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WATER PERMEATION THROUGH THE LIPID BILAYER MEMBRANE

TEST OF THE LIQUID HYDROCARBON MODEL

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Summary

According to the liquid hydrocarbon model, the lipid bilayer is viewed simply as a thin slice of bulk hydrocarbon liquid. This allows the water permeability of the bilayer to be calculated from bulk properties. In this paper the prediction of the liquid hydrocarbon model is compared with the known water permeability coefficient of the glycerol monoolein/*n*-hexadecane bilayer (Fettiplace, R. (1978) Biochim. Biophys. Acta 513, 1—10). As the alkyl chain of glycerol monoolein is equivalent to 8-heptadecene, the water permeability coefficient of 8-heptadecene/*n*-hexadecane mixtures was measured for temperatures between 20 and 35°C. The mole fraction of *n*-hexadecane in the bulk liquid was chosen at each temperature to match the known mole fraction of *n*-hexadecane in the bilayer (White, S. (1976) Nature 262, 421—422). The predicted water permeability coefficient agrees with the measured value at 32°C but is 40% above the measured value at 20°C. The apparent activation energy predicted by the liquid hydrocarbon model is 9.0 ± 0.3 kcal/mol, while the measured value is 14.2 ± 1.0 kcal/mol. The failure of the liquid hydrocarbon model probably results from a different molecular organization of the hydrocarbon chains in the bilayer and in the bulk liquid.

Introduction

A simple model has been used to predict the water permeability coefficient of unmodified lipid bilayers [1—4]. This model, the liquid hydrocarbon model, views the lipid bilayer as simply a thin slice of the appropriate bulk liquid hydrocarbon. Water passes through the membrane by dissolving into

the hydrocarbon, diffusing across it, and redissolving into the aqueous phase on the other side. The rate-limiting step is assumed to be the diffusion within the hydrocarbon. In this model the permeability coefficient is simply:

$$P = \frac{KD}{d} \tag{1}$$

where K is the partition coefficient of water into the hydrocarbon, D is the diffusion coefficient of water in the hydrocarbon, and d is the thickness of the hydrocarbon layer. The bulk liquid hydrocarbon used to represent the lipid bilayer interior has been *n*-hexadecane, as this aliphatic hydrocarbon is the closest analog to the alkyl chains of the bilayer lipids for which data on K and D exist [5,6].

While the liquid hydrocarbon model gives reasonable predictions (within a factor of about 2) for the observed lipid bilayer permeability coefficients, a stringent test of the model has not been possible for several reasons. Most measurements have been made on egg phosphatidylcholine or mixed brain lipids, with or without cholesterol. Even without cholesterol added, there is considerable variability in the measured permeability coefficients [3,7—10], probably reflecting variations in the lipid constituents and in the physical state of the bilayer [11]. The addition of cholesterol to the membrane-forming solution [1,3,4,8,9,12] leads to an unknown amount of cholesterol in the lipid bilayer; moreover the mol fraction of cholesterol in the bilayer probably varies with temperature, complicating any interpretation of the temperature dependence of water permeability in such membranes [4,12]. All the membranes mentioned also contained some solvent required for formation of the planar lipid bilayers [13]; the permeability coefficient probably depends on the type of solvent present in the lipid bilayer. Finally, while *n*-hexadecane is the best available bulk liquid hydrocarbon analog of the membrane interior, it clearly cannot represent cholesterol, and it differs from the acyl chains of the lipids both with respect to saturation and number of carbons: (the acyl chains of egg phosphatidylcholine contain from 14 to 20 carbons, with from 0 to 3 unsaturated bonds per chain; approximately half the chains are fully saturated [9]).

Recently, Fettiplace has made precise measurements on the temperature dependence of the water permeability of lipid bilayers composed of glycerol monoolein in *n*-hexadecane [14]. The thickness and the glycerol monoolein : *n*-hexadecane molar ratio are well known for this bilayer over the same temperature range [15,16]. This information allows a stringent test of the liquid hydrocarbon model, provided the appropriate bulk liquid hydrocarbon data are available. Such data were obtained in the experiments described in this paper.

Materials and Methods

Determination of water permeability coefficient for hydrocarbon liquids

According to the liquid hydrocarbon model, both the partition coefficient K and diffusion coefficient D are the same for the interior of the lipid bilayer membrane and for the appropriate bulk hydrocarbon liquid. From

Eqn. 1 the permeability coefficient of the membrane can be predicted from measurements on the hydrocarbon liquid:

$$P_m = \frac{d_h}{d_m} P_h \tag{2}$$

where the subscripts, h and m, refer to the liquid hydrocarbon and the lipid membrane, respectively.

The liquid hydrocarbon permeability coefficient is obtained from the rate of mass transport of water through a thin layer of liquid hydrocarbon, following the procedure of Schatzberg [6]. A layer of oil is floated on water in a cup of known cross-sectional area. This cup is placed on the weighing pan of a recording microbalance, while an identical cup filled with oil alone is placed on the taring pan. The microbalance system is maintained at essentially zero relative humidity and constant temperature. The rate of weight loss is equal to the mass transport of water through the oil. From Fick's first law of diffusion, this weight loss is:

$$\frac{dm}{dt} = -DA \frac{dc}{dx} \tag{3}$$

where dm/dt is mass transport rate of water, D is the diffusion coefficient of the water, A is the cross-sectional area of the liquid hydrocarbon layer and dc/dx is the concentration gradient of the water across the hydrocarbon layer. In the steady state, the concentration gradient is given by $(c_w^h - c_a^h)/d_h$, where c_w^h is the concentration of water in the hydrocarbon at the hydrocarbon/water interface and c_a^h is the concentration of water in the hydrocarbon at the air/hydrocarbon interface. Since the air is maintained at essentially zero relative humidity, c_a^h is set equal to 0. Since $K = c_w^h/c_w^w$, where c_w^w is the concentration of water in the aqueous phase below the hydrocarbon layer, Eqn. 3 can be combined with Eqn. 1 to give:

$$P_h = \frac{KD}{d_h} = -\frac{dm/dt}{Ac_w^w} \tag{4}$$

Microbalance measurements

The cylindrical weighing cups were precisely machined from custom-molded blocks of polytetrafluoroethylene (Thermech Engineering Corp., Anaheim, CA). The cross-sectional area of the cups was 0.5005 ± 0.0006 cm^2; cup volume, 250 ± 1 μl. The cup wall was made thin (0.4 mm) and smooth enough to allow the oil/water interface to be clearly visible.

The recording microbalance (Cahn Model 2000RG Electrobalance, Cahn Instruments, Cerritos, CA) was enclosed in an airtight glass bottle. The weighing cups were suspended in hangdown tubes immersed in a constant-temperature bath. The entire system was enclosed in a second thermostatically controlled box. Thermistors were located inside the left hangdown tube at the cup and at the top of the tube. These two thermistor readings were recorded continuously along with the microbalance mass signal. The temperature at the cup was maintained to within ±0.02°C throughout the run. The temperature

at the top of the hangdown tube was maintained approx. 0.15°C above the cup temperature.

The oil thickness in the weighing cup was typically 0.200 cm. To obtain this thickness, 150 μl of water were added to the cup and then 100 μl of oil were carefully layered over the water. This amount of material filled the cup to the brim producing a flat surface: (varying the amount of oil by ±1 μl gave a detectable curvature at the surface). The oil/water interface was almost flat when the cup was filled. In fact, however, the oil was found to 'wet' the polytetrafluoroethylene slightly more than the water. This led to a slightly curved oil/water interface, concave downward. As this curvature was small and in such a direction as to indicate that water was not creeping up the sides of the cup, no attempt was made to find another material for the cup. The amount of curvature of the interface was determined at the end of a microbalance run by measuring the oil thickness at the edge of the cup with a traveling microscope. A correction factor accounting for the curved interface was applied to the measured mass loss rate. Typical corrections were of the order of 2—3%.

The air in the glass enclosure was maintained at essentially zero relative humidity by placing anhydrous $CaSO_4$ in the bottom of the left and right hangdown tubes; in addition, a third hangdown tube located between the others was filled with anhydrous $CaSO_4$. As the diffusion coefficient for water through air is about 4 orders of magnitude greater than through the oil, the diffusion of water through the air to the $CaSO_4$ contributes negligibly to the mass transport time, and setting $c_a^w = 0$ introduces negligible error.

Prior to a microbalance run, the cups were rinsed with methanol, soaked at least 24 h in chromic acid/sulfuric acid and rinsed thoroughly with water. The water used for cleaning and for measurements was purified by distillation, followed by repeated recirculation through a system of particulate matter filter, mixed-bed strong acid and base ion-exchange columns, an activated charcoal column and a 0.2 μm pore filter (Milli-Q2 Reagent Grade Water System, Millipore Corp., Bedford, MA). The resistivity of the output water (monitored continuously) was 18 MΩ · cm.

Typical microbalance runs lasted about 1 week. Approx. 12 h were required for establishment of a steady rate of mass loss (primarily due to the time required to reach stable temperatures throughout the system). The microbalance output was recorded on a chart recorder, providing a continuous check on the steady rate of mass loss. At approx. 24-h intervals the balance was re-zeroed to obtain the mass loss over that period. After about three such measurements, the cups were interchanged, placing the oil/water cup in the right hangdown tube for another run of about 3 days. Mass-loss rates were stable to a few percent over the course of the run. Error bars in Figs. 1 and 2 are dominated by these day-to-day variations in the mass-loss rate.

Hexadecane tests

As a check of the experimental system and procedures, water permeation rates were measured through *n*-hexadecane for comparison with the results of Schatzberg [6]. Repeated measurements at 19 and 30°C gave results which were 15—20% below Schatzberg's values. Some observations on these studies:

(1) One major difference between the two experiments is that Schatzberg used glass cups treated with 'a very dilute aqueous solution of a soluble silicone-concentrate' [6] to render the surface slightly hydrophobic. I chose polytetrafluoroethylene because it offered a more hydrophobic surface to begin with, and it could be subjected to vigorous cleaning procedures. In order to verify that there was no problem due to the creeping of water up the side of the polytetrafluoroethylene cups, I made comparisons of the mass-loss rate for cups having cross-sectional areas of 0.500 and 1.00 cm^2. The mass-loss rate was found to be proportional to the cross-sectional areas, ruling out any detectable effect of water-creep up the sides.

(2) I found water permeation rates to be somewhat higher if the polytetrafluoroethylene cups were cleaned less vigorously than by using chromic acid/sulfuric acid. The silicone-treated glass cups Schatzberg used could not be subjected to harsh cleaning treatments without damaging the hydrophobic surface. In fact I attempted to make such glass cups coated with a similar aqueous silicone solution (Prosil-28, PCR Research Chemicals, Inc., Gainesville, FL). Prolonged exposure to pure water eventually dissolved the hydrophobic coating and no measurements were ever made with these cups.

(3) Any trace contaminants in either the water or the hexadecane would be expected to increase the water permeation rate.

While Schatzberg's measured permeation rates differ from mine, it is important to note that the activation energy I observed for water permeation through *n*-hexadecane is 11.8 ± 0.5 kcal/mol, essentially the same as Schatzberg's value of 11.2 kcal/mol. As discussed below, the activation energy provides probably the best test of the liquid hydrocarbon model.

Materials

As the alkyl chain of glycerol monoolein is 8-heptadecyl, 8-heptadecene is chosen as the liquid hydrocarbon analog of glycerol monoolein. This alkene is mixed with *n*-hexadecane in the proportions found in the glycerol monoolein/*n*-hexadecane bilayer [15].

Hydrocarbons were obtained from Chemical Samples Co. (Columbus, OH). Minimal purities, according to the manufacturer, were 99.9% for the *n*-hexadecane and 99% for the 8-heptadecene. These were further passed twice through alumina before they were used. The purity of both hydrocarbons was checked with a GC/mass spectrograph system using a 30 m glass capillary column (Finnigan 4023 GC/EI-CI MS, Finnigan Corp., Sunnyvale, CA; capillary column coated with SP2100, Supelco, Inc., Supelco Park, Bellefonte, PA). In neither case were any contaminants detected, indicating at least 99.9% purity. The 8-heptadecene eluted from the column as two closely spaced peaks. Both peaks were identified as heptadecene from their fragmentation patterns in the mass spectrograph. Comparison of the infrared absorption spectra of 8-heptadecene with *cis*-5-decene and *trans*-5-decene (Chemical Samples Co.) showed that the 8-heptadecene had both *cis* and *trans* isomers, with the *trans* isomer predominating. No 1-heptadecene contaminant was present. From the infrared and gas-liquid chromatographic data, it was inferred that approx. 85% of the 8-heptadecene was the *trans* isomer. As the alkyl chain of glycerol monoolein is in the *cis* configuration, the 8-heptadecene should be the *cis*

isomer to serve as the best analog. The difficulty of separating useable amounts of the two isomers from the sample precluded obtaining a pure *cis* preparation.

Results

Fig. 1 shows the results of the microbalance measurements on the rate of water permeation through 8-heptadecene/*n*-hexadecane mixtures chosen to match the glycerol monoolein/*n*-hexadecane proportions in the bilayer [15]. Two measurements were made at 27.5°C, one with a 0.300 cm oil layer instead of the usual 0.200 cm. The ratio of these permeability coefficients is 0.66 ±

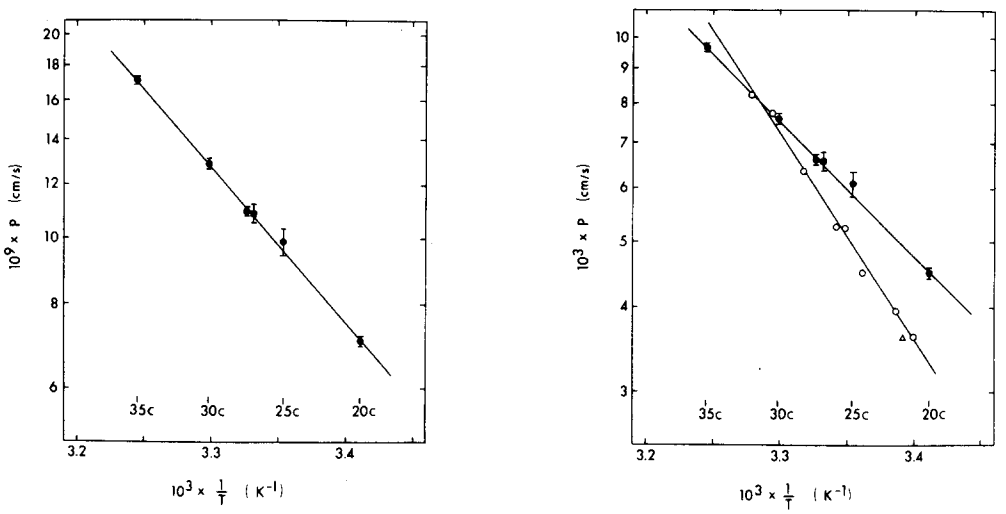

Fig. 1. Arrhenius plot of water permeability coefficient of 8-heptadecene/*n*-hexadecane. •, hydrocarbon thickness d_h = 0.200 cm; ■, d_h = 0.300 cm—measured P_h was multiplied by 1.5 to permit comparison with 0.200 cm data. Error bars indicate ±1 S.D. The volume fraction, ϕ_s, of *n*-hexadecane was chosen at each temperature to match ϕ_s for *n*-hexadecane in the glycerol monoolein/*n*-hexadecane bilayer [15]; this gives ϕ_s = 0.200, 0.223, 0.235, 0.248 and 0.275 for 20.0, 25.0, 27.5, 30.0 and 35.0°C, respectively. P_h values were obtained from the measured mass transport rate of water and Eqn. 4, with c_W^W = 1.00 gm/cm^3 and A = 0.500 cm^2. The line results from a least-squares fit to the data (r^2 = 0.997), yielding an activation energy of 10.5 ± 0.3 kcal/mol.

Fig. 2. Arrhenius plots of measured and predicted water permeability coefficients for glycerol monoolein/*n*-hexadecane bilayers. Open symbols: measured osmotic permeability coefficients; ○, data from Fettiplace [14]; △, datum from D. Petersen (unpublished result). Filled symbols: water permeability coefficients predicted from liquid hydrocarbon model; •, d_h = 0.200 cm; ■, d_h = 0.300 cm. Error bars ±1 S.D. Predictions come from Eqn. 2 and measurements in Fig. 1. Values of d_m come from capacitance measurements on the glycerol monoolein/*n*-hexadecane bilayer [15] together with the equation, $d_m = \epsilon_o \epsilon / C_g$, where ϵ_o is the permittivity of free space, ϵ is the dielectric coefficient of the hydrophobic core, and C_g is the specific capacitance of the bilayer. The dielectric coefficient, ϵ, was obtained from the mol fraction of *n*-hexadecane in the bilayer [15] together with the dielectric coefficients of 2.058 and 2.167 for *n*-hexadecane and *trans*-8-heptadecene, respectively [18,19]. Assuming the dielectric coefficients are additive on a volume fraction basis [20], ϵ = 2.14 for the glycerol monoolein/*n*-hexadecane bilayer. Bilayer thicknesses (d_m) are 31.3, 32.5, 33.2, 33.9 and 35.3 Å for 20.0, 25.0, 27.5, 30.0 and 35.0°C, respectively (Requena and Haydon [20] give slightly different values for ϕ_s and d_m for glycerol monoolein/*n*-hexadecane at 20°C. Their values of ϕ_s = 0.26 and d_m = 32.7 Å result in a predicted value for P_m at 20°C that is 6% lower than given here. They do not give ϕ_s and d_m for other temperatures). Lines result from least-squares fits to the data (△ was not used in the fit). Apparent activation energy for osmotic permeability coefficient = 14.2 ± 1.0 kcal/mol (Fettiplace [14]); apparent activation energy predicted from liquid hydrocarbon model = 9.0 ± 0.3 kcal/mol (r^2 = 0.996).

0.02, in agreement with the prediction of Eqn. 1. The values of P_h are presented as an Arrhenius plot. The slope of the line as determined by a linear least-squares fit yields an activation energy for water permeation of 10.5 ± 0.3 kcal/mol.

The predictions of the liquid hydrocarbon model for the water permeability coefficient of the glycerol monoolein/*n*-hexadecane bilayer are shown in Fig. 2. Fettiplace's measured water permeability coefficients for this bilayer [14] are also shown in the figure. Least-squares fits to the Arrhenius plots give apparent activation energies for water permeation: (note that membrane thickness varies with temperature; this variation affects the slope of the curves). The liquid hydrocarbon model predicts an activation energy of 9.0 ± 0.3 kcal/mol, whereas the observed activation energy is 14.2 ± 1.0 kcal/mol. While the predicted and observed permeability coefficients are in agreement at 32°C, at 20°C the predicted value for P_f is almost 40% above the actual value. This discrepancy in the observed and predicted activation energies is significant, and one must conclude that the liquid hydrocarbon model, while a good first-order model, does not correctly describe the actual water permeation process through the lipid bilayer.

Discussion

Where does the liquid hydrocarbon model go wrong? The model assumes: (1) the rate-limiting step for water permeation is diffusion through the hydrocarbon core of the lipid bilayer; diffusion of water through the membrane-water interface is ignored; (2) the hydrocarbon core is simply a thin slice of hydrocarbon liquid, having the same properties as the bulk liquid. Both of these assumptions will be considered.

Diffusion in the membrane vs. diffusion across the solution/membrane interface

Zwolinski et al. [21] developed an absolute rate theory of membrane permeation, in which the diffusing molecule passes through the membrane in a series of discrete jumps. In this kinetic approach the permeability coefficient can be written as:

$$\frac{1}{P} = \frac{2}{k_{sm}\lambda} + \frac{m}{k_m\lambda(k_{sm}/k_{ms})} \tag{6}$$

where k_{sm} is the rate constant for diffusion across the solution/membrane interface, k_{ms} is the rate constant for diffusion across the membrane/solution interface, k_m is the rate constant for diffusion in the membrane, λ is the length of the diffusing jump, and m is the number of jumps across the membrane. The second term can be written in a more familiar form by noting that: (1) the partition coefficient, K, is just the ratio of the rate constants for diffusion through the solution/membrane interface: $K = k_{sm}/k_{ms}$; (2) the diffusion coefficient, according to the absolute rate theory is given by $D_i = k_i\lambda^2$; (3) the membrane thickness $d = m\lambda$. Thus Eqn. 6 becomes:

$$\frac{1}{P} = \frac{2\lambda}{D_{sm}} + \frac{d}{D_m K} \tag{7}$$

If diffusion through the solution/membrane interface is ignored, the expression for P is identical in form to Eqn. 1: (note, however, that in Eqn. 7, D_m and K refer to the membrane, not to bulk hydrocarbon liquid). It is useful to consider some special cases of Eqn. 6. As the low dielectric coefficient of the hydrocarbon core provides a large barrier to a polar molecule such as water, it will be assumed that $K = k_{sm}/k_{ms} << 1$ for all cases.

Case I: $k_m >> k_{ms}$

Here the slow step is diffusion through the solution membrane interface and $P \cong D_{sm}/2\lambda$. The permeability coefficient is independent of K and d. This is clearly incorrect for bulk hydrocarbon liquids for which $P = D_m K/d$ works well [5,6]. The fact that Eqn. 2 can be used to predict the water permeability coefficient for the lipid bilayer to within a factor of 2 when the hydrocarbon thickness is decreased by 6 orders of magnitude also strongly argues against this limiting case. On the other hand, it is difficult to rule out any contribution from diffusion across the interface. Few studies have been made on the dependence of P on membrane thickness. One study compared the permeability coefficients at 30°C for an egg phosphatidylcholine/squalene bilayer and a phosphatidylcholine/decane bilayer [10]. The permeability coefficients varied approximately as the inverse of the bilayer thicknesses, which is consistent with the liquid hydrocarbon model and inconsistent with Case I. On the other hand, another study [14] found that P_f for glycerol monoolein/decane and glycerol monoolein/hexadecane bilayers at 25—26°C were the same, whereas the glycerol monoolein/decane bilayer was 50% thicker than the glycerol monoolein/hexadecane bilayer. Also in a third study at the same temperature [14], a bilayer of mixed mononervonin (24 : 1) and monoolein (18 : 1) was compared with a monoerucin (22 : 1) bilayer of the same thickness; the permeability coefficients differed by 20%. From these final two results, one cannot rule out significant contributions of the membrane/solution interface to the permeability coefficient. There are other possible inferences to draw from these results, however, as is discussed below.

Case II: $k_m << k_{ms}$

The rate-limiting step is diffusion in the membrane, and $P \cong KD_m/d$.

Case III: $k_m = k_{ms}$

Here the rate-limiting step is again diffusion through the solution/membrane interface, but with the modification that the rate constants for diffusion in the membrane and through the membrane/solution interface are equal. In this case $P \cong (m/(m+2))(KD/d)$, which is experimentally indistinguishable from Case II.

The difficulty in obtaining much useful information from Eqns. 6 and 7 comes from the fact that there are too many unknown parameters in them. From Eqn. 7 it looks as though it would be fairly easy to sort out the relative contributions of diffusion through the interface and diffusion in the membrane. One might either vary d by forming membranes of lipids having homologous alkyl chains differing in the number of CH_2 groups or vary K by altering the unsaturation of the alkyl chains (more difficult because of concomitant

variation in membrane thickness). By plotting $1/P$ vs. d or $1/K$, one could then sort out the two contributions to the permeation process. However, there is a serious problem with this approach. In varying one parameter, say d, one assumes that all other kinetic parameters, e.g., k_m, k_{ms}, k_{sm}, remain unchanged. This is almost certainly not the case for the lipid bilayer. The variety of experimental results on the dependence of P_f on d mentioned above probably reflect such unseen changes in the ultrastructure of the lipid bilayer.

The nature of the hydrocarbon core

Recent studies on the partitioning of alkanes into lipid bilayers have shown that the hydrophobic core of the bilayer is not the homogeneous, isotropic liquid found in the bulk solution [15—17,20,22,23]. In glycerol monoolein/*n*-hexadecane bilayers [16], for example, the segments of the alkyl chains near the glycerol moiety appear to be ordered more or less perpendicular to the membrane/solution interface, while alkyl chain segments near the terminal methyl group tend to run parallel to the membrane/solution interface. This configuration restricts the location and amount of the hexadecane solvent in the bilayer. While bulk alkane liquids also show some correlations of molecular orientations of *n*-alkane molecules [24], such orientational order exists only in a small fraction of the molecules [25] and is of short range, extending over only a few neighboring molecules or portions of molecules [26].

The nonhomogeneous, anisotropic structure of the hydrophobic core of the lipid bilayer would be expected to affect the permeation of molecules through the membrane. Studies on the permeability of lipid bilayers to small nonelectrolytes [27,28] give indications of the breakdown of Overton's rule (i.e., $P \propto K_{bulk}$) for the smallest of these molecules (urea, formamide, and possibly valeramide), indicating both solution/membrane interface effects and steric factors governing the diffusion of molecules in the membrane [27]. The anomalies are somewhat larger in one of these studies [27], possibly reflecting the use of tetradecane as a solvent there. In the other study [28], decane was used as the solvent. More decane than tetradecane can dissolve in the lipid bilayer, and the hydrophobic core of decane-containing bilayers appears to be more like a bulk hydrocarbon than does a tetradecane-containing bilayer [16].

It is difficult to estimate the effect of the membrane ultrastructure on water permeation from data on larger molecules, however. Studies of the translational diffusion of proteins and lipids in biological membranes yield diffusion coefficients in the $1 \cdot 10^{-9}$—$1 \cdot 10^{-8}$ cm^2/s range, from which one can infer an effective membrane viscosity of a few P (for a review of such studies, see ref. 29). Similar diffusion coefficients may be obtained from measurements on the permeation of nonelectrolytes across biological membranes [27,30] *. However, the diffusion coefficient of water in bulk hydrocarbon liquids is about

* There is controversy as to whether the permeation of nonelectrolyte molecules across a membrane is better approximated by the liquid hydrocarbon model [28] or by a model in which the membrane is viewed as a thin polymer film [30]. It may be pertinent that the polymer model is based on experiments on biological membranes, which contain no hydrocarbon solvent, whereas the liquid hydrocarbon model is compared with lipid bilayers containing decane. The presence of solvent is known to alter the bilayer structure [16,31,32] rendering it more like a bulk hydrocarbon liquid.

$1 \cdot 10^{-5}$ cm^2/s, leading to the conclusion that the diffusing water molecules see only methylene and methyl groups rather than the entire hydrocarbon molecule [6]. The diffusion coefficient of water in the membrane also appears to be about $1 \cdot 10^{-5}$ cm^2/s, as inferred from the order-of-magnitude agreement of the measured water permeability coefficient with the predictions of the liquid hydrocarbon model.

The very small size of the water molecules may make it possible for them to dissolve into the lipid bilayer without greatly altering the molecular organization of the bilayer. This assumption was made by Träuble, who developed a molecular model for the movement of water across lipid bilayers [33]. In his model, structural defects—'kinks'—in the hydrocarbon chains diffuse along the chains. Water molecules can reside in the free volume created by these kinks and thereby diffuse across the membrane. The diffusion constant for kinks was estimated to be $1 \cdot 10^{-5}$ cm^2/s, in good agreement with water permeability results.

It is interesting to compare the measured activation energy for water permeation with predictions of Träuble's kink model. The estimated activation energy for diffusion of a kink is 4.8 kcal/mol. The activation energy for the partitioning of water into the membrane may be estimated from the heat of vaporization of water, 10.5 kcal/mol. As $E_a = \Delta H^* + RT$, where E_a is the Arrhenius activation energy and ΔH^* is the enthalpy of activation [34], $E_a = 11.1$ kcal/mol for vaporization of water at room temperature. Assuming that the attraction of water molecules and hydrocarbon chains is just balanced by a reduction in chain-chain attraction on addition of water into the bilayer, the activation energy for water permeation is just $4.8 + 11.1 \cong 16$ kcal/mol. To compare this with the results of Fettiplace on the water permeability of glycerol monoolein/hexadecane bilayers, one should normalize the bilayer data to a fixed membrane thickness. If this is done, the measured activation energy increases from 14.2 to 15.6 kcal/mol, in remarkable agreement with the kink model. In contrast, the liquid hydrocarbon model predicts an activation energy of only 10.5 kcal/mol. In view of the crude estimates that went into the kink model prediction * of the activation energy, one should not overstate the significance of this apparent agreement with experiment and the disagreement with the liquid hydrocarbon model.

One final comparison of bulk hydrocarbon and lipid bilayer comes from an estimate of the entropy of activation for the permeation process. In the absolute rate theory of membrane permeation of Zwolinski et al. [21], the permeability coefficient may be given in terms of the enthalpy and entropy of activation, ΔH^* and ΔS^*, respectively:

$$P = \frac{kT}{h} \cdot \frac{\lambda^2}{d} \cdot e^{-\Delta H^*/RT} \cdot e^{\Delta S^*/R} \tag{8}$$

* Träuble's model assumes that the only sites for water molecules in the bilayer are *gauche*$^+$-*trans*-*gauche*$^-$ (or g^--t-g^+) kinks in the hydrocarbon chains. For each diffusing jump of a kink, rotation about two single C-C bonds is required. This model is probably too restrictive, as isolated *gauche* bonds, 'jogs' (g^+-t-t-t-g^-), and *cis* double bonds also exist in the bilayer [35]. Nevertheless, Träuble's estimate of the activation energy for water diffusion is probably reasonable, as the diffusing jump of a water molecule should still require, on average, the rotation of approximately two single bonds.

where k is Boltzmann's constant, h is Planck's constant, R is the gas constant, λ is the jump length for diffusion, and d is the membrane thickness. Schatzberg obtained a value for λ of 2.74 Å from the solubility of water in hexadecane [5]. As ΔS^* is quite insensitive to the value of λ, it seems safe to assume that $\lambda = 2.74$ Å for the lipid bilayer and for bulk 8-heptadecene/*n*-hexadecane mixtures as well. One can then use Eqn. 8 to obtain entropies of activation for water permeation in bulk liquids and the lipid bilayer. For *n*-hexadecane or 8-heptadecene/*n*-hexadecane mixtures, $\Delta S^* \approx 5$ cal/mol per K, while for the glycerol monoolein/*n*-hexadecane bilayer $\Delta S^* \approx 20$ cal/mol per K. For a simple unimolecular process $\Delta S^* \approx 0$ [34]. The larger value of ΔS^* for the lipid bilayer may imply that water permeation through the lipid bilayer requires a relatively greater disruption of the alkyl hydrocarbon order than is necessary in the less organized bulk hydrocarbon liquid.

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