Structural phase transitions of interacting membranes

Raymond E. Goldstein* and Stanislas Leibler

Service de Physique Théorique, Centre d'Etudes Nucléaires de Saclay, F-91191 Gif-sur-Yvette CEDEX, France
and Laboratory of Atomic and Solid State Physics and Baker Laboratory, Cornell University, Ithaca, New York 14853-2501

A thermodynamic model for phase transitions of multilamellar liquid crystals composed of phospholipid bilayers in water is developed. By means of a Landau theory of the thermally driven hydrocarbon chain-melting transition of an isolated membrane and a continuum model of the interactions between neutral phospholipid bilayers, the phenomenon of structural phase transitions induced by membrane interactions is described. The phenomenological parameters that appear in the theory may be readily determined by existing experimental techniques; an analysis of available data on several phospholipid systems is used here to estimate these quantities. Semiquantitative agreement is achieved with the experimental temperature-composition phase diagram as well as the variation of the characteristic dimensions of the lamellar crystals with water content. Possible origins of pseudocritical phenomena are suggested on the basis of the unusual topology of the phase diagram. The model highlights the central importance of the water-mediated "hydration repulsion" between membranes in the phase behavior of the lamellar systems.

I. INTRODUCTION AND SUMMARY OF RESULTS

The structure of lipid membranes immersed in water may undergo a variety of phase transitions with variations in temperature, and such internal ordering may in turn affect the fluctuations of the bilayers as well as their interactions with each other. As an example, the thermally excited undulation modes which are responsible[1] for the long-ranged "steric repulsion" between membranes are governed by a rigidity modulus which is itself related to the details of the distribution of lateral stresses within the layer. Similarly, the short-ranged membrane interactions of biologically important lipids are known to be quite sensitive to the particular molecular architecture of the lipid molecules, their conformational state, and the presence of small amounts of added or adsorbed impurities.[2]

Theories describing the statistical properties of interacting membrane systems, seen as stacks of fluctuating surfaces, usually neglect the specific aspects of the internal structure of the membranes, assuming that they are infinitesimally thin, homogeneous layers characterized by coarse-grained elastic properties. This is, of course, an oversimplification, and it is only in the recently observed[3] "hyperswollen" limit that this assumption may be justified. In ordinary multilamellar lipid bilayer arrays, however, the typical distance between lamellae is comparable to their thickness.[2] In this regime, a variety of studies[2] has shown that membrane interactions are dominated by *molecular* forces, which in turn strongly depend on the internal bilayer structure. Therefore, in the study of unswollen lamellar phases it is necessary to account for the strong coupling between the interlamellar forces and possible intralayer phase transitions. Perhaps the most well-studied example of such a phenomenon occurs in the context of the so-called *main transition*,[4,5] at which an isolated bilayer transforms from a "fluid" state, with the hydrocarbon chains of its constituents disordered and the molecules free to diffuse, to one of several possible "solid" phases in which there is collective molecular orientation, tilt, or even in-plane modulation.

In this paper we study[6] the effects of membrane interactions on a structural phase transition of lipid bilayers by means of a simplified model of the main transition of isolated membranes and a continuum treatment of the dominant molecular intermembrane forces. The phenomenological theory for isolated bilayers, in which a single, scalar order parameter distinguishes the ordered and disordered states of the membranes, does not address the different order parameters distinguishing the several low-temperature ordered phases of the membranes, but does address an essential *geometrical* feature of the chain-melting transition; it may thus serve as a prototype for more detailed studies of interacting membranes. The model is used to study the phase diagram of two of the simplest lamellar structures exhibited by hydrated neutral phospholipid membranes, making direct contact with the two most directly controllable experimental variables, temperature (T) and the chemical potential of water (μ_w). We also calculate the variation of the structural parameters of the lamellae with these thermodynamic variables. Some systematic variations with hydrocarbon chain length n in the properties of the transition between disordered and ordered planar membranes are found here to lead to corresponding changes in the phase behavior, and these are qualitatively consistent with experiment.

Central to the model is the choice of a coarse-grained order parameter ψ which distinguishes the phases and is coupled to the membrane interactions. We review in Sec. II the essential empirical observations concerning the chain-melting transition of lamellar systems which motivate the choice of a simplified order parameter related to the bilayer thickness. The proposed Landau model[7] of the transition in an isolated bilayer takes the form of

fourth-order expansion in ψ with coefficients directly related to measurable quantities. For neutral phospholipid lamellae, we construct a synthesis of this phenomenological model and a continuum description of the relevant intermembrane forces acting between bilayers. These are known to be the long-range van der Waals attraction and short-range water-mediated "hydration repulsion".[2] The thermodynamic functions of a multilamellar bilayer dispersion are then formulated in terms of the volume fraction of lipid, ϕ, T, and ψ, and it becomes clear that at the membrane spacings of interest the order parameter ψ is strongly coupled to the hydration repulsion, and that this interaction is in large part responsible for the main features of the phase diagram of some of the lamellar systems.

A variety of magnetic resonance[8] and calorimetric[9] studies, as well as ultrasonic[10] and transport measurements[11] of bilayers have shown that the melting transition of membranes is pseudocritical, with noticeable pretransitional fluctuations. Various theoretical arguments concerning the melting transition of isolated membranes have been advanced in support of these observations.[12,13] We suggest here that there is an important link between this pseudocriticality and a rather unusual topology to the temperature-composition phase diagram, one in which spinodal lines may appear extremely close to the coexistence curves. We propose that, in general, it is this property of the two-phase equilibria, rather than features of the bilayer melting transition, per se, which is central to an understanding of experimentally observed pseudocriticality.

There is, in fact, a large body of experimental data bearing on the relationship between membrane interactions and internal structure.[14] Although not all of the necessary experiments have been performed on a *single* phospholipid system, there is enough data available to allow us to estimate the theoretical parameters. In Sec. III we consider the measurements which determine those quantities, and indicate the kinds of studies which should allow a quantitative test of the theory. We conclude in Sec. IV with a brief discussion of the relationship of our results to previous studies of membrane interactions and the role of fluctuations, as well as indicating directions for future study.

II. THEORY

A. Landau theory of the main transition of an isolated bilayer

In developing a theory of phase transitions in multilamellar dispersions it is important to specify which of the characteristics of the fluid (L_α) and solid (L_β) phases will serve as an appropriate order parameter for the transition, and will also couple to the membrane interactions. In contrast to the rather universal characteristics of the high-temperature disordered phase, the nature of the low-temperature membrane configuration depends sensitively on the type of polar head group of the lipids.[15] For example, phosphatidylcholines (PC's), which have the —

$N(CH_3)_3^+$ structure in their polar headgroups, typically exhibit a tilted ($L_{\beta'}$) phase, or as has been shown recently,[16] several distinct $L_{\beta'}$ phases further distinguished by the relationship between the tilt and in-plane bond orientational order parameters. On the other hand, phosphatidylethanolamines (PE's) (—NH_3^+), appear to have untilted L_β phases. In either case, though, the L_β bilayer is significantly thicker than that of L_α as a consequence of the elongated crystalline packing of the hydocarbon chains, and the first-order jump in bilayer thickness at the transition is seen directly in X-ray scattering determinations of the multilamellar lattice constants, as well as being inferred from experiments on isolated vesicles.[17] In addition, many phospholipids exhibit an intermediate modulated phase between the L_α and L_β phase.[18] Analysis of X-ray scattering experiments[19] on multilayers of these "rippled" ($P_{\beta'}$) membranes strongly suggests a modulation of the membrane thickness in that phase as well. Clearly then, there are translational, configurational, and orientational order parameters involved in the main transition.

That the membrane thickness should enter naturally into a general coarse-grained order parameter in multilamellar arrays as well as in individual membranes can be seen as follows. We adopt the viewpoint that these lyotropic liquid crystals may be treated in analogy with conventional models of binary liquid mixtures, namely that the two components of the system, lipid and water, are assumed to be characterized by invariant molecular volumes[20] v_l and v_w and together to fill all of space. So, in a mixture of N_l lipids and N_w water molecules in volume Ω, $\Omega = N_l v_l + N_w v_w$, and the intensive thermodynamic functions of the mixture depend only on a single composition variable which we take to be the volume fraction of lipid $\phi \equiv N_l v_l / \Omega$.

Within the so-called "Luzzati approximation"[15] we invoke here, the multilamellar lattice is considered to be composed of sharply defined aqueous and membrane regions, of thickness d and δ, respectively (Fig. 1). These
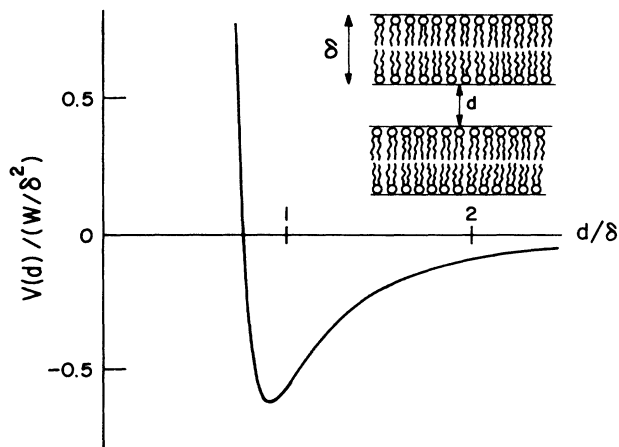

FIG. 1. The intermembrane potential per unit area acting between neutral phospholipid bilayers, normalized by the Hamaker constant W. Inset shows the geometry of the multilamellar lattice.

structural parameters are then related to the composition by

$$\frac{\delta}{d} = \frac{\phi}{1-\phi} , \tag{1}$$

and this implies that at fixed composition there is a single degree of freedom characterizing the two variables δ and d. Note also that the bilayer thickness δ and effective area per head group Σ are simply related by $\Sigma\delta = 2v_l$, so that once either of δ or d is known at a given composition, so too is the geometry of the lattice. At the level of this simple geometric description of the multilamellar structure we see that the bilayer thickness may serve as an internal degree of freedom whose value is chosen by the system in order to minimize its free energy at given values of the thermodynamic parameters T and ϕ; it thus constitutes a natural order parameter. The phenomenological Landau theory we introduce for this variable may equally well be cast in terms of Σ, as others have suggested,[7] but it proves more useful to choose δ as the independent variable and to define a dimensionless order parameter ψ which vanishes in the high-temperature disordered phase and which is nonzero below the chain-melting transition,

$$\psi(\mathbf{x}) \equiv \frac{\delta(\mathbf{x})-\delta_0}{\delta_0} , \tag{2}$$

where δ_0 is the thickness in the L_α phase, and the two-dimensional vector $\mathbf{x}$ references lateral position within the bilayer plane. Although we shall study here only uniform phases, and neglect thermal undulations within the layers, a description[21] of modulated and fluctuating structures necessitates a spatially varying $\psi(\mathbf{x})$

Note that no specific assumption about the microscopic state of the lipids is made in choosing ψ as the order parameter; it should be viewed as a hybrid coarse-grained variable which summarizes changes in various types of degrees of freedom such as tilt and conformational changes of the hydrocarbon chains. Since ψ as defined in (2) makes no explicit reference to the degrees of freedom which distinguish tilted from untilted phases, we shall refer to the low-temperature phase of the model simply as "L_β." The high-temperature bilayer thickness δ_0 is known[5] in fact to be a slowly varying function of temperature, with a large negative thermal expansion coefficient $\alpha \simeq -1\times10^{-3}$ K^{-1} due to the entropic elasticity of chain molecules. In a more detailed theory we may explicitly include this temperature dependence although for the purposes of the present work δ_0 will be assumed constant.

Experiments which are discussed in Sec. III indicate that the typical jump in ψ at the melting transition is $\Delta\psi \simeq 0.1-0.4$ depending on the number of hydrocarbons in the lipid tails, and this is sufficiently small that we may consider constructing a fourth-order[22] Landau expansion of the stretching free energy per lipid $S(\psi)$:

$$S(\psi,T) = a_0 + a_1\psi + \tfrac{1}{2}a_2(T)\psi^2 + \tfrac{1}{3}a_3\psi^3 + \tfrac{1}{4}a_4\psi^4 + \cdots , \tag{3}$$

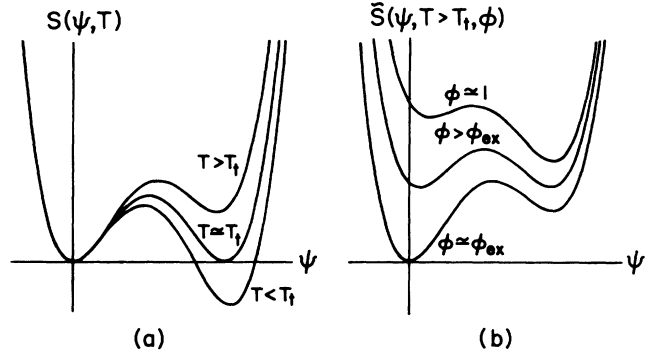where $a_4 > 0$ for stability, and by appropriate choice of

FIG. 2. (a) Thermal variation of the bare stretching energy $S(\psi,T)$, and (b) compositional dependence of the effective stretching energy $\tilde{S}(\psi,T,\phi)$ for interacting neutral bilayer membranes. Note the similar roles played by decreasing water content (increasing lipid volume fraction ϕ) and lower temperature T.

the zero of the lipid chemical potential and of ψ we may, without loss of generality, take $a_0 = a_1 = 0$ for isolated membranes. The double-well structure of $S(\psi,T)$ shown in Fig. 2(a) represents the two competing bilayer thicknesses $\psi = 0(L_\alpha)$ and $\psi > 0(L_\beta)$. As usual in such order parameter expansions, we assume that the important temperature dependence of the coefficients a_i is that of a_2, which is taken to vary linearly in the neighborhood of a reference temperature T_0,

$$a_2(T) \simeq a_2'(T-T_0) . \tag{4}$$

In the absence of a cubic term in S (and with $a_1 = 0$ by our convention), T_0 is the temperature at which the transition from L_α to L_β is critical, but with $a_3 \neq 0$ this critical point is "preempted" by a first-order "melting" transition at a temperature $T_m = T_0 + 2a_3^2/9a_2'a_4$. At T_m there is coexistence between two phases, one with $\psi = 0$, the other having $\psi = -2a_3/3a_4$, so to represent the thicker low-temperature L_β phase, we take $a_3 \leq 0$. The latent heat ΔH per molecule is just $T_t\Delta S$, where ΔS is the entropy jump; from Eqs. (3) and (4) we find $\Delta H = 2a_2'a_3^2T_m/9a_4^2$. Once the reference temperature T_0 is known, the three remaining phenomenological parameters of the Landau model (a_2', a_3, a_4) may be determined uniquely from measurements of the order parameter jump, transition temperature, and latent heat.

B. Bilayer interactions

The intermembrane potential acting between neutral nonfluctuating phospholipid bilayers consists of a long-range van der Waals attraction and the extremely strong short-range hydration repulsion. The dispersion force acting between two membranes of thickness δ separated by a slab of water of thickness d, computed under the assumption of uniformly polarizable slabs and with the neglect of retardation effects (valid[4] for membrane spacings less than ca. 1000 Å), is of the form

$$V_d(\delta,d) = -W\left[\frac{1}{d^2} - \frac{2}{(d+\delta)^2} + \frac{1}{(d+2\delta)^2}\right], \quad (5)$$

where the Hamaker constant $W \simeq 10^{-22} - 10^{-21}$ J is proportional to the square of the polarizability of the membrane. This potential is attractive for all separations d, varying like $-W/d^2$ at small spacings, and like $-6W\delta^2/d^4$ at large separations.

Osmotic stress experiments[2,23] measuring the forces between phospholipid bilayers have shown[24] that the repulsive potential per unit area of membrane is of the form

$$V_h(d) = H\exp(-d/\lambda_h), \quad (6)$$

where the amplitude $H \simeq 0.1-1$ J m^{-2} and the decay length $\lambda_h \simeq 2-3$ Å. The microscopic origin of this interaction is not well understood, but has been suggested[25] to arise from the interaction of the two profiles of induced polarization of water emanating from the polar headgroups of adjacent membranes. This notion has been developed by treating the induced dipolar order of water within the framework of a simple Landau theory that predicts a repulsive potential with an asymptotically exponential decay.[26] We adopt the form (6) for all separations, mindful of the fact that it may be inaccurate when $d \lesssim \lambda_h$.[27]

In this simple theory we take as fixed the parameters W, H, and λ_h which appear in the bilayer interactions, not allowing them to vary explicitly with the order parameter ψ. In reality this is certainly not the case, as it is known for instance that the amplitude and decay length of the hydration repulsion may be quite different in the L_β and L_α phases, as appears to be the case for dipalmitoylphosphatidylcholine (DPPC).[2] These further refinements affect the quantitative aspects of the theoretical results, and are necessary for detailed comparison with experiment, but do not change our basic conclusions.

With the sum of the van der Waals attraction and hydration repulsion as the total bilayer potential $V(d)$ (see Fig. 1), there exists a stable minimum at a separation d^* which is typically of the same order as the bilayer thickness δ, roughly 15–30 Å, and whose depth is of order W/δ^2. Since the dispersion energy as written diverges to $-\infty$ as $d \to 0$, while the hydration energy saturates in that limit at H, there is an additional minimum in the total potential at contact, but this is irrelevant to the calculations described here and may be removed by a cutoff procedure which replaces the first term in the van der Waals attraction by $-1/(d^2+\zeta^2)$, with $\zeta \simeq 0.1\delta$.

The composition at which the water layer thickness $d = d^*$ is essentially the point at which the chemical potential of the intercalated water equals that of bulk water, and at which the lamellar phase coexists with an extremely dilute and probably micellar solution [a phase referred to as[2] "excess water" (EW)]. The maximum swelling of the lattice is thus d^*. As is clear from Fig. 1 the well depth of the intermembrane potential is set by the Hamaker constant W, and as discussed below is negligible compared to the characteristic stretching energy $a_2'(T_m - T_0)$. For this reason, the state of the membranes in equilibrium with excess water is essentially identical to that of an isolated layer.

C. Thermodynamic functions of a multilamellar dispersion

Having invoked the geometrical relationship between the lamellar thickness δ, intermembrane spacing d, and lipid volume fraction ϕ, the Helmoltz free-energy density $F(N_l, N_w, \Omega, T)/\Omega$ is a function only of a single composition variable, as are all intensive thermodynamic functions of the system. F itself is approximated here as the sum of the stretching energy of the lipids, $N_l F_s(\psi,T)$, and the total energy of interaction of the membranes, $\frac{1}{2}AV(\delta,d)$, where $A = N_l\Sigma$ is the total membrane surface area. The small contributions from furtherneighbor and nonpairwise-additive forces are neglected. We define the reduced free-energy density $f(\phi,T;\psi) \equiv Fv_l/\Omega$, and the reduced parameters

$$w \equiv Wv_l/\delta_0^3, \quad h \equiv Hv_l/\delta_0, \quad \lambda \equiv \lambda_h/\delta_0. \quad (7)$$

The two variables w and h then have the units of energy. Expressing the water layer thickness d in terms of the composition and bilayer thickness using (1), and the latter in terms of δ_0 and the order parameter ψ with (2), and with (7), we obtain the free-energy density

$$f(\phi,T;\psi) = \phi S(\psi,T) - \frac{wC(\phi)}{(1+\psi)^3}$$
$$+ \frac{h\phi}{1+\psi}\exp[-D(\phi)(1+\psi)], \quad (8)$$

where $C(\phi) \equiv 2\phi^5(3-\phi^2)/(1-\phi^2)^2$ and $D(\phi) \equiv (1-\phi)/\lambda\phi$. In any equilibrium phase of the system, the order parameter ψ is found from the solution(s) to the minimization equation

$$\phi S'(\psi,T) + \frac{3wC}{(1+\psi)^4} - \frac{h\phi}{(1+\psi)^2}[1+D(1+\psi)]$$
$$\times\exp[-D(1+\psi)] = 0, \quad (9)$$

a Maxwell construction being necessary when multiple solutions exist (see Sec. II E).

We define the reduced chemical potential

$$\mu(\phi,\psi,T) \equiv \left[\frac{\partial f(\phi,T;\psi)}{\partial\phi}\right]_{T,\psi}, \quad (10)$$

and the reduced compressibility

$$\chi^{-1} \equiv \left[\frac{\partial^2 f}{\partial\phi^2}\right]_T, \quad (11)$$

from which compositional fluctuations are computed. Taking account of the compositional variations of the order parameter ψ in the free-energy density $f(\phi,T;\psi)$, we find

$$\chi^{-1} = \left[\frac{\partial^2 f}{\partial\phi^2}\right]_T - \left[\frac{\partial^2 f}{\partial\psi\partial\phi}\right]_T^2 \bigg/ \left[\frac{\partial^2 f}{\partial\psi^2}\right]_{T,\phi}. \quad (12)$$

As in ordinary binary liquid mixtures, equilibrium between two phases with compositions ϕ' and ϕ'' requires the equality of the chemical potentials, $\mu(\phi',T,\psi') = \mu(\phi'',T,\psi'')$, where we have made explicit the fact that the order parameter ψ may take on different values in the

two phases. We require further that there be a common tangent to the Helmholtz free-energy density, so that $f - \mu\phi$ is the same in the two phases, and that the common tangent form the convex envelope of the free-energy density. The computation of equilibria between a lamellar structure and excess water in principles involves the specification of the free energy of the latter (probably micellar) solution as a function of the lipid composition. It is known, that this phase is extremely dilute in lipid, so that its free energy differs little from that of pure water; thus, we take $\mu = 0$ as the approximate coexistence for equilibrium with excess water.

D. Estimates of the phenomenological parameters

Before proceeding to a discussion of the consequences of the free energy (8) we digress to consider the typical values of the parameters a_i, w, etc., which enter the model, and which we use in numerical calculations described below. Note that the equilibrium swelling spacing (and hence composition) in the L_α phase is determined by the competition between the attractive and repulsive molecular forces, with negligible contributions from the stretching energy. We thus require only the dimensionless hydration length λ and the relative amplitude of the dispersion and hydration forces, the ratio w/h, for its calculation. For phosphatidylcholines with intermediate chain lengths,[2] $\delta_0 \simeq 35$ Å, and with a hydration length of $\lambda_h \simeq 2.5$ Å, we obtain $\lambda \simeq 0.07$. In order that the equilibrium swelling composition (at which $\mu = 0$) be that observed,[2] namely $\phi \simeq 0.6$, we find numerically from Eqs. (8) and (10) that $w/h \simeq 1.5 \times 10^{-3}$. With the representative value $H = 0.1$ J m^{-2} and the lipid volume $v_l \simeq 1.1$ nm^3, we obtain $h \simeq 3.1 \times 10^{-20}$ J and $w \simeq 4.6 \times 10^{-23}$ J.

Estimates of the parameters of the stretching energy are obtained from the typical values of the order parameter jump and latent heat of intermediate chain length lipids, $\Delta\psi \simeq 0.3$,[19] and $\Delta H \simeq 25$ kJ/mol.[5] at a transition temperature $T_m \simeq 310$ K. Our estimate of the reference temperature $T_0 \simeq 260$ K involves comparing the shift in the melting temperature of multilayers upon dehydration with the melting temperature of isolated membranes, and is discussed more fully in Sec. II E. Together, these estimates imply $-a_3/a_4 \simeq 0.5$, $a_2' \simeq 2.4 \times 10^{-21}$ J K^{-1}, and $a_4 \simeq 2.2 \times 10^{-18}$ J. Scaling all quantities by the hydration energy h, we arrive at rough estimates of characteristic energy ratios:

$$w/h \simeq 1.5 \times 10^{-3} , \quad a_3/h \simeq -30, \quad a_4/h \simeq 60 . \quad (13)$$

In these units, the melting transition occurs at $a_2'(T_m - T_0)/h = \frac{10}{3}$ so with $T_m - T_0 \simeq 50$ K we find that each unit of the dimensionless temperature variable a_2/h corresponds to 15 K.

These estimates allow us to assess the relative energy scales associated with the membrane interactions and internal degrees of freedom. Note also that the curvature of the stretching energy controls the deviations of the bilayer thickness from its high-temperautre equilibrium value δ_0. Equating the typical energy of stretching with the thermal energy at the melting temperature we obtain

$\langle \psi^2 \rangle \sim k_B T_m / a_2'(T_m - T_0)$, or $\langle \psi^2 \rangle^{1/2} \simeq 0.2$ as the typical root-mean-square amplitude of relative thickness fluctuations. For the shorter lipids with lower transition temperatures, closer to T_0, the compressibility and fluctuations increase considerably. These same considerations indicate that membrane interactions can have a large effect on the melting behavior, for the characteristic energy scale of the hydration amplitude h is comparable to that of the latent heat of the noninteracting membranes.

E. Interaction-driven structural phase transitions

Returning to the free energy (8), note how the order parameter ψ appears not only in the stretching energy S but also in the interaction terms, especially in the exponential of the hydration contribution. This is the mathematical mechanism by which membrane interactions may affect the structural phase transition within the layers and indicates how ψ is a natural order parameter independent of the microscopic degrees of freedom involved in the melting transition.

Below we study the full form of f in Eq. (8) without further approximations, but it proves illuminating to consider first defining an effective stretching free-energy density $\tilde{S}$ which accounts for membrane interactions. It is defined by the relation $f = \phi\tilde{S}(\psi, T, \phi)$, and like the bare stretching energy $S(\psi, T)$, it may be expanded as a power series in ψ:

$$\tilde{S}(\psi, T, \phi) = \tilde{a}_0 + \tilde{a}_1\psi + \tfrac{1}{2}\tilde{a}_2\psi^2 + \tfrac{1}{3}\tilde{a}_3\psi^3 + \tfrac{1}{4}\tilde{a}_4\psi^4 + \cdots ,$$

$$(14)$$

the effective Landau coefficients $\tilde{a}_i$ being functions of composition. This expansion reveals most of the essential features of the interplay between membrane interactions and the main transition. First, by the existence of the linear term,

$$\tilde{a}_1(\phi) = 3wC - h(1 + D)e^{-D} , \quad (15)$$

even in the absence of one in the bare stretching energy S, we see that the interactions act as an external field on the order parameter ψ. At compositions ϕ higher than those at equilibrium with excess water, the hydration contribution dominates that from dispersion interactions, and this field varies approximately as $-(h/\lambda)\exp(-d/\lambda_h)$. This negative field acts to shift the effective free energy in a way which favors thicker membranes at lower water contents. This is the essence of Parsegian's original thermodynamic analysis[28] relating the swelling of lipids upon dehydration to the existence of a repulsive intermembrane force. Here we investigate these effects in the context of a stretching free energy (2) possessing two competing minima, rather than the single minimum assumed in that earlier work. Independent of the particular mechanism of the structural change, e.g., varying tilt or chain disordering, the existence of a linear field $\tilde{a}_1$ which shifts the minimum of the stretching potential to larger ψ explains the progressive increase in membrane thickness universally observed as the multilamellar stack is dehydrated.[2,16,19,23]

In Fig. 2(b) we show the variation in $\tilde{S}$ with ψ at a fixed temperature above the melting point of an isolated layer and for various compositions ϕ. Even though the L_β state is only metastable at high water contents, it becomes stable upon progressive dehydration. Note the similarity of this progression of curves with increasing ϕ to that seen with varying temperature for the bare stretching energy of an isolated membrane [Fig. 2(a)]. The similar roles which temperature and composition play in phospholipid systems suggest that they should be considered as an intermediate class between thermotropic and lyotropic liquid crystals.

The modification of the bare Landau coefficients also has important consequences for the degree of pseudocriticality of the melting transition, and to illustrate this it is convenient to define a new order parameter φ via $\psi = \varphi + \Delta$, and choosing the shift Δ so that the expansion of $\tilde{S}$ in powers of φ has no cubic term. This is accomplished by the choice $\Delta = -\tilde{a}_3/3\tilde{a}_4$, and results in a Landau theory like that of a ferromagnet in an external field, and *below* its critical temperature,

$$\tilde{S} \simeq \tilde{c}_0 + \tilde{c}_1\varphi + \tfrac{1}{2}\tilde{c}_2\varphi^2 + \tfrac{1}{4}\tilde{c}_4\varphi^4 + \cdots . \tag{16}$$

The effective external field is

$$\tilde{c}_1(\phi, T) \simeq \tilde{a}_1 - \frac{\tilde{a}_3}{3\tilde{a}_4}\left[\tilde{a}_2 - \frac{2\tilde{a}_3^2}{9\tilde{a}_4}\right], \tag{17}$$

and $\tilde{c}_2 < 0$. In the absence of interactions, when $\tilde{a}_1 = 0$, $\tilde{c}_1 \simeq -(a_3/3a_4)a_2'(T - T_m)$, and thus passes through zero at the main transition temperature of an isolated layer. In this sense, the L_β and L_α phases play the role of the up- and down-magnetized phases of a ferromagnet.[12] In the presence of interactions, and with $\tilde{a}_1 \sim -(h/\lambda)\exp[-(1-\phi)/\phi\lambda]$, the effective field vanishes at a temperature which deviates exponentially from the melting temperature of the isolated membranes, and inversely with the order parameter jump at the transition. Although the computation of the actual transition temperature as a function of composition requires a Maxwell construction, some indication of that variation may be obtained by assuming the tie lines to have negligible width (as appears to be the case experimentally[29,30]) and simply evaluating the temperature at which $\tilde{c}_1 = 0$. From this, we conclude that the melting temperature should vary with membrane spacing with the same form as the hydration energy, namely, exponentially with a decay length of order 2–3 Å; this is seen in hydrated phosphatidylethanolamine multilayers.[30] The well-known[5] decreasing strength of the first-order transition with decreasing chainlength n then leads to the prediction that the *shift* in transition temperature from fully hydrated down to some given spacing d should be larger for the shorter lipids, and this too is the case in the PE's.[30] These two results are essentially a consequence of a Clapeyron equation, $dT/d\mu \sim \Delta H/\Delta\psi$, with the chemical potential being dominated by the hydration energy, and the ratio of the latent heat to the order parameter jump varying inversely with $\Delta\psi$.

In the transcribed version of the Landau theory, the effective reduced "temperature" of the Ising model, $\tilde{c}_2$,

serves as a measure of the pseudocriticality and is shifted upward from its negative value of $-a_3^2/18a_4$ in the absence of interactions. This implies that the main transition becomes weaker upon dehydration of the lattice, and will be reflected in progressively decreasing order parameter jumps and latent heats which follow the form of the hydration repulsion. Such a variation in the latent heat is seen in experiments on PE's.[27] When the hydration repulsion is sufficiently strong we find (see below) that it is possible that $\tilde{c}_2$ may become positive at very low hydrations; this corresponds to a supercritical state of the analogous ferromagnet, and implies that interactions have transformed the first-order melting transition to a critical point.

F. Phase diagrams

In the construction of temperature-composition phase diagrams, appropriate Maxwell constructions may be found numerically by determining on a finely spaced mesh of compositions the solutions to the minimization equation (9). From these, we compute the two branches (L_α and L_β) of both the free energy and chemical potential. A numerical search within the range of chemical potentials defined by the extremes of the van der Waals loop is then used to locate the common tangent.

Figure 3(a) shows examples of two isotherms computed with the parameters described above, one at a value of $a_2/h = 3.5$, above that of the main transition of an isolat-
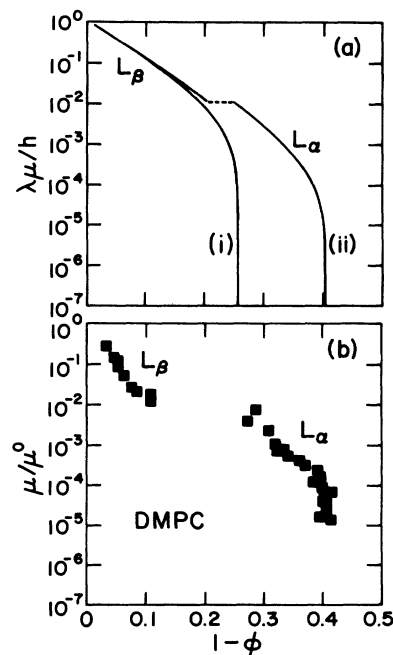

FIG. 3. (a) Chemical potential as a function of composition for the model system below (i) ($a_2/h = 3.0$) and above (ii) ($a_2/h = 3.5$) the melting temperature of the isolated membrane ($a_2/h = 3.33$). Data shown in (b) are for DMPC, from Ref. 31, with the chemical potential normalized to its extrapolated value at complete dehydration.

ed membrane ($a_2/h \simeq 3.33$), the other at a lower value, $a_2/h = 3.0$. For the low-temperature isotherm, the reduced chemical potential drops continuously toward zero with increasing water content. In contrast, the high-temperature isotherm exhibits a first-order jump at the coexistence between two lamellar phases differing both in composition and in membrane thickness, finally tending toward zero at a larger volume fraction of water than the low-temperature phase. This trend toward larger swelling in the disordered phase, a consequence of the smaller L_α bilayer thickness and consequently weaker van der Waals attraction, is in agreement with experiment.[18] As an indication of the kind of semiquantitative agreement possible between theory and experiment, we show in Fig. 3(b) the experimental data of Fuller, Parsegian, and Rand on dimyristoylphosphatidylcholine (DMPC) (Ref. 31) in water at a temperature a few degrees above its main transition, plotted in the same dimensionless units as in Fig. 3(a), with the normalization of the chemical potential, μ^0, being the extrapolated value at complete dehydration. The present theoretical model is seen to account semiquantitatively for the main properties of the isotherm, such as the coexistence value of the chemical potential relative to its saturation value μ_0, and the equilibrium composition of the L_α phase. The differing slopes of the L_α and L_β branches of the DMPC isotherm shown in the semilogarithmic plot of Fig. 3 reveals that the apparent decay lengths of the hydration repulsion differ in those two phases. The model we have studied thus far does not contain any coupling between the intramembrane order and the decay length λ_h.

Figure 4(a) shows for the same two systems as in Fig. 3(a) the thickness order parameter ψ as a function of chemical potential, highlighting the role of the membrane interactions, measured by the water chemical potential, acting as an external field on the in-plane structural transition. The curve (ii) may be viewed as analogous to a subcritical isotherm of a ferromagnet, with μ like the external magnetic field and ψ related to the magnetization. To the extent that the chosen parameters describe a moderately strong first-order phase transition, there is little indication of critical fluctuations in the neighborhood of the coexistence value of μ. The data[31] on DMPC has

not previously been plotted in the variables of Fig. 4(a), but as shown in 4(b) it displays very much the same behavior, and is semiquantitatively described by the theory.

A sequence of isotherms of the kind shown in Fig. 3(a) may be used to construct a complete temperature-composition phase diagram for the hydrated bilayer system, of which Fig. 5 is an example. There is a line of first-order $L_\alpha-L_\beta$ transitions which terminates at a triple point involving in addition the coexistence of excess water. The extreme narrowness of the tie lines, in agreement with experiment,[29,30] indicates the generally pseudocritical nature of the phase transitions in the presence of water. At the extremely dehydrated limit, we find that it is indeed possible for the interactions to have renormalized sufficiently the properties of the chain-melting transition that it has been transformed from a first-order transition to a critical point C. Beyond this liquid-gas–like critical point, the bilayer thickness changes continuously as a function of temperature. Whether this point exists in reality depends crucially on the actual symmetry differences between the two states, as we discuss below. We remark that data[31] on dilauroylphosphatidylcholine (DLPC) similar to that shown for DMPC in Fig. 4(b), but at a temperature significantly higher than T_m of an isolated membrane, indicates a pronounced degree of curvature in the $\psi-\mu$ representation. This is suggestive of a highly pseudocritical transition at low water contents.

The discussion in Sec. II E concerning an effective Landau theory which accounts for membrane interactions indicated that the hydration-induced melting temperature shifts ΔT_m are exponential in the bilayer spacing and thus approximately linear in the chemical potential μ. At the same time, $\Delta T_m \sim 1/\Delta\psi$ and so these shifts increase with more weakly first-order transitions. This is illustrated in Fig. 6 where we have plotted the $T-\mu$ phase dia-
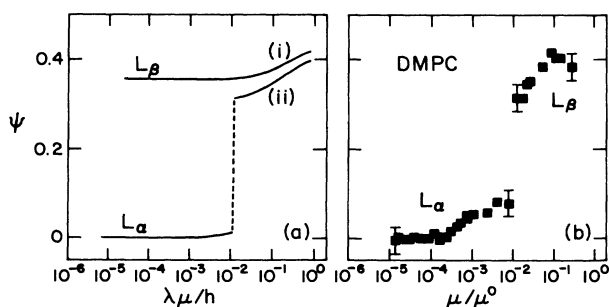

FIG. 4. Order parameter as a function of chemical potential for the two systems in (a), illustrating interaction-driven structural phase transitions. Data shown in (b) for comparison are that of DMPC, from Ref. 31.
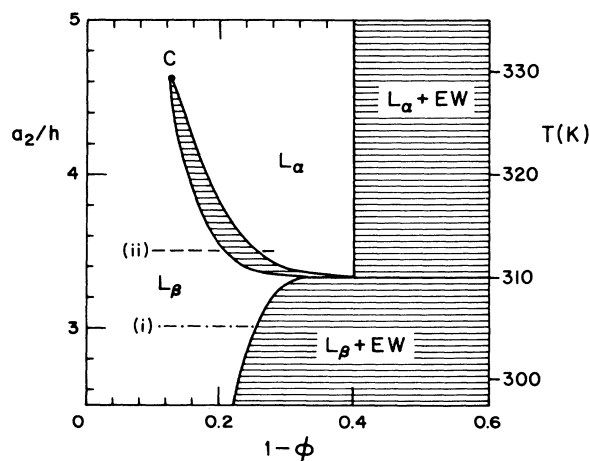

FIG. 5. Phase diagram of a multilamellar dispersion. Horizontal lines are tie-lines; equilibrium with excess water is denoted by "EW." Vertical scales are that of the bare theory (left) and in absolute degrees (right) based on the typical values of the material parameters. Isothermal cross sections labeled (i) and (ii) correspond to those in Figs. 3 and 4.

grams for three systems differing in the size of the quartic term a_4 in the stretching energy [a_4/h =90, 72, 60 in (i), (ii), and (iii), respectively], at fixed value of the cubic term, $a_3/h = -30$. The increase in transition temperature and latent heat in the sequence (i)–(iii) is qualitatively like that seen with increasing hydrocarbon chain length. Figure 6 then bears an interesting resemblance to the family of layering transitions seen in studies of adsorbed phases,[32] with hydrocarbon number corresponding to layer number, and the L_α-L_β transition being analogous to a liquid-gas transition within the layer.

For intermediate chain length lipids the shift in melting temperature upon dehydration to some 5–10 Å from its values in excess water is on the order of 10–20 K. This sets the temperature scale as $a_2/h \simeq 15$ K, so a system with melting transition at $a_2/h = \frac{10}{3}$ and $T_m \simeq 310$ K corresponds to one with the reference temperature $T_0 \simeq 260$ K.

The extreme skewing of the coexistence curve and its general narrowness have important implications for the interpretation of certain experiments which probe fluctuations within the membrane and of the water nearby. In particular, it is of interest to compute the spinodal lines of the two-phase L_α-L_β coexistence. These are defined as the loci of the vanishing of the second compositional derivative of the free energy, that is, the divergence of the osmotic compressibility $\chi \equiv (d^2 f/d\phi^2)_T^{-1}$ as given in Eq. (12).

Figure 7 is an enlargement of the neighborhood of the critical point C for a system with interaction parameters as before, but a more weakly first-order transition ($a_3/h = -30$, $a_4/h = 90$), with spinodal lines indicated. With our previous estimate of the temperature scale, $a_2/h \simeq 15$ K, we see that the spinodals may take on a remarkable relationship to the coexistence curve, coming within fractions of a degree of the phase boundaries.
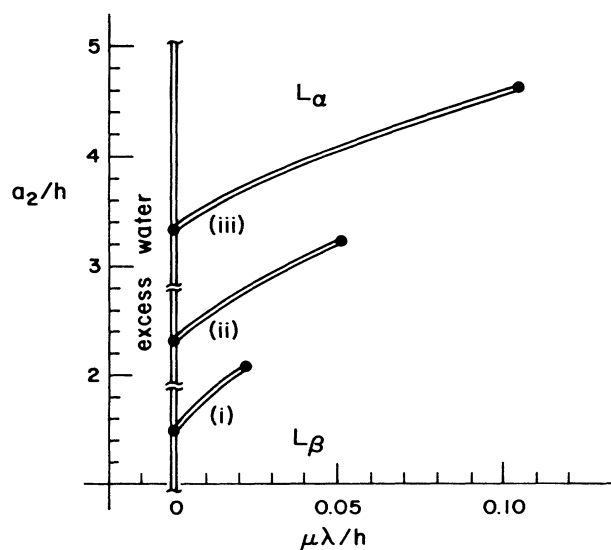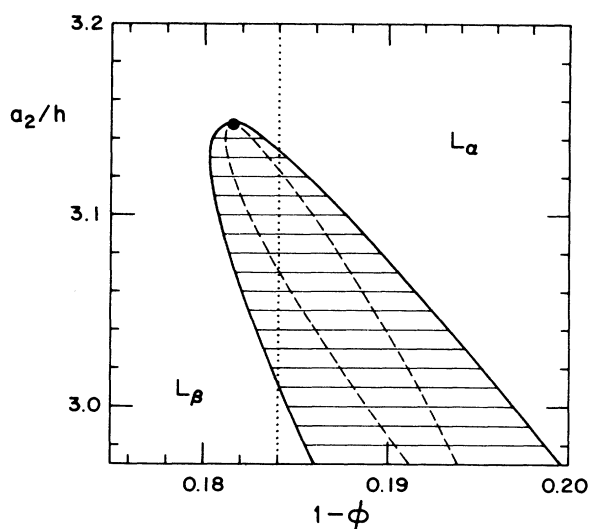

FIG. 7. Detail of a phase diagram in the region of the critical point C indicating the spinodal lines (dashed). Measurements of the compressibility along the indicated dotted path would detect two spinodal lines spaced several degrees apart.

That this should happen near a critical point in general is not surprising; what is significant here is that the coexistence curve is so narrow that such a proximity may persist to temperatures quite far from the critical point. Furthermore, the extreme skewing of the two-phase region implies that a temperature scan at fixed composition, like that indicated by a dotted line in the figure, would show the presence of *two* spinodal lines separated by several degrees.

Any experiment which is sensitive to long wavelength fluctuations of the ordering degrees of freedom of the mixture will sense these close spinodals, and appear pseudocritical, even if the in-plane transition is moderately first order. An example of this is the measurement of sound velocity,[10] which depends on the adiabatic compressibility of the mixture. We remark that a recent NMR study[8] of water order near neutral phospholipids has indeed revealed the existence of two spinodal lines along isocomposition paths, near the triple point in PC's at which L_α, P_β, and excess water coexist. The difference in temperature between the spinodals inside the P_β-L_α coexistence region was found to increase upon progressive dehydration, in much the same way as would be expected on the basis of Figs. 5 and 7. These considerations suggest that the pseudocritical effects at the main transition must be understood in the context of the thermodynamics of the coexisting lamellar phases. It is important to emphasize the distinction between on the one hand the reference temperature T_0 which enters the model of phase transitions of isolated membranes, and on the other the spinodal temperature. Unlike the latter, the former is many degrees away from the melting transition.

G. Other order parameters

The main shortcoming of the theoretical model presented here is the simplification in the choice of the

FIG. 6. Temperature-chemical potential coexistence curves for varying strength of the melting transition.

order parameter ψ. The solid L_β phase differs from the liquid L_α phase not only in the thickness of the membranes, but also in the intramembrane microscopic ordering. In fact, these two phases have different symmetries. Thus, beyond the critical points C in Figs. 5 and 6 there must exist another transition line across which the in-plane positional symmetry is broken. If the L_β phase is of hexatic nature, then such a line would be a locus of Kosterlitz-Thouless transitions. Furthermore, recent experiments by Smith et al.[16] demonstrate the existence of several different solid-like L_β phases distinguished by their tilt and positional order. One must therefore include such additional order parameters in future extensions of the present model to describe the full phase behavior of the lamellar systems.

III. REMARKS ON COMPARISON WITH EXPERIMENTS

We have already indicated in various places in the text some rough quantitative comparisons between theory and experiments, and in this section we discuss the experimental information which is necessary to ascertain the validity of the present theory. In general, the types of measurements which are readily interpreted in the framework of this paper are (i) osmotic stress measurements of the water chemical potential as a function of bilayer spacing, composition, and temperature, (ii) x-ray determinations of the bilayer thickness versus μ or ϕ and (iii) calorimetric information on the latent heat of transition at various compositions. Magnetic resonance studies of water order, permeability measurements, and ultrasonic attenuation experiments provide additional information on fluctuations and pseudocriticality. Together, these measurements overdetermine the theoretical parameters of the Landau theory and of the interactions, and so provide an important consistency check on the model.

In determining the coefficients of the Landau theory of the main transition, calorimetric and structural data on isolated bilayers are most easily interpreted. Accurate knowledge of the order parameter jump in excess water, preferably by comparison of measurements taken just above and just below the melting temperature of each lipid system, together with latent heat data determine the coefficients a_i, provided the refereence temperature T_0 is known (see below). While most of this information is available for several PC's, the presence of a modulated phases complicates the interperetation of that data. We suggest therefore that the PE's, which have no such intermediate phase and have an untilted L_β phase, constitute a simpler class of neutral lipids. Systematic measurements of the transition enthalpy in these systems are available already.[33]

Although there have been many studies of isothermal cross sections of the phase diagram in Fig. 5, in no single system is there a series of such isotherms both above and below the main transition. Such isotherms would give information on the membrane interactions completing the quantities necessary to determine the theoretical parameters. We encourage measurements of the global diagram, particularly at temperatures far above the melting tem-

perature T_m of isolated layers, for they may reveal the existence of the critical point C.

In the absence of a complete set of information on one single lipid system, we have used existing osmotic stress data and x-ray data in PC systems to estimate the material parameters. One major uncertainty is the location of the reference point T_0 of the chain-melting transition. Note that T_0 plays the role of a kind of "pseudocritical" point in the Landau theory in the sense that thermodynamic quantities of a given lipid system will show singularities upon extrapolation to T_0. Our estimates suggest that for intermediate length lipids T_m-T_0 is some tens of degrees; large pseudocritical fluctuations are thus improbable within a single layer. On the other hand, it is known that the latent heats of the main transition systematically decrease with decreasing chain length, extrapolating to zero at a critical length $n_c \simeq 9-10$. In addition, recent studies[19] of PC's have shown that the main contribution to the order parameter jump $\Delta\psi$ between L_α and L_β phases occurs at the L_α-P_β transition, that these jumps are typically in the range $0.1 < \Delta\psi < 0.4$, and that $\Delta\psi(n)$ also extrapolates smoothly to zero at $n \simeq n_c$. Most interestingly, the hypothetical melting temperature $T_m(n_c)$ lies close to our estimate of the reference temperature T_0. These two pieces of evidence suggest that the hypothetical system with $n = n_c$ would present a continuous transition at $T_m \simeq T_0$ and that hydrocarbon chain length plays the role of a thermodynamic field controlling the proximity to this second-order phase transition. If true, the above reasoning shows that one can approach the "hidden" critical point by effectively using finite-size effects analogous to those discussed in the context of capillary critical points.[34] We emphasize that more detailed experiments are needed in order to provide a critical test of this observation.

IV. CONCLUSIONS

We have shown that a simple synthesis of a phenomenological theory of intramembrane phase transitions with a continuum model of molecular forces between membranes leads to a realistic description of the phase behavior of lyotropic liquid crystals of phospholipid bilayers. Our emphasis is on the way in which membrane interactions couple to some internal order parameter of the bilayers: in the present case, the bilayer thickness. Generalizations of the present approach to include other such internal degrees of freedom are feasible, particularly with regard to the presence of impurities which may segregate within the layers. It is also of interest to study situations in which the internal state of the layers varies from one layer to the next, as one would expect for both semi-infinite systems and thin suspended films.[16] An extension of the present approach to the description of the hexagonal phase of phospholipids, and its equilibrium with lamellar phases, may prove illuminating.

In this paper, we have neglected thermal fluctuations both in the in-plane order parameter(s) and in the intermembrane spacing. Inclusion of such fluctuations is particularly important in the swollen regime at equilibrium with excess water, for new critical phenomena involving

indefinite swelling of the lattice ("unbinding"[35]) may occur. Indeed, the "hyperswollen" phases, probably driven by such fluctuations, have recently been observed.[3]

Despite all of the difficulties at present in determining the material parameters from experiment, rough fits to the data in Figs. 3(b) and 4(b) already reveal that the basic picture of the interaction influenced main transitions can be quantitatively described in the framework of the present model. Even more imortantly one can already notice some shortcomings of the accepted picture of the molecular interactions. For instance, it is clear from the data in Fig. 4(b) at low water contents that the exponential decay of the chemical potential is apparently characterized by different decay lengths in the solid and fluid phases. This disagrees with the basic assumption implicit in simple Landau theories of hydration forces, namely that λ_h is a property of bulk water, rather than also a function of the state of the polar headgroups. As suggested, a systematic and complete study of a single bilayer system may shed light on the origin of this and other possible discrepancies.

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*Present address: The Enrico Fermi Institute, The University of Chicago, 5640 Ellis Avenue, Chicago, IL 60637.

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