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The role of cholesterol in lipid membranes

Cholesterol is well recognized as a prominent lipid constituent of many biological membranes. In discussions on its function in these biological interfaces, the possible molecular interactions between cholesterol and other lipid molecules have been the subject of various theoretical considerations[1,2]. Furthermore, these interactions have been studied in various model systems, but the conclusions recently drawn by various authors seem at a first sight to be somewhat contradictory.

The investigations of DEMEL, VAN DEENEN AND PETHICA[3] on mixed monolayers of cholesterol and phospholipids demonstrate reductions in the average area per molecule compared with those in the pure films, although it was found that the extent of this effect is highly dependent on the chemical nature of the paraffin chains. Very significant condensing effects were observed with phospholipids containing mono-unsaturated fatty acids. These facts suggest that because of the presence of cholesterol, the molecular interactions can increase and that lipid expansion of the film is limited. However, the work of SHAH AND SCHULMAN[4] shows that introduction of cholesterol into a very closely packed monolayer of (dipalmitoyl)lecithin reduces the cohesive forces, as can be seen from the change from a solid to a liquid type of film.

NMR studies[5] on dispersions of egg yolk lecithin in water exhibit a selective reduction in the signal due to the polymethylene chains when cholesterol is present, which strongly suggests some reduction in the lipid chain motion. On the other hand, differential thermal analysis[6] demonstrates that addition of cholesterol to (dipalmitoyl)lecithin in an aqueous system lowers the transition temperature between the gel and the liquid crystalline phase, and decreases the heat absorbed at the transition. On the basis of these results, LADBROOKE, WILLIAMS AND CHAPMAN[6] assume that cholesterol controls the fluidity of the hydrocarbon chains of the phospholipids by disruption of the crystalline chain lattice of the gel phase and by inhibiting the flexing of the chains in the dispersed liquid crystalline phase. The various observations strongly suggest that cholesterol can cause a dual effect on phospholipids, depending on the nature of their fatty acid constituents. Observations of DE GIER, MANDERSLOOT AND VAN DEENEN[7], BANGHAM, DE GIER AND GREVILLE[8], DEMEL, KINSKY AND VAN DEENEN[9] and PAPAHADJOPOULOS AND WATKINS[10] on liposomes revealed a reduction of permeability caused by the presence of cholesterol. The present report confirms these results, but in addition demonstrates that cholesterol in liposomes from saturated phospholipids causes a more complex behaviour.

The liposome systems used were prepared by dispersion of the phospholipid or phospholipid–cholesterol mixtures into 50 mM KCl, as described earlier[7]. Because these liposomes behave as practically ideal and comparable osmometers, the permeability of non-electrolytes, such as glycerol and glycol, through the outer lipid bilayer of the structures is proportional to the initial swelling rates in isotonic solutions of the non-electrolytes[7,8]. In Fig. 1, these swelling rates are given as a function of temperature. In particular the swelling rates of the liposomes prepared from the saturated lecithins show a rapid increase with temperature. All the systems are more permeable to glycol than to the bigger molecule, glycerol. In agreement with earlier observations[7], the presence of cholesterol in the liposomes prepared from egg yolk lecithin reduces the permeability considerably, and as mentioned before, similar

results have been obtained with liposomes prepared from synthetic lecithins with unsaturated chains[7]. However, the liposomes prepared from mixtures of cholesterol and saturated synthetic lecithins reveal a different behaviour (Figs. 1b, 1d and 1e). Below a certain temperature (about 20° for (dimyristoyl)lecithin, about 36° for (dipalmitoyl)lecithin and about 44° for (distearoyl)lecithin) the presence of cholesterol in the liposomes enhances the rate of penetration of glycol as well as that of glycerol. However, above these temperatures, the reverse effect is noted, similarly to that observed with unsaturated lecithins over the whole temperature range studied. This is consistent with the idea that the thermal motion of the chains is reduced by cholesterol but that below the particular temperatures mentioned for the saturated lecithins, this sterol prevents an ordering of the chains. As a general effect, cholesterol is found to diminish the temperature dependence of the permeability of all the liposome systems.

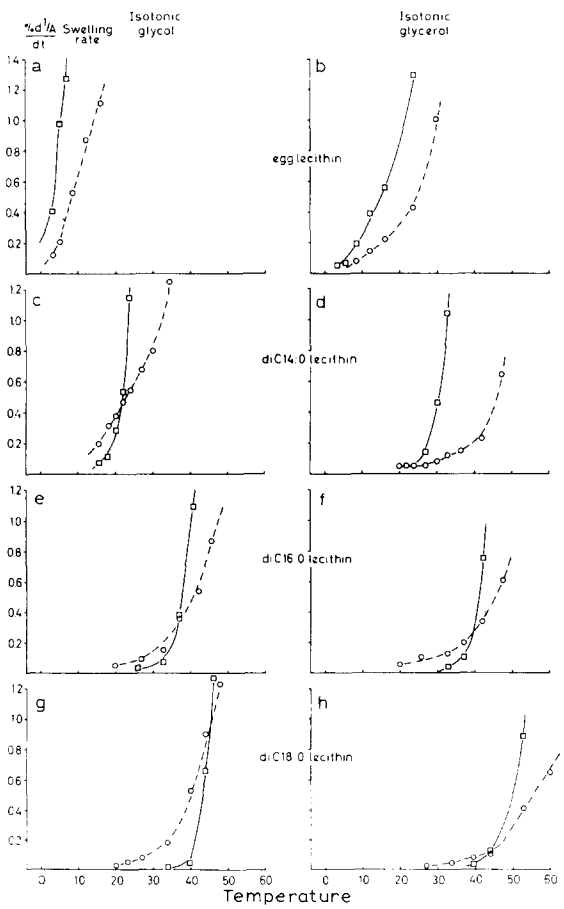

Fig. 1. Initial swelling rates of liposomes prepared from egg lecithin, (dimyristoyl)-, (dipalmitoyl)-lecithin (□—□) and of these phospholipids mixed with 30 mole% cholesterol (○---○). All the systems contained about 4 mole% phosphatidic acid prepared from egg lecithin so as to induce enough charge on the concentric bimolecular layers to give the "model cell" a suitable water content. The systems containing (dipalmitoyl)- and (distearoyl)lecithin were prepared at 55 and 60°, respectively; the others were prepared at 37°.

In monomolecular layers and liposomes of total membrane lipids the overall effect of cholesterol on phospholipids is a reduction of their average molecular area. X-ray diffraction studies of fully hydrated lipids extracted from human erythrocytes demonstrated a reduction in area of the phospholipid molecules by cholesterol which was quantitatively identical to that observed in the monolayers[11]. That the prevailing effect of cholesterol on the complex mixture of erythrocyte lecithin is a condensing one agrees with the known molecular composition of this phospholipid. Mixed acid species, such as (1-palmitoyl-2-oleoyl)lecithin, have been found to be abundant, whereas (distearoyl)lecithin, for example, is lacking. On the other hand a considerable quantity of (dipalmitoyl)lecithin has been demonstrated in this membrane, and assuming non-random distribution in the membrane, a local liquifying effect of cholesterol cannot be excluded.

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