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Tracer Flux Ratios: A Phenomenological Approach

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Summary. The kinetic behavior of tracer flows across epithelial membranes is examined and attention is called to the conditions under which unidirectional tracer flows may be described by first order rate equations. It is shown that the first order nature of the tracer rate equations when combined with simple thermodynamic constraints on tracer flow yields a relation between the *ratio* of the unidirectional rate coefficients and thermodynamic driving forces. The form of this relation is examined for the case of simple diffusion and in the presence of coupled processes.

Two phenomenological approaches have been particularly useful in characterizing the movement of matter across epithelial membranes. One, the thermodynamic approach, considers *net* flows in relation to thermodynamic driving forces. The other, referred to here as the kinetic approach, aims to characterize transepithelial transport processes by means of unidirectional rate coefficients derived from measurements of radioactive isotope (tracer) flow. The purpose of this paper is to show that a simple combination of these two phenomenological systems leads to a useful, global relation between unidirectional rate coefficients and thermodynamic driving forces, namely the well-known "flux-ratio equation" first formulated by Ussing (1949).[1]

The Thermodynamic Approach

The thermodynamic approach to membrane transport is, perhaps, best exemplified by the work of Kedem and Katchalsky (1958, 1962). They employed the formalism of irreversible thermodynamics to obtain

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[1] A preliminary report of this work was presented at the 1975 meeting of the Biophysical Society (Dawson, 1975).

a phenomenological description of the relation between the flows and forces which interact to produce observable membrane transport phenomena. One particularly useful result of this work was the introduction by Kedem (1961) of a general expression which relates the net flow of some species, *i*, across a membrane to three classes of thermodynamic forces, i.e.:

$$J_i = \frac{-1}{R_{ii}} \left(\Delta \tilde{u}_i + \sum_{\substack{j=1 \\ j \neq i}}^{n} R_{ij} J_j + R_{ir} J_r \right). \tag{1}$$

Where J_i is the net flow of *i* and $\Delta \tilde{\mu}_i$ is the difference in electrochemical potential for *i* across the membrane, the so-called "conjugate force". J_j represents other transmembrane flows of matter and J_r represents the flow of a chemical reaction within the membrane. The *R*'s are the phenomenological coefficients. This perspective is particularly useful in studies of transport by isolated epithelial sheets where $\Delta \tilde{\mu}_i$ may be conveniently reduced to zero and the possible dependence of J_i on coupled processes thereby revealed.

The Kinetic Approach

The characterization of epithelial transport processes by steady-flow, isotopic rate coefficients is closely related to the broad area of "compartmental analysis". Since the assumptions inherent in the kinetic approach have considerable bearing on the principal result of this paper, we consider them in some detail. To begin let us examine the experimental setting in which isotopic rate coefficients are obtained by the measurement of so-called "unidirectional" isotope flows. Consider a membrane of arbitrary geometry which separates two solutions of known composition. The membrane surfaces may be bounded by unstirred layers, but there must exist well-stirred regions of bulk solution on either side where it is possible to define the concentrations of the constituents of the solution and the electrical potential difference across the membrane. For the purpose of simplicity, we assume that temperature and pressure are uniform throughout the system and that the net volume flow is negligible.

To obtain the steady-flow, isotopic rate coefficient for a substance, *i*, a quantity of a radioactive isotope of *i*, *i**, is added to the solution bathing side 1 of the membrane, the "hot side". Sampling of side 2 is restricted to times greater than that required to reach steady-flow and less than that required to accumulate isotope to a degree sufficient to produce an appreciable "back-flux". During this time the isotope flow from side 1 to side 2 is, for practical purposes, unidirectional.

The operational definition of the unidirectional flux of tracer from side 1 to side 2 is given by:

$$J_{12}^* \equiv C_2^*(2) - C_2^*(1)/(t_2 - t_1)(A),$$

where, J_{12}^* is the unidirectional tracer flow from side 1 to side 2, $C_2^*(1)$ is the concentration of tracer on the cold side in counts per min per ml at time t_1, $C_2^*(2)$ is the concentration of tracer on the cold side at time t_2, and A is the area of the membrane. Experimental design is typically such that the concentration of tracer on the hot side does not change appreciably during the course of the tracer flow measurement and the cold side represents an infinite sink for tracer flow. Accordingly, we define the unidirectional rate coefficients for tracer flow, K_{12}^* and K_{21}^* as:

$$K_{12}^* = J_{12}^*/C_1^* \qquad K_{21}^* = J_{21}^*/C_2^*, \tag{2}$$

where J_{12}^* and J_{21}^* are unidirectional tracer flows measured under conditions of steady tracer flow and C_1^* and C_2^* are the concentrations of tracer on the respective hot sides.

For the tracer rate coefficient to be a useful phenomenological description of the properties of the membrane, two conditions must obtain:

(a) The properties of the membrane must be time invariant.

(b) The addition of tracer to the system must not alter the properties of the membrane.

Clearly, if the tracer rate coefficient is to be a measure of the properties of the membrane, these properties should not change appreciably during the period of time required to measure tracer flow. This condition is commonly expressed as the requirement that the system be in a "steady-state" (Solomon, 1960). Practically, this means that the composition of the bathing solutions, the transmural electrical potential and the isotopic rate coefficients do not vary with time or, if so, only very slowly with regard to the intersample interval, $t_2 - t_1$. In practice this condition is relatively easily met using a variety of isolated epithelial preparations.

The properties of the membrane which are reflected in the tracer rate coefficient may be a strong function of the total concentration of the *abundant* isotope. We assume, however, that tracer may be added to the system in such a minute quantity on a molar basis that the properties of the membrane remain virtually unchanged. Practically, this means that we may add many "counts per minute" to the hot side by adding a negligible number of moles of i^*. A large gradient of "counts" may, therefore, be introduced without disturbing the steady-state. The tracer rate coefficient thus provides a phenomenological measure of the properties of the membrane, and we assume that tracer may be introduced into the system in quantities which are so small that the tracer rate coefficient is independent of tracer concentration.

An important consequence of these assumptions is that Eq. (2) constitutes true *first order* rate equations for tracer flow. Inasmuch as the coefficients K_{12}^* and K_{21}^* relate tracer flow, J^*, to tracer concentration, C^*, they are *constants*. They remain constant as long as the properties of the membrane do not change. This is simply to assert, as pointed out by Robertson (1957) and Solomon (1960) that the rate equations for tracer movement are, in general, *first order* regardless of the actual kinetic order of the process being studied. This assumption has been examined in detail for chemical reaction kinetics by Fleck (1972). Thus, although the rate coefficients for tracer movement may depend on the concentration of the abundant isotope and the electrical potential difference, as well as other variables, the tracer rate coefficients will not depend on tracer concentrations. Ussing (1952) recognized that tracers which behave according to these assumptions can provide "new information" about the properties of a membrane. This new information arises because the properties may be measured by introducing a vanishingly small perturbation into the system.

The Flux Ratio for Simple Diffusion

In his classic paper Ussing (1949) proposed that transport by simple diffusion could be distinguished from "active transport" by examining the *ratio* of the unidirectional *tracer* fluxes across a membrane. He demonstrated that for a substance which moves by simple diffusion, the steady-state tracer flux ratio is given by:

$$J_{12}^*/J_{21}^* = (\gamma_1^* C_1^*/\gamma_2^* C_2^*) \exp\left\{\frac{zF}{RT}(\psi_1 - \psi_2)\right\}, \tag{3}$$

where J_{12}^*, J_{21}^*, C_1^*, C_2^* are unidirectional tracer flows and tracer concentrations as defined previously and γ_1^* and γ_2^* are the activity coefficients for the *tracer*. z, F, R, T have their usual significance and $\psi_1 - \psi_2$ is the electrical potential difference across the membrane. Ussing developed this useful relation by assuming that the diffusional flow of a tracer ion at any point in the membrane could be described by the Nernst-Planck equation. By taking the *ratio* of the unidirectional tracer fluxes he was able to eliminate all terms which depend on the physical properties of the membrane. Integrating this ratio across the membrane for steady-state tracer flow, he obtained the well known result that, for simple diffusional flow, the tracer flux ratio depends only on the activities of tracer in the bathing solutions and the electrical potential difference across the membrane. More recently, several authors have examined in detail the theoretical foundations of the flux-ratio equation in an effort to clearly define the necessary assumptions about tracer movement, and to generalize the flux-ratio equation to include the effects of coupled processes and membrane inhomogeneity (Hoshiko & Lindley, 1964; Kedem & Essig, 1965; Schwartz, 1971). These treatments, however, also proceed from a local description of flows and forces to a global flux-ratio equation. It is the purpose of this communication to demonstrate that the flux-ratio equation can be derived from a simple combination of the thermodynamic and kinetic approaches to transport without resort to a local description of flow.

Consider again the membrane of arbitrary geometry separating two solutions. The movement of a substance, *i*, across the membrane may be characterized by the tracer rate coefficients given in Eq. (2). In accordance with the previous discussion, we assume that these constitute *first order* equations for tracer flow, i.e., that the rate coefficients are independent of tracer concentrations. In addition, it follows that the ratio K_{12}^*/K_{21}^* does not depend on the ratio C_1^*/C_2^*.

To complete the development of the flux-ratio equation it is necessary to specify simple diffusion by some phenomenologic criterion. It is only necessary to stipulate that the flow of tracer is not coupled to any other flow of matter across the membrane (including the abundant isotope of the tracer species) or to any chemical reaction in the membrane. Specifically: in any particular stationary state of the system, regardless of the distribution of the abundant isotope, it must be possible for the *tracer* to attain thermodynamic equilibrium. At *tracer* equilibrium the electrochemical potential difference for the tracer, $\Delta\tilde{\mu}_*$, must be zero and:

$$(\gamma_1^* C_1^*/\gamma_2^* C_2^*)_{\text{equil.}} = \exp\left\{\frac{zF}{RT}(\psi_2-\psi_1)\right\}. \tag{4}$$

Inserting this relation into the expression for the ratio of the rate coefficients, K_{12}^*/K_{21}^*, and noting that at equilibrium the unidirectional tracer fluxes are equal, we obtain for the ratio of the tracer rate coefficients at *tracer* equilibrium:

$$(K_{12}^*/K_{21}^*)_{\text{equil.}} = (\gamma_1^*/\gamma_2^*)\exp\left\{\frac{zF}{RT}(\psi_1-\psi_2)\right\}. \tag{5}$$

Eq. (5) constitutes a relation between the tracer rate coefficients at *tracer equilibrium.* Clearly, it is not possible to measure the rate coefficients at tracer equilibrium. Our assumptions about tracer behavior, however, require that the tracer rate coefficients and their ratio, K_{12}^*/K_{21}^*, be independent of the distribution of the tracer, C_1^*/C_2^*. Thus, rate coefficients measured in unidirectional flux experiments, i.e., with the tracer far from equilibrium, must also conform to this relation. Eq. (5) must apply to all tracer states and constitutes, therefore, a general relation between tracer rate coefficients for simple diffusional movement. Taking the ratio of the unidirectional tracer fluxes and inserting Eq. (4), we obtain the well-known flux ratio equation in the form originally given by Ussing (1949).

$$J_{12}^*/J_{21}^* = (\gamma_1^* C_1^*/\gamma_2^* C_2^*) \exp\left\{\frac{zF}{RT}(\psi_1 - \psi_2)\right\}. \tag{6}$$

Thus, from the phenomenologic viewpoint the flux-ratio equation for simple diffusional flow is a result of the general first order nature of tracer rate equations and the stipulation that, if the tracer moves by simple diffusion, it must be able to attain thermodynamic equilibrium. Eq. (6) does not constitute the most generally useful form of the flux ratio equation because it contains the terms γ_1^* and γ_2^*, the activity coefficients for the *tracer.* As indicated by Ussing (1949) this equation is most useful when both sides of the membrane are bathed by identical solutions, in which case the ratio of the tracer activity coefficients would be unity. Eq. (6) can be cast in a somewhat more general form by introducing the postulate of "kinetic indistinguishability", i.e., by assuming that the tracer and the abundant isotope are identical in all thermodynamic and kinetic properties. With this assumption we write for the tracer activity coefficients:

$$\gamma_1^* = \gamma_1 = a_1/C_1 \qquad \gamma_2^* = \gamma_2 = a_2/C_2, \tag{7}$$

where γ_1 and γ_2, a_1 and a_2, C_1 and C_2 represent the activity coefficient, the activity and the concentration of the *abundant* isotope of the tracer species on side 1 and side 2 of the membrane. Inserting these relations into Eq. (6) we obtain the practical form of the flux-ratio equation:

$$\frac{J_{12}^*/\rho_1}{J_{21}^*/\rho_2} = \frac{a_1}{a_2} \exp\left\{\frac{zF}{RT}(\psi_1 - \psi_2)\right\}, \tag{8}$$

where ρ_1 and ρ_2 are the specific activities of the tracer on side 1 and side 2, i.e.

$$\rho_1 = C_1^*/C_1 \qquad \rho_2 = C_2^*/C_2. \tag{9}$$

The Flux Ratio in the Presence of Coupled Processes

This phenomenologic approach to tracer behavior can be extended to "coupled transport" systems by noting that, in general, the flow of tracer may be coupled to other transmembrane flows of matter or to chemical reactions in the membrane. Making use of Eq. (1) we can express the *net* flow of tracer in general as:

$$J^*_{\text{net}} = -\Delta\tilde{\mu}_*/R_* - \sum_{k=1}^{n} R_{*k} J_k/R_*, \tag{10}$$

where R_* and R_{*k} are phenomenological coefficients and J_k is the net flow of the k-th species. The summation from 1 to n is taken to include all other flows of matter across the membrane (including the abundant isotope) and the flows of any chemical reactions linked to J^*. At true tracer "steady-state", i.e., $J^*_{\text{net}}=0$, we obtain for the electrochemical potential difference of the tracer:

$$\Delta\tilde{\mu}_* = -\sum_{k=1}^{n} R_{*k} J_k. \tag{11}$$

With Eq. (2) this yields for the ratio of the tracer rate coefficients:

$$K^*_{12}/K^*_{21} = (\gamma^*_1/\gamma^*_2)\exp\left\{\frac{zF(\psi_1-\psi_2)+\sum_{k=1}^{n} R_{*k} J_k}{RT}\right\}. \tag{12}$$

Again, noting that K^*_{12} and K^*_{21} are independent of C^*_1 and C^*_2 we insert Eq. (12) into the definition for the flux ratio and obtain, with the assumption of kinetic indistinguishability, the flux ratio in the presence of coupled processes:

$$\frac{J^*_{12}/\rho_1}{J^*_{21}/\rho_2} = \frac{a_1}{a_2}\exp\left\{\frac{zF(\psi_1-\psi_2)+\sum_{k=1}^{n} R_{*k} J_k}{RT}\right\}. \tag{13}$$

This result is formally identical to that obtained by Kedem and Essig (1965), although it should be noted that the precise identification of the phenomenological coefficients will depend on the nature of the coupled processes and the presence or absence of parallel paths for transmural ion flow.

Hoshiko and Lindley (1970) have stressed the need for macroscopic specification of ideal tracer behavior. The present development provides a simple, phenomenological approach to the behavior of tracers using only the postulates of compartmental analysis and thermodynamics. The flux-ratio equation emerges from a consideration of the first-order nature of the tracer rate coefficient and the thermodynamic constraints on tracer distribution in the presence and absence of coupled processes.

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References

Dawson, D.C. 1975. A macroscopic development of the Ussing flux-ratio equation. *Biophys. J.* **15**:112*a*

Fleck, G.M. 1972. On the generality of first-order rates in isotopic tracer studies. *J. Theor. Biol.* **34**:509

Hoshiko, T., Lindley, B.D. 1964. The relationship of Ussing's flux-ratio equation to the thermodynamic description of membrane permeability. *Biochim. Biophys. Acta.* **79**:301

Hoshiko, T., Lindley, B.D. 1970. Macroscopic definition of ideal behavior of tracers. *J. Theor. Biol.* **26**:315

Katchalsky, A., Curran, P.F. 1967. Non equilibrium Thermodynamics in Biophysics. Harvard University Press, Cambridge

Katchalsky, A., Kedem, O. 1962. Thermodynamics of flow processes in biological systems. *Biophys. J.* **2**:53

Kedem, O. 1961. Criteria of active transport. *In:* Membrane Transport and Metabolism. A. Kleinzeller and A. Kotyk, editors. p. 87. Academic Press, New York

Kedem, O., Essig, A. 1965. Isotope flows and flux ratios in biological membranes. *J. Gen. Physiol.* **48**:1047

Kedem, O., Katchalsky, A. 1958. Thermodynamic analysis of the permeability of biological membranes to non-electrolytes. *Biochim. Biophys. Acta.* **27**:229

Robertson, J.S. 1957. Theory and use of tracers in determining transfer rates in biological systems. *Physiol. Rev.* **37**:133

Schwartz, T.L. 1971. The validity of the Ussing flux-ratio equation in a three dimensionally inhomogeneous membrane. *Biophys. J.* **11**:596

Solomon, A.K. 1960. Compartmental methods of kinetic analysis. *In:* Mineral Metabolism I. C.L. Comar and F. Bronner, editors. p. 119. Academic Press, New York

Ussing, H.H. 1949. The distinction by means of tracers between active transport and diffusion. *Acta Physiol. Scand.* **19**:43

Ussing, H.H. 1952. Some aspects of the application of tracers in permeability studies. *Adv. Enzymol.* **13**:31