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A THERMODYNAMIC ANALYSIS OF PARTICLE FLOW THROUGH BIOLOGICAL MEMBRANES

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SUMMARY

The methods of irreversible thermodynamics are applied to the problem of particle flow through biological membranes. Formulae are derived for the unidirectional flux and flux ratio of a permeant species under conditions where there is a coupling between flows and between flows and metabolism. The flux ratio formula is shown to be useful under appropriate conditions, for providing evidence antagonistic to the hypothesis of active transport of the permeant species. A relation is derived which specifies the conditions under which the use of tracers should lead to accurate estimates of unidirectional fluxes. When the treatment is applied to the problem of water flow through an inert semipermeable membrane a particularly simple formula is obtained for the ratio of the permeability coefficients for water as determined osmotically and isotopically.

INTRODUCTION

In recent years considerable attention has been devoted to applying the methods of irreversible thermodynamics to flows through biological membranes[1-7]. The problems investigated have included the formulation of suitable expressions for net fluxes, unidirectional fluxes and the associated flux ratio, under conditions where there is a coupling between flows and between flows and metabolism. Consideration has also been given to the permeability coefficient of a permeant species.

The present report attempts to continue this work. In its early stages attention is given to the meaning of the expression 'unidirectional flux'. The formal definition has quantum mechanical implications which restrict the development of a mathematical procedure to cases where the kinetic properties of the isotopes of the permeant species are almost identical. For this case the mathematical approach is similar to that of USSING[8] and HOSHIKO AND LINDLEY[6] in that the unidirectional fluxes of a particle species are equated to the isotopic fluxes which would result if different species isotopes were present on opposite sides of the membrane. The definition differs formally, however, from that used by KEDEM AND ESSIG[7] who defined 'unidirectional flux' in an experimental way. However, it turns out that the expressions for the one-way fluxes are identical to those of KEDEM AND ESSIG[7] so that the definitions are mutually equivalent.

Abbreviation: TEF, tracer-estimated unidirectional fluxes.

The conditions under which tracer fluxes lead to correct estimates of unidirectional fluxes are then investigated. It is shown that the criterion for negligible tracer backflux is that the ratio of the specific activities of the test species, in the cell compartments, should be much smaller than the flux ratio.

When the net flux expression is compared with the one-way flux formula a rationalisation is obtained of the discrepancy first noted by HEVESY, HOFER AND KROGH[9] between the permeability coefficients of water as determined osmotically and isotopically. Qualitative predictions of the dependence of the ratio of the permeability coefficients on pore size appear to be consistent with experimental findings.

Many of the membrane parameters present in the one-way flux formulae are eliminated in their ratio which is a fairly simple yet general expression. In the case of a single major entraining substance the flux ratio expression permits, under appropriate conditions, the deduction of no active transport of the permeant species. The systems considered in this report are the flow of water across the intestinal wall of the dog and the flow of K^+ ions across the giant algae cells of *Chara australis*.

THEORETICAL PART

The phenomenological equations

Consider an isothermal system consisting of two well mixed compartments separated by a membrane. The membrane contains a system of parallel pathways which are identical with respect to the factors influencing transport. Suppose, for the present, that there are $n-1$ diffusing species and that their chemical potentials are defineable everywhere and of values at the membrane boundary equal to those in the adjacent solution.

In the following treatment the flows are treated as steady in time and in the x direction which is taken as perpendicular to the membrane. The conservative force which drives the flow of the ith species (X_i) is its negative gradient of electrochemical potential ($-\mathrm{d}\mu_i/\mathrm{d}x$) defined by the relation

$$X_i = -\frac{\mathrm{d}\mu_i}{\mathrm{d}x} = -RT\frac{\mathrm{d}\ln a_i}{\mathrm{d}x} - \bar{v}_i\frac{\mathrm{d}P}{\mathrm{d}x} - \frac{\mathrm{d}z_iFV}{\mathrm{d}x} \tag{1}$$

where R denotes gas constant, T temperature, a chemical activity, $\bar{v}$ partial molar volume, P pressure, z valence, F Faradays number and V electric potential.

In applying the methods of irreversible thermodynamics to the system it is customary to assume that the forces (X_i) are linear functions of the flows (J_i) *i.e.*

$$X_i = R_{ii}J_i + \sum_{\substack{j=1\\ j\neq i}}^{n-1} r_{ij}J_j \tag{2}$$

where r_{ij} and R_{ii} are phenomenological coefficients.

The cross coefficients r_{ij} are related through the Onsager relation

$$r_{ij} = r_{ji} \tag{3}$$

The signs of the coefficients R_{ii} and r_{ij} have been discussed by KEDEM AND KATCHALSKY[3]. The coefficients R_{ii} are always positive. In their simple frictional interpretation the cross coefficients r_{ij} are negative. However, in the case of carrier transport r_{ij} may become positive.

As pointed out by KEDEM[10] the above formulation appears to be sufficiently general to take account of a coupling between metabolic reaction and permeation flow. In this case r_{ij} denotes the coefficient which couples the flow J_i to a metabolic reaction of rate J_j while X_j is the affinity driving the reaction.

The equation for the unidirectional flux

Consider the flow of a molecular species through a membrane. The unidirectional flux is commonly regarded as being equal in magnitude to the number of crossings per unit area of membrane per unit time of the appropriate membrane boundary which are made by particles finishing complete traversals of the membrane from one side to the other. This definition implies that if the particles were characterised according to the membrane boundary they last crossed, the net flux of each component would be constant throughout the membrane and equal to a unidirectional flow of the total test species.

This definition is classical in kind. From the standpoint of quantum mechanics, the overlap of particle wave packets during collisions produces a 'loss in identity' of the individual particles and thus precludes the possibility of a differentiation of the members of a molecular species according to the membrane boundary last crossed.

To meet this objection, and at the same time satisfy the classical interpretation of the unidirectional flux, consideration will be limited to cases where the kinetic properties of the isotopes of the species are almost identical. Then from the classical standpoint, if all other system parameters remain the same, the one-way fluxes of the total test species equal those existent when the two components of the species comprise different isotopes. From the quantum mechanical viewpoint the terms unidirectional flux and isotopic flux can, in the present context, be regarded as synonymous.

Let subscript 0 refer to the total test species and 1 and 2 to the two components (isotopes) of 0 that constitute the one-way fluxes. Then at the membrane boundaries

$$\begin{array}{lllll} x = 0: & C = C(0) & C_1 = 0 & C_2 = C(0) & V = V_m \\ x = \delta: & C = C(\delta) & C_1 = C(\delta) & C_2 = 0 & V = 0 \end{array} \tag{4}$$

The net flux of the test species is the algebraic sum of the one-way fluxes. Thus

$$J = J_1 + J_2 \tag{5}$$

We shall refer to J_1 and J_2 as the influx and efflux (per unit area of membrane) respectively. Thus Eqn. 4 implies that potentials are taken relative to an external earth.

From Eqns. 1 and 2

$$J = -\frac{1}{R_{00}}\left(\frac{\mathrm{d}\mu}{\mathrm{d}x} + \sum_{j=3}^{n} r_{0j}J_j\right) \tag{6a}$$

$$J_1 = -\frac{1}{R_{11}}\left(\frac{\mathrm{d}\mu_1}{\mathrm{d}x} + \sum_{j=3}^{n} r_{1j}J_j + r_{12}J_2\right) \tag{6b}$$

$$J_2 = -\frac{1}{R_{22}}\left(\frac{\mathrm{d}\mu_2}{\mathrm{d}x} + \sum_{j=3}^{n} r_{2j}J_j + r_{21}J_1\right) \tag{6c}$$

the terms involving r_{12} and r_{21} taking account of the interaction between the one-way fluxes.

Now since particles comprising groups '1' and '2' have almost identical physical properties, to good approximation

$$r_{1j} = r_{2j} = r_{0j} \tag{7}$$

$$\bar{v}_1 = \bar{v}_2 = \bar{v},\ z_1 = z_2 = z,\ \gamma_1 = \gamma_2 = \gamma,\ \mu_1{}^0 = \mu_2{}^0 = \mu^0 \tag{8}$$

Also the Onsager reciprocal relations require

$$r_{12} = r_{21} = r_{00}{}^* \text{ say} \tag{9}$$

Combining Eqns. 6 through 9 it is easy to show that

$$a\,(R_{00} - r_{00}{}^*)J = -a\left(\frac{\mathrm{d}\mu}{\mathrm{d}x} + \sum_{j=3}^{n} r_{0j}J_j + r_{00}{}^*J\right) \tag{10a}$$

$$a_1(R_{11} - r_{00}{}^*)J_1 = -a_1\left(\frac{\mathrm{d}\mu_1}{\mathrm{d}x} + \sum_{j=3}^{n} r_{0j}J_j + r_{00}{}^*J\right) \tag{10b}$$

$$a_2(R_{22} - r_{00}{}^*)J_2 = -a_2\left(\frac{\mathrm{d}\mu_2}{\mathrm{d}x} + \sum_{j=3}^{n} r_{0j}J_j + r_{00}{}^*J\right) \tag{10c}$$

Eqn. 10a is of the same form as Eqn. 1 in the paper of MEARES AND USSING[11]. MEARES AND USSING asserted but did not prove that their equation could be derived using the methods of irreversible thermodynamics. The present treatment validates their assertion.

Now since $a = \gamma C$ and $C = C_1 + C_2$ it follows from Eqn. 8 that

$$a = a_1 + a_2 \tag{11}$$

Thus

$$a\left(\sum_{j=3}^{n} r_{0j}J_j + r_{00}{}^*J\right) = a_1\left(\sum_{j=3}^{n} r_{0j}J_j + r_{00}{}^*J\right) + a_2\left(\sum_{j=3}^{n} r_{0j}J_j + r_{00}{}^*J\right)$$

while from Eqns. 1, 8 and 11

$$a\frac{\mathrm{d}\mu}{\mathrm{d}x} = a_1\frac{\mathrm{d}\mu_1}{\mathrm{d}x} + a_2\frac{\mathrm{d}\mu_2}{\mathrm{d}x}$$

Thus relating the sum of Eqns. 10b and 10c to Eqn. 10a

$$a(R_{00} - r_{00}{}^*)J = a_1(R_{11} - r_{00}{}^*)J_1 + a_2(R_{22} - r_{00}{}^*)J_2 \tag{12}$$

whence

$$J = \frac{a_1(R_{11} - r_{00}{}^*)J_1}{a(R_{00} - r_{00}{}^*)} + \frac{a_2(R_{22} - r_{00}{}^*)J_2}{a(R_{00} - r_{00}{}^*)} \tag{13}$$

It is convenient to rewrite Eqn. 5

$$J = J_1 + J_2 \tag{5}$$

Since Eqns. 13 and 5 hold for all values of the independent variables the coefficients of J_1 and J_2 in Eqn. 13 must be equal to unity. Thus

$$a_1(R_{11} - r_{00}{}^*) = a_2(R_{22} - r_{00}{}^*) = a(R_{00} - r_{00}{}^*) \tag{14}$$

Using Eqns. 1, 8, 10 and 14 it is easy to show that

$$a(R_{00}-r_{00}^*)J = -RT\frac{\mathrm{d}a}{\mathrm{d}x} - azF\frac{\mathrm{d}V}{\mathrm{d}x} - a\bar{v}\frac{\mathrm{d}P}{\mathrm{d}x} - a\sum_{j=3}^{n} r_{0j}J_j - ar_{00}^*J \tag{15a}$$

$$a(R_{00}-r_{00}^*)J_1 = -RT\frac{\mathrm{d}a_1}{\mathrm{d}x} - a_1zF\frac{\mathrm{d}V}{\mathrm{d}x} - a_1\bar{v}\frac{\mathrm{d}P}{\mathrm{d}x} - a_1\sum_{j=3}^{n} r_{0j}J_j - a_1r_{00}^*J_1 \tag{15b}$$

$$a(R_{00}-r_{00}^*)J_2 = -RT\frac{\mathrm{d}a_2}{\mathrm{d}x} - a_2zF\frac{\mathrm{d}V}{\mathrm{d}x} - a_2\bar{v}\frac{\mathrm{d}P}{\mathrm{d}x} - a_2\sum_{j=3}^{n} r_{0j}J_j - a_2r_{00}^*J \tag{15c}$$

Integrating

$$J = -RT\frac{a(\delta)\exp\frac{1}{RT}\left(\bar{v}P(\delta)+\int_0^\delta\sum_{j=3}^{n} r_{0j}J_j\,\mathrm{d}x+\int_0^\delta r_{00}^*J\,\mathrm{d}x\right)-a(0)\exp\frac{zF}{RT}\left(V_\mathrm{m}+\frac{\bar{v}P(0)}{zF}\right)}{\int_0^\delta a(R_{00}-r_{00}^*)\exp\frac{zF}{RT}\left(V+\frac{\bar{v}P}{zF}+\int_0^x\frac{\sum_{j=3}^{n} r_{0j}J_j+r_{00}^*J}{zF}\,\mathrm{d}x\right)\mathrm{d}x} \tag{16a}$$

$$\text{Influx } J_1 = -RT\frac{a(\delta)\exp\frac{1}{RT}\left(\bar{v}P(\delta)+\int_0^\delta\sum_{j=3}^{n} r_{0j}J_j\mathrm{d}x+\int_0^\delta r_{00}^*J\mathrm{d}x\right)}{\int_0^\delta a(R_{00}-r_{00}^*)\exp\frac{zF}{RT}\left(V+\frac{\bar{v}P}{zF}+\int_0^x\frac{\sum_{j=3}^{n} r_{0j}J_j+r_{00}^*J}{zF}\mathrm{d}x\right)\mathrm{d}x} \tag{16b}$$

$$\text{Efflux } J_2 = RT\frac{a(0)\exp\frac{zF}{RT}\left(V_\mathrm{m}+\frac{\bar{v}P(0)}{zF}\right)}{\int_0^\delta a(R_{00}-r_{00}^*)\exp\frac{zF}{RT}\left(V+\frac{\bar{v}P}{zF}+\int_0^x\frac{\sum_{j=3}^{n} r_{0j}J_j+r_{00}^*J}{zF}\mathrm{d}x\right)\mathrm{d}x} \tag{16c}$$

The unidirectional fluxes are seen to be expressible as functions of all the independent variables. Their dependence on the net flux J of the species itself is interesting for if Eqns. 6b and 6c are integrated directly, there results the following equations

$$\text{Influx } J_1 = -RT\frac{a(\delta)\exp\frac{1}{RT}\left(\bar{v}P(\delta)+\int_0^\delta\sum_{j=3}^{n} r_{0j}J_j\,\mathrm{d}x+\int r_{00}^*J_2\,\mathrm{d}x\right)}{\int_0^\delta a_1R_{11}\exp\frac{zF}{RT}\left(V+\frac{\bar{v}P}{zF}+\int_0^x\frac{\sum_{j=3}^{n} r_{0j}J_j}{zF}\,\mathrm{d}x+\int_0^x\frac{r_{00}^*J_2}{zF}\,\mathrm{d}x\right)\mathrm{d}x} \tag{17a}$$

$$\text{Efflux } J_2 = RT\frac{a(0)\exp\frac{zF}{RT}\left(V_\mathrm{m}+\frac{\bar{v}P(0)}{zF}\right)}{\int_0^\delta a_2R_{22}\exp\frac{zF}{RT}\left(V+\frac{\bar{v}P}{zF}+\int_0^x\frac{\sum_{j=3}^{n} r_{0j}J_j}{zF}\,\mathrm{d}x+\int_0^x\frac{r_{00}^*J_1}{zF}\,\mathrm{d}x\right)\mathrm{d}x} \tag{17b}$$

These equations show that a unidirectional flux can be regarded as a function of the opposing one-way flux rather than of the net flux of the species itself.

The flux ratio

The flux ratio is obtained directly from the basic equations (Eqns. 16b and 16c). Thus

$$\frac{\text{Influx}}{\text{Efflux}} = \frac{J_1}{J_2} = -\frac{a(\delta)}{a(0)} \exp \frac{zF}{RT} \left(-V_m + \frac{\bar{v}(P(\delta)-P(0))}{zF} + \int_0^{\delta} \frac{\sum\limits_{j=3}^{n} r_{0j}J_j}{zF} \mathrm{d}x + \int_0^{\delta} \frac{r_{00}^{*}J}{zF} \mathrm{d}x \right) \tag{18}$$

or in logarithmic form

$$\ln\left(-\frac{J_1}{J_2}\right) = \ln \frac{a(\delta)}{a(0)} - \frac{zFV_m}{RT} + \frac{\bar{v}(P(\delta)-P(0))}{RT} + \frac{1}{RT} \int_0^{\delta} \sum_{j=3}^{n} r_{0j}J_j \mathrm{d}x + \frac{1}{RT} \int_0^{\delta} r_{00}^{*}J \mathrm{d}x \tag{19a}$$

Meares and Ussing[11] have produced some confirmation of the validity of Eqn. 19a for flows through cation-exchange resin membranes.

Alternative expressions for the flux ratio can be derived. It follows from Eqns. 1 and 2 that

$$\int_0^{\delta} \sum_{j=3}^{n} r_{0j}J_j \mathrm{d}x = -\int_0^{\delta} \frac{\mathrm{d}\mu}{\mathrm{d}x} \mathrm{d}x - \int_0^{\delta} R_{00}J \mathrm{d}x$$

$$= -\left(RT \ln \frac{a(\delta)}{a(0)} + \bar{v}(P(\delta)-P(0)) - zFV_m\right) - \int_0^{\delta} R_{00}J \mathrm{d}x$$

Substitution for $\int_0^{\delta} \sum_{j=3}^{n} r_{0j}J_j \mathrm{d}x$ into Eqn. 19a therefore yields

$$\ln\left(-\frac{J_1}{J_2}\right) = -\frac{J}{RT} \int_0^{\delta} (R_{00}-r_{00}^{*}) \mathrm{d}x \tag{19b}$$

This equation has also been derived by Kedem and Essig[7]. Since the essential difference between their approach and that adopted here is in the formal definition used for the unidirectional flux it appears that the definitions are mutually equivalent.

Also, it is readily deduced from Eqn. 17a that when the net flux of the test species is zero

$$\frac{a(\delta)}{a(0)} = \exp \frac{zF}{RT} \left(V_m' - \frac{\bar{v}(P'(\delta)-P'(0))}{zF} - \int_0^{\delta} \frac{\sum\limits_{j=3}^{n} r_{0j}'J_j'}{zF} \mathrm{d}x \right) \tag{20}$$

where the primes denote resting values.

For experiments performed with the boundary values of a and P at their resting levels this expression can be substituted into Eqn. 19a to give the relation

$$\ln\left(-\frac{J_1}{J_2}\right) = -\frac{zF}{RT} \Delta V + \frac{1}{RT} \int_0^{\delta} \sum_{j=3}^{n} \Delta(r_{0j}J_j) + \frac{1}{RT} \int_0^{\delta} r_{00}^{*}J \mathrm{d}x \tag{19c}$$

where ΔV and $\Delta(r_{0j}J_j)$ denote the displacement in V and $r_{0j}J_j$ from their resting values. In many experiments with biological membranes Eqn. 19c is a good approximation to the more general Eqns. 19a and 19b.

Eqns. 19a–c are presented as convenient expressions for the flux ratio derivable under the fairly general circumstances depicted here. It is seen to be a function not only of coupled flows and metabolic reaction rates but also of the flow of the species itself. In later sections some applications of these formulae are discussed.

The use of tracers in flux estimations

In a typical experiment to determine an unidirectional flux the test species contains both labelled and unlabelled isotopes, the specific activity being much higher on one side of the membrane than on the other. The unidirectional flux is estimated from the net rate of accumulation of the labelled isotope on the side of lower specific activity. The estimation involves three assumptions which are:

(1) The 'back' flux of tracer through the membrane is negligible.

(2) The ratio tracer flux:unidirectional flux equals ρ where ρ is the specific activity in the 'loaded' compartment.

(3) In their flow the labelled and unlabelled particles are kinetically equivalent.

While assumption (3) is not always valid it generally represents a good approximation[12]. It is fairly easy to show that, if the assumption is acceptable, Eqn. 15a with J^* the net tracer flux substituted for J, and a^* the labelled isotope activity substituted for a, describes the flow of the labelled isotope. Upon integration the following expression is then obtained (*cf.* Eqn. 16a)

$$J^* = -RT\frac{a^*(\delta)\exp\frac{1}{RT}\left(\bar{v}P(\delta)+\int_0^\delta\sum_{j=3}^{n}r_{0j}J_j\mathrm{d}x+\int_0^\delta r_{00}^*J\mathrm{d}x\right)-a^*(0)\exp\frac{zF}{RT}\left(V_m+\frac{\bar{v}P(0)}{zF}\right)}{\int_0^\delta a(R_{00}-r_{00}^*)\exp\frac{zF}{RT}\left(V+\frac{\bar{v}P}{zF}+\int_0^x\sum_{j=3}^{n}\frac{r_{1j}J_j}{zF}\mathrm{d}x+\int_0^x\frac{r_{00}^*J}{zF}\mathrm{d}x\right)\mathrm{d}x} \tag{21}$$

where the symbol* denotes labelled isotope. In the following treatment the tracer flux expression (Eqn. 21) is subjected to assumptions (1) and (2) above and expressions are deduced for the tracer-estimated one-way fluxes.

Assumption (1) implies that in an influx experiment

$$a^*(0)\exp\frac{zF}{RT}\left(V_m+\frac{\bar{v}P(0)}{zF}\right)\ll a^*(\delta)\exp\frac{1}{RT}\left(\bar{v}P(\delta)-\int_0^\delta\sum_{j=3}^{n}r_{0j}J_j\mathrm{d}x+\int_0^\delta r_{00}^*J\mathrm{d}x\right) \tag{22a}$$

while in an efflux experiment

$$a^*(\delta)\exp\frac{1}{RT}\left(\bar{v}P(\delta)+\int_0^\delta\sum_{j=3}^{n}r_{0j}J_j\mathrm{d}x+\int_0^\delta r_{00}^*J\mathrm{d}x\right)\ll a^*(0)\exp\frac{zF}{RT}\left(V_m+\frac{\bar{v}P(0)}{zF}\right) \tag{22b}$$

Substituting these relations separately into Eqn. 21 and then applying assumption (2) above leads, since $\gamma^* = \gamma$, to the following relations for the tracer-estimated unidirectional fluxes (TEF).

$$\text{TEF influx} \approx -RT\frac{a(\delta)\exp\frac{1}{RT}\left(\bar{v}P(\delta)+\int_0^x\sum_{j=3}^{n}r_{0j}J_j\mathrm{d}x+\int_0^x r_{00}^*J\mathrm{d}x\right)}{\int_0^\delta a(R_{00}-r_{00}^*)\exp\frac{zF}{RT}\left(V+\bar{v}P+\int_0^x\sum_{j=1}^{n}\frac{r_{0j}J_j}{zF}\mathrm{d}x+\int_0^x\frac{r_{00}^*J}{zF}\mathrm{d}x\right)\mathrm{d}x} \tag{23a}$$

$$\text{TEF efflux} \approx RT\frac{a(0)\exp\frac{zF}{RT}\left(V_m+\frac{\bar{v}P(0)}{zF}\right)}{\int_0^\delta a(R_{00}-r_{00}^*)\exp\frac{zF}{RT}\left(V+\bar{v}P-\int_0^x\sum_{j=3}^{n}\frac{r_{0j}J_j}{zF}\mathrm{d}x+\int_0^x\frac{r_{00}^*J}{zF}\mathrm{d}x\right)\mathrm{d}x} \tag{23b}$$

If these equations are compared with those for the unidirectional fluxes (Eqns. 16b and 16c) it is seen that, under conditions where assumptions (1) through (3) apply, tracer experiments yield flux estimates which are reasonably accurate.

It is worthwhile to determine explicitly the criteria implied by assumption (1) above. The condition for negligible tracer backflux during an influx experiment is from Eqn. 22a

$$\frac{a^*(0)}{a^*(\delta)} \ll \exp\frac{zF}{RT}\left(-V_\mathrm{m} + \frac{\bar{v}(P(\delta)-P(0))}{zF} + \int_0^\delta \sum_{j=3}^{n} \frac{r_{0j}J_j}{zF}\mathrm{d}x + \int_0^\delta \frac{r_{00}^*J}{zF}\mathrm{d}x\right) \tag{24}$$

Eqns. 24 and 18 yield

$$\frac{a^*(0)}{a^*(\delta)} \ll -\frac{a(0)}{a(\delta)}\frac{J_1}{J_2}$$

Similarly Eqns. 22b and 18 lead to the corresponding expression for the efflux case

$$\frac{a^*(\delta)}{a^*(0)} \ll -\frac{a(\delta)}{a(0)}\frac{J_2}{J_1}$$

which, since $\gamma^* = \gamma$, give the following criteria for negligible tracer backflux.

Influx experiment

$$\frac{C^*(0)/C(0)}{C^*(\delta)/C(\delta)} \ll -\frac{J_1}{J_2} \tag{25a}$$

Efflux experiment

$$\frac{C^*(\delta)/C(\delta)}{C^*(0)/C(0)} \ll -\frac{J_2}{J_1} \tag{25b}$$

Now it is generally assumed that assumption (1) implies that the ratio of the specific activities (*i.e.* concentration ratios above) should be much smaller than unity, rather than the flux ratio. To illustrate how this can lead to erroneous flux estimates in the case of sufficiently high flux ratios consider an efflux experiment. From Eqn. 21

$$J^* = -RT\frac{a^*(\delta)\exp\left[\frac{1}{RT}\left(\bar{v}P(\delta) + \int_0^\delta \sum_{j=3}^{n} r_{0j}J_j\mathrm{d}x + \int_0^\delta r_{00}^*J\mathrm{d}x\right)\right]Z}{\int_0^\delta a(R_{00}-r_{00}^*)\exp\frac{zF}{RT}\left(V + \frac{\bar{v}P}{zF} + \int_0^x \sum_{j=3}^{n}\frac{r_{0j}J_j}{zF}\mathrm{d}x + \int_0^x \frac{r_{00}^*J}{zF}\mathrm{d}x\right)\mathrm{d}x} \tag{26}$$

where

$$Z = 1 - \frac{a^*(0)}{a^*(\delta)}\exp\frac{zF}{RT}\left(V_\mathrm{m} - \frac{\bar{v}(P(\delta)-P(0))}{zF} - \int_0^\delta \sum_{j=3}^{n}\frac{r_{0j}J_j}{zF}\mathrm{d}x - \int_0^\delta \frac{r_{00}^*J}{zF}\mathrm{d}x\right)$$

From Eqns. 18, 21, 23a and 26

$$J^* = J_1^*\left(1 + \frac{C^*(0)/C(0)}{C^*(\delta)/C(\delta)}\frac{J_2}{J_1}\right) \tag{27}$$

where J_1^* represents the true tracer influx. Thus if the specific activity ratio equals 0.01 while the flux ratio $(-J_2/J_1)$ is 60 say

$$J^* = 0.4\,J_1^*$$

and the influx estimate will be only 0.4 times the true influx. Flux ratios of order 10^2 appear to occur for K^+ ions in biological membranes at about 50 mV from the resting potential[13,14]. If the influx is estimated by the usual tracer method it seems that specific activity ratios in the 0.001 range to 0.0001 may be required.

Osmotic and isotopic permeability coefficients

One problem which arises from the use of diffusion theory in membrane transport is the discrepancy found for a number of membranous systems, between the permeability coefficients of water as determined osmotically, P_{0s} and isotopically, P_{Is} (see refs. 9, 15–18).

In recent years treatments based on diffusion theory have regarded water flow as consisting of two component flows, *viz.* a diffusion flow and a flow by filtration[19–23]. To illustrate this consider an inert semipermeable membrane which separates the two compartments of a cell and in which hydraulic flow of water occurs in response to hydrostatic and/or osmotic pressure gradients. Regard the membrane as containing a homogeneous array of parallel pores which are identical with respect to the factors influencing transport and which have a combined cross-sectional area A.

The diffusion flux J_w^D of the water is defined for the entire membranous area through the relation (see ref. 21)

$$J_w^D = -\frac{D_w A}{RT} C_w \frac{d\mu_w}{dx}$$

where D_w is the self-diffusion coefficient of water. It is generally assumed (see ref. 21) that the gradient in electrochemical potential is constant so that

$$J_w^D = -\frac{D_w A}{\delta}\frac{C_w}{RT}(\mu_w(\delta)-\mu_w(0)) \tag{28}$$

For ideal solutions

$$\mu(\delta)-\mu(0) = \bar{v}_w(P(\delta)-P(0)) - \frac{RT}{C_w^0}(C_s(\delta)-C_s(0)) \tag{29}$$

where C_s denotes solute concentration and $C_w^0 = (1-C_s\bar{v}_s)/\bar{v}_w$ or to good approximation $C_w^0 = C_w = 1/\bar{v}_w$

Combining Eqns. 28 and 29 and noting $C_w\,\bar{v}_w = 1$ gives the following expression for the diffusion flux

$$J_w^D = -\frac{D_w A}{\delta}\frac{1}{RT}[(P(\delta)-P(0))-RT(C_s(\delta)-C_s(0))] \tag{30}$$

Theoretical estimates for the 'diffusion flux' require a knowledge of the factor $D_w A/\delta$. This is obtained, for a particular membrane, from a consideration of the flow of labelled water, $H_2{}^{18}O$, through the membrane when no osmotic or hydrostatic pressure gradients are applied. The flux of labelled water $(J_w)_{H_2{}^{18}O}$ may be written as

$$(J_w)_{H_2{}^{18}O} = \frac{(D_w)_{H_2{}^{18}O}A}{\delta}[(C_w(\delta))_{H_2{}^{18}O}-(C_w(0))_{H_2{}^{18}O}] \tag{31}$$

In the treatment of MAURO[21] it is assumed that

$$D_w = (D_w)_{H_2{}^{18}O} \tag{32}$$

Thus observations of the flow of $H_2{}^{18}O$ lead to estimates of $D_w A/\delta$ which, when substituted into Eqn. 30, lead to estimates of the diffusion flux resulting from hydrostatic and/or osmotic pressure gradients. Hevesy, Hofer and Krogh[9] found ratios of the total observed flux to the theoretical diffusion flux for frog skin of 3–5, while Robbins and Mauro[16] found values for certain artificial membranes of up to several hundred.

Following Chinard[19], Pappenheimer[20] and Mauro[21], Robbins and Mauro attributed their results to the presence of a second flux, *viz.* a viscous flux J_w^V that results from a flow by filtration. Assuming that the membrane contained n pores each of radius r and that flow was laminar so that Poisseuille's law applies

$$\begin{aligned} J_w^V &= -\frac{n\pi r^2 r^2}{8\eta \bar{v}_w \delta}[(P(\delta)-P(0))-RT(C_s(\delta)-C_s(0))] \\ &= -\frac{r^2 A}{8\eta \bar{v}_w \delta}[(P(\delta)-P(0))-RT(C_s(\delta)-C_s(0))] \qquad (33) \end{aligned}$$

where η is the viscosity of the filtrate.

Taking the ratios of both sides of Eqns. 30 and 33 gives

$$\frac{J_w^V}{J_w^D} = \frac{r^2 RT}{8\eta D_w \bar{v}_w} \qquad (34)$$

which, since all other parameters are known, leads to an estimate of the pore radius r and through Eqn. 33 to the total pore area.

Recently Nims[5] has re-examined this problem using the phenomenological equations of irreversible thermodynamics in their admittance formulation. The equations derived were in a general form and applications to specific models were not considered. In the following it is shown that the application of the equations derived in this report leads to a particularly simple expression for the ratio of the osmotic and isotopic permeability coefficients for the type of membrane considered by Mauro. The expression enables qualitative predictions of the variation in their ratio as the membrane parameters are changed.

The total flux of water $J_w^T = J_w A$ which flows through a system of pathways, of combined cross-sectional area A, when there are no other permeant species present is, from Eqn. 2, given by

$$J_w^T = -\frac{A}{R_{ww}}\frac{d\mu_w}{dx}$$

In the present case the membrane is assumed to present an inert barrier to water flow thereby precluding the possibility of chemical transport of the water at the surfaces of the pores. Also since the membrane is semipermeable, the water flow is not coupled to that of other species. Under these conditions, the frictional interpretation of the phenomenological coefficients appears appropriate. The coefficient R_{ww} is then completely determined (see ref. 4) by the frictional resistance between the water and membranous particles. This frictional resistance is assumed to be independent of the relative velocity of the water and the membranous particles. It might, however, depend on their concentrations. However, in the model membrane assumed by Mauro these concentrations are constant throughout the membrane thereby justifying the assumption adopted in the following, that R_{ww} is constant with distance inside the membrane.

For the membrane model of Mauro $d\mu_w/dx$ is constant inside the membrane. Therefore if ideal solutions are assumed J_w^T may be written as

$$J_w^T = -\frac{A}{C_w R_{ww}\delta}\left[(P(\delta)-P(0)) - RT\,(C_s(\delta)-C_s(0))\right] \tag{35}$$

In the case of labelled water flow without pressure gradients the tracer flux expression (Eqn. 21) yields, if $a = C$

$$J_w^{T*} = J_w{}^* A = -\frac{RT(C_w{}^*(\delta)-C_w{}^*(0))}{C_w(R_{ww}-r_{ww}{}^*)\delta} \tag{36}$$

The osmotic and isotopic water permeabilities defined by Eqns. 35 and 36 are

$$P_{os} = \frac{1}{C_w R_{ww}\delta} \tag{37}$$

$$P_{is} = \frac{1}{C_w(R_{ww}-r_{ww}{}^*)\delta} \tag{38}$$

and their ratio is

$$\frac{P_{os}}{P_{is}} = \frac{R_{ww}-r_{ww}{}^*}{R_{ww}} \tag{39}$$

Eqn. 39 predicts that for the particular membrane model considered here the osmotic and isotopic water permeabilities should not in general be equal. The phenomenological equations of irreversible thermodynamics thus lead to a rationalisation of the discrepancy first noted by Hevesy, Hofer and Krogh[9] between the permeability coefficients of water as determined osmotically and isotopically.

In the following, a re-examination of the classical approach to the problem in the light of the irreversible thermodynamical treatment is presented.

The coefficient $r_{ww}{}^*$ is in dilute solutions related to the self-diffusion coefficient of water through the Einstein relation[*]

$$D_w = -\frac{RT}{C_w r_{ww}{}^*}$$

Thus Eqns. 30 and 35 yield

$$J_w^T - J_w^D = J_w^T\left(1-\frac{R_{ww}}{r_{ww}{}^*}\right)$$

Comparing the above expression with the viscous flux equation (Eqn. 33) it is seen that the right-hand sides cannot in general be equal since J_w^V is independent of R_{ww}. It would seem to follow that treatments (*e.g.* Mauro[21]) which regard the sum of the flows by filtration and diffusion as being equal to the total observed flux, are in error particularly when R_{ww} is significantly large as is the case for pores of sufficiently small radius.

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[*] The *minus* sign occurs because of the sign convention wherein $r_{ww}{}^*$ is negative for frictional interactions.

Some idea of the variation of $P_{\mathrm{os}}/P_{\mathrm{is}}$ with pore radius is now presented. The resistance term arising from the water membrane interaction, *i.e.* R_{ww}, is always positive and it decreases to zero as the pore radius increases to infinity. The resistance coefficient between the labelled and unlabelled water, $r_{\mathrm{ww}}{}^*$, may be negative or positive. Negative values of $r_{\mathrm{ww}}{}^*$ would be expected, in the case of pores of large radius where frictional drag is the major force. However, in cases where the pore radii are small so that the particles are restricted to moving in a single file[13] $r_{\mathrm{ww}}{}^*$ may become positive[7]. Applying these considerations to Eqn. 39 $P_{\mathrm{os}}/P_{\mathrm{is}} > 1$ for pores of radius greater than a few water molecular radii and approaches a non-finite limit as the radius approaches a non-finite limit; $P_{\mathrm{os}}/P_{\mathrm{is}} < 1$ for pores of radius less than a few water molecular radii.

These results are in agreement with the conclusions of TICKNOR[23] and the experimental findings of ROBBINS AND MAURO[16] and LAKSHMINARAYANAIAH[18] who used artificial membranes of various pore sizes.

In the context of the present discussion it is interesting to note that two groups of workers[17,24] have found that the two water permeability coefficients, *viz.* osmotic and isotopic, for artificial phospholipid bilayer membranes are different. HANAI, HAYDON AND TAYLOR[24] attributed this difference to the presence of stagnant liquid layers at the two faces of the membrane. HUANG AND THOMPSON[17] rejected this explanation. They suggested that the observed permeability ratio of about 20 might arise from either (i) single-file diffusion through long pores; or (ii) different flow mechanisms in each case, *viz.* flows by diffusion and filtration.

In the light of the preceding treatment it seems that single-file diffusion is unlikely to be the predominant flow mechanism since it has an associated permeability ratio of less than unity rather than the observed factor of 20. On the other hand the postulate of different flow mechanisms has been shown to be invalid. Thus the present treatment while not presenting specific models does lead to the rejection of the alternate hypotheses presented above.

A re-examination of the work of KOEFOED-JOHNSEN AND USSING

In the case where water flows through a semipermeable membrane and there are no pressure gradients present, the flux ratio expression obtained by using Eqn. 19a is

$$\ln\left(-\frac{J_1}{J_2}\right) = \ln\frac{a(\delta)}{a(0)} + \frac{J_{\mathrm{w}}}{RT}\int_0^{\delta} r_{\mathrm{ww}}{}^*\mathrm{d}x \tag{40}$$

In the case where flows occur in pores which occupy a combined area A the total water flux $J_{\mathrm{w}}^{\mathrm{T}}$ is defined by

$$J_{\mathrm{w}}^{\mathrm{T}} = J_{\mathrm{w}}A \tag{41}$$

Furthermore, as noted previously,

$$D_{\mathrm{w}} = -\frac{1}{C_{\mathrm{w}}r_{\mathrm{ww}}{}^*} \tag{42}$$

Combining Eqns. 40–42 and noting that $C_{\mathrm{w}}\,\bar{v}_{\mathrm{w}} = 1$

$$\ln\left(-\frac{J_1}{J_2}\right) = \ln\left(\frac{a(\delta)}{a(0)}\right) - \frac{J_{\mathrm{w}}^{\mathrm{T}}\bar{v}_{\mathrm{w}}}{D_{\mathrm{w}}}\int_0^{\delta}\frac{1}{A}\,\mathrm{d}x \tag{43}$$

Eqn. 43 is identical to Eqn. 13, in the paper of KOEFOED-JOHNSEN AND USSING[25]. In their treatment KOEFOED-JOHNSEN AND USSING assumed that the electrochemical potential gradient must include a term proportional to the net flux of the water to take account "of the escaping tendency of all substances present in the stationary solutions in contact with the membrane". In the present treatment Eqn. 44 is arrived at from the phenomenological equations of irreversible thermodynamics by taking account of the interaction between the one-way fluxes. The two treatments are not equivalent for when the net flux is zero, which in the present case implies no osmotic gradients, Eqns. 16b and 16c give for the one-way fluxes

$$J_1 = -J_2 = -\frac{RTa(\delta)}{\int_0^\delta a(R_{\mathrm{ww}}-r_{\mathrm{ww}}^*)\mathrm{d}x} \tag{44}$$

showing that both J_1 and J_2 depend on $a(\delta)$ and R_{ww}. On the other hand KOEFOED-JOHNSEN AND USSING found that

$$J_1 = -J_2 = \frac{RT}{\int_0^\delta ar_{\mathrm{ww}}^*\mathrm{d}x}$$

which is independent of $a(\delta)$ and does not involve the frictional interaction between the water and the membrane.

It appears therefore that the treatment of KOEFOED-JOHNSEN AND USSING is incompatible with the irreversible thermodynamical approach to the problem and that it leads to incorrect expressions for the unidirectional fluxes. Similar remarks apply to the treatments of USSING[8] and ANDERSON AND USSING[26].

The flux ratio expression and active transport

The flux ratio expression was originally proposed by USSING[8] as a useful tool for characterising the transport of a substance as 'active' or 'passive'. This proposal will now be re-examined using the present treatment.

The flux ratio equation (Eqn. 19a) may be rewritten in the form

$$\ln\left(-\frac{J_1}{J_2}\right) - (\mu(\delta)-\mu(0)) = \int_0^\delta \sum_{j=3}^{n} r_{0j}J_j\mathrm{d}x + J\int_0^\delta r_{00}^*\mathrm{d}x \tag{45}$$

If there is no significant coupling of the flow of the test species to other flows or metabolism, the transport is passive and the left-hand side of Eqn. 45 may vary linearly with J. However, in general, a linear plot does not necessarily imply a passive flux for there may exist a term $r_{0r}J_r$ in the summation $\sum_{j=3}^{n} r_{0j}J_j$ such that r_{0r} is the coefficient of coupling between the flow of the test species and a metabolic reaction of rate J_r. If $\int_0^\delta r_{0r}J_r\mathrm{d}x$ is greater than $\int_0^\delta r_{00}{}^*J\,\mathrm{d}x$ and approximately proportional to J the plot may be linear but the flux would be mostly active.

Nevertheless it is suggested here that linear plots, derived from experimental results, should be regarded as evidence favouring the flux being passive. This is in accord with the simplest interpretation of linearity, namely, that the coupling to other flows and metabolism is negligible and with the fact that there is, as yet, no conclusive evidence for the existence of active transport at the cellular level.

Similar remarks apply if the plot of the left-hand side of Eqn. 45 against any other species flux J_j is linear. However, in this case the simplest interpretation is that species j is the major entraining substance.

ANDERSON AND USSING[26] found that for various species flowing through toad skin the left-hand side of Eqn. 45 when plotted against the net water flux gave a straight line. The present work supports their inference that the species fluxes through toad skin are passive.

Consideration of special systems

(*a*) *Water flow across the intestinal wall of the dog.* In their studies of water flow between the gut and blood of dog VISSCHER *et al.*[27] found that the measured flux ratio was a factor of order 10^2 higher than that predicted from the ratio of the water activities. They attributed this result to active transport of the water molecules. Their results are given in Table I.

TABLE I

RESULTS OF VISSCHER *et al.*[27]

The Table No. refers to the tabulated results in ref. 27. In ref. 27 J_1 and J_2 are $R^{\text{w}}_{\text{into}}$ and $R^{\text{w}}_{\text{out}}$ respectively.

Table No.	J_1 (*ccs per 10 min*)	J_2 (*ccs per 10 min*)	J (*ccs per 10 min*)	$ln\ (-J_1/J_2) - ln\ a(\delta)/a(o)$
3	−10.8	21.0	9.7	−0.69
4	− 9.5	11.1	1.7	−0.16
5	−12.1	7.4	−4.7	0.48
6	−10.9	11.4	0.5	−0.05
7	−11.8	16.1	4.3	−0.30

For this case Eqn. 45 becomes

$$\ln\left(-\frac{J_1}{J_2}\right) - \ln\left(\frac{a(\delta)}{a(\text{o})}\right) = \int_0^\delta \sum_{j=3}^{n} r_{0j} J_j \mathrm{d}x + J_{\text{w}} \int_0^\delta r_{\text{ww}}{}^* \mathrm{d}x \tag{46}$$

Fig. 1 shows a plot of the left-hand side of Eqn. 46 against J_{w} using the results of VISSCHER *et al.*[27]. It is seen that the points are fitted by a straight line which passes through the origin. It follows from the remarks of the preceding section that the available evidence does not support the conclusion that water is actively transported across the intestinal wall.

(*b*) *The flow of K^+ ions across the cellular membranes of Chara australis.* Recently HOPE AND WALKER[14] reported efflux and influx measurements of K^+ in *Chara australis* as a function of membrane potential. The flux ratio expression most appropriate to their measurements is Eqn. 19c which may be written as

$$\ln\left(-\frac{J_1}{J_2}\right) + \frac{zF}{RT}\Delta V = \frac{1}{RT}\int_0^\delta \sum_{j=3}^{n} r_{\text{k}j} J_j \mathrm{d}x + \frac{J_{\text{k}}}{RT}\int_0^\delta r_{\text{kk}}{}^* \mathrm{d}x \tag{47}$$

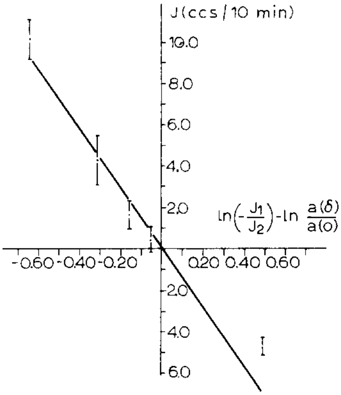
where the subscript k denotes potassium.

TABLE II

RESULTS OF HOPE AND WALKER[14] FOR *Chara australis*

V_m (mV)	J_1 $(pM \cdot cm^{-2} \cdot sec^{-1})$	J_2 $(pM \cdot cm^{-2} \cdot sec^{-1})$	J $(pM \cdot cm^{-2} \cdot sec^{-1})$	$ln\ (-J_1/J_2)$	$F\Delta V/RT$
−120	−0.25	2.10	1.85	−2.16	0.88
−130	−0.37	1.04	0.67	−1.17	0.48
−142	−0.60	0.60	0.00	0.00	0.00
−150	−0.84	0.39	−0.45	0.76	−0.32
−160	−1.30	0.22	−1.08	1.77	−0.72
−170	−1.95	0.13	−1.82	2.71	−1.12

HOPE AND WALKER's results are shown in Table II and have been used to plot the left-hand side of Eqn. 47 against J_K in Fig. 2. It is seen that all points lie close to the same straight line. Applying the criterion developed above it is concluded that, for potentials in the range indicated, the transport of K^+ is passive with little coupling to other species flows.



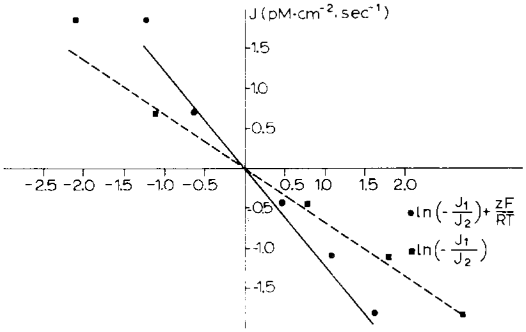


Fig. 1. Plot of J against $\ln(-J_1/J_2) - \ln a(\delta)/a(o)$ for the flow of water across the intestinal wall of the dog from the results of VISSCHER *et al.*[27] and Table I.

Fig. 2. Plots of J against $\ln(-J_1/J_2) + zF\Delta V/RT$ and $\ln(-J_1/J_2)$ for the flow of K^+ across the cellular membranes of *Chara australis*. The full and broken lines represent the respective lines of best fit. The experimental results were obtained by HOPE AND WALKER[14] and are tabulated in Table II.

Fig. 2 also shows a plot of ln $(-J_1/J_2)$ against J_K. It follows from Eqn. 19b that this curves slope equals $-1/RT \int_0^\delta (R_{KK} - r_{KK}^*)dx$. If the gradients of the two curves are compared there results

$$-\frac{\int_0^\delta r_{KK}^* dx}{\int_0^\delta (R_{KK} - r_{KK}^*)dx} \approx \frac{1}{2}$$

which if the coefficients do not change much with x implies

$$R_{KK} \approx -r_{KK}^*$$

which, since R_{KK} is positive, gives the result that r_{KK}^* is negative. In the terminology of KEDEM AND ESSIG[7] the coupling between the isotopes is therefore positive. As pointed out by KEDEM AND ESSIG positive coupling would be expected in the case of single-file diffusion[13]. However, other interpretations are possible so that while flow through narrow pores might be the dominant transport mechanism, no definitive conclusions can be reached.

DISCUSSION

In the preceding treatment the methods of irreversible thermodynamics have been employed for the purpose of gaining a better understanding of particle transport through membranes. The formalism employed is sufficiently general to ensure freedom from specific membrane models. It seems that the treatment takes account of phenomena where the particles move separately or in association with members of their own or different species. It also applies in cases where permeation flow results from active transport, single-file diffusion, or exchange diffusion.

While the one-way flux formulae cannot be integrated without a detailed knowledge of the membranous system the formula for the flux ratio is particularly simple and, irrespective of the number of independent variables in the system, is expressible in terms of only two parameters, *viz.* the net flux and the exchange resistance.

It has been shown that under the correct experimental conditions tracers can be used to provide accurate estimates of one-way fluxes. However, negligible tracer backflux is not justifiably inferred if the specific activity ratio across the membrane is much smaller than unity. Actually the specific activity ratio should be much smaller than the flux ratio (see Eqn. 25). This condition leads to rather exacting demands on the specific activities when the flux ratio becomes sufficiently large.

Numerous workers have reported discrepancies between the permeability coefficients of water as determined osmotically and isotopically. The treatment presented in this report leads to a particularly simple explanation of this effect. Thus whereas the resistance to osmotic flow of water through a semipermeable membrane is due entirely to the interaction between the water and membranous particles, the resistance to isotopic water flow also results from an interaction between the labelled and unlabelled water.

It is concluded here, on the basis of a comparison between the expressions derived for osmotic and isotopic water flow with those of MAURO[21] that the hypothesis, first proposed by CHINARD[19] and used since by numerous workers[16–18,20–23] that the water flow consists of two component flows, *viz.* flows by diffusion and filtration, is of doubtful validity.

In the case where there is only one major entraining substance the flux ratio expression is similar to that of KOEFOED-JOHNSEN AND USSING[25]. It differs, however, from their corrected formula which appears in the paper of ANDERSON AND USSING[26]. In spite of the finding here that the expressions of KOEFOED-JOHNSEN AND USSING for the individual fluxes are incompatible with those arrived by a thermodynamic treatment the flux ratio method remains useful for characterising membrane transport as active or passive. The particular cases that have been considered in this report are the flow of water across the intestinal wall of the dog and the flow of K^+ through the cellular membranes of *Chara australis*.

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