KINETICS OF DIFFUSION AND CONVECTION IN 3.2-Å PORES

Exact Solution by Computer Simulation

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ABSTRACT The kinetics of transport in pores the size postulated for cell membranes has been investigated by direct computer simulation (molecular dynamics). The simulated pore is 11 Å long and 3.2 Å in radius, and the water molecules are modeled by hard, smooth spheres, 1 Å in radius. The balls are given an initial set of positions and velocities (with an average temperature of 313° K) and the computer then calculates their exact paths through the pore. Two different conditions were used at the ends of the pore. In one, the ends are closed and the balls are completely isolated. In the other, the ball density in each end region is fixed so that a pressure difference can be established and a net convective flow produced. The following values were directly measured in the simulated experiments: net and diffusive (one-way) flux; pressure, temperature, and diffusion coefficients in the pore; area available for diffusion; probability distribution of ball positions in the pore; and the interaction between diffusion and convection. The density, viscosity, and diffusion coefficients in the bulk fluid were determined from the theory of hard sphere dense gases. From these values, the "equivalent" pore radius (determined by the same procedure that is used for cell membranes) was computed and compared with the physical pore radius of the simulated pore.

INTRODUCTION

The existence in cell membranes of water-filled channels or pores with a radius of about 4 Å explains a large number of observations more successfully than any other theory. The major success of the pore hypothesis is the fact that three relatively independent approaches all give approximately the same numerical value for the pore radius (Solomon, 1968). One difficulty with the pore hypothesis is that all of the methods used to measure the pore radius rely to some extent on the results of continuum fluid hydrodynamics, and the applicability of continuum theory to pores this small is questionable. Direct experimental investigation of this question is not possible now because cylindrical pores of uniform length and 4 Å radius determined by a method independent of continuum theory are not available. Recently,

the continuum theory has been checked in pores that were produced in mica by the etching of radiation tracks (Beck and Schultz, 1972). The pores that have been produced so far, however, have been at least 10 times larger than the postulated 4-Å pores of cell membranes. Even if a uniform cylindrical pore of 4 Å radius in mica were available, one would still need to know the details of the interaction between the mica and water. For example, if a layer of water molecules (about 2 Å in diameter) were bound to the wall, the effective radius of the pore would be reduced from 4 to 2 Å. At the present time, kinetic theory is not developed to the point where it can be used to answer the question of the validity of continuum mechanics in small pores. This is an especially difficult theoretical problem. The wall must be included directly in the calculations since the pore dimensions are only about three times the dimensions of the water molecule; but, because of the high densities involved (liquid), the approximations used in rarefied gas theory (which are primarily concerned with wall interactions) cannot be used (Cercignani, 1969; Kogan, 1969).

This paper describes an alternative approach to this question using the technique of molecular dynamics (Berne, 1971). This involves an exact computer simulation of an idealized pore and liquid. The water molecules are placed in the pore, given an initial set of positions and velocities, and the computer then calculates the actual paths of the molecules as they move about interacting with each other and the wall. Molecular dynamics has been used previously primarily to simulate equilibrium situations in the bulk liquid. In that type of problem one wants to represent an infinitely large system and the limitation in the number of atoms that can be followed by the computer is compensated for by periodic boundary conditions. In the present problem, however, the pore is small enough so that the entire system can be simulated by the computer. This problem would seem to provide an ideal application of the molecular dynamic approach.

The liquid molecules are modeled by hard, smooth spheres, 2.0 Å in diameter, that interact with each other like billiard balls. Since, in real liquids, it is the presence of an attractive potential between molecules which is responsible for the condensation from the gaseous to the liquid state, the absence of such an attractive potential in this model is a serious defect. The importance of the attractive potential in determining the pressure of the liquid is illustrated by the fact that the hard sphere fluid used in these calculations, with a density approximately the same as water, has a pressure of about 20,000 atm at room temperature. Alder and co-workers (Alder and Wainright, 1959, 1960; Dymond and Alder, 1966), however, have argued that, although the attractive potential is important in determining pressure, liquid transport properties such as diffusion, viscosity, and thermal conductivity should be relatively independent of the attractive potential and should depend primarily on the hard sphere interaction. They have verified this experimentally for atoms with relatively simple intermolecular potentials such as liquid argon, neon, and other inert atoms by comparing the theoretical transport coefficients derived from the simple hard sphere theory with the experimental values. Thus, a hard sphere liquid can be used as

a simplified model for the investigation of the general transport properties of a liquid. The hard sphere model cannot be expected to yield information which depends on the specific details of the molecular interaction. Water, for example, has an extremely complicated type of molecular interaction that deviates markedly from the assumptions of the hard sphere model (Rahman and Stillinger, 1971). The questions, however, that are being approached in this investigation are ones that should not depend critically on the details of the molecular interaction. For example, the equations that have been used by Solomon (1968) to determine the pore radius from measurements of the diffusive and hydraulic permeability of water should be valid for any liquid-hard sphere as well as water. In this paper the computer will be used to simulate an experiment in which a membrane with 3.2-Å cylindrical pores is placed between two bulk, hard sphere liquids and the diffusive and hydraulic permeability of the hard sphere liquid in the pore is measured. Then the "experimental" pore radius of this membrane will be determined by exactly the same procedure that is used to determine the pore radius of cell membranes. A direct test of the validity of this procedure of determining the pore radius is then provided by comparing this experimental radius with the actual pore radius of the simulated membrane (3.2 Å).

These computer results provide a direct test of the validity of the equations used to determine the pore radius as applied to experiments with a hard sphere liquid. Since these equations should be applicable to any liquid, however, the conclusions drawn from these hard sphere results should apply to liquids in general and can be extrapolated tentatively to water. This extrapolation must be further qualified by the assumption that water is still a liquid in pores of this size since the state of water in small pores is an unsettled question at present. In fact, the experimental results that support the pore theory provide the best available evidence that the pore water is a liquid.

The interaction between the balls and the wall is an important aspect of the modeling process. There are two basic types of interactions which represent opposite extremes. At one extreme the walls are perfectly reflecting and the balls bounce off the wall like billiard balls bouncing off the cushion (specular reflection). In this type of collision none of the momentum in the direction parallel to the wall is lost during the collision and one has complete "slip" at the wall. At the other extreme the balls are completely adsorbed on impact and reemitted in random directions (diffuse reflection). In this type of collision, all the momentum in the direction parallel to the wall is transmitted to the wall when the ball is adsorbed. In the usual analysis of boundary conditions walls are represented by a mixture of the two types. The higher the percentage of specular reflection, the less "friction" there is with the wall and the larger the flux will be for a given concentration or pressure difference. The higher the flux, the larger the calculated pore radius. In addition, if the wall condition were realistic, the ball should stick to the wall for a finite time before being reemitted. If the sticking time became long relative to the collision interval, the

pore area available to the free balls would be effectively reduced by the area occupied by the adsorbed balls. It is clear from this brief discussion that the effective pore radius depends on the dynamic interactions at the wall as well as the pore's anatomic dimensions. A purely diffusive reflection with instantaneous emission was used in these computer runs to simulate the actual pore wall. In addition, in one set of runs a purely specularly reflecting (nonadsorbing) wall was used to evaluate the effect of the wall interaction on the diffusion coefficient.

A scale drawing of the pore is shown in Fig. 1. In order to minimize the computer time it is necessary to keep the number of balls (and, therefore, the pore size) as small as possible. The pore is 11 Å long and 3.2 Å in radius. This radius is slightly less than the estimated radius of cell membrane pores and yet it is still large enough to allow the balls to pass by each other relatively freely. Two different conditions were employed at the ends of the pore. In one type, used to measure the diffusion

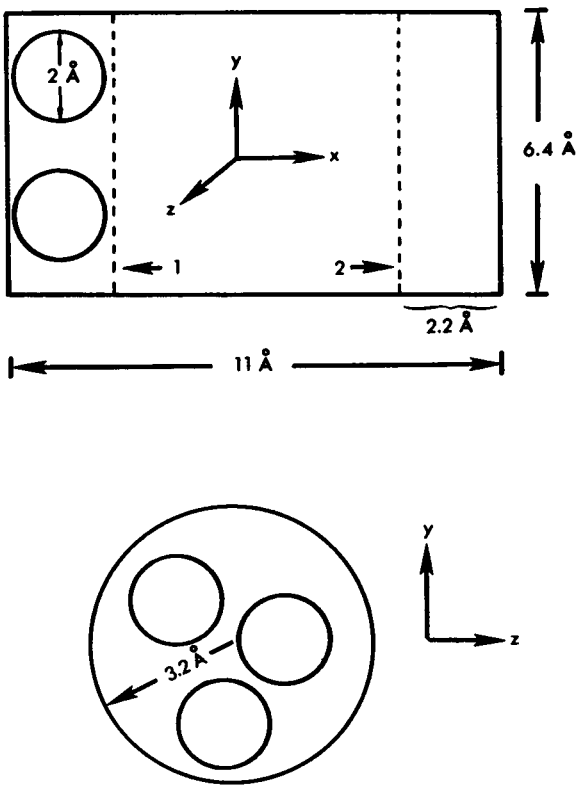

FIGURE 1 Scale drawing (side and end view) of the simulated balls and pore. The walls and ends (solid lines) are closed diffusely reflecting barriers. In the closed-end pore (see text) the one-way flux was determined by counting the balls that sequentially crossed the two dotted planes. In the open-end pore, the pore proper is the region between the dotted lines and the pressure and density was fixed in the end regions. The pressure difference measured at planes 1 and 2 produced a net flow through the pore proper.

coefficient, closed diffusely reflecting walls (indicated by the solid lines in Fig. 1) are placed at the pore ends. For this case, referred to as "closed end," the pore is a closed tube with a fixed number of balls and the diffusion coefficient can be calculated from the random motions of the balls by a procedure to be described below. In order to produce a net or mass ("convective") flow through the pore, a pressure gradient must be developed. In the real pore this pressure difference is established by the bulk solutions which are in contact with the two ends of the pore. In an attempt to model the real pore as closely as possible, a program is used which maintains a constant density, and therefore a constant pressure, in the two end regions indicated by the dotted lines in Fig. 1. For this case, referred to as "open end," the end regions are analogous to the bulk solutions and the pore proper is the region that is 6.6 Å long between the dotted lines in Fig. 1. When a pressure difference is desired, the number of balls in the two end regions are fixed and equal but the density of one region is decreased by increasing its length from 2.2 to 2.7 Å and the total pore length is increased to 11.5 Å. This end condition is probably more realistic than the closed-end condition. It will be shown that the different end conditions produce significant differences in the diffusive flux rates.

DETAILS OF THE COMPUTER CALCULATION

The numerical procedure used is almost identical with that described by Alder and Wainwright (1959). The balls are given arbitrary initial positions and velocities. The times each ball can move freely before hitting either another ball or the wall is calculated and the shortest of the times for all the balls (t_m) determined. Since no collisions occur during this interval, all the balls can be allowed to move along their free trajectories for this time period. At time t_m, a collision occurs. If a ball has hit a wall it is given a new velocity generated from a random probability distribution as described below. If the collision was between two balls the computer calculates the new velocity of each ball from the dynamics of a two-body, hard sphere collision. Then t_m is again calculated for the balls with their new velocities and the procedure is repeated.

When a ball hits a wall it is instantly reemitted with a random velocity that has the probability distribution that is most commonly used in the study of rarefied gases (Cercignani, 1969):

$$p(v, \theta, \phi)\, dv\, d\theta\, d\phi = \frac{1}{2\pi}\left(\frac{m}{kT}\right)^2 v^3 e^{-mv^2/2kT} \cos\theta \sin\theta\, dv\, d\theta\, d\phi, \qquad (1)$$

where θ is the angle between the velocity vector and the normal to the wall, ϕ is the angle of rotation about the normal, v is the absolute magnitude of the velocity, m is the mass per ball, k is Boltzmann's constant, and T is the temperature of the wall which was set at 313°K. The velocity is then transformed from the (v, θ, ϕ) coordinates to the (x, y, z) coordinates by a transformation which is a function of the ball position. The distribution given by Eq. 1 is the same as the distribution of velocities gas molecules would have if they were effusing through a small hole from a region where the molecules have a maxwellian (equilibrium) velocity distribution at temperature T. It is also the same as the distribution of velocities the balls striking the wall would have if they came from a region with a maxwellian distribution. If the balls striking the wall did not come from a region with a maxwellian distribution of velocities,

the average temperature of the balls in the pore should not be equal to that of the wall. The average ball temperature was determined from measurements of the kinetic energy per ball (which is equal to $3/2\ kT$) made at equal intervals throughout the run. The ball temperature was not significantly different from the wall temperature which is determined by the value of T (313°K) in Eq. 1 (see tables). This implies that the balls did indeed come from a region where they had a maxwellian velocity distribution.

The following procedure was used to maintain a constant density in the end compartments for the open-end case. If the center of a ball left the pore and entered the end compartment, it was removed; while if a ball left the end compartment and entered the pore, another ball was added to the end compartment. The ball was added to the far end of the compartment with the same y and z coordinates as the ball that left. The new ball's velocity was chosen randomly with the same probability distribution as for the balls leaving the wall (Eq. 1). This should equal the velocity distribution of the balls that leave the end compartment and should keep the temperature in the end compartment equal to that in the rest of the pore. The average velocity of the new balls and the balls entering and leaving the end compartments was measured and no significant differences were found. The average temperatures in the two end compartments was also measured during the run and it was found that they did not differ significantly from the average temperature for the whole pore even when the densities (or pressures) in the two ends were unequal. That is, the experiments in which a net flux was produced were isothermal and the only driving force was the pressure difference.

Since the new ball is placed in the end compartment without regard for the position of the remaining balls in the compartment, there is a good chance that the new ball overlaps another ball. When this happens the system is programmed so that the two overlapping balls do not interact with each other. They move about, colliding with the other balls, until, eventually, they no longer overlap and the normal interaction is established between the two balls.

The runs were performed at the two different densities of either about 11 or 20 balls in the whole pore (including the two end regions). With the open-end pore the number of balls in the pore proper fluctuates and is not fixed. At 20 balls per pore the density, in terms of molecules per unit volume, is several times the density of water. These two densities, however, are not directly comparable since the 2 Å ball diameter is less than the actual interaction range of water molecules and allows the balls more freedom of movement even though they are at a higher density. This is clearly seen by the fact that the diffusion coefficient of the balls even at the high density is two to three times larger than that of water.

Although the calculations were limited to a maximum of about 20 balls, the amount of computer time required limits the accuracy of the results. The computer time required per collision is proportional to N, the number of balls. Furthermore, in order to have a comparable accuracy at different densities, one needs approximately the same number of collisions per ball. Thus, the total number of collisions required is also proportional to N so that the overall time required is proportional to N^2. Since all the results are averages over the random fluctuations that occur in the molecular dynamic experiments, the problem of knowing the accuracy and statistical significance of the results is the same as in any real experiment. The longer the time of each run (experiment) and the larger the number of runs, the more accurate the results. It was decided rather arbitrarily that each experiment at the lower density would be run for 12,000 collisions and, in order to obtain approximately the same coefficient of variation, each experiment at the higher density was run for 24,000 collisions. The results for each experiment were completely independent of each other since a different random number generator was used to generate the probability distribution (Eq. 1) of the reflected balls in each run. For the case in which the walls were perfectly reflecting different initial positions of the balls were used in each run. The number of experiments and the standard error (SE,

given in the tables) provides a measure of reliability of the results. Usually, enough experiments were run to lower the SE to a value that was about 5% of the mean. The computer time on the CDC 6600 digital computer required for a single experiment was about 80 s at the lower density and about 280 s at the higher density.

RESULTS AND DISCUSSION

Accuracy of Calculation of Theoretical Pore Radius from the Diffusive Flux

In the first part of this section, a theoretical pore radius will be calculated from the flux measurements obtained in the computer experiments by exactly the same procedure that is used to obtain the pore radius of cell membranes. A comparison of this theoretical radius with the physical radius of the simulated pore should provide an estimate of the accuracy of this procedure. In the study of pores in cell membranes, the following procedure is used to derive the relationship between the one-way (diffusive) flux and the pore radius (Solomon, 1968). The diffusive flux (J_D) is described by:

$$J_D = AD\frac{\Delta c}{\Delta x}, \tag{2}$$

where A and D are the "effective" area of the pore and the effective diffusion coefficient in the pore. The most commonly used theoretical relationship between A and the physical pore radius (r) is:

$$A = \pi(r - a)^2, \tag{3}$$

where a is the radius of the ball. That is, the physical pore radius is reduced by the radius of the ball (a) so that A becomes equal to the area available to the center of the ball. The theoretical expression used to relate D to r is:

$$\frac{D}{D_b} = 1 - 2.104\left(\frac{a}{r}\right) + 2.09\left(\frac{a}{r}\right)^3 - 0.95\left(\frac{a}{r}\right)^5, \tag{4}$$

where D_b is the free diffusion coefficient in the bulk solution. Eq. 4 was derived by Faxen from a theoretical analysis of the friction exerted on a hard sphere of radius a falling under a gravitational force down the center of an infinite cylinder of radius r through a liquid of viscosity η. A more recent examination of this problem by Haberman and Sayre (1958) has shown that the Faxen equation (Eq. 4) is accurate up to values of a/r as large as 0.6, much larger than the value in these computer runs (1.0/3.2). The derivation of the Faxen equation assumes that the ball is very large relative to the molecular dimensions of the liquid which can then be treated as a continuum. These assumptions are obviously invalid in the limit of the diffusion of water in water and it is possible that Eq. 4 is not even approximately correct in this limit. The total conductance for diffusion through the pore, which is equal to the product of the diffusion and area factors, has been shown experimentally to be

accurately described by the product of Eqs. 3 and 4 in uniform circular pores as small as 80 Å (Beck and Schultz, 1972).

After substituting Eqs. 3 and 4 into Eq. 2, r can be determined if the values of J_D, Δc, Δx, a, and D_b are known. As in the cell membrane studies, the radius (a) of the molecules is given and is equal to 1 Å in the computer experiments. The experimental one-way diffusive flux (J_D) can be measured in the closed-end pore by considering the region of the pore between planes 1 and 2 (Fig. 1) and counting the number of balls entering this region across plane 1 that eventually leave the region by plane 2 and vice versa. For these two planes the value of Δx is 6.6 Å. The calculation of J_D must be corrected for the fact that the balls initially in the pore are not identifiable as actually having entered from either end. To compensate for this, the balls in the pore at the starting time are assigned a probability that they came from either end 1 or end 2 on the basis of their distance from that end. For example, if a ball leaving end 2 was one-third of the way in from end 1 at $t = 0$, this would be calculated as if two-thirds of a ball went from end 1 to end 2. This correction for the balls initially in the pore is negligible when there are 11 balls in the pore but becomes significant (about 15% of the total) at a density of 20 balls/pore. The one-way flux measurement is not begun until after a time interval long enough for each ball to have undergone about 100 collisions. The experimental values of J_D are shown in Table I.

The concentration in the pore is not well defined because the effective volume of the pore is not known. For example, should the effective radius of the pore be 2.2 Å (the area available to the center of the balls) or 3.2 Å (the physical pore area) or, more likely, some value in between. Fortunately, the concentration in the pore itself is not needed because the value of Δc that should be substituted in Eq. 2, in analogy to a cell membrane experiment, is the concentration difference in the bulk solutions that are in equilibrium with the ends of the pore. For the computer experiments in which one is measuring the one-way flux, Δc is just equal to the bulk value of the

TABLE I

CLOSED-END PORE

Wall condition	No. of runs	No. of balls in pore	Temperature	J_D	P/kT	n	D_b	Experimental			Theoretical	
								D	D/D_b	A/A_o	D/D_b (Eq. 4)	A/A_o (Eq. 3)
			K	10^{12} balls/s	$Å^{-3}$	$Å^{-3}$	10^4 cm^2/s	10^4 cm^2				
Diffuse reflection	12	11	313.2 ±1.5 (SEM)	0.167 ±0.007	0.161 ±0.002	0.0565 ±0.0006	1.84 ±0.02	1.27 ±0.03	0.69 ±0.023	0.47 ±0.04	0.405	0.472
Diffuse reflection	5	20	312.8 ±1.0	0.133 ±0.1	0.530 ±0.005	0.089 ±0.001	0.698 ±0.007	0.493 ±0.005	0.706 ±0.014	0.622 ±0.043	0.405	0.472
Specular reflection	5	11	276	0.273 ±0.005	0.167 ±0.001	0.0595 ±0.0006	1.72 ±0.02	2.23 ±0.03	1.30 ±0.02	0.44 ±0.01		

density n (as though all the balls on one side were labeled). Similarly, the value of D_b needed in Eq. 4 is the diffusion coefficient in the bulk solution in equilibrium with the pore. The following procedure is used to obtain the appropriate bulk value of the density and diffusion coefficient. From a modification of the theory of Enskog for dense gases, Dymond and Alder (1966) have obtained for the bulk, hard sphere liquid the functional dependence of the pressure, diffusion, and viscosity coefficients and the thermal conductivity on the density and radius of the hard spheres and the temperature. The dependence of the pressure (P/kT) and the diffusion (D_b/D_0) and viscosity (η/η_0) coefficient on the density (n) for the 2-Å spheres is shown in Fig. 2. D_0 and η_0 are the diffusion and viscosity coefficients of an ideal (dilute) hard sphere gas which is known from the classical Chapman-Enskog theory:

$$D_0 = 0.382\ (kT/\pi m)^{1/2}/n\sigma^2; \qquad \eta_0 = 0.317\ (mkT/\pi)^{1/2}/\sigma^2,$$

where σ is the ball diameter (Chapman and Cowling, 1970). If the pressure of the bulk fluid in equilibrium with the pore liquid is known, then n (or Δc) and D_b can be obtained from Fig. 2.

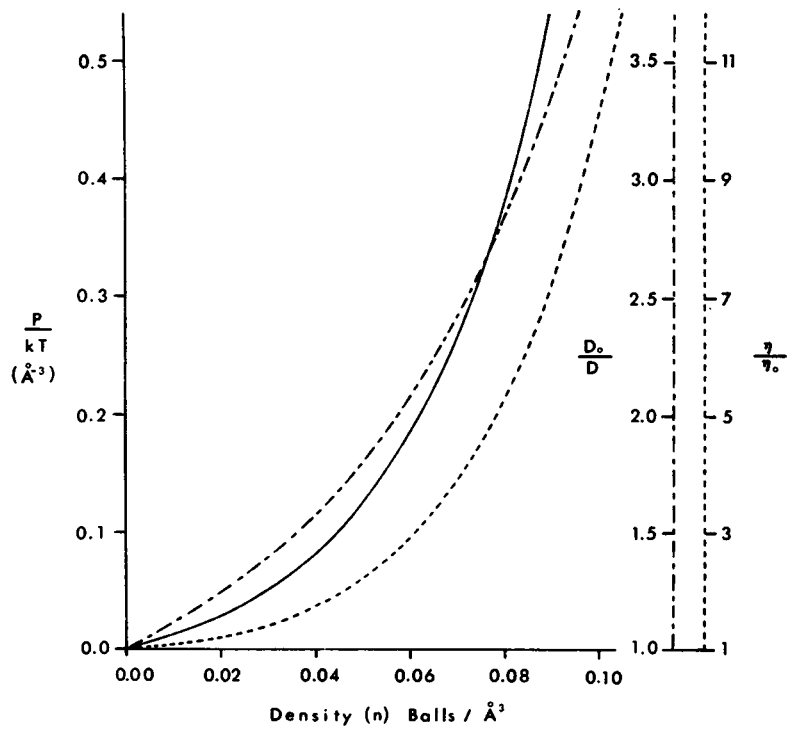

FIGURE 2 Dependence of pressure (P/kT) (——), diffusion coefficient (D_0/D) (—·—), and viscosity (η/η_0) (----) in the bulk solution on the density (n). D_0 and η_0 are the values of the diffusion coefficient and viscosity for a dilute gas (see text).

In this equilibrium case, the pressure in the bulk fluid must be equal to the pressure in the pore. The pressure in the pore can be determined by measuring the actual momentum flux in the axial (x) direction:

$$P = \frac{\text{force}}{\text{area}} = \frac{m}{A}\frac{\sum \Delta v}{t}$$

where $\sum \Delta v$ is the net flux of velocity across a fixed plane occurring in a time interval t, A is the area over which the force is exerted and is equal to $\pi \times (2.2)^2$ (since the center of the balls must remain at least 1 Å from the wall) and m is the mass of the ball which is equal to the mass of a water molecule ($18\ \text{g}/6.02 \times 10^{23}$). At the closed ends of the pore Δv is simply the difference between the incident and reflected velocity. The pressure was also measured at the two planes 2.2 Å in from the closed ends (see Fig. 1) since in later runs, when a pressure difference is maintained, it is the pressure at these two planes that will be needed. At these planes Δv is the sum of two terms: a kinetic term which represents the momentum flux due to balls actually crossing the plane, and a collision term which represents the momentum flux resulting from a collision between two balls on opposite sides of the plane. When the ends are closed, the system is at equilibrium and the pressure at the four positions should be equal. This was confirmed for the molecular dynamic experiments.

The directly measured value for the pressure (P/kT) and the corresponding value of D_b and n determined from fig. 6 are given in Table I. Now, all the values that are needed in Eqs. 2–4 to calculate the theoretical r are known. This value of r is 3.7 Å at the lower density and 4.0 Å at the higher density. These values are, respectively, 16 and 25% larger than the actual physical pore radius of 3.2 Å.

In the above calculations the pore radius was an unknown and the accuracy of the procedures used to determine the pore radius of cell membranes was evaluated. In this section the physical pore radius (3.2 Å) will be substituted into Eqs. 3 and 4 and the predicted values of the diffusion coefficient (D) and area (A) in the pore will be compared with the experimental values determined directly in the computer experiments. The one-dimensional diffusion coefficient (in the axial or x direction) in the pore can be directly determined from the following theoretical expression:

$$D = \lim_{t\to\infty} \frac{\overline{L^2}}{2t}, \tag{5}$$

where L is the displacement of the ball in the x direction that occurs in time t and the bar signifies that an average is taken over a large number of balls (Egelstaff, 1967). For Eq. 5 to be valid, the pore must extend infinitely in each direction. This is approximately satisfied by programming the computer so that when a molecule is reflected from an end wall it is treated as if it actually passed through it and entered a mirror image of the pore that it left. This should be a fairly accurate approximation

since collisions with the ends account for less than 8% of the total number of collisions. Also, if an error were introduced by this approximation, one would expect that there would be some correlation between the number of collisions a ball had with the end wall and its diffusion coefficient. No correlation was observed. For the density of 11 balls per pore L was calculated for each ball at time intervals (t) of 2, 4, 6, 8, and 10×10^{-12} s. (In 1×10^{-12} s each ball undergoes about six collisions.) At the higher density (20 balls/pore) the balls have a higher frequency of collision and appropriately shorter time intervals were used. L at each time interval was averaged for each ball and then for all the balls and the diffusion coefficient was calculated from Eq. 2 for the five different values of t. It was found that the diffusion coefficients calculated for each of the three larger time intervals were not significantly different. This implies that these times were large enough to be considered infinite and the diffusion coefficients (D) which are given in Table I are equal to the average at these three time intervals. If this experimental diffusion coefficient is substituted into Eq. 2 a value for the experimental area (A) or the ratio A/A_0, where A_0 is the physical pore area ($\pi \times 3.2^2$), can be obtained (Table I). It can be seen from Table I that although there is no significant difference between the experimental value of D/D_b at the low (0.69) and high (0.706) density, the experimental value of A/A_0 increases from a value of 0.47 at the low density to a value of 0.622 at the high density. Also shown in Table I is a comparison of the experimental values of D/D_b and A/A_0 with the theoretical values predicted by Eqs. 3 and 4 (with $r = 3.2$ Å). It can be seen that at the higher density Eq. 3 overestimates the restrictive effect of the wall on the area and, at both densities, Eq. 4 overestimates the restrictive effect of the wall on the diffusion coefficient.

The experimental value of the area that was derived above is a mathematical entity defined by Eq. 2 and it may not have any geometrical significance. To test its significance, a series of runs were performed with a pore that had specularly reflecting (nonadsorbing) walls (Table I). It can be seen from Table I that although the diffusion coefficient was almost doubled when the walls were specularly reflecting, there was no significant change in the area. This lends support to the geometrical significance of the area defined by Eq. 2.

Accuracy of Calculation of the Theoretical Pore Radius from the Convective Flux

It will be assumed that the convective flow produced by a pressure difference (ΔP) can be described by the following form of Poiseuille's equation:

$$J_b = \frac{\bar{n}\pi r_p^4 \Delta P}{8\bar{\eta}L}, \qquad (6)$$

where r_p is the hydraulic pore radius; $\bar{n}$ and $\bar{\eta}$ are the mean values of the density and viscosity, respectively; and L is the length of the pore. Since the pressure dependence

of n and η is known (Fig. 2) a more exact numerically integrated form of Eq. 6 could have been used. However, since the variation in n and η between the two ends is only about 10% of their mean value (Table II) and since they are both approximately linear over this limited range of pressure (Fig. 2) the error introduced by the use of the mean values is less than 2%. In order to establish a pressure difference, the open-end pore was used with different densities (and therefore different pressures) fixed in the two ends. The pressure at planes 1 and 2 (Fig. 1) was measured directly from the momentum flux by the previously described procedure. The pressure difference between the two ends is equal to ΔP and the length (L) over which the pressure difference is applied is 6.6 Å (Fig. 1). As in the cell membrane studies, it will be assumed that the density and viscosity in the pore are equal to the density and viscosity in the bulk fluid at the same pressure. From the measured values of the pressure at planes 1 and 2, the bulk values of n and η at the two ends can be determined from Fig. 2 and the mean values used in Eq. 6 are just the average of these end values. The convective flux (J_b) is determined simply by counting the net flux of balls across some plane in the pore. Substituting these values (shown in Table II) into Eq. 6 and solving for r_p one obtains a value of r_p of 3.24 Å at the low density and 3.26 Å at the high density. These values are in remarkable agreement with the physical pore radius of 3.2 Å.

Although the good agreement between the physical pore radius and r_p probably indicates that the continuum assumptions underlying eq. 6 are qualitatively valid in this pore, the quantitative agreement of the two radii is probably the result of a chance canceling of several effects that, individually, should have produced an error in the calculated radius. For example, the slip correction has been ignored in Eq. 6. This is an effect that becomes important in rarefied gases when the mean free path of the molecules approaches the dimensions of the pore (Kennard, 1938). The mean free path in these studies (0.67 and 0.32 Å at the low and high density, respectively) is long enough relative to the pore radius to make the slip effect important. To cor-

TABLE II

OPEN-END PORE WITH PRESSURE DIFFERENCE

No. of balls in pore	No. of runs	Temperature	P_1/kT	P_2/kT	n_1	n_2	η_1	η_2	Net flux	One-way flux ($2 \rightarrow 1$)	r_p
		K	*Å*$^{-3}$	*Å*$^{-3}$	*Å*$^{-3}$	*Å*$^{-3}$	10^{-3} *poise*	10^{-3} *poise*	10^{12} *balls/s*	10^{12} *balls/s*	*Å*
11.45	6	307.0	0.163	0.140	0.0567	0.0530	1.35	1.12	0.273	0.119	3.24
±0.04 (SEM)		±3.0	±0.002	±0.002	±0.0005	±0.0006	±0.02	±0.02	±0.013	±0.009	±0.13
19.82	4	314.7	0.448	0.394	0.0848	0.0810	6.10	5.45	0.440	0.042	3.26
±0.16		±1.0	±0.005	±0.007	±0.008	±0.0014	±0.05	±0.08	±0.039	±0.008	±0.06

rect for the slip effect in rarefied gases another term is added to Eq. 6 which, in effect, increases the pore radius by an amount that is approximately equal to the mean free path. Since, however, there is no good theoretical treatment of the wall effects in dense gases or liquids, the addition of the slip term would introduce another experimental parameter (in addition to r_p) which could not be independently measured. For this reason it was decided to use Eq. 6 directly without the slip term but with the understanding that r_p includes the slip effect. This enlargement of r_p due to the slip effect may explain why r_p is not less than the physical radius as might have been expected for the same reason that the area available for diffusion is less than the physical area (Table I).

In the determination of the theoretical pore radius of cell membranes, Solomon (1968) and others regard the radius (r_p) calculated from Eq. 6 as a restricted radius which is related to the physical pore radius (r) by the following equation:

$$r_p^2 = r^2\left[2\left(1-\frac{a}{r}\right)^2 - \left(1-\frac{a}{r}\right)^4\right]\left(\frac{D}{D_b}\right), \qquad (7)$$

where a is the radius of the balls and D/D_b is the Faxen correction (Eq. 4). The term in brackets in Eq. 7 is a correction for the reduced area available to the balls due to their finite radius. Although some correction of this form seems reasonable, it might not be necessary because, as was pointed out above, the slip effect may have already compensated for it in Eq. 6. The use of the factor (D/D_b) in Eq. 7, which is a correction for the friction between the balls and the wall, is much more questionable since the effect of the wall is already directly involved in the derivation of Poiseuille's equation. Whatever the basis of these corrections, they remain essentially empirical and these molecular dynamic experiments provide a direct test of their applicability. Since the radius calculated directly from Eq. 6 without any corrections (Table II) is already a good estimate of the physical pore radius, it is not surprising that when the value of r_p determined from Eq. 6 is substituted into Eq. 7, the value obtained for the "corrected" radius (4.6 Å) is much too large. Thus, for this hard sphere liquid, the radius calculated directly from Poiseuille's equation provides a good estimate of the physical pore radius while the procedure that is normally used for cell membranes yields a radius that is 40% too large.

If there were no pores in the membrane and the solvent molecules were simply dissolving in and diffusing through the membrane as a result of the activity difference produced by the pressure difference, then the diffusion equation should be used to describe the kinetics of net flow rather than Poiseuille's equation. For the same driving force (activity difference) the rate of flow predicted by Poiseuille's equation is much greater than that predicted by the diffusion equation. This observation provides one of the most important qualitative tests for pores (Solomon, 1968): if pores are present the flow produced by a pressure gradient (either hydrostatic or osmotic) should be larger than the flow predicted by the diffusion equation. These

molecular dynamic experiments provide a good example of this effect. The theoretical diffusive flux can be obtained by substituting into Eq. 2 the factor AD/L obtained in experiments in which there was no pressure difference (Table III, see below) and the observed difference in concentrations (n) between the two ends of the pore (Table II). The theoretical diffusive flux obtained by this procedure is only 6% (at the lower density) and 2% (at the higher density) of the observed flow. Thus even in a pore only 3.2 Å in radius the flow produced by a pressure difference is much greater than would be predicted for a diffusive type of transport.

For the determination of cell membrane pore size, Eq. 6 is usually further modified by the addition of the theoretical diffusive flux to the Poiseuille term (Solomon, 1968). Since, as was shown above, the diffusive term is at most 6% of the total flux, its omission does not significantly affect the calculation of the pore radius. Moreover, there is no good theoretical justification for adding the diffusive term. If the continuum assumptions are valid, then all the mass flux is accounted for in the derivation of Poiseuille's equation and it would be incorrect to add an additional flux. If the continuum assumptions are invalid, as they are in these small pores, then the Poiseuille equation must be modified. There is no reason, however, to expect that the addition of the diffusive term is the correct way to modify it. In any case, it should be recognized that the added diffusive term is an empirical correction that, at the present time, cannot be supported either by experimental or theoretical considerations.

Equilibrium Distribution of Balls in the Pore

One might expect that at equilibrium the center of the balls would be evenly distributed throughout the pore and the probability of finding a ball center per unit volume would be a constant, independent of position. As is shown in figs. 3–6, however, the walls of the pore actually produce a marked ordering of the ball positions in the closed-end pore. Figs. 3 and 4 show the probability per unit length (angstroms) of finding the center of a given ball at different distances from the end wall. Since the pore is symmetrical about the center, only one-half of the pore is shown. The density of ball centers shows a marked increase as the balls approach the end wall, peaking at a position 1 Å from the end wall, the closest the balls (1 Å in radius) can approach the wall. There is another much smaller but significant peak at about 3.7 Å at the lower density (Fig. 3) and two additional peaks at about 3.7 and 5.5 Å at the higher density (Fig. 4). The easiest way to understand this effect is to consider the limiting case where the balls are packed into the pore as tightly as possible. In this case the centers of the balls would only be found at a few definite distinct positions which would depend on the arrangement of the packing, and the probability of finding a center of a ball at the other positions would be zero. In this limit, the probability distribution would be a series of spikes (delta functions). As the density is decreased, the packing becomes looser and more disordered and the

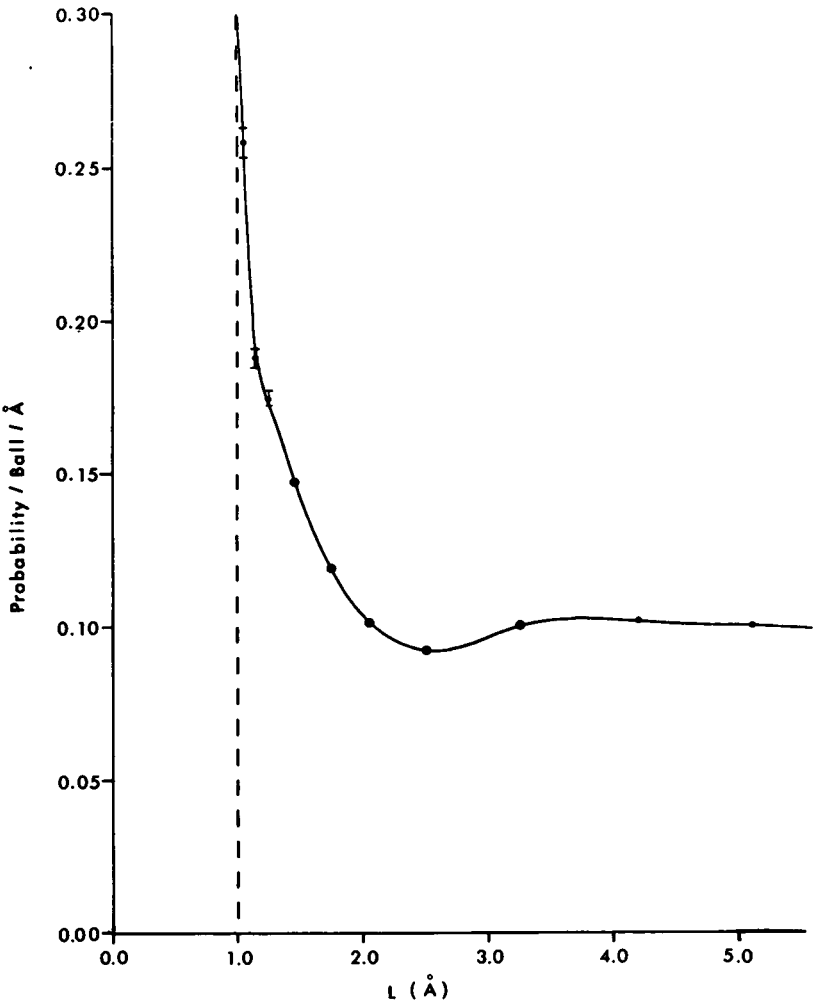

FIGURE 3 Probability distribution of finding the center of a particular ball at different distances from the end of the pore for a pore that contains 11 balls. The dotted line indicates the position of the ball center when the ball (1 Å in radius) is touching the end wall. Since the pore is symetrical, only half of the pore is shown. The cross bars bracketing the first three points indicate ±1 SE. If no cross bars are shown, then the size of the point indicates ±1 SE.

spikes tend to spread out. As is expected, since it is the wall that is responsible for the ordering, the effect decreases with increasing distance from the wall. Shown in Figs. 5 and 6 is a similar plot of the probability of finding a given ball per unit area (square angstroms) as a function of the radial position. At the lower density (Fig. 5) the ball centers tend to be concentrated at the wall of the pore while at the higher density (Fig. 6) the ball centers concentrate both at the walls and at the center of the pore. One implication of the ordering effect produced by the walls is that if

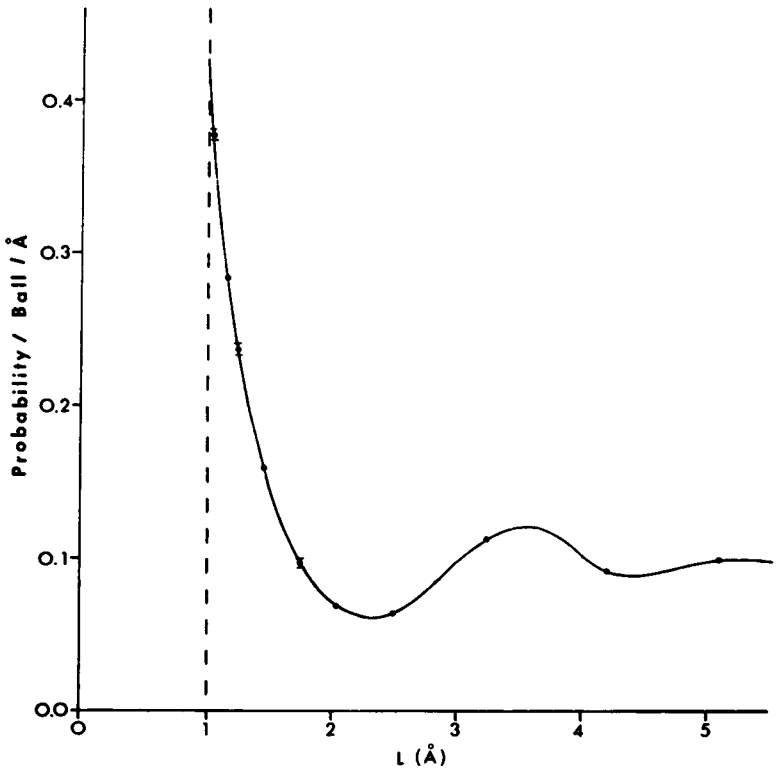

FIGURE 4 Same as Fig. 3 except that there are 20 balls in the pore.

water has a tendency to form solid-like structures, this tendency might be either increased or decreased depending on whether or not the pore size and shape was compatible with the molecular arrangement of the solid structure.

Effect of Pore End Condition on the Diffusion Coefficient

As was indicated in the introduction, two different end conditions were used. In the first, the ends were closed and the balls in the pore were completely isolated as they executed their random motions. In the second the number of balls in the two end regions (indicated by the dotted lines in Fig. 1) was kept constant by removing or adding balls as they entered or left the end regions. Since cell membrane pores would be in contact with two bulk solutions which may behave somewhat like the end regions of the open-end pore, it is of interest to compare the diffusion coefficient determined above for the closed-end pore with the diffusion coefficient in the open-end pore when the pressures in the two end regions are equal (that is, the balls are at equilibrium). The diffusion coefficient in the open-end pore cannot be determined (as it was for the closed-end pore) from Eq. 5 because balls are continually leaving and entering the system. If it is assumed, however, that the open- and closed-end pores

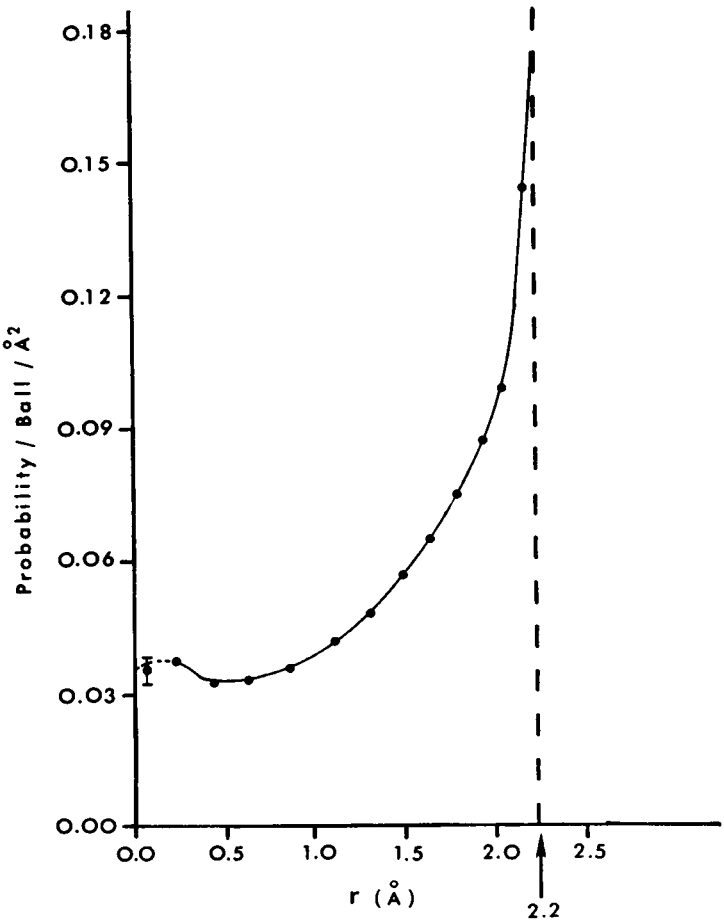

FIGURE 5 Probability per unit area of finding the center of a particular ball at different radial distances from the center of the pore for a pore that contains 11 balls. The dotted line indicates the position of the ball center when the ball is touching the side wall. Cross bars indicate ±1 SE (see Fig. 3).

with the same ball density (n) have equal effective pore areas, then the ratio of the diffusion coefficient in the two pore types is equal to the ratio of the one-way fluxes, which can be measured in both pore types. In the open-end pore, the number of balls in the whole pore is not fixed by the initial conditions as it is in the closed-end pore; and, although the experiments were designed so that the average density in the open-end pore would be similar to the density in the closed-end pore, the two densities were not exactly the same (see Table III). The measured one-way fluxes in the open-end pore have been corrected for the small effects of this density difference by using the density dependence of the flux (Table III) to extrapolate the flux to the corresponding closed-end densities (Table I). The adjustment is small (about the same size as the SE) and the conclusions do not depend on this correction.

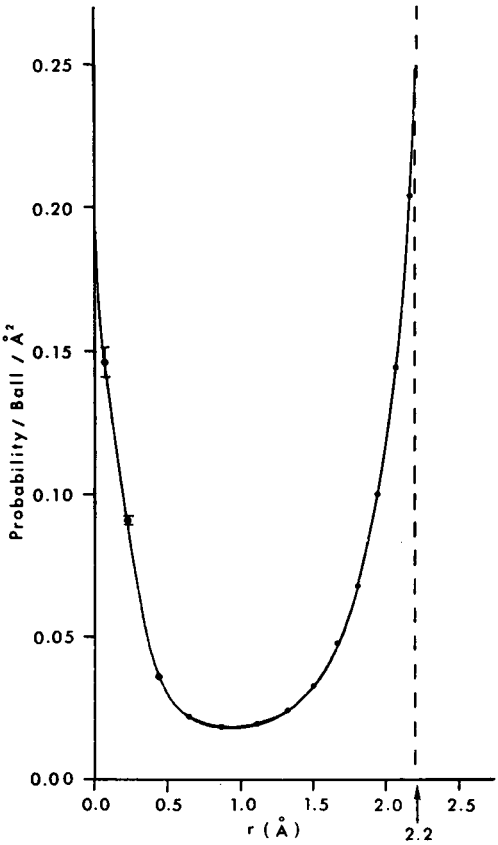

FIGURE 6 Same as Fig. 5 except that there are 20 balls in the pore.

As is seen in Table III, the ratio of the diffusion coefficients for the open- and closed-end pores as calculated from the adjusted fluxes at the lower density is 1.53. That is, the diffusion coefficient in the open pore is significantly greater than that in the closed-end pore. This is most likely due to the fact that spontaneous net fluxes can be generated in the open-end pore but not in the closed-end pore. That is, due to random fluctuations in the density (and, therefore, pressure) there will be net fluxes of balls through the open-end pore that alternate in direction so that the net flux becomes zero when it is averaged over a long enough time period. The movement of balls produced by these alternating net fluxes will be added to the movement due to the normal diffusion process and this effect could possibly produce the increase in the diffusion coefficient that is observed in the open-end pore. This interpretation is supported by the fact that at the higher density, when the fluctuations are smaller, the ratio of the fluxes is 1.12, much closer to unity (Table III). Since the end conditions of the open-end pore are similar to those that exist in a

TABLE III

OPEN-END PORE NO PRESSURE DIFFERENCE

No. of balls in pore	No. of runs	Temperature	P/kT	n	J_D, observed	J_D, adjusted	$\frac{D_{open}}{D_{closed}}$
		K	$\AA^{-3}$	$\AA^{-3}$	10^{12} balls/s	10^{12} balls/s	
12.24	6	312.7	0.179	0.0590	0.253	0.255	1.53
±0.13 (SEM)		±3.9	±0.002	±0.0006	±0.01	±0.01	±0.12
20.76	5	313.4	0.501	0.0875	0.158	0.149	1.12
±0.14		±0.4	±0.004	±0.0007	±0.01	±0.01	±0.15

real pore, this increased flux rate may have some practical significance. Such an effect would become especially important if, for some reason, the normal diffusive process became very small. For example, if the pore was narrowed so that balls could not pass each other or if the pore water assumed an essentially solid-like structure, then the normal diffusive rate would be greatly reduced, while the flux produced by the spontaneous net fluxes would still occur and could become the dominant transport mechanism.

Interaction between Diffusion and Convection

It is well known that the one-way diffusive flux will be reduced if a convective (net) flux in the opposite direction is developed (see below for derivation). This is an important effect since it provides a qualitative test for the existence of pores. That is, if diffusion and convection are occurring in the same pore, then the one-way flux determined by tracers (i.e., D_2O) should decrease when a convective flux in the opposite direction is produced, for example, by an osmotic gradient. There have been several unsuccessful attempts to observe this interaction in the red cell. Because of several experimental complications, however, the results are inconclusive (Forster, 1971). There is, however, definite evidence of an interaction in these molecular dynamic experiments. The one-way flux rate in the direction opposite the convective flux is (Table II) 0.119 and 0.042 $\times$ 10^{12} balls/s at the low and high density, respectively. The corresponding one-way fluxes that would have been expected if there were no convective flux (determined from the results shown in Table III with adjustments for the slightly different densities) are 0.25 and 0.18 $\times$ 10^{12} balls/s. That is, the convective flux reduces the diffusive flux to a value less than one-half (at the low density) and one-fourth (at the high density) its value in the absence of the convective stream.

This experimental observation can be compared with the amount of interaction that is predicted theoretically. In the steady state, the net amount of tracer entering an infinitesimal region by convection must be just balanced by the net amount entering by diffusion:

$$\frac{J_b}{\bar{n}}\frac{dc}{dx} = \bar{D}A\frac{d^2c}{dx^2}, \qquad (8)$$

where $\bar{D}$ is the average diffusion coefficient, J_b is the net flux, A is the area, $\bar{n}$ is the average density, and $c(x)$ is the tracer concentration which, in this case, is taken as the concentration of balls that have entered from end 2. The integration of Eq. 8 from 0 to L (the length of the pore) with the boundary conditions $c(0) = 0$ and $c(L) = n_2$ (the ball density at end 2) yields:

$$c(x) = \frac{n_2}{e^{\alpha L} - 1} (e^{\alpha x} - 1), \tag{9}$$

where $\alpha = J_b/(\bar{D}A\bar{n})$. The one-way flux ($J$) in the direction 2 $\rightarrow$ 1 is given by

$$J = DA \frac{dc}{dx}\bigg|_{x=0} = \frac{n_2}{\bar{n}} \frac{J_b}{e^{\alpha L} - 1}. \tag{10}$$

The factor $A\bar{D}/L$, which appears in Eq. 10, can be determined from the experiments in which there was no convective flux (Table III). The theoretical one-way flux predicted by Eq. 10 for the conditions of the experiments that had a convective flux (Table II) are 0.137 and 0.042 $\times$ 10^{12} balls/s at the low and high density, respectively. The good agreement between these predictions and the observed values (Table II) indicates that Eq. 8 is an accurate description of the interaction that occurs in small pores.

CONCLUSION

Although a hard sphere fluid may be a poor model of water, these molecular dynamic experiments provide the most direct information currently available on the behavior of a liquid in a pore of the size that is postulated to exist in cell membranes. The general overall picture suggested by the results summarized below is that the continuum hydrodynamic theory can be extrapolated, qualitatively, to pores 3.2 Å in radius. To this extent, the following results lend support to the procedures used to determine the pore radius of cell membranes.

(*a*) An examination of the kinetics of diffusion shows that the diffusion coefficient in the pore is reduced to about 70% of its value in the free solution and the available area for diffusion is about 47% (at the low density) and 62% (at the high density) of the actual physical pore area. These restrictive effects of the wall on the area and diffusion coefficient are in qualitative agreement with the continuum theory that has been used to determine the pore radius of cell membranes. Quantitatively, however, the continuum theory significantly overestimates the restrictive effects of the wall and, when applied to the molecular dynamic flux data, yields a pore radius which is larger (3.7 and 4.0 Å at the lower and higher density, respectively) than the physical pore radius (3.2 Å).

(*b*) The radius calculated from a direct application of Poiseuille's equation to the convective (bulk) flow produced by a pressure gradient does not differ significantly from the physical pore radius. The procedure normally used in the study of cell membranes is to correct the radius calculated from Poiseuille's equation for the assumed restrictive effect of the walls. If this "correction" is applied in these

experiments the theoretical radius (4.6 Å) becomes much larger than the physical radius. The flow rate produced by a pressure difference is 17 (at the low density) and 50 (at the high density) times as large as the flow that would be expected if a noninteracting diffusive mechanism were involved, as in a lipid film.

(*c*) When a pore end condition was used that allowed spontaneous fluctuations in the pore density (as occurs in real pores) the diffusion coefficient was larger than when the pore was completely isolated and the density fixed. This effect, which to my knowledge has not been described before, may have some biologic importance.

(*d*) The walls of the pore impose a marked ordering on the liquid so that at the high density the probability per unit cross-sectional area of finding the center of a ball at the wall or center of the pore is about 10 times larger than the probability of finding a ball midway between the wall and the center.

(*e*) The diffusive flux is reduced when a convective flow in the opposite direction is imposed. The magnitude of the interaction is in good agreement with the predictions of classical continuum theory.

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