ENERGETICS OF ACTIVE TRANSPORT PROCESSES

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ABSTRACT Discussions of active transport usually assume stoichiometry between the rate of transport J_+ and the metabolic rate J_r. However, the observation of a linear relationship between J_+ and J_r does not imply a stoichiometric relationship, i.e., complete coupling. Since coupling may possibly be incomplete, we examine systems of an arbitrary degree of coupling q, regarding stoichiometry as a limiting case. We consider a sodium pump, with J_+ and J_r linear functions of the electrochemical potential difference, $-X_+$, and the chemical affinity of the metabolic driving reaction, A. The affinity is well defined even for various complex reaction pathways. Incorporation of a series barrier and a parallel leak does not affect the linearity of the composite observable system. The affinity of some region of the metabolic chain may be maintained constant, either by large pools of reactants or by regulation. If so, this affinity can be evaluated by two independent methods. Sodium transport is conveniently characterized by the open-circuit potential $(\Delta\psi)_{I=0}$ and the natural limits, level flow $(J_+)_{X_+=0}$, and static head $X_+^0 = (X_+)_{J_+=0}$. With high degrees of coupling $-X_+^0/F$ approaches the electromotive force E_{Na} (Ussing); $-X_+^0/F$ cannot be identified with $((RT/F)\ln f)_{X_+=0}$, where f is the flux ratio. The efficiency $\eta = -J_+X_+/J_rA$ is of significance only when appreciable energy is being converted from one form to another. When either J_+ or $-X_+$ is small η is low; the significant parameters are then the efficacies $\epsilon_{J_+} = J_+/J_rA$ and $\epsilon_{X_+} = -X_+/J_rA$, respectively maximal at level flow and static head. Leak increases both J_+ and ϵ_{J_+} for isotonic saline reabsorption, but diminishes $-X_+^0$ and $\epsilon_{X_+^0}$. Electrical resistance reflects both passive parameters and metabolism. Various fundamental relations are preserved despite coupling of passive ion and water flows.

INTRODUCTION

Despite the intensive study of biological transport processes, many fundamental thermodynamic questions remain unanswered.

The energetics of active transport have been considered from various viewpoints (1–7). Many treatments have divided the expenditure of metabolic energy into two groups: (1) conversion into electro-osmotic work, and (2) additional expenditure necessarily associated with any real transport process (ref. 5, p. 120 and refs. 6, 7). The rate of performance of electro-osmotic work may be evaluated precisely, at

least in principle, by determining $-JX$ (where J is the rate of net transport of the test species, and $-X$ its electrochemical potential difference across the tissue). The need for the expenditure of metabolic energy at a rate greater than this value is however not precisely defined, and has therefore been variously interpreted in terms of models. Following Ussing (5), this quantity is often regarded as the work performed per mole against "internal resistance" and is evaluated by use of the flux ratio. The sum of the above quantities, defined as the "work", is often compared with estimates of "available energy" in order to evaluate the feasibility and "efficiency" of a presumed transport mechanism (refs. 5, p. 120 and 6). Heinz and Patlak (1960) and Patlak (1961) consider, in addition, the molar free energy required for the continuing supply of a "carrier" (7). They then calculate a value for the work which is equated to the total energy expenditure associated with transport.

It is appreciated that there may be methodological difficulties in determining the parameters discussed above. In addition there are fundamental conceptual problems, for implicit in such treatments is a specific thermodynamic model. For this model the maintenance of a static difference of electrochemical potential across a membrane in the absence of leak should require no expenditure of energy, since the net flow in the active transport pathway is then zero. This is possible however only if there is complete coupling between transport and metabolism, i.e. only if the flow of transport and the flow of metabolism are in a fixed stoichiometric ratio, whatever the forces; in any incompletely coupled system continuing expenditure of energy *is* required to maintain such a "static head", despite the absence of a net flow (8). An example of complete coupling is an electrochemical cell without side reactions, and it is in fact customary to interpret electrophysiological data in terms of such a system. However, adequate justification for this is as yet lacking.

The utilization of the flux ratio in evaluating energetic requirements also involves difficulties. The existence of a small shunt pathway, coupling of flows of different species, and coupling of isotopic flows of the test species (isotope interaction) will all affect the flux ratio (refs. 5, p. 49; 9, 10).

Some of these problems have been considered recently in treatments of energy conversion (8, 11) and of the kinetics of isotope flows (10, 12). In the present paper we apply linear nonequilibrium thermodynamics to simple models comprising permeability barriers and a "pump carrier". We consider in detail only "direct" active transport in two-flow linear systems, i.e. systems in which R_{ir}, the phenomenological coefficient linking transport to metabolism, is nonzero.[1] In these terms the rate of expenditure of metabolic energy is evaluated explicitly. Since the

[1] For a two-flow system it is immaterial whether we define active transport in terms of a resistance coefficient R_{ir} or a conductance coefficient L_{ir}. For three or more flows, however, the two formulations are not equivalent (11). We prefer the criterion of a nonzero R_{ir} since this represents the extent to which the ith flow is "dragged" by the flow of metabolism when no other flows are present and the force conjugate to the ith flow is zero (3). Furthermore, resistance coefficients are readily interpreted in terms of frictional coefficients, and arise naturally when integrating flow equations in systems involving conservative flows, as for example in the analysis of the kinetics of isotope fluxes (10).

degree of coupling in any instance of biological transport is unknown we examine the implications of incomplete coupling.

The fundamental question as to whether biological transport processes can indeed be characterized as linear functions of electrochemical potential differences and chemical affinities cannot be answered on the basis of available data. Chemical reactions deviate readily from linearity; a kinetic analysis shows that reaction rates are not linear in the affinity unless $A \ll RT$ (13). However, the situation may be more favorable in the case of biological reactions, which are often characterized by many consecutive steps, each of which may be near equilibrium. It has been pointed out by Prigogine (14) that if the affinities of the component reactions are sufficiently small with respect to RT, in the stationary state the reaction may be linear, although the over-all affinity may be large.

SYMBOLS

A	affinity of metabolic reaction (Kcal mole^{-1})
c, c_+, c_-	concentration, respectively of solute, cation, and anion (mole l^{-1})
E_{Na}	electromotive force of sodium transport (volt)
f	flux ratio
F	Faraday constant (Kcal volt^{-1} gram equivalent^{-1})
I	electrical current density (coulomb cm^{-2} sec^{-1})
J_+^α	flow of cation (mole cm^{-2} sec^{-1})
J_-^p	flow of anion (mole cm^{-2} sec^{-1}), restricted to passive pathway
J_r	flow of metabolism (mole cm^{-2} sec^{-1})
J_w^p	flow of water (mole cm^{-2} sec^{-1}), restricted to passive pathway
n_i	quantity of species i (mole)
q_α	degree of coupling, $-R_{+r}^\alpha/\sqrt{R_+^\alpha R_{rr}^\alpha}$, relating active transport and metabolism
q_{iw}	degree of coupling, $-R_{iw}^p/\sqrt{R_i^p R_w^p}$, relating ion flow and water flow in the passive pathway
R_+^α, R_{+r}^α, R_{rr}^α	phenomenological resistance coefficients of cation transport system (Kcal cm^2 sec mole^{-2})
R_+^p, R_-^p, R_w^p	phenomenological resistance coefficient of cation, anion, and water in the passive pathway (Kcal cm^2 sec mole^{-2})
R_{iw}	phenomenological resistance coefficient relating ion flow and water flow in the passive pathway (Kcal cm^2 sec mole^{-2})
$\mathfrak{R}$	electrical resistance of a unit area (ohm cm^2, or Kcal^{-1} volt^2 cm^2 sec)
R	gas constant (Kcal deg^{-1} mole^{-1})
T	temperature (deg K)
X_+, X_-	negative electrochemical potential difference, respectively of cation and anion (Kcal mole^{-1})
X_w	negative chemical potential difference of water (Kcal mole^{-1})
$-X_+^0$	electrochemical potential difference at static head (Kcal mole^{-1})
Z_α	$\sqrt{R_{rr}^\alpha/R_+^\alpha}$
Z_{ij}	$\sqrt{R_j^p/R_i^p}$
ϵ_{J_+}	efficacy of flow, J_+/J_rA (mole Kcal^{-1})
ϵ_{X_+}	efficacy of force, $-X_+/J_rA$ (cm^2 sec mole^{-1})

η	efficiency, $-J_+X_+/J_rA$
μ_i, $\tilde{\mu}_i$	chemical potential and electrochemical potential, respectively, of species i (Kcal mole^{-1})
ν_i	stoichiometric coefficient of species i
$\Delta\psi$	electrical potential difference (volt)
ω_+^p	permeability coefficient of cation in the passive pathway (mole cm Kcal^{-1} sec^{-1})

$\alpha = a$ for the active transport pathway.
$\alpha = p$ for the parallel passive "leak" pathway.
(Absence of the index indicates consideration of the composite observable system.)
The superscript j is used when the over-all phenomenological equations are written in terms of metabolism of species j.
Greek letter subscripts refer to "local" reactions.
We have adopted the convention that J_+^a is positive for $X_+ = 0$. Assuming positive flow to be from bath I to bath II, we consider the concentration difference to be $c^{II} - c^I$. The same convention is applied to $\Delta\psi$ and the electrochemical potential difference.

A. THE BASIC MODEL

For simplicity we limit consideration to the active transport of a single cation, uncoupled to flows of other species. It is assumed that the metabolic energy necessary for this process is derived from a single "driving" reaction. Systems in which the driving reaction forms part of a complex metabolic scheme are considered in Appendix I.

We examine first a simple "pump", exposed directly to solutions of the test species (Fig. 1). According to the concepts of linear nonequilibrium thermodynamics (13),

$$X_+ = R_+^a J_+^a + R_{+r}^a J_r , \tag{1}$$

and

$$A = R_{+r}^a J_+^a + R_{rr}^a J_r . \tag{2}$$

Here $-X_+$ and J_+^a represent respectively the electrochemical potential difference and flow of the cation, A and J_r are the affinity and flow of the metabolic reaction, and the R^a's are the phenomenological resistance coefficients of the active transport pathway. (An implicit assumption is that for a given X_+ the relative magnitudes of the chemical and electrical potential differences are immaterial.)

Since we deal with a single driving reaction the rates of consumption and production of the metabolites are related stoichiometrically. We may then take any of these processes to represent metabolism, provided that the affinity is expressed appropriately (see Appendix I). We may, for example, consider J_r to represent the rate of consumption of moles of 0_2. Then, for J_rA to represent the rate of supply of free energy, the affinity A must be expressed as the negative change in free energy

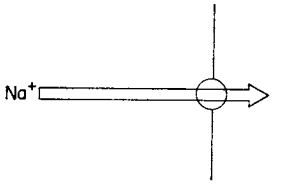


FIGURE 1 Pump.

of the metabolic reaction per mole of 0_2 consumed. For active transport the "input" J_rA must be >0, whereas the "output" $-J_+^aX_+$ may be of either sign. Since both metabolism and transport can occur in either direction there is a degree of arbitrariness in assigning a polarity to the reactions. We shall adopt the convention that J_+^a, J_r, and A are all >0 for $X_+ = 0$. Then R_{+r}^a is <0 and coupling is positive in the naturally occurring system. From the positive definite character of the dissipation function it follows that R_+^a and R_{rr}^a must be >0, and

$$(R_{+r}^a)^2 \leqq R_+^a R_{rr}^a . \tag{3}$$

For certain experimental situations and for theoretical analysis two combinations of the phenomenological coefficients are useful (8). For the basic model these are:

$$Z_a = \sqrt{\frac{R_{rr}^a}{R_+^a}} \quad \text{and} \quad q_a = -\frac{R_{+r}^a}{\sqrt{R_+^a R_{rr}^a}}; \tag{4}$$

q_a is a normalized parameter quantifying the degree of coupling. From equation 3, which is an example of a general relationship,

$$-1 \leqq q_a \leqq 1. \tag{5}$$

For coupled processes q_a is nonzero; for complete coupling $q_a^2 = 1$.[2]

The simultaneous equations 1 and 2 are readily solved for the flows, giving

$$J_+^a = \frac{R_{rr}^a X_+ - R_{+r}^a A}{R_+^a R_{rr}^a - (R_{+r}^a)^2}, \tag{6}$$

or in terms of the alternative coefficients,

$$J_+^a = \frac{X_+ + (q_a/Z_a)A}{R_+^a(1 - q_a^2)}. \tag{7}$$

Similarly,

$$J_r = \frac{(q_a/Z_a)X_+ + (1/Z_a^2)A}{R_+^a(1 - q_a^2)}. \tag{8}$$

[2] For completely coupled systems all R's become infinite, leading to indeterminacy. These systems may be treated by using the inverse phenomenological matrix (8).

Equation 6 shows that for uphill or level transport ($J_+^a > 0$, $X_+ \leqq 0$), and fixed forces, J_+^a will be increased by decreasing either of the straight resistance coefficients or by increasing $-R_{+r}^a$ (or equivalently the degree of coupling).

B. GENERALIZATIONS OF THE BASIC MODEL

Although the above model is useful for orientation, biological membranes which transport a variety of substances must be more complex. We consider therefore two generalizations. The parameters Z and q apply to the generalized models, Z_a and q_a to the active transport pathway as before. Equation 5 applies of course to q as well as to q_a.

Composite Series Membrane

The first generalized model, shown in Fig. 2, differs by the introduction of a barrier(s) in series with the pump. For this composite membrane the formal description changes only in the modification of the straight phenomenological coefficient of the test species; hence equation 1 continues to apply if R_+^a is now taken to represent the total series resistance of the active transport pathway. The degree of coupling

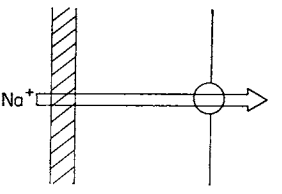


FIGURE 2 Pump and series barrier.

is decreased as a consequence of the increment in R_+^a, and thus is less than that of the pump.[3]

Composite Membrane with Parallel Elements

Another model of biological interest incorporates a parallel "leak" pathway (Fig. 3). Such a leak must exist if there is continuing active transport of only a single ion,

[3] At the barrier,

$$X_+^{\text{barrier}} = R_+^{\text{barrier}} J_+^a ,$$

and at the pump,

$$X_+ - X_+^{\text{barrier}} = R_+^{\text{pump}} J_+^a + R_{+r}^a J_r .$$

Adding,

$$X_+ = (R_+^{\text{barrier}} + R_+^{\text{pump}}) J_+^a + R_{+r}^a J_r .$$

Equation 2 is of course unchanged.

for otherwise accumulation of charge would bring transport to a stop. Formally it is immaterial whether the leak is intracellular or intercellular. If the passive flow J_+^p is uncoupled to the flow of other species,

$$X_+ = R_+^p J_+^p . \tag{9}$$

Since $J_+ = J_+^a + J_+^p$ the membrane is again characterized by linear equations. (The case of coupled ion and solvent flows is considered in Appendix II.)

It is useful to express the phenomenological coefficients of the composite membrane in terms of those of its elements. Combining equations 7 and 9,

$$J_+ = \left(\frac{1}{R_+^a(1 - q_a^2)} + \frac{1}{R_+^p}\right) X_+ + \frac{(q_a/Z_a)}{R_+^a(1 - q_a^2)} A. \tag{10}$$

For given forces, J_r is unaffected by the presence of a leak, and equation 8 continues

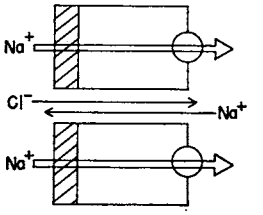


FIGURE 3 Observable composite transport system.

to apply.[4] The degree of coupling is decreased by a leak, being given by

$$q^2 = \frac{q_a^2}{1 + (R_+^a/R_+^p)(1 - q_a^2)}. \tag{11}$$

Equation 10 is useful when X_+ is fixed. Then, for $X_+ < 0$, J_+ is diminished by back flux through the leak pathway. If, as in vivo, X_+ is not fixed experimentally, the situation is different. In this case the leak will not only permit back-flux, but also partial dissipation of the adverse electrical potential difference resulting from active transport. Consider for simplicity a membrane separating identical solutions

[4] Although the phenomenological equations imply linearity, the resistance coefficients are functions of state. The effect of X_+ on intracellular concentrations is unknown. The concentration profile of the leak pathway should in general be affected more by variations of the concentration difference across the membrane than by thermodynamically equivalent variations of the electrical potential difference, particularly with coupling of ion and water flows. Therefore we might expect equation 10 to be more useful in describing the effect of $\Delta\psi$ than of Δc_+. In the absence of an electrical field and coupled flows, $J_+^p = X_+/R_+^p = -RT\Delta \ln c_+/R_+^p$, and is given also by $J_+^p \simeq -\omega_+^p RT\Delta c_+$, where for ideal dilute solutions ω_+^p is approximately constant. Thus

$$R_+^p \simeq \frac{\Delta \ln c_+}{\omega_+^p \Delta c_+}.$$

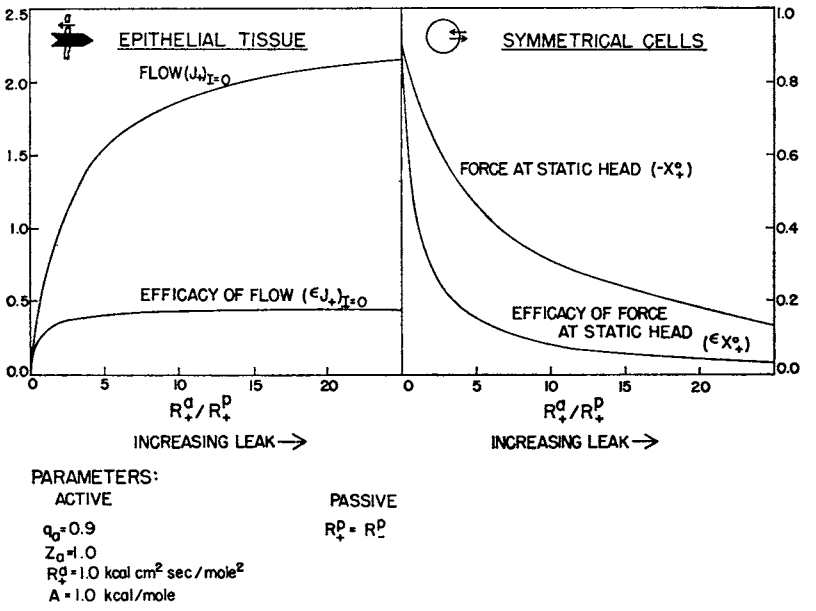


FIGURE 4 Effect of leak in vivo for an arbitrarily chosen set of parameters in the absence of coupling of ion and solvent flows. For the epithelial tissue the reabsorbate is isotonic.

of NaCl, and assume Na^+ to be transported actively and Cl^- to be representative of all passively permeant anions. If the permeability to water is very high, so that transport of NaCl is associated with transport of sufficient water to maintain near-isotonicity, the example may serve as a useful model for the proximal mammalian renal tubule. For such a system $X_w \simeq 0$, and

$$X_+ \simeq -F\Delta\psi \simeq -X_-, \tag{12}$$

where F is the Faraday and $\Delta\psi$ the difference in electrical potential across the membrane. Representing the phenomenological resistance coefficient of chloride by R^p_-, the permselectivity of the passive pathway is characterized by R^p_-/R^p_+, which we shall call Z^2_{+-}. (In the absence of coupled water flow Z^2_{+-} is the ratio of the transport numbers, τ_+/τ_-).

The chloride flux is given by

$$J^p_- = \frac{X_-}{R^p_-}. \tag{13}$$

In the steady state, when the net current is zero, equations 10, 12, and 13 give

$$(J_+)_{I=0} = \frac{(q_a/Z_a)A}{R^a_+(1 + Z^2_{+-})(1 - q^2_a) + R^p_-} \qquad (\Delta c = 0). \tag{14}$$

Equation 14 shows that when X_+ is not fixed experimentally, for a given permselectivity of the passive pathway isotonic saline reabsorption is directly related to the magnitude of the leak. (This is true even if the flows of ions and solvent are coupled, as shown in Appendix II.) An example is shown in Fig. 4.

C. THE OBSERVABLE SYSTEM

Although we may be unable to characterize all the permeability barriers, the above treatment shows that even complex systems may be treated by linear over-all relations. We therefore have for the observed system:

$$J_+ = \frac{X_+ + (q/Z)A}{R_+(1 - q^2)}, \tag{15}$$

and

$$J_r = \frac{(q/Z)X_+ + (1/Z^2)A}{R_+(1 - q^2)}. \tag{16}$$

Two stationary states of fundamental importance in the general treatment of coupled processes are "level flow" ($X_i = 0$) and "static head" ($J_i = 0$) (8). Transport across epithelial tissues often approximates level flow. This appears to be the case in the proximal tubule of mammalian kidney, where some 70–80% of the filtered sodium is reabsorbed, apparently by an active process. The associated water movement is sufficiently rapid to maintain near-isotonicity of the tubular fluid, and the electrical potential difference is small. In contrast, symmetrical cells such as red blood cells and muscle cells establish static head for a variety of solute species.

The equations which describe the two stationary states are, for cations,

$$(J_+)_{X_+=0} = \frac{(q/Z)A}{R_+(1 - q^2)} \qquad \text{(level flow)}, \tag{17}$$

and

$$X_+^0 = (X_+)_{J_+=0} = -\frac{qA}{Z} \qquad \text{(static head)}. \tag{18}$$

These relations will be discussed in section E.

The magnitude of static head is of course influenced by the magnitude of the leak, as can be shown from equation 10, which gives

$$X_+^0 = -\frac{(q_a/Z_a)A}{1 + (R_+^a/R_+^p)(1 - q_a^2)}. \tag{19}$$

As mentioned earlier, the flow of the cation is inversely related to R_+^a. On the other hand, equation 19 shows that if R_+^p is large in comparison with R_+^a, X_+^0 is insensitive to the latter, being equal to $(R_{+r}^a/R_{rr}^a)A$. The insensitivity of X_+^0 to antidiuretic

hormone, an agent which stimulates sodium transport in the toad bladder, supports the view that this substance may act by decreasing the resistance of a permeability barrier (15.)

D. EFFICIENCY OF ENERGY CONVERSION AND EFFICACY OF TRANSPORT

A fundamental consideration is the adequacy of utilization of metabolic energy for active transport. No criterion is uniquely suitable; the conditions of operation determine the appropriate criterion to be employed.

A standard means of evaluating energy utilization is the efficiency, i.e. output/input:

$$\eta = -\frac{J_+X_+}{J_vA} = -\frac{(ZX_+ + qA)X_+}{(qX_+ + (1/Z)A)A}. \tag{20}$$

Variations of resistance coefficients which increase J_+ also increase η (for fixed $X_+ < 0$ and fixed A); η increases with q^2, but for any finite J_+ it is always less than unity (8).

The efficiency function is of interest if a system converts one form of energy into another. An example is the loop of Henle, where sodium is transported against large differences of electrochemical potential at a rate which permits the osmotic water flow necessary to concentrate the urine. Near level flow or static head energy conversion becomes small and η approaches zero. In these circumstances, however, the function of a biological system is clearly not energy conversion, and therefore the efficiency is of no significance.

Near level flow the function appears to be the transport of large quantities of material. In this case a parameter of interest is the rate of transport per given rate of expenditure of metabolic energy. This quantity, which we shall call the efficacy of flow, ϵ_{J_+}, is given by

$$\epsilon_{J_+} = \frac{J_+}{J_vA} = \frac{ZX_+ + qA}{(qX_+ + (1/Z)A)A}. \tag{21}$$

As with η, for given forces in the driving region ϵ_{J_+} is increased by changes of resistance coefficients which increase the rate of transport. Unlike η, for fixed A, ϵ_{J_+} is a monotonically increasing function of X_+ (unless $q^2 = 1$), reaching qZ/A at level flow.

It was shown above that if X_+ is not fixed experimentally, isotonic saline reabsorption is directly related to the magnitude of the leak. The same is true of the efficacy of flow under these conditions (Fig. 4).

$$(\epsilon_{J_+})_{I=0} = \frac{-R^a_{+v}}{(R^a_+(1 + Z^2_{+-}) + R^2)A} \qquad (\Delta c = 0). \tag{22}$$

Again a similar result holds if ion and solvent flows are coupled (Appendix II).

Near static head the function is apparently the maintenance of an electrochemical potential difference. Here it is useful to consider the force developed per given rate of expenditure of metabolic energy,

$$\epsilon_{X_+} = -\frac{X_+}{J_r A}. \tag{23}$$

This quantity, which we shall call the efficacy of force, is a monotonically increasing function of $-X_+$, reaching its maximal value, $-R_{+r}/A$, at static head. It is of interest that $\epsilon_{X_+^0}$ is independent of the magnitude of both straight resistance coefficients of the observable system. As with X_+^0, $\epsilon_{X_+^0}$ is decreased by an increase in the magnitude of the leak (Fig. 4).

The transport of ions is often analyzed by analogy with an electrochemical cell without side reactions. For such a completely coupled system, when J_+ is zero J_r is also zero, and no expenditure of energy is required to maintain static head. For an incompletely coupled system this is no longer the case; metabolism must occur at a finite rate even in the absence of transport, as shown from equations 15 and 16, giving $(J_r)_{J_+=0} = A/R_{rr}$.

E. EXPERIMENTAL CHARACTERIZATION OF THE TRANSPORT SYSTEM

The transport system is characterized by three phenomenological coefficients which define its behavior for all values of two independent variables. In practice, however, since only X_+ can be readily controlled, the response of the system is in general unpredictable. If J_r were maintained constant, the system could be readily characterized (4). Another possibility is that the affinity of some region of the metabolic chain may be constant. This could be the case, for example, for the entire ("global") metabolic reaction if the pools of substrate and product were large. Alternatively the affinity of some more "local" region of the metabolic chain might be maintained constant by the action of a regulator. In this section we shall consider that the affinity of some region of the metabolic chain is in fact constant and shall take this region to represent the driving reaction of the transport system (see Appendix I).

Open Circuit Potential, Level Flow, and Static Head

Two epithelial tissues which conform to our model in that they actively transport only sodium are frog skin and toad bladder (9, 16). These tissues are often studied while exposed to identical saline solutions at each surface. Transport is then generally characterized in terms of the "open-circuit potential" $(\Delta\psi)_{I=0}$ and the "short-circuit current" $(I)_{\Delta\psi=0}$. From equations 12, 13, and 15,

$$(\Delta\psi)_{I=0} = \frac{qA}{FZ(1 + (R_+/R_-^P)(1 - q^2))} \qquad (\Delta c = 0), \tag{24}$$

and from equation 17,

$$(I)_{\Delta\psi=0} = F(J_+)_{\Delta\psi=0} = \frac{(Fq/Z)A}{R_+(1 - q^2)} \qquad (\Delta c = 0). \quad (25)$$

When Δc is zero, $(I)_{\Delta\psi=0}/F$ is simply level flow.

The other natural limit, static head, is also of special interest. Static head and level flow characterize the sodium transport system per se,[5] whereas the open-circuit potential reflects the magnitude of anion leakage as well. Combining equations 18 and 24 gives the relationship between the open-circuit potential and the magnitude of static head:

$$(\Delta\psi)_{\substack{I=0 \\ \Delta c=0}} = -\frac{X_+^0}{F(1 + (R_+/R_-^2)(1 - q^2))}. \quad (26)$$

From equations 17 and 18 we obtain the relationship between the short-circuit current and the magnitude of static head:

$$(I)_{\substack{\Delta\psi=0 \\ \Delta c=0}} = (I)_{X_+=0} = -\frac{FX_+^0}{R_+(1 - q^2)}. \quad (27)$$

Evaluation of R_+ and Electrical Resistance

If J_r could be maintained constant, one could measure a purely "passive" resistance coefficient (4). Neglecting activity coefficients and assuming the solutions to contain only a single uni-univalent salt, X_+ is given by

$$-X_+ = RT\Delta \ln c + F\Delta\psi.$$

From equations 15 and 16 we then obtain

$$-\frac{1}{F}\left(\frac{\partial J_+}{\partial(\Delta\psi)}\right)_{\Delta \ln c, J_r} = \frac{1}{R_+}. \quad (28)$$

If J_r is not constant the variation of J_+ with $\Delta\psi$ would reflect not only R_+, but also the degree of coupling. For example, if the affinity remains constant, equation 15 gives

$$-\frac{1}{F}\left(\frac{\partial J_+}{\partial(\Delta\psi)}\right)_{\Delta \ln c, A} = \frac{1}{R_+(1 - q^2)}. \quad (29)$$

[5] The electrical potential difference between two solutions, $\Delta\psi$, is usually measured with calomel electrodes and salt bridges. The electrochemical potential difference requires the use of reversible electrodes (13). If the two solutions are identical, $-X_+/F = \Delta\psi$, but if they differ this is not the case. In these circumstances $\Delta\psi$ is imprecisely defined owing to the uncertain contribution of diffusion potentials at the bridge tips. However, X_+ is always precisely defined, even in the presence of highly charged colloidal particles or macromolecules as in intracellular media, where estimates of $\Delta\psi$ may be seriously in error. Reversible electrodes are available for several ions; glass electrodes of high specificity for Na^+, K^+, H^+, and Ca^{++} are commercially available, and further developments are to be anticipated.

For the anion, which is not actively transported,

$$\frac{1}{F}\left(\frac{\partial J_-}{\partial(\Delta\psi)}\right)_{\Delta \ln c} = \frac{1}{R_-^p}. \tag{30}$$

Combining equations 29 and 30 we have for the electrical resistance

$$\left(\frac{1}{\mathcal{R}}\right)_{\Delta \ln c, A} = -\left(\frac{\partial I}{\partial(\Delta\psi)}\right)_{\Delta \ln c, A} = \left(\frac{1}{R^+(1-q^2)} + \frac{1}{R_-^p}\right)F^2. \tag{31}$$

Thus, in general, the electrical resistance also depends both on "passive" parameters and on the degree of coupling.

If there is linearity between I and $\Delta\psi$,

$$-\frac{\partial I}{\partial(\Delta\psi)} = \frac{(I)_{\Delta\psi=0}}{(\Delta\psi)_{I=0}}, \tag{32}$$

or

$$(\Delta\psi)_{I=0} = (I)_{\Delta\psi=0}\,\mathcal{R}. \tag{33}$$

Equation 32, which provides an experimental test for linearity, is satisfied by the frog skin and the toad bladder in standard solutions. If the derivative and the ratio differ, the latter is not a well-defined electrical resistance. If they agree, this suggests that either A or J_r is constant, or that they have a linear dependence on $\Delta\psi$.

Affinity A and its Evaluation

In general it might not be possible to vary the affinity of the driving reaction directly; however, a given hormone or drug might well exert its effect by this means. From equations 15, 16, and 18 it is seen that J_+, J_r and $-X_+^0$ are all increased by increasing A. The effects on the efficiency and efficacy parameters are however more complex, with both η and ϵ_{J_+} showing maxima. The efficacy of force at static head $\epsilon_{X_+^0}$ is inversely related to A.

If the affinity of some region of the metabolic chain is constant it can be evaluated. For example, with identical solutions on each side of the membrane, equations 15, 16, and 18 give

$$A = -\frac{Z}{q}X_+^0 = \left(\frac{\partial J_+}{\partial J_r}\right)_A F(\Delta\psi)_{J_+=0}. \tag{34}$$

If $(\partial J_+/\partial J_r)_A$ is determined in the neighborhood of static head, the correct value of A should be obtained even if the phenomenological coefficients are strong functions of state.

Equations 15 and 16 provide an independent means of evaluating the affinity;

again considering the case that the solutions on opposite sides of the membrane are identical,

$$A = -\left(\frac{\partial(\Delta\psi)}{\partial J_r}\right)_A (I)_{\Delta\psi=0} . \qquad (35)$$

Agreement between the values of A determined by these two independent methods would confirm constancy of the affinity. (However, it would not demonstrate the validity of the Onsager relation.) It should be noted that both methods are applicable even with coupling of ion and water flows in the passive pathway (Appendix II).

Stoichiometry and the Degree of Coupling

Many attempts have been made to determine the "number of equivalents transported per mole of oxygen uptake" (refs. 5, p. 120; 6; 17). If an active transport system were completely coupled, J_+/J_r would be identically equal to Z, as can be seen from equations 15 and 16. However, J_+/J_r has a unique value only if a system is completely coupled. There is no a priori reason to assume that this is the case.

Nevertheless, estimates of the "stoichiometric ratio" have commonly been made on the basis of observed linear relationships between J_+ and J_r. From equations 15 and 16 it is seen that if A is constant linearity follows directly from the linearity of the phenomenological equations, irrespective of the degree of coupling.[6] Thus, although $(\partial J_+/\partial J_r)_A$ is identically equal to Z/q, (J_+/J_r) is constant only if q is unity.

Although for effective functioning the coupling in the active transport pathway must be rather tight, and may even be complete, clearly the observable system is incompletely coupled, if only because of the leak pathway. No information is yet available which permits a calculation of q. In principle this is feasible, but the difficulties are considerable. Since coupling is incomplete, the oxygen uptake of an aerobic process must persist in the absence of transport. Therefore, the measurement of q requires a means of determining the oxygen uptake associated with other metabolic functions. Otherwise, unless there is some metabolite involved uniquely in the transport process, only a lower limit for q can be determined. (In principle it is possible to measure q if A is linearly independent of X_+ [11].)

Electromotive Force of Sodium Transport, E_{Na}, and the Flux Ratio, f

We have preferred not to use the customary notation "E_{Na}" (ref. 5, p. 120) to represent $-X^0_+/F$ for two reasons. Firstly, in referring to the active transport system in

[6] Alternatively linearity between J_+ and J_r would follow from a linear dependence of A on J_r (or equivalently, on $\Delta\psi$).

terms of an equivalent electrical circuit there is danger of the implication of complete coupling, as in an ideal electrochemical cell. Secondly, although Ussing has pointed out that the various methods for evaluating E_{Na} can determine only an "effective active transport potential," which must be influenced by such factors as leak, "exchange diffusion," and the experimental conditions (ref. 5, p. 120), this precaution has often been neglected, for example in denoting by E_{Na} both the directly measured $-X_+^0/F$ and $((RT/F) \ln f)_{\Delta c=0, \Delta\psi=0}$. Identity of these quantities has never been demonstrated experimentally and, furthermore, would be expected only in the absence of both leak and coupling of isotope flows, and only if J_r were constant (10).

F. DISCUSSION

The study of active transport has been complicated by disagreement even as to its definition. The limitations of the demonstration of a depressant effect of metabolic inhibitors are obvious.

A criterion which has often been employed is "abnormality" of the flux ratio, active transport being considered likely if the absolute magnitude of $RT \ln f$ exceeds that of the electrochemical potential difference of the test species. Various considerations which limit the usefulness of this test have been discussed (refs. 5, p. 49; 10).

A more fundamental criterion is that of Rosenberg, who considered the "demonstration of transport from a lower to a higher potential $\cdots$ the only certain criterion of active transport", but suggested also a broader definition, "movement of a substance which is influenced by other forces in addition to the chemical (or analogous) potential gradient" (18). In phenomenological terms, for two-flow linear systems Rosenberg's definition is equivalent to the requirement that the cross coefficient R_{+r} be nonzero.

Although we have treated in detail only systems in which there is no coupling between ion and water flows in the passive pathway, some implications of such coupling have been considered in Appendix II. In general the relationships become complex, but considerable simplification occurs at static head or level flow of water ($J_w^p = 0$ or $X_w = 0$). Under these circumstances all formulations relating to the observable system remain unchanged (11); the values of the over-all phenomenological coefficients now reflect the influence of coupling. Furthermore, under conditions of level flow of water qualitative statements concerning the influence of the elemental coefficient R_+^p remain valid.

A variety of histological structures and modes of solute-water interaction have been proposed to account for the observed concentrations of transported salt solutions (19–22). The possibility that solute and water might be transported in near physiological proportions by the pump seems unlikely, in view of the high specificity of active transport systems. Diamond has attributed isotonic transport in the gall bladder to active transport of neutral sodium chloride molecules, osmotic equilibration occurring during the course of passage through long channels whose

walls are highly permeable to water but not to ions (20). The variation of the concentration of the reabsorbate in different tissues depends on geometry, permeability factors, and the rate of solute transport (21). Such a scheme cannot in itself explain transport of salt solutions in tissues with ionic pumps, since electroneutrality necessitates a leak pathway. Since the details are uncertain, and since we are here concerned primarily with the active transport of solute, we have considered the simplest possible scheme, parallel pump and leak pathways. Such a system could carry out near-isotonic salt transport if its water permeability were sufficiently high as to readily dissipate osmotic gradients; alternatively, transport of solute or hypertonic solution via the active transport pathway might be compensated by electroosmotic flow of hypotonic solution through leak pathways. Clarkson has considered such a model for intestine, modified by the existence of electroosmotic flow in a membrane in series with the pump. Assuming the frictional coefficients of free solution, and that the series membrane offers the predominant resistance to water flow through the active channel, it is calculated that if the tissue is bathed by isotonic media, the solution flowing through the active channel is hypertonic, the solution in the leak pathway is hypotonic, and the combined reabsorbate is isotonic, as was in fact observed (22).

In the above treatment the validity of the Onsager reciprocal relation has been assumed. Although this relation has been widely tested, its validity for active transport processes is as yet unknown. A test would require experimental manipulation of the affinity. The problem has been studied in a model system in which the enzymatic hydrolysis of an amide to form a salt results in a current flow simulating active transport. In this system the relation was found to hold (23).

Linearity of the coupled processes would be expected to be limited to the region of small flows. In these regions, even if the resistance coefficients are strong functions of state they may or may not vary appreciably with the flows. In either case relations involving measurements over only a small range should be valid (e.g., equations 34 and 35). The definitive evaluation of the validity of a linear treatment must of course be made experimentally. Meanwhile the present formulation may facilitate the clarification of concepts and the design of experiments. In view of the many difficulties in the analysis of nonlinear systems such a tentative treatment appears justified.

In this paper we have considered the transport process to be coupled to a single driving reaction. In practice, even if this is the case, it is in general not known how the driving reaction is related to the whole complex of metabolic processes, and indeed it may not always be clear which are the pertinent reactants to be considered. This must be determined experimentally. The fundamental driving reaction may be partially or completely coupled to complicated sequences involving branches and closed loops, but the formalism may nevertheless remain appropriate (Appendix I).

Although for a two-flow system there are two independent forces, we have here

considered the possibility that the driving force A might be a constant, characteristic of the system. In principle this need not always be so. In some instances regulation of A may represent an important mechanism of response to stimuli. For example, an analysis of the Hill force velocity relation in muscle is consistent with the view that muscle is a two-flow linear energy converter whose affinity is regulated in response to the load (24).

CONCLUSIONS

1. We consider a sodium pump, coupled to a single driving reaction. The behavior of a composite system incorporating a series barrier and a parallel leak pathway may be described by the linear equations of nonequilibrium thermodynamics, providing that each element shows linearity.

2. Whether or not there is complete coupling between transport and metabolism in the active transport pathway, the composite observable system cannot be completely coupled.

3. For a system with given phenomenological coefficients the rates of transport J_+ and metabolism J_r are determined by the values of the electrochemical potential difference, $-X_+$, and the affinity of the metabolic reaction, A. The affinity is well-defined for an important class of systems, even in the presence of complex sequences of reactions and alternative closed pathways ("loops").

4. X_+ can be readily controlled. Although it may not be possible to control affinities directly, the affinity of some region of the metabolic chain may be constant, either as a consequence of large pools of reactants and products, or as a consequence of regulation. If so, and if the Onsager relation applies, this affinity can be evaluated by two independent methods, whether or not there is coupling of passive ion and solvent flow. Agreement would confirm constancy of the affinity (but not the validity of the Onsager relation).

5. The observation of a linear relationship between J_+ and J_r does not in itself imply a stoichiometric relationship, i.e., complete coupling.

6. The system is conveniently characterized in terms of the open-circuit potential $(\Delta\psi)_{I=0}$ and two natural limits. The first of these, level flow $(J_+)_{X_+=0}$, is approached physiologically in certain epithelial tissues, for example the proximal tubule of the kidney. The second, static head $X_+^0 = (X_+)_{J_+=0}$, may be found in symmetrical cells such as red blood cells.

7. $-X_+^0$ is not equivalent either to FE_{Na}, where E_{Na} is the emf of sodium transport, or to $(RT \ln f)_{X_+=0}$, where f is the flux ratio.

8. The rate of transport will be increased by agents which increase A or which decrease the magnitude of the straight resistance coefficient R_+.

9. The magnitude of static head is also proportional to A, but if leakage is small it is insensitive to the value of R_+.

10. Agents which only slightly affect the straight resistance coefficient(s) might

nevertheless influence transport by altering the extent of coupling between transport and metabolism.

11. The efficiency of energy conversion is very small near the natural limits, level flow and static head. In these circumstances the parameters of interest are respectively the efficacy of flow J_+/J_rA and the efficacy of force, $-X_+/J_rA$.

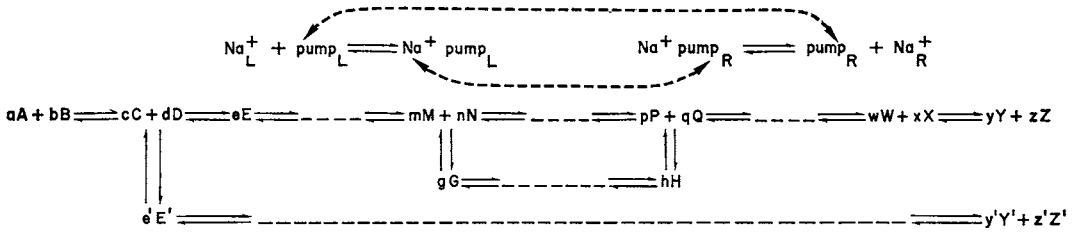
12. Leak increases the magnitude and efficacy of flow for isotonic saline reabsorption, but diminishes the magnitude and efficacy of force at static head. This is true whether or not there is coupling of passive ion and solvent flow.

13. Unless J_r is constant, the electrical resistance reflects not only "passive" parameters, but also the degree of coupling to metabolism.

14. Metabolic energy is used highly effectively at level flow and static head.

APPENDIX I

An example of the type of system with which we are concerned is as follows:



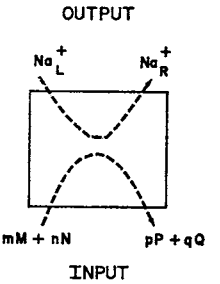
This is a scheme for the utilization of metabolic energy in the transport of Na^+ from left to right. At some point in the main sequence of reactions shown there is a subsidiary sequence of consecutive steps directly coupled to some part of the pump mechanism (it is immaterial which steps are involved). Our formalism may then be applied either to this local region or to the over-all system. The local process driving the transport of Na^+ is the reaction $mM + nN \rightleftharpoons pP + qQ$, for which the affinity[7] A is given by $(m\mu_M + n\mu_N) - (p\mu_P + q\mu_Q)$. M and N

[7] The affinity of a reaction is defined as $A = -\Sigma_i \nu_i \mu_i$ (De Donder), where the stoichiometric coefficients ν_i are considered positive for products and negative for reactants (14). Here the rate of the reaction is defined as $J_r = (1/\nu_i)\,(dn_i/dt)$, where dn_i/dt is the rate of appearance of the ith component (it is obviously immaterial which reactant or product is considered). The rate of expenditure of free energy by the reaction is $-\Sigma_i \mu_i\,(dn_i/dt) = -\Sigma_i \nu_i \mu_i\,(1/\nu_i)\,(dn_i/dt) = AJ_r$. However, experimentalists customarily choose to regard the rate of reaction as the rate of consumption or production of some easily measured species, say the jth. If we call this measured rate of reaction J_r^j, we have $J_r^j = |\nu_j| J_r$. Since the rate of expenditure of free energy is independent of the convention, the affinity must now be expressed as kcals/mole of species j. Thus $A^j = A/|\nu_j|$ and $A^j J_r^j = AJ_r$, irrespective of which species j is chosen. Furthermore, the validity of the Onsager relation is independent of the choice of species, since equations 1 and 2 become

$$X_+ = R_+^a J_+^a + (R_{+r}^a/|\nu_j|) J_r^j = R_+^a J_+^a + R_{+r}^{aj} J_r^j, \tag{A1}$$

$$A^j = A/|\nu_j| = (R_{+r}^a/|\nu_j|)\, J_+^a + (R_{rr}^a/\nu_j^2)\, J_r^j = R_{+r}^{aj} J_+^a + R_{rr}^{aj} J_r^j. \tag{A2}$$

may represent ATP and H_2O, while P and Q represent ADP and P_i. From the point of view of the local system it does not matter how the concentrations of the reactants and products are maintained. (For example, we may have a compartment in which the reactants and products are each close to equilibrium with large reservoirs.) Stoichiometric conversion of M and N to P and Q requires that there be no "branches" in this region of the chain; this is our fundamental assumption. As indicated, however, there may be a number of alternative closed pathways ("loops"). In the stationary state, consumption of M and N would still be related stoichiometrically to production of P and Q. Some of these loops may not be coupled to the pump, and it is clear that although they would not influence the validity of the formalism, they would decrease the degree of coupling between metabolism and transport. It should be appreciated that even locally the rate of dissipation of free energy must be expressed as the input minus the output. The input term is J_rA, where the affinity A which has been defined above constitutes the driving force of the process. In this view the system may be regarded as a black box, with $mM + nN \rightarrow pP + qQ$ as the input and the transport of Na^+ from left to right as the output:



It is not useful to regard the interconversion of the species pump_L, pump_R, $Na^+\ \text{pump}_L$, and $Na^+\ \text{pump}_R$ as contributing to the input.[8]

Note that the degree of coupling is also unaffected, since

$$-R_{+r}^{aj}/\sqrt{R_{++}^{a}R_{rr}^{aj}} = q_a\,.$$

Obviously, if the metabolism of oxygen is completely coupled to that of glucose ($J_r = J_r^{O_2}/|\nu_{O_2}| = J_r^{G}/|\nu_G|$), it is immaterial which is represented by j. Furthermore, since the set of stoichiometric coefficients for a reaction may be multiplied by any arbitrary factor without changing the value of J_rA, we may set $|\nu_j| = 1$ for any desired representative species j, e.g., oxygen. Then $J_r^j = J_r^{O_2} = J_r$, and $A^j = A^{O_2} = A$.

[8] The dissipation function for the black box shown is

$$\Phi = J_+X_+ + J_rA. \qquad \text{(A3)}$$

Alternatively we may consider reactions taking place within the black box:

(α) $$Na_L^+ + \text{pump}_L \rightarrow Na^+\ \text{pump}_L\,,$$

(β) $$Na^+\ \text{pump}_L \rightarrow Na^+\ \text{pump}_R\,,$$

(γ) $$Na^+\ \text{pump}_R \rightarrow \text{pump}_R + Na_R^+\,,$$

(δ) $$\text{pump}_R \rightarrow \text{pump}_L\,,$$

(ϵ) $$mM + nN \rightarrow pP + qQ.$$

Each of these reactions may of course be the sum of several subreactions. The combination of

For some purposes it is more convenient to take a "global" view and analyze the system in terms of the affinity over some larger region of the chain, providing of course that this region encompasses the reaction $mM + nN \rightleftharpoons pP + qQ$ and includes no branches which fail to return. This would be true of the whole reaction sequence if Y' were identical to Y and Z' were identical to Z, and both were in the same compartment (e.g., CO_2 and H_2O). In this case there is a single metabolic reaction, the affinity of which is given by $(a\mu_A + b\mu_B) - (y\mu_Y + z\mu_Z)$. Another possibility whereby the entire reaction sequence could represent a single metabolic input would be if E were produced wholly by breakdown of C, and E' wholly by breakdown of D. In this case the over-all reaction is $aA + bB \rightleftharpoons yY + zZ + y'Y' + z'Z'$ and the affinity is given by $(a\mu_A + b\mu_B) - (y\mu_Y + z\mu_Z + y'\mu_{Y'} + z'\mu_{Z'})$. In general, the above possibilities do not obtain and each branch would represent a different reaction, one being characterized by the affinity $(a\mu_A + b\mu_B) - (y\mu_Y + z\mu_Z)$, and the other by the affinity $(a\mu_A + b\mu_B) - (y'\mu_{Y'} + z'\mu_{Z'})$. There is no a priori reason for two such reactions to be related stoichiometrically and thus we have, in the global view, two input processes for this scheme. An analysis from this viewpoint is not carried out in this paper. However, in some systems the expenditure of free energy by one or more subsidiary flows may be small enough to be ignored. A possible example is mammalian kidney, where O_2 consumption in the absence of Na^+ reabsorption may fall to as little as 20% of that associated with high rates of Na^+ reabsorption (26).

Although we are in principle free to take either a local viewpoint or a more or less global viewpoint, the degree of coupling will depend on our choice. Consider the region $cC + dD \rightleftharpoons$

(ϵ) and one of the other reactions may possibly represent the actual mechanism of coupling. In any case, the dissipation function is given by

$$\Phi = J_\alpha A_\alpha + J_\beta A_\beta + J_\gamma A_\gamma + J_\delta A_\delta + J_r A, \tag{A4}$$

where the affinities, expressed in terms of the electrochemical potentials, are

$$A_\alpha = \bar{\mu}_{\mathrm{Na}_L} + \bar{\mu}_{\mathrm{pump}_L} - \bar{\mu}_{\mathrm{Na \cdot pump}_L},$$

$$A_\beta = \bar{\mu}_{\mathrm{Na^+ pump}_L} - \bar{\mu}_{\mathrm{Na^+ pump}_R},$$

$$A_\gamma = \bar{\mu}_{\mathrm{Na^+ pump}_R} - \bar{\mu}_{\mathrm{pump}_R} - \bar{\mu}_{\mathrm{Na^+}_R},$$

$$A_\delta = \bar{\mu}_{\mathrm{pump}_R} - \bar{\mu}_{\mathrm{pump}_L}.$$

In the stationary state,

$$J_\alpha = J_\beta = J_\gamma = J_\delta = J_+, \tag{A5}$$

and therefore

$$\Phi = J_+(A_\alpha + A_\beta + A_\gamma + A_\delta) + J_r A. \tag{A6}$$

It is readily seen that equations A3 and A6 are identical.

We have considered $J_r A$ the input and $-J_+X_+$ the output. Alternatively, since for example the terms $J_\alpha A_\alpha$, $J_\gamma A_\gamma$, and $J_\delta A_\delta$ may be positive, i.e. represent spontaneous processes, it might be possible to consider these as well as $J_r A$ as inputs. The output would then be $-J_\beta A_\beta$. This approach seems to us unnatural and unproductive, since the pump is involved in a cyclic process. As pointed out by Katchalsky and Spangler, the circulation of a species makes no *formal* contribution to the dissipation function, although it influences the phenomenological coefficients (25).

$yY + zZ$. This comprises the following partial sequences:

$$(\lambda) \qquad cC + dD \rightleftharpoons mM + nN,$$

$$(\sigma) \qquad mM + nN \rightleftharpoons pP + qQ,$$

$$(\tau) \qquad pP + qQ \rightleftharpoons yY + zZ.$$

From the local point of view we may apply equations 1 and 2, where the reaction (σ) is the metabolic input, i.e.

$$X_+ = R^a_+ J^a_+ + R^a_{+r} J_r \,, \tag{A7}$$

$$A_{(\sigma)} = R^a_{+r} J^a_+ + R^a_{rr(\sigma)} J_r \,. \tag{A8}$$

From the global point of view we must consider the additional phenomenological relations

$$A_{(\lambda)} = R^a_{rr(\lambda)} J_r \,, \tag{A9}$$

$$A_{(\tau)} = R^a_{rr(\tau)} J_r \,. \tag{A10}$$

Adding equations A8, A9, and A10, we have

$$A_{\text{global}} = A_{(\lambda)} + A_{(\sigma)} + A_{(\tau)} = R^a_{+r} J^a_+ + (R^a_{rr(\lambda)} + R^a_{rr(\sigma)} + R^a_{rr(\tau)}) J_r \,. \tag{A11}$$

Equations A7 and A11 are the phenomenological equations of the global system. The degree of coupling in the global viewpoint,

$$-R^a_{+r} / \sqrt{R^a_+ (R^a_{rr(\lambda)} + R^a_{rr(\sigma)} + R^a_{rr(\tau)})},$$

is clearly less than that in the local viewpoint except in the case of complete coupling (see footnote at end of section *A*). Although the local and global treatments lead to different values of q, Z, and A, no inconsistency arises.

In attempting to apply the present formulation experimentally, it seems natural to look at the over-all system and to consider the concentrations of exogenous substrates and of final end products. However, this may not always be possible. For example, if there is branching in the metabolic chain, then in general the system cannot be described in terms of a single input, as discussed above. Another possibility is that the affinity over some subsidiary portion of the chain is maintained constant by a self-regulatory process (which would generally imply nonlinearity in the remainder of the chain). If so, this affinity is a characteristic property of the system and would be the parameter determined by application of the linear equations 34 and 35. Even in systems in which a global view is experimentally practicable, it may be useful to analyze subsystems as well. This approach has been taken with considerable success in the case of glycerin-extracted muscle fibers which have no endogenous source of ATP (27). For such fibers it is possible in principle to regulate the affinity of the ATP hydrolysis reaction experimentally. A similar technique may be applicable to epithelial tissues.

APPENDIX II

For simplicity we have presented a detailed treatment only for the case in which there is no coupling between flows of ions and water in the passive pathway. If such coupling does exist

it is necessary to consider three phenomenological equations:

$$X_+ = R_+^p J_+^p + R_{+w} J_w^p, \tag{A12}$$

$$X_- = R_-^p J_-^p + R_{-w} J_w^p, \tag{A13}$$

$$X_w = R_{+w} J_+^p + R_{-w} J_-^p + R_w J_w^p. \tag{A14}$$

Here the subscript $_w$ refers to water. It is assumed that direct coupling between ions of opposite charge is insignificant (i.e., $R_{+-} = 0$).

We restrict consideration to systems in which there is no water flow in the active transport pathway. Then in the absence of net water flow, as for example with symmetrical cells, $J_w^p = 0$ and equations A12 and A13 reduce respectively to equations 9 and 13. Thus although coupling between ion and water flows would in general be expected to influence the magnitude of the resistance coefficients, in the absence of net water flow it will not alter the formal description of the ion flows. Hence fundamental relationships involving the over-all coefficients continue to apply.

For the more general situation it is useful to express the flows as functions of the forces. Equations A12 to A14 give

$$J_+^p = \frac{(1 - q_{-w}^2)X_+ + (q_{+w} q_{-w}/Z_{+-})X_- + (q_{+w}/Z_{+w})X_w}{R_+^p(1 - q_{+w}^2 - q_{-w}^2)}, \tag{A15}$$

$$J_-^p = \frac{(q_{+w}\, q_{-w}/Z_{-+})X_+ + (1 - q_{+w}^2)X_- + (q_{-w}/Z_{-w})X_w}{R_-^p(1 - q_{+w}^2 - q_{-w}^2)}, \tag{A16}$$

and

$$J_w = \frac{(q_{+w}/Z_{+w})X_+ + (q_{-w}/Z_{+-}\, Z_{+w})X_- + (1/Z_{+w}^2)X_w}{R_+^p(1 - q_{+w}^2 - q_{-w}^2)}, \tag{A17}$$

where

$$Z_{ij} = \sqrt{\frac{R_j^p}{R_i^p}}. \tag{A18}$$

We again examine the case of identical NaCl solutions bathing a membrane whose permeability to water is so high as to maintain near-isotonicity. (Water movement may here be either by way of the leak pathway or by some other avenue inaccessible to ions.) Then in the absence of significant pressure gradients $X_w \simeq 0$ and $X_+ \simeq -X_-$, giving

$$J_+^p = \frac{(1 - q_{-w}^2 - q_{+w}\, q_{-w}/Z_{+-})X_+}{R_+^p(1 - q_{+w}^2 - q_{-w}^2)}, \tag{A19}$$

and

$$J_-^p = \frac{(1 - q_{+w}^2 - q_{+w}\, q_{-w}/Z_{-+})X_-}{R_-^p(1 - q_{+w}^2 - q_{-w}^2)}. \tag{A20}$$

Combination of equations 7 and A19 gives the net sodium flux,

$$J_+ = \left(\frac{1}{R^a_+(1 - q^2_a)} + \frac{1 - q^2_{-w} - q_{+w}\, q_{-w}/Z_{+-}}{R^p_+(1 - q^2_{+w} - q^2_{-w})}\right) X_+ + \frac{(q_a/Z_a)}{R^a_+(1 - q^2_a)} A \quad (A21)$$

(cf. equation 10).

It is now useful to consider two consequences of the positive definiteness of the dissipation function for the passive pathway, viz.:

(1) The determinant of the matrix of the phenomenological coefficients of equations A12–A14 must be positive. Since the straight coefficients are also positive, we have

$$1 - q^2_{+w} - q^2_{-w} > 0. \quad (A22)$$

(2) Since $J^p_+X_+ + J^p_-X_- + J^p_wX_w > 0$, on setting $X_w = 0$ and $X_+ = -X_-$, equations A15–A18 give

$$(1 - q^2_{-w})Z^2_{+-} - 2q_{+w}q_{-w}Z_{+-} + 1 - q^2_{+w} > 0. \quad (A23)$$

In the absence of net current flow, $X_+ = -X_-$ is given by equations A20 and A21.

$$(X_+)_{I=0} = -\frac{(q_a/Z_a)(1 - q^2_{+w} - q^2_{-w})A}{\{1 - q^2_{+w} - q^2_{-w} + [(R^a_+/R^p_-)(1 - q^2_a) \cdot ((1 - q^2_{-w})Z^2_{+-} - 2q_{+w}\, q_{-w}\, Z_{+-} + 1 - q^2_{+w})]\}}. \quad (A24)$$

The positive definiteness conditions show that this quantity is negative. The net rate of sodium chloride reabsorption under these circumstances is

$$(J_+)_{I=0} = \frac{(q_a/Z_a)(-q_{+w}\, q_{-w}Z_{+-} + 1 - q^2_{+w})A}{\{R^a_+(1 - q^2_a)((1 - q^2_{-w})Z^2_{+-} - 2q_{+w}\, q_{-w}\, Z_{+-} + 1 - q^2_{+w}) + R^p_-(1 - q^2_{+w} - q^2_{-w})\}}. \quad (A25)$$

(Cf. equation 14.) Since both terms in the denominator are positive, the sign of $(J_+)_{I=0}$ is that of the numerator. For the positive values of interest here, isotonic saline transport is directly related to the magnitude of leak, just as in the absence of coupling between ion flow and water flow. (This statement pertains to a given membrane with given degrees of ion-water coupling and a given permselectivity. A change of the magnitude of leak induced solely by altering the number but not the caliber of the leak pathways would change all resistance coefficients proportionately, and thus the q_{iw}'s and Z_{+-} would be unaffected. For a membrane of appreciable permselectivity a change in the average pore size would in general change the q_{iw}'s and Z_{+-}).

Equations 8, A24, and A25 give the efficacy under these conditions:

$$(\epsilon_{J_+})_{I=0} = \frac{-R^a_{+r}(-q_{+w}\, q_{-w}Z_{+-} + 1 - q^2_{+w})}{\{[R^a_+((1 - q^2_{-w})Z^2_{+-} - 2q_{+w}\, q_{-w}\, Z_{+-} + 1 - q^2_{+w}) + R^p_-(1 - q^2_{+w} - q^2_{-w})]A\}} \quad (A26)$$

(cf. equation 22). Again the efficacy of isotonic saline reabsorption is directly related to the magnitude of the leak.

A consideration of equations 8 and A21 shows that the two methods for evaluation of the

affinity (equations 34 and 35) may be employed even in the presence of ion-solvent coupling, providing the concentrations on either side of the membrane are identical.

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