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THE PROPERTIES OF POLYUNSATURATED LECITHINS IN MONOLAYERS AND LIPOSOMES AND THE INTERACTIONS OF THESE LECITHINS WITH CHOLESTEROL

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SUMMARY

1. The force–area characteristics of monolayers of synthetic lecithins with one to six double bonds in one acyl chain have been studied.

2. The area per molecule increases stepwise. The most significant increase is observed after the introduction of the first double bond. The subsequent introduction of two, three or four double bonds or polyunsaturated chains at both ester positions produces some further increase.

3. The interaction with cholesterol depends on the unsaturation and the distribution of the double bonds between the acyl chains.

4. A condensing effect with cholesterol was evident for (1-stearoyl-2-oleoyl)-3-lecithin, (1-palmitoyl-2-lineoyl)-3-lecithin, (1-palmitoyl-2-linolenoyl)-3-lecithin, (1-palmitoyl-2-arachidonoyl)-3-lecithin at 22°. No effect is observed for (1,2-dilinoleoyl)-3-lecithin and (1,2-dilinolenoyl)-3-lecithin. (1-palmitoyl-2-docosahexaenoyl)-3-lecithin shows a limited effect at 22°, but no effect at 37°.

5. No significant differences in behavior are found for the two structural isomers with a mono- or disaturated chain at the 1- or 2-position.

6. The permeability of liposomes, derived from the above mentioned lecithins, to glucose, erythritol and glycerol increases in the same order as the area per molecule at the air–water interface.

7. The presence of cholesterol reduced the permeability to glucose, erythritol, glycerol only for the lecithins which showed a condensation effect.

8. The unsaturation and the distribution of the double bonds appear to be of critical importance for the barrier properties of lecithins and for the interaction with cholesterol.

INTRODUCTION

Biological membranes contain a great variety of lipids[1,2]. Two of the main lipid classes are the phospholipids and the sterols. Naturally occurring phospholipids show an enormous variation in the nature of the polar head groups. In most mammalian tissues lecithin is the most abundant phospholipid. As well as the variation in polar groups, each class of phospholipids is associated with a considerable variation

in fatty acids. The substituents on positions 1 and 2 of glycerol are derived from long-chain saturated or unsaturated fatty acids. Most phospholipids contain an appropriate amount of saturated fatty acids, having a chain length between 12 and 26 carbon atoms. In general, stearic and palmitic acid serve as the major saturated fatty acid constituents of mammalian phospholipids. Small amounts of branched and odd-numbered acids do occur naturally, but only in small percentages. The physicochemical properties of phospholipids can be drastically influenced by the presence of unsaturated fatty acids.

The *cis*-unsaturated fatty acids usually located preferentially at the 2-position in the lecithin molecule are oleic acid (monoenoic), linoleic acid (dienoic), linolenic acid (trienoic) and arachidonic acid (tetraenoic). Among the polyunsaturated fatty acid constituents of animal phospholipids, the essential fatty acid arachidonic acid is of particular interest. The fatty acids from phospholipids exhibit a certain degree of similarity in homolog tissues of different animals. This type of specificity is found in the fatty acid pattern of phospholipids from lung and brain tissue of a number of species[3].

Of the sterols found in mammalian membranes, cholesterol is by far the most commonly occurring. High proportions of cholesterol are found in the cell envelopes of liver and erythrocytes[4] as well as in myelin sheath[5]. The molar ratio of cholesterol to phospholipid is in these membranes close to unity. Smaller proportions are found in subcellular membranes such as mitochondria, microsomes and nuclei[6]. Besides cholesterol only sterols such as 7-dehydrocholesterol, lathosterol and cholestanol have been recorded in animal cells so far[6–8].

In previous papers[9,10] we have established that for the interaction of sterols with lecithin in monolayers, as well as for the reduction in permeability in liposomes the following is required: (a) a planar sterol nucleus, (b) an intact side chain, and (c) a 3β-hydroxy group.

The above mentioned sterols meet these requirements. With respect to the properties of pure synthetic lecithins, monolayer studies[11,12] showed that a decrease in chain length of saturated lecithins increases the area per molecule at the air–water interface. At the same time, the permeability properties of liposomes prepared from saturated lecithins increased with decreasing chain length[13]. The interfacial properties of lecithins are drastically influenced by the presence of double bonds in the paraffin chains. The area per molecule is strongly increased when oleic, linoleic or linolenic acid is attached to the phospholipid molecule[11]. Liposomes showed an increased permeability for glycerol, erythritol[13], and glucose[14] when the fatty acid constituents of the lecithin molecule showed an increase in unsaturation.

A decrease in area per molecule is found in mixed monomolecular films of cholesterol and saturated lecithins with fatty acid constituents of intermediate chain length[11,12], *viz.* (1-stearoyl-2-lauroyl)-3-lecithin, (1,2-ditetradecanoyl)-3-lecithin, (1,2-dilauroyl)-3-lecithin and (1,2-diundecyloyl)-3-lecithin. A small interaction is found for (1,2-didecanoyl)-3-lecithin at higher surface pressures. No change in mean molecular area is found for short chain lecithins[12], *viz.* (1,2-dinonanoyl)-3-lecithin, (1,2-dioctanoyl)-3-lecithin and long-chain lecithins, *viz.* (1,2-distearoyl)-3-lecithin. Unsaturated lecithins, particularly with monounsaturated fatty acid constituents, *viz.* (1-oleoyl-2-stearoyl)-3-lecithin, show a pronounced

condensation effect with cholesterol and small mean molecular area[9,11]. Polyunsaturated lecithins were found to be little effected by the presence of cholesterol[11]. Tinoco and McIntosh[19], on the other hand, found interactions for lecithins with linoleic acid at the 2-position[19]. Possible differences can be caused by mixing of the films at the interface. For a lecithin with two polyunsaturated chains ((1,2-dilinoleoyl)-3-lecithin) no reductions in area are found in the presence of cholesterol by the method of Goodrich[11] nor by measuring the collapse pressure[12]. In this paper the surface properties of a series of polyunsaturated lecithins will be described, including compounds with two, three, four, or six double bonds in one acyl chain. The effect of the location of the unsaturated chain at the 1-position is compared with the more natural isomer having the unsaturated chain at the 2-position. Also the less common lecithins having both at the 1- and 2-positions an unsaturated fatty acid constituent are included.

The permeability properties of liposomes formed from polyunsaturated lecithin are considered to be important in order to give a deeper understanding of the role of essential fatty acids in the biological membrane. The effect of cholesterol incorporation in liposomes formed from polyunsaturated lecithins on the glycerol, erythritol and glucose permeability is studied and compared with the properties of mixed monomolecular films.

MATERIALS

Cholesterol was obtained from Fluka, Buchs, Switzerland, and purified by recrystallization from ethanol. The fatty acids used for the synthesis of lecithins were obtained from the following sources: palmitic acid and stearic acid from Fluka, Buchs, Switzerland; oleic acid from Merck, Darmstadt, Germany; linoleic and linolenic acid from Koch-Light Laboratories, Colnbrook, Bucks, England; arachidonic acid from The Hormel institute, Austin, Minn., U.S.A.; docosahexaenoic acid from Serdary Research Laboratories, London, Canada. A natural lecithin from egg yolk containing 40 % docosahexaenoic acid[20,21] exclusively located at the 2-position was a generous gift of Mr. N. Miller (Institute of Animal Physiology, Babraham, Great Britain). A lecithin isolated from soya beans and containing as much as 67 % linoleic acid was obtained from Dr. H. Eikermann of Nattermann and Cie, Köln, Germany.

METHODS

The synthesis of lecithins containing two similar and dissimilar fatty acids, respectively, was in general carried out according to established procedures[15]. Egg lecithin was isolated according to the method of Pangborn[16]. The purity was examined by chromatography on silica gel plates with chloroform–methanol–water (65:35:4, by vol.). Subsequently the egg lecithin was hydrolyzed to *sn*-glycero-3-phosphorylcholine by a methanolic tetrabutylammonium hydroxide solution. The $CdCl_2$ adduct of *sn*-glycero-3-phosphorylcholine was reacylated with the respective fatty acid chloride. The fatty acids were first checked for purity on gas–liquid chromatography with an internal standard. The presence of peroxides was determined by ultraviolet

spectroscopy[17]. Impurities in the fatty acid starting material were eliminated by chromatography over silica gel and light petroleum (b.p. 40–60°)–diethyl ether–acetic acid (60:40:1, by vol.) as solvent system. The pure fatty acids were converted into the respective fatty acid chlorides by oxalyl chloride. During the synthesis propylgallate was used as an antioxydant. The formed lecithin was passed over a mixed amberlite (IR-45 and IRC-50) column and purified first over a silica column and then over a neutral aluminum oxide column. For the synthesis of lecithins with mixed fatty acid chains, 1-acyl-*sn*-glycero-3-phosphorylcholine was formed by phospholipase A (EC 3.1.1.4) degradation. The $CdCl_2$ adduct of lysolecithin was reacylated and purified as described above. The lecithins were checked by thin-layer and gas–liquid chromatography and ultraviolet spectroscopy before use and stored under N_2 at −80°. The experimental details for monolayer spreading[9] and the determination of glucose[14], glycerol and erythritol[13] permeability in liposomes are given elsewhere.

The following lecithins have been synthesized: (1,2-distearoyl)-3-lecithin (18:0/18:0 PC); (1-stearoyl-2-oleoyl)-3-lecithin (18:0/18:1 PC); (1-oleoyl-2-stearoyl)-3-lecithin (18:1/18:0 PC); (1-palmitoyl-2-linoleoyl)-3-lecithin (16:0/18:2 PC); (1-linoleoyl-2-palmitoyl)-3-lecithin (18:2/16:0 PC); (1-palmitoyl-2-linolenoyl)-3-lecithin (16:0/18:3 PC); (1-palmitoyl-2-arachidonoyl)-3-lecithin (16:0/20:4 PC); (1-palmitoyl-2-docosahexaenoyl)-3-lecithin (16:0/22:6 PC); (1,2-dilinoleoyl)-3-lecithin (18:2/18:2 PC); and (1,2-dilinolenoyl)-3-lecithin (18:3/18:3 PC).

Force–area[9] and liposome[10] measurements were performed according to methods described previously.

RESULTS

Figs. 1A–1C compiles the pressure–area characteristics of lecithins with an increasing number of double bonds in the fatty acid constituents, at the air–water interface at a temperature of 22°. The saturated (1,2-distearoyl)-3-lecithin shows a condensed film with an initial area per molecule of 63 Å^2 and a limiting area of 41 Å^2 (Fig. 1A). The presence of the first double bond renders the film liquid expanded at all pressures and has a dramatic effect on the pressure–area curve. The initial area per molecule for (1-stearoyl-2-oleoyl)-3-lecithin is 108 Å^2 and at a pressure of 30 dynes/cm the area per molecule is still 61 Å^2 (Fig. 1A). At an intermediate pressure of 12 dynes/cm (1-stearoyl-2-oleoyl)-3-lecithin occupies an area per molecule that is 29 Å^2 larger than that for (1.2-distearoyl)-3-lecithin. (1-Oleoyl-2-stearoyl)-3-lecithin shows a practically identical pressure–area curve as (1-stearoyl-2-oleoyl)-3-lecithin[18]. Further unsaturation of the acyl chain affects a further increase in area per molecule but to a much lesser extent than caused by the first double bond. (1-Palmitoyl-2-linoleoyl)-3-lecithin occupies an initial area of 115 Å^2 and a limiting area at the collapse pressure of 58 Å^2. At a pressure of 12 dynes/cm (1-palmitoyl-2-linoleoyl)-3-lecithin occupies an area per molecule that is 6 Å^2 larger than that for (1-stearoyl-2-oleoyl)-3-lecithin. It has to be noted that the 2 carbon atom shorter chain of the palmitic acid derivatives also causes some increase in area per molecule[11,19]. Since in natural lecithins the unsaturated chain is normally located at the 2-position, it is interesting to note that the two structural isomers, *viz.* (1-palmitoyl-2-linoleoyl)-3-lecithin and (1-linoleoyl-2-palmitoyl)-3-

lecithin show little difference in interfacial behavior. A slightly higher area per molecule is found for (1-linoleoyl-2-palmitoyl)-3-lecithin than for (1-palmitoyl-2-linoleoyl)-3-lecithin (Fig. 1A). As mentioned above the (stearoyl-oleoyl)-lecithin isomers also show no significant difference.

Lecithins containing at the 2-position fatty acid derivatives with respectively three, four or six double bonds show only a limited further increase in area per molecule (Fig. 1B). The area per molecule increases in the following order: (1-palmitoyl-2-linolenoyl)-3-lecithin, (1-palmitoyl-2-arachidonoyl)-3-lecithin and (1-palmitoyl-2-docosahexaenoyl)-3-lecithin. Of special interest is the lecithin bearing the essential fatty acid, arachidonic acid. The interfacial behavior of this lecithin is comparable with that of the other lecithins having only one polyunsaturated fatty acid constituent. The collapse pressures of these lipid species are also comparable.

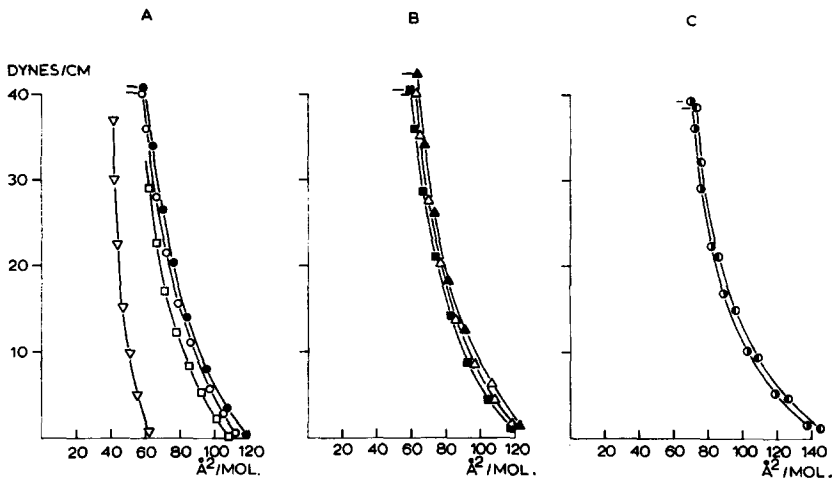

Fig.1. (A) Force–area characteristics of saturated lecithins and lecithins with one mono- or polyunsaturated fatty acid chain at 22°. ▽, (1,2-Distearoyl)-3-lecithin; □, (1-stearoyl-2-oleoyl)-3-lecithin; ○, (1-palmitoyl-2-linoleoyl)-3-lecithin; ●, (1-linoleoyl-2-palmitoyl)-3-lecithin. (B) Force–area characteristics of lecithins with one polyunsaturated fatty acid constituent at the 2-position at 22°. ■, (1-palmitoyl-2-linolenoyl)-3-lecithin; △, (1-palmitoyl-2-arachidonoyl)-3-lecithin; ▲, (1-palmitoyl-2-docosahexaenoyl)-3-lecithin. (C) Force–area characteristics of lecithins with two polyunsaturated fatty acid chains at 22°. ◑, (1,2-Dilinoleoyl)-3-lecithin; ◐, (1,2-dilinolenoyl)-3-lecithin.

Lecithin species containing two polyunsaturated fatty acid constituents occur only in small quantities in certain mammalian membranes. However, galactolipids, abundant in the membranes of chloroplast contain high amounts of polyunsaturated fatty acid constituents at both ester positions. In order to compare the effect of unsaturation in both chains with the unsaturation in one, *e.g.* (dilinoleoyl)-lecithin *versus* (palmitoyl–arachidonoyl)-lecithin, two lecithin species with two polyunsaturated chains are studied (Fig. 1C). A further increase in area per molecule is found for (1,2-dilinoleoyl)-3-lecithin and (1,2-dilinolenoyl)-3-lecithin (Fig. 1C). (1,2-Dilinoleoyl)-3-lecithin occupies an initial area of 147 Å² and a limiting area at the collapse pressure of 70.3 Å². At a pressure of 12 dynes/cm (1,2-dilinoleoyl)-

3-lecithin occupies an area per molecule that is 14 Å^2 larger than that for (1-palmitoyl-2-linoleoyl)-3-lecithin (Fig. 1A). Even slightly higher areas per molecule are found for (1,2-dilinolenoyl)-3-lecithin (Fig. 1C).

In Figs. 2 A–2C the effects of cholesterol on mixed films with polyunsaturated lecithins at a pressure of 12 dynes/cm and a temperature of 22° are given. In a previous study it was shown that a striking deviation from ideal behavior is observed when the phospholipid contains monounsaturated fatty acid constituents[11]. No interaction was found with polyunsaturated lecithins when lecithin and cholesterol were subsequently spread at the interface[11]. The absence of an interaction

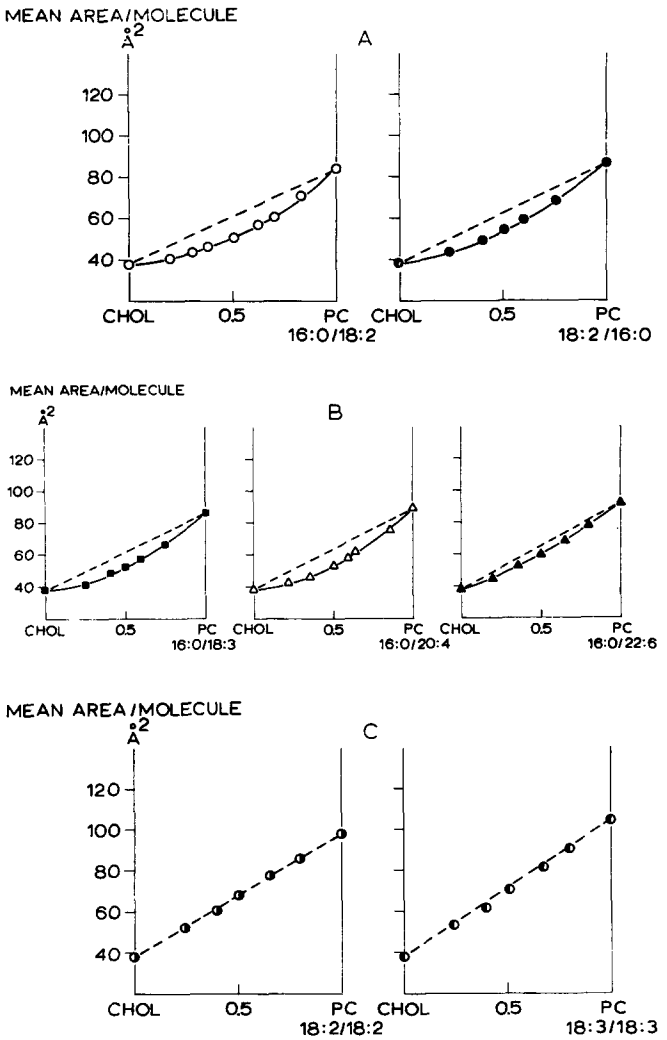

Fig. 2. Variation of the mean molecular area as a function of the composition at a pressure of 12 dynes/cm and 22° for mixed monolayers of cholesterol and, respectively: (A) (1-palmitoyl-2-linoleoyl)-3-lecithin (○), (1-linoleoyl-2-palmitoyl)-3-lecithin (●); (B) (1-palmitoyl-2-linolenoyl)-3-lecithin (■), (1-palmitoyl-2-arachidonoyl)-3-lecithin (△), (1-palmitoyl-2-docosahexaenoyl)-3-lecithin (▲), (C) (1,2-dilinoleoyl)-3-lecithin (◑), (1,2-dilinolenoyl)-3-lecithin (◐).

can be caused by a still imperfect mixing of these liquid films[12]. In the present study the lipids are mixed before spreading. Both structural isomers of (palmitoyl–linoleoyl)-lecithin show striking reductions in mean molecular area after mixing with cholesterol. At a molar ratio of 1:1 the reductions for (1-palmitoyl-2-linoleoyl)-3-lecithin and (1-linoleoyl-2-palmitoyl)-3-lecithin are 10 and 9 Å^2, respectively (Fig. 2A). Lecithins with at the 2-position a fatty acid constituent containing three or four double bonds also show striking reductions in area per molecule after mixing with cholesterol. At a molar ratio of 1:1 the reduction for (1-palmitoyl-2-linolenoyl)-3-lecithin and (1-palmitoyl-2-arachidonoyl)-3-lecithin is about 10 Å^2 (Fig. 2B). For the lecithin species bearing at the 2-position a fatty acid with six double bonds, (1-palmitoyl-2-docosahexaenoyl)-3-lecithin, a much smaller interaction is found. For this lecithin the reduction is only 5 Å^2 at a molar ratio of 1:1 (Fig. 2B). At an elevated temperature of 37° the effect of cholesterol on monomolecular films of (1-palmitoyl-2-docosahexaenoyl)-3-lecithin was completely absent. Lecithins having at both the 1- and 2-position polyunsaturated fatty acid derivatives show no detectable reduction in area per molecule after mixing with cholesterol (Fig. 2C). These results for (1,2-dilinoleoyl)-3-lecithin and (1,2-dilinolenoyl)-3-lecithin (Fig. 2C) are in agreement with earlier observations[11,12].

In order to demonstrate the effect of the molecular architecture of pure lecithins and of lecithin cholesterol mixtures on the permeability properties of lipid barriers, the permeability properties of liposomes to glucose, glycerol and Rb^+

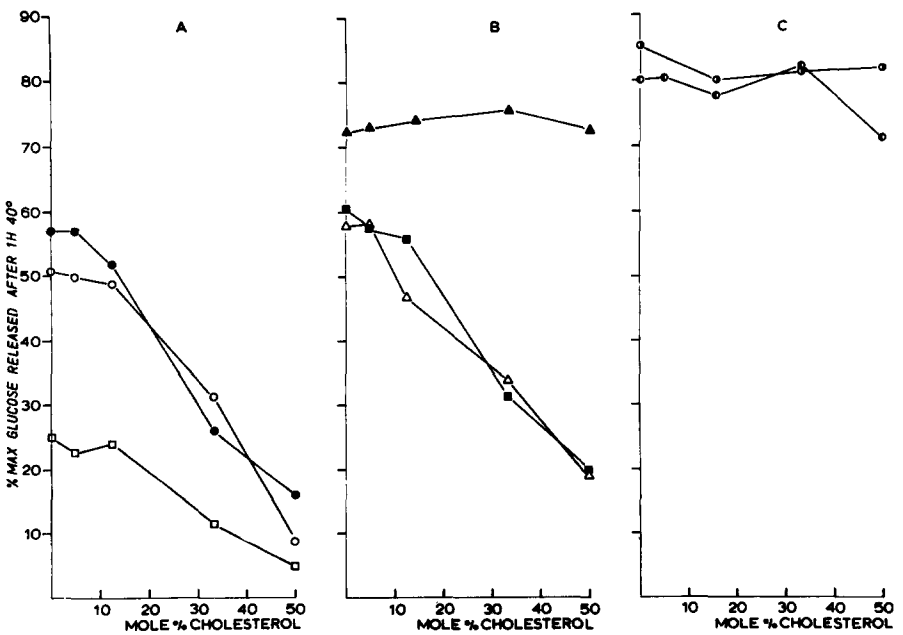

Fig. 3. Effect of cholesterol incorporation into liposomes of synthetic lecithins on the relative amount of glucose released after 1 h at 40°. Liposomes contained 4 mole % phosphatidic acid and were prepared from, respectively: (A) (1-stearoyl-2-oleoyl)-3-lecithin (□), (1-palmitoyl-2-linoleoyl)-3-lecithin (○), (1-linoleoyl-2-palmitoyl)-3-lecithin (●); (B) (1-palmitoyl-2-linolenoyl)-3-lecithin (■), (1-palmitoyl-2-arachidonoyl)-3-lecithin (△), (1-palmitoyl-2-docosahexaenoyl)-3-lecithin (▲); (C) (1,2-dilinoleoyl)-3-lecithin (◑), (1,2-dilinolenoyl)-3-lecithin (◐).

are studied. Previous studies have shown that there is a good correlation between the area per molecule occupied in pure lecithin or mixed lecithin–cholesterol films and the permeability in liposomes to different solutes[9,10,13,14]. The different liposome preparations showed a comparable osmotic behavior[13]. The results compiled in Figs. 3A–3C demonstrate that the permeability of pure lecithin liposomes towards glucose increases with increasing unsaturation of the fatty acid constituents. Monolayer studies showed already that increasing unsaturation increases the area per molecule (Figs. 1A–1C). The permeability of lecithin liposomes to glucose is particularly increased when the unsaturation is increased from one to two double bonds, (1-stearoyl-2-oleoyl)-3-lecithin *versus* (1-palmitoyl-2-linoleoyl)-3-lecithin. Comparable permeability rates are observed for (1-palmitoyl-2-linoleoyl)-3-lecithins, (1-linoleoyl-2-palmitoyl)-3-lecithin, (1-palmitoyl-2-linolenoyl)-3-lecithin and (1-palmitoyl-2-arachidonoyl)-3-lecithin (Figs. 3A and 3B). A further increase in permeability is observed for (1-palmitoyl-2-docosahexaenoyl)-3-lecithin and particularly for the lecithins with two unsaturated fatty acid chains (1,2-dilinoleoyl)-3-lecithin and (1,2-dilinolenoyl)-3-lecithin.

Monolayer studies above have shown that (1-stearoyl-2-oleoyl)-3-lecithin, (1-oleoyl-2-stearoyl)-3-lecithin, (1-palmitoyl-2-linoleoyl)-3-lecithin, (1-linoleoyl-2-palmitoyl)-3-lecithin, (1-palmitoyl-2-linolenoyl)-3-lecithin and (1-palmitoyl-2-arachidonoyl)-3-lecithin interact with cholesterol, thereby reducing the mean molecular area(Figs. 2A and 2B). The permeability of the above lecithins is strikingly reduced by the presence of cholesterol (Figs. 3A and 3B). The reduction in mean molecular

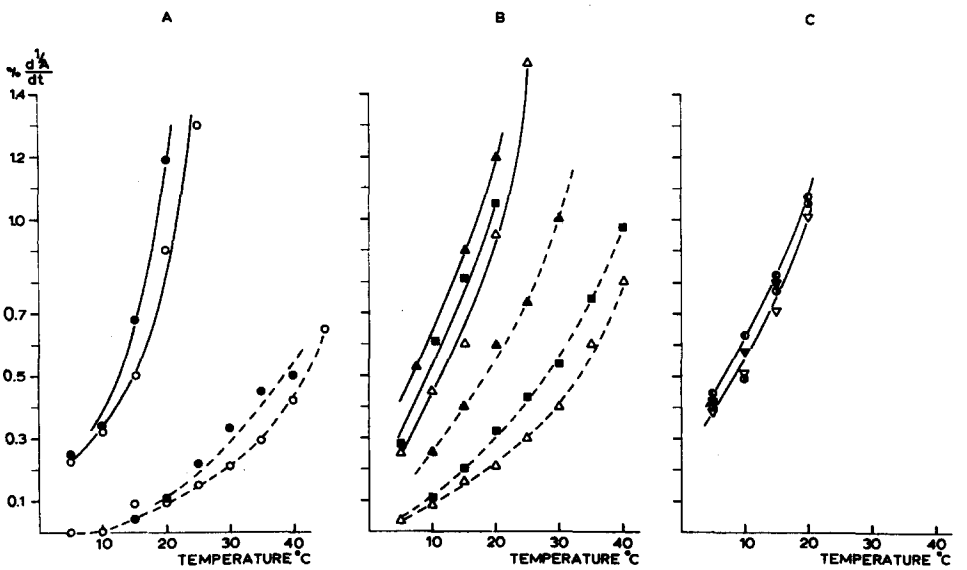

Fig. 4. Effect of cholesterol incorporation into liposomes of synthetic lecithins on the initial swelling rate in isotonic glycerol in relation to temperature. The liposome preparations contained 4 mole % phosphatidic acid. ——, swelling rate of pure lecithins; - - -, swelling rate of lecithins to which 50 mole % cholesterol is added (molar ratio of lecithin to sterol 1:1). (A) ○, (1-palmitoyl-2-linoleoyl)-3-lecithin; ●, (1-linoleoyl-2-palmitoyl)-3-lecithin. (B) ■, (1-palmitoyl-2-linolenoyl)-3-lecithin; △, (1-palmitoyl-2-arachidonoyl)-3-lecithin; ▲, (1-palmitoyl-2-docosahexaenoyl)-3-lecithin. (C) ◑, (1,2 dilinoleoyl)-3-lecithin; ◐ (1,2-dilinolenoyl)-3-lecithin; ▽, (1,2-dilinoleoyl)-3-lecithin + cholesterol; ▼, (1,2-dilinolenoyl)-3-lecithin + cholesterol.

area is reflected in reduced permeability properties of liposomes. For (1-palmitoyl-2-docosahexaenoyl)-3-lecithin a reduction of the mean molecular area in monomolecular films is found only at room temperature. At physiological temperature no deviation from ideal behavior is observed. (1,2-Dilinoleoyl)-3-lecithin and (1,2-dilinolenoyl)-3-lecithin show even no interaction at room temperature. In agreement with these findings, the permeability of liposomes to glucose at 40° formed from the preceding three lecithins is not influenced by the presence of cholesterol (Figs. 3B and 3C). The permeability to glycerol and erythritol shows qualitatively the same effects as found for glucose. Strong reductions in the swelling rate in isotonic glycerol and erythritol are caused by the addition of 50 mole % cholesterol to (1-palmitoyl-2-linoleoyl)-3-lecithin, (1-linoleoyl-2-palmitoyl)-3-lecithin, (1-palmitoyl-2-linolenoyl)-3-lecithin and (1-palmitoyl-2-arachidonoyl)-3-lecithin at all temperatures between 5 and 45° (Figs. 4A and 4B, and 5A and 5B). A significantly less pronounced cholesterol effect is found on the glycerol and erythritol swelling of (1-palmitoyl-2-docosahexaenoyl)-3-lecithin (Figs. 4B and 5B). Whereas no detectable effect is found for (1,2-dilinoleoyl)-3-lecithin and (1,2-dilinolenoyl)-3-lecithin (Figs. 4C and 5C). The swelling rates of the pure lecithins are very high towards glycerol, even at low temperatures, so that the differences between the different lecithin species are less striking than those found for glucose (*cf.* Figs. 3A–C and 4A–4C). The erythritol swelling rate is significantly higher for pure (1-palmitoyl-2-docosahexaenoyl)-3-lecithin and especially for (1,2-dilinoleoyl)-3-lecithin and (1,2-dilinolenoyl)-3-lecithin than for the other lecithin species (Figs. 5A–5C).

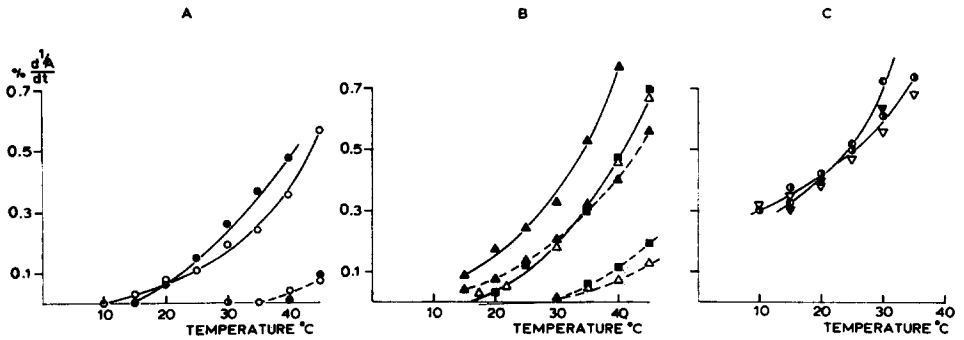

Fig. 5. Effect of cholesterol incorporation into liposomes of synthetic lecithins on the initial swelling rate in isotonic erythritol in relation to temperature. The liposome preparations contained 4 mole % phosphatidic acid. ——, swelling rate of pure lecithins; - - -, swelling rate of lecithins to which 50 mole % cholesterol is added (molar ratio of lecithin to sterol 1:1). (A) ○, (1-palmitoyl-2-linoleoyl)-3-lecithin; ●, (1-linoleoyl-2-palmitoyl)-3-lecithin. (B) ■, (1-palmitoyl-2-linolenoyl)-3-lecithin; △, (1-palmitoyl-2-arachidonoyl)- 3-lecithin; ▲, (1-palmitoyl-2-docosahexaenoyl)-3-lecithin. (C) ◑, (1,2-dilinoleoyl)-3-lecithin; ◐, (1,2-dilinolenoyl)-3-lecithin; ▽, (1,2-dilinoleoyl)-3-lecithin + cholesterol; ▼, (1,2-dilinolenoyl)-3-lecithin + cholesterol.

DISCUSSION

The data on the interfacial properties of a great number of synthetic lecithin species demonstrate that the area per molecule is increased with increasing unsaturation of the acyl moiety. The results summarized in Fig. 6A show the area per molecule at a pressure of 12 dynes/cm and a temperature of 22°. In general qualitatively

comparable results are obtained at other surface pressures. The smallest area per molecule is found for the condensed film of (1,2-distearoyl)-3-lecithin. The limiting area of 41 Å^2 per molecule of this lecithin corresponds with the cross sectional area of two saturated fatty acyl chains. The introduction of the first double bond in one of the fatty acid constituents effects an average area increase of 28.5 Å^2/mol. Unsaturation to two, three, four or six double bonds in one acyl chain effects a further but smaller area increase. Lecithins with two polyunsaturated chains (two or three double bonds) show again higher area/molecule. It is interesting to notice that a lecithin with two monounsaturated chains, *viz.* (1,2-dioleoyl)-3-lecithin, effects a much smaller area increase and occupies an area per molecule that is similar to (1-linoleoyl-2-stearoyl)-3-lecithin[11]. The area per molecule for newly synthesized lecithins given in this paper are in good agreement with results published earlier[11] and the data of Tinoco and McIntosh[19]. The data demonstrate also that there is no significant difference in spreading properties between structural isomers with the mono- or diunsaturated chain at the 1- or 2-position. There is, however, a difference between compounds having the double bonds located in one chain and compounds having the same number of double bonds distributed over the two chains. Fig. 6B compiles the initial swelling rate of the pure lecithins in

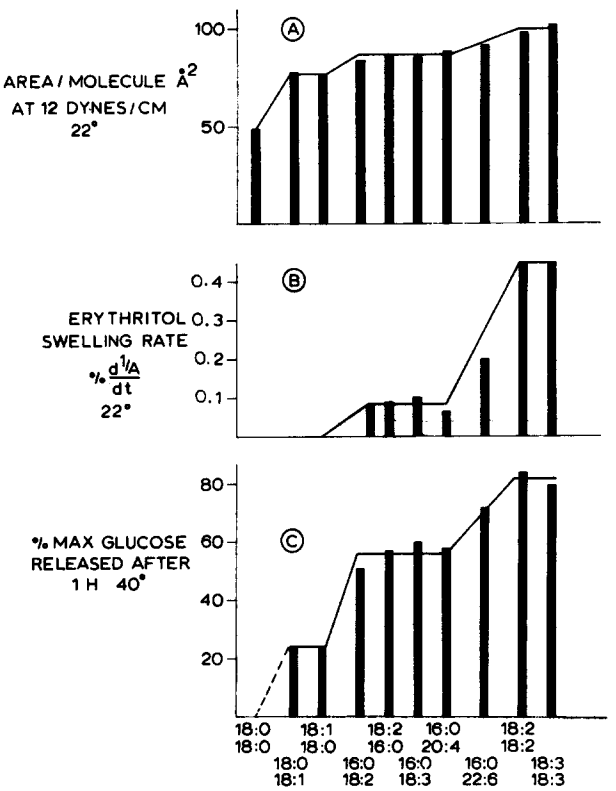

Fig. 6. (A) The area per molecule of different saturated and unsaturated synthetic lecithins at 12 dynes/cm and 22°.(B) The initial swelling rates of liposomes prepared from the corresponding pure lecithins in isotonic erythritol at 22°. (C) The relative amount of glucose released from liposomes prepared from the corresponding pure lecithins after 1 h at 40°.

isotonic erythritol at a temperature of 22°. The permeability to erythritol increases in the same order as the area per molecule (Fig. 6A). The initial swelling of monounsaturated lecithins is very low for erythritol at 22°. An average of 0.08% $d(1/A)/dt$ is shown for lecithins with two to four double bonds in one acyl chain. High swelling rates of 0.45 are found for lecithins with two polyunsaturated fatty acid chains. An intermediate value is found for (1-palmitoyl-2-docosahexaenoyl)-3-lecithin. The release of glucose measured close to the physiological temperature shows a comparable picture (Fig. 6C). Distearoyl lecithin forms a crystalline lattice at this temperature and shows no permeability[13]. The liquid bilayer structure of monounsaturated lecithins shows a release of trapped glucose of 25%, under the given experimental conditions. A sharp increase in permeability is found for the lecithin species having more than one double bond. An average value of 50% is found for the lecithins having two, three or four double bonds in one acyl chain. A significantly higher value of 72% is found for the lecithin with six double bonds in one acyl chain. Lecithin species which hold two or three double bonds in each

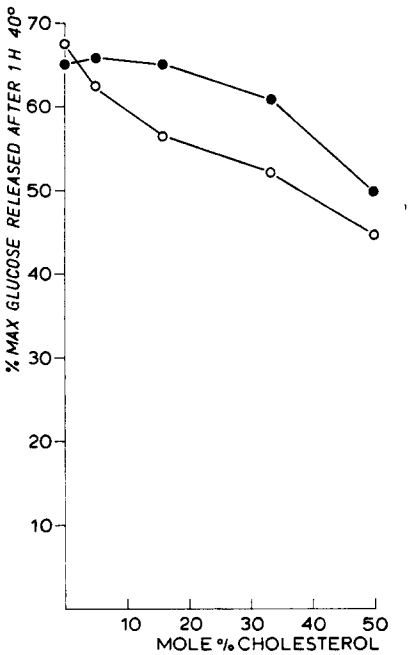

Fig. 7. Effect of cholesterol incorporation into liposomes prepared from: soya bean lecithin (containing 67% 18:2) (○), and egg yolk lecithin (containing 40% 22:6) (●) on the relative amount of glucose released after 1 h at 40°.

acyl chain show the highest permeability, 82% (Fig. 6C). For the lecithin with two monounsaturated chains, *viz.* (1,2-dioleoyl)-3-lecithin, a permeability of only 40% is found[14]. This finding is in agreement with the smaller area per molecule[11] denoted for this particular lecithin. Also with respect to the permeability properties of structural isomers having the mono- or diunsaturated chain at the 1- or 2-position, no significant differences are found (Figs. 6B and 6C). The noticed increase

in permeability for glycerol, erythritol and glucose with increasing unsaturation in synthetic lecithins is also noticed for lecithins from natural origins[20,21]. Natural products containing high amounts of polyunsaturated fatty acids showed also a high permeability for glucose. Egg lecithin containing 40 % docosahexaenoic acid[22] and soya bean lecithin containing 67 % linoleic acid, the latter containing an appreciable amount of (1,2-dilinoleoyl)-3-lecithin, give rise to a glucose leak of 65 % (Fig. 7).

Cholesterol is a generally occurring compound in many biological membranes and is found to have a marked effect on the permeability properties of liposomes[10,13,14] and biological membranes[23,24]. Monolayer studies showed that the area per molecule of saturated lecithins of intermediate chain length is reduced in the presence of cholesterol. The area per molecule of long-chain and short-

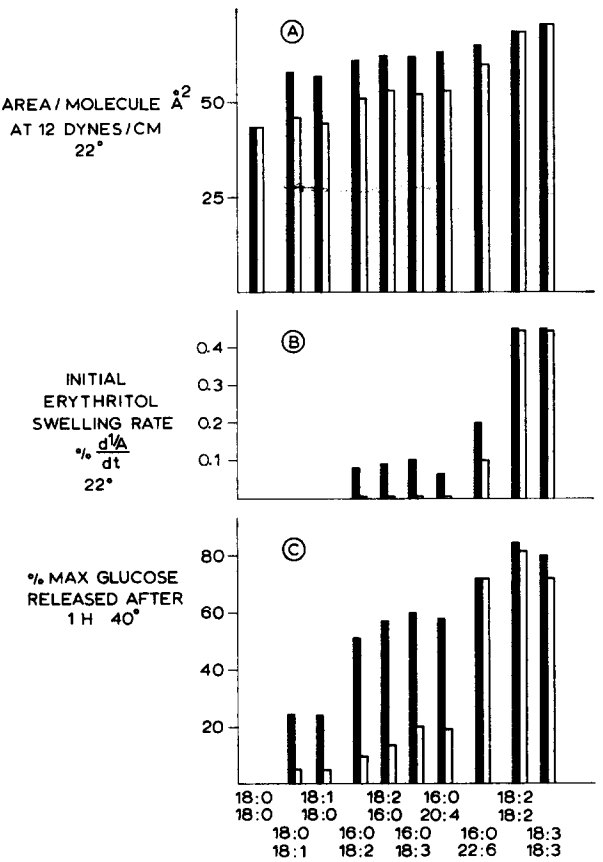

Fig. 8. (A) The area per molecule of mixed synthetic lecithin–cholesterol films, molar ratio 1:1, at a pressure of 12 dynes/cm and 22°. ■, area per molecule when the simple additivity rule is followed; □, observed mean molecular/area of the mixed lectihin–cholesterol film. The difference between the areas per molecule gives the reduction in area per molecule or the condensation effect. (B) The initial swelling rates of liposomes in isotonic erythritol at 22°. ■, liposomes prepared from the corresponding pure lecithins; □, liposomes prepared from lecithin–cholesterol (molar ratio 1:1). The difference between the swelling rates illustrates the effect of cholesterol. (C) The relative amount of glucose released from liposomes after 1 h at 40°. ■, liposomes prepared from the corresponding pure lecithins; □, liposomes prepared from lecithin–cholesterol (molar ratio 1:1). The difference between the glucose release illustrates the effect of cholesterol.

chain lecithins is not affected by cholesterol[9,11,12]. In Fig. 8 the effect of cholesterol on the mean molecular area of mono- and polyunsaturated lecithins is summarized. The most pronounced condensation effect is observed for lecithins with a monounsaturated chain at either the 1- or 2-position. Also for the lecithins with one chain containing two, three or four double bonds a significant condensation is found. Nearly identical effects are observed for the two structural isomers with a diunsaturated fatty acid at the 1- or 2-position. Although in nature the polyunsaturated fatty acids are predominantly located at the 2-position the interfacial behaviour of the two isomers shows no gross differences. In a previous study we failed to observe a condensation effect for some of these polyunsaturated lecithin species when mixed with cholesterol at the interface[11]. Surprisingly it turned out that the liquid expanded film of these lecithins and the liquid condensed film of cholesterol do not mix spontaneously. Repeated experiments showed that after subsequent spreading of (1-linoleoyl-2-palmitoyl)-3-lecithin or (1-palmitoyl-2-arachidonoyl)-3-lecithin and cholesterol no condensation effect is observed and only a small effect for (1-palmitoyl-2-linoleoyl)-3-lecithin. The effects described in this paper for premixed cholesterol and lecithins with one polyunsaturated chain are in agreement with those of Tinoco[19]. For the lecithin species with an acyl chain containing six double bonds at the 2-position a much smaller effect is observed than for the less unsaturated lecithins at 22°. At physiological temperature a deviation from ideal behaviour was even absent for this highly unsaturated lecithin. For lecithin species with two polyunsaturated fatty acid chains giving highly expanded films no condensation effects are observed and this is in agreement with earlier observations[11,12]. For the lecithin with two monounsaturated chains, which forms a less expanded film, a condensation effect is found[11]. Fig. 8B summarizes the effect of cholesterol on the erythritol permeability of liposomes of the same series of lecithins at 22°. It is obvious that for the lecithins for which a marked condensation effect is found (Fig. 8A) a strong reduction in permeability is also found (Fig. 8B)*. For (1-palmitoyl-2-docosahexaenoyl)-3-lecithin a limited reduction in permeability is observed which is in agreement with the condensation effect in monolayers at this temperature. For the lecithins with two polyunsaturated chains no permeability effect of cholesterol is observed. This corresponds with the monolayer data. The effects of cholesterol on the glucose permeability of liposomes at 40° are summarized in Fig. 8C. Dipalmitoyllecithin and distearoyllecithin have transition points above the physiological temperature. Above temperatures of about 36 and 44°, respectively, cholesterol reduces the permeability to glycerol[25]. However, at lower temperatures the permeability is increased in the presence of cholesterol[25]. As stated earlier[11] the van der Waals interaction between cholesterol and long-chain saturated phospholipids may be less than the mutual interaction of these phospholipids themselves. A prevention of the ordering and crystallization of the hydrocarbon chains of long-chain saturated lecithins by cholesterol has been observed by ESR[26] and X-ray[27] methods. *Cis*-unsaturated lecithins are liquid at physiological temperature. For lecithins with one mono-, di-, tri-, or tetra-unsaturated chain a remarkable reduction in permeability is observed at this temperature (Fig. 8C). For the monounsaturated lecithins the lowest ultimate

* For the monounsaturated lecithin species no detectable initial swelling rate can yet be detected for erythritol at this temperature.

permeability is observed. This is in agreement with monolayer data since for these lecithin species the smallest mean molecular areas are observed (Fig. 8A). It has been stated that the interaction forces of cholesterol with these naturally abundant lecithin species are governed by Van Der Waals' interactions[9,11]. Techniques such as X-ray[27], differential scanning calorimetry[24,28] and ESR[29,30] have proven that the chain mobility is reduced in the presence of cholesterol. However, recent studies on model membranes and natural membranes have shown that the 3β orientation of the hydroxyl group is also required for the interaction. In monolayers only small reductions in mean molecular area are observed in the presence of the 3α-hydroxyl structured epicholesterol[9]. No reductions in permeability are found in liposomes[10] and *Mycoplasma laidlawii* membranes[24] when epicholesterol is present. This means that also the interaction of the 3β-hydroxyl group of cholesterol with its environment is crucial for the lecithin–cholesterol interaction.

For the lecithin species with one hexaunsaturated chain no reduction in permeability is observed at 40° and this is also in agreement with monolayer data at this elevated temperature. Lecithin species with two polyunsaturated chains show no cholesterol effect in liposomes or monolayers (Figs. 8A and 8C). Apparently the bulky structure of these highly expanded lecithins prevents significant hydrophobic interactions. Also naturally occurring lecithins rich in species with hexaunsaturated chains or two polyunsaturated chains show only a limited effect of cholesterol (Fig. 7).

The studies with unsaturated lecithin species in model membranes demonstrate the importance of the degree of unsaturation with respect to the permeability properties of the lipid barrier. The unsaturation and the distribution of the double bonds is also of critical importance for the interaction with cholesterol.

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