THE ADSORPTION OF N-ALKANES INTO BIMOLECULAR LIPID LAYERS:
THEORY AND EXPERIMENT

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Abstract - It has been known for some time that the n-alkanes adsorb primarily into the central part of a lipid bilayer. In doing so they affect only slightly the area per lipid molecule but may substantially increase the bilayer thickness. Of particular interest is that the saturation adsorption of the various n-alkanes decreases strongly with increasing chain length, quite contrary to what would be expected from partitioning between bulk phases.

A theoretical treatment of the above phenomena has been developed based on the type of approach used by Marčelja to describe chain ordering in liquid crystals and lipid bilayers. At present it appears that the new theory accounts quite well for the dependence of adsorption on alkane chain length.

An offshoot of this work has been the consideration of the adsorption into single bilayers (black films) relative to that in multilayers (liposomes). The results for these two types of system may differ appreciably and the reasons for this will be explored.

INTRODUCTION

Many small inert molecules act in biological systems both locally and generally as anaesthetics. In their local action, at least, there is wide agreement that the sites of action of these molecules are the membranes of the various excitable cells. Although it is clear that certain membrane proteins are primarily responsible for the transmission of nervous impulses, it is not established that anaesthetic molecules act directly upon these proteins. An alternative possibility, that the inert molecules adsorb mainly into the lipid of the membrane and, through a structural perturbation, influence the functioning of the protein indirectly, seems at present at least as likely (1-3). For this reason, if for no other, the adsorption of non-polar molecules into lipid bilayers is of considerable interest. Of the various types of anaesthetic molecule, the n-alkanes, although of minimal practical relevance, are exceptionally convenient for mechanistic studies. Thus, they are chemically similar to the chains of the lipid bilayer molecules and they form a homologous series which is useful for interpretive purposes.

For the experimental study of the adsorption of n-alkanes into lipid bilayers two distinct types of system are available. Perhaps the most obvious is the liposomal or vesicular dispersion which is formed when phospholipids, at temperatures above their phase transition temperature, are agitated in aqueous solutions. For modest energy inputs such dispersions consist of multilamellar droplets, usually in the micron size range, of the liquid crystalline L_α phase (4,5). After prolonged sonication, such dispersions are usually found to consist of one- or two-walled vesicles some 300 Å in diameter (6). A number of studies have been made using the coarser dispersions. The techniques involve the equilibration of the aqueous solutions, with and without lipid present, with the alkane vapour at a given partial pressure followed by analysis using isotopic or gas chromatographic methods (7,8).

The second type of system is the black lipid film formed in aqueous solutions. In this system the selected lipid is dissolved or dispersed in the n-alkane of interest and the resulting liquid extended 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 elsewhere (9).

Where a comparison may be made, the results of the liquid crystalline dispersion approach do not agree with those for the black films, in that the adsorption into the former systems is much smaller than in the latter. The reasons for this will be discussed later in this paper.

Because the black film is inherently the simpler system, and because for present purposes it approximates more closely to the biological membrane bilayers, the main part of this paper will be concerned with the results and theory for this type of system.

ADSORPTION INTO MONOGLYCERIDE AND PHOSPHATIDYLCHOLINE BILAYERS: EXPERIMENTAL RESULTS

Although pure glyceryl monooleate does not form bilayers in water, it does form black films (when dissolved in n-alkanes), which have structural features closely resembling those of phospholipid films. Since this monoglyceride yields well-defined systems which are in many ways easier to study quantitatively than the phospholipids, results for both types of lipid
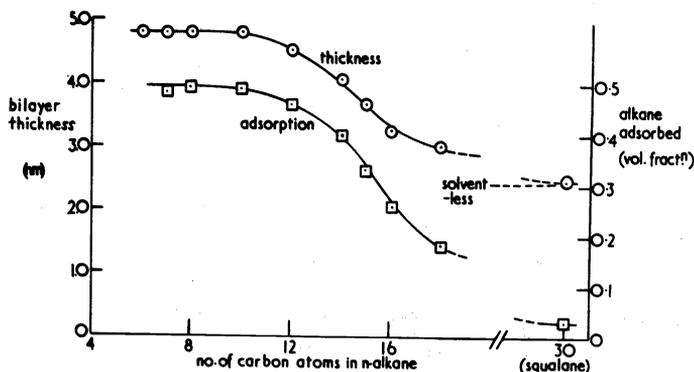
Fig. 1. Hydrocarbon layer thicknesses and alkane adsorptions for black films formed from glyceryl monooleate (ca. 8 mM) in alkanes of various chain lengths (the C_{18} result is for l-octadecene). The results have been collected from refs. 10-12, with the exception of that for squalane, which was a personal communication from J.R. Elliott. The aqueous solutions (which were O.1 M NaCl) were pre-equilibrated with the film-forming solutions and thus were nearly saturated with the alkane. The temperature was 20 ± 1°C for all systems.

are shown (Figs 1 and 2). In neither system does the chain length, or the extent of the adsorption of the alkane, have an appreciable effect on the area per molecule of the lipids (10,15). Both factors, on the other hand, markedly affect the thickness of the hydrocarbon region of the bilayer (10-15). The results of Figs 1 and 2, which are for saturated,
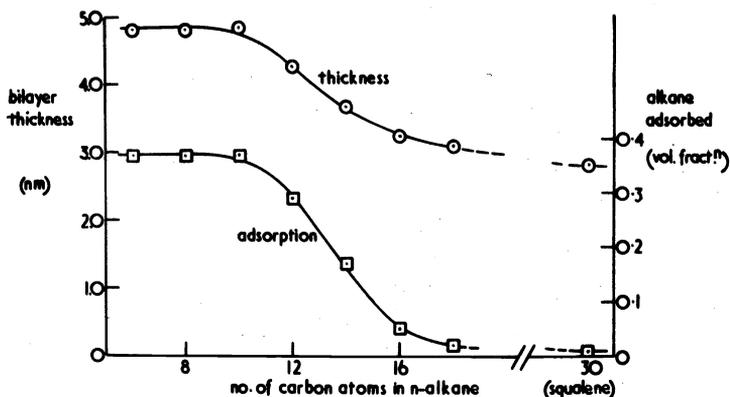
Fig. 2. Hydrocarbon layer thicknesses and alkane adsorptions for black films formed from dioleyl phosphatidylcholine or egg phosphatidylcholine (ca. 1 mg/ml) in alkanes of various chain lengths. The results are from refs. 10-14. The squalene result was a personal communication from J.R. Elliott. The aqueous solutions (O.1 M NaCl) were effectively saturated with the alkanes. With the exception of the octadecane system for which T = 33°C, the temperature was 20 ± 1°C.

or nearly saturated, equilibrium alkane concentrations, show that the maximum thickening and
adsorption occurs for the shorter homologues. As the chain length increases, the thickening
and the adsorption decline, reaching nearly zero values for the large (branched chain)
molecule squalane. The minimum and maximum values of thickness observed correspond respec-
tively to the bilayer thickness found by X-ray diffraction from multilamellar droplets of the
L_α phase (in the case of phospholipids) and to the length of two fully extended chains of the
lipid molecules. It is obvious from the fact that bilayers thicken at nearly constant area
per lipid molecule, that the alkanes tend to adsorb (or absorb) mainly into the central parts
of the leaflet. It should also be noted that the film tension increases on adsorption of the
alkane (Fig. 3) indicating a negative surface excess (i.e. the adsorption of the alkane into
a bulk solution of the lipid in the alkane is stronger than it is into the bilayer itself).
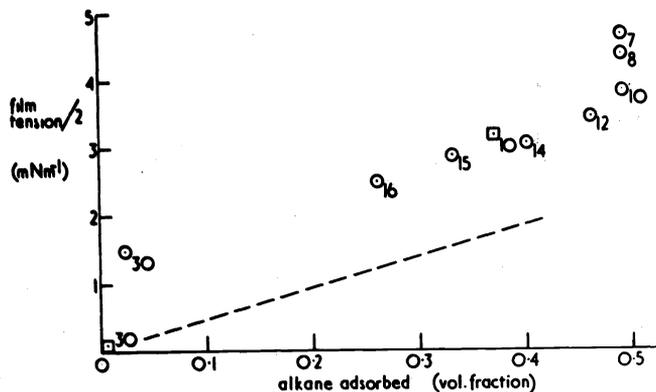
Fig. 3. Interfacial tensions (film tensions/2) for some of the systems of
Figs 1 and 2. ⊙ , glyceryl monooleate; ▣ , dioleyl phosphatidylcholine.
The numbers of carbon atoms in the alkane are indicated beside the points.
The aqueous solutions and temperature were as in Figs 1 and 2. (The dashed
line indicates the dependence of interfacial tension on adsorption
assumed for the calculations on adsorption in multilamellar systems (see
final section of the paper)).

The application of electrical potentials across the black films tends to thin them (11,15,16),
squeezing out adsorbed alkane and, presumably, also some of the lipid. The loss of lipid is
found experimentally to be insignificant compared with the loss of alkane. Hence the
thinning of a film under an electrostrictive compression represents effectively desorption of
alkane. Results for a black film of glyceryl monooleate and n-decane are shown in Fig. 4.
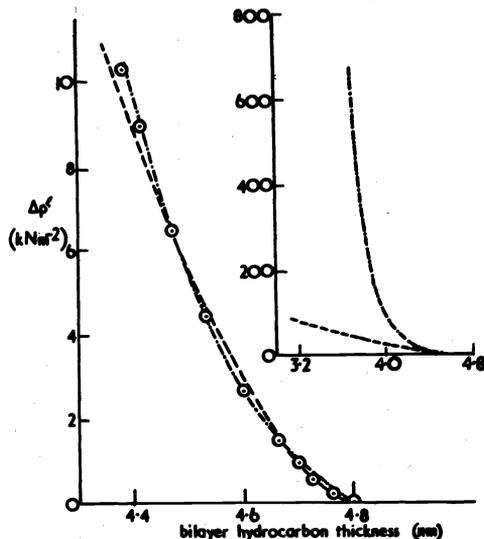
Fig. 4. The dependence of black film hydrocarbon layer thickness on applied
electrostrictive pressure for films of glyceryl monooleate + n-decane (ref.
11). The curves ----- and —·—·—·— represent empirical fits to the data
which, however, diverge sharply for smaller bilayer thicknesses. This is
shown in the inset. The units in the two graphs are the same.

At an intuitive level it is not surprising that for adsorption into a domain having the dimensions of a bilayer, small molecules are more readily accommodated than larger molecules. A quantitative description of such adsorption is, however, not easy to develop. Some progress has, nevertheless, been made in this direction and this is described in an abbreviated form in the next section. Owing to limitations of space, this description contains little detail and is intended to be an outline only of the approach.

THEORETICAL MODEL FOR THE ADSORPTION OF HYDROCARBON INTO THE BILAYER MEMBRANE

A statistical mechanical model of the solventless bilayer has been developed by Marčelja (17). The model gives good agreement with several experimental results. We have extended the model to give a more detailed description of a bilayer of dipalmitoyl-3-sn-phosphatidyl-choline (DPL) molecules (18), and to model the adsorption of alkane into this bilayer.

The extended model for a solventless bilayer

The lipid chains in the bilayer above its phase transition have considerable freedom. Apart from translational motion, this freedom comes about because each bond in the alkyl chain can take up three conformations: one trans (t), and two gauche ($g^{\pm}$) conformations. To develop the model, the assumption is made that the first CH_2 group in the chain is anchored at a plane interface (the interface between the hydrophobic interior of the membrane, and the polar exterior). All conformations of a single chain (a fully saturated C_{16} chain) are generated on a computer. (Any conformation which crosses the interface and enters the polar region is excluded.) The bilayer is assumed symmetrical in all respects about its mid-plane. The statistical weight of each conformation is evaluated by taking the following into account.

(i) Gauche bonds have an energy of 2.1 kJ/mol (19).

(ii) Van der Waals attraction between the chains lowers their energy. A molecular field is introduced which models the average behaviour of all chains other than that specifically considered. The strength of the molecular field at any position in the bilayer is set in proportion to the average order of the chains at this position. (Order is quantified by use of the order parameter η. If θ denotes the angle between the direction of a chain segment and the bilayer normal then η is related to the average orientation according to $\eta = \frac{3}{2} < \cos^2\theta > -\frac{1}{2}$. An order parameter S_{CD}, related to η, has been measured along the DPL chain, using deuterium magnetic resonance (20)).

(iii) Interaction between headgroups is modelled by assuming a simple functional relationship between headgroup free energy and area available to the headgroup (A_I), $F_{headgroup} = \alpha/A_I^2$. ($\alpha$ is a constant to be determined later.)

(iv) The free energy cost of forming hydrocarbon-water surface is assumed to be $F_{interface} = \gamma(A_I - A_{HG})$ where γ is taken as the bulk hydrocarbon-water surface tension (50 mN m^{-1}) and A_{HG} is an equivalent hard disc area taken up by the headgroup which is assumed to be the same for all chain conformations (and hence for different A_I's).

(v) The surface tension of solventless phospholipid bilayers is apparently almost zero (see Fig. 3). However, the tension of the lipid chains-water interface has been assumed to be 50 mN m^{-1}. A balancing pressure of 50 mN m^{-1} must therefore be present. We assume there are two contributions to this pressure: from the headgroups (π_{HG}), and from the chains (π_C). From the assumed dependence of headgroup free energy on interfacial area, it is possible to derive π_{HG}; i.e. $\pi_{HG} = \alpha \langle 1/A_I^3 \rangle$ (where the angled brackets denote the thermodynamic average).

The pressures π_{HG} and π_C act to decrease the interfacial area (A_I) of the molecule and the chain area (A_C) respectively. The larger π_C, the more chain conformations with small areas are preferred over those with large areas. The parameter α (which effectively determines the relative values of π_{HG} and π_C) is varied until a best fit is obtained to the experimental values of S_{CD} along the lipid chain (Fig. 5). This fit was obtained for $\pi_C = 27.3$ mN m^{-1} (and hence $\pi_{HG} = 22.7$ mN m^{-1}). Using the derived statistical distribution of chain conformations it is possible to generate a theoretical electron density profile (Fig. 6) and the average positions of different carbons in the acyl chains (Table 1).

TABLE 1. Average distance from bilayer centre (nm)

	Model (at 41°C)	Experimental (at 50°C) (22)
C_4	1.15	1.22 ± 0.15
C_5	1.05	1.05 ± 0.15
C_9	0.68	0.81 ± 0.1
C_{14}	0.30	0.36 ± 0.1
C_{15}	0.26	0.19 ± 0.1
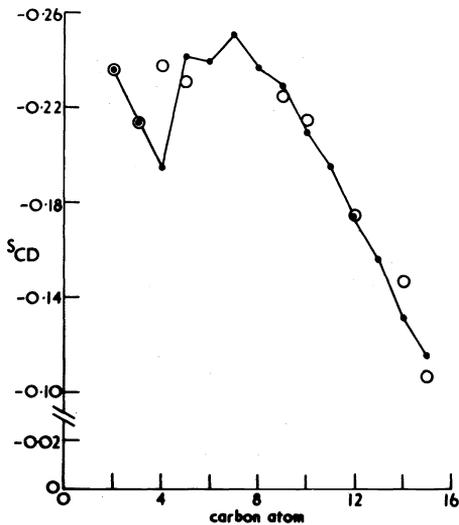
Fig. 5. Deuterium order parameters (S_{CD}) at 41°C along the lipid chain.
● , calculated from the model. ○ , data from Seelig and Seelig (20).
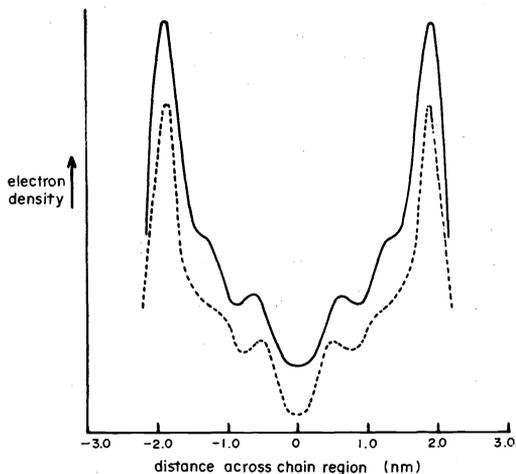
Fig. 6. Comparison of model and experimental electron density profiles.
——— , model profile at 41°C. ---- , experimental profile from Cain et al
(21) at 49°C and 8% hydration. The two curves are vertically offset for
clarity. The origin of the horizontal scale is at the centre of the
chain region.

The excellent agreement with experiment suggests that the model gives an accurate picture of
the relative probabilities of the chain conformations.

Adsorption of alkane into the bilayer
It is assumed that the density of chain packing remains constant as the bilayer takes up
alkane. Hence, any uptake must be accompanied by an increase in volume of the bilayer. The
general approach of the model is to assume, in turn, several possibilities for the dimensions
of the bilayer, evaluate the free energy of each, and look for a minimum. Changes in both
area per lipid and thickness have been considered. In all cases, the value of α derived for
the solventless system has been used.

Unlike the lipid chains, which are anchored to the bilayer interface, the n-alkanes are free
to distribute throughout the chain region of the bilayer. Hence, in generating alkane chain
conformations, it was assumed that "the beginning" of the chain could occur at several equally

spaced positions across the bilayer. As with the lipid chain, all conformations which cross
the bilayer interface and enter the polar region are excluded.

In evaluating the statistical weights of the alkane conformations, the five points mentioned
in the last section are all taken into account. The presence of the alkane changes the
order across the bilayer and hence changes the interactions of the chains with each other.
Two further points require consideration:
        (i) If the bilayer is thickened substantially, the volume fraction of lipid will fall
from unity near the interfaces to a much smaller value at the centre of the bilayer. Hence,
there are far more "holes" near the centre of the bilayer into which alkane can pack than
nearer the polar interfaces. This has been taken into account by including an extra term in
the statistical weight of the alkane. For alkane configuration i this extra term is of the
form $(1 - cV_L{}^i)$ where $V_L{}^i$ is the volume fraction of lipid at the average position of
configuration i, and c is a constant $(0 < c < 1)$ related to the compressibility of the lipid
chains and the molar volume of the alkane.
        (ii) It is not possible in a mean field model of the present type to obtain a sufficient-
ly 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 are not
always found to 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. This constraint is applied to both lipid and alkane configurations on
the basis of their positions in the bilayer.

To summarize the approach: a particular thickness and area per lipid are chosen. The
assumption of constant chain packing density then determines the volume fraction of alkane
present. An order parameter profile across the bilayer is guessed. This profile determines
the van der Waals energy and the contribution of the chain pressure (π_c) at different
positions across the bilayer. All the conformations of the lipid are generated and
statistical weights determined. The volume fraction of lipid at each position is then
determined, and a profile of "effective volume" available to the alkane evaluated. The
alkane configurations are generated and statistical weights determined. A new order
parameter profile across the bilayer can then be evaluated (taking into account both lipid
and alkane configurations) as well as the volume fraction of all chains at each depth. The
input order parameter profile and the "even distribution constraint" are adjusted and a new
iteration begun. The final result is obtained when the input and output order parameters
agree to within required accuracy at all positions in the bilayer, and the chains are evenly
packed throughout.
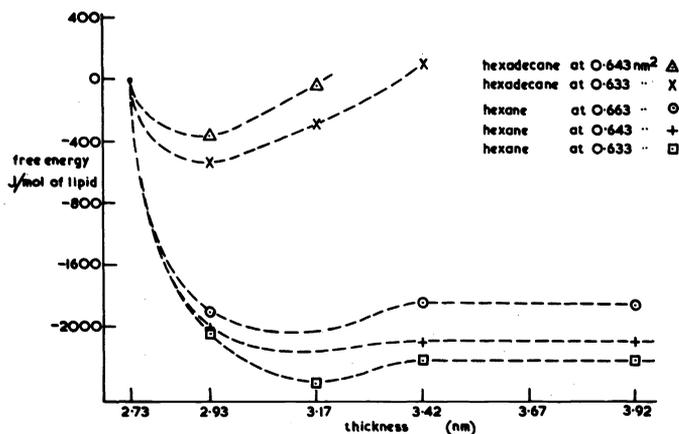
Fig. 7. Variation of free energy with thickness at three different areas
per lipid. For the solventless system the area per lipid is 0.633 nm^2 and
the thickness of the chain region is 2.73 nm. The dashed lines were drawn
by eye. The temperature as for the solventless system, is 41°C.

Figs 7 and 8 show the variation in free energy with changes in thickness and with area per
lipid. As can be seen from Fig. 7 the model predicts that the minimum free energy occurs at
the same area per lipid as the solventless system. This is understandable because of the
large free energy cost of creating hydrocarbon-water interface, and is borne out by experi-
ments. The model also predicts that the equilibrium thickness of the alkane saturated bilayer
increases as the length of alkane decreases (Fig. 8). However, the equilibrium thickness
never reaches the length of two fully extended lipid chains (in this case 3.94 nm) as the
experimental results do (Figs 1 and 2). The model predicts that the force required to
squeeze out a small volume fraction of alkane should be considerably smaller for the shorter
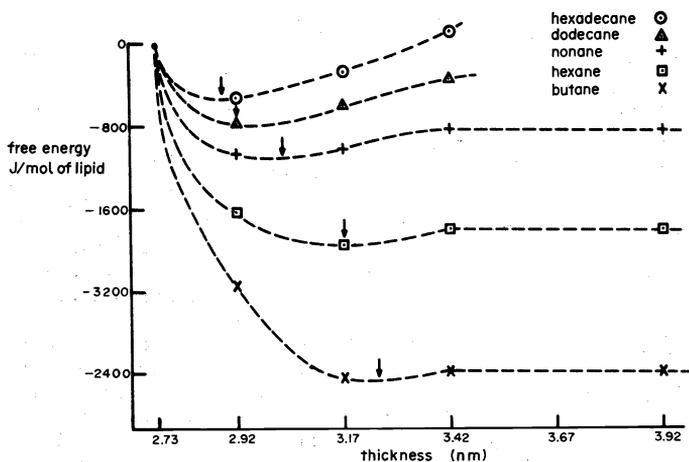
Fig. 8. Variation of free energy with thickness at constant area per lipid
(0.633 nm²). The arrows mark the approximate positions of the minima in the
free energy curves.
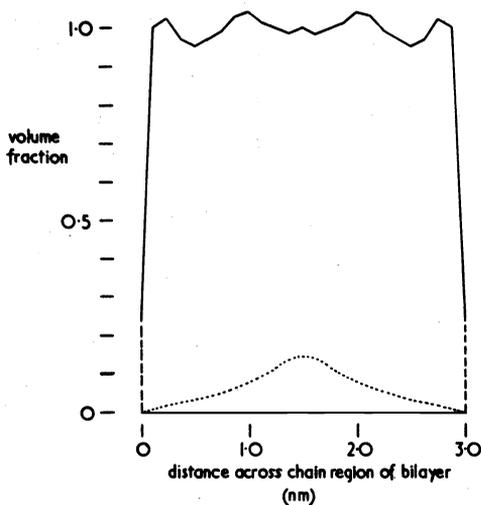
Fig. 9. ·····, distribution of hexadecane in the bilayer at approx.
equilibrium thickness and area per lipid. ———, volume weighted
distribution of hexadecane and lipid chains.

than for the longer chain alkanes. This is observed (15).

Figs 9 and 10 show how hexadecane is distributed in the bilayer. In order to accommodate the
alkane molecules in the outer regions of the bilayer, the lipid chains must straighten
somewhat. Thus the overall order parameter (which in this region depends largely on the order
of the lipid) is larger than its solventless value. Towards the bilayer centre, the volume
fraction of lipid molecules falls. The alkane is less constrained to lie parallel to the
lipid chains, and its order falls. Nevertheless, the hexadecane order parameter is always
positive suggesting that it is mainly interdigitated between the lipid chains.

In contrast to this situation, Figs 11 and 12 illustrate the behaviour of butane. (At 41°C,
butane is a gas. Hence the comparison with saturated solutions of liquid alkanes in water
is not straightforward. For the model calculations, it was assumed that the bilayer was in
equilibrium with a reservoir of pure liquid butane.) There is more butane than hexadecane
at all positions in the bilayer. In the outer regions, this requires that the lipid chains
straighten more than in the hexadecane bilayer. The overall order parameter in this region
is larger. At the centre of the considerably thickened bilayer, the volume fraction of lipid
chains has fallen to 0.12. In this region, the butane chains do not, on average, orient
parallel to the lipid chains. Rather, their orientation is almost completely random, as it
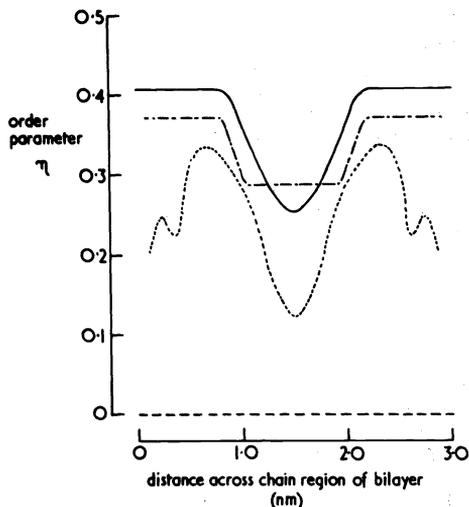
Fig. 10. ·····, order parameter profile of hexadecane in the bilayer.
————, overall order parameter profile for the hexadecane bilayer. For
comparison, —·—·—, order parameter profile for the solventless bilayer.
The order in the outer 0.75 nm of the bilayer for the latter two profiles
has been averaged. The strange behaviour of the hexadecane order
parameter in the outer regions is due to the fact that no conformations
are permitted to cross the polar interface. Only a small proportion of
the hexadecane configurations exist in these regions.
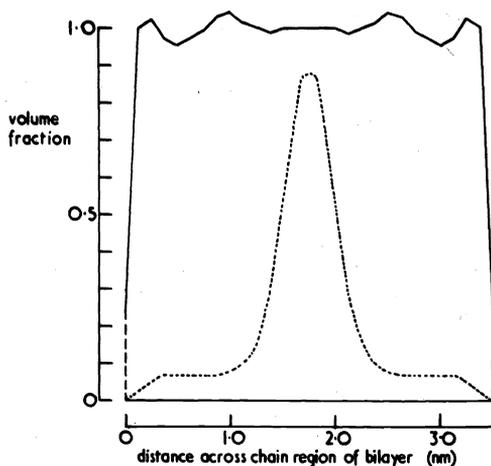
Fig. 11. ·····, distribution of butane in the bilayer at approx.
equilibrium thickness and area per lipid. ————, volume weighted
distribution of butane and lipid chains.

would be in bulk liquid alkane.

The model suggests two reasons why the shorter chain hydrocarbons thicken the bilayer
considerably more than the longer ones.
    (i) Assuming a constant area per lipid, adsorption of alkane into the outer regions of
the bilayer necessitates straightening of the lipid chains. To create room for a hexadecane
molecule in this region requires the straightening of many more lipid chains than for a
butane molecule. Hence, for a given bilayer thickness, small alkanes will mix more evenly
throughout the bilayer than large ones. While it is unfavourable, in free energy terms, to
straighten the lipid chains, this is greatly outweighed by the entropic advantage of a more
even distribution of alkane throughout the bilayer.

(ii) In bulk liquid alkane, the hydrocarbon molecules are almost free, apart from an internal 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 lies parallel to the membrane surface, are almost excluded. (Clearly, this statement applies only in regions where the volume fraction of lipid is high.) The presence of a single gauche kink in the middle of a hexadecane chain causes a long segment of the chain to be misaligned. This is not so of a much shorter chain molecule like hexane or butane.

These two effects lead to the result that the shorter chain alkanes dilute the lipid in the bilayer to a considerable extent but as the chain length increases this occurs progressively less and less.
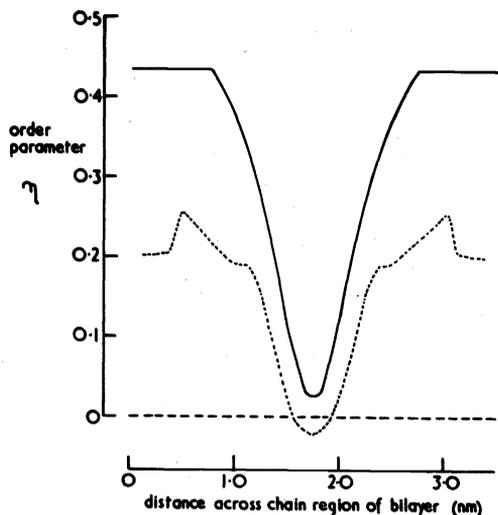
Fig. 12. ·····, order parameter profile of butane in the bilayer.
_____, overall order parameter profile for the butane bilayer (averaged in
the outer regions). The behaviour of the butane order parameter in the
outer regions is, once again, due to the presence of the polar interfaces.

HYDROCARBON ADSORPTION INTO PHOSPHOLIPID LIQUID CRYSTALLINE LIPOSOMES

Several recent papers have been concerned with the adsorption of non-polar molecules, particularly hydrocarbons, into multilamellar liposomes in suspension in aqueous media (7,8, 23). Where comparison is possible, it appears that adsorption into the bilayers of the multilamellar structures is substantially smaller than into planar bilayers or black films. The reasons for this discrepancy are of some importance since both types of system are used as indicators of the adsorption to be expected into the lipid regions of biological membranes. The purpose of this section is to draw attention to the fact that, in general, adsorption into the two systems should not be similar and to show that, owing to their curved surfaces and multilamellar structure, adsorption into liposomes could be considerably smaller than into single planar leaflets.

The chemical potential, μ_i^σ, of a hydrocarbon (i) in a lipid bilayer may be written (24,25)

$$\mu_i^\sigma = \mu_i^{+,\sigma}(p_0) + (p - p_0)\overline{V}_i^\sigma + RT\ln F(x_i^\sigma) - \sigma a_i. \tag{1}$$

$p - p_0$ is the pressure in excess of atmospheric which acts on the bilayer; $\overline{V}_i^\sigma$ is the partial molar volume of i; $F(x_i^\sigma)$ is a function of x_i^σ, the mole fraction of i, which formally includes the activity coefficient of i in the bilayer; σ is the bilayer tension (defined as $(\partial A^\sigma/\partial \mathcal{Q})_{T,V^\sigma,n_i^\sigma,n_j^\sigma}$, where A^σ is the Helmholtz free energy and $\mathcal{Q}$ is the area of the bilayer); a_i is the partial molar area of 'i' in the bilayer (defined as $(\partial \mathcal{Q}/\partial n_i^\sigma)_{T,V^\sigma,n_j^\sigma,\sigma}$). When 'i' in the bilayer is in equilibrium with 'i' at a given chemical potential in the surrounding aqueous media, $F(x_i^\sigma)$ is a function of $(p - p_0)\overline{V}_i^\sigma$ and σa_i. For present purposes σa_i will be neglected since, as mentioned previously, the near constancy of the area per molecule of the lipid molecules for different alkane adsorptions indicates that a_i is small. The pressure difference $p - p_0$ across the bilayer, on the other hand, is not negligible.

As a model for calculation it has been assumed that adsorption of a n-alkane occurs into a suspension, in an aqueous solution, of spherical multilayered liposomes of a phosphatidyl-choline. The individual bilayers will tend to thicken, their tensions will tend to increase and, because their surfaces are curved, a pressure differential will develop across them. This pressure will act on the aqueous as well as the hydrocarbon layers and thus both these spacings are likely to change. The detailed quantitative treatment of these effects is described in detail elsewhere (26), but can be summarized as follows.

The pressure differences across both the lipid and the water layers can be related to the tensions and radii of curvature of the spherical bilayers by forms of the Laplace equation. Thus the cumulative effects of pressures across layers situated outside the one in question are taken into account. The tensions, which depend on the amount of alkane adsorbed, will be assumed to be similar to those of black phospholipid films containing the same amount of alkane. This assumption neglects the van der Waals and electrostatic interaction forces which cause the bilayers to aggregate into a multilamellar structure. However, in the present systems these interactions are thought to be small and to act in the same direction as the pressure differentials. Estimates of the bilayer tensions may be obtained by considering the tensions measured for black films formed from alkanes of various chain length and hence which contain different volume fractions of hydrocarbon (Fig. 3). The most comprehensive and reliable set of results is for glyceryl monooleate films, where it can be seen that there is a roughly linear relationship between the tension and the adsorption. For dioleylphosphatidylcholine in the pure state it is widely recognized that the tension is very small, and is here assumed to be zero. For the same lipid (or for egg phosphatidylcholine), black films formed from decane have interfacial tensions of 1-3 mN m^{-1} (13,27) and it is assumed that, as for the glyceryl monooleate, there is a roughly linear dependence of tension on adsorption as indicated by the dashed line in Fig. 3.

The pressure which tends to develop between the inside and outside of each bilayer opposes the adsorption of the alkane. This is formally expressed by Eq. 1, but the precise relation-ship depends on the form of $F(x_i^0)$. As yet this information is not available but, it is known that electrostrictive pressure applied to a black lipid film can significantly reduce its thickness through squeezing out predominantly the alkane solvent (15,28,29). Two empirical expressions have been fitted to the experimental results for a glyceryl monooleate-decane black film (Fig. 4). While both expressions fit the experimental results well they diverge enormously for smaller values of the bilayer thickness. It is not at all certain which curve is physically the more realistic but since the two alternatives, between them, cover a wide range of behaviour it is likely that the actual system would have intermediate properties. The results of Fig. 4 are for a monoglyceride whereas the liposome envisaged is of phospho-lipid. Although there are no comparably detailed studies of the phospholipids, it is well-established that their response to applied pressures is similar to that of glyceryl monooleate (28,29).

The dependence of the thickness of the water layers on the pressure has been ascertained experimentally (30). Empirical relationships which describe these findings have been derived (26).

The various relationships discussed above form a set of equations which may be solved iteratively to give the hydrocarbon adsorption or thickening of each layer of a multilayered liposomal droplet. The results are shown in Fig. 13 and correspond to what should occur in an aqueous solution saturated with, say, n-hexane or n-decane. When the steeper of the two relationships in Fig. 4 is used and 2000 bilayers are assumed in the liposome, the upper curve (with dashed variants) is obtained. As can be seen the outermost bilayer adsorbs almost as much alkane as the planar bilayer (represented by the horizontal line at 1.84 nm). However, the adsorption or thickening falls rapidly over the first hundred bilayers, then more slowly, reaching about 0.5 nm in the centre. The average alkane adsorption per mole lipid, which is determined mainly by effects in the outer part of the structure, is found to be 54% of the value for the planar bilayer. The smaller 200-layer liposome, apart from a lower adsorption in the outer layers gives a very similar pattern and the average adsorption is again about 54% of the planar value. For a single-walled vesicle of radius 10 nm, where the aqueous core is assumed incompressible, the equations predict that the alkane adsorption would be approximately 50% of the planar value.

The less steep of the relationships in Fig. 4 represents a much greater sensitivity of adsorption to pressure and yields the lower curve in Fig. 13 for a 200-layer liposome. Fifty layers in from the outside the adsorption is almost zero, and the average adsorption per mole lipid is only 17% of the planar value. For a single-walled vesicle, 19% is found in contrast to the 50% given above.

In order to test the sensitivity of the results to the dependence of bilayer tension on alkane adsorption calculations were repeated, using the steeper pressure relationship but in which the slope of the dashed line in Fig. 3 was made first twice and then one-quarter of its nominal value. The average adsorptions were 48% and 65%, respectively, of the planar value.
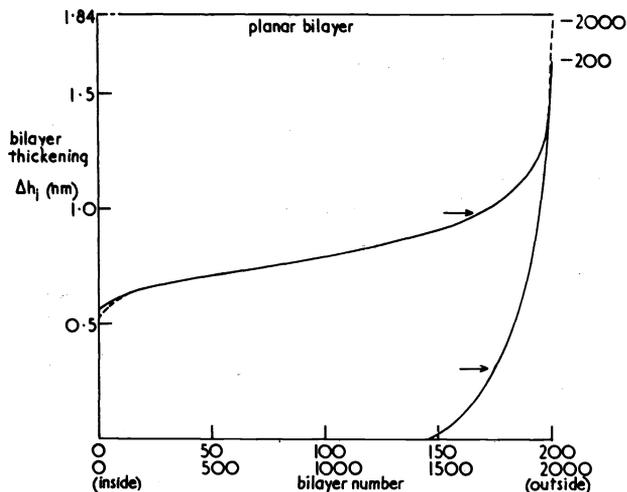
Fig. 13. The predicted thickening (which is proportional to the volume of
alkane adsorbed per unit area of membrane) of the various bilayers of 200-
(continuous curves) and 2000-layer (dashed curves) spherical liposomes in
an aqueous solution saturated with a small alkane, e.g. n-hexane. The
upper and lower curves were derived using the steeper and less steep
relationships respectively (see text). The average thickening is indicated
by the arrows. The horizontal line at the top of the diagram is the
thickening for a planar bilayer.

Two experimental results may be compared with the above predictions. Simon et al (7) report
a value for n-hexane at saturation which corresponds to roughly 5%, while Miller et al (8)
find for n-butane at 1 atmosphere about 30% of the planar value. The latter result is not
easy to compare quantitatively with the results for liquid alkanes but is certainly in the
range expected theoretically. The former value is lower than our most extreme predictions.
It is possible to argue that surface free energy changes and non-equilibrium distributions
of lipid within the liposome could have led to lower adsorption, but rough estimates suggest
that such effects are fairly small.

Most biological membranes have average radii of curvature sufficiently large that the
pressure effects mentioned are minimal. Insofar as comparisons may be made therefore, their
adsorption of non-polar molecules should correspond closely to that for planar bilayers.

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