PROGRESS IN BIOPHYSICS AND MOLECULAR BIOLOGY

VOLUME 29 30

Progress in Biophysics & Molecular Biology

Volume 29, 1975
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Prog. Biophys. Molec. Biol. 1975. Vol. 29, No. 1, p. 1. Pergamon Press. Printed in Great Britain

EDITORIAL ANNOUNCEMENT

The Editors have to inform their readers that beginning with this volume, *Progress in Biophysics and Molecular Biology* will be published by Pergamon Press Ltd in parts, three parts to form a volume. It is expected that this arrangement will in many cases permit the time between receipt of article and publication to be appreciably reduced. When a volume is completed the Publisher will provide subscribers with title and contents pages, and the subject index for the volume and the cumulative author and title indices as hitherto included, together with a binding case. Hard bound volumes will also be available for those subscribers who wish to continue to take the publication in this form.

The policy of the Editors will remain unchanged. As in the past they will aim at obtaining substantial articles concerning progress during the last few years over extensive areas of biophysics and molecular biology, which also provide personal and critical reviews of their subjects. Authors are asked to remember that their articles will be read by advanced students and others with a general interest in the subject as well as by experts in the subject. It is thus hoped that the series will provide a useful supplement to more extensive treatises and reference books. Most of the articles in previous volumes have been specially commissioned, but the Editors are always pleased to receive suggestions for or offers of articles for future volumes. Any such proposals should be made to one of the Editors at the address given below.

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Prog. Biophys. Molec. Biol. 1975. Vol. 29, No. 1, pp. 3–56. Pergamon Press. Printed in Great Britain.

FUNCTIONAL PROPERTIES OF BIOLOGICAL MEMBRANES: A PHYSICAL-CHEMICAL APPROACH

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CONTENTS

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"For an answer which cannot be expressed the question too cannot be expressed. The riddle does not exist. If a question can be put at all, then it can also be answered. For doubt can only exist where there is a question, a question only when there is an answer, and this only when something can be said" (Wittgenstein).

I. INTRODUCTION

Membrane biology has grown rapidly to become a plant of impressive size. But how to find the fruit amongst all the foliage; that is now the problem. The search is especially difficult because the recent growth in membrane studies can be attributed in no mean part to the application of a variety of spectroscopic techniques, and before such studies can provide facts of biological relevance, it is necessary to establish the limits of applicability of each technique. Since this is no trivial task for systems as complex as biological membranes, many studies have created more problems than they have solved.

There are already a large number of published reviews covering the applications of particular physical techniques to the study of membranes; enough to prompt many a reflection like those of Ecclesiastes, "Of making many books there is no end, and much study is a weariness of the flesh". The reason for adding one more is to try to put together some of the scattered observations that have been made about biological membranes, to see whether any coherent picture can be made to emerge.

The most useful spectroscopic techniques for studies of membranes are X-ray diffraction and the magnetic resonance methods, nuclear magnetic resonance (nmr) and electron spin resonance (esr). In X-ray diffraction studies, data is collected over a long period of time, so that a time-averaged picture of the membrane is obtained: such studies therefore provide information about the basic, structural organization of the membrane. On the other hand, the magnetic resonance methods can provide information about the dynamic processes in membranes, since nmr and esr data are sensitive both to the chemical environment of particular groups in a molecule, and to the motion of those groups. In esr, a free radical such as a nitroxide group (see, for example, II, p. 8) is introduced into the membrane, and it is the unpaired electron of this free radical which is observed. In nmr, magnetic nuclei such as ^{1}H and ^{13}C are observed, so that it is possible to study membranes completely unperturbed by the presence of any probe molecules. Studies of the membrane as a dynamic structure require spectroscopic methods sensitive to motions in the range from 10^{-11} to a few seconds and this is the range spanned by the magnetic resonance techniques.

Problems associated with the application of particular techniques to membrane structure will not be discussed here at any length. Reviews detailing these problems include the following: X-ray diffraction (Levine, 1973); electron spin resonance (Jost *et al.*, 1971; Keith *et al.*, 1973); nuclear magnetic resonance (Horwitz, 1972; Lee *et al.*, 1974a); fluorescence (Radda and Vanderkooi, 1972); general techniques (Chapman, 1968, 1971; Levine, 1972).

Most of these studies have been concerned with the lipid component of membranes; the study of the protein component requires highly simplified systems which have only recently become available. This means that only a rather one-sided picture of the membrane can be presented at the present time. Nevertheless, even such a picture is worthy of attention, since, as has long been recognized, lipids play a crucial role in determining membrane function:

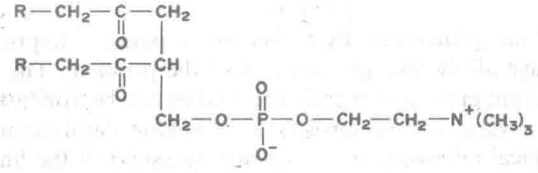
"Phosphatides (phospholipids) are the centre, life, and chemical soul of all bioplasm whatsoever, that of plants as well as animals. Their chemical stability is greatly due to the

fact that their fundamental radicle is a mineral acid of strong and manifold dynamicities. Their varied functions are the result of the collusion of radicles of strongly contrasting properties. Their physical properties are, viewed from a teleological point of standing, eminently adapted to their functions. Amongst these properties none are more deserving of further inquiry than those which may be described as their power of colloidation" (Thudichum, 1884).

II. PHOSPHOLIPIDS

A. *The Structures of Phospholipid–Water Mixtures*

Phospholipids can adopt a variety of structures in the presence of water, characterized by a long-range organization periodic in one, two or three dimensions (Luzzati, 1968). The commonest of these structures for the phosphatidylcholines (I) is the lamellar phase which



(I)

consists of bimolecular leaflets of lipid lying with their planes parallel and separated by regions of water (Fig. 1). The bimolecular leaflet is often referred to as a lipid bilayer, and contains a central core of fatty acid chains surrounded by two planes of hydrated polar groups in an aqueous environment. Aqueous dispersions of phosphatidylcholines contain fragments of the lamellar phase dispersed in excess water. These fragments are referred to as liposomes, and consist of spherically concentric bimolecular leaflets, with water trapped between the bilayers (Bangham and Horne, 1964). On irradiating suspensions of liposomes with ultrasonic (high frequency) sound, the large particles are broken down to

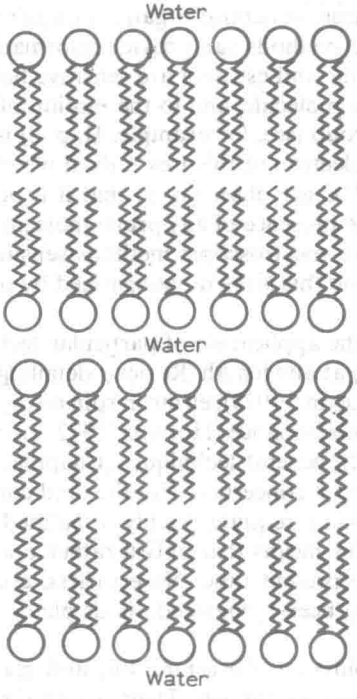


FIG. 1. The lamellar phase adopted by aqueous dispersions of phospholipids.

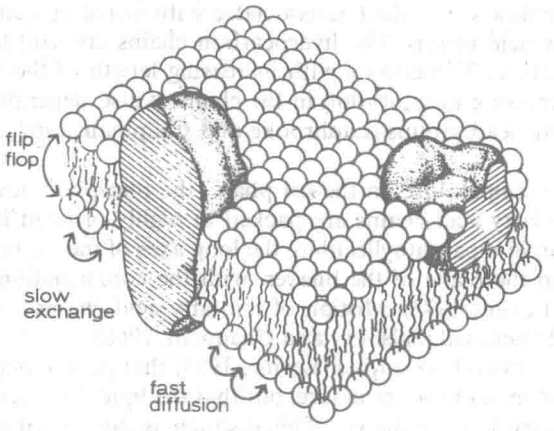


FIG. 2. The Singer–Nicolson model for the biological membrane, showing possible lipid motions (adapted from Singer and Nicolson, 1972).

give smaller more symmetrical aggregates. Prolonged ultrasonic irradiation produces vesicles which are closed spheres about 250 Å in diameter, each composed of a single continuous lipid bilayer membrane enclosing a volume of aqueous solution (Huang, 1969).

Lipid bilayers can also be formed across a small hole in a Teflon diaphragm immersed in an aqueous solution, by painting a small amount of a solution of lipid in n-decane across the hole. The film which is formed gradually thins until it becomes optically "black", at which point the membrane consists essentially of a bimolecular layer of oriented lipid molecules. Such black lipid films have been much used in studies of electrical conductivity (Lauger, 1972). Similar bilayers can be formed in the absence of the n-decane, but they tend to be very fragile (Montal and Mueller, 1972).

Lipids also form other, more complex phases in water, but these tend only to occur in the high-temperature and low water concentration regions of the phase diagram. More interestingly, it has also been shown that transitions from one phase to another can be caused by the addition of metal ions. Thus addition of Ca^{2+} to an aqueous dispersion of cardiolipin causes a phase transition from a lamellar to a hexagonal phase (Rand and Sengupta, 1972). However, in mixtures of cardiolipin with phosphatidylcholine, the lamella phase persists in the presence of Ca^{2+}. There is as yet no evidence for the presence of any lipid phase other than the lamella in biological membranes.

The most commonly accepted picture of the biological membrane is of a lipid bilayer, with some protein molecules partially embedded in the lipid matrix and some completely penetrating the bilayer (Fig. 2) (Singer and Nicolson, 1972). In this review, we will be very largely concerned with the dynamic, motional, properties of the lipid component of the membrane. Figure 2 illustrates the types of motion that can be expected. Firstly, there is the possibility of rapid internal motion within each lipid molecule. Secondly, lipid molecules might diffuse laterally in the plane of the bilayer, although the rates of this motion could be very different for those lipids associated with proteins and those lipids which make up the bulk of the membrane. Thirdly, lipid molecules may be able to rotate rapidly as a whole about their long axes. Lastly, there is the possibility of transfer of lipid molecules from one side of the bilayer to the other—a motion referred to as "flip-flop". Associated with these lipid motions, there are also various possible motions for the protein molecules: rotation and lateral diffusion in the plane of the membrane, and rotation through the membrane. Evidence has now accumulated for all these motions except the last.

B. *The State of the Lipid Fatty Acid Chains*

The physical state of phospholipids depends markedly on temperature. On heating, phospholipids undergo an endothermic transition at a temperature well below the true melting point. At this temperature, a change of state occurs from the crystalline (or gel)

state to the liquid crystalline state, which is associated with increased conformational freedom for the lipid fatty acid chains. The hydrocarbon chains are said to have "melted". The transition temperature, T_t, increases with increasing length of the fatty acid chains, and decreases with increasing unsaturation in the chain; it also depends on the chemical nature of the lipid polar head groups (Ladbrooke and Chapman, 1969; Hinz and Sturtevant, 1972b).

Below the transition temperature, in the gel phase, the phospholipids adopt a bilayer structure in which the fatty acid chains are packed in highly ordered hexagonal arrays. At maximum hydration in dipalmitoyllecithin, the long axes of the chains are tilted about 30° from the normal to the plane of the bilayer, with the conformation about all C—C b nds close to *trans* (Levine, 1973). Motion of the fatty acid chains in the gel phase is highly anisotropic and restricted (Salsbury and Chapman, 1968).

It is clear from X-ray diffraction studies (Levine, 1973) that phospholipids in the liquid crystalline phase still adopt a bilayer structure, but that the lipid fatty acid chains are considerably more disordered than in the gel. This disorder is due to rotation about C—C bonds. A potential energy diagram for rotation about the central C—C bond of an isolated butane molecule is shown in Fig. 3. The minimum potential energy occurs in the *trans* conformation, but there are also two other, higher energy minima, corresponding to the *gauche* conformations. The energy of the *gauche* conformation is *ca.* 0.5 kcal/mole above that of the *trans* conformation, and the energy barrier between the *trans* and *gauche* conformations is *ca.* 3.6 kcal/mole (Hagele and Pechold, 1970).

The relative sharpness of the minima and the quite high barriers to rotation mean that the molecule will remain for most of the time in the vicinity of the minima, carrying out torsional oscillations. Typically, C—C bond oscillation frequencies are 100–200 cm^{-1} or 3–6 $\times$ 10^{12} sec^{-1}. The energy barrier preventing rotation between *trans* and *gauche* conformations in butane is *ca.* 3.6 kcal/mole. The rate at which the molecule jumps from one conformation to the other is related to the height of the barrier by the equation

$$\nu = \frac{\mathscr{K}\, kT}{h} \exp(-E/RT) \qquad (1)$$

where E is the activation energy for the jump, and $\mathscr{K}$ is the transmission coefficient for passage over the barrier. Generally it is assumed that $\mathscr{K} = 1$ (Heatley, 1974). This then leads to a jump frequency at room temperature of *ca.* 10^{10} sec^{-1}. Thus there are approximately 500 vibrations within a given conformation for each jump between conformations.

When considering rotation about C—C bonds in the lipid fatty acid chains, it is necessary to take into account the very strong steric interactions between adjacent chains. Simple calculations show that the steric hindrance to rotation about a C—C bond will vary throughout the length of the fatty acid chain (Rothman, 1973). If the chain is in a fully extended, all *trans* conformation, then rotation of one of the C—C bonds out of its *trans* position will cause the length of the chain between that C—C bond and the terminal

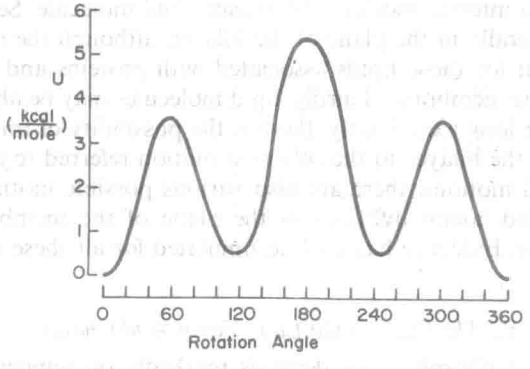


FIG. 3. The potential energy of butane as a function of the rotation angle of the centre bond C_2H_5—C_2H_5 (from Wunderlich, 1973).

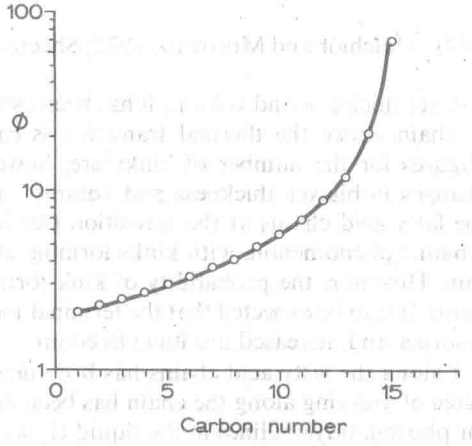


FIG. 4. The estimated maximum torsional angle ϕ about C—C bonds in a phospholipid fatty acid chain.

methyl group to rotate along the locus of a cone. If rotation about the C—C bond is too great, then the distal part of the chain will come within the van der Waal's radius of a neighbouring chain, and this will be energetically highly unfavourable. If it is assumed that only one bond per chain can be in a non-*trans* position at any one time, and if it is also assumed that the nearest-neighbour chains are in the all-*trans* conformation, then the maximum sterically allowable torsional angle for a given C—C bond can be calculated. The results obtained (Rothman, 1973) are plotted in Fig. 4 for a 16-carbon chain, where bond 1 connects the carbonyl carbon (carbon 1) to carbon 2, bond 2 connects carbon 2 to carbon 3, etc. The allowable amplitude of oscillation away from the *trans* position increases markedly towards the end of the chain.

There are two possible mechanisms for reducing steric interactions and thus allowing increased disorder and increased motion towards the beginning of the chain: both are probably important in the liquid crystalline state. In the first, motion in neighbouring chains is correlated, so that intramolecular rotation in neighbouring chains occurs to a similar extent. In the second, motions about several C—C bonds within a single chain are correlated, so that the regular packing of neighbouring chains is not greatly disrupted. This second form of correlation has been discussed in particular in connection with the formation of *gauche* conformations within the fatty acid chains.

The presence of *gauche* conformations within lipid fatty acid chains has been detected using Raman spectroscopy since the band at 1100 cm^{-1}, characteristic of the all-*trans* conformation, is absent for dipalmitoyllecithin in the liquid crystalline state (Lippert and Peticolas, 1971, 1972). To minimize the steric interactions caused by the presence of these *gauche* conformations, it has been suggested that, rather than a single *gauche* rotamer, *gauche* rotamers form about two C—C bonds, to give a so-called 2g1 kink (Trauble and Haynes, 1971). Such a kink is formed from an all-*trans* fatty acid chain by rotating about one C—C bond by an angle of 120° and then rotating about either of the two next nearest neighbouring C—C bonds by $-120°$ (Fig. 5). The result is a decrease in chain length by one CH_2 unit length (1.27 Å) and an increase in volume of *ca.* 25–50 $Å^3$. The number of kinks present in the liquid crystalline phase can then be estimated from changes in bilayer thickness and volume at the transition.

In the gel phase of dipalmitoyllecithin, the thickness of the lipid bilayer is *ca.* 42 Å and the cross-sectional area of one lipid molecule is 45 $Å^2$. At the phase transition the thickness of the bilayer decreases by about 5 Å (Chapman *et al.*, 1967; Levine, 1973). At the same time, there is an increase in volume of the lipids which can be detected by measurement of the volume of an aqueous lipid dispersion over the transition temperature. If this volume change is attributed to the fatty acid component of the lipid, then it corresponds to about a 2.5% increase in volume. This volume is probably a lower limit because it ignores any possible increase in the amounts of water bound to the lipid at the transition

(Trauble and Haynes, 1971; Melchior and Morowitz, 1972; Sheetz and Chan, 1972; Nagle, 1973).

From the change in bilayer thickness and volume, it has been estimated that the number of kinks per fatty acid chain above the thermal transition is *ca.* 0.6–2.0 (Trauble and Haynes, 1971). These figures for the number of kinks are, however, very approximate because some of the changes in bilayer thickness and volume could be associated with changes in the tilt of the fatty acid chains at the transition (see later). The formation of such kinks will be a dynamic phenomenon, with kinks forming and disappearing at any one position in the chain. However, the probability of kink formation is unlikely to be equal throughout the chain. It is to be expected that the terminal methyl ends of the chains will exhibit increased disorder and increased motional freedom.

The motional gradient along the fatty acid chains has been determined from ^{13}C nmr experiments and the degree of ordering along the chain has been determined by esr experiments. The ^{13}C data for phosphatidylcholines in the liquid crystalline phase indicate the presence of a very marked motional gradient within the lipid molecule: motion increases from the glycerol backbone of the lipid both towards the terminal methyl of the fatty acid chain and towards the $-NMe_3^+$ of the head group (Levine *et al.*, 1972a).

The ^{13}C data for the lipid fatty acid chains have been interpreted in terms of diffusion coefficients D_i for rotation about C—C bonds (Levine *et al.*, 1972b; Lee *et al.*, 1974b). In the liquid crystalline phase the rate of motion about C—C bonds at the glycerol backbone end of the fatty acid chains is considerably slower than at the terminal methyl group end. For dimyristoyllecithin up to at least carbon 7 of the chains (where carbon 1 is the carbonyl carbon) rotational diffusion coefficients are equal with $D_i = 1 \times 10^9$ sec^{-1}. At the terminal methyl end of the chain, however, motion is faster with $D_{\omega-1} = 6 \times 10^9$ sec^{-1} and $D_\omega = 8 \times 10^{10}$ sec^{-1}. It should perhaps be emphasized that although the diffusion coefficients for rotation about the C—C bonds in the first part of the chain are equal, this does not imply equal *motions* for the carbons in this part of the chain. The motion in space of any one carbon is the resultant of the motion about all the C—C bonds between it and the glycerol backbone, so that motion will increase along the chain because of the increasing number of bonds about which rotation is possible.

As well as the motion about C—C bonds within the fatty acid chains, ^{13}C nmr data provides evidence for an axial rotation of the lipid fatty acid chains as a whole. This motion could be due to a rotation of the chain with respect to the lipid glycerol group. It is, however, more likely to be due to a rotation of the whole lipid molecule in the plane

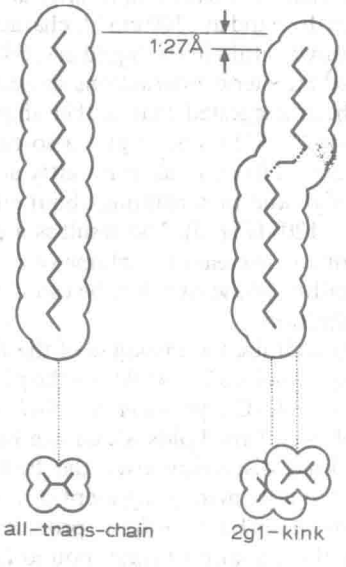


FIG. 5. The formation of a 2g1-kind in a fatty acid chain (from Lagaly and Weiss, 1971).

TABLE 1. COMPARISON OF ROTATIONAL DIFFUSION COEFFICIENTS (sec^{-1}) FOR DIMYRISTOYLLECITHIN AT 52°C AND FOR n-ALKANES AT 31°C

	D_{axial}	D_i	$D_{\omega-1}$	D_ω
Dimyristoyllecithin[a]	7×10^9	1×10^9	6×10^9	8×10^{10}
n-alkanes[b]	1×10^{11}	1×10^{10}	1.2×10^{10}	6.2×10^{10}

[a] Data from Levine *et al.* (1972b), Lee *et al.* (1974b).
[b] Average data for n-alkanes, $C_{12}H_{26}$, $C_{14}H_{30}$ and $C_{16}H_{34}$, from Birdsall *et al.* (1973), Levine *et al.* (1974).

of the bilayer. The diffusion coefficient for axial rotation is $D_{axial} = 7 \times 10^9$ sec^{-1} (Lee *et al.*, 1974b).

The data for dimyristoyllecithin can be usefully compared with that for liquid n-alkanes (Table 1). Although the rotational diffusion coefficients at the terminal methyl ends of the lipid chains are similar to those in n-alkanes, nearer the middle of the chain the diffusion coefficients are a factor of ten smaller. The rotational diffusion coefficient for axial motion of the lipids is a factor of 15 less than in the alkanes. The only motion to be grossly restricted in lipid bilayers in comparison to the liquid alkanes is rotation about an axis perpendicular to the long axis of the molecule: in lipid bilayers this motion is the so-called "flip-flop" motion in which a lipid moves from one side of the bilayer to the other, characterized by a half-time of several hours (Kornberg and McConnell, 1971).

Data for didecanoyllecithin, dilauroyllecithin and dipalmitoyllecithin are similar to that for dimyristoyllecithin. Values for D_ω and $D_{\omega-1}$ are equal to those in dimyristoyllecithin, although at the glycerol backbone end of the lipids there is probably a threefold increase in D_i for the shorter chains. Motion in the fatty acid chains of dioleoyl lecithin is comparable to that in lipids with saturated fatty acid chains, except for the olefinic carbons where motion is restricted in comparison with neighbouring C—C bonds (Lee *et al.*, 1974b).

It has been suggested (Schindler and Seelig, 1973) that the microviscosity at a particular depth within the bilayer can be estimated from the "effective diffusion coefficient" D_{eff} using the Stokes equation,

$$\eta = kT/8\pi r^3 D_{eff} \tag{2}$$

where r is the effective radius of the molecule and η is the viscosity. To the extent that microviscosity is a useful concept in a case where more than one diffusion coefficient is required to describe the motion of a group in a molecule, it is clear that the microviscosity will decrease markedly towards the centre of the bilayer. The microviscosities estimated from eq. (2) using the ^{13}C data are given in Table 2. The microviscosities for the n-alkanes can be compared with the kinematic viscosities of *ca.* $1–3 \times 10^{-2}$ P. In dimyristoyllecithin, the microviscosity at the terminal methyl end of the fatty acid chains is comparable to that in the n-alkanes. For the first half of the chain, however, η_i is approximately 15 times greater than for an n-alkane.

TABLE 2. "MICROVISCOSITIES" (POISE) ESTIMATED FROM ^{13}C nmr DATA

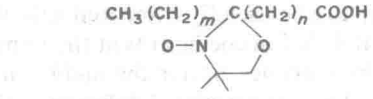
System	η_i	$\eta_{\omega-1}$	η_ω
Dimyristoyllecithin (52°C)	240×10^{-2}	30×10^{-2}	3×10^{-2}
n-alkanes (31°C)		15×10^{-2}	3×10^{-2}

Although these microviscosities and diffusion coefficients were obtained for sonicated aqueous lipid dispersions, they should apply equally well to lipids in unsonicated liposomes (Lee *et al.*, 1974b); ^{13}C nmr data are consistent with very similar motions for the lipid molecules in the two types of dispersion (Lee *et al.*, 1974b).

Attempts to attribute a single microviscosity coefficient to the bilayer from fluorescence measurements ignore the gradient present in the bilayer (Cogan *et al.*, 1973; Pownall and Smith, 1973). However, the studies with egg lecithin gave a microviscosity of *ca.* 50×10^{-2} P at 45°C (Cogan *et al.*, 1973), which is consistent with our estimate for the microviscosity towards the centre of the bilayer. Spin label studies of dimyristoyllecithin at 21.5°C (just

below the thermal transition temperature) have also been interpreted in terms of a microviscosity of *ca.* 50×10^{-2} P (Schindler and Seelig, 1973), but here the impurity effect of the spin label might be expected to be particularly important (see later). Although too much weight should not be attached to these figures, the very marked increase in microviscosity from the centre of the bilayer to the glycerol backbone region is probably genuine. The importance of these observations to the problem of the diffusion of a small molecule across the bilayer is described on p. 30.

The results of esr experiments involving the incorporation of spin labelled fatty acids (II) or lipids (III) into lipid bilayers have been interpreted in terms of an order parameter



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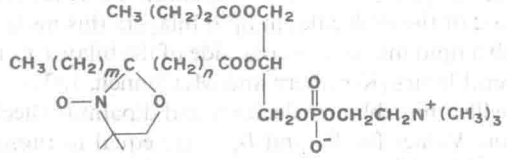

(III)

S_n. This order parameter is related to the average orientation of the nitroxide radical by the equation

$$S_n = 1/2(3\cos^2\overline{\theta}_n - 1). \qquad (3)$$

Here n is the number of carbon atoms between the carbonyl carbon and the labelled carbon, θ_n is the angle between the nitroxide $2p_\pi$ orbital and the normal to the plane of the bilayer at some time, and the bar denotes that the time average of this angle is taken. If the C—C bonds preceding the labelled carbon are all *trans* and perpendicular to the plane of the bilayer, $S_n = 1$. If the motion of the spin label is isotropic, then $S_n = 0$.

For phospholipid spin labels (III) incorporated into bilayers of dipalmitoyllecithin above the thermal transition, it has been found that log S_n shows an approximately linear decrease with increasing n up to about $n = 8$. Beyond $n = 8$ the decrease in log S_n becomes increasingly more marked (Hubbell and McConnell, 1971). These results suggest that the region of the chain up to $n = 8$ is effectively all *trans*, but that the probability of a non-*trans* conformation increases rapidly with increasing n beyond this point.

We see then that these nmr and esr results are broadly in agreement that the first half of the lipid fatty acid chain is relatively immobile, but that nearer the centre of the bilayer, disorder is considerably greater. The validity of the esr results is, however, not yet clear because of the possibility of distortions caused by the presence of the nitroxide group. Thus, unlike in dipalmitoyllecithin bilayers, the order parameters for spin labelled fatty acids incorporated into lemella of the sodium decanoate–*n*-decyl alcohol–water system decrease exponentially with distance between the nitroxide group and the carboxyl group (Seelig, 1970). In marked contrast, order parameters determined by deuterium nmr for lamella of the deuterated potassium laurate-water system are fairly constant for the first half of the chain, and then rapidly decrease (Charvolin *et al.*, 1973). Clearly, a check of the order parameters for bilayers of dipalmitoyllecithin using the less perturbed deuterated chains is necessary.

The gel to liquid crystalline phase transition has sometimes been described as a "melting" but this is somewhat misleading. In fact it has been shown that the change in volume for the lipid at the transition is about a factor of six less than the corresponding melting dilation of palmitic acid or tripalmitin, implying considerably more order in the phospholipid system (Trauble and Haynes, 1971; Melchior and Morowitz, 1972). Similarly, whereas the change in configurational entropy ΔS per CH_2 group for the melting of an

n-alkane or triglyceride is $\Delta S = 1.9$ cal mole^{-1} deg^{-1}, that for a lecithin is only $\Delta S = 1.25$ cal mole^{-1} deg^{-1} (Phillips *et al.*, 1969). Although these results have generally been taken to imply that the lipid fatty acid chains are more ordered throughout their length than are free fatty acids or alkanes, it is more likely that they reflect a relatively high degree of order for the first part of the chain in the liquid crystalline state, but with the ends of the chain having a fluidity similar to that in an *n*-alkane.

The rapidly increasing disorder towards the centre of the bilayers creates interesting packing problems. Because of its greater disorder, the volume occupied by a—CH_2—group towards the centre of the bilayer will be greater than that for a—CH_2—group near the glycerol backbone region. The increase in volume towards the centre of the bilayer can be accommodated in two ways:

(i) by a bend in the fatty acid chains, with the upper portion of the chain tilted with respect to the bilayer plane (McFarland and McConnell, 1971);

(ii) by a decreased packing density in the glycerol backbone region, perhaps with the extra space being taken up by water.

As already described, in fully hydrated bilayers of dipalmitoyllecithin in the gel phase, the fatty acid chains are fully extended (all-*trans*) and tilted with respect to the bilayer surface. This is readily shown by measurement of the area per molecule in the bilayer. Thus the cross-sectional area of two fatty acid chains in the all-*trans* conformation is 40.6 Å^2, and the area per molecule in bilayers of dipalmitoyllecithin in the gel phase at low hydration is 40.3 Å^2. At maximum hydration, however, the area per molecule increases to 44.5 Å^2 (Levine, 1973). This increase in surface area is accomplished by tilting the whole length of the fatty acid chain with respect to the surface of the bilayer. As the chains tilt, the cross-sectional area in a plane perpendicular to their axes remains constant, but the area per molecule projected at the surface of the bilayer increases.

On the basis of esr studies of oriented multilayers of egg lecithin containing spin-labelled phospholipids, it has been suggested that in the liquid crystalline phase, the upper portion of the labelled fatty acid chain is tilted *ca.* 30° from the normal to the bilayer, whereas the lower portion is nearly perpendicular to the bilayer (McFarland and McConnell, 1971). On average, the bend occurs at about bond 8, and persists for a time longer than 10^{-8} sec. Such a bent chain means that the area occupied at the bilayer surface by a—CH_2—group in the first half of the chain is greater than that occupied by a—CH_2—group in the latter part of the chain. The volume occupied by a—CH_2—group is therefore greater for the lower portion of the chain than for the upper portion, but at the same time the packing of the ordered, upper portion of the chain is still relatively tight. However, the packing cannot be as tight as in the gel phase, since the area per lipid molecule in the liquid crystalline phase is *ca.* 70 Å^2, and the area per molecule in the bent chain model with surface packing as close as in the gel phase would be only 45 Å^2. Further, as discussed by McFarland and McConnell (1971), it is not clear whether or not the bent fatty acid chain is an artifact caused by the presence of the nitroxide group. Monolayer studies (Tinoco *et al.*, 1972; Cadenhead and Muller-Landau, 1973) of spin-labelled fatty acids have shown that the nitroxide group has a tendency to orient at the air–water interface. It is therefore possible that in the lipid bilayer, when a nitroxide group is close to the beginning of the chain, it might tend to localize at the lipid–water interface, thus creating the observed bent fatty acid chain. No such bent chain could be detected by deuterium nmr in lamella of the deuterated potassium laurate–H_2O system (Charvolin *et al.*, 1973). Although this result only implies that there is no net bend in the chains with a lifetime of longer than about 10^{-6} sec, it clearly shows that in this system at least, it would be misleading to talk of any "long-lived" net bend in the chains.

Whether or not the lipid fatty acid chains can be described by a bent-chain model, it is clear that the packing in the glycerol backbone region of the lipid bilayer must be "looser" in the liquid crystalline phase than in the gel phase. It seems likely that this extra space will be taken up by water molecules, but the evidence for this is, as yet, rather scanty.

Studies using differential scanning calorimetry and proton nmr indicate that *ca.* 6–10 molecules of water are "strongly bound" per lecithin molecule in a bilayer (Ladbrooke and Chapman, 1969; Gottlieb *et al.*, 1973). This presumably includes water associated with the

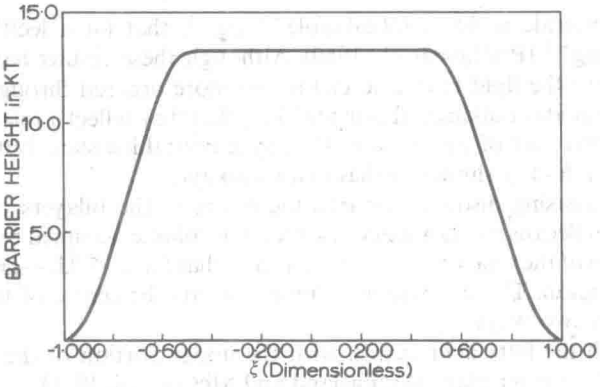

FIG. 6. A possible shape for the barrier to ion penetration presented by the lipid bilayer. The parameter $\xi = 2x/d$ where d is the membrane thickness and x is the distance from the middle of the bilayer (from Hall *et al.*, 1973).

lipid polar head groups as well as any water within the non-polar regions of the lipid bilayer. Deuteron nmr studies show that changes in water binding occur at temperatures near T_t (Salsbury *et al.*, 1972) but again these changes could be associated with alterations in the structure of the polar head group (see below) rather than being due to any changes in the glycerol backbone region of the bilayer.

Spin-label data can potentially provide information about water penetration into the bilayer, since spin label coupling constants are sensitive to the polarity of the environment. Studies with spin-labelled fatty acids and lipids incorporated into phospholipid bilayers have in fact been interpreted as showing that water molecules penetrate into the fatty acid chain region, to at least the C-2 position (Griffith *et al.*, 1974). However, the same result would be obtained if the spin label moved up into the lipid–water interface, with no water penetration into the bilayer.

To summarize, therefore, there is a marked gradient of motion within a lipid molecule in a bilayer when above the temperature of the gel to liquid crystalline phase transition. Motion increases from the glycerol backbone of the lipid both towards the polar head group and towards the terminal methyl group of the fatty acid chain. The "microviscosity" at the centre of the bilayer is similar to that for a short-chain alkane. The increasing disorder and increasing motion towards the centre of the bilayer imply that the volume occupied by a —CH_2— group at the centre of the bilayer is greater than the volume occupied nearer to the glycerol backbone. In view of the "loose" packing in the glycerol backbone region of the bilayer, it seems likely that water molecules will be able to penetrate some way into the fatty acid chain region of the bilayer. Such penetration would produce an approximately trapezoidal polarity profile for the membrane. Recent studies of the nonactin mediated K^+ transport through black lipid (phosphatidylethanolamine) films have in fact been interpreted in terms of just such an energy barrier (Fig. 6) (Hall *et al.*, 1973).

C. *The Conformation of the Lipid Head Group*

The conformation of the zwitterionic polar group of lecithin is not yet agreed. There are two schools of thought: one believes that above the thermal transition temperature, the zwitterion is oriented with its axis normal to that of the chains whereas the other

TABLE 3. TRANSITION DATA FOR AQUEOUS LECITHIN DISPERSIONS (Hinz and Sturtevant, 1972b)

Lipid	Lower transition	Upper transition		
	T_t	T_t	ΔH (kcal mole^{-1})	Co-operative unit
Dimyristoyllecithin	13.5	23.7	6.26 ± 0.18	200 ± 40
Dipalmitoyllecithin	34.0	41.7	9.69 ± 0.21	70 ± 10
Distearoyllecithin	49.1	58.2	10.84 ± 0.17	80 ± 10