THE SODIUM-POTASSIUM EXCHANGE PUMP: RELATION OF METABOLISM TO ELECTRICAL PROPERTIES OF THE CELL

I. THEORY

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ABSTRACT The Na-K exchange pump is represented as a net stoichiometrically coupled reaction, *r*, involving ATP, Na^+, and K^+, and is located in the active region of the cell membrane. The reaction rate is $J_r = L_{rr} (-\Delta F_r)$, where ΔF_r is the free energy change of the reaction. ΔF_r includes membrane potential ϕ_g in the absence of 1:1 coupling between Na^+ and K^+, and the reaction rate is potential dependent under these conditions. At the same time the pump will produce a potential *H* which is the difference between membrane potential and the diffusion potential as calculated with constant field assumptions. In the absence of 1:1 coupling, the pump is electrogenic. The feedback relation between reaction rate and membrane potential makes the membrane resistance in the presence of the pump less than or equal to the resistance in its absence, at the same membrane potential. *H* depends on stoichiometry, reaction rate, and passive ionic conductances. Experimental verification of the model will depend on the accuracy of permeability determinations. Dissipation and efficiency of transport can be calculated also.

INTRODUCTION

The Na^+ extrusion pump was postulated for muscle by Dean (1941) and Krogh (1946), and was shown later to be coupled to active inward K^+ movement in a number of tissues (Harris and Maizels, 1951; Keynes, 1954; Hodgkin and Keynes, 1955); it was also related to a Na^+–K^+ requiring ATPase (Skou, 1961; but see Conway, 1957). In the absence of 1:1 Na^+–K^+ coupling by the pump, a net electric current will be produced and the pump will be "electrogenic" (Kernan, 1962; Cross, Keynes, and Rybova, 1965; Hoshiko and Lindley, 1967). If the ratio of active (metabolically coupled) K^+ to active Na^+ flux is $\beta < 1$, the pump will hyperpolarize the membrane (make the inside membrane potential more negative); if $\beta > 1$, it will depolarize the membrane. If active coupling is 1:1, the pump is "electroneutral" and should neither hyperpolarize nor depolarize the membrane.

Work has been done to define the properties of the Na-K exchange pump, but no general formulation has been proposed which relates membrane properties—fluxes, current, potential, hyperpolarization, resistance—to the rate and stoichiometry of the metabolic reaction which constitutes the pump. This study will present a model which permits such a formulation. In part II of this series, the model will be used to consider experimental evidence of the pump.

LIST OF SYMBOLS

a_i	activity of constituent *i*
C^2_i	concentration of *i* in compartment *2*
F	Faraday
ΔF_r	free energy change of chemical reaction *r*
g_i	specific ionic conductance of *i*, mho/cm^2 (equation 17 *b*)
i	subscript which designates constituent *i*
H	$\phi_2 - \phi_{\text{diff}}$, mv
I_i	electric current carried by *i*, from side *1* to *2* of membrane, amp/cm^2, in passive region of membrane
J_i	flux of *i* from side *1* to *2* of membrane, mole/cm^2 per sec
J_r	chemical reaction rate, mole ATP/cm^2 per sec
L'_{ij}	cross conductance (mole/sec per cm^2 per joule) relating flux of *i*, (J_i), to force on *j*, (X_j), (equation 12 *a*)
L_{ii}, L'_{ii}	conjugate conductance relating flux of *i*, (J_i), to force on *i*, (X_i)
L_{rr}	conductance relating J_r to $-\Delta F_r$
P_i	permeability coefficient of *i*, cm/sec
r	subscript which designates chemical reaction of equation 1 *b*
r_j	subscript which designates chemical reaction r_j
RT	gas constant (joules/degree per mole) × absolute temperature
R	membrane resistance (ohm cm^2) in absence of electrogenic pump
R^o	observed membrane resistance in presence of pump
X_i, X_r	generalized forces
z_i	valence of *i*
β	$\nu_{\text{K}}/\nu_{\text{Na}}$
$\bar{\mu}_i$	electrochemical potential of *i* (equation 4 *b*)
$\Delta\bar{\mu}_i$	$\bar{\mu}^2_i - \bar{\mu}^1_i$
$\Delta\bar{\mu}_p$	$\bar{\mu}_{\text{ADP}} + \bar{\mu}_{Pi} - \bar{\mu}_{\text{ATP}} - \bar{\mu}_{\text{H}_2\text{O}}$
ν_i	stoichiometric coefficient of *i* (equation 1)
ϕ_2	membrane potential (electrical potential of compartment *2* with reference to *1*)
ϕ_{diff}	diffusion potential given by equation 16
ϕ_i	ionic equilibrium potential, $z_i\text{RT}/F \ln C^1_i/C^2_i$
Φ	dissipation function, joules/sec per cm^2

THEORY

Elaboration of the Coupled Transport Reaction r*; Relations between Fluxes and Forces in the Membrane*

Active Na$^+$ *and* K$^+$ *transport in a directly coupled reaction.* Let the pump take Na$^+$ from the inside to the outside of the cell and at the same time move K$^+$

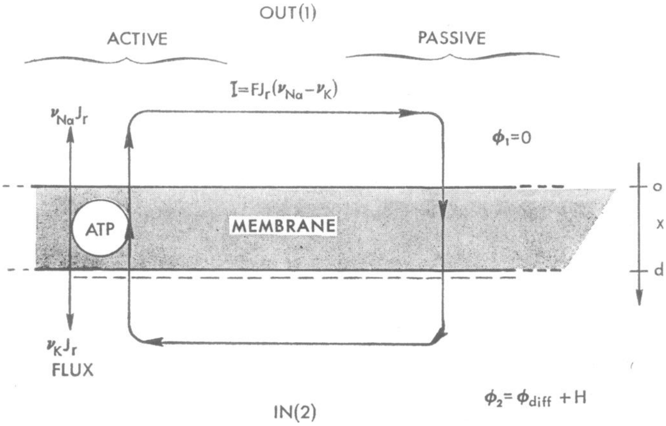

FIGURE 1 *Cell membrane model.* The membrane is anisotropic and divided into parallel active and passive regions. Breakdown of 1 mole of ATP in the active region is coupled to movement of $\nu_{Na}J_r$ mole/cm^2 per sec of Na$^+$ from inside to outside and of $\nu_K J_r$ mole/cm^2 per sec of K$^+$ from outside to inside of the cell. The net electric current $FJ_r(\nu_{Na} - \nu_K)$ leaves the cell in the active region and returns in the passive region. For $\nu_{Na} > \nu_K$, the inside becomes more negative and the membrane is hyperpolarized. Membrane potential ϕ_2 is composed of the diffusion potential and H. Positive flux direction along x is from outside, called compartment 1, to inside, compartment 2. Membrane thickness $= d$. Outside potential $\phi_1 = 0$.

in the opposite direction. We designate the cell interior compartment *2*, the exterior compartment *1*, the two compartments being separated by the cell membrane. The pump, in the active region of the membrane, is in parallel with membrane elements through which only passive diffusion can take place. Fig. 1 shows the cell model. Flux or current from compartment *1* to *2* is positive. Electrical potential of *1* is zero at reference, that of *2* is ϕ_2. The metabolic reaction produces a net electric current $-FJ_r(\nu_{Na} - \nu_K)$ which circulates through the membrane.

The general equation,

$$\text{Energy Source} + \sum_i \nu_i C^1{}_i + \sum_k \nu_k C^2{}_k \rightleftharpoons \sum_i \nu_i C^2{}_i + \sum_k \nu_k C^1{}_k\,, \qquad (1\,a)$$

represents active (metabolically coupled) movement of constituents i from compartment *1* to *2* coupled with movement of constituents k from *2* to *1*. The ν_i are stoichiometric coefficients, and the superscript designates the compartment.

Without loss of generality, let Na$^+$ and K$^+$ be the transported constituents and ATP be the energy source. For the over-all reaction r, where P_i is inorganic phosphate,

$$\text{ATP} + \text{H}_2\text{O} + \nu_K \text{K}^1 + \nu_{Na}\text{Na}^2 \rightleftharpoons \nu_{Na}\text{Na}^1 + \nu_K\text{K}^2 + \text{ADP} + P_i\,. \qquad (1\,b)$$

ν_{Na} is the net number of moles of Na$^+$ pumped from inside to outside (compartment *2* to *1*) for each mole of ATP utilized, and ν_K is the net number of moles of

K^+ pumped from outside to inside. Superimposed on equation 1 *b* we may write,

$$\mathrm{ATP} + \mathrm{H_2O} + \nu_{\mathrm{K}}\mathrm{K}^{1} + \hat{\nu}_{\mathrm{Na}}\mathrm{Na}^{1} + \tilde{\nu}_{\mathrm{Na}}\mathrm{Na}^{2}$$
$$\rightleftharpoons \nu_{\mathrm{K}}\mathrm{K}^{2} + \hat{\nu}_{\mathrm{Na}}\mathrm{Na}^{2} + \tilde{\nu}_{\mathrm{Na}}\mathrm{Na}^{1} + \mathrm{ADP} + P_{\mathrm{i}}, \quad (2)$$

which includes an exchange diffusional flux that doesn't require energy (Levi and Ussing, 1949). The net active movement of Na^+ is, for each mole of ATP utilized,

$$\tilde{\nu}_{\mathrm{Na}} - \hat{\nu}_{\mathrm{Na}} = \nu_{\mathrm{Na}} \text{ moles.} \quad (3)$$

The chemical reaction of equation 1 *b* is assumed to be reversible (cf. Garrahan and Glynn, 1967) and because of its enzymatic nature may show competitive inhibition between substrates, saturation kinetics, and variable stoichiometry. Equation 1 *b* avoids elaborating mechanism, which may be a carrier or an allosteric process. The products and reactants are localized with reference to the x axis of Fig. 1 and will be spatially separated (Rapoport, 1968). Localization is associated also with assymetrical actions of Na^+, K^+, or metabolic inhibitors (Garrahan and Glynn, 1967; Caldwell and Keynes, 1959).

The free energy change of the reaction is potential dependent for an electrogenic pump. The free energy change of r is

$$\Delta F_r = \nu_{\mathrm{K}}(\bar{\mu}^{2}_{\mathrm{K}} - \bar{\mu}^{1}_{\mathrm{K}}) - \nu_{\mathrm{Na}}(\bar{\mu}^{2}_{\mathrm{Na}} - \bar{\mu}^{1}_{\mathrm{Na}})$$
$$+ \bar{\mu}_{\mathrm{ADP}} + \bar{\mu}_{P\mathrm{i}} - \bar{\mu}_{\mathrm{ATP}} - \bar{\mu}_{\mathrm{H_2O}}, \quad (4\,a)$$

where $\bar{\mu}^{2}_{i}$ is the electrochemical potential and $\bar{\mu}^{2}_{i,o(\mathrm{T,P})}$ is the standard potential of i in compartment *2*,

$$\bar{\mu}^{2}_{i} = \bar{\mu}^{2}_{i,o(\mathrm{T,P})} + \mathrm{RT} \ln a^{2}_{i} + z_i F \phi_2 . \quad (4\,b)$$

The free energy change of equation 4 *a* is a sum of scalars, and represents differences in electrochemical potentials of the products and reactants of the net stoichiometric reaction, which is completely coupled. It is assumed (equation 6 *d*) that the reaction rate depends on how far the reaction deviates from equilibrium.

Assuming ideal solutions, the activity of i is $a^{2}_{i} = C^{2}_{i}$. We let $\bar{\mu}^{1}_{i,o(\mathrm{T,P})} = \bar{\mu}^{2}_{i,o(\mathrm{T,P})}$ and define the free energy change for the nontransported constituents averaged for the net reaction as (cf. Kushmerick, 1970),

$$\Delta\bar{\mu}_p = \bar{\mu}_{\mathrm{ADP}} + \bar{\mu}_{P_i} - \bar{\mu}_{\mathrm{ATP}} - \bar{\mu}_{\mathrm{H_2O}} . \quad (4\,c)$$

By equations 4 *a–c* the free energy change is

$$\Delta F_r = \Delta\bar{\mu}_p + \nu_{\mathrm{K}}\mathrm{RT} \ln C^{2}_{\mathrm{K}}/C^{1}_{\mathrm{K}} - \nu_{\mathrm{Na}}\mathrm{RT} \ln C^{2}_{\mathrm{Na}}/C^{1}_{\mathrm{Na}} + (\nu_{\mathrm{K}} - \nu_{\mathrm{Na}})F\phi_2$$
$$= \Delta\bar{\mu}_p - \nu_{\mathrm{K}}F(\phi_{\mathrm{K}} - \phi_2) + \nu_{\mathrm{Na}}F(\phi_{\mathrm{Na}} - \phi_2), \quad (5\,a)$$

where,

$$\phi_i = z_i \mathrm{RT}/F \ln C^1{}_i/C^2{}_i \tag{5 b}$$

$$\Delta\bar{\mu}_i = \mathrm{RT} \ln C^2{}_i/C^1{}_i + z_i F\phi_2 = Fz_i(\phi_i/z^2{}_i - \phi_2) \tag{5 c}$$

$$\Delta F_r = \nu_{\mathrm{K}}\Delta\bar{\mu}_{\mathrm{K}} - \nu_{\mathrm{Na}}\Delta\bar{\mu}_{\mathrm{Na}} + \Delta\bar{\mu}_p\,. \tag{5 d}$$

ΔF_r is the driving force of the net reaction (equation 6 *d*, below) and includes membrane potential when the pump is electrogenic ($\nu_{\mathrm{Na}} \neq \nu_{\mathrm{K}}$).

Representation of the cell membrane. The rate of the net chemical reaction is proportional to $-\Delta F_r$. The cell membrane in Fig. 1 is divided into an active region in which Na$^+$ and K$^+$ transport are coupled directly to ATP, and a passive region in which movement is caused only by diffusional forces. Neglecting cross-diffusional effects in the passive region (let $L_{\mathrm{Na,K}} = L_{\mathrm{Cl,Na}} = L_{\mathrm{Cl,K}} = 0$), a set of "generalized" fluxes can be written for the system as a whole:

$$J_{\mathrm{Na}} = L_{\mathrm{Na,Na}}(-\Delta\bar{\mu}_{\mathrm{Na}}) - \nu_{\mathrm{Na}}J_r \tag{6 a}$$

$$J_{\mathrm{K}} = L_{\mathrm{K,K}}(-\Delta\bar{\mu}_{\mathrm{K}}) + \nu_{\mathrm{K}}J_r \tag{6 b}$$

$$J_{\mathrm{Cl}} = L_{\mathrm{Cl,Cl}}(-\Delta\bar{\mu}_{\mathrm{Cl}}) \tag{6 c}$$

$$J_r = L_{rr}(-\Delta F_r), \tag{6 d}$$

where L_{ii} and L_{rr} are conductances. In the above equations all terms are scalars. The reader can write equations 6 *a* and *b* in vector notation, but if the terms are multiplied by the unit vector in the *x* direction, the scalar formulation will obtain.

Active (act) and passive (pass) fluxes are defined from equations 6 *a–d*:

$$J_{\mathrm{net},i} = J_{\mathrm{pass},i} + J_{\mathrm{act},i} \tag{7 a}$$

$$J_{\mathrm{pass},i} = L_{ii}(-\Delta\bar{\mu}_i) \tag{7 b}$$

$$J_{\mathrm{act,Na}} = -\nu_{\mathrm{Na}}J_r \qquad J_{\mathrm{act,K}} = \nu_{\mathrm{K}}J_r \tag{7 c}$$

$$J_{\mathrm{act,Cl}} = 0. \tag{7 d}$$

At the stationary state, $J_{\mathrm{net}} = 0$ and

$$J_{\mathrm{pass},i} = -J_{\mathrm{act},i}\,. \tag{8}$$

The ratio of the active fluxes is the coupling ratio of the reaction, β:

$$J_{\mathrm{act,K}}/J_{\mathrm{act,Na}} = -\nu_{\mathrm{K}}/\nu_{\mathrm{Na}} = -\beta. \tag{9}$$

Equations 6 *d*, which assumes the reaction rate J_r to be proportional to $-\Delta F_r$, may be incorrect far from equilibrium (Prigogine, 1961; Katchalsky and Spangler, 1968). L_{rr} may have to be specified for a limited region of state parameters when interpreting experimental results. Equation 1 *b* is a net stoichiometric reaction for which $\Delta\bar{\mu}_p$ in Equation 4 *c* is defined by the average concentrations of nontransported constituents. Equation 6 *d* may represent a set of series reactions of the type of equation 1 *b*. If each of the set can be written in the linear form $J_{r_j} = L_{rr_j}(-\Delta F_{r_j})$, then equation 6 *d* also is linear (Prigogine, 1961). For a series of reactions r_j, each of which can be written in the linear form and one of which involves ATP, we defined J_{r_j} by

$$J_{r_j} = \nu_{\mathrm{Na}} J_r / \nu_{\mathrm{Na}_{r_j}} . \tag{10}$$

The following equalities will obtain when Na^+ and K^+ are in the same stoichiometric ratio for each reaction:

$$\Delta F_r = \sum_j \Delta F_{r_j} \tag{11 a}$$

$$\begin{aligned} 1/L_{rr} &= \nu_{\mathrm{Na}} \sum_j 1/\nu_{\mathrm{Na}_{r_j}} L_{rr_j} \\ &= \nu_{\mathrm{K}} \sum_j 1/\nu_{\mathrm{K}_{r_j}} L_{rr_j} . \end{aligned} \tag{11 b}$$

A series of carrier steps composed of diffusional, surface-exchange, and activation terms can be written by use of equations 10–11.

Fluxes as linear homogeneous functions of independent forces, with reciprocity of cross-conductance coefficients, L'_{ij}. The flux of *i* or of the reaction *r* may be written in the form (Kedem, 1961; Fitts, 1962):

$$J_i = \sum L'_{ij} X_j + L'_{ir} X_r \tag{12 a}$$

$$J_r = \sum L'_{ri} X_i + L'_{rr} X_r . \tag{12 b}$$

The L'_{ij} are new conductance coefficients relating the flux of *i* to the force on *j*, X_j; the L'_{ir} are cross-conductance coefficients relating the flux of *i* to the force on the chemical reaction, X_r. If X_r and X_i are properly chosen and are independent, the coefficients will be reciprocal because the conductance matrix is symmetric,

$$\begin{aligned} L'_{ij} &= L'_{ji} \\ L'_{ir} &= L'_{ri} , \end{aligned} \tag{13 a}$$

and will satisfy the following inequalities (Onsager, 1931, in Fitts, 1962):

$$\begin{aligned} L'_{ii} L'_{jj} - L'^{2}_{ij} &\geqq 0 \\ L'_{ii} L'_{rr} - L'^{2}_{ir} &\geqq 0. \end{aligned} \tag{13 b}$$

By substituting J_r (equation 6 *d*) and ΔF_r (equation 5 *a*) in the expressions J_{Na} and J_{K} of equations 6 *a–b*, a set of equations with reciprocity of the conductance coefficients is derived:

$$J_{\text{Na}} = (L_{\text{NaNa}} + \upsilon_{\text{Na}}{}^2 L_{rr})(-\Delta\bar{\mu}_{\text{Na}}) - \nu_{\text{Na}}\nu_{\text{K}} L_{rr}(-\Delta\bar{\mu}_{\text{K}}) - \nu_{\text{Na}} L_{rr}(-\Delta\bar{\mu}_p) \qquad (14\,a)$$

$$J_{\text{K}} = -\nu_{\text{Na}}\nu_{\text{K}} L_{rr}(-\Delta\bar{\mu}_{\text{Na}}) + (L_{\text{KK}} + \nu_{\text{K}}{}^2 L_{rr})(-\Delta\bar{\mu}_{\text{K}}) + \nu_{\text{K}} L_{rr}(-\Delta\bar{\mu}_p) \qquad (14\,b)$$

$$J_{\text{Cl}} = L_{\text{ClCl}}(-\Delta\bar{\mu}_{\text{Cl}}) \qquad (14\,c)$$

$$J_r = -\nu_{\text{Na}} L_{rr}(-\Delta\bar{\mu}_{\text{Na}}) + \nu_{\text{K}} L_{rr}(-\Delta\bar{\mu}_{\text{K}}) + L_{rr}(-\Delta\bar{\mu}_p). \qquad (14\,d)$$

Equations 14 a–d *identify the independent driving forces of the system.* Although $-\Delta F_r$ drives the net chemical reaction (equation 1 *b*), it is not independent of the $\Delta\bar{\mu}_i$. A set of equations like 12 *a* and *b*, where $-\Delta F_r$ and $-\Delta\mu_i$ are driving forces, will not have reciprocity of the coefficients L'_{ir} and L'_{ri} as was previously assumed (Rapoport, 1968). In equations 14 *a–d* coupling between active fluxes and the free energy change $-\Delta\bar{\mu}_p$ is still in the ratio $-\beta$. Although diffusional cross effects have been neglected (equations 6 *a–d*), cross coefficients $L'_{\text{NaK}} = L'_{\text{KNa}} = -\nu_{\text{Na}}\nu_{\text{K}} L_{rr}$ do exist because of the reaction *r*. Equations 14 *a–d* represent a conductance matrix which relates fluxes to independent driving forces. Matrix inversion will give forces as functions of fluxes via a resistance matrix. Thus, each force in the equation may be represented as a sum of forces in series.

Equations 14 *a–d* may be differentiated partially to show the dependence of active fluxes on any individual variable. A common biological experiment relates the efflux of Na$^+$ tracer from a cell to membrane potential or to external K$^+$, and will be discussed in part II of this series. Since the passive Na$^+$ flux is $L_{\text{NaNa}}(-\Delta\bar{\mu}_{\text{Na}})$ (equation 7 *b*), the active flux is, from 14 *a*,

$$J_{\text{act,Na}} = \nu_{\text{Na}}{}^2 L_{rr}(-\Delta\bar{\mu}_{\text{Na}}) - \nu_{\text{Na}}\nu_{\text{K}} L_{rr}(-\Delta\bar{\mu}_{\text{K}}) - \nu_{\text{Na}} L_{rr}(-\Delta\bar{\mu}_p). \qquad (15\,a)$$

Differentiating with respect to external K^+ will give an equation which can be tested experimentally:

$$\frac{\partial J_{\text{act,Na}}}{\partial \ln C^{l}{}_{\text{K}}} = (\nu_{\text{Na}}\nu_{\text{K}} - \nu_{\text{Na}}{}^2) F L_{rr} \frac{\partial \phi_2}{\partial \ln C^{l}{}_{\text{K}}} - \nu_{\text{Na}}\nu_{\text{K}} L_{rr} RT. \qquad (15\,b)$$

Electrical Properties of Membrane

Constant-field assumptions using P_i *and* g_i *and equations for passive and active currents.* The membrane potential can be calculated from the concentration profiles and permeabilities P_i of the ions. If the ionic strength is the same on both sides of the membrane, this diffusion potential may be approximated by the constant-field "Goldman" equation (Goldman, 1943), in the absence of an electrogenic pump and when neglecting divalent ions,

$$\phi_{\text{diff}} = \text{RT}/F \ln w/y, \tag{16}$$

where

$$w = C^1{}_{\text{K}} + (P_{\text{Na}}/P_{\text{K}})C^1{}_{\text{Na}} + (P_{\text{Cl}}/P_{\text{K}})C^2{}_{\text{Cl}}$$

$$y = C^2{}_{\text{K}} + (P_{\text{Na}}/P_{\text{K}})C^2{}_{\text{Na}} + (P_{\text{Cl}}/P_{\text{K}})C^1{}_{\text{Cl}}\,.$$

We will employ specific ionic conductances g_i and permeabilities P_i in order to calculate specific ionic currents in the passive region of the cell membrane. They can be described by either of two expressions (Goldman, 1943; Hodgkin and Katz, 1949). For univalent ions,

$$I_i = z_i F J_{\text{pass},i} = P_i \frac{F^2\phi_2}{\text{RT}} \frac{C^2{}_i \exp\,(z_i\phi_2 F/\text{RT}) - C^1{}_i}{1 - \exp\,(z_i\phi_2 F/\text{RT})} \tag{17 a}$$

or

$$I_i = g_i(\phi_i - \phi_2). \tag{17 b}$$

By comparison with equation 7 *b*, the chord conductances g_i are related to the L_{ii} by,

$$g_i = F^2 L_{ii}. \tag{18}$$

The net passive membrane current at any time is

$$I_{\text{pass,net}} = \sum_i I_{\text{pass},i} = \sum_i g_i(\phi_i - \phi_2). \tag{19}$$

This must be equal and opposite to net active current in the absence of charge accumulation (Fig. 1):

$$I_{\text{act,net}} = FJ_r(\nu_{\text{K}} - \nu_{\text{Na}}) = -I_{\text{pass,net}}\,. \tag{20}$$

The current in the active region is the sum of active fluxes (equations 7 *c*) multiplied by the appropriate value of z_iF. At the stationary state $I_{\text{pass,Cl}} = J_{\text{Cl}} = 0$ (Rapoport, 1968), and for a positive J_r, passive Na^+ current is inward and passive K^+ current outward; both are equal and opposite to their respective active currents,

$$I_{\text{pass,K}} = -FJ_r\nu_{\text{K}} = -I_{\text{act,K}} \tag{21 a}$$

$$I_{\text{pass,Na}} = FJ_r\nu_{\text{Na}} = -I_{\text{act,Na}}\,. \tag{21 b}$$

J_r *is related to stoichiometric coefficients, specific conductances* g_i *and ionic* *equilibrium potentials. Membrane potential and hyperpolarization are related to pump parameters.* At the stationary state, membrane potential is obtained from equations 17 *b* and 21:

$$\phi_2 = \phi_{\mathrm{K}} + FJ_r\nu_{\mathrm{K}}/g_{\mathrm{K}} = \phi_{\mathrm{Na}} - FJ_r\nu_{\mathrm{Na}}/g_{\mathrm{Na}} \, . \qquad (22)$$

Equation 22 may be rearranged to give J_r as a function of membrane parameters, or be used to eliminate J_r :

$$J_r = \left(\frac{\phi_{\mathrm{Na}} - \phi_{\mathrm{K}}}{F}\right)\left(\frac{g_{\mathrm{K}}g_{\mathrm{Na}}}{\nu_{\mathrm{Na}}g_{\mathrm{K}} + \nu_{\mathrm{K}}g_{\mathrm{Na}}}\right) \qquad (23)$$

$$\frac{\phi_{\mathrm{K}} - \phi_2}{\phi_{\mathrm{Na}} - \phi_2} = \frac{-\nu_{\mathrm{K}}g_{\mathrm{Na}}}{\nu_{\mathrm{Na}}g_{\mathrm{K}}} = -\beta g_{\mathrm{Na}}/g_{\mathrm{K}} \qquad (24\,a)$$

$$\phi_2 = \frac{\phi_{\mathrm{K}} + \phi_{\mathrm{Na}}\beta g_{\mathrm{Na}}/g_{\mathrm{K}}}{1 + \beta g_{\mathrm{Na}}/g_{\mathrm{K}}} \, . \qquad (24\,b)$$

Equations 23 and 24 *a–b* provide a means to distinguish between an electroneutral pump ($\beta = 1$) and an electrogenic pump ($\beta \neq 1$) at the stationary state, if the conductances g_i are specified.

We define a voltage H as the difference between the observed membrane potential ϕ_2 and the diffusion potential,

$$H = \phi_2 - \phi_{\mathrm{diff}} \, . \qquad (25\,a)$$

When $\beta < 1$, H is negative and represents hyperpolarization due to the electrogenic pump. Although Kernan (1962) and Cross, Keynes and Rybova (1965) defined H as $\phi_2 - \phi_{\mathrm{K}}$, since ϕ_{K} may differ from ϕ_2 even without a pump when internal ionic concentrations are not at a stationary state because of membrane permeability to ions other than K^+, the definition of equation 25 *a* is more general, provided that the P_i ratios can be evaluated (see Discussion). Use of ϕ_{diff} also permits inclusion of divalent ion effects when necessary (Tasaki, 1968).

If $\nu_{\mathrm{Na}} > \nu_{\mathrm{K}}$ in reaction r, there will be a net outward positive current in the active region of the membrane and a net inward positive current in the passive region (equation 20). An excess of net negative charge ΔQ^- due to the pump is accumulated on side *2* (inside) of the membrane so as to hyperpolarize the membrane beyond the diffusion potential where hyperpolarization is given by (assuming zero resistance at the membrane surface),

$$H = \Delta Q^-/C, \qquad (25\,b)$$

and C is membrane capacity. The excess negative charge on the inside of the membrane drives the positive current from outside to inside in the passive region. For a depolarizing pump, positive current is driven in the opposite direction by excess negative charge on the outside of the membrane.

H can be calculated for the stationary and nonstationary states. Solving equation 19 and 20 for membrane potential gives

$$\phi_2 = \frac{\sum_i g_i \phi_i}{\sum_i g_i} + \frac{FJ_r(\nu_K - \nu_{Na})}{\sum_i g_i}. \qquad (26)$$

With use of equation 25 *a*, hyperpolarization is equal to the sum of the current due to the pump across the passive membrane resistance plus a term due to g_i dependence on membrane potential:

$$H = \left(\frac{\sum_i g_i \phi_i}{\sum_i g_i} - \phi_{diff} \right) + \frac{FJ_r(\nu_K - \nu_{Na})}{\sum_i g_i}. \qquad (27\ a)$$

When $H = 0$, diffusion potential is

$$(\phi_{diff})_{\substack{J_r = 0 \\ \text{or} \\ \nu_K = \nu_{Na}.}} = \frac{\sum_i g_i \phi_i}{\sum_i g_i} \qquad (27\,b)$$

Before it can be concluded that a metabolic inhibitor reduces *H* by reducing J_r, the inhibitor's action on the ν_i and g_i must be determined.

The apparent membrane resistance in the presence of an electrogenic pump is less than in its absence. A rectangular current pulse applied (appl) across the cell membrane by means of an intracellular microelectrode will change membrane potential from ϕ_s to $\phi_s + d\phi_s$ (Fig. 2). Membrane resistance can be obtained if the pulse is short enough not to change the P_i and concentrations, yet long enough to charge membrane capacity. Let observed resistance in the presence of the pump at potential ϕ_s be

$$R^o = -d\phi_s / dI_{appl}. \qquad (28)$$

Let resistance of the passive region at this same membrane potential ϕ_s, and with the same concentration distribution, be defined as

$$R = -d\phi_s / dI_{pass}. \qquad (29)$$

R is calculated from the potential change due to a change in net passive current, which is composed of I_{appl} and current $FJ_r(\nu_{Na} - \nu_K)$ due to the pump. Then,

$$dI_{pass} = d[FJ_r(\nu_{Na} - \nu_K)] + dI_{appl}. \qquad (30)$$

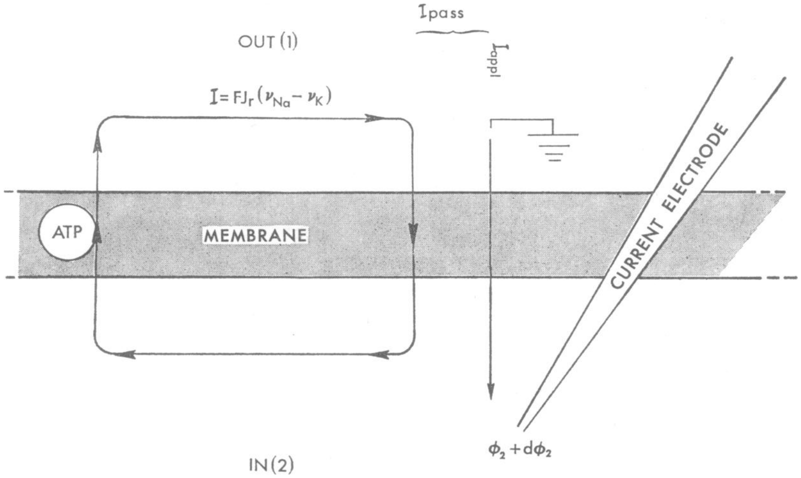

FIGURE 2 *The effect of applying current by a microelectrode.* The applied current through the passive region changes the membrane potential, which in turn changes the potential-dependent chemical reaction rate J_r and the active current. Alternately, the change in current in the active membrane region can be ascribed to passage of an equivalent applied current through that region. The net result is that observed membrane resistance is reduced in the presence of an electrogenic pump.

Assuming constant ν_i, from the relation $J_r = L_{rr}(-\Delta F_r)$ and equation 5 *a* we obtain,

$$\partial \Delta F_r/\partial \phi_2 = F(\nu_{\mathrm{K}} - \nu_{\mathrm{Na}}) \tag{31}$$

$$dI_{\mathrm{pass}}/d\phi_2 = F^2 L_{rr}(\nu_{\mathrm{Na}} - \nu_{\mathrm{K}})^2 + dI_{\mathrm{appl}}/d\phi_2 \,. \tag{32}$$

With the definitions of equations 28 and 29, we have

$$\frac{R^o}{R} = \frac{1}{1 + RF^2 L_{rr}(\nu_{\mathrm{Na}} - \nu_{\mathrm{K}})^2}\,. \tag{33}$$

We note that R is the passive resistance in the absence of the pump at the same membrane potential and ionic concentrations as in its presence. This potential would be maintained, if the pump current were withdrawn, by means of a voltage clamp which could pass a steady-state current equal to $FJ_r(\nu_{\mathrm{Na}} - \nu_{\mathrm{K}})$ through the membrane.

The pump-dependent variables in equations 30–33 might be separated from the passive variables by measuring membrane impedance as a function of frequency, if a change in the reaction rate J_r following a change in ϕ_2 is time dependent. Voltage clamp experiments might also dissect pump-dependent from pump-independent currents.

By equation 33, $R^o \leqslant R$ for both hyperpolarizing and depolarizing currents. The system illustrates Le Chatelier's principle as applied to a linear or nonlinear but stable system at a steady state (De Groot, 1952; Li, 1962). "When a system is perturbed, i.e. a characteristic parameter changes, the system undergoes such a transformation that if it could operate alone, the parameter in question would be changed in the opposite direction." For a current clamp we can say that when a current dI_{appl} is passed through the membrane, the resting current due to the pump, $FJ_r(\nu_{\mathrm{Na}} - \nu_{\mathrm{K}})$, changes so as to make the change in net passive current less than dI_{appl}. For a voltage clamp the pump rate would change so as to require more dI_{appl} to maintain $d\phi_2$ than in the absence of the pump. By equation 28 both effects would be interpreted as a reduction in resistance.

Energy dissipation and efficiency of the chemical reaction are derived. The rate of energy dissipation by the system is given by the sum of forces × net fluxes in the membrane, when the forces are linearly independent (Fitts, 1962). From the forces and fluxes of equation 14, the rate of dissipation is

$$\Phi = J_{\mathrm{Na}}(-\Delta\bar{\mu}_{\mathrm{Na}}) + J_{\mathrm{K}}(-\Delta\bar{\mu}_{\mathrm{K}}) + J_{\mathrm{Cl}}(-\Delta\bar{\mu}_{\mathrm{Cl}}) + J_r(-\Delta\bar{\mu}_p) > 0. \qquad (34\,a)$$

At the stationary state the net fluxes of all the ions vanish and

$$\Phi = J_r(-\Delta\bar{\mu}_p), \qquad (34\,b)$$

which equals the dissipation due to the breakdown of high-energy chemical bonds. Efficiency (Eff) is defined as the rate of useful (active-transport) work done divided by the rate of total energy dissipation,

$$\mathrm{Eff} = \frac{-\sum_i J_{\mathrm{act},i}(-\Delta\bar{\mu}_i)}{\Phi}, \qquad (35\,a)$$

the active or metabolically coupled fluxes divided by the dissipation. At the stationary state this reduces to (cf. Heinz and Patlak, 1960),

$$\mathrm{Eff} = \frac{-J_{\mathrm{act,Na}}(-\Delta\bar{\mu}_{\mathrm{Na}}) - J_{\mathrm{act,K}}(-\Delta\bar{\mu}_{\mathrm{K}})}{J_r(-\Delta\mu_p)}. \qquad (35\,b)$$

When J_r is eliminated by use of equation 7 *c*, efficiency may be written, as

$$\mathrm{Eff} = \frac{\mathrm{RT} \ln \dfrac{C^2{}_{\mathrm{Na}}}{C^1{}_{\mathrm{Na}}} \left(\dfrac{C^1{}_{\mathrm{K}}}{C^2{}_{\mathrm{K}}}\right)^{\beta} + F\phi_2(1 - \beta)}{\nu_{\mathrm{Na}}(-\Delta\bar{\mu}_p)}. \qquad (36)$$

ϕ_2 may also be involved in $\Delta\bar{\mu}_p$ if reaction complexes are potential dependent.

DISCUSSION

Equations 18–21 were introduced in order to analyze biological data in which P_i and g_i have been determined approximately, and to take into account variations of conductance coefficients L_{ii} with potential. The constant field assumption however is obviously incorrect in a membrane made of two layers of low and high conductivity, and its reliability in any anisotropic membrane is questionable. Yet ignorance of the exact structure of the biological membrane makes it a reasonable assumption at this time. In addition, if the P_i are determined from the relation of membrane potential to external ionic concentrations (Hodgkin, 1958) assuming $\phi_s = \phi_{\text{diff}}$, if $H \neq 0$ they are necessarily inexact. If the P_i are found by the constant-field assumption and tracer flux determinations (Hodgkin and Horowicz, 1959), they may also be incorrect (Essig, 1966).

The use in the model of these experimentally questionable parameters and the constant-field assumption means that the equations for H (27 *a*) and membrane resistance (33), among others, do not serve as exact experimental criteria for the pump, nor can H and R be precisely determined. Recognizing this limitation, we hope to find approximate solutions of the equations by using estimates of the P_i and g_i from biological data (part II of this study).

In defining H as the difference between the membrane and the diffusion potential, rather than the difference between the membrane and the potassium-equilibrium potential, we have provided a general formulation which recognizes that the membrane may be permeable to many ions and which permits, by use of equation 1 *a*, inclusion of divalent ions when necessary. We have related active fluxes and metabolism to membrane parameters in a consistent fashion, even if those parameters have not yet been determined exactly. Although the model presented is that of the Na-K exchange pump with ATP as an energy source, equation 1 *a* may be written for an active system with any transported ions or energy source.

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