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Kinetics of Tracer Flows and Isotope Interaction in an Ion Exchange Membrane

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Summary. The thermodynamic formulation of isotope interaction (coupling of abundant and tracer isotope flows) has been tested in a highly permselective anion exchange membrane in the absence of significant electroosmosis. A previous study of Cl^- permeation has now been extended to include permeation of I^-, Acetate, and SO_4^{2-} in different bath concentrations, with the use of both electrical and chemical driving forces. The flux ratios were "abnormal" according to the usual criteria for simple passive flow, but were closely predicted by the theoretical expression incorporating the influence of isotope interaction. In the absence of coupled flows of other chemical species the extent of isotope interaction can be determined either from the flux ratio or from the measurement of a single unidirectional flux at two settings of the electrochemical potential difference. Direct evidence of negative isotope interaction was presented.

Despite the common use of radioactive tracers in the study of biological transport processes, fundamental anomalies interfere with the interpretation of the data obtained by these means. Thus, it is recognized that the evaluation of permeability by the self-exchange of a tracer may give a very different result from that derived from measurements of net flow. Similarly, there are difficulties in the use of the "flux ratio" to evaluate the forces promoting net transport [4, 11, 12].

The above problems have been analyzed in terms of a variety of models [4, 11, 12]. Kedem and Essig have provided a more general thermodynamic analysis. In this formulation both of the above anomalies are attributable

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to coupling between the flows of the abundant and tracer species ("isotope interaction") [5].

Gottlieb and Sollner's recent description of anomalous electrical conductivity and tracer permeability in membranes of a high degree of permselectivity [3] has permitted a convenient test of this formalism. The results of a preliminary study were consistent with the general formulation, supporting the view that anomalous tracer permeability coefficients and flux ratios are due to coupling of isotope flows [1]. In the present study, we investigate this formulation more comprehensively and present direct evidence of negative isotope interaction.

Materials and Methods

The preparation of membranes was similar to that described previously by Gottlieb, Neihof and Sollner [2]. It involved three steps: the casting of a porous collodion membrane, activation by adsorption of the polyelectrolyte polyvinyl-benzyl-trimethyl ammonium chloride (PVBT, Dow Chemical) and irreversible shrinking by controlled drying. The dried membrane had a thickness of 50 to 75 μ.

Membranes were selected on the basis of mechanical strength, high permselectivity, low water permeability, and a convenient range of electrical resistance. Acceptable membranes produced an electrical potential greater than 55 mV in a 0.100/0.010 M KCl concentration cell, and radioactive tracer studies showed that over 99% of electrical current was carried by Cl^-. Membranes were discarded if they permitted any noticeable water flow under these same conditions. Membrane resistance was chosen to be about 100 Ω cm^2 in 0.1 M KCl.

$^{131}I^-$ was purchased from Cambridge Nuclear, Cambridge, Mass., ^{14}C-acetate, $^{36}Cl^-$, and $^{35}SO_4^{2-}$ from New England Nuclear, Boston, Mass. $^{131}I^-$ was counted on a Nuclear Chicago gamma counter. The other isotopes were counted on a Packard Tri-Carb liquid scintillation counter.

The Lucite experimental chamber consists of four 16 to 17 ml compartments. Radioactive anions were restricted to the two central regions by the use of two highly selective cation-exchange membranes separating the central and outer compartments. The transmembrane electrical potential difference $\Delta\psi$ was measured with bridges connected to calomel reference electrodes. The backing of each outer compartment was a Ag-AgCl-platinum electrode, providing near-uniform current density. A voltage clamp fixed $\Delta\psi$ with a resolution of 0.25 mV, compensating automatically for solution resistance.

Experimental Procedures

Electrical Resistance and Phenomenological Resistance Coefficient. Membranes were equilibrated overnight in the desired bathing solutions. Since the current-voltage relationship was linear, the electrical resistance was calculated from Ohm's law, transiently setting $\Delta\psi$ at ± 10 mV and recording steady-state values of the electrical current. With identical solutions at each surface, the phenomenological resistance coefficient R was evaluated from the electrical resistance $\mathscr{R}$, as discussed in Results.

Tracer Flows, Unidirectional Fluxes, and Exchange Resistance. Following equilibration, flow of tracer was determined from the rate of increase of tracer content. In most experiments the concentration of tracer on the "cold" side was always less than 1% of that on the "hot" side; the "unidirectional flux" was then given by the quotient $-J^x/\Delta\rho$, where $\Delta\rho$ is the difference in specific activity across the membrane. With dilute solutions it was sometimes necessary to correct for backflux. In the absence of net flow the same tracer permeability coefficient was obtained in both directions.

The exchange resistance and the flux ratio were evaluated from three sequential series of measurements of tracer flux J^x. For studies in which net flow was produced by an electrical potential difference the two solutions were identical except for the concentration of radioactivity. Thus, in an initial period with $\Delta\psi = 0$ there was no net flow, and the measurement of $J^x/\Delta\rho$ permitted the calculation of the exchange reistance R^x from Eq. (2) below. The cold side was then drained, rinsed, and refilled, and a positive net flow was established by setting $\Delta\psi > 0$. $-J^x/\Delta\rho$ was then taken as the influx, denoted $\overrightarrow{J}$. After three or four periods the cold side was again drained, rinsed, and refilled, and a negative net flow was produced by reversing the polarity of the potential for three or four periods. $-J^x/\Delta\rho$ was then taken as the outflux, $\overleftarrow{J}$. The quotient of the two unidirectional fluxes (influx/outflux) gave the flux ratio f.

In a few experiments, following the determination of R^x the protocol differed from that above in the use of a concentration difference to produce net flow (the mean concentration of the two solutions was the same as in the determination of R^x). Here the influx and outflux were determined by alternating the placement of the tracer between the concentrated and dilute solutions. This avoided changes resulting from reversal of the polarity of the concentration gradient across an imperfectly homogenoeus membrane.

Results

Flux Ratio – Electrical Driving Force

In the absence of a concentration difference across a membrane, the negative electrochemical potential difference of an ion is $X = -zF\Delta\psi$, where z is the valence, and F is the Faraday constant. Since the current-voltage relationship is linear in the range employed, if electroosmosis is insignificant, the phenomenological resistance to net flow is given by

$$R = X/J, \tag{1}$$

where J is the net flow of the anion under study. With values of the anion transference number τ_- greater than 0.99, $I \simeq zFJ$, where I is the electrical current. R can then be readily evaluated from a measurement of the d-c electrical resistance:

$$R = -zF\Delta\psi/J = -(zF)^2\Delta\psi/I = (zF)^2\mathscr{R}, \tag{1'}$$

where $\mathscr{R} = -\Delta\psi/I$ is the electrical resistance.

The exchange resistance is determined in the absence of net flow (i.e. with both Δc and $\Delta\psi = 0$):

$$R^x = (-\mathrm{R}T\Delta\rho/J^x)_{J=0}, \tag{2}$$

where R is the gas constant and T is the absolute temperature.

For simple passive flow in the absence of coupled flows of different chemical species the flux ratio is given by

$$f = \exp(X/\mathrm{R}T). \tag{3}$$

However, in the presence of isotope interaction

$$f = \exp[(R^x/R)(X/\mathrm{R}T)]. \tag{4}$$

These relationships (*see* ref. [5]), which were partially tested in a previous publication [1], are here examined more comprehensively.

Monovalent Anions

In the previous study we utilized membranes exposed at each surface to 0.10 M KCl solutions. In the present study we tested the behavior of membranes in KCl at lower concentrations as well.

1. *0.03* M *KCl.* In this series of experiments the electrical current remained constant for given values of $\Delta\psi$, indicating the constancy of R and X, and facilitating the application of the above formalism. The results shown in Table 1 are closely similar to those obtained in the earlier studies employing 0.10 M KCl; whereas the experimental value of the flux ratio f differed substantially from the value of Eq. (3), in all cases it was rather closely predicted by Eq. (4), the expression incorporating the effect of isotope interaction.

2. *0.01* M *KCl.* Although the above approach is in principle applicable also with more dilute solutions, it was found that despite constancy of the clamping potential the electrical current, and thus J, declined progressively. Since R was unchanged following the experiment the decline in flow must have been due to a change in bath concentrations, and hence in X; a small

Table 1. Theoretical and observed flux ratios in 0.03 M KCl (with $\Delta c = 0$, $X = -zF\Delta\psi$)

Membrane	R^x/R	$\Delta\Psi$	$\exp\left(\frac{X}{\mathrm{R}T}\right)$ (mV)	f	$\exp\left[\left(\frac{R^x}{R}\right)\frac{X}{\mathrm{R}T}\right]$
F	0.71	41.0	4.94	3.53	3.11
H	0.49	32.0	3.48	1.92	1.84
H	0.51	41.5	5.04	2.17	2.29
H	0.49	41.8	5.10	2.24	2.21
K	0.40	41.0	4.94	2.00	1.89
K	0.41	41.6	5.06	2.09	1.93

Table 2. Theoretical and observed flux ratios in various solutions

Solutions	n^x/n^+	R^x/R	$\Delta\Psi$(mV)	$f/\exp(JR/\mathrm{R}T)$	$f/\exp(JR^x/\mathrm{R}T)$ (Mean ± SE)
(a) 0.03 M KCl	6/3	0.40–0.71	32.0–41.8	0.41–0.72	1.05±0.03
(b) 0.01 M KCl	11/5	0.17–0.76	10.0–25.0	0.47–0.88	1.00±0.01
(c) 0.10 M KI	2/2	0.74–1.18	25.0–50.0	0.72–1.34	0.94±0.02
(d) 0.01 M KI	2/1	0.38–0.44	50.0–75.0	0.17–0.38	1.02±0.05
(e) 0.10 M KAc	3/2	0.47–0.72	10.0–25.0	0.60–0.89	0.99±0.01
(f) 0.01 M KAc	4/3	0.26–0.56	15.0–50.0	0.26–0.78	0.98±0.00
(g) 0.01 M K_2SO_4	6/2	0.29–0.52	25.0–50.0	0.12–0.31	0.87±0.02
(h) 0.10/0.05 M KCl	1/1	0.36	0	0.61	0.95
(i) 0.05/0.01 M KCl	6/3	0.40–0.71	0	0.31–0.66	0.88±0.04
(j) 0.10 M KCl	8/4	0.42–0.71	10.0–75.0	0.24–0.91	0.99±0.03

n^x = number of experiments, n^+ = number of membranes employed. In (b), (d) and (f), since J declined less than 3% in each period, the arithmetic mean of the initial and final values was taken to represent that of the entire period. (j) represents data reported previously [1].

flow, which altered the chemical potentials insignificantly with 0.03 or 0.10 M bathing solutions, had an appreciable effect with 0.01 M solutions. (This interpretation was confirmed by close agreement of the final open-circuit electrical potential with the value of the concentration potential predicted from the amount of salt transferred and the compartment volumes.) Because R is determined essentially by the mean concentration $\bar{c}$, being insensitive to Δc, it was unaffected (*see below*).

Since the rate of change of Δc could have been estimated it would have been possible to calculate a mean value of X appropriate for each period of each experiment. Because the electrical current and thus J were being measured continuously it was more convenient to use alternative expressions for Eqs. (3) and (4) obtained by incorporating Eq. (1), viz.: in the absence of isotope interaction,

$$f = \exp(JR/\mathrm{R}T), \tag{3'}$$

or more generally, in either the presence or absence of isotope interaction,

$$f = \exp(JR^x/\mathrm{R}T). \tag{4'}$$

The results of applying this mode of analysis to the 0.03 M KCl data of Table 1 are summarized in Table 2 (a). Table 2 (b) shows the results of the studies employing 0.01 M KCl bathing solutions. As is seen, values of $f/\exp(JR/\mathrm{R}T)$ vary appreciably in different studies and differ substantially from unity. By contrast, values of $f/\exp\ (JR^x/\mathrm{R}T)$ cluster near 1.00, in accord with the formulation incorporating the influence of isotope interaction.

3. *KI and K Acetate.* Similar results of studies employing KI or K acetate (KAc) at 0.10 M and 0.01 M are shown in Table 2 (c)-(f).

Divalent Anions

We would expect that the validity of the thermodynamic formulation would be independent of the valence of the transported species. It is of course possible that a polyvalent anion might permeate the membrane by a different mechanism than that of monovalent anions, in which case the exchange resistance R^x might not differ from R. If, however, R^x does differ from R, study of the flux ratio would further define the range of validity of the formalism.

It proved possible to use $^{35}SO_4^{2-}$ for this purpose. However, since membranes exposed to 0.10 M K_2SO_4 were insufficiently permselective to permit the direct calculation of the rate of net flow from the electrical current, for convenience and accuracy studies were performed only with 0.01 M solutions. At this concentration over 99% of the current was carried by SO_4^{2-}. Table 2 (g) shows the results of six experiments in two membranes. With low values of R^x/R and the use of a divalent anion, modest electrical potential differences resulted in marked discrepancies between the observed flux ratios and the values predicted from Eq. (3′). The values predicted from Eq. (4′) differed from f more than previously, but again were much more appropriate than those of the expression neglecting isotope interaction.

Flux Ratio — Concentration Driving Force

In principle, the above formulation should be applicable even when flow is the result of a concentration difference. Here, however, there is a possible difficulty, since R and R^x are functions of the state of the membrane, and hence of the bath concentrations, and so there may be a problem in the choice of proper bathing solutions for the evaluation of R^x/R. We reasoned that for the membranes of the present study, presuming a fairly symmetrical distribution of fixed charge and a high degree of co-ion exclusion, to a first approximation the state of the membrane would be determined by the mean bath concentration, irrespective of the magnitude or polarity of concentration gradients. Thus, it should be possible to determine R^x and R in the absence of a gradient, and to apply the resultant value of R^x/R to studies in the presence of a gradient, so long as the mean concentration

Table 3. Theoretical and observed flux ratios with concentration driving force with $\Delta\psi = 0$, $X = \mathrm{R}T \ln(c^{\mathrm{I}}/c^{\mathrm{II}})$

Membrane	R^x/R	$c^{\mathrm{I}}, c^{\mathrm{II}}$ (mole liter^{-1})	$\exp\left(\frac{X}{\mathrm{R}T}\right)$	f	$\exp\left[\left(\frac{R^x}{R}\right)\frac{X}{\mathrm{R}T}\right]$
A	0.36	0.1, 0.05	2.0	1.22	1.28
F	0.71	0.05, 0.01	5.0	3.30	3.14
F	0.64	0.05, 0.01	5.0	2.33	2.81
H	0.51	0.05, 0.01	5.0	1.94	2.28
H	0.49	0.05, 0.01	5.0	1.93	2.19
K	0.40	0.05, 0.01	5.0	1.62	1.90
K	0.41	0.05, 0.01	5.0	1.55	1.92

$\bar{c} = (c^{\mathrm{I}} + c^{\mathrm{II}})/2$ was unchanged. On this basis we carried out measurements with $\Delta\psi = 0$, one utilizing a 2:1 concentration gradient and six with a 5:1 concentration gradient (Table 3). Since control experiments had demonstrated very low water permeability, in the absence of isotope interaction the flux ratio should be given by $c^{\mathrm{I}}/c^{\mathrm{II}}$. As is seen, the experimental values differed markedly from this value, but again were close to the values predicted by incorporating the influence of isotope interaction.

Coupling of Isotope Flows ("Isotope Interaction")

According to the thermodynamic formulation being tested, both discrepancy between R and R^x, and "abnormality" of the flux ratio are manifestations of coupling of isotope flows. The above-demonstrated agreement between predicted and experimental results is consistent with this interpretation, but it would be desirable to have a more direct demonstration of coupling. Observations of the effect of the electrical potential on tracer fluxes and flux ratios are not completely satisfactory in this regard since perturbation of $\Delta\psi$ acts not only to alter net flow (and hence the possible influence of net flow on tracer flows) but alters also the thermodynamic force $(-zF\Delta\psi)$ acting directly on the tracer species. A more convincing demonstration of coupling may be provided by experiments involving concentration driving forces with $\Delta\psi$ clamped at zero. The imposition of a concentration difference for the abundant species will produce a net flow but, to the extent that the state parameters of the membrane are unaltered, in the absence of isotope interaction the tracer flows will be unaffected. In the presence of isotope interaction the situation will be quite different: with the negative coupling claimed here, a positive net flow ($J > 0$) will reduce the

Table 4. Effect of net flow on tracer flows

Membrane	n^x	R^x/R	$(\vec{J}^x)_{J>0}/(\vec{J}^x)_{J=0}$	$(\overleftarrow{J}^x)_{J>0}/(\overleftarrow{J}^x)_{J=0}$
F	4	0.71	0.84	1.27
F	4	0.64	0.77	1.66
H	4	0.51	0.77	1.99
H	3	0.49	0.73	1.90
K	4	0.40	0.72	2.21
K	4	0.41	0.70	2.26

$\vec{J}^x$ and $\overleftarrow{J}^x$ represent inward and outward tracer fluxes, respectively. R^x/R, $(\vec{J}^x)_{J=0}$, and $(\overleftarrow{J}^x)_{J=0}$ were measured with 0.03 M KCl bathing each surface of the membrane. $J>0$ represents the net flux produced by the use of 0.05 M KCl in compartment I and 0.01 M KCl in compartment II; $\Delta\psi=0$.

inward tracer flux $(\vec{J}^x)$ and increase the outward tracer flux $(\overleftarrow{J}^x)$. The existence of both of these effects in Table 4 is a convincing demonstration of coupling of abundant and tracer flows.

Effect of X on the Unidirectional Flux

The demonstration of an "abnormal" flux ratio has often been useful in pointing to special mechanisms of permeation in biological systems [4, 11, 12]. However, the measurement of two unidirectional fluxes is laborious, and in some cases unfeasible. It is possible to obtain equivalent information without measurement of the bidirectional fluxes. For unidirectional fluxes $\vec{J}$ and $\overleftarrow{J}$, $f=\vec{J}/\overleftarrow{J}$, and $J=\vec{J}-\overleftarrow{J}$. For passive flow without coupling of fluxes of different chemical species, Eqs. (1) and (4) give $\vec{J}/\overleftarrow{J}=\vec{J}/(\vec{J}-J)=\vec{J}/(\vec{J}-X/R)=\exp[(R^x/R)(X/\mathrm{R}T)]$, so that the influx is given by

$$\vec{J}=\frac{X}{R[1-\exp(-(R^x/R)(X/\mathrm{R}T))]}. \tag{5}$$

It is seen that the measurement of $\vec{J}$ at two values of X will permit the evaluation of R^x/R and R. In practice it is convenient to set one value of $X=0$. From Eq. (2), $\vec{J}_{X=0}=(-J^x/\Delta\rho)_{J=0}=\mathrm{R}T/R^x$.
Hence,

$$\frac{\vec{J}_X}{\vec{J}_0}=\frac{(R^x/R)(X/\mathrm{R}T)}{1-\exp[-(R^x/R)(X/\mathrm{R}T)]}. \tag{6}$$

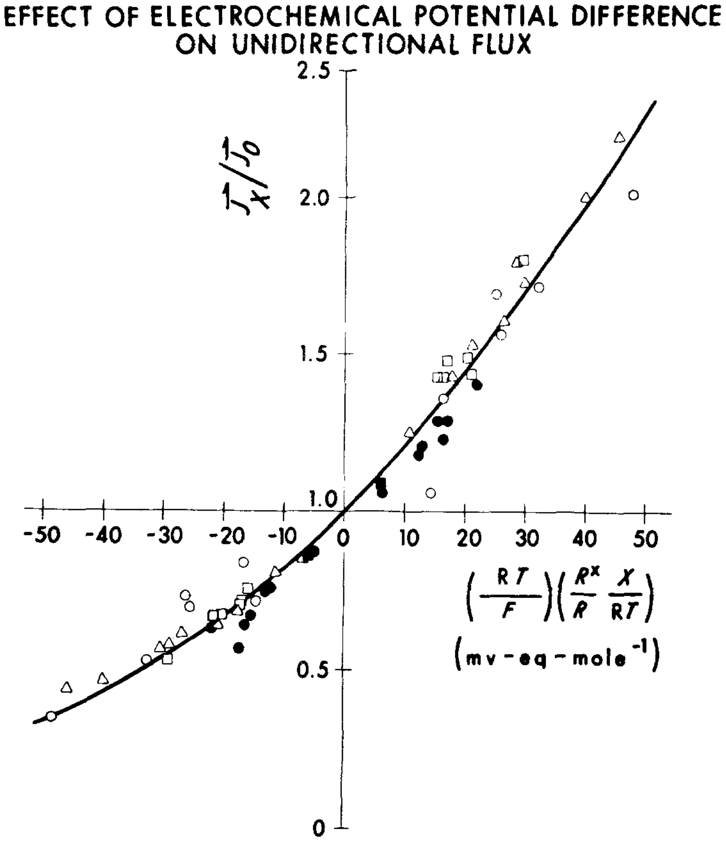

Fig. 1. Relationship between $\vec{J}_X/\vec{J}_0$ and $(R^x/R)X$. Each value of a unidirectional flux represents the mean of 3 or 4 determinations. The solid line represents the theoretical relationship of Eq. (6). In order to use the experimental electrical units (mV) the abscissa is expressed as $(\mathrm{R}T/F)\,[(R^x/R)(X/\mathrm{R}T)]$. In these terms, electrical force corresponds to $-(R^x/R)z\Delta\psi$; concentration force corresponds to $-(R^x/R)(\mathrm{R}T/F)\Delta\ln c = 25.2\ln(c^{\mathrm{I}}/c^{\mathrm{II}})$. Theoretically the ordinate $\vec{J}_X/\vec{J}_0 = \overleftarrow{J}_{-X}/\overleftarrow{J}_0$; $\vec{J}_0 = \overleftarrow{J}_0$.

Although the validity of Eq. (6) follows naturally from the above demonstration of the validity of Eqs. (3) and (4), it seemed useful to compare the experimental values of $\vec{J}_X/\vec{J}_0$ with the ratios predicted theoretically. This is done in Fig. 1 for experiments discussed above in which the force X was constant. As is seen, whether X is varied by alteration of $\Delta\psi$ or Δc, the agreement between experiment and theory is good.

Discussion

General

Because of the several anomalies commonly attendant on the use of tracers for the study of transport processes there is need for a comprehensive treatment of the kinetics of tracer flows. According to an analysis applicable to a homogeneous array of pathways with continuity of electrochemical potential [5] the net flow of the test species, its flux ratio, and the forces promoting its transport obey the relations

$$J R^x = \mathrm{R}T \ln f = (R^x/R)\left(X - \int_0^{\Delta x} \sum_j r_{0j} J_j \, dx\right). \tag{7}$$

Here the integral $\int_0^{\Delta x} \Sigma r_{0j} J_j dx$ represents the possible contribution of coupled flows of other species (e.g. "solvent drag") and metabolism (active transport) to the forces which cause "abnormality" of the flux ratio. Even in the absence of such coupled flows, anomalous flux ratios ($\mathrm{R}T \ln f \neq X$) may result from coupling of flows of the abundant and tracer forms of the test species.

The physical nature of the macroscopic resistance coefficients may be appreciated when these are related to the local phenomenological coefficients [5]. Thus,

$$R = \int_0^{\Delta x} r_{00} \, dx, \tag{8}$$

where r_{00} is the local phenomenological resistance coefficient for net flow and Δx is the thickness of the membrane, whereas

$$R^x = \int_0^{\Delta x} (r_{00} - r_{ik}) \, dx. \tag{9}$$

Here, r_{ik} is a local phenomenological resistance coefficient representing the coupling of flows of abundant and tracer isotopes of the test species ("isotope interaction"). With concentration driving forces the permeability coefficient is given by $P = -J/\Delta c = \mathrm{R}T/R\bar{c}$, where $\bar{c}$ is a logarithmic mean concentration, and the tracer permeability coefficient $P^x = (-J^x/\Delta c^x)_{J=0} = \mathrm{R}T/R^x\bar{c}$ [5]. In the absence of isotope interaction ($r_{ik} = 0$), $R^x = R$, and so a simple tracer measurement will evaluate the resistance coefficient R (and thus the permeability coefficient P). In the presence of isotope interaction a tracer measurement will not in itself quantify R or P.

The above formulation had been partially tested previously [1]. The present results further substantiate the validity of the formulation and provide a direct demonstration of negative coupling of isotope flows. Such negative coupling cannot be explained in terms of "mutual drag" or "frictional interaction". Furthermore, the unlikelihood of the presence of transmembrane mobile carriers in our system suggests that the conventional interpretation of counter-transport and abnormal flux ratios in terms of carrier transport is quite possibly often inappropriate.

Influence of Coupling of Flows of Different Chemical Species

Meares, Dawson, Sutton and Thain [7] have considered a relationship between the flux ratio and the transport number and tracer diffusion coefficient which should be applicable irrespective of the extent and nature of coupling of solute and solvent flows. Meares and Sutton [8] tested the relationship extensively with a variety of single and mixed electrolyte solutions at constant composition, with concentrations ranging from 0.01 to 1.00 equiv/liter. Agreement with theory was generally good (within ± 10%), except in dilute solutions where serious polarization occurred. With the Zeocarb 315 cation exchange membranes which were employed, exposure to 0.1 M NaBr gave an anion transport number $t_- = 0.045$, and a water transport number $t_w \sim 40$ moles equiv^{-1} [6, 8]. In view of the significant deviations from ideal permselectivity and appreciable electroosmosis, the influence of solute and solvent flows on tracer flows was undefined and hence, as was pointed out, the extent of isotope interaction could not be evaluated [8].

The approximate evaluation of the extent of isotope interaction in the present study depends on the assumption that the flows of the counter-ion test species are uncoupled to those of the co-ion and solvent. The absence of a significant influence on counter-ion flow by the co-ion flow is assured by cationic transport numbers $\leqslant 0.01$. The possible significance of electroosmosis was tested in two PVBT membranes. In the range of electrical currents employed in this study the water transport numbers were < 2 moles equiv^{-1}. It should be noted that even if electroosmosis had been significant its effect would be to lower the electrical resistance and thus the value of the phenomenological resistance coefficient R calculated from Eq. (1′). Hence values of R^x/R calculated on this basis to be less than 1 would represent negative isotope interaction, irrespective of the presence or absence of electroosmosis.[1]

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[1] The results presented here constitute a test of Eq. (7) (Eq. (29) of reference [5]). Since $\tau_+ \leqslant 0.01$ and coupling of anion and water flow appeared insignificant, we expressed Eq. (7) in the form $JR^x = \mathrm{R}T \ln f = (R^x/R)\,X$, where R was identified with $(zF)^2\,\mathscr{R}$, according to Eq. (1′). If electroosmosis were in fact significant the force promoting transport would be given by $X - \int r_{0w} J_w \, dx$. But then also $\mathscr{R} = -\Delta\psi/I = X/[(zF)^2 J_-] = XR/[(zF)^2(X - \int r_{0w} J_w \, dx)]$. Under these circumstances the finding that $\mathrm{R}T \ln f = (R^x/(zF)^2\,\mathscr{R})X$ would demonstrate that $\mathrm{R}T \ln f = (R^x/R)(X - \int r_{0w} J_w \, dx)$, as in Eq. (7).

Thus, if electroosmotic flow were appreciable the fundamental relationship would remain valid, although the true values of R^x/R would be somewhat smaller than those reported here. The precise evaluation of R^x/R would in general require, in addition to the present measurements, the careful evaluation of the water transport number and the hydraulic permeability, or equivalent information. Since we were interested only in testing the validity of the general thermodynamic formulation we did not do such measurements.

Utility of Isotope Techniques

Despite the difficulties of using tracer techniques to obtain precise knowledge of permeability coefficients and forces, with proper precautions they may be of considerable utility. By means of the present formalism it has proved possible to characterize the partial conductances for passive ion transport in epithelial tissues, despite the existence of heterogenous passive and active pathways and apparent isotope interaction. The use of a single tracer then permits the evaluation of both passive and active conductance [9, 10].

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