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

I. DEVELOPMENT OF THE MODEL

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ABSTRACT A statistical mechanical model of a bilayer of dipalmitoyl-3-*sn*-phosphatidylcholine molecules in equilibrium with an aqueous phase saturated with an *n*-alkane is presented. A mean-field approach developed in previous work on a solventless bilayer (Gruen, *Biochim. Biophys. Acta.* **595**:161–183, 1980) is extended to allow alkane chains to exist in the hydrophobic core of the membrane. As the alkane chains are chemically similar to the lipid chains, much of the analysis follows directly from the solventless model. Novel features of the present model are the inclusion of (*a*) a term which models the free energy cost of creating space for alkane conformations, (*b*) a term which constrains the chains to pack evenly in the hydrophobic region of the membrane, and (*c*) a term which estimates the free energy of mixing of the lipid and alkane molecules in the plane of the bilayer. On uptake of alkane, the dimensions of the bilayer increase. Allowance is made for an increase in thickness and/or an increase in area per lipid. A thermodynamic framework is established which allows evaluation of the free energy of a bilayer of arbitrary dimensions with a view to predicting the equilibrium structure.

INTRODUCTION

Black lipid films may be formed by dissolving or dispersing a lipid in an *n*-alkane and extending the resulting solution across a hole in an oil-wetted support. The initially thick layer of lipid solution then drains under capillary and van der Waals forces (as in aqueous soap film formation) giving ultimately a bilayer in equilibrium with an aqueous solution saturated (or nearly so) with the *n*-alkane. The amount of *n*-alkane in the lipid bilayer may be estimated by various means which have been fully discussed previously (Fettiplace et al., 1975).

Common lipids used to form black films are the phospholipids (particularly egg lecithin and dioleyl lecithin) and glyceryl monooleate. Although pure glyceryl monooleate does not form bilayers when dissolved in water (as the phospholipids do), it does form black films when dissolved in *n*-alkanes which have structural features closely resembling those of phospholipid films. In neither system does the chain length, or the extent of the absorption of the alkane, have an appreciable effect on the area per molecule of the lipids (Fettiplace et al., 1975). Both factors, on the other hand, markedly affect the thickness of the hydrocarbon region of the membrane. The maximum thickening and absorption occurs for the shorter homologues. As

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the chain length increases, the thickening and the absorption decline, reaching nearly zero values for the large (branched chain) molecule squalene (White, 1978, and J. R. Elliott, personal communication). In the case of the phospholipids, the minimum observed thickness corresponds approximately to the thickness found by x-ray diffraction from multilammellar droplets above the phase transition (i.e., in the L_α phase). The maximum value of the thickness corresponds approximately to the length of two fully extended chains of the lipid molecules.

The motivation for the present work was the desire to understand this alkane absorption cut-off effect. The approach chosen was to modify and extend an existing model of a bilayer of dipalmitoyl-3-*sn*-phosphatidylcholine (DPL) molecules (Gruen, 1980 *a*). This model is based on a mean field approach in which a single chain of a DPL molecule is embedded in a mean field which models the average behavior of other chains. Using this approach, it is possible to take account of the important factors which determine the lipids' behavior, and hence to develop a picture which agrees in many ways with experimental results.

Lipid chains in a solventless bilayer membrane are packed closely to reduce chain-water contact. They are preferentially oriented along the normal to the plane of the bilayer (see Gruen, 1980 *a*). Their freedom to orient parallel to the membrane surface is restricted because of the presence of neighboring chains. This effect (which is a surface effect and is absent in bulk alkane) results in important differences between these chains and alkane chains in a bulk phase. The lipid chains exert a lateral pressure in the plane of the bilayer. (This is a consequence of the fact that the free energy of the chains would be lowered if the area per chain were increased.) Also, because they are more ordered than in bulk alkane, the lipid chains should have slightly stronger van der Waals attractions.

In a membrane with a very small amount of alkane present, for example egg yolk phosphatidylcholine and *n*-hexadecane (see Fettiplace et al., 1975), the volume fraction of lipid is high throughout the membrane. Hence, the alkane chains present will be subject to considerable lateral pressure and will pack with similar properties to the surrounding lipid chains.

Consider, however, the center of a membrane containing a substantial mole fraction of alkane, for example glyceryl monooleate and decane at equilibrium (Fettiplace et al., 1971). The only lipid chains present are those which are fully extended. Only a small fraction of the lipid chains are in this state. This statement can be supported by many arguments. In particular: (*a*) a calculation of the osmotic pressure generated by the presence of the overlapping oleate chains in the center of the bilayer (Andrews, 1970) suggests that only a few percent are required to stabilize the film, and (*b*) measurement of the enthalpy of the chain melting transition in a solventless bilayer suggests, on average, more than one-quarter of the bonds are in a *gauche* conformation above the phase transition (Nagle and Wilkinson, 1978). The presence of a substantial mole fraction of liquid *n*-alkane will not significantly alter this picture.

Thus, at the center of such a membrane, the alkane chains sit in an environment approaching that of bulk liquid alkane. The lateral pressure acting upon them must be much smaller than for chains nearer the polar interfaces and they will be subject to van der Waals forces similar in strength to those in liquid alkane.

The present model has been designed so that when a chain (either lipid or alkane) sits in a relatively ordered environment (i.e., where surrounding chains are preferentially oriented

perpendicular to the membrane surface) it is subject to considerable lateral pressure and enhanced van der Waals forces. As the environment becomes less ordered, the chain experiences smaller lateral pressure and less van der Waals attraction. For a completely isotropic environment, the chain is subject to forces identical to those in bulk liquid alkane.

The general approach is to assume, in turn, several possibilities for the dimensions of the bilayer, evaluate the free energy of each, and look for a minimum. It is assumed that the density of chain packing is constant as the bilayer takes up alkane. As for the lipid chains, the volume of each alkane molecule is evaluated by assuming a CH_2 group takes up 27 Å^3 and a CH_3 group, 54 Å^3 (Reiss-Husson and Luzzati, 1964). Thus, for given bilayer thickness and area per lipid molecule, the volume fraction of alkane in the hydrophobic core can be evaluated. For given dimensions and a particular alkane, an order profile is guessed for all layers in the bilayer. The lipid conformations are generated and assigned statistical weights. The alkane conformations are generated and also assigned statistical weights. The order in any layer is then evaluated as the volume weighted mean order of all chain segments sitting in that layer. As with the solventless bilayer model, only one chain can be considered at any time. This chain is embedded in a mean field which models the average behavior of other chains.

Throughout this paper, and the next, much of the notation developed in Gruen (1980 *a*) will be extended to deal with alkane conformations. To avoid confusion, the subscripts *l* for lipid and *a* for alkane will precede the symbols previously defined for the solventless model. Thus, for example, ${}_aA_l^i$ is the interfacial area of alkane configuration *i*.

The chain segments in the *n*-alkane are numbered 1, 2, . . . , *n*. The order parameter n_j (Gruen, 1980 *a*) will be used for both alkane and lipid segments and is defined as $n_j = 3/2 \cos^2 \beta_j - 1/2$, where β_j is the angle between the bilayer normal and the direction of the j^{th} chain segment which, in the case of CH_2 groups, is defined as the normal of the plane spanned by the two C—H bond vectors. For CH_3 groups, this direction is defined by the terminal C—C bond vector.

As it will be necessary to refer to several of the equations in Gruen (1980 *a*), a shorthand way of doing so is appropriate. Thus, equation (*Gn*) will refer to equation (*n*) in Gruen (1980 *a*). A preliminary report of this work has been published (Gruen and Haydon, 1980).

THE ALKANE-SATURATED MODEL

Generation of Chain Conformations

Unlike the lipid chains, which are anchored to the polar interface, the *n*-alkanes can take up conformations throughout the chain region of the bilayer. The "first" CH_2 segment of the *n*-alkane (C-2, the CH_2 attached to one of the terminal methyl groups) is fixed at layers 1, 2, . . . , *l* in succession and all possible conformations generated. (Layer *l* is at the geometric center of the bilayer.) The membrane is assumed to be symmetrical about its midplane. Hence, it is only necessary to generate configurations in which the first CH_2 of the chain is on one side of the bilayer. Perfect tetrahedral symmetry of the carbon bond angles is assumed and g^+ and g^- sequences are excluded. None of the accepted configurations have segments in the polar region of the membrane (layers 0, −1, −2 . . .). (In the solventless model, all segments except C-2 were excluded from layers 0, −1, −2 . . . C-2 was permitted to lie in layer 0 to allow three initial orientations of the lipid chain.)

If C-2 has order 1 (i.e. $\eta_2 = 1$), then (using the assumption of perfect tetrahedral symmetry) there are a maximum of seven possible "orientations" for the CH_2 groups further along the chain. (If a CH_2 group is in orientation k, then after any rotation around the normal to the bilayer, the CH_2 group is still deemed to be in orientation k. Thus, for example, all CH_2 groups in an all-*trans* chain lying perpendicular to the membrane surface are in the same orientation.) These seven orientations are conveniently distinguished by assigning each orientation an ordered pair (I, K). Defining (I_j, K_j) as the ordered pair for the j^{th} segment, and D_j as the distance (in ångströms) between layer 0 and segment j, the ordered pairs have the following properties: (*a*) I_j takes the values 2, 1, 0, -1, and -2 corresponding respectively to the values 0°, 60°, 90°, 120°, and 180° for β_j. Hence, $\eta_j = 3/8I_j^2 - 1/2$. (*b*) $D_j = D_{j-1} + 1.25\,K_j$.

I_j is a measure of the direction of the j^{th} segment; K_j measures the incremental change in distance from the bilayer surface of the j^{th} segment. The seven orientations are denoted: (2,1), (1,1), (1,0), (0,0), (−1,0), (−1,−1), and (−2,−1). In the all-*trans* chain lying perpendicular to the membrane surface, each segment has a (2,1) orientation.

At each layer in which the first CH_2 in the chain is fixed, it is oriented, successively, in the seven distinct orientations described above. Thus, all subsequent segments have one of these seven orientations. One further problem remains: What relative weights should be assigned to the seven initial orientations? We can define the partition function for alkane in which the first CH_2 group is fixed in layer k (Z_k) as

$$Z_k = a_1[Z_{(2,1)} + Z_{(-2,-1)}] + a_2[Z_{(1,1)} + Z_{(-1,-1)}] + a_3[Z_{(1,0)} + Z_{(-1,0)}] + a_4 Z_{(0,0)}. \quad (1)$$

$Z_{(I,K)} = \Sigma_i \exp[-E^i/kT]$, where i runs over all chain conformations for which the first CH_2 group in the chain is fixed in layer k with orientation (I, K). The orientational partition functions have been grouped as shown for symmetry reasons. The problem is to evaluate a_1, a_2, and a_3. (We require only the relative weights. Hence, we can set one of the coefficients to unity; arbitrarily we choose $a_4 = 1$).

One of the useful properties of η is that, for randomly oriented chains, $\langle \eta \rangle = 0$. This state corresponds to bulk liquid hydrocarbon. To anticipate somewhat, the model to be described in subsequent sections has the following property: as $\langle \eta \rangle \to 0$, the energetics of the system approaches those of liquid hydrocarbon. It is important for the generated alkane configurations to mirror this property. When only the internal energy determines the probability of each conformation, we require that $\langle \Sigma_j \eta_j \rangle = 0$, where j runs over the n segments of the alkyl chain ($n - 2$ CH_2 segments and two CH_3 segments). Applying the condition, $\langle \eta_j \rangle = 0, j = 1, \ldots, 4$ to a molecule with four CH_2 groups leads to four simultaneous equations with three unknowns. An approximate solution is $a_1 = 0.508$, $a_2 = a_3 = 1.03$. To test the validity of this approach, all possible conformations of butane, hexane, and nonane were evaluated. Applying the above weightings, and using internal energies to evaluate statistical weights gives $\langle \Sigma_{j-1}^{n} \eta_j \rangle / n = 0.0006$ ($n = 4$), 0.0004 ($n = 6$), 0.0003 ($n = 9$). This was regarded as satisfactory and the above weights were applied for all alkanes, at all layers.

To evaluate the equilibrium properties of the solventless bilayer requires the running of a single computer program until the self-consistent order parameter profile across the bilayer is determined. To evaluate the equilibrium properties of an alkane saturated bilayer, requires

that many different programs be run. (It is necessary to run programs for many different membrane thicknesses and areas per lipid.)

As a result, in the case of the solventless membrane, it is possible to run through all 117,000 allowed conformations of the lipid chain. This takes an IBM 370/165 computer about ~2 min of central processor time. For the alkane saturated bilayer, however, it is possible to take only a small proportion of the lipid chain conformations. Depending on the length of the alkane being considered, it is possible to take all the conformations (for butane, hexane, and nonane) or a small fraction (for dodecane and hexadecane).

In generating the sample of conformations, it is not possible to use the "importance sampling" method introduced by Metropolis et al. (1953), as the energy of a conformation is not known when it is being generated. The self-consistent order parameter profile across the bilayer is needed to evaluate this energy. However, by assigning an approximate order profile it is possible to generate approximate statistical weights. Configurations are assigned to one of three categories on the basis of their approximate statistical weights (category one for the most likely configurations; category three for the least likely). The members of each category are then given a fixed probability of being in the sample. The most likely configurations (those in category 1) are given a high probability of acceptance; the least likely configurations are given a low probability of acceptance.

Once the sample of configurations has been generated, it is stored (on magnetic tape) and used in all subsequent computer runs. Each sample (lipid, dodecane, and hexadecane) contained ~10,000 chain conformations. We define R_K as the ratio (total number of configurations in category K)/(number of configurations in the sample from category K). (R_K can be evaluated after generation of the sample. $R_1 < R_2 < R_3$.) The statistical weight of conformation i (w^i) is then defined as

$$w_i = R_K^i \cdot \exp\left[-E^i/kT\right], \tag{2}$$

where R_K^i is the value of R_K for the i^{th} configuration (i.e., configuration i belongs to category K). E^i is the energy of configuration i. (If the configuration is an alkane configuration, the factor a_i, as defined in Eq. 1, would also appear in Eq. 2.) In this way, unbiased estimates of all thermodynamic properties can be generated. Further details of the generation of chain conformations is given elsewhere (Gruen, 1980 *b*).

To check the accuracy of the above procedure, computer runs were made in which the internal energy of the chains determined their statistical weight. In this case, it is possible to calculate theoretically the value of the partition function for any given layer, and $\langle E_{\text{int}} \rangle$ for the molecule. The calculations are similar to those in Flory (1969) chapter three and are also given in Gruen (1980 *b*). The calculations assume that none of the configurations has been excluded by entering the polar region of the membrane. Hence, comparison is only meaningful for layers far from the polar interface. Table I gives details of the comparison. The computer value for $\langle E_{\text{int}} \rangle$ was evaluated for all configurations whose first CH_2 group was fixed at the geometrical center of the bilayer. Layers l–3, . . . , l are, respectively, three, two, one, and zero layers from the center. As expected from probability theory, the small sample of configurations seems to be giving an accurate picture of the behavior of all configurations.

TABLE I
COMPARISON OF THEORETICAL AND COMPUTER GENERATED PARAMETERS

Molecule	Theoretical calculation		Deviation of sample value from theoretical value				
	$\langle E_{int} \rangle$	Partition function	$\langle E_{int} \rangle$	Partition function			
				$l-3$	$l-2$	$l-1$	l
	(*cal/mol*)		(%)	(%)	(%)	(%)	(%)
Dodecane	1,669.6	11,828.1	−1.6	−3.3	0.4	2.3	1.5
Hexadecane	2,390.3	130,388.	1.3	2.1	1.5	2.0	−1.1

Checks were also made of the butane, hexane, and nonane configurations. Exact agreement between the computer runs and the theoretical calculations was obtained in all cases.

A comparison between theoretical and computer generated results is not possible for the lipid chain conformations (because the theoretical calculation includes conformations which cross into the polar region of the membrane). It is, however, possible to compare order parameter profiles generated from all the conformations with those from the statistical sample. We wish the latter to be as close as possible to the former. A slight improvement was achieved by setting α to 7.167×10^6 cal Å^4/mol lipid (rather than 7.092×10^6 cal Å^4/mol lipid) for the sample of conformations. A comparison of order profiles is shown in Fig. 1. The agreement is excellent, and suggests that the sample is giving an accurate picture of the behavior of all chain conformations. The same sample of lipid conformations was used for all thicknesses, areas per lipid, and for each alkane present. The value 7.167×10^6 cal Å^4/mol lipid for α was used throughout. This corresponds, for the solventless membrane, to a chain pressure $\pi_c = 27.1$ dyn/cm and a headgroup (HG) pressure $\pi_{HG} = 22.9$ dyn/cm.

Incorporating Alkane Configurations in the Bilayer

The evaluation of the energy, ${}_aE^i$, of the i^{th} alkane chain conformation is in several ways identical to the corresponding evaluation for lipid conformations. The internal energy (energy

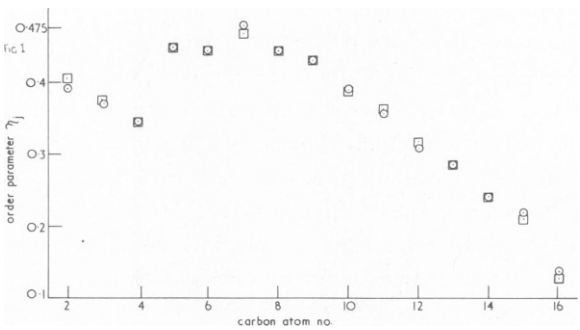

FIGURE 1 Comparison of order parameters for lipid chain conformations. (□) all conformations with $\alpha = 7.09 \times 10^6$ cal.Å^4/mol. (○) the sample of conformations with $\alpha = 7.17 \times 10^6$ cal.Å^4/mol.

of *gauche* kinking) (${}_aE^i_{\text{int}}$) is 500 n_g cal/mol, where n_g is the number of *gauche* kinks in the alkane chain. Van der Waals energy (E_{disp}) is estimated as

$$E_{\text{disp}} = -V \sum_{j=1}^{n} \eta^k \eta_j^k |\eta^k \eta_j^k|^{1/2}, \tag{3}$$

where $V = 710$ cal/mol, η_j^k is the order of segment j which happens to lie in layer k (and thus interacts with the mean field in layer k), and η^k is the volume weighted order of all segments in layer k. As will be seen, not only η_j^k, but also η^k may be negative; hence the difference between Eq. 3 and Eqs. G10, G11. For the alkane-containing bilayer, Eq. 3 is used for both lipid and alkane conformations. Eq. 3 implies that, in an environment with negative order (where, on average, there is a tendency for the chains to lie parallel to the membrane surface), a chain which lies parallel to the membrane surface will experience slightly stronger van der Waals attractions than one which lies perpendicular. This is a logical extension of the original idea that the van der Waals attractions of lipid chains in a solventless bilayer are enhanced because the chains are somewhat aligned.

The interfacial area of an alkane configuration i is defined in a similar way to that for a lipid chain configuration. Alkane configurations are not allowed to sit in layer 0. Hence, ${}_aN^i_{\text{SUR}}$ is defined as the number of chain segments of configuration i lying in layers 1 and 2 of the bilayer. (Because of their larger size, CH_3 groups were counted as "two chain segments." Thus, if three CH_2 groups and a CH_3 group lie in layers 1 and 2, ${}_aN_{\text{SUR}} = 5$.) ${}_aA^i_I$ is defined as

$${}_aA^i_I = {}_aN^i_{\text{SUR}} \cdot C^*_{\text{SUR}}. \tag{4}$$

C^*_{SUR} is a constant, independent of chain configuration, fixed so that the thermodynamic average interfacial area agrees with the chosen value. The interfacial area of lipid chain configuration i (${}_lA^i_I$) is defined as

$${}_lA^i_I = {}_lN^i_{\text{SUR}} \cdot C^*_{\text{SUR}} + {}_lA_{\text{AVE},I}. \tag{5}$$

${}_lA_{\text{AVE},I}$ is the interfacial area of the second "average" lipid chain:

$${}_lA_{\text{AVE},I} = \langle {}_lN^i_{\text{SUR}} \cdot C^*_{\text{SUR}} \rangle. \tag{6}$$

Defining n_a as the number of moles of alkane in the bilayer per mole of lipid, and $\mathcal{A}$ as the total surface area of a bilayer with m lipid molecules (and mn_a alkane molecules), C^*_{SUR} is fixed so that

$$\langle {}_lA^i_I \rangle + n_a \langle {}_aA^i_I \rangle = \mathcal{A}/m. \tag{7}$$

C^*_{SUR} must be reevaluated whenever the dimensions of the bilayer or the alkane present are changed. In all the systems considered, however, $C^*_{\text{SUR}} = 9.60 \pm 0.01$ Å^2.

For lipid configuration i, the headgroup free energy is

$${}_lF^i_{\text{HG}} = \alpha / ({}_lA^i_I + {}_aA_{\text{AVE},I} n_a)^2, \tag{8}$$

where ${}_aA_{\text{AVE},I}$ is the average interfacial area of the alkane chains in the bilayer. At equilibrium, ${}_aA_{\text{AVE},I}$ is very small, a consequence of the hydrophobic effect.

When an alkane conformation i has a nonzero interfacial area, it will increase the distance between the headgroups of surrounding lipids. (Its effect on the environment is identical to the effect produced by extra segments of a lipid chain sitting in the surface layers.) It should therefore be included in the evaluation of the statistical weight of this conformation. Thus,

$$ {}_aF^i_{HG} = \alpha[(2_lA_{AVE.l} + {}_aA^i_l)^{-2} - (2_lA_{AVE.l})^{-2}]. \tag{9} $$

${}_lF^i_{HG} > 0$, but ${}_aF^i_{HG} \leq 0$. This is a simple consequence of the fact that the lipid has a headgroup, while the alkane does not. The free energy costs of creating oil-water surface for lipid and alkane configurations are, respectively, $\gamma({}_lA^i_l - A_{HG})$ and $\gamma_a A^i_l$. As for the solventless model, $\gamma = 50$ dyn/cm.

π_{HG} is defined in an analogous way to Eq. G16. Thus,

$$ \pi_{HG} = \alpha\langle 1/({}_lA^i_l + {}_aA_{AVE.l}n_a)^3\rangle \tag{10} $$

The definition of alkane chain area (${}_aA^i_c$) is an extension of the definition, given in Eqs. G12 and G20, of lipid chain area (${}_lA^i_c$). We define ${}_aA^i$ as

$$ {}_aA^i = {}_aL_o \cdot A_o/{}_aL^i, \tag{11} $$

where ${}_aL_o$ is the fully extended length of the alkane chain (equal to $1.25\ n + 1.9$ Å; n is the number of C atoms in the alkane), A_o is the cross-sectional area of the fully extended chain (20.4 Å^2), and ${}_aL^i$ is the length (perpendicular to the membrane surface) of conformation i. ${}_aA^i$ is partitioned among the chain segments. $({}_aA^i - 20.4)/m$ Å^2 is allotted to each of the m segments which lie parallel to the membrane surface. We define segment p as the alkane segment nearest the membrane surface. ${}_aA_p$ takes the value $20.4 + [{}_aA^i - 20.4]/m$ Å^2 if segment p lies parallel to the membrane surface; ${}_aA_p = 20.4$ Å^2 if it does not. (Clearly, both p and m depend on the configuration.) All other segments are given zero area. ${}_aA^i_c$ is then given as

$$ {}_aA^i_c = \frac{{}_aL_o}{{}_lL_o} C^*_l \sum_{j=1}^{n} {}_aA_j\,\eta_{jh}, \tag{12} $$

where ${}_aA_j$ is the j^{th} segment's area, η_{jk} is the average order on the plane (plane k) on which the j^{th} segment is sitting, ${}_lL_o$ is the fully extended length of the lipid chain (19.7 Å), and C^*_l is a constant.

${}_lA^i_c$ is defined similarly to Eq. G20:

$$ {}_lA^i_c = C^*_l \sum_{j=1}^{n} {}_lA_j\eta_{jk}. \tag{13} $$

The denominator in Eq. G20 is constant for different chain conformations. In Eq. 13 it has been absorbed in C^*_l. C^*_l plays a role for chain areas identical to the role of C^*_{SUR} for surface areas. Thus,

$$ 2.\ \langle {}_lA^i_c\rangle + n_a\langle {}_aA^i_c\rangle = \mathcal{A}/m. \tag{14} $$

Eq. 12 is analogous to Eq. G20 which defined the lipid chain area. The presence of the $({}_aL_o/{}_lL_o)$ term in Eq. 12 requires discussion. Consider the situation depicted in Fig. 2. The area of the lipid chain as defined by Eq. G12 is 20.4 Å^2. If the corresponding formula (Eq. 11)

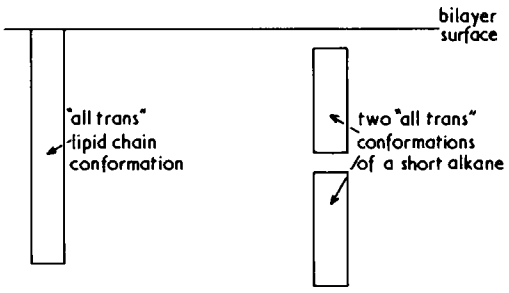

FIGURE 2 Comparison of all-*trans* conformations. The length of the lipid chain is twice the length of the alkane chain.

is applied to the alkane chain conformations, both have an area of 20.4 Å^2. However, as is clear from the diagram, the alkane chains together take up no more area than the lipid chain. In a membrane with constant order throughout the chain region, Eq. 12 gives the same area to the two alkane conformations together as Eq. 13 does to the lipid chain conformation.

Eq. 12 does have a disadvantage. It implies that, in a membrane with constant order, the area of an alkane conformation lying parallel to the membrane surface increases as the square of the length of the alkane. This is clearly unphysical. Nevertheless: (*a*) In regions of high order, conformations which lie parallel to the membrane surface are very unlikely. Errors in the evaluation of the energy of such conformations make very little difference to the average properties of the system. It is much more important that the formula gives physically reasonable areas to more likely (more aligned) conformations, which it does. (*b*) In regions of small order, Eq. 12 gives small chain areas for any chain conformation. As order approaches zero (and the environment approaches bulk alkane), the chain area for all conformations also approaches zero.

Eq. 12 was chosen because it is a straightforward extension of Eq. G20, and it embodies the important features of a definition of ${}_aA_c^i$.

We also require an expression for the chain pressure acting on a bilayer with given dimensions. Two points should be considered: (*a*) We assume that on uptake of alkane, the area per lipid either stays the same (at 63.3 Å^2/lipid) or increases. (It is conceivable, though in my view very unlikely, that the area per lipid decreases on uptake of alkane. This possibility has not been investigated.) If it remains the same, the requirement of a nonnegative surface tension, leads to the condition:

$$\pi_c \leq 27.1 \text{ dyn/cm}. \tag{15}$$

Any increase of the area per lipid should reduce the restrictions on the chains' motion, and hence Eq. 15 should always hold. (*b*) Chain pressure is intimately linked to the presence of order in the hydrophobic region. As the membrane thickens, the order in the center falls to zero (as the environment approaches liquid alkane). The contribution which the central layers make to π_c should also fall to zero. Alternately, as the area per lipid increases, both order and π_c will decrease. A simple (but clearly crude) definition for π_c is, therefore,

$$\pi_c = \min (27.1, K \sum_{x=0}^{l} \eta^x) \text{ dyn/cm}. \tag{16}$$

η^x is the order in the x^{th} layer of the bilayer. The sum is over all layers on one side of the bilayer weighted in proportion to their occupancy. The central layer (layer l) is included in the sum with 1/2 weighting. K is a constant defined so that, for the solventless bilayer, $K \Sigma_{x=0}^{l} \eta^x = 27.1$ dyn/cm. (For the solventless bilayer, $\langle \eta \rangle = 0.3378$ and there are 10.92 "layers"; hence $K = 27.1/(0.3378 \times 10.92 = 7.35$ dyn/cm. This value for K is used throughout the alkane-saturated model.)

Creating Space for the Alkane

Consider a membrane with a hydrophobic core of thickness 40 Å and an area per lipid molecule of 63.3 Å^2 (the same as the solventless membrane). For the sake of the following argument we assume that the statistical weight of all the lipid chain conformations is the same as it was in the solventless membrane. Then, in the outer 8 Å on either side of the membrane, all the available volume is filled by lipid chains. Layers nearer the bilayer center have progressively less of their available volume filled by lipid chains. Only those lipid chains which are fully extended have segments at the center of the membrane. If we wish to evaluate the free energy of a membrane with the above dimensions in an aqueous solution saturated with, say, *n*-hexane, it is necessary to evaluate the statistical weight of all possible hexane conformations in the hydrophobic core. For a hexane molecule to exist in the outer 8 Å of the bilayer it is necessary to straighten lipid chains. Further, once lipid chains have been somewhat straightened, there is only a limited proportion of space available to alkane. Contrast this with the environment an alkane molecule sees at the center of the membrane. Here, most of the space is not filled by lipid, and hence it is not necessary to straighten chains to exist in this region.

There are both adverse and favorable consequences of straightening lipid chains in the outer regions of the membrane. As already discussed in the Introduction, the free energy of the lipid chains would be lowered if the area per chain were increased. Straightening these chains more than in the solventless membrane will, therefore, have a free energy cost associated with it. The favorable consequences are twofold. The alkane chains are able to take up conformations which would otherwise not be available to them and the alkane and lipid chains can mix in the plane of the bilayer. In the present mean field model, it is necessary to introduce terms which (*a*) "inform" the alkane conformations of the free energy cost of straightening the lipid chains, (*b*) "inform" the lipid conformations of the alkane's presence and constrain the chains to pack evenly across the membrane, and (*c*) take account of the mixing of lipid and alkane chains. A description of these terms follows.

Free Energy Cost of Absorbing Alkane

We introduce an effective free energy cost of absorbing alkane configuration i (f^i). Let ${}_lV^i_{jk}$ be the volume fraction of lipid in the layer (layer k) in which the j^{th} segment of alkane conformation i sits. Also, let

$$p^i_j = (1 - {}_lV^i_{jk}) + b \cdot {}_lV^i_{jk}, \tag{17}$$

where b is a constant, related to the compressibility of the lipid chains and the molar volume of the alkane. Its value is the subject of Appendix A. Then,

$$f^i = -kT \ln \left[\prod_{j=1}^{n} (p_j^i)^{c/n+2} \right], \tag{18}$$

where $c = 1$ for CH_2 groups ($j = 2, \ldots, n-1$) and $c = 2$ for CH_3 groups ($j = 1$ and $j = n$).

As stated in Appendix A, the constant b represents the effective proportion of the space filled by lipid which is available to alkane. Thus, p_j^i is the effective proportion of space available to segment j. The term inside the square bracket of Eq. 18 is the volume weighted geometric mean of p_j^i over all segments of the chain. It is in this sense that f^i is an effective free energy cost of absorbing alkane configuration i.

Consider two hypothetical regions in the membrane, the first almost full of lipid chains, the second free of lipid. Further consider two different alkanes present, hexane or hexadecane. If either alkane were in the second region, we should require $f^i = 0$ (i.e., there is no free energy cost of absorbing into this region), and Eq. 18 gives this result. If eight CH_2 groups of the hexadecane molecule are in region 1 (and the rest in region 2), the constraints which must be met to accommodate this configuration are exactly the same as for a whole hexane molecule in region 1. Eq. 18 (coupled with Eq. A2) gives almost the same value for f^i for these two configurations. (Only when ${}_lV_{jk}^i = 1$ will the value of f^i in the two situations be exactly the same.) The reason for the form of Eq. A2 is so that the model is internally consistent in this sense.

The derivation given in Appendix A is both crude and in several respects arbitrary. The analysis which led to Eq. A1 could just as well have been repeated for each alkane considered. This approach was not chosen, because the model would not then be consistent in the above sense. Alternatively, the analysis could have been done for an alkane other than hexane. This would have given a different number in Eq. A2. The result quoted should be of the right order of magnitude. Further, because of its internal consistency, hopefully the model will show up differences in behavior of the n-alkanes. Thus, we can hope to predict an absorption cut-off effect although we cannot have confidence that it will occur at the experimentally observed n-alkane chain length.

Even Distribution of Chains and Mixing

It is not possible in a mean-field model of the present type to obtain a sufficiently accurate measure of the relative probabilities of the chain conformations of both alkane and lipid at different thicknesses and areas per lipid. As a result, the chains do not always pack evenly into the available space. It is important, if comparisons of free energy are to be made, that the chains are constrained to fill the available space as evenly as possible. If there are $l + 1$ layers in the half bilayer (layer l is the central layer) we define a set of free energies u_x ($x = 0, \ldots, l$). For lipid configuration i, we evaluate

$${}_lu^i = \sum_j u_{jk}, \tag{19}$$

where the sum is over all segments in the chain (with double weighting for the CH_3 group), and u_{jk} takes the value u_k (the j^{th} chain segment lies on layer k). For alkane configuration i,

$$_au^i = \sum_j u_{jk}, \tag{20}$$

where, once again there is double weighting for CH_3 groups. $_lu^i$ is added to $_lE^i$, and $_au^i$ is added to $_aE^i$. Thus, if a particular layer (layer y) has too many chains in it, u_y is increased. This has the effect of increasing the "energy" of all chains with segments in layer y, and this decreases the layer's occupancy. On successive computer iterations, the profile u_x is varied until the chains are distributed as required (see Gruen and Haydon, 1981).

All terms in previous equations are involved with evaluating the energy (and free energy) contributions for configuration i in an environment which is determined by the configuration's depth in the bilayer. If there was only one possible arrangement of the molecules' centers of mass, the analysis thus far would be sufficient. Mixing in the plane of the bilayer should be important if two conditions are satisfied. First, if the energy of interchanging alkane and lipid molecules in this plane is small and, second, if the mobility of the molecules is sufficiently large that such interchange can occur. The former condition should be satisfied because of the chemical and structural similarities of the chains. The latter condition should also be satisfied given the relative freedom of movement of lipids in the plane of a solventless bilayer (Edidin, 1974). The segments in the lipid chain have some ability to move perpendicular to the membrane surface (see Fig. 6 in Gruen [1980 *a*]), and hence there will be limited mixing in this direction. This effect should be very much smaller than mixing in the plane of the bilayer, and has been ignored.

It is only possible to take account of this effect in a crude way. We assume a lattice with a coordination number of six (the chains in the solventless bilayer above T_c pack in a pseudohexagonal lattice) in which a lipid molecule takes up two sites and an alkane molecule, one. (This assumption is probably good in the outer regions of the bilayer; less so towards the center.) We use a result from Guggenheim (1952), a particular case of his Eq. 10.09.6: the number of ways of arranging N_a monomers and N_ℓ dimers on a lattice with coordination number six is approximately:

$$g(N_a, N_\ell) = \frac{3^{N_t}[(N_a + 5/3N_\ell)!]^3}{N_a!N_\ell![(N_a + 2N_\ell)!]^2} \tag{21}$$

Using Stirling's formula for $n!$ (see Feller, 1968), Eq. 21 becomes

$$g(N_a, N_\ell) \simeq \frac{3^{N_t}(N_a + 5/3\,N_\ell)^{3/2}}{(2\pi N_a N_\ell)^{1/2}(N_a + 2N_\ell)}\left[\frac{(N_a + 5/3\,N_\ell)^{3(N_a+5/3N_\ell)}}{N_a^{Na}N_\ell^{N_t}(N_a + 2N_\ell)^{2(N_a+2N_\ell)}}\right] \tag{22}$$

where the ratio of the two sides approaches unity as N_a and N_ℓ increase without bound. The mixing of dimers with themselves is not required and hence we must evaluate $g(N_a, N_\ell)/g(0, N_\ell)$. Manipulation of the term [. . .] in Eq. 22 leads to

$$\frac{g(N_a, N_\ell)}{g(0, N_\ell)} \simeq \left\{\left[\left(\frac{5}{6}\right)\frac{1}{x}\right]^x\left(1 + \frac{1}{5}x\right)^{(5+x)/2}(1 - x)^{(x-1)/2}\right\}^{N_t(2+N_a/N_t)} = f(x)^{N_t(2+N_a/N_t)}, \tag{23}$$

where x is the volume fraction of monomer (alkane) in the plane. For any bilayer dimensions, it is possible to evaluate:

$$C_{\mathrm{MIX}} = \prod_k f(x_k)^{2l_k + n_a a_k}, \tag{24}$$

where x_k is the volume fraction of alkane in layer k, l_k is the proportion of total lipid in layer k, a_k is the proportion of total alkane in layer k, n_a, as previously defined, is the number of alkane molecules per lipid molecule, and Π_k is the product over all layers in the bilayer. The free energy associated with the mixing in the plane of the bilayer (F_{MIX}) is then:

$$F_{\mathrm{MIX}} = -kT \ln C_{\mathrm{MIX}}/\text{lipid molecule}. \tag{25}$$

A further discussion of the relationship f^i, u_x, and C_{MIX} appears in the next paper (Gruen and Haydon, 1981).

Energy and Free Energy

Expressions for ${}_lE^i$ and ${}_aE^i$ can now be given.

$$\begin{aligned} {}_lE^i &= {}_lE^i_{\mathrm{int}} + {}_lE^i_{\mathrm{disp}} + {}_lF^i_{\mathrm{HG}} + \gamma({}_lA^i_l - A_{\mathrm{HG}}) + \pi_c\, {}_lA^i_c + (\pi_{\mathrm{HG}} - \gamma)\, {}_lA^i_l + {}_lu^i \\ &= {}_lE^i_{\mathrm{int}} + {}_lE^i_{\mathrm{disp}} + {}_lF^i_{\mathrm{HG}} + \pi_{\mathrm{HG}}\, {}_lA^i_l + \pi_c\, {}_lA^i_c - \gamma A_{\mathrm{HG}} + {}_lu^i, \end{aligned} \tag{26}$$

and

$$\begin{aligned} {}_aE^i &= {}_aE^i_{\mathrm{int}} + {}_aE^i_{\mathrm{disp}} + {}_aF^i_{\mathrm{HG}} + \gamma_a A^i_l + \pi_c\, {}_aA^i_c + (\pi_{\mathrm{HG}} - \gamma)\, {}_aA^i_1 + {}_au^i + f^i \\ &= {}_aE^i_{\mathrm{int}} + {}_aE^i_{\mathrm{disp}} + {}_aF^i_{\mathrm{HG}} + \pi_c\, {}_aA^i_c + \pi_{\mathrm{HG}}\, {}_aA^i_l + {}_au^i + f^i, \end{aligned} \tag{27}$$

where ${}_lE^i_{\mathrm{disp}}$ and ${}_aE^i_{\mathrm{disp}}$ are given by Eq. 3, ${}_lF^i_{\mathrm{HG}}$ and ${}_aF^i_{\mathrm{HG}}$ are given by Eqs. 8 and 9, ${}_lA^i_l$ and ${}_aA^i_l$ are given by Eqs. 5 and 4, ${}_lA^i_c$ and ${}_aA^i_c$ are given by Eqs. 13 and 12, π_{HG} and π_c are given by Eqs. 10 and 16, ${}_lu^i$ and ${}_au^i$ are given by Eqs. 19 and 20, and f^i is given by Eq. 18. The statistical weight of lipid configuration i, (${}_lw^i$) is

$${}_lw^i = R^i_K \cdot \exp\,[-{}_lE^i/kT], \tag{28}$$

and of alkane configuration i,

$${}_aw^i = a_i \cdot R^i_K \cdot \exp\,[-{}_aE^i/kT] \tag{29}$$

and a_i and R^i_K are as defined in Eqs. 1 and 2. The partition functions are formed:

$$Z_l = \sum_i {}_lw^i, \tag{30}$$

and

$$Z_a = \sum_i {}_aw^i. \tag{31}$$

The probability of lipid configuration i is $\exp\,[-{}_lE^i/kT]/Z_l$, and there are R^i_K such configurations. The probability of alkane configuration i is $\exp[-{}_aE^i/kT]/Z_a$; there are $a_i \cdot R^i_K$ such configurations.

The expressions for ${}_lE^i$ and ${}_aE^i$ (Eqs. 26 and 27) are the appropriate ones because of the choice of independent variables. Thus in any bilayer the following are held constant: temperature, volume, number of alkane and lipid molecules, and total surface pressure. The presence of free energies in ${}_lE^i$ and ${}_aE^i$ comes about because there are degeneracies in the system which are not summed in Eqs. 30 and 31.

The bilayers are in equilibrium with a saturated aqueous phase. To estimate the contribution which alkane makes to the free energy it is therefore necessary to evaluate the change in free energy in the process: alkane (in bulk hydrocarbon) → alkane (in the bilayer). We define the Helmholtz free energy of alkane (F_{ALK}) as:

$$F_{ALK} = F_{ALK}(\text{bilayer}) - F_{ALK}(\text{bulk}). \tag{32}$$

F_{ALK} and $F_{LIP.C}$ (the Helmholtz free energy of the lipid chain) are derived in Appendix B.

The Helmholtz free energy for a bilayer containing m lipid and mn_a alkane molecules (F_{SYS}) is then:

$$\begin{aligned} F_{SYS} &= m[n_a F_{ALK} + 2F_{LIP.C} + F_{HEAD} + F_{INTERFACE} + F_{MIX}] \\ &= m[n_a F_{ALK} + 2F_{LIP.C} + 1/2\,\langle {}_lF_{HG}\rangle + \gamma(\langle {}_lA_l\rangle \\ &\qquad + n_a\langle {}_aA_l\rangle - A_{HG}) + F_{MIX}]. \end{aligned} \tag{33}$$

Neither the total interfacial area ($\mathcal{A}$) nor the interfacial tension ($\sigma = \gamma - \pi_c - \pi_{HG}$) are necessarily kept constant for different volume fractions of alkane in the bilayer. It is therefore necessary to minimize the following free energy:

$$G_{SYS} = F_{SYS} - \int_{\mathcal{A}-\mathcal{A}_i}^{\mathcal{A}-\mathcal{A}_f} \sigma \mathrm{d}\mathcal{A}, \tag{34}$$

where $\mathcal{A}_i$ is the initial interfacial area (area of the solventless membrane) and $\mathcal{A}_f$ is the interfacial area of the membrane for which the free energy is being evaluated. It is assumed that the alkane is absorbed infinitesimally slowly (as the concentration of alkane in the aqueous phase is increased) so that the bilayer can attain its equilibrium thickness as the area changes.

To conclude this paper, a flow diagram is presented (Fig. 3) which shows how the computer is used to generate the equilibrium distribution of lipid and alkane for different bilayer dimensions. Some amplification of the diagram should assist understanding. The "parameters for bilayer" refer to (*a*) the values of C^*_{SUR} and C^*_l (used in Eqs. 4, 5 and 12, 13), (*b*) the value of $\langle 1/({}_lA^i_l + {}_aA_{AVE.l}\,n_a)^3\rangle$ (used in Eq. 10), (*c*) the profile u_x (used in Eqs. 19 and 20), (*d*) the order parameter in each layer and (*e*) the values of ${}_aA_{AVE.l}$ and ${}_lA_{AVE.l}$ (which are required for Eqs. 8 and 9). The required accuracy with which the parameters are determined is a subject for the next paper (Gruen and Haydon, 1981).

APPENDIX A

Free Energy Cost of Creating Space for Alkane

We wish to estimate the free energy cost of creating space by straightening lipid chains. It is not possible to do this rigourously and what follows is an approximate method.

The term ${}_aA^i_c\pi_c$ occurs in ${}_aE^i$. This term takes account of the work done accommodating alkane

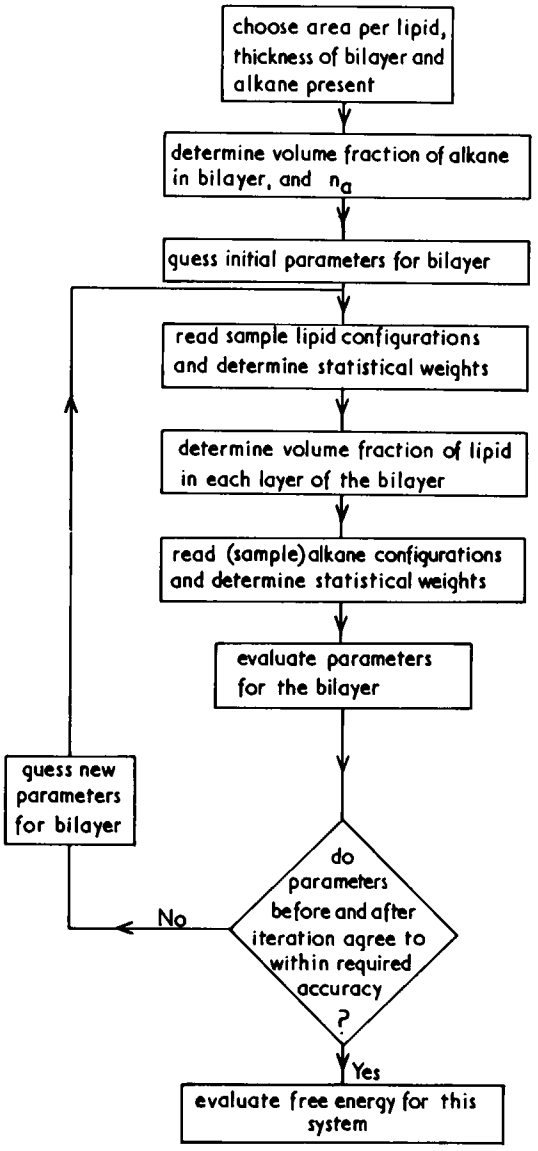

FIGURE 3 Flow diagram showing the steps taken in evaluation of the free energy of the bilayer.

configuration i in the bilayer, assuming that changes to surrounding chains are infinitesimal. However, if the area per lipid is fixed at 63.3 Å^2, any absorption of alkane into the outer 8 Å on either side of the bilayer will result in a finite distortion of surrounding chains. The aim of the following section is to estimate the extra free energy cost, over and above ${}_aA_c^i\pi_c$.

An infinitesimal compression of the lipid chains will be resisted by the chain pressure, π_c. To create 27 Å^3 of space (the volume of a CH_2 group) involves a free energy cost of $\pi_c\Delta A = 27.1 \times 27 \times 1.439/13.65 = 77.1$ cal/mol of 27 Å^3 spaces ($\pi_c = 27.1$ dyn/cm; 13.65 Å is the half thickness of the solventless chain region, and 1.439 is the conversion unit from Å^2 · dyn/cm to cal/mol).

The area per chain in the solventless membrane is 63.3/2 Å^2. If we assume that in all-*trans* chain packs with the same density as the average DPL chains above the phase transition, then the area per

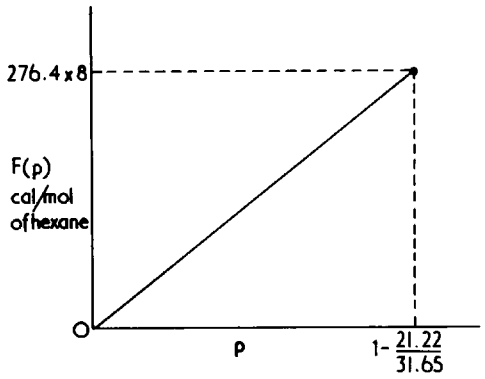

FIGURE 4 Excess free energy cost of creating space for hexane, $F(p)$, vs. proportion of available space created, p.

all-*trans* chain is 20.4 × 1.04 = 21.2 Å^2. (20.4 Å^2 is the area of an all-*trans* chain below the phase transition, and there is a 4% density decrease on melting. A very similar answer would have resulted by taking the volume of a CH_2 group in liquid hydrocarbon [27 Å^3] and dividing by the length [1.25 Å], 27/12.5 = 21.6 Å^2.)

To estimate the free energy cost of completely straightening a lipid chain we use the following argument. The Seelig and Seelig (1974) dmr experiments on which the solventless model is based, were performed just above the phase transition temperature of the DPL bilayer. At the phase transition, $F_{GEL} = F_{LIQUID\text{-}CRYSTAL}$, where F_A refers to the free energy of the A-phase. Just above the transition, the right hand side of this equation is slightly less than the left hand side. The most important changes (in terms of energy and entropy) which occur on going through the phase transition are changes in the state of the chains (Nagle and Wilkinson, 1978). Below the transition, the chains are stiff, parallel, and fully extended (Tardieu et al., 1973). (In fact, Raman spectroscopic experiments [Gaber and Peticolas, 1977] suggest that the chains are not quite stiff, with perhaps one kink on average per chain. This refinement is of little consequence for the present rough calculation.) The free energy difference between a chain below the phase transition and our fully extended chain in a liquid crystalline environment is the van der Waals energy difference between the two states. This is estimated as 171 cal/mol CH_2 by Nagle and Wilkinson (1978) on the basis of accurate density determinations of the two phases. Thus, assuming $F_{GEL} = F_{LIQUID\text{-}CRYSTAL}$, the above figure is an estimate of the free energy cost of completely straightening a lipid chain. The space created by straightening a CH_2 group is (31.65–21.2) × 1.25 = 13.06 Å^3. The free energy cost of creating 27 Å^3 of space is, therefore, 171 × 27/13.06 = 353.5 cal/mol.

As already stated, the free energy cost of compressing the lipid chains by an infinitesimal amount is already included in ${}_aE'$. The extra free energy cost of completely straightening them is 353.5–77.1 = 276.4 cal/mol. We assume a linear interpolation between the two points. The result for hexane (which has a volume of eight CH_2 groups) is shown in Fig. 4. The "effective proportion of space available to alkane," b (which appears in Eq. 17) is then defined (for hexane) as:

$$b = \int_{p-0}^{p-0.33} \exp\left[-F(p)/RT\right]dp = 0.0904. \tag{A1}$$

For an alkane with n carbon atoms,

$$b = (0.7405)^{n+2} \tag{A2}$$

(for hexane, $(0.7405)^8 = 0.0904$). A discussion of the reasons for the form of Eq. A2 is given in the text, after Eq. 18.

APPENDIX B

Details of Free Energy Calculations

For an alkane chain in bulk hydrocarbon, ${}_aE^i = {}_aE^i_{\text{int}}$, and hence, defining

$$Z_a(\text{bulk}) = \sum_i \exp\left[-{}_aE^i_{\text{int}}/kT\right], \tag{B1}$$

we have

$$F_{\text{ALK}}(\text{bulk}) = -kT \ln Z_a(\text{bulk}). \tag{B2}$$

The evaluation of $Z_a(\text{bulk})$ is given in Gruen (1980 *b*). It is similar to the derivation given by Flory (1969), chapter three.

For an alkane chain in the bilayer:

$$S = -k \sum_j p_j \ln p_j = -k/Z_a \sum_i {}_aw^i(-{}_aE^i/kT - \ln Z_a), \tag{B3}$$

where ${}_aE^i$ is defined by Eq. 27, ${}_aw^i$ by Eq. 29 and Z_a by Eq. 31. Thus,

$$TS = \langle {}_aE_{\text{int}} \rangle + \langle {}_aE_{\text{disp}} \rangle + \langle {}_aF_{\text{HG}} \rangle + \pi_c \langle {}_aA_c \rangle + \pi_{\text{HG}} \langle {}_aA_l \rangle + \langle f^i \rangle + \langle {}_au^i \rangle + kT \ln Z_a. \tag{B4}$$

The energy per chain is

$$U = \langle {}_aE_{\text{int}} \rangle + 1/2 \langle {}_aE_{\text{disp}} \rangle. \tag{B5}$$

The Helmholtz free energy of an alkane chain in the bilayer is, therefore,

$$F_{\text{ALK}}(\text{bilayer}) = U - TS, \tag{B6}$$

where U and TS are given by (B5) and (B4).

As defined in the text,

$$F_{\text{ALK}} = F_{\text{ALK}}(\text{bilayer}) - F_{\text{ALK}}(\text{bulk}) = -1/2 \langle {}_aE_{\text{disp}} \rangle - \langle {}_aF_{\text{HG}} \rangle - \pi_c \langle {}_aA_c \rangle - \pi_{\text{HG}} \langle {}_aA_l \rangle - \langle f^i \rangle - \langle {}_au^i \rangle - kT \ln [Z_a/Z_a(\text{bulk})]. \tag{B7}$$

An analysis similar to (B3) – (B6) gives:

$$F_{\text{LIP.C}} = -1/2 \langle {}_lE_{\text{disp}} \rangle - \langle {}_lF_{\text{HG}} \rangle - \pi_c \langle {}_lA_c \rangle - \pi_{\text{HG}} \langle {}_lA_l \rangle - \langle {}_lu^i \rangle - kT \ln Z_l. \tag{B8}$$

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