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EFFECTS OF LIPID-SOLUBLE SUBSTANCES ON THE THERMOTROPIC PROPERTIES OF LIPOSOME FILTRATION

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Summary

Filtration of dimyristoyl phosphatidylcholine or dipalmitoyl phosphatidylcholine liposomes at various temperatures from 3 to 60°C revealed a discontinuous change in filtration behavior centered about the gel-to-liquid crystal transition temperature. This change was continuous at temperatures immediately above or below the transition temperature. Although pure dipalmitoyl phosphatidylcholine liposomes are in the gel state at 22°C, passage of liposomes composed of dipalmitoyl phosphatidylcholine and cholesterol through the filters at 22°C gave results similar to those obtained with liquid-crystal liposomes. Low cholesterol concentrations were nearly as effective as high concentrations in producing this behavior; this observation is consistent with a shear mechanism for reduction of liposome size, since the stress induced by passage of the otherwise rigid liposome through a small pore would be relieved by fracture at a lattice imperfection. Liposomes composed of egg phosphatidylcholine and cholesterol were retained by the filters to a slightly greater extent than pure egg phosphatidylcholine liposomes; these results are consistent with the known condensing effect of cholesterol on liquid-crystal lipsomes and a shear mechanism occurring with filtration. Liposomes composed of dipalmitoyl phosphatidylcholine and either dipalmitoyl phosphatidic acid or dicetyl phosphate were filtered at 22°C; they showed a filtration characteristic similar to liquid-crystal liposomes. Inclusion of the water-soluble dyes eosin Y or Evans blue in dipalmitoyl phosphatidylcholine liposomes resulted in filtration at 22°C which was similar to that observed for liquid-crystal liposomes. The dyes, sodium fluorescein, 6-carboxyfluorescein and fluoresceinisothiocyanate dextran, did not alter 22°C liposome filtration.

Introduction

Liposomes are phospholipid bilayer membrane vesicles which enclose aqueous compartments. They are of interest as model biological membranes and may have medical potential as carriers for drugs and other water- or lipid-soluble substances [1,2]. In previous work [3], we demonstrated that vortexed liposomes in the liquid-crystal state pass through polycarbonate surface filters quite differently than do liposomes in the gel state. Gel-state liposomes are retained by pores smaller than the liposome diameter, but liquid-crystal liposomes behave somewhat like liquid droplets: passage through pores smaller than the liposome diameter results in a reduction of the liposome size. However, liquid-crystal liposomes only slightly larger than the pores may pass through unchanged. These results for liquid-crystal liposomes are in agreement with those obtained by Hunt et al. [4] and Szoka and Papahadjopoulos [5], but do not agree with those of Rahman et al. [6] or Schullery and Garzaniti [7]. Although our previous work showed differences in liposome filtration which depended on the phase (gel compared to liquid-crystal) of the liposomes, we reported results only at single temperatures above or below the transition. Therefore, it was not clearly established whether the liposome filtration properties themselves show a sharp transition or instead are subject to gradual change with temperature over a temperature range which includes the transition.

Cholesterol suppresses the gel-to-liquid crystal transition in liposomes: below the transition temperature it acts as a fluidizing agent, while above the transition it serves as a condensing agent [8]. If filtration properties of liposomes are indeed affected by the phase of the liposomes, then the presence of cholesterol in liposomes should alter filtration behavior compared to that obtained with pure phospholipid liposomes. That is, the filtration properties of liposomes composed of cholesterol and a phospholipid at temperatures below the transition temperature of liposomes composed of the pure phospholipid would be expected to approach those of liquid-crystal liposomes. Conversely, filtration properties of cholesterol-containing liposomes at temperatures above the transition temperature of the pure phospholipid liposomes would be expected to approach those of gel liposomes. Knowledge of the effects of cholesterol on liposome filtration would therefore address the question of the relationship of the transition to liposome filtration properties. Also, cholesterol is often included in liposomes, since it reduces the permeability of liquid-crystal liposomes [8], so that any effects of cholesterol on liposome filtration would be of technical interest. Since a wide variety of lipid- or water-soluble substances are often included in liposomes, effects of some of these substances on liposome filtration would be of interest.

In this paper we report: (1) the detailed temperature dependence of the filtration of dimyristoyl phosphatidylcholine and dipalmitoyl phosphatidylcholine vortexed liposomes; (2) the effect of cholesterol on liposome filtration; (3) the effects of the lipophilic compounds, phosphatidic acid and dicetyl phosphate, on liposome filtration; and (4) the effects of the water-soluble dyes, eosin Y, Evans blue, fluorescein, 6-carboxyfluorescein and fluorescein-isothiocyanate dextran, on liposome filtration.

Materials and Methods

Liposome preparation. Liposomes were prepared from egg yolk phosphatidylcholine, dimyristoyl phosphatidylcholine, or dipalmitoyl phosphatidylcholine. Cholesterol, dicetyl phosphate, or dipalmitoyl phosphatidic acid was added to dipalmitoyl phosphatidylcholine or egg phosphatidylcholine in some liposome preparations. All lipids were obtained from Sigma. Lipids were used without further purification. Nominal purities were 99% for dipalmitoyl phosphatidylcholine and 98% for dimyristoyl phosphatidylcholine; egg phosphatidylcholine was chromatographically prepared by the manufacturer.

The aqueous phase consisted of 0.01 M Tris-HCl buffered 0.9% saline, pH 7.1. For some preparations, the aqueous phase consisted of one of the following: 1.7% (w/v) eosin Y (Fisher), 0.39% Evans blue (Eastman), 3.14% sodium fluorescein (Sigma; assayed by the supplier at 6 mol water per mol dye), 0.02% 6-carboxyfluorescein (Eastman; protonated form) [9], or up to 12.5% fluoresceinisothiocyanate dextran, 20 000 daltons (FITC-dextran; Sigma), in the Tris-buffered saline. The dyes used were commercial preparations and may have contained some impurities.

Typically, 60 mg of lipid in 3 ml of chloroform were put into a 20 × 150 mm test-tube. The tube was coupled to a rotary evaporator, and the chloroform evaporated by a stream of nitrogen while the tube rotated. Tube contents were then subjected to vacuum for at least 30 min. The aqueous phase (20 ml) was added and the tube placed in a 37° C (dipalmitoyl phosphatidylcholine, 50° C) water bath for 30 to 60 min. The tube was vortexed twice (1 min bursts); between vortexings the tube contents were returned to bath temperature. The liposomes were allowed to cool to 22° C. Liposomes made with a dye solution were washed five times with 0.9% saline by centrifugation. Each wash was performed by spinning at 5000 rev./min for 5 min in a GLC-1 (Sorvall) centrifuge. Final resuspension was carried out with 30 ml of Tris-buffered saline without dye. For preparations in which no dye was employed, the suspension was brought to final volume by the addition of 10 ml of buffer.

Filtration. Polycarbonate surface-retention membrane filters (Nuclepore) were used throughout. Nominal pore sizes were 8, 5, 3, 1, 0.8, 0.6, 0.4, 0.2, 0.1 and 0.08 μm. The smallest pore sizes were not used in all experiments. The liposome suspension was placed in an Antlia hand pump (Schleicher and Schuell) and sequentially filtered under pressure through a membrane of each pore size. According to the manufacturer's specifications, maximum pressure obtainable with the Antlia pump is 7.8 atm. Pressures were not measured during filtrations, but were judged qualitatively by the number of piston strokes required to initiate flow. Flow rates during filtration were qualitatively determined by inspection. Each filtration step was conducted as a batch process and was considered complete when no suspension remained in the Antlia reservoir. Membranes were examined after filtration; no damage or breakage was observed. For filtration above 22° C, the entire filter apparatus was immersed in a heated water bath; for filtration at 3° C, the apparatus was placed in an ice-water bath. Filtration was begun after a delay of at least 30 min to allow equilibration. The filtrate was conveyed outside the bath through plastic tubing.

Phosphorus assay. The phosphorus assay was essentially that described by Chen et al. [10]. In this assay, a phosphomolybdic acid complex is formed in the presence of ascorbic acid; the absorbance of the phosphomolybdic acid complex is determined at 820 nm. Assays of serial dilutions of liposome suspensions confirmed that the Beer-Lambert law was obeyed. Two 250 μl aliquots of the liposome suspension and each of its filtrates were taken for the assay. The mean of the two readings, corrected for the reagent blank, was used to calculate the percent phosphorus present in the filtrate compared to the original unfiltered liposome suspension. The percent unfiltered phosphorus in each filtrate was plotted vs. the logarithm of the nominal filter pore diameter.

Leakage assay. Dipalmitoyl phosphatidylcholine liposomes were filtered at 22 and 50° C with sodium fluorescein or FITC-dextran encapsulated. For the 50° C filtrations, two 1 ml aliquots of the unfiltered liposome suspension were maintained at 50° C throughout the course of the filtration. Two 1 ml aliquots of the suspension of the unfiltered liposomes (with appropriate dye encapsulated) and of each of the filtrates were taken for the assay. One of each pair of aliquots was centrifuged (1 min, nominal 15 600 × *g*) in an Eppendorf model 5412 centrifuge, and the supernatant carefully drawn off with a Pasteur pipette. The pellet was resuspended in 1 ml of 10% (v/v) Triton X-100, and 1 ml of Triton X-100 was added to the remaining uncentrifuged aliquot. Both of these mixtures were incubated for 30 min at 37° C. For 50° C filtrations, both control aliquots were centrifuged and the pellets solubilized with Triton X-100 as described above. Fluorescence of the sodium fluorescein or FITC-dextran was measured with an Aminco-Bowman Spectrophotofluorometer, with excitation at 485 nm and emission at 520 nm. Samples were diluted as necessary to yield measurements unaffected by fluorescence quenching.

Results and Discussion

The phosphorus present in the filtrates, expressed as percentages of the total unfiltered liposome phosphorus, is shown for dimyristoyl phosphatidylcholine liposomes filtered at 3, 25, 37 and 50° C in Fig. 1. Filtration at 37 and 50° C was accompanied by low backpressures and high flow rates, while for filtration at 3° C, backpressures were quite high and flow rates low. The backpressures and flow rates observed at 25° C were intermediate between those observed at the temperature extremes. The main transition for dimyristoyl phosphatidylcholine liposomes occurs between 23 and 28° C [2]. Phosphorus profiles for filtrations at 37 and 50° C were similar, at least for 8 to 0.2 μm filtrates. The slopes (units are percent phosphorus per log pore diameter, in μm, for all slopes) of the two curves are rather flat: 0 to −0.0875. The phosphorus profiles for filtrations at 3 and 25° C are different from each other and from those of the higher temperature filtrations. The 3° C curve has a slope of about −0.900, while the 25° C curve has a slope of about −0.445. These results suggest that dimyristoyl phosphatidylcholine liposome filtration properties are not continuously changing functions of temperature, since the 37 and 50° C curves are identical, but do change in the neighborhood of the gel-

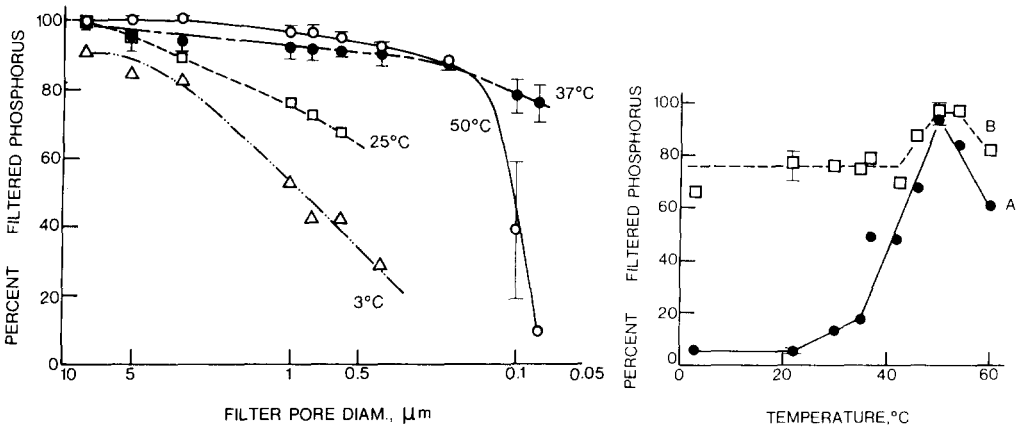

Fig. 1. Dimyristoyl phosphatidylcholine liposomes filtered at 3, 25, 37 and 50°C. Data points are means for two experiments at 37°C and for two at 50°C (except 0.08 μm filtrate, one experiment only at 50°C). Error bars show the range of results. Two different portions of the same liposome preparation were used for the 3 and 25°C filtrations. 'Percent filtered phosphorus' is the percent of initial phosphorus in the filtrate.

Fig. 2. Dipalmitoyl phosphatidylcholine liposomes filtered at various temperatures between 3 and 60°C. A, 1 μm filtrates; B, 3 μm filtrates. Data points at 22 and 50°C are the means of four and three experiments, respectively. Error bars indicate the range. Percent filtered phosphorus is the percent of initial phosphorus in the filtrate.

to-liquid crystal transition temperature. This change is not a sudden switch from the gel-phase filtration behavior (3°C) to that of the liquid-crystal phase (37 and 50°C), but is continuous, since intermediate results are obtained at the transition (25°C).

Because the dimyristoyl phosphatidylcholine used was 98% pure, while the dipalmitoyl phosphatidylcholine available was 99% pure (vendor's claims), further work continued with the dipalmitoyl phosphatidylcholine. High backpressures and low flow rates were observed during filtration of dipalmitoyl phosphatidylcholine liposomes at temperatures from 3 to 35°C, while low to moderate backpressures and relatively high flow rates accompanied filtration above 37°C. The main transition for dipalmitoyl phosphatidylcholine liposomes occurs between 41 and 43°C [2]. To simplify the presentation of the dipalmitoyl phosphatidylcholine results, the phosphorus percentages in the 3 μm filtrates (Fig. 2B) and in the 1 μm filtrates (Fig. 2A) are plotted only as a function of temperature. The distance between the curve of Fig. 2A and that of Fig. 2B, at any temperature, is proportional to the phosphorus filtration-profile slope between the phosphorus contents of the 3 and 1 μm filtrates at that temperature. The results showed a nearly constant phosphorus content (70—75%) in the 3 μm filtrates from 3 to about 42°C, followed by a rise to 95% at 50 to 54°C, and then a drop to 80% at 60°C. For the 1 μm filtrates, the phosphorus content was the same (5%) at 3 and 22°C, increased gradually to 20% at 35°C, and then increased more rapidly with temperature to 90—95% at 50°C. Finally, there was a decrease to 60% at 60°C. These results are in general agreement with those obtained for dimyristoyl phosphatidylcholine liposomes. Taking the midpoint of the rapidly ascending portion of Fig. 2A

as the transition yields a value of 42°C for the transition temperature. This is in close agreement with the value obtained by, for example, differential scanning calorimetry [8,11]. The transition, even for pure phospholipids, occurs over a range of several degrees Celsius, and often a pretransition is noted [2,8,11], so that the results are similar to those that might be expected for a transition-dependent phenomenon. The decrease in phosphorus content above 50°C is unexpected from previous results; it may indicate that another mechanism operates in dipalmitoyl phosphatidylcholine liposome filtration at high temperature.

In Fig. 3, the effect of cholesterol on dipalmitoyl phosphatidylcholine liposome filtration at 22°C is illustrated: Fig. 3B shows filtration of dipalmitoyl phosphatidylcholine/cholesterol (2 : 1, w/w) liposomes and Fig. 3C shows that for dipalmitoyl phosphatidylcholine/cholesterol (99 : 1, w/w). Fig. 3D presents the results for the filtration of pure dipalmitoyl phosphatidylcholine liposomes at 22°C, and Fig. 3A shows the results for the filtration of pure dipalmitoyl phosphatidylcholine liposomes at 50°C. Filtration of dipalmitoyl phosphatidylcholine/cholesterol (4 : 1) and dipalmitoyl phosphatidylcholine/cholesterol (9 : 1) liposomes could not be distinguished from the filtration of dipalmitoyl phosphatidylcholine/cholesterol (2 : 1) liposomes [12], nor did dipalmitoyl phosphatidylcholine/cholesterol (2 : 1) filtration results at 37°C differ from those at 22°C [12]. The filtration of cholesterol-saturated dipalmitoyl phosphatidylcholine liposomes, formed from a dipalmitoyl phosphatidylcholine/cholesterol (1 : 2, w/w) mixture in chloroform, resulted in an initial reduction (to about 50%) in the recovery of phosphorus in the 8 μm filtrate, probably due to blockage of the membrane filter pores

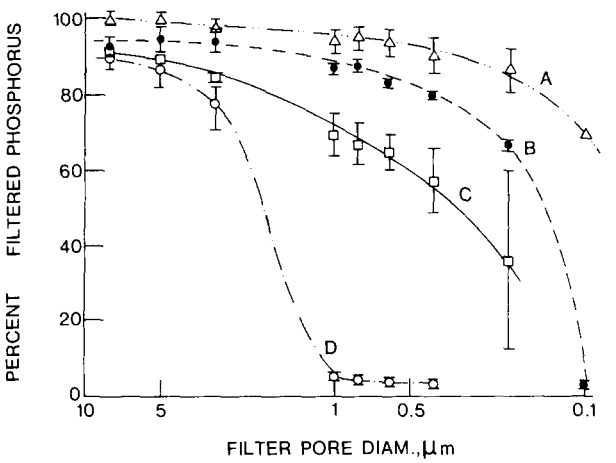

Fig. 3. Cholesterol effects on dipalmitoyl phosphatidylcholine liposomes. A, pure dipalmitoyl phosphatidylcholine liposomes filtered at 50°C; data points are the means of three experiments, except 0.4 and 0.2 μm filtrates are the means of two and 0.1 μm filtrate result is from one experiment. B, dipalmitoyl phosphatidylcholine/cholesterol (2 : 1, w/w) liposomes filtered at 22°C. Data points represent the means of two experiments. C, dipalmitoyl phosphatidylcholine/cholesterol (99 : 1, w/w) liposomes filtered at 22°C. Data points represent the means of two experiments. D, pure dipalmitoyl phosphatidylcholine liposomes filtered at 22°C. Data points are the means of four experiments, except 0.6 and 0.4 μm filtrate results are the means of two. Error bars indicate the range of results in all cases. Percent filtered phosphorus is the percent of initial phosphorus in the filtrate.

(8 μm diameter) by cholesterol crystals, which were observed with the optical microscope. The actual ratio of dipalmitoyl phosphatidylcholine to cholesterol for these liposomes is unknown, but is likely to be 2 : 1 or less by weight (1 : 1 molar), in agreement with findings on the maximum amount of cholesterol which can be included in liposomes [8].

The results obtained for the cholesterol-saturated liposomes [12] are in agreement with those for the dipalmitoyl phosphatidylcholine/cholesterol (2 : 1, w/w) liposomes (Fig. 3B). The phosphorus content of the 8 μm to 0.6 μm filtrates was essentially constant, with phosphorus content falling to near zero in the 0.8 μm filtrate. The filtration of dipalmitoyl phosphatidylcholine/cholesterol liposomes was accompanied by low backpressures and high flow rates. Optical microscopy showed the filtered liposomes reduced in diameter compared to the parent population; no aggregation was apparent. That dipalmitoyl phosphatidylcholine/cholesterol liposome filtration is like that of liquid-crystal dipalmitoyl phosphatidylcholine liposomes is in agreement with the hypothesis that liposomal membranes, when in a relatively fluid state, are sheared upon passage through pores smaller than the liposome diameter. What may at first seem anomalous is the high sensitivity of the presumed shearing action to low cholesterol concentrations: at low cholesterol concentrations, no significant effect on the transition has been noted [8]. A probable explanation for this filtration behavior is that a local fluidity, or disorder in the liquid-crystal lattice, is introduced around each cholesterol molecule; such regions would be liable to fracture when subjected to shear. At low cholesterol concentrations, these regions would be too few to have an observable effect on the population transition, which is measured by techniques such as differential scanning calorimetry and nuclear magnetic resonance.

Evidence consistent with the condensing effect of cholesterol on liquid-crystal liposomes was also obtained. The filtration results for pure egg phosphatidylcholine liposomes at 22°C are shown in Fig. 4A; these results are essentially the same as those for dipalmitoyl phosphatidylcholine liposomes filtered at 50°C (Fig. 3A) and those for dimyristoyl phosphatidylcholine liposomes filtered at 37 or 50°C (Fig. 1), as previously reported [3]. When egg phosphatidylcholine/cholesterol (2 : 1, w/w) liposomes were filtered at 22°C, the results shown in Fig. 4B were obtained. While the results for the 8 to 5 μm filtrates were the same with or without cholesterol, the cholesterol-containing egg phosphatidylcholine liposomes were retarded more by filter pores smaller than 1 μm than were the pure egg phosphatidylcholine liposomes. These results are compatible with a shear mechanism of liposome filtration. The condensing effect of cholesterol on the liquid-crystal liposomes results in only a small increase in liposome retention on the filters because, although there is an increase in membrane rigidity, there would continue to be a high probability of fracture in locally fluid regions. The effects of the lipid-soluble substances, dicetyl phosphate and dipalmitoyl phosphatidic acid on dipalmitoyl phosphatidylcholine liposome filtration at 22°C are shown in Fig. 5. The results of the filtration of dipalmitoyl phosphatidylcholine/dicetyl phosphate (11 : 1, w/w) liposomes, illustrated in Fig. 5A, are intermediate between those obtained for gel and liquid-crystal liposome filtration. Nearly

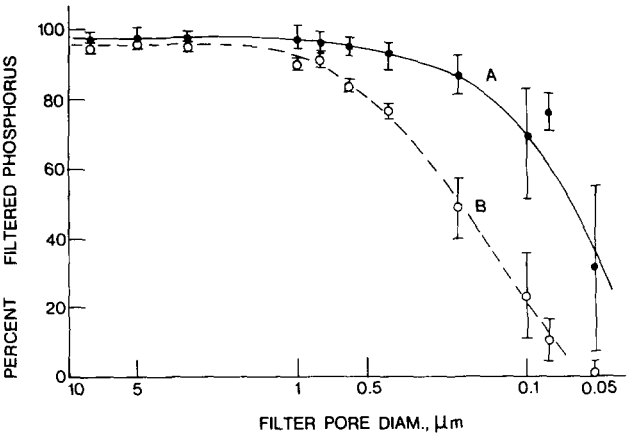

Fig. 4. Cholesterol effects on egg phosphatidylcholine liposomes. A, pure egg phosphatidylcholine liposomes filtered at 22° C. Data points are the means of four experiments, except 0.1 μm filtrate result is the mean of three, and 0.08 and 0.05 μm filtrate results are from two experiments. B, egg phosphatidylcholine/cholesterol (2 : 1, w/w) liposomes filtered at 22° C. Data points represent means of two experiments, except 0.05 μm point is from one. Error bars show the range of results. Percent filtered phosphorus is the percent of initial phosphorus in the filtrate.

the same results were obtained for the filtration of dipalmitoyl phosphatidylcholine/dicetyl phosphate (22 : 1) liposomes. No difference was noted in the filtration behavior of egg phosphatidylcholine/dicetyl phosphate compared to egg phosphatidylcholine liposomes [12]. Filtration results for dipalmitoyl phosphatidylcholine/dipalmitoyl phosphatidic acid (55 : 3 and 55 : 0.3, w/w) are presented in Fig. 5B and C, respectively. The higher dipalmitoyl phosphatidic acid concentration resulted in filtration roughly similar to that for dipalmitoyl phosphatidylcholine/dicetyl phosphate (11 : 1) liposomes, while filtra-

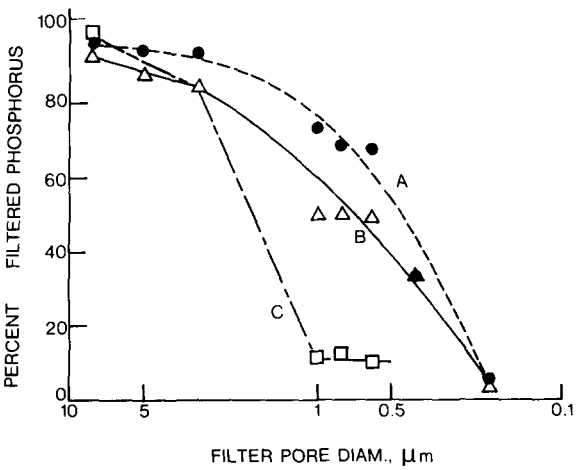

Fig. 5. Filtration at 22° C: A, dipalmitoyl phosphatidylcholine/dicetyl phosphate (11 : 1, w/w) liposomes; B, dipalmitoyl phosphatidylcholine/dipalmitoyl phosphatidic acid (55 : 3, w/w) liposomes; C, dipalmitoyl phosphatidylcholine/dipalmitoyl phosphatidic acid (55 : 0.3, w/w) liposomes. Percent filtered phosphorus is the percent of initial phosphorus in the filtrate.

tion of liposomes with the lower dipalmitoyl phosphatidic acid concentration gave results (Fig. 5C) nearly the same as those for pure dipalmitoyl phosphatidylcholine liposomes in the gel state (Fig. 3D). Filtration results for egg phosphatidylcholine/dicetyl phosphate (60 : 1, w/w) liposomes were identical to those for pure egg phosphatidylcholine liposomes [12]. Although both dicetyl phosphate and dipalmitoyl phosphatidic acid are negatively charged species, their effects on liposome filtration behavior are not necessarily related to charge. An increase in membrane fluidity seems a possible correlate of the incorporation of dicetyl phosphate or dipalmitoyl phosphatidic acid, but the effects of either on membrane fluidity are unknown. The transition temperature of dipalmitoyl phosphatidic acid has been recently determined [13] to be 67°C. We speculate that the filtration effect we have observed with this lipid is due to local disordering of the dipalmitoyl phosphatidylcholine bilayer as a result of the difference in polar head groups of the two lipids.

The effects of some water-soluble dyes on dipalmitoyl phosphatidylcholine liposome filtration at 22°C are shown in Fig. 6. Results for eosin Y (Fig. 6A) and for Evans blue (Fig. 6B) are different from those for gel-state dipalmitoyl phosphatidylcholine liposomes in buffer only (Fig. 3D). Results for protonated 6-carboxyfluorescein and fluoresceinisothiocyanate dextran were like those for gel-state dipalmitoyl phosphatidylcholine liposomes without any dye [12]. The changed filtration behavior observed with the dyes, eosin Y and Evans blue, suggests that they interact with the lipid membrane, and that this interaction alters membrane fluidity. The penetration of liposomal membranes by eosin Y has been reported [14]. These results are also interesting in terms of the biological effects of fluorescein analogs, a class of dyes including eosin Y. These biological effects increase with increasing lipid solubility of the dye [15], and Levitan [15] has suggested that such effects may be due to dye action on a general membrane property such as membrane fluidity [16].

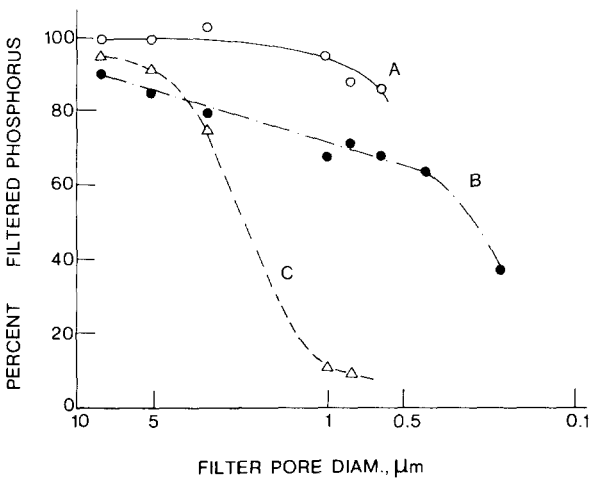

Fig. 6. Filtration at 22°C of dipalmitoyl phosphatidylcholine liposomes with one of the following water-soluble dyes encapsulated: A, eosin Y; B, Evans blue; C, sodium fluorescein. Dyes were used as received. Percent filtered phosphorus is the percent of initial phosphorus in the filtrate.

Since eosin Y is more lipophilic than fluorescein [15], and the filtration behavior at 22°C of dipalmitoyl phosphatidylcholine liposomes with eosin Y encapsulated resembles that of liquid-crystal dipalmitoyl phosphatidylcholine liposomes, the results shown in Fig. 6 tend to support the hypothesis that for lipid membranes in the presence of fluorescein-analog dyes, membrane fluidity increases with the lipophilicity of the dye. Of course, this hypothesis can be investigated with well established methods for determination of membrane fluidity.

The leakage assays, which were intended to reveal the effects of filtration on material encapsulated in liposomes, gave results which depended on the dye encapsulated. Some of the dyes originally intended as tracers for leakage assays were not suitable because they altered liposome filtration behavior, as noted earlier in this paper. For dipalmitoyl phosphatidylcholine liposomes with encapsulated sodium fluorescein (376 daltons, anhydrous) filtered at 22°C, the percentages of dye remaining in the filtrate pellets paralleled the percentages of phosphorus in the corresponding filtrates. For filtration at 50°C, however, a substantial decrease in sodium fluorescein was observed in the pellets, although the percentage of phosphorus in the filtrates was almost constant [12]. The results for dipalmitoyl phosphatidylcholine liposomes with FITC-dextran (20 000 daltons) encapsulated were unlike the sodium fluorescein results. The percentages of FITC-dextran in the pellets paralleled the percentages of phosphorus for both 22 and 50°C filtrations [12]. No fluorescence assay was performed with liposomes composed of dipalmitoyl phosphatidylcholine and cholesterol, egg phosphatidylcholine, or egg phosphatidylcholine and cholesterol. Several differing interpretations of the fluorescence results are tenable. One explanation is that considerable disruption of the liquid-crystal (50°C) dipalmitoyl phosphatidylcholine liposomes occurs during filtration, as shown by the decreased sodium fluorescein percentages present in the filtrate pellets. The results obtained with the FITC-dextran liposomes could be due to the differences in molecular weight and lipophilicity between FITC-dextran and sodium fluorescein. Only 25% of the sodium fluorescein originally present in pellets of the unfiltered liposome suspension was present in pellets of the 50°C incubated controls. In contrast, about 90% of the FITC-dextran remained in the pellets of the FITC-dextran 50°C incubated controls. The losses of sodium fluorescein accompanying filtration could be largely the result of an increased permeability of the liquid-crystal liposomes to small molecules due to stresses on the liposomal membranes during filtration. The interpretation of the FITC-dextran results would then be that minimal losses of encapsulated high molecular weight substances occur during filtrations of liquid-crystal liposomes. Resolution of these questions must await further experimentation.

The results of these studies suggest that filtration of liposomes may be a useful technique for determining the degree of fluidity or rigidity of the liposomal membranes. Determining membrane fluidity might be of particular interest when liposomes include substances which might interact with the membranes. Filtration of liposomes at various temperatures could serve as an alternative to current techniques for finding thermotropic transitions in liposomes; although a relatively time-consuming method, the filtration technique has the advantage of requiring no expensive instrumentation.

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