

SOME THEORETICAL ASPECTS OF BIOLOGICAL TRANSPORT

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Much has been said and written in recent years by many authors regarding biological transport. Yet the solutions to the problems, namely, (a) selective permeability and accumulation of ions and non-electrolytes, and (b) electrical potentials of membranes, remain to be found. Recent studies by Leaf [7] on the transepithelial transport of sodium across the mucosal and serosal surfaces clearly point out the inadequacy of widely accepted current concepts. There is enough evidence that electrolyte concentrations in various regions of the system play a crucial role. The answer should come from complete analysis of physicochemical considerations. The historical aspects of the problems have been adequately summarized in recent papers by Hechter [3] and Ling [8]. Analysis of Leaf's experimental results on sodium transport across epithelial cells resolves the over-all problems into two parts: (a) transport across the mucosal surface which includes permeability changes dependent on serosal concentration of potassium and possibly other ions, and (b) the mechanism of active transport of sodium across the serosal surface. Leaf concludes, on the basis of his experimental results, that exchange diffusion between inside potassium and intracellular sodium at the serosal side membrane does not take place and that removal of potassium from serosal medium renders the mucosal surface of epithelial cells impermeable to sodium. He suggests passive entry of sodium across the mucosal surface due to the presence of a favorable concentration gradient, in spite of the existence of a unfavorable electrical potential gradient [2]. Sodium is considered actively extruded across the serosal surface by an electrogenic transport process which does not involve coupling with potassium. In this paper we shall endeavor to present quantitative aspects of possible answers to these two parts.

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Our present theoretical knowledge of permeability comes from considerations of Kirkwood [5] and Katchalsky's group [4]. From phenomenological equations that are valid for steady state, and equations of continuity, Kirkwood defines the permeability and resistance matrices, $|\omega|$ and $|\gamma|$, by the relations

$$\begin{aligned}\omega_{\alpha\beta} &= \frac{|\gamma|_{\alpha\beta}}{|\gamma|} \\ \gamma_{\alpha\beta} &= \int_0^a R_{\alpha\beta}(x) dx \\ \Delta\mu_\alpha &= \mu_\alpha(a) - \mu_\alpha(0) = - \sum_\beta \gamma_{\alpha\beta} J_\beta \\ R_{\alpha\beta} &= \frac{|\Omega|_{\alpha\beta}}{|\Omega|} \\ J_\beta &= c_\beta u_\beta\end{aligned}\quad (1)$$

where $\Omega_{\alpha\beta}$ are the Onsager coefficients. The resistance coefficients $R_{\alpha\beta}$ are related to the local gradients of chemical potential and mass current densities J_β (expressed in moles/unit area/unit time), by the relation

$$\left(\frac{\partial\mu_\alpha}{\partial x}\right) = - \sum_\beta R_{\alpha\beta} J_\beta \quad (2)$$

The membrane is assumed as bounded by two planes normal to x -axis at $x = 0$ and at $x = a$. The membrane phase separates external fluid phases I and II containing a multicomponent electrolyte mixture of r ions and s neutral species. Kirkwood's definition of permeability matrix is restricted to a stationary state when fluxes J_α (defined with respect to external fixed frame of reference) are constant and when a local heterogeneous equilibrium at boundaries with exterior phases I and II exists such that

$$\mu_\alpha^{\text{I}} = \mu_\alpha^{(0)}; \mu_\alpha^{\text{II}} = \mu_\alpha^{(a)} \quad (3)$$

The molecular definition of local gradient in chemical potential corresponding to Eq. (2) is

$$\frac{\partial\mu_\alpha}{\partial x} = \sum_\beta C_\beta \zeta_{\alpha\beta} u_\beta - \zeta_\alpha u_\alpha \quad (4)$$

where c_β , u_β are the local concentrations and mean velocities of ions (or molecules) of kind β . In Eq. (5) $\zeta_{\alpha\beta}$ are the partial frictional coefficients related to the total frictional coefficients ζ_α of α th kind of ion in the system as

$$\zeta_\alpha = \sum_{\beta} c_\beta \zeta_{\alpha\beta} \quad (5)$$

The statistical mechanical derivation [1] of Eq. (4) involved the definition of diffusion currents j_α with reference to the center of mass velocity u .

$$\begin{aligned} j_\alpha &= c_\alpha(u_\alpha - u) \\ &= J_\alpha - c_\alpha u \\ u &= \left(\sum_{\alpha} m_\alpha c_\alpha u_\alpha / \sum_{\alpha} m_\alpha c_\alpha \right) \end{aligned} \quad (6)$$

The summation signs in Eqs. (4) and (5) extend over all neutral and ionic species present in the system. Integration of Eq. (4) over the whole length of the membrane phase yields

$$\int_0^a \left(\frac{\partial \mu_\alpha}{\partial x} \right) dx = \sum_{\beta} \theta_{\alpha\beta} J_\beta - \int_0^a \zeta_\alpha(x) u_\alpha(x) dx$$

where

$$\theta_{\alpha\beta} = \int_0^a \zeta_{\alpha\beta}(x) dx \quad (7)$$

From Eqs. (4) and (7) it is evident that the permeability matrix $|\omega|$ of Eq. (1) will involve explicitly the concentrations c_β . This dependence of permeability on concentrations was presented previously for the case of two mobile components [9, 13]. In addition, there is implicit dependence on concentrations since the partial frictional coefficients are concentration dependent. One may define the inverse of the resistance matrix $|\theta|$ as a permeability matrix $|W|$ defined on a molecular basis.

$$\begin{aligned} |W| &= |\theta|^{-1} \\ W_{\alpha\beta} &= |\theta|_{\alpha\beta} / |\theta| \end{aligned} \quad (8)$$

The advantage of defining $|W|$ in place of $|\omega|$ lies in the fact that the elements of the matrix $|W|$ can be computed quantitatively from molecular parameters such as potential energy of interactions and pair probability functions. The definition of fluxes with reference to center of mass velocity is more convenient theoretically than definition

with respect to a fixed frame of reference. Under isothermal conditions, the frictional force experienced by a molecule of kind α and the isothermal heat flux q in the membrane phase are given by

$$\begin{aligned}\nabla\mu_\alpha &= F_\alpha^{(1,1)*} = - \sum_\beta \zeta_{\alpha\beta} c_\beta (u_\alpha - u_\beta) \\ q &= \sum_\beta Q_\beta^* j_\beta\end{aligned}\quad (9)$$

The total frictional force F_α^* is related to the flux j_β and isothermal heat flux by the relations

$$\begin{aligned}F_\alpha^* &= F_\alpha^{(1,1)*} = - \sum_\beta R_{\alpha\beta}^* j_\beta - R_{\alpha 0}^* q \\ R_{00}^* q &= - \sum_\beta R_{0\beta}^* j_\beta; (\nabla T = 0)\end{aligned}$$

where

$$\begin{aligned}R_{\alpha\alpha}^* &= \sum_{\substack{\gamma \\ \gamma \neq \alpha}} \frac{c_\gamma}{c_\alpha} \zeta_{\alpha\beta} + (\gamma_{\alpha 0}^* Q_\alpha^* / kT) \\ R_{\alpha\beta}^* &= -\zeta_{\alpha\beta} + (\gamma_{\alpha 0}^* Q_\beta^* / kT); \alpha \neq \beta \\ R_{\alpha 0}^* &= -\gamma_{\alpha 0}^* / kT \\ R_{00}^* &= (1/kT) \\ \gamma_{\alpha 0}^* &= (1/c_\alpha) (\partial p_\alpha / \partial \ln T) + \gamma_{\alpha 0}^{(1)} (\neq 0 \text{ when } \nabla T = 0)\end{aligned}\quad (10)$$

p_α is the partial pressure of α and T the temperature. The phenomenological resistance coefficients $R_{\alpha\beta}^*$ are distinguished by the asterisk mark, in order to identify them as different from $R_{\alpha\beta}$ of Eq. (1). In Eq. (10) k is the thermal conductivity of the membrane phase and Q_α^* is the partial isothermal heat of transport. The matrix R^* with elements $R_{\alpha\beta}^*$ is singular. When inertial terms and viscous stresses are considered negligible, in the quasi-stationary equation of motion for the components (Eq. 5.13, Bearman and Kirkwood [1]), one obtains the linear phenomenological relations relating these coefficients $R_{\alpha\beta}^*$ with local gradients in chemical potentials and isothermal heat flux as

$$\begin{aligned}\nabla\mu_\alpha &= - \sum_\beta R_{\alpha\beta}^* j_\beta - R_{\alpha 0}^* q; (\nabla T = 0) \\ R_{00}^* q &= - \sum_\beta R_{0\beta}^* j_\beta\end{aligned}\quad (11)$$

Thus, the gradient of chemical potential of α in the membrane phase, given by the phenomenological theory, can be written as

$$\left(\frac{d\mu_\alpha}{dx}\right) = - \sum_{\beta} R_{\alpha\beta}^* j_\beta - R_{\alpha 0}^* q \quad (12)$$

The same quantity related to molecular parameters is expressed as

$$\left(\frac{\partial\mu_\alpha}{\partial x}\right) = \sum_{\beta} \zeta_{\alpha\beta} J_\beta - \zeta_\alpha u_\alpha \quad (13)$$

By substitution of the values of $R_{\alpha\beta}^*$ of Eq. (10) in Eq. (12) one may verify the equivalence of Eqs. (12) and (13). Under the steady state we note that the diffusion fluxes J_β are constants. Integration of Eq. (13) with respect to x across the membrane for isothermal stationary state yields,

$$\Delta\mu_\alpha + \int_0^a \zeta_\alpha(x) u_\alpha(x) dx = \sum_{\beta} \theta_{\alpha\beta} J_\beta \quad (14)$$

and this may be inverted to yield

$$J_\alpha = \sum_{\beta} W_{\alpha\beta} \left\{ \Delta\mu_\beta + \int_0^a \zeta_\beta(x) u_\beta(x) dx \right\} \quad (15)$$

One may obtain the expression for local mass current density from Eq. (7) as

$$J_\alpha = \sum_{\beta} \xi_{\alpha\beta} \{ \nabla_x \mu_\beta + \zeta_\beta(x) u_\beta(x) \} \\ \xi_{\alpha\beta} = |\zeta|_{\alpha\beta} / |\zeta| \quad (16)$$

where $\xi_{\alpha\beta}$ are the partial permeability coefficients which are functions of x in the membrane phase. It is demonstrated in the appendix that $\zeta_{\alpha\beta}$ and the matrix $|\zeta|$ are essentially invariant with time and that their values in (quasi-) nonsteady state equals their values in steady state and hence characteristic of the membrane phase.

The partial frictional coefficients $\zeta_{\alpha\beta}$ are given by

$$\zeta_{\alpha\beta} = \left\{ \left(\frac{1}{\beta}\right) (D_\alpha^0 + D_\beta^0)^{-1} \right\} \int \left(\frac{dV_{\alpha\beta}}{dr} \right) [\psi_{\alpha\beta} + \psi_{\beta\alpha}] g_{\alpha\beta}^{(2,0)} d^3r \quad (17)$$

where D_α^0 and D_β^0 are the self-diffusion coefficients in infinite dilution of ions α and β ; $V_{\alpha\beta}$ is the potential energy of interaction between α and β , $\psi_{\alpha\beta}$ and $g_{\alpha\beta}^{(2,0)}$ are the perturbation and equilibrium pair correlation functions related to each other by certain differential equa-

tions (Vaidhyathan [10, 11]). From the solutions of $\psi_{\alpha\beta}$ it is evident that $\xi_{\alpha\beta}$ are dependent on total concentrations (Debye-Hueckel ionic atmosphere parameter k^*) of all ions and are only implicitly dependent on individual concentrations c_α and c_β . Therefore one expects the matrices ζ and ξ to be essentially independent (explicitly) of local concentrations of individual mobile ions. This result follows intuitively by the definition of local diffusion coefficient D_α of α th ion being equal to kT/ζ_α , k being the Boltzmann constant. Herein lies the advantage of defining the permeability matrix $|W|$ whose elements are related to the elements of local permeability matrix $|\xi|$ and Kirkwood's phenomenological permeability matrix $|\omega|$ by the relations

$$W_{\alpha\beta} = \int_0^a \xi_{\alpha\beta}(x) dx \quad (18a)$$

$$J_\alpha = - \sum_\beta \omega_{\alpha\beta} \Delta\mu_\beta \quad (18b)$$

$$= \sum_\beta W_{\alpha\beta} \left\{ \Delta\mu_\beta + \int_0^a \zeta_\beta(x) u_\beta(x) dx \right\} \quad (18c)$$

$$\left(\frac{\partial W_{\alpha\beta}}{\partial c_\alpha} \right) = \left(\frac{\partial W_{\alpha\beta}}{\partial c_\gamma} \right) \cong 0 \cong \left(\frac{\partial W_{\alpha\beta}}{\partial t} \right) \quad (18d)$$

In order to compute the variation of permeability of the membrane phase to a specified ion α due to variations in concentrations of another ion γ one has to obtain the variation of J_α with respect to c_γ . Accomplishing this using Kirkwood's Eq. (18b) is complicated. On the other hand, use of Eq. (18c) reduces this complexity since $|W|$ is independent (explicitly) of concentration c_γ of a specified ion γ in a multicomponent electrolyte system.

Thus, we have

$$\begin{aligned} \left(\frac{\partial J_\alpha}{\partial c_\gamma} \right) &= \sum_\beta W_{\alpha\beta} \left(\frac{\partial}{\partial c_\gamma} \right) \left\{ \Delta\mu_\beta + \int_0^a \zeta_\beta(x) \mu_\beta(x) dx \right\} \\ &= \sum_\beta \xi_{\alpha\beta} \left\{ \frac{\partial}{\partial c_\gamma} \left(\frac{\partial \mu_\beta}{\partial x} \right) + \frac{\partial}{\partial c_\gamma} [\zeta_\beta(x) \mu_\beta(x)] \right\} \\ &\quad \left(\frac{\partial \xi_{\alpha\beta}}{\partial c_\gamma} \right) \cong 0 \end{aligned} \quad (19)$$

The chemical potential of ion (or neutral molecule) α in the system may be expressed as

$$\mu_\alpha(x) = \mu_\alpha^0 + RT \ln c_\alpha(x) \gamma_{\alpha^\dagger}(x) + z_\alpha F \varphi(x) \quad (20)$$

where γ_{α^\dagger} is the activity coefficient, $z_\alpha F$ is the charge carried by a mole of the ionic component φ , is the electrostatic potential at

x , and μ_a^0 is the chemical potential at reference state which may be taken as one at infinite dilution. We obtain

$$\frac{\partial}{\partial c_\gamma} \{ \zeta_\beta(x) u_\beta(x) \} = \zeta_{\beta\gamma} u_\beta + \zeta_\beta \left(\frac{\partial u_\beta}{\partial c_\gamma} \right) + \sum_{\substack{\sigma \neq \gamma \\ =1}} \left(\frac{\partial c_\sigma}{\partial c_\gamma} \right) \zeta_{\beta\sigma} u_\beta \quad (21)$$

Use of the definition $J_\beta = c_\beta(x) u_\beta(x)$ yields,

$$\left(\frac{\partial u_\beta}{\partial c_\gamma} \right) = \left(\frac{1}{c_\beta} \right) \left(\frac{\partial J_\beta}{\partial c_\gamma} \right) - u_\beta \left(\frac{\partial \ln c_\beta}{\partial c_\gamma} \right) \quad (22)$$

Substitution of this in Eq. (21) yields

$$\begin{aligned} \frac{\partial}{\partial c_\gamma} \{ \zeta_\beta(x) u_\beta(x) \} = u_\beta(x) \left\{ \zeta_{\beta\gamma} + \sum_{\substack{\sigma \neq \gamma \\ =1}} \left(\frac{\partial c_\sigma}{\partial c_\gamma} \right) \zeta_{\beta\sigma} - \zeta_\beta (\partial \ln c_\beta / \partial c_\gamma) \right\} \\ + (\zeta_\beta / c_\beta) \left(\frac{\partial J_\beta}{\partial c_\gamma} \right) \end{aligned} \quad (23)$$

Therefore we have

$$\begin{aligned} \left(\frac{\partial J_\alpha}{\partial c_\gamma} \right) = \sum_{\beta} \xi_{\alpha\beta} \left[RT \left(\frac{\partial^2 \ln c_\beta \gamma_\beta \dagger}{\partial c_\gamma \partial x} \right) + z_\beta F \left(\frac{\partial^2 \varphi(x)}{\partial c_\gamma \partial x} \right) \right. \\ \left. + u_\beta(x) \left\{ \zeta_{\beta\gamma} + \sum_{\substack{\sigma \neq \gamma \\ =1}} \left(\frac{\partial c_\sigma}{\partial c_\gamma} \right) \zeta_{\beta\sigma} - \zeta_\beta \left(\frac{\partial \ln c_\beta}{\partial c_\gamma} \right) \right\} \right. \\ \left. + (\zeta_\beta / c_\beta) \left(\frac{\partial J_\beta}{\partial c_\gamma} \right) \right] \\ R = \text{gas constant/mole} \end{aligned} \quad (24)$$

If one assumes that variation of concentration of component γ affects only the flux of α , i.e., $(\partial J_\beta / c_\gamma) = 0$ when $\beta \neq \alpha$ we obtain

$$\begin{aligned} \left(\frac{\partial J_\alpha}{\partial c_\gamma} \right) \{ 1 - (\zeta_\alpha \xi_{\alpha\alpha} / c_\alpha) \} = \sum_{\beta \neq \alpha} \xi_{\alpha\beta} \left[RT \left(\frac{\partial^2 \ln c_\beta \gamma_\beta \dagger}{\partial c_\gamma \partial x} \right) + z_\beta F \left(\frac{\partial^2 \varphi}{\partial c_\gamma \partial x} \right) \right. \\ \left. + u_\beta(x) \left\{ \zeta_{\beta\gamma} + \sum_{\substack{\sigma \neq \gamma \\ =1}} \left(\frac{\partial c_\sigma}{\partial c_\gamma} \right) \zeta_{\beta\sigma} - \zeta_\beta \left(\frac{\partial \ln c_\beta}{\partial x} \right) \right\} \right] \\ + \xi_{\alpha\alpha} \left[RT \left(\frac{\partial^2 \ln c_\alpha \gamma_\alpha \dagger}{\partial c_\gamma \partial x} \right) + z_\alpha F \left(\frac{\partial^2 \varphi}{\partial c_\gamma \partial x} \right) \right. \\ \left. + u_\alpha(x) \left\{ \zeta_{\alpha\gamma} + \sum_{\substack{\sigma \neq \gamma \\ =1}} \left(\frac{\partial c_\sigma}{\partial c_\gamma} \right) \zeta_{\alpha\sigma} - \zeta_\alpha \left(\frac{\partial \ln c_\alpha}{\partial c_\gamma} \right) \right\} \right] \\ \alpha \neq \beta \end{aligned} \quad (25)$$

Equation (25) is of intrinsic interest, in spite of the fact that computation of the right-hand side requires the knowledge of $c\sigma$, $\zeta_{\beta\sigma}$, and $(\partial\varphi/\partial c_\gamma)$, which are all functions of x inside the membrane phase. Such information is difficult to obtain experimentally. The molecular theory of membrane physics may evolve in the future so that one could compute all terms of Eq. (25) from molecular properties. However, one may start from Eq. (18a) instead of Eq. (18b) and obtain $(\partial J_\alpha/\partial c_\gamma)$ in terms of quantities which are easy to measure experimentally. From Eq. (18a) we obtain

$$\begin{aligned} \left(\frac{\partial J_\alpha}{\partial c_\gamma}\right) = & \sum_{\beta} W_{\alpha\beta} RT \left[\frac{\partial}{\partial c_\gamma} \left\{ \ln \left(\frac{c_\beta(a)\gamma_{\beta}^\dagger(a)}{c_\beta(0)\gamma_{\beta}^\dagger(0)} \right) + \left(\frac{z_\beta F}{RT} \right) \left(\frac{\partial \Delta\varphi}{\partial c_\gamma} \right) \right\} \right] \\ & + \sum_{\beta} W_{\alpha\beta} \left[\left\{ \frac{J_\beta(a)}{c_\beta(a)D_\beta(a)} \right\} \frac{\partial}{\partial c_\gamma} \left\{ \ln \left[\frac{J_\beta(a)D_\beta(a)}{c_\beta(a)} \right] \right\} \right. \\ & \quad \left. - \left\{ \frac{J_\beta(0)}{c_\beta(0)D_\beta(0)} \right\} \frac{\partial}{\partial c_\gamma} \left\{ \ln \left[\frac{J_\beta(0)D_\beta(0)}{c_\beta(0)} \right] \right\} \right] \\ \Delta\varphi = & \varphi(a) - \varphi(0) \end{aligned} \quad (26)$$

The quantities within parentheses denoted by (a) and (0) refer to experimentally measurable quantities at $x = a$ and at $x = 0$. One may now approximate [cf. Eq. (3)] that

$$\begin{aligned} A_\beta(a) &= A_\beta \text{II} \\ A_\beta(0) &= A_\beta \text{I}; A_\beta = c_\beta, \gamma_{\beta}^\dagger, J_\beta, D_\beta. \end{aligned} \quad (27)$$

In this manner, Eq. (26) represents quantitative variation of flux of component α , with respect to variation in concentration of component γ in either phase I and II or both. Equation (26) is susceptible for numerical computation. One should note that D_β^q ($q = \text{I, II}$) refer to tracer diffusion coefficient of β in phase q .

In order to explain the effect of change in serosal concentration of potassium ions on the mucosal permeability to sodium, one has to invoke that changes in concentration of species other than sodium occur in the mucosal side membrane. These changes are coupled either directly or through (possibly not yet investigated) another component transport. Equation (26) is applicable for both electrolyte and nonelectrolyte component transports and is valid also for nonsteady states not too far removed from steady state. Possible existence of metabolic reactions inside the membrane phase do not assure the existence of

a true steady state when all fluxes J_γ should be time invariant. As shown in a previous paper, the perturbations in concentrations C_γ do not significantly affect the partial frictional coefficients and hence the matrix $|W|$.

Returning now to the second part of the problem, namely, active extrusion of sodium against a concentration and electrical potential gradients across the serosal part of the membrane, the answer is involved though not too difficult to comprehend. It has been shown elsewhere [11, 12] that the magnitude and direction of flux of a specified component (sodium ion in this case) inside a membrane is not entirely determined by electropotential gradient. Equations (18c) and (24), as well as Eq. (26), reiterate these contentions. These equations show that the flux J_α is determined inside the membrane phase by the electropotential gradient (sum of concentration and electrical potential gradients) and another term involving the frictional coefficient and mobility of molecule (or ion) $\zeta_\beta u_\beta$ inside the membrane phase. From molecular definitions of these quantities (Eqs. 5, 17, 25) it is evident that local concentrations and mobilities of all other components (both localized and mobile) molecules and their mobilities determine $\{\zeta_\beta u_\beta(a) - \zeta_\beta u_\beta(0)\}$. Thus, the integral contribution of

$$\sum_{\beta} W_{\alpha\beta} \{\zeta_\beta(a)u_\beta(a) - \zeta_\beta(0)u_\beta(0)\}$$

to J_α could be either positive or negative. *If these terms yield a negative contribution greater in magnitude than the contribution of the term $\sum_{\beta} W_{\alpha\beta} \Delta\mu_\beta$, then net measured direction and magnitude of flux J_α will be opposite to that suggested by combined electrical and concentration potential gradient.* It is this aspect that we have explored in detail for ionic interactions; it forms the basis of our explanation for observed active transport of a specified ion. Specifically, there are two aspects to our explanation. First, the magnitude of and direction of flux of a specified ion is determined by the magnitudes and directions of fluxes of all other neutral and ionic components in the system. Second, a specified component may be facilitated to move in a direction actively determined by certain inequalities involving the ratio of local concentrations of all localized ionic and neutral species and the local concentration of the specified ion in question. We present below a simple argument on the basis of phenomenological equation to support our thesis.

Under steady-state isothermal transport of sodium across the serosal side membrane, in the absence of metabolic reactions inside the membrane phase, the flux of sodium is determined by the relation (Kirkwood [5], Eq. 1)

$$J_{Na} = - \sum_{\beta=1}^{r+s+\delta'} \Omega_{\alpha\beta} (\partial u_{\beta} / \partial x) \quad (28)$$

The origin of the Onsager coefficients $\Omega_{\alpha\beta}$ is to be found in the forces acting between molecules of the several components present in the system. This knowledge may be obtained from molecular theory. Equation 28, may be written as

$$J_{Na} = -\Omega_{NaNa} (\partial \bar{\mu}_{Na}^0 / \partial x) - \left\{ \sum_{\beta}^{r+s+\delta'} \Omega_{Na\beta} z_{\beta} F \right\} \frac{\partial \varphi}{\partial x} - \sum_{\substack{\gamma=2 \\ \neq Na}}^{r+s+\delta'} \Omega_{Na\gamma} (\partial \bar{\mu}_{\gamma}^0 / \partial x) \quad (29)$$

where γ denotes a component other than α . In the serosal side membrane, it is observed experimentally that the concentration part of the sodium chemical potential gradient ($\partial \bar{\mu}_{Na}^0 / \partial x$) and the gradient of electrical potential ($\partial \varphi / \partial x$) have signs opposite to the direction of flux of sodium J_{Na} . If one assigns a positive sign to J_{Na} , i.e., direction of flux of sodium being from intraepithelial cells to serosal side, then for Eq. (29) to hold true the right-hand side should be positive. Kirkwood [5] points out that $\Omega_{\alpha\beta}$ will be positive if the average intermolecular force is one of attraction and will be negative otherwise. If the force is one of repulsion (interaction between like ions), Eqs. (28) and (29) imply that the diffusion flux of component β induces a current of sodium in opposite direction. When all terms of ($\partial \mu_{\gamma}^0 / \partial x$) are zero in Eq. (28) we have,

$$J_{Na} = -\Omega_{NaNa} (\partial \bar{\mu}_{Na}^0 / \partial x) - \left\{ \sum_{\beta=1}^{r+\delta} \Omega_{Na\beta} z_{\beta} F \right\} \frac{\partial \varphi}{\partial x} \quad (30)$$

The terms in the right-hand side of Eq. (30) will be expected to produce a passive flux of sodium from serosal side to epithelial cells, contrary to the experimental observation. Therefore in order to ac-

count for observed results, one has to invoke the conditions that

$$-\sum_{\substack{\gamma=2 \\ \neq \text{Na}}}^{r+s+\delta} \Omega_{Na\gamma} (\partial \bar{\mu}_\gamma^0 / \partial x) > 0 \quad (31)$$

and

$$\sum_{\gamma=2}^{r+s+\delta} \Omega_{Na\gamma} (\partial \bar{\mu}_\gamma^0 / \partial x) < \left[\Omega_{\text{NaNa}} \left(\frac{\partial \mu_{\text{Na}}^0}{\partial x} \right) + \left\{ \sum_{\beta=1}^{r+\delta} \Omega_{\text{Na}\beta} z_\beta F \right\} (\partial \varphi / \partial x) \right]_{\gamma \neq \text{Na}} \quad (32)$$

The summation signs in Eqs. (31) and (32) extend over all values of γ , both ionic and neutral (mobile and localized) components which are present in the membrane phase. In an inhomogeneous membrane $(\partial \bar{\mu}_\delta^0 / \partial x)$ where δ is a localized ion or neutral molecule does not vanish. In the Koefoed-Johnson and Ussing [6] explanation $(\partial \bar{\mu}_\delta^0 / \partial x)$ is considered to produce a flux of potassium in a direction opposite to the direction of flux of sodium. Leaf's experimental results suggest that the flux of sodium across the serosal side is not coupled with fluxes of potassium, hydrogen, chloride, or other anions. The inequalities of Eqs. (31) and (32) which determine the net direction of transport of sodium from epithelial to serosal side does not specifically demand coupled transport with other ions.

One may foresee the development of a molecular explanation on the basis of equations presented. Substituting Eqs. (4) and (5) in Eq. (28), the equation for the flux of sodium inside the membrane phase is obtained as

$$J_{Na} = \sum_{\beta, \gamma=1}^{r+\delta'} \Omega_{\alpha\beta} \{ c_\gamma \zeta_{\beta\gamma} (u_\gamma - u_\beta) \} - \sum_{\beta, \delta=1}^{\delta'} \Omega_{\alpha\beta} c_\delta \zeta_{\beta\delta} u_\beta \quad (33)$$

where δ' denotes localized ions and neutral molecules. It is evident from Eq. (33) that the partial frictional coefficients of mobile ion-localized ion (and molecules) interactions and the concentrations of these localized molecules determine the direction and magnitude of the flux of sodium ions.

APPENDIX

The perturbation functions $\psi_{\alpha\delta}$, where α is a mobile ion and δ is a localized ion in the membrane phase, obey a set of differential equations

in the quasi-nonsteady states of the type [12]

$$\begin{aligned}
 L\psi_{\alpha\delta} - 4\pi\theta \sum_{\beta} F_{\alpha\beta}\psi_{\delta\beta} &= (d \ln g_{\alpha\delta}^{(2,0)}/dr) - (1/u_{\alpha}) \frac{\partial \ln g_{\alpha\delta}^{(2,0)}}{\partial t} \\
 L\psi_{\delta\alpha} - 4\pi\theta \sum_{\beta} F_{\alpha\beta}\psi_{\delta\beta} &= (d \ln g_{\alpha\delta}^{(2,0)}/dr) + (1/u_{\alpha}) \frac{\partial \ln g_{\alpha\delta}^{(2,0)}}{\partial t} \\
 F_{\alpha\beta} &= Z_{\beta}C_{\beta}Z_{\alpha}\eta_{\alpha\beta} \\
 \eta_{\alpha\beta} &= (D_{\alpha}^0 u_{\beta}/D_{\beta}^0 u_{\alpha}) \\
 \theta &= (e^2/\epsilon kT) \\
 L &= (d^2/dr^2) + (2/r)(d/dr) - (2/r^2)
 \end{aligned} \tag{A.1}$$

In Eq. (A.1), t is the time variable, r is the space variable, e is the magnitude of charge of an electron, and ϵ is the dielectric constant of the medium; u_{α} and u_{β} refer to the magnitude of velocities of α and β inside the membrane phase. The remaining quantities have been defined in the main text. Inspection of Eq. (A.1) suggests that the solutions are of the form

$$\begin{aligned}
 \psi_{\alpha\delta}(r,t) &= A(r) - B(r,t) \\
 \psi_{\delta\alpha}(r,t) &= A(r) + B(r,t) \\
 \psi_{\alpha\delta}(r,t) &\neq \psi_{\delta\alpha}(r,t)
 \end{aligned} \tag{A.2}$$

where $A(r)$ and $B(r,t)$ are solutions of differential equations

$$\begin{aligned}
 L\psi_{\alpha\delta} - 4\pi\theta \sum_{\beta} F_{\alpha\beta}\psi_{\delta\beta} &= \frac{d}{dr} \ln g_{\alpha\delta}^{(2,0)} \\
 L\psi_{\delta\alpha} - 4\pi\theta \sum_{\beta} F_{\alpha\beta}\psi_{\delta\beta} &= (1/u_{\alpha}) \left\{ \frac{\partial \ln g_{\alpha\delta}^{(2,0)}}{\partial t} \right\}
 \end{aligned} \tag{A.3}$$

respectively. From Eq. (A.2) it follows that the sum $[\psi_{\alpha\delta}(r,t) + \psi_{\delta\alpha}(r,t)]$ is time independent. Similar analysis of the differential equations that $\psi_{\alpha\beta}$ and $\psi_{\beta\alpha}$ obey in the quasi-nonsteady states (α and β are both mobile ions) yield the result that the sum $[\psi_{\alpha\beta}(r,t) + \psi_{\beta\alpha}(r,t)]$ is also independent of time. Since only these sums occur in the integrals for partial frictional coefficients $\zeta_{\alpha\delta}$ and $\zeta_{\alpha\beta}$ [Eq. (17)] it follows that $\zeta_{\alpha\delta}$ and $\zeta_{\alpha\beta}$ will be essentially independent of time [except for negligible contribution from time dependence of equilibrium pair correlation functions $g_{\alpha\delta}^{(2,0)}$ and $g_{\alpha\beta}^{(2,0)}$]. Therefore, the elements of the local permeability matrix $[\xi]$ will be time invariant. Hence, the elements $W_{\alpha\beta}$ are also independent of time for small departures of the state of the system from steady state.

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