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PART VI. ELECTRICAL AND TRANSPORT PHENOMENA OF ARTIFICIAL MEMBRANES

STUDIES OF THE PHYSICAL CHEMISTRY OF PLANAR BILAYER MEMBRANES USING HIGH-PRECISION MEASUREMENTS OF SPECIFIC CAPACITANCE*

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INTRODUCTION

A solution is, by definition, any phase containing two or more components. The cell membrane is a phase(s) distinct from the cytoplasm and interstitium containing lipids and proteins, and is therefore a solution. Simply calling the membrane a solution, however, provides few new insights into membrane architecture. My laboratory is concerned with developing quantitative methods for describing the solution properties of membrane systems that we believe will lead to a deeper understanding of membrane organization. Toward this end, we have been studying the physical chemistry of the planar bilayer membrane, which is a particularly simple membrane solution consisting of alkane molecules dissolved in a lipid bilayer. This paper describes the rationale for and the results of some of our experiments.

THE CELL MEMBRANE AS A LIPID-PROTEIN SOLUTION

The basic structural element of cell membranes is the lipid bilayer,[1-3] which is believed to act as a two-dimensional "solvent" for hydrophobic proteins.[4-7] A highly pictorial description of the membrane as a two-dimensional solution is shown in FIGURE 1. Accepting this scheme as a working hypothesis, the next logical step is to construct a quantitative physicochemical theory. This is a difficult step, however, because of our limited knowledge of solutions in volumes of molecular dimensions. Describing the solution properties of membranes is related to the problem of describing the solution properties of a very thin slice of an ordinary bulk solution. Both problems are equivalent to the notoriously intractable one of describing the behavior of solutions at the molecular level.

The complexity of describing the membrane as a lipid-protein solution can be appreciated more easily by constructing a Corey-Pauling-Koltum model of part of the hydrophobic segment[9,22] of the major glycoprotein ("glycophorin") of human red blood cell membranes and comparing it (FIGURE 2) with a CPK model

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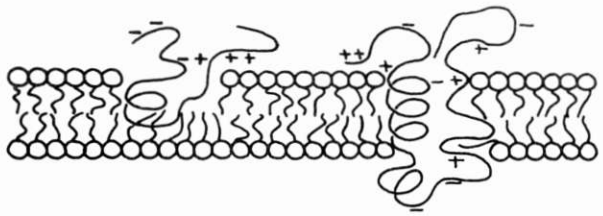


FIGURE 1. A highly schematic representation of the cell membrane as a two-dimensional solution in which the lipid bilayer acts as a "solvent" for hydrophobic proteins. Based on Singer and Nicolson.[8]

of a phospholipid. FIGURE 2 suggests that we must be cautious in describing the membrane as a simple two-dimensional solution. For one thing, at the level of a single amino acid residue, the bilayer thickness is sufficient to attribute a third dimension to the system. Perhaps the term *quasi-two-dimensional* is more appropriate. It may be reasonable to treat the membrane as a two-dimensional solution at the level of the polypeptide or protein, but then a problem arises because of the mass of the polypeptide. The approximate molecular weight of the decapeptide shown in FIGURE 2 is 1,300, and this accounts for less than half of the 23 residue hydrophobic segment[22] of glycophorin. Hexadecane, which has a molecular weight of only 226 and is ideally hydrophobic, is only slightly soluble in planar bilayers.[10,11] Heneicosane ($C_{21}H_{44}$, MW297) is probably completely insoluble (FIGURE 3). Thus, hydrophobicity alone does not assure solubility in bilayers. In one sense this comparison is unfair, because solubility of proteins will be determined in part by tertiary structure, which probably renders them amphiphilic (surface active). Nevertheless, the low solubility of the longer alkanes in planar bilayers raises a significant conceptual problem with regard to describing the membrane as a simple solution of protein in lipid bilayer. Further examination of FIGURE 2 reveals an additional problem that is usually ignored in discussions of hydrophobic proteins. The polypeptide contains numerous carbonyl oxygens that must make the polypeptide more polar than an alkane of equivalent mass. Organizing the polypeptide into a helical configuration[22] could, however, partially alleviate this problem.

Thus, the membrane is likely to be an exceptionally complex solution. It is probably proper to view the membrane as a quasi-two-dimensional solution of proteins or parts of proteins dissolved (or at least dispersed) in the bilayer. Viewed in this way, a major problem of membrane biophysics is to develop quantitative methods for describing the solubility of proteins or polypeptides in bilayers. Where shall we start? In thinking about this problem, I realized that we are largely ignorant of how even the simplest molecules interact with the bilayer. Consider, for example, hexane. This molecule should, and apparently does (S. H. White and M. Yafuso, unpublished) readily dissolve in the membrane bilayer and lipid dispersions.[41] What kind of environment does it find? Is the interior of the bilayer like a simple alkane liquid, as many people believe, or does the interior have

properties not normally observed in ordinary bulk solutions? The answers to these questions are important for understanding lipid-protein interactions in membranes. For this reason, my laboratory is engaged in studies of the interaction of alkanes with bilayers and cell membranes. The studies I describe here are concerned with the simplest possible membrane solution (compare FIGURES 2 and 8) consisting of alkane dissolved in planar lipid bilayers. Several specific questions motivated the study. 1) Is the interior of the bilayer equivalent to a bulk alkyl liquid? 2) What structural features of the lipid affect the solubility of alkanes in the bilayer? 3) What structural properties of the alkanes determine solubility? Partial answers to these questions are beginning to emerge and will be described below. I believe the results provide exciting and provocative insights into the nature of the bilayer.

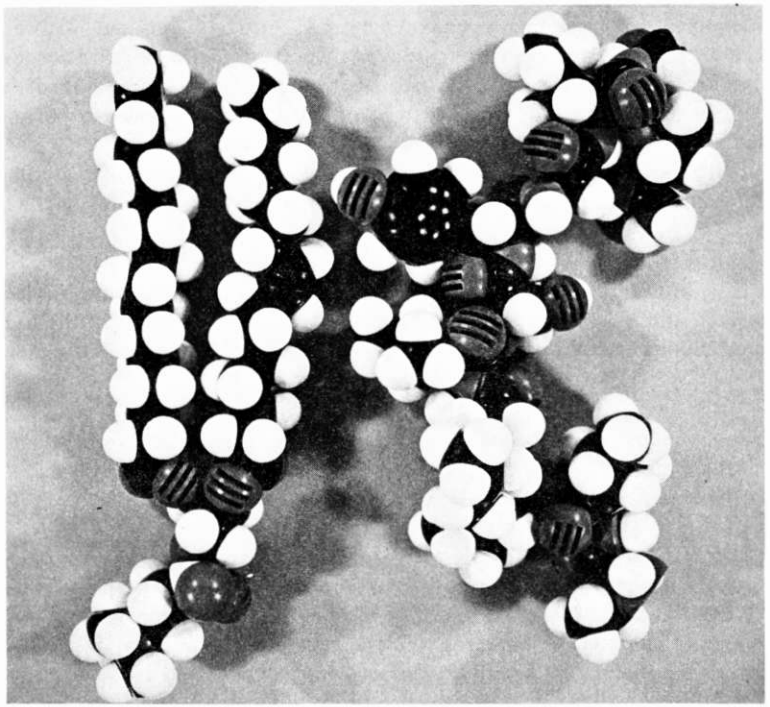


FIGURE 2. Corey-Pauling-Koltum models of a lecithin molecule (left) and a membrane polypeptide (right) consisting of a sequence of ten amino acids (numbers 87–96) of the 23 amino acid hydrophobic segment of the major red blood cell membrane glycoprotein (glycophorin).[9,22] This decapeptide has a molecular weight of about 1,300 and is far too bulky to be soluble in the bilayer in any simple sense because the 21 carbon *n*-alkane heneicosane (MW 297) is probably completely insoluble in bilayers. Relative to a single amino acid, the thickness of the bilayer is significant. It is perhaps more reasonable to refer to the membrane as a *quasi-two-dimensional* solution.

The Potential or Interactive Volume of Bilayers

The planar bilayer membrane first described by Mueller *et al.*[12] is a natural system for examining the alkane-bilayer interaction since an alkane or equivalent solvent is essential[13] for bilayer formation. Various techniques used in the formation and examination of these bilayers have been well described in two recent publications.[14,15] A measurement of particular importance is that of specific capacitance,[16] which yields information on the thickness and composition of the bilayer. The hydrophobic core of the bilayer has a specific geometric capacitance (C_g) given by

$$C_g = \frac{\epsilon_0 \epsilon_B}{\delta_B} \quad (1)$$

where $\epsilon_0 = 8.854 \times 10^{-14}$ Farads/cm, ϵ_B is the dielectric coefficient and δ_B the thickness of the alkyl interior. ϵ_B can be estimated satisfactorily[18] and C_g can be accurately measured with high precision[17] by A.C. bridge techniques, with appropriate corrections for the electrolyte-bilayer dispersion[17] and polarization-charge capacitance.[34] In my laboratory, C_g can be measured with a precision of better than 0.3% which means that changes in δ_B of about 0.2A or less can be reliably detected.

Thickness, determined from Equation 1, is a direct measure of bilayer composition.[10,23] A planar bilayer of thickness δ_B and area A will have a volume given by

$$\delta_B A = N_{AC} V_{AC} + N_A V_A$$

where N_{AC} and N_A are the total number of acyl chains and alkane molecules, respectively, and V_{AC} and V_A are the molecular volumes. In terms of the number (n) of acyl chains and alkanes per unit area of membrane, Equation 2 becomes

$$\delta_B = n_{AC} V_{AC} + n_A V_A. \quad (2)$$

For monoglyceride and phospholipid bilayers, the number of stabilizing lipid molecules per unit area of bilayer appears to be independent of the type and size of the alkane used to form the bilayer. Therefore, $\delta_B \propto n_A$, so that thick membranes have a larger volume fraction of alkane than do thin ones.[10,11]

FIGURE 3 shows the results of recent measurements in my laboratory of the thickness of bilayers formed from glycerol monooleate and *n*-alkanes at 30°C in 0.1 M NaCl solutions. The maximum thickness (48.3 Å) is obtained for *n*-decane. As the size of the alkane increases, the thickness of the bilayer decreases and approaches the theoretical limiting value of 24.9A (calculated from data in Ref. 18). This decrease is striking and, I believe, profoundly significant. First, *n*-alkanes are not equally soluble in the bilayer, for if they were, thickness would be independent of alkane type. This indicates that the interior of the bilayer is not completely equivalent to a simple bulk alkyl liquid because we know that liquid alkyls are miscible in one another. Second, the thickness of the bilayer can be changed by almost a factor of two without changing the number of stabilizing lipid molecules per unit area. This suggests that the bilayer has a *potential* volume available for accommodating proteins or other molecules that is approximately equal to the volume of the acyl chains of the stabilizing lipid molecules composing the bilayer;

that is, given a fixed amount of lipid in a bilayer, the membrane thickness can adjust to accept additional constituents without necessarily changing the interfacial organization. I like to call this potential volume the *interactive* volume of the bilayer because it is the volume which can be made available for the interaction of solute molecules with the bilayers. I emphasize that in the alkane-free bilayer this volume does not exist. It is a potential volume that is revealed only when other constituents (such as alkanes) are introduced.

Presumably, biological membranes have an interactive volume partially occupied by membrane proteins or polypeptides. The introduction of alkanes into biological membranes should reveal the available interactive volume and allow one to examine the competition between alkane and protein for the interactive volume. It may be possible in this way to quantitate the interaction of the proteins with the bilayer.

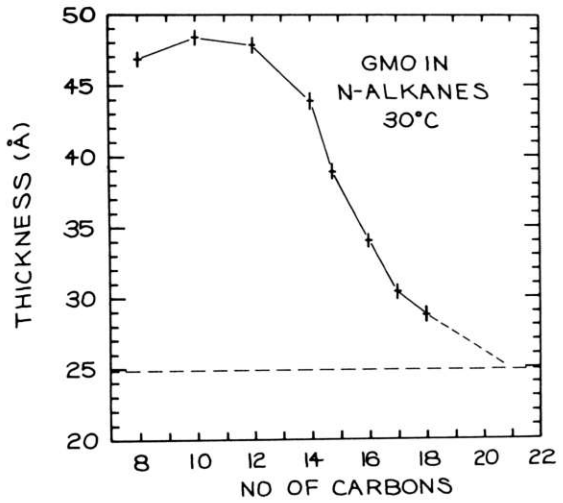


FIGURE 3. The thickness of planar bilayer membranes formed from glycerol monooleate (GMO) dispersed (10 mg/ml) in *n*-alkanes, as a function of the number of carbons in the alkane. The aqueous phase was unbuffered 0.1M NaCl (pH $\simeq$ 6); T = 30.0°C. Thickness was determined from high-precision capacitance measurements corrected for the electrolyte-membrane dispersion and the polarization-charge capacitance. Since the number of GMO molecules per unit area of membrane is independent of alkane size, thickness is a direct measure of the amount of alkane in the bilayer. Note that as the size of the *n*-alkane increases, its solubility in the bilayer decreases. The horizontal dashed line represents the thickness of a hypothetical alkane-free bilayer. The extrapolation of the curve connecting the data points shows that heneicosane ($C_{21}H_{44}$) is probably insoluble in the bilayer.

How Alkanes Occupy the Interactive Volume of the Bilayer

Why is it that the interactive volume of the bilayer cannot be occupied as easily by, say, *n*-hexadecane as by *n*-decane? That is, why is *n*-decane more soluble in the

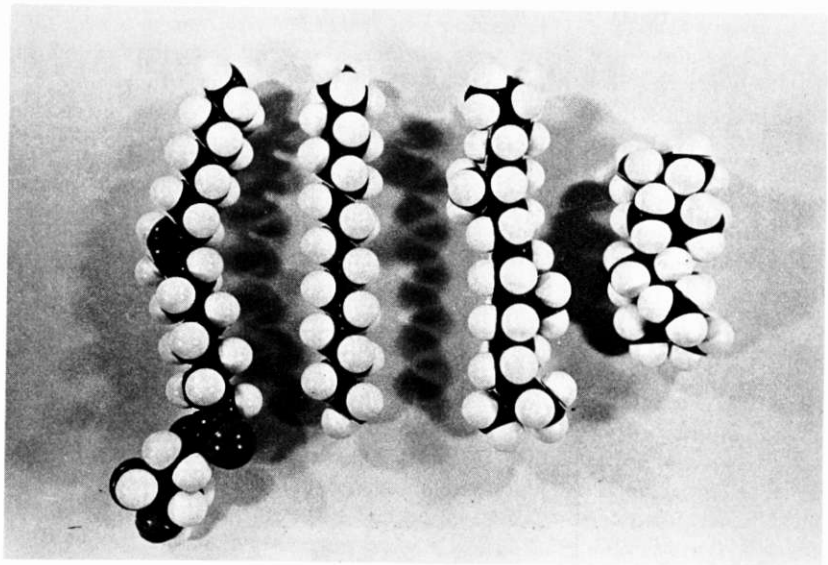


FIGURE 4. Corey-Pauling-Koltum models of (from left to right) glycerol monooleate, *n*-hexadecane, 2,6,11,15-tetramethylhexadecane, 2,2,4,4,6,8,8-heptamethylnonane. Heptamethylnonane has about the same molecular volume as hexadecane, but its length is the same as that of *n*-decane. Tetramethylhexadecane has about the same molecular volume as eicosane but the length of hexadecane. Length is the primary variable determining alkane solubility (see FIGURE 5).

bilayer than *n*-hexadecane? Before this question can be answered, we must first establish what structural property of the alkane is important in determining solubility. In FIGURE 3, thickness was plotted against the number of carbons in the alkane. Structurally, both the molecular volume and the length of the alkane vary with the number of carbons. Which of these variables is important in setting the solubility? This question can be answered by examining bilayers formed from isomers of *n*-alkanes (FIGURE 4). The results of measurements made on bilayers

TABLE 1

PHYSICAL PROPERTIES OF PLANAR BILAYERS FORMED FROM GLYCEROL MONOOLEATE AND SEVERAL ALKANES AT 30°C

Alkane	Bilayer Thickness (Å)	ΔH‡ (kcal/mol)	ΔS‡ (cal/deg/mol)
n-Hexadecane	34.0 ± 0.2	4.03 ± 0.10	13.25 ± 0.36
Tetramethylhexadecane*	32.5 ± 0.2	3.20 ± 0.25	10.65 ± 0.86
Heptamethynonane†	45.0 ± 0.2	0.37 ± 0.01	1.23 ± 0.03

*2,6,11,15-tetramethylhexadecane.

†2,2,4,4,6,8,8-heptamethylnonane.

‡ΔH is the enthalpy of transfer and ΔS the entropy of transfer of the alkane from bulk (microlenses and annulus) to the bilayer at saturation equilibrium (ΔH = TΔS).

formed from *n*-hexadecane, heptamethylnonane (a hexadecane isomer), and tetramethylhexadecane (an eicosane isomer) are shown in TABLE 1 and FIGURE 5. These data strongly suggest that the primary variable is alkane *length*, with molecular volume having a second-order effect. Heptamethylnonane has approximately the same molecular volume as *n*-hexadecane, but the bilayer has a thickness of 45.0 Å rather than 34.0 Å. A short extrapolation of the data in FIGURE 3 shows that bilayers formed from eicosane (*n*-$C_{20}H_{42}$) should be essentially alkane-free and have a thickness of about 25 Å. If molecular volume were the primary

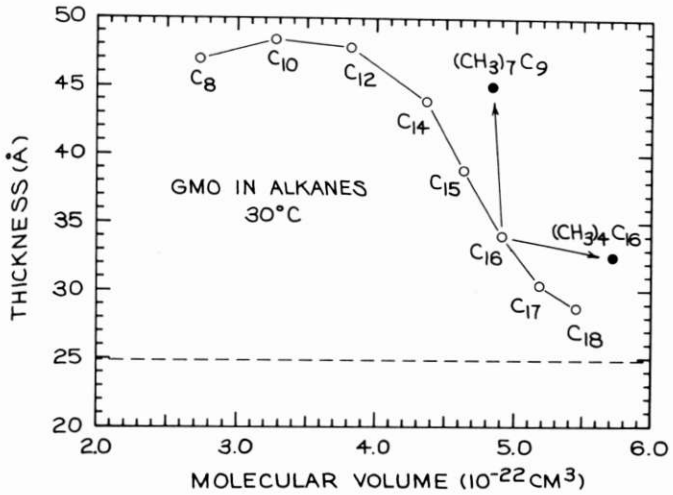
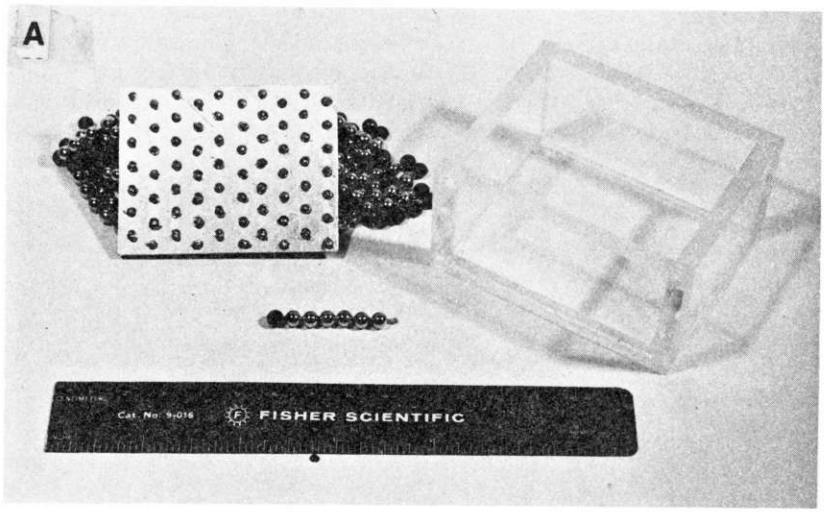


FIGURE 5. The thickness of planar bilayer membranes formed from glycerol monooleate, dispersed (10 mg/ml) in various alkanes at 30°C, as a function of the molecular volume of the alkane. The open circles (○) are values for the *n*-alkanes (C_N) from FIGURE 3, and the shaded circles (●) are values for heptamethylnonane [$(CH_3)_7C_9$] and tetramethylhexadecane [$(CH_3)_4C_{16}$]. Note that the membranes formed from $(CH_3)_7C_9$] are much thicker than from C_{16}, even though the molecular volumes of the two alkanes are about the same. $(CH_3)_4C_{16}$ gives membranes of about the same thickness as C_{16}, even though the molecular volume is about the same as C_{20}. The thickness of C_{20} membranes should theoretically give a value of thickness close to that shown by the dashed line, which represents the hypothetical alkane-free thickness of the bilayer. These data indicate that alkane length determines solubility. Molecular volume has a second-order effect.

variable, membranes formed from tetramethylhexadecane (*i*-$C_{20}H_{42}$) should give membranes of about this thickness. However, the observed thickness is 32.5 Å—close to the value of *n*-hexadecane. The length of tetramethylhexadecane is about the same as *n*-hexadecane, whereas the length of heptamethylnonane is about the same as *n*-decane (see FIGURES 4 and 8). FIGURE 5 shows bilayer thickness plotted against the molecular volume of the alkanes. The points for heptamethylnonane [$(CH_3)_7C_9$] and tetramethylhexadecane [$(CH_3)_7C_9$] are out of place in a way most easily explained by alkane length being the primary variable. Note, however, that the thicknesses are smaller than expected if length were the only vari-



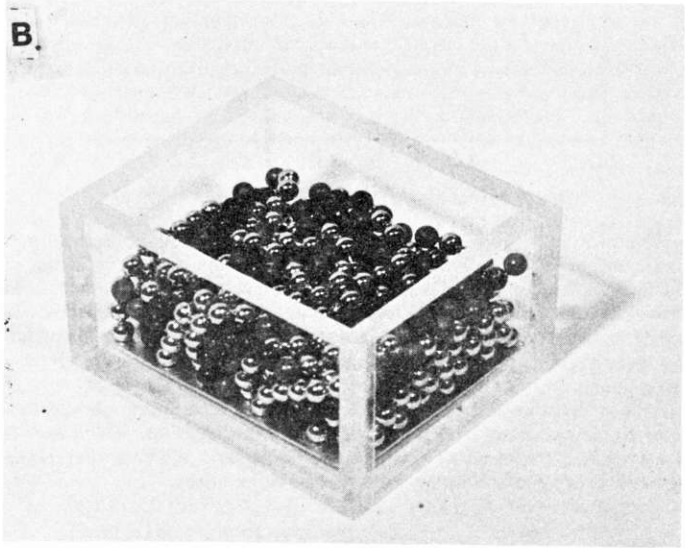


FIGURE 6, A. & B. A model for one monolayer of a bilayer constructed from number 10 nickel-plated beaded chain. A: Disassembled model showing the "acyl chains" attached to a $\frac{1}{16}$" aluminum plate in a hexagonal pattern. The spacing is scaled to give an area/acyl chain of $40A^2$, taking the cross-sectional area of a single bead as $20A^2$. The terminal "methyl groups" are painted red but appear gray in the black-and-white photo. B: Model assembled in a Plexiglass® box. The height of the box corresponds to the fully extended length of the "acyl chain."

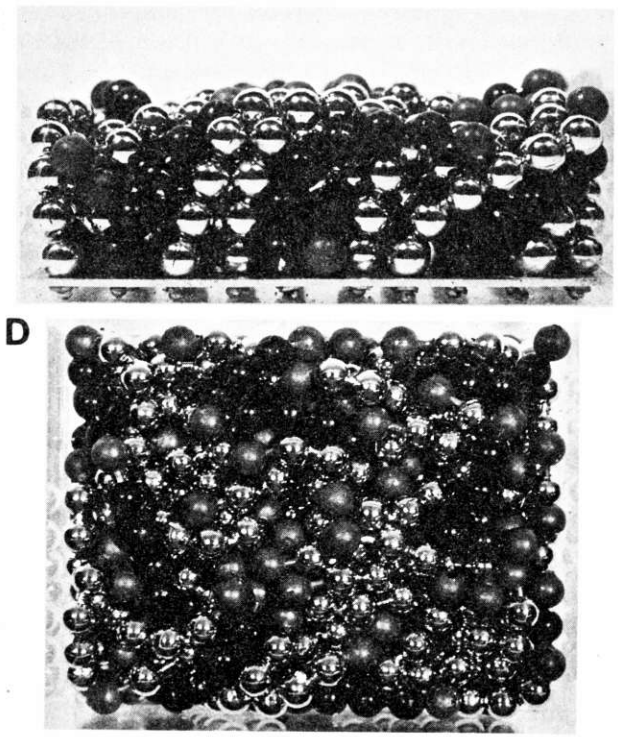


FIGURE 6, C. & D. C: Side view of the assembled model (see legend for FIGURE 6, A & B). A few chains (20%) were painted black. Note that most of the chains are bent so that the terminal methyl half of the chain *tends* to run parallel to the bilayer surface. D: Top view of the model. Note the high density of "methyl groups."

able. These deviations are probably caused by a molecular volume effect which is second order to the length effect.

Andrews *et al.*[29] have suggested that the alkane solute resides largely (but not exclusively[21]) in the central portion of the bilayer. As discussed by White,[11] this is reasonable for several reasons. First, magnetic resonance studies of phospholipid multilayers and vesicles (see e.g. Refs. 30 and 31) indicate a significant gradient of fluidity within bilayers. The methylene groups ($-CH_2-$) closer to the terminal methyl group ($-CH_3$) have more degrees of freedom (fluidity) than those near the polar group. The alkanes should fit more easily into the less constrained (more fluid) central zone than into the outer zones where the lipid acyl chains have fewer degrees of freedom. A graphic illustration of the fluidity gradient is shown in FIGURE 6, in which a model of one half of a bilayer has been constructed from beaded chain of the type used in pull-string electric lamp sockets. The model is a gross approximation to reality, but it does have the following essential constraints of lipid acyl chains in bilayers:[3] 1) The polar ends of the "molecules" are closely packed on an approximately hexagonal two-dimensional lattice. 2) The average

distance between —CH_2— groups on neighboring chains is constant throughout the bilayer (i.e., the density of the bilayer is uniform with respect to thickness). 3) Neighboring chain segments run parallel to one another. These constraints cause the bilayer to have a much smaller thickness (δ_B) than expected from the fully extended length (ℓ) of the acyl chain. Approximately, $\delta_B = 2\ (A_0/A)\ell$ where A_0 is the cross-sectional area of an acyl chain ($\sim 22A^2$, Ref. 3) and A is the area per acyl chain in the interface ($\sim 39A^2$ for glycerol monooleate, Ref. 29). For glycerol monooleate, $\ell = 22.3A$ (Ref. 11) and $\delta_B = 25A$. As a result (as the model clearly shows), each chain at any instant of time tends to be "bent" so that the segments of the chain near the terminal methyl group tend to run *parallel* to the plane of the bilayer. This means that as time runs the terminal methyl groups move about in a central zone parallel to the bilayer and sweep out much larger areas than the polar groups; hence, the increased fluidity near the center of the bilayer. In glycerol monooleate bilayers containing little solvent $\delta_B \simeq \ell$ and on the average the acyl chains will be bent near their centers, causing the chain near the polar end to tend toward the normal of the bilayer and the chain near the —CH_3 group to tend toward the parallel of the bilayer.

Second, the alkane molecules will tend to align themselves parallel to the acyl chains (see discussions in Refs. 32 and 33) just as neighboring acyl chains tend to align themselves parallel to one another. If the alkane molecules were located exclusively near the polar surfaces, they could align themselves parallel to the acyl chains only if the area per polar group increased significantly. Such an area increase would also increase the free energy (surface tension) of the bilayer and would therefore not be favored. If, on the other hand, the alkane molecules were located mostly in the center of the bilayer, they could simultaneously occupy volume and be parallel to the acyl chains without changing the area per polar group (FIGURES 7 and 8). This, coupled with the idea that the chains must be bent much of the time, explains why decane is more soluble than hexadecane (and in general why alkane length and not molecular volume is the important structural parameter). As will be explained shortly, the terminal methyl groups of the opposing monolayers must be free to make frequent contacts with one another across the bilayer midplane. All of the hexadecanes cannot simply span the distance from the polar group to the —CH_3 group without causing an energetically unfavorable increase in the area per polar group, and they therefore shift toward the fluid center of the bilayer. But, if the hexadecanes occupied a volume fraction of about 0.50, as *n*-decane does,[29] the terminal methyl groups could not come into contact at the midplane. Since this contact must occur, some hexadecanes must be excluded from the structure. The bent terminal methyl ends of the acyl chains probably swing out toward the bilayer midplane somewhat to form "slanted pockets" into which the hexadecanes can fit (FIGURE 7). In this way, the hexadecanes can be parallel to the acyl chains without occupying interfacial area, and the terminal methyls can interact. This treatment assumes that the monolayers function independently of one another; that is, a given hexadecane does not often span the midplane so that it is simultaneously mixed in both monolayers. Cross-monolayer mixing requires that space for part of the hexadecane be available in both monolayers at the same point in the midplane simultaneously. The probability of this is low unless the motions of the acyl chains in the two monolayers are highly correlated, which also seems unlikely. If the hexadecanes could span the midplane, it would be possible to have them occupy a large volume fraction (0.50) and give membranes of the same thickness as decane membranes. Since the volume fraction is actually low (0.27) and the membranes are thin, it appears that spanning does not occur, and that the monolayers act independently. This is consistent with the

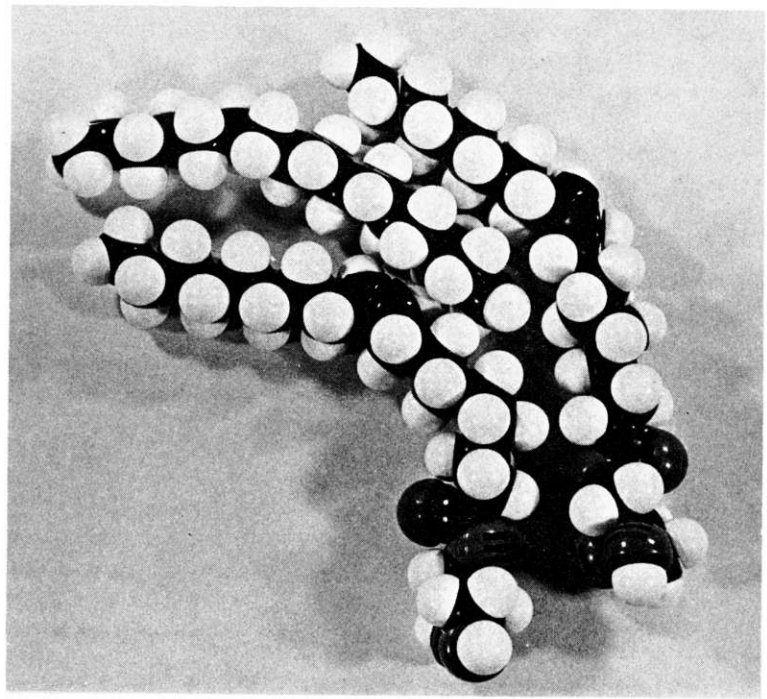


FIGURE 7. Corey-Pauling-Koltum models of *n*-hexadecane and glycerol monooleate showing how hexadecane might fit into the central zone of the bilayer. Some alkane must also be located in the interfacial zones near the polar groups but only in very limited amounts, because small increases in area per polar group lead to large increases in the interfacial free energy. It is suggested that the "bent" terminal halves of the acyl chains swing out toward the midplane slightly to generate space for accommodating the alkanes. Note that the hexadecane must be in frequent contact with —CH_3 groups from the opposed monolayer. The packing of a long alkane within the acyl chains (between planes defined by the terminal methyl groups and the double bonds) is probably not as tight as in bulk, so that some free volume is generated.

small free-energy difference between $1cm^2$ of alkane-saturated bilayer and $2cm^2$ of monolayer adsorbed at the alkane/water interface[36] and with the suggestion of Andrews *et al.*[29] that the volume fraction of alkane in the monolayer at the alkane/water interface is the same as in the bilayer saturated with alkane.

Now consider decane (FIGURE 8). The same general considerations apply,

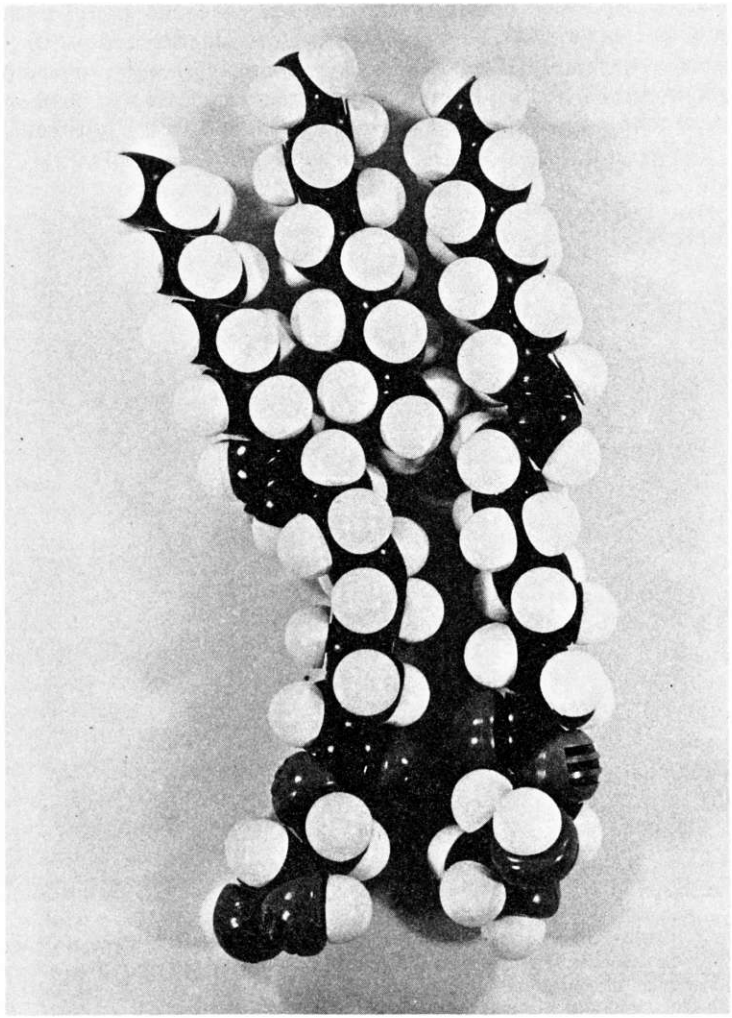


FIGURE 8. Corey-Pauling-Koltum models of *n*-decane and glycerol monooleate showing how decane might fit into the central zone of the bilayer. Because decane is shorter than *n*-hexadecane, full extension of some of the acyl chains is possible and provides enough space to accommodate a large volume fraction (about 0.5) of decane. The thickness of the bilayer is about twice the fully extended length of the acyl chain.

except that the alkane chains are shorter and can occupy a large volume fraction without occupying interfacial area or causing the terminal methyls to lose contact. The larger volume fraction (interactive volume) is achieved by having the acyl chains spend much more time in fully extended configurations. Decane bilayers, in fact, have a thickness about equal to twice the fully extended length of the acyl chain. Andrews *et al.*[29] point out that only a small percentage of the

acyl chains need to be extended at any instant of time to maintain this thickness. It can be imagined that the acyl chains are whipping back and forth between contracted and extended states. As this whipping occurs, there will be a continual exchange between space occupied by acyl chains and space occupied by alkanes.

Now consider why the terminal methyls on opposing monolayers must make frequent contact at the bilayer midplane. That is, why cannot the membrane absorb arbitrarily large amounts of alkane by growing indefinitely in thickness? As discussed by White[20] and Andrews *et al.*,[29] a compressive force acts across the bilayer due to the attractive van der Waals force between the separated aqueous phases (see Ref. 35 for an excellent discussion of this force). If the bilayer were much thicker than 2ℓ, this compressive force would cause the excess alkane to flow between the monolayers toward the annulus and microlenses until the acyl chains of the opposing monolayers came into contact and presented a significant repulsive force. Until the acyl chains meet, the only opposing force is the transient one of viscous flow of the excess alkane between the monolayers. This is precisely why the planar bilayers thin from an initially thick film in the first place.

The Position of the Acyl Chain Double Bond Affects the Interactive Volume

The acyl chains of the bilayer are, on the average, bent, causing the methyl end of the chain to have a tendency to run parallel to the bilayer. A bilayer containing little solvent has a thickness slightly greater than the fully extended length of a single acyl chain. This means that the statistical bend will occur in approximately the middle of the chain. It was proposed above that the alkanes interact predominantly with the terminal methyl half of the chain. The double bond of the glycerol monooleate is located at carbon number 9, which places it directly in the center of the chain. Since the double bond is *cis*, it favors nicely the statistical bend in the center of the chain. Thus, the alkane molecules probably spend most of the time in the central region of the bilayer defined by the double bonds of the acyl chains of the opposed monolayers. This suggests that the location of the double bond may have an effect on the way the alkanes can occupy the interactive volume of the bilayer. The data of FIGURES 3 and 5 indicate that glycerol monooleate bilayers have a maximum thickness when *n*-decane is used to form the bilayer. Bilayers formed from *n*-octane are slightly thinner. Requena *et al.*[36] have made a similar observation. Preliminary measurements in my laboratory indicate that cyclodecane and 2,2,3,3, tetramethylhexane also give thinner membranes than *n*-decane. Requena and Haydon[18] report that membranes formed from 2,2,4 trimethylpentane, cyclohexane, and *cis*-5-decene also yield thinner membranes. It thus appears that glycerol monooleate bilayers have a *maximum* thickness for *n*-decane. For alkyl solutes longer or shorter than decane, the membranes decrease in thickness. This effect might be due to the length of the alkane relative to the double-bond position. Note that in FIGURE 8 the decane molecule precisely spans the distance between carbon 9 (the double bond) and the methyl group. A reasonable hypothesis is that if the alkane can precisely span the double bond-methyl group distance, it is possible for the acyl chain to spend some time fully extended in the presence of the alkane without generating free volume. If the

TABLE 2

PHYSICAL PROPERTIES OF PLANAR BILAYERS FORMED FROM ISOMERS OF GLYCEROL MONOOLEATE AND HEXADECANE* AT 30°C

Isomer	Bilayer Thickness (Å)	ΔH (kcal/mol)	ΔS (cal/deg/mol)
18:1(Δ6)†	31.9 ± 0.2	3.01 ± 0.19	10.02 ± 0.57
18:1(Δ9)‡	34.0 ± 0.2	4.03 ± 0.10	13.25 ± 0.36
18:1(Δ11)§	32.0 ± 0.2	3.66 ± 0.22	12.15 ± 0.65

*ΔH and ΔS have same meaning as in TABLE 1.
†Glycerol monopetroselinin.
‡Glycerol monooleate.
§Glycerol monovaccenin.

alkane is shorter than the double bond-methyl distance, the acyl chain cannot spend much time fully extended without generating energetically unfavorable free volume. Therefore, the acyl chains do not extend as fully with the shorter alkanes.

To examine the role of double-bond position further, membranes were formed from *n*-hexadecane and positional isomers of glycerol monooleate. TABLE 2 shows a comparison of glycerol monooleate (18:1, Δ9) with glycerol monovaccenin (18:1, Δ11) and glycerol monopetroselinin (18:1, Δ6). When the double bond is shifted to either position 6 or 11, the membrane becomes thinner. It seems that the maximum interactive volume is achieved when the double bond is located in the center of the acyl chain. This is consistent with the work of Barton and Gunstone,[37] who have shown that the bilayer phase transition temperature (T_c) of dioctadecenoyl lecithins is minimum when the double bond is at position 9. For example, $T_c = -21°C$ for Δ9, but for Δ3 and Δ15, $T_c = +35°C$. Maximum internal fluidity is obtained when the double bond is in the center of the chain. This makes it easier to insert alkanes into the interior.

THERMODYNAMICS OF ALKANE SOLUBILITY

Not only does the thickness (and therefore the composition) of the bilayer depend upon alkane structure, it also depends strongly on temperature.[19,20] This fact is indicative of a nonideal bilayer solution and permits important thermodynamic information about the interaction of alkanes with the bilayer to be obtained.[21]

The temperature-dependence of the solubility of solid, immiscible liquid and gaseous solutes in liquids provides a means of calculating the entropy (ΔS) and enthalpy (ΔH) of solution.[25] Consider, for example, a saturated solution in which excess solid solute is present. At equilibrium, the free energy of solute in the pure solid must be the same as the partial molal free energy (chemical potential) of the solute in the saturated solution; that is, the free energy of transfer from solid to solution is zero at equilibrium. Under these circumstances, the ΔH and ΔS of transfer from solid to solution are easily calculated.[25] The planar bilayer is completely equivalent to this system; it is saturated with alkane because the bilayer is surrounded by an annulus (Plateau-Gibbs border) of the bulk solution of lipid

in alkane. The alkane is present in great excess in the annulus and can be treated for our purposes as a reservoir of pure alkane. The proof that the bilayer is saturated comes from the observation that numerous microlenses (dia. ~1 μM) of excess solvent reside in the plane of the bilayer.[26,27] Immediately after formation, the "black" film or bilayer appears completely smooth and structureless in reflected light (FIGURE 9A). Within a few minutes, however, bright "pinpoints" appear which become fully developed in 10–20 minutes (FIGURE 9B). These "pin points" are the microlenses, which are thick relative to the bilayer and scatter incident light. Exceedingly careful measurements of specific capacitance show a slight thinning of the membrane that follows the time course of appearance of microlenses. In general, however, the microlenses have less than a one-percent effect on the measured specific capacitance.[18,23,28]

Thus, the planar bilayer is saturated with alkane. The microlenses and annulus are excess solute and are completely equivalent to the excess solid found in saturated solutions of solid in liquid described earlier. If the alkane of the microlenses (M) and annulus (A) is in equilibrium with the bilayer (BI), then the enthalpy and entropy of transfer of alkane to the bilayer can be calculated by the following standard equations:[25]

$$\Delta H = \overline{H}_{BI} - H_{M,A} = -R\left[\frac{\partial \ln X_s}{\partial (1/T)}\right]_{SAT,P} \qquad (3)$$

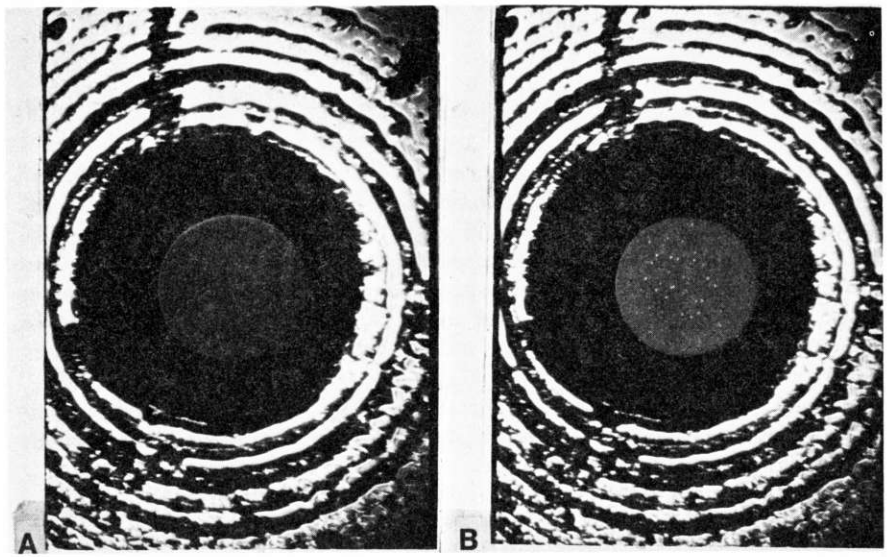
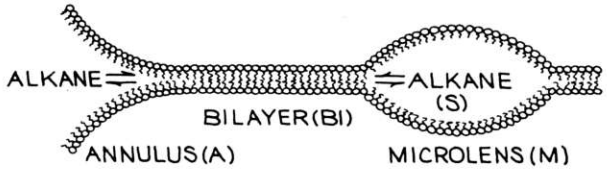


FIGURE 9. Reflected-light photographs of a planar bilayer formed from glycerol monooleate in heptamethylnonane. A: The membrane immediately after thinning. Note the smooth structureless surface. B: The membrane about twenty minutes after thinning. Numerous microlenses of excess alkane can be seen in the surface. These microlenses indicate that the bilayer is saturated with the alkane. The bright concentric rings surrounding the bilayer are due to the end-mill used in the machining of the septum.



AT SATURATION EQUILIBRIUM, $\bar{F}_{BI} = F_{M,A}$

$$\Delta H = \bar{H}_{BI} - H_{M,A} = -R\left[\frac{\partial \ln X_S}{\partial (1/T)}\right]_{SAT,P}$$

$$\Delta S = \bar{S}_{BI} - S_{M,A} = R\left(\frac{\partial \ln X_S}{\partial \ln T}\right)_{SAT,P}$$

$X_S \equiv$ MOLE FRACTION OF ALKANE IN BILAYER

FIGURE 10. Schematic drawing summarizing the structure and thermodynamics of planar bilayers saturated with alkane. At equilibrium, the partial molal free energy of the alkane in the bilayer (F_{BI}) must be the same as in the bulk alkane of the annulus and microlenses ($F_{M,A}$). The equations give the enthalpy (ΔH) and entropy (ΔS) of transfer *from* bulk *to* bilayer, assuming the activity of the alkane is a linear function of concentration, which is probably a reasonable assumption at saturation. The mole fraction of alkane solute (X_S) can be calculated from measurements of specific geometric capacitance and a knowledge of the interfacial concentration of the stabilizing lipid molecule (see text).

$$\Delta S = \bar{S}_{BI} - S_{M,A} = R\left(\frac{\partial \ln X_S}{\partial \ln T}\right)_{SAT,P} \quad (4)$$

The derivation of these equations assumes that the activity of the alkane solute in the bilayer is a linear function of concentration. In these equations, R is the gas constant, T the temperature in °K, and X_S is the mole fraction of solute in the saturated bilayer. $\bar{H}_{BI}$ and $\bar{S}_{BI}$ are, respectively, the partial molal enthalpy and entropy of the alkane solute in the bilayer, whereas $H_{M,A}$ and $S_{M,A}$ are the values in the bulk phases of microlenses and annulus. ΔH and ΔS give, therefore, the enthalpy and entropy of transfer from bulk to bilayer. At saturation equilibrium, the free energy of transfer $\Delta F = F_{BI} - F_{M,A}$ is zero, so that $\Delta H = T\Delta S$. FIGURE 10 summarizes the thermodynamics of the alkane-bilayer interaction. The beauty of this approach is that a *direct* thermodynamic comparison of the interior of the bilayer with the interior of the bulk alkane is obtained.

Procedurally, ΔS and ΔH are found from the slopes of plots of $\ln X_S$ vs. $\ln T$ and $(1/T)$. The only problem is to determine X_S, which is easily done from the measurements of specific geometric capacitance and knowledge of the concentration of the surface active lipid in the bilayer.[10,11,18,21] It is absolutely essential that the planar bilayer-annulus-microlens system (FIGURE 10) be in chemical equilibrium. That equilibrium exists can be easily verified, because the composition of the bilayer, and therefore the thickness and specific capacitance, will be invariant with time at equilibrium. Measurements as a function of time of films

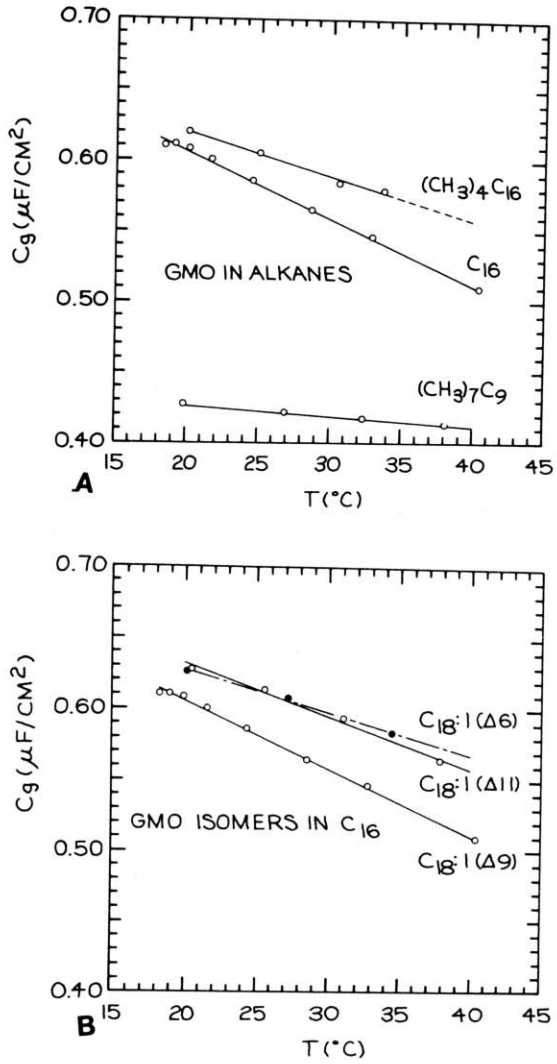


FIGURE 11. Plots of the specific geometric capacitance (C_g) as a function of temperature (T). The diameters of the data points represent the standard error of the mean ($\simeq$0.3%) of triplicate determinations on each of three membranes at each temperature. A: C_g of bilayers formed from 2,6,11,15-tetramethylhexadecane [$(CH_3)_4C_{16}$], *n*-hexadecane [C_{16}], and 2,2,4,4,6,8,8-heptamethylnonane [$(CH_3)_7C_9$]. B: C_g of bilayers formed from positional isomers of glycerol monooleate [C_{18}:1(Δ9)] and *n*-hexadecane.

formed from various lipids indicate that equilibrium is easily achieved in monoglyceride films[21] but not necessarily with films formed from lecithins.[28] The monoglycerides and shorter alkanes are slightly soluble in water,[21,29] and to have equilibrium the aqueous phase must be saturated with the bulk solution; otherwise the values of specific capacitance will be too high and time-dependent changes will be observed.

Measurements of specific geometric capacitance (C_g) of bilayers as a function of temperature for glycerol monooleate in several related alkanes and for positional isomers of glycerol monooleate in *n*-hexadecane are shown in FIGURE 11. From these data, plots of $\ln X_S$ against (1/T) and lnT were prepared as shown in FIGURE 12 for glycerol monooleate in various alkanes. A linear regression analysis on these plots, using Equations 3 and 4, yield the enthalpies and entropies of transfer (TABLES 1 and 2). The results are interesting. Consider first the data for glycerol monooleate in *n*-hexadecane (TABLE 1). The enthalpy of transfer from bulk hexadecane to saturated bilayer is +4.03 Kcal/mol and the entropy is +13.25 cal/mol/°K.‡ These values are exceptionally large. As pointed out elsewhere,[21] if the interior of the bilayer were equivalent to a simple alkyl liquid, the enthalpy would be only a few calories per mol. Therefore, the immediate and unequivocal conclusion is that the hexadecane molecules find themselves in an environment markedly different from a simple alkyl liquid. The large values of ΔH and ΔS mean that the hexadecane molecules are not bound as tightly in the bilayer as in bulk, and that they have more degrees of freedom. One explanation is that, in general, the cohesive forces in the bilayer are not as large as in bulk. The work of Gershfeld and Pagano[38] and Katz[39], however, indicates that the cohesive forces between acyl chains in monolayers and bilayers are the same as in bulk. The x-ray diffraction data of Wilkins *et al.*[3] are consistent with this conclusion. An alternative explanation was offered by White[21] and depends upon the idea outlined earlier that the alkane molecules are located largely (but not exclusively, see below) in the central zone of the bilayer. Numerous x-ray diffraction studies of bilayers (e.g. Ref. 3) reveal a zone of low electron density in the bilayer mid-plane due to the methyl ($-CH_3$) groups (FIGURE 6D illustrates this point). There is thus little interdigitation between the apposed monolayers, and a well-defined interface must exist between them. Because the methyl groups have a lower electronic polarizability than methylene groups, molecules located chiefly in the vicinity of the mid-plane should experience smaller van der Waals cohesive forces. Support for this hypothesis is given by Dean and Hayes,[40] who measured the heat of sorption of hexane vapor on close-packed stearic acid monolayers at the vapor-water interface. At low partial pressures of hexane (that is, low surface coverage by adsorbed hexanes), the heat of sorption is 4.5 Kcal/mol. At high partial pressures (high-surface coverage approaching closest packing of hexane on the stearic acid surface) the heat of sorption becomes identical to the heat of vaporization of liquid hexane (7.5 Kcal/mol). This indicates that the exposed $-CH_3$ surface of the steric acid does not attract hexanes as strongly as the fully covered hexane surface or bulk liquid.

‡These values are slightly different than previously reported[21] because of slightly different assumptions about how the area per acyl chains in the interface changes with temperature.

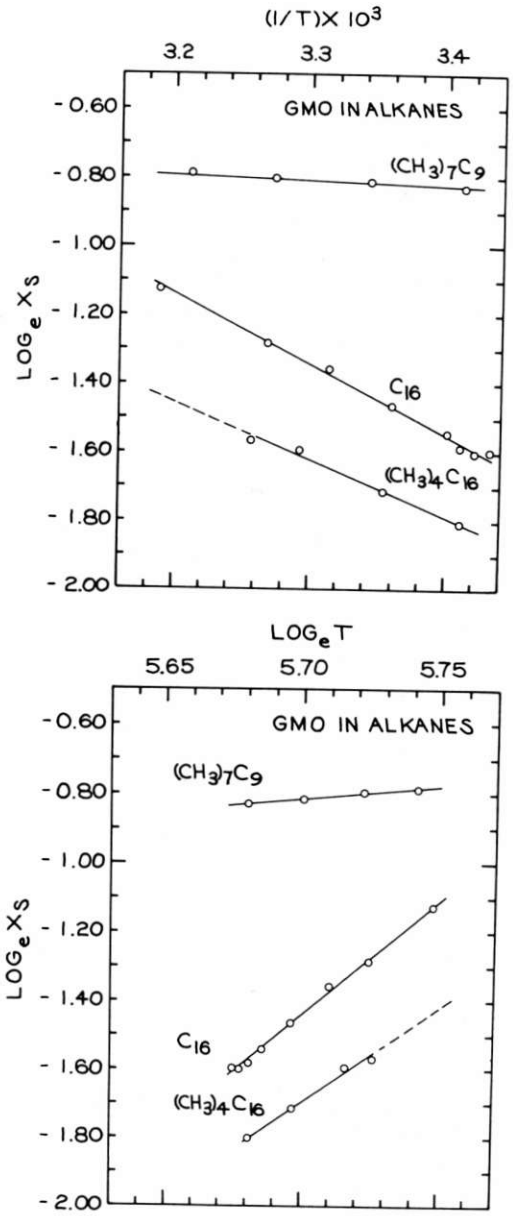


FIGURE 12. Plots from data of FIGURE 11A of $\log_e X_s$ against (A) 1/T and (B) $\log_e T$. The enthalpy and entropy of transfer, respectively, can be calculated from the slopes of these curves using Equations 3 and 4 (see also FIGURE 10).

An additional contribution to the large positive ΔH for hexadecane may come from the generation of free volume in the central zone. The insertion of hexadecane into the "slanted pockets" between the terminal methyl halves of the acyl chains probably creates some free volume because the acyl chains cannot flex enough to pack tightly about end of the hexadecane facing the interface (see FIGURE 7). This generation of free volume would increase the fluidity and therefore the entropy of the central zone. Thus, compared to bulk hexadecane where the alkane-alkane packing is tighter than the alkane-acyl chain packing of the bilayer, the cohesive energy density would be decreased. But this decrease would be compensated for by the increased entropy.

The enthalpy of transfer of heptamethylnonane into glycerol monooleate (TABLE 1) is only 0.37 Kcal/mol, compared to 4.03 Kcal/mol for hexadecane. This means that the heptamethylnonane finds an environment in the bilayer much closer to the bulk environment. This is consistent with measurements I have made on the shorter *n*-alkanes (C_{15} through C_8). The results of these measurements will be reported elsewhere, but the general conclusion is that as the length of the alkane decreases, ΔH decreases and, in fact, reverses sign at about *n*-decane or *n*-hendecane. One reason for this is the larger volume fraction (F) of the bilayer occupied by the shorter alkanes (e.g., for heptamethylnonane at 30°, F = 0.45 while for *n*-hexadecane, F = 0.27), which means more nearest neighbors of the alkanes will be alkanes rather than acyl methyl groups. This does not fully explain the difference, however, since tetradecane with F = 0.43 has a ΔH of 1.02 Kcal/mol (S.H. White, unpublished), about 3 times the heptamethylnonane value. The difference is probably due to the large number of $-CH_3$ groups on the heptamethylnonane. In bulk, this molecule is already in a $-CH_3$ rich environment and does not pack as tightly as tetradecane in bulk. Therefore the environment of the bilayer is closer to that of the bulk environment. There is also likely to be a free-volume effect for this molecule. Because the heptamethylnonane is more spherical than the neighboring acyl chains, it cannot pack as compactly in the bilayer as in bulk and consequently free volume is produced resulting in a lower cohesive energy density.

Now compare the tetramethylhexadecane data with the hexadecane data in TABLE 1. Note that ΔH is reduced to 3.20 Kcal/mol. The volume fraction of the bilayer occupied by the tetramethylhexadecane is 0.23 compared to 0.27 for hexadecane, so the lower ΔH cannot be explained by the volume fraction effect. The difference is probably due to the presence of the additional $-CH_3$ groups on the tetramethylhexadecane, which cause looser packing in bulk compared to hexadecane. The lower polarizability of the additional $-CH_3$ groups should also lower the bulk cohesive energy density compared to hexadecane. The fact that the melting point of tetramethylhexadecane is far lower than that of hexadecane supports this point of view.

The data of TABLE 2 for the bilayers formed from the glycerol monooleate positional isomers in *n*-hexadecane is more difficult to interpret. ΔH is significantly smaller for the $\Delta 6$ and $\Delta 11$ isomers, even though the volume fractions for these are smaller (0.22) than for the double bond at $\Delta 9$. The situation is not completely clear, but it may be due to a bimodal distribution of alkane. Even though the alkane is located largely in the central zone of the bilayer, there must also be some in the monolayers near the aqueous interface. Since the area per glycerol monooleate is about $39A^2$ (Ref. 10) and the cross-sectional area of the acyl chain is $22A^2$, it is possible to pack a small number of alkanes into the bilayer parallel to the acyl chain beginning at the interface, rather than at the center of the acyl chain. To accomplish this, it is only necessary for an occasional chain to be fully extended. These few alkane molecules would find an environment much more like the bulk liquid and would consequently have a lower ΔH and ΔS of transfer. I will report elsewhere that the maximum positive ΔH is achieved for *n*-hexadecane with the value decreasing slightly for *n*-heptadecane and *n*-octadecane. I believe this is because the length of the longer alkanes starts to favor insertion near the interface, rather than into the central zone. Not much can be packed in this way, of course, without increasing the free energy of the system due to an increase in the area per acyl chain. Essentially, in the general case, the central zone of the bilayer has degrees of freedom not found in bulk alkane, which favors the entry of alkane because of a positive ΔS paid for at the expense of an unfavorable positive ΔH. I propose that as the alkane chain length increases, its presence in the center of the bilayer becomes more unlikely stericly, and a more favorable enthalpy is achieved at the expense of entropy by having the alkane line up with acyl chains beginning at the interface. This cannot long continue without an unfavorable free-energy increase in the bilayer because of the interfacial changes. The amount of alkane at the interfacial zone is probably small, but it is not until the amount of alkane in the central zone is reduced that its effect on the enthalpy can be seen.

Now consider the problem of changing the location of the double bond. As the double bond is shifted to either end of the acyl chain, the acyl chain behaves more and more like an unsaturated chain, as evidenced by the thermal studies of Barton and Gunstone.[37] I believe this decreases the interactive volume and therefore favors the shifting of the alkane chains toward the interface. The increased fraction of alkane in the interfacial zone may account for the decrease in ΔH and ΔS observed for hexadecane in the two isomers of glycerol monooleate. This effect will probably not be observed for the short alkanes, because the amount of alkane in the central zone must be reduced far enough to permit the lowered ΔH of the alkane in the interfacial zone to be observed. Also, the alkane chain must be long enough to achieve a favorable enthalpy of interaction with the acyl chain. Requena *et al.*[36] report that the surface pressure of glycerol monooleate at the alkane/water interface increases as the length of the alkane increases. The insertion of a few alkanes into the absorbed monolayers should cause such an increase,[24] provided the area per glycerol monooleate in the interface does not increase. The increase in surface pressure with increasing alkane chain length is consistent with the idea that the thermodynamics favor the entrance of the longer alkanes into the interfacial zone.

CONCLUSIONS

This research represents the first attempt of my laboratory to explore systematically the solution properties of bilayers using alkane molecules as solutes. The data clearly support the notion that the interior of the bilayer is not simply

equivalent to a bulk alkyl hydrocarbon, since the enthalpy of transfer from bulk to bilayer for hexadecane is greater than 3 Kcal/mol. The solubility of alkanes depends primarily upon the length of the alkane and only secondarily upon molecular volume. The variation in solubility suggests that the bilayer has an interactive or potential volume available for interactions with other molecules roughly equivalent to the volume of the acyl chains. This potential volume can become occupied without significant changes in interfacial organization. It appears that the interactive volume is maximized by having the double bond in the center of the octadecenoyl acyl chain. It is suggested that the alkane molecules occupy primarily the central zone of the bilayer but may also occupy the interfacial zone to a limited extent, with the longer alkanes having a greater likelihood of occupying the interfacial zone than the shorter alkanes. In the central zone, it is suggested that the presence of the terminal methyl groups of the acyl chains and the greater disorder found there give rise to the positive enthalpy and entropy observed for alkanes of lengths greater than eleven carbons. The qualitative analysis of the data is consistent with having the two monolayers of the bilayer act independently; it appears unlikely that a single alkane molecule spans the bilayer midplane and mixes simultaneously in both monolayers.

Perhaps the single most important conclusion from this work is that alkane molecules do not distribute uniformly through the thickness of the bilayer. That is, the bilayer is physicochemically anisotropic normal to the plane of the bilayer. Therefore, membrane solutions are unlikely to be true two-dimensional solutions in which thickness is an irrelevant variable. The bilayer also has properties unobserved in three-dimensional solutions, and it therefore seems reasonable to refer to membranes as quasi-two-dimensional solutions.

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