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THE INFLUENCE OF LECITHIN STRUCTURE ON THEIR MONOLAYER BEHAVIOR AND INTERACTIONS WITH CHOLESTEROL

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SUMMARY

Pressure–area curves have been measured for monolayers of ten lecithins (16:0–20:0, 16:0–11-methyl-18:0, 16:0–15-methyl-18:0, 16:0–22:1, 16:0–22:6, 18:0–22:6, 20:0–18:2, 18:3–16:0, 18:2–18:2 and 18:3–18:3) and eight fatty acids (18:0, 18:1, 18:2, 18:3, 20:4, 22:6, 11-methyl-18:0 and 15-methyl-18:0).

Subtraction of the areas of the consituent fatty acids from the areas of the lecithin molecules in monolayers gave an estimate of the area contributed by the polar group. The effective areas thus obtained were found to range 3-fold in value. This result suggested that the orientation of the polar group in monolayers can vary depending upon the structures of the fatty acid chains in lecithin molecules.

Mixed monolayers of each lecithin and cholesterol were investigated in order to find the molecular features of lecithin molecules responsible for condensation in monolayers by cholesterol. Condensation by cholesterol occurred in those lecithins that form expanded films, most strongly with those molecules that have a segment of saturated hydrocarbon chain extending nine or more carbons from the carboxyl group.

The condensation between cholesterol and lecithins in monolayers has been interpreted in terms of cohesion between the saturated straight chain hydrocarbon segment of lecithin and either the ring system of cholesterol or other similar saturated segments in lecithin molecules.

INTRODUCTION

The variety in structure and composition of phospholipids in animal membranes and the interactions between phospholipids and cholesterol may be responsible for some of the diversity and specificity in the function of membranes. Effects of lipid composition on the permeability of membranes and model membrane systems have received much attention. Demel *et al.*[1] showed that permeabilities of liposomes containing pure synthetic lecithins were related to the area of the lecithin molecule in a monolayer at the air–water interface. Furthermore, incorporation of cholesterol into the liposomes decreased the permeability of the lecithins which in monolayers were condensed by cholesterol. These results imply that cholesterol–lecithin interactions may be part of the permeability control mechanism in membranes.

Interactions between lecithins and cholesterol in monolayers depend upon subtle differences in the structures of the fatty acids within the lecithin molecule[1–8].

However, the structural characteristics of the lecithin molecule necessary for strong interactions with cholesterol have not been fully defined. Therefore, to learn in more detail the nature of the interactions between cholesterol and lecithins in monolayers, we have prepared lecithins (mostly unnatural) showing a wide variation in fatty acid structure, *i.e.* saturated, unsaturated and branched chain. The pressure–area curves of the fatty acids in these lecithins were also measured to determine to what extent the areas of lecithin monolayers might be related to the areas of the constituent fatty acids.

EXPERIMENTAL

Syntheses of lecithins

L-α-Dipalmitoyllecithin (Nutritional Biochemicals Corp., Cleveland, Ohio) was the starting material for all the 1-palmitoyllecithins. It was treated with *Crotalus adamanteus* venom (Sigma Chemical Co., St. Lous, Mo.) to form 1-palmitoyllysolecithin[9] which was either used directly or converted to the $CdCl_2$ con plex[10] and treated with a fatty acid anhydride according to the procedure of Cubero Robles and Van den Berg[11]. One lecithin, 1-palmitoyl-2-arachidoyllecithin, was prepared by acylation of lysolecithin with arachidoyl chloride (Hormel Institute, Austin, Minn.)[12]. Fatty acid anhydrides were prepared from fatty acids by the procedure of Selinger and Lapidot[13]. Professor James Cason generously provided DL-11-methyloctadecanoic and DL-15-methyloctadecanoic acids (11-methyl-18:0 and 15-methyl-18:0). Palmitic, arachidic, linoleic, linolenic, erucic (22:1), and docosahexaenoic acids were obtained from Hormel Institute. Dilinolenoyllecithin was synthesized from L-α-glycerylphosphorylcholine–$CdCl_2$ complex (Sigma) and linolenic anhydride. Some of this was treated with *C. adamanteus* venom to form the lyso derivative, which was then treated with palmitic anhydride to from 1-linolenoyl-2-palmitoyllecithin. Distearoyllecithin (L-α-, Lot 00347, Calbiochem, Los Angeles, Calif.) was treated with snake venom to form the lyso derivative, which was acylated with docosahexaenoic anhydride to form 1-stearoyl-2-docosahexaenoyllecithin. Diarachidoyllecithin, synthesized from glycerylphosphorylcholine–$CdCl_2$ complex and arachidoyl anhydride, was treated with snake venom to form the lyso derivative and this was reacylated with linoleic anhydride to form arachidoyl-linoleoyllecithin. All preparations were purified by preparative thin-layer chromatography[14]. Dilinoleoyllecithin was purchased from Hormel Institute. Fatty acid compositions, as determined by gas–liquid chromatography, are given in Table I. Dilinolenoyl- and dilinoleoyllecithins contained > 99 % of linolenic and linoleic acids, respectively. Dilinoleoyllecithin produced only one spot on thin-layer chromatography[14].

Several lecithins (16:0–18:3, 16:0–20:4, 18:3–16:0 and 16:0–22:6, the first two of these taken from earlier experiments[7]) were treated with snake venom to confirm the positions of the fatty acids in the lecithin molecule. The reaction was stopped before completion, and the reaction mixture was separated by thin-layer chromatography into fatty acids, lysolecithin, and unreacted lecithin[14]. The fatty acids of each component were analyzed by gas–liquid chromatography and the results are presented in Table II. Two lecithins, 18:3–16:0 and 16:0–22:6, were also treated with all the reagents except snake venom. Under these conditions, the extent of hydrolysis was 0.8 and 1.7 %, respectively, far too little to affect the interpretation of the data from the snake venom reaction.

TABLE I

FATTY ACID COMPOSITIONS OF LECITHINS

Lecithins (fatty acid in Position 1–fatty acid in Position 2)	*Mole %*	
	Fatty acid 1	*Fatty acid 2*
16:0–20:0	48.2	51.8
16:0–15-methyl-18:0	48.6	51.4
16:0–11-methyl-18:0	46.7	53.3
16:0–22:1	49.8	50.2
20:0–18:2	50.2	49.8
18:3–16:0	51.1	48.9
16:0–22:6	49.6	50.4
18:0–22:6	50.2	49.8

TABLE II

REACTION PRODUCTS OF SYNTHETIC LECITHINS TREATED WITH *Crotalus adamanteus* VENOM

Lecithin	*Mole % fatty acids*		
	Fatty acids	*Unreacted lecithins*	*Lysolecithin*
16:0–18:3	3.2 % palmitic	51.1 % palmitic	98.0 % palmitic
	96.8 % linolenic	48.9 % linolenic	2.0 % linolenic
18:3–16:0	85.8 % palmitic	50.3 % palmitic	19.2 % palmitic
	14.2 % linolenic	49.7 % linolenic	80.8 % linolenic
16:0–20:4	11.2 % palmitic	51.7 % palmitic	93.2 % palmitic
	88.7 % arachidonic	48.4 % arachidonic	6.7 % arachidonic
16:0–22:6	8.1 % palmitic	50.5 % palmitic	92.8 % palmitic
	91.9 % docosahexaenoic	49.4 % docosahexaenoic	7.2 % docosahexaenoic

The 18:3–16:0 lecithin appears to consist of 2 isomers (Table II), as shown by the recovery of linolenic acid (14 %) in the free fatty acids released, and by the recovery of about 19 % palmitic acid in the lysolecithin. This result was unexpected and we have no clear explanation. Possibly acyl migration could occur during storage of 1-linolenoyllysolecithin, although migration apparently did not occur with 1-palmitoyl lysolecithin, nor with 1-oleoyllysolecithin[11]. Another possibility is that the enzyme specificity for the C-2 is not as absolute as formerly believed[15]. Support for this possibility is provided by the observations of Ellingboe and Steinberg[16], who showed that phytanoyllecithins are abnormally hydrolyzed by *C. adamanteus* venom. Phytanoyllecithins, like dilinolenoyllecithin, produce monolayers that are more expanded than those of normal structures[17]; these lecithins may therefore form micelles of a structure that differs from that of normal micelles in such a way that the usual specificity of the enzyme for the 2 position is reduced.

Gas–liquid chromatography

Methyl esters were prepared from fatty acids or lecithins by heating these in redistilled reagent grade methanol acidified with H_2SO_4. A measured amount of heptadecanoic acid (Eastman Organic Chemicals, Rochester, N.Y.) was added as an internal standard. Methyl esters were extracted from the reaction mixture and analyzed by gas–liquid chromatography as described earlier[5].

Pressure–area measurements

Pressure–area measurements were made at 22 ± 1 °C in a surface balance (Cenco Hydrophil Balance, Central Scientific Co., Chicago, Ill.) as described before[5,7]. A complete set of mixtures and pure components was measured within one day, and at least two films of each composition were spread. The subphase was distilled water, pH about 5.4. Before measurements were begun, the water surface was cleaned by spreading and sweeping away one or two films.

Cholesterol (Nutritional Biochemicals) was dried under vacuum over P_2O_5, weighed, and dissolved in redistilled benzene. Aliquots of this solution were mixed with aliquots of lecithin in redistilled chloroform–methanol solution for film-spreading experiments. Fatty acids were spread from redistilled benzene solutions. When each film was spread (except pure cholesterol), an equal aliquot was taken for gas–liquid chromatography measurement of the fatty acid content.

RESULTS

Pressure–area curves of fatty acid in monolayers on distilled water are shown in Figs 1a and 1b. The results for 18:0, 18:1, 15-methyl-18:0, 11-methyl-18:0, 18:3 and 18:2 are in agreement with previous work[18,19] which was done under a variety of different experimental conditions. We could not find any reports on monolayers of arachidonic acid (20:4) and 22:6.

Unsaturated fatty acids produce expanded pressure–area curves, as might be expected. Surprisingly, oleic acid with only one double bond occupied the largest area per molecule of any of the fatty acids. Both of the *n*-6 fatty acid (18:2 and 20:4) produced similar curves, which levelled off when the pressure reached about 28 dynes/cm. The

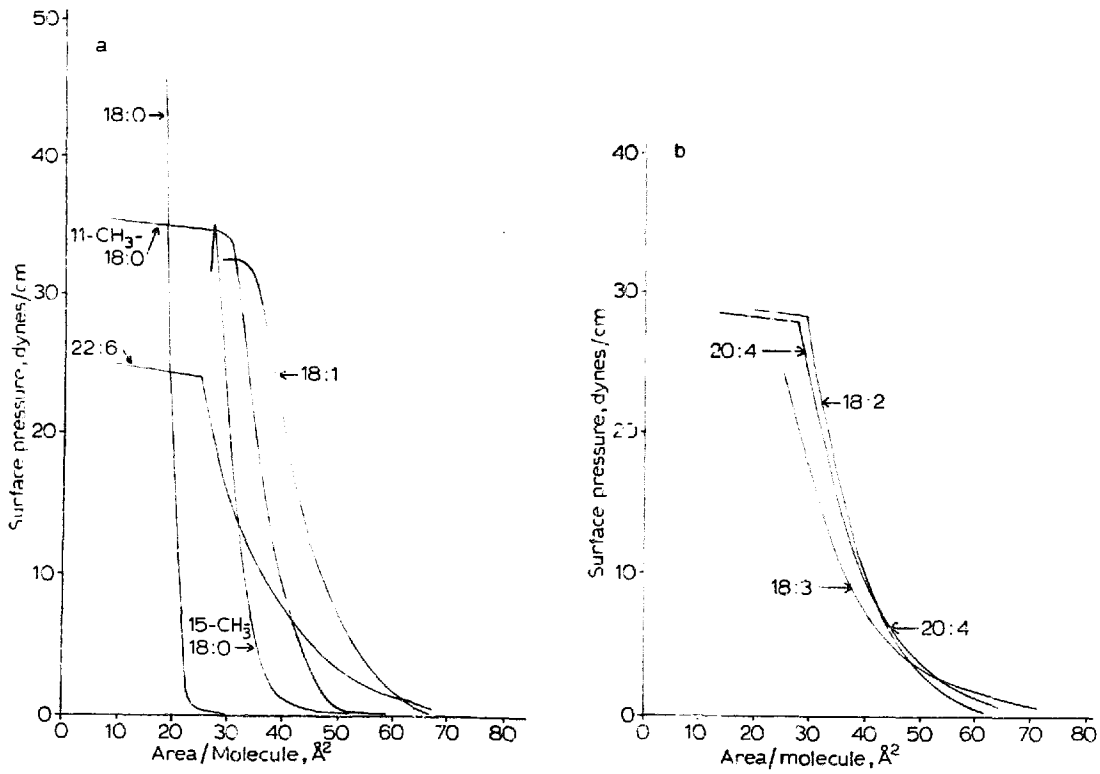

Fig. 1. Pressure–area curves of fatty acids at the air–water interface at 22 ± 1 °C. The subphase was distilled water, pH about 5.4.

n-3 fatty acids (18:3 and 22:6) also closely resembled each other, the curves of these monolayers changing at about 24 dynes/cm, either by levelling off (22:6) or by sudden loss of pressure (18:3). The levelling off or plateau phenomenon indicates that the molecules undergo a change in orientation in the monolayers, and in some cases (22:6, 20:4, 18:2, and 11-methyl-18:0) this arrangement appears stable enough to allow an orderly compression of several A^2/molecule before the film pressure is suddenly lost.

The presence of a methyl group in the C_{18} chain also caused considerable increases in molecular area, with 11-methyl-18:0 having a greater area than 15-methyl-18:0. Thus, the methyl group near the center of the chain interferes with the close packing of the backbone chain to a greater extent than does a methyl group in the 15 position. A greater expanding effect of a methyl group on the C_{18} chain in the middle position of the chain was also shown by the series of isomers studied by Weitzel *et al*[19]. The 11-methyl-18:0 reached a pressure of 34 dynes/cm before the pressure–area curve started to level off, but the 15-methyl isomer could not withstand this force (pressure), and further compression led to a loss of pressure. These curves illustrate that the behavior of fatty acids in monolayers depends upon both the number and the position of irregularities in the chain, such as a methyl group or double bond, which act as an obstacle to close packing of hydrocarbon chains.

Figs 2a and 2b illustrate the pressure–area curves of the lecithins. The values for 16:0–22:6, 18:2–18:2 and 18:3–18:3 agree with results by Demel *et al.*[1] which were reported while this work was in progress. Values for the other lecithins have not been reported. The molecules in Fig. 2a are rare or nonexistent in nature.

All lecithins except 16:0–20:0 produced expanded curves, as expected. The 16:0–20:0 molecule produced a curve that closely resembled that of 18:0–18:0 (refs.

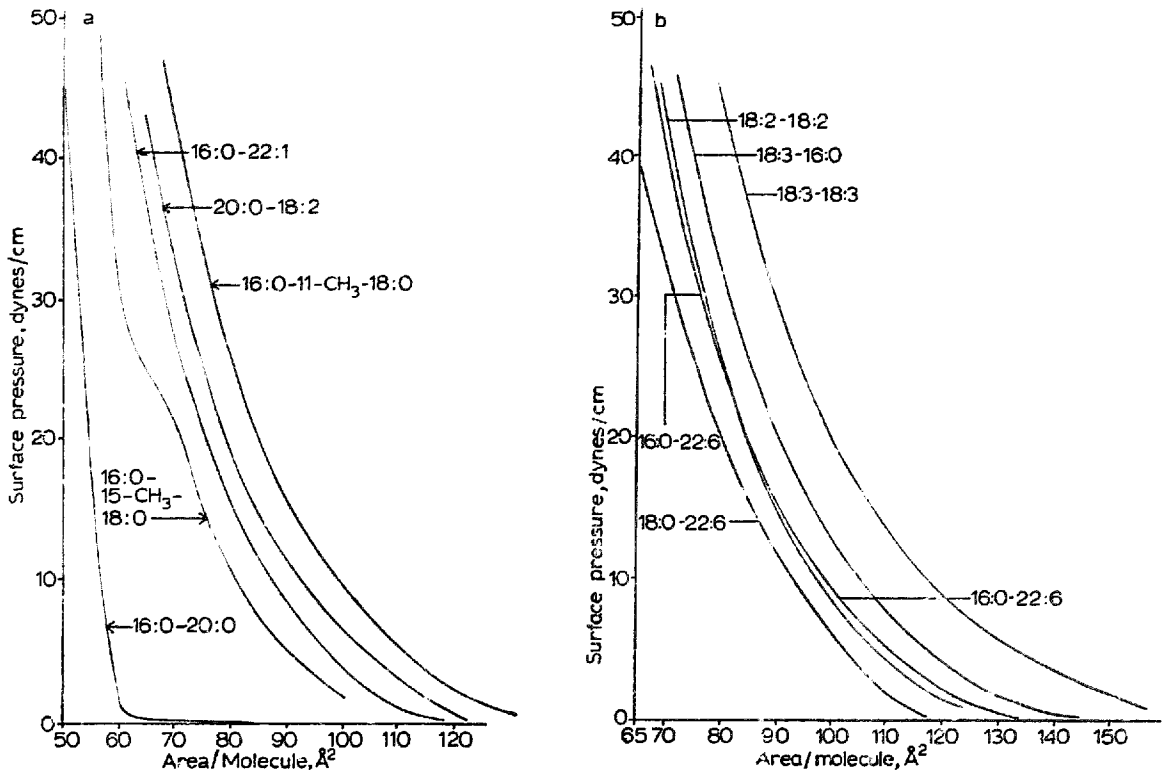

Fig. 2. Pressure–area curves of lecithins at the air–water interface at 22 ± 1 °C. The subphase was distilled water, pH about 5.4.

2, 6, 7). The pressure–area curve of 20:0–18:2 was very similar to those of the lower linoleate homologs, 16:0–18:2 and 18:0–18:2 (ref. 5). The 16:0–22:1 curve was like that of 16:0–18:1 (ref. 8) except that it occupied slightly less area per molecule. Lecithins containing branched chains also produced expanded curves, with 16:0–11-methyl-18:0 occupying considerably more area per molecule than 16:0–15-methyl-18:0, as might have been expected from the behavior of the free acids (Fig. 1a). The curve of 16:0–11-methyl-18:0 undergoes an inflection at about 22 – 30 dynes/cm, which is similar to the inflection in the pressure–area curve of 16:0–16:0 lecithin[20,21]. The inflection indicates that the molecules are undergoing a change in orientation to a more compact arrangement in the monolayer.

Fig. 2b shows the pressure–area curves of several polyunsaturated lecithins, which all exhibit the expected widely expanded curve. The curve for 18:3–16:0 matches almost exactly the earlier results with 16:0–18:3 (ref. 7). Demel *et al.*[1] have observed a similar correspondence in the curves of 16:0–18:2 and 18:2–16:0 (ref. 1). The pressure–area curve of 16:0–22:6 is very similar to that of 18:2–18:2. The 16:0–22:6 molecule also occupies less area than the 18:3–18:3 molecule, a result also obtained by Demel *et al.*[1]. The 18:0–22:6 molecule occupies less area than its palmitoyl homolog, a phenomenon observed before with the palmitoyl and stearoyl homologs of lecithins containing linoleic, linolenic and arachidonic acids in the 2 position[5,7].

Interactions between cholesterol and lecithins

All of the lecithins were examined for possible interactions with cholesterol in mixed monolayers. Table III states the interactions in the form of differences between the ideal and observed areas in Å^2. Mole fractions are given as 0.25, 0.5 and 0.75 for simplicity. The actual values varied $\pm 10\%$ of stated values. The very compact film of 16:0–20:0 lecithin was affected very little by cholesterol. The measured areas showed only a slight non-ideality which was either positive or negative depending upon the cholesterol content of the mixture. These effects are probably negligible since they are only slightly larger than the estimated experimental error of measurement (± 2Å^2 or less). The behavior of this lecithin with cholesterol in mixed monolayers is very much like that of 18:0–18:0 lecithin[6,7,22] or 22:0–22:0 lecithin[22].

Both branched chain lecithins condensed with cholesterol, especially at low surface pressures (5 dynes/cm). The lecithins containing a polyunsaturated fatty acid in each position (18:2–18:2 and 18:3–18:3 lecithins) showed small condensations, results which agree essentially with the data of Demel *et al.*[1] in which these small interactions were considered negligible.

The 16:0–22:1 and 20:0–18:2 lecithins, like their shorter chain homologs 16:0–18:1, and 16:0–18:2 and 18:0–18:2 lecithins, respectively,[8,5] condensed strongly with cholesterol. However, the additional chain length seemed to reduce the condensations with cholesterol slightly.

The inverted 18:3–16:0 lecithin possessed surface properties very much like those of the natural isomer 16:0–18:3 lecithin[7], but the condensation of the inverted isomer with cholesterol was only about two-thirds as great. The maximum condensation (about 13 Å^2) was considerably more than we had expected. However, part of the condensation may result from the possible presence of the natural isomer in the preparation (see Table II and Experimental).

Films of 16:0–22:6 showed only a moderate condensation with cholesterol, and

TABLE III

DECREASES IN AREA/MOLECULE AS A RESULT OF MIXING IN MONOLAYERS CONTAINING CHOLESTEROL AND DIFFERENT LECITHINS

Force (dynes per cm)	*Lecithin*	*Total area, ($Å^2$) (pure cholesterol)*	*$\Delta Å^2$*							*Total area ($Å^2$) (pure lecithin)*
		Mole fraction of lecithin: 0	*0.22*	*0.25*	*0.40*	*0.50*	*0.62*	*0.75*	*0.82*	*1.0*
5	16:0–20:0	40.8		−3.4		0		3.0		58.9
	16:0–15-methyl-18:0			12.4		18.1		12.4		90.7
	16:0–11-methyl-18:0			11.5		16.7		13.0		111.5
	18:2–18:2*		2.8		1.1		7.5		−4.6	108.4
	18:3–18:3			5.8		8.5		6.7		134.0
	16:0–22:1			13.3		14.9		4.5		97.5
	20:0–18:2			11.8		14.1		10.1		104.5
	18:3–16:0			11.7		12.7		6.0		117.0
	16:0–22:6			7.6		10.7		11.0		110.5
	18:0–22:6			4.1		4.5		2.9		102.8
40	16:0–20:0	38.3		1.8		1.3		5.1		51.0
	16:0–15-methyl-18:0			3.5		5.5		4.2		58.0
	16:0–11-methyl-18:0			2.6		7.8		2.5		71.2
	18:2–18:2*		0.5		0.4		5.4		−3.7	71.2
	18:3–18:3			1.5		5.1		5.4		82.1
	16:0–22:1			5.3		6.5		2.7		63.4
	20:0–18:2			4.0		7.6		5.1		66.2
	18:3–16:0			8.4		9.5		6.0		74.8
	16:0–22:6			2.5		6.2		6.7		70.3
	18:0–22:6			3.5		6.7		2.5		64.5

* Mole fractions of lecithin were 0.22, 0.40, 0.62 and 0.82, respectively.

those of 18:0–22:6 showed even less. This effect with 16:0–22:6 has also been observed by Demel *et al.*[1] who found, in addition, that condensation of this lecithin with cholesterol was completely absent at 37 °C.

DISCUSSION

Our results on the behavior of numerous lecithins and their interactions with cholesterol in monolayers agree quite well with results obtained in other laboratories. Therefore, it seems appropriate to combine our previous measurements[5,7,8] with present data in order to relate the behavior of these molecules to their structures. The following discussion will attempt (1) to identify the factors that appear to be the most important in determining the areas of lecithin molecules in monolayers and (2) to use these ideas in interpreting the interactions of cholesterol with lecithins of different structures.

In monolayers, the interactions that reveal themselves as increases or decreases in area per molecule from the ideal value are due entirely to (relatively) attractive forces between unlike molecules. Effects caused by repulsive forces between unlike molecules would not be visible in monolayers, because if two unlike molecules repelled each other (more than they repel like molecules), each molecule would simply move around until it found one of its own kind. Islands of identical molecules would then

form, and the total area of the film would be the sum of the areas of the pure islands, which would be practically the same as the ideal area.

In a monolayer of lecithin, one may imagine that the fatty acid chains are arranged in an approximately vertical direction and that the polar group is, of course, in the aqueous phase. The more the hydrocarbon chains deviate from an ordered vertical orientation, the less efficient will be the packing, and the greater the area of the film will be. Similarly, the polar group may be able to assume different orientations resulting in different areas. That the orientation of the polar group can vary from one lecithin structure to another is suggested strongly by the fact that different lecithins exhibit different surface potentials[23].

Salem[24] has described short-range attractive forces (London–Van der Waals dispersion forces) between methylene groups on adjacent hydrocarbon chains. The attractive force is extremely dependent upon the distance between interacting methylene units. For example, in monolayers of stearic acid, the dispersion energy is -8.4 kcal/mole. However, in isostearic acid (16-methyl heptadecanoic acid), the energy is only -2.8 kcal/mole, because the methyl group prevents the chains from approaching as closely as before. Attractive forces such as these are likely to be very important in monolayers of lecithins. Indeed, fully saturated long chain lecithins such as 22:0–22:0, 18:0–18:0, or 16:0–20:0 *etc.*, form very compact films at room temperature, while 9:0–9:0, 10:0–10:0, 11:0–11:0, 12:0–12:0 (ref. 4) and 14:0–14:0 (ref. 21) lecithins form expanded films. Dipalmitoyllecithin monolayers are in an intermediate condition at room temperature (about 22°C), being very expanded at low pressures, but compact at pressures above about 20 dynes/cm. These observations indicate that chain lengths of about 15 or more saturated carbons are sufficient for strong cohesion between lecithin molecules in a monolayer at 22 °C.

The polar group (which is ionically charged and therefore exerts long-range Coulombic forces) may tend to expand the film. This latter possibility is supported by the fact that dipalmitoylphosphatidylethanolamine, unlike dipalmitoyllecithin, forms a compact film at room temperature[2]. This can occur, presumably, because the smaller polar group of phosphatidylethanolamine allows closer approach of the saturated chains to one another so that the short-range cohesive forces are more effective than they are in the homologous lecithin. However, the greater cohesion allowed by a longer chain, as in 18:0–18:0 lecithin, overcomes the expanding effect of the polar group, since this lecithin forms a compact film at room temperature. In lecithins that form expanded films, it may be possible for the polar group to approach the air–water interface, while in compact films the polar group may be forced down into the aqueous phase.

In order to search for structural features of the fatty acids that are responsible for the different molecular areas of the lecithins, we have arranged the lecithins in order of increasing molecular area at 5 dynes/cm (Table IV). To see whether the amount of saturated chain length might be related to the molecular areas, we have tabulated the saturated chain lengths, 3 carbons or longer, for each molecule. The sums of the saturated chain lengths in general were inversely related to the areas of the pure films, with few exceptions. That is, those molecules with the greatest content of continuous saturated chain are also the most compact in area, an indication of greater cohesion.

Since these cohesive forces are extremely distance-dependent, any irregularity

TABLE IV

CORRELATION OF MOLECULAR AREAS IN LECITHIN MONOLAYERS WITH LENGTHS OF SATURATED CHAIN

Lecithin	*Area (Å²)*		*Numbers of carbons in saturated segments*		*Total saturated chain length*
	5 dynes/cm	*40 dynes/cm*	*Position 1*	*Position 2*	
16:0–20:0	58.9	51.0	15	19	34
18:0–18:0	59.5	51.5	17	17	34
16:0–16:0	90.2	55.3	15	15	30
16:0–15-methyl-18:0	90.7	58.0	15	13	28
16:0–22:1	97.5	63.4	15	11, 8	34
18:0–18:2	98.9	63.2	17	7, 5	29
16:0–18:1	101.8	65.8	15	7, 8	30
18:0–22:6	102.8	64.5	17		17
16:0–18:2	103.6	67.7	15	7, 5	27
20:0–18:2	104.5	66.2	19	7, 5	31
18:0–20:4	105.8	67.6	17	3, 5	25
18:2–18:2	108.4	71.2	7, 5	7, 5	24
18:0–18:3	109.0	67.4	17	7	24
16:0–22:6	110.5	70.3	15		15
16:0–11-methyl-18:0	111.5	71.2	15	9, 7	31
16:0–20:4	111.6	70.0	15	3, 5	23
18:3–16:0	117.0	74.8	7	5	22
16:0–18:3	118.0	76.3	15	7	22
18:3–18:3	134.0	82.1	7	7	14

that prevents close packing of the chains will reduce the attractive force greatly[24]. Thus, those methylene groups adjacent to an irregularity contribute less than the maximum amount to the attractive force or cohesion, because they cannot approach each other as closely as they would in adjacent straight saturated chains. This explains why 16:0–22:1 lecithin has a larger area than the fully saturated straight chain lecithins, although they all have the same length of saturated carbon chain. Similarly, a methyl group branch is even more effective in preventing close approach of adjacent chains, especially when it is in the middle of the chain (16:0–11-methyl-18:0 lecithin). As the number of double bonds increases, the length of fully saturated segments becomes shorter, and the amount of cohesion between saturated regions decreases. The decrease is reflected in increasing areas per molecule. The 22:6 lecithins are somewhat anomalous. Although they have relatively little saturated chain length to allow cohesion they occupy less area per molecule than some lecithins with long regions of saturated chain. This observation suggests that the 22:6 chain ina lecithin molecule is capable of assuming a compact conformation somewhat different from that of the other poly-unsaturated chains.

One of the reasons for measuring the pressure–area curves of fatty acids was to learn whether the areas of lecithin molecules in a monolayer could be estimated from the areas of the constituent parts, namely the fatty acid chains and the polar group. Our observations do not provide direct information on the area of the polar group, but one can roughly estimate its contribution by subtracting the measured areas per molecule of the fatty acids from that of the lecithin. One might expect that this contribution would be constant for all the lecithins, since the polar group is the same in all cases. Or alternatively, one might expect that the apparent contributions from the

TABLE V

CONTRIBUTION OF FATTY ACIDS AND POLAR GROUP TO AREAS OF LECITHIN MOLECULES IN MONOLAYERS

FA, fatty acid.

Lecithin	*Area/molecule (10 dynes/cm)*	*Areas/molecule (fatty acids, 10 dynes/cm)*			*Total area minus fatty acid area = apparent contribution of polar group*
		FA_1	FA_2	ΣFA	
16:0–20:0	57.0	23.0*	22.0*	45.0	12.0
18:0–18:0	58.5	21.3	21.3	42.6	15.9
18:2–18:2	97.2	40.0	40.0	80.0	17.2
16:0–18:1	91.0	23.0*	46.5	69.5	21.5
16:0–16:0	67.5	23.0*	23.0*	46.0	21.5
16:0–15-methyl-18:0	81.0	23.0*	33.0	56.0	25.0
18:0–18:2	89.2	21.3	40.0	61.3	27.9
16:0–18:2	92.5	23.0*	40.0	63.0	29.5
16:0–22:1	87.6	23.0*	35.0*	58.0	29.6
20:0–18:2	93.0	22.0	40.0	62.0	31.0
18:0–20:4	95.2	21.3	39.3	60.6	34.6
18:0–22:6	93.2	21.3	36.5	57.8	35.4
16:0–11-methyl-18:0	99.5	23.0*	39.3	62.3	37.2
18:0–18:3	97.0	21.3	36.5	57.8	39.2
16:0–22:6	99.0	23.0*	36.5	59.5	39.5
16:0–20:4	101.8	23.0*	39.3	62.3	39.5
18:3–18:3	117.5	36.5	36.5	73.0	44.5
18:3–16:0	105.0	36.5	23.0*	59.5	45.5
16:0–18:3	106.2	23.0*	36.5	59.5	46.7

* Areas for 16:0, 20:0 and 22:1 taken from Gaines[18] pp. 223 and 236.

polar group would decrease as the areas of the constituent fatty acids increase, since they could cover the polar group more completely. In Table V we have calculated the apparent contributions of the polar group to the total areas of the lecithins and arranged the lecithins according to the apparent area of the polar group. From this table we can see that the apparent contribution from the polar group is not constant at all, but rather varies over a 3-fold range. Furthermore, the apparent contribution of the polar group often is larger in lecithins containing fatty acids of large molecular area, a result contrary to our expectations based on the covering ability of fatty acids with large areas per molecule. Evidently, both simple hypotheses above are incorrect. It appears that in lecithin films, the fatty acid chains either behave differently from the way they behave in fatty acid films, or they influence the polar group such that it occupies more area, or both. From Table V, it appears that the polar group can occupy a large apparent area in monolayers of certain lecithins, particularly in those that have little opportunity for cohesion between saturated chains.

The preceding data show that the behavior of lecithin molecules in monolayers seems to be controlled by two main forces. One is a short-range attractive force between methylene groups on adjacent hydrocarbon chains. This can cause cohesion and hence condensation of lecithin films if the saturated regions are sufficiently long, *i.e.* about 15 carbons or more, at 22 °C. The other force, which is less well defined, is the tendency of the polar group to expand the film. These ideas can be used to interpret the condensation of lecithin films by cholesterol. Such a condensation results from

increased efficiency of packing of the molecules. This implies, in turn, a greater cohesion between unlike molecules than between like molecules.

The cholesterol molecule is comprised of a rigid ring system and a flexible side chain. In monolayers, it forms a compact film in which the area per molecule is very close to the cross-sectional area of the ring system, as determined by the use of molecular models[25]. This implies that the ring system is oriented vertically to the air–water interface. Likewise, the carboxyl ends of the fatty acid chains in lecithins are near the interface. If the ring system of cholesterol could approach closely the fatty acid chains, cohesion should result.

The length of the ring system from the polar group to the side chain is about 10.2 Å, as estimated from the molecular model of Vandenheuvel[25]. If a methylene segment of a saturated chain is about 1.2 Å long, fatty acid chain lengths of 7, 8 or 9 carbons are approximately 8.4, 9.6 or 10.8 Å long. In all expanded lecithin films so far examined, condensation is observed when a saturated straight chain of 9 carbons or more occurs next to the carboxyl group. However, the condensation is much less in those lecithins with shorter chain lengths in this position.

In Joos and Demel's[4] series of saturated short-chain lecithins, all films were expanded, but cholesterol did not condense 9:0–9:0 and condensed 10:0–10:0 only slightly. Strong condensation occurred with 11:0–11:0 lecithin and longer chain lengths All of these observations, together with the molecular dimensions given above, suggest that the ring system of cholesterol can fit well with the saturated region near the carboxyl group in lecithins if this region is 9 or more carbons long. The cohesion thus produced can overcome the expanding effect of the polar group.

In compact films, such as those of 18:0–18:0, 16:0–20:0 (Fig. 2a) or 22:0–22:0, cholesterol may approach the saturated chain closely, but no condensation is observed because the mixed films and the pure films are both packed equally efficiently. This idea also explains why much less condensation is observed in the expanded films of 18:2–18:2 or 18:3–18:3 lecithins (Table III) since these molecules have saturated chain lengths of only 7 carbons near the carboxyl.

For an expanded film of lecithin to condense strongly with cholesterol, it therefore seems necessary that there be a region of at least 9 saturated carbons adjacent to the carboxyl group on one of the fatty acid chains. Whether the saturated chain is on the 1 position or on the 2 position of the lecithin is apparently not critical since cholesterol can condense with 18:3–16:0 (Table III) and 18:2–16:0 (ref. 1). Our previous suggestion[5,7] that cholesterol can condense lecithins only of natural structures is therefore found to be too restrictive.

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