TRACER DIFFUSION AND UNIDIRECTIONAL FLUXES

PETER F. CURRAN, AUBREY E. TAYLOR, *and* A. K. SOLOMON

From the Biophysical Laboratory, Harvard Medical School, Boston, Massachusetts 2115. Dr. Curran's present address is Department of Physiology, Yale University School of Medicine, New Haven, Connecticut 06510. Dr. Taylor's present address is Department of Physiology, University of Mississippi School of Medicine, Jackson, Mississippi.

ABSTRACT Available experimental data have been utilized to examine the effects of cross-coefficients on tracer diffusion and on the estimation of unidirectional fluxes from observations on tracer flow. In free solution or in a nonselective membrane, the interaction between the flows of tracer and the unlabelled substance are small at concentrations of biological interest for the nonelectrolytes urea, alanine, and β-alanine, and for sodium and chloride ions. Under these conditions, measurement of tracer flow can be used to predict flow of the bulk substance to an accuracy of a few per cent.

In the study of transport across biological membranes, extensive use is made of radioactive tracers to estimate unidirectional fluxes. While measurement of the flow of tracer across a membrane can usually be carried out quite accurately, the calculation of unidirectional flux of the unlabeled substance is not necessarily unequivocal. In fact, Nims (1, 2) has suggested that the general approach of estimating unidirectional fluxes with tracers is incorrect and Kedem and Essig (3) and Essig (4) have called attention to possible complications in such measurements. These questions concerning certain interpretations of tracer measurements are based on the concept of interactions between flows and forces that arises within the formal framework of nonequilibrium thermodynamics. The objections raised are, in principle, correct but the possible magnitudes of the effects are not apparent from a general analysis. There are now sufficient data available to obtain some insight into these phenomena in a few simple systems and a more detailed analysis of the tracer problem seems warranted. The present paper is concerned with such an analysis via the formalism of nonequilibrium thermodynamics and with an examination of the limited experimental data available.

UNCHARGED SOLUTES

In order to obtain a clear statement of the problem, let us consider a membrane bathed on both sides by a solution containing a single uncharged solute at different concentrations. We assume that the membrane is inert and sufficiently porous and nonselective so that there is no osmotic water flow (convective flow). A sintered glass disc such as discs used in diaphragm-type diffusion cells would fulfill these requirements. The steady-state flow of the solute across this membrane can be described by the equation

$$J_1 = \mathcal{P}_1(c_1' - c_1'') \tag{1}$$

in which J_1 is solute flow in moles/cm^2 sec, $\mathcal{P}_1$ is the permeability coefficient (cm/sec), c_1 is concentration (moles/cm^3) of the solute, and the superscripts ($'$) and ($''$) denote sides of the membrane. If a tracer (component 2) for substance is introduced into the system, we assume that its flow can be described by a similar expression:

$$J_2 = \mathcal{P}_2(c_2' - c_2'') \tag{2}$$

As shown in Appendix I, equation 2 is equivalent to the kinetic expression usually used to describe tracer flow. Substances 1 and 2 are then assumed to behave in exactly the same manner in all respects so that $\mathcal{P}_1$ and $\mathcal{P}_2$ are identical. Under these conditions, measurement of J_2 can be used to estimate $\mathcal{P}_2$ (or $\mathcal{P}_1$), and the flow J_1 can be calculated for given values of c_1' and c_1''. In practice, J_2 is often measured with $c_2'' \approx 0$ so that

$$\mathcal{P}_2 = \frac{J_2}{c_2'} \tag{3}$$

and a unidirectional flow of substance 1 from the $'$ to the $''$ side of the membrane, $\vec{J}_1$, is calculated from the relation

$$\vec{J}_1 = \left(\frac{J_2}{c_2'}\right) c_1' = \mathcal{P}_1 c_1' \tag{4}$$

This operational definition of unidirectional flux follows from equation 1 which may be written

$$J_1 = \mathcal{P}_1 c_1' - \mathcal{P}_1 c_1'' = \vec{J}_1 - \overleftarrow{J}_1 \tag{5}$$

so that

$$\vec{J}_1 = \mathcal{P}_1 c_1'$$

The flux $\vec{J}_1$ cannot be measured experimentally except by using tracers, but for the present purpose it may be considered as the flux of substance 1 that would be observed if c_1'' were equal to zero. This concept obviously involves the additional assumption that $\mathcal{P}_1$ is not altered by changing c_1''. This assumption is incorrect in general, but we shall consider it further in the subsequent discussion.

The major purpose of this paper is to examine explicitly the assumption implied in equation 4 that $J_2/c_2' = \mathcal{P}_1$ when substance 2 is an ideal tracer for substance 1. By ideal tracer, we imply that all the properties of 1 and 2 are identical except for the fact that 2 is radioactive. We ignore, therefore, any effects that arise because 1 and 2 differ in mass.[1] As a first step in examining this assumption, we shall consider the behavior of the system discussed above at a point x within the completely nonselective membrane. At this point, the flow of substance 1 in the absence of substance 2 will be given by Fick's law,

$$J_1 = -D_{m1} \frac{\partial c_1}{\partial x} \tag{6}$$

in which J_1 is flow of solute relative to solvent and D_{m1} is the mutual diffusion coefficient of substance 1 in water in this frame of reference. As discussed in many publications on diffusion (6–8), exact specification of the frame of reference to which flows are referred is of considerable importance. We shall begin by considering flows of solutes relative to the solvent because the expressions are somewhat simpler, but we shall also show that similar conclusions apply for flows relative to the mean volume velocity. If tracer, (substance 2) is introduced into the system, we might assume that J_2 would be given by

$$J_2 = -D_{m2} \frac{\partial c_2}{\partial x} \tag{7}$$

and the local equivalent of equation 4 at a point in the barrier would be

$$J_1 = \frac{J_2}{\partial c_2/\partial x}\left(\frac{\partial c_1}{\partial x}\right) \tag{8}$$

Thus, in terms of these expressions the hypothesis that the tracer provides precise information on the flow of unlabeled substance involves the assumptions that equations 6 and 7 are correct and that $D_{m1} = D_{m2}$.

The theory of nonequilibrium thermodynamics indicates, however, that when substances 1 and 2 are both present, equations 6 and 7 are incorrect (6–8). In a solution containing two solutes and a solvent, the equations for solute flows relative to the solvent are

$$\begin{aligned} J_1 &= -L_{11}\frac{\partial \mu_1}{\partial x} - L_{12}\frac{\partial \mu_2}{\partial x} \\ J_2 &= -L_{21}\frac{\partial \mu_1}{\partial x} - L_{22}\frac{\partial c_2}{\partial x} \end{aligned} \tag{9}$$

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[1] Wang et al (5) observed a 4% difference in the diffusion coefficient of water measured with deuterated and tritiated water as tracers. Thau, Bloch, and Kedem (1966. *Desalination*. 1:129) were unable to detect a difference in the water permeability of an artificial membrane using these two isotopes. These observations suggest that the effects of mass differences will be insignificant in most cases.

in which μ_i is the chemical potential of component i and the L_{ij} are proportionality or phenomenological coefficients. Theoretical considerations (9–11) indicate that in general,

$$L_{12} = L_{21} \tag{10}$$

and there is now considerable experimental evidence on diffusion systems confirming this reciprocal relation (7, 12, 13). Using the explicit expressions for the dependence of μ_1 and μ_2 on concentration,[2] equations 9 may be transformed (6, 7, 14) to give

$$\begin{aligned} J_1 &= -D_{11}\frac{\partial c_1}{\partial x} - D_{12}\frac{\partial c_2}{\partial x} \\ J_2 &= -D_{21}\frac{\partial c_1}{\partial x} - D_{22}\frac{\partial c_2}{\partial x} \end{aligned} \tag{11}$$

in which,

$$\begin{aligned} D_{11} &= L_{11}\mu_{11} + L_{12}\mu_{21} \qquad & D_{12} &= L_{11}\mu_{12} + L_{12}\mu_{22} \\ D_{21} &= L_{21}\mu_{11} + L_{22}\mu_{21} \qquad & D_{22} &= L_{21}\mu_{12} + L_{22}\mu_{22} \end{aligned}$$

In these expressions,

$$\mu_{ij} = \frac{\partial \mu_i}{\partial c_j}$$

Since there is experimental evidence that D_{12} and D_{21} are nonzero (6, 11), equations 11 provide a more adequate description of the system under consideration than do equations 6 and 7. The existence of these cross-coefficients is the basis for objections to the general validity of the tracer method since they invalidate the assumptions involved in arriving at equation 8.

Comparison of equations 11 with equations 6, 7, and 8 provides the background for examination of the tracer hypothesis. Since equation 8 can be considered as summarizing the usual assumptions involved in the hypothesis, we are interested in conditions necessary to arrive at equation 8 from equations 11. Three conditions are required: (a) $D_{12}(\partial c_2/\partial x) \ll D_{11}(\partial c_1/\partial x)$; ($b$) $D_{21}(\partial c_1/\partial x) \ll D_{22}(\partial c_2/\partial x)$; and ($c$) $D_{22} = D_{11}$. In physical terms, condition a implies that the presence of tracer does not influence J_1, condition b requires that a gradient of substance 1 does not influence tracer flow (J_2), and condition c requires that the "straight" diffusion

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[2] In general, we assume uniform temperature and pressure so that

$$\frac{\partial \mu_i}{\partial x} = \sum_j \frac{\partial \mu_i}{\partial c_j}\frac{\partial c_j}{\partial x}.$$

coefficients be identical even though the concentrations of substances 1 and 2 may differ by several orders of magnitude. There is no a priori reason that any of these three conditions holds so we shall examine each of them.

Condition *a* is generally satisfied because the concentration of radioactive tracer is usually very small compared with the concentration of unlabeled substance. In typical experiments carried out with short-lived isotopes in biological systems, the concentration of tracer may be of the order of 10^{-12} M or 10^{-13} M while that of the unlabeled species is of the order of 10^{-1} M or 10^{-2} M. Further, all experimental data currently available indicate that D_{12} is smaller than D_{11} (7, 11). Thus, the term $D_{12}(\partial c_2/\partial x)$ in the expression for J_1 may be neglected.[3]

As discussed in detail by Dunlop (15), the reciprocal relation, equation 10, provides a basis for evaluating condition *c* which states that when substance 2 is a tracer for substance 1, $D_{11} = D_{22}$. The expressions for D_{ij} in terms of the L_{ij} and μ_{ij} can be solved for L_{12} and L_{21} to yield

$$L_{12} = \frac{D_{12}\mu_{11} - D_{11}\mu_{12}}{|\mu|}; \qquad L_{21} = \frac{D_{21}\mu_{22} - D_{22}\mu_{21}}{|\mu|}$$

in which $|\mu| = \mu_{11}\mu_{22} - \mu_{12}\mu_{21}$. If $|\mu| \neq 0$, equation 10 imposes the following relation on the D_{ij}:

$$D_{12}\mu_{11} - D_{11}\mu_{12} = D_{21}\mu_{22} - D_{22}\mu_{21} \tag{12}$$

We wish to examine the behavior of this expression when substance 2 is an ideal tracer for substance 1 and when 2 is present at very low concentrations ($c_2 \rightarrow 0$). The μ_{ij} can be written

$$\mu_{ij} = RT\left[\frac{\delta_{ij}}{c_i} + \frac{\partial \ln y_i}{\partial c_j}\right] = RT\left[\frac{\delta_{ij}}{c_i} + y_{ij}\right] \tag{13}$$

in which y_i is the molar activity coefficient of substance *i* and δ_{ij} is the Kronecker delta ($\delta_{ij} = 1$ for $i = j$ and $\delta_{ij} = 0$ for $i \neq j$). The quantity $y_{i,j}$ is equal to $\partial \ln y_i/\partial c_j$. Introducing equation 13 into equation 12 yields

$$D_{11} = \frac{1}{y_{12}}\left[y_{21}D_{22} + \frac{D_{12}}{c_1}(1 + c_1y_{11}) - \frac{D_{21}}{c_2}(1 + c_2y_{22})\right] \tag{14}$$

Substances 1 and 2 have identical properties so that, according to Dunlop (15),

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[3] If the tracer has a long half-life, its concentration in a typical experiment may approach 10^{-5}–10^{-4} M. As discussed subsequently, the assumption that $D_{12}(\partial c_2/\partial x) \ll D_{11}(\partial c_1/\partial x)$ remains valid in general and the effect of increasing tracer concentration gives rise to effects which are second order compared to the other effects to be considered. In the case of stable tracers such as D_2O, the relative tracer concentration may be significantly higher and the above condition may not hold.

$$y_{11} = y_{12} = y_{21} = y_{22}$$

and as $c_2 \rightarrow 0$, equation 14 becomes

$$D_{11} = D_{22} + D_{12} + \frac{1}{y_{11}} \left[\frac{D_{12}}{c_1} - \frac{D_{21}}{c_2} \right] \tag{15}$$

In writing equation 15, we have assumed that the quantity D_{21}/c_2 remains finite as $c_2 \rightarrow 0$. As discussed by Dunlop, this is a reasonable assumption. Inspection of equations 11 indicates that $D_{21} \rightarrow 0$ as $c_2 \rightarrow 0$ because J_2 must go to zero when c_2 goes to zero no matter what the value of $\partial c_1/\partial x$. Thus, D_{21}/c_2 is the limiting slope of the line obtained by plotting D_{21} against c_2 and extrapolating to $c_2 = 0$. Equation 15 may be simplified further. As shown in Appendix II for steady-state diffusion, the assumption that substances 1 and 2 have identical physical properties imposes the requirement that

$$\frac{D_{12}}{c_1} = \frac{D_{21}}{c_2} \tag{16}$$

Thus, equation 15 reduces to

$$D_{11} = D_{22} + D_{12} \tag{17}$$

Equation 17 indicates that condition *c* above is not satisfied in general and there will, therefore, be an error involved in the tracer hypothesis. Since these equations do not involve questionable assumptions, there is no doubt that the error exists but its magnitude remains uncertain.

Two recent studies by Albright and Mills (16) and by Albright (17) provide sufficient information to test the magnitude of the error introduced by the fact that $D_{11} \neq D_{22}$. They have carried out measurements of the diffusion of tracer nonelectrolyte in the presence of unlabeled substance for urea, alanine (zwitter ion form), and β-alanine. They have also shown that their data, together with data on the mutual diffusion coefficients of the unlabeled substances in water, can be used to calculate D_{11}, D_{12}, D_{21}, and D_{22} for the system: labeled substance–unlabeled substance–water. These data are given, however, in the volume fixed frame of reference and before using them, it is necessary to show that equation 17 also applies if the diffusion coefficients are expressed in this frame.[4]

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[4] Alternatively, we could convert coefficients in the volume fixed frame of reference to coefficients in the solvent fixed frame. However, the conversion requires use of partial molar volumes of the components (8) and it is simpler to make use of the published data in terms of the volume fixed frame.

Beginning with expressions of the form

$$(J_1)_v = -(D_{11})_v \frac{\partial c_1}{\partial x} - (D_{12})_v \frac{\partial c_2}{\partial x}$$

$$(J_2)_v = -(D_{21})_v \frac{\partial c_1}{\partial x} - (D_{22})_v \frac{\partial c_2}{\partial x}$$

Dunlop (15) has previously obtained the equivalent of equation 15 for the volume fixed frame. It has the form

$$(D_{11})_v = (D_{22})_v + \left[\frac{\frac{(D_{12})_v}{c_1}(1 + c_1 y_{11}) - c_0 \bar{V}_0 \frac{(D_{21})_v}{c_2}}{\bar{V}_1 + y_{11}} \right] \tag{18}$$

in which $\bar{V}_1$ and $\bar{V}_0$ are the partial molar volumes of the solute and solvent respectively and c_0 is the molar concentration of solvent; (since the two solutes are physically indistinguishable, $\bar{V}_1 = \bar{V}_2$). The subscript v denotes the volume fixed frame of reference. As might be expected and as shown explicitly in Appendix II,

$$\frac{(D_{12})_v}{c_1} = \frac{(D_{21})_v}{c_2}$$

if solutes 1 and 2 are physically indistinguishable. If we introduce this relation into equation 18 and make use of the condition

$$c_1 \bar{V}_1 + c_0 \bar{V}_0 = 1$$

which is valid when $c_2 \rightarrow 0$, we obtain

$$(D_{11})_v = (D_{22})_v + (D_{12})_v \tag{19}$$

Equation 19 indicates that the conclusions reached above are not dependent on the frame of reference and it permits easy utilization of the available experimental data to examine the magnitude of the difference between $(D_{11})_v$ and $(D_{22})_v$.

Table I gives the values of $(D_{ij})_v$ for urea, alanine, and β-alanine over a range of bulk concentrations from 0.001–4.0 M at a tracer concentration of 10^{-12} M. The $(D_{ij})_v$ were calculated from equation 14 of Albright and Mills (16) using the empirical expressions for tracer and mutual coefficients as functions of concentration given by Albright (17) and by Albright and Mills (16). Calculations using different tracer concentrations indicated that $(D_{11})_v$, $(D_{12})_v$, and $(D_{22})_v$ were altered by less than 0.5% by changing tracer concentration from 10^{-12}–10^{-4} M.[5] The data in Table I

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[5] The coefficient D_{21} is of course altered markedly because it is, to a first approximation, linearly related to the concentration of component 2 (the tracer).

indicate that the difference between $(D_{11})_v$ and $(D_{22})_v$ is quite small for bulk concentrations up to at least 0.1 M which is near the maximum usually encountered in biological experiments. At higher concentrations, however, the error becomes rather large for alanine and β-alanine. For urea, the difference between $(D_{11})_v$ and $(D_{22})_v$ is only 3.6% at a bulk concentration of 4 M. These calculations indicate that within the range of concentrations usually used in biological experiments, the error involved in assuming $(D_{11})_v = (D_{22})_v$ is well within the experimental error involved in estimating unidirectional fluxes.[6] However, significant errors are in-

TABLE I

DIFFUSION COEFFICIENTS FOR NONELECTROLYTES

c_1*	$D_{11} \times 10^5$	D_{12}	$D_{21} \times 10^{19}$	$D_{22} \times 10^5$	%Δ‡
M	*cm²/sec*	*cm²/sec*	*cm²/sec*	*cm²/sec*	
		Urea			
0.001	1.382	0.570×10^{-10}	0.570	1.382	~0
0.01	1.381	0.571×10^{-9}	0.571	1.381	~0
0.10	1.374	0.581×10^{-8}	0.581	1.373	0.04
0.50	1.344	0.314×10^{-7}	0.627	1.341	0.2
1.0	1.308	0.685×10^{-7}	0.684	1.301	0.5
2.0	1.244	0.160×10^{-6}	0.799	1.228	1.3
4.0	1.143	0.411×10^{-6}	1.028	1.102	3.6
		Alanine			
0.001	0.914	0.842×10^{-9}	8.42	0.914	0.01
0.01	0.913	0.842×10^{-8}	8.42	0.912	0.09
0.10	0.901	0.838×10^{-7}	8.38	0.892	0.9
0.50	0.848	0.410×10^{-6}	8.20	0.807	4.8
1.0	0.791	0.798×10^{-6}	7.98	0.712	10.1
2.0	0.707	0.151×10^{-5}	7.52	0.556	21.2
		β-Alanine			
0.001	0.939	0.145×10^{-10}	0.145	0.939	~0
0.01	0.937	0.201×10^{-9}	0.201	0.937	~0
0.10	0.924	0.741×10^{-8}	0.741	0.923	0.08
0.50	0.874	0.114×10^{-6}	2.88	0.860	1.3
1.0	0.828	0.499×10^{-6}	4.99	0.779	6.0
2.0	0.769	0.151×10^{-5}	7.57	0.618	19.6
4.0	0.662	0.317×10^{-5}	7.91	0.345	48.0

* Concentration of component 2 (tracer), 10^{-12} M.

‡ $\% \Delta = \left[\frac{D_{11} - D_{22}}{D_{11}}\right] \times 100.$

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[6] Note that if $c_2 y_{11}$ is not small compared to unity, equation 17 becomes

$$D_{11} = D_{22} + (D_{12} - D_{21})$$

Calculations have indicated that at tracer concentrations as high as 10^{-4} M, the error in assuming that $D_{11} = D_{22}$ is not altered significantly by including the value of D_{21}. Except at the low bulk concentrations where the error is negligible in any case, $D_{21} \ll D_{12}$.

volved at higher concentrations. Thus, further investigation of solutes of biological interest seems warranted to determine the concentration range over which the assumption introduces minimal error.

The next step in the analysis is to examine condition *b* by exploring the physical effect of the term $(D_{21})_v\ (\partial c_1/\partial x)$ on the flow of tracer. If experiments are carried out under conditions of uniform concentration of unlabeled substance, this term vanishes because $\partial c_1/\partial x = 0$. In the general case, the term cannot be neglected even though $(D_{21})_v$ is small because $\partial c_1/\partial x$ may be large, but its influence can be evaluated from equations 11 and the data in Table I. Using equation 16, the flow $(J_2)_v$ can be written

$$(J_2)_v = -\frac{(D_{12})_v c_2}{c_1}\frac{\partial c_1}{\partial x} - (D_{22})_v\frac{\partial c_2}{\partial x}$$

and in view of equation 19

$$\frac{(J_2)_v}{\partial c_2/\partial x} = (D_{12})_v\left[1 - \frac{c_2(\partial c_1/\partial x)}{c_1(\partial c_2/\partial x)}\right] - (D_{11})_v = -D_{2T} \qquad (20)$$

in which the quantity D_{2T} is the observed tracer diffusion coefficient given by the ratio $J_2/(\partial c_2/\partial x)$. Introducing equation 20 into equation 8 yields

$$(J_1)_v = \left\{(D_{12})_v\left[1 - \frac{c_2(\partial c_1/\partial x)}{c_1(\partial c_2/\partial x)}\right] - (D_{11})_v\right\}\frac{\partial c_1}{\partial x} = -D_{2T}\frac{\partial c_1}{\partial x} \qquad (21)$$

Equation 21 gives the value of $(J_1)_v$ predicted from measurement of tracer flow and the usual assumption of the tracer hypothesis that $(J_1)_v/(\partial c_1/\partial x) = (J_2)_v/(\partial c_2/\partial x)$. The observed flow $(J_1)_v$ is given by

$$(J_1)_v = -(D_{m1})_v\frac{\partial c_1}{\partial x}$$

in which we have assumed that $(D_{12})_v(\partial c_2/\partial x)$ is negligible. Under these conditions, $(D_{11})_v = (D_{m1})_v$ where $(D_{m1})_v$ is the mutual diffusion coefficient for substance 1 in water. In order to obtain some insight into the behavior of equation 21, we can consider the case of diaphragm cell experiments across a porous disk separating well stirred solutions of different composition in which flows are measured. If we assume that concentrations vary linearly with distance in the disk,

$$\frac{\partial c_i}{\partial x} = \frac{c_i' - c_i''}{\Delta x}$$

in which Δx is the thickness of the disc; a mean solute concentration, $\bar{c}_i$, can be defined by the relation

$$\bar{c}_i = \frac{c_i' + c_i''}{2}$$

Equation 21 for flow across the disc then becomes

$$(J_1)_v = \left\{(\bar{D}_{12})_v \left[1 - \frac{(c_2' + c_2'')\,(c_1' - c_1'')}{(c_1' + c_1'')\,(c_2' - (c_2'')}\right] - (\bar{D}_{11})_v\right\} \frac{(c_1' - c_1'')}{\Delta x}$$

$$= - \bar{D}_{2T} \frac{(c_1' - c_1'')}{\Delta x} \qquad (22)$$

in which $(\bar{D}_{12})_v$ and $(\bar{D}_{11})_v$ are the values of the diffusion coefficients for the concentrations $\bar{c}_1$ and $\bar{c}_2$. The quantity $\bar{D}_{2T}$ is the observed tracer diffusion coefficient under the conditions specified; it is defined by the relation

$$\bar{D}_{2T} = \frac{J_2 \Delta x}{c_2' - c_2''}$$

We wish to compare equation 22 to the expression

$$J_1 = - (\bar{D}_{m1})_v \frac{(c_1' - c_1'')}{\Delta x} \qquad (23)$$

Equation 22 gives the flow of substance 1 predicted from tracer measurements and equation 23 is the actual flow that would be observed by direct measurement of substance 1. For simplicity, we shall assume that tracer is placed on the (′) side and that measurement is made under conditions in which $c_2'' \approx 0$ so that according to equation 22,

$$\bar{D}_{2T} = (\bar{D}_{12})_v \left[\frac{c_1' - c_1''}{c_1' + c_1''} - 1\right] + (\bar{D}_{11})_v \qquad (24)$$

Using the available information on $(D_{12})_v$ and $(D_{11})_v$ for urea, alanine, and β-alanine, $\bar{D}_{2T}$ can be evaluated under a variety of conditions. Table II shows the change in $\bar{D}_{2T}$ when c_1' is kept constant at 0.001 M and c_1'' is varied from 0.001–4.0 M. As might be expected, an adverse concentration gradient of the bulk species leads to a reduction in $\bar{D}_{2T}$ from the value observed in the absence of a gradient. Thus, the unidirectional flux $\vec{J}_1$, calculated from measurement of tracer flow, will decrease as c_1'' is increased even though c_1' remains constant. It is often assumed that such an effect will not occur in an artificial system and that a variation in the flux $\vec{J}_1$ with a change in c_2'' should be ascribed to particular biological phenomena. While this "transconcentration effect" is quite marked for alanine and β-alanine at high concentrations, it is of the order of 1–2% at concentrations of biological interest. The observed effect is due in part to the interaction between diffusing species and in part to the concentration variation of diffusion coefficients. If the concentration gradient of the bulk species is in the same direction as that of the tracer, $c_1' - c_1''$ is positive and the effect on $\bar{D}_{2T}$ is much smaller because the term in brackets in equation 24 is less than unity. If $c_1' \gg c_1''$, the bracketed term approaches zero and $\bar{D}_{2T} \rightarrow (\bar{D}_{m1})_v$ so that the tracer predicts the flow of bulk species accurately under these conditions (16).

We may also ask whether measurement of tracer flow in this system can be used to predict the net flow of bulk material. Since the flow J_1 is given by equation 23,

TABLE II

EFFECT OF CONCENTRATION GRADIENT ON DIFFUSION COEFFICIENTS

c', M . . .	0.001	0.001	0.001	0.001	0.001
c'', M . . .	0.001	0.100	0.500	1.000	4.00
		Urea			
$\bar{D}_{2T} \times 10^5$	1.382	1.377	1.359	1.337	1.212
$\%\Delta_1$*	—	−0.4	−1.7	−3.3	−12.9
$(\bar{D}_{m1})_v \times 10^5$	1.382	1.378	1.362	1.344	1.244
$\%\Delta_2$‡	0	0	−0.2	−0.5	−2.6
		Alanine			
$\bar{D}_{2T} \times 10^5$	0.914	0.899	0.839	0.766	0.406
$\%\Delta_1$	—	−1.6	−8.2	−16.2	−55.5
$(\bar{D}_{m1})_v \times 10^5$	0.914	0.907	0.880	0.848	0.706
$\%\Delta_2$	0	−0.9	−4.6	−9.7	−42.6
		β-Alanine			
$\bar{D}_{2T} \times 10^5$	0.939	0.931	0.895	0.845	0.466
$\%\Delta_1$	—	−0.8	−4.7	−10.0	−50.4
$(\bar{D}_{m1})_v \times 10^5$	0.939	0.931	0.903	0.874	0.769
$\%\Delta_2$	0	0	−0.9	−3.2	−39.4

$$* \ \% \ \Delta_1 = \frac{(\bar{D}_{2T})_{c''} - (\bar{D}_{2T})_{0.001}}{(\bar{D}_{2T})_{0.001}} \times 100$$

$$‡ \ \% \ \Delta_2 = \frac{\bar{D}_{2T} - (\bar{D}_{m1})_v}{(\bar{D}_{m1})_v} \times 100$$

we compare $(\bar{D}_{m1})_v$ and $\bar{D}_{2T}$. The results are presented in Table II; the predicted and observed flows agree reasonably well for values of c_1'' up to at least 0.5 M. The effect of the concentration gradient is smaller in this case than for comparison of $\bar{D}_{2T}$ at various concentration differences because $(\bar{D}_{m1})_v$ also varies with concentration.

ELECTROLYTES

The case of diffusion of ions is made somewhat more complex by the fact that in the absence of electric current, ions cannot migrate independently of one another because of the requirement of electroneutrality. If a tracer for Na^+, for example, is present in a solution of NaCl, the migration of the tracer is determined by the mobility of Na^+ as indicated by the experimental observation that the mobility of the Na tracer approaches the limiting mobility of Na^+ at low concentrations of supporting electrolyte (18, 19). On the other hand, the movement of Na^+ in bulk solution is determined by D_{NaCl} which is dependent on the mobilities of both the anion and the cation. Thus, in free solution, measurement of tracer Na cannot be expected to predict accurately the diffusion of NaCl. In this case, a different approach must be used to examine the tracer hypothesis which states, for example, that Na tracer can be used to predict the flow of Na^+. According to the theory of nonequilibrium thermodynamics, a system containing a cation (species 1), an anion (species 3), and a tracer for either ion (species 2) can be described by the following set of equations

$$\begin{aligned} J_1 &= -L_{11}\frac{\partial\bar{\mu}_1}{\partial x} - L_{12}\frac{\partial\bar{\mu}_2}{\partial x} - L_{13}\frac{\partial\bar{\mu}_3}{\partial x} \\ J_2 &= -L_{21}\frac{\partial\bar{\mu}_1}{\partial x} - L_{22}\frac{\partial\bar{\mu}_2}{\partial x} - L_{23}\frac{\partial\bar{\mu}_3}{\partial x} \\ J_3 &= -L_{31}\frac{\partial\bar{\mu}_1}{\partial x} - L_{32}\frac{\partial\bar{\mu}_2}{\partial x} - L_{33}\frac{\partial\bar{\mu}_3}{\partial x} \end{aligned} \tag{25}$$

in which $\bar{\mu}_i$ is the electrochemical potential of component i; $\bar{\mu}_i = \mu_i + z_i\mathfrak{F}\psi$ where ψ is the electrical potential, $\mathfrak{F}$ is the Faraday, and z_i is the valence. The J_i are flows of solute relative to solvent. This system is more complex than the one discussed above because the requirement of electroneutrality necessitates introduction of a third component into the system. We assume throughout that $L_{ij} = L_{ji}$ for $i \neq j$.

We shall initially consider the simple system in which measurements are made of tracer flow, J_2, under conditions of uniform concentration of bulk electrolyte because data are available for several ions in such systems. Throughout this discussion we shall assume that the concentration of tracer is very much less than that of the bulk component ($c_2 \rightarrow 0$). Under these conditions, $\partial\psi/\partial x = 0$ (18, 19) so that

$$J_2 = -L_{22}\frac{\partial\bar{\mu}_2}{\partial x} = -L_{22}\frac{\partial\mu_2}{\partial c_2}\frac{\partial c_2}{\partial x} = -D_2{}^*\frac{\partial c_2}{\partial x} \tag{26}$$

in which $D_2{}^*$ is the diffusion coefficient for the tracer under the specified conditions.[7] We wish to compare this coefficient with the one for the unlabeled ionic species. This comparison is analogous to the case in which D_{11} and D_{22} for nonelectrolytes

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[7] Equation 26 is not exact because $\partial c_1/\partial x$ is not zero. Under the conditions specified, $\partial c_3/\partial x = 0$ if substance 2 is a tracer for 1. However, $\partial c_1/\partial x = -\partial c_2/\partial x$ and if for simplicity we assume ideal solutions, the exact form of equation 26 is

$$J_2 = -RT\left[\frac{L_{22}}{c_2} - \frac{L_{21}}{c_1}\right]\frac{\partial c_2}{\partial x} \approx -D_2{}^*\frac{\partial c_2}{\partial x}$$

If $c_2 \ll c_1$, this expression reduces to equation 26.

were compared. The appropriate experimental conditions for the comparison seem to be the following: a concentration difference for the electrolyte is set up, for example across a porous disc, and the resulting diffusion potential is short-circuited by using appropriate electrodes (13). Under these conditions, $\partial\psi/\partial x = 0$, and because substance 2 is not present, the flow of unlabeled ion, J_1 becomes

$$J_1 = -L_{11}\frac{\partial\mu_1}{\partial x} - L_{13}\frac{\partial\mu_3}{\partial x}$$

at a point x in the barrier. Making use of the condition of electroneutrality, which for uni-univalent salts takes the form $c_1 = c_3 = c$ where c is salt concentration, and the concept of mean ionic activity coefficient, this expression becomes

$$J_1 = \frac{-RT(L_{11} + L_{13})}{c}\left[1 + c\frac{\partial \ln y}{\partial c}\right]\frac{\partial c}{\partial x} = -D_1\frac{\partial c}{\partial x} \qquad (27)$$

in which y is the molar activity coefficient of the salt.[8] The flow J_1 predicted by tracer measurements made at the same uniform salt concentration would be given by the equivalent of equation 8;

$$J_1 = \frac{J_2}{\partial c_2/\partial x}\left(\frac{\partial c}{\partial x}\right) = -D_2^*\frac{\partial c}{\partial x} \qquad (28)$$

We wish to compare D_1 defined by equation 27 with D_2^*. (If substance 2 is tracer for the anion, J_3 appears in equations 27 and 28 in place of J_1, L_{33} replaces L_{11}, and a coefficient D_3 is defined as in equation 27). Since values of D_2^* are known for several systems (see Robinson and Stokes, 18, p. 317), and the L_{11} and L_{13} have been evaluated recently by Miller (20) for the same systems, such comparisons are possible. The results obtained for the system Na tracer in NaCl and for Cl tracer in NaCl and KCl are shown in Table III. Although differences between tracer (D_2^*) and bulk (D_1 or D_3) coefficients are rather large at high salt concentrations, they amount to only a few per cent at concentrations of biological interest. Further, these small differences may not be significant. The tracer diffusion coefficients are probably somewhat less accurate than the ordinary coefficients and the computation of L_{11} (or L_{33}) and L_{13} involves the use of three measured quantities. While these quantities are, in general, known quite accurately, the computed coefficients will necessarily be somewhat less exact.

The problem of evaluating the effect of a gradient of unlabeled electrolyte on the flow of tracer ion is more complex in the present case because of the presence of a third solute component in the system, and some assumptions are necessary in order to evaluate the necessary coefficients. Evaluation of the flow J_2 (equation 25) when

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[8] It can be shown that equation 27 also applies if tracer (substance 2) is present providing the concentration of 2 is small compared to the bulk electrolyte concentration.

$\partial \tilde{\mu}_1/\partial x$ and $\partial \tilde{\mu}_3/\partial x$ are nonzero requires knowledge of L_{21} and L_{23}. To obtain estimates of these quantities, we proceed as follows: the coefficient L_{23} can be estimated by the reasonable assumption that substances 1 and 2 (which are assumed to

TABLE III

COEFFICIENTS FOR ELECTROLYTE DIFFUSION

c	$\frac{10^5 RT(L_{11} + L_{13})}{c}$	$1 + cy_{ss}$‡	$D_1 \times 10^5$	$D_2^* \times 10^5$§	%Δ‖
M	cm²/sec		cm²/sec	cm²/sec	
		Na tracer in NaCl			
0.001	1.341	0.9830	1.318	1.332	1.1
0.01	1.351	0.9553	1.291	1.330	3.0
0.10	1.369	0.9164	1.255	1.299	3.5
0.50	1.351	0.9297	1.259	1.279	1.8
1.00	1.294	0.9795	1.267	1.234	−2.6
3.00	1.047	1.2760	1.330	1.030	−22.6
		Cl tracer in NaCl			
c	$\frac{10^5 RT(L_{33} + L_{31})}{c}$	$1 + cy_{ss}$	$D_3 \times 10^5$	$D_2^* \times 10^5$	%Δ‖
0.001	2.034	0.9830	1.999	2.030	1.5
0.01	2.034	0.9553	1.942	2.024	4.2
0.10	2.021	0.9164	1.851	1.953	5.4
0.50	1.973	0.9297	1.835	1.853	0.7
1.00	1.904	0.9795	1.865	1.772	−6.5
3.00	1.549	1.2760	1.977	1.449	−26.6
		Cl tracer in KCl			
0.001	2.036	0.9829	2.001	2.030	1.4
0.01	2.044	0.9540	1.950	2.018	3.5
0.10	2.072	0.9066	1.880	1.965	4.5
0.50	2.111	0.8915	1.883	1.957	3.9
1.00	2.126	0.9051	1.924	1.955	1.6
3.00	2.074	1.0370	2.151	1.849	−14.0

‡ $y_{ss} = \partial \ln y/\partial c$ where y is the activity coefficient of the salt.

§ Values of D_2^* are taken from the compilation of Robinson and Stokes (18).

‖ $\% \Delta = \frac{D_2^* - D}{D} \times 100$, where D refers to D_1 or D_3.

be physically indistinguishable) interact with substance 3 in the same manner so that[9]

$$L_{23} = \frac{c_2}{c_1} L_{13} \tag{29}$$

In order to determine L_{21}, we consider an experiment in which a mixture of un-

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[9] This relation can be demonstrated by a frictional analysis similar to that given in Appendix II. It also depends on the assumption that substances 1 and 2 are physically indistinguishable.

labeled electrolyte and tracer electrolyte diffuses into pure water. In this case, the flow of tracer per unit gradient must equal the flow of unlabeled ion per unit gradient and both flows will be determined by the mutual diffusion coefficient of the salt in water, D_{ms}, (see Albright, reference 16, for detailed discussion on this point). Under these conditions,

$$\frac{J_1}{\partial c/\partial x} = \frac{J_2}{\partial c_2/\partial x} = -D_{ms} \tag{30}$$

In such an experiment, there will be a diffusion potential so that $\partial\psi/\partial x \neq 0$ but the potential gradient will be given by

$$-\mathfrak{F}\frac{\partial\psi}{\partial x} = (2t_1 - 1)\frac{\partial\mu_1}{\partial x} \tag{31}$$

if we consider only mono-mono valent electrolytes (13); t_1 is the transference number of the cation (since the tracer is present at very low concentration, $t_2 \to 0$). If we introduce equations 29 and 31 into equation 25 for J_2 and make use of the relation

$$\frac{\partial\mu_1}{\partial x} = \frac{\partial\mu_1}{\partial c}\frac{\partial c}{\partial x} = \frac{RT}{c}(1 + c\,y_{ss})\frac{\partial c}{\partial x} = \frac{\partial\mu_3}{\partial x}$$

and the assumption that $\partial\mu_2/\partial x = (RT/c_2)\partial c_2/\partial x$ when $c_2 \to 0$, we obtain after rearrangement

$$\frac{J_2}{\partial c_2/\partial x} = -D_{ms} = -(1 + cy_{ss})\left[2(1 - t_1)\frac{RTL_{21}}{c_2} - (2t_1 - 1)D_2^* + \frac{2t_1RTL_{13}}{c}\right]\frac{c_2(\partial c/\partial x)}{c(\partial c_2/\partial x)} - D_2^* \tag{32}$$

The quantity y_{ss} is equal to $\partial \ln y/\partial c$ where y is the activity coefficient of the salt. The quantity $c_2(\partial c/\partial x)/c(\partial c_2/\partial x)$ is equal to unity under the conditions specified because the ratio c_2/c is constant at all points in the system. Since all other quantities in equation 32 are known, this expression can be used to calculate L_{21}/c_2. The data necessary for this evaluation have been taken from Miller (20) except for the values of D_2^* which are from the compilation given by Robinson and Stokes (18). If the labeled ion is the anion, so that the properties of substances 2 and 3 are identical,

$$L_{21} = \frac{c_2}{c_3}L_{31} = \frac{c_2}{c_3}L_{13} \tag{33}$$

and equation 32 takes the form

$$D_{ms} = (1 + cy_{ss})\left[2(1 - t_1)\frac{RTL_{13}}{c} + (2t_1 - 1)D_2^* + \frac{2t_1RTL_{23}}{c_2}\right]\frac{c_2(\partial c/\partial x)}{c(\partial c_2/\partial x)} + D_2^* \tag{34}$$

from which L_{23}/c_2 can be calculated. The results of these estimations of cross-coefficients between bulk and tracer ions are summarized in Table IV for several cases. Corresponding values of L_{13}/c, expressing interaction between anion and cation, are included for comparison.

Miller (21) has recently described evaluation of cross-coefficients in systems containing three ions. The coefficients describing interaction between ions of like charge such as Na-K, Li-K, and Li-Na are negative and are smaller than those between ions of opposite charge in agreement with the calculated values given in Table IV for the pairs Na-Na* and Cl-Cl*. As pointed out by Miller, negative values would be expected because of the coulombic repulsion between the ions. It is of interest to note that the values of L_{ij} for pairs of dissimilar cations are of the same order of mag-

TABLE IV

PHENOMENOLOGICAL COEFFICIENTS FOR ELECTROLYTES

c	$(L_{13}/c)^* \times 10^{12}$	(L_{21}/c_2)‡ $\times 10^{12}$	
M			
0.001	0.081	−0.048	Na tracer in NaCl
0.01	0.233	−0.145	
0.10	0.554	−0.266	
0.50	0.840	−0.546	
1.0	0.911	−0.667	
3.0	0.858	−0.788	
c	$(L_{13}/c) \times 10^{12}$	(L_{23}/c_2)‡ $\times 10^{12}$	
M			
0.001	0.081	−0.048	Cl tracer in NaCl
0.01	0.233	−0.190	
0.10	0.554	−0.282	
0.5	0.840	−0.339	
1.0	0.911	−0.423	
3.0	0.858	−0.452	
0.001	0.086	−0.060	Cl tracer in KCl
0.01	0.256	−0.101	
0.10	0.647	−0.214	
0.50	1.038	−0.415	
1.0	1.214	−0.520	
3.0	1.440	−0.532	

* L_{13}/c from Miller (20).

‡ L_{21}/c_2 and L_{23}/c_2 calculated from equations 32 or 34. All coefficients are in the solvent fixed frame of reference. In computing, $(D_{ms})_v$ and $1 + m\partial \ln \gamma/\partial m$ given by Miller have been used where m is molality and subscript v denotes volume fixed frame. No corrections for reference frame or molality are necessary (20) because

$$\frac{(D_{ms})_v}{1 + m\partial \ln \gamma/\partial m} = \frac{D_{ms}}{1 + c\partial \ln y/\partial c}$$

nitude as those obtained in the present calculations. Thus, although our estimations of L_{12} or L_{23} are based on assumptions that are not required in Miller's analysis, they should provide reasonable estimates of effects of the bulk ion on its tracer. As in the case of nonelectrolytes, the information in Table IV can be used to estimate the effect of a gradient of bulk electrolyte on tracer flow. Again, we compare the tracer flux observed when tracer diffuses in a uniform solution of bulk species at a concentration of 0.001 M with the flux predicted when tracer diffusion takes place from 0.001 M solution to one of higher bulk concentration. We have again assumed that the diffusion potential has been short-circuited so that $\partial\psi/\partial x = 0$. Under these conditions, tracer flow, J_2, is given by

$$J_2 = -L_{21}\frac{\partial\mu_1}{\partial x} - L_{22}\frac{\partial\mu_2}{\partial x} - L_{23}\frac{\partial\mu_3}{\partial x}$$

or

$$J_2 = -(1 + cy_{ss})\,RT\,\frac{(L_{21} + L_{23})}{c}\left(\frac{\partial c}{\partial x}\right) - D_2^*\frac{\partial c_2}{\partial x}$$

if the tracer concentration is small compared to c. Thus,

$$\frac{J_2}{\partial c_2/\partial x} = -D_{2T} = -(1 + cy_{ss})\,RT\left[\frac{L_{13}}{c} + \frac{L_{21}}{c_2}\right]\frac{c_2(\partial c/\partial x)}{c(\partial c_2/\partial x)} - D_2^* \qquad (35)$$

in which equation 29 has also been used. If the tracer is the anion, L_{21}/c_2 is replaced by L_{23}/c_2 in accord with equation 33. To evaluate D_{2T} in the presence of a concentration gradient we have used the same approach employed above for nonelectrolytes. Assuming that $c_2'' \approx 0$, equation 35 can be written

$$\bar{D}_{2T} = (1 + \overline{cy}_{ss})\,RT\left[\frac{\bar{L}_{13}}{\bar{c}} + \frac{\bar{L}_{21}}{\bar{c}}\right]\frac{c' - c''}{c' + c''} + \bar{D}_2^*$$

in which the bar denotes the value of the quantity at the mean salt concentration $\bar{c} = (c' + c'')/2$. Calculated values of $\bar{D}_{2T}$ for several cases are shown in Table V. As in the case of nonelectrolytes, tracer flow is reduced by the presence of an adverse concentration gradient, but the effect amounts to only a few percent at concentrations of biological interest. The difference between $\bar{D}_{2T}$ and the coefficient for flow of unlabelled ion, $\bar{D}_1$ or $\bar{D}_3$ (equation 27), is relatively small except at the highest bulk concentration. Thus, measurement of $\bar{D}_{2T}$ can be used to predict the flow of unlabeled ion to an accuracy of a few percent.

COMMENT

There are two major points regarding the analysis presented above. First, the interactions between diffusing species can give rise to errors in the interpretation of tracer fluxes. Second, for the few substances about which information is available, the effect of interactions is quite small in the range of concentrations of interest in most biological experiments (up to 0.1 or 0.2 M). Within this range, the errors arising from flow interactions in a simple physical system are probably within the over-all experimental uncertainty for most flux studies in biological systems. The immediate implication of this second point is that observed changes in tracer fluxes under varying conditions in biological systems can be ascribed to particular effects arising within the system rather than to the simple physical phenomenon of interaction of diffusing species. However, this conclusion must be considered with caution. Strictly speaking, it applies only to diffusion in free solution or within a completely nonselective barrier since these are the conditions assumed throughout the analysis. We must, therefore, pose the question of possible additional effects arising if the membrane is selective so that it retards the solute relative to the solvent. Unfortunately, a direct answer to this problem does not seem available at present. There is no suitable information on tracer flow in selective membranes to compare to that available for free solution and a clear-cut answer on a theoretical basis does not seem possible.

TABLE V

EFFECT OF CONCENTRATION GRADIENTS ON DIFFUSION COEFFICIENTS

c'', M ...	0.001	0.10	0.40	1.00	3.00
c', M ...	0.001	0.001	0.001	0.001	0.001
		Na tracer in NaCl			
$\bar{D}_{2T} \times 10^5$	1.333	1.274	1.226	1.212	1.129
$\%\Delta_1$*	—	−4.4	−8.0	−9.1	−15.3
$\bar{D}_1 \times 10^5$	1.318	1.264	1.252	1.256	1.283
$\%\Delta_2$‡	+1.1	+0.8	−2.1	−3.5	−12.0
		Cl tracer in NaCl			
$\bar{D}_{2T} \times 10^5$	2.030	1.957	1.862	1.738	1.574
$\%\Delta_1$	—	−3.6	−8.3	−14.4	−22.4
$\bar{D}_3 \times 10^5$	1.999	1.879	1.837	1.835	1.880
$\%\Delta_2$	+1.6	+4.2	+1.4	−5.3	−16.3
		Cl tracer in KCl			
$\bar{D}_{2T} \times 10^5$	2.030	1.927	1.850	1.820	1.702
$\%\Delta_1$	—	−5.1	−8.9	−10.3	−16.2
$\bar{D}_3 \times 10^5$	2.002	1.898	1.870	1.882	1.978
$\%\Delta_2$	+1.4	+1.5	−1.1	−3.3	−14.0

$$* \ \% \Delta_1 = \frac{(\bar{D}_{2T})c'' - (\bar{D}_{2T})_{0.001}}{(\bar{D}_{2T})_{0.001}} \times 100$$

$$‡ \ \% \Delta_2 = \frac{\bar{D}_{2T} - \bar{D}}{\bar{D}} \times 100, \text{ where } \bar{D} \text{ refers to } \bar{D}_1 \text{ or } \bar{D}_3.$$

An attempt to approach this problem in terms of a comparable analysis via the frictional model encounters several difficulties. First, to obtain expressions for flows relative to solvent within the membrane, we must assume that solvent flow is zero [or equal to the flow of the membrane which is usually taken as zero (22)]. Without this assumption, additional terms are introduced that make comparison with the simple nonselective barrier impossible. Using the assumption of zero solvent flow, expressions for the ratio D_{12}/D_{11} for nonelectrolytes can be obtained. As discussed above, this ratio is one measure of the error involved in the tracer hypothesis. However, the value of D_{12}/D_{11} within the membrane cannot be compared with that expected in free solution unless we have information on the behavior of, for example, the coefficient of friction between solute and water within the membrane. There is no a priori reason to assume that this coefficient is the same in the membrane as in free solution, and the observations of Ginzburg and Katchalsky (23) suggest that it may differ appreciably. Depending on the behavior of the various frictional coefficients, D_{12}/D_{11} in the membrane may be either greater than or less than D_{12}/D_{11} in free solution. Thus, a selective membrane may lead to greater discrepancies in the tracer method than those predicted for a nonselective membrane, but this cannot be proved with certainty.

The data recently reported by Kitahara et al (24) on flow of tracer Cl through cellophane membranes suggests that a substantial effect of interaction can be observed. However, their experimental arrangement is too complex for exact analysis and the necessary comparative data concerning interactions in free solution among the solutes used does not exist. Rough estimates suggest that interactions (i.e. L_{ik}) of the order of magnitude of those shown in Table IV would be sufficient to explain the observed results so that markedly greater effects than those occurring in free solution need not be postulated. In addition, Van Bruggen and his coworkers (private communication) have recently obtained evidence suggesting interactions between diffusing solutes in artificial membranes, but there is not yet sufficient information to establish whether the effect is influenced by the relative selectivity of the membrane. Thus, care remains essential in interpreting some of the apparently anomalous results observed with tracers in biological systems. Kedem and Essig (3) have suggested that some of these effects could be ascribed to interactions. This seems clearly true, but it also seems that for interactions of sufficient magnitude to exist, the physical realities of transfer in the biological system must be quite different than in free solution, and suggestions that these anomalous results are due to properties of the biological system seem reasonable. Whether the specific kinetic models advanced to explain the behavior are correct remains to be seen.

The major point emerging from this analysis is that flow interactions do not affect the interpretation of tracer measurements very markedly in simple systems at relatively low solute concentrations, so that suggestions that the tracer technique is invalid in general because of interactions is unwarranted.

APPENDIX I

We wish to show that the equations usually used in consideration of tracer kinetics (25) are equivalent to equation 4. For a closed two-compartment system with tracer initially added to the (′) side, tracer flow is described by the expression

$$\frac{1}{A}\frac{dP''}{dt} = \overrightarrow{J}_1 p_2{}^{*\prime} - \overleftarrow{J}_1 p_2{}^{*\prime\prime} \tag{A1}$$

in which P'' is the total amount of tracer in the (″) compartment (counts/minute) and p^* is specific activity of tracer (counts/minute microequivalent). Assuming constant volume and using the relation

$$p^* = \frac{P}{Vc} = \frac{p}{c}$$

where V is compartment volume, c is the concentration of bulk unlabeled substance, and p is tracer concentration (counts/minute milliliter) in the compartment, equation A1 may be written

$$\frac{V}{A}\frac{dp''}{dt} = \frac{\overrightarrow{J}_1 p_2{}'}{c_1{}'} - \frac{\overleftarrow{J}_1 p_2{}''}{c_1{}''}$$

We then define a factor

$$k = \frac{J_1}{c_1{}'} = \frac{\overleftarrow{J}_1}{c_1{}''}$$

so that

$$\frac{V}{A}\frac{dp''}{dt} = k(p_2{}' - p_2{}'')$$

Finally, both sides of equation A2 may be multiplied by a factor α which converts counts/min of tracer to moles of tracer to yield

$$\frac{V\alpha}{A}\frac{dp''}{dt} = J_2 = \alpha k(p_2{}' - p_2{}'') = k(c_2{}' - c_2{}'')$$

which is equivalent to equation 2 if k is identified with the permeability coefficient.

APPENDIX II

The relation between D_{12} and D_{21} can be evaluated using the frictional approach introduced by Spiegler (26) and discussed in detail by Kedem and Katchalsky (27). According to this concept, in steady-state diffusion, the driving force is exactly balanced by frictional forces acting on the diffusing substances. For the case of two solutes, we write

$$-\frac{\partial\mu_1}{\partial x} = f_{10}(v_1 - v_0) + f_{12}(v_1 - v_2)$$

$$-\frac{\partial\mu_2}{\partial x} = f_{20}(v_2 - v_0) + f_{21}(v_2 - {}_1) \tag{B1}$$

in which f_{ij} is the frictional coefficient between a mole of substance i and an infinite amount of substance j and v_i is the velocity of substance i. The subscript 0 denotes the solvent. Equations B1 can also be written

$$-\frac{\partial\mu_1}{\partial x} = f_{10}(v_1 - v_0) + f_{12}(v_1 - v_0) - f_{12}(v_2 - v_0)$$

$$-\frac{\partial\mu_2}{\partial x} = f_{20}(v_2 - v_0) + f_{21}(v_2 - v_0) - f_{21}(v_1 - v_0) \qquad \text{(B2)}$$

Introducing the definition of flow, $J_i' = c_i v_i$ where J_i' is the absolute flow of i relative to a fixed coordinate system, equations B2 become

$$-\frac{\partial\mu_1}{\partial x} = \frac{f_{10} + f_{12}}{c_1}\left(J_1' - \frac{c_1}{c_0}J_0'\right) - \frac{f_{12}}{c_2}\left(J_2' - \frac{c_2}{c_0}J_0'\right)$$

$$-\frac{\partial\mu_2}{\partial x} = \frac{-f_{21}}{c_1}\left(J_1' - \frac{c_1}{c_0}J_0'\right) + \frac{f_{20} + f_{21}}{c_2}\left(J_2' - \frac{c_2}{c_0}J_0'\right) \qquad \text{(B3)}$$

However, the flow $J_i' - (c_i/c_0)J_0'$ is simply the flow of solute relative to the solvent, J_i, in our notation. Equations B3 have, therefore, the form of inverted phenomenological equations:

$$-\frac{\partial\mu_1}{\partial x} = R_{11}\,J_1 + R_{12}\,J_2$$

$$-\frac{\partial\mu_2}{\partial x} = R_{21}\,J_1 + R_{22}\,J_2 \qquad \text{(B4)}$$

which are the inverse form of equations 9. The reciprocal relation, $R_{12} = R_{21}$ which is valid if $L_{12} = L_{21}$, requires that

$$\frac{f_{12}}{c_2} = \frac{f_{21}}{c_1} \qquad \text{(B5)}$$

Since the R_{ij} of equations B4 are related simply to the L_{ij} of equations 9, and the D_{ij} of equations 11 are determined by the L_{ij}, the D_{ij} can be expressed in terms of the frictional coefficients of equations B3. The L_{ij} are given by

$$L_{11} = \frac{(f_{20} + f_{21})c_1}{F} \qquad L_{12} = \frac{f_{12}\,c_1}{F}$$

$$L_{21} = \frac{f_{21}\,c_2}{F} \qquad L_{22} = \frac{(f_{10} + f_{12})c_2}{F}$$

in which $F = f_{10}f_{20} + f_{10}f_{21} + f_{20}f_{12}$. From the relation between the D_{ij} and the L_{ij},

$$D_{12} = \frac{1}{F}\left[(f_{20} + f_{21})\,c_1\,y_{12} + f_{12}\,c_1\left(\frac{1}{c_2} + y_{22}\right)\right]$$

$$D_{21} = \frac{1}{F}\left[f_{21}\,c_2\left(\frac{1}{c_1} + y_{11}\right) + (f_{10} + f_{12})\,c_2\,y_{21}\right]$$

If, however, substances 1 and 2 are physically indistinguishable, they must both interact with the solvent in the same way so that $f_{20} = f_{10}$, and $y_{11} = y_{12} = y_{21} = y_{22}$. Thus,

$$\frac{D_{12}}{c_1} = \frac{1}{F}\left(f_{10}\, y_{11} + f_{21}\, y_{11} + \frac{f_{12}}{c_2} + f_{12}\, y_{11}\right)$$

$$\frac{D_{21}}{c_2} = \frac{1}{F}\left(f_{10}\, y_{11} + f_{21}\, y_{11} + \frac{f_{21}}{c_1} + f_{12}\, y_{11}\right)$$

In view of equation B5,

$$\frac{D_{12}}{c_1} = \frac{D_{21}}{c_2} \tag{B6}$$

if substances 1 and 2 are indistinguishable. Equation B6 obviously does not hold except in this special circumstance because in general $f_{10} \neq f_{20}$.

Equation B6 is also true if diffusion coefficients in the volume fixed frame of reference are considered. For a system containing two solutes, diffusion coefficients in the solvent and volume fixed frames are related (7) by the expressions

$$(D_{12})_v = D_{12} - c_1(\bar{V}_1 D_{12} + \bar{V}_2 D_{22})$$

$$(D_{21})_v = D_{21} - c_2(\bar{V}_1 D_{11} + \bar{V}_2 D_{21})$$

in which the subscript v denotes the volume fixed frame. Unsubscripted coefficients are the solvent fixed ones discussed above and $\bar{V}_1$ and $\bar{V}_2$ are partial molar volumes of substances 1 and 2. If the two solutes are indistinguishable, $\bar{V}_1 = \bar{V}_2$ (15) so that

$$\frac{(D_{12})_v}{c_1} - \frac{(D_{21})_v}{c_2} = \frac{D_{12}}{c_1} - \frac{D_{21}}{c_2} - \bar{V}_1\,[D_{12} + D_{22} - D_{11} - D_{21}] \tag{B7}$$

The first two terms on the right hand side of equation B7 cancel in view of equation B6. The general relation between the D_{ij} when substances 1 and 2 are identical requires that the term in brackets be zero (see footnote 6) so that

$$\frac{(D_{12})_v}{c_1} = \frac{(D_{21})_v}{c_2}. \tag{B8}$$

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