Transport across epithelia: Some basic principles

STANLEY G. SCHULTZ

Department of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

The initial steps in the analysis of net transport across any epithelium are to define the thermodynamic properties of the two surrounding solutions (e.g., concentrations, or preferably activities, of solutes; electrical potential; pressure; etc.) and to measure the transepithelial flows of solutes and solvent. Then, viewing the multicellular structure (including unstirred, extraepithelial layers) as a homogeneous barrier, an attempt is made to define the forces responsible for a given flow. The theory of nonequilibrium (irreversible) thermodynamics is ideally suited for this purpose. This approach is entirely phenomenologic and does not depend upon a detailed (and often lacking) understanding of structure and function. Consequently, it can provide only limited insight into underlying mechanisms of transport. Nevertheless, by identifying the generalized forces responsible for a given flow, it provides direction for further efforts aimed at defining transport mechanisms at the molecular level in terms of membrane structure and biochemistry.

The purpose of this communication is to illustrate briefly the application of nonequilibrium thermodynamics to the analysis of flows of solute and solvent across epithelial tissues. The emphasis will be on the analysis of solute movements because it is widely accepted that, in the absence of transepithelial hydrostatic pressure differences, solvent (water) flow is dependent upon solute flow and transepithelial solute concentration differences; detailed discussions of models for the coupling between water flow and solute flow have been published elsewhere [1—5]. Because of space limitations only the more common examples of flow-force interactions will be considered and detailed derivations will be omitted. More comprehensive treatments may be found elsewhere [2, 6—12].

Flows, forces and interactions

Any homogeneous system is characterized by a set of extensive parameters and intensive parameters. The former include parameters such as total amount(s) of matter, amount of charge, amount of heat, volume, etc. which obviously depend upon the size of the system. For each extensive parameter, there is a conjugate intensive parameter whose value is independent of the size of the system and is the same at any point within the homogeneous system. The conjugate intensive parameters for the extensive parameters given above are, respectively, concentration (or chemical potential), electrical potential, temperature and pressure. When two different homogeneous systems are separated by a barrier, there may be flows or displacements of extensive parameters from one system to the other. If there are no interactions among the flows, the driving force responsible for a given flow is the difference or gradient (see footnote 4) of its conjugate intensive parameter across the barrier. Thus, for every flow (J) one may identify a conjugate driving force (X). And, from above, it follows that the conjugate driving force for the flow of uncharged matter, J, is a difference (or gradient) of concentration, \( \Delta c \) (or chemical potential, \( \Delta \mu \)); the conjugate driving force for the flow of charge (current), I, is a difference in electrical potential, \( \Delta \Psi \); the conjugate driving force for the flow of heat is a difference in temperature, \( \Delta T \); and, the conjugate driving force for the flow of volume, Jv, is a difference in pressure \( \Delta P \). Because the flow of a charged solute involves a displacement of matter as well as charge, the conjugate driving force is an expression of both the difference in chemical potential and electrical potential, i.e., the difference in electrochemical potential, \( \Delta \mu^* \).

It is empirically known that if there is only one flow across the barrier and if the conjugate driving force is "small" (i.e., the system is close to equilibrium), the flow is linearly related to the driving force so that

\[
J = L_{11} X_1,
\]

where \( L_{11} \) is the flow per unit of driving force and is expressed in units of conductance. Familiar examples of this principle are Ohm's law for the flow of cur-
rent, Fick's first law of diffusion, Poiseuille's law of volume flow, etc.

However, if a system is characterized by more than one flow, it is also empirically established that each flow may be influenced by every other flow and, hence, by forces other than its conjugate force. For a system in which there are n flows and forces, the J's may be described in terms of the X's by a set of linear phenomenologic equations as follows:

\[ J_i = L_{i1}X_1 + L_{i2}X_2 + L_{ik}X_k \ldots L_{in}X_n \]

or

\[ J_i = L_{i1}X_1 + L_{i2}X_2 + L_{i3}X_3 \ldots L_{in}X_n \]

or

\[ J_n = L_{ni}X_i + L_{nj}X_j + L_{nk}X_k \ldots L_{nn}X_n \]

where \( x \neq i \).

The \( L_{ii}, L_{ij}, \) etc. are referred to as “straight coefficients” inasmuch as they relate a given flow to its conjugate driving force. The \( L_{ik}, \) where \( x \neq i, \) are referred to as “cross coefficients” inasmuch as they reflect the extent to which the flow of \( i \) is affected by nonconjugate forces, \( X_k, \) in the system. The L's may be (and generally are) functions of the intensive parameters of the system but are independent of the flows and forces.

It should be noted that equations 2 and 3 assume linear interactions between a given flow and all of the forces in the system, and presuppose that the system is “not displaced too far from equilibrium”. The question that may be raised is: How far is too far?

Criteria for establishing the validity of the linear phenomenologic equations 2 and 3 were provided by Onsager [13, 14]. It should be noted that the product of a given flow and its conjugate driving force, \( J_1X_1, \) has units of energy per unit time. In a closed system which does not perform work on the outside environment, this product reflects the rate of energy dissipation or internal entropy production. It is the rate at which the energy inherent in a “potential difference” (e.g., a difference in chemical potential, electrical potential, pressure, etc.) is dissipated by the flow of the conjugate extensive parameter down that potential difference. For a system at constant temperature it can be shown [6] that

\[ \Phi = T(dS/dt) = \sum_i J_iX_i \]

where \( \Phi \) is the “dissipation function”, \( (dS/dt) \) is the rate of internal entropy production by the irreversible flows, and \( T \) is the absolute temperature of the system. According to the Second Law of classical thermodynamics, \( \Phi > 0 \) (positive definite) in any real system that is not at equilibrium.

Onsager [13, 14] demonstrated that if the J's and X's are properly chosen so that equation 4 is satisfied and if the system is “not displaced too far” from equilibrium, so that equation 2 and 3 are valid, then \( L_{ix} = L_{xi} \) for all \( x. \) This reciprocal relation states that the cross coefficient for the effect of \( X_k \) on \( J_i \) is equal to the cross coefficient for the effect of \( X_i \) on \( J_k. \) Thus, if the flows and forces are appropriately chosen so that their products satisfy equation 4 and if it can be experimentally demonstrated that \( L_{ix} = L_{xi}, \) then the system is not displaced too far from equilibrium and the flow-force interactions can be expressed by the set of linear phenomenologic equations 2 and 3. The validity of the linear phenomenologic relations and the reciprocal relations have been demonstrated for a number of coupled flow processes despite significant displacements from equilibrium [15]. For discussions of nonlinear systems in which the Onsager relations fail to apply, see Mason, Wendt and Bressler [16] and Sauer [17].

The relations between flows and forces given in equations 2 and 3 can be transformed into another, often more useful, set of linear phenomenologic equations that express forces in terms of flows. Thus,

\[ J_i = R_{1i}X_1 + \sum_{x \neq i} L_{ix}X_x \]

or

\[ J_i = R_{1i}X_1 + \sum_{x \neq i} L_{ix}X_x \]

where \( x = j, k \ldots n, \) but \( x \neq i. \)

Solving equation 6 for \( J_i \) we obtain the following:

\[ J_i = \left( X_i/R_{ii} \right) - \sum R_{ix}J_x/R_{ii}. \]

For the purpose of analyzing and categorizing transport across biological membranes, Kedem [18] subdivided equation 7 as follows:

\[ J_i = \left( X_i/R_{ii} \right) - \sum R_{ix}J_x/R_{ii} \]

where \( x = j, k \ldots n, \) and \( x \neq i. \)
other solutes or solvent and coupling to the flow of a chemical reaction. Kedem \[18\] proposed that the term “active transport” be restricted to those flows of i where \( R_{ir} \neq 0 \); as discussed previously \[2, 19\] this definition contrasts with the earlier classical thermodynamic criterion suggested by Rosenberg \[20\].

In general, an analysis of the flow of a given substance, i, along the lines suggested by equation 8 poses formidable experimental obstacles. Epithelia such as renal proximal tubule and small intestine transport numerous solutes as well as water so that, in principle, there are numerous \( R_{ir} \)‘s that may influence \( J_i \) and hence the complexity of an experimental analysis is staggering. However, it appears that most transepithelial movements are influenced predominantly by only three forces: (a) the conjugate driving force (diffusion), (b) coupling or interaction with water flow (solvent-drag) and (c) coupling to the flow of an exergonic chemical reaction (active transport). Other possible interactions such as solute-solute coupling, electro-kinetic phenomena, etc. appear to play a minimal role in most transepithelial transport processes under normal conditions and will not be considered further. However, these phenomena cannot be dismissed \emph{a priori} and have been observed in artificial (e.g., \[6, 12, 21\]) and biological membranes (e.g., \[22\]). For a discussion of solute-solute coupling (including interactions between tracer and abundant species) and electro-kinetic processes, see \[6, 23—25\].

\textbf{Conjugate driving forces: Diffusion}

\textit{The Nernst-Planck equation.} The flow of a substance, i, at any point in space is given by

\[ J_i = u_i c_i X_i \] (9)

where \( u_i \) is the mobility (velocity per unit driving force); \( c_i \), the concentration of i at the point; and \( X_i \), the driving force. If the flow of i is driven only by its conjugate driving force,

\[ X_i = -d\tilde{\mu}_i/dx \]

where \( \tilde{\mu}_i \) is the electrochemical potential\(^1\) and is given by

\[ \tilde{\mu}_i = \tilde{\mu}_{i}^{\circ} + RT \ln c_i + z_i \tilde{\Phi} \psi \] (10)

where \( \tilde{\mu}_{i}^{\circ} \) is the standard state electrochemical potential (which for our purpose may be considered constant), \( R \) is the gas constant, \( T \) is the absolute temperature, \( z_i \) is the valence of i, \( \tilde{\Phi} \) is the Faraday and \( \psi \) is the electrical potential at point \( x \).\(^2\)

Thus, from equations 9 and 10 we obtain the Nernst-Planck equation

\[ J_i = -u_i c_i [RT \ln c_i/dx + z_i \tilde{\Phi} d \psi/dx] \] (11)

or

\[ J_i = -D_i [dc_i/dx + (z_i c_i \tilde{\Phi}/RT) d \psi/dx] \] (12)

where \( D_i \) is the diffusion coefficient of i and is given by the relation derived by Einstein, \( D_i = u_i RT \).

For an uncharged substance equation 12 reduces to the following:

\[ J_i = -D_i (dc_i/dx) \] (13)

which is simply Fick’s first law of diffusion, and which when integrated over the thickness of the barrier yields the following:

\[ J_i = -D_i (\Delta c_i/\Delta x) \]

where \( \Delta c_i \) is the concentration difference across the membrane and \( \Delta x \) is the thickness of the membrane. It should be stressed that \( D_i \) is the diffusion coefficient \emph{within} the membrane and that \( \Delta c_i \) is the difference between the concentration of i just within the membrane at one interface and the concentration just \emph{within} the membrane at the other interface. In order to relate these concentrations to the concentrations of i in the two outer, aqueous compartments, we introduce a partition coefficient, \( \beta_i \), which is defined as the concentration just within the membrane at the interface divided by that in the outer solution. Assuming that \( \beta_i \) is the same at both interfaces, equation 13 yields

\[ J_i = -D_i \beta_i (\Delta c_i/\Delta x) \] (14)

or

\[ J_i = P_i \Delta c_i \]

where \( P_i \) now refers to the concentration difference between the two outer compartments and where \( P_i \), the permeability coefficient, is simply \( D_i \beta_i/\Delta x \).

Comparison of equations 1 and 9 indicates that \( L_{ii} \) is the rate of decrease in free energy per mole of i at constant T, P and composition. Thus, \(-d\tilde{\mu}_i/dx\) is the rate of decrease in free energy per mole per unit of path length. \( X_i \) is defined as the negative of \( d\tilde{\mu}_i/dx \) because flow \( (J_i) \) is positive in the direction of decreasing \( \tilde{\mu}_i \). Throughout this communication we will consider flows only in the x direction, orthogonal to the axis of the membrane; this assumes that the membrane is homogeneous in the y and z directions.

\( \text{\textsuperscript{1}} \) The electrochemical potential of i is the Gibb’s free energy per mole of i at constant T, P and composition. Thus, \(-d\tilde{\mu}_i/dx\) is the rate of decrease in free energy per mole per unit of path length. \( X_i \) is defined as the negative of \( d\tilde{\mu}_i/dx \) because flow \( (J_i) \) is positive in the direction of decreasing \( \tilde{\mu}_i \). Throughout this communication we will consider flows only in the x direction, orthogonal to the axis of the membrane; this assumes that the membrane is homogeneous in the y and z directions.

\( \text{\textsuperscript{2}} \) Strictly speaking, thermodynamic activities should be employed rather than concentrations. However, for illustrative purposes the use of concentrations is simpler and will suffice.
complicated when we consider the diffusion of a charged species since when \( z \neq 0 \) equation 12 cannot be integrated without additional assumptions regarding the relation between \( \psi \) and \( x \). Several assumptions have been made leading to solutions of equation 12 [8-11] but by far the simplest and most useful is the assumption that the electric field \( (d \psi / dx) \) within the barrier is constant (i.e., that the relation between \( \psi \) and \( x \) within the membrane is linear) [26]. Thus, \( \Delta \psi / \Delta x \) may be substituted for \( d \psi / dx \) in equation 12 which can then be readily integrated to give the following:

\[
J_1 = \frac{P_{i1}}{RT} \left[ \frac{c'_1 - c''_1 \exp(-z_1F \Delta \psi / RT)}{1 - \exp(-z_1F \Delta \psi / RT)} \right]
\]

where \( c'_1 \) and \( c''_1 \) are the concentrations in the two outer aqueous compartments (" and "), \( \Delta \psi = \psi' - \psi'' \) and \( P_{i1} = (\mu_{i1}RT / \beta_{i1}) = (D_{i1} / \Delta x_i) \). Equation 15 is frequently referred to as the Goldman [26] or "constant field" flux equation.

There are several interesting and useful consequences of equation 15. First, clearly, when \( c'_1 = c''_1 \),

\[
J_1 = \frac{P_{i1}z_1F}{RT} c_1/RT
\]

so that for an ion whose movement is strictly diffusional, the net flow across the membrane is a linear function of \( \Delta \psi \). (As will be discussed below, equation 16 can be derived from much more general considerations and does not depend upon the constant-field assumption. It is, in fact, the definition of \( P_1 \) when \( J_1 \) is driven solely by \( \Delta \psi \).)

Thus, studies of \( J_1 \) vs. \( \Delta \psi \) can provide a measure of \( P_1 \). Now,

\[
P_1c_i = J_1 = J''_1
\]

where \( J_{1i} \) is the unidirectional tracer flux of \( i \) from side \( ' \) to side \( " \) under "short circuit" conditions (i.e., when \( c'_1 = c''_1 \) and \( \Delta \psi = 0 \)) and \( J_{1i} \) is the unidirectional flux in the opposite direction. Further, \( J_{1i} \) expressed in \( \mu \text{Eq}/\text{cm}^2 \) hr is numerically equal to the partial ionic conductance of \( i \), \( G_{i1} \), expressed in mmhos/cm². Thus, studies of \( J_1 \) vs. \( \Delta \psi \) may indicate whether the transepithelial movement of \( i \) can be attributed entirely to diffusion and at the same time provide information regarding the permeability and partial ionic conductance of the barrier to \( i \). This approach has been employed to "dissect" the diffusional and nondiffusional components of Na transport across rat ileum [28] and \textit{Necturus} proximal renal tubule [29]. Thus, assuming that net transepithelial Na movement is comprised of a nondiffusional component and a diffusional component, and that the nondiffusional component is not affected by small, spontaneous or imposed transepithelial electrical potential differences, then when \( c_{iNa} = c_{iNa}'' \), we may write,

\[
J_{Na} = n_{JNa} + (P_{Na}z_1Fc_{iNa}/RT) \Delta \psi
\]

where \( n_{JNa} \) is the component of the net flux that is not affected by an imposed potential difference (PD). Then, a plot of \( J_{Na} \) vs. \( \Delta \psi \) should yield a straight line with an intercept (when \( \Delta \psi = 0 \)) which is equal to \( n_{JNa} \) and a slope that is a measure of \( P_{Na} \).

Often it is difficult to measure net fluxes across an epithelium chemically, and the determination of net fluxes from the difference between bidirectional tracer fluxes may pose experimental problems particularly in "leaky epithelia" where the net flux is often a small difference between two large, oppositely di-

When \( \Delta c_i \) is small,

\[
\Delta \mu_i = (RT \Delta c_i / \bar{c}_i) + z_iF \Delta \psi
\]

where \( \bar{c}_i \) is the mean concentration and is given by

\[
\bar{c}_i = (c'_i + c''_i) / 2.
\]

Thus,

\[
J_1 = L_{i1}'(RT \Delta c_i / \bar{c}_i) + z_iF \Delta \psi.
\]

We now define

\[
L_{i1}' = \bar{c}_iP_i / RT.
\]

Thus,

\[
J_1 = P_i \Delta \mu_i + L_{i1}' \Delta \psi / RT.
\]

Hence, \( P_i \) is entirely phenomenologic and includes such unknowns as the barrier thickness, the diffusion coefficient of \( i \) within the barrier and the partition coefficients. Clearly, when \( c'_1 = c''_1 \), the final equation is identical with equation 16. For a more detailed discussion of the use of \( \Delta \mu_i \) rather than \( \Delta \psi / \Delta x \), see [6], pp. 113-116.
rected tracer fluxes. Under these conditions it is easier to determine $P_i$ from measurements of the effect of $\Delta \psi$ on a unidirectional tracer flux. It can be readily shown that when $\Delta \psi$ is small (<15 mV), equation 15 may be approximated by [30, 31]

$$J_i = P_i [c_{i'} \exp(z_i \Delta \psi/2RT) - c_{i''} \exp(-z_i \Delta \psi/2RT)]$$

(18)
or

$$J_i = J_i' - J_i''$$

(19)

where $J_i'$ is the unidirectional tracer flux from side ' to side " and $J_i''$ is the unidirectional flux in the opposite direction. When $c_{i'} = c_{i''}$, equation 18 reduces to equation 16. Because $P_i c_{i'} = J_i'$ and $P_i c_{i''} = J_i''$, we may write [31–33]

$$J_i = \phi J_i' \xi^{1/2} - \phi J_i'' \xi^{-1/2}$$

(20)

where $\xi = \exp(z_i \Delta \psi/RT)$.

From equation 19 it follows that

$$J_i' = \phi J_i' \xi^{1/2} = P_i c_i' \xi^{1/2}$$

(21)

and

$$J_i'' = \phi J_i'' \xi^{-1/2} = P_i c_i'' \xi^{-1/2}.$$  

Thus, whereas equations 16 and 17 provide criteria for analyzing net transepithelial ionic fluxes ($J_i$) in terms of diffusional and nondiffusional components, equation 21 provides a similar approach for the analysis of unidirectional transepithelial ionic movements. In general, we may write

$$J_i' = \phi J_i' \xi^{1/2}$$

where $\phi J_i'$ is the nondiffusional contribution to the unidirectional flux from ' to " If a plot of $J_i'$ vs. $\xi^{1/2}$ is linear and passes through the origin, the entire unidirectional tracer flux from ' to " may be attributed to strict ionic diffusion; a nonzero intercept when $\xi^{1/2} = 0$ is a measure of that component of the unidirectional flux that is nondiffusional and uninfluenced by the transepithelial PD [32–35].

It should be stressed that these treatments assume that net and unidirectional flows are driven only by conjugate forces and that there are no interactions between the flow of tracer and that of the abundant species. The consequences of such interactions are discussed by Essig and Li (36).

The Ussing flux-ratio equation. As noted above, an exact solution of the Nernst-Planck equation requires assumptions regarding the electrical potential profile within the membrane. Consequently, the various solutions of this equation do not provide entirely rigorous criteria for strict ionic diffusion. For example, failure of $J_i$ to conform to equation 15 does not preclude strictly diffusional movements inasmuch as this could be due to the fact that the "constant-field" assumption is not valid [27]. Further, if equation 15 (or any of the other solutions of equation 12) is to be employed as a rigorous criterion for ionic diffusion, an independent measure of $P_i$ (or $u_i$) is necessary.

These problems were circumvented by Ussing [37], who demonstrated that if the transepithelial movement of an ion is strictly diffusional (i.e., driven solely by its conjugate driving force, $\Delta \mu_i$), the ratio of the bidirectional transepithelial (tracer) fluxes is given by

$$J_i'/J_i'' = (c_{i'}/c_{i''}) \exp(z_i \Delta \psi/RT)$$

(22)

where $\Delta \psi = \psi' - \psi''$. Clearly, when $c_{i'} = c_{i''}$ and $\Delta \psi = 0$, $J_i' = J_i''$ and $J_i = 0$. The assumptions underlying this equation are described in detail elsewhere [12, 19, 37–39]. However, of major importance is that the derivation makes no assumptions regarding the chemical or electrical potential profiles within the barrier or the properties of the pathways for ionic diffusion providing that the bidirectional ionic movements traverse pathways that have identical properties at any point in the direction $x$; all of the quantities appearing in equation 22 can be measured in the external solutions. Further, Schwartz [11] has demonstrated the validity of equation 22 in a three-dimensional analysis so that the assumption that the membrane is homogeneous in the $y$ and $z$ directions is unnecessary. It should be stressed that failure of the bidirectional transepithelial fluxes to conform to equation 22 does not imply that the movement is coupled to a chemical reaction (i.e., "active transport" by the Kedem definition) since interactions between the flow of tracer and the flow of abundant species or the flow of other solutes or solvent, "single-file diffusion", "exchange diffusion", etc. [2, 12, 19, 23–25] will in general lead to deviations from the behavior described by the equation. Thus, equation 22 describes a sufficient but not necessary criterion for the conclusion that the transepithelial movements of a given ion are attributable solely to external differences in concentration and electrical potential (i.e., nonconformity does not exclude simple diffusion).  

---

1 In the presence of large PD's, equation 18 is no longer an accurate approximation. Under these conditions it can be shown [31–33] that

$$J_i'/J_i'' = [z_i \Delta \psi/RT] / [\exp(z_i \Delta \psi/RT) - 1].$$

2 Rehm [40] has argued that conformity with equation 22 does not permit the conclusion that the transepithelial movements of an ion are attributable to simple diffusion. However, the crux of Rehm's argument is that because of inevitable experimental errors associated with measurements of transepithelial tracer fluxes, the flux ratio may not differ significantly from that predicted by equation 22 in spite of the fact that the flow of an ion may be influenced by
Finally, it should be noted that equation 22 can be derived from the formalism of nonequilibrium thermodynamics [23, 25] and follows directly from equations 15, 18 and 21.

**Coupling to the flow of volume: Solvent-drag**

It has been recognized for many years that trans-epithelial solute movement can result from, or be influenced by, “entrainment in a stream of solvent” or “solvent-drag” [41–43]. However, it was not until the monumental contribution of Kedem and Katchalsky [44], in 1958, that the effect of volume flow on solute flow and the effect of solute concentration differences on volume flow were described by a self-consistent formalism.

Using the linear equations of nonequilibrium thermodynamics and assuming the validity of Onsager’s reciprocal relations, Kedem and Katchalsky demonstrated that when a homogeneous membrane separates two solutions containing a solvent and an uncharged solute, i,

\[ J_i = (1 - \sigma_1) \bar{c}_i J_v + \omega_1 RT \Delta c_i \]  
(23)

and

\[ J_v = L_v(\Delta P - \sigma_1 RT \Delta c_i) \]  
(24)

where \( J_v \) is the flow of volume (solute plus solvent), \( \bar{c}_i \) is the average or mean concentration of i across the membrane, \( \omega_1 = P_1/RT \) in the absence of volume flow, \( \Delta P \) is the hydrostatic pressure difference across the membrane and \( L_v \) is the hydraulic conductivity of the membrane (a “straight coefficient” defined by the rate of volume flow per unit of pressure difference). \( \sigma_1 \) is the “reflection coefficient” introduced earlier by Staverman [45] to describe the ratio between the effective or observed osmotic pressure across a membrane which is not ideally impermeable to the solute i and the osmotic pressure predicted by van’t Hoff’s Law for an ideally semipermeable membrane. From equation 24, if \( \Delta c_i \neq 0 \) the pressure that must be applied to the more concentrated solution to prevent volume flow from the dilute solution to the concentrated solution (i.e., to make \( J_v = 0 \)) is

\[ \Delta P = \sigma_1 RT \Delta c_i. \]  
(25)

Thus, when the membrane is ideally impermeable to i, \( \sigma_1 = 1 \) and equations 24 and 25 reduce to the van’t Hoff equation. For a membrane that is not ideally impermeable to i, \( \sigma_1 < 1 \); and, if the membrane cannot distinguish between i and the solvent, \( \sigma_1 = 0' \).

According to equation 23, if \( J_v = 0 \), \( J_i \) is driven solely by its conjugate force as given by equations 14. However, if \( J_v \neq 0 \) and \( \sigma_1 < 1 \), there will be an additional contribution to the flow of \( J_i \) driven by entrainment in, or frictional interaction with, the flow of solvent. (In the example cited, the flow of i from the more concentrated solution to the more dilute solution will be slowed by solvent drag directed from the more dilute solution to the more concentrated solution.)

For the case of a charged species, \( J_i \) may be approximated by [5, 17, 47]

\[ J_i = (1 - \sigma_1) \bar{c}_i J_v + \omega_1 RT (\Delta c_i + z_i \bar{c}_i \Delta \psi/RT) \]  
(26)

where \( \omega_1 RT = P_1 \) (see footnote 4). The second term on the right of this equation essentially states that when \( c_i' \equiv c_i'' \), the diffusional flow of i is the sum of a flow driven by \( \Delta c_i \) (i.e., \( P_1 \Delta c_i \)) and a flow driven by the electrical potential difference (i.e., \( P_1 z_i \bar{c}_i \Delta \psi/RT \); see equation 16) where

\[ \bar{c}_i \equiv (c_i' + c_i'')/2. \]

Thus, equations 23 and 26 describe the flow of a solute i across a homogeneous membrane (i.e., one across which the pathways for solute and solvent flow are identical) driven by conjugate forces and by coupling to the flow of solvent. For a physical interpretation of the coefficients \( L_v \), \( \omega \) and \( \sigma \) in terms of frictional interactions, see [6, 48].

**Coupling to an exergonic chemical reaction: Active transport**

We will now consider the transport of a solute, i, that is driven by its conjugate force as well as by coupling to an exergonic chemical reaction; according to Kedem [18] such a transport process should be considered to be “active” regardless of its direction and I agree [19] that this definition is preferable to that proposed by Rosenberg [20]. The following treatment closely follows that of Essig and Caplan [49].

---

\[ J_i = (1 - \sigma_1) \bar{c}_i J_v + \omega_1 RT (\Delta c_i + z_i \bar{c}_i \Delta \psi/RT) \]

Kedem and Leaf [46] have analyzed the meaning of reflection coefficients for ions and have defined conditions when the reflection coefficient may be less than zero leading to “anomalous osmosis” (i.e., volume flow from the more concentrated to the more dilute compartment). Instances of negative reflection coefficients and anomalous osmosis for nonelectrolyte solutions are discussed by Talen and Staverman [64].
Assume that $i$, a monovalent cation, is the only species actively transported by the epithelium. Then, according to equations 5 we may write

$$X_1 = R_{ii} J_i + R_{ir} J_r$$  \hspace{1cm} (27)$$
and

$$A = R_{ri} J_i + R_{rr} J_r$$  \hspace{1cm} (28)$$
where $J_r$ is the flow of the chemical reaction in moles per unit area of tissue per unit time (e.g., the rate of $O_2$ consumption, glucose utilization, adenosine triphosphatase [ATP] hydrolysis, etc. associated with the transport of $i$), $A$ is the “affinity” or conjugate driving force for the flow of the reaction in cal/mole (when temperature, pressure, and chemical potentials are constant, as in most steady-state biological systems, $A$ is the Gibb’s free energy change, $-\Delta G$, for the chemical reaction), and $R_{ii}$ and $R_{rr}$ are the coupling coefficients reflecting the mutual interactions between $J_r$ and $J_i$. If $J_r > 0$ when $X_i \leq 0$, the flow of $i$ takes place against or in the absence of the conjugate driving force and fulfills the criterion for active transport suggested by Rosenberg [20]. However, $J_i$ may be in the same direction as its conjugate driving force (i.e., $J_i > 0$ when $X_i > 0$) but either slowed or accelerated by coupling to $J_r$; according to Kedem [18] this flow should also be considered to be “active transport”. Since one purpose of any classification of transport processes is to provide direction for future research, a definition that implies coupling with a chemical reaction (regardless of the direction of transport) is more useful than one based solely on the direction of transport.

As noted above, in theory, linear phenomenologic relations are applicable only when the driving forces for all flows are small (i.e., the system is not “displaced too far” from equilibrium). Katchalsky and Curran [6] have shown, using a kinetic analysis, that a linear relation between $J_i$ and $A$ should be expected only when the reaction is very close to equilibrium.\(^a\) However, Prigogine and Lefever [50, 51] have subsequently shown that if an overall chemical reaction is comprised of several intermediate steps each of which is close to equilibrium ($\Delta G \ll RT$), the overall reaction rate may be a linear function of each of which is close to equilibrium ($G \ll RT$), experimental data bearing on this point are limited; however, Blumenthal, Caplan and Keden [52] have verified the applicability of linear phenomenologic equations and Onsager’s reciprocal relations for coupling between a chemical reaction and current flow for affinities up to 3000 cal/mole. Further, Cussler (personal communication) has shown that symmetrical behavior similar to that predicted from considerations of microscopic reversibility can result from stoichiometric constraints and thus has a different physical basis from that of the Onsager relations. Finally, Vieira, Caplan and Essig [53, 54] have provided experimental evidence supporting the linear relations given in equations 27 and 28 for Na transport and $O_2$ consumption by isolated frog skin.

Assuming that the linear equations 27 and 28 are valid, we may define two combinations of the phenomenologic coefficients as follows:

$$Z = (R_{rr}/R_{ii})^{1/2}$$ and $$q = -R_{ir}/(R_{rr} R_{ii})^{1/2}.$$  

If the Onsager reciprocal relations hold (i.e., $R_{ir} = R_{ri}$), it can be shown [6, 49, 55] that $R_{ir}^2 \leq R_{ii} R_{rr}$; thus, it follows that $-1 \leq q \leq 1$. $q$ is defined as the “degree of coupling” [55] for the following reasons:

1. If $q = \pm 1$ the two flows are completely coupled so that $J_i/J_r = \pm Z$ and the ratio of the two flows is independent of the ratio of the two forces.\(^b\) That is, if one flow is fixed the other flow is uniquely determined. Thus, if the rate of the metabolic reaction coupled to the flow of $J_i$ is fixed (e.g., by fixed steady-state levels of substrates and products), then $J_i$ is fixed and independent of $X_i$ (or $\Delta \mu_i$). It also follows that a fixed and unique stoichiometric relation between $J_i$ and $J_r$ can only be expected when $q = \pm 1$ [49, 53]. As demonstrated by Vieira et al [53], for a given frog skin there is a linear relation between $J_{Na}$ and the rate of $O_2$ consumption; however, the ratio of $J_{Na}$ to the rate of $O_2$ consumption varies widely among different skins reflecting differences in the degree of coupling.

2. If $q = 0$, $R_{ir} = 0$, the flows are entirely uncoupled and

$$J_i/J_r = Z^2 X_i/A = (X_i/R_{ii})/(A/R_{rr}) = L_{ii} X_i/L_{rr} A.$$  

That is, the flow ratio is proportional to the force ratio, each flow is influenced only by its conjugate driving force and active transport is precluded.

From equations 27, 28 and 29 we obtain the following:

$$J_i = [X_i + (q/Z) A] / [R_{ii}(1-q^2)]$$  \hspace{1cm} (30)$$
and

$$J_r = [(q/Z)X_i + (1/Z^2) A] / [R_{rr}(1-q^2)].$$  \hspace{1cm} (31)$$

\(^a\) According to Prigogine (quoted in [6]) a linear relation between $J_r$ and $-\Delta G$ should obtain only when $\Delta G \ll RT$; that is $\Delta G$ should be much less than 600 cal/mole.

\(^b\) When $q = \pm 1$, the resistances are infinite and their ratios are indeterminate. Under these circumstances the system can be treated using conductances as described by Kedem and Caplan [55].
In the study of epithelia, two stationary states are encountered in which osmotic work is not performed. The first is termed "level flow" where the concentrations of $i$ in the two bathing solutions are essentially equal and the transepithelial PD is close to zero; examples are Na absorption by renal proximal tubule and small intestine. When $X_{Na} \equiv 0$, equations 27 and 30 reduce to
\[(J_{Na})_{x=0} \cong -(R_{r}/R_{Na})J_{r} \cong [(q/Z)A]/[R_{Na}(1-q^2)].\]
Thus, for a given A, level flow increases with decreasing resistance to the flow of Na, $(R_{Na})$, and/or increasing degree of coupling (q) between Na transport and $J_{r}$. Another example of "level flow" is a short-circuited epithelium and the short-circuit current, $I_{sc}$, is simply
\[
\mathcal{F}(J_{1})_{x=0} = [(q/Z)\mathcal{F} A] / [R_{n1}(1-q^2)] = -(R_{r}/R_{n1})J_{r} \mid_{z=0}.
\] (32)

The second is "static head" where $J_{1} = 0$ but $X_{i}$ and A are nonzero. For example, when isolated frog skin is bathed in a Na$_2$SO$_4$-Ringer's solution, transepithelial Na transport is essentially abolished because of the absence of a permeant anion [56]. Under these conditions,
\[
X_{Na} = (X_{Na})_{x=0} = -qA/Z.
\] (33)

Thus, determination of the relation between $J_{1}$ and $\Delta \psi$, together with the $I_{sc}$ permits the calculation of A. Agreement between the value of A determined using equation 35 and that determined using equation 38 would support the assumed constancy of the affinity. A linear relation between $J_{1}$ and $\Delta \psi$ has been demonstrated for isolated frog skin and equation 38 has been employed to evaluate the effect of aldosterone on A in frog skin [58].

The treatment by Essig and Caplan [49] also provides important insight into the meaning of the resistance of the epithelium to the actively transported cation and the total electrical resistance. Since
\[
-X_{i} = \Delta \psi_{i} = RT \Delta \ln c_{i} + \mathcal{F} \Delta \psi
\]
when $\Delta \ln c_{i}$ and $J_{r}$ are constant, equation 27 yields
\[
\frac{\partial (\Delta \psi)}{\partial J_{r}} = \frac{R_{n1}}{\mathcal{F}}.
\] (39)

However, if $J_{r}$ is a function of $\Delta \psi$, the relation between $J_{1}$ and $\Delta \psi$ must be influenced by the degree of coupling between $J_{r}$ and $J_{1}$. From equation 30 it can be shown that when $\Delta \ln c_{i}$ is constant and if $A$ is independent of $\Delta \psi$,
\[
\frac{\partial (\Delta \psi)}{\partial J_{1}} = -R_{n1} (1-q^2)/\mathcal{F}.
\] (40)
Thus, the greater the degree of coupling, the greater will be the influence of the variation of $J_{1}$ with $\Delta \psi$.

In order to examine the total electrical resistance of the tissue, we must consider the contribution of the anion, $j$, which is driven only by its conjugate force, so that
\[
J_{1} = X_{j}/R_{jj} = -(RT \Delta \ln c_{j} + \mathcal{F} \Delta \psi_{j})/R_{jj}
\] (41)
Thus, when $\Delta \ln c_{j}$ is constant and $z_{j} = -1$,
\[
\frac{\partial (\Delta \psi)}{\partial J_{1}} = R_{jj}/\mathcal{F}.
\] (42)

The total electrical resistance of this system, $R$, is obtained by combining equation 40 and 42, which gives
\[
R = [R_{n1} R_{jj} (1-q^2)] / [R_{n1} (1-q^2) + R_{jj} \mathcal{F}^2].
\] (43)

Clearly, if the current-voltage relation across an epithelium is linear (i.e., constant R) either A or $J_{r}$

The importance of this point can be perhaps better appreciated by writing equations 27 and 28 in terms of generalized conductances. Thus, when both solutions have identical compositions,
\[
A = -ZX_{Na}/q = (\partial J_{1}/\partial J_{r})_{x=0} \mathcal{F} \Delta \psi^0
\] (34)

Thus, determination of the relation between $J_{1}$ and $\Delta \psi$, together with the $I_{sc}$ permits the calculation of A. Agreement between the value of A determined using equation 35 and that determined using equation 38 would support the assumed constancy of the affinity. A linear relation between $J_{1}$ and $\Delta \psi$ has been demonstrated for isolated frog skin and equation 38 has been employed to evaluate the effect of aldosterone on A in frog skin [58].

The treatment by Essig and Caplan [49] also provides important insight into the meaning of the resistance of the epithelium to the actively transported cation and the total electrical resistance. Since
\[
-X_{i} = \Delta \psi_{i} = RT \Delta \ln c_{i} + \mathcal{F} \Delta \psi
\]
when $\Delta \ln c_{i}$ and $J_{r}$ are constant, equation 27 yields
\[
\frac{\partial (\Delta \psi)}{\partial J_{r}} = \frac{R_{n1}}{\mathcal{F}}.
\] (39)

However, if $J_{r}$ is a function of $\Delta \psi$, the relation between $J_{1}$ and $\Delta \psi$ must be influenced by the degree of coupling between $J_{r}$ and $J_{1}$. From equation 30 it can be shown that when $\Delta \ln c_{i}$ is constant and if $A$ is independent of $\Delta \psi$,
\[
\frac{\partial (\Delta \psi)}{\partial J_{1}} = -R_{n1} (1-q^2)/\mathcal{F}.
\] (40)
Thus, the greater the degree of coupling, the greater will be the influence of the variation of $J_{1}$ with $\Delta \psi$.

In order to examine the total electrical resistance of the tissue, we must consider the contribution of the anion, $j$, which is driven only by its conjugate force, so that
\[
J_{1} = X_{j}/R_{jj} = -(RT \Delta \ln c_{j} + \mathcal{F} \Delta \psi_{j})/R_{jj}
\] (41)
Thus, when $\Delta \ln c_{j}$ is constant and $z_{j} = -1$,
\[
\frac{\partial (\Delta \psi)}{\partial J_{1}} = R_{jj}/\mathcal{F}.
\] (42)

The total electrical resistance of this system, $R$, is obtained by combining equation 40 and 42, which gives
\[
R = [R_{n1} R_{jj} (1-q^2)] / [R_{n1} (1-q^2) + R_{jj} \mathcal{F}^2].
\] (43)

Clearly, if the current-voltage relation across an epithelium is linear (i.e., constant R) either A or $J_{r}$

The importance of this point can be perhaps better appreciated by writing equations 27 and 28 in terms of generalized conductances. Thus, when both solutions have identical compositions,
\[
A = -ZX_{Na}/q = (\partial J_{1}/\partial J_{r})_{x=0} \mathcal{F} \Delta \psi^0
\] (34)

The treatment by Essig and Caplan [49] also provides important insight into the meaning of the resistance of the epithelium to the actively transported cation and the total electrical resistance. Since
\[
-X_{i} = \Delta \psi_{i} = RT \Delta \ln c_{i} + \mathcal{F} \Delta \psi
\]
when $\Delta \ln c_{i}$ and $J_{r}$ are constant, equation 27 yields
\[
\frac{\partial (\Delta \psi)}{\partial J_{r}} = \frac{R_{n1}}{\mathcal{F}}.
\] (39)

However, if $J_{r}$ is a function of $\Delta \psi$, the relation between $J_{1}$ and $\Delta \psi$ must be influenced by the degree of coupling between $J_{r}$ and $J_{1}$. From equation 30 it can be shown that when $\Delta \ln c_{i}$ is constant and if $A$ is independent of $\Delta \psi$,
\[
\frac{\partial (\Delta \psi)}{\partial J_{1}} = -R_{n1} (1-q^2)/\mathcal{F}.
\] (40)
Thus, the greater the degree of coupling, the greater will be the influence of the variation of $J_{1}$ with $\Delta \psi$.

In order to examine the total electrical resistance of the tissue, we must consider the contribution of the anion, $j$, which is driven only by its conjugate force, so that
\[
J_{1} = X_{j}/R_{jj} = -(RT \Delta \ln c_{j} + \mathcal{F} \Delta \psi_{j})/R_{jj}
\] (41)
Thus, when $\Delta \ln c_{j}$ is constant and $z_{j} = -1$,
\[
\frac{\partial (\Delta \psi)}{\partial J_{1}} = R_{jj}/\mathcal{F}.
\] (42)

The total electrical resistance of this system, $R$, is obtained by combining equation 40 and 42, which gives
\[
R = [R_{n1} R_{jj} (1-q^2)] / [R_{n1} (1-q^2) + R_{jj} \mathcal{F}^2].
\] (43)

Clearly, if the current-voltage relation across an epithelium is linear (i.e., constant R) either A or $J_{r}$

The importance of this point can be perhaps better appreciated by writing equations 27 and 28 in terms of generalized conductances. Thus, when both solutions have identical compositions,
\[
A = -ZX_{Na}/q = (\partial J_{1}/\partial J_{r})_{x=0} \mathcal{F} \Delta \psi^0
\] (34)

The treatment by Essig and Caplan [49] also provides important insight into the meaning of the resistance of the epithelium to the actively transported cation and the total electrical resistance. Since
\[
-X_{i} = \Delta \psi_{i} = RT \Delta \ln c_{i} + \mathcal{F} \Delta \psi
\]
when $\Delta \ln c_{i}$ and $J_{r}$ are constant, equation 27 yields
\[
\frac{\partial (\Delta \psi)}{\partial J_{r}} = \frac{R_{n1}}{\mathcal{F}}.
\] (39)
must be constant, or they must be linearly related to \( \Delta \psi \). As noted above a linear relation between \( \text{O}_2 \) consumption and \( \Delta \psi \) has been demonstrated for isolated frog skin [54].

Finally, to illustrate this point, for this system in which the monovalent cation, \( i \), is actively transported and the monovalent anion, \( j \), is driven only by its conjugate force, equations 27 and 41 can be written as follows:

\[
J_i = -\frac{\Delta \mu_i}{R_{ii}} - R_{ir} \frac{J_r}{R_{ii}}
\]

and

\[
J_j = -\frac{\Delta \mu_j}{R_{jj}}.
\]

When both solutions have identical compositions \( \frac{z_1}{z_1} = \frac{z_2}{z_2} = \frac{z_3}{z_3} = \cdots \), since under the condition of zero current flow ("open-circuit") \( J_i = J_j \), we obtain [19]

\[
\Delta \psi = -\left[\frac{R_{ii} J_j}{(R_{ii} + R_{jj}) \bar{\varepsilon} j} \right] \cdot \left[\frac{R_{ir} J_r \bar{\varepsilon} r}{R_{ii}}\right].
\]

The first bracketed term in equation 46 is simply the resistance of the tissue attributable to the parallel, passive resistances \( R_{ii} \) and \( R_{jj} \). The second bracketed term is the "open-circuit" current. If \( J_r \) is not a function of \( \Delta \psi \), it follows from equation 32 that under short-circuit conditions,

\[
I_{sc} = \bar{\varepsilon} j (J_j)_{\Delta \psi = 0} = R_{ir} J_r \frac{\bar{\varepsilon} r}{R_{ii}}
\]

so that the "open-circuit" \( \Delta \psi \) (equation 46) is simply the product of the short-circuit current and the lumped resistance of the tissue due only to the parallel resistors \( R_{ii} \) and \( R_{jj} \).

However, if \( J_r \) is a function of \( \Delta \psi \) and \( A \) is constant, the "open-circuit" potential is the product of the tissue resistance, given by equation 43, and the short-circuit current, given by equation 36, i.e.,

\[
\Delta \psi = qA / \left( (R_{ii} / R_{jj}) (1 - q^2) + 1 \right) Z \bar{\varepsilon}.
\]

It should be stressed that equations 46 and 47 are equivalent statements of \( \Delta \psi \). The purpose of this exercise is simply to illustrate the important point that if \( J_r \) is a function of \( \Delta \psi \), then \( \Delta \psi / I_{sc} \) is not solely a function of \( R_{ii} \) and \( R_{jj} \) but is also dependent upon \( q \).

The analysis of the coupling of solute flow to a chemical reaction by Essig and Caplan [49] illustrates the power of the application of nonequilibrium thermodynamics to the study of transport across biological, and in particular, epithelial membranes. Thus, given some assumptions which can be tested experimentally, information regarding the driving force of transport-linked biochemical reactions can be gained from studies that treat the tissue as a "black box" (e.g., [58]).

The experimental system

To this point we have considered the description of solute flows driven by conjugate driving forces with or without coupling to either the flow of solvent or the flow of a chemical reaction. Sauer [17] has recently demonstrated that these three driving forces are formally additive so that a more inclusive equation may be written as follows:

\[
J_i = \bar{\varepsilon} j (\Delta c_i + z_i \bar{\varepsilon} i \Delta \psi/RT) + (1 - \sigma_i) \bar{\varepsilon} j J_j + L_{ir} A.
\]

The application of this equation to the analysis of solute transport across the mammalian nephron is described by Ullrich [46].

Composite membranes

All of the above considerations have been concerned with a description of the flows of solute, and to a lesser extent solvent, across a homogeneous membrane. The passive movements of solutes and solvent across such a barrier traverse pathways having identical properties. However, even the simplest epithelial tissue involves at least two different cell membranes arranged in series and paracellular (shunt) transepithelial pathways that circumvent these limiting membranes. Thus, all epithelia are characterized by series as well as parallel inhomogeneities. Further, even single cell membranes appear to be characterized by regions that are more readily traversed by hydrophilic substances in parallel with regions more readily traversed by hydrophobic substances; thus, all cell membranes must be viewed as a mosaic with parallel inhomogeneities. For this reason, although overall parameters such as \( L_0, \sigma \), and \( \omega \) have descriptive value, caution must be exercised in attempting to interpret these parameters in terms of membrane structure. For example, for a thin homogeneous membrane, when \( \omega_l \to 0, \sigma_l \to 1 \) and when \( \omega_l \) increases, \( \sigma_l \) decreases [6]. However, if a single membrane is comprised of a parallel arrangement of lipid regions and aqueous pores, a large hydrophobic molecule may have a large \( \omega \) or \( P \), but the reflection coefficient (which is, in part, a measure of the interaction of the flow of this molecule with water flow [6, 48]) may be close to unity [59].

Kedem and Katchalsky [60–62] have extended
equations 23 and 24 to (a) a single membrane crossed by two different parallel pathways [61] and to (b) a system consisting of two different homogeneous membranes arranged in series [62]. This analysis demonstrates that the “overall,” experimentally determined, $L_p$, $w_1$, and $\sigma_1$ may be rather complex functions of the elemental parameters characterizing each parallel pathway or membrane. For example, if a membrane is characterized by two different parallel pathways, the overall $\sigma$ for a given solute may be greater than or less than the reflection coefficient(s) of either or both of the individual pathways for that solute. Durbin [63] has suggested a method for relating the reflection coefficients of hydrophilic solutes to the equivalent pore radius across a homogeneous membrane. Clearly, an equivalent pore radius calculated from an “overall $\sigma$” for a composite membrane may have no physical reality.

Conclusions

Linear nonequilibrium thermodynamics provides a framework for the analysis of transepithelial solute flows in terms of responsible driving forces. In particular, it offers an approach for distinguishing between those flows that can be attributed to conjugate driving forces and/or interactions with the flow of solvent, and those flows that appear to involve direct coupling with a chemical reaction. These distinctions provide direction for further investigations aimed at gaining an understanding of transport processes at the molecular level. Nevertheless, this approach is entirely phenomenologic. Thus, while its strength derives from the fact that it requires little knowledge of membrane structure or function, its weakness is that it cannot provide detailed insight into underlying mechanisms of transport. As with any theoretical framework, important gains can result from capitalizing on its strengths but losses can result from failure to recognize its limitations.

Reprint requests to Dr. Stanley G. Schultz, Department of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261, U.S.A.

References

7. DEGROOT SR, MAZUR P: Non-equilibrium Thermodynamics. Amsterdam, North Holland, 1962
14. ONSAGER L: Reciprocal relations in irreversible processes. II. Physiol Rev 38:2265–2279, 1951
Transport across epithelia

41. Fishier RR: The absorption of water and some small solute molecules from the isolated small intestine of the rat. J Physiol (Lond) 130:665—668, 1955