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# On the Concept of Resting Potential—Pumping Ratio of the $Na^+/K^+$ Pump and Concentration Ratios of Potassium Ions Outside and Inside the Cell to Sodium Ions Inside and Outside the Cell

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**Abstract** In animal cells, the resting potential is established by the concentration gradients of sodium and potassium ions and the different permeabilities of the cell membrane to them. The large concentration gradients of sodium and potassium ions are maintained by the $Na^+/K^+$ pump. Under physiological conditions, the pump transports three sodium ions out of and two potassium ions into the cell per ATP hydrolyzed. However, unlike other primary or secondary active transporters, the $Na^+/K^+$ pump does not work at the equilibrium state, so the pumping ratio is not a thermodynamic property of the pump. In this article, I propose a dipole-charging model of the $Na^+/K^+$ pump to prove that the three $Na^+$ to two $K^+$ pumping ratio of the $Na^+/K^+$ pump is determined by the ratio of the ionic mobilities of potassium to sodium ions, which is to ensure the time constant $\tau$ and the $\tau$-dependent processes, such as the normal working state of the $Na^+/K^+$ pump and the propagation of an action potential. Further, the concentration ratios of potassium ions outside and inside the cell to sodium ions inside and outside the cell are 0.3027 and 0.9788, respectively, and the sum of the potassium and sodium equilibrium potentials is $-30.3$ mV. A comparative study on these constants is made for some marine, freshwater and terrestrial animals. These findings suggest that the pumping ratio of the $Na^+/K^+$ pump and the ion concentration ratios play a role in the evolution of animal cells.

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## Introduction

The $Na^+/K^+$ pump is a member of P-type ATPases of primary active cation transporters (Lewin et al. 2007). Its main function is to transport $Na^+$ and $K^+$ ions across the plasma membrane against their electrochemical gradients and maintain the resting potential of all animal cells. The established $Na^+$ and $K^+$ concentration gradients are essential for the excitation of muscle and nerve cells. And the $Na^+$ concentration gradient is used in most cells for the cotransport of essential nutrients by secondary active transport. In the meantime, the $Na^+/K^+$ pump functions to regulate the osmolarity and the volume of the cell (Sherwood et al. 2005; Randall et al. 1997). In each cycle, the pump transports three $Na^+$ ions out of the cell and two $K^+$ ions into the cell by hydrolysis of one ATP molecule.

We know that the $Na^+/K^+$ pump has some structural homologies and functional similarities with other P-type ATPases, such as the sarco(endo)plasmic reticulum $Ca^{2+}$-ATPase (SERCA) and the $H^+/K^+$-ATPase (Bublitz et al. 2010; Kuhlbrandt 2004; Kaplan 2002). However, unlike other primary or secondary active transporters, the $Na^+/K^+$ pump, which is driven by hydrolysis of ATP, does not work at the thermodynamic equilibrium state with the concentrations of sodium and potassium ions in the intracellular and extracellular solutions (Tanford 1981). So the pumping ratio should be special. Why does it pump three $Na^+$ out of and two $K^+$ into the cell and not, for example, three $Na^+$ and three $K^+$? This is an interesting question. To the best of

my knowledge, this issue has never been touched. This question cannot be explained by the conventional theory.

In this article, I will try to explain this ratio by physical–chemical principles, specifically, the dipole-charging model of the $Na^+/K^+$ pump, utilizing the electric circuit theory (Hodgkin and Rushton 1946). Our model is based on the electric current produced by a single $Na^+/K^+$ pump. We know that, with the pumping ratio of three $Na^+$ to two $K^+$, the $Na^+/K^+$ pump transports one net positive charge out of the cell in each cycle. This electric current of a single $Na^+/K^+$ pump is very small (Gadsby et al. 2009) and cannot be measured by experimental techniques (Apell 2004; Kaplan et al. 1978; Dempski et al. 2009). In our model, the pumping by the $Na^+/K^+$ pump is a dipole-charging process of the cell membrane and the three $Na^+$ to two $K^+$ pumping ratio can ensure the time constant $\tau$ of a single pump current as well as the related $\tau$-dependent processes, such as the normal working state of the $Na^+/K^+$ pump and the propagation of an action potential.

Concerning the process that the $Na^+/K^+$ pump transports three $Na^+$ ions out of in exchange for two $K^+$ ions into the cell in each cycle, is there any relationship related to the function of the pump which exists among the concentrations of potassium ions inside and outside the cell and the concentrations of sodium ions inside and outside the cell? We know that there is such a relationship for these four concentrations; that is, the concentration ratio of the $Na^+$ ion outside to inside the cell determines the equilibrium potential for the $Na^+$ ion, $E_{Na}$, and the concentration ratio of the $K^+$ ion outside to inside the cell determines the equilibrium potential for the $K^+$ ion, $E_K$. The $Na^+$ and $K^+$ ions are the main extracellular and intracellular cations, respectively. At steady state, the resting membrane potential is determined by the concentration gradients of sodium and potassium ions outside to inside cell, that is, $E_{Na}$ and $E_K$, and by the different permeabilities of the cell membrane to them. The principal intracellular anion is the large, negatively charged protein which is impermeable by the cell membrane and remains within the cell. The chief extracellular anion is the $Cl^-$ ion. Most cells are highly permeable to the $Cl^-$ ion, where the $Cl^-$ ion is not actively transported and only distributes passively across the cell membrane (Sherwood et al. 2005; Blaustein et al. 2004). In this article, we will derive a new relationship among the sodium and potassium concentrations. This is based on the analysis of the charging process of the cell membrane caused by a single $Na^+/K^+$ pump. We know that, in neurons, the resting potential is $-70$ mV and the cell membrane has an electric capacitance of 1 $\mu F\ cm^{-2}$. So the amount of charge required for producing this membrane potential is $(1\ \mu F\ cm^{-2})(70\ mV) = (1 \times 10^{-6}\ F/10^8\ \mu m^2)(70 \times 10^{-3}\ V) = 7 \times 10^{-16}\ C\ \mu m^{-2}$. This is equivalent to $[(7 \times 10^{-16}\ C\ \mu m^{-2})/96{,}500\ C\ mol^{-1}](6 \times 10^{23}\ mol^{-1}) \approx 4{,}352$ monovalent ions $\mu m^{-2}$, where 96,500 C $mol^{-1}$ is the Faraday's constant and $6 \times 10^{23}\ mol^{-1}$ is the Avogadro's number. This value can be used as a reference for other cell types. However, we know that the pumping rate of the $Na^+/K^+$ pump can be 100 times per second (Gadsby et al. 2009) and the density of the $Na^+/K^+$ pump can be several thousands of pumps per square micrometer on the membrane fragments isolated from kidney outer medulla (Apell 1989; Bühler et al. 1991; Stürmer et al. 1991). So a slight change in pumping efficiency will significantly affect the membrane potential. In this sense, the pumping process of each $Na^+/K^+$ pump should be precisely regulated, including the intracellular and extracellular concentrations of sodium and potassium ions. I will show that the concentration ratios of potassium ions outside and inside the cell to sodium ions inside and outside the cell are constants and that $E_K + E_{Na}$ is a constant. The evolutionary significance of these relationships in comparative animal physiology and in neurobiology will be discussed.

## Results

### Dipole-Charging Model of the $Na^+/K^+$ Pump

The electric dipole involves two charged particles of magnitude q of opposite sign and separated by a distance (Halliday et al. 2001). The electric potential at any point P in the electric field of an electric dipole can be calculated as $U(P) = (1/4\pi\varepsilon_0)[(q/r^+) + (-q/r^-)]$, where q is the charge magnitude of, for example, the positively charged particle of the dipole; $r^+$ and $r^-$ are distances between P and the positively and negatively charged particles of the dipole, respectively; and $\varepsilon_0$ is the permittivity constant. There are two points here. First, the electric potential U(P) at P is proportional to charge q; second, U(P) is more positive at the point which is nearer to the positive point charge of the dipole than at the point which is nearer to the negative point charge. So the electric dipole produces an electric potential difference around it, the magnitude of which is proportional to q and the direction of which is from the higher value of electric potential in the area near the positive point charge to the lower value of electric potential in the area near the negative point charge.

The pumping cycle of the $Na^+/K^+$ pump can be divided into two parts. In the first part, three sodium ions inside the cell bind to the pump and are transported outside the cell and released to the extracellular fluid. This will create an electric dipole of magnitude +3e, where e is the elementary charge, with the positive charges being outside the cell and the negative charges being inside the cell. The electric potential produced by this dipole is higher outside the cell than inside the cell. So this dipole will drive the positive ions outside the cell to move toward the outer surface of the cell membrane and drive the negative ions inside the cell to

move toward the inner surface of the cell membrane. Because the cell membrane has a certain capacitance, $C_m$, this process is to charge the membrane. Similarly, in the second part, the two potassium ions outside the cell bind to the pump and are transported inside the cell and released to the intracellular fluid. This will create an electric dipole of magnitude +2e, with the positive charges being inside the cell and the negative charges being outside the cell. The electric potential produced by this dipole is higher inside the cell than outside the cell. So this dipole will drive the positive ions inside the cell to move toward the inner surface of the cell membrane and drive the negative ions outside the cell to move toward the outer surface of the cell membrane. This process is to charge the membrane with the same $C_m$.

Now we consider a circuit model to simulate the two charging processes by the $Na^+/K^+$ pump. This theory was first developed by Hodgkin and Rushton (1946) based on cable theory. We assume that the membrane has an electric resistance, $r_m$, per unit area as well as an electric capacitance, $C_m$, per unit area. The resistance here refers to the resistance of the membrane to ions, which can only cross the lipid bilayer of the membrane through ion channels. So $r_m$ is a measure of the resistance by ion channels (Randall et al. 1997). We further assume that the electric resistance from the outer end of the $Na^+/K^+$ pump to the outer surface of the cell membrane is $r_o$ and that the electric resistance from the inner end of the $Na^+/K^+$ pump to the inner surface of the cell membrane is $r_i$. $r_o$ and $r_i$ are a measure of the resistances of the extracellular and intracellular fluids, respectively (Fig. 1). For simplicity, let $R_1 = r_i + r_o$, $R_2 = r_m$. $R_2$ and $C_m$ are connected in parallel, which is then connected with $R_1$ in series to the pump. Let $i_1$ denote the current through $r_o$ and $r_i$, $i_2$ denote the current through $r_m$, $i_c$ denote the current through $C_m$ and $U_c$ denote the electric potential difference across $C_m$. The electric potential difference produced by the pump dipole alone is $U_d$. This circuit can be considered equivalent to that an electric potential difference $U_d$ is applied as an energy source, and the initial condition for $U_c$ is $U_c(0) = 0$.

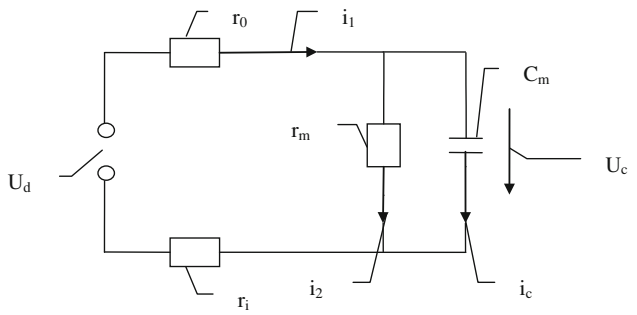


**Fig. 1** Dipole-charging model of the $Na^+/K^+$ pump shown by an electric circuit

Then, we have the following equations to describe the circuit:

$$i_1R_1 + U_c = U_d \quad (1)$$

$$i_1 = i_2 + i_c \quad (2)$$

$$i_2 = U_c/R_2 \quad (3)$$

$$i_c = C_m dU_c/dt \quad (4)$$

Substituting Eqs. 2–4 into 1, we have

$$R_1(U_c/R_2 + C_m dU_c/dt) + U_c = U_d$$

or

$$R_1C_m dU_c/dt + (R_1 + R_2)U_c/R_2 = U_d \quad (5)$$

The solution of Eq. 5 is

$$U_c = U_d R_2/(R_1 + R_2) + A\exp(-t/R_{//}C_m),$$

where

$$R_{//} = R_1R_2/(R_1 + R_2), \quad (6)$$

and A is a constant.

Using $U_c(0) = 0$, we have $A = -U_dR_2/(R_1 + R_2)$, so

$$U_c = [U_dR_2/(R_1 + R_2)][1 - \exp(-t/R_{//}C_m)] \quad (7)$$

Substituting Eq. 7 into 4, we have

$$i_c = (U_d/R_1)\exp(-t/R_{//}C_m) \quad (8)$$

$$\text{Let } \tau = R_{//}C_m, \quad (9)$$

which is the capacitive time constant of the circuit.

Next, we calculate the time − average value $I_c$ of $i_c$ over $3\tau$:

$$\begin{aligned} I_c &= \left[\int_0^{3\tau} (U_d/R_1)\exp(-t/R_{//}C_m)dt\right]/3\tau \\ &= \left[\int_0^{3\tau} (U_d/R_1)\exp(-t/\tau)dt\right]/3\tau \\ &= U_d[1 - \exp(-3)]/3R_1 \approx U_d/3R_1 \end{aligned} \quad (10)$$

According to Eq. 2, $i_1 = i_2 + i_c$. The difference between $i_2$ and $i_c$ is that $i_2$ is the part of the charging current flow which goes through ion channels, while $i_c$ is the part of the charging current flow which directly charges the membrane capacitor.

## The Pumping Ratio of the $Na^+/K^+$ Pump and the Electrolytic Conductivities of the Intracellular and Extracellular Fluids

We know that in physical chemistry the electrolytic conductivity of a solution contributed by a kind of ion is (Silbey and Alberty 2001)

$$\kappa(i) = Z(i)FU(i)C(i), \quad (11)$$

where κ(i) is the electrolytic conductivity contributed by the ion i in the solution (S $m^{-1}$), U(i) is the ionic mobility of the ion i ($m^2$ $s^{-1}$ $V^{-1}$), C(i) is the concentration of the ion i (mol $m^{-3}$), F = 96,500 J $V^{-1}$ $mol^{-1}$ is the Faraday's constant and Z(i) is the valence charge of the ion i. Thus, the electrolytic conductivity of the solution contributed by all the ions is given by (Silbey and Alberty 2001)

$$\kappa = \sum Z(i)FU(i)C(i) \tag{11'}$$

Now we consider the extracellular solution. After $n$ pumping cycles of the $Na^+/K^+$ pump, $3n$ sodium ions are pumped out and $2n$ potassium ions are pumped in. The change of $\kappa_o$ is

$$\begin{aligned}\Delta\kappa_o &= Z_{Na}FU_{Na}(C_{Na,o} + 3n/LV_o) - Z_{Na}FU_{Na}C_{Na,o} \\ &\quad + Z_KFU_K(C_{K,o} - 2n/LV_o) - Z_KFU_KC_{K,o},\end{aligned}$$

where $Z_{Na} = Z_K = 1$ are the valence charges of sodium and potassium ions, respectively; $\kappa_o$ is the electrolytic conductivity of the extracellular solution; $U_{Na}$ and $U_K$ are the ionic mobilities of sodium and potassium ions, respectively; $C_{Na,o}$ and $C_{K,o}$ are the sodium and potassium ion concentrations outside the cell, respectively; $V_o$ is the volume of the extracellular fluid; L is the Avogadro's number; and $n$ is the number of pumping cycles. Thus,

$$\begin{aligned}\Delta\kappa_o &= Z_{Na}FU_{Na}(3n/LV_o) + Z_KFU_K(-2n/LV_o) \\ &= (nF/LV_o)(3U_{Na} - 2U_K)\end{aligned}$$

As $\kappa_o$ contributed mainly by sodium and potassium ions (shown next),

$$\begin{aligned}\kappa_o &= Z_{Na}FU_{Na}C_{Na,o} + Z_KFU_KC_{K,o} \\ &= F(U_{Na}C_{Na,o} + U_KC_{K,o}),\end{aligned}$$

we have

$$\Delta\kappa_o/\kappa_o = [(n/LV_o)(3U_{Na} - 2U_K)]/(U_{Na}C_{Na,o} + U_KC_{K,o}) \tag{12}$$

If we assume that $\Delta\kappa_o/\kappa_o = 1$ %, $V_o = 100$ $\mu m^3$, $C_{Na,o} = 440$ mM and $C_{K,o} = 20$ mM for the squid axon (Nicholls et al. 2003) and substituting $U_K = 7.62 \times 10^{-8}$ $m^2$ $s^{-1}$ $V^{-1}$ and $U_{Na} = 5.19 \times 10^{-8}$ $m^2$ $s^{-1}$ $V^{-1}$ into Eq. 12, we have $n \approx 4.44 \times 10^6$. This means that 4 million pumpings can only change $\kappa_o$ by 1 %. This result shows that the pumping ratio of three $Na^+$ to two $K^+$ can ensure that the electrolytic conductivity of the extracellular fluid will not be changed by the pumping of sodium and potassium ions by the $Na^+/K^+$ pump. Consequently, $r_o$ will not change.

Similarly, when we consider the intracellular solution, we have

$$\begin{aligned}\Delta\kappa_i &= Z_{Na}FU_{Na}(C_{Na,i} - 3n/LV_i) - Z_{Na}FU_{Na}C_{Na,i} \\ &\quad + Z_KFU_K(C_{K,i} + 2n/LV_i) - Z_KFU_KC_{K,i} \\ &= Z_{Na}FU_{Na}(-3n/LV_i) + Z_KFU_K(2n/LV_i) \\ &= (nF/LV_i)(2U_K - 3U_{Na}),\end{aligned}$$

where $\kappa_i$ is the electrolytic conductivity of the intracellular solution; $C_{Na,i}$ and $C_{K,i}$ are the sodium and potassium ion concentrations inside the cell, respectively; $V_i$ is the volume of the intracellular fluid; and $n$ is the number of pumping cycles. As $\kappa_i$ is contributed mainly by sodium and potassium ions (shown next);

$$\begin{aligned}\kappa_i &= Z_{Na}FU_{Na}C_{Na,i} + Z_KFU_KC_{K,i} \\ &= F(U_{Na}C_{Na,i} + U_KC_{K,i}),\end{aligned}$$

we have

$$\Delta\kappa_i/\kappa_i = [(n/LV_i)(2U_K - 3U_{Na})]/(U_{Na}C_{Na,i} + U_KC_{K,i}) \tag{12'}$$

If we assume that $-\Delta\kappa_i/\kappa_i = 1$ % ($\Delta\kappa_i < 0$), $V_i = 100$ $\mu m^3$, $C_{Na,i} = 50$ mM and $C_{K,i} = 400$ mM for the squid axon (Nicholls et al. 2003) and substituting $U_K = 7.62 \times 10^{-8}$ $m^2$ $s^{-1}$ $V^{-1}$ and $U_{Na} = 5.19 \times 10^{-8}$ $m^2$ $s^{-1}$ $V^{-1}$ into Eq. 12', we have $n \approx 6.03 \times 10^6$. This means that 6 million pumpings can only change $\kappa_i$ by 1 %. This result shows that the pumping ratio of three $Na^+$ to two $K^+$ can ensure that the electrolytic conductivity of the intracellular fluid will not be changed by the pumping of sodium and potassium ions by the $Na^+/K^+$ pump. Consequently, $r_i$ will not change.

Then, combining Eqs. 6 and 9, we have

$$\tau = R_1R_2C_m/(R_1 + R_2) = (r_i + r_o)r_mC_m/(r_i + r_o + r_m)$$

The time constant τ depends on $r_i$, $r_o$, $r_m$ and $C_m$. The $r_m$ and $C_m$ do not change for a specific cell. So the pumping ratio of three $Na^+$ to two $K^+$ ensures the time constant τ and the τ-dependent processes, such as the normal working state of the $Na^+/K^+$ pump, regardless of the pumping rate, and guarantees the propagation of an action potential.

## Concentration Ratios of Potassium Ions Outside and Inside the Cell to Sodium Ions Inside and Outside the Cell and the $E_K + E_{Na}$

Above (see Dipole-Charging Model of the $Na^+/K^+$ Pump), we calculated the time − average value $I_c$ of $i_c$ over 3τ as

$$I_c = U_d/3R_1 \tag{10}$$

We know that $I_c$ or $i_c$ is the part of the charging current flow which directly charges the membrane capacitor. In this section, we further discuss $I_c$.

### *Asymmetric Charging by the $Na^+/K^+$ Pump*

As already stated, the pumping cycle of the $Na^+/K^+$ pump can be divided into two parts, corresponding to the pumping of sodium and potassium ions, respectively. In the sodium or the first part, after three sodium ions bind to the pump, there will be three negative charges left inside the cell. During the transportation and release of the three sodium ions to outside

the cell, the three negative charges will take the time to move to the inner surface of the membrane. When the three sodium ions begin to move to the outer surface to charge the membrane, the three negative charges may be already on the membrane. This means that the membrane charging is asymmetric. If we only consider the charging by the positive charges, that is, the three sodium ions, the electric resistance $r_i$ can be taken as zero, so $R_1 = r_o$. Conversely, in the potassium or the second part, if we only consider the charging by the potassium ions, the electric resistance $r_o$ would be zero and $R_1 = r_i$. According to Eq. 10,

$$I_c = U_d/3R_1 = U_d/3(r_i + r_o) \tag{13}$$

In the sodium or the first part,

$$I_{c,1} = U_d/3r_o \tag{14}$$

In the potassium or the second part,

$$I_{c,2} = U_d/3r_i \tag{14'}$$

The other difference between the two parts is that the potential difference $U_d$ is proportional to the transported charge, or the dipole charge. We assume that, in the sodium part, $U_d = 3\ \psi$. Then, in the potassium part, $U_d = 2\ \psi$. So, in the sodium or the first part,

$$I_{c,1} = 3\psi/3r_o = \psi/r_o \tag{15}$$

In the potassium or the second part,

$$I_{c,2} = 2\psi/3r_i \tag{15'}$$

The $r_i$ and $r_o$ can be further divided into parts contributed by different ions, as shown by Eq. 11′. The main contributions to $r_i$ and $r_o$ are made by sodium and potassium ions because they are the main extracellular and intracellular cations. Negative charges are not involved in the asymmetric charging because they move away from the membrane. For example, in the sodium part, after three $Na^+$ ions are pumped out of the cell, the dipole will drive the positive ions outside the cell to move toward the outer surface of the cell membrane while driving the negative charges outside the cell to move away from the cell membrane. In the extracellular fluid, the main positive ions are $Na^+$ and $K^+$ ions and the main negative charges are $Cl^-$ ions. At first, the $Cl^-$ ion move very quickly in the field of the electric dipole until a concentration gradient of the $Cl^-$ ion is set up which prevents them from further movement. This process takes place very quickly compared with the movement of the $Na^+$ and $K^+$ ions toward the cell membrane. This is because the movement of the $Cl^-$ ions is an electrochemical process in the solution, while the movement of the $Na^+$ and $K^+$ ions is a charging process of the cell membrane. The movement of the $Cl^-$ ions away from the cell membrane will leave behind equivalent positive charges as $Na^+$ and $K^+$ ions. These equivalent $Na^+$ and $K^+$ ions, together with the other $Na^+$ and $K^+$ ions in the extracellular fluid, will move together toward the cell membrane. So the overall charging process is made by the $Na^+$ and $K^+$ ions and the $Cl^-$ ions do not contribute to $r_o$. Similarly, in the potassium part, the overall charging process is made by the $Na^+$ and $K^+$ ions and the negatively charged proteins do not contribute to $r_i$. So we have

$$(1/r_o) = (1/r_{Na,o}) + (1/r_{K,o}) \tag{16}$$

$$(1/r_i) = (1/r_{Na,i}) + (1/r_{K,i}), \tag{16'}$$

where $r_{Na,o}$ and $r_{K,o}$ are the sodium and potassium resistances outside the cell, respectively, and $r_{Na,i}$ and $r_{K,i}$ are the sodium and potassium resistances inside the cell, respectively.

### *Calculation of Electrolytic Resistance to the $Na^+/K^+$ Pump Current*

In this part, I propose a method to calculate the electric flow of a single ion. First, we calculate the electric resistance of a spherical shell, with inner radius $x_1$ and outer radius $x_2$. Assume that the conductivity of the shell is $\kappa_s$. The electric resistance r between $x_1$ and $x_2$ is

$$r = \int_{x1}^{x2} (1/\kappa_s)(dx/4\pi x^2) = (1/4\pi\kappa_s)[(1/x_1) - (1/x_2)]$$

In the $Na^+/K^+$ pump, the ion flow is produced by a single ion. The corresponding resistance can be calculated when $x_1$ approaches the diameter of the sodium or potassium ion, about 2 Å, while $x_2$ is normally much larger than $x_1$ and can be omitted. Thus, we have

$$r = 10^{10}/8\pi\kappa_s \tag{17}$$

Next, as already noted, the pump dipole always drives the internal and the external currents toward the cell membrane, that is, occupying only half of the sphere. So the electric resistance of a spherical shell (17) should be doubled:

$$r = 10^{10}/4\pi\kappa_s \tag{17'}$$

### *$C_{K,o}/C_{Na,i}$, $C_{K,i}/C_{Na,o}$, and $E_K + E_{Na}$*

Substituting Eqs. 16 into 15 and 16′ into 15′, we have, in the sodium or the first part,

$$I_{c,1} = \psi/r_{Na,o} + \psi/r_{K,o}, \tag{18}$$

and in the potassium or the second part,

$$I_{c,2} = 2\psi/3r_{Na,i} + 2\psi/3r_{K,i} \tag{18'}$$

Substituting Eq. 17′ into 18 and 18′, we have

$$I_{c,1} = 4\pi\psi 10^{-10}(\kappa_{Na,o} + \kappa_{K,o}) \tag{19}$$

$$I_{c,2} = 4\pi\psi 10^{-10}(2/3)(\kappa_{Na,i} + \kappa_{K,i}), \tag{19'}$$

where $\kappa_{Na,o}$ and $\kappa_{K,o}$ are the electrolytic conductivities contributed by the sodium and potassium ions in the

extracellular fluid, respectively, and $\kappa_{Na,i}$ and $\kappa_{K,i}$ are the electrolytic conductivities contributed by the sodium and potassium ions in the intracellular fluid, respectively. According to Eq. 11,

$$\kappa_{Na,o} = Z_{Na}FU_{Na}C_{Na,o}, \kappa_{K,o} = Z_KFU_KC_{K,o},$$
$$\kappa_{Na,i} = Z_{Na}FU_{Na}C_{Na,i}, \kappa_{K,i} = Z_KFU_KC_{K,i},$$

where $Z_{Na} = Z_K = 1$. Substituting these relations into Eqs. 19 and 19′, we have

$$I_{c,1} = 4\pi\psi 10^{-10}F(U_{Na}C_{Na,o} + U_KC_{K,o}) \quad (20)$$

$$I_{c,2} = 4\pi\psi 10^{-10}F(2/3)(U_{Na}C_{Na,i} + U_KC_{K,i}) \quad (20')$$

We know that, in contrast to $i_2$ which is the part of the charging current flow through ion channels, $I_c$ or $i_c$ is the part of the charging current flow which goes onto the membrane. The potassium part of $I_{c,1}$ and the sodium part of $I_{c,2}$ can be used by the $Na^+/K^+$ pump, so they should be compatible with the pumping ratio, that is,

$$U_KC_{K,o} = (2/3)(2/3)\left(U_{Na}C_{Na,i}\right) \quad (21)$$

The sodium part of $I_{c,1}$ and the potassium part of $I_{c,2}$ will affect the membrane electric double layers for resting potential, so they should be equal to each other, that is,

$$U_{Na}C_{Na,o} = (2/3)U_KC_{K,i} \quad (22)$$

Substituting $U_K = 7.62 \times 10^{-8}$ $m^2$ $s^{-1}$ $V^{-1}$ and $U_{Na} = 5.19 \times 10^{-8}$ $m^2$ $s^{-1}$ $V^{-1}$ into Eqs. 21 and 22, we have

$$C_{K,o}/C_{Na,i} = 0.3027 \quad (23)$$

$$C_{K,i}/C_{Na,o} = 0.9788 \quad (24)$$

The equilibrium potential for an ion can be calculated by the Nernst equation (Nicholls et al. 2003): $E = (RT/ZF)\ln(C_o/C_i)$, where $C_o$ and $C_i$ are the concentrations of the ion outside and inside of the cell, respectively; E is the ion's equilibrium potential; $R = 8.31$ J $mol^{-1}$ $K^{-1}$ is the gas constant; and T is the absolute temperature, K. So we can further calculate $E_K+E_{Na}$. As $E_K = (RT/F)\ln(C_{K,o}/C_{K,i})$, $E_{Na} = (RT/F)\ln(C_{Na,o}/C_{Na,i})$, we have

$$\begin{aligned} E_K + E_{Na} &= (RT/F)\ln\left[\left(C_{K,o}/C_{K,i}\right)\left(C_{Na,o}/C_{Na,i}\right)\right] \\ &= (RT/F)\ln\left(C_{K,o}/C_{Na,i}\right) \\ &\quad - (RT/F)\ln\left(C_{K,i}/C_{Na,o}\right) \\ &= (RT/F)\ln(0.3027) - (RT/F)\ln(0.9788) \\ &= -30.3(\text{mV}), \text{at } T = 300\,\text{K}. \end{aligned} \quad (25)$$

This relationship is of significance in the production of the action potential in animals. We know that the membrane resting potential $\varphi$ is closer to $E_K$. For example, in neurons $E_K$ is $-90$ mV and the resting potential $\varphi$ is $-70$ mV. According to our relationship $E_K+E_{Na} = -30.3$ mV, $E_{Na}$ can be calculated as $E_{Na} = -30.3 - E_K \approx 60$ mV. So $E_{Na}$ is close to $\varphi$ in magnitude but with the opposite sign. At rest, the membrane potential is at $\varphi$. During the depolarization phase of an action potential, the membrane potential can achieve $E_{Na}$. As the membrane has a certain capacitance, $E_{Na}$ will produce the same amount of charge with opposite sign as $\varphi$ does. This can ensure the initiation and propagation of an action potential on a neuron. Without this relationship, the magnitude of $E_{Na}$ might be much smaller or much larger than that of $\varphi$. If the magnitude of $E_{Na}$ is very small, the local depolarization might not be able to cause the initiation of an action potential. If the magnitude of $E_{Na}$ is very large, the propagation of an action potential might be unstable. So this relationship may ultimately reveal another significance of the $Na^+/K^+$ pump in the evolution of the action potential in animal cells.

Some comparative data for different animals are shown in Table 1 for the values of $C_{K,o}/C_{Na,i}$, $C_{K,i}/C_{Na,o}$, and $E_K+E_{Na}$.

## Discussion

In this article, I propose a dipole-charging model to explain that the pumping ratio of the $Na^+/K^+$ pump is determined by the ratio of the ionic mobilities of potassium to sodium ions. I further find that the ratios of $C_{K,o}/C_{Na,i}$ and $C_{K,i}/C_{Na,o}$ as well as $E_K+E_{Na}$ are constants. In this section, these results will be further discussed.

### Thermodynamics of the $Na^+/K^+$ Pump: The $Na^+/K^+$ Pump Does Not Work at the Equilibrium State with $C_{K,i}$, $C_{K,o}$, $C_{Na,i}$ and $C_{Na,o}$

#### *Comparing with Other Primary Active Transporters*

The $Na^+/K^+$ pump transports three $Na^+$ ions out of the cell and two $K^+$ ions into the cell in each pumping cycle. This reaction is coupled with hydrolysis of one ATP molecule (Lewin et al. 2007). The concentrations of sodium and potassium ions in the intracellular and extracellular solutions are as follows: intracellular sodium $C_{Na,i}$, intracellular potassium $C_{K,i}$, extracellular sodium $C_{Na,o}$ and extracellular potassium $C_{K,o}$. The coupled overall reaction for the $Na^+/K^+$ pump is

$$\begin{aligned} &3Na^+(\text{in}) + 2\ K^+(\text{out}) + \text{ATP} \\ &\quad \rightarrow 3Na^+(\text{out}) + 2\ K^+(\text{in}) + \text{ADP} + \text{Pi} \end{aligned} \quad (26)$$

where "in" indicates intracellular and "out" indicates extracellular and ATP, ADP and Pi are in the intracellular solution. The equation for the equilibrium state, at membrane potential $\varphi$, was derived by Tanford (1981):

**Table 1** Typical values for intracellular and extracellular sodium and potassium concentrations from six cell types, with calculated and predicted values of $C_{K,o}/C_{Na,i}$, $C_{K,i}/C_{Na,o}$ and $E_K+E_{Na}$

| | Mammalian skeletal muscle[a] | Human[b] | Squid giant axon[c] | Predicted value |
|---|---|---|---|---|
| $C_{K,o}$ (mM) | 4 | 4.3 | 20 | |
| $C_{K,i}$ (mM) | 155 | 139 | 400 | |
| $C_{Na,o}$ (mM) | 145 | 142 | 440 | |
| $C_{Na,i}$ (mM) | 12 | 12 | 50 | |
| $C_{K,o}/C_{Na,i}$ | 0.333 | 0.358 | 0.400 | 0.3027 |
| $C_{K,i}/C_{Na,o}$ | 1.07 | 0.979 | 0.909 | 0.9788 |
| $E_K$ (mV) | −95 | −90 | −77 | |
| $E_{Na}$ (mV) | 64 | 64 | 56 | |
| $E_K+E_{Na}$ (mV) | −31 | −26 | −21 | −30.3 |
| | Crab leg nerve[d] | Frog sartorius muscle[d] | Frog muscle[e] | Predicted value |
| $C_{K,o}$ (mM) | 12 | 2.5 | 2.3 | |
| $C_{K,i}$ (mM) | 410 | 140 | 124 | |
| $C_{Na,o}$ (mM) | 510 | 120 | 109 | |
| $C_{Na,i}$ (mM) | 52 | 10 | 10.4 | |
| $C_{K,o}/C_{Na,i}$ | 0.231 | 0.250 | 0.221 | 0.3027 |
| $C_{K,i}/C_{Na,o}$ | 0.804 | 1.17 | 1.14 | 0.9788 |
| $E_K$ (mV) | −91 | −104 | −103 | |
| $E_{Na}$ (mV) | 59 | 64 | 61 | |
| $E_K+E_{Na}$ (mV) | −32 | −40 | −42 | −30.3 |

Concentrations are given in millimoles per $dm^3$, and potentials are in millivolts. Equilibrium potentials are calculated at T = 300 K

[a] Hille (2001)

[b] Yao (2000)

[c] Nicholls et al. (2003)

[d] Randall et al. (1997)

[e] Nelson and Cox (2000)

$$\left[\left(C_{Na,o}\right)^3\left(C_{K,i}\right)^2\right]/\left[\left(C_{Na,i}\right)^3\left(C_{K,o}\right)^2\right] = K_{ATP}e^{\varphi F/RT}\left[(C_{ATP})/(C_{ADP})(C_{Pi})\right], \quad (26')$$

where $K_{ATP}$ is the equilibrium constant for ATP hydrolysis, $\varphi$ is the membrane potential defined as $\varphi = \varphi(\text{in}) - \varphi(\text{out})$ and $C_{ATP}$, $C_{ADP}$ and $C_{Pi}$ denote the concentrations of ATP, ADP and Pi, respectively. A comparison can be made between the quotients on the left-hand side and the right-hand side of Eq. 26′ to reveal if the pump is working at the equilibrium state. This is because the concentrations of ATP, ADP and Pi are maintained constant by metabolic processes unrelated to the pump (Tanford 1981). Tanford made these comparisons for the ATP-driven $Na^+/K^+$ pump and the ATP-driven $Ca^{2+}$ pump, respectively. The coupled overall reaction for the latter is

$$2Ca^{2+}(\text{in}) + \text{ATP} \rightarrow 2Ca^{2+}(\text{out}) + \text{ADP} + \text{Pi} \quad (27)$$

And the equation for the equilibrium state is

$$\left(C_{Ca,o}\right)^2/\left(C_{Ca,i}\right)^2 = K_{ATP}e^{4\varphi F/RT}[(C_{ATP})/(C_{ADP})(C_{Pi})], \quad (27')$$

where $C_{Ca,i}$ is the concentration of $Ca^{2+}$ ions in the cytosol, $C_{Ca,o}$ is the concentration of $Ca^{2+}$ ions in the sarcoplasmic reticulum, and $\varphi = 0$ mV.

Note that the countertransport of two to three $H^+$ ions is now demonstrated for the SERCA pump (Niggli and Sigel 2008). But because the SR membrane is leaky to $H^+$ and other monovalent cations (Apell 2004), the $H^+$ gradient and the electrical potential are not built up. So Eqs. 27 and 27′ are valid in thermodynamics even if $H^+$ is not counted and $\varphi = 0$ mV across the SR membrane.

Let $Q_{ion}$ denote the left-hand side term in Eqs. 26′ and 27′, that is, the concentration quotient for the ions; let Q denote the right-hand side term in Eqs. 26′ and 27′; and let $Q_{ATP}$ denote the quotient $[(C_{ATP})/(C_{ADP})(C_{Pi})]$. Then, some of the results of Tanford (1981) are reproduced in Table 2, which shows that, for the $Ca^{2+}$ pump, the Q value

is close to that of $Q_{ion}$, which indicates that the $Ca^{2+}$ pump is working across the sarcoplasmic reticulum membrane of a resting skeletal muscle cell at the equilibrium state. But for the $Na^+/K^+$ pump, the Q values are much larger than those of $Q_{ion}$ for the erythrocyte and the squid axon, which indicates that the $Na^+/K^+$ pump is not working at the equilibrium state with the intracellular and extracellular ion concentrations $C_{K,i}$, $C_{K,o}$, $C_{Na,i}$ and $C_{Na,o}$.

To further test this phenomenon, I use some other sources of data to make the same calculations (Nelson and Cox 2000). The results are summarized in Table 3. The Q values are apparently larger than those of $Q_{ion}$, which is consistent with the conclusion made by Tanford (1981) that the $Na^+/K^+$ pump does not work at the equilibrium state with $C_{K,i}$, $C_{K,o}$, $C_{Na,i}$ and $C_{Na,o}$. The concentrations of ATP, ADP and Pi in Table 3 are total concentrations rather than free concentrations due to their binding to cellular proteins. The free concentrations may be much lower. For example, the concentration of free ADP in resting muscle is estimated to be between 1 and 37 μM, about one order of magnitude lower than the values in Table 3 (Nelson and Cox 2000). If the concentration of free ADP is used, the Q values in Table 3 will further increase by one order of magnitude and will be much larger than those of $Q_{ion}$. In this case, $Q_{ATP} = 30{,}000\ M^{-1}$ for the squid axon and the skeletal muscle cell in Table 2 can be explained.

From these comparisons we can see that the ATP-driven $Ca^{2+}$ pump is at the thermodynamic equilibrium state with $C_{Ca,i}$ and $C_{Ca,o}$ but the ATP-driven $Na^+/K^+$ pump is not at the thermodynamic equilibrium state with $C_{K,i}$, $C_{K,o}$, $C_{Na,i}$ and $C_{Na,o}$. The sarcoplasmic reticulum $Ca^{2+}$ pump, SERCA, is located in the sarcoplasmic reticulum membrane in muscle cells. The function of the SERCA pump is to pump $Ca^{2+}$ from the cytosol into the endoplasmic reticulum or the sarcoplasmic reticulum in contracting cells to restore the resting cytosolic $Ca^{2+}$ concentration. The calcium concentrations shown in Table 2 for the SERCA pump are for resting cells. At the resting state of a muscle cell, the SERCA pump is at equilibrium state with $C_{Ca,i}$ and

**Table 2** Physiological gradients established by the $Na^+/K^+$ pump and the sarcoplasmic reticulum $Ca^{2+}$ pump

| | Erythrocyte | Squid axon | Skeletal muscle cell |
|---|---|---|---|
| $Na^+/K^+$ pump | | | |
| $C_{Na,i}$ (mM) | 11 | 50 | |
| $C_{Na,o}$ (mM) | 138 | 440 | |
| $C_{K,i}$ (mM) | 135 | 400 | |
| $C_{K,o}$ (mM) | 4.4 | 20 | |
| $\varphi$ (mV) | −9 | −70 | |
| $Q_{ion}$ | $2 \times 10^6$ | $3 \times 10^5$ | |
| Q | $1.4 \times 10^9$ | $6 \times 10^8$ | |
| $Q_{ATP}$ ($M^{-1}$) | 5,700 | 30,000 | |
| $Ca^{2+}$ pump | | | |
| $C_{Ca,i}$ (mM) | | | $<10^{-4}$ |
| $C_{Ca,o}$ (mM) | | | 2–5 |
| $\varphi$ (mV) | | | 0 |
| $Q_{ion}$ | | | $>10^9$ |
| Q | | | $6 \times 10^9$ |
| $Q_{ATP}$ ($M^{-1}$) | | | 30,000 |

For the $Na^+/K^+$ pump, $Q_{ion} = [(C_{Na,o})^3(C_{K,i})^2]/[(C_{Na,i})^3(C_{K,o})^2]$, $Q = K_{ATP}\, e^{\varphi F/RT}\, Q_{ATP}$. $Q_{ATP}$ is directly given, not calculated from $[(C_{ATP})/(C_{ADP})(C_{Pi})]$

For the $Ca^{2+}$ pump, $Q_{ion} = (C_{Ca,o})^2/(C_{Ca,i})^2$, $Q = K_{ATP}\, e^{4\varphi F/RT}\, Q_{ATP}$. $Q_{ATP}$ is directly given, not calculated from $[(C_{ATP})/(C_{ADP})(C_{Pi})]$

Adapted from Tables I and II in Tanford (1981)

**Table 3** Comparison between different cell types on $Q_{ion}$, Q and $Q_{ATP}$ values for the $Na^+/K^+$ pump

| | $C_{ATP}$ (mM) | $C^a_{ADP}$ (mM) | $C_{Pi}$ (mM) | $Q^b_{ATP}$ ($M^{-1}$) | $Q^b$ | $Q^b_{ion}$ |
|---|---|---|---|---|---|---|
| Rat hepatocyte | 3.38 | 1.32 | 4.8 | 533.5 | $7.1 \times 10^6$ | $2.6 \times 10^6$ |
| Rat myocyte | 8.05 | 0.93 | 8.05 | 1,075.3 | $9.7 \times 10^6$ | $2.6 \times 10^6$ |
| Rat neuron | 2.59 | 0.73 | 2.72 | 1,304.4 | $1.7 \times 10^7$ | $2.6 \times 10^6$ |
| Human erythrocyte | 2.25 | 0.25 | 1.65 | 5,454.5 | $7.7 \times 10^8$ | $1.9 \times 10^6$ |

Data for $C_{ATP}$, $C_{ADP}$ and $C_{Pi}$ are from Nelson and Cox (2000, Table 14-5). For erythrocytes the concentrations are those of the cytosol (human erythrocytes lack a nucleus and mitochondria). In the other types of cells the data are for the entire cell contents, although the cytosol and the mitochondria have very different concentrations of ADP

[a] This value reflects total concentration; the true value for free ADP may be one order of magnitude lower

[b] $Q_{ATP} = [(C_{ATP})/(C_{ADP})(C_{Pi})]$, $Q = K_{ATP}\, e^{\varphi F/RT}\, Q_{ATP}$, $Q_{ion} = [(C_{Na,o})^3(C_{K,i})^2]/[(C_{Na,i})^3(C_{K,o})^2]$. For rat hepatocyte, myocyte and neuron, $Q_{ion}$ values were calculated by taking the concentrations of mammalian skeletal muscle from Table 1. That is, $C_{Na,i} = 12$ mM, $C_{Na,o} = 145$ mM, $C_{K,i} = 155$ mM and $C_{K,o} = 4$ mM. $\varphi$ is assumed to be −70 mV for the calculation of Q values of neurons and hepatocytes and −80 mV for myocytes. For human erythrocyte, the $Q_{ion}$ value was calculated using the data from Table 2. That is, $C_{Na,i} = 11$ mM, $C_{Na,o} = 138$ mM, $C_{K,i} = 135$ mM, $C_{K,o} = 4.4$ mM. $\varphi$ is taken as −9 mV from Table 2 for calculation of the Q value

The calculation of $K_{ATP}$ was as follows: the standard free energy of hydrolysis of ATP is $\Delta G_{ATP}{}'^0 = -30.5$ kJ/mol. Using $\Delta G_{ATP}{}'^0 = -RT\ln K_{ATP}$, we have $K_{ATP} = 2 \times 10^5$ M at T = 300 K

$C_{Ca,o}$ and correspondingly the pump current approaches zero. But at the resting state of a cell, there is a certain amount of potassium leakage current leaving the cell and some amount of sodium current entering the cell. The function of the $Na^+/K^+$ pump is to pump sodium ions out of and potassium ions into the cell to balance these two currents. So the $Na^+/K^+$ pump should not be at the equilibrium state with $C_{K,i}$, $C_{K,o}$, $C_{Na,i}$ and $C_{Na,o}$ because the pump current is not zero. This shows that the thermodynamic properties of the $Na^+/K^+$ pump and the $Ca^{2+}$ pump are compatible with their own functions.

The plasma membrane $Ca^{2+}$-ATPase (PMCA), which is located in the plasma membrane, works at the equilibrium state. The PMCA pump transports one $Ca^{2+}$ ion from the cytosol across the plasma membrane and one to two $H^+$ ions (Niggli and Sigel 2008) from the extracellular fluid to the cytosol by hydrolysis of one ATP, compared to the SERCA pump which transports two $Ca^{2+}$ ions from the cytosol into the sarcoplasmic reticulum and two to three $H^+$ ions from the sarcoplasmic reticulum to the cytosol by hydrolysis of one ATP. Assume that one $H^+$ is transported, then the coupled overall reaction for the PMCA pump is

$$Ca^{2+}(in) + H^+(out) + ATP \rightarrow Ca^{2+}(out) + H^+(in) + ADP + Pi \quad (28)$$

Because in most cells there is only a slight $H^+$ gradient across the plasma membrane (Milanick 1990), the $H^+$ gradient will not be considered and only the electrical gradient of $H^+$ is considered in the equation for the equilibrium state, which is

$$C_{Ca,o}/C_{Ca,i} = K_{ATP}e^{\varphi F/RT}[(C_{ATP})/(C_{ADP})(C_{Pi})], \text{ or,}$$
$$(C_{Ca,o}/C_{Ca,i})e^{-\varphi F/RT} = K_{ATP}[(C_{ATP})/(C_{ADP})(C_{Pi})], \quad (28')$$

where $\varphi$ is the membrane potential defined as $\varphi = \varphi(in) - \varphi(out)$.

Note that the membrane potential across the sarcoplasmic reticulum is zero because the sarcoplasmic reticulum membrane is leaky to monovalent cations (Apell 2004), so the right-hand side term of Eq. 27′ is equal to the right-hand side term of Eq. 28′. Because in resting muscle cells, the SERCA pump is at the equilibrium state, we can directly compare the left-hand side term of Eq. 28′ with the left-hand side term of Eq. 27′ to reveal if the PMCA pump is at the equilibrium state or not. The two terms can be written in an equation as

$$[C_{Ca,o}/C_{Ca,i}]_{PMCA}e^{-\varphi F/RT} = \left[(C_{Ca,o})^2/(C_{Ca,i})^2\right]_{SERCA} \quad (29)$$

We now know that the concentration of free $Ca^{2+}$ is about 100 nM in the cytoplasm, about 2 mM outside the cell and 100 μM in the sarcoplasmic reticulum (Hille 2001). The membrane potential for a skeletal muscle cell is $\varphi = -90$ mV. So $e^{-\varphi F/RT} \approx 33$ at T = 300 K. The two terms of Eq. 29 can be calculated as

$$[C_{Ca,o}/C_{Ca,i}]_{PMCA}e^{-\varphi F/RT} = [2 \times 10^{-3}/10^{-7}] \times 33 \approx 7 \times 10^5,$$
$$\left[(C_{Ca,o})^2/(C_{Ca,i})^2\right]_{SERCA} = [(10^{-4})^2/(10^{-7})^2] \approx 10^6.$$

This shows that the PMCA pump works at the equilibrium state.

*Comparing with Some Secondary Active Transporters*

An example of a cotransport system is $Na^+$-glucose cotransport (Hoffman and Jamieson 1997), in which one glucose molecule is transported into the cell against its concentration gradient coupling with the transport of one $Na^+$ ion down its electrochemical gradient into the cell. The overall reaction can be written as

$$Glucose(out) + Na^+(out) \rightarrow Glucose(in) + Na^+(in) \quad (30)$$

The equation for the equilibrium state at the membrane potential $\varphi$ is similar to Eq. 26′, as in Hoffman and Jamieson (1997, Equation 6-328):

$$C_{glucose,in}/C_{glucose,out} = [C_{Na,o}/C_{Na,i}]e^{-\varphi F/RT}, \quad (30')$$

where $C_{glucose,in}$ is the concentration of glucose inside the cell, $C_{glucose,out}$ is the concentration of glucose outside the cell and $\varphi$ is defined as $\varphi = \varphi(in) - \varphi(out)$. Normally, $C_{Na,o}/C_{Na,i} \approx 10$ and $\varphi$ can be taken as $-60$ mV (Hoffman and Jamieson 1997), so $e^{-\varphi F/RT} \approx 10$ at T = 300 K. Then, $C_{glucose,in}/C_{glucose,out} \approx 100$.

If the transporting ratio is one glucose/two $Na^+$, the reaction (30) will be changed to

$$Glucose(out) + 2Na^+(out) \rightarrow Glucose(in) + 2Na^+(in) \quad (31)$$

And Eq. 30′ now is, as in Hoffman and Jamieson (1997, Equation 6-328):

$$C_{glucose,in}/C_{glucose,out} = [C_{Na,o}/C_{Na,i}]^2e^{-2\varphi F/RT} \quad (31')$$

At $C_{Na,o}/C_{Na,i} \approx 10$ and $\varphi = -60$ mV, $e^{-2\varphi F/RT}$ is about 100 at T = 300 K. Then, $C_{glucose,in}/C_{glucose,out} \approx 10{,}000$.

We see that a change in transporting ratio yields a large difference in the driving force for the transport of glucose. There are two types of the $Na^+$-glucose transporter in the renal proximal tubule brush border membrane (Turner and Moran 1982). The outer cortex transporter is located in the early proximal tubule of the kidney and transports one

glucose and one $Na^+$; the outer medulla transporter is located in the late proximal tubule and transports one glucose and two $Na^+$. Because the glucose concentration is high in the early part of the proximal tubule and decreases along the proximal tubule to the late part, the arrangement of these two types of the transporter ensures a very efficient way for the kidney to reabsorb glucose.

This example shows two points. First, the two types of the $Na^+$-glucose transporter work toward their equilibrium states defined by Eqs. 30′ and 31′. These can be compared to the SERCA pump, which works toward the equilibrium state defined by Eq. 27′. All these are different from the $Na^+/K^+$ pump, which always works at a nonequilibrium state defined by Eq. 26′ as an inequality. Second, the transporting ratio is determined by the transporting function; in the $Na^+$-glucose transporter it is the glucose concentration gradient. For the $Na^+/K^+$ pump the pumping ratio is the same throughout animal cells, which suggests that the function it serves does not change.

An example of a countertransport system is the $Na^+/Ca^{2+}$ exchanger in muscle cells (Hoffman and Jamieson 1997), in which one $Ca^{2+}$ ion is transported out of the cell against its electrochemical gradient coupling with the transport of three $Na^+$ ions down their electrochemical gradient into the cell. The overall reaction can be written as

$$Ca^{2+}(\text{in}) + 3Na^+(\text{out}) \rightarrow Ca^{2+}(\text{out}) + 3Na^+(\text{in}) \tag{32}$$

The equation for the equilibrium state at the membrane potential $\varphi$ can be derived as in Hoffman and Jamieson (1997, Equation 6–360):

$$C_{Ca,i}/C_{Ca,o} = \left(C_{Na,i}/C_{Na,o}\right)^3 e^{\varphi F/RT}, \tag{32'}$$

where $\varphi$ is defined as $\varphi = \varphi(\text{in}) - \varphi(\text{out})$. If $C_{Na,o}/C_{Na,i} \approx 10$ and $\varphi = -60$ mV, there will be $e^{\varphi F/RT} \approx 0.1$ at $T = 300$ K. Then, $C_{Ca,i}/C_{Ca,o} \approx 10^{-4}$.

We know that the concentration of free $Ca^{2+}$ in the cytoplasm is 100 nM and that outside the cell is 2 mM (Hille 2001). This is compatible with $C_{Ca,i}/C_{Ca,o} \approx 10^{-4}$ and shows that the $Na^+/Ca^{2+}$ exchanger works toward the equilibrium state defined by Eq. 32′. In this sense, the $Na^+/Ca^{2+}$ exchanger is similar to the $Na^+$-glucose transporter and the SERCA pump but different compared to the $Na^+/K^+$ pump.

## The Pumping Ratio Is an Electric Property but Not a Thermodynamic Property of the $Na^+/K^+$ Pump

As we showed in Results (see The Pumping Ratio of the $Na^+/K^+$ Pump and the Electrolytic Conductivities of the Intracellular and Extracellular Fluids), the pumping ratio of the $Na^+/K^+$ pump is determined by the ratio of the ionic mobilities of potassium and sodium ions. This means that the pumping ratio is an electric property of the $Na^+/K^+$ pump. Above (see Comparing with Other Primary Active Transporters), we discussed the coupled overall reaction for the $Na^+/K^+$ pump (26) and the equation for the equilibrium state of the pump (26′):

$$3Na^+(\text{in}) + 2\ K^+(\text{out}) + \text{ATP} \rightarrow 3Na^+(\text{out}) + 2\ K^+(\text{in}) + \text{ADP} + \text{Pi} \tag{26}$$

$$\left[\left(C_{Na,o}\right)^3\left(C_{K,i}\right)^2\right]/\left[\left(C_{Na,i}\right)^3\left(C_{K,o}\right)^2\right] = K_{ATP}e^{\varphi F/RT}\left[(C_{ATP})/(C_{ADP})(C_{Pi})\right] \tag{26'}$$

We can see that the pumping ratio appears in the thermodynamic Eq. 26′. It may be argued that the pumping ratio is a thermodynamic property of the $Na^+/K^+$ pump. This is not true for the $Na^+/K^+$ pump. We have several reasons.

(1) The $Na^+/K^+$ pump always works at a nonequilibrium state (as discussed in Comparing with Other Primary Active Transporters). That is, Eq. 26′ is an inequality.

For example, we can calculate the $Q_{ion}$ and Q values for mammalian skeletal muscle. The data from Table 1 are used: $C_{Na,i} = 12$ mM, $C_{Na,o} = 145$ mM, $C_{K,i} = 155$ mM, $C_{K,o} = 4$ mM. $\varphi$ is assumed to be $-80$ mV. $K_{ATP} = 2 \times 10^5$ M, at $T = 300$ K. $Q_{ATP} = 30{,}000\ M^{-1}$ is used from Tanford (1981). We have

$$Q_{ion} = \left[\left(C_{Na,o}\right)^3\left(C_{K,i}\right)^2\right]/\left[\left(C_{Na,i}\right)^3\left(C_{K,o}\right)^2\right] = 2.6 \times 10^6, Q = K_{ATP}e^{\varphi F/RT}Q_{ATP} = 2.7 \times 10^8. \tag{33}$$

Obviously, $Q_{ion} < Q$.

We further calculate the $Q_{ion}$ and Q values for the squid axon. The data from Table 1 are used: $C_{Na,i} = 50$ mM, $C_{Na,o} = 440$ mM, $C_{K,i} = 400$ mM, $C_{K,o} = 20$ mM. $\varphi$ is assumed to be $-70$ mV. $K_{ATP} = 2 \times 10^5$ M, at $T = 300$ K. $Q_{ATP} = 30{,}000\ M^{-1}$ is used from Tanford (1981). We have

$$Q_{ion} = \left[\left(C_{Na,o}\right)^3\left(C_{K,i}\right)^2\right]/\left[\left(C_{Na,i}\right)^3\left(C_{K,o}\right)^2\right] = 2.7 \times 10^5, Q = K_{ATP}e^{\varphi F/RT}Q_{ATP} = 4.0 \times 10^8. \tag{34}$$

Obviously, $Q_{ion} < Q$.

This means that the relationship between the pumping ratio and other quantities in Eq. 26′ such as $C_{K,i}$, $C_{K,o}$, $C_{Na,i}$, $C_{Na,o}$, $K_{ATP}$, $\varphi$, ATP, ADP and Pi is indirect. By contrast, the SERCA pump, the PMCA pump, the $Na^+$-glucose cotransporters and the $Na^+/Ca^{2+}$ exchanger all work at or toward the equilibrium state, depending on the cell states. Their pumping or transporting ratios are thermodynamic properties of their own.

(2) We further discuss if the pumping ratio can be determined when Eq. 26′ is an inequality. This is

because, unlike other quantities in 26′, any change of the pumping ratio will significantly increase or decrease $Q_{ion}$, the left-hand side term of 26′.

The change in free energy per mole of the reaction (26) is

$$\Delta G_{pump} = \Delta G_{pump}{}'^{0} + RT\ln\left\{\left\{\left[(C_{Na,o})^3(C_{K,i})^2\right]/\left[(C_{Na,i})^3(C_{K,o})^2\right]\right\}\left[(C_{ADP})(C_{Pi})/(C_{ATP})\right]\right\} - (3Z_{Na} - 2Z_K)\varphi F, \quad (35)$$

where $Z_{Na}$, $Z_K$ are the valence charges of the sodium and potassium ions, respectively. So

$$\Delta G_{pump} = \Delta G_{ATP}{}'^{0} + \Delta G_{Na}{}'^{0} + \Delta G_K{}'^{0} + RT\ln\left\{\left\{\left[(C_{Na,o})^3(C_{K,i})^2\right]/\left[(C_{Na,i})^3(C_{K,o})^2\right]\right\}\left[(C_{ADP})(C_{Pi})/(C_{ATP})\right]\right\} - (3Z_{Na} - 2Z_K)\varphi F \quad (36)$$

As

$$\Delta G_{Na}{}'^{0} = 0, \Delta G_K{}'^{0} = 0, \text{ and } \Delta G_{ATP}{}'^{0} = -RT\ln K_{ATP}, \quad (37)$$

we have

$$\Delta G_{pump} = -RT\ln K_{ATP} + RT\ln\left\{\left\{\left[(C_{Na,o})^3(C_{K,i})^2\right]/\left[(C_{Na,i})^3(C_{K,o})^2\right]\right\}\left[(C_{ADP})(C_{Pi})/(C_{ATP})\right]\right\} - (3Z_{Na} - 2Z_K)\varphi F \quad (38)$$

When setting $\Delta G_{pump} = 0$ in Eq. 38 and with $Z_{Na} = Z_K = 1$, we can get 26′. Equation 38 can be rearranged as

$$\begin{aligned}\Delta G_{pump} &= -RT\ln K_{ATP} + RT\ln\left\{\left[(C_{Na,o})^3(C_{K,i})^2\right]/\left[(C_{Na,i})^3(C_{K,o})^2\right]\right\} + RT\ln\left[(C_{ADP})(C_{Pi})/(C_{ATP})\right] - (3Z_{Na} - 2Z_K)\varphi F \\ &= -RT\ln K_{ATP} + RT\ln Q_{ion} - RT\ln Q_{ATP} - (3Z_{Na} - 2Z_K)\varphi F \end{aligned} \quad (39)$$

We notice that $\Delta G_{pump}$ is a function of $Q_{ion}$, $Q_{ATP}$ and $\varphi$. To maintain a certain $\Delta G_{pump}$, several quantities can vary, such as the ion concentrations in $Q_{ion}$, $Q_{ATP}$ and $\varphi$ and the pumping ratio. We evaluate several cases.

First, we compare the $Q_{ion}$ values for mammalian skeletal muscle in Eq. 33 and for the squid axon in Eq. 34. The $Q_{ion}$ for mammalian skeletal muscle is $2.6 \times 10^6$, which is one order of magnitude higher than the $Q_{ion}$ for the squid axon, $2.7 \times 10^5$. This corresponds to the change of $\Delta G_{pump}$ in Eq. 39 about

$$\Delta(\Delta G_{pump}) \approx RT\ln 10\,\mathrm{J\,mol^{-1}} \approx 5.74\,\mathrm{kJ\,mol^{-1}}, \quad \text{at } T = 300\,\mathrm{K}, \quad (40)$$

provided that $\varphi$ is the same.

Second, we calculate the change of $\Delta G_{pump}$ for the increase or decrease of one pumping ion. Equation 38 can be rearranged as

$$\begin{aligned}\Delta G_{pump} &= -RT\ln K_{ATP} + RT\ln\left\{\left[(C_{Na,o})^3(C_{K,i})^2\right]/\left[(C_{Na,i})^3(C_{K,o})^2\right]\right\} + RT\ln\left[(C_{ADP})(C_{Pi})/(C_{ATP})\right] - (3Z_{Na} - 2Z_K)\varphi F \\ &= -RT\ln K_{ATP} - RT\ln Q_{ATP} + RT\ln\left\{\left[(C_{Na,o})^3(C_{K,i})^2\right]/\left[(C_{Na,i})^3(C_{K,o})^2\right]\right\} - (3Z_{Na} - 2Z_K)\varphi F \\ &= -RT\ln K_{ATP} - RT\ln Q_{ATP} + \left\{RT\ln\left[(C_{Na,o})^3/(C_{Na,i})^3\right] - 3Z_{Na}\varphi F\right\} + \left\{RT\ln\left[(C_{K,i})^2/(C_{K,o})^2\right] + 2Z_K\varphi F\right\} \end{aligned} \quad (41)$$

The first two terms in Eq. 41 are the free energy released by hydrolysis of 1 mol ATP under physiological conditions, which we denote as $\Delta G_{ATP} = -RT\ln K_{ATP} - RT\ln Q_{ATP}$; the third term in Eq. 41 is the free energy used for pumping 3 mol sodium ions from inside to outside the cell, which we denote as $\Delta G_{3Na} = RT\ln[(C_{Na,o})^3/(C_{Na,i})^3] - 3Z_{Na}\varphi F$; the fourth term in Eq. 41 is the free energy used for pumping 2 mol potassium ions from outside to inside the cell, which we denote as $\Delta G_{2K} = RT\ln[(C_{K,i})^2/(C_{K,o})^2] + 2Z_K\varphi F$. So, the free energy used for pumping 1 mol sodium ions and 1 mol potassium ions would be, respectively,

$$\Delta G_{Na} = (1/3)\Delta G_{3Na} = RT\ln\left[(C_{Na,o})/(C_{Na,i})\right] - Z_{Na}\varphi F, \quad \Delta G_K = (1/2)\Delta G_{2K} = RT\ln\left[(C_{K,i})/(C_{K,o})\right] + Z_K\varphi F \quad (42)$$

We calculate $\Delta G_{pump}$, $\Delta G_{Na}$ and $\Delta G_K$ as follows. First, calculate $\Delta G_{ATP}$, by using $K_{ATP} = 2 \times 10^5$ M at T = 300 K and $Q_{ATP} = 30{,}000\ M^{-1}$ and get $\Delta G_{ATP} \approx -56\ kJ\ mol^{-1}$.

For mammalian skeletal muscle, $C_{Na,o}/C_{Na,i} = 145/12$, $C_{K,i}/C_{K,o} = 155/4$, and $\varphi = -80$ mV. We have $\Delta G_{pump} = -11.5\ kJ\ mol^{-1}$, $\Delta G_{Na} = 13.9\ kJ\ mol^{-1}$ and $\Delta G_K = 1.40\ kJ\ mol^{-1}$. For the squid axon, $C_{Na,o}/C_{Na,i} = 440/50$, $C_{K,i}/C_{K,o} = 400/20$, and $\varphi = -70$ mV. We have $\Delta G_{pump} = -18.2\ kJ\ mol^{-1}$, $\Delta G_{Na} = 12.2\ kJ\ mol^{-1}$ and $\Delta G_K = 0.713\ kJ\ mol^{-1}$. The results show that $\Delta G_{Na}$ is about 10-fold larger than $\Delta G_K$. $|\Delta G_{pump}|$ can be comparable to $\Delta G_{Na}$ but much larger than $\Delta G_K$.

Third, we consider the change of $\varphi$. The membrane potential $\varphi$ in animal cells ranges from −9 mV in human

red blood cells to −90 mV in frog skeletal muscle cells. At the pumping ratio of three Na to two $K^+$, this range of $\varphi$ corresponds to the change of $\Delta G_{pump}$ about

$$\Delta(\Delta G_{pump}) = |[(-9) - (-90)] \times 10^{-3} \times 96500| = 7.82\,\text{kJ mol}^{-1}. \quad (43)$$

In these three cases, the first case shows that the difference in free energy change $\Delta G_{pump}$ due to $Q_{ion}$ between the squid axon and mammalian skeletal muscle is about 5.74 kJ $mol^{-1}$, but this does not cause any change in the pumping ratio. The second case shows that the free energy used for pumping 1 mol sodium ions is about 10-fold larger than that for pumping 1 mol potassium ions (Jorgensen 2004). This is because the potassium equilibrium potential is close to the resting potential and the resting potential favors the pumping of potassium ions from outside to inside the cell. So the pumping reaction has enough free energy to provide for pumping more potassium ions, for example, three $Na^+$ to three $K^+$. But this does not happen. The third case shows that the range of resting potentials in animal cells corresponds to the difference of 7.82 kJ $mol^{-1}$ in free energy change, and this does not change the pumping ratio. These results show that the pumping ratio of the $Na^+/K^+$ pump is not a thermodynamic property. As already shown, the pumping ratio is an electric property of the pump, due to the ratio of the ionic mobilities of potassium and sodium ions.

## Evolution of the Structure and Function of the $Na^+/K^+$ Pump

The $Na^+/K^+$ pump belongs to a large family of P-type ATPases, which are phosphorylated on a highly conserved aspartic acid residue during the transport process (Lewin et al. 2007) and establish electrochemical gradients of cations across cell membranes (Bublitz et al. 2010). They include the $Na^+/K^+$-ATPase in the plasma membrane of animal cells; the $H^+$-ATPase in the plasma membrane of plants, fungi and bacteria; the $H^+/K^+$-ATPase in acid-secreting cells of the mammalian stomach; the SERCA; and the PMCA (Lodish et al. 2004). And there are many others (Axelsen and Palmgren 1998). According to the classification of Axelsen and Palmgren (1998), all P-type ATPases are grouped into five subfamilies, I–V. The α-subunit isoforms of the $Na^+/K^+$-ATPase, together with the two isoforms of the $H^+/K^+$-ATPase, belong to the subgroup IIc of P-ATPases. Subgroups IIa and IIb include the two main types of the calcium pump, SERCAs and PMCAs (Horisberger 2004). The $H^+$-ATPase belongs to the subfamily III. The $Na^+/K^+$-ATPase in animals and the $H^+$-ATPase in plants and fungi maintain the sodium and potassium gradients as well as the proton gradient (Morth et al. 2011), respectively, to establish their own resting membrane potentials. This shows that the P-type ATPase existed before the differentiation of animals and plants.

P-type ATPases have similar protein folding and transport mechanisms. First, they share similar structures. For example, the $Na^+/K^+$-ATPase, the $H^+/K^+$-ATPase and the PMCA can be compared with the SERCA. The α-subunit, or the catalytic subunit of the $Na^+/K^+$-ATPase, has high sequence similarity with the SERCA and the gastric $H^+/K^+$-ATPase. The overall similarity of the transmembrane sequences between the $Na^+/K^+$-ATPase and the $Ca^{2+}$-ATPase is about 40 % (Ogawa and Toyoshima 2002). Second, the phosphorylation site is highly conserved among all P-type ATPases (Lewin et al. 2007). Third, the first two binding sites for the sodium ion and the two binding sites for the potassium ion in the $Na^+/K^+$ pump are homologous to the two calcium binding sites in the SERCA pump, and the third binding site for the sodium ion in the $Na^+/K^+$ pump is different from but contiguous to the first site (Ogawa and Toyoshima 2002). Other mutational studies and structural analyses identified two sites for the third $Na^+$-binding site (Morth et al. 2011). Furthermore, the ion-binding sites of the SERCA, the $H^+$-ATPase, the $Na^+/K^+$-ATPase and the $H^+/K^+$-ATPase were compared and found to be similar (Bublitz et al. 2010). These facts show that the $Na^+/K^+$ pump is not special in structure compared with other P-type pumps. But the transporting of the third sodium ion is specific because the binding sites for the other two sodium ions are homologous to the corresponding two calcium-binding sites in the SERCA. This indicates that the third sodium ion may evolve for some special functions of the cell.

As previously discussed, the pumping ratio of the $Na^+/K^+$ pump is not limited to thermodynamics. Then, a question arises concerning the structure and function of the $Na^+/K^+$ pump that whether the pumping ratio is related to the structure of the pump. That is, the fact that other pumping ratios do not appear is due to the structural obstacle of the pump. We make some further discussions as follows.

### *Further Thermodynamic Analysis*

Above (See The Pumping Ratio Is an Electric Property but Not a Thermodynamic Property of the $Na^+/K^+$ Pump), we calculated three quantities, $\Delta G_{pump}$, $\Delta G_{Na}$ and $\Delta G_K$, where $\Delta G_{pump}$ is the change in free energy per mole of the coupled overall reaction (26) for the $Na^+/K^+$ pump, $\Delta G_{Na}$ is the free energy used for pumping 1 mol sodium ions and $\Delta G_K$ is the free energy used for pumping 1 mol potassium ions. The results show that $\Delta G_{Na}$ is about 10-fold larger than $\Delta G_K$ and that $|\Delta G_{pump}|$ is comparable to $\Delta G_{Na}$ but much larger than $\Delta G_K$. This means that, under physiological conditions, the $Na^+/K^+$ pump does not work at the

equilibrium state. This is because the absolute change in free energy per mole of the overall reaction $\Delta G_{pump}$ is not zero but is comparable to $\Delta G_{Na}$. The sodium ions contribute to nearly all the free energy used by the pump, while the potassium ions contribute only a little. So how many potassium ions can be pumped in each cycle is not relevant to the thermodynamics of the pump. For example, three $Na^+$ to three $K^+$, three $Na^+$ to two $K^+$ and three $Na^+$ to one $K^+$ are nearly equivalent in thermodynamics. On the other hand, we notice that due to different $Q_{ion}$ values the free energy used by the $Na^+/K^+$ pump in mammalian skeletal muscle is more than that used in the squid axon. The difference is about 5.74 kJ $mol^{-1}$ at T = 300 K, as estimated in Eq. 40, provided that $\varphi$ is the same. So if in mammalian skeletal muscle the number of sodium ions pumped in each cycle is reduced to two, this will not cause too much excess of the free energy and the pump can be assumed to work properly. If this were the case, the pumping ratios of two $Na^+$ to three $K^+$, two $Na^+$ to two $K^+$ and two $Na^+$ to one $K^+$ would be reasonable. So thermodynamically the $Na^+/K^+$ pump allows many pumping ratios to exist.

*Mutational Analysis*

The $Na^+/K^+$-ATPase and the $H^+/K^+$-ATPase in the subgroup IIc of P-type ATPases are the two most closely related P-type ATPases and may be taken as a recent development in evolution (Axelsen and Palmgren 1998). They share 60 % amino acid identity and provide related functions in transporting $K^+$ ions into the cell and another cation, $Na^+$ ions or protons, out of the cell. The activity of the $Na^+/K^+$-ATPase is electrogenic because of exchanging three $Na^+$ ions for two $K^+$ ions. In contrast, the gastric $H^+/K^+$-ATPase performs an electroneutral exchange of two $K^+$ for two protons (Burnay et al. 2003).

A sequence comparison of the fifth transmembrane segment of the $Na^+/K^+$-ATPase and the gastric and nongastric $H^+/K^+$-ATPase α-subunits shows that there is a highly conserved neutral serine residue in the fifth transmembrane segment of the $Na^+/K^+$-ATPase, which is replaced with a single positively charged residue lysine in all known $H^+/K^+$-ATPases. Mutation at this single position shows that replacement of the neutral serine by a positively charged arginine in the $Na^+/K^+$-ATPase abolished electrogenic transport activity, whereas mutation to a neutral alanine preserved the electrogenic transport activity. Conversely, replacement of the positively charged lysine by a neutral alanine in the nongastric $H^+/K^+$-ATPase of *Bufo marinus* produced electrogenic ion pumping similar to that observed with the $Na^+/K^+$-ATPase, whereas replacing this lysine with a positively charged arginine yielded nonelectrogenic transport activity. So Burnay et al. (2003) concluded that a single positively charged residue in the fifth transmembrane segment of the α-subunit can determine the electrogenicity and the stoichiometry of cation transport by these ATPases. This work provides the evidence that the conservation of the three $Na^+$ to two $K^+$ pumping ratio of the $Na^+/K^+$ pump in all animal cells during evolution was not due to the structural obstacle of the pump but should be subjected to some functional constraints of the cell.

*Pump–Leak Model Analysis*

At the steady state of the cell, the $Na^+/K^+$ pump maintains the concentration gradients of the sodium and potassium ions across the cell membrane. The sodium ions which are pumped out of the cell will enter the cell again through sodium leakage channels or by secondary active transport. The potassium ions which are pumped into the cell will leave the cell again through potassium leakage channels. The transport of the sodium and potassium ions by the action of the $Na^+/K^+$ pump will compensate for the transport of the sodium and potassium ions down their concentration gradients into and out of the cell, respectively. This is known as the pump–leak model (Keener and Sneyd 2009; Blaustein et al. 2004). Let $I_{Na}$ be the total sodium current through channels or secondary active transporters which enters the cell under physiological conditions and $I_K$ be the total potassium current through leakage channels which leaves the cell under physiological conditions. To balance the pumping of three $Na^+$ to two $K^+$ by the pump, it should be that

$$I_{Na}/I_K = 3/2 \tag{44}$$

The relationship of Eq. 44 should not be a functional requirement of the cell because $I_{Na}$ and $I_K$ are regulated by many factors, such as $E_K$, $E_{Na}$, membrane potential and the number of channels and transporters existing in the cell membrane. Even if the normal function of the cell requires that $I_{Na} > I_K$, there should not be a ratio of 3 to 2. Furthermore, when there are mutants of the $Na^+/K^+$ pump such as two $Na^+$/two $K^+$ or two $Na^+$/three $K^+$, the mixture of them can make the $I_{Na}/I_K$ be any ratio. But we do not see any such mutants of the $Na^+/K^+$ pump in any animals or any types of animal cells. As already mentioned, structural analysis indicates that the occurrence of the third $Na^+$ should be special for some functions of the cell. The pump–leak model shows that the ionic homeostasis for $Na^+$ and $K^+$ of the cell does not require three $Na^+$ to two $K^+$. So, in this sense, we can say that the pumping of the third sodium ion by the $Na^+/K^+$ pump is not related to the function of the cell itself but rather to the function of the $Na^+/K^+$ pump. This supports our result that the pumping ratio is an electric property of the $Na^+/K^+$ pump.

**Table 4** Concentration of common ions (mM/kg water) in some marine animals

| | Sodium | Potassium | Calcium | Magnesium | Chloride | Sulphate | Protein mg/ml |
|---|---|---|---|---|---|---|---|
| Sea water standard | 478.3 | 10.13 | 10.48 | 54.5 | 558.4 | 28.77 | – |
| *Aurelia* mesogloeal fluid | 474 | 10.72 | 10.03 | 53.0 | 580 | 15.77 | 0.7 |
| *Aphrodite* coelomic fluid | 476 | 10.50 | 10.45 | 54.6 | 557 | 26.50 | 0.2 |
| *Echinus* coelomic fluid | 474 | 10.13 | 10.62 | 53.5 | 557 | 28.70 | 0.3 |
| *Mytilus* blood | 474 | 12.00 | 11.90 | 52.6 | 553 | 28.90 | 1.6 |
| *Loligo* blood | 456 | 22.20 | 10.60 | 55.4 | 578 | 8.14 | 149.7 |
| *Ligia* blood | 566 | 13.30 | 34.90 | 20.2 | 629 | 4.03 | – |
| *Maia* blood | 488 | 12.37 | 13.56 | 44.1 | 554 | 14.50 | – |
| *Carcinus* blood | 531 | 12.26 | 13.32 | 19.5 | 557 | 16.46 | 60 |
| *Nephrops* blood | 541 | 7.81 | 11.95 | 9.28 | 552 | 19.8 | 33 |
| *Myxine* blood | 537 | 9.12 | 5.87 | 18.0 | 542 | 6.33 | 67 |

From Potts and Parry (1964, Table III.2)

**Table 5** The osmotic constituents of the blood of some freshwater animals

| Animal | Na | K | Ca | Mg | Cl | $HCO_3$ | Other ions |
|---|---|---|---|---|---|---|---|
| *Rana esculenta* (mM/kg water) | 109 | 2.6 | 2.1 | 1.3 | 78 | 26.6 | Lactate 3.5 |
| *Salmo trutta* (mM/l blood) | 161 | 5.3 | 6.3 | 0.93 | 119 | n.d. | Phosphate 1.0 |
| *Potamon niloticus* (mM/l blood) | 259 | 8.4 | 12.7 | n.d. | 242 | n.d. | |
| *Astacus fluviatilis* (mM/l blood) | 212 | 4.1 | 15.8 | 1.5 | 199 | 15 | |
| *Sialis lutaria* (mM/l blood) | 109 | 5 | 7.5 | 19 | 31 | 15 | Amino acids 152 |
| *Anodonta cygnaea* (mM/kg water) | 15.6 | 0.5 | 6 | 0.2 | 11.7 | 12 | Amino acids 0.2 |

From Potts and Parry (1964, Table V.3)

## Comparative Study on the Concentration Ratios of $C_{K,o}/C_{Na,i}$, $C_{K,i}/C_{Na,o}$ and $E_K+E_{Na}$

We can see from Table 1 that the observation values of $C_{K,o}/C_{Na,i}$ and $C_{K,i}/C_{Na,o}$ of different cell types are consistent with the predicted values. It is more evident when the squid giant axon and the crab leg nerve are compared with mammalian skeletal muscle, human and the frog muscle, where the $C_{K,o}$, $C_{K,i}$, $C_{Na,o}$ and $C_{Na,i}$ values of the two groups are far from each other but the ratios $C_{K,o}/C_{Na,i}$ and $C_{K,i}/C_{Na,o}$ are all close to the predicted values.

$E_K+E_{Na}$ is less conserved from its theoretical value than $C_{K,o}/C_{Na,i}$ and $C_{K,i}/C_{Na,o}$ from theirs because the value of $E_K+E_{Na}$ is determined as a function of the ratios of $C_{K,o}/C_{Na,i}$ and $C_{K,i}/C_{Na,o}$.

For the two constants $C_{K,o}/C_{Na,i} = 0.3027$ and $C_{K,i}/C_{Na,o} = 0.9788$, there may be another explanation for $C_{K,i}/C_{Na,o} = 0.9788$. That is, $C_{K,i}/C_{Na,o} \approx 1$ can maintain the intracellular and extracellular osmotic pressures equal because the sodium and potassium ions are chief cations in the extracellular and intracellular fluids, respectively. However, as discussed by Sherwood et al. (2005), the osmotic pressure of the body fluids of most marine animals is about 1,000 mOsm, which is the same as that of seawater. The extracellular fluid of a marine osmoconformer is about 1,000 mOsm, dominated by NaCl, and sometimes contains high levels of organic osmolytes. The intracellular fluid of a marine osmoconformer is the same, about 1,000 mOsm, including 300–400 mOsm of universal solutes ($K^+$, macromolecules, and so forth) and about 600–700 mOsm of organic osmolytes. The organic

**Table 6** Composition of the blood (mM/kg, mM/l) of some land animals (selected)

| Animal | Na | K | Ca | Mg | Cl | $HCO_3$ | Amino acids | $PO_4$ |
|---|---|---|---|---|---|---|---|---|
| *Tegenaria atrica* | 207 | 9.6 | – | – | 193 | – | – | – |
| *Petrobius* sp. | 208 | 5.8 | – | – | 194 | – | – | – |
| *Aeschna* sp. *(larva)* | 135 | 5.4 | 7.5 | 6.0 | 120 | – | – | – |
| *Periplaneta* sp. | 156 | 7.7 | 4.2 | 5.4 | 144 | – | – | – |
| *Helix pomatia* | 113 | 4 | 11 | 13.4 | – | 24.2 | – | – |
| *Rana temporaria* | 104 | 2.5 | 2 | 1.2 | 74.3 | 30 | 0.7 | 3 |
| *Rattus rattus* | 140 | 6.4 | 3.4 | 1.6 | 119 | 24.3 | 3 | 2.3 |

From Potts and Parry (1964, Table VI.2)

osmolytes include carbohydrates, free amino acids, methylamine and methylsulfonium solutes and urea. There are two reasons for osmoconformers to use organic solutes as osmolytes rather than inorganic ions, such as high NaCl in the extracellular fluid. First, many cells use a sodium gradient for coupled transport and signal conduction, whereas movement of $Cl^-$ ions into the cell is unfavorable because the membrane resting potential is more negative inside the cell. Second and more universally, inorganic ions (especially $Na^+$ and $Cl^-$) at high concentrations can disrupt the structure and function of macromolecules because of charge interactions. If NaCl were to accumulate in cells to the level of the extracellular fluid and seawater, the conformation of many proteins would become abnormal and DNA would unravel. Among the common inorganic ions, $K^+$ disrupts macromolecules least, perhaps explaining why it is the universal cellular cation; but it too can be disruptive at high levels. Most organic osmolytes do not disturb macromolecules even at high concentrations; some organic osmolytes have the ability to stabilize macromolecules against disruptive forces. So, in this sense, it is unlikely that the accumulation of potassium ions in the intracellular fluid to the level of sodium ions in the extracellular fluid and seawater is to adjust the osmotic pressure because osmoconformers prefer to use organic osmolytes rather than inorganic ions.

In Table 1, the extracellular ion concentrations of the squid axon and the crab leg nerve are similar to those of seawater. This is the characteristic of the marine animal (Table 4) (Potts and Parry 1964). The frog is a freshwater animal, so the ion concentrations of the body fluid are much lower (Table 5) (Potts and Parry 1964). Mammals including human are terrestrial animals, and their body fluid ion concentrations are lower (Table 6) (Potts and Parry 1964). Potts and Parry (1964) listed the reasons for the evolution of the ion concentrations of the body fluid that "factors which may affect the blood concentration of a freshwater animal include the surface:volume ratio and hence the absolute size of the animal, the permeability of the body wall, the metabolic rate, and the period of time the group has been adapted to fresh water. The vertebrates have relatively uniform blood concentrations (between 1/4 and 1/3 sea water) and this may be related to their descent from a common freshwater ancestor. The crustaceans, in contrast, have a very wide range of blood concentration". "For example, *Eriocheir*, which has a high blood concentration, and still breeds in the sea, is probably a recent immigrant to fresh water, while the smaller branchiopods with a lower blood concentration are an ancient group of freshwater crustaceans".

Those quantities which are conserved should have significance for cell function. These include, for example, $C_{K,o}/C_{Na,i}$, $C_{K,i}/C_{Na,o}$ and $E_K+E_{Na}$. As Prosser (1950) stated, "while other branches of physiology use such variables as light, temperature, oxygen tension, and hormone balance, comparative physiology uses, in addition, species or animal type as a variable for each function." Our study shows that $C_{K,o}/C_{Na,i}$, $C_{K,i}/C_{Na,o}$ and $E_K+E_{Na}$ can be used as variables in the study of comparative physiology.

## Conclusion

Our analysis shows that, first, with the three $Na^+$ to two $K^+$ pumping ratio, the $Na^+/K^+$ pump will not change the electrolytic conductivities of the intracellular and extracellular fluids so that the time constant $\tau$ does not change and the $\tau$-dependent processes can be ensured, such as the normal working state of the $Na^+/K^+$ pump and the propagation of an action potential. Second, the concentration ratios of potassium ions outside and inside the cell to sodium ions inside and outside the cell are proved to be constants, that is, $C_{K,o}/C_{Na,i} = 0.3027$, $C_{K,i}/C_{Na,o} = 0.9788$, which are supported by comparative study of different animals, including marine animals, freshwater animals and terrestrial animals. As a direct consequence, $E_K + E_{Na} = -30.3$ mV can ensure initiation and stable propagation of an action potential on a neuron. So these results reveal the characteristic events in the evolution of animals, specifically the reason for animal cells to use the $Na^+/K^+$ pump to maintain resting potential during evolution.

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