion on both sides of the membrane, the charge on the ion, and the membrane potential. The free energy change can be calculated as

$$\Delta\mu = \mu_{\text{final}} - \mu_{\text{initial}}$$

where

$$\mu = \mu^0 + RT \ln C + z\mathfrak{F}\psi$$

where R is the gas constant = 8.314 J mol^{-1} K^{-1}, z is the charge on the ion, $\mathfrak{F}$ is the faraday = 9.649×10^4 C mol^{-1}, and T is the temperature in Kelvin.

At rest in muscle cells, the free energy per mole ($\Delta\mu$) for Na^+ entry into the cell is negative, meaning that it occurs spontaneously. Similarly, $\Delta\mu$ for K^+ exit is negative, whereas $\Delta\mu$ for Cl^- is near zero, implying that Cl^- distribution is near equilibrium. Cells maintain the Na^+ and K^+ gradients by actively pumping out Na^+ ions and pumping in K^+ ions. This movement of ions requires free energy that is supplied by the energy in the terminal phosphate bond of ATP. The Na,K-ATPase couples the outward movement of three Na^+ ions and the inward movement of two K^+ ions to the hydrolysis of 1 ATP molecule. The enzyme mechanism is responsible for this coupling. Under cell conditions, the overall $\Delta\mu$ for the Na,K-ATPase is negative because $\Delta\mu$ for ATP hydrolysis is more negative than the combined positive $\Delta\mu$ for Na^+ and K^+ transport "uphill."

The Na,K-ATPase is an example of primary active transport in which the transport of ions is directly linked to the hydrolysis of ATP. Other transporters couple the positive $\Delta\mu$ for solute transport with the negative $\Delta\mu$ for Na^+ entry into the cell. These are secondary active transporters, because they use energy to concentrate materials but the energy is derived directly from the Na^+ gradient and indirectly from ATP hydrolysis. Examples of secondary active transport include the surface membrane Na–Ca exchanger, Na–glucose cotransport, and Na–amino acid cotransport.

Transporters carrying materials in the same direction are called symports or cotransporters. Those carrying materials in opposite directions are called antiports or exchangers. Multiple examples of both classes occur in the body.

Clinical Applications: SGLT2 Inhibitors and Diabetes Mellitus

Diabetes mellitus is characterized by an abnormally high plasma glucose concentration caused by insufficient production of insulin by the pancreas. Insulin is a protein hormone that increases glucose transporters in peripheral tissues (GLUT4) that remove glucose from the circulation. There are two major classifications of persons with diabetes. Those with Type 1 diabetes require insulin injections and have little or no production of insulin, generally caused by a destruction of the beta cells in the pancreas that produce the hormone. Persons with Type 2 diabetes generally produce insulin but the body cells are resistant to the hormone and the circulating levels of insulin are inadequate to lower plasma glucose levels. High blood glucose causes glycosylation of proteins, as measured clinically by HbA_{1c}, glycosylated hemoglobin. Long-term control of plasma glucose is monitored by the HbA_{1c} level. Diabetes mellitus is described in more detail in Chapter 9.4.

The kidney produces urine by filtering large volumes of blood and then reabsorbing the desired materials and discarding the rest. Glucose is reabsorbed in the kidney in the proximal tubule of the nephron, the functional unit of the kidney, by SGLT2 present in the apical membrane of the tubule (see Figure 2.6.5). Final transport of glucose into the blood occurs over GLUT2, a facilitated transport mechanism. 90% of glucose reabsorption in the kidney occurs via the SGLT2 glucose uptake mechanism. A relatively new class of drugs inhibits the SGLT2 so that not all of the filtered glucose is reabsorbed, and glucose appears in the urine. These drugs include empagliflozin, canagliflozin, and dapagliflozin. The chemical structures of these drugs are shown in Figure 2.6.6.

The term "diabetes" derives from the Greek meaning "to siphon" and this refers to the increased urinary flow in diabetic persons. This is caused by the inability of the kidney to reabsorb all of the glucose, and the excretion of this glucose in a larger volume of water. The effect of the SGLT2 inhibitors is to (1) lower blood glucose levels, (2) reduce HbA_{1c} levels, (3) generally cause a slight loss of weight, and (4) tend to cause dehydration due to increased urine flow. These drugs are of limited value to persons with dysfunctional kidneys (Whalen K, Miller, S, and Onge E, The role of sodium–glucose co-transporter 2 inhibitors in the treatment of type 2 diabetes, *Clin. Therap.* **37**:1150–1166, 2015).

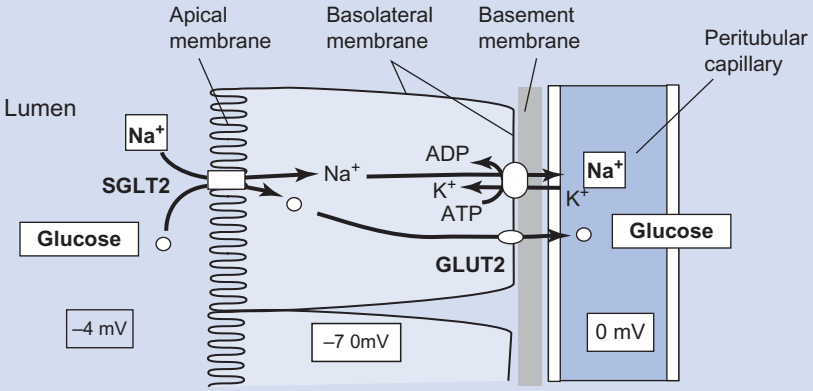

FIGURE 2.6.5 Mechanism of reabsorption of glucose from the ultrafiltrate in the lumen of the nephron proximal tubule. SGLT2 transports 2 Na^+ ions into the cell along with glucose. The energy is supplied by the electrochemical gradient for Na^+ that is established by pumping Na^+ out of the cell at the basolateral membrane. Glucose enters the blood through GLUT2, a facilitated diffusion carrier on the basolateral membrane.

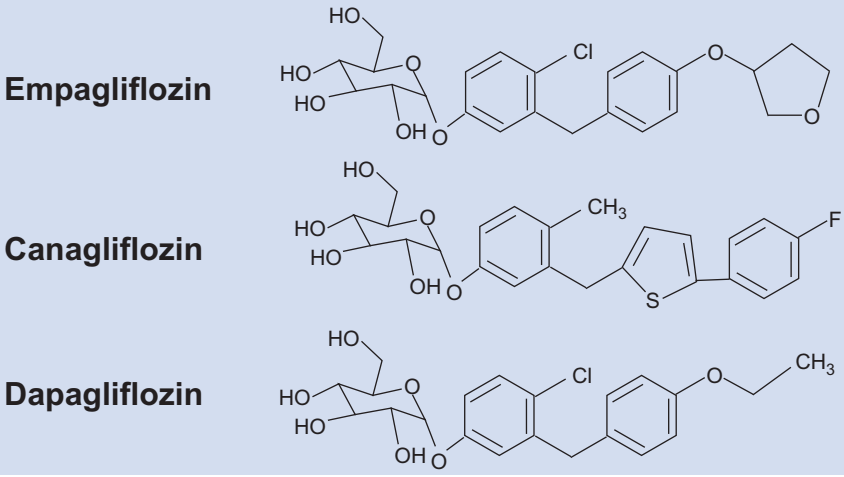

FIGURE 2.6.6 Chemical structure of the SGLT2 inhibitors.

Clinical Applications: Oral Rehydration Therapy

One of most important public health issues in the world is the availability of clean drinking water. Contaminated water supplies carry cholera and other infectious agents that result in diarrhea and vomiting that cause dehydration that can be fatal, especially in children. Prior to the introduction of oral rehydration therapy (ORT), diarrhea was the leading cause of infant mortality in developing nations. ORT is estimated to have reduced world-wide infant deaths from 5 million per year to 3 million per year (2006 figures). However, diarrhea remains the second leading cause of death in children less than 5 years old (18%, after pneumonia at 19%).

The WHO (World Health Organization) and UNICEF (United Nations Children's Fund, shortened from the original United Nationals International Children's Emergency Fund) jointly publish guidelines for the composition of oral rehydration solution (ORS). Its current formulation is: 2.6 g NaCl; 2.9 g trisodium citrate dihydrate ($Na_3C_6H_5O_7$•2 H_2O); 1.5 g KCl; 13.5 g glucose per L of solution.

The molar ratio of sodium to glucose in this ORS is 1.0, and it is slightly hyposmotic. It should be made with clean water, but when clean water is not available other fluids may be substituted—but not sugar-containing fluids like fruit juices.

The effectiveness of ORT relies on the SGLT1 system in the intestine that transports glucose along with 2 Na^+ ions into the enterocyte and then into the blood. The transport of nutrients creates an osmotic reabsorption of water along with the solutes. This is enough to counteract the loss of fluid through diarrhea or vomiting. ORT was not used until the 1960s. Before that time, rehydration was accomplished by intravenous fluid administration. In the developing world, IV therapy is not widely available and ORT is a low-technology solution that is much more readily available.

REVIEW QUESTIONS

1. If a Na channel was to open on the surface of a cardiac cell, at rest, in which direction would Na ions travel? If the channels were K-specific, in which direction would K ions travel? If it were Ca^{2+}-specific, which way would Ca^{2+} go? If it was a Cl^- channel, which way would Cl^- ions go?
2. Which part of Eqn [2.6.4] gives the energy due to diffusion? Which part gives the energy due to electrical forces? For Na^+, which is bigger, the part due to concentration differences or the part due to electrical forces? Which part of the total energy, concentration or electrical, is bigger for Ca^{2+}?
3. Where does the energy come from for the movement of materials by passive transport?
4. Where does the energy come from for the movement of materials by primary active transport?
5. Where does the energy come from for the movement of materials by secondary active transport?
6. Do you think that the NCX can go in reverse?
7. Why cannot thermodynamics predict reaction rates?
8. What is a symport? What is an antiport? What is a cotransporter?

APPENDIX 2.6.A1 DERIVATION OF THE USSING FLUX RATIO EQUATION

The passive flux of an ion in a solution is given by Fick's First Law of Diffusion with an external force, which was introduced in Eqn [1.7.19]. The one-dimensional form of this equation is reproduced here:

$$[2.6.A1.1] \qquad J_s = -D\frac{\partial C}{\partial x} - \frac{D}{RT}Cz\mathfrak{F}\frac{\partial \psi}{\partial x}$$

where J is the flux, in mol cm^{-2} s^{-1}, D is the diffusion coefficient in cm^2 s^{-1}, C is the concentration (converted to units of mol cm^{-3}), R is the gas constant ($=8.314$ J mol^{-1} °K^{-1} $= 1.987$ cal mol^{-1} °K^{-1}), T is the absolute temperature (°K), z is the integer of the charge on the ion (± 1 or 2, generally), $\mathfrak{F}$ is the Faraday ($=96{,}489$ coulomb mol^{-1}) and ψ is the electrical potential, in joules (=volt-coulomb). The quantity $\partial C/\partial x$ is the magnitude of the gradient of C, and $\partial\psi/\partial x$ is the magnitude of the electric field. The flux can be obtained by integrating this equation. To begin, we multiply both sides of the equation by an integrating factor, ρ, and we choose ρ so that the right-hand side of the equation becomes an exact differential:

$$[2.6.A1.2] \qquad J_s\rho = -D\left[\rho\frac{\partial C}{\partial x} + \frac{Cz\mathfrak{F}\rho}{RT}\frac{\partial \psi}{\partial x}\right]$$

We choose ρ so that the terms in brackets are an exact differential. Thus we want

$$[2.6.A1.3] \qquad \rho\frac{\partial C}{\partial x} + \frac{Cz\mathfrak{F}\rho}{RT}\frac{\partial \psi}{\partial x} = \frac{\mathrm{d}(\rho\, C)}{\mathrm{d}x} = \rho\frac{\partial C}{\partial x} + C\frac{\partial \rho}{\partial x}$$

From comparing the left of Eqn [2.6.A1.3] to the right, we see that multiplication by ρ transforms the equation into an exact differential if

$$[2.6.A1.4] \qquad \frac{\partial \rho}{\partial x} = \frac{z\mathfrak{F}\rho}{RT}\frac{\partial \psi}{\partial x}$$

We rearrange this to get

$$[2.6.A1.5] \qquad \frac{\partial \rho}{\rho} = \frac{z\mathfrak{F}}{RT}\partial\psi$$

A solution to Eqn [2.6.A1.5] is

$$[2.6.A1.6] \qquad \rho = e^{\frac{z\mathfrak{F}}{RT}\psi}$$

We may insert this result back into Eqn [2.6.A1.2], with Eqn [2.6.A1.3], to obtain

$$[2.6.A1.7] \qquad J_s e^{\frac{z\mathfrak{F}}{RT}\psi} = -D\,\frac{\mathrm{d}\left(Ce^{\frac{z\mathfrak{F}}{RT}\psi}\right)}{\mathrm{d}x}$$

which may be rewritten as

$$[2.6.A1.8] \qquad J_s e^{\frac{z\mathfrak{F}}{RT}\psi}\mathrm{d}x = -D\,\mathrm{d}\left(Ce^{\frac{z\mathfrak{F}}{RT}\psi}\right)$$

If we are considering passive transport across a membrane, we can determine the ***passive*** flux by integrating this equation from $x = 0$ (one side of the membrane) to $x = \delta$, the other side of the membrane for a membrane with thickness δ:

$$[2.6.A1.9] \qquad \int_0^{\delta} J_s e^{\frac{z\mathfrak{F}}{RT}\psi}\mathrm{d}x = -D\int_0^{\partial}\delta\left(Ce^{\frac{z\mathfrak{F}}{RT}\psi}\right)$$

Here we limit ourselves to the steady-state condition. In this case, J_s does not vary with distance across the membrane—it is constant. Therefore, J_s may be removed from the integral and we get

$$[2.6.A1.10] \qquad J_s = \frac{-D\int_0^{\partial}\delta\left(Ce^{\frac{z\mathfrak{F}}{RT}\psi}\right)}{\int_0^{\delta} e^{\frac{z\mathfrak{F}}{RT}\psi}\mathrm{d}x}$$

The numerator in this equation is the integral of an exact differential and can be immediately evaluated between the boundaries. This gives

$$[2.6.A1.11] \qquad J_s = \frac{-D\left[C(\delta)e^{\frac{z\mathfrak{F}}{RT}\psi(\delta)} - C(0)e^{\frac{z\mathfrak{F}}{RT}\psi(0)}\right]}{\int_0^{\delta} e^{\frac{z\mathfrak{F}}{RT}\psi}\mathrm{d}x}$$

The denominator in this equation can be evaluated only if $\psi(x)$ is known. However, generally $\psi(x)$ is unknown. Ussing made the observation that the presence of an active transport mechanism would not obey Eqn [2.6.A1.11], because the flux would not be passive, and this equation describes the passive flux. He further made the observation that we don't need to know how ψ varies with x if we take the ratio of the unidirectional fluxes. The unidirectional flux is the flux that you would observe if the concentration on the other side was zero.

We define here the unidirectional flux $J_{i\to o}$ to be the flux from inside to outside, with $x = 0$ on the inside and $x = \delta$ on the outside. This flux is given from Eqn [2.6.A1.11] by setting $C(\delta) = 0$, and we obtain

$$J_{i\to o} = \frac{D\,C(0)\,e^{\frac{z\mathfrak{F}}{RT}\psi(0)}}{\int_0^\delta e^{\frac{z\mathfrak{F}}{RT}\psi}\mathrm{d}x} \quad [2.6.A1.12]$$

We further define the unidirectional flux $J_{o\to i}$ to be the unidirectional flux from outside with $x = \delta$ to the inside, with $x = 0$. This flux is also given from Eqn [2.6.A1.11] by setting $C(0) = 0$; we obtain

$$J_{o\to i} = \frac{-D\,C(\delta)\,e^{\frac{z\mathfrak{F}}{RT}\psi(\delta)}}{\int_0^\delta e^{\frac{z\mathfrak{F}}{RT}\psi}\mathrm{d}x} \quad [2.6.A1.13]$$

Here the minus sign conveys a convention that the outward flux is taken as positive. Thus a negative flux simply means that the flux is directed inward, and a positive flux is directed outward. The magnitude of the fluxes is given by the absolute values of the fluxes. If we take the ratio of the unidirectional fluxes, the denominators in each cancel each other out, and we don't need to do the integration that requires knowledge of $\psi(x)$. Taking the ratio of the two unidirectional fluxes, we arrive at

$$\frac{J_{i\to o}}{J_{o\to i}} = \frac{D\,C(0)\,e^{\frac{z\mathfrak{F}}{RT}\psi(0)}}{-D\,C(\delta)\,e^{\frac{z\mathfrak{F}}{RT}\psi(\delta)}} \quad [2.6.A1.14]$$

Using the notation that $C(0) = C(\mathrm{i})$, the inside concentration, and $C(\delta) = \mathrm{C(o)}$, the outside concentration, this equation can be simplified to

$$\frac{J_{i\to o}}{J_{o\to i}} = -\frac{C(\mathrm{i})}{C(\mathrm{o})}e^{\frac{z\mathfrak{F}}{RT}\psi(0)-\psi(\delta)} \quad [2.6.A1.15]$$

Using the definition that the difference in potential across the membrane, $\psi(0) - \psi(\delta) = E_m$, the membrane potential, this last Eqn [2.6.A1.15] becomes

$$\frac{J_{i\to o}}{J_{o\to i}} = -\frac{C(\mathrm{i})}{C(\mathrm{o})}e^{\frac{z\mathfrak{F}}{RT}E_m} \quad [2.6.A1.16]$$

This last equation is the **Ussing Flux Ratio Equation**. It describes the expected ratio of the unidirectional fluxes ***if the ions are transported passively across the membrane***. Deviations from the flux ratio equation are taken to indicate that the fluxes are not transported passively. That is, deviations from the expected flux ratio can be taken to indicate that an active transport mechanism is present. Ussing considered other possible deviations from the expected flux ratio such as single-file transport.

Hans Ussing (1911–2000) derived his flux ratio equation in the late 1940s, soon after radioactive isotopes became available to measure unidirectional ion fluxes. He was the first to prove the existence of active transport mechanisms, using the frog skin as a model. The Na,K-ATPase was later discovered, in 1957, by Jens Skou, who earned a Nobel Prize for the discovery.

APPENDIX 2.6.A2 NOMENCLATURE OF TRANSPORT PROTEINS

HUGO NOMENCLATURE

Proteins have "trivial" or common names that were generally first provided by their discoverers. These names have often been changed when a new function for the protein was discovered or its relationship to other proteins was discovered. Since these proteins derive from genes, it is now increasingly useful to use the name for the gene to also describe the resulting protein. The **HUGO Gene Nomenclature Committee (HGNC)** provides a unique identifier for each gene. This committee is a part of the **Hu**man **G**ene **O**rganization (HUGO). Full and updated databases are available at www.genenames.org.

CARRIER CLASSIFICATIONS

The transport proteins as described in Chapters 2.5 and 2.6 can be generally classified as belonging to a small number of types. These are:

Passive Transporters:
- Facilitated transporters
- Ion channels
- Water channels

Active Transporters:
- Secondary active transporters: exchangers and cotransporters
- ATPase pumps (P, V, and F-types)
- ABC (ATP-binding cassette) transporters.

These functional classifications do not map precisely onto the HUGO nomenclature. The HUGO classification lumps facilitated transporters and secondary active transporters into one group, the solute carriers, SLC. Carriers are often grouped into families based on sequence homology rather than functionality. An example of this is the sodium–iodine exchanger located in the thyroid gland, which is part of the sodium–glucose cotransport family.

SOLUTE CARRIERS

The solute carriers include the facilitated diffusional carriers such as GLUT1 and GLUT2 and the secondary active transport carriers such as NCX, NHE, SGLT, NIS, and AE. All members of the SLC superfamily are named according to

SLC *n* X *m*

where SLC indicates the superfamily, *n* is an integer that denotes the family, X is a letter that denotes the subfamily, and *m* is a second integer that denotes the isoform. As an example, the facilitated glucose carriers are all members of the SLC2A subfamily of which there are 14 members: SLC2A{1...14}. These correspond to their common names, as shown in Table 2.6.A2.1.

There are an enormous number of these SLC genes. There are at least 52 families (n) with a total of 396 genes (Σm_i) that encode for transporter proteins. It is

TABLE 2.6.A2.1 Nomenclature of the Facilitated Glucose Transporters (Augustin, R. "The protein family of glucose transport facilitators; It's not about glucose after all." *Life* **62**:315–333, 2010)

HUGO Name	Common Name	Location and Function
SLC2A1	GLUT1	K_m for glucose = 1.5 mM; ubiquitous but important for erythrocytes and uptake of glucose into the cerebrospinal fluid (CSF)
SLC2A2	GLUT2	K_m for glucose = 17 mM; liver, kidney, intestine, β cells of pancreas; transports glucose out of intestine and kidney cells
SLC2A3	GLUT3	K_m for 2-deoxyglucose = 1.4 mM; present in neurons, spermatozoa, placenta
SLC2A4	GLUT4	K_m for glucose = 5 mM; skeletal and cardiac muscle, adipose tissue; rate-limiting step in insulin-stimulated glucose uptake into tissues
SLC2A5	GLUT5	K_m for fructose = 6 mM; mainly jejunum of small intestine but also found in kidney, brain, muscle and fat; primarily a fructose transporter
SLC2A6	GLUT6	brain, spleen, and leukocytes
SLC2A7	GLUT7	K_m for glucose = 0.3 mM; apical membrane of intestine and colon; also transports fructose
SLC2A8	GLUT8	K_m for glucose = 2 mM; testis, cerebellum, liver, spleen, lung, fat; intracellular without insulin-stimulated translocation to the surface
SLC2A9	GLUT9	K_m for glucose = 0.6 mM but also transports urate; kidney and liver and β cells of pancreas; exchanges urate for glucose or fructose
SLC2A10	GLUT10	Present in heart, lung, brain, liver, muscle, pancreas, placenta, and kidney; may be intracellular
SLC2A11	GLUT11	Three isoforms (GLUT11{A,B,C}); in humans GLUT11 is exclusively expressed in slow-twitch muscle fibers
SLC2A12	GLUT12	
SLC2A13	GLUT13; HMIT	Does not transport sugar; H^+-coupled myoinositol symporter; K_m = 0.1 mM for myoinositol; located intracellularly; highest in brain
SLC2A14	GLUT14	

entirely unreasonable to attempt memorization of all of them. The list of these and other gene families is available in tabular format on the HUGO website.

Note that some designations within a family can be confusing in that the function of the carrier is not related to its stated family! For example, GLUT 9 (SLC2A9) is primarily regarded as a urate transporter rather than a glucose transporter, but it is a member of the facilitated diffusion carriers for glucose.

A second example of this is found in the sodium–glucose transport family. SGLT1 and SGLT2 are part of the SLC5A subfamily that includes 12 members (SLC5A {1…12}. SLC5A1 corresponds to SGLT1 and SLC5A2 corresponds to SGLT2, but SLC5A5 corresponds to the sodium–iodine exchanger (NIS), shown in Figure 2.6.4, that does not transport glucose! The NIS is grouped in this way because of structural similarities in the transport proteins that leads researchers to suppose that they belong to the same subfamily of transporters. SGLT1 is the main transporter in the intestine whereas SGLT2 is the main transporter in the kidney, although SGLT1 is also found in the kidney.

The amino acid transporter shown in Figure 2.6.4 has three members ($B^0AT1–3$) that correspond to genes SLC6A19, SLC6A15, and SLC6A18, respectively. These all carry neutral amino acids. Because there are so many amino acids and also a variety of neurotransmitters and other solutes that need carrying, there are a large number of these kinds of carriers. They belong to the SLC1, SLC3, SLC6, SLC7, SLC36, SLC38, and SLC43 subfamilies.

The anion exchanger shown in Figure 2.6.4, AE, also has three subtypes (AE1–3) that correspond to SLC4A1–3. These are all electroneutral Cl^- $-HCO_3^-$ exchangers. The SLC4 family contains 10 members (SLC4A{1…5; 7…11} that include Na^+ and HCO_3^- or CO_3^{-2} cotransporters.

The $Na^+–Ca^{2+}$ exchanger (NCX) of Figure 2.6.4 also has three members (NCX1–3) encoded by genes SLC8A1–3, respectively. There is also a mitochondrial $Na^+–Ca^{2+}$ exchanger, NCLX, as a gene product of SLC8B1. There are additional transporters for Ca^{2+} including NCKX ($Na^+–Ca^{2+}–K^+$ exchanger, SLC24 family) and CCX (Ca^{2+}-cation exchanger).

The $Na^+–H^+$ exchanger (NHE) of Figure 2.6.4 has several members. NHE1–5 are all present on the surface membrane, and NHE3 and NHE5 recycle between the surface and intracellular membranes. They correspond to SLC9A1–5, respectively. NHE6, NHE7, and NHE9 appear to be located in intracellular membranes along the secretory pathway. They correspond to SLC9A6, SLC9A7, and CLC9A9, respectively.

The Na–K–2Cl cotransporter shown in Figure 2.6.4 comes in two forms, NKCC1 and NKCC2, corresponding to genes SLC12A1 and SLC12A2.

There are a large number of other transporters of the SLC gene superfamily that transport neurotransmitters or ions, or vitamins, or metabolites across plasma membranes or internal membranes. A complete listing is available on the HUGO website.

ATP-DRIVEN ION PUMPS

Names for the genes for the ATP-driven ion pumps have the form

ATP *n* X *m*

where ATP indicates the superfamily, *n* is an integer that denotes the family, X is a letter that denotes the subfamily, and *m* is an integer that denotes the member. Many of the ATP-driven pumps consist of multiple subunits, and these are generally organized by having the same family integer, *n*, and a different subfamily name. For example, the Na,K-ATPase has an α and a β subunit, and these are indicated as ATP1A and ATP1B. There are four varieties of each, so that the α subunit of the Na,K-ATPase corresponds to ATP1A{1...4} and the β subunit has genes ATP1B{1...4}.

The Ca^{2+} pumps are members of the ATP2 family. ATP2A{1,2,3} correspond to SERCA1, SERCA2, and SERCA3, respectively, that are located on the internal membranes of the cell, the endoplasmic reticulum, or the sarcoplasmic reticulum. The plasma membrane Ca-ATPases, PMCA{1...4} are encoded by the ATP2B {1...4} genes, respectively. SPCA1 and SPCA2 are encoded by ATP2C1 and ATP2C2. The gastric H^+-K^+-ATPase is encoded by ATP4A (the α subunit) and ATP4B (the β subunit}. All of the above-mentioned pumps constitute the P-class of ATP-driven ion pumps.

The F-type and V-type ATPase ion pumps are much more complex, consisting of multiple subunits and multiple copies of some of these subunits. Therefore, there is no one gene that encodes the entire operating complex. The mitochondrial F-type ATPase consists of an F_1 and an F_O subunit that themselves are complexes of additional subunits. These are encoded by the ATP5 family of genes, with subfamilies denoted by the letters A, B, C, D, E, F, G, H, I, J, L, and O.

Somewhat differently, the V-type ATPase that acidifies lysosome contents by pumping in H^+ ions is designated ATP6V0 and ATP6V1 for the V0 and V1 subunits, and letters corresponding to the subunits within V0 and V1.

ABC TRANSPORTERS

ABC transporters hydrolyze ATP to transport a wide variety of substrates. There are 48 transporters classified in 7 families denoted in this case by letters alone:

ABC X m

where ABC denotes "ATP-binding cassette", X is a letter indicating the family, and *m* is an integer indicating the member.

AQUAPORINS

Aquaporins are proteins that increase water movement across biological membranes. They have a molecular weight of around 30 kDa and associate as tetramers, although each monomer has a water channel. Several of the aquaporins will transport other small molecular weight, electrically neutral substrates such as glycerol or urea. There are a variety of aquaporins named AQP *n* where *n* is the member of the family. There are 14 members of the family, named AQP{1...12}. AQP0 has been renamed MIP for "major intrinsic protein" of the lens, and AQP12 has A and B subtypes. In the HUGO classification, aquaporins are considered to be a subtype of ion channels.