Studies on Phospholipid Mono- and Bilayers

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Abstract

Lipids play a fundamental part in the lipid-protein network of biological membranes. Some of the variations in biological membranes may be brought about by differences in the chemical structure of the lipid constituents. A study was made by the interfacial force-area characteristics of saturated and highly unsaturated phospholipids and lyso phosphatidyl compounds. The action of some of these phospholipids on lipid bilayers has also been studied. Comparisons were made between the interfacial behavior of individual phospholipid species which were chemically synthesized and phospholipids from natural sources. The influence of diets on the force-area characteristics of liver lecithins has been studied. From studies of mixed monolayers of cholesterol and phospholipids it was found that the mean area per molecule in mixed films of cholesterol with (1,2-distearoyl)-3-lecithin and (1,2-didecanoyl)-3-lecithin at 22C followed practically the additivity rule. A condensing effect of cholesterol was evident with films of (1-stearoyl-2-lauroyl)-3-lecithin, (1,2-ditetradecanoyl)-3-lecithin, (1-stearoyl-2-oleoyl)-3-lecithin, and the corresponding ethanolamine analogue as well as with (1,2-dioleoyl)-3-lecithin at 22C. At 5C the condensation effect with (1,2-ditetradecanoyl)-3-lecithin was much reduced.

The expanded films of synthetic lecithins and phosphatidylethanolamines containing linoleic or linolenic acid showed no appreciable condensation effects with cholesterol. The behavior of the mixed-phospholipid films is governed by a number of factors, including van der Waals interactions, configurational entropy effects, and alterations in the structure of water adjacent to the monolayers. These factors depend on chain length and degree of unsaturation.

Polyene antibiotics are found to lyse fungi, protozoa, and erythrocytes, but bacteria protoplasts and blue-green algae are not. Cholesterol as well as lecithin were found by some authors to reduce the effective polyene antibiotic concentration. It therefore seemed desirable to determine whether polyene antibiotics can interact with lipids other than sterols. Filipin, nystatin, amphotericin B, etruscomycin, and pimaricin readily penetrate monolayers of cholesterol and ergosterol at initial surface pressures greater than the collapse pressure of the antibiotics. Under the same conditions there was essentially no interaction with a variety of pure synthetic phospholipids unless sterol was present. Filipin did not penetrate monolayers prepared from polyene-insensitive bacteria. The increase in surface pressure of mixed films of phospholipid and cholesterol after the injection of filipin was highly dependent on the relative quantity of sterol as well as on the molar ratio lipid-polyene antibiotic. From the results of mono- and bilayer experiments which are in good agreement with the physiological experiments, it is concluded that cholesterol is a necessary membrane constituent for the polyene antibiotic action.

Introduction

LIPIDS ARE KNOWN to be main membrane constituents of a lipid-protein network. It is well known that natural phospholipids contain a great variety of fatty acid constituents. The effect of phospholipid composition, also of chain length and unsaturation of the paraffinic chains can bring about some of the variations in the properties of biological membranes (1). Some of these effects can be studied at the air-water interface since well-defined phospholipids have become available by chemical synthesis in the author's laboratory by the groups of de Haas, Bonsen, and Slotboom (2).

Force-area characteristics of different saturated phospholipids are shown in Fig. 1. It can be seen that the area per molecule increases by decreasing the chain length. Diheptanoyl lecithin monolayers however became unstable and water-soluble at low surface pressures. In Fig. 2 the force (π)-area (A) characteristics of different unsaturated lecithin are compiled. According to expectation, the force-area (π-A)-curves exhibit a shift from a condensed to an expanded type of film when the number of unsaturated bonds in the paraffinic chains increases. The highest area increase is acquired by the introduction of the first double bond. The same type of expansion can be shown for the ethanolamine derivatives (3).

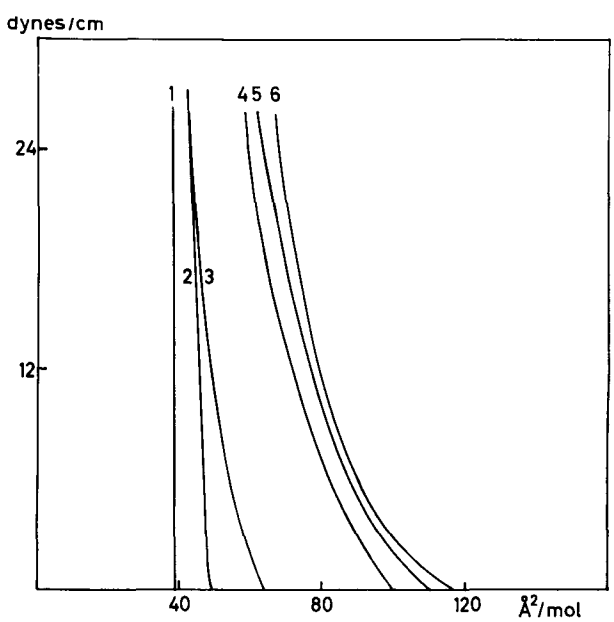

FIG. 1. Force-area characteristics of various saturated phospholipids and cholesterol. 1) cholesterol, 2) (1,2-dipalmitoyl)-3-phosphatidylethanolamine, 3) (1,2-distearoyl)-3-lecithin, 4) (1,2-ditetradecanoyl)-3-lecithin, 5) (1-stearoyl-2-lauroyl)-3-lecithin, 6) (1,2-didecanoyl)-3-lecithin.

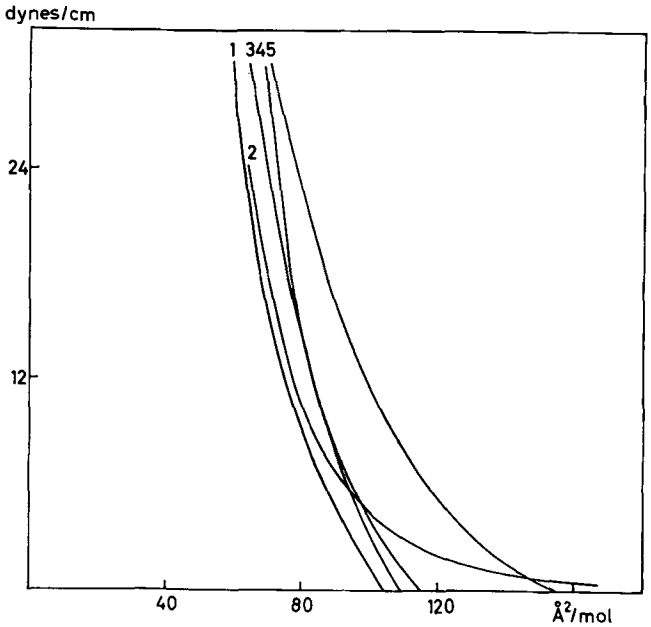

FIG. 2. Force-area characteristics of various unsaturated lecithins (phosphatidylcholines). 1) (1-stearoyl-2-oleoyl)-3-lecithin, 2) (1-butyryl-2-oleoyl)-3-lecithin, 3) (1-linoleoyl-2-stearoyl)-3-lecithin, 4) (1,2-dioleoyl)-3-lecithin, 5) (1,2-dilinoleoyl)-3-lecithin.

Phospholipids of natural origin generally provide a liquid expanded type of film although some differences may exist, depending on their source.

Environmentally- and dietary-induced alterations in the fatty acid composition of membrane lipids may be regulated by living organisms in such a manner that the physical behavior is preserved to a great extent. The question arises: what can be illustrated, for example, with lecithins isolated from the liver of rats kept on a fat-free diet which is essential fatty acid-deficient, coconut diet which is highly saturated, and corn-oil diet which is highly unsaturated? These lecithins show big shifts in the fatty acid composition (4,5).

In liver lecithin of essential fatty acid-deficient rats only a little arachidonic acid is found, but high percentages of eicosatrienoic and oleic acid occur. Surprisingly, only small shifts in the force-area plots at the air-water interface appear (Fig. 3). Detailed chemical analysis by van Golde showed that this effect may be attributed to a) a replacement of fatty acids of related unsaturation and b) shifts in the relative proportions of several molecular lecithin species (5).

Also interesting are the observations of Meyer and Bloch (6) on the change in fatty acid composition of phospholipids from yeast grown aerobically and anaerobically. Under anaerobic conditions the absence

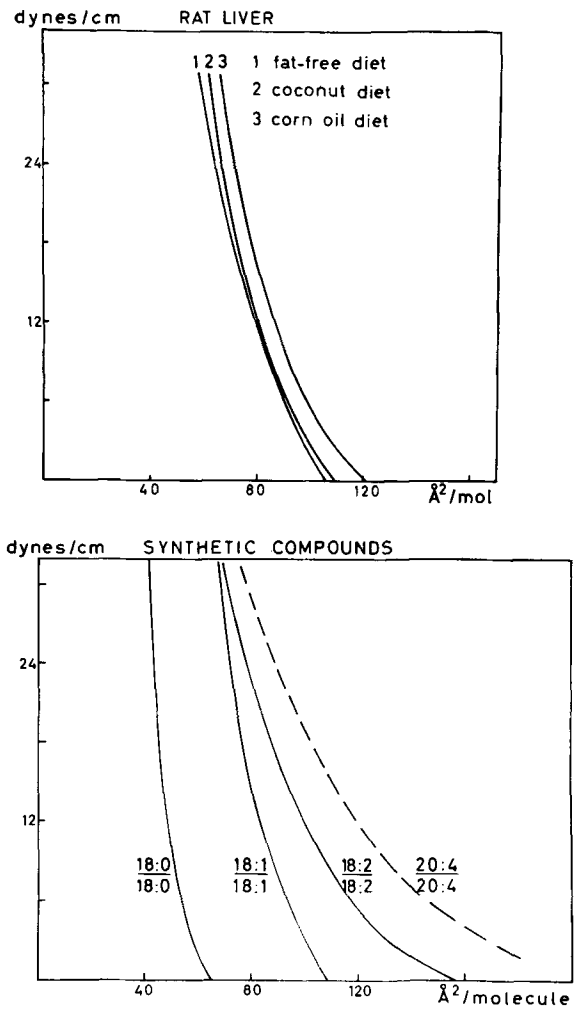

FIG. 3. Comparison of the force-area characteristics of monolayers from lecithins isolated from liver of rats kept on 1) fat-free diet, 2) coconut diet, and 3) corn-oil diet.

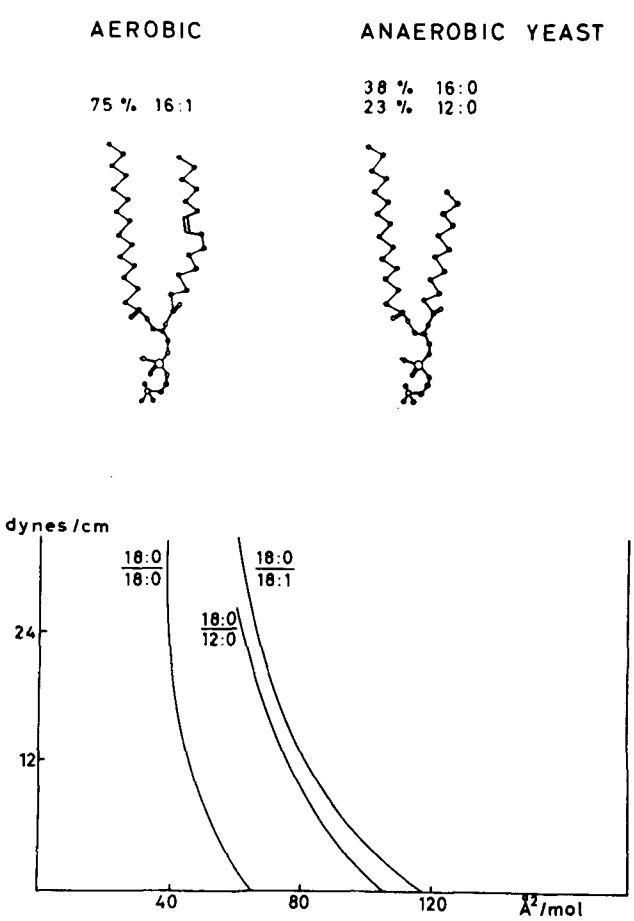

FIG. 4. Comparison of the force-area characteristics of monolayers from synthetic lecithins, viz., (1,2-distearoyl)-3-lecithin (18:0/18:0); (1-stearoyl-2-lauroyl)-3-lecithin (18:0/12:0); (1-stearoyl-2-oleoyl)-3-lecithin (18:0/18:1). (Meyer and Bloch, Ref. 6).

of unsaturation is compensated for by the appearance of saturated fatty acids with shorter chain-length as capric (C_{10}), lauric (C_{12}), and myristic (C_{14}) acid. It is striking that the unsaturated as well as the saturated short-chains preferentially occupy the two-ester position. By comparing the π-A characteristics of (1-stearoyl-2-oleoyl)-3-lecithin and (1-stearoyl-2-lauroyl)-3-lecithin (Fig. 4), it can be suggested that this organism made a successful attempt to produce a phospholipid with the same physico-chemical properties as synthesized under aerobic conditions.

Another example is the appearance of cyclopropane fatty acids. In several species of bacteria a phosphatidyl ethanolamine, isolated by Law (7,8) from *E. coli*, contained 40% of C_{17} and C_{19} cyclopropane fatty acids located with about 10% unsaturated fatty acids at the 2-ester position while the straight saturated chains occupied the 1-ester position (Fig. 5). The cyclopropane containing phosphatidyl ethanolamine preparation revealed a π-A curve, hardly distinguishable from the (1-stearoyl-2-oleoyl)-3-phosphatidyl ethanolamine curve. It is known that the presence of isomethyl and cyclopropane groups can reduce the total London-van der Waals dispersion forces (9). Thus, in membranes practically devoid of unsaturated fatty acid residues, the branched fatty acids may take over the function for regulating the distance between the paraffinic chains of lipids in the membrane.

Cholesterol is known to be an important membrane constituent. With the aid of monomolecular layers interactions of phospholipids and cholesterol (3) were studied. It is well known that the physical properties of monomolecular layers formed by mixed lipids may be quite different from the films of the single lipid components. In this way the interactions between several classes, e.g., fatty acids and triglycerides, fatty acids and cholesterol, and glycerides and phospholipids have been established by Dervichian and De Bernard (10), Adam and Jessop (11), Desnuelle, Molines, and Dervichian (12) respectively.

In regard to the influence of cholesterol on the behavior of lecithin in biological systems, Leathes (13) has already shown that the presence of cholesterol, which is assumed to have a practically invariant molecular cross-section, causes a decrease of the apparent area occupied by lecithin molecules. The study of this co-called condensing effect of cholesterol was extended to mixtures of egg lecithin and cholesterol by De Bernard (14), who determined the variation of the mean molecular area at constant sur-

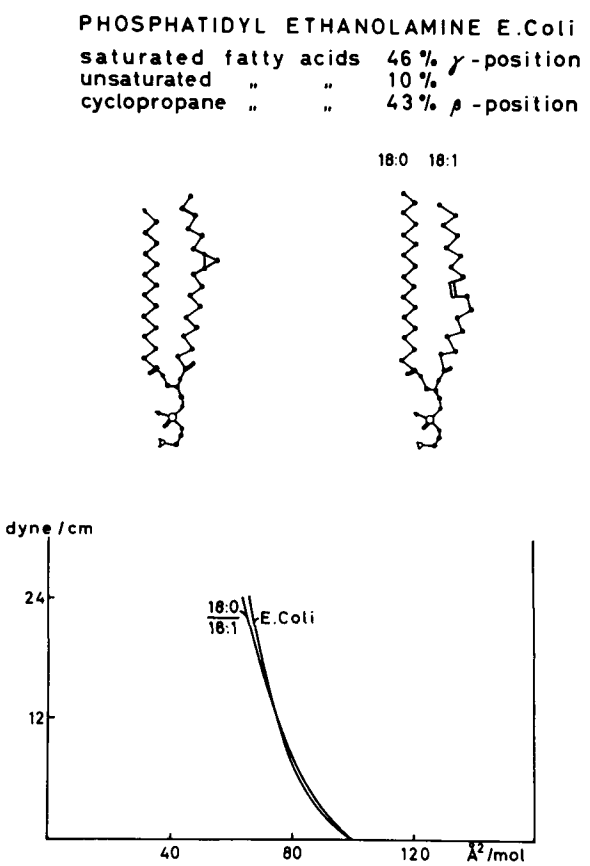

FIG. 5. Force-area characteristics of films from (1-stearoyl-2-oleoyl)-3-phosphatidylethanolamine, isolated by Law from *E. coli*. The latter compound contained 43% of palmitic acid located at the 1-position and 44% of C_{17} and C_{19} cyclopropane fatty acids at the 2-position. (J. Law, see References.)

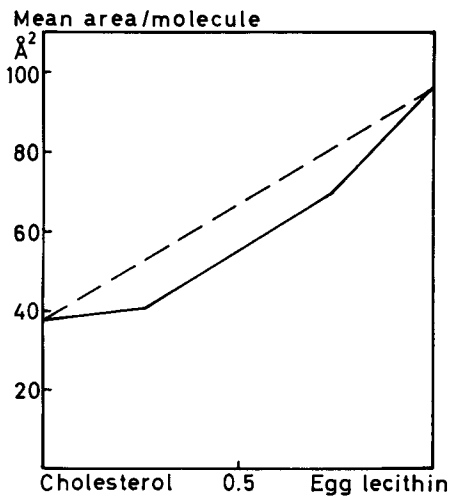

FIG. 6. Variations of the mean molecular area, as a function of the composition, for mixed monolayers of cholesterol and egg-lecithin as reproduced from the paper of de Bernard (14). The straight dotted line represents the simple additivity rule of the molecular areas of cholesterol and egg-lecithin.

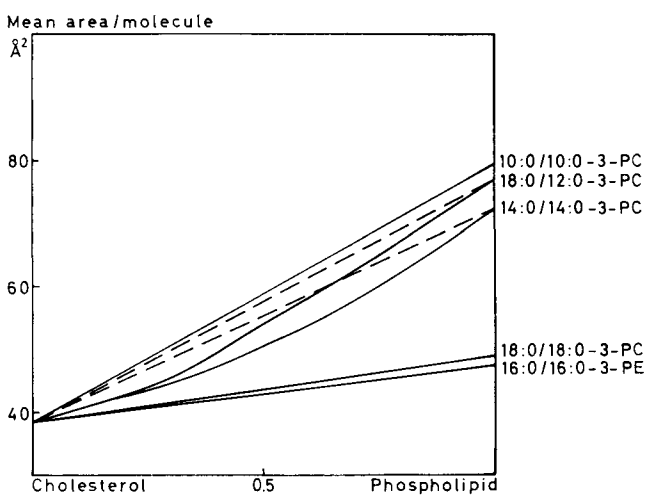

FIG. 7. Variation of the mean molecular area, as a function of the composition, for mixed monolayers of cholesterol and respectively (1,2-dedecanoyl)-3-lecithin, (1-stearoyl-2-lauroyl)-3-lecithin, (1,2-ditetradecanoyl)-3-lecithin, (1,2-distearoyl)-3-lecithin, (1,2-dipalmitoyl)-3-phosphatidyl ethanolamine. PC, phosphatidyl cholines; PE, phosphatidyl ethanolamines.

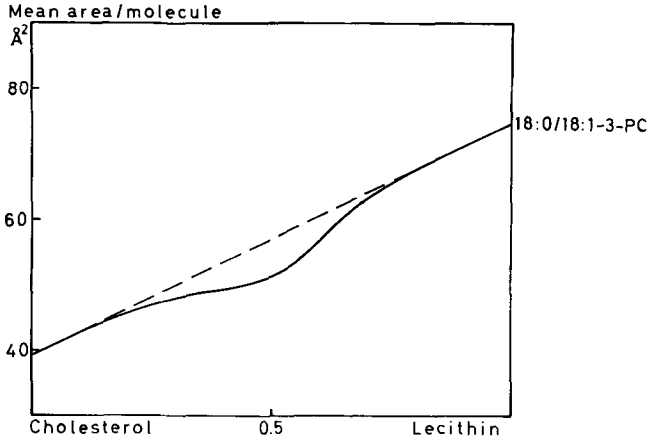

FIG. 8. Variation of the mean molecular area, as a function of the composition, for mixed monolayers of cholesterol on (1-stearoyl-2-oleoyl)-3-lecithin (18:0/18:1–3–PC).

face pressure as a function of the mole ratios of lecithin and cholesterol in mixed monolayers at the air-water interface. Because of the abundance of cholesterol in a number of cell membranes, this phenomenon was considered to have biological relevance. A natural phospholipid, such as egg lecithin, is known to consist of a great number of molecular species differing with regard to the composition of their fatty acid constituents.

For mixed films, the surface pressure was plotted against the mean area per molecule, by which is understood the total area divided by the total number of cholesterol and phospholipid molecules at the air-water interface. For a quantitative evaluation of the effect of cholesterol, the variation of the mean molecular area at a given constant surface pressure was plotted as a function of the mole fraction. In the figures the mean molecular areas are shown at a pressure of 12 dynes/cm. In general, the same qualitative form for the curves was found at other surface pressures at the same temperature although of course some quantitative differences appear. Particularly, where the condensation effect occurs, the fractional condensation for a given mixture diminishes as the surface pressure increases.

In order to facilitate a comparison between the author's results on the monolayers of individual

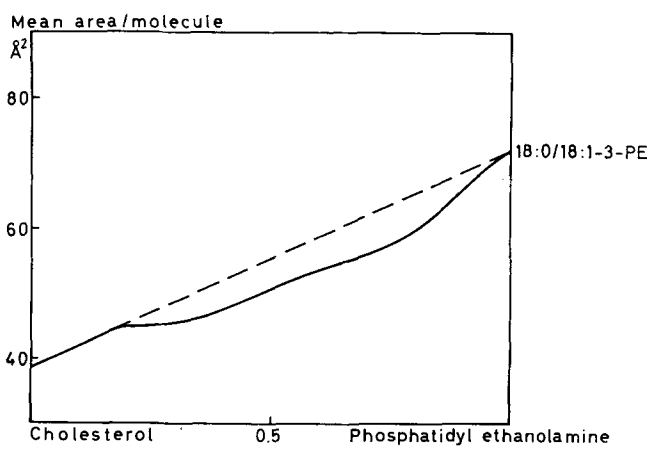

FIG. 9. Variation of the mean molecular area, as a function of the composition, for mixed monolayers of cholesterol and (1-stearoyl-2-oleoyl)-3-phosphatidyl ethanolamine (18:0/18:1–3–PE).

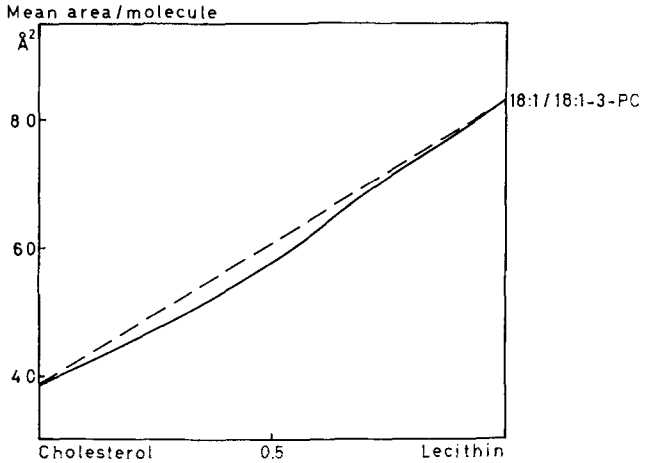

FIG. 10. Variation of the mean molecular area as a function of the composition, for mixed monolayers of cholesterol and (1,2-dioleoyl)-3-lecithin (18:1/18:1–3–PC).

phosphoglycerides and the observations obtained by De Bernard, the results of this investigator on egg lecithin are reproduced in Fig. 6. The curve shows that the mean molecular area of the lipid molecules in the mixed films falls below the proportionate average of the areas in the pure films (condensation). The curve shows two breaks at mixtures corresponding to cholesterol-egg lecithin ratios 3:1 and 1:3 respectively. The area of cholesterol undergoes little variation either on compression or on change in temperature (11,15), and De Bernard assumed that the reduction of the total area can be attributed to a decrease of the molecular area of egg lecithin.

Results on the various synthetic compounds in principle confirmed this condensing effect of cholesterol, but considerable differences were found to exist between phosphoglycerides containing different fatty acid chains (Fig. 7). A number of long-chain saturated synthetic phospholipids, when mixed with cholesterol in various proportions, did not reveal any measurable reduction of the cross-sectional area. The mean area per molecule in mixed films of cholesterol with (1,2-distearoyl)-3-lecithin and (1,2-dipalmitoyl)-3-phosphatidyl ethanolamine (16) practically follow the simple additivity rule as indicated by the straight lines which were obtained. These results are not surprising inasmuch as these saturated phospholipids form rather condensed films. The interactions between the molecules is strong in the pure phospholipid films, and the addition of cholesterol gives only small, barely measurable changes in packing.

When mixed films of cholesterol, with saturated lecithins which give expanded films, are considered, the picture is more confused. The (1,2-didecanoyl)-3-lecithin, which gives an expanded film, shows no condensation effect with cholesterol. Yet the (1,2-ditetradecanoyl)-3-lecithin and (1-stearoyl-2-lauroyl)-3-lecithin, which also give expanded films, exhibit marked condensation effects. In turning to the expanded films of unsaturated phosphoglycerides, it will be seen that cholesterol causes condensation with phospholipids containing oleic acid (Fig. 8). The film of (1-stearoyl-2-oleoyl)-3-lecithin shows a particularly striking condensation effect at a mole ratio of about 1:1 with much less interaction at low and high mole ratios. The corresponding ethanolamine compound also showed a significant condensation with

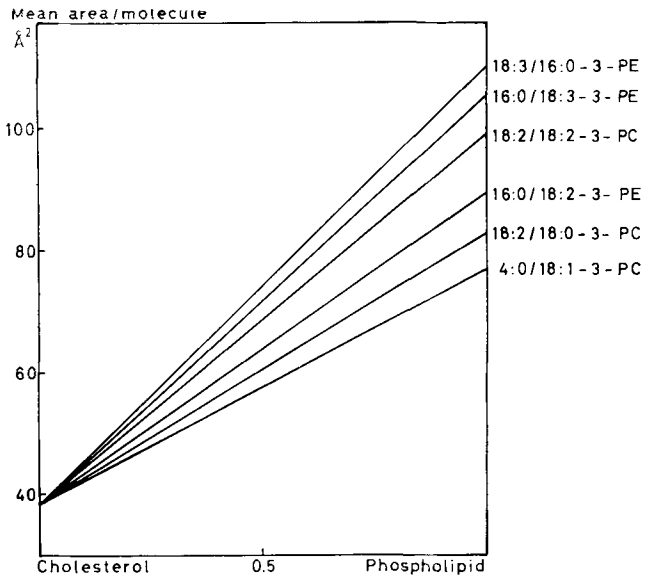

FIG. 11. Variation of the mean molecular area, as a function of the composition, for mixed monolayers of cholesterol and respectively (1-linolenoyl-2-palmitoyl)-3-phosphatidyl ethanolamine, (1-palmitoyl-2-linoleoyl)-3-phosphatidyl ethanolamine, (1,2-dilinoleoyl)-3-lecithin, (1-palmitoyl-2-linoleoyl)-3-lecithin, (1-butyryl-2-oleoyl)-3-lecithin.

cholesterol but over a more extended range of mole ratios (Fig. 9).

The effects observed for the oleoyl compounds show that two oleoyl chains in the phospholipid cause less condensation with cholesterol than one oleoyl chain with another saturated chain of similar length (Fig. 10). With (1-butyryl-2-oleoyl)-3-lecithin no condensation is observed, or with the phosphoglycerides containing the polyunsaturated linoleic or linolenic acids at 7°, 22°, or 37° (Fig. 11). In agreement with De Bernard, purified lipids from natural sources were found to be reduced in molecular area by cholesterol (Fig. 12). In contrast to the results of De Bernard on egg lecithin, there is no evidence in these experiments of phospholipid-cholesterol complexes at 1:3 and 3:1 mole ratios for human red-cell lecithin, chromatographically pure egg lecithin, human plasma sphingomyelin, or any of the pure synthetic phospholipids which were studied.

The data on the monolayer interactions of phospholipids and cholesterol show that these interactions are far from simple. There is no direct correlation, for example, between the state of expansion of the phospholipid and its interaction with cholesterol. Nor is there a specific interaction between cholesterol and the oleoyl chain of the phospholipids in view of the results for (1-butyryl-2-oleoyl)-3-lecithin and in view of the lack of interaction between cholesterol and lyso oleoyl lecithin (17).

Van der Waal's interactions will play a part in the condensation effect and may partially account for the fact that several saturated and oleoyl phosphoglycerides condense with cholesterol whereas the polyunsaturated molecules do not. The polyunsaturated molecules, at the temperatures under study, will be unable to approach closely to the cholesterol molecule because of the double bond-induced distortion of the chain, and the CH_2 interactions with cholesterol will be accordingly reduced (18).

Similarly, short-chain saturated phosphoglycerides will also undergo smaller van der Waals interactions and therefore be less able to give condensation effects.

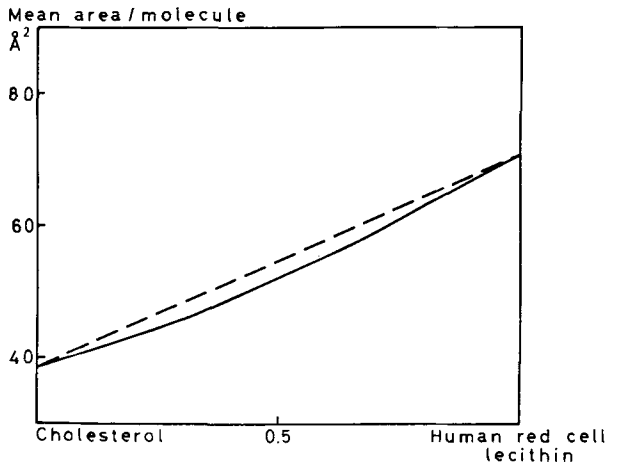

FIG. 12. Variation of the mean molecular area, as a function of the composition, for mixed monolayers of cholesterol and lecithin extracted from human red cells.

Further, information on the nature of the interactions can be obtained from the effect of temperature on the force-area curves, and on the condensation effect itself. By using the thermodynamic approach of Goodrich (19) for a mixture of equimolar amounts of cholesterol and (1,2-ditetradecanoyl)-3-lecithin at 12 dynes/cm, it can be calculated from the force-area curves of the pure and mixed films that the compression of the monolayer is accompanied by a positive change in heat content and that, over the temperature range which was studied, the excess entropy of mixing is positive ($T\Delta S$ = approx. 725 cal/mole), with an associated positive excess enthalpy of mixing (approx. 650 cal/mole).

Mixing phospholipid and cholesterol would be expected to lead to a reduction of the configurational entropy of the hydrocarbon chains. The positive excess entropy of mixing suggests that the expected negative contribution arising from chain-configurational factors is swamped by a substantial positive entropy contribution from another source. One such possible source of positive entropy would be net structure-breaking effects in the water adjacent to the monolayer. The condensation phenomenon, where it occurs, is thus seen as the result of a balance of effects, notably chain-configurational terms, van der Waals interaction, and water-structure changes. All three will depend on temperature, the length and shape of the chains, and their consequent ability to interact with each other, with cholesterol, and with the substrate water.

Although the validity of extrapolation from monolayer experiments at the air-water interface to complex biological structures is debatable, it is worth

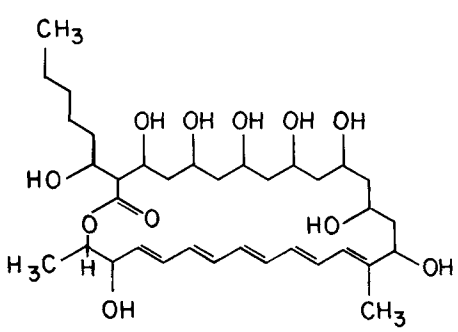

FIG. 13. The structure of filipin (Ceder and Ryhage, 1964).

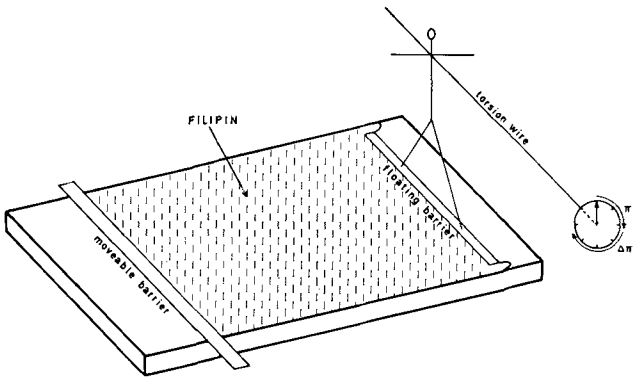

FIG. 14. Langmuir-Adam trough to measure the surface pressure increase, owing to the injection of polyene antibiotics in the subphase, at constant film area.

noting that several phospholipid species (e.g., those containing oleic acid), giving pronounced effects with sterol in monolayers, are indeed abundant in a number of membranes, such as myelin sheath and erythrocyte membranes.

Another topic studied with the aid of monolayers and bilayers was the selective toxicity of polyene antibiotics. Some polyene antibiotics, such as nystatin, pimaricin, and amphotericin B, are of clinical importance because of their fungocidic or fungostatic action. The polyene antibiotics are characterized by a large macrolide ring containing a conjugated chromophore. Fig. 13 shows the structure of the potent pentaene, filipin. Previous studies have already shown that polyene antibiotics alter permeability in sensitive fungi and thus lead to the loss of essential cytoplasmic constituents, which ultimately culminates in cell death (20–22). Subsequent investigations have led to the contention that the selective toxicity of the polyene antibiotics is attributable to the interaction with a component present only in the membrane of sensitive

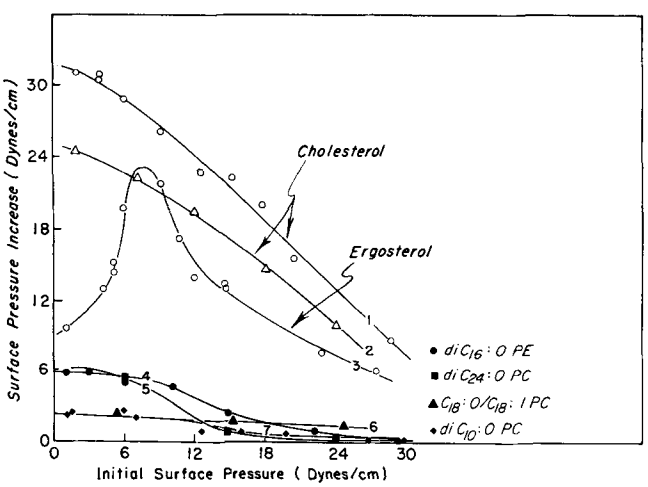

FIG. 15. Penetration of pure lipid monolayers by filipin. Monolayers were prepared with the indicated amounts (in micromoles) of lipids and compressed to give the initial surface pressures indicated on the abscissa. Then $6.38.10^{-3}$ μmole of filipin was injected underneath (except for curve 2, where $3.2.10^{-3}$ μmole of filipin was used). Curves 1 and 2, cholesterol 0.054; Curve 3, ergosterol 0.056; Curve 4, (1,2-dipalmitoyl)-3-phosphatidyl ethanolamine (di C_{16}:0 PE), 0.055; Curve 5, (1,2-ditetracosanoyl)-3-lecithin (di C_{24}:0 PC), 0.052; Curve 6, (1-stearoyl-2-oleoyl)-3-lecithin (C_{18}:0/18:1 PC), 0.038; Curve 7, (1,2-didecanoyl)-3-lecithin (di C_{10}:0 PC), 0.049.

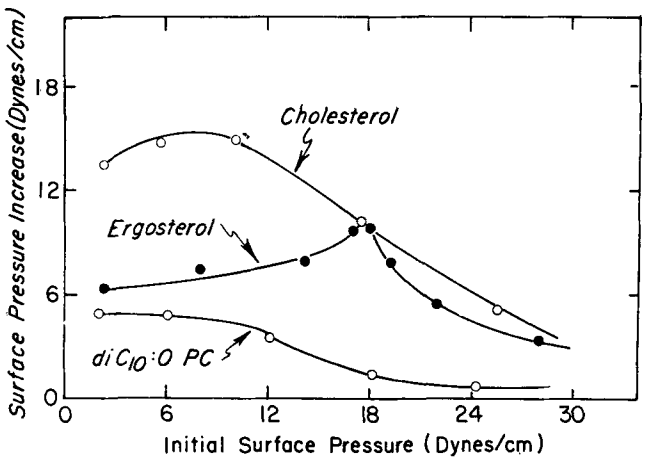

FIG. 16. Penetration of pure lipid monolayer by nystatin. The procedure was similar to that described for Fig. 3 except that 6.38 10^{-3} μmole of nystatin was injected underneath monolayers prepared with the following amounts (in micromoles) of lipids. Curve 1: cholesterol, 0.054; Curve 2: ergosterol, 0.056; Curve 3: (1,2-didecanoyl)-3-lecithin (di C_{10}:0 PC), 0.049.

organisms. This hypothesis was based on the observation that whole cells and isolated membrane fractions of polyene-sensitive fungi bind appreciable quantities of nystatin, amphotericin B, and filipin whereas cells, protoplasts, and membrane fractions of polyene-sensitive bacteria do not bind any of the antibiotics (23,24).

Sterols, as well as lecithin, satisfy the requirement of a membrane component present only in polyene-sensitive organisms since Goldfine and Ellis (25) showed that bacteria, in general, are not able to synthesize choline derivatives. Several reports have, in fact, indicated that polyene activity may be nullified by the addition of sterols, as well as by the addition of phosphatides, to the culture medium. A reduction of the effective antibiotic concentration owing to complex formation (26–28) is suggested.

To study the interaction of polyene antibiotics with different membrane components, first the monolayer technique (29) at the air-water interface was used (Fig. 14). The monolayer is compressed in a Langmuir-Adam trough to a certain pressure (π), after which the water-soluble antibiotic is injected under the film and, at constant film area, the pressure increase ($\Delta\pi$) is measured. It is also possible to measure the area increase at constant pressure by displacement of the movable barrier. The term monolayer penetration is used to denote interaction of a soluble surface-active compound with an insoluble material spread at the phase boundary. The polyene antibiotics and derivatives, in general are water-soluble at the concentrations which are used and, insofar as these substances are oriented at the air-water interface, they have collapse pressures of about 14 dynes/cm.

These observed collapse pressures are operationally significant in regard to all subsequent experiments concerning monolayer penetration. If injection of a polyene or derivative into the aqueous subphase produces an increased pressure in monolayers which have been previously compressed to surface pressures at, or above, the collapse pressure of the antibiotic, then this increase is interpreted as indicating specific interaction with some constituent of the monolayer.

Penetration of pure lipid monolayers by filipin showed that this antibiotic gave an extremely

strong interaction with cholesterol (Fig. 15). At an initial surface pressure of 2 dynes/cm the pressure increase owing to the antibiotic was 31 dynes/cm. The pressure change was less at higher initial pressures but was still significant at pressures much greater than the collapse pressure of the antibiotic. The magnitude of these pressure changes was dependent upon the initial antibiotic concentration. Filipin also interacts with ergosterol monolayers although the effect was less when compared with cholesterol, especially at low initial pressures. The interaction of a variety of phospholipids with filipin only was very limited.

The less potent antibiotic nystatin was also able to penetrate monolayers of cholesterol and ergosterol but to a smaller extent than did equimolar quantities of filipin (Fig. 16). The penetration of phospholipid films was again small.

Also for amphotericin B, etruscomycin, and pimaricin the same specificity pattern could be shown, that is, these antibiotics also preferentially interacted with sterol monolayers (30). At initial surface pressures above 14 dynes/cm, the relative order of cholesterol film penetration is filipin > etruscomycin > amphotericin B > pimaricin > nystatin. This is exactly the order in which the polyenes are able to lyse mammalian erythrocytes or fungal protoplasts. Filipin causes the most rapid and extensive membrane damage, and nystatin the least. This correlation between sterol monolayer penetration and physiological effects suggests that the polyenes have different affinities for sterol. A similar correlation exists with the modified filipin derivatives. Filipin derivatives, such as perhydrofilipin, saponified filipin, or irradiated filipin, which have little or no biological activity, do not produce "pits" in lipid dispersions (31) and also are less able to interact with cholesterol monolayers when compared with the parent antibiotic (11).

Bacterial protoplasts are not lysed by polyene antibiotics. This observation is in perfect accord with the present finding that filipin cannot penetrate a monolayer prepared from a lipid extract of bacteria (Fig. 17). But filipin does interact with monolayers of lipid extracts of beef erythrocytes which can be lysed. This interaction was most probably owing to the presence of cholesterol because filipin was able to interact with the neutral lipid fraction of the erythrocytes, which consists primarily of cholesterol, but gave no pressure increase with the phospholipid, which was obtained from the same lipid extract.

Although sterols may be responsible for the interaction of polyene antibiotics with cell membranes, Kinsky et al. (32) found that mitochondria of *Neurospora crassa,* which contain only 4% ergosterol, are not affected by filipin. Therefore not only the presence of sterols but also the phospholipid/sterol ratio may be important in determining whether or not a membrane is sensitive to polyenes. Consistent with the above conclusions, filipin produces a much smaller increase in the surface pressure of mixed monolayers containing cholesterol and lecithin (30). In mixed cholesterol-lecithin monolayers containing 20% of lecithin, the effect of filipin is decreased 55% at an initial film pressure of 18 dynes/cm.

In the above experiments, interaction between lipids and polyene antibiotics was examined by measuring the increase in surface pressure that occurred when the monolayer was kept at constant area. The change in area necessary to maintain the initial surface pressure was only 1–5% for steroids as well as for phospholipids when filipin or nystatin was used in a 6.10^{-9} mole concentration (29). The physical basis for the polene-steroid interaction is not yet shown. The surface pressure increase may be caused by actual penetration of the polyene into the monolayers (in that case only a few polyene molecules would need to penetrate) and/or may reflect accumulation of the antibiotic directly underneath the monolayer with a subsequent spacial reorientation of the sterol molecules. The results of polyene antibiotics on bimolecular layers are in good agreement with the monolayer results and biological effects, as seen from the data in Table I (33).

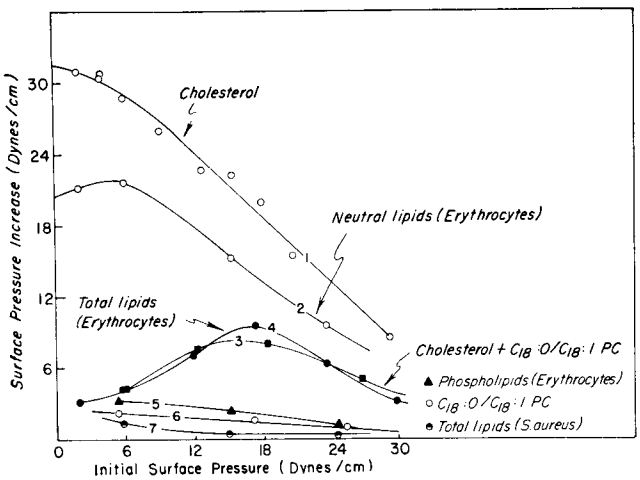

FIG. 17. Penetration of mixed lipid monolayers. The procedure was similar to that described for Fig. 3 except that $6.38.10^{-3}$ μmole of filipin was injected underneath monolayers prepared with the indicated amounts (in micrograms) of lipids. Curve 1: cholesterol, 21; Curve 2: neutral lipids from erythrocytes, 21 to 30; Curve 3: equimolar mixture of cholesterol and C_{18}:0/18:1 PC, 35; Curve 4: total lipids from erythrocytes, 45; Curve 5: phospholipids from erythrocytes, 36; Curve 6: C_{18}:0/18:1 PC, 30; Curve 7: total lipids from *S. aureus,* 33.

Filipin and nystatin had no effect on the stability of films containing only lecithin at a concentration of 4.10^{-5} M. But at the same concentration these antibiotics were able to disrupt films prepared from an equimolar solution of lecithin and cholesterol. As discussed above, the phospholipid/sterol ratio probably determines whether or not a membrane is sensitive to polyenes. This conclusion is supported by the observation that a 10-fold increase in lecithin/cholesterol ratio results in a bilayer with longer survival times in the presence of filipin. Perhydrofilipin which has approximately 1/100 of the potency of filipin was without effect on bilayer films, which are rapidly disrupted by polyene antibiotics. The bilayer data suggest again that the differences among the polyenes can be revealed to affiinity for sterol.

TABLE I

Effect of polyene antibiotics on the stability of lipid bilayers.

Lipid	Addition	Concentration	Survival Time Film (Min)
Lecithin	None		>60
	Filipin	4×10^{-5}	>60
	Nystatin	4×10^{-5}	>60
	Perhydrofilipin	4×10^{-5}	>60
Cholesterol-Lecithin 1:1 molar	Filipin	4×10^{-5}	< 1
		4×10^{-6}	1.5 (1–3)
		4×10^{-7}	>60
	Nystatin	4×10^{-5}	1.5 (1–2)
		4×10^{-6}	8 (5–15)
		4×10^{-7}	>60
	Perhydrofilipin	4×10^{-5}	>60
	Filipin	4×10^{-5}	1.5 (1–2)
1:10 molar		4×10^{-6}	20—>60

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Discussion

DR. CHARLES PAK (National Institutes of Health, Bethesda, Maryland): I would like to make two comments. One, we have observed the condensing effect of stearyl alcohol on monooctadecyl phosphate monofilm at the air-water interface. The second point I would like to make pertains to your use of the total change in surface pressure to determine the interaction between filipin and cholesterol. Your data are so clear-cut that my comments probably will not alter your conclusions. But I would like to point out that at pressures below 14.9 dyne/cm (i.e., the collapse pressure for filipin monofilm), you will be encountering the problem of "nonspecific" interaction. If one injects a sufficient quantity of filipin to the subsolution below a monomolecular film formed of the same fiilipin molecules, he will observe an increase in pressure or area of the film. This increase must be "nonspecific," since the interaction occurs between the same filipin molecules. The total change in pressure or area of cholesterol monolayer, shown on injection of filipin, thus reflects the combined influence of the nonspecific interaction and the specificity existing between the two dissimilar molecules. This is what Dr. Arnold and I were trying to point out on our presentation last Monday.

DR. WILLIAM W. DAVIS (Eli Lilly and Company, Indianapolis, Indiana): I wonder if you have actually spread filipin along with your cholesterol, in small amounts, to see whether the filipin would be retained in the film and to determine directly its area per molecule, from the spread mixed film? While it is relatively quite soluble it should, perhaps, with this interaction, remain in the film at intermediate pressures.

DR. DEMEL: Spreading of mixtures of cholesterol and filipin showed π-A curves practically identical to the curve for pure cholesterol. This indicates that at the air-water interface the cholesterol-filipin complex most probably is formed by filipin which is adsorbed underneath the cholesterol monolayer.

DR. MYSELS: It may be worth pointing out the simple fact that an additive such as filipin is strongly adsorbed on a film. This is sufficient to account for the fact that it promotes the rupture of the film. One should remember that most defoamers act precisely in this way by lowering locally the surface tension in the film which causes this part of the film to expand and therefore to thin down and thus to become increasingly unstable with respect to the action of van der Waals forces. The classical example is the effect of ether vapor upon a foam or even upon the surface of water in a shallow dish which may be seen to recede at the point where the vapors contact it until the bottom is exposed. One of the reasons why the surface tension is lowered more in the film than in the Plateau border surrounding it, is that the additive may dissolve (even if ever so little) in the oil phase and thus accumulate more rapidly in the film than in the border.

DR. DAVIS: Your experiments showing extensive complexation of the filipin toward the cholesterol film right up to the point of collapse might suggest that the filipin and cholesterol form a complex and do collapse together. Now, when you collapse a cholesterol film alone, it is quite irreversible in that it will not respread, but if cholesterol films are collapsed with some other agents, the collapsed film may respread on release of pressure. If a complex of cholesterol and filipin do crystallize and collapse together, I would not expect the complex to respread reversibly. If they collapse together without crystallization, they may respread. I think it would be significant to examine whether these mixed films do reversibly respread after collapse.

DR. DEMEL: No, we did not study the reversibility of the cholesterol-filipin films. It is a very good suggestion for the future.

DR. SMALL: One other thing that might be observed would be the viscosity of the film. As you know even though cholesterol has a very steep isotherm the film is liquid. Is the film solidified with filipin underneath?

DR. DEMEL: No, the cholesterol film remains liquid after injection of filipin underneath the monolayer.