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STRUCTURAL REQUIREMENTS OF STEROLS FOR THE INTERACTION WITH LECITHIN AT THE AIR–WATER INTERFACE

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SUMMARY

1. The force area characteristics of monolayers of 3β-, 3α-hydroxysterols and ketosteroids have been studied.

2. The 3β-hydroxysterols cholesterol, cholestanol, lathosterol, 7-dehydrocholesterol, and B-norcholesterol are perpendicularly oriented at the air–water interface and show high collapse pressures. Ergosterol, stigmasterol, and androstan-3β-ol demonstrate a similar behavior at high pressures, but the collapse pressure is reduced. The *cis*-structured coprostanol shows an increased area per molecule. 3α-Hydroxysterols (epicholesterol and androstan-3α-ol) reveal strongly reduced collapse pressures when compared with the respective 3β-hydroxysterols. All ketosteroids show high areas per molecule.

3. The force–area curves show little temperature effect.

4. The interaction of 18:1/18:0 lecithin with sterols is dependent on: (a) planar sterol nucleus, (b) 3β-hydroxy group, (c) intact side chain.

5. Besides 3β-hydroxysterols, 3α-hydroxysterols and ketosteroids can also show some condensation effect. However, minimal area per molecule can be achieved only with 3β-hydroxysterols.

6. The interaction of lecithin and sterol in monolayers is governed by a number of factors including van der Waal's interactions, but also hydrogen bonding between the 3β-hydroxy group and environment is considered to be important.

INTRODUCTION

Sterols are widely occurring compounds in many biological membranes, cholesterol being the main sterol of animal organisms. The amount of cholesterol is characteristic for a given type of membrane and appears to vary greatly between various types of biomembranes. Membranes of liver cells, erythrocytes[1] and myelin[2] contain high proportions of cholesterol (molar ratio phospholipid–cholesterol, 1:1) whereas subcellular membranes, *e.g.* mitochondria, microsomes, and nuclei[3] are low in sterol. Besides cholesterol, other unesterified sterols such as 7-dehydrocholesterol, lathosterol, cholestanol[3–5] have been detected in appreciable amounts in various tissues. Coprostanol is excreted in feces. The two saturated substances differ in the configuration of the asymmetric center C-5 at the juncture of rings A and B. In cholestanol rings A and B

are *trans*, and in coprostanol (the 5β-epimer of cholestanol) *cis* oriented. Sterols of animal origin all have the same skeletal structure of 27 carbon atoms. The principal plant sterols, typified by ergosterol and stigmasterol, are either C_{28} or C_{29} compounds. These substances have both the 3β-hydroxyl group and the 5,6-double bond characteristic of cholesterol; they differ from cholesterol in having a side chain with a double bond at C-22,23 (*trans*) and an extra alkyl group, methyl and ethyl, respectively, at C-24. Bacteria, however, generally do not require sterols as membrane constituents. Some microorganisms such as T-strain mycoplasma have to be grown on media containing sterols[6]. The sterol requirement can be met by cholesterol and β-sitosterol and, to a lesser extent, by 7-dehydrocholesterol, cholestanol, stigmasterol and ergosterol, but not by cholesterollaurate or cholestan-3-one. Coprostanol, epicoprostanol and epicholesterol even inhibited cell growth. For strain 07 of mycoplasma[7] it was observed that only cholesterol and cholestanol support growth.

Mycoplasma laidlawii strain B are not dependent on sterol and can grow on media depleted of sterols, but when added to the growth medium, sterols are incorporated into the cell membrane[8]. Recently DE KRUYFF[9] *et al.* showed that epicholesterol can be incorporated to a similar extent as cholesterol in the cell envelop of strain B mycoplasma. Similar observations have been made for the mould Pythium[10]. Incorporation of cholesterol, stigmasterol, sitosterol or ergosterol rendered mycelial discs sensitive to the sterol complexing antibiotic filipin.

Earlier studies[11] have demonstrated that aqueous dispersions of egg lecithin are able to remove cholesterol from erythrocyte membranes and that cholesterol can exchange between lecithin–cholesterol dispersions and erythrocyte ghosts. It was also shown[36] that large proportions of membrane sterol could be replaced by other lecithin solubilized sterols. In a further study, the presence of some 3-ketosteroids greatly affected the barrier properties of erythrocyte membranes[12]. Studies on monomolecular layers have been used by many investigators[13–17] to gain information on the interfacial behavior of cholesterol and the interaction of cholesterol with different phospholipid species, particularly lecithin.

In this report the interfacial properties of naturally occurring 3β-hydroxysterols, as well as some 3α-hydroxysterols and 3-ketosteroids are compared with cholesterol. To understand why some sterols are not able to replace cholesterol in the biological membrane, the interaction of the above mentioned sterols was studied with a synthetic lecithin which is known to interact with cholesterol[17]. Hormone-like compounds are not included in this study since these compounds with more than one keto and/or hydroxyl group show a completely different interfacial behavior[18].

MATERIALS AND METHODS

Sterols

The sterols were obtained from the following sources: cholest-5-en-3β-ol (cholesterol), cholest-5,7-dien-3β-ol (7-dehydrosterol), cholest-5,7,22-trien-24-methyl-3β-ol (ergosterol), cholest-4-en-3-one and 5α-cholestan-3β-ol (cholestanol) from Fluka AG, Buchs, Switzerland; cholest-5-en-3-one, 5α-cholestan-3-one and cholest-5,22-dien-24-ethyl-3β-ol (stigmasterol) from Koch-Light Laboratories, Colnbrook, Bucks, England; 5β-cholestan-3β-ol (coprostanol), cholest-3,5-dien-7-one from K. and K. Laboratories, Hollywood, Calif., U.S.A.; cholest-7-en-3β-ol (lathosterol), androstan-

3β-ol, androstan-3α-ol from Ikapharm, Ramat-Gan, Israel; cholest-4,6-dien-3-one from British Drug Houses, Poole, England; cholest-5-en-3α-ol (epicholesterol) from Mann Research Lab., New York, N.Y., U.S.A. A sample of cholest-B-nor-5-en-3β-ol (B-norcholesterol) was kindly provided by Dr. J. Joska (Institute of Organic Chemistry and Biochemistry, Prague, Czechoslovakia). The chemical structure of most of these sterols is given in a previous paper[12]. The steroids were examined on Silica gel plates with a light petroleum (b.p. 40–60°)–ether-formic acid (50:50:2, by vol.); chloroform–acetone (98:2, by vol.); benzene–ethyl acetate (5:1, by vol.); cyclohexane–ethyl acetate–water (600:400:1, by vol.); solvent systems and also checked by gas–liquid chromatography[12]. A high degree of purity was indicated in most samples. Those requiring purification were recrystallized several times from ethanol or purified by preparative thin-layer chromatography.

Phospholipid

1-Oleoyl-2- stearoyl-*sn*-glycero-3-phosphorylcholine (18:1/18:0 lecithin) was synthesized according to established procedures[19]. The purity was always verified by thin-layer chromatography with chloroform–methanol–water (65:35:4, by vol.) as developers. The fatty acid distribution was checked by degradation with phospholipase A. The lipid constituent at the 2-position was found to be exclusively stearic acid.

Determination of force area curves

Force–area measurements were performed at the air–water interface in a paraffin-coated silica trough, 60 cm long × 14 cm wide. The total capacity of the trough was 1500 ml. The trough was filled with unbuffered water (pH 5.4) that had been distilled from alkaline permanganate and then redistilled. The aqueous surface was swept clean with a teflon bar. Surface pressures were determined with a conventional Langmuir–Adam surface balance[20]. Known amounts of pure lipids dissolved in chloroform were carefully released onto the air–water interface from an Agla micrometer syringe. Mixed lecithin–sterol monolayers were formed from premixed solutions. The initial area was 560 cm^2. This area was then compressed and the surface pressure determined. The measurement of an entire force–area curve took about 10–15 min. Experiments were carried out at room temperature (22°) and physiological temperature (37°). The trough was enclosed in a chamber containing nitrogen in an attempt to prevent the possibility of oxidation. Collapse pressures were additionally checked by the Wilhelmy plate method. For mixed films, the surface pressure was plotted against the mean area per molecule, that is, the total area divided by the total number of sterol and phospholipid molecules at the air–water interface. For a quantitative evaluation of the interaction, the variation of the mean molecular area at a given constant surface pressure was plotted as a function of the mole fraction according to the Goodrich method[15,17]. In the data given, the molecular cross-sections concern a pressure of 12 dynes/cm. In general, equivalent results were found at other surface pressures, though of course some quantitative difference may exist.

RESULTS

Fig. 1 gives the surface characteristics of 3β-hydroxysterols at 22°. The pressure–area plots show a very steep incline indicating a perpendicular orientation at all

surface pressures[16]. The deflection at high pressures indicates the collapse pressure. Cholestanol, cholesterol, lathosterol and 7-dehydrocholesterol (fig. 1A) show a pressure increase between 39,5 and 36.7 Å²/molecule and high collapse pressures between 35 and 40 dynes/cm. These sterols are naturally occurring animal sterols with a saturated ring system, a C-5,6 double bond, a C-7,8 double bond and C-5,6–C-7,8 double

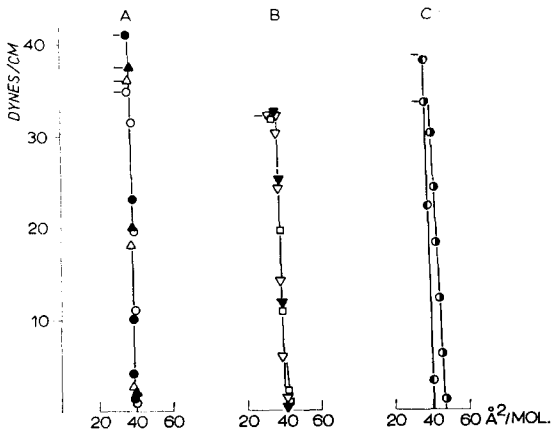

Fig. 1. Force area characteristics of various 3β-hydroxysterols at 22°. A. ▲, cholesterol ○, cholestanol; ●, lathosterol; △, 7-dehydrocholesterol; B. □, ergosterol; ▼, stigmasterol; ▽, androstan-3β-ol; C. ◐, B-norcholesterol; ◑, coprostanol.

bond, respectively. Fig. 1B compiles results of sterols with changed side chain structures. The plant sterols, stigmasterol and ergosterol with double bonds at the C-5,6 and C-5,6–C-7,8 positions, respectively also have an extra double bond at C-22, 23 as well as an ethyl or methyl group at C-24. The area per molecule is, compared with the animal sterols (Fig. 1A), slightly increased at low surface pressures whereas the collapse pressure is reduced to about 32 dynes/cm. Androstan-3β-ol (Fig. 1B) has a saturated, trans oriented ring system, but lacks the eight-carbon side chain. The interfacial behavior of this compound resembles that of the plant sterols. Fig. 1C compares the effect of sterols with changed B ring structure. In B-norcholesterol one CH_2 group at C-8 is omitted so that a five carbon atom B ring is formed. The surface properties of this compound are, however, in agreement with those found for cholesterol. Coprostanol, detected in feces but not in animal cell membranes, has a cis oriented A/B ring structure which is reflected by an increased area per molecule of 3 to 7 Å² at high and low pressures, respectively. Also the collapse pressure is lower than found for cholesterol. 3α-Hydroxysterols (Fig. 2A) show practically the same orientation and pressure–area dependence at the air–water interface as 3β-hydroxysterols. However, the collapse pressure is considerably reduced: compare cholesterol *vs.* epicholesterol and androstan-3β-ol *vs.* androstan-3α-ol In Fig. 2B and 2C results are compiled of sterols where the hydroxy group is replaced by a keto group. All ketosteroids studied show remarkable increases in the area per molecule which are most significant for cholest-4-en-3-one cholest-5-en-3-one and cholest 4,6-dien-3-one. The collapse pressures of the ketosteroids are well below 30 dynes/cm and vary from 20–28 dynes/cm. It is worth noting that it was not possible to form monolayers of cholest-5-en-chloride. The polarity of this compound is too small to orient the molecule at the interface. In Table I the date for

the collapse pressure are summarized. High collapse pressures are noted for the 3β-hydroxysterols (32–41 dynes/cm), whereas the collapse pressures of the 3α-hydroxysterols (12–26 dynes/cm) and 3-ketosteroids (20–28 dynes/cm) are considerably lower. In Table I also the area per molecule, at an intermediate pressure of 12 dynes/cm, are compared at 22° and 37°. At 20° the area per molecule for 3β-hydroxysterols with a planar sterol nucleus varies from 39.5 to 36.5 Å². The area occupied by coprostanol, having a

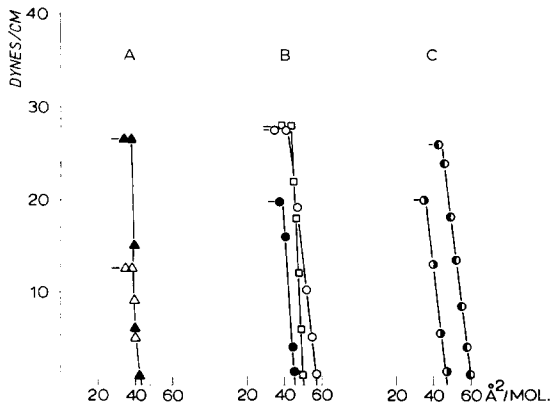

Fig. 2. A. Force area characteristics of 3α-hydroxysterols at 22°. ▲, epicholesterol; △, androstan-3α-ol. B. Force area characteristics of various ketosteroids at 22°. ●, cholestan-3-one; ○, cholest-4-en-3-one; □, cholest-5-en-3-one. C. Force area characteristics of various ketosteroids at 22°. ◐, cholest-4,6-dien-3-one; ◑, cholest-3,5-dien-7-one.

TABLE I

COLLAPSE PRESSURES OF 3β-HYDROXYSTEROLS AND KETOSTEROIDS AT 22°.

Area per molecule of these sterols at a pressure of 12 dynes/cm at 22° and 37°.

	Sterol	*Collapse pressure (dynes/cm) 22°*	*Å²/molecule at 12 dynes/cm*	
			22°	*37°*
3β-Hydroxy	Cholesterol	37.2	39.0	39.5
	Cholestanol	34.7	39.5	42.0
	Lathosterol	41.0	38.5	39.4
	7-dehydrocholesterol	36.1	36.5	37.7
	Ergosterol	31.7	38.5	39.2
	Stigmasterol	32.7	39.3	40.6
	Androstan-3β-ol	32.0	38.0	39.0
	B-norcholesterol	38.6	39.0	39.8
	Coprostanol	33.6	43.8	45.8
3α-Hydroxy	Epicholesterol	26.5	40.2	44.0
	Androstan-3α-ol	12.5	38.5	39.0
Keto	Cholestan-3-one	19.8	42.0	42.9
	cholest-4-en-3-one	27.4	50.0	52.0
	cholest-5-en-3-one	28.0	47.2	53.8
	Cholest-4,6-dien-3-one	26.1	52.5	53.0
	Cholest-3,5-dien-7-one	20.1	40.0	40.5

TABLE II

MEAN MOLECULAR AREA OF 18:1/18:0 LECITHIN–STEROL MIXTURES 1:1 MOLAR RATIO IN Å² AT 22° AND 37°.

Deviation from the simple additivity rule in Å² at 22° and 37°.

	Sterol	*Mean area/molecule (lecithin–sterol, 1:1)*		*Deviation from ideal behavior (Å²)*	
		22°	*37°*	*22°*	*37°*
3β-Hydroxy	Lecithin only	78.0	82.1		
	Cholesterol	45.7	48.9	12.3	11.5
	Cholestanol	48.0	50.8	10.0	11.2
	Lathosterol	45.5	50.8	12.5	9.9
	7-dehydrocholesterol	45.0	49.9	12.5	10.1
	Ergosterol	50.0	52.0	8.7	7.8
	Stigmasterol	49.2	54.0	9.5	7.2
	Androstan-3β-ol	58.0	59.5	0	0
	B norcholesterol	49.0	51.0	10.0	10.0
	Coprostanol	52.8	58.0	7.7	5.5
3α-Hydroxy	Epicholesterol	51.1	60.0	6.9	3.0
	Androstan-3α-ol	58.5	60.5	0	0
Keto	Cholestan-3-one	51.3	56.2	8.7	5.8
	Cholest-4-en-3-one	56.7	66.4	7.8	0.3
	Cholest-5-en-3-one	50.1	57.0	12.9	10.5
	Cholest-4,6-dien-3-one	58.2	67.0	6.6	0
	Cholest-3,5-dien-7-one	55.1	66.0	4.0	0

cis-oriented sterol nucleus, is 43.8 Å². The area per molecule of the 3α-hydroxysterols is not much different from that found for 3β-hydroxysterols. Some of the ketosteroids show a larger area up to 52.5 Å². A temperature increase of 15° has only a small effect on the area per molecule (0.5–2.5 Å²) of the 3β-hydroxysterols and ketosteroids. This is in agreement with observations of PETHICA[16] who found an area increase of 1.2 Å² for cholesterol for 15°.

A significantly greater area increase was noted for epicholesterol (3.8 Å²) and cholest-5-en-3-one (5.6 Å²). Table II summarises the interaction data of sterols with 18:1/18:0 lecithin. The interfacial behavior and the interaction with cholesterol of this lecithin is essentially the same as for the more natural compound 18:0/18:1 lecithin[21]. Also for the structural isomers 16:0/18:2 lecithin and 18:2/16:0 lecithin no significant differences were noted[22]. The increase in area per molecule of 18:1/18:0 lecithin exhibiting a liquid expanded type of film, is over a range of 15° only 4.1 Å² at a pressure of 12 dynes/cm. At least five different lecithin–sterol ratios were tested *viz.* lecithin mole fractions of 0, 0.2, 0.4, 0.5, 0.6, 0.8, and 1.0. The spreading of each curve was repeated 3 times using different amounts of spreading solvent. In Figs. 3A, 3B, 3C, and 3D the effects of respectively cholesterol, ergosterol, epicholesterol, and cholest-4,6-dien-3-one on mixed films with 18:1/18:0 lecithin at a pressure of 12 dynes/cm and 37° are given. The observed mean molecular area of a mixed monolayer molar ratio lecithin–sterol 1:1, at a pressure of 12 dynes/cm are given in columns 1 and 2 of Table II. In columns 3 and 4 the deviation from ideal behavior is given, indicating the

reduction in mean area per molecule. At 22° the mean molecular area of mixed films containing cholesterol, cholestanol, lathosterol and 7-dehydrocholesterol, varies from 44.0 to 48.0 Å² and at 37° from 48.9 to 50.8 Å². At both temperatures these 3β-hydroxysterols show a remarkable interaction with 18:1/18:0 lecithin indicated by

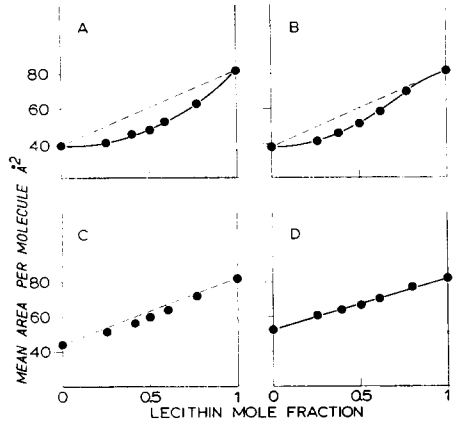

Fig. 3. Variation of the mean molecular area as a function of the composition for mixed monolayers of 1-oleoyl-2-steroyl-*sn*-glycero-3-phosphorylcholine and, respectively, A, cholesterol; B, ergosterol; C, epicholesterol; D, cholest-4,6-dien-3-one, at a pressure of 12 dynes/cm and 37°.

a reduction in area per molecule of 12.5 to 9.9 Å². Corresponding results were observed for B-norcholesterol. The plant sterols ergosterol and stigmasterol show a slightly increased mean area per molecule and at physiological temperature a less pronounced reduction in mean area per molecule, 7.2–7.8 Å². Androstan-3β-ol shows a high mean molecular area and no reduction in area per molecule at 22° or at 37°. Coprostanol shows a significant effect at 22° but at 37° the mean area per molecule is higher than for the other 3β-hydroxysterols, 58.0 *vs.* 50.8, and the reduction in area is less marked.

Of the 3α-hydroxysterols androstan-3α-ol shows no interaction. Epicholesterol still shows some effect at 22° but little at 37°. The 3α-hydroxysterols show especially at physiological temperature a remarkably high mean molecular area. The ketosteroids reduce the area per molecule at room temperature and to some extent also at physiological temperature; but it should be noted that at 37° the mean area per molecule is significantly higher for the ketosteroids (56.2–67.0) than for cholesterol, cholestanol, lathosterol, or 7-dehydrocholesterol (48.9–50.8).

DISCUSSION

The data on the interfacial properties of 3β-, 3α-hydroxysterols and ketosteroids show that the 3β-hydroxysterols, *viz.* cholesterol, cholestanol, lathosterol, 7-dehydrocholesterol and B-norcholesterol are perpendicularly oriented at all surface pressures and exhibit high collapse pressures (Figs. 1A, 1C), 3β-Hydroxysterols with a changed side chain structure, *viz.* the plant sterols stigmasterol and ergosterol as well as androstan-3β-ol, the latter lacking the side chain, show slightly increased area per molecule at low surface pressures and reduced collapse pressures (Fig. 1B). The nonplanar nucleus of the *cis* A/B structured coprostanol causes an increase in the area per

molecule as well as a reduction in collapse pressure (Fig. 1C). 3α-Hydroxysterols, *viz.* epicholesterol and androstan-3α-ol, show essentially the same orientation and at pressures above 5 dynes/cm they occupy the same area per molecule as the respective 3β-hydroxysterol (Fig. 2A). The collapse pressure of the 3α-hydroxysterols is, however, significantly reduced. This may indicate a different extent of hydration of the polar part and orientation of the surrounding water molecules. The ketosteroids all show increased area per molecule and reduced collapse pressures (Figs. 2B, 2C). These properties of the ketosteroids can be explained by the reduced polarity of the keto moiety. The temperature dependence of the studied sterols is small with the exception of epicholesterol and cholest-5-en-3-one (Table I).

The data on the interaction of 3β-, 3α-hydroxysterols and ketosteroids with 18:1/18:0 lecithin show that the 3β-hydroxysterols, *viz.* cholesterol, cholestanol, lathosterol, 7-dehydrocholesterol and B-norcholesterol interact to a significant extent with this lecithin (Table II). The interaction is indicated by the deviation from the simple additivity rule (also defined as condensation effect). The mean molecular area of the above-mentioned sterol–lecithin mixtures is reduced to extremely low values. At a molar ratio of 1:1 (37° and a pressure of 12 dynes/cm) the mean molecular area is 48.9–51.0 Å^2. The limiting area for the sterol under these circumstances is 37.7–42.0 Å^2 for the pure lecithin film 82.1 Å^2. To acquire this mean molecular area, van der Waals' interaction forces are thought to be of significant importance[17]. This opinion is supported by X-ray data which show that the thermal motion of the paraffin chains is restricted by the presence of cholesterol[23] and by NMR data which show a reduction in the signal due to the polymethylene chains when cholesterol is added[24]. Also more recent techniques such as ESR lead to the same conclusion. It was demonstrated that cholesterol reduces the motional freedom of the spin label in hydrated egg lecithin and dipalmitoyl lecithin multilayers[25–27]. The demonstrated interaction is also reflected in the barrier properties of lecithin–sterol membranes. Liposomes showed a drastic reduction in permeability for glucose[28], glycerol, and erythritol[29], black lipid membranes a reduction in water permeability[30] when cholesterol is present.

On the basis of the monolayer data it can be demonstrated that both at 22° and at 37°, cholestanol, lathosterol, 7-dehydrocholesterol and B-norcholesterol are as effective as cholesterol in achieving a minimal mean molecular area. The mixtures of 18:1/18:0 lecithin with the plant sterols ergosterol and stigmasterol show a slightly higher mean molecular area and especially at 37° a smaller condensation effect. The less effective interaction in monomolecular layers must be due to the double bond at C-22, 23 and the extra methyl and ethyl group respectively at C-24. The sterol lacking the eight carbon atom side chain, *viz.* androstan-3β-ol, shows no condensation effect and a high mean molecular area, 58.0 Å^2 at 22° and 59.5 Å^2 at 37°. The interfacial properties of this sterol have been shown to be identical to that of the plant sterols, which do show an interaction. The smaller molecule, androstan-3β-ol would be expected to act as an excellent space filler. SHAH AND SCHULMAN[13] and SHAH[31] stated that the deviation in molecular area in mixed monolayers is not due to an interaction but rather to steric factors, such as unsaturation and length of the hydrocarbon chains of lecithin. Molecules with dimensions smaller than or equal to those of the cavity added to the lecithin monolayer are thought to occupy these cavities without causing a proportional increase in the area of the monolayer[31]. The results with androstan-3β-ol

clearly demonstrate that the condensation effect cannot be explained simply by steric factors only, but that interaction forces are involved as stated already for the 3β-hydroxysterols above. CADENHEAD AND PHILLIPS[32] also concluded from the ability of cholesterol to erect a horizontally oriented estradiol acetate film, that molecular interactions play a significant role in condensation effects. Coprostanol occupying a higher area per molecule than the other 3β-hydroxysterols studied still shows some condensation effect; however, the mean molecular area is significantly increased. The condensation effect does result in only a slight effect on the motional freedom of the paraffin chains in ESR studies[27]. This result is in agreement with the observed high mean molecular area. The 3α-hydroxysterol, epicholesterol shows an interaction at room temperature, but at physiological temperature the effect is minimal whereas the mean molecular area is high. For androstan-3α-ol no interfacial effects could be measured (Table II). D.s.c. measurements showed that cholesterol has dramatic effects on the phase transition of lecithin[34]. This phase transition is characteristic for the conversion of lecithin from the Lβ crystalline phase to the Lα liquid crystalline phase. Both 3α-hydroxysterols, epicholesterol[9] and androstanol[9,34] showed only a slight effect on the phase transition of lecithin.

For the ketosteroids studied varying condensation effects are observed at room temperature. It has to be noted that the observed mean molecular area are significantly higher than for the 3β-hydroxysterols. At 37° only cholestan-3-one and cholest-5-en-3-one still show a condensation effect, but even these two sterols show high area per molecule. KWONG *et al.*[33] found a condensing effect for cholesterol acetate, but not for other cholesterol esters. ESR studies showed that cholesterol derivatives with a methoxy, carbonyl oxygen or chloro substituents at the 3-position of the nucleus[25,27] contribute little to increase the rigidity of the alkyl chains. The effects on the spin label are in agreement with the presented interfacial studies. Although some ketosteroids still show a condensation effect, the observed mean molecular area are too high to effect the fluidity of the alkyl chains. BRUCKDORFER *et al.*[12] showed that after replacement of part of the erythrocyte membrane cholesterol by cholestan-3-one, cholest-4-en-3-one, cholest-4,6-dien-3-one, respectively, the membrane permeability for glycerol was increased. The permeability of egg lecithin liposomes for glucose was not influenced by the presence of cholesterol acetate[28]. In a following paper the effects of 3α-, 3β-hydroxysterols and ketosteroids on the permeability properties of egg lecithin liposomes will be described. Finally it can be concluded that for a condensation effect and a minimal mean molecular area, a planar sterol nucleus with an intact side chain and a 3β-hydroxy group are required. Coprostanol, 3α-hydroxysterols and ketosteroids show especially at lower temperature condensation effects. However, the mean molecular area of mixtures of these sterols and lecithin are significantly higher than for 3β-hydroxysterols. Monolayer, NMR, X-ray and ESR studies, indicate that van der Waals' forces are involved in lecithin–cholesterol interaction. Also hydrogen bonding between the 3β-hydroxy group and oxygen anchored to phosphorus or with water molecules are considered to be important since a 3β-hydroxy group is required for a maximal effect. ESR studies by LONG *et al.*[25] lead to the same conslucion. Earlier studies already pointed to this possibility. X-ray data by RAND AND LUZZATI[23] showed that part of the cholesterol nucleus is located between the polar groups of the phospholipid molecules, while infrared studies[35] indicated that the hydroxy group of cholesterol is inserted into the polar region of the lecithin molecule.

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