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THE EFFECT OF STEROL STRUCTURE ON THE PERMEABILITY OF LIPOMES TO GLUCOSE, GLYCEROL AND Rb^+

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SUMMARY

1. The effect of 3β-, 3α-hydroxysterol and ketosteroids on the permeability properties of (egg lecithin) liposomes towards glucose, glycerol and Rb^+ has been studied.

2. The 3β-hydroxysterols, cholesterol, cholestanol, lathosterol, 7-dehydrocholesterol and B-norcholesterol affect the most pronounced reduction in permeability of glucose, glycerol and Rb^+.

3. The plant sterols, ergosterol and stigmasterol are less effective whereas compounds lacking the side chain (androstan-3β-ol) or with a non-planar sterol nucleus (coprostanol) show no effect.

4. The 3α-hydroxysterols, epicholesterol and androstan-3α-ol reveal no significant effect on the permeability.

5. The ketosteroids either do not affect the permeability or increase the permeability of the lipid barrier.

6. The reduction in permeability, as found for cholesterol, is dependent on: (a) planar sterol nucleus, (b) 3β-hydroxy group, (c) intact side chain.

7. The effect of sterols on the permeability properties of liposomes are in good agreement with the effect on the mean molecular area measured in monolayers, and the effect of these sterols on biological membranes such as erythrocytes and Mycoplasma.

INTRODUCTION

It is well known that cholesterol is abundant in many biological membranes. High proportions are found in the cell envelopes of liver and erythrocytes[1] as well as in myelin sheath[2]. Smaller proportions are found in subcellular membranes such as mitochondria, microsomes and nuclei[3]. It is not yet understood why cholesterol is such a unique membrane substituent. Besides cholesterol only sterols such as 7-dehydrocholesterol, lathosterol and cholestanol are recorded in animal cells so far[3–5]. In plant cells particular sterols typified by ergosterol and stigmasterol are found. Some Mycoplasma strains can utilize a variety of sterols as membrane constituents. T-strain Mycoplasma[6] can incorporate cholesterol and β-sitosterol and, to a lesser extent, 7-dehydrocholesterol, stigmasterol, ergosterol and cholestanol. For strain 07 of Mycoplasma[7] it was observed that only cholesterol and cholestanol support growth. In *Mycoplasma laidlawii* strain B cells[8], besides cholesterol also epicholesterol was

incorporated into the cell membrane. However, the incorporation of epicholesterol, in contrast to cholesterol, does not result in a reduced permeability for a number of solutes. In erythrocytes part of the membrane cholesterol can be exchanged by several 3β-hydroxysterols and ketosteroids[9]. Incorporation of some ketosteroids resulted in an increased permeability for glycerol through the erythrocyte membrane. In a previous paper[10] on the interaction of sterols with 18:1/18:0 lecithin in monolayers, it was shown that optimal effects are observed with sterols having a planar sterol nucleus, an intact side chain and a 3β-hydroxy group. Earlier studies have shown that the interaction measured in monomolecular films[11] is reflected in reduced permeability properties of liposomes[12,13]. In this paper the effect of 3β-, 3α-hydroxysterols and ketosteroids on the permeability of egg lecithin liposomes to glucose, glycerol and Rb^+ is studied and compared with the properties of mixed monomolecular films. The effects of hormone-like compounds[23,24] are not included in this study since those compounds with more than one keto and/or hydroxyl group show a completely different interfacial behaviour.

MATERIALS AND METHODS

Sterols

Sterols were obtained from the same sources and purified according to methods denoted in the preceding paper[10]. Sterols were estimated by gas-liquid chromatography[9]; only 7-dehydrocholesterol was estimated from its ultraviolet absorption spectrum in ethanol. The chemical structure of most of these sterols is given in a previous paper[9].

Phospholipid

Egg lecithin was isolated according to established procedures[14]. The purity was routinely examined by chromatography on silica gel plates with chloroform–methanol–water (65:35:4, by vol.). Phosphatidic acid was obtained from egg lecithin by degradation with a crude phospholipase D preparation obtained from Savoy cabbage.

Measurement of glucose permeability

For the preparation of liposomes 2 μM of egg lecithin, 0.1 μM of phosphatidic acid and, respectively, 0, 0.1, 0.28, 1.0, and 2.0 μM sterol, dissolved in chloroform were taken to dryness under vacuum. 0.2 ml of 0.3 M glucose was added to the dried lipid film, whereupon the film was dispersed on a vortex mixer under a N_2 atmosphere. Most untrapped glucose was dialysed away against 300 ml of a solution containing 0.075 M KCl and 0.075 M NaCl. The remaining untrapped glucose was estimated by measuring the absorption at 340 nm in a system containing: Tris buffer, pH 8, magnesium acetate, ATP, NAD^+, hexokinase and glucose-6-phosphate dehydrogenase. In a control cuvette the enzymes were omitted. The reaction was started by the addition of 10 μl of liposome preparation. The total amount of glucose was estimated by measuring the absorption at 340 nm after lysing the liposomes by the addition of Triton X-100. The difference in absorption before and after the addition of Triton indicates the amount of glucose trapped in the liposome. After incubating the liposomes for 1 h at 40°, the remaining trapped glucose was assayed

again. From the glucose trapped before and after incubation, the percent maximum glucose released can be estimated. Further details of the assay are described elsewhere[12]. The presented data are the average of at least three experiments. Most experiments agreed within 5 %.

Measurements of glycerol permeability

Because bimolecular lipid layers are fairly impermeable to inorganic salts, the liposomes behave like ideal osmometers and the osmotic swelling has been found to obey the Boyle–van 't Hoff law[15]. The liposome preparations to which the present experiments refer were prepared in 50 mM KCl from egg lecithin to which 4 mole % phosphatidic acid is added. The final lipid concentration was 15 mM. 50 μl of liposome solution was added to 5 ml of an isotonic solution of the permeating glycerol. The rate of the subsequent volume increase is proportional to the penetration rate of glycerol, assuming that the permeability constant of glycerol is much lower than for water. The swelling process of liposomes is complicated by the fact that, with time, more and more bimolecular layers become involved, but at the beginning of the swelling process there is only a concentration gradient over the first barrier. Therefore, the initial swelling rate is considered to give a fair indication of the penetration rate through the outer lipid barrier. The changes in absorption were measured at 450 nm[15].

Measurement of $^{86}Rb^+$ permeability

The original procedure was described by BANGHAM, STANDISH AND WATKINS[16]. Liposomes of egg lecithin and 4 mole % phospatidic acid were prepared in a 50 mM KCl/^{86}RbCl solution. The original radioactivity was approx. $5 \cdot 10^5$ counts/min. The liposomes were freed of untrapped label by passing the liposomes over a G_{50} Sephadex column and subsequently dialysed against 50 mM KCl solution. 0.5 ml quantities of liposomes were transferred to dialysis bags contained in stirred tubes of 10 ml 50 mM KCl and kept at the respective temperature. After 1 h 1.9 ml of the dialysis solution was counted as well as the remaining radioactivity in the dialysis bag. The radioactivity of the aqueous suspensions was determined in a Packard Tricarb liquid scintillation counter employing a dioxane counting solution containing 0.3 g of dimethyl POPOP, 7 g of PPO and 100 g naphtalene per l of dioxane.

RESULTS

The data compiled in Table I show that the 3β-hydroxysterols, *viz.*, cholesterol, cholestanol, lathosterol, B-norcholesterol and coprostanol are well incorporated in egg lecithin liposomes (containing phosphatidic acid) up to molar ratios of approximately 1. 7-Dehydrocholesterol is incorporated to a lesser extent. In the absence of phosphatidic acid, more lipid material is centrifuged down at 48000 $\times$ *g* but the ratio of lecithin:sterol is essentially the same as in the presence of 4 mole % of phosphatidic acid. The plant sterols ergosterol and stigmasterol are more poorly solubilized by egg lecithin liposomes than animal sterols. The 3α-hydroxysterols and keto steroids,*viz.*, epicholesterol, cholest-4,6-dien-3-one, cholestan-3-one, cholest-4-en-3-one and cholest-5-en-3-one are solubilized to a molar ratio of steroid: lecithin of 0.30 to 0.59. This is about 1/3 to 1/2 of the amount solubilized by the respective

TABLE I

THE SOLUBILIZATION OF STEROLS BY SONICATION WIHT EGG LECITHIN

Mixtures of lecithin (10 mg) and one sterol (6 mg) were sonicated in 6 ml 30 mM saline for 7 min and centrifuged at 48000 × *g* for 1 h. The phospholipid and sterol contents were analysed in the lipid extracts.

Dispersed sterol	*Molar ratio sterol:lecithin*
Cholesterol	1.04:1
Cholestanol	1.11:1
Lathosterol	1.07:1
7-Dehydrocholesterol	0.55:1
Ergosterol	0.35:1
Stigmasterol	0.57:1
Androstan-3β-ol	0.72:1
B-Norcholesterol	1.16:1
Coprostanol	1.04:1
Epicholesterol	0.30:1
Androstan-3α-ol	0.58:1
Cholestan-3-one	0.43:1
Cholest-4-en-3-one	0.59:1
Cholest-5-en-3-one	0.57:1
Cholest-4,6-dien-3-one	0.55:1

3β-hydroxysterols. Remarkable high proportions of the side chain lacking sterols (androstan-3β-ol and androstan-3α-ol) are solubilized by lecithin. The ratios presented in Table I are found to be essentially the same for sonicated and unsonicated liposomes. The osmotic behaviour of the liposomes was not changed by the presence of different sterols. Currently the incorporation of sterols in lecithin liposomes is under investigation by X-ray diffraction. It is found that up to 25 mole % of epicholesterol is homogeneously distributed in the lipid bilayer, which is in agreement with the ratio found by the centrifugation procedure. Epicholesterol which is incorporated in liposomes to a lesser extent than cholesterol (Table I), is incorporated in *Mycoplasma laidlawii* membranes to the same extent as cholesterol[8]. Fig. 1A, B, and C illustrates the effect of 3β-hydroxysterols on the glucose release from liposomes prepared with egg lecithin. Incorporation of increasing amounts of animal sterols such as cholesterol[12], cholestanol, lathosterol and 7-dehydrocholesterol (Fig. 1A) results in a practically linear decrease of the glucose permeability. The glucose release of pure egg lecithin liposomes after incubation for 1 h at 40° is 30–33 % of the marker originally trapped. Addition of 50 mole % of the above mentioned sterols reduces this release to 1–8% of the amount trapped.

Incorporation of plant sterols such as ergosterol and stigmasterol also results in a reduction of the glucose permeability (Fig. 1B). The maximal obtainable effect on the glucose permeability is, however, much less than for the animal sterols. Androstan-3β-ol which lacks the eight carbon side chain has no measurable effect on the glucose permeability (Fig. 1B). Also the A/B ring *cis*-structured coprostanol (Fig. 1C) shows no significant effect on the permeability. B-norcholesterol (Fig. 1C) having a planar sterol nucleus, but missing one CH_2 at C-8, shows a permeability-reducing effect comparable with that obtained for the animal sterols (Fig. 1A).

Figs. 2A, B, and C shows the effects of 3α-hydroxysterols and ketosteroids on

the glucose release from egg lecithin liposomes. The 3α-hydroxysterol epicholesterol (Fig. 2A) has only an insignificant effect on the glucose release whereas the respective 3β-hydroxy compound, cholesterol, shows the most pronounced effect (Fig. 1A). As also expected the 3α compound lacking the side chain, *viz.*, androstan-3α-ol, shows no permeability reducing effect. None of the keto steroids bring about a reduction in permeability whereas cholest-7-en-3-one, cholest-4,6-dien-3-one and cholest-3,5-dien-7-one can even significantly increase the permeability of glucose (Figs. 2B and C).

Figs. 3 and 4 compile results on the glycerol swelling rate of egg lecithin liposomes and of liposomes formed from equimolar amounts of egg lecithin and various

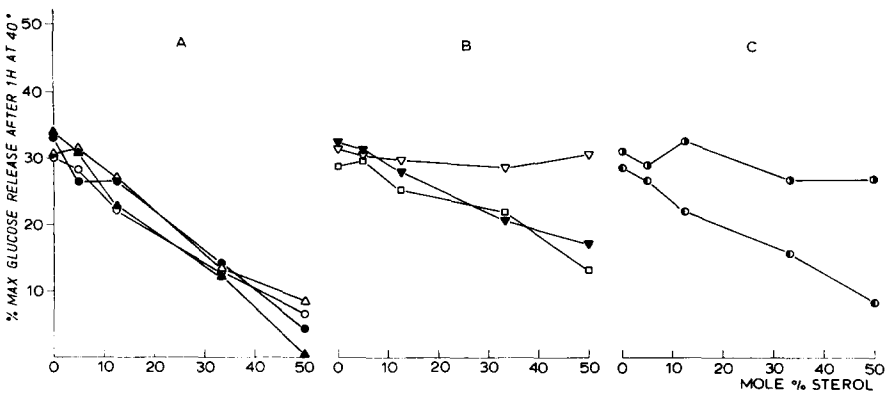

Fig. 1. Effect of 3β-hydroxysterols in liposomes prepared with egg lecithin on the relative amount of glucose released after 1 h at 40°. Liposomes are prepared from egg lecithin and 4 mole % phosphatidic acid with varying amounts respectively of: A. ▲, cholesterol; ○, cholestanol; ●, lathosterol; △, 7-dehydrocholesterol; B. □, ergosterol; ▼, stigmasterol; ▽, androstan-3β-ol; C. ◑, coprostanol; ◐, B-norcholesterol.

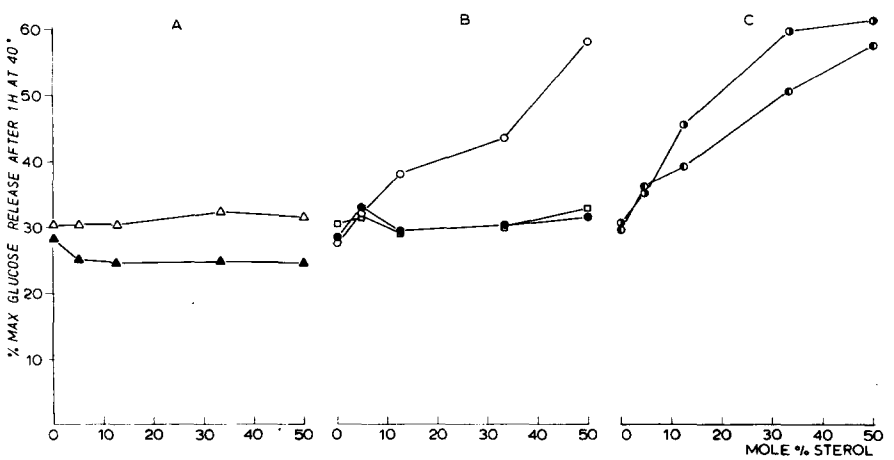

Fig. 2. Effect of 3α-hydroxysterols and ketosteroids in liposomes prepared with egg lecithin on the relative amount of glucose released in 1 h at 40°. Liposomes were prepared from egg lecithin and 4 mole % phosphatidic acid with varying amounts respectively of: A. ▲, epicholesterol; △, androstan-3α-ol; B. ●, cholestan-3-one; ○, cholest-4-en-3-one; □, cholest-5-en-3-one; C. ◐, cholest-4,6-dien-3-one; ◑, cholest-3,5-dien-7-one.

sterols at different temperatures. Glycerol being a smaller solute than glucose permeates much faster and has to be studied at lower temperatures. The observed effects on the glycerol swelling rate are qualitatively in agreement with the effects on the glucose release. Again the addition of the animal sterols, cholesterol, cholestanol, lathosterol and 7-dehydrocholesterol bring about a striking reduction in glycerol

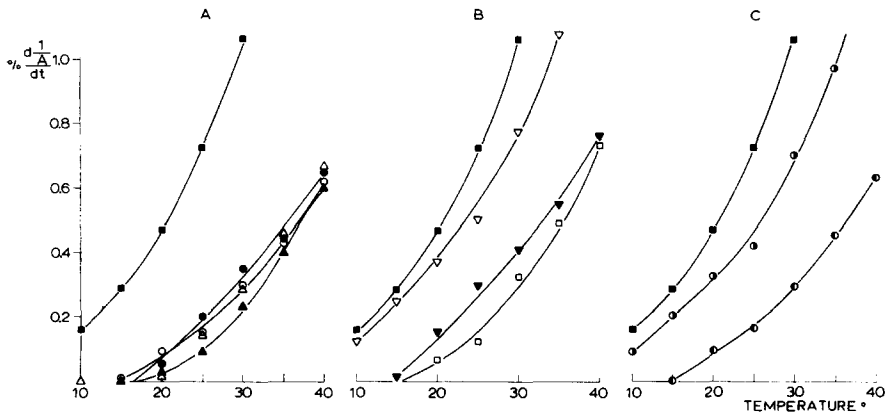

Fig. 3. Effect of 3β-hydroxysterols in liposomes prepared with egg lecithin on the initial swelling rate in isotonic glycerol in relation to temperature. Liposomes are prepared from egg lecithin and 4 mole % phosphatidic acid with 50 mole % (mole ratio lecithin to sterol, 1 : 1), respectively of: A. ▲, cholesterol; ○, cholestanol; ●, lathosterol; △, 7-dehydrocholesterol; B. □, ergosterol; ▼, stigmasterol; ▽, androstan-3β-ol; C. ◑, coprostanol; ◐, B-norcholesterol. ■ Denotes the swelling rate of egg lecithin liposomes in the absence of sterol.

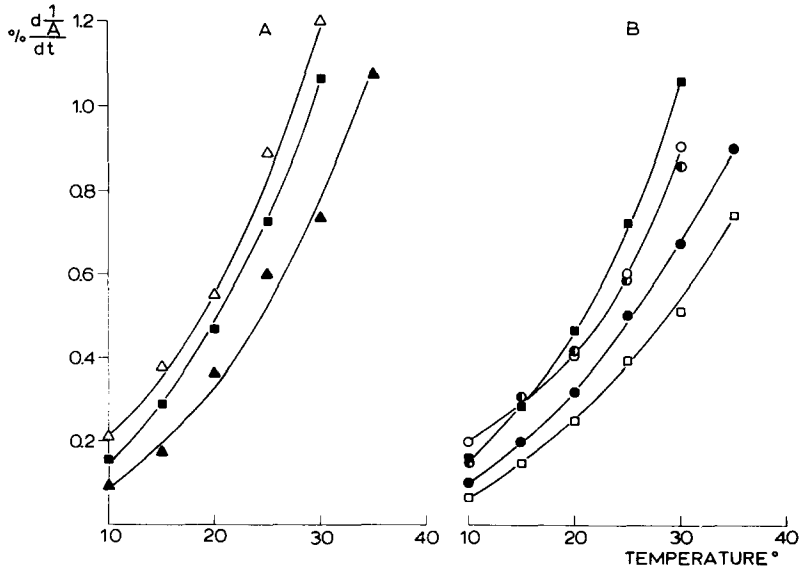

Fig. 4. Effect of 3α-hydroxysterols and ketosteroids in liposomes prepared with egg lecithin on the initial swelling rate in isotonic glycerol in relation to temperature. Liposomes are prepared from egg lecithin and 4 mole % phosphatidic acid with 50 moles % (mole ratio lecithin to steroid, 1 : 1), respectively of: A. ▲, epicholesterol; △, androstan-3α-ol; B. ●, cholestan-3-one; ○, cholest-4-en-3-one; □, cholest-5-en-3-one; ◐, cholest-4,6-dien-3-one. ■ Denotes the swelling rate of egg lecithin liposomes in the absence of sterol.

permeability at all temperatures between 10 and 40° (Fig. 3A). The plant sterols ergosterol and stigmasterol (Fig. 3B) also affect the permeability of egg lecithin liposomes but to a somewhat lesser extent than the animal sterols. The glycerol swelling rate is not significantly affected by androstan-3β-ol and coprostanol (Fig. 3B, 3C). These sterols were also unable to affect the glucose permeability as demonstrated in Figs. 1B and C. The presence of B-norcholesterol (Fig. 3C) restricts the glycerol permeability to values which were also denoted for cholesterol, cholestanol, lathosterol or 7-dehydrocholesterol.

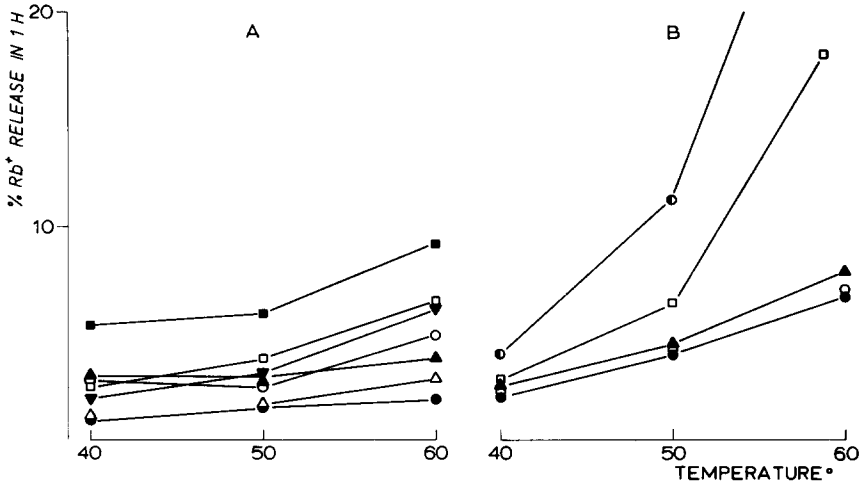

Fig. 5. The effect of 3β-, 3α-hydroxysterols and ketosteroids in liposomes prepared with egg lecithin on the $^{86}Rb^+$ release after 1 h in relation to temperature. Liposomes are prepared from egg lecithin and 4 mole % phosphatidic acid with 50 mole % (mole ratio lecithin to steroid, 1:1), respectively of: A. ▲, cholesterol; ○, cholestanol; ●, lathosterol; △, 7-dehydrocholesterol; □, ergosterol; ▼, stigmasterol; B. ▲, epicholesterol; ●, cholestan-3-one; ○, cholest-4-en-3-one; □, cholest-5-en-3-one; ◐, cholest-4,6-dien-3-one. ■ Denotes the $^{86}Rb^+$ release of egg lecithin liposomes in the absence of sterol.

3α-Hydroxysterols, *viz.*, epicholesterol and androstan-3α-ol do not appreciably affect the swelling rate of egg lecithin liposomes in glycerol (Fig. 4A). Of the ketosteroids cholest-4-en-3-one and cholest-4,6-dien-3-one do not alter the permeability for glycerol (Fig. 4B). Cholestan-3-one and cholest-5-en-3-one even show some reduction in swelling rate. This is in contrast to the glucose permeability where these sterols did not affect the release of glucose. Since some of the ketosteroids can increase the permeability for Rb^+, the reduction in swelling rate can be explained by a loss of the trapped KCl so that the ion concentration inside the liposomes is lowered.

The permeability for ions through bimolecular lipid membranes is generally very low at temperatures below 40°. In order to measure some effect of sterols on the ion permeability, temperatures above the physiological temperature had to be used. Cholesterol, cholestanol, lathosterol and 7-dehydrocholesterol reduced the permeability for Rb^+ by a factor of 2 (Fig. 5A). This is in agreement with the observations of DE GIER *et al.*[22]. Stigmasterol and ergosterol are only less effective at a temperature of 60° (Fig. 5A). Also epicholesterol, cholestan-3-one and cholest-4-en-3-one still show some reduction in Rb^+ permeability. However, cholest-5-en-3-one and cholest-4,6-dien-3-one strongly increase the ion permeability (Fig. 5B).

DISCUSSION

Sterols detected in animal membranes, *viz.*, cholesterol, cholestanol and lathosterol, are incorporated in egg lecithin liposomes up to a molar ratio of sterol to lecithin close to unity. Also B-norcholesterol and coprostanol are solubilized to a similar extent as cholesterol. 7-dehydrocholesterol shows a lower sterol:lecithin ratio. The 3α-hydroxysterols and ketosteroids are incorporated in liposomal structures of egg lecithin to approximately half the amount observed for cholesterol. KELLAWAY AND SAUNDERS[17] estimated the solubilization of steroids by weight and found that also the ketosteroids, cholest-5-en-3-one, cholest-4-en-3-one and cholestan-3-one are solubilized to the same high extent as cholesterol. As stated already, epicholesterol is solubilized less by egg lecithin liposomes than cholesterol. In the Mycoplasma membrane, however, epicholesterol is as well incorporated as cholesterol[8]. On the other hand cholestanol is rather poorly incorporated in erythrocyte membranes despite the high solubilization by egg lecithin[9]. It is also difficult to explain why the plant sterols are more poorly solubilized in the lecithin dispersions whereas the *cis* form coprostanol is well incorporated in liposomes and erythrocyte membranes[9]. It is hoped that experiments which are presently in progress will show the location and distribution of the sterol in the liposome and the biological membrane. The reduction in permeability for glucose, glycerol and Rb^+ in liposomes containing cholesterol, cholestanol, lathosterol and 7-dehydrocholesterol (Figs. 1A, 3A and 5A) is in perfect agreement with the interaction and small mean molecular area found in mixed monolayers[10]. The above mentioned sterols show the most pronounced effect on the permeability properties of bimolecular lipid membranes whereas these sterols also showed the strongest reduction in area per molecule and smallest mean molecular area (ref. 10, Table II). Plant sterols, stigmasterol and ergosterol, show less pronounced effects on the permeability properties of liposomes, especially at high sterol concentrations (Figs. 1B, 3B and 5A). These sterols are found to be less readily solubilized in liposomes, and monolayer studies showed that the interaction is less than with the animal sterols[10]. The presence of the double bond at C-22 and/or the ethyl or methyl respectively at C-24 restrict the interaction with lecithin and this is reflected in the permeability properties of liposomes. Androstan-3β-ol shows absolutely no effect on the glucose or glycerol permeability in liposomes (Figs. 1B and 3B). This sterol lacking the side chain was also unable to interact with 18:1/18:0 lecithin in mixed monomolecular layers. The sterol side chain is obviously of critical importance for the sterol lecithin interaction and therefore for the reduction of the permeability properties of liposomes. B-Norcholesterol shows a permeability reducing effect that is comparable with that observed for the animal sterols (Fig. 1C and 3C). Monolayer studies showed that the interaction of B-norcholesterol with 18:1/18:0 lecithin is almost identical to the effect observed for cholesterol. The A/B *cis*-structured coprostanol fails to affect significantly the permeability for glucose and glycerol in liposomes (Figs. 1C and 3C). In monolayer studies both at room temperature and at physiological temperature a moderate reduction in area was observed. However, the mean molecular area noticed is still high and of the same order as for the non-interacting adrostan-3β-ol (ref. 10, Table II). A planar sterol nucleus appears to be necessary for an interaction and a permeability reducing effect as is observed for cholesterol.

Epicholesterol, the 3α-hydroxy epimer of cholesterol, has little or no effect on

the glucose, glycerol or Rb^+ leak from liposomes (Figs. 2A, 4A and 5B). This sterol is incorporated in liposomes to a lower extent than cholesterol but also higher epicholesterol concentrations as achieved in the Mycoplasma membrane do not result in reduced membrane permeabilities to glycerol and erythritol[8]. Monolayer studies show a significant interaction of epicholesterol with 18:1/18:0 lecithin at 22° only, whereas at physiological temperatures the condensation effect is strongly reduced[10]. The experiments on model membranes (monolayers, liposomes) and biological membranes (*Mycoplasma laidlawii*) demonstrate that the orientation of the -OH group at the 3 position is important for the interaction between sterol and phospholipid. Androstan-3α-ol shows, as is demonstrated already for androstan-3β-ol, no effects on the permeability in liposomes (Figs. 2A and 4A) nor on the interaction with 18:1/18:0 lecithin in monolayers[10]. Ketosteroids show no effect or even an increase in the permeability for glucose, glycerol and Rb^+ in liposomes (Figs. 2B, 2C, 4B and 5B). Erythrocyte membranes, when part of the cholesterol in the erythrocyte membrane is replaced by cholestan-3-one, cholest-4-en-3-one or cholest-4,6-dien-3-one, also showed an increased permeability for glycerol[9]. Monolayer studies show reductions in area for ketosteroids at room temperature[10,20] and for cholestan-3-one and cholest-5-en-3-one even at physiological temperatures[10]. However, for all ketosteroids high mean molecular areas are found which could account for the fact that they are not able to reduce the permeability of lipid bilayers. KAMEL *et al.*[20] concluded from monolayer studies that in spite of the large area per molecule, ketosteroids would have similar effects to cholesterol in biological membranes. On the other hand, this study demonstrates that the increased area per molecule of ketosteroids has dramatic effects on the properties of liposomes and biological membranes[9].

It can be concluded from the present data that for a permeability reducing effect as is observed for cholesterol there is required: (a) a planar sterol nucleus, (b) an intact side chain, (c) a 3β-hydroxy group. Compounds studied which fulfil these criteria are cholesterol, cholestanol, lathosterol, 7-dehydrocholesterol and B-norcholesterol. Sterols with a different side chain structure, stigmasterol and ergosterol, cause a smaller reduction in permeability. Compounds with no side chain (androstan-3β-ol) with a non planar sterol nucleus (coprostanol) or a 3α-hydroxy group (epicholesterol) show no effect on the permeability properties of liposomes. Ketosteroids show either no effect or increase the permeability. Monolayer studies showed that sterols with a planar sterol nucleus, an intact side chain and a 3β-hydroxy group reveal a strong interaction with lecithin[10]. Electron spin resonance studies[18,19] indicated that cholesterol and cholestanol increased the degree of order of the lipids in the lamellar structure. Compounds with a 3α-hydroxy or keto group or lacking the side chains appeared to have only a very small ordering effect[18,19]. By the method of spin labeling the maximal effect observed for ergosterol was also ultimately less than that obtained with cholesterol[18]. Differential scanning calorimetry measurements showed that cholesterol strongly affected the phase transition of lecithin[21]. This phase transition is characteristic for the conversion of lecithin from the Lβ crystalline phase to the Lα liquid crystalline phase. Epicholesterol[8], androstan-3β-ol[8,21] and cholest-5-en-3-one[8] showed only a slight effect on the phase transition of lecithin. It is obvious that the reduction in the permeability is strongly influenced by a reduction in motional freedom of the hydrocarbon region as shown by monolayer, X-ray, electron spin resonance and differential scanning calorimetry methods.

However, as is demonstrated in this paper, the interaction of the sterol hydroxyl group with its environment is also of crucial importance in determining the permeability properties of the lipid barrier. The effects of sterols in model membranes such as monolayers and liposomes are in good agreement with the effects in biological membranes such as erythrocytes and Mycoplasma.

Replacement of cholesterol in erythrocyte membranes by lathosterol, 7-dehydrocholesterol or B-norcholesterol, by exchange with lecitin–sterol dispersions, shows that these sterols have a comparable effect on membrane permeability as cholesterol[9]. Liposome experiments show that cholesterol, lathosterol, 7-dehydrocholesterol and B-norcholesterol have similar effects on the permeability. Replacement of cholesterol by cholestan-3-one, cholest-4-en-3-one or cholest-4,6-dien-3-one in erythrocyte membranes results in an increased permeability[9]. The permeability of liposomes is not affected or increased when these steroids are incorporated. In erythrocytes coprostanol showed as much effect as cholesterol. In liposomes, however, coprostanol does not reduce the permeability as much as cholesterol.

Incorporation of cholesterol in *Mycoplasma laidlawii* membranes[8] results in a decrease in permeability as is found in liposomes. Incorporation of epicholesterol in these cell envelopes[8] does not affect the permeability whereas the permeability of liposomes is also not affected by epicholesterol. Further experiments are in progress to study the effects of other sterols on the properties of Mycoplasma membranes.

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