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Pace of Diffusion Through Membranes

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Summary. Although membranes are often viewed as barriers to diffusing particles, in many cases their presence does not slow down diffusion. Investigations of the transit time (mean diffusion time) for cases where the source and the target of diffusing particles are separated by various arrangements of membranes reveal the following facts: (i) The transit time is composed of the sum of the times to diffuse each of the membrane and aqueous regions separately and terms representing the time spent at the vicinity of the interfaces between these regions. (ii) In cases of one dimensional diffusion between aqueous and membranal phases, the transit time is governed by the parameter $\beta\sqrt{D_m/D_w}$ where D_m and D_w are the diffusion coefficients in the membrane and water, respectively, and β is the membrane/water partition coefficient of the particles. While the former ratio depends mostly on the viscosities of the two phases, the latter parameter is very strongly dependent on the identity of the particles. The diffusion from water to the membrane is faster than from the membrane to water whenever $\beta\sqrt{D_m/D_w}>1$. The opposite is true when this parameter is smaller than 1. (iii) In case of one dimensional transmembranal diffusion, the transit time shows a minimum when $\beta\sqrt{D_m/D_w}=\sqrt{l_{w1}/l_{w2}}$ where l_{w1} and l_{w2} are the net diffusion distances in the aqueous phases on both sides of the membrane. In this case, if the diffusion proceeds through pores in the membrane, β represents the fraction of membrane area that is occupied by the pores.

The transit times for three dimensional diffusion into and from a spherical cell are also presented in a simple form. In addition, some of the relations between transit times and other measurable time parameters, such as the course of the decay of gradients and the time lag to establish steady states, are discussed briefly.

The conclusions emerging from this analysis, together with the simple expressions for the transit times can make these investigation useful for the understanding of diffusion in systems containing natural or artificial membranes.

Occasionally particles must diffuse to and through membranes if they are to reach their assigned target. These diffusion processes may take place while organisms perform their vital functions as well as within man-made nonliving systems. In many cases, however, the diffusional drift is too slow to transport particles at the required speed and the

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facilitation of diffusion is needed. If we now want to be in a position to decide under what conditions does diffusion proceed fast enough, we must learn about the timing of diffusion in such systems.

We can intuitively perceive that properties of the particles as well as the arrangement of membranes would influence the pace of diffusion. For example, it is reasonable to expect that the relative affinity of the particles to the membrane and aqueous phases would strongly affect diffusion times. Also, the diffusion coefficients and the net diffusion distances in each of these phases would govern the pace of diffusion.

But let us first define what we refer to here as the diffusion time. We must recollect that diffusion proceeds by the random movements of particles in solution. Consequently, particles that leave the source together would not all reach the sink simultaneously. However, when we consider the timing of diffusion, it is sufficient in many cases to obtain the mean of these times of arrival ("first passage") to the sink. This mean time is denoted the *transit time*.

It should be mentioned here that, although the subject of diffusion through membranes has been investigated for well over a century, transit times for these processes had never been calculated. The main reason for this lies in the extreme mathematical complexity which one encounters when attempting to obtain transit times by the formal method available. This is because this formal method requires the knowledge of solutions to the time-dependent diffusion equation and these solutions are discouragingly difficult or occasionally even impossible to obtain for the cases of diffusion in heterogeneous systems.

In this paper, it is our purpose to introduce and investigate the properties of transit times in structures with membranes. Here the transit time is obtained by a simple straightforward approach. This approach is based on the fact that the transit time can be extracted directly from the behavior of the system at its steady state.

The results obtained in this paper are presented in a simple form. This, we believe, would help make them better understood and hence readily usable by researchers who follow the pace of diffusion in structures with membranes. The implications of the results presented here to the study of the strategies adopted by living organisms to minimize diffusion delays are discussed elsewhere (Hardt & Cone, 1979)[1].

If we now examine for a moment, the time parameters measured in typical diffusion experiments, we immediately realize that the transit time

[1] Hardt, S.L., Cone, R.A. 1979. Diffusion in small structures *(unpublished manuscript)*.

is not always the measured quantity. Occasionally, one observes experimentally other time parameters such as, for example, the time course of the decay of an initially prepared concentration gradient or the pace of approach of a system to its steady state. We must recollect, however, that all the transient concentration changes observed come about by the independent movements of single particles. Therefore, it is reasonable to expect that a close link exists between these times and the transit time. Fortunately, these links permit us to use the insight provided by our transit time analysis when examining other measured time parameters. We come back to this subject with some specific examples in the last section.

We begin our presentation by introducing a few key facts about the relations between transit times and net diffusion distances for the cases of diffusion in one and in three dimensions. We believe that the understanding of these basic relations is essential to the study of transit times in heterogeneous media.

Diffusion Adds Distances Not Times

As it was first observed by Einstein (1956), the mean diffusion time τ, for one dimensional diffusion does not bear a simple linear dependence on the diffusion distance l, but rather, it depends on the square of this distance, namely,

$$\tau = l^2/2D \tag{1}$$

where D is the diffusion coefficient.

One of the straightforward outcomes of this relation is that if we now divide in our mind the distance l into two subdistances l_1 and l_2 (as is shown schematically in Fig. 1*a*), the transit time remains unchanged and equal to

$$\tau = (l_1 + l_2)^2/2D = l_1^2/2D + l_2^2/2D + l_1 l_2/D. \tag{2}$$

The first two terms in Eq. (2) can be easily identified as the transit times for diffusion in separate regions of length l_1 and l_2. The third term, however, is of special interest. It can be regarded as reflecting the fact that even after the particles have crossed the border between the two regions for the first time, their random movements can still bring them back into the first region and away from their target. Therefore we can say that the third term in Eq. (2) represents the contribution to the

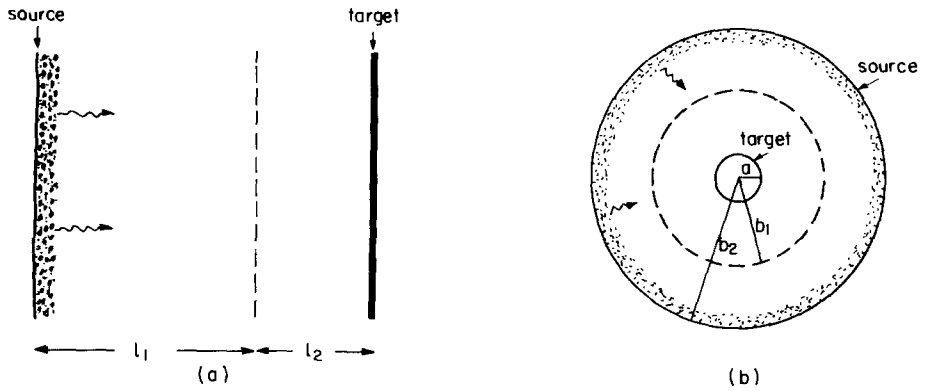

Fig. 1. "Thought experiments" designed to illustrate the special dependence of the transit time on the diffusion distance when the latter is divided into subregions. (*a*): The case of one-dimensional diffusion. (*b*): The case of three-dimensional diffusion from a source to a small surrounded sink

transit time from these diffusional trajectories that cross the border region. We shall denote this term *interference term.*

In the case of diffusion through membranes, the distance between the source and the sink is naturally divided into subregions (layers of membranes and aqueous solutions). However, in that case, since the subregions differ in their physical properties, they are distinguishable by the diffusing particles. But, as we shall see, even for this more complicated case, the transit time retains the simple structure of Eq. (2), with the only difference being that now the interference term depends on the relative affinity of the particles to the two phases.

If we now consider the case of diffusion in three dimensions, we would observe, in principle, the same behavior of transit times as we have seen demonstrated by Eq. (2). For example, in the case of diffusion from a source at a radius b_2 to a surrounded sink of a radius a (Fig. 1*b*), the transit time is given by (*see* Hardt & Cone, 1979[2]):

$$\tau = [(b_2 - a)^2/2D]\,(2b_2 + a)/3a. \tag{3}$$

If we now divide the diffusion distance $(b_2 - a)$ into two subregions of length $(b_2 - b_1)$ and $(b_1 - a)$ as is shown schematically in Fig. 1*b*, the transit times for these two regions would be, respectively (using Eq. (3)),

$$\tau_1 = [(b_2 - b_1)^2/2D]\,(2b_2 + b_1)/3b_1$$

and

$$\tau_2 = [(b_1 - a)^2/2D]\,(2b_1 + a)/3a.$$

[2] *See* footnote 1, p. 300.

Therefore, the transit time from the source to the sink, as given by Eq. (3), can be expressed in terms of the transit times for the subregions as

$$\tau = \tau_1 + \tau_2 + (b_1 - a)(b_2^3 - b_1^3)/3ab_1 D. \tag{4}$$

As may have been expected, the interference terms in this case do not assume the simple form found in Eq. (2). The reason for this lies in the special geometry effects presented in this transit time which emerges due to the arrangement of the source and the sink.

Another case of interest here is the case of diffusion from a small source of radius a to a surrounding sink at a radius b_2 (diffusion in the direction opposite to what is shown in Fig. 1*b*). For this case, the transit time is given by (*see* Hardt & Cone, 1979[3]):

$$\tau = [(b_2 - a)^2/2D] \cdot (2a + b_2)/3b_2. \tag{5}$$

Upon the division of the diffusion distance, one observes a behavior which is similar to what is expressed by Eq. (4). We shall come back to the properties of the three dimensional diffusion when we investigate the transit times of transmembranal diffusion into and from a spherical cell.

In the next section we shall introduce briefly the simple method used to calculate transit times. Since this method requires the knowledge of the behavior of the system at its steady state, we shall also introduce some of the essential features of steady states in cases where diffusion occurs either by partitioning into the membrane or, alternatively, when it proceeds through pores.

How to Obtain the Transit Time

As was mentioned in the introduction, diffusing particles that leave the source together do not arrive at the sink simultaneously; however, their arrival times can be described by a distribution function $f(t)$. Here we are mainly interested in the transit time, which is the mean of the arrival times, and which is formally related to $f(t)$ by

$$\tau = \int_0^\infty t f(t)\, dt. \tag{6}$$

The problem with this approach, which is used in the literature, is the extreme difficulties that one encounters when trying to obtain $f(t)$ for all

[3] *See* footnote 1, p. 300.

but the simplest diffusion problems. However, there is an alternative way to obtain the mean diffusion time without the need to know the distribution function. It can be shown that the transit time may be extracted directly from the behavior of the system at its steady state.

A steady state in the system is attained when we allow the source to release particles continuously. In the steady state there is a constant flux of particles, F, that both enter through the source and leave through the sink. Consequently, the number of particles in the system, N, is a constant. It can be shown that this total number of particles in the system in cases where the concentration at the sink is zero equals the flux of particles into the system multiplied by their mean diffusion time from the source to the sink. Hence

$$\tau = N/F. \tag{7}$$

(For a proof and a discussion of this approach, *see* Hardt, 1979. A scheme of the proof is also introduced in *Appendix I.*)

It must be clear that the transit time obtained by Eq. (7) reflects the behavior of a single diffusing particle, and that the steady-state concentrations of particles are here only to permit the calculation of this single-particle-mean-diffusion-time. Moreover, since the movements of single particles are mutually independent, it can be anticipated that the behavior of a single particle can be deduced in principle from various other diffusion problems.

Some Features of Steady-State Diffusion through Membranes

Equation (7) permits the calculation of transit times provided that we know the steady-state concentration profile for our particular problem. Therefore, we shall now discuss the process of steady-state diffusion through membranes and demonstrate how to obtain the concentration profile. Of course, this problem has been treated extensively in the literature. References to the available work can be found in Crank (1975).

In the steady state, the flux of particles from the source and into the sink reaches a constant value. If we now assume that the diffusion process in systems such as the one shown in Fig. 2 is a one-dimensional process, we can state that the flux through the system is equal at every point along the distance from the source to the sink. The assumption that we have just made about the unidirectionality of the flux may not be valid, especially in cases where diffusion proceeds through pores in the membrane. We will discuss this point later.

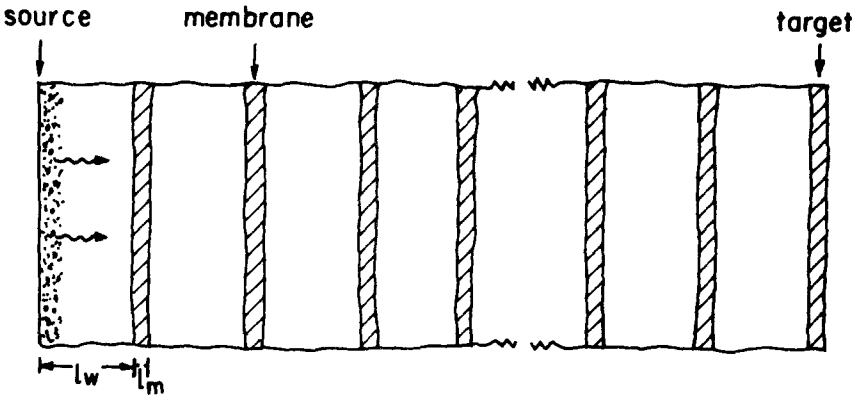

Fig. 2. A case of one-dimensional diffusion when the source and the sink are sparated by successive layers of the aqueous solution and the membrane. The diffusion coefficients of the particles in the two phases are D_w and D_m, respectively. The partition coefficient membrane/water or the fraction of membrane area occupied by pores is β

Obviously, the type of steady-state behavior that we are to observe depends strongly on the type of mechanism by which the diffusing particles penetrate into the membrane. One possible mechanism is that the particles diffuse in the membranes after dissolving into them. In this case the problem is strictly one dimensional and can be treated exactly (the same holds when diffusion is facilitated by diffusable carriers.) Another possibility for transmembranal diffusion is through pores in the membrane. In this case, even the steady-state problem is not always solvable exactly, and the type of approximation used would strongly depend on the distribution and the shapes of the pores.

In the following, we shall analyze the steady-state behavior for these two cases. The results summarized here are used in the next sections to calculate the transit time.

a) Dissolution in the Membrane

In this case we assume that the particles penetrate into the membraneous phase by dissolving into it. For this mechanism, the affinity of the diffusing particles to the membrane, comparative to their affinity to the aqueous solution, is represented by the partition coefficient β. This constant is equal to the ratio of the concentrations in the membrane and in the solution at the interface between these two phases, when a state of equilibrium or a steady-state has been attained. If we denote these

concentrations c_m and c_w, respectively, then $\beta = c_m/c_w$. It is interesting to note that, in the case of facilitated diffusion, in which the particles have to bind to their carrier molecule in order to diffuse in and across the membrane, c_m in the definition of β may stand for the concentration of bound carrier molecules at the interface.

The steady-state concentration profile for this case of diffusion can be obtained in a simple straightforward manner. For example, in the one-dimensional case represented by Fig. 2, the steady-state flux through each phase would equal (Fick's first law) $D\Delta c/l$ where Δc is the concentration drop across that phase and l is the net diffusion distance in it.

If we now assign numbers to the pairs of water-membrane layers starting from 1 at the source and reaching j at the sink, and if we further denote by D_w and D_m the diffusion coefficients of the particles at the water and membrane regions, we obtain for the flux the following relation

$$\begin{aligned} F &= D_w A_w (c_0 - c_{w1})/l_w = D_m A_m (c_{m1} - c_{m2})/l_m \\ &= D_w A_w (c_{w2} - c_{w3})/l_w = \ldots D_m A_m c_{mj-1}/l_m \end{aligned} \tag{8}$$

where A_w and A_m are the cross-sectional areas of the water and membrane phases, respectively, and c_0 is the concentration at the source. We can now use the definition of the partition coefficient and substitute everywhere

$$c_m = \beta c_w \tag{9a}$$

and also in our case of dissolution in the membrane

$$A_w = A_m. \tag{9b}$$

Equations (8) and (9) define the slope of the concentration in each region and permit the calculation of the concentration function itself.

Figure 3 demonstrates a typical concentration profile. In this figure we have chosen $D_w/D_m = 100$, which is in the order of this parameter for the case of biological membranes. Also in this figure, $l_w/l_m = 10$. The particles in this case have $\beta = 2$, which means that they dissolve twice as much in membranes than they dissolve in water (notice the twofold concentration jump at the interface). The relative slope of the concentration gradient in the two phases is, according to Eqs. (8) and (9), D_m/D_w, which in our example, as shown by Fig. 3, is equal to 0.01.

We can use a graphical presentation such as Fig. 3 or solve Eq. (8) to obtain explicitly the concentation function in the steady state. By integrating this function over the distance between the source and the sink,

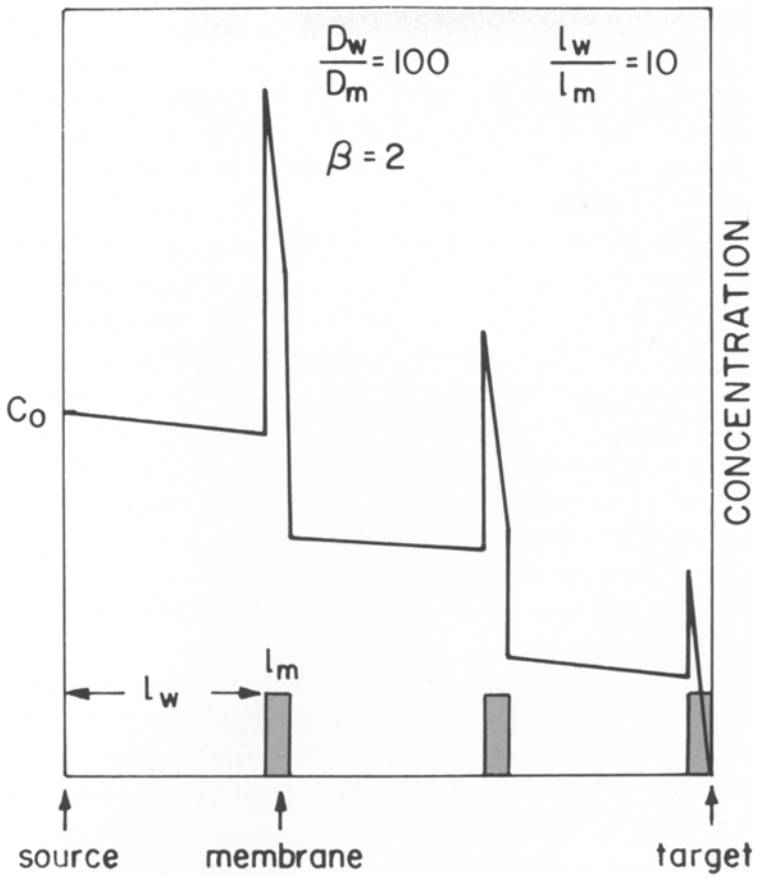

Fig. 3. The concentration profile in a case of steady-state diffusion through three successive water-membrane regions. In this case the particles penetrate into the membrane by dissolution. For the case drawn, they partition twice as well in the membrane than they do in water ($\beta = 2$), and hence one can observe "jumps" in the concentration at the interface. The ratio between the slopes of the gradients in the water and membrane region is governed by the diffusion coefficients and given by D_m/D_w

we obtain that the total number of particles in the system is

$$N = A c_0 (j/2) \{l_w + \beta l_m + [l_w l_m (D_w/\beta - \beta D_m)/j(l_w D_m + l_m D_w/\beta)]\}. \quad (10)$$

The flux in this case, as given by Eq. (8), is

$$F = A c_0 / j(l_w/D_w + l_m/D_m \beta). \quad (11)$$

The knowledge of these two parameters now permits calculation of the transit time for this diffusion process which according to Eq. (7) is equal simply to N/F.

b) Diffusion through Pores

If we imagine the process of diffusion in which particles cross the membranes through pores, it becomes immediately clear that the timing of this process should be influenced by the time it takes a particle to locate such pores when it approaches the membrane.

It is helpful for this case of diffusion through pores, to think about each pore as surrounded by its "range of influence". By this range, we mean a region in the close vicinity of the pore in which it is extremely easy to find the pore by diffusion. It also should be mentioned that the range of influence can be clearly observed in a steady state as the region surrounding the pore in which the concentration is drastically reduced. It is interesting to notice that the range of influence of a small circular pore is in the order of its radius, while the range of influence of an elongated pore is in the order of its long linear dimension. Therefore, the influence of the latter can be much greater than that of a circular pore with the same cross-sectional area (*see* fuller discussion of this point in Hardt & Cone, 1979[4]).

There are several approximated treatments to solve the problem of diffusion through pores [*see*, for example, Prager & Frisch (1975) and Bell & Crank (1974); *see also* Crank (1975)]. However, we would adopt here the case where the ranges of the influence of adjacent pores interfere to the extent that in the steady state the flux is essentially undirectional. Hence to this end, no entrance or exit effects of the pore should be considered. This treatment is valid in cases where the pores are located a few large diameters apart. It should be also mentioned here that if one takes the opposite extreme and assumes the entrance and exit effects to be so marked as to make the diffusion flux nonzero only at limited portions of the aqueous phase, one does not get a much different result for the transit time than we get with our approximation. (These two approximations for the steady-state flux are denoted by Bell and Crank (1974), as the series-parallel and parallel-series approximations. Their degree of accuracy for the case of two-dimensional diffusion was investigated numerically by these authors. *See also* Crank (1975), pp. 281–285.)

Now, if we take the reasonable assumption of the undirectionality of the flux, we get for this flux the same expression as given in Eq. (8), only this time the cross-sectional area is not equal in the two regions. Also D_m represents in this case the diffusion coefficient in the pore and may or

[4] *See* footnote 1, p. 300.

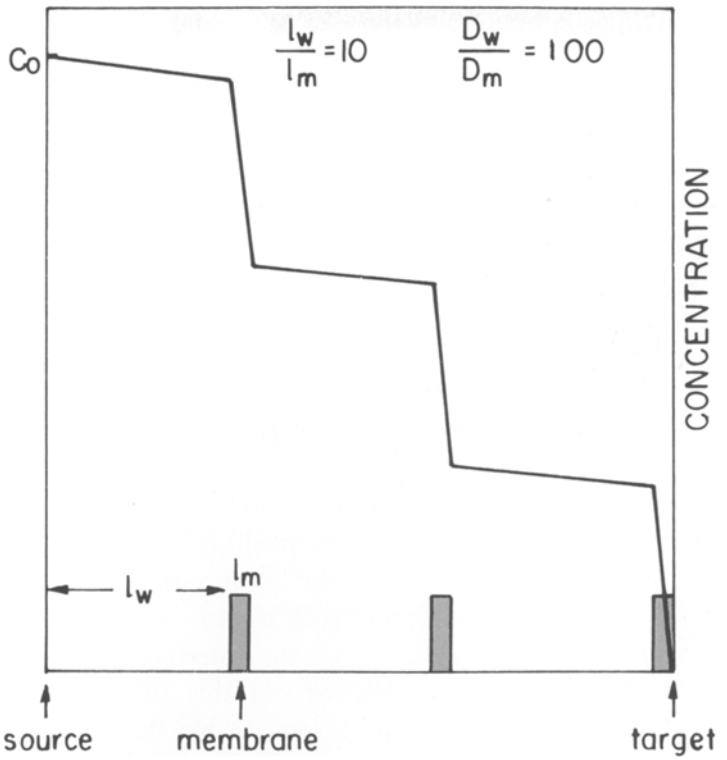

Fig. 4. The concentration profile in a case of steady-state diffusion through three successive water-membrane regions. The particles are assumed to make their way across the membrane by diffusing through pores. The concentration drawn in the membrane region is the concentration within the pores. If we were to draw the averaged concentration in the membrane region, it would assume "jumps" similar to those shown in Fig. 3. Here β denotes the fraction area occupied by the pores

may not equal D_w, depending on the size and internal properties of the pore as experienced by the diffusing particles.

If we define for this case β as A_m/A_w and in addition use the fact that at the interface the concentrations near the pore are equal from both sides ("partition coefficient" of 1), we can obtain the steady-state flux from Eq. (8). The steady-state concentration profile will typically look like what is shown in Fig. 4. In this figure the concentration in the membrane region is the actual concentration in the pores. The number of particles and the flux for this case will assume a form identical to the one obtained in the previous case, as shown by Eqs. (10) and (11).

With the above analysis we have concluded the part of the paper dealing with the general background necessary to calculate the mean diffusion times to and through membranes.

Diffusion between Water and Membrane Regions

We will now use the relations derived in the previous section to obtain the transit time for the simple arrangement of a source and a sink which are separated by a single pair of aqueous and membrane phases. This case is shown schematically in Fig. 5.

By substituting $j=1$ in Eqs. (10) and (11), we obtain the steady-state number of particles and flux for this case. Now, if we take the ratio between these two quantities, as is suggested by Eq. (7), we obtain the transit time for this case as given by

$$\tau = l_w^2/2D_w + l_m^2/2D_m + (l_w\, l_m/\sqrt{D_w\, D_m})\,(\beta\sqrt{D_m/D_w})^{-1}. \tag{12}$$

The first two terms in Eq. (12) are easily recognized as the transit times to diffuse across the water and the membrane phases, respectively. The third term, however, contains properties of both phases. As we have demonstrated in Eq. (2), the origin of such a term in the transit time are those diffusional trajectories that cross the interface between the two diffusion subregions. We have factored out this third term into two groups of parameters for a special reason. As we shall see in the next

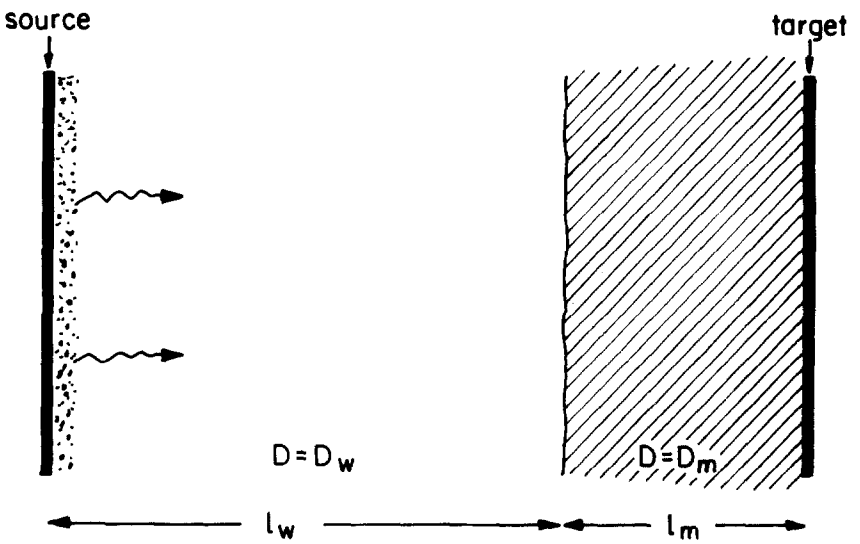

Fig. 5. Diffusion across a water-membrane interface. As is concluded in the text, the direction in which the particles have to cross the interface to reach the sink may significantly influence the transit time

section, the parameter $\beta\sqrt{D_m/D_w}$ always appears in the transit time and has a significant meaning.

If we now calculate the transit time for diffusion in the case where the source is located in the membrane and the sink is in the water, we clearly obtain a different transit time. This transit time can be calculated simply by interchanging the parameters of the two phases in Eq. (12). Alternatively, this transit time for the reverse diffusion can be obtained by calculating for this new arrangement the number of particles and the flux in the steady-state. While the latter is the same as in the previous case, the former quantity assumes a different value and hence yields a transit time different than the one given in Eq. (12).

The transit time for the diffusion in a direction reversed to that shown in Fig. 5 is then

$$\tau = l_m^2/2D_m + l_w^2/2D_w + (l_m\, l_w/\sqrt{D_m D_w})\,(\beta\sqrt{D_m/D_w}). \tag{13}$$

Equations (12) and (13) can be very helpful if one wants fast diffusion and hence wonders in what phase to locate the sink. These relations clearly suggest that the parameter $\beta\sqrt{D_m/D_w}$ is the key to the answer. If it is greater than 1, Eq. (12) yields a shorter transit time than Eq. (13). The opposite holds in the case where this parameter assumes values smaller than 1. Hence, since D_w/D_m is on the order of 100 for biological membranes, this observation suggests that for particles with partition coefficients greater than 10, delays for diffusion from water to the membrane can be significantly shorter than delays for diffusion in the opposite direction.

The origin of the assymetry in transit times across an interface lies in the random nature of the diffusive movements. Once reaching the boundary between the two phases, the particles tend to spend more time in the phase to which they have a higher affinity. Now, if the sink is located in that phase, its probability to be found by the particles there is much higher than if it is located in the unfavorable phase.

We can further conclude from Eqs. (12) and (13) that in certain cases transit times given by these equations can be significantly shorter than the transit time for the same net distance in homogeneous media. This conclusion can be based intuitively on the same reasonings as above. If in the homogeneous case the particles diffuse without special preference to any of the subregions, here they prefer the high affinity phase. As a result in cases where the sink is located in this phase, it may be found faster than in cases where the medium is homogeneous.

Transmembranal Diffusion between Two Aqueous Solutions

In the previous section we established the main features of the transit time for diffusion in the interface region. With this knowledge in hand, we can now easily perceive the nature of transit times in more complicated structures. In this section we treat the case of transmembranal diffusion. This case is of great importance both for interpreting diffusion experiments performed on cells or vesicles and for understanding transport problems encountered by living organisms. We will examine the transit times for the cases shown schematically in Fig. 6. The details of the solution are essentially the same as in the previous section and therefore will be stated only briefly.

Case a: A Transmembranal Diffusion in One Dimension

The transit time for this case (*see* Fig. 6*a*) can be obtained in the following way. First we write the equations for equal flux in the three regions. This yields an equation similar to Eq. (8). From that we obtain the number of particles and the flux as

$$N=(C_0/2)\{l_{w1}[\beta D_m(l_{w1}+2l_{w2})+2D_w l_m] + l_m\beta(D_w l_m+2D_m\beta l_{w2})+l_{w2}^2 D_m\beta\}/\gamma \tag{14a}$$

$$F=D_w D_m \beta C_0/\gamma \tag{14b}$$

where

$$\gamma=D_w l_m+D_m\beta(l_{w1}+l_{w2}).$$

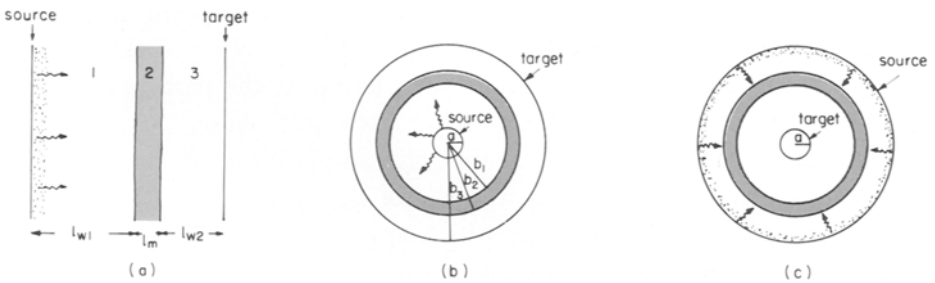

Fig. 6. Transmembranal diffusion in one and three dimensions. In these cases the source and the sink are both located in water and are separated by a membrane. The case where the source and the sink are in membranes and are separated by a layer of water can be easily obtained by interchanging the parameters for these regions in the equations in the text

Using Eq. (7) we obtain for the transit time

$$\tau = l_{w1}^2/2D_w + l_m^2/2D_m + l_{w2}^2/2D_w + l_{w1}\, l_m/D_m\, \beta + l_m\, l_{w2}\, \beta/D_w + l_{w1}\, l_{w2}/D_w. \tag{15}$$

Upon examining each of the terms in Eq. (15) we can easily identify the first three terms as the transit times for diffusion in each of the separate subregions. The last three terms, however, are the interference terms.

It is of interest to observe the conditions under which the transit time in Eq. (15) exhibits a minimum. This condition can be derived by differentiating Eq. (15) with respect to β. This yields that the minimal transit time is attained when

$$\beta^2(D_m/D_w) = (l_{w1}/l_{w2}). \tag{16}$$

Notice that the square root of the term on the left is the same expression that appears in the interference term of Eq. (13).

If we want to possess an intuitive insight into the process of diffusion through membrane, we must be able to establish what the condition represented by Eq. (16) actually means in terms of the behavior of single diffusing particles. As we shall see in the next section, this very same condition for minimum in the transit time also holds for diffusion through arrangements of successive membranes.

The meaning behind Eq. (16) may become apparent if we recollect what has been said in previous sections about the origin of the interference terms present in the transit time. As has been stated, the essence of these additional terms is to add a delay to the diffusing particles. This delay comes about because even though the particles crossed the boundary between two regions, they can still diffuse back to their original starting point away from their target.

With this picture in mind, we can now examine the first two interference terms in Eq. (15). The first term, $l_{w1}\, l_m/D_m\, \beta$, represents the diffusional interference between the source aqueous region and the membrane. The second term, $l_m\, l_{w2}\, \beta/D_w$ represents the interference between the membrane and the sink aqueous region. If we now compare these two terms to the condition stated in Equation (16), we immediately realize that the latter implies the equality of the former. To put it in other words, the minimal transit time is attained when

$$l_{w1}\, l_m/D_m\, \beta = l_m\, l_{w2}\, \beta/D_w.$$

We are now in a position to understand what Eq. (16) states. It says that the fastest diffusion occurs when the delays introduced by the interference terms at the two interfaces are equal. What this in fact implies is that, in order to achieve a state of minimal diffusion delays, the diffusing particles should not prefer one phase over the other in terms of the amount of time they spend in the vicinity of the different boundaries.

One can further investigate the value that β should assume in order to make negligibly small the first interference terms discussed above. Such a condition can easily be achieved in cases where l_m is much smaller than l_{w1} or l_{w2}, as is the case if the membrane is a biological membrane. This and other conditions relevant to the understanding of the design of biological diffusion systems are discussed elsewhere (*see* Hardt & Cone, 1979[5]).

We shall now investigate the transit time for diffusion to and from a spherical cell as shown in Fig. 6*b* and *c*. The transit times for these three dimensional diffusion processes assume a slightly more complicated form than in the one-dimensional case; however, it retains the latter general structure.

Case b: Transmembranal Diffusion from a Spherical cell

The case of interest is illustrated in Fig. 6*b* where the system dimensions are also specified.

To obtain the transit time, we have, as before, to obtain the steady-state parameters. The details of the calculation for this case and for the following one (Fig. 6*c*) are given in *Appendix II*.

The resulting transit time emerging from the analysis is given as

$$\begin{aligned}\tau = {} & [(b_3-b_2)^2/6D_w](2b_2+b_3)/b_3+[(b_2-b_1)^2/6D_m](2b_1+b_2)/b_2 \\ & +[(b_1-a)^2/6D_w](2a+b_1)/b_1 \\ & +[(b_1-a)(b_3-b_2)/6D_w][(b_1+a)^2+b_1^2+a^2]/b_2 b_3 \\ & +[\beta(b_3-b_2)(b_2-b_1)/6D_w][(b_1+b_2)^2+b_1^2+b_2^2]/b_1 b_2 \\ & +[(b_2-b_1)(b_1-a)/6\beta D_m][(b_1+a)^2+b_1^2+a^2]/b_1 b_2.\end{aligned} \tag{17}$$

The first three terms represent the transit time to diffuse across each phase separately (*compare* Eq. (5)). The last three terms represent here again the diffusional interference between the regions. Here these terms

[5] *See footnote* 1, *p.* 300.

assume a more complicated structure because of the geometry factors involved. It can be easily perceived that the three-dimensional transit time given by Eq. (17) is easily reduced to the one-dimensional case (as given by Eq. (15)) in cases where the curvature of the surfaces is negligible. This happens when $(b_1-a)\ll a$, $(b_2-b_1)\ll b_1$ and $(b_3-b_2)\ll b_2$.

Case c: Transmembranal Diffusion into a Spherical Cell

This case is shown schematically in Fig. 6*c*. The transit time is again of the same general structure as in Eq. (17) and is given by (*see Appendix II* for details)

$$\begin{aligned}\tau=&[(b_3-b_2)^2/6D_w](2b_3+b_2)/b_2+[(b_2-b_1)^2/6D_m](2b_2+b_1)/b_1\\&+[(b_1-a)^2/6D_w](2b_1+a)/a\\&+[(b_1-a)(b_3-b_2)/6D_w][(b_1+a)^2+b_1^2+a^2]/b_2b_3\\&+[\beta(b_3-b_2)(b_2-b_1)/6D_w][(b_2+b_1)^2+b_2^2+b_1^2]/b_1b_2\\&+[(b_2-b_1)^2(b_1-a)/6\beta D_m][(b_1+a)^2+b_1^2+a^2]/b_1b_2.\end{aligned}\tag{18}$$

As in the previous cases, the first three terms in Eq. (18) represent the transit times for the three diffusion regions (*compare* Eq. (3)). This three-dimensional transit time given in Eq. (18) reduces to the one-dimensional one whenever the curvature of the surface is negligibly small.

It is of interest to note that in cases where b_3 is much larger than b_2, b_1 and a, the diffusing particles view the cell as a small target and the actual process of penetration through the membrane can be negligible, provided that β is on the order of unity. In this particular case the transit time reduces to

$$\tau=[b_3^2/2D_w][2b_3/3b_2]$$

which is the transit time to locate a small target of radius b_2 by diffusing from a distance b_3.

Diffusion through Successive Membranes

After the observation we have made in previous sections, the derivation and the understanding of transit times for the case of diffusion through successive membranes becomes almost a trivial matter. We have encountered the parameter $\beta\sqrt{D_m/D_w}$ representing surface properties and understood the condition for minimal diffusion delays for transmem-

branal diffusion. Now we shall see how these facts are nicely confirmed by the case investigated in this section.

We shall consider here a case of diffusion through couples of water-membrane layers as shown by Fig. 2. The transit time can be easily obtained for this case by dividing the number of particles at a steady state by the flux. These two steady-state parameters for our case are given by Eqs. (10) and (11), and the resulting transit time is

$$\tau = j^2 \{ l_w^2/2D_w + l_m^2/2D_w + l_m l_w/2\sqrt{D_m D_w} \\ [(1-1/j)\beta\sqrt{D_m/D_w} + (1+1/j)/\beta\sqrt{D_m/D_w}]\}. \tag{19}$$

Note the essential identity between this equation and Eq. (12) for the case $j=1$.

We shall investigate τ for the case where $j \gg 1$. In this case τ assumes the form

$$\tau = j^2 \{ l_w^2/2D_w + l_m^2/2D_m + l_w l_m/2\sqrt{D_w D_m}\,[\beta\sqrt{D_m/D_w} + 1/\beta\sqrt{D_m/D_w}]\} \tag{20}$$

and has a minimum when

$$\beta\sqrt{D_m/D_w} = 1. \tag{21}$$

This condition for a minimum is identical to the condition formulated by Eq. (16) since here we have $l_{w1} = l_{w2} = l_w$. Hence, the meaning of this condition for minimal diffusion delays is identical to what is discussed in connection with Eq. (16).

Doubtlessly, there is a wealth of conclusions that one can deduce from Eq. (20) concerning aspects of diffusional transport in living organisms, but this is left to a separate paper (*see* Hardt & Cone, 1979[6]).

Since our original attempt has been to present and investigate the transit times for systems with membranes and, in addition, to make the conclusion drawn also useful for analyzing data usually obtained in diffusion experiments, in the following and last section we introduce some remarks which may turn out to be useful in this regard.

Some Relations between Transit Times and other Measurable Times for Diffusion

The theoretical observations obtained by our analysis can be applied to various real experimental observations. For example, Eqs. (15) or (19) can serve to clarify the nature of the observed deviations from Collander

[6] *See* footnote 1, p. 300.

plots in measurements of nonelectrolytic permeability in epithelia cells (Hingson & Diamond, 1972). Also, Eq. (20) provides a means to obtain "geometry factors" for measurements of diffusion times in media with irregularly shaped boundaries. An example of such measurement is that of the lateral diffusion time of rhodopsin in the photoreceptor disc membrane (Poo & Cone, 1974). For this particular experimental observation, Eq. (20) yields a "geometry factor" (i.e., a ratio between τ with and without the boundary irregularities) of $\sim 2-3$, which is similar in value to that obtained by the above authors, after performing analogue heat flow measurements. (For the calculation here, the values $\beta \simeq 0.05 - 0.1$, $D_m = D_w$ and $l_w \simeq 10\, l_m$ were taken.) A third example of experimental systems to which the previously derived relations may be useful is that where the diffusion of growth factors into cells is considered (e.g., Whittenberger & Glaser, 1978). Here parameters such as the thickness of the unstirred layer may greatly affect diffusion times, and this possible effect can well be estimated by Eqs. (15) or (18).

It is our purpose in this section to briefly demonstrate how the transit time may be related to measured time courses in some typical diffusion experiments. Three processes are analyzed here: (i) The decay of concentration gradients in systems containing sinks; (ii) the equilibration of concentration gradients in closed systems; and (iii) the establishment of a steady-state gradient in a system with zero initial concentration. We believe that, although partly qualitative, this analysis is important, especially since it allows us to practice in real experiments the conclusions and the insight acquired by our basic investigation on transit times.

a) The Decay of Gradients into Sinks

We will consider here the diffusion of particles into sinks in cases where these particles initially participate in a concentration gradient. This diffusion problem differs from what we have analyzed before in that here the particles do not all diffuse the same net distance to the sink, but rather possess different "release" points as dictated by the initial concentration gradient.

We will address ourselves to the problem of finding the mean lifetime of particles in the system following the introduction of the sink. We can immediately say that this mean lifetime equals the transit time in the case of one release point (a δ function concentration gradient).

For simplicity of presentation, we treat here a case of one-dimensional diffusion. The treatments for two and three-dimensional diffusion can, however, be obtained in an identical manner.

Consider a system in which a one-dimensional concentration gradient of diffusing particles exists. This concentration is represented by $c(x)$ which measures the concentration as a function of the distance from the origin, and the origin in this case is taken as an impermeable boundary.

Suppose now that at time $t=0$ a sink is introduced into the system and is located at distance b from the origin. We want to derive the average time it takes particles in the region between $x=0$ and $x=b$ to find the sink.

To derive this mean lifetime, we must know the transit time for diffusion from a point x_0 between 0 and b to the sink at b. This transit time will then be weighted according to the concentration function (which reports at each point x_0 how many particles start their diffusion from that point).

The transit time from a point x_0 to the sink is (*see* Hardt & Cone, 1979[7])

$$\tau(x_0)=(b^2-x_0^2)/2D. \tag{22}$$

Note that for a release at $x_0=0$, Eq. (22) gives the Einstein relation (Eq. (1)) and for $x_0=b$ (release at the sink) the transit time is obviously equal to 0.

The mean lifetime for particles in a concentration gradient $c(x)$ is

$$\tau=(1/N)\int_0^b \tau(x)c(x)dx=(1/N)\int_0^b [(b^2-x^2)/2D]c(x)dx \tag{23}$$

where

$$N=\int_0^b c(x)\,dx.$$

For the case of an initially uniform concentration, $c(x)=$constant, and from Eq. (23) $\tau=b^2/3D$. For the case of a linear concentration gradient $c(x)=1-x/b$, Eq. (23) yields $\tau=b^2/2.4D$.

b) The Relation between Mean Lifetimes and the Transient Behavior

Formally, the transient behavior of a decaying diffusion gradient can be described as an infinite sum of decay modes (*see*, for example, Crank,

[7] *See* footnote 1, p. 300.

1975). For the case of one dimensional diffusion, the behavior in time and space can be expressed as

$$c(x,t) = \sum_{n=0}^{\infty} b_n(x) \exp(-t/\tau_n). \tag{24}$$

It can be easily shown that, since the rate at which particles evacuate the system through the sink determines how many particles are still left in it, the mean life time of the particles after the introduction of the sink is simply (see Adam & Delbruck (1968) for the derivation).

$$\tau = \sum_{n=0}^{\infty} B_n \tau_n / \sum_{n=0}^{\infty} B_n \tag{25}$$

where B_n is related to $b_n(x)$ of Eq. (24) by $B_n = \int_0^b b_n(x)\, dx$.

Equation (25) suggests that the mean lifetime is in fact a weighted average of the modes of decay τ_n of Eq. (24). Moreover, Eq. (25) implies that in cases where one τ_n dominates Eq. (25), the mean lifetime of the particles would be equal to that mode.

Indeed, what one finds in many cases [*see* Adam & Delbruck (1968) for the treatment of the transient behavior for two and three-dimensional diffusion, and Meyer & Kostin (1976) for the treatment of transients in diffusion through membrane] is that the mean lifetime in fact equals the largest (slowest) mode of decay. We can, at this point, say that the processes of diffusion in which one mode of Eq. (24) dominates is mostly those in which diffusion to the sink is restricted. For instance, this behavior is not found for most one-dimensional processes in homogenous media. Clearly the last remark is basically intuitive, and its establishment needs further investigation.

c) The Timelag and the Transient Time

The timelag is a parameter that has proven useful in measuring various diffusion parameters in nonhomogeneous systems [*see* Ash, Barrer & Plamer (1965) and Crank (1975), p. 222].

Here we wish to emphasize how the timelag can be related to the transit time. We base our claim on the formal definition of the timelag which states that for the case of a system which is empty of diffusing particles at time 0, the steady state will be established after a time period

L, which is given for a case of one-dimensional diffusion by

$$L=(1/b\ F)\int_0^b x\,c(x)\,dx \tag{26}$$

where b is the separation between the source and the sink, F is the steady-state flux and $c(x)$ is the concentration function at the steady state.

Using the definition of the transit time by Eq. (7), it can be easily shown that the timelag of Eq. (26) is just

$$L=\tau\int_0^b (x/b)\big(c(x)/N\big)\,dx. \tag{27}$$

We can conclude from Eq. (27) that the timelag is equal to the transit time multiplied by a reduced distance parameter. The existence of such a parameter is expected intuitively since, to establish a steady-state concentration, $c(x)$, not all the particles must diffuse the distance b. A detailed account the special form of this distance parameter as well as a further investigation on the relation between the transit time and various measurable time parameter will be given elsewhere.

Conclusion

We have attempted in this paper to provide an analysis of the peculiar properties of the pace of diffusion in systems containing membranes. The basic features of diffusion emerging from our investigation may help strengthen our intuition about this rather old, but yet not so fully understood, transport process.

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Appendix I.

Here we shall introduce the scheme of the proof of Eq. (7). A detailed discussion of this matter can be found in Hardt (1979). We wish to show that the transit time obtained from the steady-state parameters N and F

is identical to that obtained by averaging the "first passage" times of individual particles to the sink. To formulate this claim mathematically

$$N/F = \int_0^\infty t f(t)\, dt$$

(*see* text for definitions of parameters).

The proof is straightforward if we proceed along the following line of thought. In the steady state, at every time, there are N particles in the system. We know, however, that those particles present at any particular time, e.g., $t=0$, will eventually evacuate the system through the sink and be substituted by new particles that enter continuously through the source. If we try to formulate this last observation, we shall obtain the desired proof for the above relation.

The number of particles that leave the system at time t but entered at times later than $t=0$ is $F\int_0^t f(T)dT$. Therefore, to obtain the number of particles that were already present in the system at $t=0$ and leave at time t, we subtract the above number from the total number of leaving particles. Hence now $N=F\int_0^\infty \left(1-\int_0^t f(T)\, dT\right) dt$ represents the fact that, if we sum the number of particles that leave at t and were present at 0 over all t, we should obtain N. Integrating this relation by parts and using the fundamental features of the Brownian movements which guarantee that $\int_0^t f(T)\, dT=1$ as $t\to\infty$ and that this integral approaches 1 faster that t approaches ∞ (e.g., Karlin & Taylor, 1975), one obtains $N=F\int_0^\infty t f(t)\, dt$. Now, since in the case of free diffusion the movements of individual particles are mutually independent (Einstein, 1956), the same distribution function $f(t)$ that describes the behavior observed in a steady state is also obeyed in general during nonsteady-state processes (and in particular during the process of diffusion from an instantaneous source, which is used to formally define τ). With these theoretical observations, our claim is proven.

Appendix II.

Here we shall show how to obtain the steady-state flux and number of particles for cases of three-dimensional diffusion from and into a spherical cell. These two cases are shown schematically in Fig. 6*b* and *c*.

1. Transmembral Diffusion from a Spherical Cell

In the steady for this case (Fig. 6*b*), the equality of the flux in every subregion dictates [*see* Crank (1975), pp. 267]

$$\begin{aligned} F &= 4\pi D_w a b_1 (c_0 - c_1)/(b_1 - a) = 4\pi D_m b_1 b_2 \beta (c_1 - c_2)/(b_2 - b_1) \\ &= 4\pi D_w b_2 b_3 c_2/(b_3 - b_2) \end{aligned} \tag{A1}$$

where c_0 is the concentration at the source and c_1 and c_2 the concentrations in the aqueous solution at the two interfaces. From Eq. (A1) we obtain

$$\begin{aligned} F = 4\pi D_w D_m a b_1 b_2 b_3 c_0 / [&a b_1 (b_3 - b_2) \beta D_m \\ &+ a b_3 (b_2 - b_1) D_w + b_2 b_3 (b_1 - a) \beta D_m]. \end{aligned} \tag{A2}$$

To obtain the number of particles, we have to obtain c_1 and c_2 from Eq. (A1) and then integrate the concentration function over the volume.

2. Transmembral Diffusion into a Spherical Cell

In this case (Fig. 6*c*) the equality for the flux yields

$$\begin{aligned} F &= 4\pi D_w b_3 b_2 (c_0 - c_1)/(b_3 - b_2) = 4\pi D_m b_2 b_1 \beta (c_1 - c_2)/(b_2 - b_1) \\ &= 4\pi D_w b_1 a c_2/(b_1 - a). \end{aligned} \tag{A3}$$

Here c_1 and c_2 are the concentrations in the aqueous phases at the interface. (The first interface is the one closest to the source.) This condition yields for the flux the same expression as given by Eq. (A2). The number of particles in this case is again obtained by deriving c_1 and c_2 from Eq. (A3) and integrating the concentration function over the volume. Obviously in this case N assumes a value different from that in the previous case.

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