Applied Microbiology, University of Tokyo, which seemed to contain a single-protein component free of carbohydrates, did not show similar dissociating activity even at 50 μg/ml.

Our recent investigations have suggested that rapid dissociation of DNA from the membrane complex (measured by the modified Sarkosyl method[4]) with little DNA degradation also occurred at the early stage of colicin E_2 challenge in the intact sensitive cells. The ATP requirement for the *in vitro* reaction of colicin E_2 could possibly be related to the fact that an energy-producing system of the sensitive cells was necessary for the killing action of colicin E_2 in the intact cells[5]. Further experiments are needed to ascertain whether the *in vitro* reaction of colicin E_2 observed here is involved in the *in vivo* transmission mechanism of colicin action from receptor sites across the cell membrane. However, the fact that a very low concentration of colicin E_2 but not colicin K was sufficient for the dissociation reaction of the membrane complex seems to be the consequence of a specific interaction of colicin E_2 protein with the cytoplasmic membrane of *E. coli*.

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Sterol structure and ordering effects in spin-labelled phospholipid multibilayer structures*

Cholesterol is a common component of vertebrate cellular membranes[1]. Sterols with a similar structure (3β-OH group, hydrocarbon chain at position 17) are also found in vascular plants, algae, fungi[2], and microorganisms[2,3]. The biological function of these compounds is not completely understood. We have investigated the effects of steroid structure on the degree of order in multibilayer structures of polar membrane lipids[4,5] using a spin label technique[6–9]. The results indicate that cholesterol and structurally related sterols increase the degree of order of the spin label, and hence that of the lipids in the lamellar structure. The term "degree of order" is used to denote the extent to which the long axes of the lipids orient preferentially in a

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direction perpendicular to the lamellar plane. The results are taken to suggest that the biological function of cholesterol and structurally related sterols in membranes is related to the ability to produce this preferred orientation of lipids in a bilayer structure.

The technique used is based on the fact that the electron spin resonance spectra of nitroxides depend on the angle they make with the magnetic field of the spectrometer[10]. When the long axis of the cholestane spin label, 3-spiro[2'-(*N*-oxyl-4',4'-dimethyloxazolidine)]cholestane[6,7], is perpendicular to the plane of the lipid lamellae (because of constraints imposed by the polar lipids) the hyperfine splittings are 19 gauss and 6.5 gauss when the multibilayer films are parallel and perpendicular to the spectrometer magnetic field, respectively. When the long axis of the spin label and other bilayer lipids assume angles differing from 90°, the degree of spectral anisotropy decreases. Thus, the degree of spectral anisotropy (the difference in separations between the three lines of the electron spin resonance spectra taken with the multibilayer films parallel and perpendicular to the spectrometer magnetic field; see Fig. 1) can be used as a measure of the degree of order of the lipid multibilayers.

The experiments were carried out with fraction I (ref. 11) of the lipids of the white matter of bovine brain and the lipids of human erythrocyte ghosts from which cholesterol had been removed by chromatography on silica gel. An acidic lipid fraction isolated from vegetatively grown morning glory cells was also used in a number of instances. Sterols were incorporated in the lipid mixture prior to making the films[7]. The sterol concentration range investigated ranged from 0 to 40 mole %. The films were hydrated with mammalian Ringer's solution.

In the case of brain lipids, cholesterol caused an increase in order of the spin label as shown by the spectra in Fig. 1. 20–25 mole % produced the maximum effect.

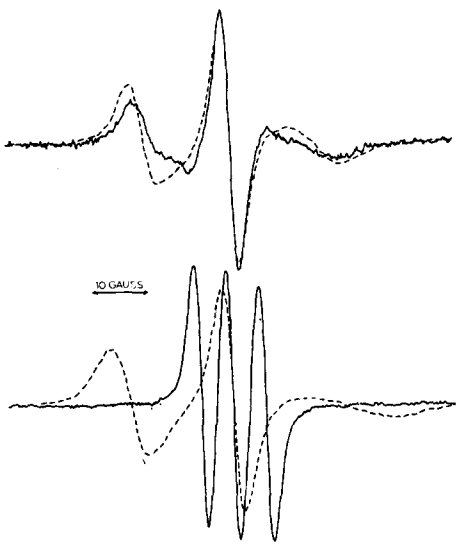

Fig. 1. Effect of cholesterol on orientation of spin label. Solid and dashed lines denote spectra obtained with the films perpendicular and parallel to the magnetic field, respectively. Upper spectra, beef brain lipids without cholesterol. Lower spectra, the same lipids *plus* 33 mole % cholesterol.

β-Sitosterol, the 24β-ethyl derivative of cholesterol commonly found in plants, was equally effective. Ergosterol, another plant sterol, which has two more double bonds than cholesterol (C-7–C-8 and C-22–C-23) and a 24β-methyl group, was more effective than cholesterol in the 5–10 mole % range, but the maximal order achieved ultimately was less than that obtained with cholesterol. This may partly be due to the light-induced formation of ergocalciferol, a compound which was found in these studies to be totally ineffective at inducing order.

A C-5 double bond is not essential for the induction of order since cholesterol and cholestanol were equally effective. The hydrocarbon tail is important, but not crucial, since 5-androsten-3β-ol and 5α-androstan-3β-ol promoted ordering, but to a lesser extent than the tail-bearing analogues, cholesterol and cholestanol. The presence of the hydroxyl group in the 3-position is important since cholestanone caused only a low degree of order, while cholestane was without effect. However, the orientation of the hydroxyl group is crucial, since 5β-androstan-3α-ol was totally ineffective, in contrast to 5β-androstan-3β-ol. In addition, whereas 5β-cholestane-3β-ol was slightly effective, 5β-cholestane-3α-ol was completely ineffective. The presence of a polar group in the region of the hydrocarbon tail renders the steroids totally ineffective in producing order as demonstrated by pregnanalone, 5-pregnen-3β-ol-20-one, testosterone, cholic acid, and cortisone; in fact these compounds may possibly decrease whatever order was previously present.

The over-all geometry of the steroid nucleus is important as both 5-cholestene-3β-ol and 5α-cholestane-3β-ol, which are both planar, are highly effective in promoting order, whereas 5β-cholestane-3β-ol, which has a bent steroid nucleus, is only slightly effective.

Precursors of cholesterol were also investigated. Lanosterol (8,24-(5α)-cholestadien-4,4,14α-trimethyl-3β-ol) produced a small amount of order (less than did 5-androsten-3β-ol), and squalene produced no order.

Both α-tocopherol and vitamin K consist of conjugated rings and one long hydrocarbon tail. In addition, α-tocopherol, like cholesterol, has a hydroxyl group on the side of the ring structure opposite to the hydrocarbon chain. Neither of these compounds caused any order.

Essentially identical results were obtained with human erythrocyte ghost lipids. In addition, ordering was induced in films prepared with the morning glory cell lipids by β-sitosterol, one of the sterols found in these cells (N. H. TATTRIE, personal communication).

The possibility that the ordering effects are due to the formation of a separate microdisperse sterol phase may be ruled out for two reasons. The first is that films of pure cholesterol, one of the sterols most effective in producing order, evinced only a very slight spectral anisotropy. The second is that from the solubility data for 3-hydroxy sterols in phospholipid dispersions[12,13] it may be inferred that there would be little tendency for the sterols which produce order to form a separate phase under the conditions of the experiment.

In summary, the ordering effect of steroids on cellular lipids depends on their structure and requires a planar steroid nucleus and a single hydroxyl group in the 3β-position. The presence of a hydrocarbon chain at C-17 increases the effectiveness in promoting order. Since these structural properties are shared by many nonhormonal naturally occurring sterols, it is suggested these compounds have the common function

of ordering the lipids in membranes of the organisms in which they are found. It is considered highly significant that there is a strong correlation between the effects of sterol structure in a biological system and in inducing order in lipid multibilayers. One nutritional type of mycoplasma[14], whose external envelope encompassing the cell is a typical unit membrane composed almost entirely of lipoprotein, requires sterols for growth. Growth is supported by those sterols which the present study indicates promote order, but not by those which do not promote order.

It is suggested also that the effect of cholesterol in controlling the spatial location of a spin label in liposomes[15], the effects of sterols in changing some permeability properties of human erythrocytes[12] and model membranes[16], as well as in altering the nuclear magnetic resonance spectra of liposomes[17], are related directly to their influence on order. It is expected that additional studies using the spin-labelled multibilayer technique will aid in the elucidation of the details of the various relationships.

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