A MEAN-FIELD MODEL OF THE ALKANE-SATURATED LIPID BILAYER ABOVE ITS PHASE TRANSITION

II. Results and Comparison with Experiment

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ABSTRACT Equilibrium properties of a model lipid bilayer saturated with an *n*-alkane are presented. The model exibits a cut-off in absorption as the chain length of the alkane increases which is similar to that observed with black lipid films. The reasons for this cut-off are explored in detail. The model provides qualitative agreement with the experimental enthalpies of transfer of the various alkanes from bulk pure liquid to the bilayer, and with results of electrical compression experiments on black films. Distributions of alkane across the bilayer for different volume fractions in the membrane are presented. For small volume fractions of alkane, its distribution is fairly even across the bilayer and the alkane chains line up essentially parallel to the lipid chains. For larger volume fractions, the alkane distribution is strongly peaked in the center of the membrane. The alkane chains in the outer regions of the membrane line up essentially parallel to the lipid chains, while those in the center are almost completely disordered. The model suggests that the chains (both lipid and alkane) are in an essentially liquid state with no well defined interface between opposing monolayers. It gives a possible explanation for the discrepancy between the experimental free energy of thinning of some lipid membranes formed from the longer chain length alkanes and the theoretical values estimated from Lifshitz's theory.

INTRODUCTION

In the preceding paper (Gruen, 1981), the details of the alkane-saturated bilayer model were presented. The model is an extension of a solventless model (Gruen, 1980). As *n*-alkane chains are chemically similar to the lipid chains, much of the analysis in paper I follows directly from the solventless model. Three features of the alkane-saturated model are novel (the terms f^i, u_x, and C_{MIX}, see Eqs. 18, 19, 20, and 24 in paper I). These features arise because of the nature of the mean-field approach and concern, respectively, the provision of holes for the alkane, the even packing of the chains of both lipid and alkane at different positions in the bilayer, and the entropy of mixing of the lipid and alkane. They are necessary for the development of a physically reasonable model.

In the present paper, the results of the model are presented and compared with experiment. Equations from paper I will be referred to by preceding the equation number by I. Thus, Eq. 15 in paper I becomes Eq. I15. The results shown in this paper differ from those in the preliminary report of this work (Gruen and Haydon, 1980 *a*) because, in the preliminary report, no account was taken of mixing of molecules in the plane of the bilayer.

RESULTS FOR THE ALKANE-SATURATED MODEL

Results for five *n*-alkanes (butane, hexane, nonane, dodecane, and hexadecane) were generated. All the experimental black films which will be discussed in this paper were in equilibrium with bulk liquid alkane and, to mirror the experimental situation as closely as possible, it is assumed that all theoretical bilayers are in equilibrium with bulk liquid alkane. Thus, it is necessary to assume that butane forms a bulk liquid phase at atmospheric pressure and 41°C with the same van der Waals energy as other alkanes. The results of the model should not be taken as predictive of the absolute values of absorption of any gases into the bilayer. However, as discussed in paper I, because of internal consistency, the model should predict changes in absorption with chain length of the alkane.

For given bilayer dimensions, it is necessary to run ~15–20 iterations before the parameters are determined with the required accuracy. After each iteration the results are examined and changes made for the next iteration. Thirty-three different "bilayers" were completed (each with a unique "dimensions of bilayer-alkane" combination). The required accuracy with which the parameters of the bilayer were chosen is the subject of Table I. A discussion of this table follows.

The parameters under discussion were referred to in Fig. 3 of paper I and in the final paragraph of that paper. Once the dimensions of the bilayer have been chosen, the expected value of the first three quantities in the table are fixed. Bilayers with an area/lipid of 63.3, 64.3, and 66.3 Å^2 were considered. The standard deviations shown in the final column are the root mean square (rms) deviations of the values in the final iteration from the expected values averaged over the 33 different bilayers considered. The values 4.6×10^{-6}, 4.4×10^{-6}, and 4.0×10^{-6} Å^{-6} for $\langle 1/({}_aA_{\text{AVE},l}n_a + {}_lA_l^i)^3 \rangle$ correspond respectively to the above areas per lipid. They imply values of the pressure in the headgroup region π_{HG} of 22.91, 21.91, and 19.92 dyn/cm. The actual values of the above quantity deviate from the expected values by an average of 0.0264×10^{-6} Å^{-6} (see Table I). This corresponds to an error in π_{HG} of ~0.1 dyn/cm, which is very small.

As emphasized in Gruen (1980), the lipid chains in the solventless membrane fill the space available to them very evenly. Their distribution was shown in Fig. 7 in Gruen (1980). The distribution of the sample of lipid conformations is very similar. The solventless membrane has exactly 23.5 layers including the "0 layers" on the two sides of the bilayer. (Hence, the

TABLE I
ACCURACY OF BILAYER PARAMETERS

Quantity of interest	Expected value	Parameter(s) which alter(s) value	Standard deviation from expected value
Avg. of surface areas/lipid	63.3–66.3 Å^2	C^*_{SUR}	0.033 Å^2
Avg. of chain areas/lipid	63.3–66.3 Å^2	C_l^*	0.338 Å^2
$\langle 1/({}_aA_{\text{AVE},l} \cdot n_a + {}_lA_l^i)^3 \rangle$	4.0×10^{-6} Å^{-6} to 4.6×10^{-6} Å^{-6}	—	0.0264×10^{-6} Å^{-6}
Volume fraction of chains in most uneven layer	1.0	u_x	0.00168
Order parameter in "worst" layer	−0.1 to 0.45	Order parameter profile	0.00072

distance between "layer 0" on the two sides of the bilayer is 22.5 × 1.25 = 28.125 Å. The thickness of the chain region, defined as the average distance between the C1—C2 bonds on the two sides of the bilayer, is 27.3 Å). This arose from the overlapping of fractional parts of the 12th layer from the two sides of the membrane. When alkane is added, it is possible to choose convenient thicknesses for the free energy calculations. Membranes with exactly 25, 27, 29, 31, and 33 layers were considered. These correspond, respectively, to chain region thicknesses of 29.2, 31.7, 34.2, 36.7, and 39.2 Å. For the outer 12 layers on either side of the bilayer, the distribution of chains was fitted to the solventless distribution. For any remaining layers, the chains are constrained to fill the available space as evenly as possible. (This procedure enables straightforward comparison of free energies of all alkane containing bilayers with that for the solventless bilayer.) On successive iterations, the profile u_x (as defined in Eqs. I19 and I20) is varied, and the distribution of chains approaches the required distribution. A completely even distribution of chains has a volume fraction of 1.0 in all layers (except layer 0). It was possible for all 33 bilayers to get an average deviation from the required distribution of <0.001. As a specific example, consider the most uneven layer in each bilayer, i.e., the layer with the largest deviation from the required distribution. The rms value of the largest deviation (averaged over the 33 bilayers) is, from Table I, 0.00168.

It is possible to compare the order parameter profile fed in at the beginning of the final iteration with that derived after the iteration. As for the solventless model (and for the same reasons), the order parameters in the outer seven layers on either side of the bilayer are averaged. All other layers are treated individually. The average difference between the order parameters before and after the final iteration for all the bilayers considered is ~0.0002. Table I shows the rms value of the largest difference of all the layers in a bilayer (0.00072).

As long as the order parameter profile is determined within the above limits, the most important factor in determining the free energy of a given bilayer was found to be the distribution of the chains. A numerical example should assist understanding. Two successive iterations for a hexane-containing bilayer of thickness 34.2 Å and area per lipid 63.3 Å^2, produced the results shown in Table II. For both iterations errors in the order parameter profile fall within the limits given in the last paragraph. The first iteration was not deemed final because the most uneven layer had a chain distribution which differed by 0.0023 from the required distribution. The second iteration was deemed final; the most uneven layer differed from the required distribution by 0.0011. The difference in free energy (as calculated from Eq. I34) between the two iterations is 0.6 cal/mol of lipid molecules. As the rms deviation from the required distribution almost halves between iterations, the error in the

TABLE II
VALUES OF PARAMETERS IN TWO SUCCESSIVE ITERATIONS FOR A HEXANE-CONTAINING BILAYER

	First	Second
Thermodynamic avg. of surface areas (Å^2)	63.31	63.30
Thermodynamic average of chain areas (Å^2)	63.52	63.52
$\langle 1/(_aA_{AVE,l} \cdot n_a + {}_lA_l^i)^3 \rangle$ ($\times 10^{-6}$ Å^{-6})	4.616	4.617
Rms difference in order parameters: before—after	0.00006	0.00019
Rms deviation from required distribution of chains	0.00069	0.00039

estimation of the free energy of the bilayer (from the value for a bilayer with perfect agreement of all parameters) is $\sim \pm 1$ cal/mol of lipid.

Fig. 1 shows the variation of the free energy of the system G_{SYS} (as given by Eq. I34) with thickness at an area per lipid of 63.3 Å^2. No account is taken of the van der Waals attraction of the water layers on either side of the membrane, or of the capillary pressure on the bilayer (which is present because of the Plateau-Gibbs border around it), or of electrical double layer interaction. The latter two forces are apparently negligibly small (Fettiplace et al., 1975). The van der Waals attraction will be dealt with in Fig. 3. The points in Fig. 1 for butane at 34.2, 36.7, and 39.2 Å were computed with extra care (average rms deviation from the required distribution of 0.00027) and are therefore known to within $\sim \pm 0.5$ cal/mol lipid. A small number of the results far from equilibrium (dodecane at 34.2 and 36.7 and hexadecane at 34.2 Å) are somewhat less accurately known, with errors of possibly ± 3 cal/mol lipid. Had error bars been drawn for the points, they would all lie inside the symbols on the figure.

In Fig. 2 the variation of free energy with area per lipid is investigated. To do this it is necessary to take account of the surface energy $\int \sigma d\mathcal{A}$ as in Eq. I34. For points with an area per lipid of 63.3 Å^2 this integral is zero. For other areas per liqid, the integral can be estimated roughly from experimental data. Thus, the experimental gradient of a $\pi - A$ curve of a monolayer of egg lecithin at the hexadecane-water interface at a surface pressure of 50 dyn/cm is ~ -1.5 dyn/cm per Å^2 (see Fettiplace et al., 1975). This leads to $\int \sigma d\mathcal{A} = 1.1$ cal/mol lipid for $\mathcal{A}_f = 64.3$ Å^2, and $\int \sigma d\mathcal{A} = 9.7$ cal/mol lipid for $\mathcal{A}_f = 66.3$ Å^2. The points in Fig. 2 have been drawn assuming these values for the integral. An alternative approach would have been to use the theoretical estimate for σ ($\sigma = \gamma - \pi_{HG} - \pi_c$). As already stated, π_{HG} decreases by 1 and 3 dyn/cm as the area per lipid is increased to 64.3 and 66.3 Å^2, respectively. However, the determination of π_c (as defined in Eq. I16) is crude and it was felt that the experimental result (even though on a different system) provides a more reliable estimate of the variation of σ with $\mathcal{A}$. Table III gives the theoretical values of σ for the

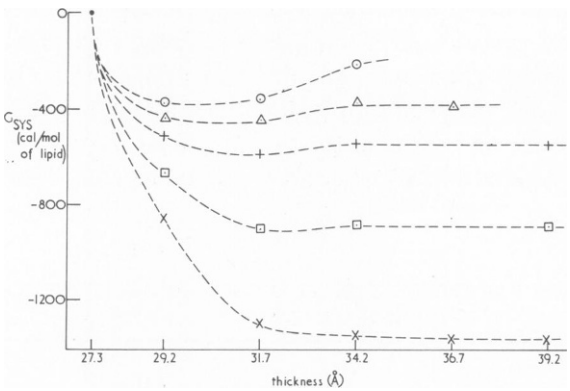

FIGURE 1 Free energy G_{SYS} of the system bilayer + alkane (see Eq. I34) vs. thickness at an area/lipid of 63.3 Å^2. Saturating alkane: ⊙ hexadecane, (△) dodecane, (+) nonane, (⊡) hexane, and (×) butane. The dashed lines were drawn by eye. The free energy of the solventless membrane was set at 0 cal/mol of lipid.

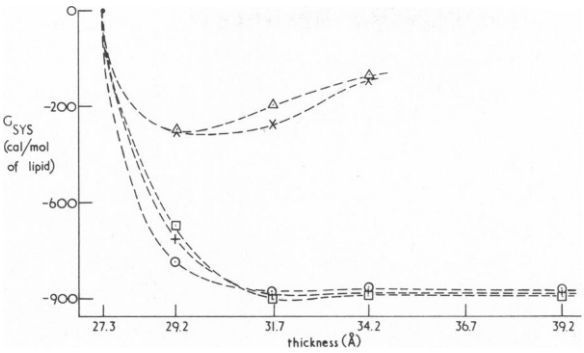

FIGURE 2 Free energy G_{SYS} of the system bilayer + alkane (see Eq. I34) vs. thickness at different areas per lipid. Saturating alkane and area per lipid: (△) hexadecane at 64.3 Å^2, (×) hexadecane at 63.3 Å^2, (⊙) hexane at 66.3 Å^2; (+) hexane at 64.3 Å^2, and (⊡) hexane at 63.3 Å^2. The estimation of $\int \sigma da$ is described in the text. The dashed lines were drawn by eye. The free energy of the solventless membrane is set at 0 cal/mol of lipid.

bilayers in Fig. 2. In fact the magnitude of $\int \sigma d\mathcal{A}$ is sufficiently small that had the theoretical estimates of σ been used, the results would be indistinguishable from those shown in Fig. 2.

Fig. 3 shows, on an expanded free energy scale, the variation of free energy G_{SYS} with thickness for the butane-containing bilayer and the van der Waals (vW) free energy of attraction of the water layers across the chain region. The latter has been taken as:

$$F_{vW} = A/12\pi h^2, \tag{1}$$

where A is the Hamaker constant for the chain region of the film (taken as 0.956×10^{-21} cal) and h is its thickness. (See Requena et al., 1975, for a discussion of the assumptions involved in Eq. 1.) For thicknesses $\gtrsim$39.2 Å, the free energy (excluding van der Waals attraction of the water layers) vs. thickness curve must become horizontal for all alkanes. This is a consequence of the fact that the environment at the center of the membrane rapidly approaches that of bulk alkane as the thickness increases beyond twice the fully extended chain length of the

TABLE III
THEORETICAL ESTIMATES OF σ (BILAYER TENSION/2)

Alkane	Area/lipid	Thickness	σ
	(Å^2)	(Å)	(*dyn/cm*)
Hexane	63.3	29.2–39.2	0.0
Hexane	64.3	29.2–39.2	1.0
Hexane	66.3	29.2	3.0
Hexane	66.3	31.7	3.6
Hexane	66.3	34.2	4.6
Hexane	66.3	39.2	4.8
Hexadecane	63.3	29.2–31.7	0.0
Hexadecane	63.3	34.2	2.5
Hexadecane	64.3	29.2	1.0
Hexadecane	64.3	31.7	2.5
Hexadecane	64.3	34.2	4.8

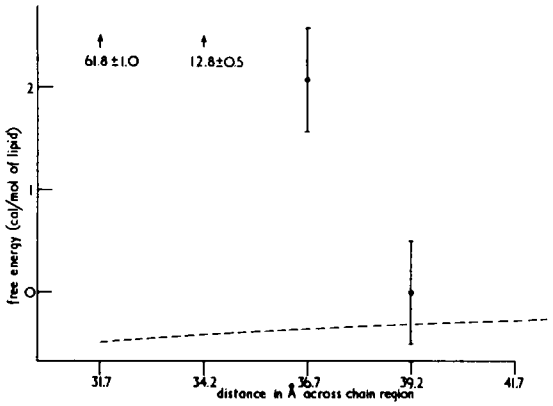

FIGURE 3 Free energy G_{SYS} vs. thickness for the butane-containing bilayer at an area/lipid of 63.3 Å^2. The points come from evaluation of Eq. I34. The errors shown are discussed in the text. The dashed line represents the van der Waals attraction of the water layers across the chain region as given by Eq. 1. The free energy of the 39.2-Å bilayer has been chosen as zero.

lipid. As should be clear from Fig. 3, the theoretical model is not sufficiently sensitive to give an exact value for the equilibrium thickness of this bilayer. While it seems highly probable that the equilibrium thickness is at least 39.2 Å, the upper limit is less certain. Equilibrium thicknesses corresponding to other chain length alkanes are also shown in Fig. 4. The error bars for nonane, dodecane, and hexadecane (in Fig. 4) were derived by inspection of Fig. 1. The result for hexane was less clear cut; hence the large error bar.

Figs. 5–8 show the distribution of alkane and order parameter profiles for bilayers of different dimensions. The shape of the "volume fraction of all chains" curves are almost identical in the outer 12 layers on either side of the bilayer. (As previously stated, they have been fitted to the solventless profile of the sample of lipid chain conformations.) Any remaining layers have a chain distribution which is as even as possible.

The irregular behavior of the alkane order parameters in the outer layers of the membrane invites comment. The presence of an interface which the chain conformations cannot cross leads to a sample of allowed conformations biased in favor of those which lie parallel to the membrane surface. Hence the tendency of the order parameter to drop as the interface is

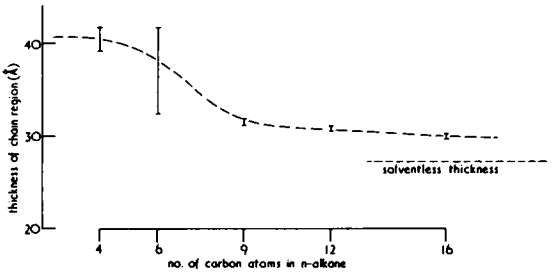

FIGURE 4 Equilibrium thicknesses derived from Figs. 1 and 3 where the area per lipid molecule is 63.3 Å^2. The error bars are discussed in the text. The dashed curve was drawn by eye.

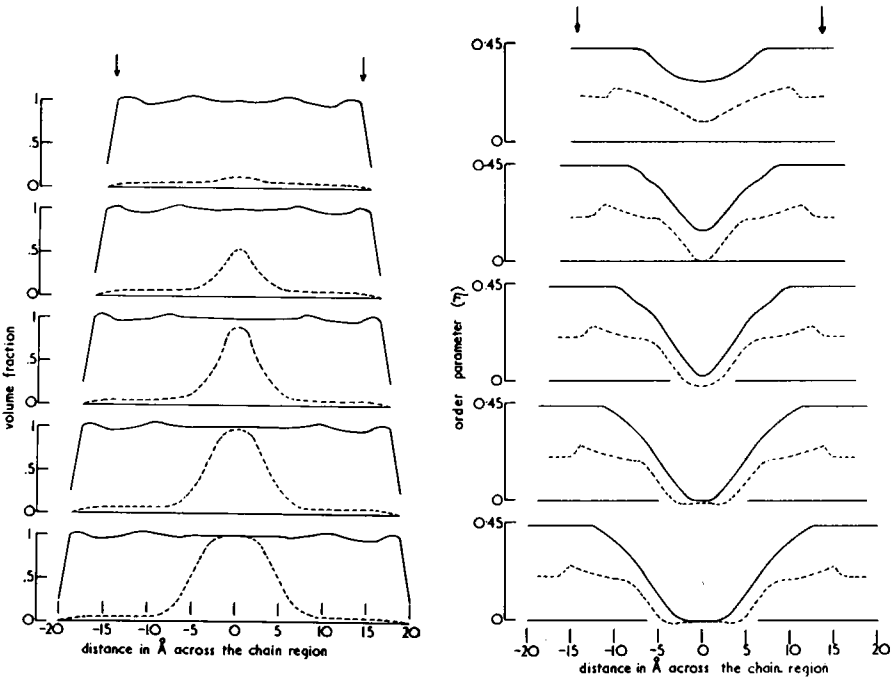

FIGURE 5 Volume fractions and order parameter profiles across butane-containing bilayers at different thicknesses (area/lipid 63.3 Å^2). The bilayers have chain region thicknesses of 29.2, 31.7, 34.2, 36.7, and 39.2 Å, respectively. The arrows represent the positions of the 0 layers on the two sides of the solventless membrane. (*a*) (----) volume fraction of butane across the bilayer. (—) volume fraction of all chains. (*b*) (----) butane order parameters across the bilayer. (—) volume weighted order parameters for all chains. As discussed in the text, the latter order parameters are averaged in layers 0–6 on both sides of the bilayer. Note that the butane order parameters do not extend to layer 0 on both sides of the bilayer; all alkanes were excluded from these layers.

approached. The irregular behavior extends further into the bilayer as the length of the alkane increases. This is simply because the direct influence of the interface (in disallowing conformations) can only extend the length of the alkane. It should be emphasized that in all bilayers considered, the irregular behavior is experienced by a very small volume fraction of alkane.

Figs. 9 and 10 show, respectively, the averaged order profiles along the lipid and alkane chains of particular bilayers. Fig. 11 shows two representative profiles of u_x, the free energy parameter invoked to constrain the chains to pack at constant density (see paper I).

For a given area per lipid, the energy of the bilayer per lipid molecule is defined as:

$$U_{\text{bilayer}} = 2\langle {}_lE_{\text{int}}\rangle + \langle {}_lE_{\text{disp}}\rangle + n_a(\langle {}_aE_{\text{int}}[\text{bilayer}]\rangle - \langle {}_aE_{\text{int}}[\text{bulk}]\rangle + 0.5\langle {}_aE_{\text{disp}}\rangle). \quad (2)$$

${}_aE_{\text{int}}[\text{bulk}]$ is the average internal energy of the alkane in a bulk hydrocarbon environment. Its value for dodecane and hexadecane has been given in Table I of paper I. Fig. 12 plots the value of U_{bilayer} for all bilayers with an area per lipid of 63.3 Å^2. The assumption of constant chain packing density as the bilayer takes up alkane implies that changes in energy and enthalpy between systems are identical.

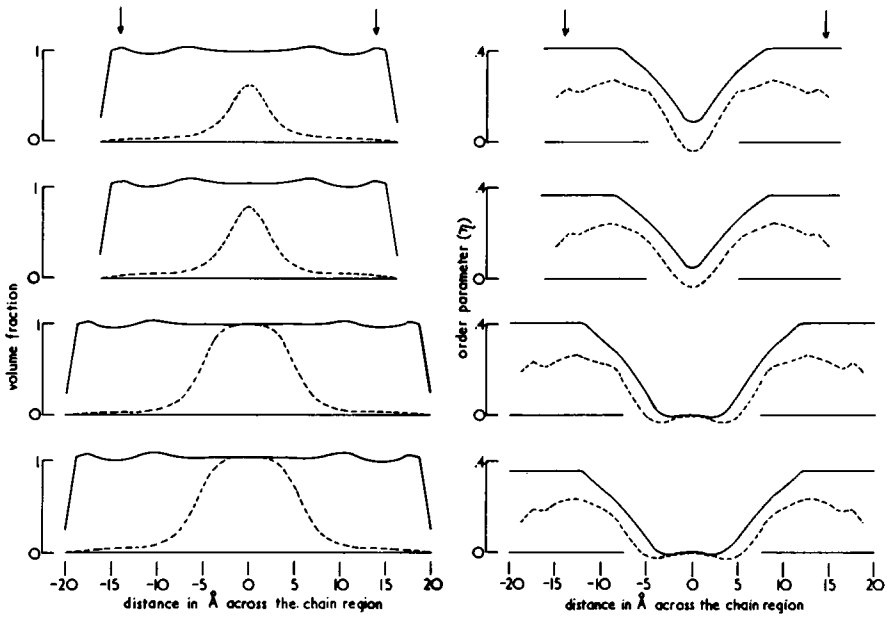

FIGURE 6 Volume fractions and order parameter profiles across hexane-containing bilayers at different thicknesses and areas per lipid. The bilayers have the following dimensions (from the top of the figure to the bottom): thickness 31.7 Å, area/lipid 63.3 Å^2; thickness 31.7 Å, area/lipid 66.3 Å^2; thickness 39.2 Å, area/lipid 63.3 Å^2; thickness 39.2 Å, area/lipid 66.3 Å^2. The arrows represent the positions of the 0 layers on the two sides of the solventless membrane. (*a*) (----) volume fraction of hexane across the bilayer. (—) volume fraction of all chains. A volume fraction of 1.0 was deemed to correspond to an area/lipid of 63.3 Å^2. The mean volume fraction for the second and fourth profiles is 66.3/63.3 = 1.047. The height of all curves therefore corresponds to the volume fraction per lipid molecule. (*b*) (----) hexane order parameters across the bilayer). (—) volume weighted order parameters for all chains (averaged in layers 0–6 on both sides of the bilayer).

DISCUSSION

Assumptions

The approach used in the development of the alkane-saturated model is as follows. The results of dmr experiments (Seelig and Seelig, 1974) performed on unsonicated dispersions of DPL (48.5 wt%) and water (51.5 wt%) are used to develop a model of the solventless bilayer. The model is then extended to allow alkane into the hydrophobic core of the membrane, with the aim ultimately of understanding how the absorption of alkane perturbs the structure of the lipid bilayer regions of biological membranes.

The majority of experimental results with which the predictions of the model may be compared are those for black films. It is thus necessary to argue that the near-solventless black film is similar to a single lipid leaflet in a planar lamellar phase. This argument requires justification. (*a*) There are van der Waals and electrostatic interaction forces between bilayers in the lamellar phase which are absent in the single black film. Provided the lamellar phase is sufficiently hydrated, these forces are small (LeNeveu et al., 1977). (*b*) An obvious difference between the two structures is the presence of a border around the black film. This border (as already discussed) leads to a capillary pressure on the membrane and exerts a

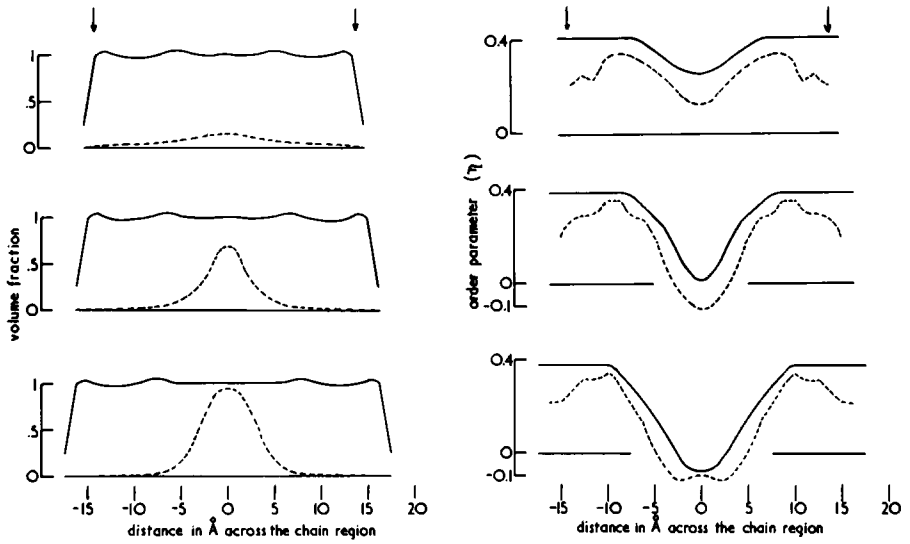

FIGURE 7 Volume fractions and order parameter profiles across hexadecane-containing bilayers at different thicknesses and an area/lipid of 63.3 Å^2. The bilayers have thicknesses of 29.2, 31.7, and 34.2 Å, respectively. The arrows represent the positions of the 0 layers on the two sides of the solventless membrane. (*a*) (----) volume fraction of hexadecane across the bilayer. (—) volume fraction of all chains. (*b*) (----) hexadecane order parameters. (—) volume weighted order parameters for all chains (averaged in layers 0–6 on both sides of the bilayer).

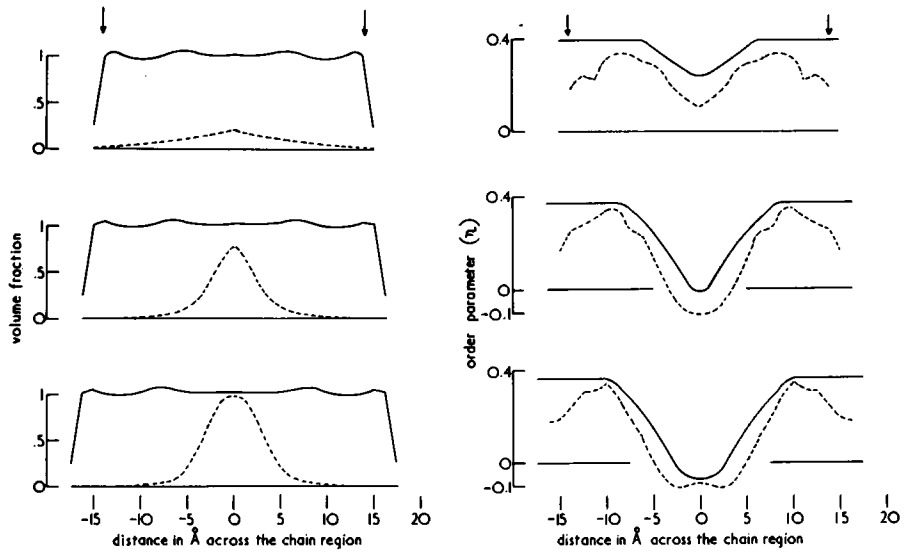

FIGURE 8 Volume fractions and order parameter profiles across hexadecane-containing bilayers at different thicknesses and an area/lipid of 64.3 Å^2. The bilayers have thicknesses of 29.2, 31.7, and 34.2 Å, respectively. The arrows represent the positions of the 0 layers on the two sides of the solventless membrane. (*a*) (----) volume fraction of hexadecane across the bilayer. (—) volume fraction of all chains. The mean volume fraction for all profiles is 64.3/63.3 = 1.016. (*b*) (----) hexadecane order parameters. (—) volume weighted order parameters for all chains (averaged in layers 0–6 on both sides of the bilayer).

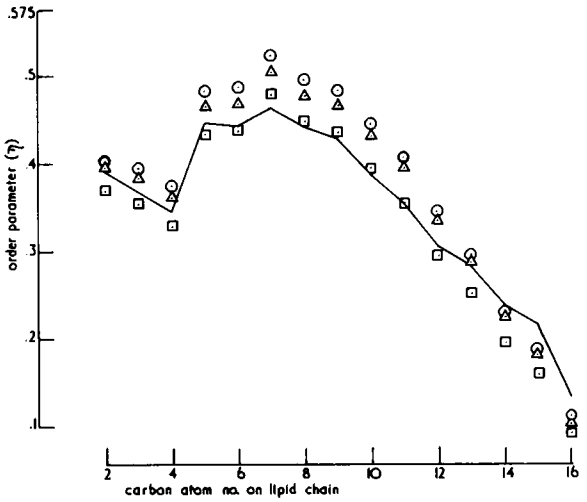

FIGURE 9 Lipid order parameters in different bilayers. (⊙) hexane-containing bilayer at a thickness of 39.2 Å and an area/lipid of 63.3 Å^2. (△) hexadecane-containing bilayer at a thickness of 31.7 Å and an area/lipid of 63.3 Å^2. (—) solventless bilayer. (⊡) hexane-containing bilayer at a thickness of 39.2 Å and an area/lipid of 66.3 Å^2.

tension on it as well. The former is very small (Fettiplace et al., 1975) and in the case of near-solventless lecithin films, so is the latter (Fettiplace et al., 1971). (*c*) The agreement, to within experimental error, of the dimensions (thickness and area per lipid) of near-solventless black films (Fettiplace et al., 1975) and lecithin dispersions (Reiss-Husson, 1967; Small, 1967; Lecuyer and Dervichian, 1969) suggests strongly that the lipids are in a similar physical state in the two membranes.

The assumption of constant chain packing density as the bilayer takes up alkane should also be reasonable. The fact that the density of the hydrophobic region of a DPL membrane above T_c is the same (to within experimental error) as liquid alkane (Nagle and Wilkinson, 1978) suggests that packing of the chains in the solventless and alkane-saturated states is similar. As

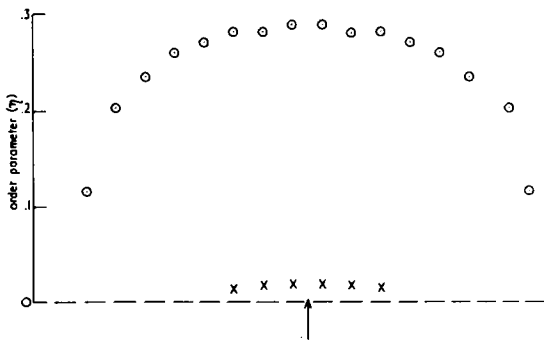

FIGURE 10 The thermodynamically averaged order parameters for the alkane chain. (⊙) hexadecane order parameters in a bilayer of thickness of 29.2 Å and an area/lipid of 63.3 Å^2. (×) hexane order parameters in a bilayer of thickness 39.2 Å and an area/lipid of 63.3 Å^2. The arrow marks the center of the molecule.

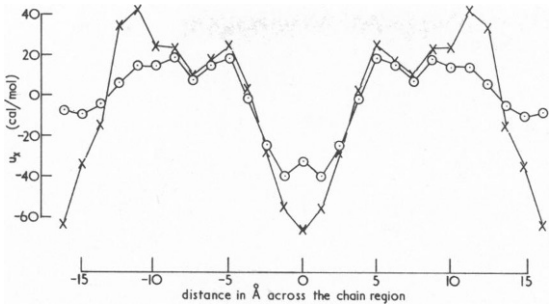

FIGURE 11 The profile of u_x (see text) for two bilayers of thickness 31.7 Å and area/lipid 63.3 Å^2. (×) butane-containing bilayer. (○) hexadecane-containing bilayer. The profiles have been drawn so that the volume weighted average of u_x over all layers in the bilayer is zero. (Any shift of the whole curve up or down makes no change to any properties of the bilayer.)

stated in Gruen (1980), the solventless model predicts a difference in packing density of 0.7%. Further, the observed density change when alkanes of different lengths are mixed is very small. For example, when equimolar amounts of hexane and hexadecane are mixed at 20°C and atmospheric pressure, the volume change is 0.2% (see Stoeckli et al., 1966).

Some of the assumptions inherent in the development of the alkane-saturated model follow directly from assumptions in the solventless model. These were discussed in Gruen (1980) and will not be dealt with further. The important new features of the alkane-saturated model are the terms f^i (Eq. I18), u_x (Eqs. I19 and I20), and C_{MIX} (Eq. I24). These three terms, taken together, represent a crude attempt to model a complicated situation. Compare two bilayers with identical dimensions, the first, saturated with a long chain alkane, the second with a short chain alkane. The presence of f^i will lead in the first instance to a very small fraction of alkane in the outer regions of the bilayer whereas in the second, there will be a somewhat larger fraction of alkane in these regions. Without u_x, the lipid chains in the two bilayers would behave almost the same (as the order parameter profiles would be similar). In reality, the lipid chains in the second bilayer must straighten significantly more than in the first. The profile u_x achieves this result. Fig. 11 illustrates the point. In the outer parts of the membrane (except for the outermost three layers on either side[1]) the value of u_x for the butane-bilayer is larger than for the hexadecane-bilayer. The opposite is true for the innermost three layers. This has the effect of straightening lipid chains more in the butane-bilayer than in the hexadecane one. Thus, the profile u_x plays an important, physically realistic role.

Both f^i and u_x change the relative probabilities of configurations in the bilayer but they do not take account of the degeneracy of those configurations. C_{MIX} is evaluated for the

[1]The behavior of u_x in the outer three layers on either side of the butane-bilayer is irregular for two reasons. First, the volume fraction of butane in these layers is falling to zero as the interface is approached (see Fig. 5 *a*). Second, for this bilayer, ${}_aA_{AVE}/n_a$ = 2.4 Å^2 and hence relative headgroup free energies for different lipid configurations (as defined by Eq. I8) are changed from their solventless value. As the occupancy of these layers is determined by the relative energies of the conformations, (and we are fitting the solventless chain packing profile), u_x compensates for these changes. For the hexadecane-bilayer, the volume fraction of hexadecane is virtually zero in the outer six layers on either side of the bilayer (see Fig 7 *a*). ${}_aA_{AVE}/n_a$ = 0.3 Å^2 and hence there is little change from the solventless bilayer in these regions.

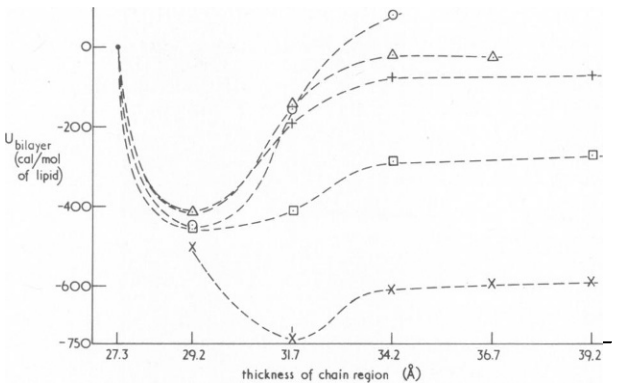

FIGURE 12 Energy, $U_{bilayer}$ (as defined in Eq. 2), vs. thickness for alkane-saturated bilayers at an area/lipid of 63.3Å^2. Saturating alkane: (⊙) hexadecane, (△) dodecane, (+) nonane, (⊡) hexane, (×) butane. The curves were drawn by eye. For the sake of clarity, the butane curve was not drawn for thicknesses <29.2 Å. The energy of the solventless membrane was set to zero.

equilibrium distribution of alkane and lipid for each bilayer. It estimates the combinatorial factor which is appropriate for the membrane.

The chains of DPL are fully saturated, while those of the experimental lipids contain one (or in the case of a small proportion of the chains of egg lecithin, more than one) double bond per chain. Using the former system to model the latter therefore, requires justification. As discussed in Gruen (1980), the results of dmr and x-ray diffraction experiments suggest that the packing of the chains in systems with some unsaturation is very similar to their packing for DPL. (The experiments are performed at temperatures an equal distance above the respective phase transition temperatures.) The factors taken into account in the present model would apply with little change had a double bond been introduced in the model lipid chains. Further, x-ray diffraction experiments on fully hydrated lamellar dispersions of dilauroyl phosphatidylcholine (C_{12}, fully saturated chains) in excess alkane have been performed (McIntosh et al., 1980). An alkane absorption cut-off, similar to that in black lipid films, is observed. This suggests strongly that bilayers with fully saturated lipid chains and those with limited unsaturation are comparable in their alkane uptake behavior.

Predictions of the Model

On the basis of Fig. 2, we assume that the model predicts, for all alkanes, that the equilibrium area per lipid is 63.3 Å^2. (A discussion of the implications of an increase in area per lipid is presented later.) For a small alkane (butane) the behavior of the bilayer as it thickens is described by Fig. 5. The five profiles shown in the figure represent equilibrium distributions for increasing aqueous concentrations of butane. (The fifth profile represents the approximate distribution for a saturated aqueous phase.) For a chain region thickness of 29.2 Å (top profile), the volume fraction of (relatively ordered) lipid chains is high throughout the membrane. Hence, the butane chains are constrained to lie essentially parallel to the lipid chains and the butane order parameter is positive throughout the membrane. The lipid chains in the outer regions of the membrane have been straightened considerably and the overall order parameter in these regions has increased from 0.371 (its solventless value) to 0.422. As

the membrane thickens, the volume fraction of butane in the outer regions remains at ~0.07. These butane chains are relatively well ordered and lie essentially parallel to the lipid chains. The center of the membrane becomes increasingly disordered as the volume fraction of alkane rises. For intermediate thicknesses (34.2 and 36.7 Å), the butane chains at the center show a slight tendency to lie parallel to the membrane surface (and hence have a negative order parameter). In this way, they can "stay out of the way" of lipid chains. At the center of the 39.2 Å bilayer, the volume fraction of lipid is zero, and the butane chains appear to be randomly arranged. If these chains are truly in their bulk state, the membrane will not thicken further. This would simply represent a transfer of alkane from a bulk liquid reservoir to a bulk liquid interior, a process resisted by the van der Waals attraction of the two water layers (see Fig. 3). It is therefore understandable that the experimental equilibrium thickness of bilayers formed with small alkanes is approximately the double extended lipid chain length. This point has been appreciated for some time (see Taylor and Haydon, 1966, and White, 1970).

The behavior of the hexadecane bilayer is depicted in Fig. 7. At a thickness of 29.2 Å, hexadecane behaves qualitatively like butane. The chains are aligned essentially parallel to the lipid chains, although they do not distribute as evenly across the bilayer as butane chains. At thicknesses of 31.7 and 34.2 Å their behavior is quite different. Consider the latter thickness. The hexadecane chains must "choose" between the center of the bilayer, where the volume fraction of lipid is small and the environment is relatively disordered, and the outer regions of the membrane where lipid chains must be straightened, and where the environment is relatively ordered. There are a vast number of possible conformations available to a hexadecane molecule in liquid alkane. At the center of the 34.2 Å membrane, a substantial proportion of these are possible, whereas in the outer regions of the membrane the molecule is limited, almost completely, to those conformations which are essentially parallel to the lipid chains. The hexadecane chains therefore partition very strongly into the center of the membrane. The volume fraction of hexadecane in the outer regions falls substantially between a bilayer thickness of 29.2 and 34.2 Å.

Summarizing, the two most important effects which determine the absorption of alkane, and hence the equilibrium thickness are, in order: (*a*) In bulk liquid alkane, the hydrocarbon molecules are free to take up any spatial orientation and, apart from an internal energy barrier, to undergo *trans* and *gauche* conformations. In the bilayer, there is an extra constraint, which, especially for longer alkanes, limits their freedom and reduces their internal entropy. The presence of the relatively ordered lipid chains means that alkane conformations in which a large part of the chain lines parallel to the membrane surface are almost excluded. (This statement applies progressively less in regions with smaller and smaller volume fractions of lipid.) A single *gauche* kink in the middle of a hexadecane molecule causes a long segment of the chain to be misaligned. This is not so of a much shorter molecule like hexane or butane. (*b*) Assuming a constant area per lipid, absorption of alkane into the outer regions of the membrane necessitates straightening of the lipid chains. To create room for a large molecule in this region requires the straightening of many more lipid chains than for a small molecule. Hence, for given bilayer thickness, small alkanes will distribute more evenly throughout the bilayer than large ones. While it is unfavorable, in free energy terms, to straighten the lipid chains, this is outweighted by the entropic advantages of a more even distribution of alkane throughout the bilayer and improved mixing in the plane of the bilayer.

Comparison with Experiment

The short alkanes lose only a small amount of internal entropy. This is more than made up by the entropic advantages of diluting the lipid molecules. Hence, they thicken the bilayer until no more dilution is possible (at a thickness of twice the extended lipid chain length). For longer alkanes, the loss of internal entropy is more severe, and their ability to dilute the lipid (at any given thickness) is decreased. The result is a smaller equilibrium thickness. As the size of the alkane increases, the equilibrium thickness asymptotes to the solventless value. Although this assertion cannot be tested with *n*-alkanes (as beyond hexadecane they are solid at room temperature), White (1978) has shown that the absorption of squalene ($C_{30}H_{50}$) into glyceryl monooleate (gmo) bilayers is very small.

White (1977) has measured the absorption of 2,2,4,4,6,8,8-heptamethylnonane and 2,6,11,15-tetramethylhexadecane into gmo bilayers at 30°C. The volumes of these two alkanes are, respectively, similar to and considerably greater than the volume of hexadecane. His results are displayed in Table IV. The extent to which an alkane will lose internal freedom by sitting in the bilayer is related to its length and to the number of bonds which can undergo *trans* and *gauche* isomers (and thereby misalign segments of the alkane with respect to surrounding lipid chains). The extent to which it can distribute evenly in the bilayer is inversely related to its molecular volume. By introducing methyl groups along the alkane chain, the most important effect is to increase substantially the volume of the molecule. A secondary effect is to sterically hinder neighboring C—C bonds. As White explains, there are further complications. The presence of the methyl groups may hinder chain packing, and lower van der Waals attractions. Nevertheless, his results can (tentatively) be explained by the present model. Thus, heptamethylnonane will, presumably, have slightly less internal entropy in the bulk state than nonane and substantially less than hexadecane. Incorporated into a bilayer, its loss of internal entropy will be comparable to nonane and much smaller than hexadecane. It should therefore, thicken the bilayer slightly less than nonane (as it is larger) but substantially more than hexadecane. The same logic leads to the conclusion that tetramethylhexadecane should thicken the bilayer slightly less than hexadecane.

TABLE IV
TRANSFER OF ALKANE FROM BULK RESERVOIR TO GMO BILAYER AT 30°C (WHITE, 1977)

Alkane	Thickness	ΔH (alkane)
	(Å)	(*kcal/mol*)
Octane	≈47	
Decane	48.3	≈0.0
Heptamethylnonane	45.0 ± 0.2	0.37 ± 0.01
Tetradecane	≈44	1.02
Hexadecane	34.0 ± 0.2	4.03 ± 0.10
Tetramethylhexadecane	32.5 ± 0.2	3.2 ± 0.25
Heptadecane	≈30.2	Slightly less than hexadecane
Solventless	≈25	

The results come from figures, tables, and the text of White's paper. Hence the differences in accuracy with which they are quoted.

An increase in the area per lipid has the following three consequences: (*a*) The lipid headgroups, which repel, are on average further apart. This is a favorable change. (For an increase in area of 3 $Å^2$/lipid molecule, the model suggests a headgroup free energy gain of 80 cal/mol of lipid.) (*b*) In the hydrophobic region of the membrane, two effects tend to occur, both of them favorable. As previously stated, the free energy of the lipid chains would be lowered if the area per chain were increased. There is therefore a tendency for the lipid chains to take up any extra space available in the outer regions of the membrane. The alternative is for alkane chains to take up the extra interfacial area. At an area per lipid of 63.3 $Å^2$, the volume fraction of alkane in the outer regions of the membrane is, for all alkanes and at all thicknesses, <0.1. Hence, this alternative will improve distribution of alkane and mixing in the plane of the bilayer. (*c*) An increase in hydrocarbon-water surface results. If we assume a surface free energy equal to that of an aliphatic hydrocarbon-water interface, the free energy cost is 216 cal/mol of lipid for an increase of 3 $Å^2$/lipid molecule; i.e., this effect is strongly unfavorable.

The present model is not sufficiently sophisticated to take account accurately of the interplay between the two effects described in (*b*) above. As should be clear from Figs. 6 through 9, the former predominates. In reality however, as the alkane chain length decreases, the latter should become increasingly important. (This follows from what has been said about internal entropy.) Hence, it is tempting to speculate that, as the length of the alkane decreases, the equilibrium area per lipid should increase. There is experimental evidence that this is so. White (1978) gives values for the area per lipid which show this general trend. Requena et al. (1975) give data which show a monotonic increase in bilayer tension as the length of the saturating alkane decreases, suggesting the same trend. It should be stressed that the proposed increases are of the order of 1–3 $Å^2$/lipid. The formation of chain-water surface is very costly, and will quickly outstrip all other factors. For such increases in area per lipid, the two most important effects which determine the equilibrium thickness (given above), still apply.

Andrews et al. (1970) found a substantial difference in the compressibility of glyceryl monooleate black films of different thicknesses. Thin films (those formed with long alkanes) were very incompressible whereas successively thicker films were more and more compressible. The shape of the curves in Fig. 1 implies the same trend and suggests (as do the experiments) that the effect is large. A more quantitative comparison is not possible. For thin films, the experiments do not produce a large enough change in thickness. For thick films, the model free energies are not known with sufficient accuracy (see Fig. 3).

Comparison with Previous Descriptions of the Near-Solventless Bilayer

As Fettiplace and coworkers (1971) have argued, the lipid chains in a black film containing a very small volume fraction of alkane (for example, egg lecithin and hexadecane) are in a state very similar to their state in a hydrated lamellar phase (with no alkane present). However, the picture presented by these authors (and by Andrews et al., 1970; Brooks et al., 1975; and White, 1977) of the state of the lipid chains in these systems differs significantly from the present model. These authors assume that the lipid chains are coiled down into a nearly close-packed state, and that overlap of the lipid chains in the center of the membrane is small (see for example Fig. 6 *a* in Andrews et al., 1970). It also suggests that those lipid chain conformations which extend beyond the midplane of the bilayer are almost excluded.

In contrast, the present model predicts that ~38% of lipid chains in the solventless DPL membrane extend beyond the midplane of the bilayer. (The presence of a very small volume fraction of alkane will change this number only slightly.) It also suggests that the transition region between lipid chains on the two sides of the membrane is substantially broader (see Fig. 6 in Gruen, 1980). The model does imply a slightly smaller average length for the lipid chains in hexadecane at equilibrium (~13.56 Å) than for the same in butane at equilibrium (~13.85 Å).

Density measurements (Nagle and Wilkinson, 1978), x-ray diffraction experiments (Tardieu et al., 1973) and calorimetric data (Phillips et al., 1969) suggest that the lipid chains in a solventless membrane above T_c are in a state similar in many ways to liquid alkane. The picture implied by the present model seems more consistent with this experimental evidence than previous pictures of the bilayer.

In explaining how hexadecane packs into a gmo bilayer, White (1977) argues that "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."

The present model implies that at equilibrium, a large proportion of the hexadecane molecules in the bilayer span the midplane (see Figs. 7 and 8). This is a consequence of the fact that the model does not impose any handicap on a configuration (either alkane or lipid) which exists in both monolayers of the bilayer. Such a handicap was not felt of sufficient importance given the liquid-like nature of the chains.

Consider bulk liquid hexadecane. If a molecule is to change its conformation, there must be room for it to do so. This may involve many molecules around it. In liquid alkane, the molecules are thought to be rotamerically completely disordered (Flory, 1969), and hence changes in conformation must occur frequently. It is in this light that we believe the interior of the bilayer must be viewed, though admittedly with the proviso that there are surface constraints (e.g., a lateral pressure) which are absent in the bulk. Thus, mixing across the geometric midplane of the bilayer occurs as a consequence of the liquid-like nature of the lipid and alkane chains. It is not necessary to postulate any more correlation of acyl chains in opposing monolayers than for neighboring chains in bulk alkane.

Enthalpy of Transfer from Bulk Alkane to the Bilayer

White (1977) has measured the enthalpy of transfer of various alkanes from a bulk reservoir to the bilayer at equilibrium. His results are summarised in Table IV. Fig. 13 shows the same quantity derived from the theoretical model. As stated in its legend, the curves in Fig. 12 were drawn by eye. There is no reason why the minima of the hexane-hexadecane curves should lie exactly at 29.2 Å (as drawn). Without extra points near 29.2 Å, however, it seems unreasonable to place the minima elsewhere. To generate Fig. 13 we require the

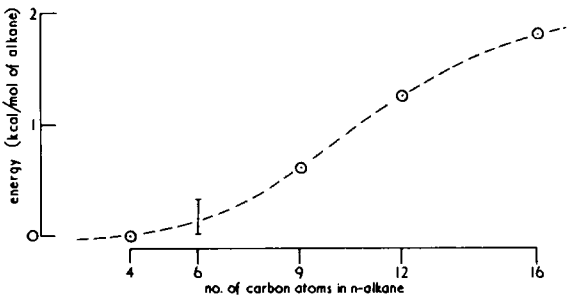

FIGURE 13 Energy of transfering alkane from bulk reservoir to bilayer at equilibrium. The results in this figure were derived by linear interpolation between points in Fig. 12. The following points (from Fig. 12) were used for the interpolation. For butane, the 36.7 and 39.2 Å points. For hexane, the error bar spans the interpolations 31.7–34.2 Å and 34.2–39.2 Å. For nonane, the points 29.2 and 34.2 Å and for dodecane and hexadecane, the points 29.2 and 31.7 Å. The dashed line was drawn by eye.

gradient of each curve at equilibrium. In the cases of dodecane and hexadecane, the exact position of the minima will affect (to a considerable extent) the gradient of the curve at equilibrium. For this reason, the points in Fig. 13 were generated as described in the legend to the figure. It should be noted, however, that these points may be in some error. If the equilibrium area per lipid is, in fact, slightly greater than the solventless value, the curves in Fig. 12 would be shifted upwards. This would further complicate the analysis, especially for the longer chain length alkanes. Nevertheless, the agreement, both in order of magnitude and trend, between White's data and the theoretical model's predictions is good. The comparisons must be made on membranes with similar fractions of alkane present. For example, White's gmo-decane membrane and the theoretical DPL-butane membrane represent membranes with a thickness of twice the extended chain length of the respective lipids and hence maximal uptake of alkane.

The explanation for the shape of the curves in Fig. 12 follows from what has been said previously. The state of the lipid chains between 36.7 Å and 39.2 Å (or between 39.2 Å and 41.7 Å) changes only fractionally as butane is added. The butane is being transferred from a bulk reservoir to a region of the membrane which is almost pure butane. Thus, the energy cost of transfer is minimal. This explanation is similar to that presented by White (1977) for the gmo-decane membrane. For the hexadecane-DPL bilayer, the following occurs. Initially, as the bilayer thickens, the hexadecane molecules must line up essentially parallel to the lipid chains as the volume fraction of lipid throughout is high. The lipid chains straighten (the model suggests that there is a loss of 0.16 *gauche* bonds/chain from the solventless membrane to the hexadecane-DPL membrane at 29.2 Å) as do the hexadecane chains (there is a loss of 0.37 *gauche* bonds/chain from bulk hexadecane to the hexadecane-DPL membrane at 29.2 Å). Also, there are somewhat enhanced van der Waals attractions (between the chains) as the order in the membrane increases. As the membrane thickens further, the volume fraction of lipid falls in the center of the membrane and the hexadecane partitions strongly into this region, at the expense of the outer regions of the membrane. The above changes reverse with the result that, at equilibrium, there is a huge energy cost of transfer of hexadecane. The model suggests that 86% of the energy cost (between thicknesses 29.2 Å and 31.7 Å) is due to

changes in the lipid chains. The hexadecane does not experience smaller cohesive forces than in bulk alkane (as the large value of ΔH might lead one to believe). Rather, as extra hexadecane molecules are added, the environment of the interior of the membrane changes.

For successively longer alkanes at equilibrium, the energy transfer cost should go through a maximum, decrease, and eventually go strongly negative. White's data show a decrease in ΔH for alkanes larger than hexadecane. Simon et al. (1977) measured the uptake of hexane into multilayered vesicles of egg lecithin and dioleoyl lecithin. The volume fraction of hexane in the hydrophobic core of the bilayers was 0.019 for a saturated aqueous phase. Reasons for such a small uptake of hexane have been discussed by Gruen and Haydon (1980 *b*). Briefly, we argued that by absorbing hexane, the bilayers of the vesicle would develop tensions, which would lead to progressively larger pressure exerted on bilayers near the center of the vesicle. This pressure would tend to "squeeze out" hexane. Simon et al. (1977) found that the enthalpy transfer from bulk hexane to dioleoyl lecithin and egg lecithin bilayers is respectively, -1.7 and -2.2 kcal/mol of hexane. The fact that the bilayers of the multilayered structure may be subject to elevated pressure and that they are curved means that any comparison with the present model should be viewed with caution. Nevertheless, it is interesting that the model gives a very similar result. A linear interpolation between the energies of the solventless bilayer and the hexane-bilayer at 29.2 Å (from Fig. 12) leads to an energy transfer cost of -1.7 kcal/mol of hexane.

Further experimental evidence that at very small volume fractions of alkane the alkane chains line up essentially parallel to the lipid chains, and increase the order of the membrane, comes from the x-ray diffraction studies of McIntosh et al. (1980).

White (1977) suggests that the huge ΔH for long alkanes may be a consequence of their spending a large proportion of time in a region of the membrane with a high concentration of CH_3 groups (and hence experiencing smaller van der Waals cohesive forces). The volume fraction of CH_3 groups at the center of the solventless model membrane is 0.5 (see Fig. 7 in Gruen, 1980). This is the same as in bulk liquid hexane and hence the magnitude of this effect can be estimated by examining the energetics of mixing long alkanes and hexane. Experimentally, ΔH for mixing equimolar quantities of hexane and hexadecane at 40°C is ~33 cal/mol of hexadecane (Holleman, 1965). For a mixture with the smallest mole fraction of hexadecane measured by Holleman (0.1174), $\Delta H = 46$ cal/mol of hexadecane. The present model does not take account of this effect (it would predict a zero heat of mixing of two alkanes); nevertheless these values are two orders of magnitude smaller than White's value for partitioning into a gmo bilayer.

Free Energy of Thinning of Membranes

In a series of papers, Requena, Haydon, and others (Requena et al., 1975; Requena and Haydon, 1975; Brooks et al., 1975) compare the experimental free energy of thinning of membranes (from infinite thickness to their black state) with the van der Waals free energy calculated from Lifshitz theory. In the first and second papers of the series the authors assume, for calculation of the van der Waals free energy, that the chain region may be regarded as an isotropic layer of hydrocarbon. They find for the gmo-decane system that the Hamaker constant required for the fit agrees "remarkably well with the results of calculations from Lifshitz theory." Thinner membranes, formed in both glycerol and aqueous solutions,

require (on average) a larger Hamaker constant to fit the experimental free energy of thinning. The explanation for this observation presented in the final paper of the series (Brooks et al., 1975) relies on assuming, for the thinner films, that "the solvent (alkane) and lipid chains are particularly well segregated into layers." The present model suggests that, for the volume fractions considered by Brooks et al., the segregation is less pronounced than they assumed.

For both thick, e.g., gmo-decane, and thin, e.g., gmo-hexadecane, films, Requena et al. (1975) assumed that the free energy (excluding van der Waals attraction across the film; i.e., that given by Eq. I34) is constant for all thicknesses greater than the equilibrium thickness plus 5 Å (see Fig. 7 of Requena et al.). For progressively smaller thicknesses, they assumed that the free energy increased monotonically. As should be clear from Figs. 1 and 3, the model gives this result for the thickest films; but for progressively thinner films, there is a minimum of increasing depth in the curve. (The depth of the minimum refers to the difference in free energy from a very thick membrane, say 100 Å, to the equilibrium black membrane.) In comparing the experimental free energy of film thinning with its theoretical estimation, this minimum must be taken into account. The present model therefore predicts for progressively thinner membranes an increasing discrepancy between the experimental free energy of thinning and the Lifshitz van der Waals free energy (in the direction found by Requena et al., 1975). Reasons for this minimum in the free energy vs. thickness curve were presented above.

The thinnest membrane examined by Requena et al. (1975) (monolinolein and *n*-hexadecane) has an estimated volume fraction of 0.21 hexadecane present. Assuming the experimental Hamaker constant from the gmo-decane system (3.92×10^{-21} J), the van der Waals free energy of thinning for the monolinolein membrane is 11.4 $pJmm^{-2}$, while the experimental free energy of thinning is 42.11 ± 1.12 $pJmm^{-2}$.

The DPL-nonane system has ~0.12 vol fraction of nonane at equilibrium. This membrane is therefore the most closely comparable of the theoretical membranes. The depth of the free energy minimum (as measured by the difference in the free energies of the 31.7 Å and 39.2 Å membranes) is 33.8 cal/mol of lipid, or assuming 63.3 $Å^2$/lipid, 740 $pJmm^{-2}$. The depth of the minima in Fig. 1, and also the required Hamaker constants in Requena et al. (1975), increase sharply for thinner membranes. Thus, some of the difference between 740 and $42.11 - 11.4 = 30.7$ may be explained by the fact that the theoretical system has a substantially smaller volume fraction of alkane than the experimental one. More sceptically, it is unrealistic to expect quantitative agreement, given the difference in systems and the nature of the model.

Finally, it is possible to make a qualitative statement on the effect of cholesterol on the uptake of alkanes. The presence of 33-mol percent cholesterol straightens the chains of egg lecithin considerably (Stockton and Smith, 1976). The order throughout the membrane increases. An absorbed alkane will lose more internal entropy and will not be able to distribute as evenly as in a cholesterol-free membrane. Thus, the cut-off in absorption should move towards smaller alkanes. This was observed by Haydon et al. (1977).

David Gruen wishes to thank Shell Australia Limited for a Postgraduate Scholarship and Unilever Limited, Port Sunlight, England for a maintenance grant.

Received for publication 24 April 1980 and in revised form 25 September 1980.

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