PRELIMINARY NOTES

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Valinomycin-induced permeation of $^{86}Rb^+$ of liposomes with varying composition through the bilayers

The importance of the chemical nature of the hydrophobic core of a membrane for the permeation of nonelectrolytes has been demonstrated using the liposome system. The permeability of the lipid bilayers for glycol, glycerol and erythritol[1] and also for glucose[2] increases strongly with increasing unsaturation of the fatty acid chains esterified to the constituting lipid molecules. In this communication we show that also the valinomycin-mediated transport of $^{86}Rb^+$ is strongly dependent on the chemical composition of the hydrophobic barrier.

Liposomes were made with $^{86}Rb^+$ trapped inside the bilayer structures. Using methods described earlier, we prepared liposome systems from various lecithins with identical microscopic appearance and comparable behaviour as osmometers[1]. The leak of the radioactive ions from the inside to the outside was measured according to the method of BANGHAM *et al.*[3].

Fig. 1 demonstrates the spontaneous leak of $^{86}Rb^+$ out of the various liposome systems. In agreement with the observations of BANGHAM *et al.*[3] on $^{42}K^+$ and $^{22}Na^+$, the leak of this $^{86}Rb^+$ is very low. Of the mechanism by which the ions are released nothing is known, but it is likely that at least part of the leak is due to the rupture of a few of the bilayers[4]. During experiments with polyunsaturated systems we observed that peroxidation, which is likely to reduce the stability of the lipid bilayer,

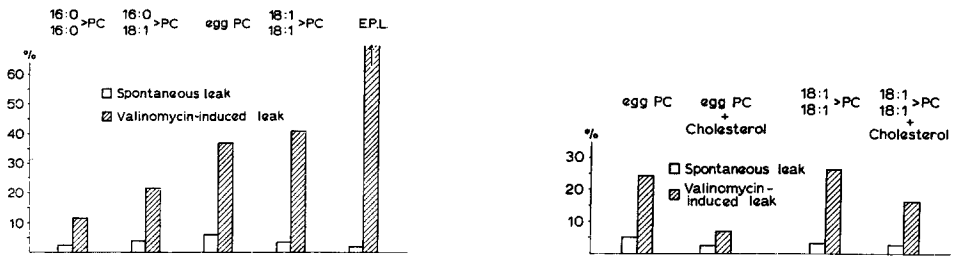

Fig. 1. The spontaneous and valinomycin-induced exchange of $^{86}Rb^+$ trapped in liposomes, measured over a period of 30 min at 40°. The model systems were prepared from pure lecithins after addition of 4 mole% of phosphatidic acid. In the given order we used (dipalmitoyl)lecithin, (1-palmitoyl-2-oleoyl)lecithin, egg yolk lecithin, (dioleoyl)lecithin and E.P.L. E.P.L. is an isolated lecithin with 70% of the fatty acid chains being linoleate, which was kindly donated to us by Dr. H. Eikermann of Nattermann and Cie. The liposomes of (dipalmitoyl)lecithin were prepared at 40°, the others at room temperature.

Fig. 2. The effect of cholesterol on the $^{86}Rb^+$ exchange. The liposomes were prepared from pure egg yolk lecithin or (dioleoyl)lecithin and from mixtures of the pure lipids with 30 mole% of cholesterol. The leak was measured over a period of 30 min at 25°.

strongly enhanced the spontaneous leak. There is no apparent correlation between the spontaneous leak and the degree of unsaturation. The addition of valinomycin at a concentration of 4.5 μmoles/mole of lipid resulted in a marked increase in the leak of the rubidium out of all the liposome systems. It has been demonstrated that such a valinomycin-induced leak of ions exhibits a high ionic specificity. The exchange over the bilayer for Rb^+ and K^+ is highly promoted but not that for Na^+ (ref. 5). The present result demonstrates that the promoting effect of valinomycin is also determined by the degree of unsaturation of the paraffin chains in the hydrophobic layer. The correlation between the valinomycin-induced $^{86}Rb^+$ permeation and the chemical composition of the lipids in the bilayer is comparable with that found for nonelectrolyte permeability and bilayer composition[1]. Accepting a carrier function for the valinomycin, the increase in $^{86}Rb^+$ permeation with unsaturation could be explained either by a easier complex formation at the border of the unsaturated barrier or by an increased diffusion rate of the $^{86}Rb^+$–valinomycin complex in the hydrophobic layer. In this respect it is relevant to mention the investigations of JOHNSON AND BANGHAM[8] on the combined action of valinomycin and anaesthetics. The presence of an anaesthetic which is supposed to increase the disorder in the membrane also enhances the valinomycin-induced cation permeability.

Fig. 2 illustrates that the introduction of cholesterol in the lipid bilayer reduces the valinomycin-induced permeability of the $^{86}Rb^+$. This reduction is also comparable with the decrease of the penetration rate of the nonelectrolytes after addition of the sterol[1]. The experiment in Fig. 2 is comparable with the observations of SZABO *et al.*[6] on the decrease in membrane resistance as a consequence of the addition of the macrotetralide antibiotics to black films prepared of various mixtures of lecithin and cholesterol. Furthermore, our observations rule out the possibility that the cholesterol effect might be the result of a displacement of the solvent (decane), a restriction which these investigators[6] had to make using the black film system.

In addition to the apparent role of the hydrophobic layer, the polar headgroups can also play a role in the ion transport. BANGHAM[7] described a very strong restriction of the spontaneous leak of cations from the liposomes in a case in which there is a net positive charge on the bilayers. Our current experiments demonstrate that the valinomycin-mediated exchange is strongly reduced in liposomes containing the positively charged lysyl-*O*-phosphatidylglycerol, which is a major phospholipid of certain Gram-positive bacteria.

The results indicate that the chemical make-up of membrane lipids not only controls the simple diffusion across the membranes but may also govern the rate of facilitated diffusion to a certain extent.

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