The Flux Ratio Equation under Nonstationary Conditions

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Summary. The time dependent (i.e., nonstationary) unidirectional fluxes through a multilayered system consisting of sandwiched layers of arbitrary composition and exhibiting arbitrary potential and resistance profiles have been calculated, assuming that the flux is governed by the Smoluchowski equation (i.e., a flux resulting from a diffusion process superimposed upon a migration and/or a convection process, where part of the latter may arise from an active transport process). It is shown that during the building up of the concentration profile of the isotope inside the system towards the stationary value the ratio between the two oppositely directed, time-dependent unidirectional fluxes is, from the very first appearance of the isotope in the surrounding solutions, equal to the value of the stationary flux ratio. The practical implications of this result are discussed.

Key words: Flux ratio equation, nonstationary, time invariance, isotope, tracers.

If the passive movement of an ionic species through a membrane results exclusively from a process of electrodiffusion, i.e., a diffusion process superimposed upon a migration process due to the presence of an electric field in the membrane, the ratio between the two oppositely directed unidirectional fluxes is given by the flux ratio equation, which states that this ratio is solely determined by the electrochemical potential difference across the membrane for the ion in question. This equation was first derived by Ussing (1949) and by Teorell (1949), the derivation by the latter being restricted to the case of a homogeneous membrane. The flux ratio equation has been widely used in biological studies since it can disclose the presence of active transport, ex-

change diffusion, and other types of interactions which may contribute to the transport of the isotope used in the study of the membrane transport of the ion in question. The interaction resulting from solvent flux has been treated repeatedly (Koefoed-Johnsen & Ussing, 1953; Meares & Ussing, 1959a, b; Hoshiko & Lindley, 1964; Kedem & Essig, 1965). Although the fundamental principles chosen by these authors as the basis of their treatment differed, the various derivations had one feature in common, viz. the physical and theoretical situation was considered at times sufficiently large to allow for stationary (i.e., time independent) conditions to prevail in the membrane.

In a biological object of minute thickness like a cell membrane, the time to establish a stationary concentration profile for the ionic radio isotope tracer species in question is negligibly small for all practical purposes, and the above restriction on the applicability of the flux ratio equation is here of no consequence. The same does not always apply, however, in the case where the experimental object is a composite structure of a considerable thickness like an epithelium. In such a system the flux of, for example, radio-sodium becomes constant within a period of about 15 min. In the case of potassium, however, the pool is large and the transport rate is low. In such a case it may take hours for the fluxes to reach a steady-state condition. In fact, it may take so long that the preparation has changed its properties (potential, resistance, etc.) in the meantime. The same complication may arise when the preparation is subject to the action of hormones or drugs. Such experimental conditions would favor a determination of the flux ratio before the isotope flux had become stationary, i.e., at a time when the experimental system was still being loaded with the isotope. This procedure, however, could be open to criticism since it implies the use of the flux ratio

equation under circumstances which are outside its original range of validity. This naturally poses the problem of extending the range of validity of the flux ratio equation into that time domain where non-steady state conditions prevail.

It has been suggested by one of us (Ussing, 1978) that the value of the flux ratio remains the same whether stationary conditions prevail or not. The mathematical approach employed, however, was incomplete since, although it indicated that the hypothesis was correct as soon as a quasi-stationary state was established, it was not clear whether or not the hypothesis was also correct in the very early stages of equilibration. In this paper we shall attempt to resolve this problem by calculating the time-dependent unidirectional fluxes through a multilayered system consisting of sandwiched layers of arbitrary composition and exhibiting arbitrary potential and resistance profiles. It will be shown that if the flux of the isotope is governed by the Smoluchowski equation (i.e., a flux resulting from a diffusion process superimposed upon a migration process and/or a convection process where part of the latter may arise from an active transport process), the ratio between the time-dependent unidirectional isotopic fluxes is, from the very first appearance of the isotopes in the surrounding solutions, equal to the value of the stationary flux ratio. A preliminary report of some of the results was made at a meeting on Membrane Transport in Biology held at Sandbjerg Castle in May, 1977.

Results

The transport process of the pertinent isotopic tracer ion is considered to take place both as the result of a concentration gradient in the region in question and as the result of each particle being influenced by an outer driving force, e.g., an electrical or pressure gradient which imparts to each of the particles a stationary velocity v which is superimposed upon their Brownian movements. Furthermore, the transport process is considered to take place through a stratified barrier of total thickness h which is delineated by planes perpendicular to the x-axis at $x = 0, x_1, x_2, \ldots, x_j, \ldots, x_n = h$. The diffusion coefficient D for the isotope in question and its velocity v caused by the external field may vary from layer to layer but are assumed to have constant values in each layer. In the layer between x_{j-1} and x_j these quantities are denoted $D^{(j)}$ and $v^{(j)}$, respectively. The constancy of $D^{(j)}$ and $v^{(j)}$ inside each layer implies that the external driving force, e.g., the potential gradient or pressure gradient, respectively, remains constant within each layer. If no other mechanisms

are present the isotope transport in each layer will be governed by the time-dependent Smoluchowski equation (Smoluchowski, 1915)

$$\frac{\partial C^{(j)}}{\partial t} = D^{(j)} \frac{\partial^2 C^{(j)}}{\partial x^2} - v^{(j)} \frac{\partial C^{(j)}}{\partial x} \tag{1}$$

where $C^{(j)}(x, t)$ is the concentration of the isotope inside the layer $x_{j-1} \leqq x \leqq x_j$ at the time t. The flux $J^{(j)}(x, t)$ corresponding to the concentration profile $C^{(j)}(x, t)$ in the layer is

$$J^{(j)} = -D^{(j)} \frac{\partial C^{(j)}}{\partial x} + v^{(j)} C^{(j)} \tag{2}$$

because of the condition of mass conservation: $\partial C^{(j)}/\partial t = -\partial J^{(j)}/\partial x$. For an elementary derivation of Eqs. (1) and (2), see, e.g., Sten-Knudsen (1978). For the sake of convenience it is assumed that no isotope is present in either the membrane system of in the surrounding bathing solutions before zero time. At the time $t = 0$ two different concentrations or the same isotope are established instantaneously in the two solutions which surround the system. Consequently, the concentration profile inside the membrane system will grow steadily with time, approaching a stationary profile. The determination of the time-dependent concentration profile of the isotope requires a solution of the n partial differential equations corresponding to Eq. (1) with the initial condition

$$C(x, t) = 0, \quad \text{for } 0 \leqq x \leqq h \quad \text{and} \quad t = 0 \tag{I}$$

and the boundary conditions

$$\left. \begin{array}{ll} C = C(0, t), & \text{for } x = 0 \quad \text{and} \quad t > 0 \\ C = C(h, t), & \text{for } x = x_n = h \quad \text{and} \quad t > 0 \end{array} \right\} \tag{II}$$

together with the conditions

$$C^{(j)}(x_j, t) = C^{(j+1)}(x_j, t)$$
$$J^{(j)}(x_j, t) = J^{(j+1)}(x_j, t)$$

for $j = 1, 2, \ldots, n-1$ and $t > 0 \tag{III}$

which expresses that the concentrations and fluxes are continuous at the interfaces between the layers inside the stratified system. The set of conditions (II) and (III) provide a total of $2n$ equations which allows a determination of the $2n$ constants which appear from the solution of the n Smoluchowski equations. Thus the problem can in principle be solved, but the algebra becomes increasingly heavy as the number of layers increases beyond $n = 2$. Therefore, we shall restrict ourselves to the solution of the more tractable problem which suffices for our purpose, viz. to find how the "input quantities" $C(0, t)$

and $J(0, t)$ at the plane $x = 0$ are interrelated to the "output quantities" $C(h, t)$ and $J(h, t)$ at the plane $x = h$.

1. Concentrations and Fluxes

To solve the problem raised we begin by considering the jth layer with its interfaces situated at x_{j-1} and x_j, when the concentration and flux have the values $C^{(j)}(x_{j-1}, t)$ and $J^{(j)}(x_{j-1}, t)$, respectively, at $x = x_{j-1}$ and time t. We seek a solution of the Smoluchowski equation Eq. (1) inside the region $x_{j-1} \leqq x \leqq x_j$. Because of the conditions (I), (II), and (III), this solution must satisfy both the initial

$$C^{(j)}(x, 0) = 0; \quad \text{for } x_{j-1} \leqq x \leqq x_j \quad \text{and} \quad t = 0 \qquad \text{(A)}$$

as well as the boundary conditions

$$C^{(j)}(x, t) = C^{(j)}(x_{j-1}, t);$$
$$\text{for } x = x_{j-1} \quad \text{and} \quad t > 0 \qquad \text{(B)}$$

and

$$J^{(j)}(x, t) = J^{(j)}(x_{j-1}, t),$$
$$\text{for } x = x_{j-1} \quad \text{and} \quad t > 0. \qquad \text{(C)}$$

The solution of Eq. (1) in conjunction with the boundary conditions (A), (B) and (C) is most easily obtained by using the method of Laplace transforms. The transform $\bar{C}^{(j)}(x, s)$ of $C^{(j)}(x, t)$ is defined by

$$\bar{C}^{(j)}(x, s) = \int_0^\infty C^{(j)}(x, t) e^{-st} dt \qquad (3)$$

(see, e.g., Sneddon, 1972). The transform of the flux is obtained from Eq. (2) as

$$\bar{J}^{(j)}(x, s) = -D^{(j)} \frac{d\bar{C}^{(j)}(x, s)}{dx} + v^{(j)} \bar{C}^{(j)}(x, s). \qquad (4)$$

Equation (1) is multiplied by e^{-st} and integrated from 0 to ∞ with respect to t. This gives

$$\int_0^\infty \frac{\partial C^{(j)}}{\partial t} e^{-st} dt = D^{(j)} \frac{d^2}{dx^2} \{\int_0^\infty C^{(j)} e^{-st} dt\}$$
$$- v^{(j)} \frac{d}{dx} \{\int_0^\infty C^{(j)} e^{-st} dt\}$$

after interchanging the order of integration and differentiation on the right-hand side. The left-hand side is integrated once by parts. This gives

$$s\bar{C}^{(j)}(x, s) - C^{(j)}(x, 0) = s\bar{C}^{(j)}(x, s)$$

because of the initial condition (A). Thus the Laplace transform of Eq. (1) satisfying the initial condition is

$$\frac{d^2\bar{C}^{(j)}(x, s)}{dx^2} - (v^{(j)}/D^{(j)}) \frac{d\bar{C}^{(j)}(x, s)}{dx}$$
$$- (s/D^{(j)}) \cdot \bar{C}^{(j)}(x, s) = 0. \qquad (5)$$

The solution of this equation must satisfy the boundary conditions below

$$\bar{C}^{(j)}(x, s) = \bar{C}^{(j)}(x_{j-1}, s); \quad \text{for } x = x_{j-1} \qquad \text{(Bb)}$$

and

$$\bar{J}^{(j)}(x, s) = \bar{J}^{(j)}(x_{j-1}, s); \quad \text{for } x = x_{j-1} \qquad \text{(Cc)}$$

which are the transforms of Eqs. (B) and (C). To facilitate printing, the transforms of concentration and flux will from now on be written as $\bar{C}^{(j)}(x)$ and $\bar{J}^{(j)}(x)$, excluding the transform parameter s. The general solution of Eq. (5), which is most easily applicable in the interval $x_{j-1} \leqq x \leqq x_j$ when both x_{j-1} and x_j differ from zero and which already satisfies the boundary condition Eq. (Bb), is of the form

$$\bar{C}^{(j)}(x) = \bar{C}^{(j)}(x_{j-1}) \exp \{\alpha^{(j)}(x - x_{j-1})\}$$
$$\cdot \cosh q^{(j)}(x - x_{j-1}) + B^{(j)} \exp \{\alpha^{(j)}(x - x_{j-1})\}$$
$$\cdot \sinh q^{(j)}(x - x_{j-1}). \qquad (6)$$

In this expression the parameter $\alpha^{(j)}$ is defined by

$$2\alpha^{(j)} = v^{(j)}/D^{(j)} \qquad (7)$$

whereas the transform parameter s now is contained in

$$q^{(j)} = \{(\alpha^{(j)})^2 + s/D^{(j)}\}^{\frac{1}{2}}. \qquad (8)$$

(As to the supplementary algebraic steps, see Appendix). B in Eq. (6) is a constant to be found by applying Eq. (4), which defines the transform of the flux, to Eq. (6) and invoking the boundary condition Eq. (Cc) for $\bar{J}^{(j)}$ (see Appendix A). The transforms for the concentration and flux in the region $x_{j-1} \leqq x \leqq x_j$ then take the form

$$\bar{C}^{(j)}(x) = \exp \{\alpha^{(j)}(x - x_{j-1})\} \left[\cosh q^{(j)}(x - x_{j-1}) \right.$$
$$\left. + \frac{\alpha^{(j)}}{q^{(j)}} \sinh q^{(j)}(x - x_{j-1}) \right] \bar{C}^{(j)}(x_{j-1})$$
$$- \frac{1}{D^{(j)} q^{(j)}} \exp \{\alpha^{(j)}(x - x_{j-1})\} \sinh q^{(j)}(x - x_{j-1}) \cdot \bar{J}^{(j)}(x_{j-1}) \qquad (9)$$

and

$$\bar{J}^{(j)}(x) = D^{(j)} \frac{(\alpha^{(j)})^2 - (q^{(j)})^2}{q^{(j)}}$$
$$\cdot \exp \{\alpha^{(j)}(x - x_{j-1})\} \sinh q^{(j)}(x - x_{j-1}) \cdot \bar{C}^{(j)}(x_{j-1})$$
$$- \frac{1}{q^{(j)}} \cdot \exp \{\alpha^{(j)}(x - x_{j-1})\} [\alpha^{(j)} \sinh q^{(j)}(x - x_{j-1})$$
$$- q^{(j)} \cosh q^{(j)}(x - x_{j-1})] \bar{J}^{(j)}(x_{j-1}). \qquad (10)$$

The relations sought for between the "input values" $\bar{C}^{(j)}(x_{j-1})$ and $\bar{J}^{(j)}(x_{j-1})$ to the layer at $x=x_{j-1}$ and the "output values" $\bar{C}^{(j)}(x_j)$ *and* $\bar{J}^{(j)}(x_j)$ from the layer at $x=x_j$ are obtained by putting $x=x_j$ in Eqs. (9) and (10). This gives

$$\bar{C}^{(j)}(x_j)=\exp\{\alpha^{(j)}\Delta^{(j)}\}\left[\cosh\beta^{(j)}+\frac{\alpha^{(j)}}{q^{(j)}}\sinh\beta^{(j)}\right]\cdot\bar{C}^{(j)}(x_{j-1})$$
$$-\frac{\exp\{\alpha^{(j)}\Delta^{(j)}\}\sinh\beta^{(j)}}{D^{(j)}q^{(j)}}\bar{J}^{(j)}(x_{j-1}) \qquad (11)$$

and

$$\bar{J}^{(j)}(x_j)=D^{(j)}\frac{(\alpha^{(j)})^2-(q^{(j)})^2}{q^{(j)}}\cdot\exp\{\alpha^{(j)}\Delta^{(j)}\}\sinh\beta^{(j)}\cdot\bar{C}^{(j)}(x_{j-1})$$
$$-\frac{1}{q^{(j)}}\cdot\exp\{\alpha^{(j)}\Delta^{(j)}\}[\alpha^{(j)}\sinh\beta^{(j)}-q^{(j)}\cosh\beta^{(j)}]\bar{J}^{(j)}(x_{j-1}) \qquad (12)$$

where

$$\Delta^{(j)}=x_j-x_{j-1} \qquad (13)$$

is the thickness of the layer $x_{j-1}\leqq x\leqq x_j$ and the transform parameter is now contained in

$$\beta^{(j)}=q^{(j)}\Delta^{(j)}. \qquad (14)$$

It appears from Eqs. (11) and (12) that both "outputs" $\bar{C}^{(j)}(x_j)$ and $\bar{J}^{(j)}(x_j)$ from the layer inside the region $x_{j-1}\leqq x\leqq x_j$ are linear functions of the "inputs" $\bar{C}^{(j)}(x_{j-1})$ and $\bar{J}^{(j)}(x_{j-1})$ to the same layer, *viz.*

$$\bar{C}^{(j)}(x)=A^{(j)}\bar{C}^{(j)}(x_{j-1})+B^{(j)}\bar{J}^{(j)}(x_{j-1}) \qquad (15)$$

and

$$\bar{J}^{(j)}(x)=E^{(j)}\bar{C}^{(j)}(x_{j-1})+F^{(j)}\bar{J}^{(j)}(x_{j-1}) \qquad (16)$$

where

$$\left.\begin{array}{l} A^{(j)}=\exp\{\alpha^{(j)}\Delta^{(j)}\}\left[\cosh\beta^{(j)}+\frac{\alpha^{(j)}}{q^{(j)}}\sinh\beta^{(j)}\right] \\[2mm] B^{(j)}=-\frac{\exp\{\alpha^{(j)}\Delta^{(j)}\}\sinh\beta^{(j)}}{D^{(j)}q^{(j)}} \\[2mm] E^{(j)}=D^{(j)}\frac{(\alpha^{(j)})^2-(q^{(j)})^2}{q^{(j)}}\exp\{\alpha^{(j)}\Delta^{(j)}\}\sinh\beta^{(j)} \\[2mm] F^{(j)}=-\frac{1}{q^{(j)}}\cdot\exp\{\alpha^{(j)}\Delta^{(j)}\}[\alpha^{(j)}\sinh\beta^{(j)}-q^{(j)}\cosh\beta^{(j)}]. \end{array}\right\} \qquad (17)$$

It will be convenient to write Eqs. (15) and (16) in their matrix form as

$$\left\{\begin{array}{l}\bar{C}^{(j)}(x_j)\\ \bar{J}^{(j)}(x_j)\end{array}\right\}=\begin{bmatrix}A^{(j)} & B^{(j)}\\ E^{(j)} & F^{(j)}\end{bmatrix}\cdot\left\{\begin{array}{l}\bar{C}^{(j)}(x_{j-1})\\ \bar{J}^{(j)}(x_{j-1})\end{array}\right\}. \qquad (18)$$

Thus, the layer in the region $x_{j-1}\leqq x\leqq x_j$ can be described by a characteristic square matrix

$$Q^{(j)}=\begin{bmatrix}A^{(j)} & B^{(j)}\\ E^{(j)} & F^{(j)}\end{bmatrix}, \qquad (19)$$

the transfer matrix, which connects the "input values" $\bar{C}^{(j)}(x_{j-1})$ and $\bar{J}^{(j)}(x_{j-1})$ at $x=x_{j-1}$ to the "output values" $\bar{C}^{(j)}(x_j)$ and $\bar{J}^{(j)}(x_{j-1})$ from the layer at $x=x_j$. But according to the fundamental continuity condition (III), the "input values" $\bar{C}^{(j)}(x_{j-1})$ and $\bar{J}^{(j)}(x_{j-1})$ to the layer $x_{j-1}\leqq x\leqq x_j$ at $x=x_{j-1}$ are also the "output values" $\bar{C}^{(j-1)}(x_{j-1})$ and $\bar{J}^{(j-1)}(x_{j-1})$ from the adjacent layer $x_{j-2}\leqq x\leqq x_{j-1}$ at $x=x_{j-1}$, i.e.,

$$\left\{\begin{array}{l}\bar{C}^{(j)}(x_{j-1})\\ \bar{J}^{(j)}(x_{j-1})\end{array}\right\}=\left\{\begin{array}{l}\bar{C}^{(j-1)}(x_{j-1})\\ \bar{J}^{(j-1)}(x_{j-1})\end{array}\right\}.$$

Thus, Eq. (18) can also be written as

$$\left\{\begin{array}{l}\bar{C}^{(j)}(x_j)\\ \bar{J}^{(j)}(x_j)\end{array}\right\}=Q^{(j)}\cdot\left\{\begin{array}{l}\bar{C}^{(j-1)}(x_{j-1})\\ \bar{J}^{(j-1)}(x_{j-1})\end{array}\right\}. \qquad (20)$$

If the argument leading to Eq. (18) had been applied to the region $x_{j-2}\leqq x\leqq x_{j-1}$, the following system of equations would have been obtained

$$\left\{\begin{array}{l}\bar{C}^{(j-1)}(x_{j-1})\\ \bar{J}^{(j-1)}(x_{j-1})\end{array}\right\}=Q^{(j-1)}\cdot\left\{\begin{array}{l}\bar{C}^{(j-1)}(x_{j-2})\\ \bar{J}^{(j-1)}(x_{j-2})\end{array}\right\}.$$

Inserting this into Eq. (20) above gives

$$\left\{\begin{array}{l}\bar{C}^{(j)}(x_j)\\ \bar{J}^{(j)}(x_j)\end{array}\right\}=(Q^{(j)}\cdot Q^{(j-1)})\cdot\left\{\begin{array}{l}C^{(j-1)}(x_{j-2})\\ J^{(j-1)}(x_{j-2})\end{array}\right\}. \qquad (21)$$

Thus, the "input values" $\bar{C}^{(j-1)}(x_{j-2})$ and $\bar{J}^{(j-1)}(x_{j-2})$ at $x=x_{j-2}$ are now connected to the "output values" $C^{(j)}(x_j)$ and $\bar{J}^{(j)}(x_j)$ at $x=x_j$ by a transfer matrix which is the product of the two transfer matrices which are associated to the layers between x_{j-2} and x_{j-1} and between x_{j-1} and x_j respectively. By extending this argument through the whole stratified system, i.e., across the region $0\leqq x\leqq x_n=h$, it follows that the "output values" $\bar{C}(h)$ and $\bar{J}(h)$ to the one bathing solution at $x=h$ corresponding to the "input values" $\bar{C}(0)$ and $\bar{J}(0)$ from the other solution at $x=0$ are described by the expression

$$\left\{\begin{array}{l}\bar{C}(h)\\ J(h)\end{array}\right\}=(Q^{(n)}\cdot Q^{(n-1)}\cdots\cdots Q^{(1)})\cdot\left\{\begin{array}{l}\bar{C}(0)\\ J(0)\end{array}\right\}. \qquad (22)$$

The product of two square matrices of the second order is again a square matrix of the second order. Consequently,

$$Q^{(n)}\cdot Q^{(n-1)}\cdots\cdots Q^{(1)}=\begin{bmatrix}A(s) & B(s)\\ E(s) & F(s)\end{bmatrix} \qquad (23)$$

where each of the elements $A(s)$, $B(s)$, $E(s)$ and $F(s)$ are complicated functions of the transform parameter s. Equation (22) can therefore be written in the more compact form

$$\left\{ \begin{matrix} \bar{C}(h) \\ \bar{J}(h) \end{matrix} \right\} = \begin{bmatrix} A(s) & B(s) \\ E(s) & F(s) \end{bmatrix} \cdot \left\{ \begin{matrix} \bar{C}(0) \\ \bar{J}(0) \end{matrix} \right\} \qquad (24)$$

which is equivalent to the following system of equations

$$\left. \begin{matrix} \bar{C}(h) = A(s)\,\bar{C}(0) + B(s)\,\bar{J}(0) \\ \bar{J}(h) = E(s)\,\bar{C}(0) + F(s)\,\bar{J}(0) \end{matrix} \right\} \qquad (25)$$

which shows that the Laplace transforms of the "output values" $\bar{C}(h)$ and $\bar{J}(h)$ from the stratified object at $x=h$ both are linear functions of the "input values" $\bar{C}(0)$ and $\bar{J}(0)$ to the experimental system at $x=0$. However, it would be equally natural to regard the values $\bar{C}(h)$ and $\bar{J}(h)$ as the input to the system and those of $\bar{C}(0)$ and $\bar{J}(0)$ as output. This relation can be obtained from Eq. (25) according to the rules of matrix algebra (see, e.g., Heading, 1958) as

$$\left\{ \begin{matrix} \bar{C}(0) \\ \bar{J}(0) \end{matrix} \right\} = \frac{1}{\det Q} \begin{bmatrix} F(s) & -B(s) \\ -E(s) & A(s) \end{bmatrix} \cdot \left\{ \begin{matrix} \bar{C}(h) \\ \bar{J}(h) \end{matrix} \right\} \qquad (26)$$

where the quantity $\det Q = A(s)F(s) - B(s)E(s)$ is the determinant of the matrix

$$Q = Q^{(n)} \cdot Q^{(n-1)} \cdot \ldots \cdot Q^{(1)}$$

corresponding to Eq. (23). Since the determinant of the product of two square matrices equals the product of the determinants of the two matrices (see, e.g., Heading, 1958) it follows that

$$\det Q = \det Q^{(n)} \cdot \det Q^{(n-1)} \cdots \det Q^{(j)} \cdots \det Q^{(1)}. \qquad (27)$$

It is shown in appendix B that

$$\det Q^{(j)} = \exp\{2\alpha^{(j)} \varDelta^{(j)}\} \qquad (28)$$

where $\alpha^{(j)}$ and $\varDelta^{(j)}$ are defined by Eqs. (7) and (13), respectively. In view of the complicated elements $A^{(j)}$, $B^{(j)}$, $E^{(j)}$ and $F^{(j)}$ which constitute the matrix of $Q^{(j)}$ [cf. Eq. (17)], the appearance of this simple result is in itself surprising. But the more important property of Eq. (28) is that none of the determinants of the transfer matrices $Q^{(j)}$ contain the Laplace transform parameter s. Insertion of Eq. (28) into Eq. (27) gives

$$\det Q = \exp\{2\sum \alpha^{(j)} \varDelta^{(j)}\}.$$

Accordingly, Eq. (26) can be written as

$$\left\{ \begin{matrix} \bar{C}(0) \\ \bar{J}(0) \end{matrix} \right\} = \exp\{-2\sum \alpha^{(j)} \varDelta^{(j)}\} \cdot \begin{bmatrix} F(s) & -B(s) \\ -E(s) & A(s) \end{bmatrix} \cdot \left\{ \begin{matrix} \bar{C}(h) \\ \bar{J}(h) \end{matrix} \right\}$$

which again is equivalent to the system of equations

$$\begin{matrix} \exp\{2\sum \alpha^{(j)} \varDelta^{(j)}\}\,\bar{C}(0) = F(s)\,\bar{C}(h) - B(s)\,\bar{J}(h) \\ \exp\{2\sum \alpha^{(j)} \varDelta^{(j)}\}\,\bar{J}(0) = -E(s)\,\bar{C}(h) + A(s)\,\bar{J}(h). \end{matrix} \qquad (29)$$

In addition to demonstrating the linear dependence between the input and output concentrations and fluxes, this system of equations taken together with the system of Eq. (25) provides a convenient means of calculating the time-dependent unidirectional fluxes through the stratified object.

2. Unidirectional Fluxes

The unidirectional flux was defined by Ussing (1948, 1978) as the flux of the substance in question when its concentration is alyways maintained at zero in one of the phases surrounding the membrane. In general, we have the option of measuring either the value of the input flux of the isotope to the experimental object or the output flux from it. When the flux is stationary and the substance is conserved during its transport across the membrane the input flux equals the output flux. In the nonsteady state, however, where the concentration profile is being built up towards the stationary value the input flux will exceed the output flux. Furthermore, if the values of the transport parameters vary across an asymmetric stratified object, the input fluxes at either membrane surface may have a different time dependence according to which of the two sides of the object is being loaded with the isotope during the unidirectional transport. In flux ratio considerations we are only interested in keeping an account of those particles which in one way or the other have permeated the whole system in question. Accordingly, as time-dependent unidirectional fluxes we shall consider the flux of the radioactive tracer ions at the plane where the tracer ions *leave* the biological object and enter the surrounding medium having virtually zero concentration for the tracer ion in question.

We begin by considering the unidirectional flux in the direction $(0) \rightarrow (h)$, i.e., the flux $\bar{J}(h)$ at the position $x=h$, when $\bar{C}(h)=0$. Using the upper equation of Eq. (29) and putting $\bar{C}(h)=0$ gives

$$\bar{J}(h) = -\exp\{2\sum \alpha^{(j)} \varDelta^{(j)}\} \frac{\bar{C}(0)}{B(s)}.$$

If the concentration $C(0)$ is kept constant we have

$\bar{C}(0) = C(0)/s$ since the Laplace transform of unity equals $1/s$, and the above expression can be written

$$\bar{J}(h) = -\exp\{2\sum \alpha^{(j)} \Delta^{(j)}\}\frac{1}{sB(s)} \cdot C(0). \qquad (30)$$

But $C(0)$ is a constant and $\sum \alpha^{(j)} \Delta^{(j)}$ does not contain the Laplace transform parameter s. Thus, the time-dependent part $G(t)$ of the unidirectional flux $J(h,t)$ is contained in the complex inversion integral

$$G(t) = \frac{1}{2\pi i}\int_{\gamma-i\infty}^{\gamma+i\infty}\frac{\exp\{tz\}}{zB(z)}dz$$

which in general can be transformed into a closed contour integral – the Bromwich integral – and evaluated making use of the calculus of residues (see, e.g., Sneddon, 1972). The time-dependent unidirectional flux $J^{(0h)}(t)$ in the direction $(0)\to(h)$ at the position $x=h$ can thus be written

$$J^{(0h)}(t) = -C(0)\exp\{2\sum \alpha^{(j)} \Delta^{(j)}\}G(t). \qquad (31)$$

$J^{(0h)}$ is directed along the positive direction of the x-axis and is therefore always positive. This implies that $G(t)$ must be a negative quantity, i.e., $-G(t) = |G(t)|$.

Next we consider the unidirectional flux in the opposite direction, $(h)\to(0)$, i.e., the flux $\bar{J}(0)$ at the position $x=0$ when $C(0)=0$. Invoking this condition in the upper equation of Eq. (25) gives

$$\bar{J}(0) = \frac{\bar{C}(h)}{B(s)} = \frac{C(h)}{sB(s)} \qquad (32)$$

if the concentration at $x=h$ has the constant value $C(h)$. Comparison between Eqs. (32) and (39) shows that, apart from the appearance of different contants, the two transforms are identical. Hence the inversion of Eq. (32) can be written as

$$J(0,t) = C(h)G(t)$$

from which it follows that the time-dependent unidirectional flux in the direction $(h)\to(0)$ is

$$J^{(h0)}(t) = C(h)|G(t)| \qquad (33)$$

bearing in mind that $G(t)$ is negative and that unidirectional fluxes always are considered as positive quantities. Division of Eq. (33) into Eq. (31) gives

$$\frac{J^{(0h)}(t)}{J^{(h0)}(t)} = \frac{C(0)}{C(h)}\exp\{2\sum \alpha^{(j)} \Delta^{(j)}\} \qquad (34)$$

since $G(t) = -|G(t)|$. Substituting Eq. (7) into the above expression, we obtain the following expression for the time dependent flux ratio equation

$$\frac{J^{(0h)}(t)}{J^{(h0)}(t)} = \frac{C(0)}{C(h)}\exp\left\{\sum \frac{v^{(j)}}{D^{(j)}}\Delta^{(j)}\right\}. \qquad (35)$$

The right-hand side does not contain the time t. Thus the ratio between the two time-dependent unidirectional fluxes (i.e., output fluxes) is time invariant even in a stratified medium where the transport parameter $v^{(j)}$ and $D^{(j)}$ may vary from one layer to the next.

The input component of the unidirectional fluxes are $\bar{J}(0)$ for $C(h)=0$ and $\bar{J}(h)$ for $C(0)=0$. Applying these boundary conditions to Eqs. (25) and (29) gives

$$\frac{\bar{J}(0)}{\bar{J}(h)} = -\frac{A(s)\,C(0)}{F(s)\,C(h)}$$

which shows that the ratio between the time-dependent *input fluxes* is *not time invariant* unless $A(s) = F(s)$, which in general is not the case.

3. Flux Ratio for Electrodiffusion and Convection

The exponential term in Eq. (35) bears little resemblance to those usually appearing in flux ratio equations. It will now be shown that Eq. (35) is identical with the expressions obtained by Ussing (1949, 1978), assuming stationary conditions.

In general the migration velocity $v^{(j)}$ is given by

$$v^{(j)} = B^{(j)}X^{(j)} \qquad (36)$$

where $B^{(j)}$ is the mechanical mobility of the single isotope molecule in question in the region $x_{j-1}\leqq x\leqq x_j$, and $X^{(j)}$ is the driving force acting on each particle. $X^{(j)}$ may vary from one layer to the next but is assumed to have a constant value inside each layer $x_{j-1}\leqq x\leqq x_j$. The mobility $B^{(j)}$ and the diffusion coefficient $D^{(j)}$ in each layer are interconnected by Einstein's relation

$$D^{(j)} = kTB^{(j)} \qquad (37)$$

where k is Boltzmann's constant and T is the absolute temperature. Thus the exponent in Eq. (35) can be written as

$$\sum \frac{v^{(j)}}{D^{(j)}}\Delta^{(j)} = \sum \frac{B^{(j)}X^{(j)}}{D^{(j)}}\Delta^{(j)} = \frac{1}{kT}\sum X^{(j)}\Delta^{(j)}.$$

But the sum of products $X^{(j)}\Delta^{(j)} = X^{(j)}(x_j - x_{j-1})$ represents the work, $W^{(0h)}$, which the driving force does on each isotope molecule in moving it through the stratified medium from the position $x=0$ to $x=h$. Hence Eq. (35) can be written as

$$\frac{J^{(0h)}(t)}{J^{(h0)}(t)} = \frac{C(0)}{C(h)}\exp\{W^{(0h)}/kT\} \qquad (38)$$

which from the physical point of view perhaps is more easily understood than Eq. (35).

If the isotope is an ion with charge number z and the electrical potential is $\psi(0)$ at $x=0$ and $\psi(h)$ at $x=h$, the work done against the electric field when moving the ion in question from $x=0$ to $x=h$ is

$$zq_e[\psi(h)-\psi(0)]$$

where q_e is the elementary electronic charge. Since the particle is assumed to move with constant velocity, this work is numerically equal to and of opposite sign to the work, $W^{(Oh)}$ done by the electric field on the ion. That is to say $W^{(Oh)}, = -zq_e[\psi(h)-\psi(0)]$. Inserting this into Eq. (38) gives

$$\frac{J^{(Oh)}(t)}{J^{(hO)}(t)}=\frac{C(0)}{C(h)}\exp\{zq_e[\psi(0)-\psi(h)]/kT\} \qquad (39)$$

or, since Faraday's number $F=N_A q_e$ and the gas constant $R=N_A k$, where N_A is Avogadro's number,

$$\frac{J^{(Oh)}(t)}{J^{(hO)}(t)}=\frac{C(0)}{C(h)}\exp\{zF[\psi(0)-\psi(h)]/RT\}. \qquad (40)$$

The right-hand side is identical to that obtained in the original treatment by Ussing (1949) where the system was considered to be in the stationary state only.

The velocity $v^{(j)}$ in Eq. (35) could also result from a convection process where the solvent and the dissolved particles move with the same speed through the stratified medium of thickness h. Alternatively, the velocity $v^{(j)}$ could be ascribed to an active transport being present in the jth layer. If both the convection velocity and the diffusion coefficient for the isotope in question assume the constant values v and D everywhere in the region $0 \leq x \leq xh$, Eq. (35) takes the particular simple form

$$\frac{J^{(Oh)}(t)}{J^{(hO)}(t)}=\frac{C(0)}{C(h)}\exp\{hv/D\}. \qquad (41)$$

The right-hand side is identical to the expression obtained by Sten-Knudsen (1978), assuming that stationary conditions hold.

If the convection velocity is constant but the diffusion coefficients $D^{(j)}$ vary from layer to layer, we have

$$\frac{J^{(Oh)}(t)}{J^{(hO)}(t)}=\frac{C(0)}{C(h)}\exp\left\{\frac{v}{\langle P\rangle}\right\} \qquad (42)$$

where $\langle P \rangle$ is the average permeability defined by

$$1/\langle P\rangle=\sum 1/P^{(j)}=\sum 1/(D^{(j)}/\Delta^{(j)})$$

(cf., e.g., Sten-Knudsen, 1978).

If $v^{(j)}$ varies from layer to layer the condition of mass conservation must still prevail. This can be written as

$$v^{(j)}S^{(j)}=\Phi$$

where Φ is the volume flow through the system, and $S^{(j)}$ the area available for flow in the jth layer. If the diffusion coefficients $D^{(j)}$ all have the same value, D, Eq. (35) assumes the form

$$\frac{J^{(Oh)}(t)}{J^{(hO)}(t)}=\frac{C(0)}{C(h)}\exp\left\{\frac{\Phi}{D}\sum\frac{\Delta^{(j)}}{S^{(j)}}\right\}.$$

If the process of summation passes into one of integration we have

$$\frac{J^{(Oh)}(t)}{J^{(hO)}(t)}=\frac{C(0)}{C(h)}\exp\left\{\frac{\Phi}{D}\int_0^h\frac{dx}{S(x)}\right\} \qquad (43)$$

where the right-hand side is the expression obtained by Ussing (1952, 1978) and Koefoed-Johnsen and Ussing (1953), assuming stationary conditions to hold.

Discussion

1. The Time Invariance of the Flux Ratio

The main result of the preceding analysis is the demonstration of the time invariance of the flux ratio equation. This result holds irrespective of whether the transport process considered takes place through a single barrier of homogeneous composition or through a multilayered barrier consisting of sandwiched layers of homogeneous barriers in which the transport parameters for the particles in question vary from one layer to the next. This implies that the order in which the individual barriers are connected in cascade is immaterial for the time dependence of the flux of those particles which have passed the whole system. The transport processes considered were those which could be described within the framework of the Smoluchowski equation.

$$\frac{\partial C}{\partial t}=D\frac{\partial^2 C}{\partial x^2}-v\frac{\partial C}{\partial x}$$

which describes the motion of a collection of particles whose random movements are being dislocated unidirectionally by some mechanism which imparts to each particle a constant drift velocity v. Since the values of both the diffusion coefficient D and the drift velocity v inside each layer are assumed to be constant, the Smoluchowski equation is a *linear* partial differential equation of the second

order. It is this property of linearity and not the particular from of the Smoluchowski equation which results in the linear interrelationship between the "input values" of concentration, $\bar{C}^{(j)}(x_{j-1})$, and flux, $\bar{J}^{(j)}(x_{j-1})$, and the "output values" $\bar{C}^{(j)}(x_j)$ and $\bar{J}^{(j)}(x_j)$, viz.

$$\begin{Bmatrix} \bar{C}^{(j)}(x_j) \\ \bar{J}^{(j)}(x_j) \end{Bmatrix} = \begin{bmatrix} A^{(j)}(s) & B^{(j)}(s) \\ E^{(j)}(s) & F^{(j)}(s) \end{bmatrix} \cdot \begin{Bmatrix} \bar{C}^{(j)}(x_{j-1}) \\ \bar{J}^{(j)}(x_{j-1}) \end{Bmatrix}.$$

This result is analogous to that which is obtained in an electrical four-terminal network where the potential and current at the input side are linear functions of the potential and currents at the output side. In the four-terminal network the value of the determinant of the transfer matrix is unity. This can be shown to be a consequence of the reciprocity relation which holds for any linear electrical network and which follows from the more general reciprocal theorem for conductors (Green, 1828). A reciprocity relation similar to that of the four-terminal network will hold in a homogeneous slab for any transport process provided only linear operators in space and time are involved. As a result, the determinant of the transfer matrix in the above equation will always turn out to be a pure number, not containing the transform parameter s, although its value in general will differ from unity (cf. Eq. (27), except when the transport of the particles results solely from a diffusion process. In the case of a cascade connection of several homogeneous slabs, the over-all transfer matrix of the whole system is the matrix product of the matrices of the individual slabs taken in the order of connection. Since the determinant of the product of any number of square matrices is equal to the product of the determinants of those matrices, it follows that the determinant of the over-all matrix also is a pure number. This property, which also is a consequence of linearity prevailing throughout the system, results in two unidirectional fluxes, both having the same time dependence although they may not approach the same final stationary value.

It was essential for our demonstration of the time invariance of the flux ratio equation that the following parameters were constant during the time considered: (i) The concentrations $C(0)$ and $C(h)$ of the substance in question in the two surrounding solutions, and (ii) the diffusion constant D and the drift velocity v for the particles in each of the cascaded layers. The first condition can be readily assumed but the second cannot. For example, a deterioration of the preparation would gradually cause a change in the resistance and potential profiles through the preparation and thus in the value of the flux ratio. But even with a stable nondeteriorating

preparation the values of D and v may change during the experiment because they both may depend upon the concentration profile. In that case the condition for establishing the flux ratio equation would require that both the concentration profile for all the permeating substances and the potential profile remained stationary throughout the preparation during the entire duration of the experiment. In general, this requirement can only be fulfilled by measuring isotope fluxes alone provided the isotope is used in such a small concentration that the progress of the tracer concentration profile through the preparation towards its stationary state does not perturb the existing stationary concentration profile for the corresponding nonradioactive substance.

The procedure of considering a large number of slabs of variable thickness $\Delta^{(j)}$ arranged in cascade led to an alternative formula for the flux ratio equation, where the weight factor to the concentration ratio $C(0)/C(h)$, which at the first glance might appear somewhat strange, is an exponential of the following form

$$\exp \{\Sigma v^{(j)} \Delta^{(j)}/D^{(j)}\}.$$

Nevertheless, this form of the exponential seems to be the more general. This is so because a great number of transport processes in biology can be described within the framework of the Smoluchowski equation by assigning a partial drift velocity, v_p, which is characteristic for each particular process. Thus, if the drift velocity is caused by an electric field acting on ionic tracers, the above expression was easily transformed into Eq. (40) which is the well known expression of Ussing (1949), which allows for a precise numerical evaluation of the flux ratio under a process of pure electrodiffusion. Similarly, if a convective flow causes a solvent drag on the particles, Eq. (35) will lead to the various expressions for the flux ratio which depending upon the geometry of the flow, have been derived assuming steady-state conditions to hold (Ussing, 1952; Koefoed-Johnsen & Ussing, 1953; Meares & Ussing, 1959 a, b; Hoshiko & Lindley, 1964; Kedem & Essig, 1965; Ussing, 1978). Analogously, if the particles are subject to exchange diffusion, to co-transport with another molecular species, or even to an active transport, a component to the drift velocity can be assigned to each type of transport. Thus, the exponential in the flux ratio equation appropriate to these processes will again be given by Eq. (35), although it may be impossible in these cases to evaluate with precision the numerical value of the exponential. Nevertheless, even in these cases the two oppositely directed unidirectional tracer fluxes have the same time de-

pendence, i.e., the steady-state value of the flux ratio can be estimated from the nonsteady-state value.

2. Experimental Application of the Principle of Constant Flux Ratio

From the foregoing it is clear that deviations from the rule of time-invariant flux ratio can occur only if one or more of our original assumptions are not fulfilled. We shall briefly discuss two such cases which are directly applicable to biological studies.

In the first place, the substance in question may pass the experimental object by more than one pathway. Granted that the pathways are characterized by different flux ratios as well as different mean passage times, the flux ratio observed for the whole object may change with time. To illustrate this point, let us assume that sodium can pass an epithelium by a slow cellular route where it undergoes active transport and a fast intercellular shunt path where the transport is strictly passive. Under these conditions it may well be that immediately after addition of the tracers to the bathing solution the flux ratio will be close to that of passive transport, whereas that of active transport will dominate later on. In fact, a recent study has shown this to be the case. Under the assumption that there are only two significant pathways with different flux ratios and different mean passage times, it is possible to resolve the experimental data into two sets of fluxes, each belonging to one of the two pathways (Ussing, Eskesen & Lim, 1981).

The second application of the principle of constant flux ratios is concerned with obtaining reliable flux ratios in experiments where the steady flux ratio cannot be obtained either because of a limited viability for the biological specimen or because the experimental conditions themselves lead to changes in the flux ratio before the stationary state has been reached. If only one pathway is involved one can usually obtain the correct flux ratio by extrapolating the sequentially measured flux ratios back to zero time. The rationale for this procedure is that a correct measurement of the flux ratio only requires that the properties of the specimen have not changed measurably within the period required for the tracer experiment. Now, the time it takes for the first appearance of the tracer passing the specimen can be reduced almost at will by an increase in the specific activity. Thus, formally at least, one can reduce the duration of the experiment until the specimen has not changed measurably during the passage of the tracer. The extrapolated zero-time flux ratio is thus the best possible estimate of the steady-state value. For multiple pathways each one must be considered

separately, but for more than two pathways the method is hardly warranted.

Finally, a word about experimental procedure: Ideally one should use two tracers for the same substance added simultaneously on the two sides. Alternatively, one can measure two fluxes sequentially on the same specimen or in parallel on two specimens. In the latter case it is mandatory that all measurable physical properties should be as similar as possible during the corresponding measurement of forward and backward flux.

Appendix A

The Evaluation of the Transforms $\bar{C}(x,s)$ and $\bar{J}(x,s)$ Suitable for the Interval $x_{j-1} \leqq x \leqq x_j$, when Both Transforms Must Conform to Boundary Conditions for $x = x_{j-1}$

We begin by adjusting the parameter ξ in the function

$$\bar{C} = e^{\xi x}$$

so that the function satisfies the Laplace transform of the Smoluchowski equation

$$\frac{d^2 \bar{C}}{dx^2} - 2\alpha^{(j)} \frac{d\bar{C}}{dx} - (S/D^{(j)}) \bar{C} = 0 \tag{A1}$$

where $2\alpha^{(j)} = v^{(j)}/D^{(j)}$. Inserting $\bar{C} = \exp\{\xi x\}$ into the above equation gives the following quadratic in ξ

$$\xi^2 - 2\alpha^{(j)} \xi - s/D^{(j)} = 0$$

the roots of which are

$$\left.\begin{matrix} \xi_1 \\ \xi_2 \end{matrix}\right\} = \begin{cases} \alpha^{(j)} + \{(\alpha^{(j)})^2 + s/D^{(j)}\}^{\frac{1}{2}} = \alpha^{(j)} + q^{(j)} \\ \alpha^{(j)} - \{(\alpha^{(j)})^2 + s/D^{(j)}\}^{\frac{1}{2}} = \alpha^{(j)} - q^{(j)} \end{cases}$$

A solution of Eq. (A1) will therefore be of the form

$$\bar{C}(x,s) = A \exp\{\alpha^{(j)} x\} \exp\{q^{(j)} x\} + B \exp\{\alpha^{(j)} x\} \exp\{-q^{(j)} x\}$$

where A and B are arbitrary constants. By putting $A/2 = B/2$ and $A/2 = -B/2$, respectively, we obtain

$$\bar{C} = A \exp\{\alpha^{(j)} x\} \cosh q^{(j)} x + B \exp\{\alpha^{(j)}\} \sinh q^{(j)} x \tag{A2}$$

which is another general form of the solution. Since $\sinh 0 = 0$ and $\cosh 0 = 1$ and, furthermore, $\bar{C}$ in the above equation must assume the value $\bar{C}(x_{j-1})$ for $x = x_{j-1}$, an appropriate form of Eq. (A2) to serve this end is

$$\bar{C}(x) = \bar{C}(x_{j-1}) \exp\{\alpha^{(j)}(x - x_{j-1})\} \cosh q^{(j)}(x - x_{j-1})$$
$$+ B^{(j)} \exp\{\alpha^{(j)}(x - x_{j-1})\} \sinh q^{(j)}(x - x_{j-1}) \tag{A3}$$

which is Eq. (6) in the text.

Next, the constant $B^{(j)}$ is determined by using the second boundary condition to be satisfied, *viz.* that $\bar{J}$ must assume the value $\bar{J}(x_{j-1})$ for $x = x_{j-1}$. We invoke the transform for the flux

$$\bar{J} = -D^{(j)}\frac{d\bar{C}}{dx} + v^{(j)}\bar{C}$$

and insert Eq. (A3). This gives the expression

$$\begin{aligned}\bar{J}(x) = D^{(j)}&\exp\{\alpha^{(j)}(x - x_{j-1})\}\\ &\cdot[\alpha^{(j)}\cosh q^{(j)}(x - x_{j-1}) + q^{(j)}\sinh q^{(j)}(x - x_{j-1})]\,\bar{C}(x_{j-1})\\ &+ D^{(j)}\exp\{\alpha^{(j)}(x - x_{j-1})\}\\ &\cdot[\alpha^{(j)}\sinh q^{(j)}(x - x_{j-1}) - q^{(j)}\cosh q^{(j)}(x - x_{j-1})]\,B^{(j)}\end{aligned}$$

(A4)

from which the constant $B^{(j)}$ is determined by invoking the condition $\bar{J} = \bar{J}(x_{j-1})$ for $x = x_{j-1}$. The result is

$$B^{(j)} = \frac{\alpha^{(j)}}{q^{(j)}}\bar{C}(x_{j-1}) - \frac{1}{D^{(j)}q^{(j)}}\bar{J}(x_{(j-1)})$$

and inserting this value into Eqs. (A3) and (A4) gives, respectively, Eqs. (9) and (10) in the text.

Appendix B

Evaluation of the Determinant of the Transfer Matrix Q

We have from Eq. (19)

$$\det Q^{(j)} = A^{(j)}F^{(j)} - B^{(j)}E^{(j)}.$$

In this expression we insert the values given by Eq. (17), dropping the superscripts in $\alpha^{(j)}$, $\varDelta^{(j)}$, $q^{(j)}$, $\beta^{(j)}$, thus

$$\begin{aligned}\det Q^{(j)} &= e^{\alpha\varDelta}\left[\cosh\beta + \frac{\alpha}{q}\sinh\beta\right]\cdot(-1)\cdot e^{\alpha\varDelta}\left[\frac{\alpha}{q}\sinh\beta - \cosh\beta\right]\\ &\quad - D\,[\alpha^2/q - q]\,e^{\alpha\varDelta}\cdot\sinh\beta\cdot(-1)\,e^{\alpha\varDelta}\cdot\sinh\beta/Dq\\ &= -e^{2\alpha\varDelta}(\alpha/q)^2\sinh^2\beta + e^{2\alpha\varDelta}\cosh^2\beta\\ &\quad + e^{2\alpha\varDelta}(\alpha/q)^2\sinh^2\beta - e^{2\alpha\varDelta}\sinh^2\beta\\ &= e^{2\alpha\varDelta}(\cosh^2\beta - \sinh^2\beta) = \exp\{2\alpha^{(j)}\varDelta^{(j)}\}.\end{aligned}$$

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