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MONOLAYER INTERACTIONS OF INDIVIDUAL LECITHINS WITH NATURAL STEROLS

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SUMMARY

In order to find the features of molecular structure responsible for interactions between sterols and lecithins in monolayers at the air–water interface, we have measured mixed monolayers containing one pure sterol and one molecular species of lecithin in different mole ratios. Four naturally-occurring sterols and nine synthetic lecithins were used. Cholesterol, β-sitosterol, dihydrocholesterol, and ergosterol were examined in mixtures with 1,2-dipalmitoyl-, 1-palmitoyl-2-oleoyl-, 1-palmitoyl-2-linoleoyl-, 1-palmitoyl-2-linolenoyl-, 1-palmitoyl-2-arachidonoyl-, 1,2-distearoyl-, 1-stearoyl-2-linoleoyl-, 1-stearoyl-2-linolenoyl- and 1-stearoyl-2-arachidonoyllecithins. In addition, the interactions of 5-α-androstane-3-β-ol with the first three palmitoyllecithins were investigated.

All sterols condensed strongly with 1,2-dipalmitoyllecithin at low pressures (5 dynes/cm) and this condensation was much less at 40 dynes/cm.

Cholesterol and β-sitosterol produced the greatest condensations in all lecithin films, and these condensations were greater at low pressures. These results suggest that differences in the aliphatic side chain have little influence on the behavior of these sterols in mixed monolayers with lecithins.

Dihydrocholesterol (cholestanol), ergosterol and 5-α-androstane-3-β-ol produced much smaller condensations in lecithin monolayers, and in these cases also, condensations were usually less at 40 than at 5 dynes/cm. The difference between these sterols and the other sterols is mainly in the B-ring, which suggests that a B-ring with a double bond at C_5 favors condensation much more than either a saturated B-ring (dihydrocholesterol and androstanol) or one with double bonds at C_5 and C_7 (ergosterol).

INTRODUCTION

In living cells, unesterified sterols and other lipids with polar groups are found at the interface between polar and non-polar media. These lipids collect at such interfaces because of their amphiphilic structures, and therefore are responsible for some of the surface properties of membranes and soluble lipoproteins. Cholesterol is the sterol of major quantitative importance in the animal kingdom. In yeasts and fungi, ergosterol is an important sterol, and in plants, β-sitosterol is abundant. Dihydrocholesterol is a minor component of the sterol fraction in most animal tissues,

but it comprises one-fourth of the total sterol in the testis of the White Carneau pigeon[1] and in certain brain fractions from patients with cerebrotendinous xanthomatosis[2]. The functions *in vivo* of these sterols remain to be learned.

Lecithins of plants and fungi mainly contain acyl chains of 18 carbons or less, but in animals the chain length may reach 22 or more. Most natural lecithins (1,2-diacylglycerylphosphorylcholines) possess a saturated acyl group of 16 or 18 carbons at the 1-position and an unsaturated acyl group of 16–22 carbons at the 2-position. Enzyme systems which control this specific arrangement of fatty acids have been described, but the reason for, or function of this arrangement is not yet known.

The first studies on surface properties of individual molecular species of lecithins indicated that cholesterol would interact (condense) in a monolayer with lecithins containing one double bond per acyl chain, and that those lecithins with more than one double bond per chain would not condense[3–5]. Later work showed that lecithins with two or more double bonds per chain would condense with cholesterol, provided that the unsaturated chain was in the 2-position, as occurs in nature[6,7]. These experiments suggested that the 1-saturated-2-unsaturated structure of lecithins might be an adaptation to provide maximum interaction of these phospholipids and sterol at interfaces. Also, these results indicated that the geometry of the molecules is most important in determining the fit between them, in a monolayer.

In order to learn more about the geometrical fit between membrane sterols and individual lecithin species we have measured interactions between pure sterols and lecithins in mixed monolayers at the air–water interface. These sterols are cholesterol, β-sitosterol, dihydrocholesterol, ergosterol, and 5-α-androstane-3-β-ol. The lecithins are 1,2-dipalmitoyl-(16:0-16:0), 1-palmitoyl-2-oleoyl- (16:0-18:1), 1-palmitoyl-2-linoleoyl- (16:0-18:2), 1-palmitoyl-2-linolenoyl- (16:0-18:3), 1-palmitoyl-2-arachidonoyl- (16:0-20:4), 1,2-distearoyl- (18:0-18:0), 1-stearoyl-2-linoleoyl- (18:0-18:2), 1-stearoyl-2-linolenoyl- (18:0-18:3), and 1-stearoyl-2-arachidonoyl- (18:0-20:4) lecithins.

MATERIALS AND METHODS

Pressure–area measurements were made with a Cenco Hydrophil Balance (Central Scientific Co., Chicago, Ill.) at 22 ± 1° as described before[6,7]. Lecithins were synthesized by acylation of glycerylphosphorylcholine [$CdCl_2$ complex) with an acyl chloride, removal of the fatty acid in the 2-position with snake venom, and reacylation of the 2-position with another acyl chloride[6,7]. The amount of fatty acid in a lecithin was measured by gas chromatography[6,7]. Each lecithin preparation contained at least 97 mole % of the desired molecular species[7].

β-Sitosterol was recrystallized from 95 % ethanol, and ergosterol and dihydrocholesterol from absolute ethanol. Cholesterol and 5-α-androstane-3-β-ol were used as received. Sterols were dried over P_2O_5 at 0.5 mm Hg (room temperature, 24 h) and their melting points (uncorrected) were measured (Table I). Only one component was visible by thin-layer chromatography (Table I). For film-spreading experiments, a weighed sample of sterol was dissolved in a redistilled benzene–chloroform mixture.

Aliquots of sterol solution and lecithin solution were combined to provide mixtures of various mole ratios. Spreading solutions were applied to the water surface with a Hamilton syringe (Hamilton Co., Whittier, Calif.), and at the same time an

TABLE I

DESCRIPTIONS OF STEROLS USED IN MONOLAYER EXPERIMENTS

Sterol	*Source*	*Reference to thin-layer chromatographic system*	*Melting point*		
			Observed	*Literature*	*(ref.)*
Cholesterol	Nutritional Biochemicals, Cleveland, Ohio	8	148	148.5	(10)
Dihydrocholesterol (cholestanol)	Calbiochem, San Diego, Calif.	9	141	142	(11)
Ergosterol	Calbiochem	8	169	166–183	(10)
				165	(11)
β-Sitosterol	Calbiochem	8	139–140	140	(10, 11)
5-α-Androstane-3-β-ol	Schwarz–Mann Orangeburg, N.Y.	8	149–150	151*	

* Stated on label.

equal aliquot was taken for gas-chromatographic analysis to confirm the concentration of lecithin.

RESULTS

Dipalmitoyllecithin interacted strongly with the natural sterols at 5 dynes/cm, and much less strongly at higher pressures. For example, interaction curves for this lecithin and β-sitosterol are shown at 5 and 40 dynes/cm, in Fig. 1. This illustration is typical of the interaction of these sterols with di-16:0-lecithin. (Numerical data for these are given in Table II.)

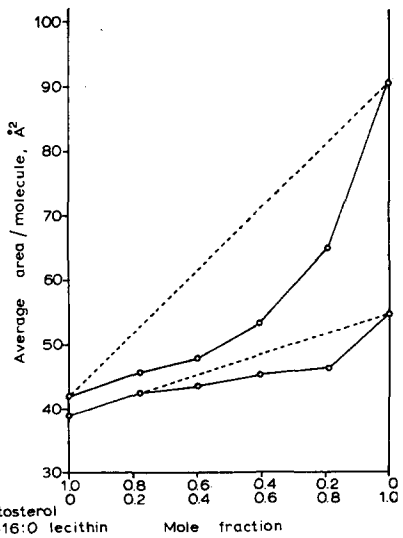


Fig. 1. Average molecular area as a function of composition in monolayers of di-16:0-lecithin and β-sitosterol at 5 dynes/cm (upper curve) and 40 dynes/cm (lower curve).

TABLE II

DECREASES IN AREA/MOLECULE IN MIXED MONOLAYERS CONTAINING INDIVIDUAL STEROLS AND LECITHINS

Ideal areas *minus* observed areas are given in Å²/molecule. Mole fractions varied within ± 10% of stated values.

Sterol	*Lecithin*	*Total Å²*	*ΔÅ²*				*Total Å²*
	Mole fraction of lecithin:	*0*	*0.2*	*0.4*	*0.6*	*0.8*	*1.0*
		5 dynes/cm					
Cholesterol	16:0-16:0	41.0	5.3	8.8	18.2	14.5	90.2
β-Sitosterol		41.8	7.2	13.6	18.0	16.7	
Dihydrocholesterol		38.5	4.8	14.5	18.7	11.3	
Ergosterol		39.3	1.3	11.0	11.5	11.6	
Cholesterol	16:0-18:1	41.0	9.4	17.3	14.7	11.6	101.8
β-Sitosterol		41.8	9.2	15.0	17.3	10.9	
Dihydrocholesterol		38.5	7.4	9.6	5.5	3.6	
Cholesterol	16:0-18:2	41.0	9.4	16.0	13.5	12.1	103.6
β-Sitosterol		41.8	4.0	10.0	9.0	5.7	
Dihydrocholesterol		38.5	5.8	3.4	7.3	5.5	
Ergosterol		39.3	4.0	6.0	4.7	3.1	
Cholesterol	16:0-18:3	41.0	9.4	18.5	21.1	14.0	118.0
β-Sitosterol		41.8	8.3	8.0	10.5	5.2	
Dihydrocholesterol		38.5	5.3	12.3	8.0	5.5	
Ergosterol		39.3	3.5	−0.9	2.9	1.7	
Cholesterol	16:0-20:4	41.0	6.8	14.4	9.8	7.0	111.6
β-Sitosterol		41.8	7.8	10.0	9.9	4.5	
Dihydrocholesterol		38.5	4.3	3.3	4.5	3.1	
Ergosterol		39.3	1.5	2.0	1.2	4.0	
Cholesterol	18:0-18:0	41.0	−1.2	1.9	2.9	0	59.5
β-Sitosterol		41.8	1.0	4.0	3.0	2.6	
Cholesterol	18:0-18:2	41.0	10.5	16.2	11.5	13.1	98.9
β-Sitosterol		41.8	6.7	9.0	7.5	3.0	
Cholesterol	18:0-18:3	41.0	5.5	13.8	12.3	5.1	109.0
β-Sitosterol		41.8	9.0	12.7	14.0	10.9	
Dihydrocholesterol		38.5	1.4	6.7	6.4	3.0	
Cholesterol	18:0-20:4	41.0	5.7	10.4	8.4	7.8	105.8
β-Sitosterol		41.8	6.5	9.0	7.0	4.7	
Dihydrocholesterol		38.5	1.0	3.0	9.1	7.4	
		40 dynes/cm					
Cholesterol	16:0-16:0	38.6	0.6	1.4	4.2	3.2	55.3
β-Sitosterol		38.9	0	1.7	3.3	5.5	
Dihydrocholesterol		36.6	−1.2	3.2	4.0	4.9	
Ergosterol		36.4	−2.7	0.4	3.0	0	
Cholesterol	16:0-18:1	38.6	3.3	7.7	6.8	5.5	65.8
β-Sitosterol		38.9	3.2	7.3	12.8	6.7	
Dihydrocholesterol		36.6	2.8	5.4	5.6	2.4	
Cholesterol	16:0-18:2	38.6	4.0	7.5	6.8	5.9	67.7
β-Sitosterol		38.9	0.7	4.0	5.5	2.7	
Dihydrocholesterol		36.6	3.0	3.4	7.4	5.2	
Ergosterol		36.4	2.8	4.3	4.0	1.0	
Cholesterol	16:0-18:3	38.6	4.9	12.6	12.7	7.7	76.3
β-Sitosterol		38.9	3.2	6.7	7.0	4.0	
Dihydrocholesterol		36.6	0.7	5.8	4.7	1.5	
Ergosterol		36.4	5.0	4.3	6.0	4.5	
Cholesterol	16:0-20:4	38.6	3.0	7.0	7.0	4.4	70.0
β-Sitosterol		38.9	2.5	6.7	6.2	2.8	
Dihydrocholesterol		36.6	2.2	6.4	7.2	6.2	
Ergosterol		36.4	4.5	5.5	3.5	2.5	

(*continued on p. 45*)

Table II (*continued*)

Sterol	*Lecithin*	*Mole fraction of lecithin:*	*Total Å²* 0	*ΔÅ²* 0.2	0.4	0.6	0.8	*Total Å²* 1.0
			40 dynes/cm					
Cholesterol	18:0-18:0		38.6	−1.1	1.1	3.0	2.0	51.5
β-Sitosterol			38.9	1.4	2.3	3.7	3.2	
Cholesterol	18:0-18:2		38.6	4.4	6.8	5.6	5.2	63.2
β-Sitosterol			38.9	1.4	4.1	3.5	0.5	
Cholesterol	18:0-18:3		38.6	0.6	6.5	6.7	2.7	67.4
β-Sitosterol			38.9	4.0	7.7	8.8	3.2	
Dihydrocholesterol			36.6	−2.5	2.2	2.2	−1.2	
Cholesterol	18:0-20:4		38.6	1.4	5.2	4.4	4.3	67.6
β-Sitosterol			38.9	3.0	6.4	10.6	5.4	
Dihydrocholesterol			36.6	−2.4	5.2	5.7	3.7	

Data for the other mixture are presented in Table II. The numbers, ΔÅ², are the differences between the ideal area for a given composition, determined graphically by drawing a straight line through the points for pure sterol and pure lecithin as in Fig. 1, and the observed area for this composition. These values are therefore the losses in area produced when the two components are mixed. The difference between replicate measurements of a given mixture was $\pm$1 Å² or less, so that the uncertainty in ΔÅ² is $\pm$ 2 Å² or less. For simplicity, mole fractions are given as 0.2, 0.4, *etc.*, but actual values varied $\pm$ 10% of the indicated value. The largest interactions were usually found at 0.4 or 0.6 mole fraction of lecithin.

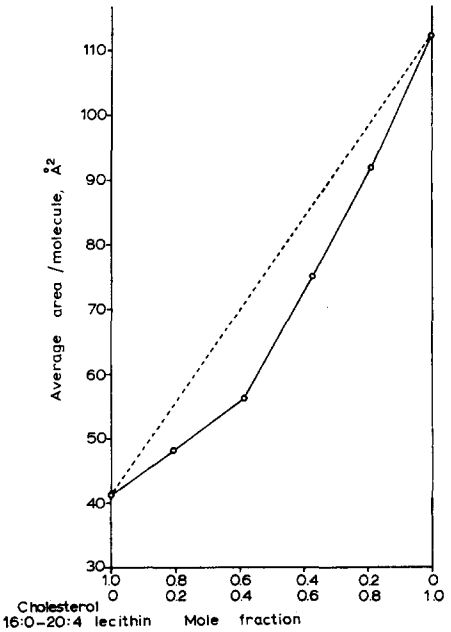
Palmitoyllecithins

16:0-18:1-lecithin, which forms an expanded film as do all the unsaturated lecithins given here, condenses considerably with cholesterol and β-sitosterol, especially at 5 dynes/cm. The condensation is much less with dihydrocholesterol at 5 dynes. At 40 dynes/cm, condensation with each sterol is reduced by about 50% in comparison with that at 5 dynes. Thus this lecithin appears to fit best with those sterols that contain a double bond at the 5-carbon of the B-ring.

16:0-18:2-lecithin shows the same general pattern as 16:0-18:1 in that it condenses more with cholesterol and β-sitosterol than with dihydrocholesterol or ergosterol at 5 dynes/cm. This again indicates a better fit with a double bond at C-5 of the sterol. At 40 dynes/cm, however, these differences between sterols do not appear.

16:0-18:3-lecithin forms the most expanded film, and interacted strongly with cholesterol, β-sitosterol and dihydrocholesterol at 5 dynes/cm, and with cholesterol at 40 dynes/cm. There was very little interaction with ergosterol at either pressure.

16:0-20:4-lecithin was similar to 16:0-18:3-lecithin in its behavior with β-sitosterol, dihydrocholesterol and ergosterol at both pressures. However, with cholesterol, the condensation was somewhat less than with 16:0-18:3-lecithin. Figs. 2-5 illustrate the interactions of the four sterols with 16:0-20:4-lecithin at 5 dynes per cm. Cholesterol and β-sitosterol, with double bonds at C_5 of the B-ring, condense much more than either dihydrocholesterol or ergosterol.



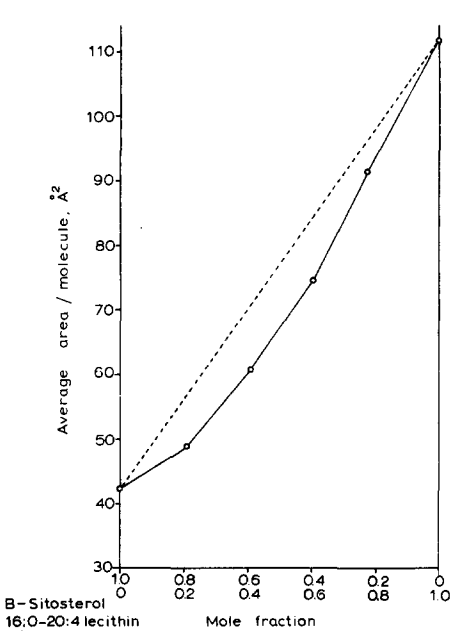
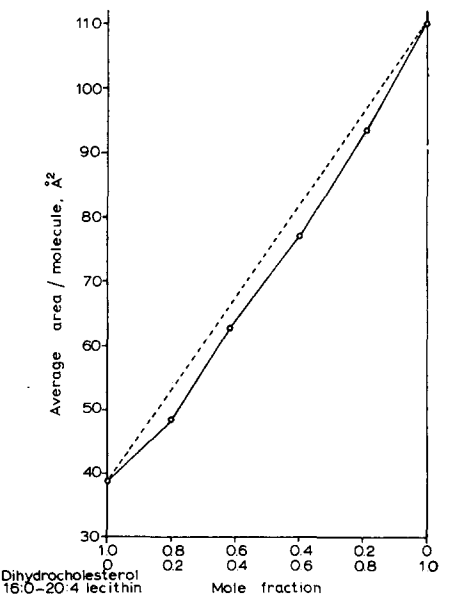


Fig. 2. Average molecular area as a function of composition in monolayers of 16:0-20:4-lecithin and cholesterol at 5 dynes/cm.

Fig. 3. Average molecular area as a function of composition in monolayers of 16:0-20:4-lecithin and β-sitosterol at 5 dynes/cm.



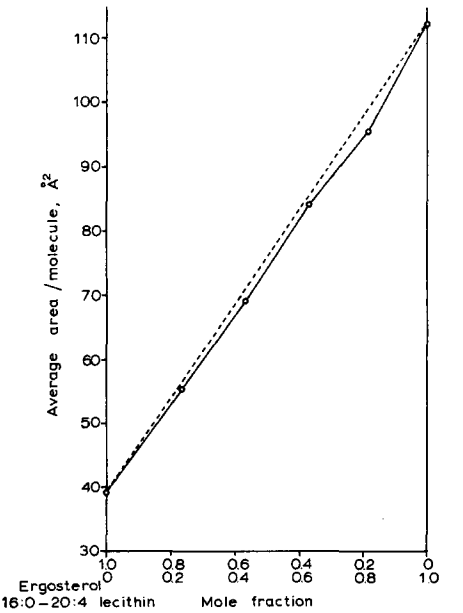


Fig. 4. Average molecular area as a function of composition in monolayers of 16:0-20:4-lecithin and dihydrocholesterol at 5 dynes/cm.

Fig. 5. Average molecular area as a function of composition in monolayers of 16:0-20:4-lecithin and ergosterol at 5 dynes/cm.

Stearoyl lecithins

Di-18:0-lecithin forms a compact film at 22°, and condenses little, if at all, with either cholesterol or β-sitosterol at both 5 and 40 dynes/cm.

18:0-18:2-lecithin forms a slightly smaller film than 16:0-18:2-lecithin, and condenses considerably with cholesterol at 5 dynes/cm, as does 16:0-18:2. However, 18:0-18:2-lecithin condenses only to a small extent with β-sitosterol at 5 dynes/cm and, at 40 dynes/cm, the interaction with either sterol is greatly reduced.

18:0-18:3-lecithin condenses strongly with both cholesterol and β-sitosterol, and much less with dihydrocholesterol at 5 dynes/cm. Interactions with all sterols are smaller at 40 dynes/cm.

18:0-20:4-lecithin condenses somewhat with cholesterol and β-sitosterol and a little less with dihydrocholesterol at 5 dynes/cm. Condensations were smaller at 40 dynes/cm.

In general, cholesterol and β-sitosterol gave greater condensations than did ergosterol and dihydrocholesterol. This indicates that a double bond at the 5-carbon of the sterol produces more condensation than either a fully saturated B-ring, or one containing double bonds at carbons 5 and 7. Consequently, the B-ring is evidently an important factor in the interaction between lecithins and sterols in monolayers.

Comparison of the results with cholesterol and β-sitosterol shows the effect of changes in the side chain of the sterol. These two sterols behave very similarly with lecithins in most cases, an indication that the difference in their side chains had no great effect on their monolayer behavior. In order to see if the side chain had any effect at all, we measured mixtures of 5-α-androstane-3β-ol, which is dihydrocholesterol without a side chain, with the first three palmitoyllecithins (Table III). This sterol produced smaller interactions than those produced by cholesterol or β-sitosterol, and more closely resembled dihydrocholesterol or ergosterol. These obser-

TABLE III

DECREASES IN AREA/MOLECULE IN MIXED MONOLAYERS CONTAINING 5-α-ANDROSTANE-3-β-OL AND PALMITOYL LECITHINS

Ideal areas *minus* observed areas are given in Å²/molecule. Mole fractions varied within ± 10% of stated values.

Sterol	*Lecithin*	*Mole fraction of lecithin:*	*Total Å²* 0	*ΔÅ²* 0.2	0.4	0.6	0.8	*Total Å²* 1.0
			5 dynes/cm					
5-α-Androstane-3-β-ol	16:0-16:0		41.6	2.5	7.8	10.7	6.8	90.2
5-α-Androstane-3-β-ol	16:0–18:1			5.0	5.5	5.4	2.6	101.8
5-α-Androstane-3-β-ol	16:0-18:2			5.1	9.4**	4.0	0	103.6
			*40 dynes/cm**					
5-α-Androstane-3-β-ol	16:0-16:0		38.3	1.4	1.6	4.6	5.5	55.3
5-α-Androstane-3-β-ol	16:0-18:1			3.3	4.7	5.6	2.4	65.8
5-α-Androstane-3-β-ol	16:0-18:2			4.1	6.3**	4.0	0.5	67.7

* It was not possible to compress films of 5-α-androstane-3-β-ol above pressures of about 35 dynes/cm; a short linear extrapolation was made from the values at lower pressures to obtain a value for 40 dynes/cm.

** The mole fraction of lecithin was 0.5 in this sample.

vations indicate that the side chain has only a small effect under these conditions, and that the structure of the B-ring is much more important.

As would be expected, condensation with sterols was decreased at higher pressures in most cases. In three cases, however, an increase may have occurred at 40 dynes/cm. These were the interactions between ergosterol and 16:0-18:3- and 16:0-20:4-lecithins, and dihydrocholesterol with 16:0-20:4-lecithin. These changes in condensation were at the limit of detection.

DISCUSSION

Monolayer studies provide information concerning the interaction between the molecules in the monolayer, particularly the geometric arrangements of the molecules. The use of spin labels produces independent, but closely related data on the geometric arrangements of molecules in multilayered systems. Butler, *et al.*[12] have found that in cholesterol–brain lipid multibilayers, the addition of either cholesterol or β-sitosterol increases the order of the spin label (cholestane spin label, 3-spiro-[2′-(*N*-oxyl-4′,4′-dimethyloxazolidine)] cholestane). In our monolayer experiments, these sterols both gave large condensations. Butler *et al.*[12] found that ergosterol as well was effective at ordering the bilayer, but only at concentrations of 5–10 mole %. We found very little condensation of ergosterol–lecithin films, but did not examine concentrations below about 20 mole %.

With respect to cholesterol and dihydrocholesterol, Butler *et al.*[12] found that these sterols were equally effective in producing ordering of the spin label in their multibilayers, and concluded that the double bond at C_5 in the B-ring is not essential for the ordering effect. In monolayers, dihydrocholesterol produced much less condensation than either cholesterol or β-sitosterol, which both have one double bond at C_5. Also, ergosterol, with 2 double bonds in the B-ring, produced little condensation. In multibilayers, Butler *et al.*[12] found that the hydrocarbon chain is "important but not crucial, since 5-α-androstane-3-β-ol promoted ordering but to a lesser extent" than either cholesterol or dihydrocholesterol. In multibilayers, the side chain seems to have a greater effect than the structure of the B-ring, while in monolayers, the structure of the B-ring seems to be of major importance.

In related work, using 3-spin-labelled cholestane, Hsia, *et al.*[13] have calculated that cholesterol increases the order in multibilayers of dipalmitoyllecithin or egg lecithin. They suggest that cholesterol causes the fatty acyl chains to become more nearly perpendicular to the plane of the polar groups. Such an effect could correspond to a condensation in a mixed monolayer.

The geometry of the spin-labelled cholestane molecule is different from that of cholesterol, and this difference will affect the orientation and possibly also the signal obtained from this probe molecule in a multibilayer. Therefore, apparent discrepancies between information derived from spin-label studies of multibilayers and that obtained by monolayer observations may be the result of (a) actual differences in geometry between multibilayers and monolayers; (b) differences in the compositions of phospholipids used in the two types of work; or (c) perturbations of the geometry in multibilayers by the bulky spin-label group itself. It is to be expected that there will be differences in geometry between monolayers and multibilayers, because in the latter system, hydrocarbon chains will presumably have considerable

end-to-end interaction in addition to the side-to-side interaction found in monolayers. As for the effect of phospholipid composition, no qualitative differences would be expected between results obtained from pure lecithins or mixtures, since all of the natural lecithins behaved similarly. To check this point, however, we measured the interactions of cholesterol and dihydrocholesterol with mixed rat liver lecithins (mole ratio, 1:1). Cholesterol produced condensations of 11.5 and 7 $Å^2$ at 5 dynes/cm and 40 dynes/cm, respectively, and dihydrocholesterol produced corresponding condensations of 10 and 5 $Å^2$. These measurements indicated that the properties of mixed lecithins are what would be expected from the properties of the individual components. The fact that natural mixed lecithins interact with cholesterol and dihydrocholesterol in a manner very similar to that of individual synthetic lecithins indicates that the differences between results obtained from monolayer and multilayer structures is probably caused by differences between the structures of monolayers and multilayers themselves, and are much less affected by differences in fatty acid composition.

We have no information concerning the possibility that spin-label probe molecules may perturb their environment sufficiently to produce misleading results. The study of these molecules in monolayers should produce useful information on their behavior at interfaces, and on their interactions with other lipid molecules.

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