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CHAIN ORDERING IN LIQUID CRYSTALS

II. STRUCTURE OF BILAYER MEMBRANES

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SUMMARY

The ordering of the hydrocarbon chain interior of bilayer membranes has been calculated using the molecular field approximation developed in previous work on liquid crystals. Different statistical averages are evaluated by exact summation over all conformations of a single chain in the field due to neighboring molecules. The internal energy of each conformation, as well as contributions arising from interaction with the molecular field and from a lateral pressure on the chain have been included.

The results describe properties of both lipid monolayers and bilayers. For monolayers, the calculated pressure-area relationships are in good agreement with experimental observations. The order parameter for hydrocarbon chains in bilayers (or monolayers) as a function of temperature, lateral pressure and position along the chain, is shown and compared with the available NMR data. Combining the results of calculation and NMR measurements we obtain the value for intrinsic lateral pressure within bilayer membranes, in excellent agreement with direct measurements on surface monolayers.

The calculation also gives average length of hydrocarbon chains, thermal expansion coefficient and fraction of bonds in *gauche* conformations. The effect of cholesterol and proteins within the bilayer is qualitatively described, and the contribution of the bilayer interior to membrane elasticity is determined.

INTRODUCTION

Lipid chains in the interior of natural and artificial bilayer membranes are preferentially oriented along the normal to the plane of the membrane. Depending on chemical composition and external factors this order can assume almost any value, giving rise to a large variety of phenomena. After numerous experimental studies, the information on the related properties of bilayer membranes is now fairly complete. The most important experimental techniques, listed together with several representative studies, are X-ray scattering [1–3], NMR [4, 5], ESR [6], calorimetric studies [7, 8] and studies of transport across the membrane [9].

Lipid monolayers at the air–water interface exhibit significant similarity with the behavior of bilayers formed in aqueous dispersions of lipids. This is well established [10] by the study of the relation between surface pressure and the area of a monolayer.

In contrast, theoretical description [11–15] of the ordering of the interior of bilayers, together with the related phase transition, is still fragmentary. This is not surprising in view of the uncertainties in our understanding of the simpler problem of freezing of polymers. An exception in this situation is the recent exact solution of a two dimensional model for phase transition in membranes [13] which clearly indicates some features of polymer condensation. A review of the current understanding of polymer melting is given by Nagle [16].

In this work we present for the first time a molecular field calculation of the hydrocarbon chain order within bilayer membranes. Compared to the exact solutions of simpler model systems, molecular field calculations have one well-known deficiency. In the immediate vicinity of the phase transition temperature the results of the molecular field approximation deviate from the behavior of real systems because the fluctuations in the value of the molecular field are neglected. On the other hand, molecular field approximation enables one to consider more realistic models, and in this way a large number of experimental results can be correlated within a single physical picture. In this sense, exact solutions of simple models and molecular field calculations with more realistic models complement each other as a way to obtain information on the behavior of complex systems.

Here we use a version of the simple model for interaction of lipid chains, introduced in the first part of this work [17], where end-chain ordering in nematic liquid crystals is described. The theory is introduced in the next section, while the following sections discuss various aspects of the results. A preliminary report, describing a simplified version of the present model was published in an earlier letter [15].

THEORETICAL MODEL

The orientation of hydrocarbon chains forming the interior of a membrane will be characterized by the value of the order parameter calculated separately for each carbon atom site. Let z axis be along the normal to the plane of the membrane, pointing away from polar ends. For each carbon atom C_i, we define the angle between the normal to the plane spanned by C–H bonds and the z axis as ϑ_i. The order parameter at the site of the carbon atom C_i is defined as a thermodynamic average

$$\eta_i = \langle \tfrac{3}{2} \cos^2 \vartheta_i - \tfrac{1}{2} \rangle, \tag{1}$$

while the order parameter for the whole chain η is obtained as an average over η_i. With this definition, η_i correspond to order parameters measured by ESR and NMR [18].

In the mean field approximation, interaction of a hydrocarbon chain with its neighbors is described by the energy of a single chain in the molecular field. This energy consists of three contributions

$$E = E_{\mathrm{int}} + E_{\mathrm{disp}} + PA \tag{2}$$

The first term is the internal energy of a single chain in a given conformation. It depends on the particular sequence of the *trans*, *gauche*$^+$ and *gauche*$^-$ orientations along the chain. This contribution is well known, and evaluated as described in the first part of this work [17].

The second term describes dispersive or Van der Waals interaction of the chain with its neighbors, via the molecular field Φ,

$$E_{\mathrm{disp}} = -\Phi(n_{\mathrm{tr}}/n)\sum_{\mathrm{i}}(\tfrac{3}{2}\cos^2\vartheta_{\mathrm{i}}-\tfrac{1}{2}) \tag{3}$$

The strength of the molecular field depends on the average order of the system

$$\Phi = V_0\langle(n_{\mathrm{tr}}/n)\sum_{\mathrm{i}}(\tfrac{3}{2}\cos^2\vartheta_{\mathrm{i}}-\tfrac{1}{2})\rangle \tag{4}$$

As discussed in the first part of this work, this expression for E_{disp} represents an effective attractive interaction energy due to dispersive forces between the chains. The coupling constant [17] $V_0 = 680$ cal/mole is determined from the freezing energy of polyethylene.

Compared to the corresponding expression in the first part of this work, here we have introduced an extra factor of n_{tr}/n, which gives the fraction of bonds in the *trans* state. This has been done because in bilayer membranes hydrocarbon chains freeze during the phase transition, while in nematic liquid crystals the molecules only acquire a common average orientation. The full attractive interaction in the frozen state is achieved when neighboring molecules assume both common orientation and an all-*trans* conformation. Should the factor n_{tr}/n be omitted, the results for the liquid state of the hydrocarbon chain system would not be much affected. However, the resulting order in the condensed phase would be too small.

The last contribution to Eqn 2 is due to lateral pressure on each chain. Its origin is in the steric repulsion among hard cores of each atom. The energy associated with the lateral pressure on a chain is proportional to the value of the pressure and the average cross sectional area of the chain. In surface monolayer experiments the steric repulsion is balanced by the applied external pressure. In the case of the lamellar phase in lipid-water dispersions, the intrinsic pressure on hydrocarbon chains is dictated by the packing of polar heads required to satisfy much stronger hydrophobic forces. Therefore, the area per polar head is almost independent of lipid chain length, while it changes with the concentration of dispersed lipids [19]. Compared to free surface-expanded monolayers, the area per polar head is greatly reduced. The intrinsic lateral pressure on hydrocarbon chains depends on the available area per polar head, and increases with the increasing concentration of lipids in the dispersion. Even in the case of complete hydration at lower lipid concentrations, intrinsic lateral pressure is still significant. As a result, hydrocarbon chains, although in fluid phase, are still relatively well ordered [2].

The statistical mechanical expressions are constructed in the usual way [15], with the partition function

$$Z = \sum_{\text{all conformations}} \exp\left[-E(\Phi, P)/k_{\mathrm{B}}T\right]. \tag{5}$$

The sum runs over all conformations of a hydrocarbon chain. The self-consistent equation for the molecular field Φ is

$$\Phi = \sum_{\text{all conformations}} \{[(n_{tr}/n) \sum_{i} (\tfrac{3}{2}\cos^2 \vartheta_i - \tfrac{1}{2})] \exp[-E(\Phi, P)/k_B T]\}/Z. \tag{6}$$

Thermal averages of different functions, like η_i, area per polar head etc., are calculated in the standard way. The internal energy, the entropy and the free energy of the hydrocarbon chain system are given by (in units of the gas constant R_0, per mole)

$$U = E_{int} - (N-2)(\Phi^2 - \Phi_0^2)/2V_0, \tag{7}$$

$$TS = E_{int} - (N-2)(\Phi^2 - \Phi_0^2)/V_0 - \ln Z, \tag{8}$$

$$F = U - TS. \tag{9}$$

Here, Φ_0 is the value of Φ with no interaction between the molecules, E_{int} being the average value of the internal energy of the chain and N, the number of carbon atoms in the chain. If the orientation of the initial chain segment is fixed, $N-2$ is the number of segments free to orient in the molecular field. The temperature of the first order transition between the ordered and the disordered phase is obtained as a point at which the free energy of both phases is equal.

The numerical results are obtained [17] by generating on the computer all conformations of a single lipid chain with N carbon atoms and solving Eqns 5–9. The conformations where the chain bends backwards to enter the polar region are eliminated. The results depend on the assumed order of the initial chain segment, C_1–C_2. This will in turn depend on the apriori order of polar heads, i.e. the orientational order of polar heads with no lipid chains. Although not much experimental information is presently available on this point, it seems that polar heads are disordered at physiological temperatures. The experiments [20] indicate that the order parameter measured at the site of the first carbon atom is not higher from that further along the chain. The polar heads apparently order at the so called "lower transition", below the freezing transition of the chains [13].

In generating different chain configurations to be used in Eqns 5–6 we have used three initial orientations, with angles (0, 0), (ϑ, 0) and (ϑ, π), as described in the first part of this work. The value $\vartheta = 60°$ corresponding to the order parameter value $\eta_1 = 0.25$ at the first carbon atom site was used throughout the calculation. Any small value for η_1 would lead to similar results. However, for values of η_1 larger than about 0.4 the calculated values of order parameter along the chain resemble the results of older spin label measurements [21], i.e. the order parameter decreases along the chain. Since such a profile was shown [5] to be a result of the perturbing effect of the spin label group it was not studied further.

RESULTS

Relation between lateral pressure and area per polar head

The statistical sums described in the previous section are evaluated using an average cross sectional area for each configuration of a hydrocarbon chain. The cross sectional area is estimated from the calculated length of the chain in the z direction as

$$A = A_0 L_0 / L \tag{10}$$

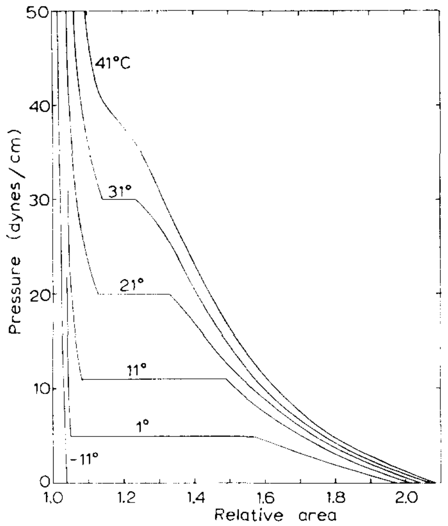


Fig. 1. Calculated relation between lateral pressure and area per polar head for surface monolayers at different temperatures. The area is measured relative to its value in the perfectly ordered state.

A_0 and L_0 are the area and the length, respectively, of the chain in the frozen all-*trans* state. The value of A_0 is 20.4 Å^2 [1].

Numerical results for the relation between the lateral pressure and the area per polar head have been obtained for lipid molecules with $N = 10$ carbon atoms in each chain. The results should approximately correspond to the pressure–area per polar head curves for lecithins measured by Phillips and Chapman [10].

The agreement between the results shown in Fig. 1 and the measurements [10] is very satisfactory. The qualitative features of different measured isotherms as well as the absolute values of pressure and temperature in the condensation region are well reproduced by the theory. The major remaining difference is that the data show clearly the beginning of the condensation while the end is relatively rounded, and in calculated curves both the beginning and the end of condensation is well defined. It is possible that with increased precision and high purity samples the experiments would yield sharp transitions, as is the case with liquid–gas transition in surface monolayers [22], or the more recent data on bilayers [8, 23].

Experimental isotherms of the condensed phase at zero external pressure show larger area per polar head than the corresponding calculation. This is probably due to tilted chains [24], a possibility which was not included in the calculation.

The calculated relative area depends weakly on chain length. For example, at 20 dynes/cm the change from 10 to 12 carbon atoms in the chain results in an increase of relative area by 3 %. The corresponding experimental curves for lecithins are almost identical. The difference is partly due to the fact that measured dependence of transition temperatures on chain length is somewhat stronger than indicated by the calculated values.

Order within the membrane

Let us now consider the lamellar phase of aqueous dispersions of amphiphilic molecules. In the high temperature phase, with fluid hydrocarbon chains, the area per polar head is determined by the degree of hydration. For lecithins [25], this area is typically between 60 and 70 Å^2. The relatively small values of area per polar head in bilayer membranes indicate that, even in the fluid phase, hydrocarbon chains have a considerable order normal to the plane of the membrane.

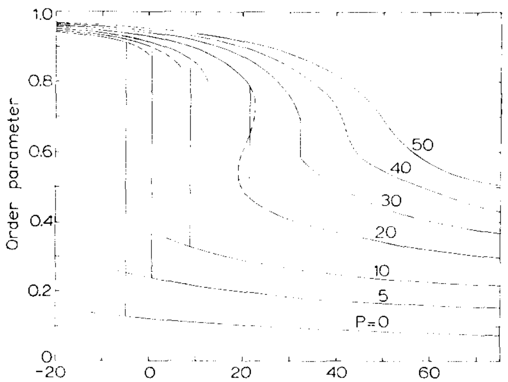
On the other hand, in surface expanded monolayers, in the absence of applied external pressure, the area per polar head is much larger. Since water molecules cannot enter the hydrophobic region of the monolayer, looser arrangement of polar heads allowing for less order in hydrocarbon chain conformations is energetically favorable.

Significant ordering within the fluid interior of a bilayer, first inferred from the X-ray data [2], is confirmed by direct NMR measurements [4, 5]. Using materials with deuterated chains, the order parameter is obtained as a function of the position along the chain. NMR experiments indicate that the order parameter is essentially constant along the chain, except for the region near the center of the bilayer.

The constant value of the order parameter across the bilayer is imposed by the packing of polar ends [26], while the central layer with smaller value of the order parameter is the region containing free ends of chains.

The steric repulsion between neighboring chains results in an internal lateral pressure experienced by each chain. This pressure, brought about by the required close packing of polar heads, has the same effect as the external lateral pressure applied in surface monolayer experiments. In the following, the full equivalence between the two systems will be established.

Order parameter for membranes built of molecules with 10 carbon atoms in the end chains has been calculated as a function of temperature and pressure. The results are shown in Figs 2 and 3. Fig. 2 shows the order parameter, averaged over the length of the chain, as a function of temperature. For low lateral pressures, typical first order transition between the ordered and the disordered phase is obtained. Higher



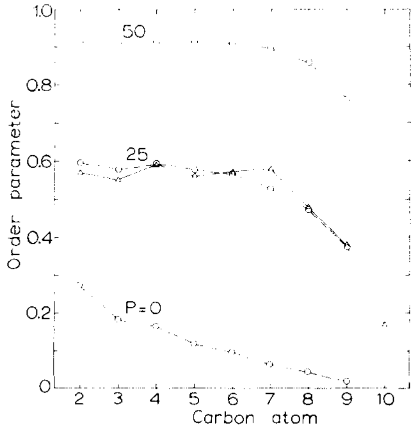


Fig. 2. Order parameter, averaged over the chain length, as a function of temperature. The values of lateral pressure (in dynes/cm) used in the calculation are shown with the corresponding curves.

Fig. 3. Order parameter, at 28 °C, shown as a function of a position along the chain. – – –, calculation for different values of lateral pressure (in dynes/cm). ———, data by Seelig and Niederberger [5].

lateral pressures impose increasing order on the chains, resulting in a continuous change of order with temperature.

The results calculated for the frozen phase of hydrocarbon chains are less reliable because of deficiencies in the applied approximations. Nevertheless, we believe that the order in the condensed phase is not perfect, and some very restricted motion of molecules is still possible. This conclusion is supported by experimental results which show small but finite values of diffusion in the condensed phase [6].

In Fig. 3 we compare the calculated and the measured [5] values of the order parameter as a function of the position along the chain. In both experiment and theory the temperature is 28 °C and the chains have 10 carbon atoms. The order parameter for the terminal methyl group was not calculated.

The measured values of the order parameter coincide almost perfectly with the calculation at 25 dynes/cm lateral pressure. This value can be compared with monolayer measurements. Typically, the area per polar head in bilayers with fluid chains is 1.5–1.7 times larger than the value in the frozen state [25]. From Fig. 3 of ref. 10, this corresponds to lateral pressures between 25 and 15 dynes/cm, which is in excellent agreement with the result obtained combining theory and NMR data. Thus, we conclude, that typical lateral pressures in the hydrocarbon chain interior of bilayer membranes are of the order of 20 dynes/cm for each half of the bilayer.

In Fig. 4 we have shown the values of the order parameter as a function of the position along the chain, calculated for chains with 12 carbon atoms and a lateral pressure of 20 dynes/cm. Except for the profiles just above and below the freezing temperature, several intermediate cases are also shown. For $P = 20$ dynes/cm these correspond to the physically inaccessible region of the phase transition. However, for larger values of lateral pressure, such profiles correspond to physical states of the system at different temperatures.

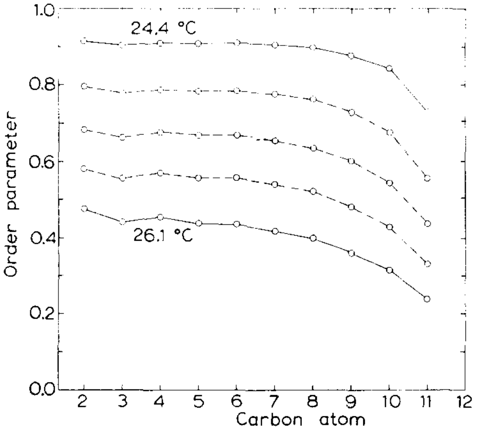


Fig. 4. Order parameter at different temperatures shown as a function of a position along the chain. – – –, Described in the text, unstable region of the phase transition.

Membrane thickness

The thickness of the hydrophobic interior of the membrane has been calculated along with other thermodynamic quantities. In Fig. 5, the average dimension of

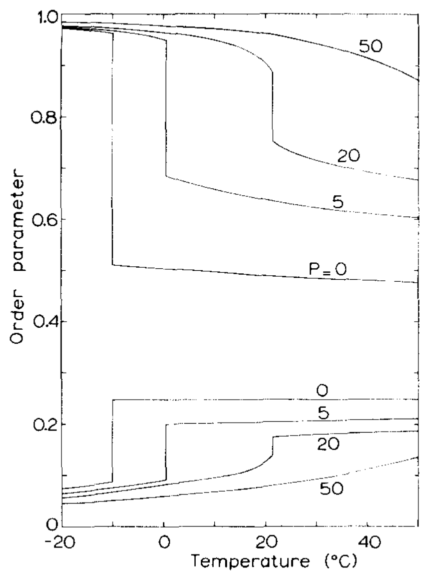


Fig. 5. Top lines: thickness of the hydrocarbon interior of the membrane (relative to its value in the ordered state) at different values of lateral pressure (in dynes/cm), shown as a function of temperature. Bottom lines: thickness of the central boundary layer at different values of lateral pressure. Units are the same as in the top part of the diagram.

hydrocarbon chains in the direction perpendicular to the plane of the membrane is plotted as a function of temperature and lateral pressure. The length is measured relative to the length of the fully extended all-*trans* conformation. The calculation was done for chains with $N = 10$.

The value of the linear thermal expansion coefficient in z direction for hydrocarbon interior of the membrane can be determined from Fig. 5. For typical membranes in the fluid state, with $P = 20$ dynes/cm, one obtains α_z between $-2 \cdot 10^{-3}/°\mathrm{C}$ and $-3 \cdot 10^{-3}/°\mathrm{C}$.

Calculations of change in membrane thickness upon freezing and linear thermal expansion in the fluid state can be compared with X-ray measurements, resulting in excellent agreement. The measured change in thickness is 7 Å for α-L-dipalmitoyllecithin [25] between 25 and 50 °C, and 8 Å for palmitate enriched *Mycoplasma laidlawii* membrane [3] between 0 and 40 °C. Theoretically, at 20 dynes/cm, the relative thickness of the hydrocarbon region changes from about 0.7 to 0.9 throughout the transition, corresponding to the change of 8 Å in total thickness. The experimentally determined linear thermal expansion coefficient is $-2.7 \cdot 10^{-3}/°\mathrm{C}$ for the lamellar phase of potassium soaps [19] and $-2 \cdot 10^{-3}/°\mathrm{C}$ for egg lecithin multibilayers [27].

Both measurements [4, 5] and calculations [26] indicate that the order parameter is constant across the membrane, except for the boundary layer near the center, containing ends of individual chains. The thickness of this layer can be estimated by calculating the value of $\sigma^2 = \langle Z^2 \rangle - \langle Z \rangle^2$, where Z is the coordinate of the last carbon atom. The value of σ, in the same units as the length, is shown in the bottom part of Fig. 5. Except for the highest pressure, σ shows almost no temperature dependence in the fluid phase.

The fraction of bonds in *trans* conformation has also been calculated as a function of temperature and pressure. The result is numerically almost identical to the calculated length in z direction, and for that reason was not shown as a separate figure. For example, reading from Fig. 5, one finds that for a palmitate membrane in the fluid state, at $P = 20$ dynes/cm, on average, there are about 9 C–C bonds in the *trans* conformation and 4 bonds in *gauche* conformations. This agrees well with previous estimates [5, 13].

Temperatures and heats of transition

The temperatures and latent heats of the transition between the fluid and the condensed phase of the bilayer interior calculated at $P = 20$ dynes/cm are listed in Table I. The chain lengths between 9 and 12 carbon atoms are too short for direct comparison with experiments. For example, the available data on lecithins [7] cover the region between 14 and 22 carbon atoms in the chain. Nevertheless, several trends of the calculated values can be established. Without any adjustable parameters, both transition temperatures and entropies are roughly of the correct magnitude. The increase of transition temperatures with increasing chain length is somewhat slower than indicated by the experiment. At $P = 20$ dynes/cm the latent heats of the transition are only slightly higher than the values obtained by extrapolation of the experimental data. Undoubtedly, for a slightly higher value of lateral pressure the calculated latent heats would coincide with the extrapolation of the data.

TABLE I

CALCULATED TEMPERATURES AND HEATS OF TRANSITION

	Number of carbon atoms in chain			
	9	10	11	12
Transition temperature (°C)	18.6	21.6	23.1	25.2
Latent heat (kcal/mole)	0.90	3.51	4.77	6.20

We have preferred to leave the calculated latent heats slightly higher than the experimental values because this is consistent with the applied molecular field approximation. In this approximation, fluctuations in the value of the order parameter near the transition temperature are neglected. A well-known consequence is that the theoretical latent heat is too large, while the specific heat anomaly near the phase transition (as well as the anomalous change of other quantities) is suppressed.

Effect of cholesterol and proteins

Numerous studies have shown that cholesterol and protein molecules incorporated into the phospholipid bilayer have profound influence on the order of hydrocarbon chains. Upon the addition of increasing amounts of cholesterol, the latent heat of the phase transition in dipalmitoyllecithin dispersions decreases, and the sharp phase transition gradually broadens [28]. More than 20 mole% of cholesterol causes a decrease of transition temperatures. As a result of the incorporation of cholesterol into the bilayer, the order of hydrocarbon chains increases when the bilayer is in the fluid phase, while the opposite is true in the condensed phase. This can be described as a condition of "intermediate fluidity" [29]. The membrane integral proteins have qualitatively similar effect on the neighboring phospholipid molecules [30].

A relatively rigid cholesterol or protein molecule restricts the orientational freedom of the neighboring chains. In the fluid state, the restriction of the number of available rotational isomeric states increases the molecular order. However, for steric reasons, the interaction between fatty acid chains and cholesterol cannot be as strong as the interaction among ordered all-*trans* chains. The order in the condensed phase is therefore decreased.

If the volume fraction of cholesterol within the bilayer is f and the interaction between the hydrocarbon chain segment and neighboring cholesterol molecules is V_1, the molecular field in the interior of the bilayer will be

$$\Phi_1 = (1-f)\Phi + fV_1, \tag{11}$$

where Φ is given by Eqn 5. The interaction V_1 is smaller than the chain–chain interaction V_0 and independent of the order within the bilayer.

To illustrate the effect of changed molecular fields, Eqn 9, we have choosen $V_1 = 0.5\ V_0$. The resulting average order parameter for $N = 10$, $P = 20$ dynes/cm and different values of f is shown in Fig. 6. All the main qualitative effects observed experimentally are reproduced by the model calculation. With increasing fraction of foreign molecules in the phospholipid bilayer the dependence of order parameter on temperature becomes less pronounced and the cooperative phase transition gradually disappears.

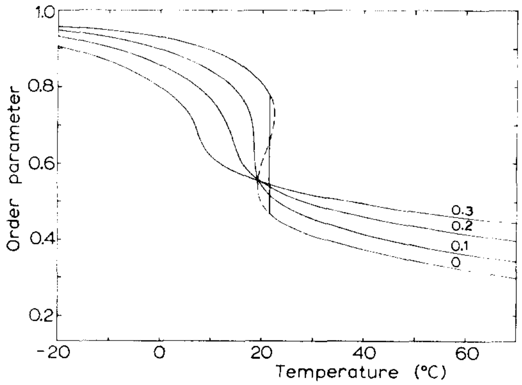


Fig. 6. Average order parameter as a function of temperature. The parameter f, describing the fraction of non-lipid molecules within the bilayer, is shown with the corresponding curves.

CONCLUSION

The model calculation presented in previous sections is successful in describing the structure and static properties of bilayer membranes. The only exception, included in the theory only indirectly [17], is the total volume change upon the phase transition. This description was accomplished without any free parameters introduced into the model. The main novel result of this work, i.e. the value of lateral pressure within the lipid interior of the membrane, can therefore be accepted with some confidence.

The value of lateral pressure within the interior of bilayers of different composition can be estimated from Figs 2–4 if the value of the order parameter is measured, or read directly, if the surface monolayer data are available. The information on lateral pressure should prove valuable in considerations concerning the insertion of proteins into the bilayer or the assembly of membrane components.

APPENDIX

Elasticity of the bilayer

The unperturbed bilayer assumes the optimum value of area per polar head. Any lateral expansion or compression away from that value (e.g. to increase the volume of a cell) will result in an increase of the energy of the bilayer. For small deformations, the response is elastic and the contribution of lipid chains to the corresponding elastic modulus [31] can be determined using the results described previously. Let us assume that initially, hydrocarbon chains are in equilibrium with the lateral pressure due to polar heads. To first order accuracy, the pressure exerted by either heads (h) or chains (c) will be a linear function of the relative area per polar head

$$P_h = P_h^0 + k_h(A/A_0 - 1)$$

$$P_c = P_c^0 + k_c(A/A_0 - 1).$$

At the equilibrium, $A = A_0$, $P_h = -P_c$, and therefore, $P_h{}^0 = -P_c{}^0$. The change in energy per unit area for a small change in relative area is

$$E = \int (P_h + P_c)\mathrm{d}(A/A_0) = \tfrac{1}{2}(k_h + k_c)(A/A_0)^2.$$

The elastic modulus is then

$$k_s = k_h + k_c = \frac{\partial P_h}{\partial(A/A_0)} + \frac{\partial P_c}{\partial(A/A_0)}.$$

The value of $\partial P_h/\partial(A/A_0)$ is not known. However, the contribution of lipid chains to the elastic modulus, $\partial P_c/\partial(A/A_0)$, can be determined from Fig. 1. Alternatively, $\partial P_c/\partial(A/A_0)$ at a given value of area per polar head can be read from the surface monolayer data. In either case, the value has to be doubled, corresponding to the two sides of a bilayer. At $P = 20$ dynes/cm, both calculations and experiments [10] indicate that the lipid chain contribution to the elastic modulus of a bilayer is between 150 and 200 dynes/cm. This value is somewhat smaller than the earlier estimate [31].

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