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Minireview

Water transport across biological membranes

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Abstract

The rate of the lateral diffusion of *straight-chain* phospholipids predicts the rate of water diffusion through bilayers. A new model of lipid dynamics integrates these processes. Substances such as cholesterol that reduce water diffision proportionally reduce lateral diffusion. The model yields a number of predictions about the dynamics of the lipids at the T_m and suggests different mechanisms for how water diffuses across bilayers of other-than-straight-chain lipids, and how proteins bind to membranes. A second recent development in water transport across biological membranes is the discovery of a ubiquitous family of water transport proteins that facilitate large-volume water translocation. Like water diffusion through lipid bilayers, water transport by these proteins is directed by osmosis and is therefore under the control of ATP and ion pumps. The presence of water transport proteins in membranes is often regulated by hormones.

Key words: Water transport; Lipid lateral diffision; Bilayer; Liquid crystal; Molecular dynamics; Water channel; CHIP28

1. Introduction

Since Pfeffer [1] first described it in 1877, osmotic behavior of living cells has been a central focus in Biology. In 1908 Nernst [2], recognizing that water could pass through biological membranes freely but ions could not, introduced the concept of the 'semipermeable' membrane. Just *how* water passes through membranes has only begun to come clear in the last five years. Water moves through living membranes through both the lipid bilayer and through specific water transport proteins. The rate through the lipid bilayer depends on lipid structures and the presence of sterols. Water flow through water transport proteins is controlled by the number of copies of the proteins in the membrane. In both cases water flow is *passive* and directed by osmosis. Water transport therefore depends on ion pumps, ion channels and ion exchange proteins. It is ultimately determined by the bioenergetics of the cell. In most cases the energy derives from ATP.

There are three mechanisms of water transport through biological membranes. This Minireview will cover only the first (water diflision through bilayers).

The most general water transport mechanism is diffusion through lipid bilayers (2 to 50×10^{-4} cm/s). Diffusion occurs only above the T_m of the bilayer. Living organisms adjust their membrane lipid composition to keep the T_m of the lipids of their membranes some 10 degrees below ambient. They do this by changing the structures of the lipid chains, not the headgroups. Presumably one important reason for this is the need for water diffusion through the bilayer. A recent random walk calculation [3] suggests that the lateral diffusion of the lipids and the water diffision through the bilayer is a single process. Cholesterol proportionately reduces both the lateral diffusion of the lipids and the water diffusion through bilayers.

Water transport also occurs through certain membrane transport proteins such as the glucose transporter [4,5] or the anion channel of erythrocytes [6]. Despite numerous investigations of such transport proteins including ion channels, investigators have concluded that they transport little water for two reasons. First, there are few copies of the proteins present in the membrane. Second, although certain channels such as the CFTR-chloride channel conduct when open, they are only briefly open. Oocytes expressing CFTR-chloride channels displayed only an increase of water permeability of 4×10^{-4} cm/s [7] The flow rates through such proteins are greater than bilayer diffusion but are probably not physiologically significant.

The highest volume of water transport (200×10^{-4} cm/s) passes through a class of apparently ubiquitous water transport proteins. The archetypal member of this family is CHIP28 (channel-forming integral protein, 28 kDa) an erythrocyte protein that is sensitive to sulfhydryl inhibitors. Since its isolation and characterization as an integral protein of unknown biological function [8], it has been found to be a water channel when expressed in *Xenopus* oocytes [9] and in rat kidney [10] These proteins are a large family. of which three have been found in human tissues. They have been sequenced, reconstituted into lipid bilayers, and two-dimensional crystals have

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been obtained of these proteins. Some are sensitive to $HgCl_2$ while others are not. Members of the family have been identified in *E. coli*, yeast, *Drosophila*, plants and mammals. Water transport proteins are present in a membrane in proportion to the overall water transport activity of the membrane. Finally, in tissues where water transport is regulated, the proteins are stored in intracellular vesicles which fuse with the plasma membrane upon a signal from a hormone such as vasopressin [10].

The following are important recent reviews on water transport across biological membranes. For water channels proteins there are those of Verkman [11] and Nielson et al. [12]. The most recent prior reviews are those by Harris Strange and Zeidel [13] by Bacic Srejic and Ratkovic [14] by Finkelstein [15] in two volumes edited by Benga [16], and by Macey [17]. Water permeability through lipid bilayer preparations has been studied since the 1960's. Bilayer vesicles prepared by Bangham and co-workers [18] permitted measurements of vesicular water flow. This was later used by Carruthers and Melchior [19]. The preparation of planar lipid bilayers immediately permitted direct measurements of water movement through bilayer sheets [20–22].

Lawaczeck [23] has developed a fluorescence technique for measuring water permeability through phospholipid vesicles. See also a review by Fettiplace and Haydon [24]. Various models for the movement of water through bilayers of phospholipids have been reviewed by Deamer and Bramhall [25], de Gier [26], and Disalvo et al. [27]. These reviews [18–27] cite many measurements of water diffusion through pure phospholipid bilayers ranging from $2–200 \times 10^{-3}$ cm/s.

In this minireview I shall cover the mechanism of water transport across lipid bilayers derived from the connection between the lateral diffusion of the lipids and water diffusion through chain lipid bilayers at the T_m Readers interested in water transport proteins are referred to the above reviews.

2. Diffusion of water through chain lipid bilayers

Physical chemists treat water diffusion through lipid bilayers as solubility-diffusion. On the basis of measurements of the solubility of water and molecules such as N_2, O_2, Finkelstein [15], Hanai and Haydon [28], and Reeves and Dowben [29], and others have predicted water flow through bilayers from its solubility in liquid hydrocarbons. Given the entropic energy that maintains the intrinsic oil–water interface, it is surprising that water passes so easily through bilayers. Deamer and Bramhall [25] have proposed a defect model in which the lipids of the bilayer molecules (only two molecules thick) separate just enough at some discrete frequency to allow water to pass through the membrane.

In 1971, Träuble [30] 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 [31] as low energy motion for chain polymers. 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.

3. The gel-to-liquid-crystal phase transition (T_m)

Water transport through lipid bilayers occurs when the bilayer is in the liquid crystal state (above the T_m) A molecular dynamics model for water diffusion must account for the molecular dynamics of this state. At present we lack a coherent molecular dynamics model of the T_m for even a one-component phospholipid bilayer [32,33].

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

1. The lateral diffusion of phospholipids was first measured in 1972 [34–36]. The rate varies slightly depending on the technique, the structure of the lipids, and conditions, such as temperature, but are within an order of magnitude of about 10^{-8} cm^2/s. The rate is unchanged when measured on lipid bilayers or cellular membranes [37].

2. Water diffuses through bilayers above the T_m within an order of magnitude of 10^{-3} cm/s, the rate found in the early measurements of van Deenen [38] and of Bangham [18,39].

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 [40] used deuterium quadrupole coupling NMR to obtain 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 carne from the spin-label work of Hubbell and McConnell [41]. The order parameter plateau of Seelig and Seelig has been confirmed by a wide variety of measurements during the last two decades [42]. Recently developed infrared studies on deuterolabelled chains [43] provide, in addition to the order parameter, specific information on the chain rotamers.

4. The bilayer expands 30–40% laterally at the T_m, varying with phospholipid structure [44]. 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 expan-

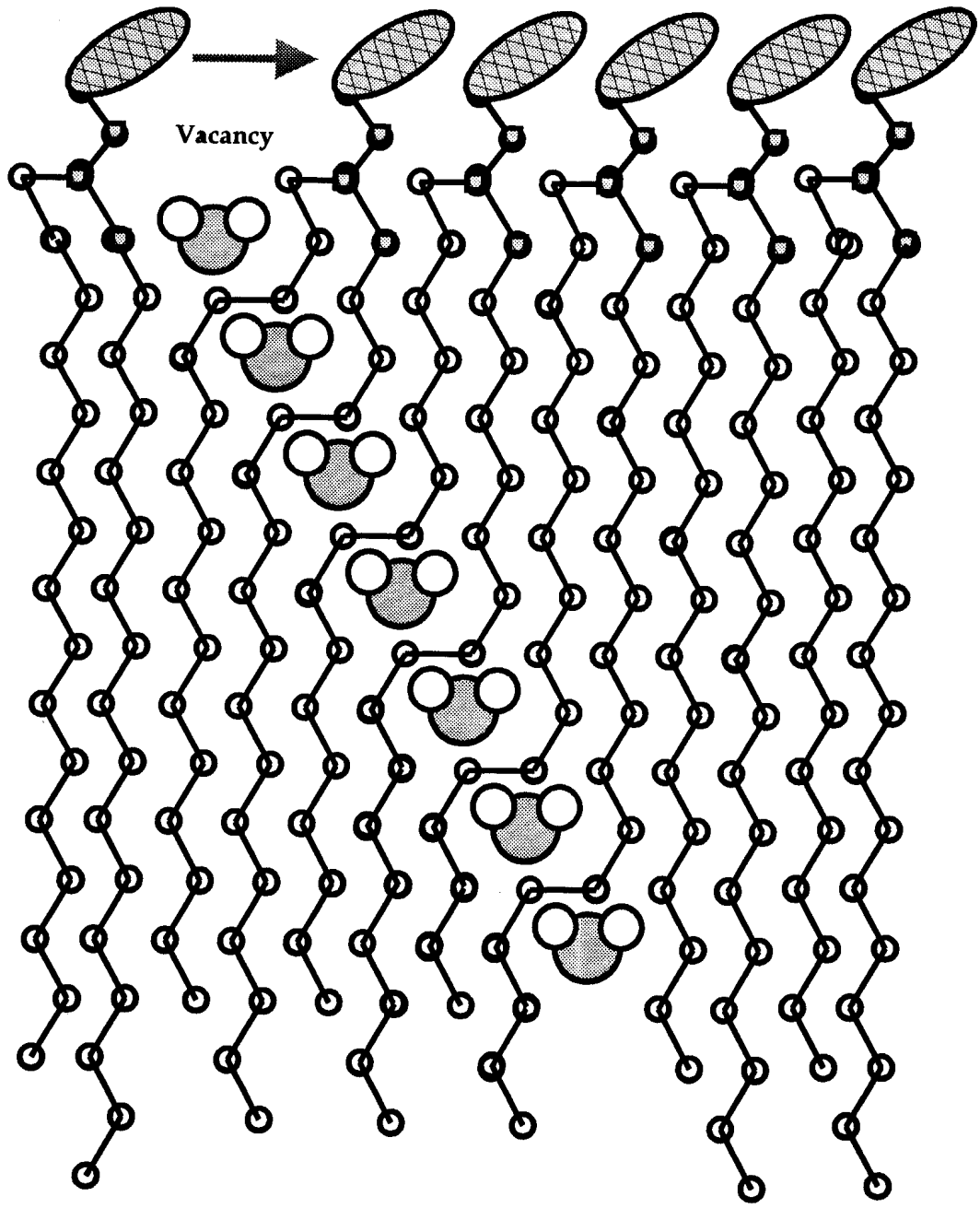

Fig. 1. A 2-dimensional cartoon displaying the progress of an isolated water molecule into a bilayer. The water molecule enters the bilayer at a 'vacancy' in the headgroup lattice that was created as the bilayer expanded upon passing through the T_m. It is suggested that the headgroup on the left 'jumps' into the vacancy covering the water molecule. The series of water molecules are actually the same water molecule moving down the same chain. The chain moves laterally to the right as the water molecule proceeds down the chain. The water molecule is nested between *g-t-g* kinks on adjacent chains as suggested by Träuble [30]. The rate of kink diffusion is orders of magnitue faster that the rate of headgroup jumps. The random walk calculation suggests that the rate of headgroup jumps is the rate-limiting step in water transport.

sion 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 [45] in 1968. Bilayers of DPPC, for example, are 47Å thick in the gel state but are 35Å thick above the T_m [46].

4. The Haines–Liebovitch–Träuble model for bilayer water transport

These 5 features may be viewed as aspects of a single process (Fig. 1). The lateral expansion of the bilayer introduces 'vacancies' in the phospholipid headgroup 'lattice'. Water fills each vacancy as it appears. An adja-

cent 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. Thus *the lateral motion of the lipid molecules is linked to the water migration through the bilayer*. A two-dimensional random walk calculation predicts the rate of water flow from the rate of the lateral diffusion of the phospholipids and vice versa.

As early as 1966, Chapman recognized that phospholipid *monolayers* assumed a 'condensed state' and an 'expanded state'. [47,48] as did bilayers. The monolayer condensed state is the bilayers' gel state. The expanded state is the liquid crystal state. A 30 to 40% expansion at the T_m increases the average distance between the chains less than 10%. Hence 30–40% expansion in the bilayer means a 20–30% vacancy rate in the headgroup lattice. 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 depends on both the water solubility in the hydrocarbon [15] and by the kinetics of the lipids due to thermal energy (kT).

Flory [31] used statistical mechanical calculations to predict many physical properties of linear polymers. He showed that in hydrocarbon chains the lowest energy defects that migrated down the chains were *g-t-g* (*gauche-trans-gauche*) kinks. He made the following three important points regarding the chain dynamics ofthese kinks that directly apply [3] to phospholipid bilayers in the liquid crystal state. (1) For a linear hydrocarbon kinks may only be initiated at either end of the polymer chain. Kinks cannot begin in the middle of the chain for steric reasons. *Applicalion to lipids:* Kinks must begin either at the headgroup end, or the methyl end of a lipid chain. (2) This lowest energy (*g-t-g*) kink, which propagates down the polymer chain at rates of 10^9/s or faster, displays momentum. *Application to lipids:* Once started at the headgroup end, or the methyl end, kinks must continue to the other end unless impeded by an external force. (3) Polyethylene chains may be viewed end on as an approximate hexagonal lattice. When a *g-t-g* kink has moved down the length of the chain, the chain has moved over one lattice unit in the hexagonal array. *Application to lipids:* Kinks (such as those nesting water molecules as suggested by Träuble [30]) are coupled to the lateral movement of a phospholipid because they displace the chain by a lattice unit [3]. Transbilayer water movement (along the hydrocarbon chain) thus moves the chain laterally to a neighboring lattice unit.

Seelig and Seelig [40] using deuterium quadrupole coupling NMR, described a surprising order parameter profile for DPPC. (1) 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. (2) They found a low (0.4) order parameter of the plateau. An order parameter near 0.4 implies 60% disorder. Are the kinks in the C_2–C_9 region are associated with transverse water movement and lateral headgroup movement? Such kinks would go the full length of the chain. (3) They found that the order parameter plateau of this saturated lipid ended at C_9. The Seeligs' discovery of a break at C_9 is intriguing because of the structures of lipids in 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^8 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. Recent IR studies on saturated chains by Mendelsohn [49] suggest that the C_{10}-to-methyl region is rich in *g-g* and other conformers in contrast to the C_2–C_9 region which is known to have a high proportion of *g-t-g* kinks. Although eukaryote lipids have more variant chains, including polyunsaturated lipids, they contain sterols. As described by Rothman and Engleman, [50] 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 [51] who had predicted such a curve assuming that the chains were tethered to immobile headgroups. Haines and Liebovitch [3] assume that the lateral movement of a headgroup-initiated kink moves the length of the chain. Thus the order parameter plateau associated with the lateral movement of the headgroup (headgroup-initiated kinks). Ionic interactions between the headgroups are the strongest interactions between the chains. Lateral headgroup movement is presumably associated with a kink diffusing the length of the chain. Methyl-end-initiated kinks lack the energy to push the headgroups aside unless they are accompanied by a water molecule.

Because the dynamics and the dimensions of the molecules are known, the Haines–Liebovitch–Träuble model yields highly specific predictions. One can calculate selected measurements from others within an order of

magnitude. The lateral diffusion of the lipids may be calculated from the water permeability by a random walk calculation on a 2-dimensional surface. A water molecule that leaves the bulk water enters the low dielectric as a gas molecule. Its kinetic energy is restrained by the movements of the aliphatic chains. If the rate limiting step in water transport is headgroup jumps, then it may not *leave* the bilayer without such a lateral jump. A random walk calculation is only possible if: (1) the molecular motion is organized and orderly, and (2) if 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.

A random walk calculation begins with the conversion of the diffusion coefficient into steps of discrete size in a random walk. If there is no drift velocity, then the equation for a two-dimensional diffusion shows that:

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

Let's assume each lipid headgroup jump entrains a single water molecule into the hydrophobic domain of the bilayer (Fig. 1). The diffusion coefficient, D, of phospholipids in bilayers above the T_m has been measured using many methods to be approximately 10^{-8} cm^2/s. The measurements actually vary from 1.0 to 25×10^{-8} cm^2/s. Although the calculation applies best when the measurements are made just above the T_m, this is not the case for most literature measurements.

Let us assume that the lattice distance between headgroups, L, is 8Å or 8×10^{-8} cm (the common measurement obtained from monolayer experiments). We may then calculate the rate that the headgroups jump into empty lattice sites [52].

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 $\Delta t = 1/r$, where r is the rate of a headgroup jump. We find that:

$$r = \frac{D}{L^e} = \frac{10^{-8}(\text{cm}^2/\text{s})}{(8 \times 10^{-8}\text{cm})^2} = 1.6 \times 10^6 \text{ s}^{-1}$$

The headgroup jump-rate is approximately 10^6. This is a direct calculation from the measured diffusion rate. This is an important result because the chain motions (kink diffusion, etc.) is about 10^9.

The water permeability P is the transmembrane 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 ρ = 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

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

$$= 7 \times 10^{-3} \text{ cm/s}$$

Many measurements have been made on passive water permeability [15]. 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.*

5. The cholesterol effect

Cholesterol inhibits the water permeability of phospholipid bilayers. It reduces [53] 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 [54] of the effect of cholesterol on *lateral diffusion* found it inhibited D for lecithin from 4×10^{-8} cm^2/sec 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.

A recent study of the cholesterol effect by Saito et al. [55] 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 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 [56] have studied the kinetics of water permeability in the presence of cholesterol. Mendelsohn et al. [57] have shown, using FTIR, that the chain motions of bilayers of DPPC/cholesterol (2:1) have been reduced by a factor of 6–9 compared to DPPC alone.

6. Some implications of the model

This model of water transport has implications for the understanding of both lipid dynamics and biological systems. Although the most familiar biological membranes are made of straight-chain phospholipids, there are common variations in chain structure. So long as the lipid has straight chain 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. Those biological membranes with a high proportion of saturated fatty acid chains have, in addition, a large fraction of chains with 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 [31], 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–C_3 bonds and the C_7–C_8 bonds have low energy transitions and may allow the C_3 to C_7 segment to be all-*trans*. Such low-energy tilting of this rigid all-*trans* rod may explain why monounsaturated chains often display affinity for cholesterol.

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

Cis-monounsaturated fatty acids reduce the T_m to below freezing. *Trans*-double bonds do not reduce the T_m significantly because they fit into the all-*trans* conformations of saturated chains *in the gel state. Cis*-double bonds have approximately the same size and shape of *g-t-g* kinks thus they reduce the T_m. The introduction of more double bonds cannot usefully further reduce the T_m. Most double bonds in *polyunsaturated* fatty acid chains occur in the C_{10}-to-terminal-methyl region, although in some, such as arachidonic acid, a double bond occurs in the C_{2-9}region. Methylene-interrupted double bonds are essentially hinged planes. Lagaly et al. [58] have reviewed double bonds and their effect on chain conformations in bilayers. A *cis*-double bond is approximately the same size and shape as a *g-t-g* kink. It would seem that *cis*-double bonds, whether methylene interrupted or isolated as in $\Delta 9$ may replace *g-t-g* kinks to facilitate the movement of water molecules across the bilayer. Fewer steps for water transport would be required since each ci 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 not hasten water transport but might increase proton leakage.

Chain lipids made of *iso* or *anteiso* lipids are straight-chain lipids in the C_2–C_9 region of the bilayer. They have methyl branches in the ω-1 and ω-2 terminal positions, respectively. These distal methyls presumably serve to prevent them from forming a stable gel state, i.e. they reduce the T_m No water permeability studies, order parameter studies nor lateral diffusion studies have been conducted on bilayers of these lipids.

The conformation of *polyisopranes* or phytanyl chains of the Archaebacteria is radically different from that of the aliphatic chains. Models of a bilayer of this lipid suggests that the water molecules in this case move in a helix down each molecule around the central chain. This pattern of dynamics that the methyl groups on the polyisoprane chains move more rapidly than the central strand. This was observed by Lindsey, Petersen and Chan [59] for diphytanoyl phosphatidyl choline (DΦPC). Little or no lateral motion of the lipids in the plane of the bilayer would be expected associated with water transport. To our knowledge a study of the lateral diffusion of diphytanyl lipids has not been reported. If the mechanism below is correct for proton permeability of straight-chain lipids, then the proton permeability through these lipids should also be significantly lower than through the chain lipids; closer to that of the alkali cations. Very recently this has been observed for DΦPC [60] *Proton leakage* across lipid bilayers is commonly viewed today as a defect phenomenon [61]. The Haines–Liebovitch–Träuble model raises another mechanism for proton leakage [62]. As each water molecule enters the bilayer it rides a *g-t-g* kink two carbons at a time. As a sixteen carbon chain moves from one location in the lattice (looking down on the bilayer) to another, a kink moves down the chain from the ester group to the methyl end in seven steps. With some frequency water molecules will be on two adjacent chains. Likewise there will be three adjacent, four, etc. However infrequent, it is also inevitable that a proton-carrying Grotthus mechanism may result when there 10 to 15 adjacent water-carrying chains. Thus, a cluster of chains, each chain escorting a water molecule, must line up so that the water molecules form a hydrogen bonded diagonal wire. The frequency of the formation of such a proton wire depends upon two properties of the bilayer, the amount of water in the bilayer, and the thickness of the bilayer.

Membrane-associated, or *extrinsic proteins may not*

bind to lipid headgroups as this would prevent lateral diffusion of the lipids and therefore water transport. Measurements using spin or fluorescent labels have shown that the lipids in the membranes of living cells diffuse laterally at the same rates as they do in synthetic lipid bilayers in the absence of proteins. Thus the model suggests that cytoskeletal proteins must attach to membranes in cells via transmembrane proteins and not directly to the lipid headgroups. An interesting exceptional biological membrane where the lipids do bind to protein is the myelin sheath. Basic protein in the myelin sheath binds to the acidic lipids as it does to acidic lipid bilayers in vitro. If this membrane is permeable to water, the model suggests that it must have water channel proteins that connect the lamellar sheets.

Finally, this model suggests many *experiments*. These include the study of water transport and lateral diffusion (and proton leakage) in bilayers made of defined lipid chains. For example varying the chainlength should not alter the rate of water transport, nor should it alter the lateral diffusion of the lipids. However it predicts that the proton leakage should fall off logarithmically with the increased chainlength. A study of the binding of myelin basic protein (or polylysine) to a bilayer of anionic lipids should decrease both the water transport and the lateral diffusion of the lipids.

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