Chapter 6

A Molecular Mechanism for the Transport of Water Across Phospholipid Bilayers

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CONTENTS

I. INTRODUCTION

In 1908 Nernst[1] introduced the concept of biological membranes as "semipermeable." To Nernst this meant that water could pass through them but ions could not. *How* water passed through them was as mysterious as was the membrane's molecular structure and constitution. Indeed membrane structure remained intensely controversial until 1971 when David E. Green organized a meeting[2] at the New York Academy of Sciences. At this meeting a consensus emerged that biological membranes are *liquid-crystal bilayers* displaying lateral diffusion of both the phospholipids[3,4] and the embedded proteins.[5] At the same meeting Fleischer[6] suggested that membrane proteins could be sorted out as intrinsic or membrane associated. The consensus expressed at this meeting was immediately summarized and graphically fluidized by Singer and Nicolson.[7]

Devaux and McConnell[3] and Trauble and Sackmann[4] had recently discovered that membrane lipids diffused laterally in the plane of bilayers. This added an important insight into the properties of lipid bilayers above the transition state. It also provided a method of measuring this diffusion quantitatively. We now propose that this process is directly connected to water transport for straight-chain lipid bilayers.

Water transport through biological membranes has been reviewed by Bacic, Srejic, and Ratkovic,[8] by Finkelstein,[9] and in two volumes edited by Benga.[10] An important review of the recently characterized water transport proteins is that of Verkman.[11] The latter reference describes a class of water channels exemplified by CHIP28 (Channel-forming Intrinsic Protein, 28kD). This protein is representative of more than a dozen highly conserved, homologous, water-channel proteins found in mammals, plants, Drosophila, *E. coli*, and yeast.

Water diffusion through biological membranes occurs in three ways: (1) by diffusion through the lipid bilayers, (2) through transport proteins such as channels and some occlusion transport proteins

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such as the glucose transporter,[12] and (3) through water-channel proteins specifically expressed by cells for that purpose. In all cases water flow through the membrane is *passive*, directed by osmosis. The water diffusion is therefore controlled by ion pumps, channels and exchange proteins. Ultimately it is determined by the bioenergetics of the cell; in most cases this means the energy comes from ATP.

Here we focus on the movement of water directly through bilayers of phospholipids. Pure phospholipid bilayers go through as many as four states as the temperature is raised: crystal, gel, liquid crystal, and liquid. Substantial water diffusion begins as the bilayer enters the liquid-crystal state (T_m).

Water permeability in lipid bilayers has been measured since they were first prepared in the laboratory in the 1960s. The preparation of *planar lipid bilayers* immediately permitted direct measurements of water movement through bilayer sheets.[13-15] The production of *bilayer vesicles* in the laboratory by Bangham and co-workers[16] permitted measurements of water flow through this form of bilayer as well, as shown for example by Carruthers and Melchior.[17] A fluorescence technique for measuring water permeability of phospholipid vesicles was also developed by Lawaczeck.[18] See also a review by Fettiplace and Haydon.[19] Various models for the movement of water through bilayers of phospholipids have been reviewed by Deamer and Bramhall,[20] Finkelstein,[9] de Gier,[21] and Disalvo et al.[22] These reviews[13-22] cite many measurements of water transport from 2 to 200 $\times$ 10^{-3} cm/s for pure phospholipid bilayers.

II. THE MODELS

A. THE SOLUBILITY-DIFFUSION VIEW

The solubility-diffusion view is a physical chemist's description of water diffusion rather than a molecular model. It is based on solubility and experiments that show that molecules such as N_2, O_2, and other molecules the size of water diffuse through bilayers at about the same rate. It has been described by Finkelstein,[9] Hanai and Haydon,[23] Reeves and Dowben,[24] and others. Given the entropic energy that maintains the intrinsic oil-water interface, it is surprising that water passes so easily through bilayers. A water molecule needs high energy to break the hydrogen bonds with its neighbors and enter the hydrocarbon phase (see Chapter 3).[25,26] Finkelstein[9] predicted water flow through bilayers from its hydrocarbon solubility. Interfacial water has been examined by McIntosh et al.[27]

B. THE DEFECT MODEL

In the *defect model* cracks appear between the bilayer molecules (only two molecules thick) at some discrete frequency to allow water to pass through the membrane. This model has been explored experimentally by extrapolating from measurements of the bilayer diffusion barriers. Deamer and Bramhall[20] explored this model with studies on the permeability of protons and monovalent inorganic ions. They find that at least two varieties of transient defects are required to explain the permeation of water and ionic solutes, one for water and another for ions. In their study on the relationship between water permeability and the bilayer physical state, Carruthers and Melchior[17] discovered that the rate of water permeability was not affected by the chainlength of the phospholipids. They concluded that their data supports the defect model.

C. THE TRAUBLE MODEL

In 1971, Trauble[28] proposed a specific molecular mechanism for the movement of water through chain-lipid bilayers. He noted that a water molecule fits neatly between two chains that have *g-t-g* kinks in the adjacent chains calculated by Flory[29] as low-energy motion for chain polymers. Trauble recognized in his original proposal a distinct difference between the center of the bilayer and the region close to the headgroups. He doubted that his mechanism applied to the C_{10}-to-terminal-methyl region. Trauble did not extend his proposal beyond explaining the interaction of the water molecule with the hydrocarbon. However, his model has stimulated and/or formed the basis for much theoretical and experimental work.

D. GENERALIZED MOLECULAR DYNAMICS MODELS

Pace and Chan[30] have examined the molecular dynamics of various solutes in bilayers. Day and Willis,[31] Nagle,[32-34] and Marcelja[35] are among those that have built theoretical models of the molecular dynamics of the phase transition. Molecular dynamics *computer simulations* of bilayers have also been attempted during the last decade (see Chapters 3 and 6).[36,37] These may be on too small a time scale to be useful at this time as will be seen below.

III. THE GEL-TO-LIQUID-CRYSTAL PHASE TRANSITION (T_m)

In the last 20 years over 2000 publications have appeared describing the molecular dynamics of the liquid-crystal state. Yet we lack a coherent molecular dynamics model for even a one-component phospholipid bilayer.[18,39] Before attempting this daunting task, let us summarize some key experimental observations.

A. THE PHASES OF PHOSPHOLIPID BILAYERS

The "melt" of the bilayer occurs at the transition between the gel and the liquid crystal (T_m). The stability of the bilayer in the liquid-crystal state displays a decrease of some 10% of van der Waals interactions without much change in the ionic or hydrophobic forces.[40] Some experimental features of the liquid-crystal state of bilayers such as the enthalpy[41] at the T_m is sufficient to explain the introduction of two *g-t-g* conformers (kinks) per chain.[18,42] For a general discussion of permeability at the gel-to-liquid-crystal phase transition see Chapter 8.

B. EXPERIMENTAL DESCRIPTION OF THE LIQUID-CRYSTAL STATE

Five features of the liquid crystal state are essential for modeling water transport across bilayers.

1. *Phospholipids diffuse laterally* in the plane of the bilayer. The rate varies slightly depending on the technique, the structure of the lipids, and conditions, such as temperature. Since the first measurements in 1972[3,4,43] diffusion rates have been consistently measured to within an order of magnitude of about 10^{-8} cm^2/s, for cellular membranes[44] as well as for lipid bilayers.
2. *Water diffuses through bilayers* above the T_m much as do gases and uncharged molecules the size of water. The rate of water permeability through bilayers is within an order of magnitude of 10^{-3} cm/s, the rate found in the early measurements of van Deenen[45] and of Bangham.[16,46]
3. *The segmental order parameter* is a measure of a combination the rotational (*gauche-trans*) isomerization and the tilt of rod-like (all-*trans*) chains of each segment ($-CH_2-$) of the hydrocarbon chain. In 1974 Seelig and Seelig[47] used deuterium quadrupole coupling NMR to study the order parameter profile of DPPC (dipalmitoyl phosphatidyl choline). They found that the ($-CH_2$) segments from C_2 to C_9 display a plateau in the order parameter. The concept of the order parameter in a bilayer had actually come from the spin-label work of Hubbell and McConnell.[48] The order parameter plateau of Seelig and Seelig has been confirmed by a wide variety of measurements during the last two decades.[49] Recently developed infrared studies on deuterolabeled chains[50] provide, in addition to the order parameter, specific information on the chain rotamers.
4. *The bilayer expands by 30 to 40% laterally at the T_m*, varying with phospholipid structure.[51] DPPC in the liquid-crystal state occupies 136% of its original gel-state area. A clear implication of this bilayer expansion has received little attention. If the chains in the gel state are in a hexagonal array in the gel state and the area expands, what happens in the headgroup sheet? Assuming a lattice of headgroups exist in the gel state, the expansion necessarily implies *vacancies appear in the lattice of headgroups* at the bilayer surface.
5. *The phospholipid bilayer thins* as it passed through the T_m. This was recognized in Luzatti's X-ray studies[52] in 1968. Bilayers of DPPC, for example, are 47 Å thick in the gel state but are 35 Å thick above the T_m.[53]

IV. A MOLECULAR DYNAMICS MODEL FOR BILAYER WATER TRANSPORT

A. OUTLINE OF THE HAINES-LIEBOVITCH-TRÄUBLE MODEL

We propose that a single process accounts for these five features as follows: The lateral expansion of the bilayer introduces "vacancies" in the phospholipid headgroup "lattice." Water fills each vacancy as it appears. An adjacent ester group moves into the lattice vacancy occluding (covering) a single water molecule separating it from the bulk. The water molecule occupies a defect (*g-t-g* kink) in the chain. A second chain assumes a *g-t-g* kink above the water molecule in conjunction with its lateral headgroup jump. As the water molecule, nested between both kinks, moves down their respective chains the *chains* move laterally one lattice unit. The *lateral motion of the lipid molecules is linked in the water migration through the bilayer.* We use a two-dimensional random walk to calculate the water diffusion rate from the lateral diffusion of the phospholipids and *vice versa.*

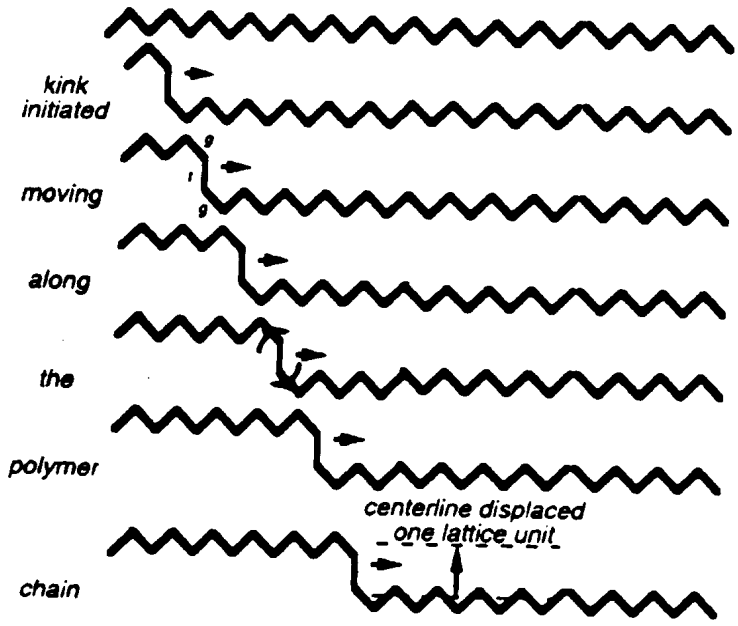


Figure 1 Depiction of *g-t-g* conformer (kink) moving along a polyethylene chain after Paul Flory. There are three features of the movement emphasized in this depiction. (1) The kink must be initiated at the end of the chain (for steric reasons). (2) The kink has momentum because its lowest-energy state, once initiated, is to continue down the chain. (3) A result of the kink moving down to the other end of the chain is that it has moved over a lattice unit. For lipids, this means that the molecule moves laterally in the plane of the bilayer. This occurs every time *g-t-g* kinks move up or down the chains.

B. KINK DIFFUSION IN ALIPHATIC CHAINS

Flory,[29] in his calculations on linear polymers, showed that low-energy defects or *g-t-g* kinks in hydrocarbon chains migrated down the chains. His statistical mechanical calculations allowed him to predict many physical properties of polymers. He made several assumptions as illustrated in Figure 1.

1. For a linear hydrocarbon in the gel state, kinks begin at either end of the chain. Kinks cannot begin in the middle of the chain for steric reasons.
2. The lowest energy kink consists of a *g-t-g* (*gauche-trans-gauche*) kink which propagates down the chain at rates of 10^9/s or faster. Kinks display momentum: Once started, they continue to the end of the chain unless impeded by an external force.
3. Kinks displace the chain by a lattice unit. Chains may be viewed end on as an approximate hexagonal lattice. A consequence of the water motion along the chain is to move the chain from one lattice unit to a neighboring one.

Application of these three principles to the bilayer chains implies (1) that if *g-t-g* kinks are introduced they must begin either at the headgroup end or at the methyl end, (2) that the lowest-energy kinks may sequester water molecules, and (3) that kinks are coupled to the lateral movement of a phospholipid chain in a bilayer.

C. TRÄUBLE REVISITED

Träuble's suggestion[28] of nested water molecules was based on CPK models as shown in Figure 2. It assumes that if the headgroups are proximal (same "height" with respect to the bilayer) kinks create vacancies between the chains. The water molecule just fits a vacancy (vacuum) that would be energetically unfavorable.

D. THE BILAYER EXPANDS AT THE T_m

For many years it was known that phospholipid monolayers assumed a "condensed state" and an "expanded state." In 1968, Chapman[54,55] recognized a specific correspondence between the monolayer

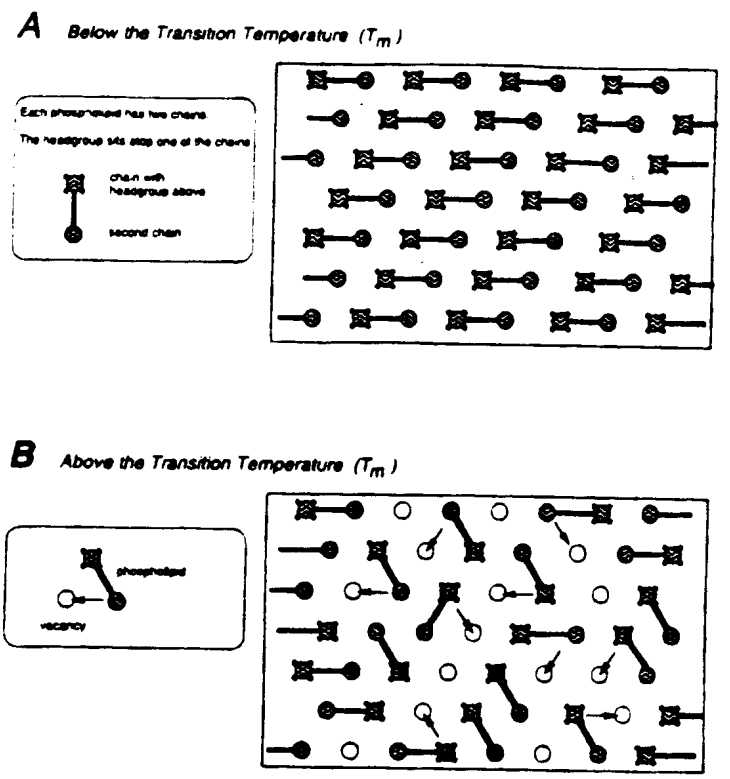


Figure 2 A, A hexagonal array of the chains of a phospholipid as viewed from above the plane of the bilayer below the T_m. Circles surrounded by four rings represent the chain beneath a phosphate headgroup; plain circles represent the chains of the secondary esters of the glycerol. Although the lipids are arranged in rows the reader is cautioned that the tethered pairs are in random arrays and orientations in the gel state. B, The same hexagonal array of chains above the T_m. The figure shows random "vacancies" (open circles) in the lattice resulting from expansion of the bilayer. These represent the area of direct contact of water with the hydrocarbon.

and the bilayer. The condensed state in the monolayer corresponds to the gel state in bilayers, and the expanded state in the monolayer corresponds to the liquid-crystal state in the bilayers of the same chainlength.[51] In the gel state the chains assume a hexagonal array (Figure 2A). At the T_m, it expands laterally by 30 to 40%, yet the average distance between the chains increases by less than 10%. The chains may accommodate the expansion by tilt and rotational isomerization but the headgroups cannot. Hence the 30 to 40% expansion in the bilayer means a 20 to 30% vacancy rate in the headgroup lattice (Figure 2B). The expansion exposes the tops of the chains so that cavities of hydrocarbon come into direct contact with the water. Headgroups (ester) that jump into a vacancy cut the isolated water molecule off from the bulk water. The energetics in the bilayer is determined by both the water solubility in the hydrocarbon[9] and by the kinetics of the lipids due to thermal energy (kT).

E. THE ORDER PARAMETER PROFILE OF THE CHAIN METHYLENES

Using deuterium quadrupole coupling NMR, Seelig and Seelig[47] described an order parameter profile (Figure 3) for the carbon chain of DPPC in the liquid-crystal state. The Seeligs' experiments produced several surprising results.

First, they observed a plateau in the order parameter profile for carbons 2 to 8 in the chains of the phospholipid. For each rotational isomerization at C_2, one occurs at C_4, C_6, and C_8. In contrast, segments C_{10} to C_{16} show increased frequency of rotational isomerization toward the end of the chain.

The *second* surprise was the low (0.4) order parameter of the plateau. An order parameter near 0.4 implies 60% disorder. We suspect that kinks in the C_2 to C_9 region are associated with transverse water movement and lateral headgroup movement. These kinks would go the full length of the chain.

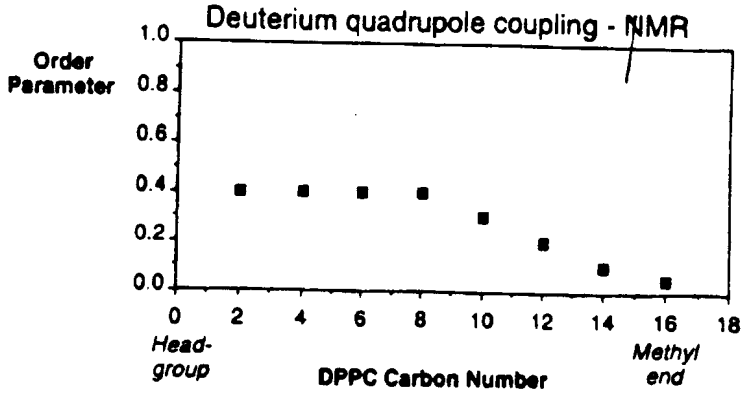


Figure 3 The order parameter for each segment of the chains of DPPC established by deuterium quadrupole coupling NMR as established by Seelig and Seelig.[47]

The *third* surprise was that the *length* (C_2–C_9) of the order parameter plateau of this saturated lipid corresponded to properties of biological membranes. Both prokaryotes and eukaryotes have structural lipid features suggesting a division in chain dynamics at the 9–10 position.

Prokaryote lipids are monounsaturated with the double bond in the 9–10 position. The dynamics of the chain between C_2 and C_9 necessarily differ from the dynamics of the chain from carbon C_{10} to the end of the chain. The *g-t-g* conformers (kinks) cannot pass through the double bond at carbon 9. Additionally, in the bilayer, saturated-chain conformers are constrained by unsaturated neighbors.

Eukaryote lipids have more variant chains, including polyunsaturated lipids which contain double bonds, mostly at the distal end of the chain. Eukaryotes contain sterols in their membranes. As described by Rothman and Engleman,[56] the sterol ring system lies in the membrane between C_2 and C_9, whereas its flexible side chain rests between C_{10} and the terminal methyl. This means that the motions of the C_2 to C_9 region are more constrained than those of the C_{10}-to-terminal-methyl region.

The Seeligs interpreted the order parameter plateau according to statistical mechanical calculations made by Marcelja,[55] who had predicted such a curve assuming that the chains were tethered to immobile headgroups. Our model does not conflict with Marcelja's conclusions. The headgroups move three orders of magnitude more slowly than do the chains. For purposes of statistical mechanical calculations the chains are effectively tethered.

In 1975, Barton and Gunstone[57] measured the T_m's of a series of monounsaturated 1,2-dioctadec-*cis*-enoyl phosphatidylcholines into which they had inserted the single *cis*-double bond at positions C_2 through C_{17}. They obtained the lowest T_m with the double bond at position 9. These results have been widely misinterpreted to mean that the double bond in position 9 biological membranes serves to increase the "fluidity" of the membrane. The position of the Δ9 double bond does not affect the "fluidity," it merely lowers the T_m. The qualities of a molecule that affect T_m relate to the stability of the *gel state*. The word *fluidity* (formally the converse of viscosity) applies to the motion *above the* T_m. Barton and Gunstone's experiments have no bearing on the fluidity of the bilayer above the T_m.

Figure 4 depicts the data of Seelig and Seelig, interpreted according to our water transport model. Since kinks must begin at the ends of the chains, there should be two kinds of kinks in lipid bilayers: the headgroup end and the methyl end.

Headgroup kinks are associated with the lateral movement of the headgroup. The ionic interactions between the headgroups are significantly greater than van der Waals interactions between the chains. Hence, the headgroup movement itself should be the rate-limiting step for water transport. Each ester group jump is presumably associated with a kink diffusing the length of the chain.

Methyl end kinks may be of many *different* varieties. Recent IR studies by Mendelsohn[58] suggest that the C_{10}-to-methyl region is rich in *g-g* and other conformers that apparently do not occur under the conditions of steric constraint of the C_2 to C_9 region. We speculate that, in contrast to the headgroup-jump-initiated *g-t-g* kinks, methyl-end-initiated kinks lack the energy to push the headgroups aside unless they are accompanied by a water molecule.

The central, freeze-fracture-cleavage region of the bilayer has been described by D. Chapman as the "pliant" domain of a phospholipid bilayer. Our model suggests that the C_{10}-to-terminal-methyl region

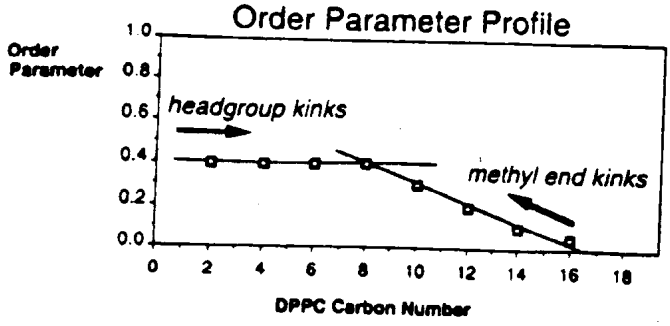


Figure 4 The order parameter of Figure 3 illustrating the proposed model using the premises discussed in Figure 1. The figure is not intended to explain the order parameter solely by kinks, but it suggests that the order parameter profile may be enhanced by these two classes of *g-t-g* kinks.

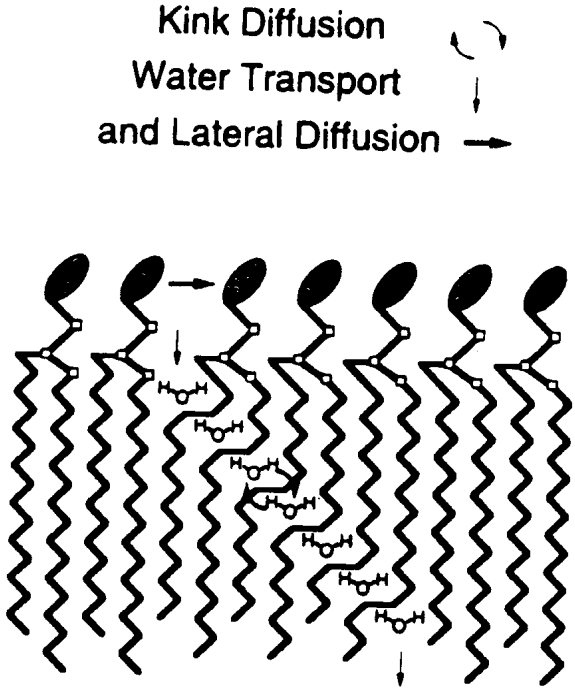


Figure 5 A two-dimensional cartoon describing the path of an isolated water molecule into a bilayer nested between two kinks on adjacent lipid chains.

is more disordered or "liquid-like" than the orderly C_2 to C_9 region. The cooperative motions of the liquid crystal are lateral and apply to both domains in each monolayer. Outside of a small attractive interaction between the two monolayers[39] the motion between them is uncoupled. If the composition of the two monolayers is different, as it is in biological membranes, their physical properties will be different.

"Wavelike" motions *perpendicular* to the plane of the membrane are inevitable. This is because diffusing kinks in adjacent chains must first thin the bilayer and then extend the chain as they hit the end of it, thickening the bilayer again. The most direct evidence for this comes from the very detailed and elegant studies of Wiener and White (Chapter 1).[40]

F. SUMMARY OF THE MODEL

The cartoon in Figure 5 depicts an integrated and comprehensive molecular model of the liquid-crystal state of the phospholipid bilayer just above the T_m. The figure shows the lateral movement of a phospholipid chain due to the diffusion of a kink down the chain. *The diagonal feature of this motion is for illustration purposes only; it is assumed that each of the positions shown occurs on the same*

chain that is shown to move laterally at the top. The water molecule moves down the chain nested between two kinks on adjacent chains. The van der Waals forces help to stabilize the water molecule in the hydrophobic domain. The rate-limiting step for the cooperative motion is the headgroup jump. The model suggests that the many observations made on the bilayer in the liquid-crystal state are each measuring different aspects of but a few processes. Our model suggests that water diffusion and lateral diffusion of the phospholipids, together with tilt, describe most of the chain dynamics in the C_2 to C_9 region. The model suggests that motion is less organized in the C_{10}-to-terminal-methyl region. The lipid motion in this region facilitates cooperative alteration of the thickness of the bilayer, generating a wave motion at the surface.

G. SUPPORTING EVIDENCE

Because the dynamics and the dimensions of the molecules are known, the Haines-Liebovitch-Trauble model yields highly specific predictions. We can calculate selected measurements from others within an order of magnitude. We link the lateral diffusion of the headgroups to the permeability of water. If these processes are linked then a random walk calculation should predict water permeability from lateral diffusion. This calculation *sets a maximum rate* for water permeability. If it predicts that the headgroup jumps exceed the measured water transported, then the model suggests a *possible* mechanism. However, *the model fails if the measured water transported exceeds the headgroup jumps.* We must make an order-of-magnitude calculation since we must rely on literature values conducted in different laboratories on different preparations. Future measurements may be made on a series of single systems.

Once a water molecule leaves the bulk water and enters the low dielectric it is effectively a gas molecule. Its kinetic energy is restrained by the movements of the aliphatic chains. In the frame of this model, it may not *leave* the bilayer without the lateral jump of a headgroup. We calculate the water permeability from the lateral diffusion of the phospholipids as a random walk on a two-dimensional surface. A random walk calculation is only possible if (1) the molecular motion is highly organized and orderly, and (2) the phospholipid headgroup makes discrete random jumps of a fixed length. As for (1), it is the nature of a liquid crystal that the motions are highly ordered *and not fluid.* As for (2), ionic interactions dominate the dynamics of the headgroups.

1. A Random Walk Calculation

We determine the diffusion coefficient, D, from the size and rate of the steps in a random walk.

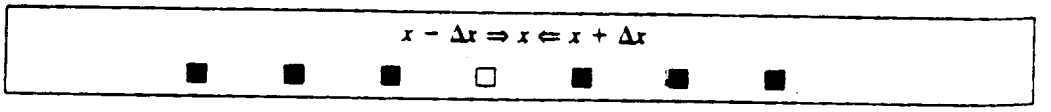
First consider a random walk in one dimension. At each step which takes time Δt, a lipid moves from x to $x - \Delta x$ or $x + \Delta x$. $P(t,x)$ is the probability that the lipid headgroup at time, t, is at position x. p_+ is the probability that the lipid moves forward; p_- is the probability that the lipid moves backward; and

$$p_+ + p_- = 1$$

The probability that the headgroup is at position x equals the sum of the probabilities that the headgroup has arrived from $x - \Delta x$ plus the probability that the lipid has arrived from $x + \Delta x$, *or*

$$P(t + \Delta t, x) = p_+ P(t, x - \Delta x) + p_- P(t, x + \Delta x)$$

as illustrated in the following diagram:



Using a Taylor expansion:

$$P(t + \Delta t, x) = P(t, x) + \Delta t \frac{\partial P(t, x)}{\partial t}$$

$$P(t, x - \Delta x) = P(t, x) - \Delta x \frac{\partial P(t, x)}{\partial x} + \frac{(\Delta x)^2}{2} \frac{\partial^2 P(t, x)}{\partial x^2}$$

$$P(t, x + \Delta x) = P(t, x) + \Delta x \frac{\partial P(t, t)}{\partial x} + \frac{(\Delta x)^2}{2} \frac{\partial^2 P(t, x)}{\partial x^2}$$

we find that

$$\frac{\partial P(t, x)}{\partial t} = -(p_+ - p_-) \frac{\Delta x}{\Delta t} \frac{\partial P(t, x)}{\partial x} + \frac{\Delta(x)^2}{2\Delta t} \frac{\partial^2 P(t, x)}{\partial x^2}$$

In this equation we can now identify c as the drift velocity of a lipid headgroup:

$$c = (p_+ - p_-) \frac{\Delta x}{\Delta t}$$

and D as the rate of diffusion:

$$D = \frac{(\Delta x)^2}{2\Delta t} \qquad \Delta t << 1$$

Thus the random walk can be described by the *Fokker-Planck* equation:

$$\frac{\partial P(t, x)}{\partial t} = -c \frac{\partial P(t, x)}{\partial x} + D \frac{\partial^2 P(t, x)}{\partial x^2}$$

If there is no drift velocity, then $c = 0$ and $p_+ = p_-$, so the equation for one-dimensional diffusion becomes:

$$\frac{\partial P(t, x)}{\partial t} = D \frac{\partial^2 P(t, x)}{\partial x^2} \quad \text{where} \quad D = \frac{(\Delta x)^2}{2\Delta t}$$

A similar derivation for two-dimensional diffusion shows that:

$$\frac{\partial P(t, x)}{\partial t} = D\left(\frac{\partial^2 P(t, x)}{\partial x^2} + \frac{\partial^2 P(t, y)}{\partial y^2}\right) \quad \text{where} \quad D = \frac{(\Delta x)^2}{4\Delta t}.$$

We now apply this calculation to a model in which each lipid headgroup jump entrains a single water molecule into the hydrophobic domain of the bilayer. The *diffusion coefficient*, D, of phospholipids in bilayers above the T_m has been measured using a wide variety of methods[11-39] and found to be approximately 10^{-8} cm^2/s. The measured values actually vary from 1.0 to 25 $\times$ 10^{-8} cm^2/s. Although the calculation should apply best when the measurements are made just above the T_m, this is not the case for the literature measurements.

Let us assume that the *lattice distance between headgroups*, L, is 8 Å or 8 $\times$ 10^{-8} cm (the common measurement obtained from monolayer experiments). We may then calculate the *rate that the headgroups jump* into empty lattice sites.[61]

Since there are many uncertainties in the values of the measurements, we will use the approximate relationship: $D = (\Delta x)^2/\Delta t$. Thus, allowing $\Delta x = L$ and $\Delta t = 1/r$, we find that

$$r = \frac{D}{L^2} = \frac{10^{-8}(\text{cm}^2/\text{s})}{(8 \times 10^{-8}\ \text{cm})^2} = 1.6 \times 10^8\ \text{s}^{-1}$$

The water permeability P is the volume flow per time per area,

$$P = \frac{V}{(\Delta t)A}$$

Each headgroup movement allows one water molecule to enter in time Δt through an area, $A = L^2$.

The volume associated with one water molecule is the mass of one water molecule divided by the density of water $\rho = 1$ g/cm^3. The mass of one water molecule is equal to MW/N_A, where MW = 18 g is the molecular weight and $N_A = 6 \times 10^{23}$ is Avogadro's number.

Therefore, the predicted permeability is given by

$$P = \frac{\left(\frac{MW}{N_A\rho}\right)D}{L^4} = \frac{\left(\frac{18\text{ gm}}{6 \times 10^{23}(1\text{ g/cm}^3)}\right)1 \times 10^{-8}\text{ cm}^2/\text{s}}{(8 \times 10^{-8}\text{ cm})^4} = 7 \times 10^{-3}\text{ cm/s}$$

Many measurements have been made on passive water permeability.[9] These values range from 2 to 20×10^{-4} cm/s. We calculate a value of 70×10^{-4}. Furthermore, the calculation assumes a water molecule enters the bilayer with each jump. This number must be *halved*, since every molecule that enters must also leave with a phospholipid headgroup jump. The result is therefore 35×10^{-4}, a value remarkably close to the observed values. *This estimate is a direct calculation with no adjustable parameters.*

H. THE CHOLESTEROL EFFECT

Cholesterol inhibits the water permeability of phospholipid bilayers. It reduces[62] the *water permeability* of egg lecithin bilayers from 4.2×10^{-3} cm/s to 0.75×10^{-3} in bilayers containing a cholesterol:phospholipid ratio of 1:4, a reduction of about 80%. A measurement[63] of the effect of cholesterol on *lateral diffusion* found it inhibited D for lecithin from 4×10^{-8} cm^2/s to D for lecithin/cholesterol 1/1 of 1.8×10^{-8} cm^2/s, a reduction of about 55%. The random walk calculation above again gives a number within the range of the observed value. The Haines-Liebovitch-Trauble model connects permeability and lateral diffusion and predicts that any bilayer permeant that inhibits one of these properties will inhibit the other proportionately.

A recent study of the cholesterol effect by Saito et al.,[64] using a combination of headgroup and chain fluorescent probes, reveals and confirms many NMR experiments on some of its familiar and yet puzzling molecular dynamics. Cholesterol both restrains the motions of the lipid chains and increases the headgroup spinning motion. These investigators found, using fluorescent headgroup probes, that the order of magnitude of the rate of the headgroup rotor motion was 2 to 3×10^9/s. They observed *increased* headgroup motion of DPPC on addition of cholesterol. In contrast, cholesterol significantly *decreased* the chain motion. That cholesterol only constrains chain motion suggests that water transport, which it inhibits, *may be due to chain motion.* They found that the addition of cholesterol to DPPC bilayers permeated with DPH (the fluorescent probe contained diphenylhexatriene replacing the *sn*-1 chain of DPPC) decreased the quenching of the DPH. This means that the chain probe was exposed to less water in the presence of the cholesterol. These findings suggest that cholesterol not only decreases water permeability, but simultaneously reduces the motion of the hydrocarbon chains in bilayers *and reduces the amount of water in the hydrocarbon domain.*

Bittman and Blau[65] have studied the kinetics of water permeability in the presence of cholesterol. Mendelsohn et al.[66] have shown, using FTIR, that the chain motions of bilayers of DPPC:cholesterol (2:1) have been reduced by a factor of from 6 to 9 compared to DPPC alone. McIntosh et al. have established that on average water does not penetrate deeply in bilayers on addition of cholesterol.

V. SOME IMPLICATIONS OF THE MODEL

Our model has important implications for the understanding of biological systems. Many of these are discussed elsewhere.[70] A few points of chemical and biophysical interest are mentioned here.

Although the most familiar biological membranes are made mostly of straight-chain phospholipids, there are common variations in chain structure. The model predicts that the headgroups participate in water transport by moving laterally. So long as the chain is a straight chain of methylenes then the rules that Flory worked out for polyethylene apply. In nature, biological membranes do not consist of lipids made *only* of methylene chains. Biological membranes with a high proportion of saturated fatty acid chains have, in addition, a large fraction of chains that have at least one double bond in the 9–10 position. Kinks cannot diffuse through a double bond because it is rigid and flat. With rare but important exceptions double bonds in natural lipids are always *cis*. According to Flory, the CH_2–CH_2 bond

adjacent to a double bond or a carbonyl has a very low *g-t* isomerization energy. This means that for monounsaturated chains the C_2 to C_9 segment may be in a low-energy state when it is all-*trans*. This is because *g-t* isomerization at both ends of such a rod is low energy. Such low-energy tilting of this rigid all-*trans* rod may explain why monounsaturated chains may allow cholesterol-like dynamics in the lipid chains.

Although DPPC is not a natural lipid, it has been extremely useful as an archetype. The reason that DPPC does not occur naturally is presumably due to its high T_m. If water transport and motion within bilayers was important in the evolution of biological membranes, then clearly the T_m had to be below ambient. If chain lipids permitted the dynamics described in our model, then lipid structures were needed that reduced the T_m and yet permitted water transport.

Nature has developed at least three chemical methods for altering the chains that reduce the T_m of bilayers:

- The monounsaturated fatty acid chain (and the polyunsaturated variation).
- The *iso* and *anteiso* chains of certain prokaryotes.
- The phytanyl chains of the *Archaebacteria*.

Monounsaturated fatty acids reduce the T_m to below freezing. The introduction of more double bonds cannot usefully further reduce the T_m. *Cis* double bonds have the same shape as *g-t-g* kinks. Fewer steps for transport would be required for the sequence of events shown in Figure 5 since each *cis* double bond requires three, not two, carbons. Only four *cis* double bonds fit into a 16-carbon chain, whereas six *g-t-g* kinks may fit. Their presence would hasten water transport according to the model.

Lagaly et al.[68] have reviewed double bonds and their effect on chain conformations in bilayers.

Chain lipids made of *iso or anteiso lipids* are straight-chain lipids in the C_2 to C_9 region of the bilayer. They have methyl branches in the ω-1 and ω-2 terminal positions respectively. The role of these distal methyls is presumably to prevent them from forming a stable gel state, i.e., to reduce the T_m. To our knowledge no water permeability studies, order parameter studies, or lateral diffusion studies have been conducted on bilayers of these lipids.

The conformation of *polyisopranes* is radically different from that of the aliphatic chains. One of us (T.H.H.) has made models of a bilayer of this lipid and drawn the conclusion that the water molecules in this case move in a helix down each molecule around the central chain. This model implies the following:

- Methyl groups on the chain move more rapidly than the central strand. This was observed by Lindsey, Petersen, and Chan[69] for diphytanoyl phosphatidyl choline (DΦPC).
- There is little or no lateral motion of the lipids in the plane of the bilayer associated with water transport. To our knowledge the lateral diffusion of diphytanyl lipids has not been reported.
- Proton permeability through these lipids should be significantly lower than through the chain lipids; closer to that of the alkali cations. Very recently this has been observed for DΦPC.[70]

The amount of water in the bilayer is the subject of some controversy. Measurements by Miller[71] indicate remarkably high water content in the low dielectric.

This model of water transport immediately suggests that protons may cross the bilayer along a diagonal proton wire. Unlike water permeability, proton permeability should be chainlength dependent, since it is based on the probability of wire formation at each step in the water transport mechanism. Readers are referred to Chapter 9 for a thorough discussion of the data on the permeability of protons across bilayers.

VI. SUMMARY AND CONCLUSIONS

We have summarized the transport of water through phospholipid bilayers. In general water permeability measurements vary from 2 to 20 $\times$ 10^{-3} cm/s. Measurements have been made by tritium labeling, NMR, and a variety of other techniques. Biological membranes display similar levels of water permeability unless they contain protein water channels or in some cases ion channel proteins that also conduct modest amounts of water.

The mechanisms for water permeability through lipid bilayers include the solubility-diffusion view, the defect model, and a molecular dynamics diffusion model in which each water molecule is nested with two *g-t-g* kinks between adjacent chains of the phospholipids.

We propose a molecular model consistent with the first and last of the above proposals. In our model, the lateral diffusion of the phospholipids both produces *g-t-g'* kinks and allows water permeability. We discuss our model in molecular detail. We present a random walk calculation showing how measurements of the lateral diffusion of the phospholipids predict the water permeability within an order of magnitude.

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