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PERMEABILITY PROPERTIES OF LIPOSOMES PREPARED FROM DIPALMITOYLLECITHIN, DIMYRISTOYLLECITHIN, EGG LECITHIN, RAT LIVER LECITHIN AND BEEF BRAIN SPHINGOMYELIN

KEIZO INOUE

Department of Chemistry, National Institute of Health, Kamiosaki, 2-10-35, Shinagawa-ku, Tokyo (Japan)

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SUMMARY

Liposomes have been prepared from dipalmitoyllecithin, dimyristoyllecithin, egg lecithin, rat liver lecithin and beef brain sphingomyelin.

Permeability properties of liposomes thus prepared were studied toward glucose. The glucose permeability of liposomes with saturated lecithins (dipalmitoyllecithin and dimyristoyllecithin) and sphingomyelin appears to be more strongly temperature dependent than that of liposomes with lecithin containing unsaturated fatty acyl chains (egg and rat liver lecithins). The permeability of glucose through vesicles of dipalmitoyllecithin or dimyristoyllecithin was enhanced drastically at their transition temperatures, while the incorporation of about 25 mole% of egg lecithin into liposomes of saturated lecithins suppressed the enhanced permeation rates of glucose above the transition temperatures.

The incorporation of small amounts of cholesterol enhanced the temperature-dependent permeability of glucose through the bilayer of saturated lecithins or sphingomyelin. This tendency was best shown in the case of dipalmitoyl-lecithin, in which 20 mole% of cholesterol had the most stimulating effect on the temperature-dependent permeability. The introduction of more than 33 mole% of cholesterol showed, however, reduced effects on the temperature-dependent permeability through liposomes with saturated lecithins or sphingomyelin. It was also shown that cholesterol had a much larger effect on the regulation of the temperature-dependent permeability of liposomes prepared with saturated lecithins or sphingomyelin than on that of liposomes prepared with phospholipids containing unsaturated fatty acids.

INTRODUCTION

A barrier to diffusion between the interior of organelles and their surroundings is thought to be one of the most important functions of membranes. The function as a barrier mostly depends on phospholipids which have markedly hydrophobic chains, preventing the unregulated passage of water-soluble materials through membranes.

The variety in structure of lipids and the composition of fatty acid chains on the phospholipids may be responsible for regulating membrane fluidity.

The importance of fluidity of membrane lipids in regulating permeability was suggested by a number of recent studies on the temperature dependence of membrane transport [1–7].

The model systems of lipids can also provide valuable information on the relation between permeability properties and the lipid composition of membranes. Since Bangham et al. [8] found that closed vesicles consisting of liquid crystals of phospholipids were formed spontaneously when phospholipids were swollen in salt solution. Attempts to correlate the permeability and the lipid composition of membranes have been carried out in many laboratories [9–16]. Few systematic studies, however, have been carried out on the permeability properties of membranes of saturated lecithins [13–16] or sphingomyelin.

In this paper it will be described that as a barrier, saturated phospholipids are rather different from phospholipids having unsaturated acyl chains.

It has been suggested that cholesterol also has the function of controlling the fluidity of hydrocarbon chains in the lipid bilayer of membranes. The condensing and fluidizing effect of cholesterol in model membrane systems has been studied by a variety of techniques such as monolayer studies [17, 18], X-ray diffraction [19, 20], nuclear magnetic resonance [21, 22], electron spin resonance [23–25] and calorimetry [26].

The observations of de Gier et al. [9], Kinsky et al. [10], Demel et al. [11] and Papahadjopoulos et al. [12] on liposomes revealed a reduction of permeability caused by cholesterol. De Gier et al. [13] demonstrated further that cholesterol incorporation into liposomes of saturated phospholipids caused a complex effect on "highly permeable" non-electrolytes such as glycol and glycerol. Therefore, it is of interest to determine how cholesterol effects the permeation of "non-permeable" glucose through the saturated lecithin or sphingomyelin bilayer.

The results obtained are discussed in terms of the role of unsaturated fatty acids in the structure and the function of membranes.

MATERIALS AND METHODS

Materials

The chemicals and enzymes were purchased from the following companies: Boehringer-Mannheim Japan K. K., Tokyo (hexokinase, glucose-6-phosphate dehydrogenase and NADP); Oriental Yeast Company, Tokyo (hexokinase, glucose-6-phosphate dehydrogenase and NADP); Sigma Chemical Company, St. Louis, Mo. (hexokinase, cholesterol and β, γ-dipalmitoyl-D, L-α-lecithin); K and K Laboratories Inc., Plainview, N. Y. (dicetyl phosphate); Pierce Chemical Company, Rockford, Ill. (sphingomyelin); Calbiochem., San Diego, Calif. (β,γ-dimyristoyl-L-α-lecithin).

Lecithins of egg yolk and rat liver were prepared in our laboratory by chromatography on alumina and silicic acid. The fatty acid composition of egg lecithin used throughout the present work is as follows; 38.7% of palmitic acid, 11.8% of stearic acid, 34.2% of oleic acid, and 12.4% of linoleic acid. The purity of the phospholipids was checked by thin-layer chromatography on silica gel plates with chloroform–methanol–water (70:30:5, by vol.) as solvent systems. Stock solutions of the phospho-

lipids, dicetyl phosphate and cholesterol was prepared in chloroform and stored at −20 °. The concentration of the phospholipids was determined by the method of Gerlach and Deuticke [27] for total phosphate.

Preparation of liposomes

Liposomes having lecithin, dicetyl phosphate and cholesterol in the desired molar ratio were prepared by the same method developed by Kinsky et al. [28]. The molar ratio of lecithin:dicetyl phosphate in liposomes was always 1:0.1.

Assay for permeation rate

Assays for the permeation rates of glucose were carried out by the method described by Kinsky et al. [28] in which the glucose released from liposomes is measured enzymatically. The amount of glucose which is trapped in liposomes was determined by adding Triton X-100 [28].

In the case of liposomes which did not show complete leakage of glucose in the presence of Triton X-100, the "organic solvent method" developed by Kinsky et al. [28] was applied. Liposomes containing saturated lecithins (Inoue, K., unpublished) or sphingomyelin [28] are resistant to the action of Triton X-100, when prepared with more than 30 mole% of cholesterol.

The effect of the temperature on the amount of trapped glucose released from liposomes was determined by the following procedure. Appropriate aliquots of the liposomes (about 2.5 μl) were added to cuvettes containing complete systems, and were incubated for about 10 min at room temperature or an appropriate temperature to obtain the value due to untrapped glucose in the preparations. After the amount of untrapped glucose in the preparations was measured, the whole cuvettes were incubated for various times and at varying temperatures.

Enzyme activity in the experimental condition did not change significantly over the range of temperature from 10–40 °C. Below 10 °C, enzyme systems are slightly affected in the initial rate. However, even at 1 °C, the reaction proceeded and reached a plateau within 10 min. Therefore, it was possible to measure the temperature dependence of the permeation of liposomes by the enzyme systems. It was practically impossible to obtain the rates of glucose permeability above 42 °C by the present assay procedure, because the enzymes were inactivated above 42 °C.

RESULTS

Capacities of various liposomes to trap glucose

The experiments described below are concerned with the effect of the lipid composition on the amount of glucose which is trapped inside the liposome vesicles. Liposomes of rat liver lecithin, egg yolk lecithin, synthetic dipalmitoyllecithin, synthetic dimyristoyllecithin and beef sphingomyelin were prepared without or with cholesterol in various molar ratios. The systems containing dipalmitoyllecithin were prepared at 55 °C or at room temperature (20–25 °C). Liposomes containing beef sphingomyelin were prepared at 50 °C or at room temperature, while those containing dimyristoyllecithin were usually prepared at 37 °C. Liposomes thus prepared were dialyzed against salt solution (the solution containing 0.075 M KCl and 0.075 M NaCl) at room temperature except in the case of liposomes containing dimyristoylleci-

TABLE I

AMOUNTS OF TRAPPED GLUCOSE IN LIPOSOMES

Lecithin or sphingomyelin liposomes were prepared with various amounts of cholesterol at different temperatures. The amount of glucose which was trapped in liposome preparations was assayed enzymatically, dialyzed and kept at 4 °C or at room temperature. The amount of trapped glucose is expressed as moles glucose/mole lipid phosphorus.

Phospholipids		Rat lecithin	Egg lecithin	Dimyristoyllecithin				Dipalmitoyllecithin		Beef brain sphingomyelin			
Temperature at preparation (°C):		20–25	20–25	>20		37		20–25	55	20–25		50	
Temperature at dialysis (°C):		20–25	20–25	4	20–25	4	20–25	20–25	20–25	4	20–25	4	20–25
Quantities of cholesterol	0	1.87	2.10	0.07*	0.08*	2.86	0.26	0.1*	4.45	0.01*	0.04*	1.03	0.433
incorporated	0.1	1.80	2.23	–	0.02*	2.41	0	0.94*	5.26	0.05*	–	2.50	–
(molar ratio of cholesterol	0.2	–	2.56	0.21*	0.04*	2.64	0.04	5.74	5.05	–	0.96	3.62	–
to phospholipid)	0.3	2.05	2.76	–	0.35	2.95	0.40	5.92	4.89	–	0.98	–	–
	0.4	–	2.87	–	2.49	3.20	2.73	6.02	–	–	2.16	–	–
	0.5	2.14	3.16	–	3.90	–	–	6.05	4.43	–	5.85	–	–
	0.75	2.71	3.55	–	5.34	–	–	5.78	–	–	7.73	–	–
	1.0	3.22	3.78	–	5.45	–	–	6.31	–	–	–	–	–

* These samples were not suspended well.

thin or sphingomyelin. In the latter case, the suspension of liposomes was first chilled, and then dialyzed against the salt solution at 4 °C. Liposomes of dimyristoyllecithin or sphingomyelin were also dialyzed at room temperature when prepared with more than 30 mole% of cholesterol. Usually the assay for determining the amount of trapped glucose was performed at room temperature, while liposome preparations containing dimyristoyllecithin or sphingomyelin were also assayed below 10 °C. As seen in Table I, liposomes of egg and rat liver lecithin could trap glucose when prepared at room temperature even without cholesterol, while those of dipalmitoyllecithin, dimyristoyllecithin or sphingomyelin trapped no glucose when prepared in the absence of cholesterol at room temperature.

Dipalmitoyllecithin could not be swollen well in 0.3 M glucose solution at room temperature, but it could be swollen very well and could trap glucose even at room temperature if more than 15 mole% of cholesterol was incorporated. As mentioned already by de Gier et al. [9], it is possible to make dipalmitoyllecithin swell in aqueous solution by elevating the temperature of the solution to 55 °C. Liposome preparations prepared at 55 °C showed the ability to trap a large amount of glucose. Dimyristoyllecithin could not be suspended well below 20 °C, but well suspended at 37 °C or even below 20 °C with more than 30 mole% of cholesterol. However, liposomes prepared with dimyristoyllecithin and less than 20 mole% of cholesterol trapped only a small amount of glucose when dialyzed and assayed at room temperature. The liposomes glucose showed the ability to trap when they were dialyzed and kept below 10 °C.

Effect of the incubation temperature on the diffusion of glucose from liposomes prepared with dipalmitoyl- or dimyristoyllecithin

Liposomes containing dipalmitoyllecithin and dicetyl phosphate were incubated at various temperatures for 10 min. This time interval was chosen, because as seen in Fig. 1, the amount of glucose diffused within 10 min provides an approximate measure of the extent to which glucose is released.

The rates of glucose permeability through dipalmitoyllecithin liposomes were enhanced drastically at the temperature range from 38–40 °C (Fig. 2). This critical temperature range almost corresponds to the phase transition temperature of 41 °C

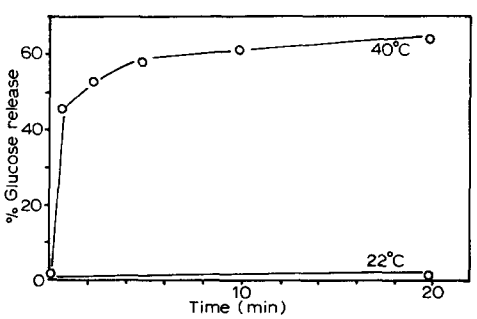

Fig. 1. Time course of diffusion of glucose from liposome of dipalmitoyl lecithin. Liposomes were prepared from dipalmitoyllecithin and dicetyl phosphate in the molar ratio of 1–0.1. About 2.5 μl of liposome preparations were incubated in cuvettes containing a Tris-buffered solution of enzymes and cofactors at 40 or 22 °C. Changes of absorbance at 340 nm were determined at intervals of 1 min.

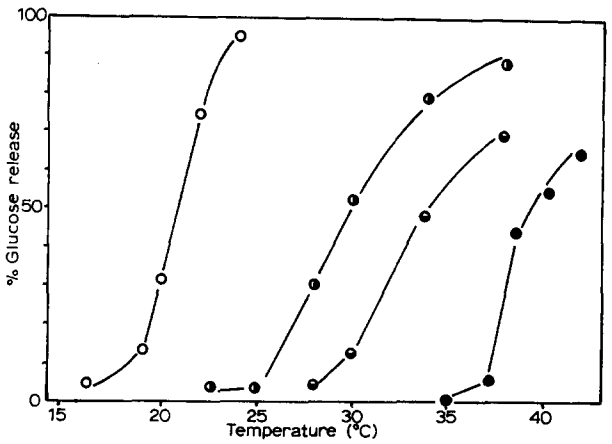

Fig. 2. Temperature dependence of glucose permeability through liposomes prepared with dipalmitoyllecithin, dimyristoyllecithin and mixtures of dipalmitoyl- and dimyristoyllecithins. Glucose release from various liposomes was assayed 10 min after incubating at different temperatures. Liposome composition (molar ratios): ●, dipalmitoyllecithin, dicetyl phosphate (1:0.1); ○, dimyristoyllecithin, dicetyl phosphate (1:0.1); ◑, dipalmitoyllecithin, dimyristoyllecithin, dicetyl phosphate (0.5:0.5:0.1); ◒, dipalmitoyllecithin, dimyristoyllecithin, dicetyl phosphate (0.75:0.25:0.1).

which is reported for dipalmitoyllecithin by differential thermal analysis and X-ray diffraction [26]. Fig. 2 also illustrated that glucose permeability through dimyristoyllecithin liposomes remarkably increased at the temperature range from 20–23 °C. This temperature range is close to the phase transition temperature reported for dimyristoyllecithin (23 °C) [26]. At 24 °C, these liposomes released glucose extremely fast, losing 100% of the trapped glucose within 5 min. This is the reason why liposome preparations dialyzed and kept at room temperature did not trap any detectable amounts of glucose (Table I).

Liposomes prepared from mixtures of dipalmitoyl- and dimyristoyllecithins also showed strong temperature dependence. Temperature ranges which caused drastic leakage of glucose from liposomes prepared with mixed lecithins varied roughly in proportion to the molar ratio of both lecithins. Liposomes containing equal quantities of dipalmitoyl- and dimyristoyllecithins showed a permeability change at around 30 °C, while those containing 3 parts of dipalmitoyllecithin and 1 part of dimyristoyllecithin showed the change at around 34 °C. Permeability of glucose through liposomes derived from mixtures of the two different saturated lecithins showed a little broader temperature dependence than that through the bilayer containing homogeneous saturated lecithin. The permeability through liposomes of egg lecithin was rather insensitive to the temperature, because, at most, 10% of the glucose was released after incubating for 10 min at 37 °C. This temperature is apparently much higher than the transition temperature of egg lecithin. The lack of the severe temperature dependence of the permeability in egg lecithin liposomes might be due to the heterogeneous fatty acid composition in egg lecithin. However, homogeneous synthetic 1-stearoyl-2-oleoyllecithin showed almost the same temperature dependence of permeability for glucose as egg lecithin according to the observation by Demel et al. [11].

It is, therefore, quite reasonable to assume that the liquid–solid transition of the paraffin chains has a striking effect on permeability rates of glucose through the liposomes of saturated phospholipids. The bilayer in liposomes of saturated lecithin completely loses the function as a barrier to glucose at about the transition temperature.

Influence of cholesterol incorporation on diffusion rates of glucose from dipalmitoyllecithin and dimyristoyllecithin liposomes

Liposomes prepared with dipalmitoyllecithin, dicetyl phosphate and various quantities of cholesterol were incubated for 10 min at different temperatures. Glucose permeability through liposomes with 15 mole%, 20 mole% and 30 mole% of cholesterol increased markedly above 30 °C (Fig. 3).

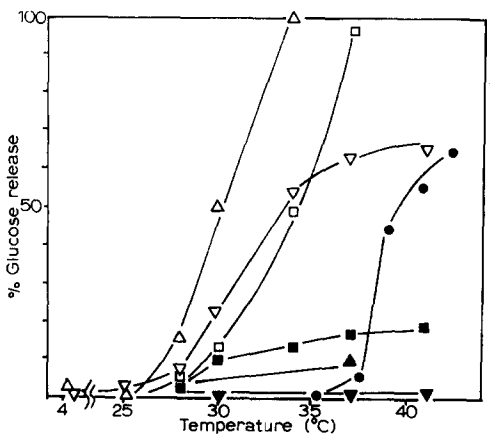

Fig. 3. Effect of temperature on glucose diffusion after incubation for 10 min from liposomes of dipalmitoyllecithin with and without cholesterol. The experiment was similar to that described in the legend to Fig. 2 except that the liposomes were prepared with various amounts of cholesterol. Liposome compositions are as follows: ●, dipalmitoyllecithin, dicetyl phosphate (1:0.1); □, dipalmitoyllecithin, dicetyl phosphate, cholesterol (1:0.1:0.2); △, dipalmitoyllecithin, dicetyl phosphate, cholesterol (1:0.1:0.3); ▽, dipalmitoyllecithin, dicetyl phosphate, cholesterol (1:0.1:0.4); ■, dipalmitoyl lecithin, dicetyl phosphate, cholesterol (1:0.1:0.5); ▲, dipalmitoyl lecithin, dicetyl phsophate, cholesterol (1:0.1:0.75); ▼, dipalmitoyl lecithin, dicetyl phosphate, cholesterol (1:0.1:1).

Permeability rates of glucose through liposomes with 20 mole% of cholesterol at 37 °C was so fast that the liposomes lost almost 100% of the trapped glucose within 5 min.

With the incorporation of more than 33 mole% of cholesterol, the permeation of glucose through dipalmitoyllecithin liposomes was almost independent of the temperature. In liposomes having 50 mole% of cholesterol, the rates of glucose diffusion were less than 1% per h at 41 °C. Glucose was apparently less permeable at 40 °C through liposomes with more than 33 mole% of cholesterol than through liposomes without cholesterol (Fig. 3).

The leakage of glucose from liposomes prepared with dimyristoyllecithin, dicetyl phosphate and various quantities of cholesterol at different temperatures is demonstrated in Fig. 4. In agreement with the experiments for dipalmitoyllecithin,

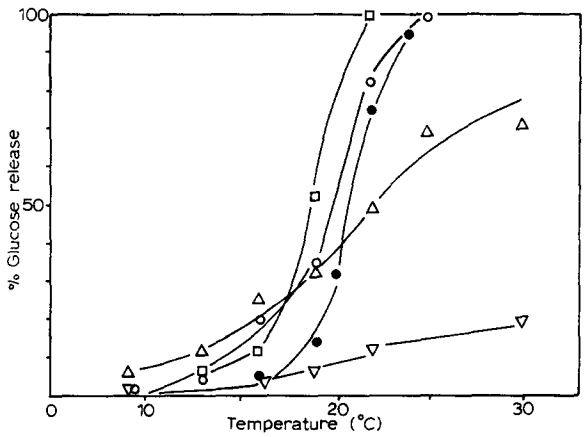

Fig. 4. Effect of temperature on glucose release from liposomes of dimyristoyllecithin with and without cholesterol. Experiment was similar to that described in the legend to Fig. 3 except that the liposomes were prepared with dimyristoyllecithin. Cholesterol contents of liposomes (molar ratios to phospholipid): without (●), 0.1 (○), 0.2 (□), 0.3 (△), 0.4 (▽).

the incorporation of 10–15 mole% of cholesterol enhanced temperature-dependent glucose permeability, while the incorporation of more than 30 mole% of cholesterol apparently reduced the permeation of glucose. The enhancing effect of cholesterol incorporation on permeation of glucose through dimyristoyllecithin liposomes was found to be much less than the effect on the permeation through dipalmitoyllecithin liposomes. In contrast to dipalmitoyllecithin liposomes, the enhancing effect of cholesterol seemed maximum at around 15 mole%, and furthermore the degree of reduction of the permeation rates by introducing 30 mole% of cholesterol was much more significant in dimyristoyllecithin liposomes.

Egg lecithin liposomes were also affected by cholesterol incorporation as reported by Demel et al. [11]. It should be emphasized, however, that cholesterol had a much smaller effect on liposomes prepared with egg lecithin. In addition, no enhancing effect of cholesterol on glucose permeability was observed in liposomes with egg lecithin.

Effect of lecithin fatty acid substituents on glucose permeability

To determine how the nature of fatty acid substituents influences the temperature dependence of the permeability, an experiment was performed with liposomes prepared from mixtures of saturated dipalmitoyllecithin and egg lecithin. Fig. 5 indicates that the incorporation of more than 25 mole% of egg lecithin reduced the permeability of glucose at 40 °C. On the contrary, the permeability of glucose at 4–10 °C was enhanced when egg lecithin was incorporated into liposomes of dipalmitoyllecithin. Since liposomes with dipalmitoyllecithin and 20 mole% of cholesterol were more sensitive to temperature than liposomes without cholesterol. The effect of fatty acid substituents on the temperature dependence of the permeability was determined by using liposomes prepared from a mixture of dipalmitoyllecithin, egg lecithin and 20 mole% of cholesterol. The permeability at 37 °C was completely suppressed by incorporating 25 mole% of egg lecithin, while the permeability at 1 °C increased

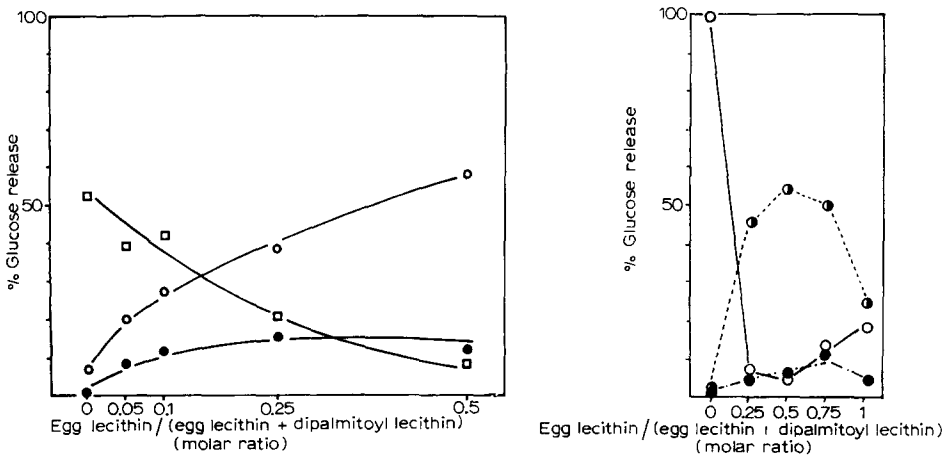

Fig. 5. Effect of fatty acid composition of lecithin on the diffusion rate of glucose through liposomes at various temperatures. Liposomes were prepared from mixtures of dipalmitoyllecithin and egg lecithin in varying molar ratios as indicated. They were incubated for 10 min 4–10 °C (○), 26 °C (●) and 40 °C (□). Release of glucose was determined by the standard procedure.

Fig. 6. Effect of fatty acid composition of lecithin on the diffusion rate of glucose through liposomes with cholesterol at various temperatures. Experiments were performed using an almost similar procedure as that described in the legend to Fig. 5. Liposomes with cholesterol (molar ratio to phospholipid:0.3) were prepared with various mixtures of dipalmitoyllecithin and egg lecithin as indicated. The incubation temperature was 37 °C (○), 21 °C (●) and 1 °C (◑).

through liposomes with the mixture of dipalmitoyllecithin and egg lecithin (Fig. 6). It was also observed that the rate of permeation of glucose at 1 °C was maximal in liposomes having an equimolar mixture of dipalmitoyl- and egg lecithins. In this regard, some observations by Kinsky et al. [10] and Demel et al. [11] appear relevant. They observed that more marker (glucose or phosphate) was released from liposomes prepared with egg lecithin or synthetic 1-stearoyl-2-oleoyllecithin at 2–5 °C that at 20–30 °C. It was also reported by the same authors that such phenomena did not occur in liposomes prepared with either dioleoyl- or dilinoleoyllecithins.

It was confirmed in the present study that more glucose diffused through liposomal membranes of egg lecithin at 1–10 °C than at 15–25 °C. The enhanced permeability at 1 °C was not observed in liposomes of dipalmitoyl- or dimyristoyllecithin without cholesterol.

It was further shown that liposomes prepared with mixtures of dipalmitoyl- and egg lecithins were much more sensitive to low temperature than liposomes prepared with egg lecithin.

Influence of cholesterol incorporation on glucose release from liposomes with mixtures of egg lecithin and dimyristoyllecithin

The preceding experiments with liposomes of the mixture of dipalmitoyllecithin and egg lecithin were repeated with liposomes containing dimyristoyllecithin and egg lecithin. The permeability through liposomes with equal quantities of egg lecithin and dimyristoyllecithin was quite insensitive to temperature between 20–35 °C. However, the liposome preparations released about 60–70% of trapped glucose within

10 min when incubated below 10 °C. In liposomes with 30 mole% of cholesterol, the enhancement of glucose release at a low temperature was negligible.

Permeability property of liposome with beef sphingomyelin

Beef brain sphingomyelin, the acyl chains of which were reported to be mainly stearic acid and nervonic acid, is one of the main components of myelin membranes. Therefore, it is interesting to determine the permeability property of sphingomyelin.

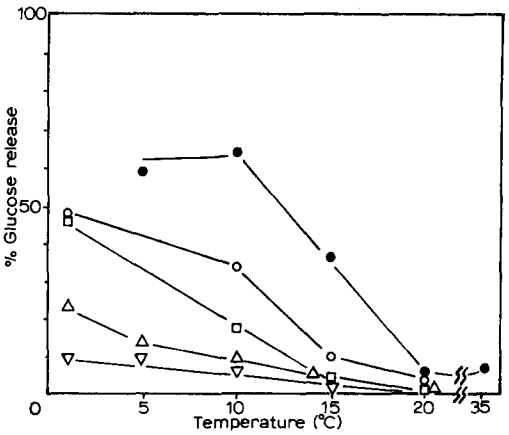

Fig. 7. Effect of temperature on the percent of trapped glucose released after incubation for 10 min from liposomes prepared with a mixture of dimyristoyllecithin, egg lecithin and dicetyl phosphate in the molar ratio of 1:1:0.2 and varying amounts of cholesterol. The cholesterol contents of the liposomes (molar ratio to phospholipid) were as follows: 0.1 (○), 0.2 (□), 0.3 (△) and 0.4 (▽). The diffusion rate of glucose through liposomes without cholesterol was also illustrated (●).

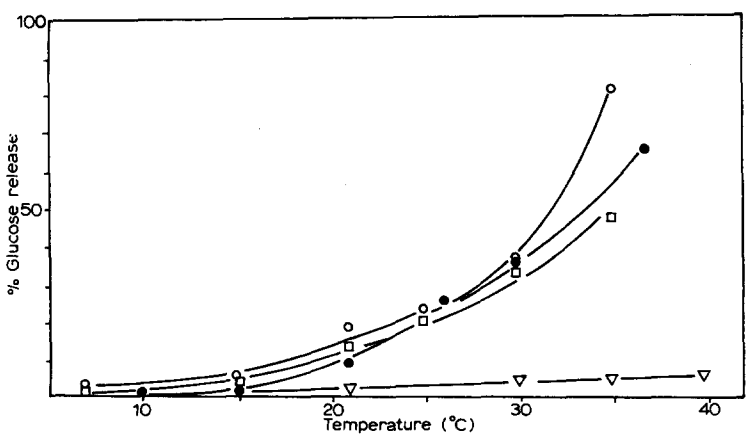

Fig. 8. Temperature sensitivities of glucose permeability through liposomes with beef brain sphingomyelin. Liposomes were prepared with beef brain sphingomyelin and dicetyl phosphate in the molar ratios of 1:0.1 (●), and with cholesterol (in the molar ratio to phospholipid) of 0.1 (○), 0.2 (□) and 0.4 (▽). The diffusion rate of glucose through the liposomes was determined by the same procedure as described in experiments using lecithins.

Fig. 8 demonstrates that liposomes prepared with beef brain sphingomyelin also showed that the permeability showed a strong temperature dependence. The slope of the temperature-dependent curves of the permeation rates through sphingomyelin liposomes was much smaller than that of the permeation rates through dipalmitoyllecithin or dimyristollecithin liposomes. The difference might be partly due to the heterogeneity of fatty acyl chains in the sphingomyelin preparation.

The effect of cholesterol incorporation into sphingomyelin liposomes was found to be similar to the effect observed on liposomes of dimyristoyllecithin, that is, introduction of 10 mole% of cholesterol slightly enhanced the temperature-dependent permeability, while more than 15 mole% of cholesterol incorporation showed reductive effects on the permeability rates. Liposomes with more than 30 mole% of cholesterol were quite insensitive to temperature.

DISCUSSION

There have been several reports [9–15] concerning the strong temperature dependence of the permeation of solutes through lipid bilayer. Apparently, an increased thermal mobility of the chains in the lipid barrier enhances the permeation of glycol, glycerol, erythritol, glucose and ions. Also, introducing double bonds into the paraffin chains and shortening the chain length of the fatty acid residues of phospholipid increases the fluidity of the lipid barrier and consequently gives an increased permeability.

In this paper, it has been described that saturated and homogeneous chains of dipalmitoyl- and dimyristoyllecithins were extremely sensitive to temperature and liposomes composed of them dramatically lost trapped glucose at about the transition temperatures (40 and 20 °C, respectively). In liposomes of egg or rat liver lecithin, both of which have unsaturated fatty acyl chains, dependence of the permeability on temperature was much weaker than in liposomes of dipalmitoyl- and dimyristoyllecithins.

In this connection, it is interesting to note that relatively steep drops in the swelling rate of liposomes at the transition temperatures were observed by de Gier et al. [9, 13]. Their results suggest that glycerol and glycol freely penetrate liposomal membranes with either saturated or unsaturated phospholipids above the transition temperatures. In the present paper, it was shown that the temperature-dependent permeability of glucose through saturated lecithin bilayers was similar to that of "fast-moving" solutes like glycerol or glycol. In contrast, the permeability of glucose through a bilayer of lecithin having unsaturated fatty acyl chains is completely different from that of glycerol or glycol. Bilayers of lecithin having unsaturated fatty acyl chains (egg lecithin) could function as a barrier toward glucose even above the transition temperatures, at which glycerol or glycol could permeate freely.

Quite recently, it was reported by Papahadjopoulos et al. [14] that the self-diffusion rates of Na^+ and sucrose through dipalmitoyl phosphatidyl glycerol or dipalmitoyllecithin vesicles also exhibited a dramatic increase between 30 and 40 °C. It was also observed that the permeability rates of Na^+ decreased between 40 and 46 °C. They postulated that the observed maximum in the diffusion rates could be related to increased permeability through microscopic regions of disorder, which were formed in the membrane plane during the process of phase transition.

It is a generally accepted concept that the close regulation of the ratio of unsaturated fatty acid to saturated acid could be of vital importance to living organisms. Unsaturated fatty acids are believed to be essential for the growth of *Escherichia coli* and for the membrane function [29–33]. The minimum amount of unsaturated fatty acids required for the growth of the unsaturated fatty acid auxotroph of *E. coli* was estimated to be 11–15% of the phospholipid fatty acid by Akamatsu et al. [33]. Cronan and Gelman [31] recently obtained similar results by using unsaturated the fatty acid auxotroph of *E. coli* .In their experiments, it was suggested that 15–20% of unsaturated fatty acid was essential for the normal membrane function of *E. coli*. These estimations are quite interesting because the existence of about 15% of unsaturated fatty acyl chains in liposomes suppressed a dramatic leakage of trapped glucose through lipids membranes above the transition temperatures. It may be possible to assume that the properties of saturated phospholipids described above are responsible for the necessity of unsaturated fatty acid for most of the cells.

The unexpectedly dramatic release of glucose was noted when liposomes with a mixture of saturated lecithin and egg lecithin were incubated at the low temperature range. McElhaney et al. [4] described that intact *Acholeplasma* cells were lysed spontaneously when the temperature was lowered to the point where most of the membrane lipids existed in the gel state. Haest et al. [15] observed that cooling *E. coli* cells or liposomes prepared with dimyristoyllecithin and 5 mole% of egg phosphatidic acid below their transition temperatures resulted in a release of small molecules. In the present investigation, liposomes of saturated lecithins did not show any significant glucose release upon cooling. The permeability increase upon cooling was only demonstrated with liposomes containing a certain amount of unsaturated acyl chains. Therefore, some faults in the lipid membrane may be formed when membrane lipids having both unsaturated and saturated fatty acid residues partially approach the crystalline state. It is reasonable to assume that cholesterol incorporation may suppress formation of these faults.

It was also demonstrated here that the incorporation of cholesterol into liposomes of saturated lecithins or sphingomyelin had complicated effects on the permeability of glucose. Introducing 10–20 mole% of cholesterol into dipalmitoyllecithin liposome enhanced the temperature-dependent permeability of glucose. Ladbrook et al. [26] observed by differential scanning calorimetry and X-ray diffraction that cholesterol tended to liquify saturated lecithin membranes which were normally below their liquid–crystalline transition temperatures. Thus, the permeability enhanced by cholesterol incorporation into the liposomes of dipalmitoyllecithin could be explained by the increase in mobility of the acyl chains of lecithin. De Gier et al. [13] reported that the presence of 30 mole% of cholesterol in the liposome preparation with dipalmitoyllecithin enhanced the rate of penetration of glycol and glycerol below 36 °C. They also observed that the reverse effect of cholesterol was noted above 36 °C. In the present study, the incorporation of more than 30 mole% of cholesterol reversed the reduction of the temperature-dependent permeability of glucose. The suppressive effect was apparent when more than 33 mole% of cholesterol was incorporated, because the glucose release from liposomes with more than 33 mole% of cholesterol was rather small at 40 °C, while liposomes without cholesterol were very leaky at this temperature. The effect of incorporating more than 33 mole% of cholesterol is, therefore, similar to that observed constantly in the egg lecithin liposomes.

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REFERENCES

1 de Kruyff, B., Demel, R. A. and van Deenen, L. L. M. (1972) Biochim. Biophys. Acta 255, 331–347
2 McElhaney, R. N., de Gier, J. and van Deenen, L. L. M. (1970) Biochim. Biophys. Acta 255, 321–330
3 de Kruyff, B., de Greef, W. J., van Eyk, R. V. W., Demel, R. A. and van Deenen, L. L. M. (1973) Biochim. Biophys. Acta 298, 479–499
4 McElhaney, R. N., de Gier, J. and van der Neut-Kok, E. C. M. (1973) Biochim. Biophys. Acta 298, 500–512
5 Wilson, G., Rose, S. P. and Fox, C. F. (1970) Biochem. Biophys. Res. Commun. 38, 617–623
6 Overath, P., Hill, F. F. and Lamnek-Hirsch, I. (1971) Nat. New Biol. 234, 264–267
7 Esfahani, M., Ioneda, T. and Wakil, S. J. (1971) J. Biol. Chem. 246, 50–56
8 Bangham, A. D., Standish, M. and Watkins, J. C. (1965) J. Mol. Biol. 13, 238–252
9 de Gier, J., Mandersloot, J. G. and van Deenen, L. L. M. (1968) Biochim. Biophys. Acta 150, 666–675
10 Kinsky, S. C., Luse, S. A., Zopf, D. A., van Deenen, L. L. M. and Haxby, J. A. (1967) Biochim. Biophys. Acta 135, 844–861
11 Demel, R. A., Kinsky, S. C., Kinsky, C. B. and van Deenen, L. L. M. (1968) Biochim. Biophys. Acta 150, 655–665
12 Papahadjopoulos, D., Nir, S. and Ohki, S. (1971) Biochim. Biophys. Acta 266, 561–583
13 de Gier, J., Mandersloot, J. G. and van Deenen, L. L. M. (1969) Biochim. Biophys. Acta 173, 143–145
14 Papahadjopoulos, D., Jacobson, K., Nir, S. and Isac, T. (1973) Biochim. Biophys. Acta 311, 330–348
15 Heast, C. W. M., de Gier, J., van Es, G. A., Verkleg, A. J. and van Deenen, L. L. M. (1972) Biochim. Biophys. Acta 288, 43–53
16 HsuChen, C. and Feingold, D. S. (1973) Biochem. Biophys. Res. Commun. 51, 972–978
17 Demel, R. A., van Deenen, L. L. M. and Pethica, B. A. (1967) Biochim. Biophys. Acta 135, 11–19
18 Shah, D. O. and Schulman, J. H. (1967) J. Lipid Res. 8, 215–226
19 Lecuyer, H. and Dervichian, D. G. (1967) J. Mol. Biol. 45, 39–57
20 Levine, Y. K. and Wilkins, M. H. F. (1971) Nat. New Biol. 230, 69–72
21 Chapman, D. and Penkett, S. A. (1966) Nature 211, 1304–1305
22 Darke, A., Finer, E. G., Flook, A. G. and Fillips, M. C. (1972) J. Mol. Biol. 63, 265–279
23 Hsia, J. C., Long, R. A., Hiruska, F. E. and Gesser, H. D. (1972) Biochim. Biophys. Acta 290, 22–31
24 Boggs, J. M. and Hsia, J. C. (1972) Biochim. Biophys. Acta 290, 32–42
25 Schreier-Muccillio, S., Marsh, D., Dugas, H., Schneider, H. and Smith, Ian C. P. (1973) Biochim. Biophys. Acta 298, 133–144
26 Ladbrooke, B. D., Williams, R. M. and Chapman, D. (1968) Biochim. Biophys. Acta 150, 333–340
27 Gerlach, E. and Deuticke, B. (1963) Biochem. Z. 337, 477–479
28 Kinsky, S. C., Haxby, J. A., Zopf, D. A., Alving, C. R. and Kinsky, C. B. (1969) Biochemistry 8, 4149–4158
29 Silbert, D. F. and Vagelos, P. R. (1967) Proc. Natl. Acad. Sci. U.S. 58, 1579–1586
30 Kass, L. R. (1968) J. Biol. Chem. 243, 3223–3228
31 Cronan, J. E. and Gelman, E. P. (1973) J. Biol. Chem. 248, 1188–1195
32 Esfahani, M., Limbrick, A. R., Knutton, S., Oka, T. and Wakil, S. J. (1971) Proc. Natl. Acad. Sci. U.S. 68, 3180–3184
33 Akamatsu, U., Imai, M. and Nojima, S. (1972) Seikagaku (in Japanese) 44, 666