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A Thermodynamic Analysis of the Correlation between Active Na^+ Transport and the Rate of Oxygen Consumption in Epithelia

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Summary. Active transport in epithelia is discussed in terms of the relationships between oxygen consumption and sodium flux as affected by each of the two corresponding thermodynamic forces. Analysis is presented of the use of nonequilibrium thermodynamics as a tool in elucidating coupling and stoichiometry, and in evaluating drug action in the system. The analysis leads to the quantitative characterization of active transport in "two-flow" systems in terms of two plots: oxygen consumption and sodium flow, each as a function of electrical potential difference, at constant affinity and constant concentrations. The relevant characteristic parameters are then shown to be represented by the slopes and intercepts of the two plots, the ratios of the slopes and of the intercepts, and by the difference – as well as the ratio – of the ratios. Distinction is made between experimental conditions in which the phenomenological coefficients remain constant and those in which these coefficients undergo appreciable changes. In terms of the above analysis, an examination is made of the effect of commonly used drugs. It is shown that while drugs may affect both the affinity and the phenomenological coefficients, they invariably affect the latter – at least in the cases hitherto reported.

The rates of active sodium transport J_+ and of oxygen consumption J_r in a transporting tissue depend on characteristic parameters of the system, such as the permeabilities and the rate coefficients of the chemical reactions involved, and on the forces operative in the system [4, 7]. Any change in one or more of the characteristic parameters will entail a change in the fluxes even when there are no changes in the driving forces. Analysis of the system therefore requires distinction between changes of fluxes stemming from a change in the characteristics of the system and those stemming from a change in the imposed forces. In this paper we discuss the relevant theoretical expressions required for the description of active transport according to nonequilibrium thermodynamics and apply them to the case of the frog abdominal skin. We shall first consider conditions under which the characteristic parameters are not altered so that all changes in

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fluxes are caused by changes in the imposed forces. Subsequently, we shall discuss conditions which lead to alteration of the characteristic parameters. Consideration will be given to characterization of the system following both spontaneous changes and changes brought about by drugs.

It will be assumed throughout that, at given characteristic parameters, both sodium transport and oxygen consumption are completely determined by two forces – the transepithelial electrochemical potential of Na^+, X_+, and the chemical affinity of the driving reaction, A [19, 21]. In epithelia it is possible to change X_+ in a controllable way, while the chemical affinity cannot yet be changed in a satisfactorily controlled fashion, such that all the characteristic parameters of the system are not affected. This experimental difficulty, however, does not prevent a full characterization of the system; this includes information on the value of the affinity under the given conditions and on the effect of the variation of A on the fluxes, provided that the system is linear and that Onsager's symmetry relationship is valid. Certain aspects of the relationship between flows and forces in transepithelial active transport have been discussed earlier [2, 4, 7, 11, 16, 19, 21, 24]; these are included in the general analysis presented here.

Theoretical

The rates of net Na^+ transport across the membrane and of O_2 consumption by the membrane have been assumed to depend on two thermodynamic forces only, i.e., the transmembrane electrochemical potential difference and the affinity of the chemical reaction involved in utilizing the free energy of substrate oxidation by O_2 in order to drive active transport [16, 19, 21]. Since both the flow of Na^+ and of the chemical reaction depend on two forces, the ratio of the two flows may assume any value, depending on the values of the two forces. However, when one of the forces is maintained constant, the ratio is uniquely determined by one variable only, and can thus be expressed as a single valued function of the chosen independent variable – either the other force or one of the flows.

For a system obeying a linear relationship between flows and forces, we have [8]:

$$J_+ = L_+ X_+ + L_{+r} A \tag{1}$$

$$J_r = L_{r+} X_+ + L_r A \tag{2}$$

where J_+ and J_r are the net transmembrane flow of Na^+ and the rate of sodium-dependent O_2 consumption, respectively, and X_+ and A are the electrochemical potential difference across the membrane and the affinity of the chemical reaction driving transport, respectively. The L's are phenomenological coefficients. The straight coefficients L_+ and L_r are related to the electrical conductance of the active pathway and the rate coefficient of the chemical reaction, respectively. The cross coefficients L_{+r} and L_{r+} are equal according to Onsager's symmetry relations, and indicate the extent of dependence of each flow on the nonconjugate force.

The derivatives $(\partial J_+/\partial J_r)$, at constant A or at constant X_+, are expressed as ratios of phenomenological coefficients in the following manner:

$$\left(\frac{\partial J_+}{\partial J_r}\right)_A = \frac{(\partial J_+/\partial X_+)_A}{(\partial J_r/\partial X_+)_A} = \frac{L_+}{L_{r+}} \tag{3}$$

and

$$\left(\frac{\partial J_+}{\partial J_r}\right)_{X_+} = \frac{(\partial J_+/\partial A)_{X_+}}{(\partial J_r/\partial A)_{X_+}} = \frac{L_{+r}}{L_r}. \tag{4}$$

The values of the derivatives are constant for the linear case discussed here, namely, when the phenomenological coefficients are not changed concomitantly with the changes in the flows. As can be seen, these derivatives differ from one another: the relative change of the flows caused by an electrical potential difference is different from the relative change caused by a variation of the chemical affinity.

The integral relationship between J_+ and J_r at constant A follows directly from Eq. (3):

$$J_r = \frac{L_{r+}}{L_+} J_+ + C(A) \tag{5}$$

where $C(A)$ is a constant of integration depending on the value of A. By rearrangement we get:

$$\frac{J_+}{J_r} = \frac{L_+ J_+}{L_{r+} J_+ + L_+ C(A)}. \tag{6}$$

This equation can be derived algebraically from Eqs. (1) and (2). Similarly, for $X_+ = \text{constant}$, we obtain from (4):

$$J_r = \frac{L_r}{L_{+r}} J_+ + C''(X_+) \tag{7}$$

where $C''(X_+)$ is an integration constant depending on the value of X_+. When working with identical concentrations on both sides of the mem-

brane and varying only the electrical potential difference $\Delta\psi$, we have $X_+ = -F\Delta\psi$ and thus $C''(X_+) = C'(\Delta\psi)$. The choice of positive direction of X_+ is discussed below in connection with the sign of L_{+r}. Rearrangement gives:

$$\frac{J_+}{J_r} = \frac{L_{+r}J_+}{L_r J_+ + L_{+r}C'(\Delta\psi)}. \tag{8}$$

The quantities $C(A)$ and $C'(\Delta\psi)$ have the following significance: According to Eqs. (5) and (7) (replacing $C''(X_+)$ by $C'(\Delta\psi)$) we have,

$$(J_r)_{J_+=0} = C(A) \tag{9}$$

$$(J_r)_{J_+=0} = C'(\Delta\psi) \tag{10}$$

where $(J_r)_{J_+=0}$ is the rate of oxygen consumption at static head [8] – when the two driving forces are such as to bring the flow of sodium to a halt. Equation (9) gives J_r as a function of A when $\Delta\psi$ is adjusted to give $J_+ = 0$, while Eq. (10) gives J_r as a function of $\Delta\psi$ when A is adjusted to give $J_+ = 0$. The explicit expressions for $C(A)$ and $C'(\Delta\psi)$ follow directly from Eqs. (1) and (2):

$$(J_r)_{J_+=0} = \frac{L_r L_+ - L_{+r}L_{r+}}{L_+} A = C(A) \tag{11}$$

$$(J_r)_{J_+=0} = F\frac{L_+ L_r - L_{+r}L_{r+}}{L_{+r}} \Delta\psi = C'(\Delta\psi). \tag{12}$$

Under the specific conditions of $A = 0$ or $\Delta\psi = 0$, $C(A) = 0$ or $C'(\Delta\psi) = 0$, respectively. The right hand sides of Eqs. (6) and (8) are, in this case, identical with the right hand sides of Eqs. (3) and (4), respectively. The familiar result follows that the derivatives of the flows are equal to the ratios of the flows:

$$\left(\frac{\partial J_+}{\partial J_r}\right)_A = \left(\frac{J_+}{J_r}\right)_{A=0} \tag{13}$$

$$\left(\frac{\partial J_+}{\partial J_r}\right)_{\Delta\psi} = \left(\frac{J_+}{J_r}\right)_{\Delta\psi=0}. \tag{14}$$

The difference between the two derivatives is given, according to Eqs. (3) and (4), by the following:

$$\left(\frac{\partial J_+}{\partial J_r}\right)_A - \left(\frac{\partial J_+}{\partial J_r}\right)_{\Delta\psi} = \frac{L_+}{L_{r+}} - \frac{L_{+r}}{L_r} = \frac{L_+ L_r - L_{+r}L_{r+}}{L_{r+}L_r}. \tag{15}$$

Applying Onsager's reciprocal relations, $L_{+r}=L_{r+}$, we obtain:

$$\left(\frac{\partial J_+}{\partial J_r}\right)_A - \left(\frac{\partial J_+}{\partial J_r}\right)_{\Delta\psi} = \frac{L_+}{L_{+r}}(1-q^2) \tag{16}$$

where the degree of coupling q is defined as $q^2=\frac{L_{+r}^2}{L_+ L_r}$ [8]. When there is complete coupling, we have $L_+ L_r = L_{+r}^2$ [8] and therefore:

$$\frac{J_+}{J_r} = \text{constant} = \left(\frac{\partial J_+}{\partial J_r}\right)_A = \left(\frac{\partial J_+}{\partial J_r}\right)_{\Delta\psi} \tag{17}$$

and also, according to Eqs. (11) and (12), $C(A)=0$ and $C'(\Delta\psi)=0$, irrespective of the values of the forces.

Analysis of the dependence of J_+/J_r on J_+ (Eqs. (6) and (8)) predicts a "saturation" pattern for $C(A)\neq 0$ or $C'(\Delta\psi)\neq 0$, respectively. At decreasing values of J_+ the ratio J_+/J_r decreases from its maximal asymptotic value to zero. In the case of complete coupling, however, the value of the ratio stays constant as shown above. It will be noticed that the ratio also stays constant in the special case of $A=0$ or $\Delta\psi=0$.

The dependence of J_+/J_r on J_+ is mathematically identical with the Michaelis-Menten equation and the Langmuir adsorption isotherm, and may similarly be expressed in the form of a linear relationship between J_r/J_+ and $1/J_+$, as follows:

$$\frac{J_r}{J_+} = \frac{L_{r+}}{L_+} + C(A)\frac{1}{J_+} \qquad \text{at constant } A \tag{18}$$

$$\frac{J_r}{J_+} = \frac{L_r}{L_{r+}} + C'(\Delta\psi)\frac{1}{J_+} \qquad \text{at constant } \Delta\psi \tag{19}$$

where the slope gives $C(A)$ or $C'(\Delta\psi)$ and the intercept L_{r+}/L_+ or L_r/L_{r+}, respectively.

Materials and Methods

Frogs (*Rana pipiens*) were obtained from Carolina Biological Supply Co., Burlington, North Carolina, and were kept at room temperature in a spacious tank containing tap water and dry rocks. Prior to the experiment, the animals were doubly pithed, the abdominal skin cut along the midline, and resected providing two paired hemiskins. The hemiskins were washed twice with Ringer's solution (consisting of 110 mM NaCl, 2.4 mM $KHCO_3$, 1.0 mM $CaCl_2$, 10 mM glucose and 40 mg/liter gentamicine sulfate (Schering)) and mounted in modified Ussing-Zerahn Lucite chambers of 7.1 cm^2 cross-sectional area. The same Ringer's solution was added on both sides of the hemiskin.

The electrical potential difference, $\Delta\psi$ ($\Psi_{\text{inside}} - \Psi_{\text{outside}}$), was regulated with an automatic voltage clamp, and the current, I, was recorded continuously. Oxygen consumption was measured polarographically with Clark electrodes (Yellow Springs Instrument Co., Yellow Springs, Ohio) in a closed system as previously described [20]. After an initial equilibration period of 1.5 hr in the short circuited state, the potential difference across the tissue was clamped sequentially at $\Delta\psi = \pm 40$, ± 80 mV, for periods of 6 min each. To ensure that the tissues would be in a steady state, measurements were made during the final two minutes of each period[1]. Following two such series, amiloride (kindly supplied by Merck, Sharp and Dohme, N.J.) was added to the mucosal solution to a concentration of 1×10^{-4} M, in order to depress the short-circuit current I_0, and oxygen consumption was again measured. For measurements of spontaneous changes in the system, no amiloride was added, and the system was maintained aerated and short circuited for an additional period of 2 hr. Subsequently, measurements of oxygen consumption were repeated. In all cases, both with and without amiloride, the electric current was continuously monitored. Every 5 min throughout all experiments, a potential difference of ± 20 mV was imposed for 15 sec in order to evaluate the electrical conductance, κ, of the system. The passive conductance κ^p was evaluated by plotting κ *vs.* I_0 for the untreated skin and for the maximally inhibited skin, and extrapolating the straight line connecting the two points to $I_0 = 0$ [11]. This method was adopted since, in the frog skin, amiloride inhibits I_0 to 0.06 ± 0.02 ($n = 10$) of the initial value rather than to zero [10]. Estimates of κ^p by this technique differ insignificantly from those obtained by the measurement of tracer $^{22}Na^+$ backflux [6].

The rate of active Na^+ transport J_+ was determined (*see* footnote on page 7) using the equation:

$$J_+ = \frac{I}{F}(1 + \kappa^p \Delta\psi).$$

The rate of suprabasal oxygen consumption, J_r, was determined by subtracting the rate of oxygen consumption in the amiloride-treated skin from the total rate in the same skin before treatment.

The value of the affinity, A, was calculated according to [4, 21]: $A = -I_0/(\partial J_r/\partial \Delta\psi)_A$.

List of Symbols

J_+ = transmembrane Na^+ flux not including intercellular pathways
J_r = rate of supra-basal (pump-related) O_2 consumption
X_+ = transmembranal electrochemical potential difference of Na^+
A = affinity of the chemical reaction driving transport
L_+ = phenomenological coefficient, proportional to the electric conductance of the active pathway
L_r = phenomenological coefficient, proportional to the rate constant of the chemical reaction driving transport
L_{+r}, L_{r+} = phenomenological cross coefficients
$C(A)$ = rate of O_2 consumption at static head as a function of A
$C'(\Delta\psi)$ = rate of O_2 consumption at static head as a function of $\Delta\psi$
$\Delta\psi$ = transepithelial electrical potential difference
F = Faraday constant

1 Discussion of "memory" and of conditions for obtaining steady-state values is given in references 3, 9, 10, 20, 23.

κ = electric conductance
κ^p = passive conductance
I = electric current
I_0 = electric current in the short circuited state
J_{+0} = net transepithelial Na^+ flux in the short circuited state
J_{r0} = rate of pump-dependent O_2 consumption in the short circuited state
q = degree of coupling

Results and Discussion

According to the theoretical analysis, it is convenient to obtain all the terms needed for our discussion from two plots: the dependence of J_+ on the transepithelial electrical potential $\Delta\psi$, and the dependence of J_r on $\Delta\psi$, both at constant A. These plots present in a straightforward manner readily obtainable experimental parameters[2].

Figures 1 and 2 show the change of J_+ and of J_r with $\Delta\psi$ in a representative skin. In accordance with previous reports [16, 19, 21] both plots are linear, the slopes (divided by $-F$) giving the conductance L_+ and the cross coefficient L_{r+}, respectively. (The linearity of these plots substantiates the assumed constancy of L_+ and L_{r+}, as well as that of the chemical affinity A.) The ratio of the two slopes gives the change in active Na^+ transport with change in the rate of oxygen consumption at constant A, $(\partial J_+/\partial J_r)_A$. Values of the slopes, as well as of the slope ratios, determined in 11 skins, are presented in Table 1. As can be seen, the values of both L_+ and L_{r+} are positive. This finding is in accordance with expectations since, in the absence of a chemical driving force, L_+ represents the proportionality between J_+ and $\Delta\psi$. For L_{+r} to be positive, the flux of Na^+ from the outer side to the inner side (which is the direction of the short-circuit current) is designated as positive. As both L_{+r} and L_+ are positive, the derivative $(\partial J_+/\partial J_r/_A$ is always positive (Eq. 3), indicating that for any change in one of the fluxes, at constant A, there is a corresponding change in the other flux *in the same direction.*

The intercepts of the two lines presented in Figs. 1 and 2 give the values of J_+ and J_r in the short circuited state. As shown in Eq. (13), the ratio of the two intercepts represents the change of J_+ per unit change of J_r at any constant value of $\Delta\psi$ (including the special case of $\Delta\psi = 0$). The values of the fluxes at short circuit and of their ratio, as measured in the same 11 skins, are included in Table 1.

2 It has been shown that ions other than sodium are transported only passively in the frog skin [1, 10, 22]. For the isotonic conditions maintained in the system, the driving force of water is zero. Under the above conditions the two-flow representation is valid (*see also* [4]).

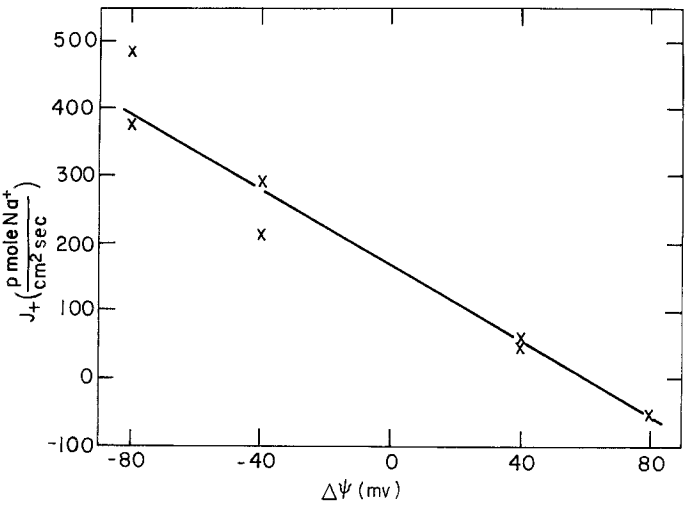

Fig. 1. Flow of Na^+, J_+, as function of the transmembrane electric potential difference, $\Delta\psi$, in a representative skin, with identical solutions on both sides of the skin

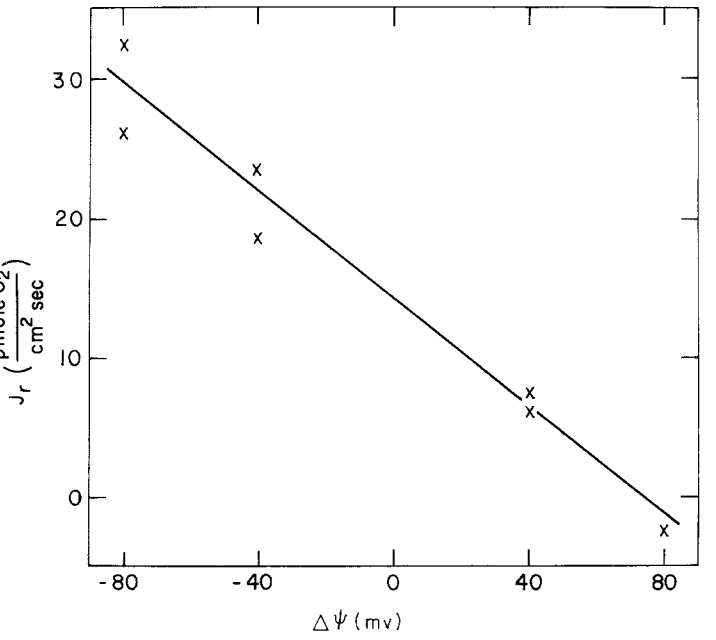

Fig. 2. Supra-basal rate of oxygen consumption, J_r, as function of the transmembrane electric potential difference, $\Delta\psi$, in a representative skin, with identical solutions on both sides of the skin

Table 1. Thermodynamic parameters of the frog skin[a]

Column No.	1	2	3	4	5	6	7
Skin No.	$FL_+ = -\left(\frac{\partial J_+}{\partial \Delta\psi}\right)_A$	$FL_{r+} = -\left(\frac{\partial J_r}{\partial \Delta\psi}\right)_A$	$\left(\frac{\partial J_+}{\partial J_r}\right)_A = \frac{L_+}{L_{r+}}$	J_{+0}	J_{r0}	$\left(\frac{\partial J_+}{\partial J_r}\right)_{\Delta\psi} = \frac{J_{+0}}{J_{r0}}$	q
	$\left(\frac{\text{pmole } Na^+}{\text{cm}^2 \text{ sec mV}}\right)$	$\left(\frac{\text{pmole } O_2}{\text{cm}^2 \text{ sec mV}}\right)$	$\left(\frac{\text{mole } Na^+}{\text{mole } O_2}\right)$	$\left(\frac{\text{pmole } Na^+}{\text{cm}^2 \text{ sec}}\right)$	$\left(\frac{\text{pmole } O_2}{\text{cm}^2 \text{ sec}}\right)$	$\left(\frac{\text{mole } Na^+}{\text{mole } O_2}\right)$	
1	3.35	0.149	22.5	181	9.6	18.8	0.84
2	7.07	0.189	37.4	450	34.9	12.9	0.35
3	3.10	0.126	24.6	226	15.5	14.6	0.59
4	2.43	0.125	19.4	219	16.6	13.2	0.68
5	2.72	0.070	38.8	162	4.7	34.5	0.90
6	4.84	0.280	17.3	337	15.7	21.4	1.23
7	2.05	0.169	12.1	158	14.8	10.7	0.88
8	2.46	0.166	14.8	242	25.9	9.3	0.63
9	3.46	0.080	43.2	84	7.7	10.9	0.25
10	1.00	0.104	9.6	109	15.9	6.8	0.71
11	1.65	0.089	18.5	171	19.4	8.8	0.48
Mean	3.10	0.140	23.5	213	16.4	14.7	0.69
SE	0.50	0.018	3.4	31	2.5	2.4	0.08

[a] Experimental values of the rate coefficient for active transport, L_+, and of the cross coefficient, L_{+r}, are given in columns 1 and 2, respectively. The change of active transport J_+ with change in oxygen consumption J_r at constant affinity (column 3) was obtained from the ratio of the two rate coefficients. The experimental values of active transport J_{+0} and of the rate of oxygen consumption J_{r0} in the short circuited state, and the change of J_+ with the change in J_r at constant $\Delta\psi$ as obtained from the ratio of these two fluxes are given in columns 4, 5 and 6, respectively. The value of the degree of coupling between metabolism and active transport, q, (column 7) was calculated from the two ratios (column 6/column 3).

According to Eq. (4), the change of J_+ with the change in J_r at constant $\Delta\psi$ equals the ratio of the cross coefficient L_{+r} to the chemical reaction rate coefficient L_r. Thus, the ratio of the intercepts when divided by the slope of J_r *vs.* $\Delta\psi$ provides the value of $L_r \cdot (-1/FL_r)$. It may be pointed out that the values of L_r and L_{+r} have been obtained from the two plots representing results measured at constant A, nevertheless they provide information on the effect of variation in A on changes in J_r and in J_+. Evidently, such information can be obtained because of the validity of the Onsager symmetry relations.

Consider now the ratio of the two slopes and that of the two intercepts. Both ratios represent changes in the same fluxes, the difference between them lying in the force causing the changes. Since L_{+r} is positive, both ratios are positive. The question arises as to the conditions under which they are equal, and whether there is an *a priori* reason to assume that, when not equal, one of the ratios should be larger than the other. Equation (15) indicates that when the two fluxes are not completely coupled, i.e., their ratio is not constant under all conditions, the ratio of slopes should be different from the ratio of intercepts. Comparison of columns *3* and *6* in Table 1 shows clearly that in each skin the ratios differ significantly from one another, indicating that the two fluxes are not completely coupled. In all cases both ratios are positive and we find:

$$\left(\frac{\partial J_+}{\partial J_r}\right)_A > \left(\frac{\partial J_+}{\partial J_r}\right)_{\Delta\psi}.$$

The one exception to this inequality is discussed below.

The positive values of the derivatives as well as the observed inequality are consistent with the thermodynamic formalism. According to expressions (3) and (4) the two ratios must have the same sign, determined by the sign of L_{+r} (since both L_+ and L_r are positive). By convention the positive direction of J_+ is chosen so that $L_{+r} > 0$, hence the positive value of the derivatives.

The finding that the derivative at constant A is larger than that at constant $\Delta\psi$ follows from Eq. (15), since for all linear systems obeying Onsager's reciprocal relation $L_+ L_r \geqq L_{+r}^2$, and the denominator in Eq. (15) is positive. This important relation states that a change in one flux (per unit change of the other flux) due to its conjugate force is always larger than a change in the corresponding flux due to the nonconjugate force. In view of this discussion, any experiment giving $(\partial J_+/\partial J_r)_A < (\partial J_+/\partial J_r)_{\Delta\psi}$ is thermodynamically inconsistent. One such result has been

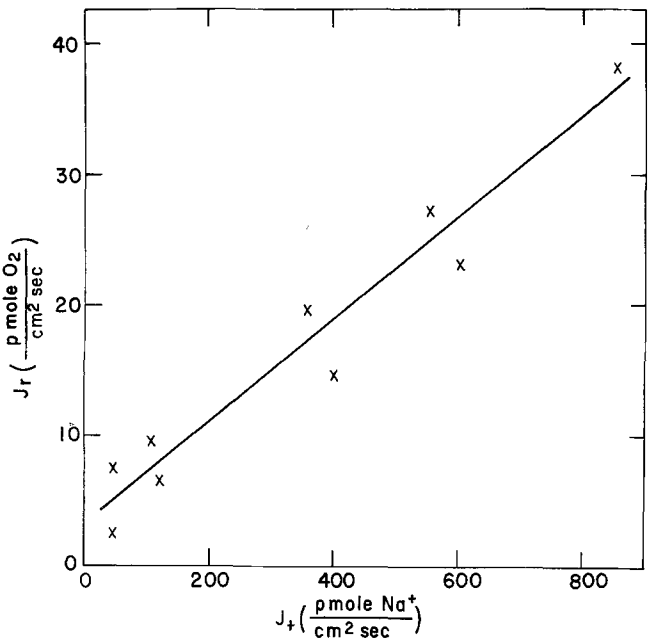

Fig. 3. Supra-basal rate of oxygen consumption, J_r, as function of Na^+ flux, J_+, in a representative skin

obtained in the present study (Table 1, Skin No. 6). This inconsistent value is nevertheless included in the calculation of the mean since a reasonable scatter in the results may be expected.

Another parameter to be considered is the ratio of the two derivative forms, given by the ratio of the intercepts divided by the ratio of the slopes. This ratio equals the square of the degree of coupling q referred to earlier. It is a dimensionless parameter and a sensitive measure of the mutual dependence of the two fluxes J_+ and J_r. When $q^2=1$, we have $(\partial J_+/\partial J_r)_A = (\partial J_+/\partial J_r)_{\Delta\psi}$ and the fluxes are completely coupled; when $q^2=0$, $(\partial J_+/\partial J_r)_{\Delta\psi}=0$ and the change in J_r at constant $\Delta\psi$ does not affect J_+ in any way.

As can be seen from column 7 in Table 1, the two fluxes in frog skin are coupled ($q\neq 0$), but not completely since q is significantly different from unity. Inspection of the results reported by M.A. Lang *et al.* [13] shows that the toad bladder is very similar in its degree of coupling to the frog skin (however, *see* [9]).

Since both J_+ and J_r are linear in $\Delta\psi$, it follows that J_r plotted as a function of J_+ will be linear when the only cause for a change in either is a change in $\Delta\psi$. This is shown in Fig. 3 for a representative skin. Another

Table 2. Comparison of the values of J_r at static head given by $C(A)$ according to Eq. (5) and by the method of Lahav *et al.* [11] (pmole O_2/cm^2 sec)

Skin No.	$C(A)$	$(J_r)_{J_+=0}$
1	3.07	2.75
2	14.76	9.86
3	28.72	25.14
4	4.83	5.01
5	12.06	6.12
6	1.05	1.33
7	0.65	1.80
8	9.99	7.13
9	22.45	24.1
10	−2.56	3.0
Mean	9.50	8.62
SE	3.21	2.79

feature, evident in Fig. 3, is the fact that the intercept of the straight line is significantly different from zero. This intercept gives the value of $C(A)$, which is the rate of oxygen consumption when the skin is hyperpolarized to such an extent that all active transport is stopped, i.e., J_r at static head (Eq. 9). Had the intercept been at the origin ($C(A)=0$), the system would have been completely coupled. That would have meant no pump-related oxygen consumption when the pump is stopped. In our case $q^2 \neq 1$ ($C(A) \neq 0$) and, according to expression (11), the value of $C(A)$ depends on the value of A of the system (as well as on the characteristic rate constants).

It is of interest to compare the values of $C(A)$ calculated by the method presented in this paper with those calculated according to a different method reported earlier [10, 11], using the same experimental raw data. Table 2 presents values of J_r at static head for 10 different skins, calculated by the two methods. As can be seen, the values obtained are not identical, but there is a close correspondence between the two sets of data, so that the differences found in both the mean and the standard error are insignificant.

Having described one flux as a function of the other, the dependence of their ratio on one of the fluxes is readily obtained. This ratio is constant if the plot of J_r vs. J_+ goes through the origin, i.e., when $q^2=1$ [Eq. (6)]. When $q^2 \neq 1$, J_+/J_r is not constant, but shows a "saturation" pattern as observed experimentally by us (Fig. 4) and by others [13, 14]. This pattern is characterized by two parameters [Eq. (6)], the initial slope $1/C(A)$ and the maximal ("saturation") value given by L_+/L_{r+}. As mentioned above,

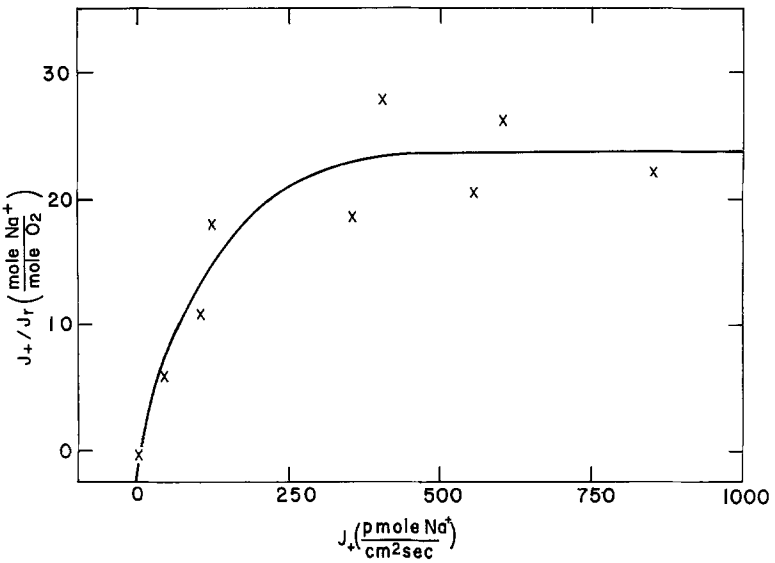

Fig. 4. The ratio of Na^+ flow to the supra-basal rate of O_2 consumption as function of Na^+ flow in a representative skin

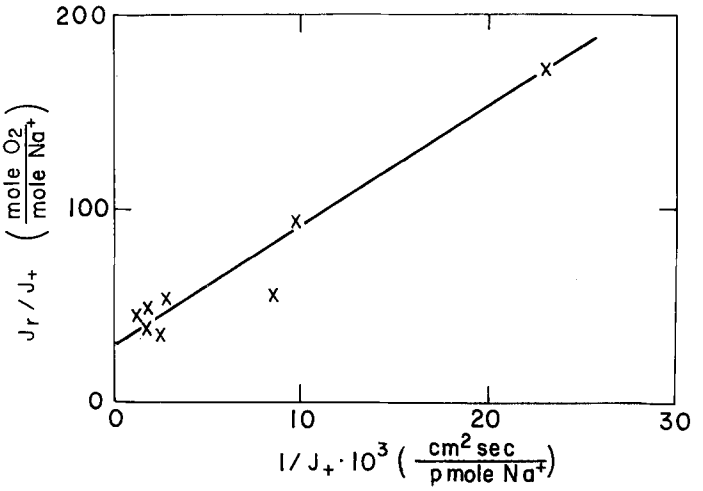

Fig. 5. Inverse flow ratio as function of the inverse of Na^+ flow in a representative skin

this pattern is mathematically analogous to that described by Langmuir's isotherm and by the Michaelis-Menten reaction-rate equation, and like those equations can be expressed linearly by plotting the reciprocal values of both J_+/J_r and J_+ (a Lineweaver-Burke type plot). Such a plot is shown in Fig. 5.

It is convenient to summarize the discussion of the relationships between the flows in terms of the notion of "stoichiometry". Both the ratio and the derivatives have been referred to in the literature as the "stoichiometric coefficients" of the system. This may be fully justified in the case of complete coupling when the ratio of the integral values and the two derivatives are all given by the same constant. In any system with $q^2 < 1$, the parameter expressing the relationship between the number of sodium ions transported and the number of oxygen molecules consumed is not uniquely defined, since it can be any one of the three. Moreover, if it is defined in the integral form, the value is not a constant but depends on the experimental conditions. As seen in Fig. 4, when $\Delta\psi$ is varied at constant A, the stoichiometric coefficient follows a saturation pattern. The plot of the reciprocal values given in Fig. 5 provides a convenient way to characterize the variation of the integral stoichiometric coefficient in a linear representation.

Unlike $\Delta\psi$, or more generally X_+, A cannot be set at will in this system. It is therefore not possible to measure the values of J_+/J_r for different values of A at constant X_+. The differential parameter $(\partial J_+/\partial J_r)_{\Delta\psi}$, however, can be obtained, since it is given by the ratio of the current and the rate of oxygen consumption at short circuit.

We now turn to a discussion of conditions which lead to changes in the characteristics of the system. As will be seen, much of the information concerning such changes stems from studies of spontaneous variations of the fluxes at constant X_+ and from attempts to bring about changes in the chemical affinity A.

The short-circuit current and oxygen consumption decrease slowly with time [2, 14, 20]. In our experiments the variation with time has been determined by computing the values of each of the parameters in Eqs. (1) and (2) at the beginning of each experiment and 2.5 hr later. We find that there is a significant change in some, if not all, of the rate parameters, but no change in A. Table 3 summarizes changes observed by us as well as changes computed from results reported by others [15]. It indicates that there is a large change in L_+, a small change in L_{+r}, and no change in L_r. There is a disagreement between our work and that of Owen *et al.* [16] regarding the spontaneous change in A, which calls for further study. Irrespective of the variation in A, changes in the two fluxes evidently cannot be regarded as functions of the changes in the driving forces only.

Another common practice for measuring the variation of J_+ and J_r with change of A at constant X_+ is the use of drugs that change the fluxes in the short circuited system. In view of the above discussion, however,

Table 3. Spontaneous changes in fluxes, affinity, and rate coefficients at $\Delta\psi = 0$ in 2.5 hours[a]

Source	$\frac{(J_{+0})_{fin}}{(J_{+0})_{init}}$	$\frac{(J_{r0})_{fin}}{(J_{r0})_{init}}$	$\frac{(A)_{fin}}{(A)_{init}}$	$\frac{(L_{+})_{fin}}{(L_{+})_{init}}$	$\frac{(L_{r})_{fin}}{(L_{r})_{init}}$	$\frac{(L_{+r})_{fin}}{(L_{+r})_{init}}$	$\frac{(J_{+0}/J_{r0})_{fin}}{(J_{+0}/J_{r0})_{init}}$	$\frac{q_{fin}}{q_{init}}$
This work	0.78 ± 0.10	0.89 ± 0.04	1.01 ± 0.19	0.59 ± 0.15	0.97 ± 0.26	0.86 ± 0.10	0.88	1.30 ± 0.09
Owen [15]	0.58 ± 0.08	0.70 ± 0.04	0.67 ± 0.07	–	1.04	0.86 ± 0.05	0.83	–

[a] The results are reported as ratios of final to initial values.

observation of changes in the fluxes alone does not necessarily mean that these changes are brought about by variation in A since a concomitant change in the rate coefficients may take place. When the latter occurs the conditions of measurement at the beginning of the experiment are different from those at its end. A careful analysis of the effect of the agent used for changing the fluxes is therefore necessary before any conclusion about the nature of the correlation between the fluxes can be drawn.

The agents most often used and their effect on the parameters of the system are summarized in Table 4. This table presents data computed from results reported by various investigators and represents an attempt to make as complete an analysis as possible of the available data. As can be seen, the mode of action of the various drugs is different, some affecting mainly one parameter, and some changing more than one. In all cases L_+ does not remain constant. The same holds for L_r in the three cases where its changes can be calculated. The cross coefficient L_{+r} is seen to vary significantly in two cases and only slightly in two others. While both L_+ and L_{+r} are significantly affected by ouabain, the differential stoichiometric parameter L_+/L_{+r} [Eq. (3)] remains constant. This is not the case with the other drugs. It should be remarked that some of the values cited in Table 4 for changes in L_+ are taken from the toad bladder [6, 18], and it is not improbable that different epithelia react differently to some of the drugs, as was indeed found when using ouabain [10].

Use of aldosterone and of sub-maximal doses of ouabain indicates that these drugs affect the rate coefficient of the driving chemical reaction, L_r. Since these drugs, at the concentrations used, are specific to the transport system, it is assumed that they do not affect basal J_r. With 2-deoxy-D-glucose (2 DG) the case is not as clear-cut as with the other drugs, since 2 DG affects the overall mechanism for ATP production and therefore may lead to changes in basal J_r. If basal J_r is affected no conclusions can

Table 4. The effect of several drugs on the fluxes, affinity, and rate coefficients of transepithelial active transport[a]

Agent	Final concentration	$\frac{(J_{+0})_{tr}}{(J_{+0})_{un}}$	$\frac{(J_{r0})_{tr}}{(J_{r0})_{un}}$	$\frac{(A)_{tr}}{(A)_{un}}$	$\frac{(L_{+})_{tr}}{(L_{+})_{un}}$	$\frac{(L_{r})_{tr}}{(L_{r})_{un}}$	$\frac{(L_{+r})_{tr}}{(L_{+r})_{un}}$	$\left(\frac{J_{+0}}{J_{r0}}\right)_{tr} \Big/ \left(\frac{J_{+0}}{J_{r0}}\right)_{un}$	Source	Animal
Ouabain	10^{-7} M	0.45	0.84	0.85		0.99	0.54	0.54	[15, 16]	Frog
	10^{-4} M	0.41			0.52				[6]	Toad
2-deoxy-D-glucose (2 DG)	16×10^{-3} M (in 10^{-3} M glu)	0.58	0.92	0.53		1.73	1.09	0.63	[15, 16]	Frog
	7.5×10^{-3} M (in 10^{-3} M glu)	0.31			0.34				[6]	Toad
Amiloride	$10^{-7} - 10^{-5}$ M	0.34[b]	0.65[b,c]	0.92[b]			0.32[b]		[19]	Frog
	5×10^{-7} M	0.41			0.21				[6]	Toad
Aldosterone	5×10^{-7} M	1.67	1.26[c]	2.28		0.53	0.94	1.16	[19]	Frog
	5×10^{-7} M				2.3				[18]	Toad
	5×10^{-7} M	3.00							[12]	Frog
Vasopressin (ADH)	100 mU/ml	2.16			2.36				[6]	Toad

[a] The values cited are the fractions remaining after administration of the drug. (All calculations were performed using the mean values of the parameters involved.) Subscripts *un* and *tr* designate the values of the parameters before and after treatment, respectively; glu indicates glucose.

[b] The skins were pretreated with $5 \cdot 10^{-7}$ M aldosterone.

[c] The change reported is in total (including basal) oxygen consumption.

be drawn concerning the value of J_r and therefore concerning the effect of 2 DG on L_r, unless the effect of 2 DG on basal J_r is determined. If there is an effect on L_r and/or on basal J_r, the value of $(\partial J_+/\partial J_r)_{X_+}$ cannot be established by 2 DG. Inspection of Table 4 shows that the average change in J_{r0} is very slight, thereby implying that basal J_r is not changed significantly by 2 DG. The affinity A, however, decreases significantly with 2 DG; therefore, it should be concluded that L_r increases [Eq. (2)]. Unlike L_r, the cross coefficient L_{+r} is not affected by 2 DG though affected by all other agents. The effect of 2 DG on the parameters of the system requires further elucidation since the information available is insufficient for a complete description.

Table 4 clearly expresses the fact that, apart from their common effect in reducing J_+ (at given $\Delta\psi$), inhibitors of active transport have widely differing effects on the other parameters of the system. The same is true for the drugs enhancing J_+. The table also shows that changes in J_{r0} are not necessarily due to changes in the chemical driving force, but may be due to changes in the chemical rate coefficient. The changes caused by aldosterone encompass both the change in A and in the L's [12], indicating that this hormone affects the system in more than one way, as had been suggested previously [5, 17].

The analysis presented above distinguishes between different types of changes which may occur in the system. It outlines experimental methods for obtaining the required information, and shows possible ways of correlating sodium flux and rate of metabolism.

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