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Novel Surface Phase Containing Cholesteryl Esters. 1. Structural Characteristics Determined from Surface Pressure-Area Measurements†

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ABSTRACT: The behavior of cholesteryl myristoleate in mixtures with dioleoylphosphatidylcholine was investigated at the air-water interface. In addition to the previously described monolayer phase [Smaby, J. M., Baumann, W. J., & Brockman, H. L. (1979) *J. Lipid Res. 20*, 789-795], a second surface phase has been identified. Analysis of surface pressure and molecular area data as a function of composition shows that the molecules in the second phase can exist in two miscible, double-layer states or packing arrangements, only one of which contains lecithin. The mixed double-layer state is preferentially formed and has stoichiometry ranging between 2.0 and 9.5 molecules of cholesteryl ester for each lecithin molecule. The structure of this state resembles a mixed monolayer of pressure-dependent composition and area which is covered by a second layer of cholesteryl ester at 38.2 Å^2/molecule. The cholesteryl myristoleate/lecithin ratio of the layer in contact with the aqueous phase ranges from 0 to 2.8 between 39 and 0 mN/m. The second double-layer state is equivalent to a monolayer of cholesteryl ester at the lipid-water interface, covered by a layer of cholesteryl ester molecules at 38.2 Å^2. Overall, our data show that the presence of lecithin at a lipid-water interface has a definite ordering effect on cholesteryl ester molecules at least 30-50 Å from the interface.

Models of lipoproteins and arterial lipid deposits normally depict all of the cholesteryl ester in a bulk lipid phase, surrounded by a monolayer of more polar lipids [e.g., Shen et al. (1977)]. Such models are based on known bulk properties of cholesteryl esters, in particular their insolubility in water and low solubility in lamellar phospholipid phases in the presence of excess water (Janiak et al., 1974). It is important biologically to know if cholesteryl esters are present in finite amounts in the surface phase surrounding the cores of lipoproteins or arterial lipid deposits and in what state(s) they exist. We have previously studied the properties of cholesteryl esters in mixtures with other lipids (colipids) at the air-water interface. A mixed monolayer phase was formed provided the colipid had fluid acyl chains and the cholesteryl esters contained 9-cis unsaturation in the acyl moiety. The stability of this phase depended upon its composition; as the mole fraction of cholesteryl ester approached 0.5, the collapse pressure of the monolayer approached 0 (Smaby et al., 1979). Such low collapse pressures were of interest because it has been reported that in pure form the cholesteryl esters employed exhibit small, but finite, collapse pressures (Kwong et al., 1971; Lundberg & Bergstrom, 1974; Cadenhead & Phillips, 1967). To clarify this apparent anomaly, we have studied the surface behavior of mixtures of cholesteryl myristoleate and dioleoyl-

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phosphatidylcholine over the entire range of mole fractions. Our results indicate the formation of a novel surface phase containing colipid and cholesteryl ester which is immiscible with the monolayer phase and can exist over a wide range of compositions and pressures. The analysis of the phase diagram and force–area data described in this paper suggest a double-layer arrangement of molecules in this phase. The accompanying paper (Smaby & Brockman, 1980) describes how the properties of this phase are dependent upon colipid and acyl structure of the cholesteryl ester.

Experimental Procedures

Materials

Reagents. Lipids. Cholesteryl myristoleate was purchased from NuChek Prep, Elysian, MN. The purity of the ester was checked by thin-layer chromatography (TLC) and showed only one spot after detection with sulfuric–chromic acid. From measured detection limits, the lipid was shown to be 99.5% pure. The dioleoyllecithin (1,2-dioleoyl-*sn*-glycero-3-phosphocholine) was from Supelco and was repurified by thin-layer chromatography in a solvent system of chloroform–methanol–H_2O, 65:25:4 (v/v/v). The product showed a single spot when analyzed by TLC. The concentration of dioleoyllecithin was determined by assaying aliquots for phosphorus after perchlorate digestion (Bartlett, 1959).

Spreading Solvent. Petroleum ether (bp 60–70 °C) was stirred with 98% sulfuric acid for 20 h and washed once with water, once with 0.1 M sodium bicarbonate, and twice with water. The organic layer was dried overnight over calcium chloride and distilled (65–68 °C) from calcium hydride. The product was tested for surface-active impurities by spreading 100 μL on a clean air–buffer interface and recording its force–area curve (see below). No surface pressure could be measured, even at the minimum useable trough area, indicating the absence of surfactant.

Water. Water was purified by reverse osmosis, deionization, and distillation from alkaline permanganate.

Other Chemicals. All other compounds were reagent grade and were used without further purification.

Methods

Force–Area Measurements. Surface pressure–area determinations were made at 24 °C by using a Lauda recording film balance (Brinkman Instruments, Westbury, NY). It is a Langmuir-type balance with which surface pressure is measured by using a floating barrier attached to an inductive linear transducer. The balance has been interfaced with a microcomputer to provide simplified calibration and operation and to record data in digital form for subsequent calculations (Brockman et al., 1980). In all cases, lipids were spread in 50 μL of petroleum ether onto a 10 mM potassium phosphate–0.1 M sodium chloride subphase, pH 6.6, 24 °C. After standing at a large molecular area for 2–4 min, the monolayer was compressed at rates varying from 1–5 $Å^2$ min^{-1} $molecule^{-1}$ to an area/molecule less than the area of collapse of the colipid. Phase transitions were identified by using the second and third derivatives as previously described (Brockman et al., 1980).

Results

The expansion of dioleoyllecithin films by cholesteryl myristoleate is shown in Figure 1. The data show two sets of phase transitions, each of which approximately describes an envelope curve. The phase transitions in the curves obtained at 0.104, 0.259, and 0.367 mole fraction of cholesteryl myristoleate (curves 2, 3, and 4 from the left) arise from the "collapse" of the mixed monolayer (Smaby & Brockman,

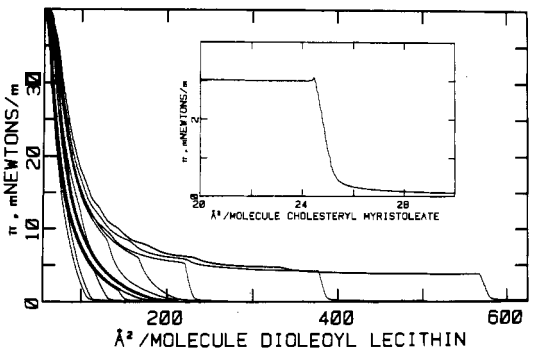

FIGURE 1: Force–area curves for cholesteryl myristoleate–dioleoyllecithin mixtures. The subphase was 0.01 M potassium phosphate and 0.1 M NaCl, pH 6.6, 24 °C. The mole fractions of cholesteryl myristoleate were, from left to right, 0.0, 0.104, 0.259, 0.367, 0.511, 0.602, 0.650, 0.677, 0.699, 0.751, 0.807, 0.848, 0.893, 0.937, and 0.959. The inset shows the force–area curve for pure cholesteryl myristoleate.

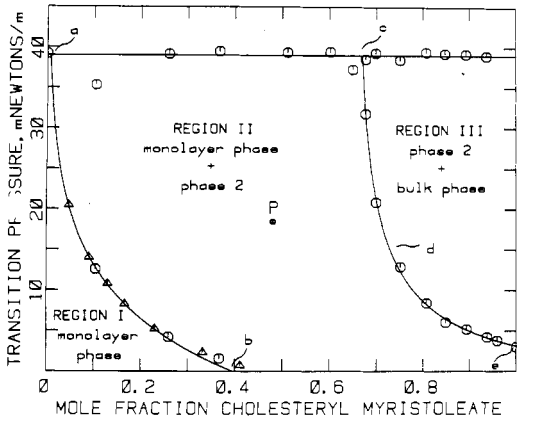

FIGURE 2: Composition dependence of phase transition pressures for dioleoyllecithin–cholesteryl myristoleate mixtures. Transition pressures (○) were obtained from data shown in Figure 1. (△) Manually determined phase transitions obtained earlier (Smaby et al., 1979).

1978; Smaby et al., 1979). The data clearly show a second set of phase transitions at larger areas which change in a composition-dependent manner, indicating the formation of a second lecithin–cholesteryl ester surface phase. At mole fractions ≥ 0.9, the force–area curves were somewhat irregular at surface pressures above the first phase transition. At these pressures and compositions, a bulk phase is visible on the surface, and the irregularities in the force–area curves probably result from the slow collapse of cholesteryl ester from the surface to the bulk phase. This is supported by our observation that in this region clearly defined phase transitions could only be obtained at compression rates of 1.0 $Å^2$ min^{-1} $molecule^{-1}$. Also, force–area curves obtained at mole fractions > 0.6 were not rapidly reversible above the first phase transition; however, if the surface was increased to a large area and then recompressed, the original compression curves could be regenerated. Below and slightly above the first phase transition at each composition, the force–area curves were readily reversible at the compression rates employed. The inset in Figure 1 shows the force–area curve for pure cholesteryl myristoleate. It exhibits a low, but finite, collapse pressure of 3.0 mN/m at a molecular area of 24.5 $Å^2$. The curve is reversible but is not stable over long periods of time (30–60 min), suggesting slow collapse to a bulk phase.

The composition dependence of the phase transition pressures is summarized in Figure 2. Because few data points were obtained at low mole fractions in this study, the set of manually determined phase transitions which we earlier described is also shown (Smaby et al., 1979). The points are in excellent agreement with those obtained in the present study

using our automated system. In region I of Figure 2 the lecithin and cholesteryl ester exist in a mixed monolayer phase (Smaby et al., 1979). It was previously assumed that the collapse of this phase was to a bulk cholesteryl ester phase, but the availability of the complete phase diagram shows the existence of a second, novel surface phase. At any point, P, in region II the surface consists of monolayer phase with a composition corresponding to that pressure on line ab and novel surface phase, designated phase 2, with a composition corresponding to that pressure on line cde. The line cde denotes the boundary between phase 2 and pure cholesteryl ester. That the pure phase is a bulk phase at pressures and compositions in region III is indicated by inspection of the surface.

For ideal miscibility behavior, points along line ab should be described by

$$\pi_c = \frac{RT}{A} \ln X \qquad (1)$$

where π_c is the phase transition pressure observed with a mixture of X mole fraction of cholesteryl ester and A is the apparent molecular area of the cholesteryl ester at the phase transition (Crisp, 1949). For the monolayer phase–phase 2 boundary with lecithin and cholesteryl myristoleate, such a plot is linear between 4.2 and 20.5 mN/m, and the parameters obtained were used to draw the solid line ab shown in Figure 2. Such a plot (not shown) is not linear for the phase transition pressures along cde. Note also that the transition pressures along cde do not vary continuously between 0 and 1 mole fraction of cholesteryl oleate but approach an asymptotic limit at ~0.67 mole fraction. This suggests not simple miscibility, but the formation of a preferred packing arrangement between the cholesteryl ester and the lecithin with a limiting stoichiometry, m, of about 2:1, cholesteryl ester/lecithin. The change in transition pressure from c to e results from miscibility with increasing amounts of "excess" cholesteryl ester within phase 2. Along cde, the mole fraction of this noncomplexed cholesteryl ester should vary from 0 to 1 as the mole fraction of cholesteryl ester in the system varies from $m/(m + 1)$ to 1 and the data should be consistent with eq 1 if X is replaced by X' where

$$X' = \frac{\text{moles of noncomplexed CE}}{\text{moles of noncomplexed CE + moles of colipid}} = \frac{X(m+1) - m}{m(X-1) + 1} \qquad (2)$$

Although m can be estimated to be 2 from the phase diagram, the data along cde were fit to eq 1 as modified by eq 2 to determine the value of m which gave the best coefficient of correlation. The best fit was obtained with $m = 1.95$ ($r = 0.999$) and the apparent molecular area of the cholesteryl ester at collapse, A from eq 1, was 29.6 Å^2. That the entire data set yields the same m as suggested by the points near c on the phase diagram (Figure 2) supports the hypothesis of miscibility between the components with a minimum stoichiometry. The slope and intercept of the fitted line were used to compute the solid line cde which, as shown in Figure 2, is in good agreement with the raw data over the entire range.

As shown qualitatively in Figure 1, the cholesteryl ester expands the lecithin monolayer at all compositions. The expansion in the monolayer phase has been previously described and quantitatively analyzed (Smaby et al., 1979). Such analysis is useful for characterizing the arrangement of the molecules in the surface phase and can be applied to data from region II of the phase diagram. Figure 3 shows representative plots of the average molecular area of the surface as a function

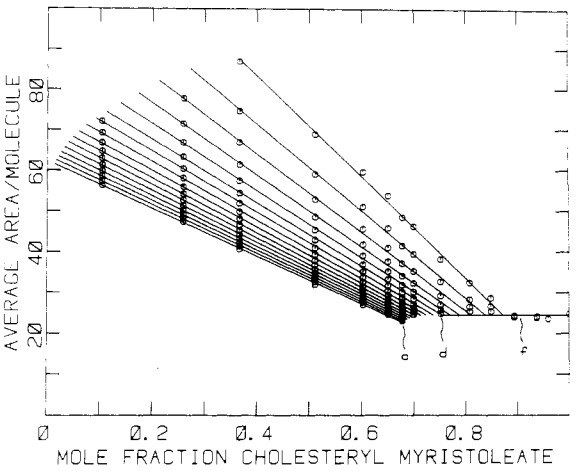

FIGURE 3: Average molecular area in Å^2 as a function of composition for cholesteryl myristoleate–dioleoyllecithin mixtures from 2–32 mN/m (top to bottom) shown every 2 mN/m. Data are from Figure 1, and the least-squares lines were calculated by using data between lines ab and cde in Figure 2, with the exception of average molecular areas < 30 Å^2 and mole fractions ≥ 0.8.

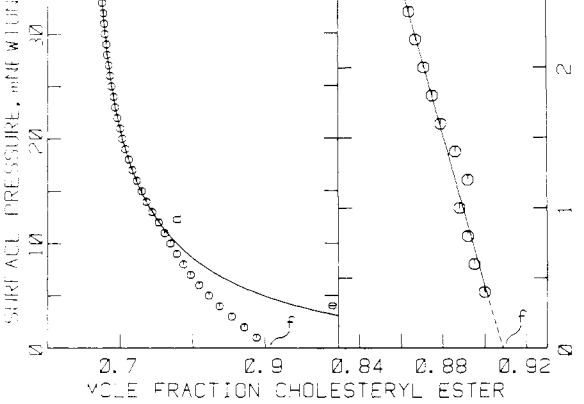

FIGURE 4: State boundary limits for the m–n state in phase 2. Points were calculated from data of the type shown in Figure 3 as described in the text. For comparison, line cde from Figure 2 is shown (solid line). (b) is an enlargement of the low-pressure region of Figure 5a showing the extrapolation to a limiting mole fraction from which n is calculated.

of composition. At higher pressures the data corresponding to region II of the phase diagram give linear average area plots between the phase boundary lines, but they are biphasic below 12 mN/m. Furthermore, the biphasic plots arise because below 12 mN/m the average molecular area never goes below 24–25 Å^2. As shown in the inset to Figure 1, this is the observed collapse area of pure cholesteryl ester. The region of constant molecular area and biphasic average area plots is indicated by the horizontal line in Figure 3 between d and f. Above 12 mN/m the linear segments are not biphasic and terminate at areas of 24–25 Å^2/molecule or to lesser areas at pressures above 18 mN/m.

To define the limits of the packing arrangement which give rise to this average area behavior, we plotted the surface pressure at which each average area plot was obtained vs. the mole fraction at which the right hand termination of the line occurred or the line became biphasic at 24.5 Å^2/molecule. This corresponds to the points defined by cdf in Figure 3 which are plotted every 1 mN/m in part a and every 0.2 mN/m in part b of Figure 4. As shown in part a and the enlargement in part b of Figure 4, the data describe a curve, cdf, which extrapolates (Figure 4b) to a limiting mole fraction of 0.909.

Thus, the particular packing arrangement which gives rise to the linear average area plots can exist between cholesteryl ester/lecithin ratios of m and n which in this case are 1.95 and 9.99. For comparison, line cde from the phase diagram (Figure 2) is shown on Figure 4a. At higher surface pressures the data coincide (by definition) with the phase diagram, whereas they differ significantly below 12 mN/m. This implies that above 12 mN/m the addition, at constant pressure, of cholesteryl ester to a mixture with a composition on the line will result in formation of a cholesteryl myristoleate bulk phase. Below 12 mN/m the excess cholesteryl ester remains on the surface in a form which is miscible with the m–n packing arrangement and has a molecular area of 24.5 Å^2, as does pure cholesteryl myristoleate. In effect, our results indicate that the data of Figure 4a along df describe a pseudo phase boundary line or state boundary line. For any mixture at a composition and pressure in region II, but to the left of df (Figure 4a), the surface consists of a mixture of monolayer phase and phase 2 in the m–n packing arrangement or state. At pressures and compositions below de and to the right of df, i.e., the lower right-hand corner of the phase diagram, the monolayer phase no longer exists, and the surface contains only one phase made up of a mixture of cholesteryl ester and lecithin in the m–n state and pure cholesteryl ester in the 24.5 Å^2/molecule state.

The state of the molecules in the m–n packing arrangement can be investigated by constructing an apparent force–area curve for the m–n state, as has been done for data in the monolayer phase, which also exhibits linear average area plots (Smaby et al., 1979). At any surface pressure and composition in region II, the surface is composed of two immiscible phases each containing cholesteryl ester and lecithin. If A_i denotes the apparent molecular area of lecithin and M_i denotes the molecules of lecithin in the ith phase and two phases are present, then the total surface area is given by

$$\text{area} = M_1A_1 + M_2A_2 \tag{3}$$

From the lever rule

$$M_1(X_2 - X) = M_2(X - X_1) \tag{4}$$

where X is the mole fraction of cholesteryl ester in the system and X_1 and X_2 are the mole fractions in phases 1 and 2; if the ratios of cholesteryl ester to lecithin in the phases are R_1 and R_2, then

$$X_1 = R_1/(1 + R_1) \qquad X_2 = R_2/(1 + R_2) \tag{5}$$

By use of these relationships, it can be shown that at any pressure

$$\bar{A} = \frac{X[(1 + R_1)A_2 - (1 + R_2)A_1] + R_2A_1 - R_1A_2}{R_2 - R_1}$$

where $\bar{A}$ is the average molecular area, i.e., the total surface area divided by the total number of molecules in the film. This equation predicts the observed linear relationship between $\bar{A}$ and X shown in Figure 3 with

$$\text{slope} = S = \frac{(1 + R_1)A_2 - (1 + R_2)A_1}{R_2 - R_1} \tag{6}$$

and

$$\text{intercept} = I = \frac{R_2A_1 - R_1A_2}{R_2 - R_1} \tag{7}$$

Simultaneous solution of (6) and (7) to eliminate A_1 gives

$$A_2 = SR_2 + I(1 + R_2) \tag{8}$$

which allows calculation of the apparent molecular area of lecithin in the m–n state of phase 2, A_2, from the slope, intercept, and R_2 at each pressure. This latter parameter can be empirically determined from data of the type shown in Figure 4a, and S and I can be obtained from lines of the type shown in Figure 3. In this manner we calculated A_2 at each 0.1 mN/m pressure increment between 1.0 mN/m and the collapse pressure of dioleoyllecithin, and the results are shown in Figure 5. This apparent force–area curve is essentially the apparent molecular area of lecithin as one proceeds along the line cdf described by the data in Figure 4a, i.e., where the surface consists solely of lipid in the m–n state. For comparison we have also shown the force–area curve for dioleoyllecithin alone. At low surface pressures cholesteryl myristoleate makes a large contribution to the area of the phase. However, as the collapse pressure of the lecithin (39.4 mN/m) is approached, the curves become parallel and the contribution of the cholesteryl ester to the area of phase 2 is small. This near identity of the curves occurs in the region of the phase diagram near point c where the surface consists entirely of phase 2 and at which R_2 equals its limiting value of m.

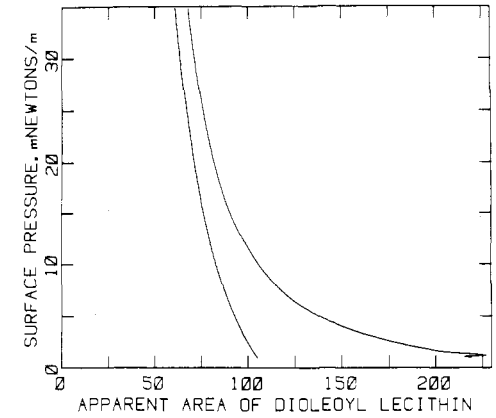

FIGURE 5: Apparent molecular area in Å^2 of dioleoyllecithin as a function of surface pressure in pure monolayer phase (left) and in phase 2 (right). Data for the pure lecithin are from Figure 1. In phase 2 molecular areas were calculated every 0.1 mN/m between 1 and 0.5 mN/m below the collapse pressure of pure dioleoyllecithin as described in the text.

Discussion

The apparent expansion of lecithin force–area curves by cholesteryl myristoleate in all binary mixtures and the resultant phase diagram (Figure 2) show clearly that mixed surface phases can exist at all compositions and pressures below the collapse pressure of the colipid and confirm earlier reports that pure cholesteryl esters could form surface films at low packing densities. Because of the unusually small collapse area of the pure cholesteryl ester it was previously assumed that the surface film resulted from oxidation of a small percentage of the lipid (Kwong et al., 1971). However, this did not explain the reproducibility of this area. As shown by this work, the collapse area and pressure of the pure ester are but the limits of a continuous pattern of collapse areas and pressures. Such behavior could not be caused by a trace contaminant unless it had an unusually large molecular area. More reasonably, the metastable packing arrangement of pure cholesteryl myristoleate yields a collapse area of 24.5 Å^2/molecule.

From the decidedly biphasic nature of the average molecular area data at low pressures in region II (Figure 3), it is apparent that the molecules in phase 2 exist in discrete packing arrangements or states, the distribution of which depends on surface pressure and composition. In particular, the m–n packing arrangement is more surface active than the 24.5 Å^2/molecule state of the pure ester and is preferentially formed. This gives rise to a pseudo phase or state boundary

line df (Figure 4a) to the left of which the 24.5 Å^2/molecule state of cholesteryl ester is not observed. The state which does exist in phase 2 to the left of df (Figure 4a) and in equilibrium with the monolayer phase was designated the *m–n* state or packing arrangement and exhibits a stoichiometry between 2 and 10 cholesteryl myristoleate molecules per lecithin. It can exist at pressures up to the collapse pressure of the lecithin itself. The term "complex" was not used to describe this packing arrangement because it does not exhibit a fixed stoichiometry as a function of surface pressure.

The heretofore unrecognized existence of this state or packing arrangement and its confinement to a limited range of stoichiometries prompted a more detailed analysis of its surface pressure–area characteristics (Figure 5). Comparison of the apparent force–area curve for lecithin in this state with that of lecithin alone in a monomolecular film shows a striking feature. At high surface pressure, at which there are two cholesteryl myristoleate molecules associated with each lecithin, the area of the lecithin plus cholesteryl myristoleate is only ~10% greater than that of lecithin alone. This implies that the structure of the surface is more than monomolecular with the cholesteryl ester located mostly or entirely above the lecithin molecules, i.e., away from the aqueous bulk phase.

If we envision a layered arrangement, then the apparent area of lecithin, which is 66.7 Å^2/molecule at 34.8 mN/m, must also be the area occupied by the two cholesteryl ester molecules in the layer above it. Thus, the molecular area of cholesteryl myristoleate in the upper layer is 33.4 Å^2/molecule. At a pressure of 2 mN/m the apparent area of lecithin is considerably expanded relative to lecithin alone (189.9 vs. 101.5 Å^2/molecule). The increase in area is not, however, sufficient to accommodate all the molecules of cholesteryl ester per molecule of lecithin at the interface at that pressure (R_2 = 6.75 at 2 mN/m) in any reasonable arrangement. More likely, the expansion arises from part of the cholesteryl ester molecules residing at the interface and the remainder in a layer above the interfacial layer. At present we have no direct way to determine precisely the distribution of cholesteryl ester between the upper and interfacial layers, but it can be estimated in the following way. If it is assumed that the expansion of lecithin arises from cholesteryl myristoleate molecules at the interface occupying approximately the same area as they do in monolayer films with dioleoyllecithin (69.4 Å^2/molecule at 2 mN/m; Smaby et al., 1979), then the number of molecules of cholesteryl myristoleate in the interfacial layer can be determined [(189.9 − 101.5)/69.4 = 1.27]. Subtracting this number from the total number of cholesteryl myristoleate molecules per molecule of lecithin at that pressure gives the number of cholesteryl ester molecules comprising the upper layer (6.75 − 1.27 = 5.48). As at high surface pressures, dividing this into the apparent area of the lecithin at this pressure (189.9/5.48) gives 34.7 Å^2/molecule of cholesteryl myristoleate in the upper layer. Repeating this calculation at 10 mN/m gives 36.9 Å^2. Note that this value is essentially constant, supporting the assumptions employed. Thus, over its range of existence the *m–n* packing arrangement can be envisioned as an interfacial layer consisting of dioleoyllecithin and cholesteryl myristoleate arranged as in a mixed monolayer phase but covered with a continuous upper layer of cholesteryl myristoleate molecules occupying ~35 Å^2/molecule.

If this molecular arrangement is correct, it should be possible to describe the state boundary line qualitatively and to directly calculate both *n* and the molecular area of the cholesteryl ester in the upper layer from the data below 18 mN/m shown in Figure 4. To accomplish this, we view the surface as composed of lecithin and cholesteryl ester in the interfacial layer covered with a layer of pure cholesteryl ester at constant molecular area, A_u. The mole fraction of the cholesteryl ester "species" relative to lecithin is, in effect, the mole fraction of cholesteryl ester in the interfacial layer, and use of this mole fraction in eq 1 should yield a linear plot. At any pressure the total number of cholesteryl ester molecules associated with each lecithin is R_2. These can be divided into those in the upper layer, U, and those at the interfacial or lower layer, $R_2 - U$. The areas occupied by the upper and interfacial layers, expressed per molecule of lecithin, are by definition the same and equal A_2. Below 18 mN/m the average molecular area is constant at 24.5 Å^2, the collapse area of the pure cholesteryl ester (Figure 3). Thus

$$A_2 = \bar{A}(1 + R_2) = UA_u \tag{9}$$

This equation can be solved for U, and it can be readily shown that the mole fraction of cholesteryl ester in the interfacial layer along the state boundary line, $X'' = (R_2 - U)/(1 + R_2 - U)$, is given by

$$X'' = \frac{A_u R_2 - \bar{A}(1 + R_2)}{(A_u - \bar{A})(1 + R_2)} \tag{10}$$

To fit the experimental data of Figure 4 below 18 mN/m, we substituted X'' for X in eq 1 and 24.5 Å^2/molecule for $\bar{A}$ and determined the value of A_u which gave the best fit of the data. This value was 38.2 Å^2/molecule, similar to that estimated above by using monolayer areas, and the fit was excellent with a coefficient of correlation >0.999. The intercept of this line was 0.905 mole fraction of cholesteryl ester which corresponds to an *n* of 9.53. These values are in excellent agreement with the values of 0.909 and 9.99 determined empirically from Figure 4b and support the model that the state boundary line behaves mathematically like a phase boundary line. Indeed, the observed instability of pure cholesteryl myristoleate at its collapse pressure over long time periods suggests that the pure cholesteryl ester state of phase 2 may be metastable and that the state boundary line would be the phase boundary line if the raw data were collected over hours instead of minutes. Evaluation of X'' at 0 surface pressure gives a limit of 2.8 molecules of cholesteryl ester associated with each dioleoyllecithin at the lipid–water interface. For comparison, the limit is 1.0 for most cholesteryl esters in monolayer phases (Smaby et al., 1979).

The slope of the log plot described above allows calculation of the apparent molecular area of cholesteryl myristoleate as it is "collapsed" out of the mixed state with lecithin to the pure cholesteryl myristoleate state. This is 29.8 Å^2 and is identical with the value calculated from the similar plot of the data along cde shown in Figure 3. It is also consistent with the measured collapse area of pure cholesteryl myristoleate of 24.5 Å^2/molecule, suggesting that the cholesteryl ester in region II which is not associated with lecithin has essentially the same packing as that which is in the *m–n* state. With the assumption that the cholesteryl ester in the interfacial layer is approximately that observed in monolayer films (see above), these values of 25–30 Å^2/molecule correspond to about 1.5–2 cholesteryl ester molecules above each interfacial molecule at collapse.

Throughout, phase 2 is thicker than a monolayer and consists of miscible components in an interfacial layer covered by a layer of cholesteryl myristoleate occupying 38.2 Å^2/molecule. Compression of this phase results in a decrease in the number of cholesteryl ester molecules associated with lecithin in the interfacial layer and in the molecular areas of the interfacial molecules, whereas packing in the upper layer is essentially

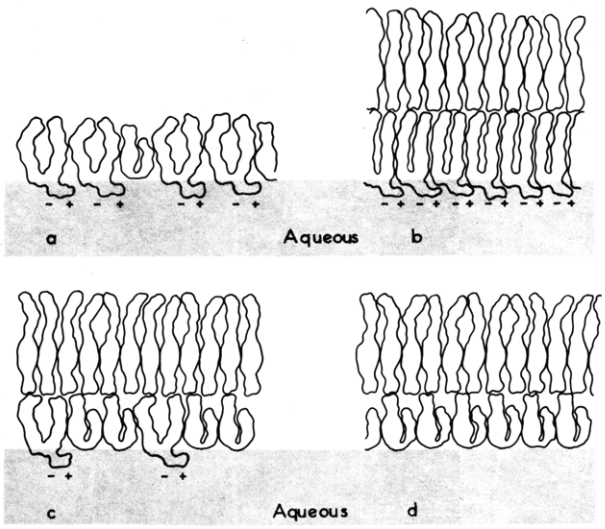

FIGURE 6: Hypothetical models for states in mixed monolayer and in double-layer phases in different surface pressure–composition regions. (a) 0.44:1 cholesteryl myristoleate/dioleoyllecithin, ~2 mN/m; (b) 2:1 cholesteryl myristoleate/dioleoyllecithin, ~38 mN/m; (c) 8:1 cholesteryl myristoleate/dioleoyllecithin, ~1 mN/m; (d) pure cholesteryl ester, ~3 mN/m.

constant. The packing arrangement of the cholesteryl ester molecules in the upper layer is suggested by comparison of our results with structural studies of lipoproteins. On the basis of X-ray small-angle scattering it has been shown that both LDL and HDL_c from atherosclerotic swine have a layer of in register steroid nuclei immediately adjacent to the apolar region of the surface lipid–apoprotein layer (Atkinson et al., 1978). The molecular area of 38.4 $Å^2$ which we obtained for cholesteryl myristoleate in the upper layer is consistent with such an arrangement because it essentially equals the value for the cross-sectional area of the steroid nucleus of 36.6 $Å^2$/molecule (Craven & DeTitta, 1976). From the X-ray analysis it was not possible to determine the orientation of the steroid nuclei in the layer adjacent to the surface. However, the small apparent expansion of dioleoyllecithin by cholesteryl myristoleate in phase 2 at high surface pressure (Figure 5) indicates that there cannot be significant interdigitation of myristoleate and oleate acyl groups in the interfacial layer. The small expansion is more likely due to interaction of the ends of the isooctyl moieties of the cholesterol with the lecithin acyl groups. Combining these observations leads to the schematic model shown in Figure 6a–d. Panel a shows the monolayer phase in which the cholesteryl esters are oriented with their ester functions toward the aqueous phase (Smaby & Brockman, 1978; Smaby et al., 1979). On the basis of the analysis presented herein, these same structural features are incorporated into the interfacial layer of phase 2 (Figure 6b,c). Adjacent to the apolar region of the interfacial layer is a layer of cholesteryl myristoleate molecules oriented with their acyl moieties away from the bulk aqueous phase. Panel b of Figure 6 represents the surface near the collapse pressure of dioleoyllecithin where the mole fraction of cholesteryl myristoleate is 0.67, and panel c shows phase 2 at a lower surface pressure of 1 mN/m and a mole fraction of 0.9. The pure cholesteryl ester state shown in Figure 6d at 3 mN/m resembles panels b and c except that no lecithin is present. As mentioned above, this metastable packing arrangement is miscible with those shown in Figure 6b,c. A basic difference between our model system and small lipoproteins is radius of curvature. It is possible that the constraints we have imposed from the X-ray analysis of LDL and HDL_c on molecular packing in the cholesteryl ester adjacent to the interfacial layer are not applicable to large lipid inclusions with essentially planer surfaces. In this case, alternative packing arrangements can be proposed based on the crystal structures determined for various cholesteryl esters (Craven & Guerina, 1979a,b; Dahlen, 1979; Guerina & Craven, 1979). For example, the molecules could be arranged in an antiparallel array as observed with cholesteryl nonanoate (Guerina & Craven, 1979). The major differences between this and the packing shown in Figure 6c,d is extensive chain ring overlap.

Regardless of the actual structure of the miscible states within phase 2, our data clearly indicate that the presence of a lipid–water interface can have a definite ordering effect on cholesteryl myristoleate molecules above the interfacial layer. In the accompanying paper (Smaby & Brockman, 1980), the formation and properties of this phase are related to colipid head group and cholesteryl ester acyl group structure.

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