

THEORY OF THE MAIN LIPID BILAYER PHASE TRANSITION

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INTRODUCTION

Fortunately, the day when it was necessary to argue the biological relevance of fundamental physical and chemical studies on pure synthetic lipid bilayers is past, and space will not be devoted to flogging that dead horse. The lipid bilayer phase transition (to be abbreviated LBPT), often called the gel to liquid crystal phase transition, appears to have direct biological relevance (1-5). Even if it did not, it is nevertheless a striking physical event, the study of which leads to a better understanding of the structure of biological membranes. In addition, the theoretical study of phase transitions in lipid bilayers is an interesting chemical physics problem in its own right and not just a problem for routine application of theoretical methods developed for simpler systems.

The emphasis of this review is upon the lipid systems for which the most detailed and quantitative experimental and theoretical studies can be performed; the lecithin (phosphatidylcholine, abbreviated PC) bilayers with saturated and homogeneous hydrocarbon chains are the preeminent system, although a number of studies involving variations in the lipid molecules are mentioned. This review is not concerned with the proliferation of phases that occur at low water content (6a) or with the theory of self-assembly of the lipids into a bilayer in contrast to micellar forms (6b). Furthermore, this review is more concerned with equilibrium properties of the LBPT than with the dynamics of molecular motions. Even with these restrictions, the subject is a large one, and the author apologizes for omissions due to lack of space or oversight.

Because this is an interdisciplinary area that attracts readers and authors from a variety of backgrounds with different perspectives, there has been confusion in evaluating the advances of different theories and

in comparing them to each other. This review proposes to clarify the discussion by dividing the theory into two aspects. The first, called *General Modeling*, involves interpretation of experiments, back of envelope calculations, and knowledge of intermolecular interactions in simpler systems. Its goal is the writing down of the Hamiltonian of the system, which is to say, the elucidation of the relevant degree of freedom and the development of formulas that give the energies of the states of the system. The second aspect of the theory consists of *Specific Model Calculations*. Because the lipid bilayer system is quite complicated, good statistical mechanical calculations for completely general models are not feasible. Therefore, theoreticians simplify the General Model, retaining what they think is most important and tractable. It is not uncommon that two theoreticians who largely agree upon a General Model may nevertheless do considerably different Specific Model Calculations, with different specific models and different degrees of rigor in the calculations. Conversely, many papers employ the ubiquitous mean field approximate calculation, but the authors may have different Specific Models in mind and sometimes even disagree upon the General Model.

In terms of our division of the theory into these two aspects, it is easier to preview the status of theory in this LBPT field. There is widespread, though not complete, agreement upon the main features of a General Model; there is less agreement upon some secondary features and further development may be expected with continuing experimentation. The General Model is reviewed in the next section.

In contrast to the relative accord for the General Model, there are many, superficially discordant, Specific Model Calculations. As will be discussed in the later section entitled Philosophy of Statistical Mechanical Calculations, this multiplicitous development is more harmonious than it appears. Indeed, it is essential that a variety of Specific Model Calculations be done, from exact calculations on highly simplified models for a rigorous establishment of the existence of the phase transition in such models, to highly approximate calculations on more realistic models to obtain in a convenient way semiquantitative results for comparison with experiment. Much more of the philosophy of Specific Model Calculations is discussed below. Now we turn to General Modeling.

GENERAL MODELING

Rotameric Degrees of Freedom

For temperatures below the main transition temperature T_M in lipid bilayers in which the lipid molecules have uniform, saturated hydro-

carbon chains, the wide angle X-ray diffraction gives a sharp ring at 4.2 Å. As the temperature is raised through the transition, this ring becomes diffuse and moves to larger spacings. This behavior is very similar to the behavior of long chain alkanes, C_nH_{2n+2} , at their melting transitions. From this Tardieu and co-workers (6a) concluded that the phase transition in lipid bilayers results from "melting" or disordering of the hydrocarbon tails of lipids. (See Figure 1.) This conclusion is also indicated by the main (M) transition temperatures $T_M(n)$ of the series of phosphatidylcholines (lecithins DC_nPC) with n carbons in each fully saturated hydrocarbon chain. As n increases $T_M(n)$ increases in a very systematic way, which is revealed especially well when $T_M(n)$ is plotted versus $1/(n-3)$ as shown in (7). If the transition is the result of hydrocarbon chain melting, then as the chain length becomes longer, the system should become similar to polyethylene because the relative influence of the head groups becomes smaller. Indeed, in the $T_M(n)$ versus $1/(n-3)$ plot (2), the transition temperatures $T_M(n)$ extrapolate to the transition temperature of polyethylene, $T_M(\infty) = 138^\circ C$, as $n \rightarrow \infty$. A similar kind of extrapolation has been used by Zacharis (8), although he does not actually use the $1/(n-\delta)$ plot.

The theoretical implication of the preceding experimental evidence is that the most important degrees of freedom required in a model are the different conformations of the hydrocarbon chains. In principle this requires a continuous rotation angle θ_i for each C-C bond, $i = 1, \dots, n-3$. However, extensive polymer studies (9a) have shown that a three-state model suffices, with a trans-ground state, $\theta_i = 0$, and doubly degenerate gauche states $\theta_i = 120^\circ$, because the energies of intermediate angles are

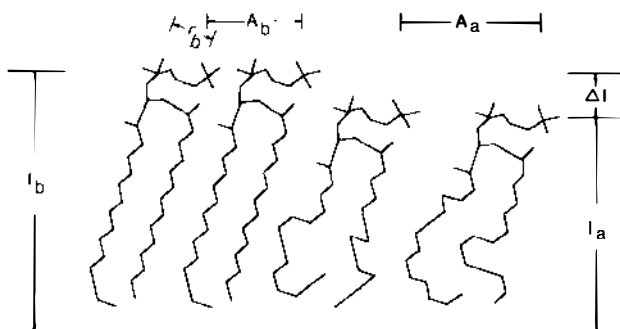


Figure 1 A schematic picture of one half of a lipid bilayer of DMPC with 14 carbons/chain. The horizontal dashed line marks the midplane of the bilayer. The hydrocarbon chains of the two molecules on the left are in the all-trans state below the transition (subscripts b) except for the ends of the 1-chain. The hydrocarbon chains of the two molecules on the right have considerable rotameric disorder characteristic of chains above the transition (subscripts a). The thinning of the membrane is shown by $\Delta l = l_b - l_a$ and the areas A_a and A_b are indicated.

large compared to kT . The polymer studies (9a) and liquid alkane studies (9b) have determined the trans-gauche energy difference ϵ (strictly speaking, free energy difference due to different shapes of the potential minima) to be

$$\epsilon = 0.5 \pm 0.1 \text{ kcal/mole} \quad 1.$$

for each C-C bond, although there is an interaction energy of about 2 kcal/mole for two gauche rotations of the same sign on adjacent bonds. Studies on shorter hydrocarbons (10) give $\epsilon = 0.6$ kcal/mole.

EFFECTIVE DIMENSIONALITY OF THE MODEL OF THE TRANSITION Currently there is high interest in phase transitions in low dimensional materials and one might hope that the LBPT would be a good candidate for two-dimensional melting. However, the basic disordering element is the trans to gauche configurational change for each C-C bond, and there are on the order of $n-2=14$ of these units per chain. Therefore, in comparison to an Ising model or a lattice gas model one has a system whose size is $n \times \infty \times \infty$. Calculations on pseudo-two-dimensional $n \times \infty$ Ising models show that for $n=16$ the thermal behavior is very close to the $\infty \times \infty$ two-dimensional system, except very near the critical point (11). Therefore, it seems most likely that the pseudo-three-dimensional $n \times \infty \times \infty$ LBPT will behave like a three-dimensional system, except very close to a critical point where, with very careful experimentation and sample preparation, one might hope to see a crossover to two-dimensional behavior, although this seems unlikely to be realized for the LBPT. Consequently, studies of strictly two-dimensional models of melting (12,

would seem to be largely irrelevant for the LBPT. In particular, such models clearly are unable to account for the very large transition enthalpies, $\Delta H \simeq 9$ kcal/mole, and transition entropies, $\Delta S \simeq 30$ cal/deg/mole.

An Important Feature of the LBPT Different From Ordinary Hydrocarbon-Melting

Although the LBPT (lipid bilayer phase transition) is clearly closely related to the bulk melting phase transition in hydrocarbons, such as the long chain alkanes, there are important differences. This was first realized by Chapman and co-workers (14) from their calorimetric studies, which show lower entropy changes ΔS in lipid bilayers by about a factor of two as compared to the alkane systems. Also, our studies show that the volume changes per CH_2 group are smaller in lipid bilayers by

about the same factor, although the volume changes do extrapolate with chain-length n to the volume change of the melting transition in long chain hydrocarbons (7). The differences in ΔH and ΔV emphasize an obvious though important fact for modeling the LBPT that distinguishes the General Model from models appropriate for other kinds of hydrocarbon melting. Whereas general hydrocarbon melts are isotropic, in lipid bilayers (even in the high temperature phase) there is a constraint, namely, one end of each chain is attached to a head group, which is anchored at the water interface. From this anchoring point the chains must proceed, on the average, in the direction perpendicular to the bilayer surface, thereby introducing an anisotropy. This anisotropic constraint, which is an important effect of the water on the main transition, would be expected to reduce the amount of disordering occurring at the phase transition as compared to the isotropic melting in alkanes. It certainly reveals a feature (the anisotropic constraint) necessary to a General Model of LBPT that is considerably different from models of polymer or alkane melting.

Other Intramolecular Features

The general picture of the phase transition that has developed consists of a low temperature phase, in which the hydrocarbon chains are parallel and in the all-trans conformation, going to a phase where the chains are disordered by trans to gauche rotation of some of the C-C bonds. Although this picture remains largely valid, NMR studies (15-17) indicate that above the phase transition the two hydrocarbon chains in each lipid molecule are not equivalent. Recent Raman spectroscopy by Peticolas and co-workers (18) indicates that in the low temperature phase, "The 1-chain, although it is probably more all-trans near the glycerol backbone, is distorted near the terminal methyl group, whereas the 2-chain, though more distorted near the glycerol backbone, is more all-trans near its terminal methyl group." Together with the picture proposed by Dennis and co-workers (17), which in turn is consistent with single crystal X-ray studies of phosphatidyl ethanolamines (19), one has the picture shown in Figure 1. The 1-chain is buried more deeply in the bilayer than the 2-chain in accord with the NMR model. In addition, it is proposed here that the terminal methyl end of the 1-chain may have one or more gauche rotations, in accord with the Raman data, which tucks the terminal methyl under the 2-terminal methyl of the same or a neighboring molecule. If so, this would avoid the necessity of interdigitation of extended all-trans 1-chains at the midplane of the bilayer and preserve the independence of the two monolayers in a bilayer (see below).

Recent studies of Keough & Davis (20) on lipids with saturated chains of different lengths support this picture. They synthesized PMPC, which has the longer Palmitoyl (C_{16}) chain in the 1-position and the shorter Myristoyl chain (C_{14}) in the 2-position, and MPPC, which has the chains reversed. PMPC has a T_M at 27.2°C and MPPC has a T_M at 35.5°C . The most relevant temperature for comparison is the T_M of DC_{15}PC , which is 33.7°C , because DC_{15}PC , PMPC, and MPPC all have the same number of CH_2 groups, and the odd-even effect in lecithins is less than 0.5°C (7). (Less relevant is the midpoint of the two-phase transition region of equimolar DMPC–DPPC mixtures, which, nevertheless, has nearly the same temperature.) According to the picture presented above, the fact that PMPC has a lower T_M than DC_{15}PC is consistent with a greater degree of disordering in the low temperature phase because of packing perturbations due to the greater disparity in penetration of the 1 vs 2-chains, as compared to DC_{15}PC . In contrast, in MPPC the 1 and 2-chains should penetrate a nearly equal distance into the bilayer. Accordingly, T_M for MPPC should be greater than for DC_{15}PC , as observed. The consistency of these results is very encouraging. Equally encouraging is the fact that the inequivalence of the 1 and 2-chains in DC_nPC clearly plays only a secondary, perturbative role in the phase transition, as evidenced by the small changes in T_M which are only about 2 K, compared to a base of about 300 K. Keough & Davis (20) also point out that the transition enthalpy ΔH is not drastically affected by the inequivalence of the chains. Finally, because of the closer equivalence of the chains, MPPC might be the experimental system of choice to help simplify theoretical analysis.

Another potential perturbation on anisotropic chain models of the phase transition is that the chains are tied together in pairs by the glycerol backbone. However, Kreissler & Bothorel (21) have done a conformational analysis that shows that the glycerol moiety is quite flexible and allows, with little increase in energy, the pair of chains on a molecule to increase their separation by the same distances as do average neighboring pairs of chains on different molecules during the LBPT. Thus, this pairing would appear not to be a serious constraint and, indeed, a rigid pairing constraint may be a poorer model for the phase transition than no pairing constraint at all. However, the pairing constraint does help to anchor the ends of the chains to a narrower interfacial region than would be the case with single-chain molecules because the increase in water-hydrocarbon contact area, and hence free energy (see below), is greater for di-chain molecules undergoing fluctuating displacements perpendicular to the bilayer than for single-chain molecules.

Role of H_2O in the Transition

Because water is so vital to biological membranes and the hydrophobic effect is so important in maintaining lipid bilayer integrity, the idea that water plays only a minor role in models of the phase transition may seem unbalanced. However, the bilayer remains intact on both sides of the transition and so the hydrophobic interactions are nearly, although not entirely, unchanged by the transition. Their main effect is to keep the bilayer intact and this is automatically included in an anisotropic chain-melting model without explicit inclusion of a water term. An analogy to this is provided by theories of magnetic phase transitions in insulating solids. There, the strongest interactions are the chemical bonds that form the crystal structure. Theories of magnetic phase transitions assume this structure, much as we assume the bilayer structure in the LBPT, and concentrate upon the much weaker magnetic interactions that cause the transitions (22).

One important role played by water in the LBPT of multilamellar dispersions is to keep adjacent bilayers apart so as to reduce interactions between bilayers. The fact that fully hydrated multilamellar dispersions and single bilayers have the same phase transition temperature (23, 24) supports the assumption that interactions between bilayers can be neglected in models of the phase transition in dispersions. The theory of long-range van der Waals forces also supports this conclusion. For typical water spacings d_w of 20 Å, the Hamaker constants and interaction formulae given by Nir (25) yield a total interaction of about -0.07 kcal/mole. From X-ray studies (26) this spacing d_w only changes by about $+5$ Å during the transition so the total change in van der Waals interaction energy between adjacent bilayers is only about $+0.02$ kcal/mole. The criterion for declaring this to be a negligible interaction in models of the phase transition is that this change in energy is very small compared to the observed transition enthalpy ΔH of about 8.7 kcal/mole for dipalmitoyl lecithin (14). Furthermore, with a water layer ~ 20 Å thick between bilayers, dipolar interactions between headgroups on adjacent bilayers will be negligible compared to ΔH . Of course, as the system is dehydrated, such interactions as well as hydration forces will become stronger as evidenced by the observed changes in T_M (6a, 14), which are nevertheless modest on an absolute temperature scale.

There remains a residual effect of water that requires a specific energy contribution in models. As the bilayer is heated through the transition, the disordering of the hydrocarbon chains decreases the thickness of the bilayer, d . According to X-ray studies (26, 27), d decreases by about 20% for the lecithins. Our density studies find a

volume increase of about 3 to 4%, which can be ascribed wholly to about a 5% increase in the volume of the hydrocarbon chain region (7). Therefore, the area per lipid molecule must increase by about $\Delta A \sim 25\%$ at the main transition (28). Using a surface tension argument one would suppose that the surface free energy would increase by

$$\Delta F = \gamma \Delta A. \quad 2.$$

The quantity γ was interpreted by this author (29) as the surface tension γ_{BLM} , which has been measured as about 1 to 2 dyn/cm in black lipid membranes. However, this is not appropriate because in the BLM measurements the annulus contains a reservoir of lipid, so the measurement of γ_{BLM} only gives the free energy increase of new surface with constant area/molecule, A . Evans & Waugh (30) have suggested a way to derive γ in Eq. 2. Their calculation uses the experimentally determined (31) free energy of transfer of hydrocarbons to water of 25 cal/mole/Å², although this value has been criticized as giving only 18 dyn/cm for the surface tension of an oil/water interface instead of the observed 50 dyn/cm (6b). The idea is that increases in A expose hydrocarbons to water. Evans & Waugh assume that area increases expose hemispherical cavities, which make the exposed area equal to $2\Delta A$. However, such cavities are not very large and hydrostatic pressure alone will not force water into them, so I would replace the factor of 2 obtained from the hemispherical geometry by 1. This yields $\gamma \approx 18$ dyn/cm for each surface. Moreover, the area exposed may be occupied by carbonyl groups rather than hydrocarbons, so even this value of γ may be an overestimate. To obtain a feeling for the importance of Eq. 2 in a model of the LBPT, consider the magnitude of its change during the transition. For transition changes $\Delta A = 10 \text{ Å}^2/\text{molecule}$ one obtains

$$\begin{aligned} \Delta F &= (15 \text{ dyn/cm})(10 \text{ Å}^2/\text{molecule}) N_{\text{Avogadro}} \\ &= 0.2 \text{ kcal/mole} = \Delta H_W - T\Delta S_W. \end{aligned} \quad 3.$$

According to Tanford (32) ΔH_W is smaller than ΔF_W and negative and most of ΔF_W comes from the $-T\Delta S_W$ term. Thus, both ΔH_W and $T\Delta S_W$ are considerably smaller than the observed $\Delta H = T_M \Delta S \approx 9$ kcal/mole and so it seems that the water term of Eq. 2 plays a fairly modest role in models of the phase transition. This conclusion is supported by specific model calculations (to be discussed below in the section entitled A Simplified Rigorously Solvable Model) that show that Eq. 2 does have a nonnegligible, though secondary, effect upon T_M and the size of the transition.

Terms of the type, Eq. 2, are a source of great confusion because γ represents different interactions in different theories. Parsegian (33)

used it as a catch-all for everything except head group interactions and the energy of the Gouy-Chapman double layer. Marcelja (34) uses it in a much different way, excluding the rotameric energies and the dispersion interactions between chains. Curiously, some authors (35) find the agreement in the two values of $\gamma \approx 18$ dyn/cm to be reassuring. But we would not suggest that the closeness of these two values and of our value of γ obtained above has any significance, because γ has such a different meaning in the different theories.

Intermolecular Interactions

EXCLUDED VOLUME INTERACTIONS At very low temperatures each hydrocarbon chain is most likely to be in the low energy all-trans conformation, even if the molecules were isolated. If there were no intermolecular interactions, then as T is increased each chain would rotamerically disorder only very gradually; this behavior is not a phase transition which, by definition, has sharp anomalies in thermodynamic functions. However, lipid molecules that are packed into a bilayer are not free to disorder gradually. In particular, if one molecule attempts to rotate one of its hydrocarbon chains about a C-C bond, then the free, terminal methyl, end of the chain will bump into neighboring chains, which are only about 4.8 Å distant in the low T phase of the bilayer. Thus, chain rotation is forced to be a cooperative event, involving many molecules. This gives rise to a sharp anomaly, i.e. phase transition. In contrast, phospholipid bilayers with high cholesterol concentration do not have a phase transition, but only exhibit gradual disordering. This is understandable because the fairly rigid backbone of cholesterol molecules effectively isolates the phospholipid molecules from each other. From this description it is clear that the excluded volume interaction between hydrocarbon chains is crucial in order to obtain a sharp, cooperative phase transition. So saying, it then follows that this is a very difficult statistical mechanical problem.

VAN DER WAALS INTERACTIONS BETWEEN HYDROCARBON CHAINS Although the hydrophobic hydrocarbon-water interaction is primarily responsible for forming the bilayer, once it is formed other intermolecular interactions are more important in determining molecular packing. The excluded volume effect places a lower bound on the volume, but this lower bound is not achieved except at $T=0$ because of thermal agitation, which tends to expand the volume. This expansion is constrained, not only by the hydrophobic effect, but by attractive van der Waals interactions between the chains. Indeed, for the densities of the bilayer in the vicinity of the phase transition, the van der Waals

interaction dominates the hydrophobic effect. In particular, our calculations (7) of the contributions of the van der Waals interactions to the enthalpy of transition ΔH_{vdW} show that $\Delta H_{vdW} \approx 5.5$ kcal/mole for DPPC whereas ΔH_{H_2O} (see above) is only about 0.2 kcal/mole.

The calculation (7, 82) of ΔH_{vdW} is a key to a detailed understanding of the thermodynamics of the bilayer phase transition as we show in the section, Tests of the General Model. The calculation does not use any sophisticated statistical mechanics. The primary input is the measurement (7) of the hydrocarbon volume per CH_2 group v_{CH_2} in bilayers, which we have shown to be separable from the headgroup volume. From v_{CH_2} we can estimate *average* (see below) intermolecular chain separations, r_b below and r_a above T_M . These average separations are then used in a formula for van der Waals energies, such as the one given by Salem (36)

$$U_{vdW} = (1.84 \text{ kcal/4}) \left[(r_0/r)^{25} - 5(r_0/r)^5 \right] \quad 4.$$

which conforms to the very thorough experimental work of Billmeyer (37) on long chain alkanes, which establishes the overall strength of the parameter

$$a_{vdW} = 1.84 \text{ kcal/mole CH}_2. \quad 5.$$

Because Salem's derivation of the van der Waals interaction has been criticized (38, 39), we emphasize that Eq. 4 can be thought of as a phenomenological equation with a_{vdW} determined by experiment. Consideration of more sophisticated theories, discussed by Langbein (40), gives only minor corrections to the r^{-5} attractive term in Eq. 4. The most uncertain feature of this formula is the repulsive $(r_0/r)^{25}$ part. However, due to the larger number of close contacts in the high temperature disordered phase, where the chains become entangled, we do not use the repulsive part in computing ΔH_{vdW} .

Although our procedure is straightforward, it is an approximation, especially above T_M in the disordered phase, because of the use of average intermolecular distances instead of detailed intermolecular correlation functions, which are not available from experiment. However, this calculational procedure passes a very important, independent test: It balances the energy equation for polyethylene and the long chain alkanes for which all the other terms in the equation are known. This is discussed further below.

Marsh (87, 88) has also performed calculations of ΔU_{vdW} , which are similar in spirit to our calculations (7, 82) but which involve considerable differences in detail. He uses an average separation of chains in the high temperature state, $r_a = 5.31 \text{ \AA}$ (87) or $r_a = 5.47 \text{ \AA}$ (88), the latter

value being obtained from the ratio of areas/molecule, namely $(r_a/r_b)^2 = A_a/A_b = 1.25$. This corresponds to a rather unlikely high temperature reference state consisting of straight trans-chains with volumes $V_a/V_b = A_a/A_b = 1.25$ instead of the measured $V_a/V_b = 1.04$. With this choice the change in just the attractive part of the van der Waals energy $\Delta U_{vdW, att}$ is then about 20 kcal/mole for DPPC. To reduce this to a more reasonable value, Marsh neglects the repulsive part of the interaction for the high temperature state but not for the low temperature state, which reduces ΔU_{vdW} to a value ~ 6 kcal for DPPC quite close to ours (7, 82). However, if the repulsive interactions for the high temperature reference state were included, as seems proper, then Marsh's ΔU_{vdW} would be increased by about 4 kcal, which would make it even larger than ΔH . Thus, the appropriate way (7, 82) to obtain r_a for an all-trans reference state is from the volume change, $(r_a/r_b)^2 = V_a/V_b$, rather than from the area change. Although our high temperature reference state has the wrong area/molecule, actual disordered states with the same volumes as the reference state will have the correct area. Because van der Waals internal energy should depend more upon a bulk property like density than upon a surface property like area, the energies of the actual high temperature states should be more accurately approximated by using our reference state than ones like Marsh's, which have different densities.

INTERACTIONS BETWEEN HEAD GROUPS; CHAIN TILTING Besides the interactions between the polyethylene hydrocarbon chains, there are clearly a number of interactions between the other parts of the lipid molecules, which include the carbonyl groups, the glycerol linkage, and, in lecithins, the phosphatidylcholine head group. These interactions include 1. electrostatic dipolar and, in the case of charged lipids, monopolar interactions, 2. excluded volume interactions, 3. weak hydrogen bonding between phosphate, ethanolamine, etc groups, and 4. nonspecific van der Waals interactions, which, however, when the water is considered, are likely to be more or less constant as the bilayer state changes. Comparative studies of lipid bilayers with different headgroups illuminate the kinds of effects such interactions can have. The T_M s of lipids with different headgroups (41) typically change by 20 K, which is important biologically. However, this is small on a fundamental Kelvin temperature scale: it is less than is obtained by unsaturating one C-C bond in the hydrocarbon chain and it is comparable to adding only two CH_2 groups to the hydrocarbon chains. Therefore, one should not overestimate the importance of headgroup interactions to the phase transition.

Probably the most thoroughly studied head group variation is the removal of the head group methyls of the PCs (phosphatidylcholines) to form PEs (phosphatidylethanolamines) when all methyls are removed or intermediate compounds when removal is incomplete. This has the effect of raising T_M by about 7 K for each methyl removed [Vaughan & Keough (42)], or about 20 K difference in T_M between PCs and PEs with the same length hydrocarbon chains. (The changes in ΔH and ΔV are less systematic.) A few years ago it was believed that the T_M difference between PCs and PEs was due to differences in head group conformations (43) which would then make a difference in the dipolar interactions 1 between *PE* and *PC*. However, evidence is now very strong that the headgroup conformations are nearly the same in PCs and PEs with the zwitterionic dipole lying in the plane of the bilayer (44, 45). With this conformation the head groups in the gel phase are in close contact and this suggests that the repulsive interactions 2 may be important. In particular, in the gel phase of PCs the tilt in the molecules seen by X-ray studies can be attributed to this interaction. The argument is that the repulsive headgroup interaction prevents molecules that are perpendicular to the bilayer from coming close enough together to minimize the van der Waals interactions between the hydrocarbon chains; however, by tilting the molecules the headgroups remain separated but the all-trans chains can be brought closer together. Recent X-ray studies (46) show that there is no tilt in the PEs, which is consistent with the smaller headgroup, and indicate that it is the headgroup region and not, as I once suggested (29), the glycerol region that is responsible for the tilt.

This chain tilting below T_M for the PCs and the excluded volume interaction 2 leads to a general model of the phase transition only slightly different from one in which the chains are perpendicular to the bilayer. In particular, the chain packing as measured by X-ray studies (46) and dilatometry (7, 47) is much the same in PEs and PCs. Thus, although the larger excluded volume interaction in the PCs explains the chain tilt, it does not explain the lower T_M compared to the PEs. It has been my contention (29) that the T_M difference is due to weak and transient hydrogen bonding (only about 3% of all molecules bonded at any one time, so there is only a small effect on lateral mobility) between the phosphate and ethanolamine groups in the PEs, which inhibits the lateral expansion of the bilayer (a necessary feature of the phase transition); this hydrogen bonding thereby raises T_M in the PEs compared to the PCs. Therefore, in this statistical model of the phase transition the excluded volume interaction of the headgroups in the PCs and the tilt below T_M are not highly significant variables, but hydrogen

bonding (interaction 3) is a significant variable in the PEs. Unfortunately, hydrogen bonding is a rather specific and highly directional interaction that is hard to model in a way amenable to calculation. This reviewer (29) has used a simple approximate form

$$U_{\text{head}} = -a_{\text{head}} A^{-c} \quad 6.$$

where A is area/molecule and a large value of c in the range 4–7 mimics the short-range nature of hydrogen bonding. In contrast, for electrostatic monopole interactions c is chosen to be 1/2, and for dipolar interactions 3/2.

In contrast, above T_M a cooperative tilting of the chains near the glycerol groups *could* be a highly significant variable in modeling the transition. However, above T_M the area/molecule A is increased by about 25% (28), so such a tilt would no longer be due to repulsive interactions between groups. The suggestion for such a cooperative tilt was made by McFarland & McConnell (48) in connection with their ESR measurements, which indicated a flexibility gradient with the terminal methyl end of the chains being more disordered than the end anchored to the glycerol groups at the interface. If one assumes (*a*) that the terminal methyl ends are all located nearly in the central plane of the bilayer and (*b*) that the chains run perpendicular to the membrane with no cooperative tilt, then the chain density must be greater nearer the more disordered center than near the more ordered interface. This absurd conclusion can be neatly avoided by dropping assumption *b* and invoking a cooperative tilt in the more ordered portions of the chains. A second way to avoid the absurd conclusion is to drop assumption *a* as has been recently discussed by Gruen (49). [Dropping assumption *a* allows the variation in the order parameter along the chain to be different from the variation in order across the bilayer. In particular, the disorder across the bilayer may be nearly constant whereas the disorder along the chain may increase toward the terminal methyl end; the density may remain constant and no tilt is required. This may be accomplished by intimate mixing of highly disordered chains with terminal methyl ends close to the glycerol group and more ordered chains with terminal methyls further from the glycerol groups (49).] However, the experimental flexibility gradient and chain-tilting result has been disputed by Seelig and co-workers on the basis of NMR of selectively deuterated lipids. Seelig (50) found a nearly constant amount of disorder along most of the chain, and this is consistent with assumptions *a* and *b*, and a constant density, as was pointed out by de Gennes (51). But McConnell (52) and more recently Petersen & Chan (53) have countered with the argument that the cooperative tilt reorients on a time

scale that is fast for NMR studies, but slow enough to be seen by ESR studies. The question of tilting is a thorny one and most theorists have ignored it, partly from lack of belief or, in this reviewer's case, because it complicates an already difficult theoretical problem.

Another interesting way to vary headgroup interactions is to titrate lipids with dissociable headgroups, such as dipalmitoyl phosphatidic acid, from the singly charged state DPPA^{1-} to the doubly charged state DPPA^{2-} [Trauble & Eibl (54), Jacobson & Papahadjopoulos (55)]. This lowers T_M by about 20 K (56). Trauble & Eibl (54) offered a theory for this change in T_M based on the Gouy-Chapman double layer energy. However, if one uses the proper free energies per mole instead of the free energy per cm^2 used by Trauble & Eibl, then the surface energy does not change with A , area/molecule, and so their explanation is irrelevant to the lowering of T_M . Subsequent papers [Jähnig (57), Trauble, Teubner, Woolley & Eibl (58a)] found a dependence of the double layer entropy upon A . But a more relevant interaction that gives a larger free energy change at the transition is the direct repulsive electrostatic charge-charge interaction between the discrete charges of the lipid molecules in the membrane (29, 58b). Another revealing feature of the phosphatidic acid PA systems is that the T_M of even doubly ionized PA^{2-} s are higher than the PCs and comparable to the T_M s of the PEs with identical hydrocarbon chains. This implicates weak and transient hydrogen bonding between the phosphates, perhaps mediated by water molecules, for the PAs (29). Such hydrogen bonding would be reduced in the PCs by the steric restrictions imposed by the choline groups.

Interactions Between the Two Monolayers of a Bilayer: Connection to Monolayer Studies

After temperature and entropy, the most important thermodynamic variable for the LBPT is the area/molecule, A , which is strongly and intimately affected by the phase transition. The conjugate thermodynamic variable to A is the surface pressure, $\pi = -(\partial F / \partial A)_T$. We emphasize that π is an externally imposed surface pressure. Unfortunately, it has not been possible thus far to impose an external π upon lipid bilayers and so $\pi=0$ identically. The reason this is unfortunate is that it prevents exploration of the full phase diagram, which could reveal critical points, or other features that could test theories and suggest analogies to phase transitions in other systems. In this regard, the conjugate pair (pressure, volume) is rather uninteresting; raising the pressure P merely increases T_M according to the Clausius-Clapeyron equation (59). As we shall discuss in more detail later, A is likely to be closely connected to the order parameter of the system whereas V is not.

[We use the term "order parameter" in the sense that it was introduced by Landau for phase transition (60) where it is identically zero in the high T disordered phase; this is different from the way it is used in resonance work (61).]

In contrast to work with bilayers, it is standard practice to impose variable surface pressures π on lipid monolayers at air/water interfaces (62–65) and more recently at oil/water interfaces (66, 67). A wealth of phase transition behavior is observed. It has long been felt that the monolayer studies reveal important information relevant to the bilayer phase transition, although this has been challenged by Gershfeld (68, 69) on the ground that lipid monolayers below T_M are metastable or even unstable for $\pi > \pi_{\text{equilibrium spreading}} \approx 0$ dyn/cm. However, metastable systems (such as, for a trivial example, xenon in a pressure cell) can be very useful to thermodynamic investigation provided that one does not wait too long to do the experiment (i.e. before the gas leaks out) and provided, of course, that the metastable behavior lasts long enough to perform measurements. Most workers have found this to be the case for lipid monolayers, and so we will not be concerned with Gershfeld's criticism.

Because monolayers and bilayers are quite different systems, it is not a priori obvious that their behavior should have more than a qualitative similarity. But let us try a hypothesis (29) that will allow a quantitative correspondence to be made and tested. Suppose that a bilayer is just two weakly coupled monolayers in the following sense. Imagine a dividing plane in the middle of the bilayer that separates the two monolayers; that is, the chains in the lipid molecules are assumed to cross the dividing plane only very seldom. Thus, the interaction of a hydrocarbon chain with chains in the opposite monolayer can be replaced by a nearly constant nonspecific interaction energy that depends primarily upon density that is not strongly varying even at T_M . This assumption is clearly suspect for mixtures of lipids with different chain lengths or for molecules where the chains are very much inequivalent with respect to penetration into the bilayer in the low T phase, but let us pursue it for lipids such as DPPC or MPPC (see Other Intramolecular Features, above). Next, consider a monolayer at the air/water (a/w) interface. The interface between the air and the hydrocarbon chains should be fairly smooth, in order to minimize the energy (maximize van der Waals contacts), and so should look like those monolayers which form half a bilayer. Thus, it is possible that there are corresponding states in the two systems. But what is the value of the surface pressure, which we call π_B , that must be applied to the monolayers in order that the state correspond to the bilayer at $\pi=0$? Clearly, the

monolayer at the a/w interface has an extra hydrocarbon/air interface not shared by the bilayer. It can be estimated that this interface costs about $20 \text{ ergs/cm}^2 = 20 \text{ dyn/cm}$ from octane-hexane/air interfaces, although the hydrocarbon in the lipid monolayer is somewhat more ordered than liquid oils. Thus, with our assumption of weakly coupled monolayers, the interaction between the two monolayers in a bilayer can be mimicked in a monolayer at the a/w interface by $\pi_B = -20 \text{ dyn/cm}$. However, this is not the whole story, because monolayer surface pressures also include the surface tension of the a/w interface on the other side of the barrier, which is about 70 dyn/cm , so (29),

$$\pi_B = +70 - 20 = +50 \text{ dyn/cm}.$$

[Also, if one assumes that monolayers at the o/w (oil/water) interface are analogous to lipid bilayers, the same analysis may be used to give $\pi_B = +50 - 0 = 50 \text{ dyn/cm}$.] The result, $\pi_B = 50 \text{ dyn/cm}$, has been used by a number of authors (29, 49, 70, 71a, 71b).

The conclusion that $\pi_B = 50 \text{ dyn/cm}$ in order that monolayer states correspond to bilayer states passes one very important test, namely, the transition T_M for monolayers at π_B is essentially the same as T_M for bilayers (64, 72, 73). Unfortunately, it fails another test, namely, at π_B the transition changes ΔA , ΔH are much smaller in monolayers than in bilayers. This has prompted an alternative choice of correspondence between monolayers and bilayers (34, 65), namely, choosing $\pi_B = 20 \text{ dyn/cm}$ such that ΔA and ΔH are the same, even though the transition temperatures do not agree. However, this latter procedure is somewhat uncertain because the monolayer isotherms are far from flat and ΔA , and especially ΔH , are hard to measure.

The most challenging obstacle in establishing a correspondence between monolayer and bilayer phase transitions is the nonflat monolayer isotherms, which, taken literally, are inconsistent with the first order transition seen in bilayers made with the purest lipid (74). Albrecht et al (65) (in Sec. 4.5 of their paper) argue that the isotherms are not flat because of limited regions of cooperativity and they calculate about 80 to 190 molecules in a cooperative unit. Although the theory behind the calculation is very crude, the idea that some nonessential irregularity associated with the surface phase disrupts the cooperativity of the transition is appealing.

In contrast to lipid monolayers at the a/w interface, monolayers at the o/w interface exhibit much more nearly flat isotherms (66, 67) and it has been argued that the o/w films are better models for bilayers than a/w films (75). A priori this would not seem to be reasonable because

the very important van der Waals interactions between hydrocarbon chains, which are attractive and decrease A , are effectively reduced at the o/w interface. This follows because oil molecules can enter between two adjacent chains that are pulled apart laterally and the energy of such a state is not increased as much as if no oil molecules could enter, as at the a/w interface or in bilayers. Accordingly, the disordered phase of monolayers at the o/w interface ought to be much more expanded than at the a/w interface, and this is observed (66, 67). On the other hand, it is interesting that the ΔA (extrapolated) at $\pi_B = 50$ dyn/cm is much closer to that for bilayers. However, the T_M (extrapolated) is about 15°C too low and so monolayers at the o/w interface are not phenomenologically ideal analogues either, and they suffer from the theoretical complication of having another molecular species in the system. Indeed, there is a considerable difference in the $\pi-A$ isotherms if *n*-heptane is used for the oil phase (66, 67) or if 2, 2, 4 trimethylpentane is used (76).

Although the connection between monolayer and bilayer measurements is certainly not unequivocal, the fact that both undergo a phase transition at roughly similar temperatures and surface pressures is consistent with the working hypothesis that neglects specific interactions (in contrast to surface free energy terms of the type already discussed) between the two monolayers that comprise a bilayer when the two chains are nearly equivalent.

Communal Entropy, Vibrational Energies, and Kinetic Effects

In this section, we briefly argue that many degrees of freedom of the lipid bilayer are unimportant for the phase transition. For example, the fact that lipid molecules are much more laterally mobile in the disordered biological phase is an important and much studied characteristic (77). However, this extra degree of freedom of the lipid molecules is not a primary determinant of the phase transition, but a consequence of it. Historically, the concept of communal entropy has been used to quantify this degree of freedom. It is doubtful that the communal entropy is important even for simple monatomic liquids (78) and, for molecules with large numbers of internal degrees of freedom such as lipids or polymers, it is clear that it is not important (79).

Next, one might argue that the molecular vibrational energies are greater in the disordered phase than in the ordered phase because the volume expansion allows the molecules to vibrate with larger amplitudes before suffering hard collisions. To obtain an estimate of this effect

consider a harmonic potential for just one degree of freedom,

$$U(x) = \begin{cases} ax^2, & |x| \leq x_o \\ \infty, & |x| > x_o \end{cases} \quad 7.$$

with a hard barrier at x_o . It is easy to show that

$$\bar{U} = (kT/2) \left\{ 1 - e^{-y} \left[\sum (-y)^n / n! (2n+1) \right]^{-1} \right\} \quad 8.$$

where $y = ax_o^2/kT$. At the phase transition a volume increase of 4% corresponds to an increase Δx_o of 4/300. $\Delta \bar{U}$ depends upon the size of y . If y is large, which is the case for most vibrations, then $\Delta \bar{U} \approx 0$. But even a small rotation around a C-C bond near the head group will result in a hard collision, so for the rotameric degrees of freedom ax_o^2 is small compared to kT in which case

$$\Delta U_{vib} \approx (a/e) \Delta x_o^2 < (2/3)(4/300) \approx 4.8 \text{ cal.}$$

Even for 28 degrees of freedom $\Delta U_{vib} \approx 0.15$ kcal which is small compared to $\Delta H = 8.7$ kcal.

Another possible objection is that the trans and gauche rotameric basis states are not quantum mechanical eigenstates. A simple estimate of the differences in energy of the true eigenstates compared to the rotameric basis states if $\Delta E = \hbar/\tau_c$ where τ_c is the lingering time in the rotameric basis state and should be related to the correlation time for trans-gauche isomerization. Even if τ_c is as short as 10^{-10} s, ΔE is less than 1/100 of $\epsilon = 0.5$ kcal, so the rotameric model is virtually unaffected by this consideration.

Finally, it has been suggested (80, 81) that there is a difference in translational free energy in the two phases on the grounds that in the high temperature disordered phase the molecules have a larger free area, $A_{free} = A - A_o$, in which to move. This leads Belle & Bothorel (80, 81) to the addition of a term $F_{translational}$, which is the free energy of a particle moving in a two-dimensional box of size $A - A_o$. A criticism of this procedure is that A_o in the disordered state with gauche rotations is clearly larger than A_o in the all-trans state, which suggests that A_{free} is not much different for the two condensed phases and would only become a significant factor for more dilute vapor phases such as those that occur in monolayers.

TESTS OF THE GENERAL MODEL

In this section two simple thermodynamic tests of the General Model and a third test that appears to rule out a pure kink model are

described. Although tests automatically follow from a complete statistical mechanical solution, in practice the approximations employed obscure the validity of the tests. Consequently, it is advisable (and common practice in many areas of research) in modeling phase transitions to employ as many simple tests as possible before expending considerable effort in extensive statistical mechanical calculations. Furthermore, as we shall see, additional information about the states of the system emerges from such tests.

The Energy Test

The measured transition enthalpy ΔH is related to the various internal energy changes ΔU_i of a model by an energy equation,

$$\Delta H = P\Delta V + \sum_i \Delta U_i \quad 9.$$

where the $P\Delta V$ term is negligible for the LBPT. For molecules as complicated as lipids there are many different internal energies, U_i , $i=1, \dots$. Omission of important terms in the Hamiltonian of the system, such as the omission of the van der Waals interactions between hydrocarbon chains, can make it impossible to balance Eq. 9 (82). Furthermore, the relative size of the ΔU_i (not the U_i 's themselves) is an indication of the importance of the i th term of the Hamiltonian to the LBPT. (Hard core repulsions are an exception which give $\Delta U_{\text{hard core}}=0$, but these are still vitally important because they restrict degrees of freedom and keep the system ordered in the low temperature phase.)

The most obvious ΔU_i is due to the increase in rotameric degrees of freedom, $\Delta U_{\text{rot}} = \epsilon \Delta n_g$ where Δn_g is the increase in numbers of gauche rotamers with energy $\epsilon=0.5$ kcal/mole (9). In addition our calculations [see section on Van der Waals interactions between hydrocarbon chains and (7)], based on measured volume changes, show that ΔU_{vdW} for DPPC is about 5.5 kcal/mole. Other internal energy changes, ΔU_{other} , such as those arising from headgroup interactions, are hard to estimate accurately, but it is clear (see above) that they are much smaller than ΔU_{vdW} or ΔU_{rot} . In our study (7) we set $\Delta U_{\text{other}}=0.4$ kcal/mole for DPPC and this balances the energy Eq. 9 with $\Delta n_g=5.6$. This value of Δn_g is safely less than the value of $n_g=12$ for DPPC molecules in the vapor. Thus, the general model passes the energy test. Furthermore, this test provides an estimate of Δn_g which is an important characteristic of the difference between the states of the lipid bilayer above and below the LBPT (83).

Although the general model passes the energy test, the test itself required a rough guess of ΔU_{other} and a crude calculation of ΔU_{vdW} .

Therefore, to test the test, it was applied to phase transitions in long chain alkanes (paraffins) and polyethylene (7). The advantage of these two systems over lipids is the lack of headgroups and water interactions and the result that Δn_g is very nearly the same as in the vapor (9). In these simpler systems the energy equation balanced to within 5%, thereby supporting our calculation of ΔU_{odw} .

The Entropy Test

Even though the transition entropy ΔS and the transition enthalpy ΔH are closely related by $\Delta H = T_M \Delta S$, the entropy test is quite different from the energy-enthalpy test. The most obvious contribution to the entropy increase at the transition comes from the rotameric disorder. Let $W(n_g)$ be the number of ways of placing n_g gauche rotations on the hydrocarbon chains of a single molecule and the related entropy is $S_{rot}(n_g) = R \ln W(n_g)$. If one assumes complete freedom in placing the two gauche rotations on each of the 28 rotatable bonds of DPPC, then

$$W(n_g) = 2^{n_g} \frac{28!}{n_g! (28 - n_g)!} \quad 10.$$

A first approximation to the transition entropy is $[S(n_g = 7.6) - S(n_g = 0)]/R \simeq 20$ where the value of $n_g = 7.6$ in the high temperature phase comes from the sum of $\Delta n_g = 5.6$ obtained from the energy test (7) and $n_g = 2$ in the low temperature phase from Raman studies (84). The reason we do not use $S(n_g = 2)$ for the low temperature phase is that the gauche rotations there are probably closely coupled in the form of kinks and these are probably restricted to localized parts of the chains, especially near the ends (18). This first approximation to ΔS is safely larger than the experimental DPPC value $\Delta S/R \simeq 14$, thus indicating that the model has enough degrees of freedom. The fact that it is larger is not surprising because the approximation neglected excluded volume interactions between neighboring molecules which will considerably reduce the actual number of permissible configurations below that given by Eq. 10, although one must also consider that the possibility that neighboring molecules having different n_g s will increase ΔS . Unfortunately, these corrections are extremely difficult to estimate short of a complete statistical mechanical calculation and this circumstance reduces the value of the entropy test.

In addition there may be other contributions ΔS_{other} to the transition entropy, e.g. from different vibrational states of disordered molecules (which is probably a small effect) and the larger volume in which they can move. A rough way to estimate ΔS_{other} for polymer systems has been developed by Tonelli (85), who followed a general procedure of

Slater (86). The idea is to use the volume as the independent variable upon which ΔS_{other} depends, so that ΔS_{other} is proportional to ΔV . The coefficient of proportionality is approximated from the single phases as

$$\left(\frac{\partial S}{\partial V}\right)_T = \left(\frac{\partial P}{\partial T}\right)_V = -\left(\frac{\partial V}{\partial T}\right)_P \left(\frac{\partial P}{\partial V}\right)_T = \alpha/\beta$$

so

$$\Delta S_{other} = (\alpha/\beta) \Delta V. \quad 11.$$

In the case of polyethylene, Tonelli (85) estimated that ΔS_{other} amounts to about 25% of ΔS . For lipids below T_M , $\alpha \approx 10^{-3} \text{ cm}^3/\text{deg}$ (7). Unfortunately, β is not well determined, but from measurements of Liu & Kay (59) it is about $4 \times 10^3 \text{ atm/ml}$ for DPPC, which is comparable to the value of $7 \times 10^3 \text{ atm/ml}$ for polyethylene. Thus, one can estimate $\Delta S_{other}/R = 2.3$ or about 16% of $\Delta S_{total}/R$. Therefore, the amount of rotameric entropy $\Delta S_{rot}/R$ need only be comparable to $[\Delta S - \Delta S_{other}]/R = 12$. Incidentally, this term ΔS_{other} would appear as an extra contribution to the free energy in a way which is qualitatively similar (as a function of V rather than A) but smaller than the F_{trans} proposed by Belle & Bothorel (80, 81). However, again it must be emphasized that this may be an overestimate of F_{trans} because a few close contacts in the disordered phase would prevent translational motion even though there is a larger volume per molecule. In conclusion the model still appears to pass this rather inexact entropy test whether ΔS_{other} is considered or not.

Test of the Kink Model

The general model of lipid bilayers was modified by Trauble (89, 90), influenced by Blasenbrey & Pechhold's theory of polymers (91). The hypothesis is that the excluded volume interaction restricts the disordered rotameric states to those which have only "kinks" consisting of three successive $g \pm tg \mp$ rotations. This hypothesis clearly simplifies the rotameric description. Trauble (89, 90) based his proposal of the kink model on measurements for DPPC of the smallness of the volume change ΔV , which has since been shown to be over 2.5 times as large, and upon a 5 Å thinning, Δl of the membrane, which is now given by Büldt et al (45) as 6–8 Å and an even larger $\Delta l \sim 8\text{--}13 \text{ Å}$ is shown in Figure 6 in the paper of Janiak et al (26). In the kink theory this thinning Δl is related to the number of kinks k because each kink shortens a straight chain by 1.27 Å. Therefore, if θ_a and θ_b are the tilt angles of the chains above and below the transition, then

$$\Delta l = 1.27 \text{ Å} \times k \times \cos \theta_a + 31 \times 1.27 \text{ Å} \times (\cos \theta_a - \cos \theta_b)$$

where there are effectively 31 C-C intervals for DPPC. Taking $\Delta l = 8 \text{ \AA}$ [from GC3 in Table I of Reference (45)], $\theta_b = 30^\circ$ from X-ray studies (26) and $\theta_a = 30^\circ$ from spin label studies (48) gives $k = 7.3$ kinks or $n_g = 14.6$ gauche rotations (even more than in the vapor phase!) for a $\Delta U_{rot} = 7.3$ kcal. The first problem with this is that there is only 1.4 kcal left in ΔH to go into other forms of internal energy such as ΔU_{vdw} , which is about 5 to 6 kcal. The situation is further worsened, i.e. k increases to 10.5, if one takes Seelig's NMR result that $\theta_a = 0^\circ$ (50). To decrease k to 4, i.e. $n_g = 8$, requires $\theta_a = 40^\circ$. The second problem with the model is that coupling the gauche rotations in the form of kinks considerably reduces ΔS_{rot} , which for isolated molecules of DPPC is given by:

$$S_{rot}/R = \ln[2^k \cdot 24 \cdot 21 \cdots (27 - 3k)/k!]$$

which only equals 14.5 for $k = 7.3$ and is reduced to 11.4 for $k = 4$. Again, these values should be overestimates because kinks on neighboring molecules are not completely independent but will be constrained to fit into a kink-block structure (91, 92) in order to maintain only 4% increase in V .

Thus, it is difficult to see how the pure kink model can simultaneously pass all three tests of energy, entropy, and thinning of the bilayer. The latter test is easily passed by the general model because single gauche rotations are far more effective in shortening the chains. This criticism does not imply that kinks are not present. Indeed, they undoubtedly are the most numerous, though not dominant, type of rotational conformation and one should certainly not proceed to the opposite extreme of assuming that they do not exist, as was done by Rothman (109). In the same spirit as in the kink model Marsh (87) also restricts, although less severely, the permissible isomeric states.

PHILOSOPHY OF STATISTICAL MECHANICAL MODEL CALCULATIONS

The theoretical basis of equilibrium statistical mechanics is as certain as any paradigm in science. Therefore, in principle, given a general model of a system, the phase transition and all thermal properties follow mathematically. Often, there is an uncertainty in the magnitude of some of the basic parameters or interaction strengths and then comparison of the statistical mechanical calculation to experiment can help to refine these, although it must always be kept in mind that a set of experimental thermodynamic data does not uniquely specify a model, especially if the data are limited and if there is a little noise in it. However, in

practice the problem of doing the mathematics to obtain the phase transition properties for interacting systems with phase transitions has only been solved for about a dozen models. It is deemed very unlikely that the mathematics for the general model of the LBPT with rotameric degrees of freedom, excluded volume interactions, and van der Waals interactions can ever be done without resorting to approximations that are essentially uncontrolled in their accuracy. Unfortunately, the history of approximations in statistical mechanics is a rather abysmal one, as judged by comparisons to the few exact solutions and to critical phenomena experiments (93–95). Usually the approximations are quantitatively and qualitatively incorrect and sometimes they even predict phase transitions for systems that do not have them. Thus, there are two uncertainties in the theoretical studies of the LBPT: These may be called the *computational uncertainty* and the *model uncertainty*. At this point a critic might well ask, what value can be derived from statistical mechanical calculations for the LBPT? More precisely, with enough parameters in a complicated interaction Hamiltonian and a large choice of uncontrolled approximations, is there any value in elaborate fitting of experimental data?

In contrast to the preceding phenomenological approach, the modern approach to statistical mechanical calculations for phase transitions is to simplify the general model into a particular model that can be solved exactly or by controlled approximations or at least for which qualitative existence proofs of behavior of interest can be constructed. The success of this approach, which is discussed at some length and very clearly by Fisher (93), depends upon being able to keep the essential features of the general model in the particular model. If this is done, then the particular model is like a cartoon or caricature of the general model. It is easy to study in the sense of being mathematically tractable and, like a good cartoon, it reveals important features even though it may distort them quantitatively. This approach to the LBPT will be reviewed in the next section. The value of it is twofold. First, it gives confidence that the phase transition produced is inherent in the general model and is not just an artifact of the approximation method. Second, as Fisher (93) emphasizes, approximate methods nearly always give so-called classical phase transition behavior, which is an artificially restricted subset of possible behavior only infrequently realized in nature. In contrast, the modern approach to the LBPT reveals unsuspected possibilities for this transition.

The reader will have gathered that this reviewer prefers the modern approach of the last paragraph to the phenomenological approach of fitting data with multiparametered models solved approximately. However, instead of detracting from the phenomenological approach, the

modern approach, by answering fundamental questions, prepares the way for approximate methods by reducing some of the uncertainties in their use, such as the probable existence of a phase transition in the general model. Another value of the modern approach is that the rigorous solution for simple models can be used to test approximation methods by comparing the results for the same simple model. Such comparisons and contrasts should be helpful in gauging the artifacts produced by approximate methods, which can then be applied with more understanding to the unsimplified general model. The results of such comparisons may not be pleasing to practitioners of approximate methods, and so researchers such as Priest (96) and Wiegel (97) who confront the issue are to be commended.

A SIMPLIFIED RIGOROUSLY SOLVABLE MODEL

This reviewer (29, 82, 98, 99) has developed a model of long hydrocarbon chains on a planar lattice in which each C-C bond may be in a ground (trans) state or two excited (gauche \pm) states where the states are distinguished by different directions with respect to the bilayer normal, so the anisotropic constraint discussed above in the General Modeling section is simulated in the rotameric description (see Figure 2). Priest (96) uses this simplified model in two dimensions as the basis for a more phenomenological model applicable to three dimensions. However, Priest chooses to reinterpret the model into a kink model. This gives a transition near 700 K, far higher even than the extrapolated $T_M = 410$ K for infinitely long chains (7), thereby supporting my interpretation that the smallest excitation in the model should correspond to the smallest excitation of the hydrocarbon chain, which is a single gauche rotation and not the doubly rotated kink. The excluded volume interaction is that no two chains occupy the same lattice site in Figure 2. Similar models for polymer systems have been discussed by Flory (100), but his

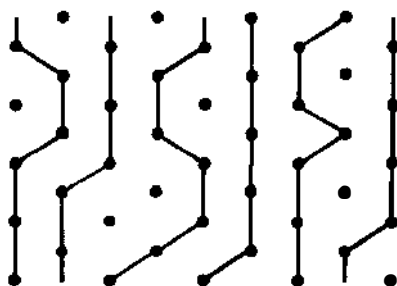


Figure 2 Exactly solvable model of hydrocarbon chains (solid lines) on a hexagonal lattice (dots).

approximate solution has been shown to be substantially in error (101, 102). The difficulty in solving such models is to account for the excluded volume interaction properly; this is done in the simplified LBPT model (82) as well as for some other models of isotropic chain melting (101). The reason that this was possible for these models is that they were designed to be isomorphic to dimer and six-vertex models (22, 95), which may be solved by two of the nontrivial exact computational techniques in the statistical mechanics of cooperative phenomena.

Even when the LBPT model is constrained to maximum density by infinitely strong van der Waals attractive forces the model exhibits a rotameric disordering phase transition. The model also allows for volume expansion, although the attractive van der Waals interactions and also the water interactions must then be treated in an approximate way. The approximation is similar to mean field theory in that it expresses the interaction energy as ensemble averages rather than by the energies of the individual fluctuating microstates. In particular, a term

$$U_{vdW} = -a_{vdW}\rho^b \quad 12.$$

where ρ is the average density is added to the free energy. If $b=1$ then this is equivalent to mean field theory. As Cotter (103) has emphasized, other values of b in Eq. 12 are not strictly the same as self-consistent mean field theory, but such terms are very similar in spirit in the sense of ignoring fluctuations. The reasons I chose to generalize the values of b was to make this ΔU_{vdW} the same as the one used in the energy test (see above). However, the question is not a crucial one because varying b does not change the solution significantly (82). The parameter $a_{vdW} = 5.2$ kcal/mole CH_2 was obtained from the experimental heat of sublimation of hydrocarbon chains, 1.84 kcal/mole CH_2 , extrapolated to $T=0$ K. (By convention $\rho = 1/2$ at 0 K.) However, as Gruen (49) points out, this neglected the volume expansion between 0 K and T_M , which has already done much work to separate the hydrocarbon chains. For a reference state with $\rho = 1/2$ just below T_M the effective a_{vdW} should be reduced by a factor

$$(r_{bo}/r_b)^{2b} = (4.48 \text{ \AA}/4.85 \text{ \AA})^{2b} = 0.89$$

for $b=3/2$ or 0.82 for $b=5/2$. However, this still does not make a_{vdW} as small as the value used by Marcelja (34) and Gruen (49).

Although it would be desirable to treat the long-range van der Waals interactions exactly, this is currently an impossible problem for even a one-dimensional Ising system (104). Wiegell (97) investigates this question in some detail for interactions of the form $\gamma \exp(-\gamma r)$, but in the

end he is only able to recover the general Kac-Uhlenbeck-Hemmer-Baker result, which is identical to mean field theory (94, 95). Incidentally, the chain model with excluded volume interactions in Wiegel's paper (97) is a special case of the rather unrealistic Model B of (82). The development of the statistical mechanics of $a\gamma \exp(-\gamma r)$ potentials is very useful because it emphasizes that mean field theory is exact for very long-range interactions and may be expected to be poorest for short range, e.g. excluded volume interactions, so if it is necessary to use it, the least error will be made if it is used for the attractive van der Waals interactions. Another mitigating circumstance concerns the overall strength of the van der Waals interactions. It is intuitively clear that if an interaction is very weak compared to kT_M , then it is not essential to the phase transition. It is also true that if an interaction is very strong, then it may not play a sensitive role in the transition and can be treated in a less exact fashion. The extreme example is the very strong chemical bonds in a solid that play a very minor role in magnetic transitions. In the LBPT, whereas ΔU_{vdW} is a substantial fraction of ΔH , it is only about 10% of its maximum value, $\Delta U_{sublimation}$. Because the attractive chain-chain interaction is so strong, its primary role is as a cohesive force to keep the bilayer intact. In any case the effect of variations in a_{vdW} and b in the mean field form does not change T_M as much as proportional variations in the trans-gauche energy ϵ . Therefore, several workers have expended more effort to refine the treatment of the excluded volume interactions than of the long-range interactions (80, 82, 92, 105, 106).

Statistical mechanicians often view complex problems such as the LBPT as a one-way street from fundamental theory to application. It was very pleasurable for me to discover (98) that the LBPT illuminated a fundamental problem concerning the apparent lack of an order parameter in the Kasteleyn dimer model, which is the basis of the simplified solvable model of the LBPT. The question arose in the LBPT context because of the analogy to monolayer transitions where the $\pi-A$ variables are so important, and this presented the question of how such variables could be included in the simplified solvable model. Once this was done these $\pi-A$ variables could then be transformed via the isomorphism to the Kasteleyn dimer model in which context they were not obvious enough to have been previously noticed.

Figure 3 shows schematically the monolayer isotherms for the LBPT model in a $\pi-A$ graph; a separable and passive lattice compressibility and expansibility has been assumed as suggested but not shown in (29). The first remarkable feature of Figure 3 is the wedged shape of the coexistence region, which ends in a sharp point instead of being rounded.

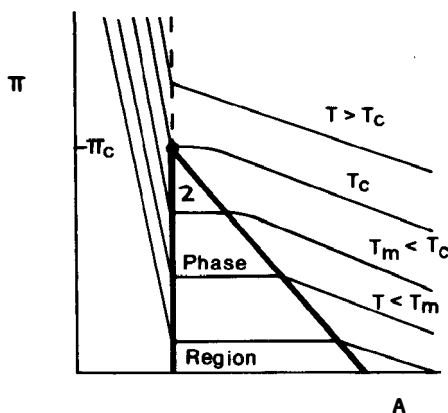


Figure 3 The π - A phase diagram for the model in Section 5. The light solid lines are isotherms, the dashed line is the weak second-order phase line and the wedge shaped two-phase region is delineated by heavy solid lines.

The second remarkable feature is that the transition does not simply end at a typical critical point marked by the end of the coexistence region but continues as a weak second-order transition with discontinuities in the lateral compressibility $K_T = -(\partial A / \partial \pi)_T / A$. Technically, the point labeled T_c, π_c is called a tricritical point (22, 107) although this one is much different from the usual sort (107). [It is interesting that Albrecht et al (65) propose a tricritical point based on their monolayer experiments, although their experimental and theoretical evidence for it is far from compelling.] I have called this point a 3/2 order critical point (83) and its critical exponents are thoroughly discussed in (98, 99). This special point is clearly a product of the all or nothing, infinitely hard excluded volume interaction and the conservation of chain flow (22); this latter property simply means that chains do not end arbitrarily (51). Most likely with a more realistic, softer hard core the second-order line would disappear and the 3/2 order critical point would become an ordinary critical point (29). However, the coexistence curve ought to remain wedge shaped until close to the critical point where it would finally become rounded. Thus, lipid monolayers may be close to having 3/2 order critical points but not quite succeed. This behavior of the model in a π - A diagram is in strong contrast to its behavior in a P - V diagram (99) in which the transition continues as first order with no critical point and with only small increases in T_M as P increases. This contrast conforms very nicely to reality (59, 99). These results also show that the order parameter for the phase transition is the area A . This result is assumed in the completely phenomenological Landau theory of Owicki et al (110), but only with specific model calculations can one show that A is the order parameter and not V or something else. Indeed,

Priest (111) assumes that the order parameter is the number of gauche rotations n_g in his version of a phenomenological Landau theory. However, in the simple model n_g and A are strongly related so the difference in the two phenomenological Landau theories is not so great as might appear. Also, Priest (111) makes considerable contact with more detailed theories in order to obtain the correct form for his parameters.

Where does the LBPT appear in Figure 3? In first approximation lipid bilayers are back-to-back lipid monolayers at surface pressures $\pi_B = 50$ dyn/cm, as given above. Thus, the LBPT occurs when the constant π_B curve in Figure 3 crosses a phase transition line. If $\pi_B < \pi_c$, then the transition is first order. This is different from my first paper (82) where the introduction of the $\pi-A$ variables had not been made and it appeared that the transition required going through the 3/2 order critical point. However, even if $\pi_B > \pi_c$ the effect of the 3/2 order critical point will be felt just as for $\pi_B < \pi_c$ because the transition is very asymmetric with larger tails in the thermodynamic functions in the disordered phase. One piece of evidence for such asymmetric tails appears in the sodium permeability which is related to the lateral compressibility (108). Another is the measured $(\partial V/\partial T)_P$ for DMPC, which shows a gradual decrease (in contrast to the usual increase for most materials) from 10^{-3} ml at 26°C just above T_M to half that at 50°C (7). A gradual decrease in this broad temperature range is much more in keeping with the model results than the original measured asymmetry (83) in the V vs T curve very near T_M , which now appears to be largely (but perhaps not completely) sample dependent (7, 74).

The introduction of the $\pi-A$ variables allows incorporation of mean field type terms that depend upon area in the form of Eq. 6, which is similar to Eq. 12 but with ρ replaced by A . Headgroup interactions and the water term, Eq. 2, have this form. The values of π_c and T_M have significant biological variations with variations in headgroup interactions (even though ΔH_{head} remains less than 0.5 kcal/mole) and so the model easily accounts for variations in the transition for different lipids (29). Also, the water term in Eq. 2 decreases π_c by about 15 dyn/cm (see Role of H_2O in the Transition, above). However, these terms are hard to estimate and, because our statistical mechanical treatment of them is approximate, the most important conclusion that can be drawn is that π_c can be tuned in the range of 0 to 100 dyn/cm with small changes in headgroup interaction strengths and T_M is as close to the T_M of DPPC as one could expect given the 20% uncertainty in ϵ . However, the quantities ΔH , ΔA , and ΔV obtained for this model are much too small (although using the smaller a_{vdW} discussed earlier would improve these

somewhat). Thus, the primary value of this model is to show that rigorous treatment of excluded volume interactions does lead to a rotameric disordering transition and that the transition may have features of $3/2$ order type.

OTHER MODELS AND APPROXIMATE SOLUTIONS

A number of statistical mechanical calculations on simplified models based upon the General Model are reviewed in this section. This reviewer focuses upon the computational methods, the number of phenomenological parameters and their physical content, and the model simplifications and fundamental disagreements with experiment. Quantitative agreement with experiment is usually well discussed in the original papers.

One of the simplest approximate calculations that gives phase transitions is the mean field theory. The theory was developed by Maier & Saupe (112) for liquid crystalline systems that exhibit orientational order. The mean field interaction term was described as an anisotropic van der Waals dispersion energy. However, estimates of the strength of the attractive anisotropic dispersion interaction are too small by an order of magnitude to account for liquid crystal transition temperatures (113, 114). Thus, the justification of the Maier-Saupe calculations is that they also include anisotropic excluded volume interactions. This problem of identifying the terms in mean field Hamiltonians with real interactions in models also arises in Marcelja's adaptation of the Maier-Saupe theory to the LBPT (34). The principal mean field interaction is given as $-V_o(n_{trans}/n_{total})^2\eta^2$, where V_o sets the scale of the interactions and η is the orientational order parameter. The $(n_{trans}/n_{total})^2$ factor has no molecular basis and is added to give agreement with experiment at low temperatures; its presence means that the calculation is not self-consistent mean field theory in the sense of Cotter (103). Although this term has the form of an anisotropic interaction, Marcelja uses it to account for the change in the isotropic van der Waals interaction caused by volume changes (115a). However, this involves a nonobvious and untested relation between the volume change and the order parameter. Furthermore, V_o is evaluated from phase transition properties of alkanes and polyethylene, thus it is less fundamental than if it were evaluated from molecular interactions; indeed this bases the theory of the LBPT upon the similar alkane phase transition. Marcelja (34) introduced a term, PA , where P is determined to be 20 dyn/cm by

fitting the theoretical results to experiment. Although there is a comment in (34) that this term has "its origins in the steric repulsion among hard cores," another comment in (34) and Marcelja's current interpretation relate it to an interfacial water term. Furthermore, the numerical value of P is in general agreement with our analysis in the role of H_2O section, above. However, by elimination the mean field term must then account for the excluded volume interactions: this is open to the objection that mean field theory is best for very long-range interactions and poorest for excluded volume interactions. Despite these criticisms there are two very strong points to Marcelja's paper (34). First, because of the ease of doing mean field theory and because its limitations are well known, such calculations should be performed as a benchmark for any new transition such as the LBPT. Second, the statistics of the individual hydrocarbon chains, though only 10 carbons long, are calculated precisely by Marcelja.

In a recent paper Jahnig (115b) also uses the mean field treatment for interchain interactions and introduces a continuous elastic model to approximate the rotational isomerism in the chains. Jahnig establishes a reasonable case for the validity of his elastic approximation. However, the remainder of the theory is rather unusual because only the order parameter S is allowed to change as a function of T whereas the area A , the thickness $\langle x \rangle$, and the hydrocarbon density ρ are constrained not to change, which is certainly quite different from what happens in the LBPT.

A common theme of many of the remaining papers reviewed in this section is the attempt to treat the excluded volume interaction better than can be done in mean field treatments. Whittington & Chapman (116) performed the very first calculation for lipid chain statistics by placing three chains of length 7–10 carbon atoms on a two-dimensional hexagonal lattice (the same one used for my later calculation in the previous section) with periodic boundary conditions. The chain statistics were sampled using a Monte Carlo technique with area/chain being the primary independent variable. Unfortunately, the calculation did not include any trans-gauche energy ϵ or any attractive interactions, which are necessary for a transition. Much more comprehensive work along the same line, but with the preceding deficiencies repaired, has been performed by Belle & Bothorel (80, 81) and Lemaire (117). Lemaire has devised a cleverly efficient Monte Carlo technique (117) to sample the configurations of a single three-dimensional hydrocarbon chain that interacts with images of itself, i.e. it is a single chain in a periodic three-dimensional box. This approach obviously exaggerates correlations over long distances by eliminating states that have different

conformations on neighboring molecules. But it very specifically takes into account all interactions between nearest neighboring molecules, including both attractive van der Waals and excluded volume interactions, and so it provides the strongest contrast to mean field theory. It is my feeling that the two methods of calculation might reasonably be expected to provide opposing bounds for (i.e. to bracket) various thermodynamic quantities of interest, although neither can be trusted in a critical region. Unfortunately, the two methods have not been applied to the same specific model [e.g. F_{trans} in (80, 81) (see Communal Entropy, Vibrational Energies, and Kinetic Effects, above) is not the same as PA in (34)]. Such a systematic comparison could be rewarding. Another problem with the F_{trans} term used in (80, 81) is that it includes a term $-RT \ln A_{free}$ which becomes infinite for configurations with small A_{free} . Such behavior can only be justified as simulating the effects of repulsive excluded volume interactions. However, because these interactions are already accounted for in the interchain interactions, it would appear that they are being counted twice in (80, 81). It might also be noted that Scott (118, 71b) has also performed Monte Carlo calculations with more than one chain in a periodic box; however, these studies were limited to the high temperature phase. One important conclusion drawn by Scott is that the excluded volume interactions are more important for determining the NMR order parameters than attractive van der Waals interactions.

A noteworthy attempt to treat the excluded volume problem has been made by Kox & Wiegel (119) who use a diffusion equation formalism to show that the problem is isomorphic to the problem of hard quantum disks. Unfortunately, the latter problem is also unsolved and only low density approximations are available. Nevertheless, it is always a step forward to reduce two unsolved problems to one. However, it does seem curious that all traces of the rotational isomerism drop out of the final pressure, density relations, Eq. 23 and 31 of (119).

The procedure of altering the General Model so that the effect of the excluded volume interactions can be related to better understood reference systems has been adopted by Jacobs, Hudson & Andersen (105, 120). The reference they use is that of continuum hard disks in a plane. Although this system has not been solved exactly, molecular dynamics calculations give reasonable perturbation series for the lateral pressure P in terms of the variable $\alpha = (A - A_0)/A_0$. The excluded volume interaction is then accounted for by a term $P\Delta A$. This is similar to Marcelja's form (34); however, here P varies strongly with A in the hard disk reference system, which is an improvement. In the first paper (105) the long-range attractive van der Waals interactions are given by an area-

dependent mean field type term, but this is improved to a density-dependent term in (120), which also includes an area-dependent term to account for head groups and end effects. One sacrifice that was made in the calculation is that the area of a molecule is parameterized from the position of only the first gauche rotation. This could be improved somewhat by a Monte Carlo calculation of individual chains. However, a more fundamental difficulty is that the projected areas of two neighboring molecules may be different from the sum of the individual projected areas because of specific fitting together of kinks and jogs; in this sense the calculation of the excluded volume interaction is similar in spirit to mean field theory. These points and a full discussion of the parameters, including several free ones, are given in the original papers.

Another set of model calculations that attempts to do more justice to the excluded volume interactions while sacrificing some realism in the chain statistics has been performed by Scott (106, 121, 122). Instead of considering all rotameric chain states, a small subset is chosen with each state weighted according to the number of actual states it represents. Furthermore, the states are idealized chains on a square lattice. The excluded volume interaction between chains is treated using the Flory approximation (100). Although this reviewer has criticized this approximation as a mean field type of calculation when compared to the superior Huggins calculation (101), it is nevertheless quite different from the usual mean field approximation for soft interactions and more appropriate for excluded volume interactions. For the attractive van der Waals interactions Scott uses a density-dependent mean field type term. In (121) Scott obtains nonflat isotherms similar to monolayer experiments. As is explained more fully in (122) this is only possible by adding a variable head group pressure after the phase transition has been found by a Maxwell construction; this procedure is not legal in equilibrium statistical mechanics and, to justify it, Scott argues that the head groups do not equilibrate with the hydrocarbon chains during the time course of the monolayer measurements. This seems implausible to this reviewer. In a more recent paper Scott & Cheng (71a) have followed Jacobs et al (105) by using hard disk mixtures as a reference system to account for the excluded volume interactions of the weighted subset states. In an earlier paper (123) Scott studied a model where each chain had only three rotameric states, but which incorporated positional or translational disorder. Although the model is very primitive and could only be solved in a completely mean field approximation, it has the interesting feature of having two transitions, reminiscent of the two transitions in lecithins (but not in ethanolamines), although this requires the rotameric energies to be much larger than the excluded volume

energies. Incidentally, the lower transition has been neglected in this review; the nature of it is still disputed (26, 124), but Doniach (125) has an interesting theory based upon the ripple picture (26).

In the above section on the kink model we argue that the kink model is not a good general model of the LBPT. However, as a simplified model for specific calculations the kink simplification is certainly as good as many others that have been proposed. The chief advantage of it is that one can do a more precise analysis of the fitting together of neighboring molecules into a kink block structure, as is shown by Jackson (92). However, the occurrence of close contacts as shown in Jackson's Figure 1, which are disfavored only by a soft, i.e. slowly varying repulsive potential, $nw/(A - A_o)$, where w is a completely arbitrary parameter used to fit the experiments, is suspect. Therefore, even though Jackson obtains ΔH in agreement with experiment, one should not regard that as proof of the kink model as a general model.

The process of simplifying the rotameric chain statistics has often been carried to the extreme of distinguishing only whether a lipid molecule is "solid," which usually means no gauche states or kinks, or "fluid" (126–129). One motivation for this extremely grotesque caricature is that the ensuing model is just a spin 1/2 Ising model about which more is known than for any other model. However, in addition to grossly oversimplifying the chain statistics, this approach also eliminates the special role played by excluded volume interactions. Indeed, the entire interaction energy is a "soft" one between neighboring spins (i.e. it is of order only of KT_M for any pair of molecules) and is a reasonable model only for the attractive van der Waals interactions, which are least important for the transition. In connection with the universality hypothesis of phase transitions, this reviewer has argued that the combined effect of "hard" excluded volume interactions and conservation of chain flow (22) makes it most likely that the LBPT is in a different universality class than the Ising phase transition as indicated by the exactly solvable model discussed above. Nevertheless, it is interesting to use the Ising model as a contrasting basis for interpreting data. From it Kanehisa & Tsong (126) and Doniach (127) suggest that the LBPT is in the vicinity of a critical point and that this could account for the peak in passive permeability at T_M . Kanehisa & Tsong (126) related the permeability peak to boundary lipid obtained from a cluster calculation whereas Doniach (127) takes a more fundamental approach and relates it to fluctuations.

With the exception of (127), the Ising model papers (126, 128, 129) and also a paper by McCammon & Deutch (130) employ a cluster picture as an aid to approximate calculations and to present results.

The cluster theory of condensed phases and their phase transitions has historically been appealing because it is an intuitive, pictorial description with which nonexperts often feel comfortable. Unfortunately, real systems are more complex. Instead of clusters consisting of entirely fluid or solid molecules, there is usually gradual spatial, and temporal, variation that makes the drawing of sharp cluster boundaries impossible. Therefore, the modern description has concentrated upon correlation functions that are much more appropriate to describe the degree of order of a system. One simple case where sharp cluster boundaries may be defined unambiguously is for spin $1/2$ Ising models; however, even for this model the correlation function description is the more fruitful and respected (131). Nevertheless, an interesting paper by Fisher (132), which reviewed and extended the older theories, showed that the idea was useful for discussing the analyticity at a first order transition, although Fisher is very cautious with regard to the assumption of neglect of interactions between clusters as regards the overall faithfulness of the calculation. Kanehisa & Tsong (126) adopt the notation of Fisher and they also try to account for finite size effects by limiting the cluster sums to l_{\max} . Unfortunately, this removes Fisher's essential singularity, which is the herald of the phase transition. In order to obtain a transition in their treatment Kanehisa & Tsong (126) match the high temperature cluster expansions against low temperature cluster expansions. This procedure is guaranteed to give a sharp transition as shown in Figure 1 of (126), but it is also likely to be an artifact of this approximate procedure.

A two-state, noninteracting cluster model has been used by Freire & Biltonen (128) to interpret their calorimetric measurements in terms of average size and number of gel and liquid clusters as a function of temperature. This is pure curve-fitting and cannot be construed as a calculation for a phase transition. Nevertheless, it is important to address the questions of whether this yields a real picture of the LBPT and whether this picture is fundamental. Given the statistical model, which is highly oversimplified (two-state) and approximate (noninteracting clusters), the picture is certainly nonquantitative. Furthermore, the results for average size and number of clusters are extremely sensitive to impurities that broaden the transition. Indeed, the temperature course of these quantities just gives another, more complicated, way to describe broadening effects. With the measurements of Albon & Sturtevant (74) on purer DPPC samples, it is clear that the transition is truly first order and that the calculated cluster statistics (128) are describing artifacts and not fundamental features of the LBPT. I think a similar comment should be made to Lee's lengthy review (129), which stresses prefreezing and premelting effects to the near exclusion of the mechanisms that

cause the transition. This is not to say that such secondary phenomena should be completely ignored. In particular, the premelting phenomena are important in interpreting NMR data, as was demonstrated by Gent and Ho (133), who showed that there is a broad line below T_M , which can be attributed to defects in the solid state and which is quite different from the sharp line that appears at T_M . Such defects are always present in the solid state, especially in polymer systems (134), but to greater or lesser degree depending upon the sample preparation. However, to characterize the transition in terms of defects and/or clusters is like saying the tail wags the dog.

McCammon & Deutch (130) were the first to employ the cluster idea to the LBPT. They took an eclectic approach to the chain statistics by permitting only 2–4 mobile bonds on the terminal methyl ends of the chains and only kinks on the remainder. The attractive van der Waals interaction was a function only of numbers of gauche rotations within a cluster; no independent density variation was permitted. All the molecules in each cluster were assumed to have the same number of kinks and interactions between clusters were completely ignored. The solid phase was specified to have only clusters with no kinks, the fluid phase was specified to have only clusters with one or more kinks [Eq. 2.17 in (130)], and to obtain a transition the relation $\mu_{fluid}(T_M) = \mu_{solid}(T_M)$ was used. This procedure is strictly illegal because the complete partition function must consist of all clusters with 0 or more kinks. For example, suppose one considers a system of noninteracting "clusters" in which each cluster consists of a molecule with one "ordered" solid state with zero energy and m "disordered" fluid states with energy E . The procedure employed by McCammon & Deutch (130) yields

$$\mu_{solid} = \ln(1) \quad \text{and} \quad \mu_{fluid} = \ln(m \exp(-E/kT))$$

and predicts an order-disorder transition when

$$1 = m \exp(-E/kT_m), \quad \text{i.e.} \quad kT_m = E/\ln m.$$

However, no such transition exists because the complete partition function is $1 + m \exp(-E/kT)$.

Marsh et al (135) apply the Zimm-Bragg computational procedure to a simplified model with three kinds of lipid: solid, liquid, and boundary lipid. The transfer matrix employed is tailor-made for one dimensional problems such as the helix-coil biopolymer transition but is totally inappropriate for two-dimensional phase transitions, even for this oversimplified model of the LBPT. Furthermore, the fundamental parameters in the theory are highly sensitive to the measured breadth of the transition and thus the theory is artifactual.

CONCLUDING REMARKS

There is general agreement that the main lipid bilayer phase transition is primarily one of cooperative rotameric disordering of the hydrocarbon chains, which are frozen into an ordered state at temperatures below T_M by the combined excluded volume interactions and attractive van der Waals interchain interactions. This conclusion is supported by numerous calculations of varying degrees of rigor on specific models of varying degrees of simplification. Given the difficulties and uncertainties inherent in statistical mechanical calculations on complex and highly cooperative transitions, this multiplicitous theoretical development is proper. However, more detailed and critical comparison of different techniques of calculation upon identical specific models could be even more helpful in understanding and quantifying the shortcomings of various theories. This might lead to a state where the value of the theoretical development is greater than the value of the sum of the individual contributions. In any case it appears that the stage has been set and the play has begun to extend theoretical calculations to tackle a variety of biologically relevant complicating factors, such as protein, cholesterol, unsaturated hydrocarbon bonds, and variations in chain lengths and head groups. But a detailed discussion of these developments must be left to another review.

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