THE TIME DEPENDENCE OF SINGLE FILE DIFFUSION

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ABSTRACT The single file diffusion of particles through a narrow pore membrane separating two media is treated as a stochastic birth and death process. A set of differential-difference equations is derived to describe the probability of finding n particles in the pore at any time whose source is the left-hand medium. Explicit time-dependent solutions for an arbitrary number of sites are obtained. These can be used to calculate both one-way and net flux as a function of time. Parameters are estimated from steady state permeability data, and the results of some numerical calculations are presented to illustrate the time required to approach a steady state. In many cases, significant time delays can occur.

INTRODUCTION

The single file passage of molecules through narrow pores was first suggested by Hodgkin and Keynes (1955) to explain the anomalous K^+ flux ratios measured in poisoned axons. According to this hypothesis, solute molecules occupy positions in isolated rows which extend throughout the membrane (Fig. 1). Each time a molecule enters a row from the left (from solution L), each molecule in the row moves to the right, the last member on the extreme right being discharged into the solution R; similar results hold for discharges into solution L as a result of entries from the solution R. Molecules from solution L can only enter solution R if the row is completely filled with L-molecules, and vice versa.

In many cases, it is likely that slippage of one molecule past another occasionally occurs. However, the rigid coupling assumed in the above model is attractive because it is mathematically tractable with minimal specification of membrane parameters. The magnitudes of energy barriers, for example, at each point in the membrane need not be specified because their contributions to the transport process is lumped entirely within a single parameter, the frequency of "successful" collisions. The phenomena of simple free diffusion and single file diffusion occupy opposite extremes of a spectrum where coupling ranges from no interaction (complete slippage) to maximal interaction with no slippage.

Following the suggestion of Hodgkin and Keynes, similar mechanisms have been

proposed to account for a variety of phenomena in different tissues. For example, Diamond (1962) has suggested that single file transport of water molecules occurs through the isolated gall bladder with approximately 50 sites/row. Hope and Walker (1960) suggest that single file diffusion with 10 sites/row may account for the discrepancy between the calculated and observed electrical resistance of the tonoplast membrane in *Nitella*. Passow (1964) listed single file diffusion as a possible explanation for the unusually high activation energies involved in the transport of SO_4 and PO_4 in red blood cells. Dantzig (1965) and Heckmann (1965 *b*) have pointed out that uphill transport may result if more than one molecular species is allowed to interact with the same channel, and Kuhn and Ramel (1959) have suggested that single file transport may play a role in the excitation process.

Although a number of detailed studies of steady state kinetics for single file transport have been published [e.g. Dantzig (1965), Lea (1963), Heckmann (1965 *a*, *b*), Hladky (1965)], little or no attention has been paid to the time dependence of these phenomena. A comparison of the kinetics of single file transport with the kinetics of simple diffusion is particularly interesting. In the case of simple diffusion of nonelectrolytes through a membrane of finite thickness, the steady state net flux is proportional to concentration difference across the membrane. Under non–steady state conditions, the concentration profile within the membrane has a time dependence which is given by a sum of decaying exponentials whose most prominent term is generally of the order of exp $(\pi^2 Dt/\delta^2)$, where D is the diffusion coefficient and δ is the width of the membrane. For biological membranes, estimates of D are unavailable. Nevertheless, because of the small value of δ (approximately 100 A), the sum of exponentials is usually considered to be negligible. Consequently, most problems which involve changing extra- or intracellular concentrations are treated by assuming that the membrane is in a steady state at all times. In the case of single file passage, the steady state net flux is also proportional to the concentration difference and is thus indistinguishable from simple diffusion. However, to date, there has been no corresponding justification for dismissing membrane transients under non–steady state conditions. The purpose of this paper is to give the complete time-dependent solution for single file transport with an arbitrary number of membrane sites. In many cases, the theory predicts significant time delays in reaching the steady state.

DERIVATION OF EQUATIONS

Fig. 1 illustrates a single file passage containing a total of N sites. We define the state of the row as the number of sites occupied by molecules which have originated from the left-hand side. In general, there are a total of $N + 1$ possible states ranging from 0 to N and numbered from left to right. Hence the state corresponds to the number of L-molecules in the row. If the row is in state n, then it can advance to state $n + 1$ by an "effective" collision from the left. On the other hand, an "effective" collision from the right will move the row from state n to state $n - 1$.

Let $P_n(t)$ represent the probability that the row is in state n at the time t. In the

time interval between t and $t + dt$, let $\lambda\, dt$ equal the probability of an effective collision from the left and $\mu\, dt$ equal the probability of an effective collision from the right. If the time interval dt is so small that a maximum of one effective collision can occur within it, then the probability that the passage is in state n at time $t + dt$ can be written as the following sum of three mutually exclusive probabilities,

$$P_n(t + dt) = P_{n-1}(t)\lambda\, dt + P_n(t)[1 - (\lambda + \mu)\, dt] + P_{n+1}(t)\mu\, dt, \qquad (1)$$

where it is assumed that $n \neq 0$ and $n \neq N$. The first term in expression (1) represents the probability that the row is in state $n - 1$ at time t and within the interval dt an effective collision occurs from the left. The second term covers the case where the row is in state n at time t and no effective collisions occur within dt. The last terms represent the probability that the row is in state $n + 1$ and an effective collision occurs from the right within dt. Dividing equation (1) by dt and passing to the

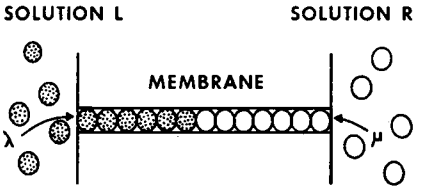

FIGURE 1 A single file row with $N = 13$ sites in state $n = 6$.

limit (1) can be written as

$$\frac{dP_n(t)}{dt} = \lambda P_{n-1}(t) - (\lambda + \mu)P_n(t) + \mu P_{n+1}(t). \qquad (2)$$

When $n = 0$, $P_{n-1} = 0$, and the contribution of μ to the second term on the right-hand side of equation (1) drops out because the row will remain in state 0 even after a collision from the right. Accordingly, we have the boundary equation

$$\frac{dP_0(t)}{dt} = -\lambda P_0(t) + \mu P_1(t). \qquad (3)$$

Similarly, when $n = N$, $P_{n+1} = 0$ and the contribution of λ to the second term of the right-hand side of equation (1) vanishes, so that

$$\frac{dP_N(t)}{dt} = \lambda P_{N-1}(t) - \mu P_N(t). \qquad (4)$$

Equations (2)–(4) represent a simple birth and death process (Feller, 1950). In more compact matrix notation, equations (2)–(4) can be written

$$\frac{d\bar{p}(t)}{dt} = A\bar{p}(t), \qquad (5)$$

where $\bar{p}(t)$ represents the column vector $[P_0(t), P_1(t) \cdots P_n(t) \cdots P_N(t)]$, and A represents a coefficient matrix whose only nonzero elements lie on the main diagonal and on two codiagonals—one immediately above, the other immediately below the main diagonal, i.e.

$$A = \begin{pmatrix} -\lambda & \mu & & & & & \\ \lambda & -(\lambda+\mu) & \mu & & & & \\ & \ddots & \ddots & \ddots & & & \\ & & \lambda & -(\lambda+\mu) & \mu & & \\ & & & \ddots & \ddots & \ddots & \\ & & & & \lambda & -(\lambda+\mu) & \mu \\ & & & & & \lambda & -\mu \end{pmatrix} \tag{6}$$

The solution to equation (5) as derived in the appendix can be written in terms of $\bar{p}(0)$, a column vector which specifies the initial values of $\bar{p}(t)$, and a square matrix Z (defined below) as follows:

$$\bar{p}(t) = Z\bar{p}(0). \tag{7}$$

Letting $\rho = \lambda/\mu$, the elements z_{jn} of the matrix Z are defined by the following expressions:

$$z_{jn} = \left[\frac{1-\rho}{1-\rho^{N+1}}\right]\rho^j + \frac{2\mu\rho^{(j+1-n)/2}}{N+1}\sum_{k=1}^{N}\frac{e^{\omega_k t}}{\omega_k} \cdot \left[\sin\frac{jk\pi}{N+1} - \rho^{1/2}\sin\frac{(j+1)k\pi}{N+1}\right]\left[\sin\frac{(n+1)k\pi}{N+1} - \rho^{-1/2}\sin\frac{nk\pi}{N+1}\right] \tag{8}$$

and

$$\omega_k = -(\lambda+\mu) + 2\sqrt{\lambda\mu}\cos\frac{k\pi}{N+1} \leqq 0. \tag{9}$$

Note that the magnitudes of the decay constants ω_k defined in equation (9) are restricted by the inequality

$$-(\lambda + \mu) \leqq \omega_k \leqq -(\lambda + \mu) + 2\sqrt{\lambda\mu} \tag{10}$$

so that all ω_k are contained within a range of $2\sqrt{\lambda\mu}$, which is independent of N.

Most applications involve the two unidirectional fluxes and the net flux through the membrane. Let φ_f denote the unidirectional forward flux defined here for each solute as the flow of solute through the *entire* membrane from left to right, and let φ_b denote the unidirectional back flux. Then

$$\varphi_f(t) = \lambda P_N(t), \qquad \varphi_b(t) = \mu P_0(t) \tag{11}$$

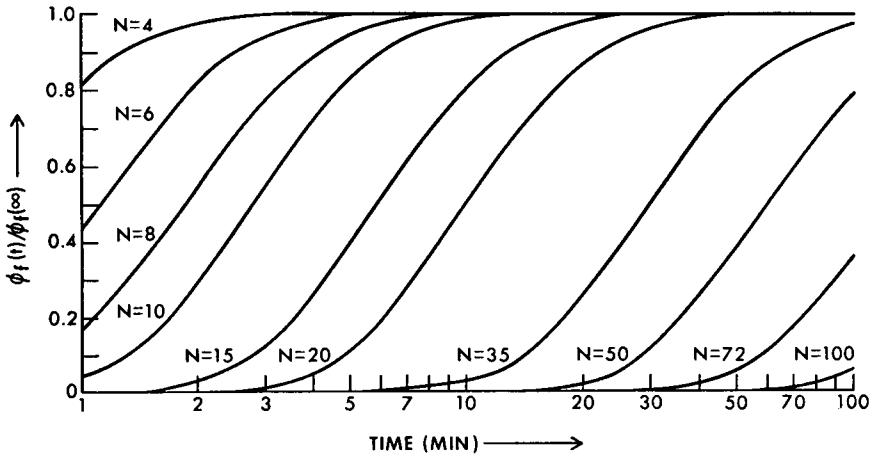

FIGURE 2 The time dependence of single file diffusion calculated for various values of N. In each case the initial condition is $P_0(0) = 1$ and the parameters are $\lambda = \mu = 6.0\ \text{min}^{-1}$.

and the net flux $\varphi = \varphi_f - \varphi_b$ is given by

$$\varphi(t) = \lambda P_N(t) - \mu P_0(t) = \sum_n (\lambda z_{Nn} - \mu z_{0n}) P_n(0). \tag{12}$$

Some notion of the size of λ may be obtained from consideration of the steady state, where equations (8) and (12) reduce to

$$\varphi(\infty) = \lambda - \mu. \tag{13}$$

To relate the order of magnitude of λ and μ to that of the conventional permeability constant, consider the simple case where λ and μ are proportional to the density of solute particles on the left- and right-hand sides, respectively, of the membrane; i.e. let

$$\lambda = \alpha\beta C_L, \qquad \mu = \alpha\beta C_R, \tag{14}$$

where C_L and C_R represent the concentrations (in moles per cubic centimeter) on the left- and right-hand sides, respectively, α is Avogadro's number, and β is a constant. Let γ equal the surface density of rows (number of rows per square centimeter) and J the next flux density (moles per square centimeter per second). Then, in a steady state,

$$J = \frac{\gamma}{\alpha}\varphi(\infty) = \frac{\gamma}{\alpha}(\lambda - \mu) = \gamma\beta(C_L - C_R). \tag{15}$$

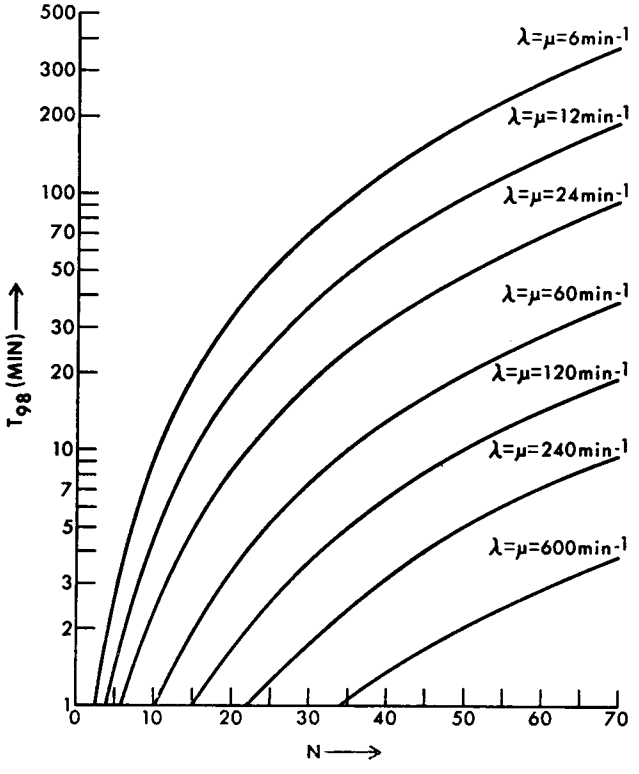

FIGURE 3 The time required for $\varphi_f(t)$ to reach 98%of its steady state value, plotted as a function of N. In each case $P_0(0) = 1$ and $\lambda = \mu$ as indicated in the figure.

If h represents the permeability constant, i.e. $J = h(C_L - C_R)$, it follows from equation (15) that $\beta = h/\gamma$ and, from equation (14),

$$\lambda = \frac{h\alpha}{\gamma} C_L, \qquad \mu = \frac{h\alpha}{\gamma} C_R. \tag{16}$$

The value of γ is not known with certainty for any cell. However, if we take the red blood cell as an example, the number of water channels has been estimated at 10^5 channels/cell (Solomon, 1960), so that γ might be of the order of 10^{11} rows/cm^2. The permeability of the red cell to Na is of the order of 10^{-10} cm/sec, and if we take a concentration of 100 mM, then λ will be of the order of 10^{-1} sec^{-1}, or 6.0 min^{-1}.

As an illustration of the time lag involved in reaching the steady state, consider the case with initial conditions $P_0(0) = 1$, $P_n(0) = 0$ for $n \neq 0$. This corresponds to the case where a tracer is suddenly introduced into solution L and the ensuing tracer flux is followed. Putting these values into equations (7) and (11), we have

$$\varphi_f(t) = \frac{\lambda\rho^N(1-\rho)}{1-\rho^{N+1}} + \frac{2\lambda\rho^{N/2}}{N+1}\sum_{k=1}^{N}\frac{(-1)^k \sin^2 k\pi/(N+1)}{\rho^{1/2}+\rho^{-1/2}-2\cos k\pi/(N+1)}\, e^{\omega_k t}. \quad (17)$$

In Fig. 2, values of $\varphi_f(t)/\varphi_f(\infty)$ are plotted against log t with $\lambda = \mu = 0.1$ sec^{-1} = 6.0 min^{-1} for various values of N. As N increases, the time lag becomes more significant. Similar results are illustrated in Fig. 3, where the log of T_{98}, the time required for $\varphi_f(t)$ to reach 98% of its steady state value, is plotted as a function of N for various values of λ. Taking $\lambda = 6.0$ min^{-1}, we see that a row with 5 sites requires almost 3 min before its flux reaches 98% of its steady state value; with 20 sites, this time is increased to 35 min, while for 50 sites it becomes 200 min. If the permeability is increased 100-fold so that $\lambda = \mu = 600$ min^{-1}, the time required to reach the steady state may still be of some significance. Under these conditions, and with $N = 4$, about 1.1 sec are required before the flux reaches 98% of its steady state value. With $N = 50$, T_{98} equals 126 sec.

All of the above numerical calculations are based on the assignment of the value 10^{11} rows/cm^2 to γ, which was derived from the estimate that there are approximately 10^5 water channels per cell. However, the calculation of the density of water channels was in turn based on the assumption that the diffusion coefficient for water has the same magnitude within the membrane as in free solution. If the channels are narrow, then interactions between the membrane and the water may alter this parameter and create a higher resistance to movement. In this case, γ will be larger, and from equation (16) λ and μ will be smaller. The net result will be to increase the duration of the transients beyond the numerical values estimated above.

APPENDIX

The solution of equation (5) can be written in terms of the eigenvalues and eigenvectors associated with the matrix A. Let the eigenvalues of A be given by $\omega_k (k = 0, 1, \cdots N)$ and let U represent the modal matrix whose kth column $\bar{u}_k = (u_{0k}, \cdots, u_{jk}, \cdots, u_{Nk})$ represents the kth eigenvector associated with ω_k. Then the formal solution to equation (5) is given by (Goertzel and Tralli, 1960)

$$\bar{p}(t) = [Ue^{\Omega t}U^{-1}]\bar{p}(0), \quad (18)$$

where $e^{\Omega t}$ is a pure diagonal matrix with elements $\exp(\omega_k t)$, U^{-1} is the inverse matrix of U, and $\bar{p}(0)$ is a column vector giving the initial values of $\bar{p}(t)$.

To find the eigenvalues ω_k and corresponding eigenvectors $\bar{u}_k$, we begin with the definition

$$A\bar{u}_k = \omega_k\bar{u}_k, \quad (19)$$

which leads to the following difference equations:

$$-(\lambda + \omega_k)u_{0k} + \mu u_{1k} = 0, \quad (20)$$

$$\lambda u_{j-1,k} - (\lambda + \mu + \omega_k)u_{jk} + \mu u_{j+1,k} = 0, \tag{21}$$

$$\lambda u_{N-1,k} - (\mu + \omega_k)u_N = 0. \tag{22}$$

The solution to equation (21) is given in terms of $\rho = \lambda/\mu$ as (Goldberg, 1961)

$$u_{jk} = \rho^{j/2}(a_k \sin j\theta_k + b_k \cos j\theta_k), \tag{23}$$

where θ_k is defined by

$$\cos \theta_k = (\lambda + \mu + \omega_k)/2\sqrt{\lambda\mu} \tag{24}$$

and a_k and b_k are constants (independent of j). If equation (24) is used to eliminate ω_k in favor of $\cos \theta_k$, the value of b_k obtained by substituting equation (23) into equation (20) becomes

$$b_k = -\frac{a_k \rho^{1/2} \sin \theta_k}{1 - \rho^{1/2} \cos \theta_k} \tag{25}$$

and substituting equation (25) into equation (23) leaves

$$u_{jk} = \frac{a_k}{1 - \rho^{1/2} \cos \theta_k} [\rho^{j/2} \sin j\theta_k - \rho^{(j+1)/2} \sin (j + 1)\theta_k]. \tag{26}$$

Permissible values of θ_k and hence ω_k can be obtained by substituting equation (26) into the boundary condition (22). After some manipulation we arrive at

$$[\rho^{N/2} \sin (N + 1)\theta_k](\rho^{-1/2} - 2 \cos \theta_k + \rho^{1/2}) = 0. \tag{27}$$

The solutions to equation (27) are given by

$$\theta_k = \frac{k\pi}{N + 1} \qquad 0 \leqq k \leqq N \tag{28}$$

and

$$\cos \theta_k = \tfrac{1}{2}(\rho^{-1/2} + \rho^{1/2}). \tag{29}$$

Inserting the value $k = 0$ into equations (28) and (26) results in the trivial solution $u_{j0} = 0$ so that equations (28) and (24) yield only N eigenvalues, given by

$$\omega_k = -(\lambda + \mu) + 2\sqrt{\lambda\mu} \cos \frac{k\pi}{N + 1}, \qquad 1 \leqq k \leqq N \tag{30}$$

The other eigenvalue, ω_0, determined by equations (29) and (24), corresponds to the steady state solution and is simply

$$\omega_0 = 0. \tag{31}$$

The jth component of the kth eigenvector ($1 \leqq k \leqq N$) corresponding to the eigenvalues specified by equation (30) are given by equation (26). The eigenvector corresponding to ω_0, obtained by setting $\omega_0 = 0$ and solving equations (20)–(22), is given by

$$u_{j0} = a_0 \rho^j. \tag{32}$$

Equations (26), (28), and (32) specify the modal matrix U. If the eigenvalues ω_k are all distinct, the rows of the inverse matrix U^{-1} can be constructed from the eigenvectors of A^T, the transpose of A. This follows because the set of eigenvectors of A is orthogonal to the set of eigenvectors of A^T and can be made orthonormal by simple adjustment of the arbitrary constants a_k (Lanczos, 1956). It follows that if V represents the modal matrix whose columns are eigenvectors of A^T (orthonormal to the eigenvectors of A), then V is the inverse transpose of U, i.e.

$$V^T = U^{-1}. \tag{33}$$

The eigenvalues of A^T are identical with the eigenvalues of A given by equations (30) and (31). The eigenvectors $\bar{v}_k = (v_{0k}, v_{ik}, \cdots \bar{v}_{Nk})$ of A^T are found in the same manner as $\bar{u}_k$. Instead of equations (20)–(22), we now have

$$-(\lambda + \omega_k)v_{0k} + \lambda v_{1k} = 0, \tag{34}$$

$$\mu v_{j-1,k} - (\lambda + \mu + \omega_k)v_{jk} + \lambda v_{j+1,k} = 0, \tag{35}$$

$$\mu v_{N-1,k} - (\mu + \omega_k)v_{Nk} = 0. \tag{36}$$

The solutions to equation (34)–(36) determine the eigenvectors of A^T and are given by

$$v_{n0} = c_0 = \text{constant} \tag{37}$$

and

$$v_{nk} = c_k[\rho^{-n/2} \sin (n + 1)\theta_k - \rho^{-(n+1)/2} \sin n\theta_k], \quad 1 \leqq k \leqq N \tag{38}$$

where c_k are arbitrary constants.

The values of a_k in equations (26) and (32) and the values of c_k in equations (37) and (38) can be adjusted to normalize the eigenvectors $\bar{u}_k$ and $\bar{v}_k$ by requiring

$$\sum_{j=0}^{N} u_{jk}v_{jk} = 1. \tag{39}$$

Equation (39) will be satisfied with

$$c_k = 1, \qquad 0 \leqq k \leqq N \tag{40}$$

$$\left.\begin{aligned} a_0 &= \frac{1 - \rho}{1 - \rho^{N+1}} \\ a_k &= \frac{2\mu\rho^{1/2}(1 - \rho^{1/2} \cos \theta_k)}{(N + 1)\omega_k}, \end{aligned}\right\} \qquad 1 \leqq k \leqq N \tag{41}$$

Returning to our solution (18), we see that the required matrix U is completely specified by equations (26), (28), (30), (32), and (41); the diagonal matrix $e^{\Omega t}$ is specified by equations (30) and (31); the inverse matrix U^{-1} is specified by the transpose of V, whose elements are given by equations (37), (38), (28), and (40). If we define the matrix Z by

$$Z = Ue^{\Omega t}U^{-1} = Ue^{\Omega t}V^T \tag{42}$$

then the elements z_{jn} are given by

$$z_{jn} = \sum_{k=0}^{N} u_{jk} e^{\omega_k t} v_{nk} = \left[\frac{1-\rho}{1-\rho^{N+1}}\right]\rho^j + \frac{2\mu\rho^{(j+1-n)/2}}{N+1}\sum_{k=1}^{N}\frac{e^{\omega_k t}}{\omega_k}$$
$$\left[\sin\frac{jk\pi}{N+1} - \rho^{1/2}\sin\frac{(j+1)k\pi}{N+1}\right]\left[\sin\frac{(n+1)k\pi}{N+1} - \rho^{-1/2}\sin\frac{nk\pi}{N+1}\right], \quad (43)$$

where

$$\omega_k = -(\lambda+\mu) + 2\sqrt{\lambda\mu}\cos\frac{k\pi}{N+1}.$$

From equations (18) and (42), the solution $\bar{p}(t)$ is

$$\bar{p}(t) = Z\bar{p}(0). \quad (44)$$

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