Tracer Flow, Permeability, and Partial Conductance

A. Essig and J. H. Li

Department of Physiology, Boston University School of Medicine,
Boston, Massachusetts

Summary. It is often not possible to evaluate a permeability coefficient for net flow P from the small flows produced by physiological gradients of concentration or electrical potential. The common use of a tracer permeability coefficient P^x for this purpose, under the assumption that $P^x = P$, requires that the species be transported passively, and that there be no significant coupling between its flow and that of other chemical species, and between the flows of its tracer and abundant isotopes (isotope interaction). These conditions are often not satisfied. However, for passive transport in the absence of coupling of flows of different chemical species the measurement of tracer flow at two values of electrical potential difference evaluates (P^x/P) and thus P. In the presence of coupling of flows of different chemical species, although these measurements no longer evaluate P, they evaluate the partial conductance G. A graphical method of evaluating (P^x/P), P, and G is presented.

A fundamental problem in the characterization of membrane transport processes is the evaluation of the permeability coefficient:

$$P_i = -J_i/\varDelta c_i, \tag{1}$$

where J_i is the net flow and $\varDelta c_i$ the concentration difference of the species of interest. When the flow is produced by an electrical driving force the common assumption of equivalence of chemical and electrical forces [6][1] leads to an alternative expression:

$$P_i = -R\,TJ_i/c_i\,z_i\,F\varDelta\psi, \tag{2}$$

Send correspondence to: Dr. A. Essig, Department of Physiology, Boston University School of Medicine, 80 East Concord Street, Boston, Mass. 02118.

1 This assumption is broadly applicable for ion transport in solutions but may require experimental verification in a biological membrane; it will be most appropriate when $\varDelta \ln c$ is small.

R being the gas constant, T the absolute temperature, z_i the ionic valence, F the Faraday constant, and $\Delta\psi$ the electrical potential difference across the membrane. (Our polarity conventions are that $\Delta c = c(2) - c(1)$, $\Delta\psi = \Psi(2) - \Psi(1)$, and flow is taken as positive from region (1) to region (2). We will include the subscript or superscript referring to species i only when its omission would lead to confusion.)

In practice it is often impractical to evaluate P by the above means. Physiological flows may be absent or very small. The measurement of flows by chemical techniques may be impossible, and evaluation of the difference of inward and outward unidirectional fluxes may be laborious and imprecise. Attempts to make Δc and J large enough for accurate measurement may alter the state of the system, with poorly defined effects on P.

For such reasons, permeability is often evaluated from the measurement of the tracer flow J_i^x induced by a tracer concentration difference Δc_i^x in the absence of net flow of the test substance and other species:

$$P_i^x = (-J_i^x / \Delta c_i^x)_{J_{i, j} = 0}. \tag{3}$$

Implicit in this approach is the assumption that P^x evaluated by means of Eq. (3) has the same value as would be obtained for P, if P could be evaluated directly by means of Eq. (1) or (2). As has been discussed previously, this can be expected to be the case if, and only if, there is (a) no active transport, (b) no significant coupling of the flow of the test species with flows of other solutes or solvent, and (c) no significant coupling of flows of the tracer and abundant forms of the test species ("isotope interaction") [4, 5, 7, 11].

A test for the satisfaction of these conditions is provided by the measurement of the ratio of unidirectional fluxes across a membrane [4, 5, 7, 11]. Placement of tracer "outside" a membrane permits the evaluation of the influx $\vec{J} = -J^x / \Delta\rho$, where $\Delta\rho$ is the difference in specific activity $\Delta(c^x/c)$ of the tracer across the membrane, initially $\simeq -\rho^{out}$. Placement of tracer on the "inside" similarly permits the evaluation of the outflux $\overleftarrow{J} \simeq -J^x / \rho^{in}$. If conditions (a)–(c) are satisfied the flux ratio is said to be "normal", being given by

$$f = \vec{J} / \overleftarrow{J} = \exp[X/RT], \tag{4}$$

where X is the negative electrochemical potential difference of the test species across the membrane. With an electrical driving force,

$$f = \exp[-zF\Delta\psi/RT] \quad (\Delta c = 0). \tag{5}$$

Unfortunately, in many circumstances it may not be possible to measure both unidirectional fluxes, or the measurements may be inaccurate. It may however be possible to measure a single unidirectional flux at two settings of $\Delta\psi$, and thereby obtain equivalent information. It is convenient to choose one setting of $\Delta\psi$ to be zero. Then, combining Eqs. (2), (3) and (5), and remembering that $J = \vec{J} - \overleftarrow{J}$,

$$\vec{J}_{\Delta\psi}/\vec{J}_0 \equiv \overleftarrow{J}_{-\Delta\psi}/\overleftarrow{J}_0 = \frac{zF\Delta\psi/RT}{\exp[zF\Delta\psi/RT]-1}. \tag{6}$$

(In the absence of rectification the current voltage relationship is linear and $\vec{J}_{\Delta\psi} = \overleftarrow{J}_{-\Delta\psi}$ over some range $-x < \Delta\psi < x$. Since for passive flows $\vec{J}_0 = \overleftarrow{J}_0$, in this range $\vec{J}_{\Delta\psi}/\vec{J}_0 = \overleftarrow{J}_{-\Delta\psi}/\overleftarrow{J}_0$.) Good agreement with Eq. (6) suggests that conditions (a)–(c) are satisfied [10]. On the other hand, if these conditions can be assumed on the basis of independent evidence, Eq. (6) may be used to evaluate fundamental parameters of a system.

Frizzell and Schultz applied such an analysis to rabbit ileum, for fluxes comprising parallel active and passive components [3]. For passive fluxes induced by small values of $z\Delta\psi$, Eq. (6) was approximated by

$$\vec{J}_{\Delta\psi}/\vec{J}_0 \simeq \{\exp[zF\Delta\psi/RT]\}^{-1/2} \equiv \xi^{-1/2}, \tag{7}$$

where the identity at the right indicates Frizzell and Schultz's nomenclature. Unidirectional fluxes of Na^+, K^+, and Cl^- at various settings of $\Delta\psi$ were plotted as a function of $\xi^{-1/2}$. The intercept of the least-squares line at $\xi = 0$ then represented the active influx of species i across the mucosal membrane, presumed insensitive to $\Delta\psi$, and the slope represented the unidirectional passive shunt flux at short circuit $_0J_d^i$ (corresponding in our terms to $\vec{J}_0 = \overleftarrow{J}_0$). $_0J_d^i$ is simply related to the partial conductance $G_i = -I_i/\Delta\psi$ since $_0J_d^i = -(J^x/\Delta c^x)_{J_i=0}c = P^x c = Pc$, and with Eq. (2), since the current $I_i = z_i F J_i$,

$$G_i = (z_i^2 F^2/RT)_0 J_d^i.$$

Clarkson's studies in rat ileum [1] support the validity of conditions (a)–(c) here, but this should not be assumed always to be the case. More generally it is necessary to consider the possible influence of isotope interaction ($P \neq P^x$) and the contribution of coupled material flows and metabolism to the forces determining transport of species i. We shall designate this latter quantity $\Sigma R_{ij} J_j$, where J_j represents a flow, and R_{ij} the corresponding phenomenological resistance coefficient [5]. In the presence of these influences the flux ratio becomes "abnormal", being given by

$$f = \exp[(P/P^x)(X - \Sigma R_{ij} J_j)/RT]. \tag{8}$$

For electrical driving forces the combination of Eqs. (2) and (8) gives

$$\vec{J}_{\Delta\psi}/\vec{J}_0 = \frac{(P/P^x)(zF\Delta\psi + \Sigma R_{ij}J_j)/RT}{\exp[(P/P^x)(zF\Delta\psi + \Sigma R_{ij}J_j)/RT] - 1} \qquad (\Delta c = 0). \qquad (9)$$

With passive coupled flows proportional to the electrical potential difference, we may introduce the proportionality constant α, defined by the relation $\Sigma R_{ij}J_j = \alpha zF\Delta\psi$. It is then useful to define a quantity which incorporates all of the factors which may produce abnormality of the flux ratio: $\Theta = (P/P^x)(1 + \alpha)$. This permits rewriting Eq. (9) in the convenient form

$$\vec{J}_{\Delta\psi}/\vec{J}_0 = \frac{\Theta zF\Delta\psi/RT}{\exp[\Theta zF\Delta\psi/RT] - 1} \qquad (\Delta c = 0). \qquad (10)$$

For sufficiently small values of $\Theta\Delta\psi$,

$$\vec{J}_{\Delta\psi}/\vec{J}_0 \simeq \{\exp[\Theta zF\Delta\psi/RT]\}^{-1/2},$$

and   (11)

$$\ln(\vec{J}_{\Delta\psi}/\vec{J}_0) \simeq (-1/2)\Theta zF\Delta\psi/RT.$$

The finding that the slope of the plot of $\ln(\vec{J}_{\Delta\psi}/\vec{J}_0)$ (or $\ln\vec{J}_{\Delta\psi}$ or $\ln\vec{J}_{-\Delta\psi}$) against $zF\Delta\psi/RT$ differs from $-1/2$ (i.e. $\Theta \neq 1$) indicates coupling of flows of different species (i.e. $\alpha \neq 0$) and/or isotope interaction ($P \neq P^x$). In general it may not be possible to distinguish between these two effects. For example, $\Theta > 1$ might result from either positive isotope interaction (with $P/P^x > 1$) or $\alpha > 0$. In some cases however a distinction will be possible. Thus, the recent demonstration of $\Theta < 1$ in a highly permselective ion exchange membrane [2] is unequivocal evidence for negative isotope interaction (i.e. $P/P^x < 1$), irrespective of the possible influence of electroosmotic water flow J_w, since in this case $\alpha = R_{iw}J_w/zF\Delta\psi > 0$. (For electroosmotic coupling R_{iw} is < 0 and J_w is opposite in sign to $zF\Delta\psi$ [8].)

In contrast to Eq. (11), Eq. (10) is exact and can be applied over a wide range of values of $\Theta\Delta\psi$, provided only that net flows are linear functions of $\Delta\psi$ in this range. Because Eq. (10) cannot be solved explicitly for Θ it is useful to present a graph representing the dependence of $\vec{J}_{\Delta\psi}/\vec{J}_0 \equiv \vec{J}_{-\Delta\psi}/\vec{J}_0$ on the dimensionless quantity $\Theta zF\Delta\psi/RT$ (which we will call η). This will allow the graphical evaluation of η (or Θ) from the value of $\vec{J}_{\Delta\psi}/\vec{J}_0$ determined experimentally at any setting of $z\Delta\psi$. Depending on the circumstances, Fig. 1 may be used for the different purposes discussed below.
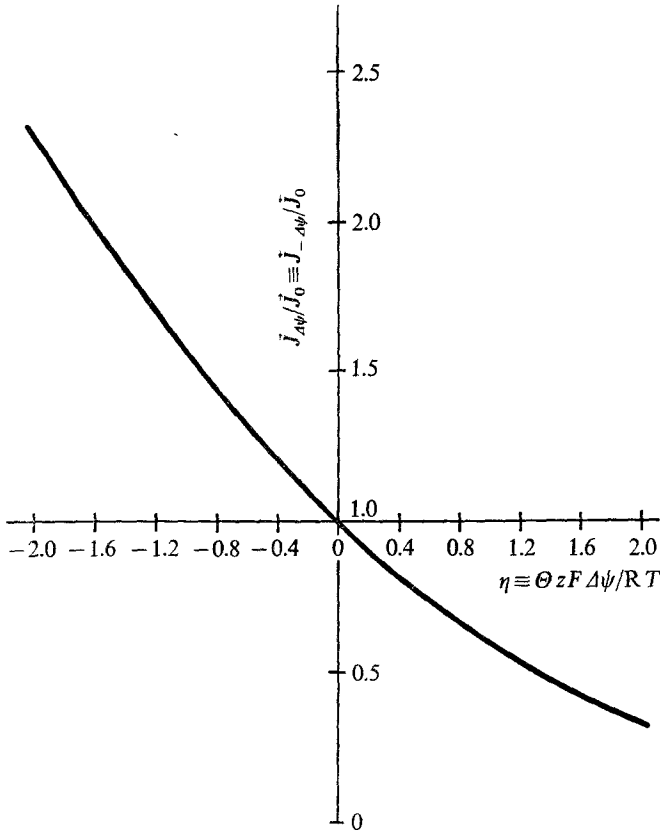
Fig. 1. Relationship between $\vec{J}_{\Delta\psi}/\vec{J}_0 \equiv \overleftarrow{J}_{-\Delta\psi}/\overleftarrow{J}_0$ and $\eta \equiv \Theta zF\Delta\psi/RT$. For passive flow due to electrical force in the absence of coupled flows of other species this relation permits evaluation of the extent of isotope interaction. If $\Delta\psi$ is expressed in mV, $(P/P^x) = \Theta = (25.3/z\Delta\psi)\eta$. The application of Eq. (3) then permits the evaluation of P^x and P. For passive flows in either the presence or absence of isotope interaction or coupled flows of other species it is possible to evaluate the partial conductance. For $\Delta\psi$ expressed in mV, $\vec{J}_0 = P^x c$ expressed in mM cm^{-2} sec^{-1}, and G expressed in mmho cm^{-2},

$$G = 9.65 \times 10^7 \, (z/\Delta\psi)\vec{J}_0\eta$$

For passive flows in the absence of coupled flows of different chemical species, $\alpha = \Sigma R_{ij}J_j = 0$ and $P/P^x = \Theta$, thus quantifying the extent of isotope interaction. The concomitant application of Eq. (3) will then evaluate P, the intrinsic membrane permeability coefficient for net flow. This approach, which has been validated in the study of a tight highly permselective ion exchange membrane [2], has permitted the estimation of P/P^x and P for passive Na$^+$ and Cl$^-$ flows in the toad bladder, presuming that both inter-action between chemically different solute species and electroosmosis are minimal [9, 10].

In the presence of both isotope interaction and other coupled flows the interpretation of Θ becomes complex, and it may not be practical to evaluate (P/P^x) and P. Nevertheless, for passive transport it remains possible to utilize unidirectional tracer fluxes to characterize the membrane in terms of its partial conductances, as was pointed out by Krämer and Meares [7]. This may be seen by considering that for a passive system in the absence of concentration driving forces the net flow of a test species

$$J = -Pc(zF\Delta\psi + \Sigma R_{ij}J_j)/RT$$
$$= -P^x c(P/P^x)(zF\Delta\psi + \Sigma R_{ij}J_j)/RT = -P^x c\,\Theta\,zF\Delta\psi/RT$$

can also be expressed in terms of the partial conductance G: $J = -(1/zF)G\Delta\psi$. Substituting for $P^x c$ the unidirectional flux $\vec{J}_0$, these relations give

$$G = [(zF)^2/RT]\,\Theta\vec{J}_0 \equiv [zF/\Delta\psi]\,\vec{J}_0\eta \quad (\Delta c = 0). \tag{12}$$

Clearly, if $\Theta \neq 1$ is not attributable to isotope interaction, an analogous approach to those above permits the evaluation of the influence of coupled material flows and metabolism, characterized by the proportionality constant α.

References

1. Clarkson, T. W. 1967. The transport of salt and water across isolated rat ileum. Evidence for at least two distinct pathways. *J. Gen. Physiol.* **50**:695

2. De Sousa, R. C., Li, J. H., Essig, A. 1971. Flux ratios and isotope interaction in an ion exchange membrane. *Nature* **231**:44

3. Frizzell, R. A., Schultz, S. G. 1972. Ionic conductances of extracellular shunt pathway in rabbit ileum. *J. Gen. Physiol.* **59**:318

4. 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

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

6. Kedem, O., Leaf, A. 1966. The relation between salt and ionic transport coefficients. *J. Gen. Physiol.* **49**:655

7. Krämer, H., Meares, P. 1969. Correlation of electrical and permeability properties of ion-selective membranes. *Biophys. J.* **9**:1006

8. Li, J. H., De Sousa, R. C., Essig, A. 1974. Kinetics of tracer flows and isotope interaction in an ion exchange membrane. *J. Membrane Biol.* **19**:93

9. Saito, T., Essig, A. 1973. Effect of aldosterone on active and passive conductance and E_{Na} in the toad bladder. *J. Membrane Biol.* **13**:1

10. Saito, T., Lief, P. D., Essig, A. 1974. Conductance of active and passive pathways in the toad bladder. *Amer. J. Physiol.* **226**:1265

11. Ussing, H. H. 1960. The Alkali Metal Ions in Biology. Springer-Verlag, Berlin