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Influence of Membrane Heterogeneity on Kinetics of Nonelectrolyte Tracer Flows

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Summary. In a composite membrane with heterogeneous channels, prevention of net volume flow with hydrostatic pressure differences and/or impermeant osmotic solutes may induce positive isotope interaction (coupling of isotope flows) consequent to circulation of volume flow. The permeability coefficient for net flow will then exceed the tracer permeability coefficient. A permeant osmotic solute will induce either positive or negative isotope interaction, according to whether membrane heterogeneity is more marked for the test solute or the osmotic solute, respectively. Thus membrane heterogeneity may account for phenomena commonly attributed to "single file diffusion" or "exchange diffusion". For sufficiently small flows the general flux ratio relationship for homogeneous membranes will continue to apply.

In the study of membrane transport processes, anomalous characteristics of tracer isotope fluxes are often interpreted in terms of specific mechanisms. Thus discrepancies between permeability coefficients determined by different means, as well as "abnormality" of the flux ratio, are commonly attributed either to "exchange diffusion" by means of a mobile carrier, or "single file diffusion" through a narrow channel [4, 8, 12]. In principle, however, the observed anomalies are explicable in terms of a variety of mechanisms [5, 13]. Indeed it can be shown that both apparent exchange diffusion and single file diffusion can be the consequence of the heterogeneity of synthetic and biological membranes.

Results and Discussion

One useful means of investigating mechanisms of passive membrane transport is the examination of possible interactions between flows of different species. Of special interest is the case where the solutes are isotopes of the same chemical species, since then they must traverse identical pathways. Such studies are carried out under a variety of conditions, as considered below.

Absence of Hydrostatic Pressure Difference ($\Delta P = 0$)

For convenience, studies of transport are often carried out in the absence of a significant hydrostatic pressure difference across the membrane ($\Delta P = 0$). Thus, for nonelectrolytes only concentration driving forces are operative. Using this approach, Ussing and Johansen showed that in the toad skin net flow of urea enhanced the unidirectional flux of tracer sucrose in the same direction (influx), and retarded the efflux [14]. Similar interaction between urea and mannitol was demonstrated by Biber and Curran in toad skin [1], and by Franz, Galey, and Van Bruggen in frog skin [3]. Lief and Essig found analogous interaction between macroscopic and radio-active tracer urea fluxes in the toad bladder [10]. In all of these cases, osmotic water flow would interfere with the observed effects, since it would depress tracer influx and enhance tracer efflux in all pathways. Hence the above-described demonstrations of the influence of macroscopic solute flow on tracer flows has been taken as evidence of direct molecular interaction as the cause of abnormality of the flux ratio.

There are, however, significant ambiguities associated with transport measurements at $\Delta P = 0$, in that osmotic water flow interferes with the evaluation of the true extent of solute interaction. If sufficiently rapid, solvent flow may prevent the demonstration of positive coupling, or even lead to an erroneous impression of negative coupling between solute flows. Thus, Franz *et al.* have demonstrated reversal of the direction of net tracer flux on changing the experimental constraint from zero hydrostatic pressure difference ($\Delta P = 0$) to zero volume flow ($J_v = 0$), and have emphasized the importance of this factor in attempts to evaluate the nature of solute interaction precisely [3]. However, Patlak and Rapoport have shown that, for flows of two chemical species, even studies at $J_v = 0$ may lead to ambiguity, owing to the possibility of circulation of volume flows in heteroporous membranes [11]. We shall consider here the influence of circulation on the apparent interaction between the abundant and tracer forms of a single chemical species ("isotope interaction" [5]). In order to demonstrate the effects of circulation in pure form we assume that there is no interaction between the isotope flows in any individual pathway.

Absence of Volume Flow ($J_v = 0$)

In this case the two bathing solutions are identical except for a concentration difference for the solute of interest. The tendency of the osmotic

pressure difference to produce net volume flow is compensated by the application of a hydrostatic pressure difference just adequate to make $J_v=0$. Each solution may contain a different tracer isotope of the test solute, permitting the simultaneous determination of influx, efflux, and net flux. Alternatively, if only a single tracer isotope is available, equivalent information may be obtained from the measurement of influx and efflux in separate experiments.

In order to treat the possibility of membrane heterogeneity we consider the characteristics of each pathway individually. For the i-th pathway let ω_i^* and ω_i be the tracer permeability coefficient and the permeability coefficient for net flow of the test species respectively, σ_i its reflection coefficient, and L_{pi} the hydraulic conductivity[1]. The parameters of different pathways may differ owing either to heteroporosity or other factors. (For simplicity, we shall normalize all flows relative to unit total membrane area; hence $J=\sum_i J_i$ for all flows.)

In a tracer self-exchange experiment there is no net macroscopic flow in any pathway. Hence the tracer permeability coefficient is obtained from the quotient of the tracer flow J^* and the tracer concentration difference Δc^*:

$$\begin{aligned}\omega^* &\equiv (-J^*/RT\Delta c^*)_{J_j=0} = -(1/RT\Delta c^*)\sum_i (J_i^*)_{J_{ji}=0} \\ &= -(1/RT\Delta c^*)\sum(-\omega_i^* RT\Delta c^*) = \sum \omega_i^*.\end{aligned} \tag{1a}$$

(We shall omit the subscript i in the summation $\sum_i$, except when its omission would be confusing.) In the absence of isotope interaction in any pathway, $\omega_i^* \equiv \omega_i$ [5], and we have also

$$\omega^* = \sum \omega_i. \tag{1b}$$

Thus, the tracer permeability coefficient is given simply by the sum of the permeabilitycoefficients of the individual pathways.

The permeability coefficient for net flow, however, may be quite different. This is obtained from the flow and concentration difference of the macroscopic species, in the absence of volume flow:

$$\omega \equiv -(J/RT\Delta c)_{J_v=0} = -(\sum J_i/RT\Delta c)_{J_v=0}.$$

1 In conformity with our earlier publications, ω designates the permeability coefficient for net flow, and ω^* represents the tracer permeability ("self-diffusion") coefficient [5]. This usage is in contrast to that of Patlak and Rapoport, for whom ω designates the permeability coefficient for a heteroporous membrane, and ω^* that for a homogeneous membrane [11].

The flows in the individual pathways are given by [6]

$$J_i = -\omega_i RT\,\Delta c + \bar{c}(1-\sigma_i)\,J_{vi}, \tag{2}$$

where $\bar{c}$ is the logarithmic mean concentration ($\bar{c} = \Delta c/\Delta \ln c$).

In general the volume flow in any pathway is not zero, but rather

$$J_{vi} = L_{pi}(\sigma_i RT\,\Delta c - \Delta P). \tag{3}$$

Summing over all pathways and setting $J_v \equiv \sum J_{vi} = 0$ give the value of ΔP which would abolish net volume flow:

$$(\Delta P)_{J_v=0} = (\sum \sigma_i L_{pi} RT\,\Delta c)/\sum L_{pi}.$$

Therefore, we have

$$J = \sum J_i = \sum\{-\omega_i RT\,\Delta c + \bar{c}(1-\sigma_i)\,J_{vi}\} = \sum\{-\omega_i RT\,\Delta c - \bar{c}\,\sigma_i J_{vi}\}$$
$$= \sum\{-\omega_i RT\Delta c - \bar{c}\,\sigma_i[L_{pi}(\sigma_i RT\Delta c - (\sum \sigma_i L_{pi} RT\Delta c)/\sum L_{pi})]\} \quad (J_v = 0),$$

and

$$\omega = -(J/RT\Delta c)_{J_v=0} = \sum \omega_i + \bar{c}[\sum \sigma_i^2 L_{pi} - (\sum \sigma_i L_{pi})^2/\sum L_{pi}].$$

Comparison with Eq. (1b) shows that

$$\omega = \omega^* + \bar{c}\,\gamma, \tag{4a}$$

where

$$\gamma \equiv -\sum(1-\sigma_i)\,J_{vi}/RT\,\Delta c = \sum \sigma_i^2 L_{pi} - (\sum \sigma_i L_{pi})^2/\sum L_{pi}. \tag{4b}$$

Thus it is seen that the two coefficients ω and ω^* differ whenever $\gamma \neq 0$. This will occur whenever the σ_i's are not all equal. On the other hand, if σ_i is the same for each pathway, variation in L_{pi} would not cause γ to differ from zero.

Since all the L_{pi} are positive, it is readily shown that γ must be $\geqq 0$, irrespective of the values of σ_i. Hence $\omega \geqq \omega^*$, i.e., the permeability coefficient derived from the measurement of net flow must be equal to or exceed that derived from tracer exchange. In the absence of a net volume flow across the membrane, this discrepancy between ω and ω^* would appear phenomenologically as positive isotope interaction, despite the absence of isotope interaction in the individual pathways.

This phenomenological coupling of isotope flows would also lead to "abnormality" of the flux ratio [5, 13]. It can be shown (*Appendix*) that the unidirectional fluxes are given by

$$\vec{J} = RT\,c^{\mathrm{I}}(\omega^* - \gamma\,\Delta c/2), \tag{5a}$$

$$\overleftarrow{J} = RT\,c^{\mathrm{II}}(\omega^* + \gamma\,\Delta c/2), \tag{5b}$$

where $\vec{J}$ is the influx (from bath I to bath II), $\overleftarrow{J}$ is the efflux, and $\Delta c = c^{II} - c^{I}$. The flux ratio is therefore given by

$$f = \frac{\vec{J}}{\overleftarrow{J}} = \frac{c^{I}(\omega^* - \gamma\,\Delta c/2)}{c^{II}(\omega^* + \gamma\,\Delta c/2)} = \frac{c^{I}}{c^{II}}\,\frac{(1-\gamma\,\Delta c/2\omega^*)}{(1+\gamma\,\Delta c/2\omega^*)}. \tag{6}$$

Clearly, for $\gamma \neq 0$ the flux ratio is abnormal, since $f \neq c^{I}/c^{II}$. It is also of interest to examine the logarithm of the flux ratio. For sufficiently small Δc, $|\gamma\,\Delta c/2\omega^*| \ll 1$, and

$$\ln f = \ln \frac{c^{I}}{c^{II}} + \ln \frac{(1-\gamma\,\Delta c/2\omega^*)}{(1+\gamma\,\Delta c/2\omega^*)} \approx \ln \frac{c^{I}}{c^{II}} - \frac{\gamma\,\Delta c}{\omega^*}.$$

Since $\Delta c = \bar{c}(\Delta \ln c)$,

$$\ln f = (1+\gamma\,\bar{c}/\omega^*)\ln(c^{I}/c^{II}), \quad \text{and with Eq. (4a)},$$

$$\ln f = (\omega/\omega^*)\ln(c^{I}/c^{II}) = (\omega/\omega^*)(X/RT). \tag{7}$$

Hence, the general flux ratio relation for homogeneous membranes continues to apply [5]. Since $\omega \geqq \omega^*$, $|RT \ln f| \geqq |X|$, as in single file diffusion (Fig. 1).

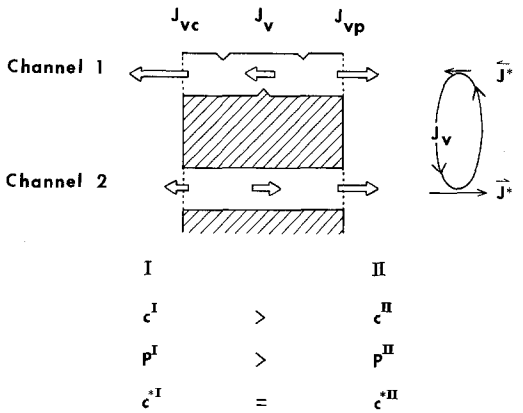

Fig. 1. Effect of circulating volume flow on unidirectional fluxes (net volume flow abolished by hydrostatic pressure difference). Channels 1 and 2 are taken as representative of a heterogeneous membrane, with $\sigma_1 > \sigma_2$. (Although the membrane is pictured as heteroporous, differences in σ may equally well be attributable to other factors. L_p is assumed the same for all channels.) We consider $c^{I} > c^{II}$, $P^{I} > P^{II}$. Volume flow J_{vc} attributable to Δc is from II to I and is of larger absolute magnitude in channel 1 (with higher σ). Flow J_{vp} attributable to ΔP is from I to II and equal in both channels. With appropriate setting of ΔP, $J_{v1} < 0$, $J_{v2} > 0$, and net volume flow $J_v = J_{v1} + J_{v2} = 0$. The effect of the counter-clockwise circulating volume flow on tracer solute flux is to markedly enhance $\vec{J}^*$ from I to II through channel 2 (of lower σ) and to slightly enhance $\overleftarrow{J}^*$ from II to I through channel 1 (of higher σ); hence with equal initial concentrations of tracer in each bath, net tracer flux $J^* > 0$, and the flux ratio $f = [\vec{J}^*/(c^{*I}/c^{I})]/[\overleftarrow{J}^*/(c^{*II}/c^{II})] > c^{I}/c^{II}$, as in single file diffusion

In the above development a hydrostatic pressure difference was employed to make $J_v=0$. As in Patlak and Rapoport's study, it is immaterial whether J_v be made zero by this means or by the combined effects of hydrostatic pressure and concentration gradients of any number of impermeant species. If, however, additional permeant species are present, new phenomena may be observed.

Absence of Volume Flow and Hydrostatic Pressure Difference ($J_v=0$; $\Delta P=0$; $\Delta c\neq 0$) (*2 permeant chemical species*)

In this case the two bathing solutions differ not only in the concentration of the test species, but also in the concentration of a second permeant species, designated by a superscript $'$. We consider the situation in the absence of a hydrostatic pressure difference, with the concentration difference of the second ("osmotic") species adjusted so as to make $J_v=0$. Following the same general approach as above, it can be shown that now

$$\omega=\omega^*+\bar{c}\,\varepsilon, \tag{8a}$$

where

$$\varepsilon=\sum\sigma_i^2 L_{pi}-(\sum\sigma_i\sigma_i' L_{pi})(\sum\sigma_i L_{pi})/(\sum\sigma_i' L_{pi}). \tag{8b}$$

(We have here assumed that in any individual pathway there is no isotope interaction, and no interaction between the flows of the chemically distinct solute species.) Clearly, in order for the osmotic solute to have effects different from those of an impermeant solute it is necessary that σ_i' not be identical in all pathways. Otherwise, $\sigma_i'\equiv\sigma'$, and $\varepsilon=\gamma$.

More generally, the nature of the relationship between ω and ω^* will depend on whether membrane heterogeneity is more significant for the test solute or the osmotic species. This is most readily seen by considering, for simplicity, that L_{pi} is the same for all pathways. Then $L_{pi}\equiv L_p$, and

$$\begin{aligned}\varepsilon&=L_p\{\sum_i\sigma_i^2-(\sum_i\sigma_i\sigma_i')(\sum_i\sigma_i)/(\sum_i\sigma_i')\}\\&=(L_p/2\sum_i\sigma_i')[\sum_i\sum_j(\sigma_i-\sigma_j)(\sigma_i\sigma_j'-\sigma_j\sigma_i')].\end{aligned} \tag{9}$$

It is clear that with reflection coefficients $\geqq 0$ we have $L_p/2\sum_i\sigma_i'>0$, and thus $\varepsilon>0$ when finite $(\sigma_i-\sigma_j)$ and $(\sigma_i\sigma_j'-\sigma_j\sigma_i')$ are of like sign (for all i, j) and $\varepsilon<0$ when they are of unlike sign. The significance of these relationships is clarified by examining for a representative pair of pathways $\{i,j\}$ the quantity

$$\theta_{i,j}\equiv(\sigma_i-\sigma_j)(\sigma_i\sigma_j'-\sigma_j\sigma_i')/(\sigma_j^2\sigma_i')=(\sigma_i/\sigma_j-1)[(\sigma_i/\sigma_j)/(\sigma_i'/\sigma_j')-1]. \tag{10}$$

It can be seen that if heterogeneity of pathways is more significant for the test solute than for the osmotic solute (i.e., the σ's differ more than the σ''s), we have for $(\sigma_i/\sigma_j)>1$, $(\sigma_i/\sigma_j)/(\sigma'_i/\sigma'_j)>1$, and for $(\sigma_i/\sigma_j)<1$, $(\sigma_i/\sigma_j)/(\sigma'_i/\sigma'_j)<1$, so that $\theta_{i,j}>0$. On the other hand, if heterogeneity is more marked for the osmotic solute than the test solute, $\theta_{i,j}<0$. With all $\theta_{i,j}>0$ we have $\varepsilon>0$ and with $\theta_{i,j}<0$, $\varepsilon<0$. Thus, in the absence of either single file or carrier mechanisms, we may have either $\omega>\omega^*$ (positive isotope interaction) or $\omega<\omega^*$ (negative isotope interaction), according as membrane heterogeneity is more marked for the test solute or the osmotic solute respectively (Fig. 2). With a more random pattern of pathway heterogeneity, isotope interaction might be positive, negative, or absent. Clearly the nature of the interaction may vary markedly with change of either the test solute or the osmotic solute employed. In all cases, for sufficiently small flows the general flux ratio relationship of Eq. (7) will continue to apply.

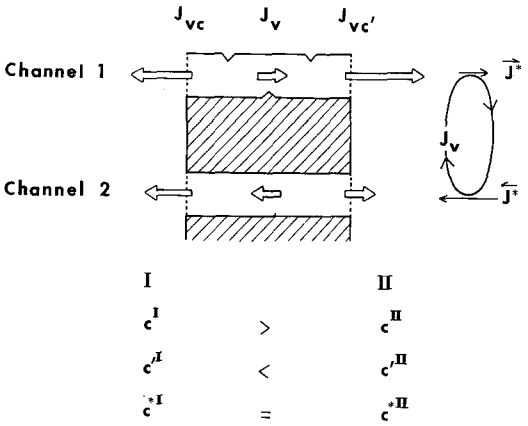

Fig. 2. Effect of circulating volume flow on unidirectional fluxes (net volume flow abolished by concentration difference of a permeant "osmotic" solute). Channels 1 and 2 are taken as representative of a heterogeneous membrane such that for the test solute $\sigma_1>\sigma_2$ and for the osmotic solute $\sigma'_1>\sigma'_2$. For the example pictured it is assumed that membrane heterogeneity is more pronounced for the osmotic solute than for the test solute, i.e., $\sigma'_1/\sigma'_2>\sigma_1/\sigma_2$. We consider $c^I>c^{II}$, $c'^I<c'^{II}$. Volume flow J_{vc} attributable to Δc of the test solute is from II to I and of slightly greater magnitude in channel 1. Volume flow $J_{vc'}$ attributable to $\Delta c'$ of the osmotic solute is from I to II and is of substantially greater magnitude in channel 1. With appropriate setting of $\Delta c'$, $J_{v1}>0$, $J_{v2}<0$, and net volume flow $J_v=J_{v1}+J_{v2}=0$. The effect of the clockwise circulating volume flow on tracer solute flux is to slightly enhance $\vec{J}^*$ from I to II through channel 1 (of higher σ) and to markedly enhance $\overleftarrow{J}^*$ from II to I through channel 2 (of lower σ); hence with equal initial concentrations of tracer in each bath, net tracer flux $J^*<0$, and the flux ratio $f=[\vec{J}^*/(c^{*I}/c^I)]/[\overleftarrow{J}^*/(c^{*II}/c^{II})]<c^I/c^{II}$, as in exchange diffusion. Alternatively, if membrane heterogeneity were more pronounced for the test solute than for the osmotic solute, $f>c^I/c^{II}$, as in single file diffusion

The above analysis of the kinetics of isotope flows demonstrates that, as for other transport phenomena, heterogeneity of membrane structure can lead to a variety of interesting effects, which may be strongly influenced by experimental conditions. Thus, the absence of hydrostatic pressure gradients permits the unequivocal demonstration of positive interaction of solute flows, but with underestimation of its magnitude if volume flow is appreciable. On the other hand, the abolition of net volume flow may lead to the appearance of interaction, attributable in fact to circulation of volume flows. If the solutes used to regulate osmotic pressure are themselves permeant, the resultant phenomenological isotope interaction may be either positive or negative, according to whether membrane heterogeneity is more significant for the test solute or the osmotic solute.

The precise demonstration of the nature and extent of isotope interaction requires that net coupled flows of other chemical species be absent, despite appreciable flows in individual pathways. For solvent flow between dilute solutions, this state is readily approximated by the abolition of volume flow. The control of net flow of permeable solute species is more difficult; however, with biological systems in the steady state, absence of net solute flow is often achieved by the combined effects of parallel "pump-leak" pathways. Such could be the case, for example, in studies of tracer sugar exchange across red blood cell membranes [7] or tracer sodium exchange in muscle [8, 12].

In biological systems, positive isotope interaction is often attributed to single file diffusion. Negative isotope interaction is often attributed to exchange diffusion via a membrane carrier. However, it is appreciated that other mechanisms may be operative [4, 5, 8, 13]. Experimental studies of ion transport have demonstrated negative isotope interaction in synthetic membranes which are unlikely to be traversed by carriers [2, 9]. The present theoretical study demonstrates that both positive and negative isotope interaction can be the simple consequence of membrane heterogeneity, even for uncomplicated passive transport of nonelectrolytes. It is to be anticipated that active transport, and the influence of electrical fields and unstirred layers, would lead to still more diverse effects [15].

Appendix

Unidirectional Fluxes with $J_v=0$; Single Permeant Chemical Species

Unidirectional fluxes cannot be calculated by the direct application of Eq. (2) since this describes net flow; furthermore, for the case of unidirec-

tional flux of a species, the appropriate mean concentration is not well-defined. We can, however, perform a "thought-experiment", in which we add tracer quantities of two different isotopes, species a and b, to baths I and II respectively. With sufficiently large sinks the tracers will be much diluted on crossing the membrane, and we can conceive that throughout the experiment the total tracer concentration in bath I is $c^{*\,\mathrm{I}} = c_a^{\mathrm{I}} + c_b^{\mathrm{I}} \simeq c_a^{\mathrm{I}}$ and that in bath II is $c^{*\,\mathrm{II}} = c_a^{\mathrm{II}} + c_b^{\mathrm{II}} \simeq c_b^{\mathrm{II}}$. If the tracer concentrations in the two baths are nearly equal, $\Delta c^* = c^{*\,\mathrm{II}} - c^{*\,\mathrm{I}}$ is small and

$$\bar{c}^* = \Delta c^* / \Delta \ln c^* \simeq (c^{*\,\mathrm{I}} + c^{*\,\mathrm{II}})/2.$$

For small Δc^*, and well-defined $\bar{c}^*$, in the absence of isotope interaction in the individual pathways, it is valid to apply Eq. (2), giving

$$J_i^* = -\omega_i^* \, \mathrm{R}T \Delta c^* + \bar{c}^* (1 - \sigma_i) J_{vi}, \tag{A.1}$$

i.e. in the absence of isotope interaction in the i-th pathway J_i^* is not explicitly dependent on the concentration difference or flow of the abundant species across the membrane; for a given mean concentration $\bar{c}$, altering Δc without altering J_{vi} will affect J_i but not J_i^* (*see* Eq. (2)). (ω_i^*, σ_i, and J_{vi} may of course, however, be functions of $\bar{c}$.)

Adding the J_i^*'s and introducing Eqs. (1a) and (4b) gives

$$J^* = -\omega^* \, \mathrm{R}T \Delta c^* - \bar{c}^* \, \gamma \, \mathrm{R}T \Delta c \qquad (J_v = 0). \tag{A.2}$$

Thus it is seen that, in contrast to the tracer fluxes in the individual pathways at constant J_{vi}, the total tracer flux is affected by perturbation of Δc, again consistent with the phenomenological interaction of tracer and abundant isotope flows.

The tracer flux J^* must be associated with unidirectional tracer fluxes of the form

$$\vec{J}^* = \omega^* \, \mathrm{R}T \, c^{*\,\mathrm{I}} - (c^{*\,\mathrm{I}}/2) \, \gamma \, \mathrm{R}T \Delta c + \alpha \tag{A.3}$$

$$\overleftarrow{J}^* = \omega^* \, \mathrm{R}T \, c^{*\,\mathrm{II}} + (c^{*\,\mathrm{II}}/2) \, \gamma \, \mathrm{R}T \Delta c + \alpha \tag{A.4}$$

in order that $J^* \equiv \vec{J}^* - \overleftarrow{J}^*$. Since we deal with tracer fluxes, $\vec{J}^*$ must be proportional to $c^{*\,\mathrm{I}}$ and independent of $c^{*\,\mathrm{II}}$, and $\overleftarrow{J}^*$ must be proportional to $c^{*\,\mathrm{II}}$ and independent of $c^{*\,\mathrm{I}}$. Hence $\alpha = 0$. Dividing each tracer flux by the specific activity in the appropriate bath gives the unidirectional fluxes

$$\vec{J} = \vec{J}^* / (c^{*\,\mathrm{I}}/c^{\mathrm{I}}) = \mathrm{R}T \, c^{\mathrm{I}} (\omega^* - \gamma \, \Delta c/2), \tag{A.5a}$$

$$\overleftarrow{J} = \overleftarrow{J}^* / (c^{*\,\mathrm{II}}/c^{\mathrm{II}}) = \mathrm{R}T \, c^{\mathrm{II}} (\omega^* + \gamma \, \Delta c/2). \tag{A.5b}$$

Clearly these relationships apply irrespective of the actual concentrations of tracer and abundant isotope employed experimentally, provided that we operate in the range of applicability of Eq. (2).

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