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ACKNOWLEDGMENTS AND ADDRESSES

Received November 12, 1974, from *The Squibb Institute for Medical Research, New Brunswick, NJ 08902*

Accepted for publication February 11, 1975.

The authors are indebted to Dr. P. T. Funke and Dr. M. S. Puar for the mass spectrometry and NMR spectroscopy, respectively, to Mr. H. Lerner for the radiolabeled recovery experiment, and to Miss A. Hoffman for the IR spectrophotometry. The technical assistance of Mrs. V. Woolard is also gratefully acknowledged.

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Molecular Arrangement in Monolayers Containing Cholesterol and Dipalmitoyl Lecithin

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Abstract □ The molecular arrangement of dipalmitoyl lecithin and cholesterol in mixed monolayers was investigated with the aid of a physical model. The two lipids are miscible at the surface, but there is no indication of a specific interaction. In equimolar mixed monolayers at 25 and 37°, the lipids are in tail contact. Lecithin molecules are able to remain hydrated in the mixed monolayers at high values of surface pressure.

Keyphrases □ Monolayers—cholesterol and dipalmitoyl lecithin, molecular arrangement, force–area isotherms, model □ Cholesterol–dipalmitoyl lecithin monolayers—molecular arrangement, force–area isotherms, model □ Dipalmitoyl lecithin–cholesterol monolayers—molecular arrangement, force–area isotherms, model □ Films—molecular arrangement in cholesterol–dipalmitoyl lecithin mixed monolayers, model

The notion that monomolecular films might serve as a physical model for cellular membranes was first suggested by Langmuir (1) in 1917. Like a natural membrane, the monolayer is a coherent, organized, interfacial structure. Consequently, it is a particularly useful model system for obtaining information on the orientation and arrangement of membrane components.

Special significance has been ascribed to the behavior of monolayers containing both cholesterol and a phospholipid, such as lecithin, since these materials are known to be important constituents of biological membranes. Usually, investigators have reported the mean area per molecule (at constant surface pressure) for the mixture as a function of monolayer composition (2–7). In some cases, the mean molecular area has been a linear function of composition, indicating that each component occupied essentially the same molecular area in the mixed system as in a monolayer of the pure material.

However, deviation from linearity has been observed. The mean molecular area in such systems is less than that expected on the basis of the monolayer properties of the pure materials (2–7). These deviations have been interpreted in various ways. One view is that they are evidence of a significant interaction between the two lipids as a result of van der Waals' forces, configurational entropy effects, and alteration in water structure (3), leading to the conclusion that cholesterol must strengthen or stabilize the monolayer. The implication is that cholesterol also stabilizes biological membranes.

Another explanation of the reduction in mean area per molecule was put forth by Shah and Schulman (4), who attributed the effect to the formation of cavities between phospholipid molecules as a result of thermal motion of the fatty acyl chains. Cholesterol molecules were believed to be accommodated in the cavities. These authors noted that monolayers of pure dipalmitoyl lecithin at a surface pressure of 35 dynes/cm underwent a transition to the gel state and that compression to 40 dynes/cm resulted in the formation of a two-dimensional solid. The transitions were not observed in mixed monolayers containing cholesterol, which remained fluid at all values of surface pressure. It was concluded that cholesterol decreases monolayer cohesion and, by inference, that it acts as a "biological plasticizer" in natural membranes (4).

Gershfeld and Pagano (8) reviewed these theories and, based on the results of adsorption experiments, concluded that dipalmitoyl lecithin is immiscible with cholesterol at the surface and that a rational interpretation of the mixed monolayer experiments is not possible.

A physical model (the "heads-or-tails" model), which should help clarify the role of cholesterol, was recently suggested (9). It is based on the assumptions that lipids with long hydrocarbon chains are essentially vertically oriented and that the surface area of each molecule in a coherent monolayer conforms either to the area of the hydrocarbon tails (tail contact) or the hydrated polar head (head contact). According to this model, phospholipids in tail contact (*i.e.*, in a condensed surface state) do not exhibit a reduction in the mean molecular area in the presence of cholesterol, a result in accord with published findings (4, 5). But if the phospholipid molecules are extensively hydrated, cholesterol molecules may find free space between the hydrocarbon tails and thus require little

Table I—Calculation of Mean Molecular Area in Mixed Monolayers of Cholesterol and Dipalmitoyl Lecithin Using the Heads-or-Tails Model[a]

Case	Criterion	A_m =		
1	$(A_2 - A_{HC}) \approx 0$	$A_1X_1 + A_{HC}X_2$		
		for $X_2 \leq 0.5$		for $X_2 \geq 0.5$
2	$A_1 > (A_2 - A_{HC}) > 0$	$A_1X_1 + A_{HC}X_2$		$(A_1 + A_{HC})X_1 + A_2(X_2 - X_1)$
3	$(A_2 - A_{HC}) = A_1$	$A_1X_1 + A_{HC}X_2$		A_2X_2
		for $X_2 \leq 0.333$	for $X_2 \geq 0.333$	
4	$2A_1 > (A_2 - A_{HC}) > A_1$	$A_1X_1 + A_{HC}X_2$	$A_2(2X_2 - X_1) + (2A_1 + A_{HC}) \cdot (X_1 - X_2)$	A_2X_2

[a] A_m = mean per area per molecule, X_1 = mole fraction of cholesterol, A_1 = area per molecule in cholesterol monolayer, X_2 = mole fraction of lecithin, A_{HC} = area per molecule of lecithin hydrocarbon tail, and A_2 = area per molecule in lecithin monolayer.

additional area. Therefore, in the presence of cholesterol, the effective area of a phospholipid molecule is the area of its hydrophobic tail rather than that of the bulkier polar portion, provided that the monolayer components are miscible.

An advantage of the proposed model is that it permits the derivation of quantitative relations for the mean area per molecule in a mixed monolayer as a function of the surface properties of the pure components, the composition of the mixed monolayer, and the area of the hydrocarbon tail of the phospholipid. This report deals with results obtained with mixtures of cholesterol and dipalmitoyl lecithin at two temperatures.

EXPERIMENTAL

Cholesterol[1] and dipalmitoyl lecithin[1] were reportedly 99% pure. Organic solvents were spectrograde, and sodium chloride was reagent grade. Water was double distilled; the final distillation took place in an all-glass still. The apparatus used was described previously (10).

The subphase contained 0.9% sodium chloride. Dipalmitoyl lecithin and lecithin–cholesterol mixtures were spread from solution in hexane–ethanol (4:1), and cholesterol was spread from solution in hexane. A thermostat[2] was used to maintain the subphase temperature constant within 0.1°. Surface pressure (π) was measured by the Wilhelmy plate method.

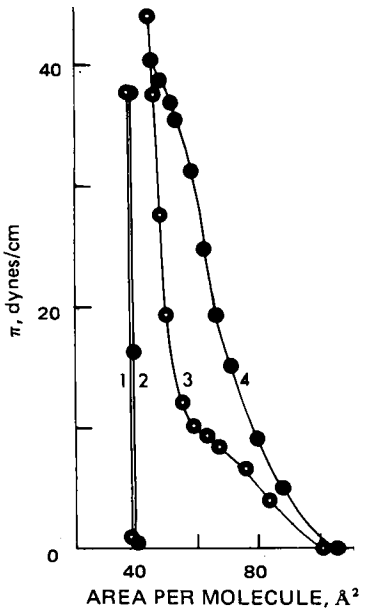

Figure 1—*Surface pressure as a function of available surface area of pure lipids. Key: curve 1, cholesterol at 25°; curve 2, cholesterol at 37°; curve 3, dipalmitoyl lecithin at 25°; and curve 4, dipalmitoyl lecithin at 37°.*

RESULTS AND DISCUSSION

Force–area isotherms for cholesterol and dipalmitoyl lecithin monolayers at 25 and 37° are plotted in Fig. 1. The cholesterol monolayer at 25° is in a highly condensed state at surface pressure values above 1 dyne/cm. An increase in the subphase temperature to 37° has no effect on the state of the monolayer. The entire isotherm is displaced to slightly higher values of surface area per molecule. Based on its low compressibility and the correspondence of the experimental surface area per molecule with the cross-sectional area of the hydrocarbon portion of cholesterol (11), it was concluded that the hydrocarbon tail determines the surface area required for each cholesterol molecule (9).

The π–A curve for dipalmitoyl lecithin at 25° (Fig. 1) is of the expanded type below a surface pressure of about 20 dynes/cm. In terms of the model, the molecules are described as being in head contact. In other words, the area occupied by each molecule is that of the hydrated polar portion. With increasing surface pressure, a change of state takes place and the area per molecule approaches that of the hydrocarbon tails (11). In contrast to the behavior of cholesterol, dipalmitoyl lecithin monolayers undergo marked changes in surface state as a function of temperature. At 37° (Fig. 1), the monolayer is considerably more expanded than at 25°. A state of tail contact is not realized until the surface pressure reaches 40 dynes/cm.

The equations previously derived (9) permit calculation of A_m, the mean area per molecule at a given surface pressure of the

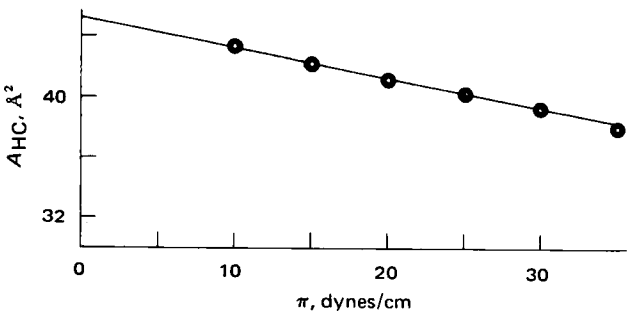

Figure 2—*Area per molecule of the hydrocarbon tail of dipalmitoyl lecithin in the 1:1 mixed monolayer with cholesterol as a function of surface pressure at 25°.*

[1] Schwartz-Mann, Orangeburg, N.Y.

[2] Lauda K-2.

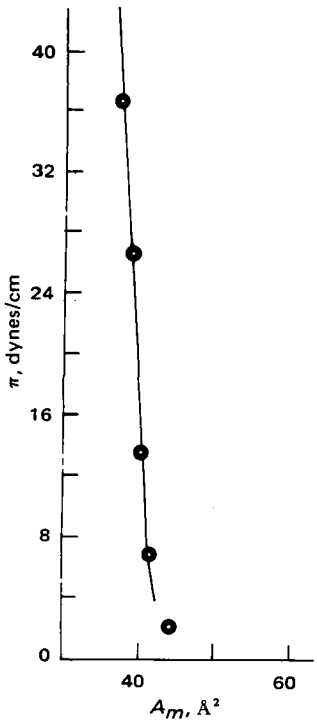

Figure 3—*The π–A curve for mixed monolayers of cholesterol–dipalmitoyl lecithin (1:1) at 25°. Key: —, calculated from heads-or-tails model; and O, experimental points.*

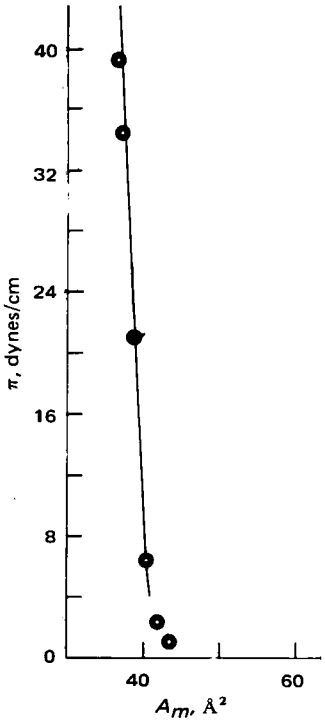

Figure 4—*The π–A curve for mixed monolayers of cholesterol–dipalmitoyl lecithin (2:1) at 25°. Key: —, calculated from heads-or-tails model; and O, experimental points.*

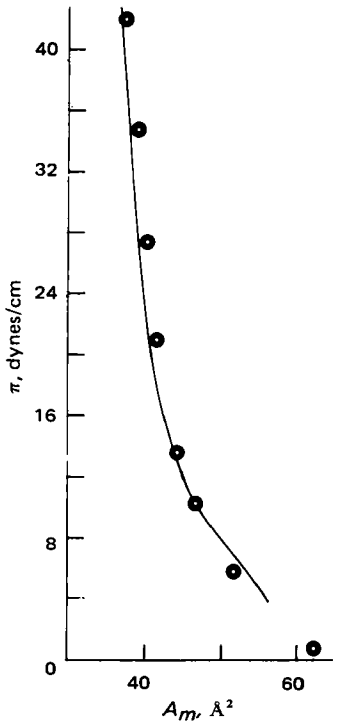

Figure 5—*The π–A curve for mixed monolayers of cholesterol–dipalmitoyl lecithin (1:2) at 25°. Key: —, calculated from heads-or-tails model; and O, experimental points.*

mixed monolayer, from the surface properties of the pure components. These relations are summarized in Table I. The only parameter needed for the calculation that may not be obtained directly from experiment is A_{HC}, the area of the hydrocarbon tail of the phospholipid. It is possible to estimate A_{HC} from molecular models and data from π–A curves of simple lipids. However, to obtain a more accurate approximation and also to see the effect of surface pressure on A_{HC}, another method was chosen.

The 1:1 mixed monolayer at 25° represents a Case 2 situation (above a surface pressure of about 5 dynes/cm), so Eq. 1 applied:

$$A_m = A_1X_1 + A_{HC}X_2 \qquad \text{(Eq. 1)}$$

where X_1 and X_2 are the mole fractions of cholesterol and lecithin, respectively; and A_1 is the area per molecule in a monolayer of pure cholesterol at the same surface pressure. This equation may be rearranged to:

$$A_{HC} = \frac{A_m - A_1X_1}{X_2} \qquad \text{(Eq. 2)}$$

where A_1, X_1, and X_2 are known. By using the experimental values of A_m at each surface pressure for the 1:1 mixed monolayer, A_{HC} can be calculated. As shown in Fig. 2, A_{HC} is a linear function of π, obeying the relationship:

$$A_{HC} = 45.2 - 0.2\pi \qquad \text{(Eq. 3)}$$

The compressibility of the hydrocarbon tails corresponds to what one would expect for a liquid condensed monolayer (12) and thus supports the notion that the tails are vertically oriented.

The π–A data for the pure monolayers at 25° and values of A_{HC} from Eq. 3 were used to calculate A_m at various values of surface pressure for each mixed monolayer at that temperature. For each system, the theoretical points were joined to produce a continuous curve. These curves and the corresponding experimental data are presented in Figs. 3–5. It is not surprising that the fit to experimental data is so good for the 1:1 monolayer (Fig. 3), since the values for A_{HC} were derived from this system. However, the correspondence between theory and experiment is also quite good for the other mixtures (Figs. 4 and 5).

The values of A_m for a 1:1 mixed monolayer at 37° were also calculated. However, since A_{HC} is expected to vary with temperature, the values at 25° from Eq. 3 could not be used directly at the higher temperature. The effect of temperature on A_{HC} was estimated

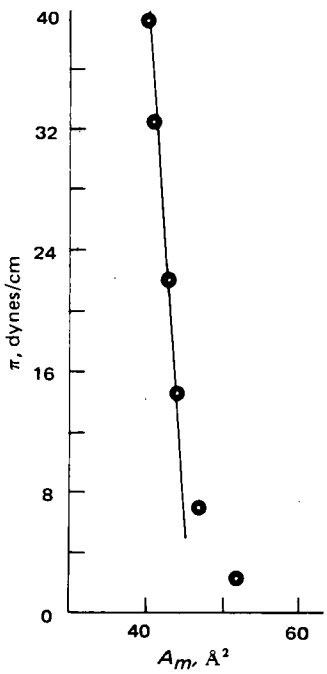

Figure 6—*The π–A curve for mixed monolayers of cholesterol–dipalmitoyl lecithin (1:1) at 37°. Key: —, calculated from heads-or-tails model; and O, experimental points.*

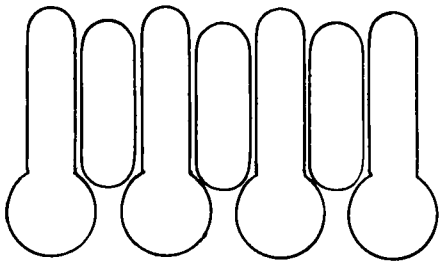

Figure 7—*Representation of molecular arrangement in equimolar mixed monolayers of cholesterol (short rod) and dipalmitoyl lecithin at surface pressure values above 5 dynes/cm.*

from the surface properties of palmitic acid, since its hydrocarbon chain is saturated and contains 16 carbon atoms, as do each of the hydrocarbon chains of our lecithin. An adjustment was made based on the observation that a comparable increase in temperature causes an expansion of 2.8 Å^2/molecule of palmitic acid in the condensed state (13).

Therefore, A_{HC} of dipalmitoyl lecithin at 37° was assumed to be 2.8 Å^2/hydrocarbon chain, or 5.6 Å^2/molecule, higher than at 25° at the same surface pressure. The values of A_m were calculated for various surface pressures, and the theoretical points were joined to produce a continuous curve. The curve and corresponding experimental data are compared in Fig. 6.

The good agreement found in these experiments between calculations based on the heads-or-tails model and the experimental results strongly supports the validity of the assumptions of the model, at least with respect to mixtures of dipalmitoyl lecithin and cholesterol. Therefore, it is concluded that these substances are miscible in monomolecular films (and presumably in biological membranes). Furthermore, there is no indication of a specific interaction between the two lipids. Shah and Schulman (4) arrived at the same conclusion, based on surface potential measurements.

In the mixed monolayers, lecithin molecules are in tail contact with cholesterol molecules (Fig. 7). Since the hydrocarbon portions of the molecules are area determining, lecithin molecules in the mixed monolayers may remain hydrated even at high values of surface pressure. In contrast, compression of pure lecithin monolayers to surface pressures above 20 dynes/cm severely restricts the space available to the molecules (Fig. 1), thereby limiting the ability of the polar groups to surround themselves with water molecules. This difference in the extent of hydration may explain the liquefaction of monolayers of dipalmitoyl lecithin by cholesterol.

The mechanical properties of surface films depend not only on the size and shape of the hydrocarbon chains but also on interactions among polar groups and between polar groups and the subphase (14). In fact, the high values of surface viscosity found for monolayers of long chain alcohols and amides have been ascribed to the existence of two-dimensional networks as a result of intermolecular hydrogen bonding (15). If the formation of similar networks is responsible for the rheological changes that occur in monolayers of dipalmitoyl lecithin at 25° (4), then the effect of cholesterol in permitting interactions of lecithin molecules with the subphase rather than with each other can account for the lower viscosity of monolayers containing cholesterol.

The force–area isotherms for the equimolar mixed monolayers at the two temperatures studied are quite similar (Figs. 3 and 6) and, as shown, differences between them are accounted for by the dependence of the hydrocarbon tail area on temperature. The arrangement of the lipids in the 1:1 mixed monolayers is practically independent of temperature, while the packing of dipalmitoyl lecithin in monolayers of the pure material is a function of temperature. This observation may have some significance in the extrapolation of work with model membranes containing dipalmitoyl lecithin at room temperature to a biological system at some other temperature.

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ACKNOWLEDGMENTS AND ADDRESSES

Received November 11, 1974, from the *College of Pharmacy, Rutgers—The State University, New Brunswick, NJ 08903*

Accepted for publication February 4, 1975.

Supported by a Merck Grant for Faculty Development to J. L. Zatz. The authors are also grateful for the Johnson and Johnson Pharmacy Fellowship awarded to G. W. Cleary.

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