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Author(s)	KWAN, Takao
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ORDERLY ARRANGEMENT AND TRANSPORT OF THE MEMBRANE

By

Takao KWAN*)

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Abstract

The liquid crystalline behaviour of phospholipid, in biological science, is of considerable importance and growing steadily in recent years. The fluidity, orientation and phase separation of biomembranes are believed to affect ion transport and excitation of the membrane hence to control living cells. With respect to a newly constructed multilayer planar membrane, a certain correlation exists between the orderly character of the membrane and its permeability property: a less ordered arrangement of the membrane seems to be associated with the promotion of the membrane permeability.

1. Introduction

The physical chemistry at liquid interfaces, initiated by I. Langmuir and developed by E. Rideal and his school, still constitutes a significant basis

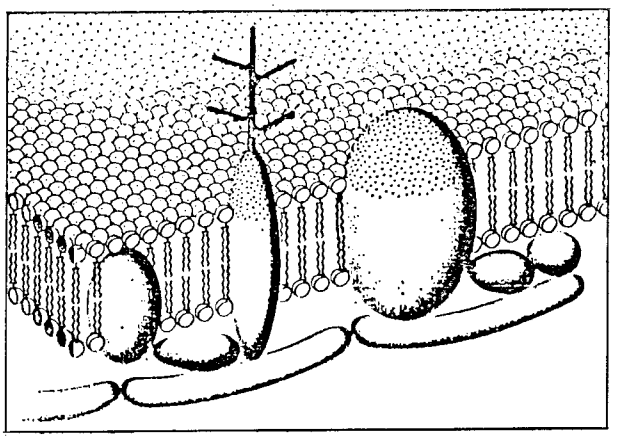

Fig. 1. The Singer-Nicholson model for the biomembrane (Erythrocyte membrane as modified by Capaldi (1974)).

*) Faculty of Pharmaceutical Sciences, Teikyo University, Sagamiko, Kanagawa, 199-01 Japan.

for studying biological surfaces (biomembranes) of a living cell. The biomembrane is known to contain long chain phospholipids, proteins, carbohydrates, cholesterol, *etc.*, as best illustrated by the Singer-Nicholson model.

The phase diagram of phospholipid and related biomaterials were extensively investigated by Chapman and others.[1] It has been shown that phospholipids —the main component of the biomembrane— form a lamellar phase in water with their polar groups oriented so as to be adjacent to the water layers, and their hydrocarbon chains arrange in some orderly lattice. Indeed, electron microscopic studies have indicated that the constituent molecules of the biomembrane are orderly arranged when frozen and fractured at liquid nitrogen temperature. It might be interesting to see whether the biosurface looks like the polycrystalline surface of metal oxides. It must be remembered, however, that in the biological world 1) water is present in abundance and 2) the constituents of a cell membrane are in a semi-solid state, that is, in a gel or lamellar liquid crystalline phase, at least, *in vivo*.

To get an insight into the physical aspect of the biomembrane, simplified model membranes have been proposed. Mono-, bi- and multilayer films have been prepared on a glass plate or on a liquid surface. A black membrane was also prepared in a pin-hole and proposed as a model suitable to a biomembrane although it proved to be less stable. Liposomal membranes appeared to be useful to some extent.

In the present paper, the author would like to investigate how the physical character of the biosurface correlates with its transport phenomena, apart from the subtle biological functions, on the basis of a newly constructed multilayer planar membrane, an approach advanced by Setaka *et al.*[2] For such a membrane, it was confirmed by the ESR technique that some orderly arrangement of the membrane is obtainable, while at the same time the permeation property of the membrane is readily accessible to investigation. Special attention will be given to the question of how orderly arrangements can be correlated with the transport process across the multilayer planar membrane.

2. Lamellar Phase of Phospholipids as Investigated by the ESR Technique

A spin labeled ESR technique has been utilized to get information about the orientation of the model membrane: a spin probe, 5-doxylstearic acid, was synthesized and employed at a concentration below 1 mole % of the host lipids. Synthetic 1, 2-dipalmitoyl-*sn*-glycero-3-phosphocholine (abbrevi-

ated as DPPC), egg-yolk lecithin (egg PC) and cholesterol were purified as usual and stored in chloroform. The spin probe was also kept in chloroform and mixed with the host lipids prior to the preparation of membrane.

Commercial cellulose sheet was placed on a glass plate and slightly wet with water. A chloroform solution containing lipids and the spin probe was dropped on the sheet. The solvent was then evaporated in air. The lipid preparation thus obtained (6～7 mm in diameter) was then covered with another wet cellulose sheet.

The sample holder, specially designed for the ESR measurement, consisted of two methacrylate plates with a 6 mm diameter hole at the center and with a rod through an axially movable device to fix the direction of the plate in the ESR cavity. The lipid preparation attached to the center of methacrylate plate was kept for about 12 hours at room temperature in a container fully saturated with water vapour

ESR MEASUREMENT A JEOL PE-1 spectrometer (x-band) with 100 kHz magnetic field modulation was used. The DPPC and egg PC with or without cholesterol were subjected to membrane formations. The egg PC preparations gave little orientation after chloroform had been evaporated, that is, in the absence of water. They were remarkably oriented, however,

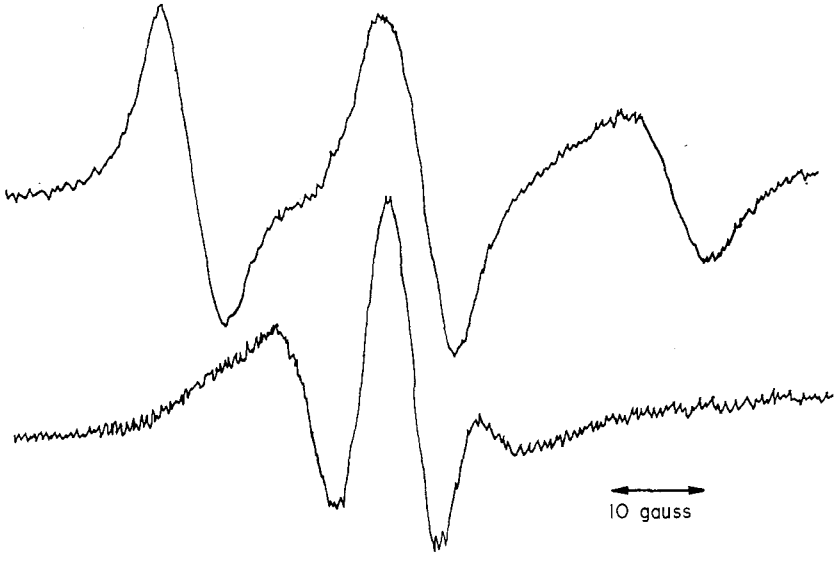

Fig. 2. ESR spectra of 5-doxylstearic acid in a membrane of egg PC-cholesterol mixture (1.0 : 0.67). The upper and lower spectra were obtained when the plane of the membrane was perpendicular and parallel, respectively, to the magnetic field.

when the lipid sample was kept in contact with saturated water vapour at room temperature over night. The orientation appeared to be pronounced, as expected from the condensing role of cholesterol, when cholesterol was added to the egg PC.

In Fig. 2 are shown typical ESR spectra of the spin probe in the sample of egg PC-cholesterol mixture (1 : 0 : 0.67) when the plane of the membrane was perpendicular (upper) and parallel (lower), respectively, to the direction of magnetic field.

It is apparent from Fig. 2 that the hyperfine splitting constant is larger when the membrane is perpendicular than when it is parallel to the magnetic field. This result implies that the hydrocarbon chains of the spin probe and probably also those of the lipid molecules are oriented in a direction perpendicular to the cellulose sheet, in accordance with earlier works done with lipid films on a glass plate.

The extent of orientation may now be expressed by the well-known parameter, S, as defined by

$$S=\frac{A_{/\!/}-A_{\perp}}{A_{zz}-A_{xx}}$$

where A_{zz} and A_{xx} are principal values of the hyperfine splitting tensor along the symmetry axis of the nitrogen P orbital and along the x and y axes perpendicular to z, where axial symmetry of the tensor around z is assumed to be $A_{zz}=30.8$ and $A_{xx}=A_{yy}=5.8$ gauss.[3] Accordingly, S may vary from zero to unity.

The parameter, S, was determined with the multilayer planar membrane composed of egg PC and also with the membrane composed of egg PC plus cholesterol. The comparative values are shown in Table 1.

TABLE 1. Order Parameters for Membranes

	Egg PC	Egg PC-Cholesterol	
		1.0 : 0.15	1.0 : 0.67
S	0.41	0.49	0.68

3. Permeability Property of the Multilayer Planar Membrane

One advantage of the multilayer planar membrane presented here is that the permeation of a simple biological substance can readily be studied for a defined surface area. The apparatus for this measurement consisted of

two concentric cylinders of methacrylate: inner 19 mm and outer 32 mm diameter. Phospholipid preparations with or without cholesterol sandwiched between two cellulose sheets were fastened onto the bottom of the inner cylinder. Prior to the permeation experiment, the membrane was brought into contact with a Tris-HCl buffer solution (pH=8.0) for about 12 hours at room temperature.

A Tris-HCl buffer solution containing a known amount of glucose was placed initially in the inner cylinder, and then every 20 minutes a small portion of the solution was taken out of the outer cylinder and subjected to a determination of the glucose content.

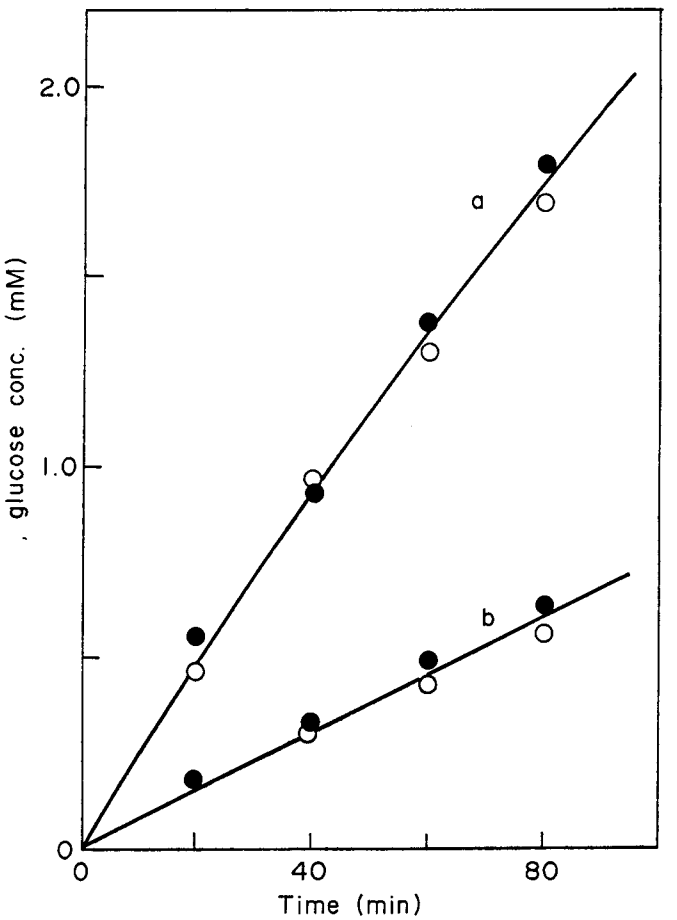

Fig. 3. Time dependence of glucose permeation across the membrane of egg PC (a) and of egg PC-cholesterol (1.0 : 1.0) (b).

The rates of glucose permeation thus obtained for the membrane of egg PC and that of egg PC — cholesterol mixture (1.0 : 1.0 molar ratio) are shown in Fig. 3.

The multilayer planar membranes were found to be so stable and reproducible that two runs, as reporesented by the open and closed circles in Fig. 3, carried out with independently constructed membranes coincided with each other. A further significant result of Fig. 3 is that the rate of glucose permeation is lowered by the addition of cholesterol to egg PC. Since the cholesterol addition to the egg PC brought about an increase in the order of the membrane (Table 1), one may infer, therefore, that membrane permeability can be enhanced by a less ordered arrangement. Similar results were also obtained with the permeation of Ca ions.[4]

4. Discussion

While limited experimental data are presented here, the results led us to conclude without ambiguity that a highly ordered arrangement of the membrane suppresses the transport of simple molecules and ions across it. Since the addition of cholesterol to a lipid monolayer has long been known to condense the film, a similar role of cholesterol would be expected to give rise to an orderly packed state for the multilayer film of egg PC. If so, the suppression of glucose permeation can probably be interpreted in terms of less molecular space available for the permeation.

Now, let us refer to the DPPC membrane prepared between the cellulose sheets. The membrane has shown little orientation of the lipids when they were prepared *at room temperature*. However, they turned out to be oriented after incubating the membrane at 70°C for 3 hours in an atmosphere of saturated water vapour as judged from ESR measured at room temperature.

In the case of a liposomal membrane consisting of DPPC,[5] where the extent of incorporation of a spin probe into the membrane was taken as a measure of permation, the effect of cholesterol addition on the parameter S appeared to be reversed to that obtained with egg PC (Table 1); that is, S *descreased* with the addition of cholesterol while the incorporation (permeation) of the spin probe was increased. This apparent contradiction may be attributed to the fluidizing role of cholesterol that is effective below the critical temperature of DPPC (41°C). In other words, the addition of cholesterol to the DPPC membrane prepared around room temperature is expected to fluidize the membrane and, in consequence, to give rise to some disorderlyness of the membrane.

Finally, it is worth mentioning the temperature-dependence of the uptake and release of Tempo-choline (2, 2, 6, 6 tetramethyl piperidinyl-1-oxycholine) by DPPC liposomes.[6] The rates of uptake and release were determined from the change of the ESR signal intensity and were found to give a distinct peak around the critical temperature of the DPPC, namely 41°C. This means that neither gel nor the liquid crystalline state of the DPPC is favoured for the permeation of the spin probe : the permeation property is enhanced only when both phases coexist.

If the coexistence of different phases is indicative of a less ordered state of the membrane, the pronounced permeation around the critical temperature can be understood along the line already inferred or in terms of the phase boundary. Incidentally, the importance of the phase boundary in heterogeneous catalysis has been emphasized by G. -M. Schwab half a century ago.

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References

1) D. Chapman (Ed.), *Biological Membrane*, Academic Press, London, p. 125 (1968).
2) M. Setaka, M. Yano, T. Kwan, H. Shimizu, J. Biochem., **86**, 355 (1979).
3) W. L. Hubbel, H. M. McConnel, J. Am. Chem., Soc., **93**, 314 (1971).
4) T. Yamamoto, K. Tahara, M. Setaka, M. Yano, T. Kwan, J. Biochem., **88**, 1819 (1980).
5) H. Utsumi, K. Inoue, S. Nojima, T. Kwan, Chem. Pharm, Bull., **24**, 1219 (1976).
6) M. Setaka, T. Ichiki, H. Shimizu, J. Biochem., **83**, 1299 (1978).