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## A Theory of Phase Transitions and Phase Diagrams for One- and Two-Component Phospholipid Bilayers†

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**ABSTRACT:** A statistical mechanical partition function for phospholipid bilayers is constructed to obtain a theoretical description of the chain melting phase transition in lipid bilayer membranes and of the phase diagrams for two-component bilayers. In addition to providing an accurate representation

of the transition temperatures and enthalpies of one-component bilayers composed of 1,2-diacylphosphatidylcholines, the theory can also account for the shapes of the phase diagrams observed for bilayers which are binary mixtures of these compounds with two different hydrocarbon chain lengths.

Aqueous dispersions of phospholipid bilayers can undergo a phase transition which involves a disordering of the hydrocarbon chains in the interior of the bilayer (Ladbroke and Chapman, 1969; Engleman, 1970; Hubbell and McConnell, 1971; Lippert and Peticolis, 1971; Hinz and Sturtevant, 1972; Nagle, 1973a,b; Scott, 1975; Marčelja, 1974; Marsh, 1974; Ranck et al., 1974; Träuble and Eibl, 1974; Sklar et al., 1975, 1976, 1977; Jacobs et al., 1975; McCammon and Deutch, 1975; Mabrey and Sturtevant, 1976). The same type of transition occurs in biological membranes (Steim et al., 1969; Reinert and Steim, 1970; Melchior et al., 1970; Ashe and Steim, 1971; Schechter et al., 1972; Overath et al., 1975; Linden and Fox, 1975; Morrisett et al., 1975; Cronan and Gelmann, 1975; Melchior and Steim, 1976; Thilo and Overath, 1976; Tecoma et al., 1977), and many properties of membranes, such as transport (Overath et al., 1967; Wilson et al.,

1970), enzyme function (Chapman and Urbina, 1970; Papahadjopoulos et al., 1975a), drug susceptibility (Jain et al., 1975), and interaction with anesthetics (Papahadjopoulos et al., 1975b; Trudell et al., 1975), are affected by the transition. In this paper, we develop a statistical thermodynamic model for a bilayer which accounts for many of the phase-transition properties observed in a homologous series of 1,2-diacylphosphatidylcholines (DLPC,<sup>1</sup> DMPC, DPPC, DSPC, DBPC) as well as for the qualitative features of the phase diagrams observed for binary mixtures of these lipids.

In the following section, the experimental observations on one- and two-component phosphatidylcholine bilayers are reviewed. This is followed by a discussion of the motivation for the present three-dimensional model in terms of an earlier two-dimensional model (Jacobs et al., 1975). The present model is developed in detail in the next section and the theoretical results are then compared with the experiment.

### Experimental Data

There is a wealth of experimental data for the 1,2-diacylphosphatidylcholines with two saturated acyl chains. The

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<sup>1</sup> Abbreviations used are: DLPC, dilauroylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; DBPC, dibehenoylphosphatidylcholine. The fatty acids in these esters have the formula  $C_nH_{2n}O_2$ , where  $n$  is 12, 14, 16, 18, and 22, respectively.

TABLE I: Transition Temperatures for Saturated Diacylphosphatidylcholines (°C).

Lipid	<i>n</i>	Calori- metric <sup>a</sup>	Calori- metric <sup>b</sup>	Calori- metric <sup>c</sup>	ESR spin label <sup>d</sup>	ESR spin label <sup>e</sup>	<i>cis</i> - Parinaric acid fluorescence <sup>f</sup>	Dilatometric value <sup>g</sup>	Diphenyl- hexatriene fluorescence depolarization <sup>h</sup>
DLPC	12	0	-1.8						
DMPC	14	23	23.9	23	23.6	23.2	23.0		24.4
DPPC	16	41	41.4	41.5	41.8	40.5	42.0	41.5	41.1
DSPC	18	58	54.9		54.4	54.0	54.0		54.0
DBPC	22	75							

<sup>a</sup> Ladbrooke and Chapman (1969). <sup>b</sup> Mabrey and Sturtevant (1976). <sup>c</sup> Van Dijck et al. (1976). <sup>d</sup> Plachey, private communication. <sup>e</sup> Shimshick and McConnell (1973). <sup>f</sup> Sklar et al. (1977). <sup>g</sup> Nagle (1973). <sup>h</sup> Lentz et al. (1976).

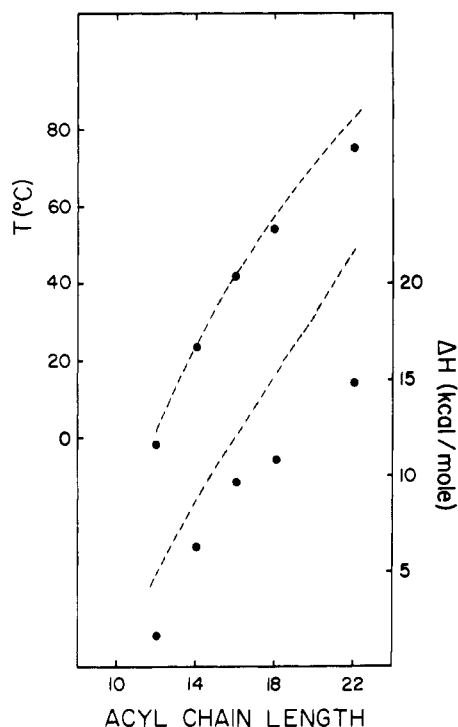


FIGURE 1: Transition temperatures and enthalpy changes for phosphatidylcholines. The circles are the experimental values from Tables I and II as summarized in column 3 of Table III. The dashed line is drawn through the theoretical values calculated using parameter set b ( $\delta = 0$ ,  $k_f = 56$ ) given in column 5 of Table III.

transition temperatures observed by a variety of techniques are given in Table I. Excellent agreement is found for measurements from different laboratories, often using very different techniques. The data presented in Table I refer to the main (higher temperature) transition of the phosphatidylcholines. A pretransition is also observed for phosphatidylcholines at a temperature 3 to 12 °C below the main transition. The enthalpies and volume changes associated with the pretransitions are much smaller than for the main transitions (Mabrey and Sturtevant, 1976; Nagle, 1973a,b; Plachy, 1977, private communication). The nature of this pretransition has been discussed recently (Janiak et al., 1976; Rand et al., 1975). It should also be pointed out that the data of Table I refer to fully hydrated phospholipid bilayer preparations. The chain-length dependence of the transition temperature is shown graphically in Figure 1.

The calorimetric excess enthalpies observed for the main transition of the 1,2-diacylphosphatidylcholines are given in Table II. The recent values of Mabrey and Sturtevant (1976) are presumably the most reliable. This reference should be

TABLE II: Transition Enthalpies for Saturated Diacylphosphatidylcholines (kcal/mol).

Lipid	<i>n</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>
DLPC	12			1.7	4.3
DMPC	14	6.6	6.3	5.4	
DPPC	16	8.7	9.7	8.7	
DSPC	18	10.7	10.8	10.6	
DBPC	22	14.9			

<sup>a</sup> Ladbrooke and Chapman (1969). <sup>b</sup> Hinz and Sturtevant (1972). <sup>c</sup> Mabrey and Sturtevant (1976). <sup>d</sup> Ververgaert et al. (1975).

consulted for a discussion of the two reported values for DLPC. The data of Table II are plotted in the lower half of Figure 1.

The fractional increase in volume ( $\Delta V/V$ ) accompanying the transition from the low to the high temperature phases is less than 5%. There is some disagreement as to the exact value for DPPC (Träuble and Haynes, 1971; Melchior and Morowitz, 1972; Sheetz and Chan, 1972; Nagle, 1973a,b; Srinivasan et al., 1974; Plachy, 1977, private communication). The values of 2.3, 2.6, and 3.6% for DMPC, DPPC, and DSPC reported by Melchior and Morowitz (1972) indicate an increase with increasing chain length in agreement with the results of Plachy (1977, private communication) using the Clausius-Clapeyron equation (2.9, 3.8, and 4.3%).

This small volume change is associated with much larger, but compensating, area and thickness changes. The fractional decrease in the thickness of the hydrocarbon region ( $-\Delta L/L$ ) is believed to be about 20% (Chapman et al., 1967; Luzzati, 1968; Träuble and Haynes, 1971). From  $\Delta V/V$  and  $\Delta L/L$ , it has been estimated that the fractional area increase accompanying the transition ( $\Delta A/A$ ) is approximately 25% (Träuble and Haynes, 1971; Engleman, 1971).

Phase diagrams for binary mixtures of phosphatidylcholines whose chain lengths differ by two, four, or six carbons have been measured by calorimetric (Hinz and Sturtevant, 1972; Mabrey and Sturtevant, 1976) and spin-label (Shimshick and McConnell, 1973; Wu and McConnell, 1975) techniques. Both methods give qualitatively the same phase diagrams. The experimental data for DMPC-DPPC, DPPC-DSPC, DMPC-DSPC, and DLPC-DSPC mixtures are shown in Figures 2 through 5 as open circles for calorimetric and squares for spin-label experiments.

In all the binary mixtures that have been examined, the *fluidus* curve (i.e., the boundary between the two-phase region and the region of a single fluid phase) lies closer to a straight line drawn through the end points of the phase diagram than does the *solidus* curve (which is the boundary between the two-phase region and the region of a single solid phase). This

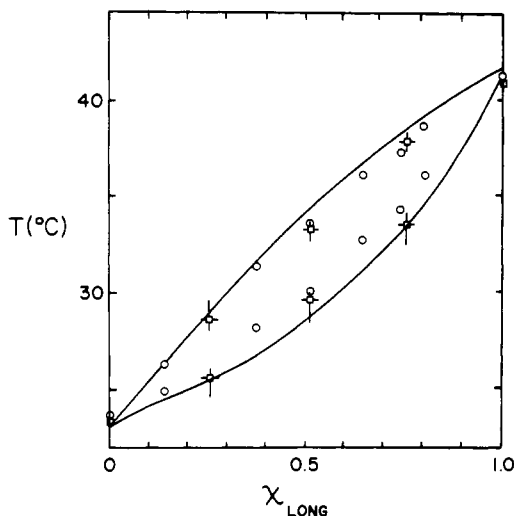


FIGURE 2: The DMPC/DPPC phase diagram. In this and all subsequent figures the open circles represent the calorimetric data of Mabrey and Sturtevant (1976) and the open squares represent the data of Shimshick and McConnell (1973).  $\chi_{\text{LONG}}$  is the mole fraction of the phosphatidylcholine with the longer acyl chain. The solid curves in Figures 2-5 are the phase boundaries calculated with parameter set b ( $\delta = 0$ ,  $k_f = 56$ ).

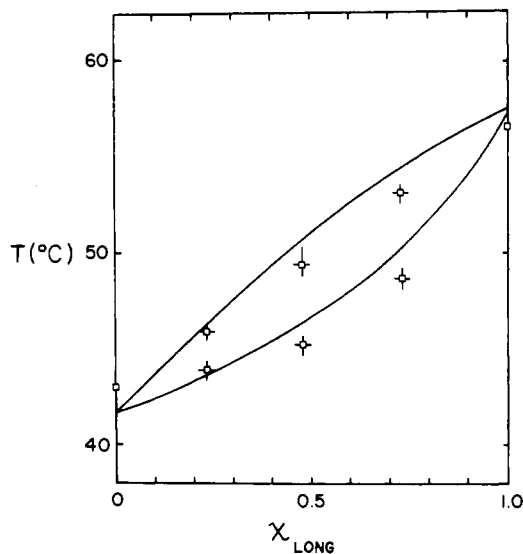


FIGURE 3: The DPPC/DSPC phase diagram. The experimental data have been shifted up by 1.7 °C.

asymmetry is slight in the DMPC-DPPC and DPPC-DSPC phase diagrams (see Figures 2 and 3) where the two species have a chain-length difference of only two carbons. It is quite pronounced in the DMPC-DSPC mixture (chain-length difference of four carbons), while the DLPC-DSPC mixture exhibits the extreme asymmetry characterized as monotectic behavior. The various asymmetries are important qualitative features of the phase diagrams, and one would expect an adequate theory of the transition to provide an explanation of these asymmetries.

#### Previous Calculations and Motivation for the Present Model

In a previous paper (Jacobs et al., 1975), we constructed a model for a one-component phospholipid bilayer. The head groups of the lipid molecules were assumed to lie in a two-dimensional plane separating the hydrocarbon chain region and the aqueous region. The thermodynamic variables which

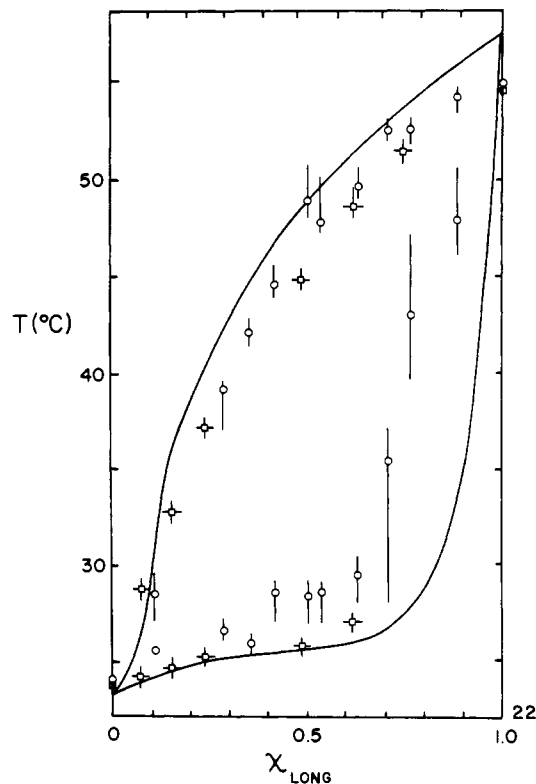


FIGURE 4: The DMPC/DSPC phase diagram.

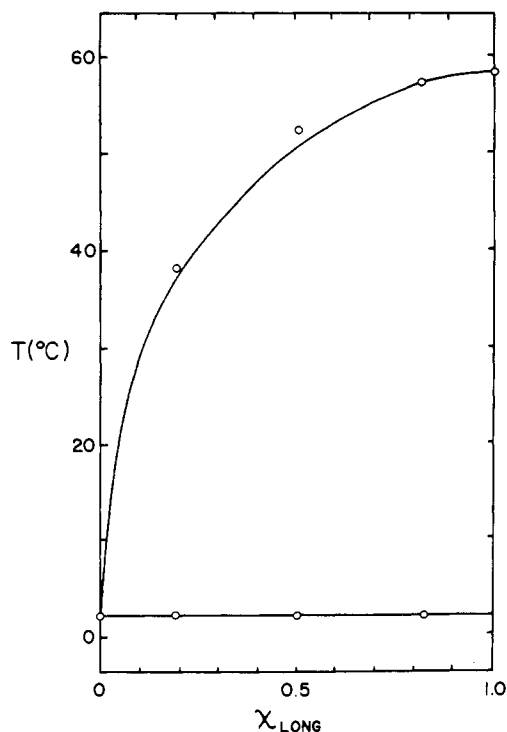


FIGURE 5: The DLPC/DSPC phase diagram. The experimental data have been shifted upward by 3.8 °C.

characterized this system were  $N$ , the number of molecules,  $T$ , the temperature, and  $A$ , the two-dimensional area occupied by the head groups. The canonical partition function, which is a function of  $N$ ,  $T$ , and  $A$ , was calculated assuming an approximate Hamiltonian that contained the following interactions: short-ranged excluded volume forces between head groups and between chains, the conformational energy asso-

ciated with gauche rotations of the bonds in the hydrocarbon chains, long-range dispersion forces between chains, and long-ranged attractive or repulsive forces between head groups. The molecular degrees of freedom taken into account in the calculation of the partition function were the coordinates of the head groups in their two-dimensional plane and the internal rotational state (which could be either trans, gauche (+), or gauche (-)) for each carbon-carbon bond of the chains. For reasonable choices of the two free parameters in the Hamiltonian, this model predicts a first-order phase transition with transition temperatures and enthalpies in reasonable agreement with the experimental data for aqueous dispersions of 1,2-diacylphosphatidylcholines of various hydrocarbon chain lengths.

Such a simple model cannot provide an accurate calculation of the partition function and thermodynamic properties of either of the two phases involved in the transition. However, it can provide a reasonable description of the phase transition itself, provided it takes into account those degrees of freedom and those molecular interactions which are most strongly affected by the transition. The simple model is successful presumably because the degrees of freedom and molecular interactions which it does not take into account affect the free energy of both phases to approximately the same extent and because the approximations in the model are adequate for calculating differences in the free energy.

In an attempt to understand the chain-melting transition in two-component phospholipid bilayers, it seemed reasonable to begin by generalizing the one-component theory described above. To calculate phase diagrams for mixtures of cholines with different chain lengths using this type of theory, we must first make some assumptions about the relative vertical position of the two species and the effect of the relative vertical position on the interaction between the opposing monolayers which comprise the bilayer. (The vertical direction is the direction normal to the bilayer surface.) We tried three different sets of assumptions: (1) The head groups of the two different species in the same monolayer were assumed to lie in two different planes, such that the interior surface of each monolayer was flat. Hence, the interaction between the two monolayers in the mixed bilayer would be the same as in a single-component bilayer. (2) All the head groups in the same monolayer were assumed to lie on the same plane, the ends of the long chains in one monolayer tending to fill in the voids under the short chains in the opposing monolayer. In other words, we assumed that the opposing monolayers tended to interpenetrate each other. (3) All the head groups in a monolayer were assumed to lie in the same plane and the monolayers were assumed not to interpenetrate each other.

As noted above, the experimental phase diagram for the DPPC-DSPC mixture is slightly asymmetric in shape. Phase diagrams calculated for this system using either of the first two sets of assumptions were completely symmetrical. The phase diagram calculated using the third set of assumptions has a maximum in its freezing-point curve at about 90% DSPC. Thus, none of these sets of assumptions, when used with the theory discussed above, could lead to a correct description of the phase diagram for DPPC-DSPC mixtures. The qualitative discrepancy between the theory and experiment is even greater for DMPC-DSPC mixtures, where the experimental phase diagram is even more asymmetric.

It is reasonable to suppose that the failure of the theory to describe correctly the phase equilibria in bilayers composed of molecules of different chain length is related to the fact that the theory does not take into account the vertical motion of the molecules in calculating the partition function. Instead, arbitrary

assumptions had to be made about the vertical positions of the molecules. There are several competing effects which might influence the vertical motion of the molecules. The attractive dispersion interactions between chains would inhibit the formation of voids or cavities below the short chains and thus would tend to pull the shorter molecules away from the aqueous layer to fill in the voids. However, these voids will be favored by the extra entropy or configurational freedom they give to the ends of the longer chains which are near them. Interaction between the head groups and water will probably inhibit a short-chain molecule from being pulled into the bilayer. This will also be resisted by the hydrophobic interaction between the water and that part of the longer hydrocarbon chains which would be exposed to water as a result of pulling the short-chain molecules into the bilayer. If these effects are important, they might be expected to contribute to the free-energy difference between solid and fluid phases.

The theory presented below is a generalization of our previous theory and is designed to take into account the effect of the motion of the molecules in the direction perpendicular to the bilayer surface. Many of the approximations which proved useful in the previous model are employed in the present model with only minor changes. This new statistical mechanical model of a bilayer does indeed account for the observed asymmetry in the phase diagrams of mixtures of cholines with different chain lengths.

#### Description of the Model

**Molecular Degrees of Freedom.** For each monolayer in a bilayer, we construct a Cartesian coordinate system with the  $xy$  plane parallel to the surface of the monolayer. The  $+z$  direction points from the head groups toward the acyl chain ends of the molecules. (We will ultimately position the origin of the coordinate system so that the average  $z$  coordinate of the head groups is 0.)

A phosphatidylcholine molecule consists of a head group (containing a glycerol backbone, a phosphate group, and a choline group) and two identical saturated fatty acid chains. We use a rotational isomeric state model for the hydrocarbon chains. Each chain has a certain number ( $m$ ) of carbon-carbon bonds which can exist in either a gauche or trans conformation. (If the fatty acid from which the chains are derived has the molecular formula  $C_nH_{2n}O_2$ , then  $m = n - 2$ .) We imagine a vector directed along the glycerol backbone of the head group. At all times, we suppose that the head group is oriented so that the chains, if all their bonds were trans, would be pointing in the  $z$  direction. The molecule can rotate about the  $z$  axis while keeping this orientation, and so the azimuthal angle,  $\varphi$ , of the vector along the glycerol backbone can take on any value from zero to  $2\pi$ .

In this model, the coordinates necessary to specify the state of a molecule are the three-dimensional position,  $r$ , of its head group, the angle  $\varphi$ , and a set of labels  $\alpha^{jk}$ , where  $j = 1, 2$  and  $k = 1, 2, \dots, m$ . Here  $\alpha^{jk}$  is  $+1, 0$ , or  $-1$  if the  $k$ th bond on the  $j$ th chain is gauche (+), trans, or gauche (-), respectively. The collection of  $2m$  labels for a molecule  $i$  will be denoted as  $\alpha_i$ .

**The Model Hamiltonian for the Bilayer.** The important types of interaction energies in this model are the same as in the original model with one addition. They are the excluded volume interactions between chains and between head groups, the long-range dispersion interactions between chains, the long-ranged attractive or repulsive interactions between head groups, the intramolecular energy associated with chain conformations, and an energy associated with the relative vertical positions of the molecules. The last energy contribution arises,

at least partially, from hydrocarbon-water interactions which may take place when the head group of one molecule is displaced from the average vertical position of the head groups. We shall call this the "roughness" energy because it arises when the head groups are not in one smooth flat plane. All the other energy contributions were employed in the original model and have been used by other investigators (Nagle, 1973a,b; Marčelja, 1974; Scott, 1975). Thus, we write the energy of a monolayer (in a bilayer) with  $N$  molecules as:

$$H(\mathbf{r}^N, \boldsymbol{\varphi}^N, \alpha^N) = H_{\text{exv}}(\mathbf{r}^N, \boldsymbol{\varphi}^N, \alpha^N) + H_{\text{conf}}(\alpha^N) + H_{\text{rough}}(\mathbf{r}^N, \boldsymbol{\varphi}^N, \alpha^N) + H_{1r}(\mathbf{r}^N, \boldsymbol{\varphi}^N, \alpha^N) \quad (1)$$

where the four terms are the excluded volume interactions (involving both chains and head groups), the chain conformation energy, the roughness energy, and the long-range interactions (which also involve both the chains and the head groups). The various approximations we shall make for these contributions to the energy will be described below.

**The Partition Function.** The canonical partition function for a one-component monolayer is a function not only of  $N$ ,  $A$ , and  $T$ , where  $N$  is the number of molecules,  $A$  is the area, and  $T$  is the temperature. (The generalization to two-component monolayers will be discussed below.) It will also depend on some variables describing the thickness of the monolayer. For reasons to be discussed below, we will calculate the following partition function:

$$Q(N, T, A, \zeta, \tau, \Delta) = (N!)^{-1} \int_A d\mathbf{x}^N d\mathbf{y}^N \int_{-\infty}^{\infty} dz^N \int_0^{2\pi} d\boldsymbol{\varphi}^N \times \sum_{\alpha^N} \exp[-H(\mathbf{r}^N, \boldsymbol{\varphi}^N, \alpha^N)/kT] \quad (2)$$

$$\delta \left[ N^{-1} \sum_{i=1}^N z_i - \zeta \right] \delta \left[ (2N)^{-1} \sum_{i=1}^N \sum_{j=1}^2 h_j(\alpha_i, z_i) - \tau \right] \times \delta \left[ (2N)^{-1} \sum_{i=1}^N \sum_{j=1}^2 (h_j(\alpha_i, z_i))^2 - \Delta \right]$$

Here  $h_j(\alpha_i, z_i)$  is the maximum  $z$  coordinate of any part of the  $j$ th chain of the  $i$ th molecule when the molecule has conformation  $\alpha_i$  and has its head group at  $z_i$ . It is given by

$$h_j(\alpha_i, z_i) = z_i + l_j(\alpha_i) \quad (3)$$

where  $l_j(\alpha_i)$  is the vertical length of the  $j$ th chain on the  $i$ th molecule. We shall call  $h_j(\alpha_i, z_i)$  the height of the chain. In eq 2  $\delta$  denotes the Dirac  $\delta$  function.

This partition function is the sum and integral of the Boltzmann factor,  $\exp(-H/kT)$ , over all states of the monolayer, subject to a number of constraints. First, there must be  $N$  molecules and their head groups must be within the area  $A$ . Secondly, the average  $z$  coordinate of the head groups must be  $\zeta$ . Thirdly, the mean height of the chains must be  $\tau$ , and the mean square height must be  $\Delta$ .

Some comments about this choice of partition function are in order. One might have thought that the appropriate additional variable to choose, beside  $N$ ,  $A$ , and  $T$ , would be the thickness of the monolayer,  $L$ . Thus, one would sum over all states of the monolayer, consistent with the fact that all molecules are confined within imaginary walls parallel to the  $xy$  plane and separated by a distance  $L$ . This choice is inappropriate (and certainly inconvenient) for monolayers which are parts of bilayers. Neither the aqueous region nor the opposite monolayer provides a flat boundary which constrains the molecules in a particular monolayer. We expect that under most conditions at equilibrium there will be fluctuations in the thickness of a monolayer, in the flatness of the head group-

water interface, and in the flatness of the interface between the two monolayers in a bilayer. These fluctuations may make an important contribution to the relative stability of the solid and fluid phases, and it seems reasonable to construct an ensemble which allows for such fluctuations. Thus, in the ensemble whose partition function is  $Q(N, T, A, \zeta, \tau, \Delta)$ , only the average  $z$  coordinate of the head groups,  $\zeta$ , and the average height of the chains,  $\tau$ , are specified, leaving some freedom for fluctuations to take place.

In an effort to account for the interactions between the two monolayers in a bilayer, we have also constrained the mean square height,  $\Delta$ , of the chains. The quantity  $\Delta - \tau^2$ , or  $(\Delta - \tau^2)^{1/2}$ , is a measure of the lack of flatness in the hydrocarbon side of a monolayer. If this quantity is small, most of the chains in the monolayer have the same height, and we might expect that the monolayer will be able to make close contact with the opposing monolayer in the same bilayer. If  $(\Delta - \tau^2)^{1/2}$  is large, the hydrocarbon surface will be very rough and only a poor contact, with the presence of many holes or voids, will be made between opposing monolayers. It is reasonable to suppose that two monolayers, each characterized by the same value of  $\Delta$  and  $\tau$ , will be able to approach each other and mesh their hydrocarbon surfaces so that the average distance between the head-group layers is

$$2[\tau - \zeta + a(\Delta - \tau^2)^{1/2}]$$

where  $a$  is a quantity of the order of unity. That is, the effective thickness of a bilayer is greater than twice the average thickness,  $\tau - \zeta$ , of a monolayer by an amount proportional to the roughness of the hydrocarbon surface,  $(\Delta - \tau^2)^{1/2}$ . We will use the symbol  $L$  to denote the effective thickness of a monolayer (half the thickness of a bilayer), and assume for simplicity that  $a = 1$ . Hence

$$L = \tau - \zeta + (\Delta - \tau^2)^{1/2} \quad (4)$$

is the effective thickness of a monolayer.

$Q(N, T, A, \zeta, \tau, \Delta)$  is the canonical partition function we wish to calculate. The negative of its logarithm is proportional to a Helmholtz free energy, which can be used to calculate all the desired thermodynamic properties. The proof that this partition function is directly related to a Helmholtz free energy is straightforward. (See the discussion of generalized ensembles in Hill (1956).) We will choose to demand that  $\zeta = 0$ . This is just a definition of the origin for measuring heights. In an experiment,  $A$ ,  $\tau$ , and  $\Delta$  are not controlled, and so at equilibrium they will adopt the values which maximize  $Q$  or minimize the Helmholtz free energy. (Experiments performed at atmospheric pressure are at essentially zero pressure.) Thus, we will calculate  $Q$  as a function of  $N$ ,  $T$ ,  $A$ ,  $\tau$ , and  $\Delta$ , keeping  $\zeta = 0$ , and then vary  $A$ ,  $\tau$ , and  $\Delta$  at fixed  $N$ ,  $T$ , and  $\zeta$  to obtain the experimental Helmholtz free energy as a function of  $N$  and  $T$ . This minimum Helmholtz free energy is also equal to the Gibbs free energy at zero pressure and is a function of  $N$  and  $T$ .

**Approximations Which Simplify the Intermolecular Interactions.** All of the terms in the Hamiltonian, with the exception of  $H_{\text{conf}}$ , involve interactions between molecules. Such interactions, in general, make it impossible to evaluate the partition function exactly, even if the form of the interactions is precisely known. A standard procedure for obtaining approximate partition functions is to replace the exact intermolecular interactions by effective potentials which act on the individual molecules separately. These effective potentials are functions of the macroscopic or average variables of the system. The van der Waals theory of fluids and the Weiss mean field theory of magnetism are examples of this approach. We now

want to apply this procedure to the various terms in the Hamiltonian to enable us to obtain an approximate partition function.

First, we make the assumption that for fixed values of  $N$ ,  $A$ ,  $\zeta$ ,  $\tau$ , and  $\Delta$  the structure of the system is determined by the first three terms in eq 1, while the longer ranged attractions and repulsions in  $H_{lr}$  merely provide some cohesion. The same type of idea was employed in our original model (Jacobs et al., 1975) and in other theories (Nagle, 1973a,b; Marsh, 1974; Scott, 1975). This assumption is a generalization of the van der Waals idea for nonpolar fluids which gives a qualitatively correct description of phase-transition phenomena in simple liquids (Longuet-Higgins and Widom, 1964). Moreover, as in the van der Waals theory of fluids, the  $H_{lr}$  term is then approximated in a mean field form, i.e., as a function of the macroscopic variables,  $N$ ,  $A$ ,  $\zeta$ ,  $\tau$ , and  $\Delta$ , and not as detailed functions of the microscopic state. Thus, we let

$$H_{lr} = Ne_{lr}(N/A, \zeta, \tau, \Delta) \quad (5)$$

to indicate that the long-range interaction energy is extensive (proportional to  $N$ ) and is a function of the two-dimensional density and of the parameters describing the thickness of the bilayer. The functional form for  $e_{lr}$  which we use is discussed below.

Next we consider the second term in the Hamiltonian, namely, the chain conformational energy. We assume that the only important contribution is an energy  $\epsilon$  for each bond which is in a gauche conformation. This energy is known to be about 500 cal/mol for hydrocarbon chains (Flory, 1969). Thus,

$$H_{\text{conf}}(\alpha^N) = \sum_{i=1}^N \sum_{j=1}^2 \sum_{k=1}^m |\alpha_i^{jk}| \epsilon \quad (6)$$

The third term in the Hamiltonian is the head-group roughness energy which is expected to be nonzero when the relative vertical displacement of adjacent head groups is nonzero. We shall model this interaction by assuming that the net result is a harmonic restoring force that tends to drive each head group back to the  $xy$  plane, where  $z = 0$ . Thus,

$$H_{\text{rough}} = \sum_{i=1}^N k_f z_i^2 / 2, \quad (7)$$

where  $k_f$  is the harmonic force constant. We will assume for simplicity that  $k_f$  is a constant that depends only on the nature of the head group. Equation 7 is the simplest form for  $H_{\text{rough}}$  which gives an effective force which is continuous and which is zero for a completely flat head-group region.

The first term in the Hamiltonian is the excluded volume interaction between chains and between head groups, and it depends in principle on head-group positions and orientations as well as on the chain conformations. A Hamiltonian containing such a term is so complicated that the partition function cannot be calculated exactly with currently available techniques. Hence, it is necessary to make some statistical approximations to decouple the head-group positions from the chain conformations. We use the following procedure, which is essentially equivalent to the one used in our previous calculation. First, we note that when the chains are in their all-trans conformation, the molecules will pack together well at high density, and this represents the conformation in which the molecules interfere with each other the least. Let  $\alpha^*$  represent the all-trans conformation. Hence, we can write

$$H_{\text{exv}}(\mathbf{r}^N, \varphi^N, \alpha^N) = H_{\text{exv}}(\mathbf{r}^N, \varphi^N, \alpha^{*N}) + \Delta H_{\text{exv}} \quad (8)$$

where the first term on the right represents the excluded volume interactions when all the molecules are in the all-trans conformation and  $\Delta H_{\text{exv}}$  is the change or increase in the in-

teractions as a result of the gauche rotations. Furthermore, we assume that  $\Delta H_{\text{exv}}$ , which actually depends in a very complicated way on the position of each head group and the conformation of each chain, can be approximated as an effective interaction which acts individually on the chains of each molecule. A chain which is not in the all-trans conformation has a larger effective cross-sectional area when viewed from above. When a molecule changes its conformation, we imagine that it in effect changes its area and hence changes the area available to the other molecules. This area increase will be resisted by some average pressure caused by the other chains. Thus, we assume that  $\Delta H_{\text{exv}}$  can be approximated as

$$\Delta H_{\text{exv}} = \sum_{i=1}^N \sum_{j=1}^2 P(N/A, \zeta, \tau, \Delta, T) \Delta a_j(\alpha_i) \\ = \Delta H_{\text{exv}}(\alpha^N, P) \quad (9)$$

where  $\Delta a_j(\alpha_i)$  is the effective area increase, relative to an all-trans chain, of the  $j$ th chain on molecule  $i$ , and  $P$  is an effective pressure, about which we will have more to say below. We note only that it is assumed to be a function of the macroscopic and average properties of the ensemble and not of the detailed microscopic state of the system. Equations 8 and 9 accomplish the decoupling of head-group and chain conformational degrees of freedom in the excluded volume interaction.

With the various approximations we have made, it follows that the only part of the integrand in  $Q$  which depends on the  $x$  and  $y$  coordinates of the head group and the  $\varphi$  angles is

$$\exp[-H_{\text{exv}}(\mathbf{r}^N, \varphi^N, \alpha^{*N})/kT]$$

This factor is independent of the chain conformation variables,  $\alpha^N$ . It is even independent of the  $z$  coordinates of the molecules, if we assume that the effective area, in the  $xy$  plane, of a head group is the same as (or less than) that of the two chains on the molecule. Under such conditions, moving a molecule up or down will not affect its excluded volume interactions with its neighbors. Hence, the integral over  $x^N$ ,  $y^N$ , and  $\theta^N$  can be factored out of the partition function. The factor containing these integrals is of the form

$$(N!)^{-1} \int_{\text{area}} d\mathbf{x}^N d\mathbf{y}^N \int_0^{2\pi} d\varphi^N \\ \times \exp[-H_{\text{exv}}(\mathbf{r}^N, \varphi^N, \alpha^{*N})] \quad (10)$$

This is the partition function of a two-dimensional fluid of particles interacting via excluded volume forces. As we did in previous work, we assume that the problem of the packing of straight-chained molecules in a single layer is equivalent to that of the packing of hard disks in two dimensions. (This ignores the fact that the straight-chain phospholipids have a somewhat elliptical cross section.) Hence, we shall call this integral  $Q_{\text{HD}}(N, A, T)$ , which is the configuration integral for hard disks in two dimensions. Computer simulation studies (Alder et al., 1968; Hoover and Ree, 1968; Ree and Hoover, 1964) yielded the following expressions for the configuration integral and pressure of a hard-disk solid:

$$Q_{\text{HD}} = (\alpha^2/\rho_0)^N \exp[N(0.06 - 0.12\alpha + 0.382\alpha^2 \\ + 0.243\alpha^3 + \dots)] \\ P_{\text{HD}}^{\text{solid}} A_0 / NkT = (2/\alpha + 1.90 + 0.67\alpha \\ + 1.5\alpha^2 + \dots)/(1 + \alpha) \quad (11)$$

where  $\alpha = (A - A_0)/A_0$ ,  $\rho_0 = N/A_0$ , and  $A_0$  is the area at closest packing for  $N$  disks. The expansions were truncated and used as written.

Thus, our partition function can be written in the following form

$$Q(N, A, T, \zeta, \tau, \Delta) = Q_{HD}(N, A, T) \times \exp[-Ne_{lr}(N/A, \zeta, \tau, \Delta)/kT] Q_{zc}(N, A, T, \zeta, \tau, \Delta) \quad (12)$$

where  $Q_{zc}$  is the part of the partition function that arises from the  $z$  integrations and the sum over chain conformations.

$$\begin{aligned} Q_{zc}(N, A, T, \zeta, \tau, \Delta) &= \int_{-\infty}^{\infty} dz^N \sum_{\alpha^N} \exp[-H_{\text{conf}}(\alpha^N)/kT] \\ &\times \exp[-\Delta H_{\text{exv}}(\alpha^N; P)/kT] \exp[-H_{\text{rough}}(z^N)/kT] \\ &\times \delta \left[ N^{-1} \sum_{i=1}^N z_i - \zeta \right] \delta \left[ (2N)^{-1} \sum_{i=1}^N \sum_{j=1}^2 (h_j(\alpha_i, z_i)) - \tau \right] \\ &\times \delta \left[ (2N)^{-1} \sum_{i=1}^N \sum_{j=1}^2 h_j(\alpha_i, z_i)^2 - \Delta \right] \quad (13) \end{aligned}$$

In going from our original definition of  $Q$  to this result, we made three important simplifying assumptions. (1) The long-range interactions have been expressed as a mean field energy. (2) The excluded volume interaction between molecules has been expressed as the sum of two parts:  $H_{\text{exv}}(\mathbf{r}^N, \varphi^N, \alpha^N)$ , which is independent of chain conformation and is assumed to be equivalent to the Hamiltonian for the packing of hard disks in two dimensions; and  $\Delta H_{\text{exv}}$ , which is assumed to be a sum of terms which act individually on each chain. (3) The head-group roughness energy has been expressed as a sum of terms which act individually on each head group. Thus, many of the intermolecular interactions, which make evaluation of a partition function difficult or impossible, have been approximated as effective potentials acting on individual molecules, and the remaining interactions have been assumed equivalent to those in the problem of hard disks.

**Evaluation of  $Q_{zc}$ .** The factor  $Q_{zc}$  involves integration over the  $z$  coordinates of the head groups of all the molecules and summation over all the conformational states of all the molecules. The Hamiltonians in the exponents of the integrand are the sums of contributions from each individual molecule, and thus  $Q_{zc}$  would factorize into the  $N$ th power of a single molecule partition function except for the presence of the Dirac  $\delta$  functions, which in effect couple different molecules together.

Because of the nature of the  $\delta$  functions,  $Q_{zc}$  can be easily evaluated by making use of a standard trick in statistical mechanics. We consider the Laplace transform of  $Q_{zc}$  with respect to  $\zeta, \tau$ , and  $\Delta$ .

$$\begin{aligned} \Psi(N, T, A, \pi_\zeta, \pi_\tau, \pi_\Delta) &\equiv \int_{-\infty}^{\infty} d\zeta \int_{-\infty}^{\infty} d\tau \int_0^{\infty} d\Delta \\ &\times Q_{zc}(N, T, A, \zeta, \tau, \Delta) \exp[-N\pi_\zeta\zeta - 2N\pi_\tau\tau - 2N\pi_\Delta\Delta] \quad (14) \end{aligned}$$

$\Psi$  can be regarded as a partition function in a new ensemble characterized by particular values of  $\pi_\zeta, \pi_\tau$ , and  $\pi_\Delta$ , which are the thermodynamic pressures conjugate to  $\zeta, \tau$ , and  $\Delta$ , respectively. The relationship between  $\Psi$  and  $Q_{zc}$  is analogous to the relationship between the partition functions in the isothermal-isobaric ensemble and in the canonical ensemble (Hill, 1956; McQuarrie, 1973) except that we have three size parameters and pressures rather than one.

$\Psi$  can be expressed in a much simpler form. When eq 13 for  $Q_{zc}$  is substituted into eq 14 for  $\Psi$ , the integrals over  $\zeta, \tau$ , and  $\Delta$  can be performed immediately because of the  $\delta$  functions. The resulting integral can then be factored into one part for each molecule, and we obtain

$$\Psi(N, T, A, \pi_\zeta, \pi_\tau, \pi_\Delta) = q(T, \pi_\zeta, \pi_\tau, \pi_\Delta; P)^N \quad (15a)$$

where

$$\begin{aligned} q(T, \pi_\zeta, \pi_\tau, \pi_\Delta; P) &= \int_{-\infty}^{\infty} dz \sum_{\alpha} \exp \left[ - \left( k_f z^2 / 2 \right. \right. \\ &\quad \left. \left. + P \sum_{j=1}^2 \Delta a_j(\alpha) + \sum_{j=1}^2 \sum_{k=1}^m |\alpha^{jk}| \epsilon \right) / kT \right] \\ &\times \exp \left[ - \pi_\zeta z - \pi_\tau \sum_{j=1}^2 h_j(\alpha, z) - \pi_\Delta \sum_{j=1}^2 (h_j(\alpha, z))^2 \right] \quad (15b) \end{aligned}$$

This expression, involving a one-dimensional integral and a sum over the conformational states of the two chains on a single molecule, is simple enough to be evaluated directly.

All that remains is to derive the relationship between  $\Psi$  and  $Q_{zc}$ . Using the standard methods for relating the partition functions for different ensembles, it can easily be shown that

$$\begin{aligned} Q_{zc}(N, T, A, \zeta, \tau, \Delta) &= \Psi(N, T, A, \pi_\zeta, \pi_\tau, \pi_\Delta) \\ &\times \exp[+N\pi_\zeta\zeta + 2N\pi_\tau\tau + 2N\pi_\Delta\Delta] \quad (16) \end{aligned}$$

where  $\pi_\zeta, \pi_\tau$ , and  $\pi_\Delta$  are now functions of  $N, T, A, \zeta, \tau$ , and  $\Delta$  which are solutions of the following set of equations

$$\begin{aligned} \zeta &= -\frac{1}{N} \left( \frac{\partial \ln \Psi(N, T, A, \pi_\zeta, \pi_\tau, \pi_\Delta)}{\partial \pi_\zeta} \right)_{N, T, A, \pi_\tau, \pi_\Delta} \\ &= -\frac{\partial \ln q}{\partial \pi_\zeta} \quad (17a) \end{aligned}$$

$$\begin{aligned} \tau &= -\frac{1}{2N} \left( \frac{\partial \ln \Psi(N, T, A, \pi_\zeta, \pi_\tau, \pi_\Delta)}{\partial \pi_\tau} \right)_{N, T, A, \pi_\zeta, \pi_\Delta} \\ &= -\frac{1}{2} \frac{\partial \ln q}{\partial \pi_\tau} \quad (17b) \end{aligned}$$

$$\begin{aligned} \Delta &= -\frac{1}{2N} \left( \frac{\partial \ln \Psi(N, T, A, \pi_\zeta, \pi_\tau, \pi_\Delta)}{\partial \pi_\Delta} \right)_{N, T, A, \pi_\zeta, \pi_\tau} \\ &= -\frac{1}{2} \frac{\partial \ln q}{\partial \pi_\Delta} \quad (17c) \end{aligned}$$

The physical meaning of these equations is straightforward. For particular values of  $N, T, A, \zeta, \tau$ , and  $\Delta$ , we must find the values of  $\pi_\zeta, \pi_\tau$ , and  $\pi_\Delta$  for which the average values of  $z_i, h_j$ , and  $h_j^2$  in the  $\Psi$  ensemble are  $\zeta, \tau$ , and  $\Delta$ .

The final result for  $Q$  is

$$\begin{aligned} Q(N, A, T, \zeta, \tau, \Delta) &= Q_{HD}(N, A, T) \\ &\times \exp[-Ne_{lr}(N/A, \zeta, \tau, \Delta)/kT] q(T, \pi_\zeta, \pi_\tau, \pi_\Delta; P)^N \\ &\times \exp[N\pi_\zeta\zeta + 2N\pi_\tau\tau + 2N\pi_\Delta\Delta] \quad (18) \end{aligned}$$

where  $\pi_\zeta, \pi_\tau$ , and  $\pi_\Delta$  are solutions of eq 17, and  $P$  is a function of  $N, T, A, \zeta, \tau$ , and  $\Delta$ .

**The Long-Ranged Part of the Mean Field Energy,  $e_{lr}$ .** In the bilayer solid phase of 1,2-diacylphosphatidylcholines with saturated chains at temperatures well below the bilayer transition temperature, the hydrocarbon chains form a hexagonal close-packed lattice quite similar to that found in long-chain saturated hydrocarbon crystals (Chapman et al., 1967). Hence, the cohesive energy due to the chains at their close-packed density should be similar to that found in long-chain saturated hydrocarbons. As the density decreases the cohesive energy will likewise decrease. We shall assume that the cohesive energy per methylene and methyl segment is proportional to the three-dimensional density of such segments. We also expect there to be some long-range interactions between the head groups and perhaps other end effects which can be modeled in a mean field way. This last part of  $H_{lr}$  we assume to be

proportional to the number of head groups and to the two-dimensional surface density of the system.

Let  $a_0$  be the area per chain for a close-packed bilayer in which the chains are assumed to be perpendicular to the bilayer surface, and let  $l_0$  be the distance between bonded carbon atoms in a direction perpendicular to the bilayer surface. (The values of  $a_0$  and  $l_0$  are approximately  $25 \text{ \AA}^2$  and  $1.24 \text{ \AA}$ , respectively.) The reduced two-dimensional density of chains or molecules of a bilayer is defined as

$$\rho^{(2)} = 2Na_0/A \quad (19)$$

This quantity is proportional to the number of molecules per unit area (or the number of chains per unit area) and its value is unity at closest packing. The reduced three-dimensional density of chain segments ( $\text{CH}_2$  and  $\text{CH}_3$  groups) in a bilayer is defined as

$$\rho^{(3)} = 2N(n-1)a_0l_0/AL \quad (20)$$

This quantity is proportional to the number of carbon atoms per unit volume and its value is unity at closest packing. Using these measures of density, we assume that the long-ranged energy per molecule is

$$e_{lr}(\rho^{(3)}, \rho^{(2)}, n) = -\Delta H_{\text{sub}}[2n\rho^{(3)} + \rho^{(2)}\delta] \quad (21)$$

where  $\Delta H_{\text{sub}}$  is the heat of sublimation per  $\text{CH}_2$  group of a long-chain hydrocarbon crystal at 0 K (taken to be  $1.84 \text{ kcal/mol}$  in accordance with experimental determinations (Billmeyer, 1957; Salem, 1962; Zwanzig, 1963; Shapiro and Ohki, 1974)), and  $\delta$  is a dimensionless number defined so that  $-\Delta H_{\text{sub}}\delta$  is the mean field energy per molecule due to the head groups and end effects at closest two-dimensional packing of the molecules.

*The Effective Length and Area of a Molecule.* To evaluate the chain partition function  $q$ , we need to know  $\Delta a_j(\alpha)$ , which is the effective area increase of a chain when its conformation changes from the all-trans conformation to the conformation  $\alpha$ . We also need  $l_j(\alpha)$ , which is the vertical length of a chain as a function of its conformation.

We have assumed that the head group of each molecule is oriented so that the chains, if all their bonds are trans, would be pointing in the  $z$  direction. If a chain  $j$  has a single gauche rotation at the  $i$ th bond from the head group, the part of the chain below this bond no longer points in the  $z$  direction. The length of this lower segment is proportional to  $m-i+1$ . It follows that

$$\Delta a_j(\alpha) = \gamma(m-i+1)2a_0 \quad (22)$$

where  $\gamma$  is a dimensionless number. For example, see Figure 1 of our previous paper (Jacobs et al., 1975). It also follows that the vertical length of an otherwise all-trans chain with one gauche bond at position  $i$  is

$$l(\alpha) = [i + (m-i+1)\cos\theta]l_0 \quad (23)$$

with  $\theta = 60^\circ$ , or

$$l(i) = \frac{1}{2}(m+i+1)l_0 \quad (24)$$

If another gauche rotation is performed about a bond further from the head group, then the vertical length and the area may either increase or decrease. We shall assume that on the average these additional gauche rotations have no effect on the vertical length and the effective area of the chain. Hence, we assume that  $l_j(\alpha)$  and  $\Delta a_j(\alpha)$  are given by eq 24 and 22, where  $i$  is the position of the gauche bond nearest the head group.

*The Effective Pressure Acting on a Chain.* The quantity,

$P(N/A, \zeta, \tau, \Delta, T)$ , introduced in eq 9, is the effective two-dimensional pressure, due to short-ranged repulsive steric interaction, acting to inhibit each chain from adopting conformations other than the all-trans conformation. It is reasonable to suppose that  $P$  depends on both  $N/A$  and on  $L$ . Thus, a decrease in the area per molecule, at constant  $L$ , will increase the packing of the chains and is likely to increase this effective pressure. A decrease in  $L$ , at constant  $N/A$ , is expected to have the same effect. Thus, it is reasonable to assume that  $P/kT$  can be expressed as some function of the three-dimensional segment density.

To estimate what this function is, consider a monolayer in which all the chains are straight and  $\Delta = 0$ , i.e., the ends of all the chains in the monolayer are on one flat plane. Under such circumstances, if one chain increases its effective area it will have to displace other chains in the same bilayer. If all the other chains were to stay straight and vertical, the system would in effect be a two-dimensional system of hard disks in which each molecule corresponds to one disk. It is reasonable to assume that under these conditions the effective pressure resisting a conformational change is this two-dimensional hard-disk pressure.

Combining these two assumptions, we obtain the following. We assume that  $P(N/A, \zeta, \tau, \Delta, T)$  is the hard-disk pressure corresponding to a hypothetical bilayer which has the same temperature and the same three-dimensional segment density, which has  $\Delta = 0$ , and which has a length corresponding to all the chains being straight. For a hard-disk solid, the equation of state is given in eq 11. In that equation,  $(1+\alpha)^{-1}$  is the reduced two-dimensional density, and for our hypothetical bilayer it is equal to  $\rho^{(2)}$ . The hypothetical system has a thickness  $L$  which is equal to  $(n-1)l_0$ . Thus, it has a three-dimensional reduced density of

$$\rho^{(3)} = \rho^{(2)}(n-1)l_0/L = \rho^{(2)} \quad (25)$$

This reduced three-dimensional density of our hypothetical system must be chosen so that it has the same value as the reduced three-dimensional density as our bilayer of interest. Thus, our assumption about  $P$  can be restated in the following form. The effective pressure acting on a chain in a bilayer that has a certain three-dimensional reduced density  $\rho^{(3)}$  is the same as that of a hypothetical hard-disk fluid whose two-dimensional reduced density is numerically equal to  $\rho^{(3)}$  and whose area per disk at closest packing is the same as the area per phospholipid molecule in a close-packed bilayer. (This area is  $2a_0$ , where  $a_0$  is the area per chain at closest packing.) Hence, we find

$$\frac{2Pa_0}{kT} = (2/\alpha + 1.90 + 0.67\alpha + 1.5\alpha^2)/(1+\alpha) \quad (26)$$

where  $\alpha = (1/\rho^{(3)}) - 1$ .

The arguments leading to eq 26 are plausible but certainly not based on any fundamentally correct theory of the steric interactions. This expression will actually be used in connection with eq 9 for  $\Delta H_{\text{exv}}$  and 22 for  $\Delta a_j(\alpha)$ . Because of the crudeness of these approximations, we shall treat the dimensionless number  $\gamma$  in eq 22 as an adjustable parameter. It will be assumed to be the same for all acyl chain lengths.

*Generalization to Two-Component Bilayers.* The discussion above has been presented for the special case of a one-component bilayer. The generalization to two-component systems is straightforward. We are concerned with the case in which the two components have the same head group, have saturated chains, and differ only in the number of carbon atoms in their chains. The partition function for the mixture becomes, instead of eq 18,



$$Q(N_1, N_2, A, T, \zeta, \tau, \Delta) = (N_1! N_2! / N!) Q_{HD}(N, A, T) \\ \times \exp[-N_1 e_{lr}(\rho^{(3)}, \rho^{(2)}, n_1)/kT] q_1(T, \pi_\zeta, \pi_\tau, \pi_\Delta; P)^{N_1} \\ \times \exp[-N_2 e_{lr}(\rho^{(3)}, \rho^{(2)}, n_2)/kT] q_2(T, \pi_\zeta, \pi_\tau, \pi_\Delta; P)^{N_2} \\ \times \exp(N\pi_\zeta\zeta + 2N\pi_\tau\tau + 2N\pi_\Delta\Delta) \quad (27)$$

Here  $N_1$  and  $N_2$  are the number of molecules of type 1 and 2, respectively, and  $N = N_1 + N_2$ . The two-dimensional reduced density is still given by eq 19, but the three-dimensional reduced density is

$$\rho^{(3)} = 2[N_1(n_1 - 1) + N_2(n_2 - 1)]a_0 l_0 / AL \quad (28)$$

which replaces eq 20. Equation 21 for  $e_{lr}$  and 26 for  $P$  still hold, with the new definition of  $\rho^{(3)}$ . Also,  $q_1$  and  $q_2$ , the chain partition functions for molecules of type 1 and 2, are each given by eq 15b. Equations 17a-c for  $\zeta$ ,  $\tau$ , and  $\Delta$  become

$$\zeta = -\frac{\partial}{\partial \pi_\zeta} (X_1 \ln q_1 + X_2 \ln q_2) \quad (29a)$$

$$\tau = -\frac{1}{2} \frac{\partial}{\partial \pi_\tau} (X_1 \ln q_1 + X_2 \ln q_2) \quad (29b)$$

$$\Delta = -\frac{1}{2} \frac{\partial}{\partial \pi_\Delta} (X_1 \ln q_1 + X_2 \ln q_2) \quad (29c)$$

where  $X_i = N_i/N$ ,  $i = 1, 2$ . For fixed values of  $N_1$ ,  $N_2$ , and  $T$ , the procedure is to find the values of  $A$ ,  $\zeta$ ,  $\tau$ , and  $\Delta$  which maximize  $\ln Q$  subject to the restriction that  $\zeta = 0$ . The resulting  $\ln Q$ , when multiplied by  $-kT$ , is the Gibbs free energy of the bilayer at zero pressure, as a function of  $N_1$ ,  $N_2$ , and  $T$ .

**Phase Transitions and Phase Diagrams.** For this model, certain choices for the free parameters gave free-energy surfaces which indicated first-order phase transitions. We will now briefly describe how the properties of the phase transitions were obtained.

For one-component bilayers,  $\ln Q$  often had two different maxima at the same temperature (see Figure 2 of Jacobs et al., 1975). One occurred for relatively small values of  $A$  and large values of  $L$  and the other occurred for larger  $A$  and smaller  $L$ . At each temperature, the larger of the two values for  $\ln Q$  corresponds to the actual state of the bilayer, but at one temperature the two maxima would have the same value. This corresponds to the transition temperature, and the two thermodynamic states corresponding to the two maxima can coexist at zero pressure. Typically, at lower temperatures the small  $A$ , large  $L$  maximum would be the higher one, with the situation just reversed at higher temperatures. The properties of the coexisting phases, and hence the enthalpy, area, and length changes for the transition, can be obtained in the usual way.

For mixtures, phase diagrams were calculated in the usual way (Findlay, 1951). At fixed  $T$  and  $N$ , the (highest) maximum in  $\ln Q$  was obtained for each value of  $X_1$ . A graph of this maximum value as a function of  $X_1$  was constructed, and coexisting phases, if any, were determined by a double-tangent construction.

### Comparison of Theory with Experiment

For the model presented above, there are three parameters,  $\gamma$ ,  $k_f$ , and  $\delta$ , whose values are not known. The first,  $\gamma$ , is a dimensionless parameter characterizing the strength of the interchain steric repulsions. The second,  $k_f$ , is a force constant for the motion of a phospholipid molecule in a direction perpendicular to the plane of the bilayer. The third,  $\delta$ , is a dimensionless parameter characterizing the strength of the head-group repulsions and any other "end" effects which cause

the mean field cohesive energy to be offset from a strict proportionality with the number of carbons. Other parameters in the theory, such as  $\epsilon$  and  $\Delta H_{vap}$ , are known from experiments on systems unrelated to bilayers. (A numerical value for  $a_0$ , the area per chain at closest packing in a bilayer, is not needed, because we used reduced rather than absolute areas in our calculations.)

The parameters  $\gamma$ ,  $k_f$ , and  $\delta$  were treated as free parameters and varied to see if the theory could be fit to experiment. The physical meaning of all three leads us to assume that they are independent of chain length (but  $k_f$  and  $\delta$  may be dependent upon the nature of the head group). Thus, if the model is correct, one set of values for these three parameters should be able to fit the experimental phase-transition data for phosphatidylcholine bilayers of all hydrocarbon chain lengths and for any mixtures of these molecules.

Calculated transition temperatures, enthalpies, volume changes, thickness changes, and area changes for various values of  $\delta$  and  $k_f$  are shown in Table III. In each case the area parameter,  $\gamma$ , has been determined by the condition that the 16 carbon chain species have a zero pressure transition at 41.75 °C. Increasing either  $\delta$  or  $k_f$  (while keeping the other constant) generally has the effect of decreasing the chain-length dependence of the transition temperatures.

Several sets of values for  $\delta$  and  $k_f$  give transition temperatures which agree equally well with the experimental data in terms of the magnitude, slope, and curvature of  $T_m$  vs. chain length. The root mean square standard deviation for the calculated and experimental values of  $T_m$  is 3 to 7° for all six sets of parameters. The calculated values of  $T_m$  and  $\Delta H$  for parameter set b ( $\delta = 0$ ,  $k_f = 56$  dyn/cm) are shown in Figure 1. The calculated values of  $\Delta H$  are uniformly high by several kilocalories for all parameter sets and are quite similar to the values calculated with our previous model (Jacobs et al., 1975). The calculated values of  $\Delta L/L$  are all in reasonable agreement with the experimental estimate of -20%. The calculated area change  $\Delta A/A$ , and hence the derived quantity  $\Delta V/V$ , is somewhat larger than the experimental values.  $\Delta V/V$  increases with increasing chain length in agreement with the experimental trend. For parameter sets c and f,  $\Delta A/A$  is particularly large. For parameter set e, no transition is observed for DMPC at zero pressure. Overall, however, we must conclude that a comparison of calculated and experimental data for one-component systems does not yield a unique set of parameter values.

Parameter sets a-d were used to calculate phase diagrams for one or more of the two-component lipid mixtures for which experimental data are available. Qualitatively different phase diagrams were obtained. For sets a and d ( $\delta = 0$ ,  $k_f = 0$  and  $\delta = +2$ ,  $k_f = 0$ ) the calculated two-phase regions were symmetric and for parameter set d the two-phase regions were much thinner than in the experimental diagrams. With set c ( $\delta = 0$ ,  $k_f = 560$  dyn/cm), the calculated results for the DMPC-DPPC phase diagram had a eutectic at about 22 °C and 20 mol % DPPC.

For parameter set b ( $\delta = 0$ ,  $k_f = 56$  dyn/cm), we calculate the phase diagrams shown in Figures 2 through 5. These calculated phase diagrams exhibit the same qualitative behavior as the corresponding experimental phase diagrams. If we take the liberty of shifting the experimental phase diagrams up slightly in temperature so that the calculated and experimental end points for the pure components agree, then there is reasonable quantitative agreement for most of the mixtures.

The DMPC-DSPC calculated phase diagram exhibits the same flat solidus curve as is observed experimentally. The calculation predicts that the solids will be miscible in all pro-

TABLE III: Calculated Phase Transition Properties.

Lipid	<i>n</i>	exp <sup>g</sup>	$\delta = 0$ $k_f = 0^a$	$\delta = 0$ $k_f = 56^b$	$\delta = 0$ $k_f = 560^c$	$\delta = +2$ $k_f = 0^d$	$\delta = +2$ $k_f = 56^e$	$\delta = -2$ $k_f = 56^f$
Transition temperatures (°C)								
DLPC	12	-1.8	7	2	6	5		
DMPC	14	23.3	25	23	25	25	Nt <sup>h</sup>	21
DPPC	16	41.4	(41.75)	(41.75)	(41.75)	(41.75)	(41.75)	(41.75)
DSPC	18	54.3	56	58		55	54	
DBPC	22	75	80	83	77	76		87
RMSΔ <sup>i</sup>			5	4	4	3		7
Transition enthalpies, Δ <i>H</i> (kcal/mol)								
DLPC	12	1.7	6.7	4.8	7.4	5.3		
DMPC	14	5.4	10.0	8.7	10.1	8.0	Nt	10.5
DPPC	16	8.7	12.6	12.0	12.9	11.0	10.6	13.3
DSPC	18	10.6	15.4	15.3		14.8	14.1	
DBPC	22	14.9	21.3	21.6	21.4	20.9		22.0
Volume increase, Δ <i>V</i> <sub>v</sub> <sup>j</sup>								
DLPC	12		0.13	0.07	0.11	0.08		
DMPC	14	0.029	0.16	0.12	0.13	0.11	Nt	0.16
DPPC	16	0.038	0.17	0.14	0.15	0.13	0.11	0.17
DSPC	18	0.043	0.18	0.16		0.15	0.13	
DBPC	22		0.20	0.20	0.19	0.17		0.22
Thickness change, Δ <i>L</i> / <i>L</i> <sup>j</sup>								
DLPC	12		-0.07	-0.14	-0.21	-0.02		
DMPC	14		-0.14	-0.19	-0.26	-0.05	Nt	-0.32
DPPC	16		-0.17	-0.23	-0.29	-0.08	-0.17	-0.34
DSPC	18		-0.20	-0.26		-0.14	-0.21	
DBPC	22		-0.24	-0.31	-0.36	-0.17		-0.39
Area change, Δ <i>A</i> / <i>A</i> <sup>j</sup>								
DLPC	12		0.22	0.24	0.41	0.10		
DMPC	14		0.35	0.38	0.53	0.17	Nt	0.71
DPPC	16		0.41	0.48	0.62	0.23	0.34	0.77
DSPC	18		0.48	0.57		0.34	0.43	
DBPC	22		0.58	0.74	0.86	0.41		1.00

<sup>a</sup>  $\delta = 0$ ,  $k_f = 0$ ,  $\gamma = 0.05993044$ . <sup>b</sup>  $\delta = 0$ ,  $k_f = 56$  dyn/cm,  $\gamma = 0.05898532$ . <sup>c</sup>  $\delta = 0$ ,  $k_f = 560$  dyn/cm,  $\gamma = 0.051608089$ . <sup>d</sup>  $\delta = +2$ ,  $k_f = 0$ ,  $\gamma = 0.052891989$ . <sup>e</sup>  $\delta = +2$ ,  $k_f = 56$  dyn/cm,  $\gamma = 0.0498097724$ . <sup>f</sup>  $\delta = -2$ ,  $k_f = 56$  dyn/cm,  $\gamma = 0.062764539$ . (For  $k_f = 56$  dyn/cm, a displacement of a chain by one methylene unit (1.25 Å) increases the energy by  $kT$  for  $T = 41.75$  °C.) <sup>g</sup> The experimental values for  $T_m$  are averages of the values given in Table I; for Δ*H*, the experimental values are from column *c* of Table II except for  $n = 22$  which is from column *a*. <sup>h</sup> The entry Nt means that no transition occurs for zero pressure. <sup>i</sup> RMSΔ is the root mean square deviation between calculated and observed transition temperatures. <sup>j</sup> Fluid-phase value minus solid-phase value divided by solid-phase value. Δ*V*/*V* is calculated from Δ*A*/*A* and Δ*L*/*L*.

portions. Monotectic behavior is found in the calculated DLPC–DSPC phase diagram as is found experimentally. For this mixture, our calculations predict that the two solids should be essentially immiscible.

### Discussion

In the preceding sections we have presented an explicitly three-dimensional model for the phospholipid bilayer. There are three adjustable parameters in this model:  $k_f$ ,  $\delta$ , and  $\gamma$ . Using optimum values for these parameters, the agreement between the calculated and experimental phase-transition properties for both binary mixtures and pure 1,2-diacylphosphatidylcholine bilayer is quite reasonable.

The physical picture of the phase transition in mixed bilayers that emerges from the theoretical calculation is the following. Both the solid and fluid mixed phases are characterized by a relatively flat interface between the two monolayers in a bilayer. (This is caused by the relatively large dispersion energy penalty that would result from the formation of voids.) In the solid, this means that the head groups on the molecules with longer chains must penetrate further into the aqueous surroundings than do the head groups on the smaller molecules, despite the energy penalty that results from this roughness of the head-group layer. In the liquid, however, the head-group

layer can remain relatively flat by having the longer molecules make more “bends” or internal rotations than the shorter molecules. This change in the relative positions of the head groups of the two types of molecules on going from the solid to the fluid phase is the cause of the asymmetries in the calculated phase diagrams and is therefore a plausible explanation for the experimentally observed asymmetries.

In conclusion, we have found that a theory which takes into account the degrees of freedom corresponding to molecular motion perpendicular to the bilayer surface can provide an explanation of the observed asymmetries in the phase diagrams for two-component bilayers.

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