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THE AFFINITY OF CHOLESTEROL FOR PHOSPHATIDYLCHOLINE AND SPHINGOMYELIN

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Summary

Erythrocyte ghosts were incubated with sonicated vesicles and the uptake of cholesterol by vesicles allowed to proceed to equilibrium. The experiments were carried out for a series of phospholipids at different temperatures. The equilibrium partition of cholesterol between ghosts and single shelled vesicles provided a measure of the relative affinities of cholesterol for the different phospholipids studied. It was found that the affinity of cholesterol for dipalmitoyl phosphatidylcholine was the same as that for *N*-palmitoyl sphingomyelin both at temperatures above and below the gel to liquid crystalline transition temperature of these phospholipids.

Introduction

Cholesterol is a major constituent of many biological membranes. Numerous studies have established that cholesterol can affect the packing of the paraffin chains of membrane lipids and that it plays a role in maintaining membrane fluidity [1]. However, very little is known about the localization of cholesterol in the membrane and about its interaction with other membrane constituents. This lack of information is due in part to the non-reactivity of the cholesterol molecule. Thus, the techniques which made possible the elucidation of the disposition of proteins and phospholipids in the erythrocyte membrane cannot be applied to the study of cholesterol. However, cholesterol exchanges freely between erythrocytes and sonicated phospholipid vesicles [2] and furthermore the cholesterol content of the membrane can be modified over a considerable range [3,4]. In the studies reported here, these features of membrane cholesterol have been exploited to obtain information about the interaction of cholesterol with phospholipids.

Recent studies using differential scanning calorimetry have led to the suggestion that in mixtures of phospholipids which show phase separation, cholesterol interacts preferentially with certain phospholipids [5,6,7]. One such study showed that cholesterol has a preferential affinity for sphingomyelin in mixtures of sphingomyelin and phosphatidylcholine where sphingomyelin was either the higher or the lower melting phospholipid [7]. In the present study we have compared the affinity of cholesterol for dipalmitoyl phosphatidylcholine to its affinity for *N*-palmitoyl sphingomyelin. These two phospholipids were chosen as they have the same acyl chain composition and the same gel to liquid crystalline transition temperature and therefore permit a precise comparison of the relative affinities for cholesterol of phosphatidylcholine and sphingomyelin. In addition, we have studied the affinity of cholesterol for two naturally occurring phospholipids, egg phosphatidylcholine and bovine brain sphingomyelin.

Erythrocyte ghosts were incubated with sonicated phospholipid vesicles and the uptake of cholesterol by the vesicles allowed to proceed to equilibrium. The equilibrium partition of the cholesterol in the system between ghosts and vesicles provided a measure of the relative affinities of cholesterol for the different phospholipids studied. These experiments showed that the affinity of cholesterol for dipalmitoyl phosphatidylcholine was the same as that for *N*-palmitoyl sphingomyelin both at temperatures above and below the gel to liquid crystalline transition temperature of these phospholipids. The affinity of both phospholipids for cholesterol was found to depend strongly on the physical state of the phospholipid.

Materials and Methods

Bovine brain sphingomyelin and egg phosphatidylcholine, both grade I, were purchased from Lipid Products (Redhill, Surrey, U.K.). Dipalmitoyl phosphatidylcholine was purchased from Sigma (St. Louis, MO). *N*-Palmitoyl sphingomyelin was synthesized according to the method of Kaller [8]. Cholesterol (purity >99%) was purchased from Nu Chek Prep. (Elysian, MN). All lipids gave a single spot after thin layer chromatography on silica gel. [^{3}H]-Cholesterol, bovine brain [^{14}C]sphingomyelin and [^{14}C]dipalmitoyl phosphatidylcholine were purchased from New England Nuclear (Boston, MA). Egg [^{14}C]phosphatidylcholine was synthesized from enzymically prepared phosphatidic acid and [*Me*-^{14}C]choline chloride [9]. Aquasol (New England Nuclear, Boston, MA) was used as scintillation solvent.

Blood from healthy human donors was collected in heparin (4000 U.S.P. units/l blood). Plasma and the 'buffy coat' were separated from the cells after 15 min centrifugation at 1500 × *g*. The red cells were washed 3 times in 10 vols. of the following buffer: 150 mM NaCl/5 mM KCl/5.5 mM Na_2HPO_4/0.8 mM NaH_2PO_4/0.5 mM $CaCl_2$/5 mM glucose (pH 7.4). Plasma was preheated for 50 min at 56°C to destroy lecithin : cholesterol acyltransferase activity [10] and then labeled with [^{3}H]cholesterol as described by Murphy [11]. Labeling of erythrocytes by exchange from plasma was accomplished by incubating them at 37°C at a hematocrit of 30% in buffer containing approx. 4% labeled plasma for 15—17 h. Unsealed ghosts were prepared from labeled

erythrocytes using the technique described by Steck [12].

For preparation of sonicated vesicles approx. 20 μmol of ^{14}C-labeled phospholipid with the addition of 3.5—5.0 μmol cholesterol in some experiments, was lyophilized from benzene. The lyophilized lipids were dispersed in approx. 6 ml 150 mM NaCl/5 mM sodium phosphate (pH 8.0) and sonicated for 30—45 min under N_2 in a water-jacketed cell, using a Branson sonifier. The sonication temperature was maintained at 4°C for the preparation of egg phosphatidylcholine vesicles and at 25°C in the case of all the other lipids. The sonicate was centrifuged at 15 000 × *g* for 20 min at 4°C to remove Ti particles shed from the probe. Vesicles were used immediately after preparation.

Incubations of [^{3}H]cholesterol-labeled ghosts and ^{14}C-labeled vesicles were carried out under N_2 at a hematocrit of approx. 20% in phosphate-buffered saline in a temperature-controlled water bath. In some experiments streptomycin sulfate was added as an antibacterial agent. Similar results were obtained in the presence and absence of antibiotic. The ghost to vesicle phospholipid ratio varied between 10 and 0.3. Aliquots of the incubation mixture were removed at various times and the ghost membranes separated from the vesicles by a 5 min centrifugation at 12 000 × *g* in a Brinkmann model 3200 centrifuge. Aliquots of the total incubation mixture and of the supernatant were placed in scintillation solvent for the determination of radioactivity. Incubations were terminated after 36—50 h by centrifuging the mixture at 39 000 × *g* for 20 min at 4°C. The supernatant containing the vesicles was concentrated under N_2 in a magnetically stirred cell using an Amicon Diaflo ultrafilter. In some experiments approximately one-half of the concentrated vesicles was extracted for assay and the remaining vesicles were fractionated by gel filtration through a Sepharose 4B column. The radioactivity in aliquots of the eluted fractions was determined. Fractions from the training edge of the elution profile were pooled, concentrated and extracted for assay of chemical composition and radioactivity.

The lipids from ghost membranes and vesicles were extracted using the procedure of Folch et al. [13]. The possibility that some phospholipid breakdown could have occurred in the vesicles during the lengthy incubations with ghosts was checked by thin-layer chromatography. No lysophosphatidylcholine or fatty acids were detected indicating that contamination by these breakdown products was less than 5%. Furthermore, the presence of small amounts of lysoderivatives in phospholipid vesicles has no effect on cholesterol movement between the vesicles and erythrocytes [14].

Cholesterol and phospholipid were measured using the techniques of Parekh and Jung [15] and Gomori [16], respectively. Since large amounts of phospholipid can interfere with the color development in the cholesterol assay, the lipids were saponified and cholesterol was extracted by hexane prior to assay.

Calculations

The cholesterol and phospholipid content and the cholesterol-specific activity of the ghosts at the end of an experiment were determined directly by mass assay and the cholesterol/phospholipid molar ratio calculated. The cholesterol/phospholipid molar ratio of the vesicles was determined in various ways as the experiments progressed. Initially, at the end of the experiment, the vesicles

were fractionated by gel filtration and the ^{3}H and ^{14}C content of the eluted fractions was determined. The fractions constituting the trailing edge of the second peak which contained homogeneous, single shelled vesicles were then pooled, extracted and the ^{3}H, ^{14}C and phospholipid content was measured. Cholesterol mass assay of the vesicles was not possible due to sample-size limitations. Vesicle cholesterol therefore was determined from ^{3}H radioactivity content and the measured ghost cholesterol specific activity, using the relation: cholesterol mass = radioactivity/specific activity. The cholesterol/phospholipid molar ratio of each of the fractions eluted from the column was calculated from the [^{14}C]phospholipid specific activity in the vesicles and the [^{3}H]cholesterol specific activity of the ghosts. For all the different phospholipid vesicles studied it was found that the mol cholesterol/mol phospholipid value determined by extraction and assay of the total vesicle population agreed with the value calculated for the single shelled vesicles eluted from the column to within 10—15%. Furthermore, the cholesterol/phospholipid molar ratio determined for the fractions eluted in the void volume was never significantly different from that of the single shelled vesicles. Therefore, in later experiments the vesicles were not fractionated and the whole population was assayed instead.

Results

The equilibrium distribution of cholesterol between ghost membranes and sonicated vesicles prepared from each of four phospholipids: dipalmitoyl phosphatidylcholine, *N*-palmitoyl sphingomyelin, egg phosphatidylcholine and bovine brain sphingomyelin was studied. The interpretation of these studies in terms of relative cholesterol affinities for the different phospholipids depended on the validation of two essential assumptions: (1) equilibrium of cholesterol distribution was attained during the course of the incubation of ghosts with vesicles, (2) the vesicles after acquiring cholesterol from ghosts were single shelled, that is they did not contain internal layers of phospholipid not available for cholesterol exchange. Equilibrium was demonstrated by kinetic analysis of the time course of uptake of cholesterol by the vesicles. The presence of single shelled vesicles was demonstrated by gel filtration. After the incubation of sonicated phospholipid vesicles with ghosts was terminated, the vesicles were fractionated and the cholesterol/phospholipid molar ratio determined in the separated fractions. In this way, it was possible to measure the mol cholesterol/mol phospholipid at equilibrium in those vesicles that were still homogeneous in size and unilamellar at the end of the period of incubation with ghosts.

Time course of cholesterol uptake by vesicles: proof of equilibration

In order to be certain that the data taken in these experiments involved ghosts and vesicles close to equilibrium with respect to cholesterol movement, it is necessary to show that the rate of this process is sufficiently rapid compared to the duration of the incubations (40—50 h). A representative time course of [^{3}H]cholesterol uptake from ghosts into egg [^{14}C]phosphatidylcholine vesicles is shown in Fig. 1. Previous studies [17] have shown that erythrocyte membrane cholesterol is in two pools, with approx. 10% in a more

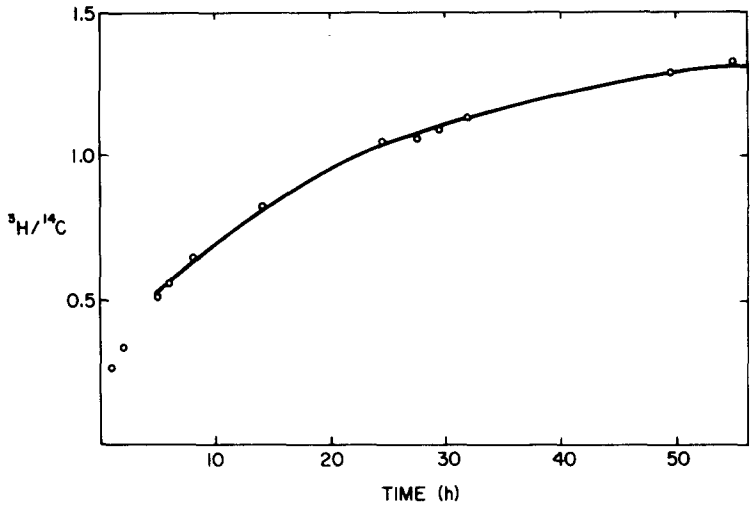

Fig. 1. Time course of uptake of cholesterol by egg phosphatidylcholine vesicles from ghosts at 46°C. In this experiment approximately equal amounts (in terms of lipid phosphorus) of [^{3}H]cholesterol-labeled ghosts and ^{14}C-labeled vesicles were incubated together. Aliquots were sampled at different times and the vesicle radioactivity determined. The curve shows the exponential function $y = 1.398 - 1.102\ \exp(-t/22)$ which best fits data points taken after the second hour of the experiment.

rapidly exchangeable pool than the remaining 90%. In that study it was shown that the rapidly exchangeable cholesterol pool was depleted after 2—3 h incubation of erythrocytes with sonicated phospholipid vesicles. Therefore, if we use only the data points obtained after the initial 2 h of incubation we can fit with a function

$$y(t) = y_f + (y_i - y_f)\ \exp(-t/\tau)$$

where $y(t)$ is the $^3H/^{14}C$ ratio in the vesicles at time t. This function describes a single exponential process of half-time τ. y_i, the value of the function at $t = 0$, incorporates the completed fast exchange and y_f is the value of the function at infinite time. The determination of the parameters y_i, y_f, and τ to provide the best fit to the experimental data and the determination of σ_τ, the variance giving the uncertainty in the determination of τ were performed as described previously [4]. The parameters of best fit to the experiment shown in Fig. 1 were: $y_i = 0.296$, $y_f = 1.398$ and $\tau = 22.0 \pm 1.9$ h.

The excellence of fit of the exponential function to the data is apparent from Fig. 1. A quantitative expression of this is the fact that the variance per data point is less than, but comparable to the experimental uncertainty of a typical data point. An exponential process with a τ of 22 h is clearly near its equilibrium limit at times on the order of 50 h. Using the fit to the data at 50 h, vesicle cholesterol content was 92% of its true equilibrium value.

The recovery of sonicated vesicles in the supernatant after centrifugation was measured by the recovery of ^{14}C-labeled phospholipid. In all experiments, about 90% of the labeled phospholipid remained in the supernatant indicating that vesicle fusion with ghosts was negligible. Some of the loss of ^{14}C radioactivity from the supernatant could be accounted for by the slow exchange of

phospholipid between ghosts and vesicles. When the data were expressed as the uptake of ^3H radioactivity in the supernatant, the fitting procedure gave the same parameters as those determined for the data expressed as the $^3H/^{14}C$ radioactivity ratio.

Similar data were obtained from sphingomyelin vesicles incubated with ghosts. However, it was found that when ghosts were incubated with either dipalmitoyl phosphatidylcholine or *N*-palmitoyl sphingomyelin vesicles, massive loss of vesicles from the supernatant occurred between 4 and 20 h of incubation. Vesicles incubated under identical conditions in the absence of ghosts were stable and therefore the instability observed in the presence of ghosts was attributed to the lysis of vesicles containing 15 mol% cholesterol which has been reported by other authors in studies of dipalmitoyl phosphatidylcholine vesicles [18]. This problem was circumvented by incorporating approx. 20 mol% cholesterol into the dipalmitoyl phosphatidylcholine and *N*-palmitoyl sphingomyelin vesicles during their preparation. It was found that the presence of cholesterol in the vesicles at the outset of the incubation with ghosts did not affect the final equilibrium distribution of cholesterol between ghosts and vesicles.

In all the experiments described here the distribution of cholesterol between ghosts and vesicles was measured under conditions such that cholesterol movement was within at least 90% of equilibrium.

Gel filtration of sonicated vesicles

When the sonicated phospholipid vesicles were fractionated by gel filtration through a Sepharose 4B column immediately after preparation, two lipid peaks were observed. Over 95% of the lipid phosphorus was made up of fraction II [19] homogeneous vesicles. The vesicles were prepared by sonication at 25°C, a temperature below the gel to liquid crystalline transition temperature (T_c) of sphingomyelin. To assess the stability of the vesicles, they were incubated at 45°C for 24 h and then subjected to gel filtration once more. It was found that the elution profile was not modified by this procedure. A profile of a fractionation of [^{14}C]sphingomyelin vesicles which had been incubated with [^3H]-cholesterol-labeled ghosts for 40 h is shown in Fig. 2. The cholesterol/phospholipid molar ratio values for eluted fractions were all similar as can be seen from the small variability of the $^3H/^{14}C$ ratio in the different fractions (Fig. 2). In the experiment illustrated in Fig. 2 there appears to be a systematic decrease in $^3H/^{14}C$ in the fractions from the trailing edge of the elution profile. This was not a consistent feature of the experimental results and the variation in $^3H/^{14}C$ in the different fractions never exceeded 20% and was often less than 10%. Similar results were obtained for all the other phospholipids studied. In each case the value of the cholesterol/phospholipid molar ratio determined for each of the eluted fractions showed almost no variation.

Equilibrium distribution of cholesterol between ghosts and vesicles prepared from different phospholipids

The dependence of the vesicle cholesterol/phospholipid molar ratio on the ghost cholesterol/phospholipid molar ratio at equilibrium was investigated for all four phospholipids. In order to study this equilibrium over a range of ghost

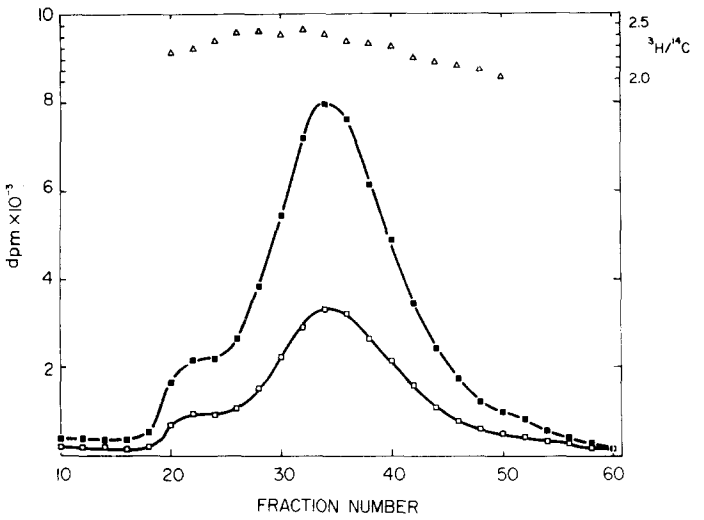

Fig. 2. Elution pattern of bovine brain [^{14}C]sphingomyelin vesicles after 40 h incubation at 46°C with [^{3}H]cholesterol-labeled ghosts. ■, ^{3}H radioactivity; □, ^{14}C radioactivity; △, ratio of ^{3}H to ^{14}C radioactivity. Column size: 10 X 250 mm.

cholesterol contents, incubations were carried out using different proportions of ghosts and vesicles. From a single preparation of ghosts and vesicles, three mixtures containing different amounts of ghosts and vesicles were prepared and allowed to reach equilibrium. In these experiments simultaneous incubations of mixtures containing ratios of ghost phospholipid to vesicle phospholipid of, for example, 10 : 1, 1 : 1 and 0.3 : 1 were carried out. In the case of high ratios of ghosts to vesicles, equilibrium was attained after only a small loss of ghost cholesterol and the vesicle cholesterol/phospholipid molar ratio was high. Conversely, at low ratios of ghosts to vesicles, the ghosts lost a lot of cholesterol and the equilibrium vesicle cholesterol/phospholipid molar ratio was low. In these experiments the incubations were carried out at a temperature above the gel to liquid crystalline transition temperatures of the phospholipids (T_c). The results of these experiments for dipalmitoyl phosphatidylcholine and *N*-palmitoyl sphingomyelin at 46°C are shown in Fig. 3. The equilibrium distribution of cholesterol between ghosts and vesicles was very similar for both phospholipids. The solid line in Fig. 3 is the line of equal ghost and vesicle cholesterol/phospholipid molar ratios. Taking each phospholipid separately, the data points for dipalmitoyl phosphatidylcholine and *N*-palmitoyl sphingomyelin can be fit by straight lines. The lines of best fit to the data points for dipalmitoyl phosphatidylcholine and *N*-palmitoyl sphingomyelin have slopes of 1.37 ± 0.10 (r^2 = 0.89) and 1.59 ± 0.18 (r^2 = 0.92), respectively. These slopes are not significantly different. The dashed line in Fig. 3 is the straight line of best fit (r^2 = 0.90) to the data points of both phospholipids taken together.

The temperature dependence of cholesterol distribution between ghosts and vesicles

These experiments were carried out exactly as those described in the previ-

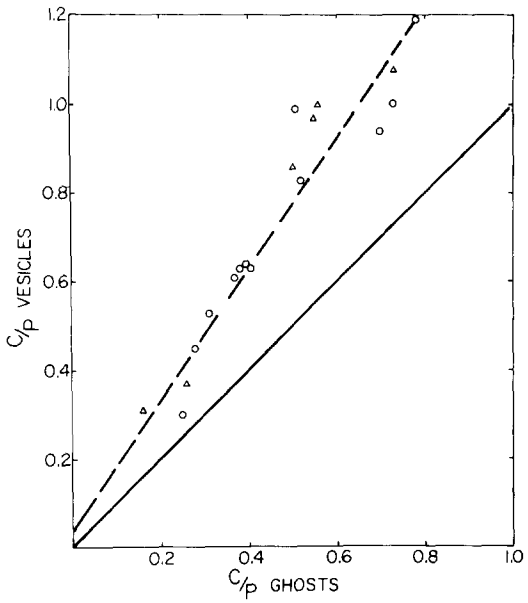

Fig. 3. Equilibrium distribution of cholesterol between ghosts and vesicles prepared from dipalmitoyl phosphatidylcholine (○) and *N*-palmitoyl sphingomyelin (△) at 46 ± 1°C. The solid line is the line of equal ghost and vesicle cholesterol/phospholipid molar ratio. The broken line is the line of best fit to the data points. C/P is the cholesterol/phospholipid molar ratio.

TABLE I

EQUILIBRIUM DISTRIBUTION OF CHOLESTEROL BETWEEN GHOSTS AND VESICLES OF DIFFERENT PHOSPHOLIPID COMPOSITIONS

r is the ratio of vesicle C/P to ghost C/P. Multiple data for a given temperature were obtained using different ratios of ghosts to vesicles in the incubation. C/P is the cholesterol/phospholipid molar ratio.

Phospholipid	*T* (°C)	C/P at equilibrium		*r*	Mean *r* ± S.D.
		Ghosts	Vesicles		
Dipalmitoyl phosphatidylcholine	22	0.71	0.24	0.31	0.35 ± 0.05 (n = 2)
		0.65	0.26	0.40	
	46	Data given in Fig. 3.			1.49 ± 0.22 (n = 12)
N-palmitoyl sphingomyelin	22	0.69	0.19	0.28	0.32 ± 0.04 (n = 2)
		0.58	0.21	0.36	
	46	Data given in Fig. 3.			
Bovine brain sphingomyelin	22	0.64	0.24	0.38	0.33 ± 0.05 (n = 2)
		0.73	0.21	0.29	
	46	0.64	1.01	1.59	1.54 ± 0.14 (n = 5)
		0.66	1.15	1.74	
		0.76	1.07	1.42	
		0.82	1.15	1.40	
		0.35	0.54	1.54	
Egg phosphatidylcholine	46	0.76	0.77	1.00	0.79 ± 0.15 (n = 5)
		0.63	0.55	0.87	
		0.48	0.29	0.60	
		0.64	0.45	0.70	
		0.77	0.59	0.76	

ous section with the difference that for a single preparation of ghosts and vesicles, mixtures having identical proportions of ghosts and vesicles were prepared and incubated at different temperatures until equilibrium was attained. The equilibrium partition of cholesterol between ghosts and vesicles at a particular temperature depends on the affinity of cholesterol both for the ghost membrane and for the phospholipid vesicle. Because these two affinities could depend on temperature in different ways, it is the ratio, r, of vesicle mol cholesterol/mol phospholipid to ghost mol cholesterol/mol phospholipid which must be compared for the different phospholipids to assess their cholesterol affinity. Table I shows the values of r determined in these experiments.

The temperature of 22°C was chosen as it is below the gel to liquid crystalline transition temperature of dipalmitoyl phosphatidylcholine and *N*-palmitoyl sphingomyelin. The transition temperature of naturally occurring sphingomyelins varies widely depending on their fatty acid composition. For the bovine brain sphingomyelin used in the present study 22°C was below the onset temperature of the transition.

Discussion

The data shown in Fig. 3 and in Table I give the equilibrium partition of cholesterol between ghosts and the various phospholipids studied. Two different kinds of phospholipid vesicles which separately are at equilibrium with ghosts having a particular cholesterol content must be at equilibrium with each other. Therefore, the data permit conclusions to be drawn about the relative affinities of cholesterol for the different phospholipids. Two of the phospholipids studied are phosphorylcholines, have the same fatty acyl chain(s), have a gel to liquid crystalline transition at the same temperature (41°C), but differ in their glycerol or sphingosine backbone. The fact that the data points shown in Fig. 3 for dipalmitoyl phosphatidylcholine and *N*-palmitoyl sphingomyelin taken separately can be fit by straight lines whose slopes are not significantly different, indicates that both phospholipids have the same affinity for cholesterol at 46°C, the temperature of these experiments. Experiments carried out at 22°C showed that also at this temperature, which is below the gel to liquid crystalline transition temperature of the phospholipids, the affinity of cholesterol is essentially the same for dipalmitoyl phosphatidylcholine and *N*-palmitoyl sphingomyelin (Table I).

Demel and coworkers [7] reported on the basis of differential scanning calorimetry studies that there is a preferential affinity of cholesterol for sphingomyelin in mixtures of sphingomyelin and phosphatidylcholine when the sphingomyelin was either the higher or the lower melting phospholipid. In that study, comparison was not made of phospholipids having the same fatty acyl chain composition. Our experiments have shown that the affinities of cholesterol for phosphatidylcholine and for sphingomyelin are the same if both phospholipids have the same fatty acyl composition. Thus, the substitution of the sphingosine backbone for the glycerol backbone does not affect the affinity of cholesterol for the phospholipid.

The affinity of phospholipids for cholesterol was found to depend on the physical state of the phospholipid. Below the gel to liquid crystalline transi-

tion temperature of dipalmitoyl phosphatidylcholine and *N*-palmitoyl sphingomyelin the parameter, *r*, had a value of approx. 0.3 whereas above the transition temperature, *r* was approximately 5-fold greater (Table I).

We included two widely used naturally occurring phospholipids in our study, bovine brain sphingomyelin and egg phosphatidylcholine. The affinity of cholesterol for bovine brain sphingomyelin was similar to that for *N*-palmitoyl sphingomyelin, however egg phosphatidylcholine had a lower affinity for cholesterol than did the other three phospholipids studied (Table I). The finding that egg phosphatidylcholine had a lower affinity for cholesterol than did both the sphingomyelins studied, is in accord with the observations of Demel and coworkers [7].

Vesicles prepared from all four of the phospholipids studied were able to take up cholesterol in amounts of approximately 1 mol per mol phospholipid (Fig. 3 and Table I) which suggests that both halves of the vesicle bilayer were available for the incorporation of cholesterol. It follows that transmembrane movement of cholesterol must occur in the vesicles. This result is consistent with the recent observation that transmembrane cholesterol movement takes place in sonicated phosphatidylcholine vesicles which have different amounts of cholesterol in the two halves of the bilayer [20]. It is worthy of comment that when vesicles prepared from dipalmitoyl phosphatidylcholine, sphingomyelin, or *N*-palmitoyl sphingomyelin were incubated at 46°C with ghosts in excess, the equilibrium cholesterol content of the vesicles reached a value as high as 1.2 mol cholesterol/mol phospholipid (Fig. 3). There exists evidence that vesicles containing up to 2 mol cholesterol/mol phospholipid can be prepared by cosonication of the lipids [21] or by drying from a common organic solvent [22], however, it is likely that these vesicles are in a metastable state [21]. The results of the present study provide the first demonstration, to our knowledge, that phospholipid vesicles can take up cholesterol in an amount greater than 1 mol cholesterol/mol phospholipid at equilibrium.

Using the cholesterol/phospholipid molar ratio of the ghosts with which they are at equilibrium as a reference, we have demonstrated that the affinity of cholesterol for dipalmitoyl phosphatidylcholine, sphingomyelin, and *N*-palmitoyl sphingomyelin is the same and greater than the affinity of cholesterol for egg phosphatidylcholine. Another feature of our data, worthy of interpretation, is the finding that egg phosphatidylcholine vesicles contained less cholesterol expressed as mol per mol phospholipid than did the ghosts with which they were at equilibrium (Table I). On the other hand, the other three phospholipids had more cholesterol per mol phospholipid than did the ghosts with which they were at equilibrium. The value of the cholesterol/phospholipid molar ratio for vesicles prepared from dipalmitoyl phosphatidylcholine, sphingomyelin, or *N*-palmitoyl sphingomyelin reached almost twice the value of the cholesterol/phospholipid molar ratio of the ghosts with which they were at equilibrium (Table I). These results could indicate that the affinity of cholesterol for the phospholipids in the ghost membrane is greater than the affinity of cholesterol for egg phosphatidylcholine and less than the affinity of cholesterol for the other three phospholipids. However, it also is a possibility that the presence of membrane proteins plays a role in determining the equilibrium partition of cholesterol between ghosts and vesicles.

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References

1 Chapman, D. (1973) in Biological Membranes (Chapman, D. and Wallach, D.F., eds.), Vol. 2, pp. 91—144, Academic Press, New York
2 Bruckdorfer, K.R., Edwards, P.A. and Green, C. (1968) Eur. J. Biochem. 4, 506—511
3 Bruckdorfer, K.R., Graham, J.M. and Green, C. (1968) Eur. J. Biochem. 4, 512—518
4 Lange, Y. and D'Alessandro, J.S. (1977) Biochemistry 16, 4339—4343
5 De Kruyff, B., Van Dijck, P.W.M., Demel, R.A., Schuijff, A., Brants, F. and Van Deenen, L.L.M. (1974) Biochim. Biophys. Acta 356, 1—7
6 Van Dijck, P.W.M., de Kruijff, B., Van Deenen, L.L.M., de Gier, J. and Demel, R.A. (1976) Biochim. Biophys. Acta 455, 576—587
7 Demel, R.A., Jansen, J.W.C.M., Van Dijck, P.W.M. and Van Deenen, L.L.M. (1977) Biochim. Biophys. Acta 465, 1—10
8 Kaller, H. (1961) Biochem. Z. 334, 451—456
9 Sears, B., Hutton, W.C. and Thompson, T.E. (1976) Biochemistry 15, 1635—1639
10 Lichtman, M.A., Marinetti, G.V. and Gordesky, S.E. (1974) Nouv. Rev. Fr. Hematol. 1, 14, 5—24
11 Murphy, J. (1962) J. Lab. Clin. Med. 60, 571—578
12 Steck, T.L. (1974) in Methods in Membrane Biology (Korn, E., ed.), Vol. 2, pp. 245—281, Plenum Press, New York
13 Folch, J., Lees, M. and Stanley, G.H.S. (1957) J. Biol. Chem. 226, 497—509
14 Bloj, B. and Zilversmit, D.B. (1977) Biochemistry 16, 3943—3948
15 Parekh, A.C. and Jung, D.H. (1970) Anal. Chem. 42, 1423—1427
16 Gomori, G. (1942) J. Lab. Clin. Med. 27, 953—960
17 Lange, Y. and D'Alessandro, J.S. (1978) J. Supramol. Struct. 8, 391—397
18 Hunt, G.R. and Tipping, L.R.H. (1978) Biochim. Biophys. Acta 507, 242—261
19 Huang, C. (1969) Biochemistry 8, 344—351
20 Poznansky, M.J. and Lange, Y. (1978) Biochim. Biophys. Acta 506, 256—264
21 Lundberg, B. (1977) Chem. Phys. Lipids 18, 212—220
22 Freeman, R. and Finean, J.B. (1975) Chem. Phys. Lipids 14, 313—320