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Flux Ratio Theorems for Nonstationary Membrane Transport With Temporary Capture of Tracer

L. BASS, A. J. BRACKEN AND J. HILDEN†

Department of Mathematics, University of Queensland, Brisbane, Australia

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It has been shown recently that the ratio of unidirectional tracer fluxes, passing in opposite directions through a membrane which has transport properties varying arbitrarily with the distance from a boundary, is independent of time from the very first appearance of the two outfluxes from the membrane. This surprising proposition has been proved for boundary conditions defining standard unidirectional fluxes, and then generalized to classes of time-dependent boundary conditions. The operational meaning of all the resulting theorems is that when any of them appear to be refuted experimentally, the presence of more than one parallel transport pathway (that is, of membrane heterogeneity transverse to the direction of transport) can be inferred and analyzed. Recent experimental data have been interpreted accordingly. However, the proofs of the theorems given so far have not taken into account the possibility of temporary capture of tracer at sites fixed in the membrane (including also entrances to microscopic *culs-de-sac*). The possible presence of such a process, which would not affect fluxes in the steady state, left a fundamental gap in the aforementioned inferences. It is shown here that all the theorems previously proved for the flux ratio under unsteady conditions remain valid when temporary capture of tracer is admitted, no matter how the rate of capture, and the probability distribution of residence times of tracer at capture sites, may depend on the distance from a membrane boundary. The validity of the aforementioned inferences from observed time-dependence of the flux ratio is thereby extended to a much wider class of membrane transport processes.

1. Introduction

The ratio of unidirectional tracer fluxes passing in opposite directions through a membrane (called the *flux ratio*) has proved to be a powerful tool for studying transport properties of biological membranes (Ussing, 1978). In this context, unidirectional flux is defined and observed as the outflux of tracer from the membrane into a bathing solution maintained at zero tracer concentration, following a sudden imposition and subsequent maintenance of a non-zero tracer concentration in the solution bathing the other side of the (initially tracer-free) membrane.

A salient feature of the flux ratio is that important conclusions about membrane transport can be drawn from it without specifying the dependence of local transport parameters within the membrane (such as tracer diffusion coefficient and drift velocity) on the distance from a membrane boundary. For charged tracers this means that the spatial distribution of the potential difference measured across the membrane need not be known or postulated.

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† Permanent address: Institute of Medical Genetics, University of Copenhagen, Denmark.

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The uses of the flux ratio are quite different in the steady and in the unsteady states. The flux ratio under *steady-state* conditions has long been used to distinguish between passive, facilitated and active transport mechanisms (Ussing, 1978). We shall not be concerned here with this aspect of the flux ratio.

The use of the flux ratio under *unsteady* conditions has arisen recently from a surprising conjecture of Ussing (1978): after the sudden imposition of constant boundary concentrations, the flux ratio is independent of time from the very first appearance of each of the two unidirectional outfluxes from a plane membrane, no matter how the diffusion coefficient and the drift velocity of the tracer may depend on the distance from a membrane boundary. This conjecture has recently been proved (Sten-Knudsen & Ussing, 1981; Bass & Bracken, 1983) and extended to time-dependent boundary conditions on concentrations (Bass & Bracken 1983), and to fluxes into and out of a hollow circular cylinder (Bass & McAnally, 1984). As these results hold in general only for a membrane with a single transport pathway (that is, with no heterogeneity transverse to the direction of transport) it has been concluded that whenever a time-dependence of the flux ratio is detected experimentally, the presence of at least two kinds of parallel transport pathways can be inferred and analysed (Ussing *et al.*, 1981; Lim & Ussing, 1982).

However, a major gap has been left in the foregoing reasoning. Tracer may be trapped temporarily at sites distributed through the membrane. Such sites may be interpreted in terms of molecular kinetics, or as entrances to microscopic *culs-de-sac* within the membrane. This possibility has not been taken into account in the proofs of Ussing's conjecture (and of its extensions), so that an observed unsteadiness of the flux ratio might be due to temporary capture of tracer rather than to heterogeneity of transport pathways transverse to the direction of transport. In the present paper we close this gap. We show that, no matter how the density of capture sites may depend on the distance from a membrane boundary, and no matter how the form of the distribution of residence times of trapped tracer may vary with that distance, Ussing's conjecture and its aforementioned extensions remain true. We shall show that effects of temporary capture of tracer increase greatly the variety of possible unidirectional flux transients, and cannot be described in general by a mere redefinition of the functions representing the arbitrary space-dependence of the tracer diffusion coefficient and drift velocity. We shall show also that temporary capture of tracer has no observable effects on fluxes in the steady state, so that the aforementioned gap could not be detected by steady-state studies.

We are surprised by the generality of circumstances under which the flux ratio is steady under unsteady conditions and, more generally, by the kinetic and mathematical depth of the concept of flux ratio. In this regard we note that the constancy of the flux ratio in the one-dimensional diffusion-drift problem (Sten-Knudsen & Ussing, 1981; Bass & Bracken, 1983) implies, with the use of Bäcklund transformations, related ratio theorems for boundary value problems for certain non-linear partial differential equations (Rogers & Bracken, 1986).

The validity of flux ratio theorems under unsteady conditions, so surprising at first sight, is related to characteristic features of classical diffusional transport, and in particular to the fact that the speed of such transport is in principle infinite. It is for this reason that arbitrary *finite* convection advancing the one unidirectional flux and retarding the other does not cause the ratio theorems to fail even at first appearances of the tracer outfluxes. In practice the observed speed of diffusion is limited only by the sensitivity of detectors of the diffusing particles. The concept of "flux ratio at first appearance of the unidirectional outfluxes" has, therefore, the operational meaning of the ratio taken when the smaller outflux first becomes reliably measurable, using instruments of the same accuracy on both sides of the membrane.

2. Transport of Tracer With Temporary Capture

We represent the membrane by a slab in the spatial interval $0 \leq x \leq h$, in which a tracer has diffusion coefficient $D(x)$ and drift velocity $v(x)$. Both D and v are independent of time, but they can depend arbitrarily on position except that $D(x) > 0$ for all x. The free tracer concentration $c(x, t)$ and flux $j(x, t)$ depend also on the time t, and are related by

$$j = -D(x)\frac{\partial c}{\partial x} + v(x)c. \tag{1}$$

Here $v(x)$ may be the velocity of convective flow, or of the drift of tracer in an electric field associated with the membrane. The continuity (mass balance) equation for the free tracer is

$$\frac{\partial c}{\partial t} + \frac{\partial j}{\partial x} = q(x, t), \tag{2}$$

where the term $q(x, t)$ accounts for capture and release of tracer. It is the presence of this term that generalizes the previous considerations of the flux ratio problem.

As tracer does not saturate the sites, capture occurs by first-order kinetics with a rate constant $k(x)$ which varies with position as the density of sites does. Hence the capture contribution to q is $-k(x)c(x, t)$. If $g(x, \tau)\,\mathrm{d}\tau$ is the probability that a tracer molecule captured at x will remain there for a time between τ and $\tau + \mathrm{d}\tau$, then the contribution to q at x due to release of previously captured tracer is

$$k(x)\int_0^t c(x, t-\tau)g(x, \tau)\,\mathrm{d}\tau$$

the upper limit has been set equal to t because the membrane was initially tracer-free: $c(x, t \leq 0) = 0$. The probability density $g(x, \tau)$ is non-negative and normalized at any x

$$\int_0^\infty g(x, \tau)\,\mathrm{d}\tau = 1. \tag{3}$$

Altogether

$$q = -k(x)c(x, t) + k(x)\int_0^t c(x, t-\tau)g(x, \tau)\,\mathrm{d}\tau. \tag{4}$$

If we introduce the new integration variable $\theta = t - \tau$ (time of capture) in place of τ, we can write equation (4) in the form

$$q = -k(x)c(x, t) + k(x)\int_0^t c(x, \theta)g(x, t-\theta)\,\mathrm{d}\theta. \tag{4a}$$

We wish to establish the validity of flux ratio theorems (previously proved for $q = 0$) for the set of equations (1)-(4) or (4a). Before doing so in section 3, we elucidate four aspects of this generalization of membrane transport.

(A) THE ROLE OF BOUND TRACER

If we denote the concentration of bound (captured) tracer by $c_b(x, t)$ for $0 \le x \le h$ and $t > 0$, we have evidently

$$\frac{\partial c_b}{\partial t} = -q \tag{5}$$

so that total tracer $c + c_b$ satisfies the continuity equation

$$\frac{\partial}{\partial t}(c + c_b) + \frac{\partial j}{\partial x} = 0 \tag{2a}$$

in accord with equation (2).

We now elucidate the general problem by using bound tracer explicitly to construct an important example of the probability density $g(x, \tau)$. Suppose that bound tracer is released at any x by a first-order process with the rate $\tilde{k}(x)c_b(x, t)$, where the local rate constant $\tilde{k}(x)$ may depend arbitrarily on the position x. Then $q = -kc + \tilde{k}c_b$, and equations (2) and (5) become

$$\frac{\partial c}{\partial t} + \frac{\partial j}{\partial x} = -k(x)c(x, t) + \tilde{k}(x)c_b(x, t) \tag{6}$$

$$\frac{\partial c_b}{\partial t} = k(x)c(x, t) - \tilde{k}(x)c_b(x, t). \tag{7}$$

We can solve equation (7) for c_b by using the integrating factor $\exp(\tilde{k}(x)t)$, and the circumstance that the membrane is initially tracer-free ($c_b(x, 0) = 0$)

$$c_b(x, t) = k(x)\int_0^t c(x, \theta)\,\mathrm{e}^{-\tilde{k}(x)(t-\theta)}\,\mathrm{d}\theta. \tag{8}$$

Substituting in equations (6) and (7) and comparing with equations (2) and (5) we see that

$$q(x, t) = -k(x)c(x, t) + k(x)\int_0^t c(x, \theta)\tilde{k}(x)\,\mathrm{e}^{-\tilde{k}(x)(t-\theta)}\,\mathrm{d}\theta. \tag{9}$$

Comparison with equation (4a) shows that in this special case

$$g(x, \tau) = \tilde{k}(x)\,\mathrm{e}^{-\tilde{k}(x)\tau}. \tag{10}$$

Using the general definition of the mean residence time

$$\bar{\tau}(x) = \int_0^\infty \tau g(x, \tau)\, \mathrm{d}\tau \tag{11}$$

and substituting from equation (10), we find readily

$$g(x, \tau) = \mathrm{e}^{-\tau/\bar{\tau}(x)}/\bar{\tau}(x), \qquad \bar{\tau}(x) = 1/\tilde{k}(x). \tag{12}$$

Thus the kinetics described in equations (6) and (7) results at each x in the standard exponential distribution of the residence times of captured tracer, with the mean $1/\tilde{k}(x)$. It is easy to show, conversely, that the exponential distribution of residence times implies the capture-release kinetics described in equations (6) and (7).

(B) THE STEADY-STATE LIMIT

If the unidirectional fluxes are due to the imposition, at $t = 0$, of boundary concentrations that are kept *constant* in time (as in Ussing's original conjecture), then it may be expected that a steady state of fluxes and of concentrations of free and bound tracer is approached as time tends to infinity. (This need not be the case under the time-dependent boundary conditions pertaining to the generalized flux ratio theorems). From equation (5) it follows that $q = 0$ in the steady state. From equation (2) we conclude that, in the steady state, fluxes and concentrations of free tracer are unaffected by temporary capture of tracer. We can see this also directly from equation (4) without reference to bound tracer. Temporary capture means that $g(x, \tau)$ tends to zero as τ tends to infinity. As t tends to infinity in equation (4), the contributions to the integral from large values of τ tend to zero with $g(x, \tau)$, so that only $c(x, \infty)$ contributes to the integrand. Because of equation (3), equation (4) yields $q(x, \infty) = 0$. The presence of temporary capture of tracer cannot be discovered by the study of steady-state fluxes.

The presence of captured (bound) tracer does increase the radioactivity of the membrane even in the steady state. In the special case of an exponential distribution of residence times we see immediately, from equations (7) and (12), that

$$c_b(x, \infty) = k(x)\bar{\tau}(x)c(x, \infty). \tag{13}$$

We show in the Appendix that equation (13) holds for any probability density $g(x, \tau)$. It follows that, at any x, the steady-state radioactivity of the membrane is increased by the factor $(1 + \bar{\tau}k)$ as compared with the case of no capture $(k = 0)$. However, as all tracer can be washed out of the membrane, this observable effect could be interpreted in terms of a tracer partition coefficient between the membrane and the bathing solutions, without specific consideration of a process of temporary capture.

(C) MODIFICATION OF TRANSPORT PARAMETERS

It might seem possible intuitively that the effects of capture and release of tracer upon transport could be described by modifying suitably the arbitrary functions

$D(x)$, $v(x)$. We consider briefly to what extent this is the case. For this purpose we suppose that $g(x, \tau)$ is distributed narrowly about its mean $\bar{\tau}$ given by equation (11). Then, for times very much greater than $\bar{\tau}$, the value of the integral in equation (4) is not affected significantly when its upper limit is replaced by infinity. For such times t we expand $c(x, t-\tau)$ in the Taylor series

$$c(x, t-\tau) = c(x, t) - \tau \frac{\partial c(x, t)}{\partial t} + \tfrac{1}{2}\tau^2 \frac{\partial^2 c(x, t)}{\partial t^2} + \cdots \tag{14}$$

where dots denote terms of order τ^3 and higher. Substituting the expansion in the integral in equation (4) and using equation (3) we obtain

$$q = -k\bar{\tau}\frac{\partial c}{\partial t} + \tfrac{1}{2}k\overline{\tau^2}\frac{\partial^2 c}{\partial t^2} + \cdots \tag{15}$$

where

$$\overline{\tau^2} = \int_0^\infty \tau^2 g(x, \tau)\, \mathrm{d}\tau. \tag{16}$$

Using q from equation (15) in equation (2) and re-arranging, we have

$$(1+k\bar{\tau})\frac{\partial c}{\partial t} + \frac{\partial j}{\partial x} = \tfrac{1}{2}k\overline{\tau^2}\frac{\partial^2 c}{\partial t^2} + \cdots. \tag{17}$$

Suppose for simplicity that D, v, $\bar{\tau}$ and k are all independent of x. Then, using equation (1) in equation (17) and dividing through with $(1+k\bar{\tau})$ we find

$$\frac{\partial c}{\partial t} - \tilde{D}\frac{\partial^2 c}{\partial x^2} + \tilde{v}\frac{\partial c}{\partial x} = \frac{k\overline{\tau^2}}{2(1+k\bar{\tau})}\frac{\partial^2 c}{\partial t^2} + \cdots \tag{18}$$

with

$$\tilde{D} = \frac{D}{1+k\bar{\tau}} < D, \qquad \tilde{v} = \frac{v}{1+k\bar{\tau}}, \qquad |\tilde{v}| < |v|. \tag{19}$$

We see that the intuitive expectation is borne out to order $\bar{\tau}$ if we replace D, v with $\tilde{D}$, $\tilde{v}$: the right-hand side of equation (18) is negligible to this order of approximation, and the equation is then of the same form as if there was no temporary capture, but with modified D and v. But if the $\overline{\tau^2}$-term on the right-hand side of equation (18) is retained, we have a new mathematical situation which cannot be grasped by any re-definition of the transport parameters D, v even in the simplified circumstances used in this discussion. (The $\overline{\tau^2}$-term changes the character of the partial differential equation from parabolic to elliptic.) The use of $\tilde{D}$ and $\tilde{v}$ accounts for the capture-release effects only under linear changes of $c(x, t)$ with time ($\partial^2 c/\partial t^2 = 0$). If $\partial^2 c/\partial t^2 > 0$ at some time t, then at closely preceding times c was higher at x than accounted for by the use only of $\tilde{D}$, $\tilde{v}$, so at time t more tracer is being released than accounted for. In that way the $\overline{\tau^2}$-term acts as a positive source of tracer at x. (If $\partial^2 c/\partial t^2 < 0$, the $\overline{\tau^2}$-term acts as a sink for analogous reasons). The profound effect of temporary capture of tracer on the non-steady tracer transport is apparent from this example: in general, the variety of unidirectional transients is much richer than in the case $k = 0$.

(D) PRESENCE AND ABSENCE OF SYSTEM MEMORY

In equations (4) and (4a) the process of tracer capture is described probabilistically whereas, for the release, the unspecified probability density $g(x, \tau)$ admits probabilistic as well as causal mechanisms. A simple causal mechanism is described by setting

$$g(x, \tau) = \delta(\tau - \hat{\tau}) \tag{20}$$

with δ an impulse (Dirac delta) function, imposing a single standard residence time $\hat{\tau}$ on all captured tracer. By contrast, the release described by equations (12) is a purely probabilistic (Poisson) process at each position x. If $g(x, \tau)$ includes a causal element, the membrane-tracer system can be said to have a temporary memory of past states. If the release is purely probabilistic, as in equations (12), the system is memoryless (Markovian).

The possibility of memory is reflected in the mathematical circumstance that equations (1)-(4) for the free tracer are an integro-differential system in the time variable. The absence of memory is reflected in the reducibility of this system to a differential one in the purely probabilistic case, as can be shown by substituting from equations (12) in equation (4a)

$$q = -kc + (k/\bar{\tau})\,\mathrm{e}^{-t/\bar{\tau}} \int_0^t c(x, \theta)\,\mathrm{e}^{\theta/\bar{\tau}}\,\mathrm{d}\theta. \tag{21}$$

Multiplying equations (2) and (21) through with $\exp(t/\bar{\tau})$ and using the new dependent variable $c \exp(t/\bar{\tau})$ in place of c, we can remove the integral by differentiation with respect to time (because t no longer occurs in the integrand). We thus arrive at a differential system (of higher order), still greatly enriched (complicated) by temporary capture of tracer, but now without a memory.

In the next section we prove flux ratio theorems for a general probability density $g(x, \tau)$. As we are interested primarily in the validity of these theorems under the most general conditions, we shall not consider particular forms of $g(x, \tau)$ any further.

3. Generalized Flux Ratio Theorems

We consider equations (1), (2), (3) and (4a), with the initial condition $c(x, 0) = 0$, under the two sets of boundary conditions which bring about the two unidirectional fluxes passing through the membrane in opposite directions. We distinguish the two resulting cases by subscripts 1, 2. Thus, when $c_1(h, t) = 0$ and $c_1(0, t) > 0$, the concentration is $c_1(x, t)$, the flux is $j_1(x, t)$ and the unidirectional outflux from the membrane is $j_1(h, t)$. When $c_2(0, t) = 0$ and $c_2(h, t) > 0$, the unidirectional outflux from the membrane is $-j_2(0, t)$. The ratio theorems concern the ratio $j_1(h, t)/j_2(0, t)$. The oldest of the theorems (see for example Ussing, 1978) pertains to the steady state ($t \to \infty$ and hence $q = 0$): if $c_1(0, t) = c_1(0)$ and $c_2(h, t) = c_2(h)$ are constants, then

$$\frac{j_1(h, \infty)}{-j_2(0, \infty)} = \frac{c_1(0)}{c_2(h)} \exp\left(\int_0^h \frac{v(x)}{D(x)} \, \mathrm{d}x\right). \tag{22}$$

In order to consider the flux ratio under unsteady conditions, we first transform the equations for either suffix by the method of Bass & Bracken (1983). Throughout this section, equation (2) is understood as having q expressed explicitly by substituting from equation (4a). We introduce the function

$$U(x) = \exp\left(\int_0^x \frac{v(x')}{D(x')} \, \mathrm{d}x'\right) \tag{23}$$

which plays a key role in what follows. We call $U(x)$ the Ussing function. We assume that the integral in the exponent exists for all x in the interval $0 \leq x \leq h$; in particular we assume that $D(x) > 0$. Clearly $U(x)$ is positive, and for real membranes it is greater than zero and less than infinity. Hence $1/U$ is likewise greater than zero and less than infinity.

We introduce the new dependent variable $u(x, t)$ by writing

$$c(x, t) = U(x) u(x, t). \tag{24}$$

In terms of $u(x, t)$, equation (1) becomes

$$j = -D \frac{\partial u}{\partial x} U \tag{25}$$

and equation (2) becomes

$$\frac{\partial u}{\partial t} + \frac{\partial j}{\partial x} U^{-1} = -ku + k \int_0^t u(x, \theta) g(x, t - \theta) \, \mathrm{d}\theta. \tag{26}$$

The initial condition $c(x, 0) = 0$ becomes

$$u(x, 0) = 0. \tag{27}$$

We shall use Laplace transforms of u, j and g. For example

$$\bar{u}(x, p) = \int_0^\infty \mathrm{e}^{-pt} u(x, t) \, \mathrm{d}t \tag{28}$$

and similarly for the transforms $\bar{j}$ of j and $\bar{g}$ of g. The transform of equation (25) is

$$\bar{j} = -D \frac{\mathrm{d}\bar{u}}{\mathrm{d}x} U. \tag{29}$$

In transforming equation (26) we note that the integral term is a convolution, whose Laplace transform is the product of transforms of the convolved functions. Using also equation (27), the transform of equation (26) is therefore

$$p\bar{u} + \frac{\mathrm{d}\bar{j}}{\mathrm{d}x} U^{-1} = k(\bar{g} - 1)\bar{u}. \tag{30}$$

We now consider any two solutions of equations (25), (26) and (27), in particular (u_1, j_1) and (u_2, j_2). We envisage writing equation (30) first for $(\bar{u}_1, \bar{j}_1)$, and then again for $(\bar{u}_2, \bar{j}_2)$. Multiplying the first of these through with $\bar{u}_2$, the second with $\bar{u}_1$, and subtracting, we cancel the terms involving $\bar{u}_1\bar{u}_2$ and find

$$0=\bar{u}_2\frac{\mathrm{d}\bar{j}_1}{\mathrm{d}x}-\bar{u}_1\frac{\mathrm{d}\bar{j}_2}{\mathrm{d}x}=\frac{\mathrm{d}}{\mathrm{d}x}(\bar{u}_2\bar{j}_1-\bar{u}_1\bar{j}_2)-\left(\bar{j}_1\frac{\mathrm{d}\bar{u}_2}{\mathrm{d}x}-\bar{j}_2\frac{\mathrm{d}\bar{u}_1}{\mathrm{d}x}\right) \tag{31}$$

where the last equality is an identity. Using $\bar{j}_1$ and $\bar{j}_2$, respectively, as given by equation (29), we see that the last bracket in equation (31) vanishes, so that $\bar{u}_2\bar{j}_1-\bar{u}_1\bar{j}_2$ is independent of x. In particular, at the boundaries we have

$$\bar{u}_2(0)\bar{j}_1(0)-\bar{u}_1(0)\bar{j}_2(0)=\bar{u}_2(h)\bar{j}_1(h)-\bar{u}_1(h)\bar{j}_2(h). \tag{32}$$

Next we consider boundary conditions. For the most general case of unidirectional fluxes we take for $t>0$

$$c_1(0,t)=f_1(t)>0, \qquad c_1(h,t)=0 \tag{33}$$

and

$$c_2(0,t)=0, \qquad c_2(h,t)=f_2(t)>0. \tag{34}$$

Transforming equations (33), (34) first by equation (24), and then by the Laplace transformation, we find readily

$$\bar{u}_1(0)=\bar{f}_1, \qquad \bar{u}_1(h)=0 \tag{33a}$$

$$\bar{u}_2(0)=0, \qquad \bar{u}_2(h)=\bar{f}_2/U(h). \tag{34a}$$

Using equations (33a) and (34a) in equation (32), we obtain

$$-\bar{f}_1\bar{j}_2(0)=\bar{f}_2\bar{j}_1(h)/U(h). \tag{35}$$

From here we proceed in two steps.

(i) Suppose first that the two boundary concentration have a time-independent ratio r

$$\frac{c_1(0,t)}{c_2(h,t)}=\frac{f_1(t)}{f_2(t)}=r=\text{const.} \tag{36}$$

Since $f_1(t)$ and $f_2(t)$ are finite and positive, $\bar{f}_1(p)$ and $\bar{f}_2(p)$ are finite and positive for all $p>0$. As $\bar{f}_1(p)=r\bar{f}_2(p)$ from equation (36), equation (35) is reduced to

$$-r\bar{j}_2(0)=\bar{j}_1(h)/U(h). \tag{37}$$

As equation (37) holds for the Laplace transforms $\bar{j}_1(h)$ and $\bar{j}_2(0)$ for all $p>0$, it holds for $j_1(h,t)$ and $j_2(0,t)$ themselves by the uniqueness (Lerch) theorem for Laplace transforms. Hence

$$\frac{j_1(h,t)}{-j_2(0,t)}=rU(h)=r\exp\left(\int_0^h\frac{v}{D}\,\mathrm{d}x\right), \qquad t>0. \tag{38}$$

This is the flux ratio theorem for unsteady states with boundary conditions constrained by equation (36). The theorem evidently includes the case of steady boundary conditions discussed by Ussing (1978).

(ii) If we drop the constraint equation (36) and permit quite different (positive) functions of time $f_1(t)$ and $f_2(t)$ in equations (33), (34), we return to equation (35) and recall that the product of two Laplace transforms is the transform of a convolution. Equation (35) implies therefore that, for all times $t > 0$,

$$\frac{\int_0^t c_2(h, t-\tau) j_1(h, \tau)\, \mathrm{d}\tau}{-\int_0^t c_1(0, t-\tau) j_2(0, \tau)\, \mathrm{d}\tau} = U(h) = \exp\left(\int_0^h \frac{v}{D}\, \mathrm{d}x\right). \tag{39}$$

This result, obtained by Bass & Bracken (1983) for the case $q = 0$, completes the proof that all the results of Sten-Knudsen & Ussing (1981) and of Bass & Bracken (1983) hold even in the presence of temporary capture of tracer described by equations (4) or (4a).

Another generalization of interest concerns ratio theorems for unidirectional tracer fluxes passing into and out of a hollow circular cylinder, such as is formed by the membrane of a single perfused capillary (Bass & McAnally, 1984). It is not difficult to show, by adapting the calculations of the present section to cylindrical symmetry, that this generalization too remains valid in the presence of temporary capture of tracer described by equations (4) or (4a).

Applications of the flux ratio in the presence of chemical transformations of the free tracer have been considered by Patlak *et al.* (1980). In this context we note that if a permanent sink (for example, a metabolic one) of the form $-w(x)c(x, t)$ is added to the right-hand sides of equations (4) and (4a), then fluxes are affected even in the steady state: writing $c(x, \infty) = c(x)$ and using equation (1), we have

$$\frac{\mathrm{d}}{\mathrm{d}x}\left[-D(x)\frac{\mathrm{d}c}{\mathrm{d}x} + v(x)c\right] = -w(x)c. \tag{40}$$

Moreover, incomplete recovery of the original tracer may be noticed experimentally, so that the applicability of the flux ratio theorems might be doubted. However, a review of the calculations of the present section shows readily that all the theorems obtained above remain valid. If therefore any of the flux ratio theorems (for the original tracer) appeared to be refuted experimentally, the inference of heterogeneity transverse to the direction of transport would still be valid.

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APPENDIX

Bound and Free Tracer in the Steady State

In the case of a general distribution of residence times of captured tracer, described by a density function $g(x, \tau)$ as in equation (4a), the equations governing the concentrations $c(x, t)$ and $c_b(x, t)$ of free and bound tracer in the membrane are, in place of equations (6) and (7)

$$\frac{\partial c}{\partial t}+\frac{\partial j}{\partial x}=-k(x)c+k(x)\int_0^t c(x, \theta)g(x, t-\theta)\,\mathrm{d}\theta \tag{A1}$$

$$\frac{\partial c_b}{\partial t}=k(x)c-k(x)\int_0^t c(x, \theta)g(x, t-\theta)\,\mathrm{d}\theta. \tag{A2}$$

Here j is the flux of free tracer as in equation (1): there is no corresponding flux for the (immobile) bound tracer.

Integration of equation (A2) gives, with the initial condition $c_b(x, 0)=0$,

$$c_b(x, t)=k(x)\int_0^t c(x, \theta)\,\mathrm{d}\theta-k(x)\int_0^t\left[\int_0^\tau c(x, \theta)g(x, \tau-\theta)\,\mathrm{d}\theta\right]\mathrm{d}\tau. \tag{A3}$$

The second term on the right hand side can be regarded as a double integral of $c(x, \theta)g(x, \tau-\theta)$ over the triangular region $0\leq\theta\leq\tau, 0\leq\tau\leq t$ in the (θ, τ)-plane. This region, which is bounded by the straight lines $\theta=0$, $\theta=\tau$ and $\tau=t$, can also be described by the inequalities $\theta\leq\tau\leq t, 0\leq\theta\leq t$, enabling us to see that the double integral can be rewritten so that equation (A3) becomes

$$c_b(x, t)=k(x)\int_0^t c(x, \theta)\,\mathrm{d}\theta-k(x)\int_0^t\left[\int_\theta^t c(x, \theta)g(x, \tau-\theta)\,\mathrm{d}\tau\right]\mathrm{d}\theta. \tag{A4}$$

Then

$$\begin{aligned} c_b(x, t)&=k(x)\int_0^t c(x, \theta)\left[1-\int_\theta^t g(x, \tau-\theta)\,\mathrm{d}\tau\right]\mathrm{d}\theta \\ &=k(x)\int_0^t c(x, \theta)\left[1-\int_0^{t-\theta} g(x, \alpha)\,\mathrm{d}\alpha\right]\mathrm{d}\theta \end{aligned}$$

on setting $\alpha=\tau-\theta$ in the inner integral. Or, on setting $\theta=t-\tau$ in the outer integral

$$c_b(x, t)=k(x)\int_0^t c(x, t-\tau)\left[1-\int_0^\tau g(x, \alpha)\,\mathrm{d}\alpha\right]\mathrm{d}\tau. \tag{A5}$$

In this form, the equation for $c_b(x, t)$ has an illuminating interpretation in probabilistic terms: $k(x)c(x, t-\tau)$ is the amount of free tracer at x which is captured at time $t-\tau$; and $\int_0^\tau g(x, \alpha)\,\mathrm{d}\alpha$ is the probability that tracer bound at x at any one time will be released in a subsequent time interval of length τ, so that $1-\int_0^\tau g(x, \alpha)\,\mathrm{d}\alpha$ is the probability that such tracer will remain bound after that time interval. Thus the right-hand side of equation (A5) expresses the probable amount of tracer, captured at any time between 0 and t, which remains bound at time t: this equals $c_b(x, t)$.

Suppose now that $g(x, \alpha)$ approaches zero sufficiently rapidly as α approaches infinity, so that the mean $\bar{\tau}$ in equation (11) is finite; and that $c(x, t)$ approaches $c(x, \infty)$ as t approaches infinity. By going to the latter limit in equation (A5) and integrating by parts, we find

$$\begin{aligned} c_b(x, \infty) &= k(x)c(x, \infty)\int_0^\infty\left[1-\int_0^\tau g(x, \alpha)\,\mathrm{d}\alpha\right]\mathrm{d}\tau \\ &= k(x)c(x, \infty)\left\{\left[\tau\left[1-\int_0^\tau g(x, \alpha)\,\mathrm{d}\alpha\right]\right]_{\tau=0}^{\tau=\infty}+\int_0^\infty \tau g(x, \tau)\,\mathrm{d}\tau\right\} \\ &= k(x)c(x, \infty)\int_0^\infty \tau g(x, \tau)\,\mathrm{d}\tau \\ &= k(x)\bar{\tau}(x)c(x, \infty). \end{aligned} \tag{A6}$$

We have thus obtained equation (13) for any choice of the density function $g(x, t)$. At each position x in the membrane, the fractional concentrations of free and bound tracer are therefore, respectively

$$\frac{k(x)^{-1}}{k(x)^{-1}+\bar{\tau}(x)}, \qquad \frac{\bar{\tau}(x)}{k(x)^{-1}+\bar{\tau}(x)}. \tag{A7}$$

The fraction $k(x)^{-1}/\bar{\tau}(x)$ is the ratio of the mean waiting times: for the free tracer to become bound, and for the bound tracer to become free.