Phenomenological Description of Active Transport of Salt and Water

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ABSTRACT The phenomenological definition of active transport by Kedem and the methods of Kedem and Katchalsky have been used to obtain practical equations describing active transport in the single salt and bi-ionic systems. Procedures were devised to evaluate the required set of 10 coefficients for the single salt case and 15 for the bi-ionic. Three of these coefficients are unusual. They express the effects of active transport, i.e. of entrainment between metabolism and the conventional transport flows: active salt transport coefficient, a volume pump coefficient, and an electrogenicity coefficient. In the bi-ionic case a new passive coefficient, λ, was used to express the linkage between the fluxes of the two salts. However, if primary active transport involves only one ion, for example in the bi-ionic case, 12 coefficients suffice and certain relations can be predicted between the practical coefficients. Particular types of primary active transport could be identified by this means. The relation of active transport to membrane electrogenesis was also examined and the flux ratio equation was rederived in terms of the practical coefficients. Applications to specific parallel and series membrane systems have been analyzed.

INTRODUCTION

The full characterization of biological membranes requires a clear operational definition of active transport. Recently, Kedem (1) offered a definition of active transport as an entrainment between a transport flux and a metabolic reaction; she also described a number of consequences of her definition in the simple case of a single permeant ion.

In the present work it has been possible to use Kedem's definition to obtain practical equations describing active salt transport and to examine the consequences of certain postulates concerning the active transport mechanism. Unambiguous answers, independent of the use of poisons, can now be obtained to questions concerning active transport and coupling between transport fluxes.

Throughout this work we have used the thermodynamics of irreversible processes, as applied to electrolyte solutions by Kedem and Katchalsky (2).

Thus, all terms and definitions, when possible, are used in a fashion consistent with that paper. (See List of Symbols preceding References.)

COUPLING BETWEEN CHEMICAL REACTIONS AND TRANSPORT PROCESSES

The question of the application of Curie's principle to allow for a relation between chemical reactions and vectorial transport flows has been discussed before (1, 3, 4). It was concluded that appropriate anisotropy in the system allows the existence of vectorial coefficients. Such coefficients may be only a phenomenological disguise for a complicated mechanism such as entrainment by a carrier possessing a "diffusion coefficient" strongly dependent upon position, the carrier being produced or consumed in the chemical reaction. On the other hand, they may express the fact that the molecular transformation of the reaction itself is spatially directed.

Most real active transport systems are probably rather nonlinear; i.e., transport fluxes may not necessarily be linear functions of all forces and other system parameters. There is no known case of ATP generation from an ionic gradient; there are pronounced structural heterogeneities in most membranes of interest. Many transport processes are nonlinear such as the effect of osmotic gradients on frog skin potential (5). Nonetheless, nonlinear systems may be approached through application of linear approaches, which should be valid for small deviations from equilibrium.

A further question is the applicability of the linear rate laws of irreversible thermodynamics to chemical reactions. It is well known that the rate of a chemical reaction is proportional to the affinity (as defined by DeDonder, 6) only for very small displacements from equilibrium. However, the dissipation function contribution is still given by the product of reaction rate and affinity outside the linear range (7). Thus all forces and fluxes for transport and metabolism even in the active transport system can be identified by means of the appropriate dissipation function. All features of the phenomenological equations except for the precise relationship of chemical reaction rate to affinity can hold if all processes except the reaction itself are linear. The relation we shall write below exploits this premise, which must be considered an additional postulate. In other words we suggest that the transport fluxes in the system can remain in linear relation to the *rate* of the driving metabolic reaction even if the metabolic reaction itself is nonlinear with respect to the affinity. This postulate is credible in view of the linear relation found by Zerahn (8) between active Na transport and O_2 consumption in frog skin.

More specifically, all steps beyond equations (3) in which a parameter contains R_{rr}, are invalid to the extent that A_r is not linear in J_r.

Carrier models for active transport (9) quite often assume that the combination of carrier and carried species is at equilibrium. Thus, for this class of model, the affinity may indeed be very small, and the linear rate law may be expected to hold.

I. SINGLE SALT WITH ACTIVE TRANSPORT

The approach is essentially that of Kedem and Katchalsky (2). Briefly, it is given by the following questions.

1. What are the appropriate forces and fluxes for characterizing a system? The forces and fluxes are to be extracted from the dissipation function.

For the present case (see List of Symbols),

$$\Phi = J_r A_r + J_1 \Delta\tilde{\mu}_1 + J_2 \Delta\tilde{\mu}_2 + J_w \Delta\mu_w \tag{1}$$

$$\Phi \cong J_r A_r + J_v(\Delta p - \Delta\pi) + IE + J_s \Delta\mu_s^c \tag{2}$$

Here the system consists of two solutions, each containing the same permeant electrolyte plus nonpermeant substances, separated by a membrane (of unit area) capable of active transport.

$J_v = \bar{v}_s J_s + \bar{v}_w J_w$ is volume flow. $J_s = J_1/\nu_1$ is salt flow
$\Delta\tilde{\mu}_s = \nu_1 \Delta\tilde{\mu}_1 + \nu_2 \Delta\tilde{\mu}_2$ is the difference in total chemical potential for the salt.
$\Delta\mu_s^c = RT\Delta\ln a_s$ is the difference in the concentration-dependent part of the chemical potential.
$\Delta\tilde{\mu}_1 = \bar{v}\Delta p + z_1 F \Delta\phi + RT\Delta\ln a_1$ is the difference in electrochemical potential of ion 1.
$E = \Delta\tilde{\mu}_2/z_2 F$ is the potential difference measured with electrodes reversible for ion 2.

In this communication, all Δ's are taken in such a sense (initial minus final) that a positive Δ gives a positive flux.

2. Which forces and fluxes can be controlled easily? These are taken as independent variables.

$$\Delta\mu_s^c, I, J_v, E, (\Delta p - \Delta\pi)$$

are all easily set to zero. Although J_r and A_r are difficult to control, either one must be used. Because of the linearity considerations given earlier, J_r is taken.

3. What are the appropriate linear phenomenological equations? From the dissipation function, we write the phenomenological equations

$$\begin{aligned}
\Delta\mu_s^c &= R_{ss}J_s + R_{si}I + R_{sv}J_v + R_{sr}J_r \\
E &= R_{is}J_s + R_{ii}I + R_{iv}J_v + R_{ir}J_r \\
\Delta p - \Delta\pi &= R_{vs}J_s + R_{vi}I + R_{vv}J_v + R_{vr}J_r \\
A_r &= R_{rs}J_s + R_{ri}I + R_{rv}J_v + R_{rr}J_r
\end{aligned} \tag{3}$$

The Onsager reciprocal relations are assumed:

$$R_{kj} = R_{jk}$$

The resistance formulation is written here, although a conductance formulation could equally well be used.[1]

Several consequences are immediately apparent. If the active transport process acts upon, say, ion 1, none of the vectorial entrainment coefficients, R_{sr}, R_{ir}, R_{vr}, can be zero since each of the corresponding fluxes, J_s, I, and J_v, contains J_1. As a result it is not possible to define any membrane parameter of this set without explicitly considering the metabolic factor, either A_r or J_r. For example, the resistance R_{ii} can be defined only by holding the metabolic factor J_r, as well as J_s and J_v, zero.

$$R_{ii} = (E/I)_{J_s, J_v, J_r}$$

The same consideration applies to the remaining nine independent parameters which are required to characterize the system fully. Biologists have long recognized this difficulty and have attempted to meet the problem by using metabolic inhibitors such as cyanide or dinitrophenol or "specific uncoupling agents" such as ouabain. However, the use of such agents has also been recognized as unsatisfactory since their use presumes knowledge of their actions on the tissue to be studied.

4. Which coefficients are especially desired? Ten are necessary; the desirable ones are those which can be easily measured, such as L_p. In order to transform the above equations into a more usable form incorporating the desired practical coefficients, the procedure of Kedem and Katchalsky (2) is followed, but with the additional restriction of zero metabolic reaction rate. The rate constant used for the driving metabolic reaction is not new. However, three practical coefficients which describe the effect of active transport are introduced:

1. an electrogenicity coefficient

$$\epsilon = (I/J_r)_{\Delta\mu_s^c, E, J_v}$$

2. an active salt-pumping coefficient

$$U = (J_s/J_r)_{\Delta\mu_s^c, I, J_v}$$

The letter "U" was chosen to honor Professor H. H. Ussing.

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[1] Ohki (10) presented a related set of equations using the conductance formulations and a different set of variables.

3. a volume pump coefficient

$$V = (J_v/J_r)_{\Delta\mu_s^c, I, (\Delta p - \Delta\pi)}$$

The letter "*V*" was chosen to honor Professor M. B. Visscher.

The practical equations are simply a linear arrangement of equations (3):

$$J_s = a_{11}\Delta\mu_s^c + a_{12}I + a_{13}J_v + a_{14}J_r$$

$$I = a_{21}\Delta\mu_s^c + a_{22}E + a_{23}J_v + a_{24}J_r$$

$$J_v = a_{31}\Delta\mu_s^c + a_{32}I + a_{33}(\Delta p - \Delta\pi) + a_{34}J_r$$

$$J_r = a_{41}\Delta\mu_s^c + a_{42}I + a_{43}J_v + a_{44}A_r$$

Note that the definition of any membrane parameter requires *three* restrictions. For example, if we define the conductance as a_{22}, it is measured by the ratio of current to potential E when $\Delta\mu_s^c$, J_v, and J_r are zero. We thus proceed to establish the definitions of the *a*'s in terms of *R*'s, and to give descriptive names to these former phenomenological quantities, the *a*'s, which are unique.

Salt permeability

$$a_{11} = (J_s/\Delta\mu_s^c)_{I, J_v, J_r} = \omega c_s = 1/R_{ss}$$

Conductance

$$a_{22} = (I/E)_{\Delta\mu_s^c, J_v, J_r} = \kappa = R_{ss}/(R_{ii}R_{ss} - R_{is}{}^2)$$

Mechanical permeability

$$a_{33} = (J_v/(\Delta p - \Delta\pi))_{\Delta\mu_s^c, I, J_r} = L_p = R_{ss}/(R_{ss}R_{vv} - R_{vs}{}^2)$$

Transference number, τ_1,

$$a_{12} = (J_s/I)_{\Delta\mu_s^c, J_v, J_r} = \frac{\tau_1}{\nu_1 z_1 F} = \tau_1{}^r = -(E/\Delta\mu_s^c)_{I, J_v, J_r} = -R_{si}/R_{ss}$$

τ_1^r is Staverman's reduced transport number (11).

Reflection coefficient, σ,

$$a_{13} = (J_s/J_v)_{\Delta\mu_s^c, I, J_r} = c_s(1 - \sigma) = -([(\Delta p - \Delta\pi)/\Delta\mu_s^c])_{I, J_v, J_r} = -R_{sv}/R_{ss}$$

Salt-pumping coefficient

$$a_{14} = (J_s/J_r)_{\Delta\mu_s^c, I, J_v} = U = -(A_r/\Delta\mu_s^c)_{I, J_v, J_r} = -R_{sr}/R_{ss}$$

Electroosmotic pressure

$$\begin{aligned} a_{23} &= (I/J_v)_{\Delta\mu_s^c, E, J_r} = -P_E = -[(\Delta p - \Delta\pi)/E]_{\Delta\mu_s^c, J_v, J_r} \\ &= (R_{sv}R_{si} - R_{ss}R_{iv})/(R_{ss}R_{ii} - R_{is}{}^2) \end{aligned}$$

Electrogenicity coefficient (compare N. P. of Zerahn, 8)

$$\begin{aligned} a_{24} &= (I/J_r)_{\Delta\mu_s^c, E, J_v} = \epsilon = -(A_r/E)_{\Delta\mu_s^c, J_v, J_r} \\ &= (R_{sr}R_{is} - R_{ir}R_{ss})/(R_{ss}R_{ii} - R_{is}^2) \end{aligned}$$

Volume pump coefficient

$$\begin{aligned} a_{34} &= (J_v/J_r)_{\Delta\mu_s^c, I, \Delta p - \Delta\pi} = V = -[A_r/(\Delta p - \Delta\pi)]_{\Delta\mu_s^c, J_r, I} \\ &= (R_{sr}R_{sv} - R_{ss}R_{vr})/(R_{ss}R_{vv} - R_{sv}^2) \end{aligned}$$

Metabolic rate coefficient

$$\begin{aligned} a_{44} &= (J_r/A_r)_{\Delta\mu_s^c, I, J_v} = k \\ &= R_{ss}/(R_{rr}R_{ss} - R_{sr}^2) \end{aligned}$$

The remainder of the coefficients can be expressed in terms of these by using the appropriate reciprocal relations.

Practical Equations

$$\begin{aligned} J_s &= \omega c_s \, \Delta\mu_s^c + \frac{\tau_1}{\nu_1 z_1 F} I + c_s(1 - \sigma)J_v + UJ_r \\ I &= \frac{\kappa\tau_1}{\nu_1 z_1 F} \Delta\mu_s^c + \kappa E - P_E J_v + \epsilon J_r \\ J_v &= L_p c_s(1 - \sigma)\Delta\mu_s^c - \frac{P_E L_p}{\kappa} I + L_p(\Delta p - \Delta\pi) + VJ_r \\ J_r &= kU\Delta\mu_s^c + \frac{k\epsilon}{\kappa} I + \frac{kV}{L_p} J_v + kA_r \end{aligned} \tag{4}$$

Note that $c_s \equiv RT \, \Delta c_s/\Delta\mu_s^c$

When current, volume flow, and active transport (U) are zero, the equation for J_s is the familiar permeability equation from Fick's law. When P_E and ϵ are zero, the current equation is the chord conductance equation (compare Ohm's law, 12). When $\Delta\mu_s$, P_E, and V are zero, the Starling equation for volume flow results. Thus, the limiting case of vanishing interactions takes equations (4) into the classical equations used in transport studies.

Measurement of Parameters

The measurement of the coefficients in equations (4) involving active transport is straightforward. For example, U would be the ratio J_s/J_r measured when $\Delta\mu_s^c$, I, and J_v are zero. However, J_r cannot be set to zero independent of other effects and the remaining coefficients must be measured without interfering with J_r or A_r. A solution is suggested by the common definition of resistance as the open-circuit potential divided by the short-circuit current. From equations (4) it is seen that both these quantities involve the simultaneous metabolic rate. If the measurements of both open-circuit potential and short-circuit current are corrected by the corresponding metabolic rates, the resistance (or $1/\kappa$) is obtained. Thus appropriate choice of measurements, always including the metabolic rate, made under different sets of restrictions, should yield the desired coefficients.

4 sets of experimental conditions, with a total of 14 measurements, give all 10 coefficients.

A. ZERO VOLUME FLOW, OPEN CIRCUIT In this condition I, $\Delta\mu_s^c$, and J_v are all held at zero; E, J_s, $(\Delta p - \Delta\pi)$, J_r, and A_r are measured.

$$\frac{\epsilon}{\kappa} = -\left|\frac{E}{J_r}\right|_{I,\Delta\mu_s^c,J_r} \qquad U = \left|\frac{J_s}{J_r}\right|_{I,\Delta\mu_s^c,J_v}$$

$$\frac{V}{L_p} = -\left|\frac{\Delta p - \Delta\pi}{J_r}\right|_{I,\Delta\mu_s^c,J_v} \qquad k = \left|\frac{J_r}{A_r}\right|_{I,\Delta\mu_s^c,J_v}$$

B. ZERO VOLUME FLOW, SHORT CIRCUIT In this condition E, $\Delta\mu_s^c$, and J_v are zero; I, J_s, $(\Delta p - \Delta\pi)$, and J_r are measured.

$$\epsilon = \left|\frac{I}{J_r}\right|_{E,\Delta\mu_s^c,J_v} \qquad \frac{\tau_1\,\epsilon}{\nu_1\,z_1\,F} + U = \left|\frac{J_s}{J_r}\right|_{E,\Delta\mu_s^c,J_v}$$

$$-\frac{\epsilon P_E}{\kappa} + \frac{V}{L_p} = -\left|\frac{\Delta p - \Delta\pi}{J_r}\right|_{E,\Delta\mu_s^c,J_v}$$

C. ZERO SALT FLOW, OPEN CIRCUIT In this condition, I, J_v, and J_s are zero; $\Delta\mu_s^c$, $\Delta p - \Delta\pi$, and J_r are measured.

$$-\frac{U}{\omega c_s} = \left|\frac{\Delta\mu_s^c}{J_r}\right|_{I,J_v,J_s} \qquad \frac{V}{L_p} - \frac{Uc_s(1-\sigma)}{\omega c_s} = -\left|\frac{\Delta p - \Delta\pi}{J_r}\right|_{I,J_v,J_s}$$

This condition will be automatically reached when the stationary state between the bulk solutions is achieved. Then all fluxes corresponding to nonfixed forces vanish (see deGroot, 13, p. 200). Experimentally, it may be more convenient to make measurements of J_s at several $\Delta\mu_s^c$ and interpolate, since the stationary state may not be reached rapidly. The c_s's, though appearing in both numerator and denominator, are retained since the measured quantities are ωc_s and $(1 - \sigma)\, c_s$.

D. ZERO PRESSURE, OPEN CIRCUIT In this condition, $\Delta\mu_s^c$, $(\Delta p - \Delta\pi)$, and I are zero; J_v and J_r are measured.

$$V = \left|\frac{J_v}{J_r}\right|_{\Delta\mu_s^c, (\Delta p - \Delta\pi), I}$$

By simple algebra the membrane parameters can be obtained. Note that no further assumptions beyond the original ones of linearity have been necessary. It is seen that, for example, the conductance is obtained by

$$\kappa = -\left|\frac{I}{J_r}\right|_{E, \Delta\mu_s^c, J_v} \bigg/ \left|\frac{E}{J_r}\right|_{I, \Delta\mu_s^c, J_v}$$

or is the "short-circuit current" divided by the "open-circuit potential," the whole corrected by the ratio of metabolic rates.

The parameters above have been defined so as to correspond to general usage. However, we can, along with Kedem and Katchalsky (2), define an alternative set with $(\Delta p - \Delta\pi)$ as an independent variable rather than J_v. These parameters (denoted by primes) and their evaluation are presented below for the bi-ionic case and can be easily reduced to the single salt case.

Two points of potential ambiguity should be mentioned. The first point is that the dissipation function (equation 2) given above is an approximation. However, the dissipation function may be made exact by defining a new variable $\Delta\mu_x^c$ as

$$\Delta\mu_x^c = \Delta\mu_s^c\left(1 + \frac{\Delta\pi}{\Delta\pi_s}\frac{\theta_s}{\theta_w}\right)$$

and using it in place of $\Delta\mu_s^c$. θ_s and θ_w are the volume fractions of solute and water respectively. Problems may arise in "translating" coefficients if this approximation is ignored. The second point is that the definition of J_v is $J_s\bar{v}_s + J_w\bar{v}_w$ and not $J_1\bar{v}_1 + J_2\bar{v}_2 + J_w\bar{v}_w$. This leads to no ambiguities in the further theoretical development. However, among the practical coefficients, V for example, can be expressed as a function of just U and not ϵ when there is no primary active transport of water. The present definition of J_v as used in equation (2) in most circumstances closely approximates the exact definition.

Determination of Primary Active Transport

The practical coefficients used above have the advantage of being simply and directly measurable. However, molecular transport models are usually not in the same form. Instead, models are most conveniently approached from the molar flux equations, based on dissipation function (1) rather than on (2).

$$\begin{aligned}
\Delta\tilde{\mu}_1 &= R_{11}J_1 + R_{12}J_2 + R_{1w}J_w + R_{1r}J_r \\
\Delta\tilde{\mu}_2 &= R_{21}J_1 + R_{22}J_2 + R_{2w}J_w + R_{2r}J_r \\
\Delta\tilde{\mu}_w &= R_{w1}J_1 + R_{w2}J_2 + R_{ww}J_w + R_{wr}J_r \\
A_r &= R_{r1}J_1 + R_{r2}J_2 + R_{rw}J_w + R_{rr}J_r
\end{aligned} \tag{5}$$

These coefficients will be called molar coefficients to distinguish them from the subsidiary coefficients of equations (3) and from the practical coefficients of Equation (4). Kedem defined active transport in terms of the molar coefficients R_{1r}, R_{2r}, R_{wr}.

Primary active transport of a substance is defined as the ability to generate a gradient of that substance with no fluxes except that due to the driving metabolic reaction. Primary active transport of ion 1 means

$$\left|\frac{\Delta\tilde{\mu}_1}{J_r}\right|_{J_1, J_2, J_w} = R_{1r} \neq 0$$

Similar definitions can be given for primary active transport of ion 2 or of water. Entrainment between transport fluxes will mean that a given substance may move against its conjugate gradient without being directly entrained by the pump mechanism. Such a case can be considered *secondary* active transport; it is expressed by the ordinary cross-coefficients which do not involve metabolism. However, the observed practical coefficients—U, V, ϵ—cannot be given simple molecular interpretations. Thus, the problem is to define R_{1r}, R_{2r}, and R_{wr} in terms of ϵ, U, V, so that direct experiments can be used to test for primary active transport.

If R_{1r}, R_{2r}, or R_{wr} vanishes, one of the practical coefficients from equations (4) can be eliminated. This dependence among the measured quantities is the experimental sign of the lack of primary active transport.

The molar coefficients can be defined directly in terms of the practical coefficients:

$$R_{1r} = -\frac{z_1 F\epsilon}{\kappa} - \frac{V\bar{v}_s}{\nu_1 L_p} - \frac{\left(1 + \frac{\theta_s}{\theta_w}\right)}{\nu_1 c_s \omega} U + \frac{\tau_1 U}{\nu_1 c_s \omega} + U \frac{\bar{v}_s(1 - \sigma)c_s}{\nu_1 c_s \omega} \tag{6 a}$$

$$R_{2r} = \frac{z_2 F\epsilon}{\kappa} - \frac{U\tau_1}{\nu_2 c_s \omega} \tag{6 b}$$

$$R_{wr} = -\frac{V\bar{v}_w}{L_p} + U \frac{(1 - \sigma)c_s \bar{v}_w}{c_s \omega} \tag{6 c}$$

The practical consequences of the various cases can now be examined.

1. If there is no primary active transport of *water*, $R_{rw} = 0$, and

$$V = \frac{U}{\omega c_s}(1 - \sigma)c_s L_p \tag{7}$$

2. If there is no primary active transport of *ion (2)*, $R_{2r} = 0$, and

$$\epsilon = \frac{U}{\omega c_s} \frac{\tau_1 \kappa}{\nu_1 z_1 F} \tag{8}$$

3. If there is no primary active transport of *ion* (*1*), $R_{1r} = 0$, and

$$\epsilon = \frac{U}{\omega c_s} \frac{(\tau_1 - 1)\kappa}{\nu_1 z_1 F} + \frac{\bar{v}_s \kappa}{\nu_1 z_1 F} \left[U \frac{(1 - \sigma) c_s}{c_s \omega} - \frac{V}{L_p} \right] \tag{9}$$

There are certain interactions. If neither ion (1) nor ion (2) is actively transported,

$$U = \frac{V \bar{v}_s \, \omega c_s}{L_p[\bar{v}_s \, c_s(1 - \sigma) - 1]} \quad \text{and} \quad \epsilon = \frac{\tau_1 \kappa V \bar{v}_s}{v_1 z_1 F L_p[\bar{v}_s \, c_s(1 - \sigma) - 1]}$$

If none of the three is actively transported, V, ϵ, and U are obviously all zero and equations (4) assume the form given by Kedem and Katchalsky (Equations (46) in reference 2).

There are thermodynamic limitations on the sizes of the coefficients. Thus, in order for the entropy production to be positive, all cross-coefficients have maximum sizes such that (see reference 6, p. 46)

$$R_{jk}^2 \leqq R_{jj} R_{kk}$$

When $\Delta\mu_s^c$, J_v, and A_r are zero, Φ must be positive. The necessary consequence for the coefficients is a maximum size for ϵ

$$\epsilon^2 \leqq \frac{\kappa}{k} \tag{10}$$

The condition $\Delta\mu_s^c$, I, and A_r, all zero, indicates that

$$V^2 \leqq \frac{L_p}{k} \tag{11}$$

Although these restrictions were uncovered by examining special cases, they must hold in general for the linear region. No such relation holds for U in the form of equations used.

Relations (10) and (11) suggest a possible index of tightness of coupling of the pumping mechanism.

$$\eta_\epsilon = \text{electrogenic coupling coefficient} = \epsilon\sqrt{k/\kappa} \tag{12}$$

$$\eta_v = \text{volume pumping coefficient} = V\sqrt{k/L_p} \tag{13}$$

These quantities should be independent of the flows and forces and therefore they have unambiguous definitions. They can also be translated into terms of a specific model.

Electrogenic Effect of Active Transport

The nature of the mechanism whereby living tissues can generate electric potential or current (i.e., electrogenic action) has been the subject of many experimental studies. In general the basis for interpretation of such studies has been thus far rather uncertain. However, the present formulation makes it possible to proceed with a theoretical analysis of electrogenesis and to define criteria for the nature of the coupling between the production of electricity and the metabolic energy source.

TABLE I

DEFINITIONS OF ELECTROGENICITY

Electricity	Salt	Volume	Criterion
I	$\Delta\mu_s^c$	J_v	$\epsilon \neq 0$
E	$\Delta\mu_s^c$	J_v	
I	J_s	J_v	$\frac{\tau_1^r U}{\omega c_s} - \frac{\epsilon}{\kappa} \neq 0$
E	J_s	J_v	
I	$\Delta\mu_s^c$	$\Delta p - \Delta\pi$	$\epsilon' \neq 0$
E	$\Delta\mu_s^c$	$\Delta p - \Delta\pi$	
I	J_s	$\Delta p - \Delta\pi$	$\frac{\tau_1^{r\prime} U}{\omega c_s} - \frac{\epsilon'}{\kappa'} + \frac{c_s(1-\sigma)}{\omega c_s}\left\{\tau_1^r V - L_p c_s(1-\sigma)\frac{\epsilon}{\kappa}\right\} \neq 0$
E	J_s	$\Delta p - \Delta\pi$	$\frac{\tau_1^{r\prime} U}{\omega c_s} - \frac{\epsilon'}{\kappa'} \neq 0$

Two manners of looking at transport systems may be adopted in discussions of electrogenicity. On the one hand, we have the strictly phenomenological question of whether a membrane system can produce either an electric current or a potential difference, with several variations according to the restrictions imposed. On the other hand, there are the intuitive notions concerning the sort of coupling involved in a molecular model, where nonelectrogenicity is felt to arise from strict ion coupling. These two ways of using the word "electrogenicity" can lead to confusion, for there is not a simple correspondence between the two, in general.

Table I summarizes the simplest phenomenological definitions of electrogenicity. The entries in the first three columns are the quantities held at zero in order to define the conditions. If either a current in a zero potential case or a potential difference in a zero current case occurs, the membrane can be said to be electrogenic in that sense. The entries in the last column give the criteria for the kinds of phenomenological electrogenicity in terms of practical coefficients.

In terms of specific relations between the phenomenological coefficients, the number of possible ways in which metabolic entrainment could generate electric potentials and current is enormous because of the various combinations which are possible. However, from an intuitive standpoint we may distinguish three classes of mechanisms according to how closely metabolism is coupled to the transport of electric charge.

1. Primary electrogenesis is the case in which metabolism exerts an unequal force on anion and cation. From the molar phenomenological equations, it is readily shown for the single salt case that if metabolic entrainment of anion and cation is finite and not equal, i.e., $R_{1r} \neq R_{2r}$, $\Delta\phi$ cannot be zero when all transport fluxes are zero. Conversely, neutral salt primary active transport simply means $R_{1r} = R_{2r}$.

It is conceivable that although anion-cation metabolic entrainment is unequal, electric potential does not develop with zero ionic and pressure gradients. This would result if the unequal metabolic coupling were exactly offset by a conversely matching friction of anion and cation with water, another solute, or the membrane matrix proper. The net result would be in effect the transport of a neutral salt. This mechanism will be called latent primary electrogenesis. A number of different physical mechanisms could be envisioned which would give the appropriate effect. For example, an uncharged cation-selective carrier operating in a low dielectric medium would result in transport of ion pairs. Thus a membrane which develops neither current nor potential in ordinary circumstances of short circuit or open circuit may be capable of exhibiting a true electrogenic action given the proper circumstances of J_v, I, $J_s = 0$.

2. Secondary electrogenesis is the case in which primary active transport involves neutral salt, water, or nonelectrolyte. An asymmetric anion-cation friction with other solutes, water, or membrane matrix would be responsible for the electrogenic effect which would be seen even in the absence of external concentration or pressure gradients. Thus ϵ' must show a finite value.

3. Tertiary electrogenesis is the case in which a current or potential difference appears only in the presence of an external concentration or pressure gradient imposed by external forces or established by the active transport of neutral salt, water, or nonelectrolyte. Thus $\epsilon' = 0$. This type of electrogenic effect which depends upon the generation of an external ion gradient would appear to be the simplest type and has been used to explain the resting potential of nerve and muscle (14) and of frog skin (15). Strictly speaking, this third type is not "intrinsically" electrogenic since it depends upon the development of an external gradient.

From the classification of electrogenesis outlined above, three types of electroneutral or nonelectrogenic membrane systems with active transport mechanisms may be distinguished. First is the type which is completely nonelectrogenic in all circumstances, even in the presence of concentration and pressure differences. This would encompass all mechanisms not included in the previous classification. Actually such a completely nonelectrogenic membrane would conduct no electric charge and would be a perfect insulator. A second type involving direct metabolic entrainment of only neutral molecules produces a potential with application of concentration or pressure differences. This class is identical with tertiary electrogenesis. Finally, the third class is the latent type of primary electrogenesis.

Tonicity of Transported Fluid

A question of common interest in studies on epithelial membranes such as intestine (16), gall bladder (17), and frog skin (18) is the relative rate of salt and water transport. Using the above equations, an expression for the salt concentration of the transported fluid can be obtained for the "short-circuit case" ($\Delta\mu_s$, $\Delta p - \Delta\pi$, E all zero).

If water is the *only* species with primary active transport,

$$\left|\frac{J_s}{J_v}\right|_{\Delta\bar{\mu}_1,\Delta\bar{\mu}_2,\Delta\mu_w} = \left|c_s(1-\sigma) - \frac{P_E\,\tau_1}{\nu_1\,z_1\,F}\right| \tag{14}$$

Since the reflection coefficient as well as P_E may be of either sign, the transported fluid may be either hypotonic or hypertonic. However, the reflection coefficient is rarely negative (19) and the last term is usually rather small. Therefore, the resultant solution will probably be slightly hypotonic in most instances.

The operational definition of pinocytosis is $(\mathrm{I})_{E,\Delta\mu_s^c,(\Delta p-\Delta\pi)} = 0$ and $(J_s/J_v)_{E,\Delta\mu_c^s(\Delta p-\Delta\pi)} = c_s$. The first restriction means $\epsilon' = 0$ as seen from the previous section and the second means $U' = Vc_s$.

Active Transport of Water

A criterion often used for active water transport (see, for example, Windhager et al. 20) is a nonzero intercept of a plot of volume flow against osmolar flux.

When there is no electrical current and no pressure difference,

$$J_s = J_v\left[\frac{\omega c_s}{L_p\,c_s(1-\sigma)} + c_s(1-\sigma)\right] + J_r\left[U - \frac{\omega c_s\,V}{L_p\,c_s(1-\sigma)}\right] \tag{15}$$

From equation (7), the J_r term vanishes if there is no primary active transport of water. An equivalent finding results from analysis of the bi-ionic case. Consequently, the presence of extra coupling terms does not vitiate the intuitive criterion previously used. However, the presence of series heterogeneitv can complicate the interpretation in molecular terms.

II. BI-IONIC SYSTEM WITH ACTIVE TRANSPORT

The results given above for a single salt can be generalized to the case of two salts with one common ion (taken as the reference for the electrodes) and in the presence of nonpenetrating ions. One new coefficient appears, λ, the salt linkage coefficient. It should be emphasized that λ does not represent just the frictional interaction between the two ions themselves, but involves contributions from the way the ions share the role of carrying current.

The dissipation function is

$$\Phi = J_r A_r = J_1 \Delta\tilde{\mu}_1 + J_2 \Delta\tilde{\mu}_2 + J_3 \Delta\tilde{\mu}_3 + J_w \Delta\mu_w$$

or

$$\Phi \cong J_r A_r + J_v(\Delta p - \Delta\pi) + IE + J_a \Delta\mu_a^c + J_b \Delta\mu_b^c \tag{16}$$

Here ion (3) is the electrode reference.

$$\Delta\tilde{\mu}_a = \nu_1^a \Delta\tilde{\mu}_1 + \nu_3^a \Delta\tilde{\mu}_3\,; \qquad \nu_1^a J_a = J_1\,; \qquad \nu_1^a z_1 = -\nu_3^a z_3$$

$$\Delta\tilde{\mu}_b = \nu_2^b \Delta\tilde{\mu}_2 + \nu_3^b \Delta\tilde{\mu}_3\,; \qquad \nu_2^b J_b = J_2\,; \qquad \nu_2^b z_2 = -\nu_3^b z_3$$

$$J_3 = I/z_3F + \nu_3^a J_a + \nu_3^b J_b$$

$$J_v = \bar{v}_a J_a + \bar{v}_b J_b + \bar{v}_w J_w$$

$$E = \Delta\tilde{\mu}_3 / z_3 F$$

The 15 practical coefficients required are defined in operational terms as follows.—

Straight coefficients:

$$\omega_a\, c_a = \left| \frac{J_a}{\Delta\mu_a^c} \right|_{I, J_v, \Delta\mu_b^c, J_r}$$

$$\omega_b\, c_b = \left| \frac{J_b}{\Delta\mu_b^c} \right|_{I, J_v, \Delta\mu_a^c, J_r}$$

$$L_p = \left| \frac{J_v}{\Delta p - \Delta\pi} \right|_{\Delta\mu_a^c, \Delta\mu_b^c, I, J_r}$$

$$\kappa = \left| \frac{I}{E} \right|_{\Delta\mu_a^c, \Delta\mu_b^c, J_v, J_r}$$

$$k = \left| \frac{J_r}{A_r} \right|_{\Delta\mu_a^c, \Delta\mu_b^c, I, J_v}$$

Entrainment coefficients:

$$\frac{\tau_1}{\nu_1^a\, z_1\, F} = \left|\frac{J_a}{I}\right|_{\Delta\mu_a^c,\Delta\mu_b^c,J_v,J_r} = -\left|\frac{E}{\Delta\mu_a^c}\right|_{\Delta\mu_b^c,I,J_v,J_r}$$

$$c_a(1-\sigma_a) = \left|\frac{J_a}{J_v}\right|_{\Delta\mu_a^c,\Delta\mu_b^c,I,J_r} = -\left|\frac{\Delta p - \Delta\pi}{\Delta\mu_a^c}\right|_{\Delta\mu_b^c,I,J_v,J_r}$$

$$U_a = \left|\frac{J_a}{J_r}\right|_{\Delta\mu_a^c,\Delta\mu_b^c,I,J_v} = -\left|\frac{A_r}{\Delta\mu_a^c}\right|_{\Delta\mu_b^c,I,J_v,J_r}$$

Three analogous coefficients for the salt *b* are defined in the same way.

$$P_E = -\left|\frac{I}{J_v}\right|_{\Delta\mu_a^c,\Delta\mu_b^c,E,J_r} = \left|\frac{\Delta p - \Delta\pi}{E}\right|_{\Delta\mu_a^c,\Delta\mu_b^c,J_v,J_r}$$

$$\epsilon = \left|\frac{I}{J_r}\right|_{\Delta\mu_a^c,\Delta\mu_b^c,E,J_v} = -\left|\frac{A_r}{E}\right|_{\Delta\mu_a^c,\Delta\mu_b^c,J_v,J_r}$$

$$V = \left|\frac{J_v}{J_r}\right|_{\Delta\mu_a^c,\Delta\mu_b^c,I,(\Delta p-\Delta\pi)} = -\left|\frac{A_r}{\Delta p - \Delta\pi}\right|_{\Delta\mu_a^c,\Delta\mu_b^c,J_r,I}$$

$$\lambda_{ab}\, c_a\, c_b = \left|\frac{J_a}{\Delta\mu_b^c}\right|_{\Delta\mu_a^c,I,J_v,J_r} = \left|\frac{J_b}{\Delta\mu_a^c}\right|_{\Delta\mu_b^c,I,J_v,J_r}$$

The last coefficient, $\lambda_{ab}c_ac_b$, is called the *salt linkage* coefficient. It is subject to the restriction from the second law

$$(\lambda_{ab}c_ac_b)^2 \leqq \omega_ac_a\omega_bc_b$$

Practical Equations—Bi-Ionic Case

The practical equations are obtained by the same methods used to obtain equation (4).

$$J_v = L_p(\Delta p - \Delta\pi) - \frac{P_E\, L_p}{\kappa} I + L_p\, c_a(1-\sigma_a)\Delta\mu_a^c + L_p\, c_b(1-\sigma_b)\Delta\mu_b^c + VJ_r$$

$$I = -P_E\, J_v + \kappa E + \frac{\kappa\tau_1}{\nu_1\, z_1 F}\Delta\mu_a^c + \frac{\kappa\tau_2}{\nu_2\, z_2 F}\Delta\mu_b^c + \epsilon J_r$$

$$J_a = c_a(1-\sigma_a)J_v + \frac{\tau_1}{\nu_1\, z_1 F} I + \omega_a\, c_a\, \Delta\mu_a^c + \lambda_{ab}\, c_a\, c_b\, \Delta\mu_b^c + U_a\, J_r \qquad (17)$$

$$J_b = c_b(1-\sigma_b)J_v + \frac{\tau_2}{\nu_2\, z_2 F} I + \lambda_{ab}\, c_a\, c_b\, \Delta\mu_a^c + \omega_b\, c_b\, \Delta\mu_b^c + U_b\, J_r$$

$$J_r = \frac{kV}{L_p} J_v + \frac{k\epsilon}{\kappa} I + kU_a\, \Delta\mu_a^c + kU_b\, \Delta\mu_b^c + kA_r$$

The parameters for the bi-ionic case can be evaluated in a manner exactly similar to that above, replacing condition C, given for the single salt case with condition E, in which J_v, I, J_a, and $\Delta\mu_b^c$ are all zero and condition F, in which J_v, I, J_b, and $\Delta\mu_a^c$ are all zero.

Measurements with Zero Pressure Gradient

An alternative formulation given by Kedem and Katchalsky (2) uses $(\Delta p - \Delta\pi)$ as the independent variable rather than J_v. Then the practical equations become the following:

$$
\begin{aligned}
J_v &= L_p(\Delta p - \Delta\pi) + \beta I + L_p c_a(1 - \sigma_a)\Delta\mu_a^c + L_p c_b(1 - \sigma_b)\Delta\mu_b^c + VJ_r \\
I &= \kappa'\beta(\Delta p - \Delta\pi) + \kappa' E + \frac{\kappa'\tau_1'}{\nu_1 z_1 F}\Delta\mu_a^c + \frac{\kappa'\tau_2'}{\nu_2 z_2 F}\Delta\mu_b^c + \epsilon' J_r \\
J_a &= c_a(1 - \sigma_a)L_p(\Delta p - \Delta\pi) + \frac{\tau_1'}{\nu_1 z_1 F} I + \omega_a' c_a \Delta\mu_a^c \\
&\qquad + \lambda_{ab}' c_a c_b \Delta\mu_b^c + U_a' J_r \qquad (18) \\
J_b &= c_b(1 - \sigma_b)L_p(\Delta p - \Delta\pi) + \frac{\tau_2'}{z_2 \nu_2 F} I + \lambda_{ab}' c_a c_b \Delta\mu_a^c \\
&\qquad + \omega_b' c_b \Delta\mu_b^c + U_b' J_r \\
J_r &= k'V(\Delta p - \Delta\pi) + \frac{k'\epsilon'}{\kappa'} I + k'U_a' \Delta\mu_a^c + k'U_b' \Delta\mu_b^c + k'A_r
\end{aligned}
$$

Here, unprimed quantities are defined for $J_v = 0$ the same as those in equations (17). Primed quantities are defined for $(\Delta p - \Delta\pi) = 0$ and must be carefully distinguished. In this paper mainly unprimed quantities (for zero volume flow) are used. However, all active transport criteria, etc., can be translated from one nomenclature to the other by the following relations.

$$
\beta = -P_E L_p/\kappa; \qquad \kappa' = \kappa/(1 + P_E\beta); \qquad \epsilon' = (\epsilon - P_E V)/(1 + P_E\beta);
$$

$$
k' = k/(1 - kV^2/L_p); \qquad \lambda_{ab}' c_a c_b = \lambda_{ab} c_a c_b + L_p c_a c_b(1 - \sigma_a)(1 - \sigma_b);
$$

$$
\frac{\tau_1'}{\nu_1 z_1 F} = \frac{\tau_1}{\nu_1 z_1 F} + \beta c_a(1 - \sigma_a); \qquad \omega_a' c_a = \omega_a c_a + L_p c_a^2(1 - \sigma_a)^2;
$$

$$
U_a' = U_a + Vc_a(1 - \sigma_a).
$$

Coefficients analogous to the last three are defined for the salt b in a similar way. Note that the two sets of coefficients partially merge under certain conditions, such as $P_E = 0$, $(1 - \sigma_a) = 0$, $(1 - \sigma_b) = 0$, etc.

Now, the evaluation of the primed quantities proceeds exactly parallel to that for the unprimed quantities. Here one has, besides conditions (A) and (D) from above,

condition (G): $\Delta p - \Delta\pi$, E, $\Delta\mu_a^c$, $\Delta\mu_b^c$, all zero
condition (H): $\Delta p - \Delta\pi$, I, J_a, $\Delta\mu_b^c$, all zero
condition (I): $\Delta p - \Delta\pi$, I, J_b, $\Delta\mu_w^c$, all zero.

At first thought the zero pressure condition may appear to be easier to impose compared to the zero volume flow condition. However, there is little difference in difficulty of measurement between $(J_v)_{(\Delta p - \Delta\pi)}$ compared to $(\Delta p - \Delta\pi)_{J_v}$ if one is to control solution composition or solute flow at the same time.

Some of the single salt active transport criteria appear simpler when expressed in terms of the zero pressure (primed) coefficients. Thus, if there is no water primary active transport,

$$V = \frac{L_p c_s(1 - \sigma_s)U'}{\omega' c_s} \tag{19}$$

The open-circuit potential is now

$$(E)_{\Delta\mu_s^c,(\Delta p - \Delta\pi),I} = -\frac{\epsilon'}{\kappa'}(J_r)_{\Delta\mu_s^c,\Delta p - \Delta\pi,I} \tag{20}$$

Bi-Ionic Active Transport Criteria

From an extension of the system of equations (5), we can determine the consequences of zero values of R_{1r}, R_{2r}, R_{3r}, and R_{wr}. For example, we examine the situation when J_1, J_2, J_3, and J_w are all zero. If R_{wr} is zero, then $\Delta\mu_w = (\Delta p - \Delta\pi)/c_w$ is also zero. From applying these values to equations (17) we find the consequence in terms of a dependence among the practical coefficients.

The relations between the practical coefficients turn out to be quite involved. The number of variables involved is so large that the required precision for a meaningful test is beyond that attainable in the usual biological experiment. However, the condition of zero transport flows as mentioned above in the case of water, leads to the prediction that if one metabolic entrainment coefficient (e.g. R_{wr}) is zero, the corresponding force ($\Delta\mu_w$) must also be zero. Thus, to test whether R_{wr} is finite, we measure $(\Delta p - \Delta\pi)$ with zero transport flows. If $(\Delta p - \Delta\pi)_{J_a,J_b,I,J_v} = 0$, then we conclude that there is no active transport of water. An equivalent operational measurement is $(J_v)_{J_a,J_b,I,(\Delta p - \Delta\pi)} = 0$. In practice, the procedure of plotting J_v against J_s discussed above would be used. Similarly, if R_{3r} is zero,

$$(E)_{J_aJ_bIJ_v} = 0 \quad \text{and} \quad (I)_{J_aJ_bJ_vE} = 0$$

If R_{1r} is zero, $(\Delta\tilde{\mu}_1)_{J_a,J_b,I,J_v}$ must be zero and therefore

$$(\Delta\mu_a^c)_{J_aJ_bIJ_v} + \nu_1 z_1 F(E)_{J_aJ_bIJ_v} + \bar{v}_w(\Delta p - \Delta\pi)_{J_aJ_bIJ_v} = 0$$

These relations are summarized in Table II together with the corresponding results for the cases in which only one substance is actively transported. The relations (between the practical coefficients) which result from the latter conditions are quite complex and are not given. Of course, when no ions or water is actively transported, U_a, U_b, ϵ, and V are all zero and the equations describe the bi-ionic case for a passive membrane.

TABLE II

TESTS OF ACTIVE TRANSPORT

Condition of active transport (A.T.)	Zero entrainment Coefficients	Operational Tests
1. No A.T. of ion 1	$R_{1r} = 0$	$(\Delta\mu_a^c)_{J_j} + \nu_1 z_1 F(E)_{J_j} + \bar{v}_w(\Delta p - \Delta\pi)_{J_j} = 0$
2. No A.T. of ion 3	$R_{3r} = 0$	$(E)_{J_j} = 0$ or $(I)_{J_a J_b J_{vE}} = 0$
3. No A.T. of water	$R_{wr} = 0$	$(\Delta p - \Delta\pi)_{J_j} = 0$ or $(J_v)_{J_a J_b I(\Delta p - \Delta\pi)} = 0$
4. A.T. of ion 1 only	R_{2r} $R_{3r} = 0$ R_{wr}	$(\Delta p - \Delta\pi')_{J_j} = 0$ $(E)_{J_j} = 0 \quad (\Delta\mu_a^c)_{J_j} \neq 0$ $(\Delta\mu_b^c)_{J_j} = 0$
5. A.T. of ion 3 only	R_{1r} $R_{2r} = 0$ R_{wr}	$(\Delta p - \Delta\pi)_{J_j} = 0$ $(\Delta\mu_a^c)_{J_j} = -\nu_1 z_1 F(E)_{J_j} \neq 0$ $(\Delta\mu_b^c)_{J_j} = -\nu_1 z_1 F(E)_{J_j} \neq 0$
6. A.T. of water only	R_{1r} $R_{2r} = 0$ R_{3r}	$(\Delta p - \Delta\pi)_{J_j} \neq 0 \quad (E)_{J_j} = 0$ $(\Delta\mu_a^c)_{J_j} = -\bar{v}(\Delta p - \Delta\pi)_{J_j}$ $(\Delta\mu_b^c)_{J_j} = -\bar{v}(\Delta p - \Delta\pi)_{J_j}$

Note: J_j refers to J_a, J_b, I, J_v all kept at zero.

Electrogenesis and Ion Transport Coupling

The definitions of electrogenesis given earlier are applicable to the simple membrane with active transport in the bi-ionic case. The phenomenological tests remain the same. However, the required relationships between the phenomenological coefficients are involved and will not be presented. Instead we examine the same relationships in terms of their effect on the coupling ratio between the ions. Such ratios, for example, can be easily obtained in terms of Onsager coefficients analogous to those in equations (5).

Two types of coupling ratios may be distinguished. The degree of coupling due to direct metabolic entrainment itself would be the ratio of the appropriate molar metabolic entrainment coefficients. The coupling ratios will be represented by the symbol ρ_{12} :

$$\rho_{12(\text{direct})} = \left| \frac{\Delta\tilde{\mu}_1}{\Delta\tilde{\mu}_2} \right|_{J_1 J_2 J_3 J_w} = \frac{R_{1r}}{R_{2r}} \tag{21}$$

The comparative effect of active transport on two ions as an over-all process will be called the effective coupling ratio and defined as the ratio of the fluxes during complete short circuit (i.e., $\Delta\tilde{\mu}_j = 0$ for all j):

$$\rho_{12(\text{effective})} = \left|\frac{J_1}{J_2}\right|_{\Delta\tilde{\mu}_1\Delta\tilde{\mu}_2\Delta\tilde{\mu}_3\Delta\tilde{\mu}_\omega} = \frac{L_{1r}}{L_{2r}} \tag{22}$$

However, inasmuch as the R's and L's in these expressions are complicated combinations of the practical coefficients, it is preferable to take a shortcut to look at the case of greatest interest, a "one-to-one" coupling; however, any value of ρ_{12} can be experimentally uncovered by going through the straightforward algebra involved.

In the case of the effective ratio, equation (22), the shortcut consists of placing the restrictions of zero $\Delta\tilde{\mu}_1$, $\Delta\tilde{\mu}_2$, $\Delta\tilde{\mu}_3$, and $\Delta\mu_\omega$ on the practical equations (18) by means of the definitions of these quantities. Then, we find the simultaneous values of J_1 and J_2 through their definitions.

$$J_1 = \nu_1\left(\frac{\tau_1'\epsilon'}{\nu_1 z_1 F} + U_a'\right)(J_r)_{\Delta\tilde{\mu}_1\Delta\tilde{\mu}_2\Delta\tilde{\mu}_3\Delta\tilde{\mu}_\omega}$$

$$J_2 = \nu_2\left(\frac{\tau_2'\epsilon'}{\nu_2 z_2 F} + U_b'\right)(J_r)_{\Delta\tilde{\mu}_1\Delta\tilde{\mu}_2\Delta\tilde{\mu}_3\Delta\tilde{\mu}_\omega}$$

Then,

$$\rho_{12(\text{effective})} = \frac{\nu_1\left(\dfrac{\tau_1'\epsilon'}{\nu_1 z_1 F} + U_a'\right)}{\nu_2\left(\dfrac{\tau_2'\epsilon'}{\nu_2 z_2 F} + U_b'\right)} \tag{23}$$

For the case in which the pump fluxes of (1) and (2) carry equal amounts of charge in opposite directions,

$$\epsilon' = -\frac{U_a' + U_b'}{\dfrac{\tau_1'}{\nu_1 z_1 F} + \dfrac{\tau_2'}{\nu_2 z_2 F}} \tag{24}$$

In a similar fashion ρ_{13} and ρ_{23} can be evaluated.

Analogous expressions for the direct coupling ratio are very involved. Direct evaluation of the ratio defined in equation (21) would be necessary.

Nonelectrolyte—Electrolyte Interaction

Problems such as glucose-sodium interaction in the gut can be handled by a simple extension. In the dissipation function we replace $J_b\Delta\mu_b^c$ by $J_u\Delta\mu_u^c$ (for "uncharged") and drop z_2FJ_2 from the current definition. Given a nonelectrolyte which in any way associates with ions it is quite plainly true that current flow with all other gradients (except pressure) and volume flow at zero can cause a flow of an uncharged species. In this sense, the reduced transference number (11) of the nonelectrolyte, τ_u^r, cannot reasonably be set at zero ($\tau_u^r = (J_u/I) \neq 0$). Of course, the transference number defined as $(zFJ_s/I) = \tau$ is always zero for a nonelectrolyte. Thus we replace $\tau_2/\nu_2 z_2 F$ in the bi-ionic equations with τ_u^r. As one consequence, we might point out that

$$(E)_{IJ_vJ_r} = -\left(\frac{\tau_1}{\nu_1 z_1 F}\Delta\mu_a^c + \tau_u^r\,\Delta\mu_u^c\right)$$

In other words, the membrane potential may be a function of the concentration gradient of nonelectrolyte. The change in membrane potential with glucose concentration was recently investigated by Barry et al. (21). The series system of the intestine complicates the matter a bit in that the potential difference is sensitive to the glucose concentration on one surface only.

Flux Ratio Equation for Actively Transported Salt

Starting from equation (17) and using a very straightforward extension of the procedure used by Hoshiko and Lindley (equations 21 and 22 in reference 22), we arrive at the expression

$$RT\ln\left(-\frac{J_a}{J_b}\right) = \frac{1}{c\omega - c^2\lambda}\left[c(1-\sigma)J_v + \frac{\tau\kappa'}{\nu_1 z_1 F}E + \left(U + \frac{\tau\epsilon'}{\nu_1 z_1 F}\right)J_r\right] \quad (25)$$

where a and b now refer to two isotopic species. Algebraic elimination of J_v for the case $(\Delta p - \Delta\pi) = 0$ results in

$$RT\ln\left(-\frac{J_a}{J_b}\right) = \frac{1}{c\omega - c^2\lambda}\left[\frac{\tau_1'\kappa'}{\nu_1 z_1 F}E + \left(U' + \frac{\tau_1'\epsilon'}{\nu_1 z_1 F}\right)J_r\right] \quad (26)$$

In the present case, it should be noted that the quantity $(c\omega - c^2\lambda)$ does not approximate $c\omega$. Unless the membrane is nearly impermeable to one ion, $c^2\lambda$ cannot be assumed to be small. It may also be noted that the volume pump coefficient, V, does not appear in this expression. The reason is probably related to the definition of J_v which does not include the contribution of J_3 except in the open-circuit case. However, it may be noted that when only water is actively transported, both U and ϵ can be expressed as functions of V.

When the membrane is completely short-circuited and E is zero, equation (26) when divided by zF becomes the "active transport potential" or "E_{Na}" defined by Ussing and Zerahn (23).

$$z_1 FE_{\text{Na}} = RT\ln\left|-\frac{J_a}{J_b}\right| = \frac{U' + \dfrac{\tau_1'\epsilon'}{\nu_1 z_1 F}}{c\omega - c^2\lambda}(J_r)_{\Delta\mu_s^c, E, \Delta p - \Delta\pi}$$

It is difficult to attempt a comparison of this expression with the open-circuit potential of equation (20). However, it is readily seen that neither expression gives a unique membrane parameter—each incorporates a metabolic flux. A change in E_{Na} does not necessarily mean an effect on the active transport mechanism. Therefore the conclusion that pituitrin does not affect the active transport mechanism of frog skin (23) cannot be made on the basis of a lack of effect on E_{Na}. Actually skin oxygen consumption increases on exposure to vasopressin. If E_{Na} remains constant, some membrane parameter must have changed. The problem is actually more complex since the skin represents at the very least two membranes in series (15).

"*Pump-Leak*" *Mosaic Membrane*

As pointed out by Kedem and Katchalsky (24), parallel elements combine Onsager coefficients (conductance formulation) in a simple fashion; however, a given over-all practical coefficient is not a simple weighted mean of those for the constituent elements.

Erythrocyte electrolyte transport has been discussed in terms of parallel pump and leak fluxes (see Tosteson, 25). The present treatment can be readily extended to such a formulation, and the form of the practical equations will be invariant. We consider only the extreme case in which the pump portion of the membrane has no passive permeability properties.

$$R_{kj}^{P} = 0, \qquad j \neq r.$$

Also, we assume that the leak portion of the membrane has no active transport properties.

$$R_{kj}^{L} = 0, \qquad j = r.$$

This being the case, none of the leak membrane coefficients will depend on the pump parameters. We can proceed to evaluate the coefficients simply by adding the fluxes of the two parts and establishing the new parameters in terms of those of the parts. It is found that only the salt pump, electrogenicity, and volume pump coefficients of the over-all system differ from the sum of those of the constituents. We have over-all coefficients (asterisk).

$$\begin{aligned} U^* &= U^P - \frac{\tau_1}{\nu_1 z_1 F}\,\epsilon^P - c_s(1 - \sigma)V^P \\ \epsilon^* &= \epsilon^P + P_E V^P \qquad (27) \\ V^* &= V^P + \frac{P_E L_p}{\kappa}\,\epsilon^P \end{aligned}$$

This represents the simplest possible mosaic membrane combination of pump and leak elements; with more complicated systems all coefficients would start to intertwine. Nonetheless, under a model hypothesis it is apparently possible to separate out the contributions from the phenomenological elements. The generalization from equations (27) to the bi-ionic case is straightforward.

Series Systems—Epithelial Membranes

In recent years, considerable attention has been paid to nonlinear properties of series systems of linear membranes (26, 27). These systems are especially interesting as models for epithelial membranes. Kedem and Katchalsky point out many of the difficulties involved in producing a general description in even the case of a single salt; these apply *a fortiori* in the present cases.

An additional complication in the present instance is that although the membranes are in series, the metabolic component is, in effect, in parallel. Thus, with two membranes, α and β, in series, each over-all transport force will be the sum of the forces across each membrane.

$$X^{\alpha\beta} = X^{\alpha} + X^{\beta}$$

The transport flux in the steady state will be the same across both membranes. However, the over-all metabolic rate will be the sum of the metabolic rates in the two membranes. Consequently, although in a passive system, the over-all resistance coefficient is the sum of those across each membrane, in the active transport system, such a simple relationship does not exist. In fact the general definition of any given over-all coefficient is a function of the metabolic flux or affinity. However, we may as an approximation, assume that the affinities in the two membranes are the same. If we consider the affinities to be proportional to the partial pressure of oxygen or the substrate concentration in the bathing medium, the assumption may be reasonable. With this assumption of $A^{\alpha} = A^{\beta}$, the over-all resistance definitions are

$$R_{rr}^{\alpha\beta} = R_{rr}^{\alpha} R_{rr}^{\beta} / (R_{rr}^{\alpha} + R_{rr}^{\beta}) \tag{28 a}$$

$$R_{ri}^{\alpha\beta} = (R_{ri}^{\alpha} R_{rr}^{\beta} + R_{ri}^{\beta} R_{rr}^{\alpha}) / (R_{rr}^{\alpha} + R_{rr}^{\beta}) = R_{ir}^{\alpha\beta} \tag{28 b}$$

$$R_{ij}^{\alpha\beta} = R_{ij}^{\alpha} + R_{ij}^{\beta} - \frac{R_{ir}^{\alpha}(R_{rj}^{\alpha} - R_{rj}^{\beta}) + R_{ir}^{\beta}(R_{rj}^{\beta} - R_{rj}^{\alpha})}{R_{rr}^{\alpha} + R_{rr}^{\beta}} = R_{ji}^{\alpha\beta} \tag{28 c}$$

Examination shows that the over-all metabolic entrainment coefficient, $R_{ir}^{\alpha\beta}$, will be zero if the two component coefficients, R_{ir}^{α} and R_{ir}^{β}, are each zero. Thus, the practical tests for the simple membrane can be applied directly to test for the metabolic entrainment of the three ions and water.

Consistency Checks

It is considerably more difficult to measure metabolic rates in most commonly studied transport systems than it is to measure the other fluxes. Although a complete characterization, or even the isolation of any single parameter, requires metabolic measurements or special assumptions about the absence of interactions, some tests are possible. Simple tests for the existence of metabolic entrainment have been given above. Just as important are systematic tests of consistency. These may be stated in the form of two theorems for the single salt active transport case:

$$\left|\frac{X_i}{J_k}\right|_{J_iJ_jX_k}\left|\frac{J_i}{X_k}\right|_{X_iJ_jJ_k} = \left|\frac{J_i}{J_k}\right|_{X_iJ_jX_k}\left|\frac{X_i}{X_k}\right|_{J_iJ_jJ_k} \tag{29 a}$$

$$\left|\frac{J_i}{X_k}\right|_{X_iX_jJ_k}\left|\frac{X_i}{J_k}\right|_{J_iX_jX_k} = \left|\frac{X_i}{X_k}\right|_{J_iX_jJ_k}\left|\frac{J_i}{J_k}\right|_{X_iX_jX_k} \tag{29 b}$$

Similarly, for the bi-ionic active transport case, we have two theorems:

$$\left|\frac{X_i}{J_l}\right|_{J_iJ_jJ_kX_l}\left|\frac{J_i}{X_l}\right|_{X_iJ_jJ_kJ_l} = \left|\frac{J_i}{J_l}\right|_{X_iJ_jJ_kX_l}\left|\frac{X_i}{X_l}\right|_{J_iJ_jJ_kJ_l} \tag{30 a}$$

$$\left|\frac{J_i}{X_l}\right|_{X_iX_jX_kJ_l}\left|\frac{X_i}{J_l}\right|_{J_iX_jX_kX_l} = \left|\frac{X_i}{X_l}\right|_{J_iX_jX_kJ_l}\left|\frac{J_i}{J_l}\right|_{X_iX_jX_kX_l} \tag{30 b}$$

These theorems are readily proven from the resistance equations and the corresponding conductance equations together with the reciprocal relations. In the single salt case, if $i = i$, $j = s$, and $k = v$, the required relation is

$$\left|\frac{I}{\Delta p - \Delta\pi}\right|_{E,\Delta\mu_s^c,J_v}\left|\frac{E}{J_v}\right|_{I,\Delta\mu_s^c,(\Delta p-\Delta\pi)} = \left|\frac{E}{\Delta p - \Delta\pi}\right|_{I,\Delta\mu_s^c,J_v}\left|\frac{I}{J_v}\right|_{\Delta\mu_s^c,E,\Delta p-\Delta\pi}$$

A total of 6 such relationships exists for the single salt case and 12 for the bi-ionic case. Additional relationships may be predicted for cases in which certain entrainment coefficients are zero. Compliance with these consistency checks is necessary before the other tests (e.g., for electrogenesis) can be used with confidence. One should not assume that the tissue under study obeys the basic phenomenological equations without some test. Furthermore, it is always conceivable that alternative systems, in particular, complex membranes with elements of particular properties, could give the behavior predicted by the present basic equations. The whole set of consistency checks (equations 29) should not hold for series or parallel systems since in general the reciprocal relations do not hold for the over-all coefficients in complex systems. Other evidence, especially the detailed morphological and chemical characterization of the membrane, should also be carefully considered before use of the practical equations.

Short Cuts

It is possible to gain some information about certain combinations of parameters without making metabolic measurements or special assumptions about the absence of interactions. These relations can be obtained from measurement methods suggested above but we summarize a few here. The most common experimental conditions for epithelial membranes are zero pressure gradient at open circuit (condition D) and zero pressure gradient at zero voltage (condition G). The primed quantities give expressions of simpler form in these cases.

$$\left|\frac{J_a}{J_b}\right|_D = \frac{U_a'}{U_b'}; \qquad \left|\frac{J_v}{E}\right|_D = -\frac{V\kappa'}{\epsilon'}; \qquad \left|\frac{J_v}{J_a}\right|_D = \frac{V}{U_a'}; \qquad \left|\frac{E}{J_a}\right|_D = -\frac{\epsilon'}{\kappa' U_a'} \tag{31 a}$$

$$\left|\frac{J_a}{I}\right|_G = \frac{\tau_1'}{\nu_1 z_1 F} + \frac{U_a'}{\epsilon'}; \qquad \left|\frac{J_v}{I}\right|_G = \frac{V}{\epsilon'} + \beta \tag{31 b}$$

The above are for the bi-ionic case. For the cases in which volume flow is prevented, we have for the single salt case such relations as

$$\left|\frac{E}{J_s}\right|_A = -\frac{\epsilon}{\kappa U}; \qquad \left|\frac{\Delta p - \Delta\pi}{J_s}\right|_A = \frac{V}{L_p U}; \qquad \left|\frac{\Delta p - \Delta\pi}{E}\right|_A = \frac{V\kappa}{L_p \epsilon} \tag{32 a}$$

$$\left|\frac{J_s}{I}\right|_B = \frac{\tau_1}{\nu_1 z_1 F} + \frac{U}{\epsilon}; \qquad \left|\frac{\Delta p - \Delta\pi}{I}\right|_B = \frac{V}{L_p \epsilon} - \frac{P_E}{\kappa} \tag{32 b}$$

In these relations, as throughout this paper, the form presented assumes that no denominator goes to zero.

DISCUSSION

The phenomenological description of active transport has a dual aim. On the one hand it is intended as a tool for those whose primary interest is simply the pragmatic question of what substances are moved against gradients without the intermediation of other substances. A related physiological question concerns the control and modification of transport processes in the animal. It is with these straightforward operational terms that irreversible thermodynamics deals most comfortably.

On the other hand, investigation of subcellular and molecular mechanisms of transport requires the availability of meaningful system parameters which can be evaluated in terms of specific kinetic models. Because of the Onsager relations and the explicit consideration of entrainment processes the thermodynamic approach again affords a convenient and desirable framework.

Thus, the present work has sought to establish coefficients with operational definitions of such terms as coupling, electrogenicity, etc. This set of coefficients can be measured under varying conditions—different temperatures, different average concentrations, with poisons—in order to uncover mechanisms. For example, the concentration dependence of U should reflect the occurrence of a saturating mechanism and of competition between transported species.

All measurements suggested in this approach are stationary-state measurements, for it is in the stationary states that we have available the Onsager relations and thermodynamic restrictions.

No special assumptions have been made about the nature of the chemical reactions which drive active transport. The transport behavior of the substrates and products of the metabolic reaction is unspecified. This omission may be considered equivalent to assuming (*a*) a low entrainment coefficient between the metabolites and the transported ion and water or (*b*) that the required fluxes of metabolites are small. In the case of sodium transport by frog skin, Zerahn (8) estimated that approximately 3 Na ions are transported per ATP molecule. This ratio is such that for over-all entrainment to be low, the entrainment coefficient must be low. In the isolated anaerobic turtle bladder, however, Klahr and Bricker (28) estimate 15 Na ions are transported per ATP. Passive entrainment of metabolites and transported fluxes is of the type called stationary coupling by Prigogine (6). This process may be related to the concept of carrier-mediated transport envisaged by Ussing (29).

In complex membranes the region of linearity may be quite restricted. Within this region the operational definitions are applicable. However, interpretation of mechanisms may require "dissection" of the system. Thus, access to the intracellular compartment of an epithelial membrane by micropuncture, chemical analysis, or other means becomes necessary. Let us consider the question of whether a pharmacological agent or hormone affects the active transport mechanism only, the passive properties only, or both in an epithelial membrane. From the considerations used to derive equations (28) above, it can be shown that the over-all U for a series system can be altered by a change in ω or λ, without either of the constituent U's being altered. Of course, the over-all ω would also be altered. Thus, one would conclude that an agent which "in reality" had changed only the passive permeability, ω, of one constituent membrane has altered both active and passive properties. In fact, *this is operationally true*; such an agent does alter the active transport properties of the membrane as a whole. The correct answer is obtained by the person interested in broad levels of integration, and it is only the person interested in the kinetic mechanism who might be misled if he did not proceed to a more detailed analysis, looking at the component ω's and U's.

These considerations apply with equal force to possible alternative general approaches to transport and cannot be considered a weakness of this approach.

It may be thought that the use of the full equations with allowance for entrainment is overblown, unnecessary elaboration. The danger of assigning a priori zero values to any of the practical cross-coefficients is indicated by the fact that they are not simply related to frictional interactions (e.g., the fact that absence of frictional interaction between ions of the same sign is not a sufficient condition for $\lambda = 0$). Direct experimental evidence is the only suitable criterion for setting any of the practical coefficients to zero. Let us examine the orders of magnitude of some coefficients in commonly studied biological systems.

As a matter of fact, the only coefficients which one could reasonably expect to be very small in many cases are P_E, $(1 - \sigma)$, λ (salt linkage), and the active transport coefficients ϵ, U, V. The importance of including the reflection coefficient in biological studies is now well established. The active transport coefficients are precisely the things wanted from the experiment. Thus, the coefficients to consider are λ and P_E.

It was mentioned in the flux ratio section that λc^2 will in many cases be on the order of ωc and cannot be neglected in a priori formulations. Therefore the salt linkage coefficient, λ, must be included in the description of biological membranes. λ incorporates the isotope effect if any.

The simplest way to check the order of P_E is to look at the magnitude of $P_E\beta$ compared to 1. This gives an estimate of the relative magnitude of the cross-coefficients compared to the straight ones. For the artificial ion exchange membrane described by Kedem and Katchalsky (24), $(P_E\beta)$ was about 0.04. However, Diamond's (17) gall bladder data give a value for $(P_E\beta)$ of about 0.015. House's (18) data indicate a similar small magnitude of $P_E\beta$ in the frog skin. The potential changes seen with osmotic gradients across the frog skin (5) would indicate an impossibly high value for $P_E\beta$, but those changes cannot be considered true streaming potentials, since they were highly nonlinear. Indeed, those effects of osmotic gradients indicate the extent to which series systems can depart from simple expectations. It appears that often P_E may be small in animal systems, in contrast to the situation in artificial ion exchange membranes. Nonetheless, it would be desirable to have definitive experimental tests in each case of interest. None of the biological measurements cited here was done with control of the metabolic terms. However, the estimates should be a useful first approximation.

It has long been suspected that the "resistance" of an actively transporting membrane would be dependent on the rate of active transport (Linderholm, 30). This has been confirmed in the present work, and we can estimate the probable extent of the error entailed by the neglect of metabolism. If the resistance measurement is taken as the ratio of open-circuit potential to short-circuit current, the "true" passive resistance $(1/\kappa')$ is

$$\frac{1}{\kappa'} = -\frac{(J_r)_{\Delta\mu_s^c(\Delta p-\Delta\pi),E}}{(J_r)_{\Delta\mu_s^c,(\Delta p-\Delta\pi),I}} \cdot \frac{(E)_{\Delta\mu_s^c,(\Delta p-\Delta\pi),I}}{(I)_{\Delta\mu_s^c,(\Delta p-\Delta\pi),E}} \tag{33}$$

If $P_E\beta$ is very small, $1/\kappa'$ is about the same as $1/\kappa$. For frog skin, the data of Zerahn (8) can be used to estimate that the passive resistance is 20–30% greater than the ratio of open-circuit potential to short-circuit current. The metabolic factors are consequently important in estimating even the passive properties of biological membranes.

A question always lurking in the background, especially with biological membranes, is the necessary precision of measurements for making desired inferences. The problem is that the direct experimental measurements are not of the coefficients L_p, ω, λ, ϵ, etc. Consequently the precision of these parameters must be estimated by propagation of error techniques using the estimates of error obtained with the directly measured quantities. It is these latter quantities that one might expect to have normally distributed variation. When the tests involve many coefficients, such as the bi-ionic active transport criteria, the precision attainable in practice may be inadequate. Fortunately a series of relatively simple operational tests are available (Table II) which can be applied even to some of the series membrane cases.

This work was supported in part by grants from the United States Public Health Service AM 05865; 5TI GM 899–08 and the National Science Foundation, GF-164.

Dr. Hoshiko is a United States Public Health Service Career Development Awardee, 5-K3-GM-15,467-05. Part of this work was done while Dr. Hoshiko was a guest in the Department of Physiology, Tokyo Medical and Dental University, and he is grateful for the hospitality afforded by his hosts, Professors Watanabe and Katsuki and their staff.

Dr. Lindley was a National Science Foundation Fellow.

Received for publication 21 February 1966.

LIST OF SYMBOLS

a_{11}, a_{12}, etc.	dummy coefficients to be defined in terms of practical coefficients
a_s, a_1, a_2	chemical activity of salt "*s*," ions 1 and 2
A_r	affinity of driving metabolic reaction (6)
c_s ; c_a ; c_b	mean salt concentration defined by $c_s = \Delta\pi_s/RT\Delta \ln a_s$; mean salt concentration for *a* and *b*
Δc_j	difference in concentration of component *j*
E	potential difference measured with electrodes reversible for ion 2 in single salt case, and for ion 3 in bi-ionic case
F	Faraday's constant
I	electric current density
J_s, J_a, J_b	flux of salt *s*, *a*, *b*
J_w, J_1, J_2, J_3	flux in gram-moles or gram ions $\text{cm}^{-2}\ \text{sec}^{-1}$ of water, and ions 1, 2, 3
J_v	volume flow
J_r	metabolic flow rate
k	metabolic rate coefficient
L_p	mechanical permeability
L_{jk}	phenomenological conductance coefficients
Δp	hydrostatic pressure difference
P_E	electroosmotic pressure
R_{jk}	phenomenological resistance coefficients
R	gas constant

T	absolute temperature
U	salt-pumping coefficient—in honor of Professor H. H. Ussing
V	volume pump coefficient—in honor of Professor M. B. Visscher
$\bar{v}_1$, $\bar{v}_2$, $\bar{v}_s$, $\bar{v}_a$, $\bar{v}_b$, $\bar{v}_w$	partial molar volume of ions 1 and 2, of salts *s*, *a*, *b*, and of water
z_1, z_2, z_3	valence of ion 1, 2, 3
β	electroosmotic permeability
ϵ	electrogenicity coefficient
η_ϵ, η_v	electrogenic and volume pump coupling coefficients
θ_s, θ_w	volume fraction of solute and water
κ	electrical conductance
λ	salt linkage coefficient
$\Delta\tilde{\mu}_1$, $\Delta\tilde{\mu}_2$, $\Delta\tilde{\mu}_s$, $\Delta\tilde{\mu}_a$, $\Delta\tilde{\mu}_b$	difference in total chemical potential of ions 1 and 2, and of salts *s*, *a*, *b*
$\Delta\mu_s^c$	difference in concentration-dependent part of the chemical potential of salt *s*
ν_1, ν_2 ; ν_1^a, ν_3^a ; ν_2^b, ν_3^b	stoichiometric coefficients of ions 1 and 2 in salt *s*; ions 1 and 3 in salt *a*; ions 2 and 3 in salt *b*
$\Delta\pi$	total osmotic pressure difference
ρ_{12}	transport coupling ratio
σ	reflection coefficient
τ_1, τ_2	transference number
$\tau_1^r = (\tau_1/\nu_1 z_1 F)$	reduced transference number (11)
Φ	dissipation function
$\Delta\phi$	electric potential difference
ωc_s	salt permeability

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