RESEARCH PAPERS

MONOMOLECULAR LAYERS OF SYNTHETIC PHOSPHATIDES*

BY L. L. M. VAN DEENEN, U. M. T. HOUTSMULLER, G. H. DE HAAS AND E. MULDER

From the Laboratory of Organic Chemistry, State University of Utrecht, The Netherlands

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In studying the functions of phosphatides in biological membranes, investigations have been made into monomolecular layers of synthetic phosphatides. Force-area curves of monolayers of phosphatidic acid, phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine, all containing identical fatty acids, showed small differences, obviously to be attributed to differences in size and charge of the end groups. The shifts of the force-area curves within one class of phosphatides were more pronounced and are brought about by variations of the apolar moiety. Shortening of the chain length and particularly unsaturation of the fatty acid constituents greatly expanded the films of the L-α-lecithins, thereby increasing the closest stable packing attainable. Force-area curves of structurally isomeric mixed-acid L-α-phosphatides, carrying dissimilar fatty acids in different positions, were identical. Mixed films consisting of phosphatides and cholesterol in molar equivalents—at proportions also found in red cell membranes—revealed a condensing effect of cholesterol on the film of phosphatides containing certain unsaturated fatty acid constituents.

IN theories formulating the structure of biomembranes, bimolecular layers of phosphatides and other lipids are presumed to be associated on both sides through their polar groups with layers of proteins, thus forming the framework of the biological interfaces (Booij and Bungenberg de Jong, 1956; Stein and Danielli, 1956). Electron microscopic studies on natural objects as well as on artificially produced systems for instance myelins, formed from isolated phosphatides, appear to sustain the idea that a bimolecular phosphatide layer represents the backbone of the numerous cell membranes and boundaries inside the cells (compare Robertson, 1959; Stoeckenius, 1959, 1960; Stoeckenius, Schulman and Prince, 1960; Engström and Finean, 1960). Although the numerous biomembranes may be constructed according to a general pattern (a "unit membrane"), it is likely that differences exist in their fine structure, accounting for the apparent variations in properties between various membranes. Perhaps some of these variations may be brought about by differences in the chemical structure of the membranous lipid constituents.

Studies from this laboratory, made on the chemical composition of

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lipids of a number of animal tissues, membranes and membranous fragments, have already supplied information on how far variations in the lipid moiety of the membrane play an essential part in the properties of membranes. We found from results of comparative fatty acid analyses made on lipid fractions of a number of mammalian tissues that neutral lipids of the species studied possessed animal specificity; the fatty acid patterns differed from animal to animal, but resembled each other for different tissues within one species. On the other hand, the group of phosphatides revealed some tissue specificity, inasmuch as their fatty acid patterns differed for various tissues within one animal, but showed some degree of similarity in several homologous tissues (e.g. lung and brain) of different species of mammals (Veerkamp, Mulder and van Deenen, 1962). Furthermore, analyses on phosphatides extracted from whole tissues, may supply information about the fatty acid composition of lipids from intracellular membranes, for example mitochondrial membrane fragments. These results suggested that, beside the proportions of various lipid groups, a specific composition of the apolar part of the phosphatides may also play an essential part in the function of phosphatides in membranes. This view was supported by investigations on lipids from red cell membranes from different animal species. These lipids appeared to differ significantly. In the sheep, ox, pig, man, rabbit, rat, the lecithin percentage of the membranous phosphatides increases from about 1 to 58 per cent. A considerable shift in the sphingomyelin content compensates this effect, thereby giving a nearly equal content of choline-containing phosphatides in the red cells of the species studied (de Gier and van Deenen, 1961). Moreover, the fatty acid patterns of red cell lipids were found to be highly specific for each animal species and to vary between the red cells of different mammals. After alkaline saponification of the lipids from red cell ghosts, a decrease of arachidonic acid and palmitic acid was observed in the sequence rat, man, rabbit, pig, ox, sheep, whereas in that order the oleic acid content was found to be significantly increased with these groups of lipids (Kögl, de Gier, Mulder and van Deenen, 1960; de Gier, Mulder and van Deenen, 1961).

A comparison of these lipid characteristics with data on the permeability properties of the red cells from these animals, as determined by other investigators (Jacobs, Glassman and Parpart, 1935, 1950; Höber and Orskow, 1933), surprisingly showed that the shift observed in the lipid composition varies in the same sequence of animals as does the permeability behaviour when determined by osmotic means. This coincidence suggested that lipid composition and permeability properties of biomembranes may be closely related. One possible approach to elucidate such a relation, in progress now in our laboratory, involves the induction of changes in the chemical composition of membranous lipids by dietary means and the study of consequent changes in the permeability characteristics of the membranes.

Another approach will be by the perhaps devious but necessary route of making artificial membrane models composed of defined phosphatides and to search for differences in structure and properties of these

models in connection with the chemical composition of their lipid constituents.

During introductory attempts in this direction it became desirable to study mono-molecular layers of defined phosphatides composed of different, but known, fatty acids.

Studies on Monolayers of Phosphatides

Although it is unlikely that a phosphatide film at the air/water interface bears a very close resemblance to a cell membrane, studies on such monolayers certainly have improved the insight into the structure of biomembranes. This was demonstrated as early as 1925 by Gorter and Grendel, who deduced, from the spreading of lipids from red cell ghosts, that these bio-interfaces contain sufficient lipid material to constitute a bimolecular layer covering the red cell surface.

Leathes (1923, 1925) found that, when lecithin was spread on the surface of water, an expanded film was formed, while hydrolecithin gave a more condensed film. The possibilities offered by studying the action of phospholipases on monolayers of phosphatides was recognised by Hughes (1935). Recently, Dawson and Bangham (1959; compare also Bangham and Dawson 1960) developed this method in an attractive way, using labelled substrates. Beside studies on the complex formation of phosphatides with cholesterol, glycerides and fatty acids (Leathes, 1925; Dervichian and Pilet, 1944; Dervichian and Joly, 1946; Guastalla, 1949; Croes and Ruyssen, 1950; de Bernard, 1958) the interaction of monolayers of phosphatides with proteins was also investigated (Eley and Hedge, 1956, 1957; Schulman, 1957; Payens, 1960). These studies appear to be very important to the understanding of the binding between different types of lipids and between lipids and proteins, combinations which probably exist in membranes. The results of the studies on the interaction between proteins and phosphatides, however, have been interpreted in different ways.

As a consequence of Rideal's (1939) valuable suggestions, various attempts have been made to obtain information about the possible action of biologically active compounds on biomembranes by studying their effects on monolayers of phosphatides, for example sulphonamides (Veldstra and Havinga, 1947), local anaesthetics (Skou, 1961; Shanes, 1960), antibiotics (Few and Schulman, 1953), chemotherapeutic agents inhibiting neoplasms (Hirt and Berchtold, 1961), plant growth hormones (Veldstra and Havinga, 1948). Although many interesting results were obtained it is not possible, at the present, to draw definite conclusions about the mechanism of action of these substances in the living cell from the results of studies on phosphatide films at the air/water interface or other artificial systems. Evaluation of membrane models, which in composition and structure more closely resemble living membranes, probably will furnish further knowledge about the mechanism of drug action in the future. Encouraging possibilities arise from the work of Saunders (1953), who succeeded in studying stable films of phosphatides formed between two aqueous liquids, bearing a close resemblance to

boundaries separating a living cell from a liquid environment. Furthermore the possibilities offered by electron microscopy of artificial boundary systems (compare Trurnit and Schidlovsky, 1960), may give fresh information on the ultra structure of these synthetic membrane systems in comparison with biomembranes.

Most results in this field have been obtained by using isolated lecithin specimens of biological origin, whereas only few studies were made on reliable synthetic compounds (Anderson and Pethica, 1955; Eley and Hedge, 1957). Because of the differences in the lipid composition of biomembranes, presumably related to differences in membrane properties, it appeared desirable to carry out future experiments with well-defined phosphatides.

Materials and Methods

Synthetic Phosphatides

To verify the effects of the chain length and the presence of double bonds of the fatty acid constituents on the interfacial behaviour of phosphatides, a method for the synthesis of a series of L-α-lecithins in a simple way was developed (Kögl, de Haas and van Deenen, 1960). By this method, involving a reacylation of L-α-glyceryl-phosphorylcholine prepared from egg lecithin, defined L-α-lecithins were obtained, which are composed of two identical fatty acids per molecule (I). The fatty acid chain length varied from C_2 to C_{24}, while also compounds composed of an unsaturated acyl chain are included (Table I).

TABLE I

SYNTHETIC L-α-LECITHINS CARRYING TWO IDENTICAL FATTY ACIDS (Formula I)

L-α-Lecithins	R	Abbreviations†
L-α-(Diacetyl)lecithin	CH_3—	2°/2°-L-α-PC
L-α-(Dibutyryl)lecithin	CH_3—CH_2—CH_2—	4°/4°-L-α-PC
L-α-(Diheptanoyl)lecithin	CH_3—$(CH_2)_5$—	7°/7°-L-α-PC
L-α-(Didecanoyl)lecithin	CH_3—$(CH_2)_8$—	10°/10°-L-α-PC
DL-α-(Ditetradecanoyl)lecithin*	CH_3—$(CH_2)_{12}$—	14°/14°-DL-α-PC
L-α-(Di-pentadecanoyl)lecithin	CH_3—$(CH_2)_{13}$—	15°/15°-L-α-PC
L-α-(Di-stearoyl)lecithin	CH_3—$(CH_2)_{16}$—	18°/18°-L-α-PC
L-α-(Di-tetracosanoyl)lecithin	CH_3—$(CH_2)_{22}$—	24°/24°-L-α-PC
L-α-(Di-undecenoyl)lecithin	CH_3=CH—$(CH_2)_8$—	11^1/11^1-L-α-PC
L-α-(Di-oleoyl)lecithin	CH_3—$(CH_2)_7$—CH=CH—$(CH_2)_7$—	18^1/18^1-L-α-PC

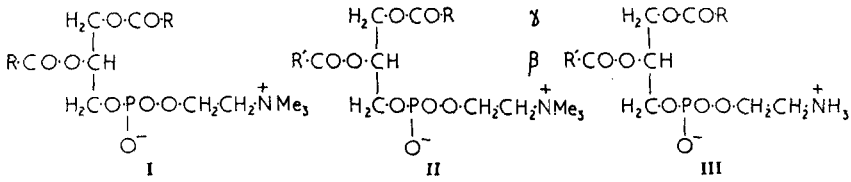
* This lecithin was synthesised by an unpublished procedure involving a preparation of dimyristoyl-DL-α-glycerylphosphoryl(*NN*-dimethyl)ethanolamine according to de Haas and van Deenen (1961a). The latter compound was converted into the corresponding lecithin by quaternisation with methyliodide.

† The various groups of phosphatides are abbreviated in the usual way, as follows: PC, phosphatidylcholine (lecithin); PE, phosphatidylethanolamine; PS, phosphatidylserine; PA, phosphatidic acid.

The fatty acid constituents are indicated by means of the number of carbon atoms and double bonds; e.g. 18^1 stands for oleic acid. The abbreviation of the fatty acid in γ-position of the phosphatide molecule preceeds that of the β fatty acid.

Most naturally occurring lecithins are known to be composed of both saturated and unsaturated fatty acid consituents. From the recent proof of the β-specificity of the mono fatty acid-releasing phospholipase present in snake venom (Tattrie, 1959; Hanahan, Brockerhoff and Barron, 1960; de Haas, Mulder, and van Deenen, 1960; de Haas and van Deenen, 1961), it is now concluded that saturated fatty acids are preferentially

located in the γ-ester position, whereas the unsaturated ones are predominantly esterified at the β-position. The biological significance of this asymmetrical distribution of the fatty acids in this most abundant type of phosphatides is not yet elucidated. The synthesis of mixed-acid lecithins (II) was first achieved by de Haas and van Deenen (1960; 1961a),



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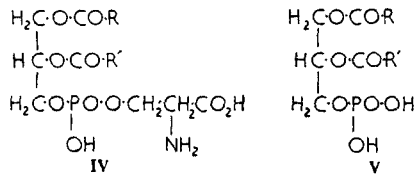

IV V

according to a fully synthetic method being suitable for the preparation of compounds having the naturally occurring L-α-configuration, as well as for D isomers (Table II). After elucidating the β-specificity of snake venom phospholipase with these compounds, a less elaborate partial synthesis of mixed-acid L-α-lecithins was developed, implying the specific mode of action of this enzyme (de Haas and van Deenen, 1960a). The synthetic mixed-acid lecithins contained either two saturated fatty acids of unequal chain length or one saturated and one unsaturated fatty acid in different positions (Table II).

TABLE II

SYNTHETIC MIXED-ACID LECITHINS (Formula II)

Compounds	Fatty acid position		Abbreviations*
	R	R′	
(γ-Stearoyl-β-lauroyl)-L-α-lecithin	Stearic	Lauric	18°/12°-L-α-PC
(γ-Stearoyl-β-lauroyl)-D-α-lecithin	Stearic	Lauric	18°/12°-D-α-PC
(γ-Stearoyl-β-lauroyl)-DL-α-lecithin	Stearic	Lauric	18°/12°-DL-α-PC
(γ-Lauroyl-β-stearoy)-DL-α-lecithin	Lauric	Stearic	12°/18°-DL-α-PC
(γ-Oleoyl-β-stearoyl)-L-α-lecithin	Oleic	Stearic	18^1/18°-L-α-PC
(γ-Stearoyl-β-oleoyl)-L-α-lecithin	Stearic	Oleic	18°/18^1-L-α-PC
(γ-Stearoyl-β-oleoyl)-D-α-lecithin	Stearic	Oleic	18°/18^1-D-α-PC

* For explanation see Table I.

In addition, mixed-acid L-α-phosphatidylethanolamines (III) (de Haas and van Deenen, 1961a; Daemen, de Haas and van Deenen, 1962), and a DL-α-phosphatidylserine (IV) (de Haas and van Deenen, 1961b) were prepared fully synthetically. Besides these mixed-acid cephalins, DL-α-(distearoyl)phosphatidylethanolamine, DL-α-(distearoyl)phosphatidyl

serine and DL-α-(distearoyl)phosphatidic acid (V) were also synthesised accordingly by comparable methods (Table III).

TABLE III

SYNTHETIC CEPHALINS AND PHOSPHATIDIC ACID (Formulae III, IV and V)

Compounds	Fatty acid position		Abbreviations*
	R	R′	
Formula III,			
DL-α-[Distearoyl]-phosphatidylethanolamine	Stearic	Stearic	$18^0/18^0$DL-α-PE
(γ-Stearoyl-β-oleoyl)-L-α-phosphatidylethanolamine	Stearic	Oleic	$18^0/18^1$-L-α-PE
(γ-Oleoyl-β-stearoyl)-L-α-phosphatidylethanolamine	Oleic	Stearic	$18^1/18^0$-L-α-PE
Formula IV			
DL-α-(Distearoyl)phosphatidylserine	Stearic	Stearic	$18^0/18^0$-DL-α-PS
(γ-Stearoyl-β-lauroyl)-DL-phosphatidylserine	Stearic	Lauric	$18^0/12^0$-DL-α-PS
Formula V			
DL-α-(Distearoyl)phosphatidic acid	Stearic	Stearic	$18^0/18^0$-DL-α-PA

* For explanation see Table I.

Spreading Methods

A conventional Langmuir-Adam trough assembly was used for surface pressure measurements. The phosphatides were spread from a 5×10^{-4} molar solution in highly purified chloroform on a phosphate buffer pH 7·4, ionic strength 0·14, at room temperature (21–24°). In a number of experiments a citrate-phosphate buffer pH 4·0, ionic strength 0·14, was used as substrate. The initial surface per molecule was about 200 Å^2, at which the pressure varied between 1 to 2·5 dynes/cm. The measurement of a force area-curve lasted for 1·5 to 2 hr., depending on the compressibility of the film and the closest stable packing attainable.

Force area curves are generally averages of at least three measurements.

RESULTS AND DISCUSSION

Force-area Curves of Monolayers of Several Types of Glycerol Phosphatides

Effects brought about in force-area characteristics of phosphatides by different polar end groups are demonstrated in Fig. 1. For comparison, films of phosphatides containing identical fatty acids were spread. Apparently the molecular areas occupied by the various types of phosphatides at the various pressures differ only to a limited extent. The closest stable packing for the distearoyl homologues of DL-α-phosphatidic acid and DL-α-phosphatidylethanolamine approaches at pH 7·4 near to 36 Å^2/molecule. Since this value is in keeping with the molecular dimensions of two fatty acid chains, it appears that the area per molecule of these groups of phosphatides is determined mainly by the apolar moiety of their molecules. The closest stable packing of L-α-(distearoyl)lecithin, however, was found to be about 39–40 Å^2/molecule, being in agreement with the data reported by Anderson and Pethica (1955). The differences in molecular area occupied in the films of L-α-lecithin and DL-α-phosphatidic acid and DL-α-phosphatidylethanolamine respectively, might be caused by differences in steric configuration. But it is not unlikely that the three methyl groups of the choline moiety or its charge may be involved

in determining the packing of the lecithin molecules. In fact, the force-area curves of more expanded films of mixed-acid L-α-lecithins and L-α-phosphatidylethanolamines, (Figs. 4 and 5), showed a comparable difference. With DL-phosphatidylserine, values observed for the closest stable packing attainable at pH 7·4 amounted to 39 Å².

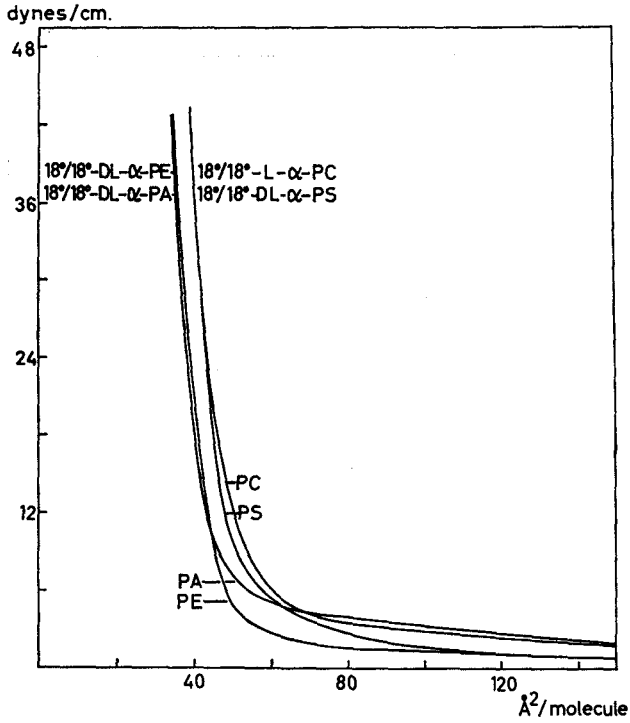

FIG. 1. Force-area characteristics of distearoyl homologues of lecithin (phosphatidylcholine; PC), phosphatidylethanolamine (PE), phosphatidic acid (PA) and phosphatidylserine (PS) on a phosphate buffer substrate of pH 7·4 (abbreviations are also indicated in Tables I–III).

Furthermore some differences were noted in the response of the various phosphatide films to changes in pH of the substrate medium. At pH 4·0 the films of phosphatidylethanolamine, phosphatidylserine and particularly of phosphatidic acid, showed a decreased area per molecule at low pressures if compared with the films spread at pH 7. The lecithin films were identical at both pH values. The closest stable packing of phosphatidyl ethanolamine and phosphatidic acid was not altered appreciably by this pH variation, while phosphatidylserine films revealed a slight decrease to 37 Å². However, (γ-stearoyl-β-lauroyl)-DL-phosphatidylserine, at pH 7·4, gave a more expanded film than the distearoyl homologue, and showed, when spread on an underlayer of pH 4·0, a significant phase transition at a pressure of about 24 dynes/cm., resulting

in the closest stable packing comparable to the value of the distearoyl compound (Fig. 2). In addition, Fig. 2 demonstrates that the force-area characteristics of (γ-stearoyl-β-lauroyl)-DL-lecithin did not respond to variations of pH 7·4 to 4·0. At pH 7·4, the differences between the film characteristics of the DL-α-lecithin and DL-α-phosphatidylserine, both having an identical fatty acid composition, appeared to be small.

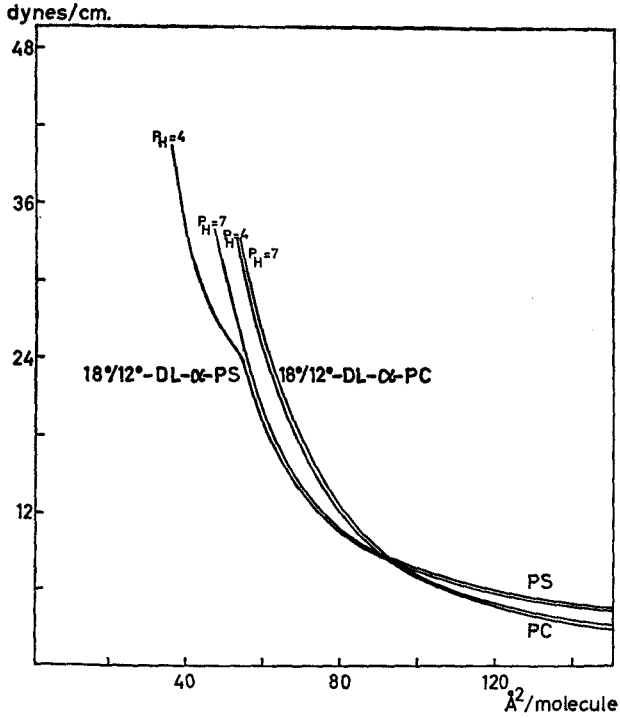

FIG. 2. Effect of pH on force-area characteristics of (γ-stearoyl-β-lauroyl)-DL-α-phosphatidylserine and (γ-stearoyl-β-lauroyl)-DL-α-phosphatidylcholine (Compare also Table II and III.)

Effect of Fatty Acid Chain Length on Force-area Characteristics of Lecithins

The pioneering studies of Adam (1930) on monolayers of fatty acids, alcohols and derivatives, clearly indicated that the force-area curves of amphiphatic molecules generally are highly dependent on the chain length of the apolar part, which determines the van der Waals' forces involved. In this respect, however, information about the behaviour of the films of the more complex phosphatide molecules is lacking. Representative characteristics of a number of synthetic lecithins, containing different fatty acids, are given in Fig. 3. The long-chain compounds, L-α-(ditetracosanoyl)lecithin and L-α-(distearoyl)lecithin, produce monolayers of the liquid condensed type. Shortening of the chain length of

the fatty acid constituent results in far more expanded films, like those of L-α-(dipentadecanoyl)lecithin and L-α-(didecanoyl)lecithin. Furthermore, the compressibility of both foregoing long-chain lecithins was about 45×10^{-4} cm./dyne, whereas the latter short-chain lecithins revealed a value of 90×10^{-4} cm./dyne (compare Harrap, 1954).

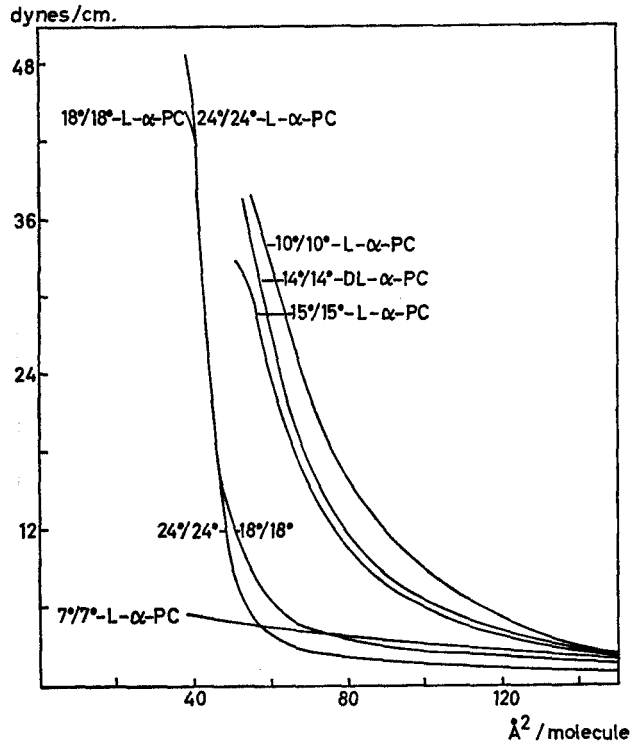

FIG. 3. Force-area characteristics of various saturated lecithins (phosphatidyl choline; PC) on a phosphate buffer substrate, pH 7·4. All compounds contain two equal fatty acid constituents per molecule. The nature of the fatty acid constituents is indicated by the number of carbon atoms (compare Table I).

As expected, the fairly water-soluble L-α-(diheptanoyl)lecithin and the highly water-soluble L-α-(dibutyryl)lecithin and the L-α-(diacetyl)lecithin failed to give a stable film.

Effect of Unsaturated Fatty Acid Constituents on Force-area Characteristics of Phosphatides

Naturally occurring phosphatides, for example from red cell membranes, often show great variation in their content of unsaturated fatty acids. Introduction of one mono-unsaturated fatty acid (oleic acid) in the lecithin molecule causes the monomolecular film of the phosphatide to be more expanded than the film of the corresponding saturated lecithin (Fig. 4), and changes the compressibility from 45×10^{-4} cm./dyne to 83×10^{-4}cm.

/dyne. Consequently, the presence of two oleic acid chains in the lecithin molecule effect a further shift of the force-area curve, increasing the expanded character of the lecithin film. The presence of oleic acid within the phosphatidylethanolamine molecule causes effects similar to those noted for the film characteristics of lecithin (compare Fig. 5). Apparently, the presence of unsaturated bonds in the acyl chain of the phosphatides greatly influences their interfacial properties. Taking into account the significant amounts of poly-unsaturated fatty acids, such as arachidonic acid, present in membranous phosphatides, it seems important to extend these studies to these types of compounds. The synthesis of phosphatides composed of highly unsaturated fatty acids is in progress in our laboratory.

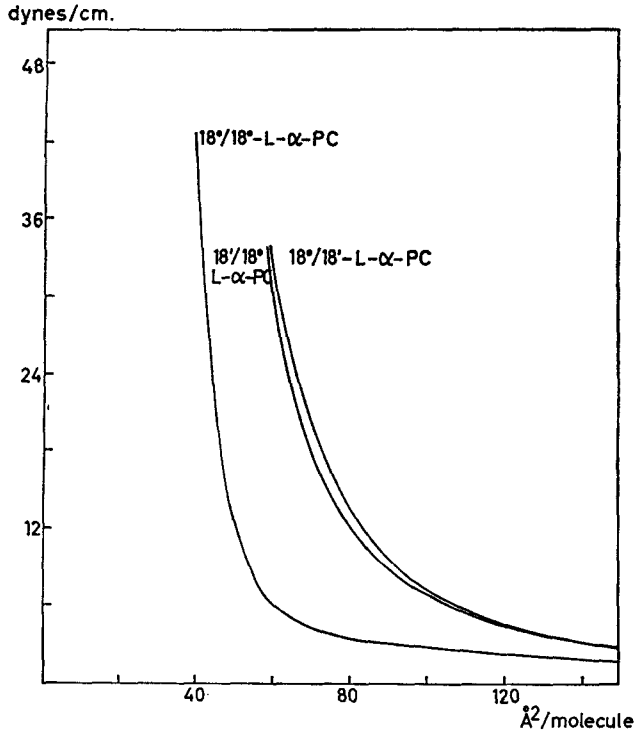

FIG. 4. Force-area characteristics of a saturated lecithin (PC) (stearic acid) and of two lecithins containing one mole of stearic acid and one mole of oleic acid in different positions (compare Table I and Table II).

Monolayers of Structurally Isomeric Mixed-acid Phosphatides

The significance of the asymmetrical distribution of fatty acids in naturally occurring lecithins viz. the preferential location of saturated acyl groups in the γ-position, and of the unsaturated ones in the β-position, has not yet been elucidated. Force-area curves of structurally isomeric mixed-acid phosphatides, having the two dissimilar fatty acids attached in different positions, appear to be identical.

Examples presented in Figs. 4 and 5 show that the curves of two mixed-acid L-α-lecithins and two L-α-phosphatidylethanolamines respectively,

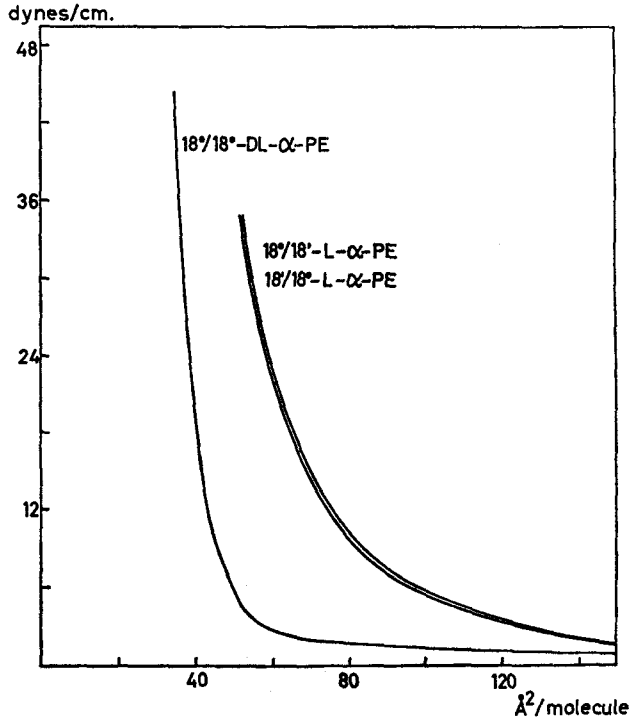

FIG. 5. Force-area characteristics of a saturated (stearic acid) phosphatidyl ethanolamine (PE) and of two phosphatidylethanolamines containing one mole of stearic acid and one mole of oleic acid in different positions (compare Table III).

composed of one oleic and one stearic acid chain, are equally situated for each group of phosphatides. This is true also when two saturated fatty acids of unequal chain length are located in different positions (Fig. 6). These findings do not preclude the asymmetrical distribution of fatty acids in the phosphatides from having a structural significance, since in membranes phosphatides are associated with other lipids and with proteins, forming complicated complexes. On the other hand the possibility exists that this asymmetrical fatty acid distribution is merely a result of the biosynthetic pathways of phosphatides. Generally, our knowledge of the fundamental background of the specific configuration in natural compounds for example L-amino acids, is rather restricted.

Furthermore, the curves of the expanded films of D- and L- isomers of lecithins composed with stearic and lauric acid, were found to be identical, while the DL compounds gave slightly decreased areas per molecule (Fig. 6).

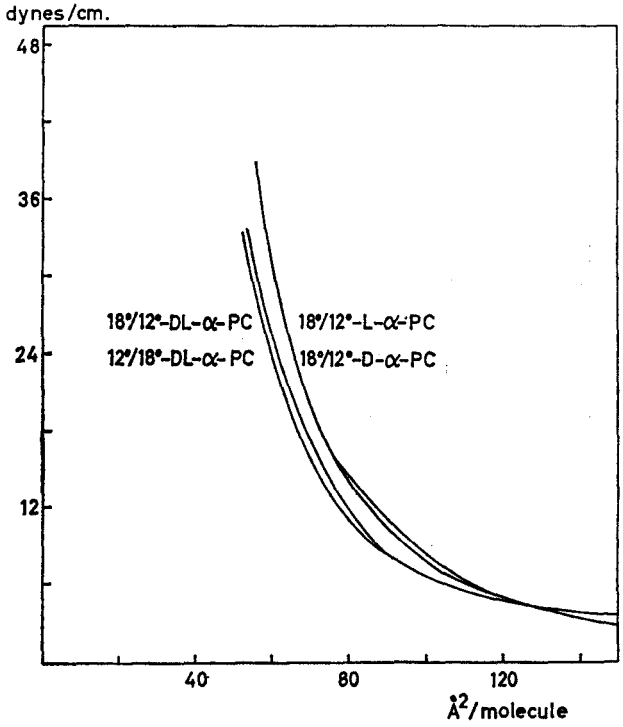

FIG 6. Force-area characteristics of D, L and DL isomers of α-lecithins (pH 7·4) The two DL compounds contain stearic acid and lauric acid in different molecular positions (compare Table II).

Mixed Films of Cholesterol and Synthetic Lecithins

Various investigations (de Bernard, 1958; Dervichian, 1958; Finean, 1953, 1961; Willmer, 1961) have emphasised the importance of cholesterol -phosphatide interaction because of the occurrence of such complexes in living tissues, such as myelin sheaths. Since our analyses of red cell membranes from different species of mammals (de Gier and van Deenen, 1961) demonstrated, that the molecular proportion of cholesterol to phosphatides is approximately 1 : 1, mixed films were prepared of cholesterol and various lecithins in this ratio. Fig. 7 indicates that even the already condensed film of L-α-(distearoyl)lecithins undergoes a small shift in the presence of cholesterol. Assuming that the molecular area of cholesterol, being about 35 Å² per molecule at 33 dynes/cm., is not changed in the mixed film, it can be derived from the mixed film area (37 Å² per molecule), that the molecular area of lecithin in the mixed film was 39 Å² per molecule at 33 dynes/cm., whereas L-α(distearoyl)lecithin, when spread alone, had a value of 41·5 Å² at this pressure. Presumably the molecular area of L-α-(distearoyl)lecithin in the mixed film with cholesterol is no longer attributed to the dimensions of the choline moiety, as was supposed to be likely in the single lecithin film.

The effect of cholesterol on the expanded film of a lecithin containing one oleic acid constituent, however, is quantitatively much more pronounced (Fig. 8). The presence of an equimolar amount of cholesterol resulted in a significantly more condensed film giving a decrease of the molecular area per lecithin molecule, when compared with the spreading characteristics of the lecithin alone. Taking into account the above presumption, the molecular area of (γ-stearoyl-β-oleoyl)-L-α-lecithins in the mixed film was calculated to be 49 Å^2 per molecule, while the lecithin alone had a value of 59 Å^2 at the same pressure of 33 dynes/cm. This

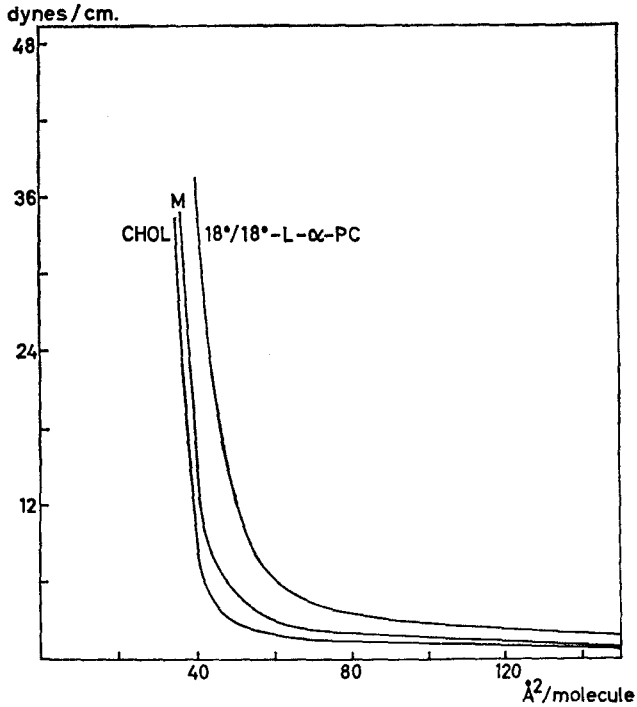

FIG. 7. Force-area characteristics of cholesterol (Chol), L-α-[distearoyl]lecithin (PC) and a mixed film (M) of both compounds in equimolar amounts.

effect was also met in experiments on mixed films of cholesterol with L-α-lecithins containing two unsaturated fatty acid chains. Apparently the presence of cholesterol in the monolayers of these lecithins causes a re-orientation of the unsaturated fatty acids, thereby partly abolishing the expanded character of lecithin films attributed to unsaturated fatty acid constituents. Endorsing previous reports on the importance of cholesterol-phosphatide complexes (Dervichian, 1958; Finean, 1953), the observed effects may be of interest for the understanding of the intermolecular arrangements of the phosphatide and the cholesterol at biological interfaces, e.g. red cell membranes.

Comments

Thus it can be concluded that the interfacial behaviour of various types of glycerol phosphatides is a function of the nature of the apolar moiety. Because of the significant differences brought about in the film characteristics of the monolayers of phosphatides by variation of the chain length and particularly by unsaturation of the fatty acid constituents, it may be imagined that the fatty acid composition of membranous phosphatides plays an important part in the fine-architecture and properties of biomembranes. Studies from our laboratory have demonstrated that the lipid characteristics of red cell membranes from various mammals differ greatly, and their differences could be tentatively related to variations in the permeability behaviour of the membranes. It is not yet advisable

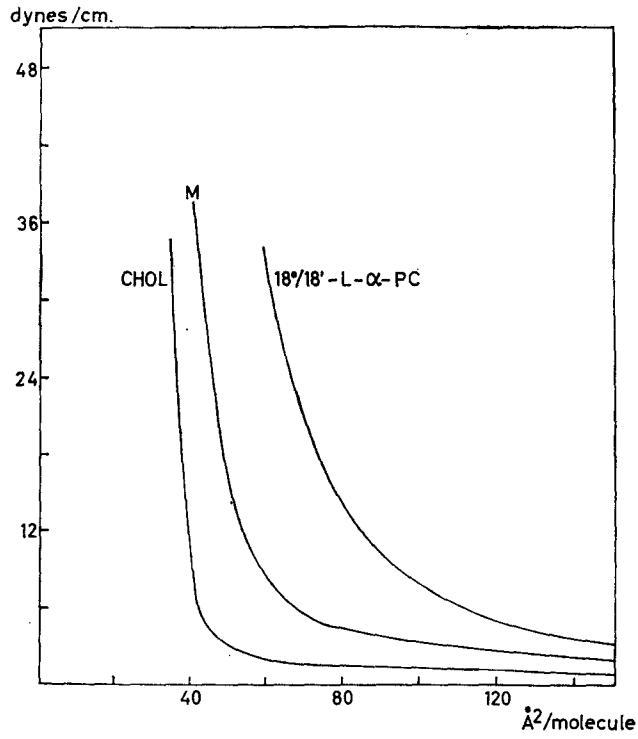

FIG. 8. Force-area characteristics of cholesterol (Chol), (γ-stearoyl-β-oleoyl)-L-α-lecithin (PC) and a mixed film (M)of both compounds in equimolar amounts.

to attempt an interpretation of this relationship between lipid composition and biophysical properties of membranes on the basis of the results presented on phosphatide monolayers. Apart from the fact that a comparison of data obtained at an air/water interface, with the complex framework of a cell membrane separating two aqueous phases, is always imperfect, the variations in the lipid composition of the red cell membranes

are complicated. As well as the variation in fatty acid composition, the sphingomyelin content and the amount of plasmalogen also differed greatly; data on monolayers of the latter phosphatides are not available at present. But the results obtained so far, prompt us to consider further attempts, to formulate the structure and the functional mechanism of biomembranes, and to study the influence of variations of fatty acid composition of the membranous constituents.

The better understanding of the relationship between monolayer properties and fatty acid composition in the phosphatides, will probably facilitate further studies on more complex systems in which phosphatides are combined with other lipids and proteins. In particular, extension of the experiments on mixed films of several types of phosphatides with cholesterol may supply further information about the precise orientation of these molecules. It should also help in elucidating the stabilising effect in biomembranes, such as in red blood cells, produced by the condensation of cholesterol with phosphatides. Furthermore, the fresh information obtained on monolayers of defined phosphatides, together with the new analytical values of the lipid composition of red cell membranes will stimulate the investigation of monolayers of lipids extracted from red cells of various animal species, to ascertain whether the earlier work of Gorter and Grendel (1925, 1926), leading to the bimolecular lipid theory, is consistent with present data.

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