EQUILIBRIUM STUDIES OF LECITHIN-CHOLESTEROL INTERACTIONS

II. PHASE RELATIONS IN SURFACE FILMS: ANALYSIS OF THE "CONDENSING" EFFECT OF CHOLESTEROL

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ABSTRACT From measurements of the equilibrium spreading pressure π^e for dispersions of lecithin—dimyristoyl (DML) or dioleoyl (DOL)—and cholesterol (CHOL) in water, we have deduced the phase relations in both the aqueous dispersions and the equilibrium surface films. At 29.5°C, when the mole fraction of cholesterol in the dispersion x(CHOL) is $0 < x(\text{CHOL}) < 0.33$, π^e is constant and equal to the value for pure lecithin (DOL or DML). The phase rule predicts that two bulk lipid phases coexist; these are pure lecithin and lecithin:cholesterol 2:1 complex. The equilibrium surface film contains only lecithin and therefore lecithin and 2:1 complex are immiscible in surface films. When $0.33 < x(\text{CHOL}) < 1.0$, π^e is also constant with a value intermediate between that for pure lecithin and cholesterol. In this range of lipid composition two bulk lipid phases also coexist: lecithin:cholesterol 2:1 complex and pure cholesterol. However, the equilibrium surface film contains only the 2:1 complex and, therefore, 2:1 complex is also immiscible with cholesterol in surface films. When $\pi < \pi^e$, as in the case of spread films, we deduce that two surface phases may coexist; the composition of the phases will depend on x(CHOL). When $0 < x(\text{CHOL}) < 0.33$, both lecithin and 2:1 complex coexist, and when $0.33 < x(\text{CHOL}) < 1.0$, 2:1 complex and cholesterol coexist. The "condensing" effect of cholesterol in lecithin surface films is reexamined. The effect is attributed to formation of the lecithin:cholesterol 2:1 complex and nonequilibrium conditions in the two-phase surface film.

INTRODUCTION

Evidence for the formation of a lecithin:cholesterol 2:1 complex, and for phase relations in which the 2:1 complex is immiscible when condensed phases of lecithin and cholesterol has been presented (1). Even though the methods employed in that study helped identify equilibrium properties of the lipid dispersions, they could only provide

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indirect verification of the stoichiometry of the 2:1 complex. More direct evidence is attainable from the colligative properties of the lipid dispersions, but the solubilities of lecithin and cholesterol in water are too low for measurement of these thermodynamic properties to be feasible. However, even for extremely dilute aqueous lipid solutions, properties of the equilibrium surface film can often be readily measured. Therefore, in the present study, surface pressures (π) of equilibrium lecithin-cholesterol dispersions were used as the basis for a rigorous thermodynamic method to verify both the existence of the 2:1 complex and the validity of the phase relations given in part I (1). Implicit in this approach is the possibility of also obtaining the phase relations in the adsorbed surface film in equilibrium with the dispersion.

Adsorbed films, in principle, are identical to those formed by spreading a solution of the lipids in a volatile solvent, provided that equilibrium conditions are established (2–4). Surface films composed of lecithin and cholesterol, formed by spreading, have been used to study the interactions of these lipids in condensed surface states on water. Cholesterol's "condensing" effect on lecithin, originally observed by Leathes (5), has been considered a fundamental property of surface film interactions, and has been interpreted by various molecular models (6–9).

However, those film studies have recently been criticized because they may not have been made under equilibrium conditions (10); moreover, the films may have been heterogeneous and contained more than one surface phase (11, 12). In more recent studies of equilibrium films of lecithin and cholesterol at very low surface pressures, it was shown that homogeneous two-component films are formed, but that phase separation must occur with increasing surface pressure (13). This conclusion was confirmed by studies (14) in which spread films containing both lecithin and cholesterol, apparently homogeneous and showing the condensing effect, were allowed to stand for prolonged periods with the surface pressure (π)-area (A) isotherms being monitored periodically. Spontaneous expansion of the π-A isotherm was observed and the expansion was attributed to demixing of the lipid components, but the nature of the separated phases was not established. In view of the uncertain significance of the condensing effect as a fundamental measure of molecular interactions in spread films, we have also reexamined the nature of the interactions in mixed films of lecithin and cholesterol using the properties of adsorbed films.

In this study, dispersions of lecithin and cholesterol in water were prepared by procedures known to yield equilibrium systems (1). From the π-composition dependence we show that the dispersions conform to the phase relations presented in the preceding study (1). The surface film compositions of the equilibrium films are evaluated, and these results are used to reinterpret the condensing effect of cholesterol.

EXPERIMENTAL METHODS

Equilibrium dispersions of lipid in water were prepared by procedures described previously (1). The majority of the dispersions in this study were prepared by vortexing at room temperature, but some were prepared by bath sonication at temperatures below T_c, the gel-liquid crystal

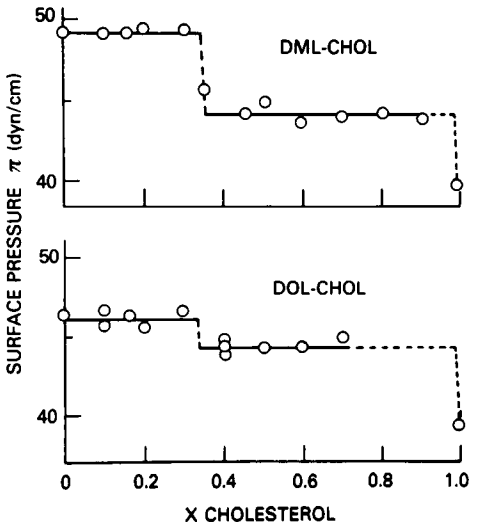

FIGURE 1 Surface pressures (π) for dispersions of DML-cholesterol (DML-CHOL) and DOL-cholesterol (DOL-CHOL) in water as a function of the mole fraction x of cholesterol in the dispersion; x(cholesterol) + x(lecithin) = 1. Temperature is 29.5°C.

transition temperature of the lecithin. L-α-dimyristoyl lecithin (DML), L-α-dioleoyl lecithin (DOL), and cholesterol (CHOL) were the same samples used previously (1).

Surface pressures were measured at 29.5°C by the Wilhelmy plate technique with an apparatus described earlier (15). All measurements were performed in an atmosphere of nitrogen to prevent oxidation of cholesterol. Precision of the measurements was ±0.2 dyn/cm; temperature control was ±0.1°C. Dispersions were prepared with lipid concentrations varying from 0.1 to 1.0 mg/ml; no surface pressure differences were detected in this range of concentrations. For most of the dispersions, equilibrium, as determined by unchanging values of π with time, was reached within 1 h, and measurements were continued for an additional hour to assure that the surface pressures remained constant. Dispersions with high cholesterol content (mole fractions > 0.75) were followed for 24 h because these systems took somewhat longer to reach equilibrium (4 h). Water evaporation was not significant in these longer experiments because nitrogen saturated with water vapor was constantly flowing through the measuring chamber.

RESULTS AND DISCUSSION

Equilibrium Phase Relations in Surface and Bulk: Verification of the Lecithin:Cholesterol 2:1 Complex

Surface pressure-composition diagrams for DML- and DOL-cholesterol dispersions in water at 29.5°C are presented in Fig. 1; similar results were obtained with both lecithins. In the range of cholesterol mole fractions x(CHOL) from 0 to 0.33, the surface pressure is constant and equal to the value for the pure lecithin (x[CHOL] = 0). When $0.33 < x(\text{CHOL}) < 1.0$, π is again constant but the value is different from either the value for pure lecithin or for pure cholesterol.

The phase rule for this system at atmospheric pressure and constant temperature may be written (16, 17)

$$F = C - P^b - P^s + 1, \quad (1)$$

where C is the total number of components in the system (four: water, lecithin, cholesterol, and nitrogen), P^b is the number of bulk phases, P^s is the number of surface phases, and F is the number of independent intensive variables that may be altered without changing the phase relations. Because temperature and pressure are constant, the only intensive variable is the composition, and when π is independent of composition, $F = 0$. Because $P^s = 1$,[1] the phase rule predicts that $P^b = 4$ (water, nitrogen, and two lipid phases). The composition of each of the coexisting bulk lipid phases is given by the limits of the π-composition plots (Fig. 1) in which the surface pressure is independent of composition. Thus for $0 < x(\text{CHOL}) < 0.33$ the composition of one of the phases is 2 mol of lecithin to each 1 mol of cholesterol, and the other lipid phase contains pure lecithin. When $0.33 < x(\text{CHOL}) < 1.0$, two bulk lipid phases also coexist: free cholesterol and the phase containing lecithin:cholesterol in the molar ratio 2:1. In each case the 2:1 lecithin:cholesterol phase is in equilibrium with either a pure lecithin or a pure cholesterol phase. These results indicate that the 2:1 lecithin:cholesterol phase is a molecular complex because it is stable in the presence of excess amounts of each of the pure constituents. The result also agrees with the conclusions of the independent studies of the preceding paper (1).

The composition of the equilibrium surface films may also be evaluated from the phase diagram in Fig. 1. When $x(\text{CHOL}) = 0$, the system contains only lecithin dispersed in water, and $\pi = \pi^e$, the equilibrium spreading pressure (18, 19) of a saturated lecithin solution. As cholesterol is added to the system, 2:1 complex forms and it, too, is present as bulk phase because all the lipids are present far in excess of their solubilities in water. However, the surface pressure remains constant at the value of π^e for pure lecithin despite the presence of the 2:1 complex phase. With further increase in cholesterol content, more 2:1 complex is formed with a corresponding decrease of the pure lecithin phase until the amount of lecithin falls below the saturation value of lecithin in water. With the disappearance of bulk phase lecithin, the phase rule predicts that $F = 1$ (Eq. 1), and π, now a function of the lecithin concentration in water, decreases as the lecithin concentration decreases. This is noted by the dashed line in Fig. 1 where $x(\text{CHOL})$ approaches 0.33. The concentration of lecithin ultimately becomes zero at $x(\text{CHOL}) = 0.33$, and only the lecithin:cholesterol 2:1 complex is present. The surface pressure is π^e for the 2:1 complex. With further increase of $x(\text{CHOL})$ two lipid phases reappear, the 2:1 complex and a pure cholesterol phase. The surface pressure is again independent of composition and remains at the value of π^e for the 2:1 complex until $x(\text{CHOL}) \approx 1.0$. When $x(\text{CHOL}) \approx 1.0$, the concentration of 2:1 complex falls below the saturation value in water and π decreases until only cholesterol is present. The surface pressure is now π^e for a saturated solution of cholesterol.

Thus when $0 < x(\text{CHOL}) < 0.33$, the surface film is composed only of lecithin de-

[1] When bulk lipid phase is present, the phase rule (Eq. 1) requires that $P^s = 1$. For example, when $P^b = 3$, only one bulk lipid phase is present whose composition will vary. Therefore, $F = 1$ and $P^s = 1$.

TABLE I

EQUILIBRIUM SPREADING PRESSURES π^e OF DML, DOL, CHOLESTEROL, AND THE LECITHIN:CHOLESTEROL 2:1 COMPLEX

Lipid	π^e
	dyn/cm ±0.2
CHOL	39.3
DML	49.0
DML:CHOL 2:1 complex	44.1
DOL	46.3
DOL:CHOL 2:1 complex	44.4

T = 29.5°C.

spite the presence of 2:1 complex in bulk and in solution. This can only occur if the 2:1 complex is immiscible with lecithin in surface films. When $0.33 < x(\text{CHOL}) < 1.0$, the surface film is composed exclusively of the 2:1 complex, even though cholesterol is also present, and the complex is therefore also immiscible with cholesterol in surface films. Preliminary studies in which the surface concentrations were measured with radiotracers of lecithin and cholesterol have confirmed these conclusions (K. Tajima and N. L. Gershfeld, unpublished results).

Because the 2:1 complex is immiscible with either lecithin or cholesterol, the composition of the surface film in saturated lipid dispersions is determined by the component with the higher π^e (17). Table I lists the values of π^e for each of the compounds that have been measured at 29.5°C. It should be noted that π^e will vary with temperature. For example π^e for DML is almost zero at temperatures below 23.5°C, the gel-liquid crystal transition temperature, T_c (20), whereas π^e for cholesterol increases with decreasing temperatures (15). Thus with variation of temperature the composition of the surface film in saturated lipid dispersions will depend on the temperature variation of π^e for each of the components in the aqueous dispersion.

Distinction between Adsorbed and Spread Films

Before examining the condensing effect observed with spread films of lecithin and cholesterol, it is important to identify the conditions under which adsorbed and spread films are equivalent. This question has been examined in some detail previously (4, 12), and aspects of that discussion will be summarized briefly.

The main distinction between adsorbed and spread films lies in the fact that lipid is distributed throughout the system (surface and bulk) for the former, whereas for the latter the lipid is assumed to be confined to the surface. Asserting that the two film types are equivalent presents an apparent paradox that spread films are to be considered insoluble, and yet equilibrium conditions require that some of the surface film lipid be distributed in the underlying aqueous phase. However, it has been established that immediately upon spreading a lipid film, a portion of the lipid material desorbs into a thin region of the aqueous phase just beneath the surface (21). The amount that desorbs will depend on a number of factors: the rate of desorption and the rate

of diffusion away from the surface region to the bulk aqueous phase. The maximum amount of lipid desorption will occur when the rate of diffusion away from the surface is much less than the rate of desorption; the ratio of the surface concentration to the amount desorbed then approaches that represented by the Gibbs absorption isotherm. Under these conditions, spread and adsorbed films are essentially equivalent. Using rates of film desorption for slightly soluble lipids, an experimental test for establishing the presence of these conditions has been reported (22, 23).

Lecithin and cholesterol have very low solubilities in water; hence the amount of lipid that desorbs from spread films of these lipids is very small, and the rate of diffusion of desorbed lipid away from the surface is concomitantly low. These films appear to be insoluble, and it is difficult to verify whether they are equivalent to equilibrium films formed by adsorption. However, there are obvious conditions where the spread films are metastable and therefore not equivalent to adsorbed films.

It has been demonstrated that the use of spreading solvents with lecithin results in the formation of supercooled films at temperatures below the gel-liquid crystal transition temperature T_c (20). These spread films may be compressed to surface pressures that exceed the equilibrium spreading pressure π^e, the value at which the aqueous phase is saturated with lipid. The supercompressed states that form are metastable, and they owe their stability in part to the slowness of bulk lipid formation compared with the rate at which the films are compressed (4, 12, 24–26).

When two lipid components are present, similar considerations apply as for the single component films. However, additional complications may arise, analogous to those observed in the formation of bulk dispersions of these lipids (1). Thus the spreading solvent may form metastable surface mixtures whose properties are inconsistent with the equilibrium surface phase relations expected with adsorbed films. For example, spreading the film components may result in the formation of apparently homogeneous films where heterogeneous ones are predicted. This may arise when the rate of demixing is slower than the rate at which the films are compressed. Recent results of Cadenhead et al. (14) have demonstrated that when mixed films of dipalmitoyl lecithin and cholesterol, spread from a solvent, are allowed to equilibrate at $\pi = 0$ for various periods, the π-A isotherms expand with time. Isotherm expansions of 10–20% were observed after 100-min equilibration at $\pi = 0$, and the results suggest that even larger expansions would have been observed if longer equilibration times had been used. They deduced that the expansion was due to demixing of the components into discrete surface phases.

In virtually all the lecithin-cholesterol mixed film studies reported in the literature, either π^e was exceeded or the time allowed for preconditioning the films at $\pi = 0$ was invariably much shorter than necessary to permit complete demixing (e.g. 27–33). For those systems in which condensing effects have been reported, it is obvious that an arbitrary choice of equilibration times for demixing would result in various degrees of film condensation. Therefore, it is reasonable to question the significance of the condensing effect and the general use of spread mixed films to study molecular interactions. Despite these limitations, it would be instructive to examine how the

equilibrium phase relations might be used to interpret equilibrium spread film data. To this end, we have utilized π-A isotherms from the literature. Our analysis is within the context of the film condensing effect of cholesterol, and we demonstrate how an arbitrary choice of models to represent the surface phase relations can be misleading about the nature of the interactions between lecithin and cholesterol.

The "Condensing" Effect of Cholesterol in Spread Films

The majority of spread film studies have been for surface pressures that are intermediate between the values of π^e reported in the present study and the very low surface vapor pressure π_v of our earlier study (13). For the surface vapor pressure range ($\pi_v < 0.1$ dyn/cm) spread films of DML or DOL and cholesterol form homogeneous surface solutions, whereas at the very high range of surface pressures ($\pi^e \approx 40$ dyn/cm), we report the existence of lecithin:cholesterol 2:1 complex in the surface film, and in free solution in the presence and absence of the bulk lipid phase. Therefore we deduce that 2:1 complex will also form at some intermediate value of π ($\pi^e > \pi > \pi_v$). At these intermediate surface pressures, bulk lipid phase is absent and the phase rule may be written $F = 3 - P^s$. Thus when $F = 1$, π is a function of A as is the case for most of the π-A isotherms of lecithin-cholesterol films, and $P^s = 2$; two surface phases may therefore coexist. The presence of two coexisting surface phases in spread films need not be manifested by any obvious discontinuity in the π-A isotherms because $F = 1$ when $P^s = 2$; it is therefore virtually impossible to distinguish between a homogeneous and heterogeneous two-component film from the properties of the isotherm.[2] Deductions of molecular interactions based solely on the behavior of the π-A isotherms will consequently depend strongly on the choice of model to represent the surface phase behavior, i.e., whether the surface film is homogeneous or heterogeneous. In this section we examine data from the literature to illustrate this point, comparing the homogeneous surface solution model—used by most investigators (5–9, 26–28, 30–33)—to the heterogeneous model in which 2:1 complex surface phase coexists with a second lipid phase in the surface, where the composition of the second phase will depend on the mole fraction of cholesterol.

In typical spread film experiments, components i,j are confined to a fixed surface area A_t at surface pressure π. The conventional representation (17) of the data is of the general form

$$\bar{A}_{ij} = A_i + x_j(A_j - A_i), \tag{2}$$

where $\bar{A}_{ij}$, the average area/molecule in the surface, is calculated by

$$\bar{A}_{ij}(n_i + n_j) = A_t. \tag{3}$$

[2] Crisp (17) has suggested a test for distinguishing between the two: homogeneous surface films can be compressed to values of π which exceed π^e of the components, whereas for heterogeneous films bulk phase separation occurs at π^e of one of the components. However, given the likelihood that spread films containing lecithin and cholesterol are metastable, this test may not be reliable for this system.

A_i and A_j are the molecular areas of the pure components at π, n_i and n_j are the number of molecules of each component in A_t, and x_j is the mole fraction of component j in the spreading solution. Eq. 2 will hold when the components form either an "ideal" surface solution or if the components are completely immiscible (17).

In the majority of the lecithin-cholesterol spread film studies it has been assumed that homogeneous surface solutions are formed and that deviations from the linear relation predicted by Eq. 2 may be interpreted as "nonideal" solution behavior (9, 27). An example of "ideal" mixing behavior, taken from the literature (31), is given in Fig. 2 *a*, where $\bar{A}_{lc}$, the average molecular area in mixed films of dilinoleoyl lecithin (component *l*) and cholesterol (component *c*), is plotted against the mole fraction of cholesterol in the surface. A negative deviation from the behavior predicted by Eq. 2 is the experimental basis for the condensing effect. Many lecithin compounds conform to this behavior, and an example is given in Fig. 2 *c* for DML-cholesterol mixed films (27). However, molecular models have not been able to account for the

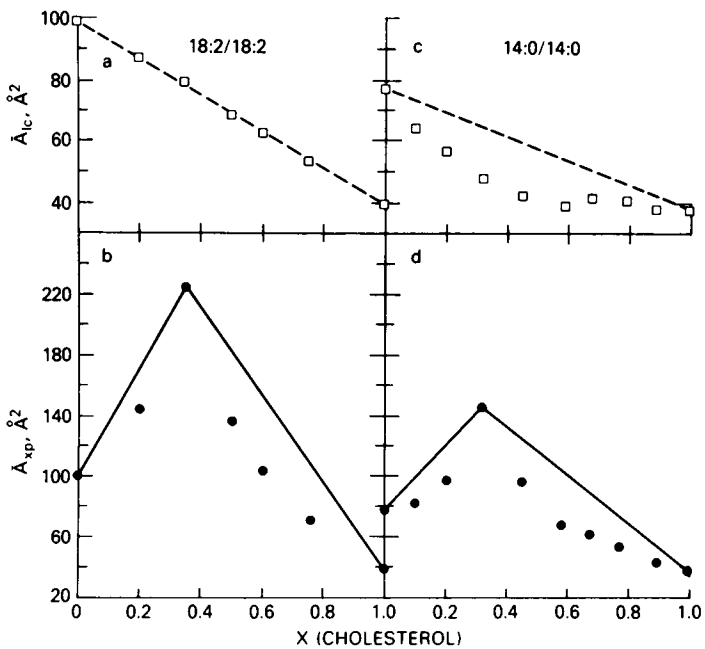

FIGURE 2 Comparison of homogeneous and heterogeneous film models for calculating average molecular areas in spread films. □, homogeneous film ($\bar{A}_{lc}$). (*a*) Dilinoleoyl lecithin (18:2/18:2), π = 12 dyn/cm, T = 22°C; data of Demel et al. (31). (*c*) Dimyristoyl lecithin (14:0/14:0), π = 5 dyn/cm, T = 23 ± 1°C; data of Cadenhead and Phillips (27). Dashed lines indicate "ideal" mixing behavior. •, heterogeneous film ($\bar{A}_{xp}$). (*b* and *d*) Data in *a* and *c* recalculated using Eq. 4. Solid lines are drawn assuming that lecithin:cholesterol 2:1 complex coexists with either pure lecithin or cholesterol in the surface film. $\bar{A}_{lc}$ calculated by dividing total film area by the number of lecithin and cholesterol molecules in surface; $\bar{A}_{xp}$ obtained by dividing total film area by the number of 2:1 complex and pure lipid (lecithin or cholesterol) molecules present. x(cholesterol) + x(lecithin) = 1.

condensing effect because no systematic relation has been found between the type of surface solution formed (ideal or condensed) and the chemical nature of the lipid (32, 33). In contrast, the concept that lecithin-cholesterol mixed films are heterogeneous provides a self-consistent analysis of the data that does not require *ad hoc* molecular modeling.

From the adsorbed film study (Fig. 1), it is deduced that the composition and phase behavior of lecithin-cholesterol films will depend on the mole fraction of cholesterol in the spreading solution. Thus when $0 < x(\text{CHOL}) < 0.33$, the 2:1 complex and lecithin coexist, and when $0.33 < x(\text{CHOL}) < 1.0$, the 2:1 complex phase coexists with a pure cholesterol surface phase. The relative amounts of each phase will change as x(CHOL) is varied, but the molecular area within each phase will remain constant. Eq. 2 may be applied to this system where the components are 2:1 complex (x) and either lecithin or cholesterol as a pure phase (p), depending on the value of x(CHOL) in the spreading solution. It is predicted that a linear relation between the average molecular area $\overline{A}_{xp}$ and x(CHOL) will exist in the composition range from 0 to 0.33 and another from 0.33 to 1.0. This must follow if the phases when x(CHOL) equals 0.0, 0.33, and 1.0 are pure lecithin, pure 2:1 complex, and pure cholesterol, respectively, as predicted from our equilibrium studies. To illustrate this approach we utilize the data of Fig. 2 *a* and *c* and calculate the average molecular area in the heterogeneous film using Eq. 4,

$$\overline{A}_{lc}(n_l + n_c)/(n_x + n_p) = \overline{A}_{xp}, \tag{4}$$

to calculate $\overline{A}_{xp}$, the average area in the film containing complex and pure lipid from the values of $\overline{A}_{lc}$ given in the literature. When x(CHOL) = 0.33, $n_l = 2$, $n_c = 1.0$, n_x (2:1 complex) = 1.0, and $n_p = 0$; thus $3\overline{A}_{lc} = \overline{A}_{xp}$. When x(CHOL) equals 0.0 and 1.0, $\overline{A}_{xp}$ will be equal to the molecular area for pure lecithin and cholesterol, respectively.

Plots of $\overline{A}_{xp}$ as a function of the mole fraction of cholesterol in the spreading solution are given in Fig. 2 *b* and *d* using the same data shown in Fig. 2 *a* and *c*. The straight lines in Fig. 2 *b* and *d* were drawn between cholesterol mole fractions of 0.0 and 0.33, and 0.33 and 1.0 in accordance with the surface phase relations predicted by our adsorption studies (Fig. 1). In drawing these lines it was assumed that homogeneous films of lecithin, 2:1 complex, and cholesterol (x[CHOL] = 0, 0.33, and 1.0) do not undergo the film expansion process noted by Cadenhead et al. (14) for intermediate mole fractions of cholesterol.

Two features of Fig. 2 *b* and *d* are noteworthy: (*a*) at all mole fractions where heterogeneous films are predicted, the values of $\overline{A}_{xp}$ are 15–35% less than predicted, and (*b*) DML and dilinoleoyl lecithin behave similarly. The smaller-than-predicted values for $\overline{A}_{xp}$ are to be expected because these data were obtained under conditions that did not allow enough time for demixing, and the decrement (15–35%) is the correct magnitude observed by Cadenhead et al. (14) for expansion due to demixing. Inasmuch as there are no a priori reasons for expecting DML- and dilinoleoyl lecithin-cholesterol mixed

films to exhibit different surface film properties (cf. DML and DOL, Fig. 1), the similarity of the two lipid systems when treated as heterogeneous films is in marked contrast with the analysis of these systems when treated as homogeneous films; in the former, similar behavior is anticipated, whereas for the latter *ad hoc* modeling in terms of negative deviations or "ideal" solution behavior is necessary to explain the data.

Thus we have identified two factors likely to contribute to the condensing effect of cholesterol: the interaction between lecithin and cholesterol to form a 2:1 complex, and lack of sufficient time to allow demixing of the 2:1 complex in solvent spread films. If arbitrary equilibration times for demixing are chosen, an entire spectrum ranging from negative to positive deviations from ideal homogeneous solutions might be postulated for the same lecithin-cholesterol mixed film. The contribution of the 2:1 complex formation to the condensing effect may vary as a function of the specific chemical features of the lecithin employed. We have estimated the contribution of this effect for a variety of lecithin:cholesterol 2:1 complexes by applying the heterogeneous model to data from other lecithin-cholesterol mixed film studies. The values for the molecular areas of the 2:1 complexes are listed in Table II, and were obtained at 40 dyn/cm, near π^e, and hence they represent nearly the closest packing areas for these compounds. These data indicate that the packing area for the complex is about 10% less than the area of the composite of the pure components at the same value of π. Therefore, the formation of the 2:1 complex will also contribute to the condensing effect, but this contribution is smaller than that resulting from the absence of demixing equilibrium. It should be noted that the decrease in packing area of the complex over that for the composite of the pure components is consistent with the increased density of the 2:1 complex in the dispersion compared to the densities of the bulk states of lecithin and cholesterol reported previously (1).

TABLE II

MOLECULAR AREAS OF LECITHIN:CHOLESTEROL 2:1 COMPLEXES AND OF PURE LECITHINS IN FILMS AT 40 DYN/CM, 22°C

Lecithin* Fatty acids	Molecular area (Å^2)	
	Lecithin	2:1 Complex
16:0/18:1	65.8	152
16:0/18:2	67.7	155
16:0/18:3	76.3	173
16:0/20:4	70.0	159
18:0/18:2	63.2	147
18:0/18:3	67.4	156
18:0/20:4	67.6	161

Molecular area of pure cholesterol is 38.6 Å^2. Calculations based on data of Ghosh and Tinoco (33).

*Fatty acid composition of lecithin indicated by number of carbons followed by the number of double bonds in the aliphatic hydrocarbon chain.

Although the equilibrium phase relations provide a consistent molecular interpretation of the spread film system, the equilibrium state represents only the limit that the metastable films approach. Clearly, nonequilibrium states, such as those that form when solvents are used to prepare dispersions of these lipids (1), may be present as well as the 2:1 complex in the spread films.

In summary, we have established, by a rigorous thermodynamic method, that a lecithin:cholesterol 2:1 complex is stable in bulk dispersions and in surface films. The phase relations in bulk reported previously have been verified (1). Phase relations in equilibrium mixed films have also been presented. However, application of these phase relations to lecithin-cholesterol spread films is limited, as the majority of these spread films are likely to be metastable. The concept that the condensing effect signifies an interaction between lecithin and cholesterol may be qualitatively correct, but the nature of the interaction is obscured by complicating surface phase relations, and the arbitrary choice of transient demixing states. The use of the heterogeneous surface phase relations obviates the need for the *ad hoc* molecular models proposed to rationalize the homogeneous film concept.

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