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Mixed Monolayers Containing Phosphatidylcholine, Cholesterol, Oleic Acid, Mono- and Triolein

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The properties of egg phosphatidylcholine, cholesterol, monoolein, oleic acid, and triolein in mixed monolayers at the air-water interface have been studied. Phosphatidylcholine, monoolein, and oleic acid generate miscible two- and three-component systems in all combinations and proportions.

Triolein forms partially miscible films with all the other lipids studied. The partial molar area and the limiting solubility of triolein in the mixed monolayers were calculated. The results show that triolein has a relatively high two-dimensional miscibility in mixed monolayers with monoolein and oleic acid.

Cholesterol generates miscible monolayers with phosphatidylcholine, monoolein, and oleic acid in all proportions in both two-, three- and four-component systems. Cholesterol has a condensing effect on the liquid expanded monolayers, which is at maximum when the ratio between cholesterol and fatty acid chains reaches 1:1. Cholesterol forms partially miscible films with triolein.

The systems studied may provide a model for studying the molecular organization of the lipids in, *e.g.*, intestine emulsion particles, and serum lipoproteins, and the enzymatic attack on these.

Water insoluble amphiphilic lipids spread at air/water or oil/water interfaces forming mono- and multilayers with the polar group in contact with water and the hydrophobic part in air or oil. The dual properties of the amphiphilic lipids make them suitable as structural elements in, *e.g.*, biological membranes, serum lipoproteins and emulsion particles. The surface balance technique is a relevant and reliable method to study molecular interactions of lipids adequate in such structures.

The surface phase rule developed by Crisp [1] can be used to establish miscibility in multi-component monolayers. The surface area occupied by immiscible components will be the sum of the areas of the individual components. Deviations from this ideal behaviour, positive or negative, indicate miscibility, as well as some sort of molecular interaction. However, films with miscible components may also conform to an ideal behaviour but the phase rule states that the surface pressure must vary with the composition. If the components are immiscible the equilibrium spreading pressure is independent of composition and the component with the lower one is squeezed out of the monolayer.

Of the lipids studied in this paper phosphatidylcholine, cholesterol, monoolein and oleic acid have pronounced polar groups and form stable monolayers.[2–5] Triolein has weak polar groups and forms a film with low surface pressure. This lipid is ejected from a mixed monolayer with polar lipids at higher pressures.[6] Phosphatidylcholine and cholesterol are important components in biological membranes and mixed monolayers of these lipids have long been a popular membrane model system. The most evident feature of this system is the condensing effect of cholesterol on expanded phosphatidylcholines.[7] The same effect has also been found for mixed monolayers of cholesterol with oleic acid [8] and monoolein.[9]

Studies of the lipid-lipid interactions in serum lipoproteins with physicochemical methods [10,11] have led to a structural model where neutral lipids are assumed to be located in the core while the polar lipids build up the interfacial region. However, many details in this model regarding molecular interaction and organiza-

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tion have to be elucidated before this model is definite. Mixed monolayers are suitable model systems for studying interactions between relevant lipids.

The actual systems are also relevant for the conditions in the intestine where triglycerides are hydrolyzed to partial glycerides and fatty acids by lipases and where phosphatidylcholine and cholesterol are involved as natural emulsifiers. Similar systems are also common in food [12] and pharmaceutical [13] preparations.

MATERIALS AND METHODS

The cholesterol used was obtained from Merck AG and recrystallized three times from 1,2-dichlorethane. Triolein and oleic acid were purchased from Fluka AG and monoolein from California Corporation for Biochemical Research, Los Angeles. They were purified by Florisil column chromatography. The egg phosphatidylcholine employed was a highly purified product prepared in this laboratory.[14] The lipids were found to be chromatographically pure by silica gel TLC and GLC. All organic solvents were Merck *p.a.* The water used was quartz distilled.

Surface pressure-area determinations were made using a surface balance apparatus of the Wilhelmy-Dervichian type which was the same as previously used.[15] The subsolution consisted of 0.01 M NaCl containing 0.04 M phosphate buffer (pH 7.0) and with 0.001 % 4-methyl-2,6-*tert*-butylphenol (BHT) added as an antioxidant. The lipids were dissolved in chloroform–methanol–hexane 1:1:3 and added to the substrate surface with an "Agla" micrometer syringe. The monolayer was compressed at a rate of approximately 5 Å² min⁻¹ per molecule. All measurements were carried out at 25 °C.

RESULTS

Pure monolayers. The π-A isotherms of the individual lipids: Egg phosphatidylcholine (EPC), cholesterol (CH), monoolein (MO), oleic acid (OA) and triolein (TO) are shown in Fig. 1A. EPC, MO, OA and TO all generate liquid-expanded films with collapse point molecular areas of 57.5, 29.2, 25.5, and 102.5 Å²/molecule, respectively. The cholesterol films is of the condensed type with a collapse point area of 37.5 Å²/molecule. It is notable that TO has a much lower collapse pressure than the other lipids,

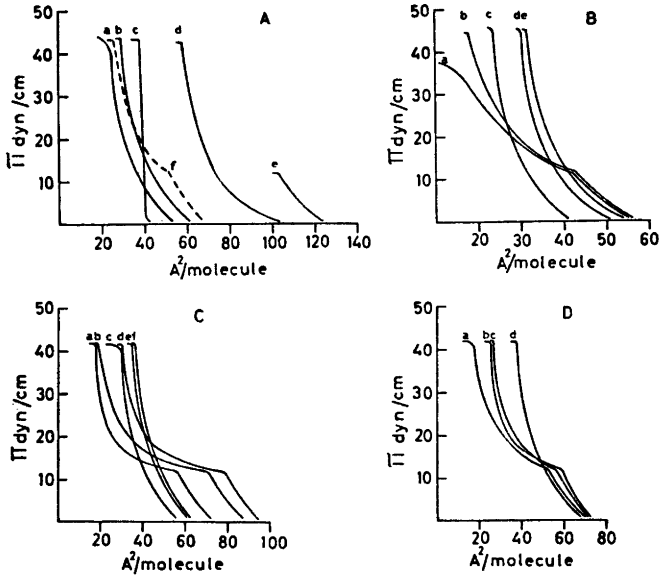

Fig. 1. π-A isotherms for: (A) OA (a), MO (b), CH (c), EPC (d), TO (e), and an equimolar mixture of all these (f, broken line); (B) two component systems with 1:1 molar mixtures of: OA–TO (a), MO–TO (b), OA–MO (c), OA–EPC (d), and MO–EPC (e); (C) three component systems with equimolar mixtures of: MO–CH–TO (a), OA–MO–TO (b), OA–MO–CH (c), OA–EPC–TO (d), OA–MO–EPC (e), and MO–EPC–CH (practically identical with OA–EPC–CH) (f); (D) four component systems with equimolar mixtures of: OA–MO–CH–TO (a), OA–EPC–CH–TO (b), OA–MO–EPC–TO (c), and OA–MO–EPC–CH (d).

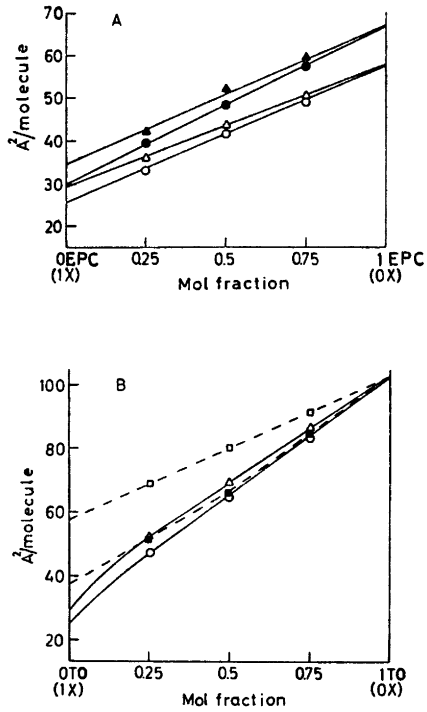

Fig. 2. Variation of average molecular area with composition for mixed films of: (A) OA-EPC (○), and MO-EPC (△) at the collapse pressure and at 20 dynes cm^{-1} (●), and (▲), respectively (X stands for OA and MO in the figure); (B) OA-TO (○), MO-TO (△), CH-TO (■), and EPC-TO (□) at the collapse pressure (X stands for OA, MO, CH, and EPC).

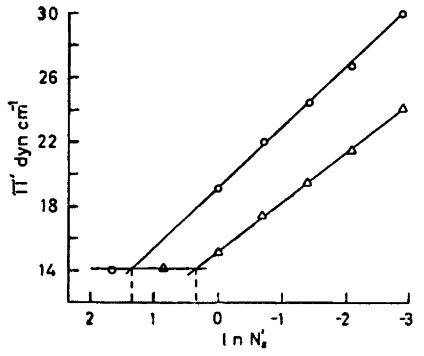

Fig. 3. Graph of the collapse pressure of mixed films of: OA-TO (△), and MO-TO (○) *versus* ln mole fraction of TO in order to obtain the limiting solubility and partial molar areas for the mixed monolayers.

which all have similar collapse pressures.

Two component monolayers. EPC, MO and OA generate miscible two component monolayers with an ideal behaviour at all combinations and proportions (Fig. 1B and 2A). In mixed monolayers with TO, these lipids, and CH also, form partially miscible films (Fig 1C). If the mean molecular areas of the mixed monolayers at the "ejection pressure" of triolein are plotted against mol fractions of TO, approximately straight lines are obtained, which connect the collapse area of pure TO with those of OA, MO, CH, and EPC (Fig. 2B). The partial molar area and the limiting solubility of TO in MO and OA are obtained from the plot ln N'_s (N'_s = mol fraction of TO) *versus* the collapse pressure of the mixed film (the pressure where triolein is ejected). The plots which are shown in Fig. 3 give values for solubility of 3.8 and 1.4 (mol fraction) and partial molar areas of 106 Å² and 134 Å² for TO-MO and TO-OA monolayers, respectively.

CH forms miscible films with EPC, MO, and OA, and has a marked condensing effect on these lipids. In Fig. 4 the effect is presented as "per cent condensation" (P_c).[15] In the calculation of this parameter it was assumed that the area of CH is constant (37.5 Å²) and the area per fatty acid chain of EPC, MO, and OA is 19.5,[2] 19.5,[16] and 18.3 Å²,[17] respectively.

Three component monolayers. The expanded polar lipids EPC, MO, and OA form ideally miscible films in all proportions (Fig. 1C). The condensing effect of CH on these lipids is restored in three-component systems (Table 1). TO generates partially miscible films in all combinations with two of the other lipids studied (Fig. 1C).

Four component monolayers. Some representative force-area isotherms of four-component mixed monolayers are shown in Fig. 1D. CH forms miscible four-component films with EPC, MO, and OA where the condensing effect of CH is the same as in two- and three-component systems calculated on the fatty acid chain basis (Table 1). TO generates partially miscible mixed monolayers in all combinations with three of the other lipids studied.

Five component monolayers. Mixed monolayers with all five lipids studied EPC, MO, OA, CH, and TO showed the same characteristics as the simpler systems (Fig. 1A) TO is squeezed out

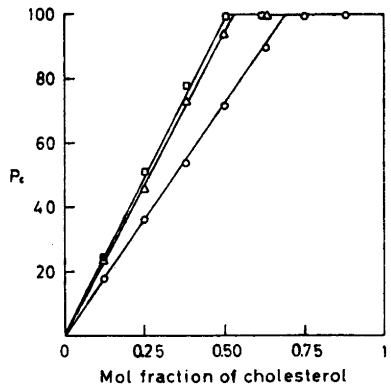

Fig. 4. Per cent condensation (P_c) *versus* mol fraction of cholesterol for mixtures of: OA-CH (□), MO-CH (△), and EPC-CH (○).

of the mixed film at its collapse pressure and the condensing effect of CH is restored.

BIOLOGICAL CONCLUSIONS

The mixed monolayers studied in this paper are relevant as models for several physiological systems, *e.g.* organization and lipolysis of lipids in the intestinal emulsified oil phase, serum lipoproteins, biological membranes, and atherosclerotic plaques.

Dietary fat largely consists of triglycerides with a small proportion of phospholipids and sterols, and, in the case of processed foods, of surface active additives such as monoglycerides. After ingestion the first process is the formation of an oil-in-water emulsion [18] probably stabilized by phospholipids, monoglycerides, and other amphiphilic compounds. It can be predicted that the conditions in the mixed monolayers are analogous to those in the lipid-water interface of an emulsion particle. The surface-balance measurements show that TO is ejected from a mixed film of polar lipids such as EPC, MO, OA, and CH. It has also been shown that EPC and CH are scarcely soluble in TO.[19,20] The conclusion is that the amphiphilic lipids form mono- or multilayers around the triglycerides, which make up the core of the emulsion particle. Further, it can be assumed that the polar end-products from the lipolytic attack on the oil phase, *i.e.* monoglycerides and free fatty acids, accumulate in the interfacial region where the partition between emulsified oil phase and micellar bile salt solution [21] can take place.

The mechanism of the lipolysis of the triglycerides in the emulsified oil phase is an intricate problem because the enzyme is water soluble but the substrate is not. The surface balance results show that the triglycerides do not penetrate a monolayer of polar lipids and thus the ester bonds are not exposed to the water phase where the enzyme could attack them. A possible explanation to this problem is that the enzyme has a hydrophobic penetration site, which penetrates the interfacial layer before the catalytic action is fulfilled.[22]

The proposition above, made for the emulsion particle in the small intestine, may also generally be applied to serum lipoproteins. The results from this study are in accordance with the hypothesis that the neutral lipids are located in the particle core while the polar lipids together with the proteins would be organized in the interfacial region.[10] The lipolytic attack by lipoprotein lipases is likely to involve some kind of hydrophobic penetration. The end products accumulate in the surface film and can be distributed to different tissues.

Table 1. The actual mean molecular areas and the same parameters as calculated from the area of CH (37.5 Å²) and the area of EPC, MO, and OA in mixed two component films with CH.

Monolayer components in equimolar concentrations	Measured value of mean molecular area/Å²	Calculated value of mean molecular area/Å²
EPC – MO – CH	35.9	36.1
EPC – OA – CH	34.8	34.7
MO – OA – CH	27.5	26.9
EPC – MO – OA – CH	33.3	32.0

Phospholipids and cholesterol are important structural components of biological membranes. The role of cholesterol in membranes has been much studied and discussed.[23] The maximal solubilizing capacity in bulk systems has been reported to be a 1:1 molar ratio for phosphatidylcholine [24] and a 1:2 molar ratio for monoolein.[9] These relations would indicate a maximal interaction at a ratio of 2:1 between fatty acid chains and cholesterol. It has also been reported that ultrasonicated dispersions at low concentrations [25,26] and also membranes under special conditions [27] can hold a 2:1 molar ratio of cholesterol to phospholipid. The surface organization of the lipid molecules in these systems can be assumed to correspond to that of a cholesterol–phosphatidylcholine mixed monolayer at the 2:1 molar ratio where the condensing effect is maximal (see Fig. 4).

The contents of glycerides and free fatty acids in biological membranes are low, but to the extent they occur it can be assumed that monoglycerides and fatty acids are incorporated into the pallisade layer while tryglycerides are solubilized in the hydrocarbon part of the bilayer.

One of the most striking features of the atherosclerotic lesions is a great accumulation of lipids. It has been proposed by several authors [28–30] that there is a connection between the physicochemical properties of lipids and their deposition in atherosclerotic arteries. The lipids accumulate in the form of intracellular droplets [31] or extracellular plaques.[32] Parallels can be drawn to transport and storage forms of lipids such as serum lipoproteins and adipocytes. Morphological and chemical studies [33] seem to indicate that the lipids are organized with a gross neutral lipid core and an interfacial layer of polar lipids. The accumulation of lipids in the atherosclerotic lesion is thus greatly dependent on the exchange of neutral lipid molecules between the interior and surface of the lesion and on the accessibility of the neutral lipids for enzymatic attack. Mixed monolayer systems may be valuable as models for studying these aspects of lipid accumulation in atherosclerotic arteries.

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