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A NEW THEORY OF TRANSPORT FOR CELL MEMBRANE PORES

II. EXACT RESULTS AND COMPUTER SIMULATION (MOLECULAR DYNAMICS)

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SUMMARY

An exact expression for the solvent diffusion coefficient in a pore that is so narrow that two molecules cannot get around each other is derived. The theory states that the diffusion coefficient for a pore that contains n molecules is simply equal to the diffusion coefficient a molecule would have if it were all alone in the pore (D°) divided by n. The theory is very general. Two different models that can be used to determine D° are described. An exact experimental test of the theory is carried out by the use of computer simulation (molecular dynamics). It is shown that as long as the density of molecules in the pore is not too high, the experimental results are not significantly different from the theoretical predictions. These experimental results also test the theory presented in the first paper of this series and provide strong support for those results.

INTRODUCTION

The general theory for the transport kinetics of a pore that is so narrow that the solute and solvent molecules cannot get around each other was presented in the first paper of this series [1]. The basic idea of this theory is that one can apply to the pore the fundamental Einstein equation which relates the bulk diffusion coefficient (D) to the frictional drag coefficient (β):

$$D = \frac{kT}{\beta} \tag{1}$$

By use of this assumption, it was shown for the case where the pore is so narrow that the molecules cannot get past each other that the solvent diffusive permeability (ω) can be related to the hydraulic permeability (L_p) and the number of solvent molecules per pore (n) by the expression ($\bar{V}$ is the molar volume of the solvent):

$$\omega\bar{V} = \frac{L_p}{n} \tag{2}$$

Eqn 2 provides a general relation between three experimental parameters and no assumptions about the molecular interactions in the pore or the pore shape are assumed in its derivation.

In the first part of this paper it will be shown that in a certain limit the same assumptions that lead to Eqn 2 can be used to derive the following exact expression for the solvent diffusion coefficient (D) in a pore that contains n molecules:

$$D = \frac{D^0}{n} \tag{3}$$

where D° is the pore diffusion coefficient that a solvent molecule would have if it were the only molecule in the pore. Eqn 3 reduces the many body problem of determining D to the much easier one body problem of finding D° for which some solutions are already available. For example, if the solvent is approximated by hard smooth spheres and the pore has hard diffusely reflecting walls (see below for details), then the problem of determining D° reduces to the case of Knudsen flow for which exact kinetic solutions are known [2]. Another approach to finding D°, which may be more applicable to the actual cell membrane pore, assumes that the pore consists of a series of binding sites; and D° is calculated from reaction rate theory [3]. Eqn 3 combined with a theory for D° provides a description of the kinetics of transport in narrow pores that is much more complete and detailed than any previous theory. In fact, it is one of the very few exact results in all of kinetic theory.

In the second part of this paper, the range of validity of Eqn 3 will be tested by comparing it with exact results obtained by computer simulation (molecular dynamics). In the molecular dynamic calculation, solvent molecules (modeled by n hard smooth spheres) are given a random set of initial positions and velocities in the pore (modeled by hard diffusely reflecting walls), and the computer then calculates the exact trajectories of the spheres as they move about colliding with each other and with the pore walls. From this exact solution it is easy to determine the diffusion coefficient D which can be compared with the prediction of Eqn 3 using the Knudsen value for D°. It will be shown that if the pore density is not too high, Eqn 3 provides a very good description of the molecular dynamic results. Since the assumptions used in the derivation of Eqn 3 include those used in the derivation of the general case of Eqn 2, these computer experiments also test the validity of Eqn 2 and the other results obtained in the first paper of this series (at least for one special case).

THEORETICAL DERIVATION OF DIFFUSION COEFFICIENT IN THE PORE

This derivation is based on the assumption that the collisions between the solute molecules in the narrow pore can be approximated by one-dimensional hard sphere collisions [4, 5]. The effect of this assumption is illustrated in Fig. 1 where the x positions (axial distance) of the molecules are plotted as a function of time. It is easy to show that when two hard spheres moving in only one dimension (x direction) collide, they simply exchange velocities. Thus, if the molecule–molecule collisions in the pore are assumed to be one-dimensional, the trajectories of the spheres behave as if they jump the distance between the ball centers during a collision and then continue as if there were no collision. This is illustrated in Fig. 1A. The kinks in the sphere

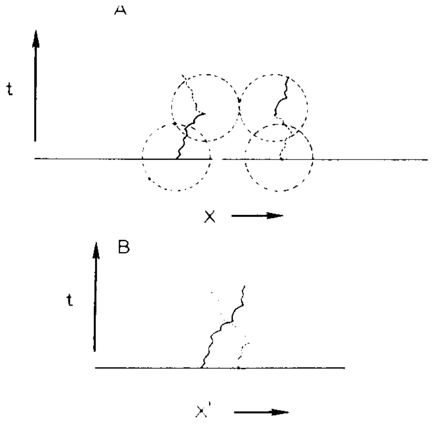

Fig. 1. (A) The axial (x) positions of the centers of two spheres as a function of time. The kinks in the trajectories represent collisions with the pore walls. If the sphere–sphere collisions are one dimensional then the trajectories simply jump the intersphere distance and continue unchanged during a collision. (B) Equivalent system of points with the sphere diameters removed.

trajectories result from the collisions with the wall. It is assumed in Fig. 1 that when a sphere collides with the wall it is instantaneously absorbed and emitted in a random direction (diffuse wall condition).

The diagram in Fig. 1A can be replaced by an equivalent diagram (Fig. 1B) in which the system now consists of point particles and the pore length (l) is reduced to:

$$l' = l - nb' \tag{4}$$

where b' is the average separation between the centers of the two colliding particles at the time of a collision. If the pore was really one-dimensional (the same diameter as the spheres) then b' would be equal to the sphere diameter b. However, in the actual pore b' will be somewhat less than b. As is shown in Fig. 1B, the point trajectories simply pass through each other and when a point trajectory has gone a distance l', the sphere trajectory has traveled the whole pore length (l).

The derivation of Eqn 3 is again based on the basic idea that the Einstein relation can be extended to the pore. First β is found by considering the flux that results from a pressure difference, and then the diffusion coefficient in the pore is determined from Eqn 1. The net flux that results from a pressure difference can be determined easily from the diagram shown in Fig. 1B. Since the trajectories simply pass through each other (Fig. 1B) they behave as if they do not interact with other particles and are all alone in the pore. Thus the net flux of trajectories (J) produced by a given pressure or density difference ($\Delta\rho'$) is given by:

$$J = \frac{D^0 \Delta\rho'}{l'} \tag{5}$$

where $\Delta\rho'$ is the density difference (per unit length) of point particles across the pore ends and D° is the diffusion coefficient of a single trajectory which is equal to the pore diffusion coefficient that a molecule would have if there were no other molecules in the pore. Since the pore is so narrow that the molecules cannot get past one another a pressure difference across the ends of the pore is equivalent to a force (F) dragging one of the molecules [1]:

$$J = \rho'\bar{v} = \rho'\frac{F}{\beta} \tag{6}$$

where ρ' is the density of points per unit length (n/l'). In Eqn 6, $\bar{v}$ is the drift velocity of the point particles (Fig. 1B) which is identical to the drift velocity of the spheres (Fig. 1A) so that β in Eqn 6 is equivalent to the β for the system of spheres. Since a system of point particles is equivalent to a dilute ideal gas, F is equal to $\Delta\rho' kT$ so that:

$$J = D^0\frac{\Delta\rho'}{l'} = \rho'\frac{\Delta\rho' kT}{\beta} \tag{7}$$

or

$$\beta = \frac{kT\rho' l}{D^0} = \frac{nkT}{D^0}$$

Substituting this value of β into the Einstein relation (Eqn 1) yields the final result:

$$D = \frac{D^0}{n} \tag{3}$$

If the molecule–molecule collisions were not one-dimensional, the trajectories would be bent as they crossed in Fig. 1B and they would not pass straight through each other. However, if the wall collisions were much more frequent than the intermolecular collisions as in Fig. 1, the trajectories of the different molecules would not be significantly altered by the few extra kinks that result from the intermolecular collisions which are not one dimensional. Thus, one would predict that as the fraction of the collisions that are with the wall increased, the agreement between the experimental results and the theory would improve. It will be shown that this prediction is supported by the molecular dynamic experiments.

The system described in Fig. 1B can also be solved for an infinitely long pore [5]. The most interesting result of this analysis is that the probability that a point that was at the origin at time zero will be at position x after a sufficiently long time t is given by:

$$P(x, t) = \frac{\sqrt{\rho'}}{2(\pi D^0 t)^{\frac{1}{4}}} \exp\{-\rho' x^2[\pi/(16D^0 t)]^{\frac{1}{2}}\} \tag{8}$$

It can be seen from Eqn 8 that the spread in x in the pore goes at $t^{\frac{1}{4}}$ which is much slower than what one would expect for the solution to the usual diffusion equation ($t^{\frac{1}{2}}$). This is an illustration of the fact that for an infinite pore n becomes infinite (constant ρ') and therefore D becomes zero.

In the next section, D will be determined experimentally for a cylindrical, diffusely reflecting pore of diameter d and length l and the molecules will be modeled by hard, smooth spheres of diameter b and mass m. For this system, the value of D° is exactly equal to the Knudsen diffusion coefficient [2]:

$$D^0 = \tfrac{2}{3}(d-b)\left(\frac{2kT}{\pi m}\right)^{\frac{1}{2}} \tag{9}$$

It is important to note here that since periodic boundaries are used at the pore ends in the molecular dynamic experiments, the value of D° for the infinite pore (Eqn 9) should be used. If one wanted to apply Eqn 3 to a pore that separated two well mixed compartments, it would be necessary to correct D° for the effects of the finite length [6]. The theoretical value of the diffusion coefficient (D_{th}) that will be compared with the experimental value is obtained by substituting Eqn 9 into Eqn 3:

$$D_{\mathrm{th}} = \frac{2(d-b)}{3n}\sqrt{\frac{2kT}{\pi m}} \tag{10}$$

MOLECULAR DYNAMIC EXPERIMENTS

Since these computer experiments are almost identical to those carried out previously [7], only the modifications of the previous methods will be described here. The most important modification is the use of periodic boundaries at the pore ends. This, in effect, closes the pore on itself so that, for example, if there are n spheres in the pore, sphere No. n interacts with sphere No. 1 and No. $(n-1)$. The experimental value of the diffusion coefficient (D_{exp}) is calculated from a direct application of the expression [8]:

$$D = \lim_{t\to\infty} \frac{\overline{X^2}}{2t} \tag{11}$$

where X is the displacement of the ball in the x direction that occurs in time t and an average is taken over many periods of duration t. Eqn 11 is valid only for an infinite system. This condition is satisfied in the molecular dynamic experiments by the use of periodic end conditions. In order to use Eqn 11 it is necessary to show that t is long enough to satisfy the limit in Eqn 11. Fig. 2 shows the value of $\overline{X^2}/2t$ as a function of

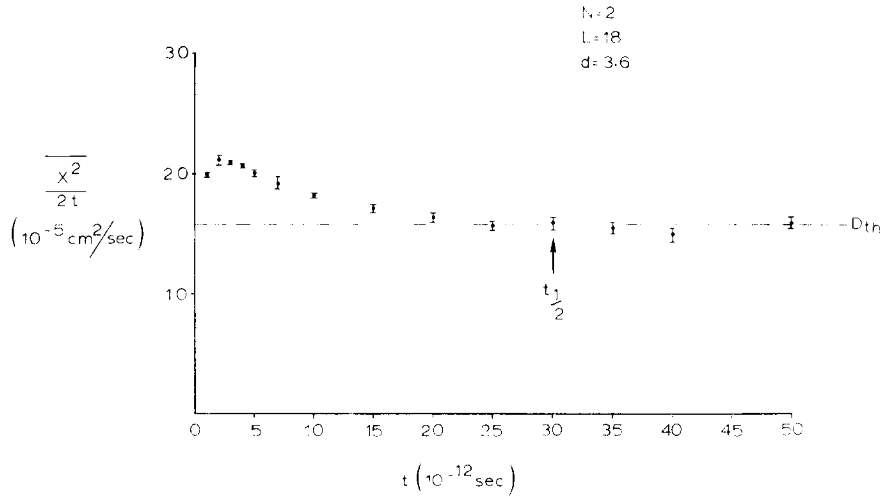

Fig. 2. Average value of $X^2/2t$ (± S.E.) as a function of t for the case $n = 2$, $l = 18$ Å, $d = 3.6$ Å. The arrow marks the value of t at which D_{exp} was determined (see text). The value of D_{th} is shown by the dotted line.

time for one set of conditions ($n=2$, $l=18$ Å, $d=3.6$ Å). It can be seen that $\overline{X^2}/2t$ has reached its limiting value after a time of about 20×10^{-12} s. The arrow in Fig. 1 marks the time ($t_{\frac{1}{2}}$) at which the sphere would have drifted an average distance of $l/2$ ($t_{\frac{1}{2}} = l^2/8D$, where the limiting value of D is used). In the cases studied, D had reached its limiting value by time $t_{\frac{1}{2}}$ and the D_{exp} was determined for this time period. As a test of the molecular dynamic computer program, D° was determined by this procedure and the experimental result agreed well with the theoretical value (Eqn 9).

The experiments were carried out on a CDC 6600 digital computer. The computer time was proportional to n, the time period ($t_{\frac{1}{2}}$) and the accuracy that was required (which depends on the number of time periods in which X is measured). For example, 10 % accuracy for a pore 24 Å long and 2.8 Å in diameter that contains 4 spheres required 17 min of computer time.

Values for $D_{\text{exp}}/D_{\text{th}}$ and the percentage wall collisions are tabulated in Table I for a variety of combinations of pore diameters (d), lengths (l) and number of spheres in the pore (n). In all the experiments the ball diameter (b) was 2 Å, the mass was $3.0114 \cdot 10^{-23}$ g, and the temperature was 300 °K. In Fig. 3 the ratio $D_{\text{exp}}/D_{\text{th}}$ is plotted for the three values of the parameter bn/l (which is proportional to the sphere density). It can be seen that the experimental results do not differ significantly from the theoretical values for values of bn/l of 0.33 or less. This agreement between theory and experiment is not significantly correlated with either the number of

TABLE I

RESULTS OF MOLECULAR DYNAMIC EXPERIMENTS

The sphere diameter (b) is 2 Å.

Pore diameter (d) (Å)	Nos of spheres per pore (n)	Pore length (l) (Å)	$\frac{bn}{l}$	D_{th} (Eqn 10) (10^{-5} cm³/s)	$\frac{D_{\text{exp}}}{D_{\text{th}}}\pm$S.E.	Wall collisions (%)
2.2	2	6	0.67	1.973	0.98±0.065	96
2.4	4	12	0.67	1.973	0.87±0.069	90
2.8	2	6	0.67	7.8902	0.89±0.045	88
2.8	2	12	0.33	7.8902	1.00±0.038	96
2.8	2	18	0.22	7.8902	1.01±0.020	98
2.8	4	12	0.67	3.9451	0.91±0.053	83
2.8	4	24	0.33	3.9451	1.03±0.084	95
2.8	4	36	0.22	3.9451	0.98±0.087	97
2.8	6	18	0.67	2.6300	0.85±0.072	82
3.6	2	6	0.67	15.7804	0.81±0.025	80
3.6	2	12	0.33	15.7804	0.98±0.040	93
3.6	2	18	0.22	15.7804	1.01±0.052	96
3.6	4	12	0.67	7.8902	0.79±0.040	73
3.6	4	36	0.22	7.8902	1.03±0.115	94
4.2	4	12	0.67	10.849	0.84±0.031	70
4.2	4	36	0.22	10.849	0.98±0.119	90
5	4	12	0.67	14.7904	1.290±0.047	70
5	4	36	0.22	14.7904	2.347±0.046	90
6	4	12	0.67	19.726	1.718±0.059	72
6	4	36	0.22	19.726	2.735±0.138	90

spheres (n) or the pore diameter (d). At the higher density ($bn/l = 0.67$) there is a significant deviation of the results from the theory. At the higher density the percentage of molecule–molecule collisions increases (Table I) so that the deviation of the collisions from true one-dimensional dynamics becomes more important. This is illustrated by the fact that, for $bn/l = 0.67$, as the radius decreases the percentage of wall collisions increases and there is less deviation from the theory.

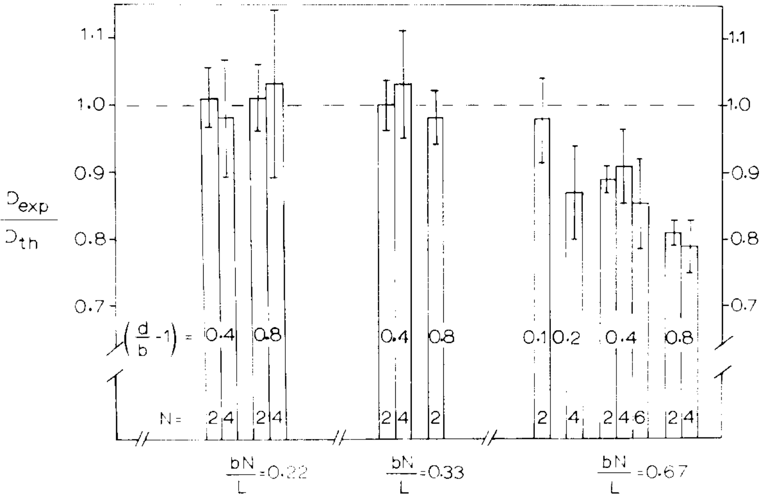

Fig. 3. Bar graph of $D_{\mathrm{exp}}/D_{\mathrm{th}}$ for three values of the parameter bn/l. At the base of the bar the values, are given for n (the number of spheres per pore) and $(d/b-1)$ which is proportional to the diameter of the pore available for movement of the center of the sphere (b, the ball diameter, is fixed at 2 Å while d, the pore diameter, is varied).

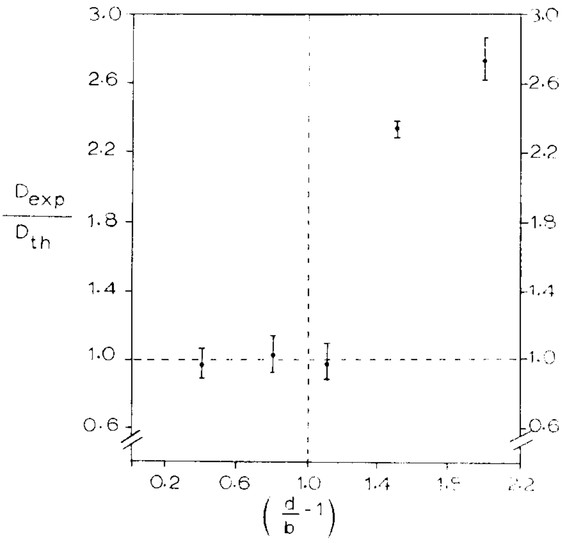

Fig. 4. Plot of $D_{\mathrm{exp}}/D_{\mathrm{th}}$ as a function of the relative pore diameter available to the sphere center $(d/b-1)$. For values of this relative diameter greater than one (dotted line) the pore is large enough for the spheres to get past each other.

Fig. 4 shows a plot of the ratio D_{exp}/D_{th} as a function of the reduced pore radius $(d/b-1)$ for an l of 36 Å with 4 spheres per pore. It can be seen that as long as the pore is narrow enough so that two balls cannot get past each other $(d/b-1 < 1)$ the experimental value of D does not differ significantly from the theoretical value. However, as expected, the experimental value of D gets much larger than D_{th} as the pore becomes large enough to allow two spheres to get past each other. In the limit as the pore diameter becomes very large, the spheres will be able to pass through the entire pore without colliding with any other spheres and D_{exp} will approach D°.

DISCUSSION

The general theoretical result obtained in this paper is that the diffusion coefficient (D_{th}) of the solvent in a pore that is so narrow that two molecules cannot get past each other is simply equal to the diffusion coefficient a molecule would have if it were the only molecule in the pore (D°) divided by the average number of molecules per pore $(D_{th} = D^{\circ}/n)$. Although this result is apparently very simple, it is a new and, in our opinion, important advance. It is very general. The basic assumption required is that the collisions between the solvent molecule in the pore can be approximated by one-dimensional collision dynamics. As was discussed above, even if the collisions are not one dimensional the theory will still be correct if the wall collisions are much more frequent than the molecule–molecule collisions. This is well supported by the molecular dynamic experimental results where, for example, in the 3.6-Å pore with a bn/l of 0.33 (a case in which one would expect the collisions to deviate significantly from the one-dimensional case) there was no significant difference between the theoretical and experimental values of D when only 7 % of the collisions were between spheres. Furthermore, the molecular dynamic results demonstrate that even for conditions when one would not expect the theory to be adequate (i.e. $d = 3.6$ Å, $n = 4$, $l =$ 12 Å, 73 % wall collisions) the experimental result is only about 20 % smaller than the theoretical prediction.

The molecular dynamic experiments confirm Eqn 3 for the special case in which D° is given by the Knudsen value. However, Eqn 3 is much more general than this and should be valid for other types of diffusion processes. For example, one would expect that if there are aqueous pores in cell membranes there should be strong chemical forces between the water and the pore wall so that the movement may be by a jump diffusion process which could be estimated from reaction rate theory [3]. Eqn 3 should be applicable to this case where D° is the diffusion coefficient calculated for the assumption that there are no other water molecules in the pore and n is the average number of water molecules per pore. One would expect that the one-dimensional assumption would be very good for this case because the strong chemical interactions between the water and the wall would dominate the paths of the trajectories and the details of the water–water collisions would not be important.

The derivation of Eqn 3 is based on the same assumptions that were used to derive the general results in the first paper of this series [1] (e.g. Eqn 2). Thus the agreement between the theoretical predictions of Eqn 3 and the molecular dynamic experiments is an important experimental test of a special case of the general theoretical results.

ACKNOWLEDGEMENTS

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