THE VALIDITY OF THE USSING FLUX RATIO EQUATION IN A THREE-DIMENSIONALLY INHOMOGENEOUS MEMBRANE

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ABSTRACT Derivations of the Ussing flux ratio equation have, until now, required the membrane to be both bounded by parallel planes and homogeneous, except in the transmembrane direction. These constraints have been necessary for the theoretical demonstration that the equation is independent of membrane parameters in the absence of carriers, coupling, solvent drag, or "single-file" diffusion. In a new derivation, the flux ratio equation is shown to be valid in this kind of diffusion regime without regard to the three-dimensional structure of the membrane. Thus the constraints on both membrane homogeneity and membrane geometry are shown to be unnecessary. The general use of this equation to differentiate between simple, uncoupled diffusion and other membrane transport phenomena is thus placed on a firmer base. However, as in earlier derivations, it is necessary that isopotential, isobaric, constant concentration surfaces exist sufficiently close to the membrane on both of its sides.

INTRODUCTION

Ussing (1949) has demonstrated that tracers can be used to distinguish between simple diffusion and all other transport phenomena in membranes. The steady-state expression that he derived for this purpose is known as the flux ratio equation. It has proven quite useful and its theoretical foundations have therefore been re-examined on several occasions (see, for instance, Ussing, 1952; Meares and Ussing, 1958; Hoshiko and Lindley, 1964; Kedem and Essig, 1965), but an important weakness seems to have been overlooked.

Ussing's (1949) derivation as well as Teorell's (1949) and those of other authors have all required an assumption of homogeneity in the plane of the membrane (Fig. 1 *a*). Inhomogeneity was allowed while crossing the membrane, that is, in the x-direction only. Indeed, the membrane phase was also conceived to be bounded by two parallel planes. These requirements are annoying in general; but, in particu-

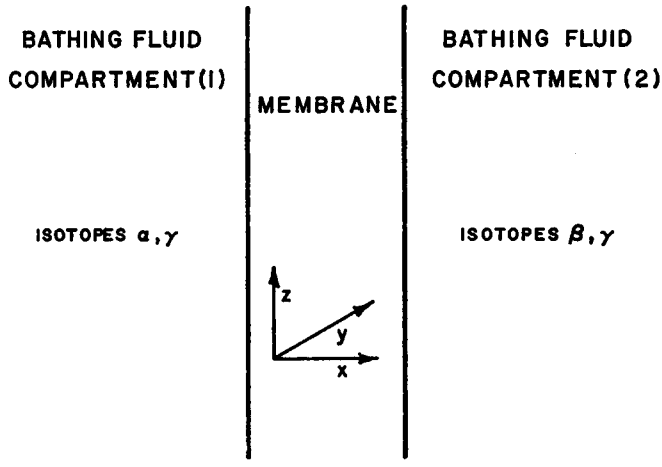

FIGURE 1 *a* The membrane as previously visualized in derivations of the unidirectional flux ratio. Compartments were well stirred and infinite. The membrane was bounded by parallel planes. Inhomogeneity was allowed only in the *x*-direction. The *y*-direction is into the plane of the figure.

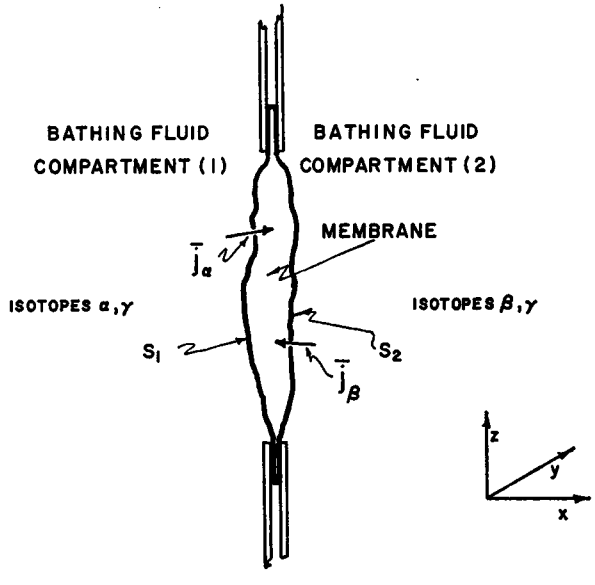

FIGURE 1 *b* Inhomogeneous, irregularly shaped membrane, mounted for the measurement of the unidirectional flux ratio. Compartments are well stirred and infinite.

lar, one cannot imagine an epithelial membrane to display such homogeneity. Then this constraint could be catastrophic. The flux ratio equation has nevertheless often been applied to the analysis of diffusion phenomena in such membranes. It will be

demonstrated that these restrictions on membrane geometry and homogeneity can fortunately be relaxed.[1]

DISCUSSION

Suppose the isolated inhomogeneous, mounted membrane and its infinite, well stirred, bathing media (Fig. 1 *b*) to be in a steady state, the system to be isothermal, thermodynamic coupling between flows to be negligible,[2] and the solutions to be ideal. The membrane is taken to be nonporous so that diffusion can result only if the diffusing substance dissolves in the membrane phase. Suppose further that the transported species has three isotopes that are identical for all purposes except tracing. Let compartment 1 contain only isotope α of this ion while compartment 2 contains only isotope β. The vector flux densities, j_α and j_β in the membrane will then be

$$j_\alpha = -\omega_\alpha c_\alpha [RT \nabla \ln c_\alpha + zF \nabla \varphi + \bar{v} \nabla p],$$

and

$$\bar{v}_\beta = -\omega_\beta c_\beta [RT \nabla \ln c_\beta + zF \nabla \varphi + \bar{v} \nabla p]. \quad (1)$$

Here R is the gas constant, T the absolute temperature, z the valence, F the Faraday constant, φ the local electrical potential, c the local concentration, $\bar{v}$ the partial molal volume, and p the hydrostatic pressure. The ω's are ionic mobilities. Because of the identical diffusion properties of the isotopes

$$\omega_\alpha = \omega_\beta \equiv \omega. \quad (2)$$

With the vector identity

$$\nabla c_i + \frac{c_i}{RT}[zF\nabla\varphi + \bar{v}\nabla p] = \exp -\left(\frac{zF\varphi + \bar{v}p}{RT}\right) \nabla \left[c_i \exp\left(\frac{zF\varphi + \bar{v}p}{RT}\right)\right], \quad (3)$$

equations 1 can be rewritten as

$$j_\alpha = -\omega RT \exp\left(-\frac{zF\varphi + \bar{v}p}{RT}\right) \nabla \left[c_\alpha \exp\left(\frac{zF\varphi + \bar{v}p}{RT}\right)\right], \quad (4\,a)$$

and

$$j_\beta = -\omega RT \exp\left(-\frac{zF\varphi + \bar{v}p}{RT}\right) \nabla \left[c_\beta \exp\left(\frac{zF\varphi + \bar{v}p}{RT}\right)\right]. \quad (4\,b)$$

Taking the dot product of the left side of equation 4 *a* with the right side of equa-

[1] A preliminary report on this work was included in a group of lectures given at the Marine Biological Laboratory, Woods Hole, Massachusetts during the summers of 1969 and 1970 (Schwartz, 1970).
[2] The reference here is to the coupling phenomena typical of purely diffusive processes as opposed to those occurring, for example, in carrier-mediated transport.

tion 4 *b*, and the left side of equation 4 *b* with the right side of equation 4 *a*, yields the expression

$$\bar{j}_\alpha \cdot \nabla \left[c_\beta \exp\left(\frac{zF\varphi + vp}{RT}\right)\right] = \bar{j}_\beta \cdot \nabla \left[c_\alpha \exp\left(\frac{zF\varphi + vp}{RT}\right)\right]. \qquad (5)$$

A steady state in the membrane means that

$$\frac{\partial c_i}{\partial t} = -\nabla \cdot \bar{j}_i = 0; \qquad i = \alpha, \beta, \qquad (6)$$

so that mass is conserved. But

$$\nabla \cdot \left[c_\beta \exp\left(\frac{zF\varphi + vp}{RT}\right)\bar{j}_\alpha\right] = c_\beta \exp\left(\frac{zF\varphi + vp}{RT}\right)\nabla \cdot \bar{j}_\alpha + \bar{j}_\alpha \cdot \nabla \left[c_\beta \exp\left(\frac{zF\varphi + vp}{RT}\right)\right]. \qquad (7)$$

Thus

$$\bar{j}_\alpha \cdot \nabla \left[c_\beta \exp\left(\frac{zF\varphi + vp}{RT}\right)\right] = \nabla \cdot \left[c_\beta \exp\left(\frac{zF\varphi + vp}{RT}\right)\bar{j}_\alpha\right] \qquad (8)$$

in the steady state. It can similarly be shown that

$$\bar{j}_\beta \cdot \nabla \left[c_\alpha \exp\left(\frac{zF\varphi + vp}{RT}\right)\right] = \nabla \cdot \left[c_\alpha \exp\left(\frac{zF\varphi + vp}{RT}\right)\bar{j}_\beta\right]. \qquad (9)$$

Substitution of equations 8 and 9 into equation 5 then yields

$$\nabla \cdot \left[c_\beta \exp\left(\frac{zF\varphi + vp}{RT}\right)\bar{j}_\alpha\right] = \nabla \cdot \left[c_\alpha \exp\left(\frac{zF\varphi + vp}{RT}\right)\bar{j}_\beta\right]. \qquad (10)$$

Integration of equation 10 over the volume of the membrane followed by the use of Gauss's divergence theorem gives

$$\int_S c_\beta \exp\left(\frac{zF\varphi + vp}{RT}\right)\bar{j}_\alpha \cdot d\bar{S} = \int_S c_\alpha \exp\left(\frac{zF\varphi + vp}{RT}\right)\bar{j}_\beta \cdot d\bar{S}, \qquad (11)$$

where S is a closed surface bounding the membrane phase. This surface may be divided into two portions, S_1 and S_2, facing compartments 1 and 2 respectively (Fig. 1 *b*). If φ, p, and c are uniform along each of these portions of S, it follows from equation 11 and the experimentally imposed boundary conditions

$$c_\beta(1) = 0$$

and (12)

$$c_\alpha(2) = 0,$$

that

$$c_\alpha(1) \exp\left(\frac{zF\varphi(1) + \bar{v}p(1)}{RT}\right) \int_{S_1} \bar{j}_\beta \cdot d\bar{S} = c_\beta(2) \exp\left(\frac{zF\varphi(2) + \bar{v}p(2)}{RT}\right) \int_{S_2} \bar{j}_\alpha \cdot d\bar{S}. \quad (13)$$

A restriction on the choice of S is evident here. It was indicated that uniform concentrations, pressures, and potentials must exist along the appropriate portions of this surface for equation 13 to hold[3]; but, to avoid problems, S should lie within the connective tissue or other unstirred layers along the membrane boundaries. Stirring could otherwise introduce nondiffusive transport into the region of the membrane. This would invalidate equations 1 in those locales. In particular, difficulties may arise if the membrane is so inhomogeneous that local circulating currents prevent the development of a constant potential surface close enough to the membrane.

The integral on the left side of equation 13 is the net flux of isotope β into compartment 1, while that on the right is the net flux of isotope α into compartment 2. If

$$J_\alpha \equiv \int_{S_2} \bar{j}_\alpha \cdot d\bar{S} \quad (14)$$

and

$$J_\beta \equiv \int_{S_1} \bar{j}_\beta \cdot d\bar{S},$$

it follows that

$$\frac{J_\alpha}{J_\beta} = \frac{c_\alpha(1)}{c_\beta(2)} \exp\left(\frac{zF\Delta\varphi + \bar{v}\Delta p}{RT}\right), \quad (15\,a)$$

where

$$\Delta\varphi \equiv \varphi(1) - \varphi(2)$$

and (15 *b*)

$$\Delta p \equiv p(1) - p(2).$$

The membrane surface–solution interfaces may be continua, as Ussing (1949)

[3] Equation 11 may be rewritten as

$$\int_s \exp\left(\frac{\mu_\beta}{RT}\right) \bar{j}_\alpha \cdot d\bar{S} = \int_s \exp\left(\frac{\mu_\alpha}{RT}\right) \bar{j}_\beta \cdot d\bar{S},$$

where μ is the electrochemical potential. A requirement of constant μ along the proper portions of S might, at first glance, appear to be sufficient. The use of the boundary conditions in equation 12, however, really calls for the more stringent constraint: uniform c, p, and φ.

assumed, but it is also possible that discontinuities, such as those at lipid-water interfaces, exist in these regions. The relationship between the c, φ, and p in equations 15, which occur on the membrane sides of the interfaces, and these same quantities on the solution side of the interfaces, must then be clarified. Since diffusion through the membrane is the rate-limiting step in this well stirred regime, local equilibrium will exist at the boundaries (Kirkwood, 1954). It follows that the right side of equation 15 a is independent of whether it is evaluated just inside the membrane phase, or just outside it in the solution phases (Meares and Ussing, 1958).

During a real double-label experiment, the tracer isotopes are present only in small amounts and are used to trace the movement of an isotope present in bulk. The specific activities of the tracer isotopes, α and β, can then be defined as

$$a_\alpha(1) \equiv \frac{c_\alpha(1)}{c_\alpha(1) + c_\beta(1) + c_\gamma(1)},$$
$$a_\beta(2) \equiv \frac{c_\beta(2)}{c_\alpha(2) + c_\beta(2) + c_\gamma(2)}, \tag{16}$$

where c_γ refers to the concentration of the bulk isotope. Since γ is present in great excess, and because of equations 12,

$$a_\alpha(1) \simeq \frac{c_\alpha(1)}{c_\gamma(1)},$$

and (17)

$$a_\beta(2) \simeq \frac{c_\beta(2)}{c_\gamma(2)}.$$

Thus

$$\frac{[J_\alpha/a_\alpha(1)]}{[J_\beta/a_\beta(2)]} = \frac{c_\gamma(1)}{c_\gamma(2)} \exp\left(\frac{zF\Delta\varphi + v\Delta p}{RT}\right). \tag{18}$$

CONCLUSIONS

This is the usual general expression for that portion of the flux ratio equation which was also found to be independent of membrane parameters in earlier derivations (see, for instance, Hoshiko and Lindley, 1964, equation 18). The applicability of those derivations, however, is restricted because of their requirements with regard both to membrane homogeneity and membrane geometry. In the present paper equation 18 has been demonstrated to be independent of the three-dimensional structure of the membrane interior provided that the specified constraints at the membrane surface are met. In practice these constraints should not be too troublesome. They were also required in the earlier, more restrictive, derivations.

Equation 18 is valid for steady-state diffusion processes in the absence of thermodynamic coupling between flows, or the solvent drag phenomena peculiar to porous

membranes, or the "single-file" diffusion defined by Hodgkin and Keynes (1955), or of interactions with carriers. It can be used for nonideal solutions if activity coefficients are known. Activities have then simply to be substituted for concentrations. Solvent drag cannot occur if there are no transmembrane osmotic or hydrostatic pressure differences.[4] The restriction to nonporous membranes can then be relaxed; but in the presence of any of these complicating transport phenomena a modified expression results. This expression, in general, contains terms dependent on membrane structure. This has been amply demonstrated for the membrane of Fig. 1 *a* in earlier derivations. It appears also to be true of the more general membrane of Fig. 1 *b* discussed in the present derivation.

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[4] Thermodynamic coupling between solvent and solute flows can occur during diffusion through nonporous membranes. The term "solvent drag" has sometimes been applied to the resultant effects on solute fluxes. The historical usage of this term, however, seems to be restricted to phenomena occurring in porous membranes, and, in particular, to the effect of "bulk" or nondiffusive components of solvent flow (Ussing, 1952, p. 35; Meares and Ussing, 1958, p. 143; Ussing, 1960, p. 46; Park, 1960). This is the sense in which I have used it.