Factors Contributing to the Distribution of Cholesterol among Phospholipid Vesicles*

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The distribution of cholesterol between vesicles of different lipid composition at equilibrium has been determined. Small, sonicated unilamellar vesicles and large unilamellar vesicles were incubated at a defined temperature, and aliquots were then obtained at selected times for analysis. Inclusion of a small amount of phosphatidylycerine or phosphatidylinositol in the membrane does not appreciably affect the distribution of cholesterol at equilibrium by these measurements. A membrane in the gel state is a poor acceptor of cholesterol. The length of the hydrocarbon chain on the phospholipid may also play a role. Bovine brain sphingomyelin dramatically slows the kinetics of cholesterol transfer, and the equilibrium distribution of cholesterol among vesicles containing sphingomyelin is therefore not observable in these experiments. Data obtained with vesicles containing phosphatidylethanolamine indicate a preference of cholesterol for vesicles composed of phosphatidylcholine compared to vesicles consisting primarily of phosphatidylethanolamine, at equilibrium. Experiments with a chaotropic agent indicate that the nature of the surface of the phosphatidylethanolamine bilayer, and its hydration, are important factors in the distribution of cholesterol among membranes in which phosphatidylethanolamine is present. These data suggest that membrane lipid content may play a role in the distribution of cholesterol among the membranes of a cell.

One of the interesting questions of cell biology concerns the role of cholesterol in cells requiring that sterol for growth and proper function. One of the important issues within that question is understanding the nonhomogeneous distribution of cholesterol among the membranes of the cell. It is well known that cholesterol is found in relatively high concentrations in mammalian plasma cell membranes, much lower in endoplasmic reticulum, and lower yet in mitochondrial membranes. The factors controlling the establishment and maintenance of this cholesterol distribution are important to understand.

Some limited data have been reported concerning factors affecting the distribution of cholesterol among membranes. It was suggested on the basis of calorimetry data that cholesterol has a preference for sphingomyelin over phosphatidylcholine (1) and for phosphatidylcholine over phosphatidylethanolamine (2). However, such an apparent preference could only be observed when a phase transition to the gel state from the liquid crystal state occurred. In contrast, Blume (3) reported no calorimetric evidence for preference of cholesterol for phosphatidylcholine in saturated phosphatidylcholine/phosphatidylethanolamine mixtures. Calhoun and Shipley (4) also reported a lack of calorimetric evidence for a preference of cholesterol for sphingomyelin in dimyristoylphosphatidylcholine/N-palmitoylsphingomyelin mixtures. Another group interpreted exchange experiments to suggest that there was no preference between sphingomyelin and dipalmitoylphosphatidylcholine for cholesterol (5).

More recently, investigators addressed the question whether the observed differences in cholesterol concentration in the various cellular membrane fractions were thermodynamically stable (6). The results indicated that the composition of the membranes did have an effect on cholesterol distribution. Therefore, one viable factor to explain the non-uniform distribution of cholesterol in cellular membranes might be effects of membrane composition on the thermodynamics of the partitioning of cholesterol. However, the composition of biological membranes is of sufficient complexity that identification of specific factors controlling the distribution of cholesterol is difficult in such a study.

The above results indicate the importance of a systematic study of the factors which might exert thermodynamic control over cholesterol distribution in cellular membranes. The experiments reported here constitute the first stage in such a study. Our approach does not require a phase transition to detect cholesterol distribution as did earlier studies. Furthermore, our approach does not require addition to the membranes of lipids not directly under investigation.

To investigate the question of cholesterol distribution, we incubated membranes of defined composition and determined the cholesterol/phospholipid ratio in each of the membranes at equilibrium where possible. In this report, the role of phospholipid components in controlling cholesterol distribution is examined in membranes of purified lipids.

**EXPERIMENTAL PROCEDURES**

**Materials—** All phospholipids, including 16:0,18:1 phosphatidylethanolamine (PC), egg phosphatidylcholine, 14:0,14:0 phosphatidylcholine (DMPC), 16:0,18:1 phosphatidylethanolamine (PE), 18:1,18:1 phosphatidylethanolamine, transphosphatidylated (from egg PC) phosphatidylethanolamine, 16:0,18:1 phosphatidylserine, bovine brain sphingomyelin, and bovine liver phosphatidylinositol, were obtained from Avanti Polar Lipids (Birmingham, AL) in pure form. The fatty acid content of the synthetic phospholipids was checked by gas chromatography, performed as described elsewhere (7). Oxytetracycline was obtained from Calbiochem-Behring. Trinitrobenzenesulfonate was purchased from Pierce Chemical Co. Phosphate was assayed

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by the method of Bartlett (8), as modified (9). Cholesterol was assayed enzymatically by the method of Allain et al. (10).

Preparation of Vesicles—Lipids were dissolved in chloroform to ensure complete mixing. When cholesterol was incorporated, this sterol was included at approximately 20 mol per cent in most cases. Cholesterol was evaporated under a stream of nitrogen gas and then under high vacuum. The dry lipid film was then hydrated and vortexed to suspend the lipid. Small unilamellar vesicles were formed by sonication of the lipid suspension for four 5-min periods on a Branson W350 sonifier in an ice bath except where noted. The vesicles were then centrifuged as described below. Large unilamellar vesicles were formed by octylglucoside dialysis at 4 °C as described previously (11), regardless of the phospholipid composition, except as noted below.

The large vesicles were further purified by centrifugation in a 50 rotor using a Beckman L5-50 ultracentrifuge. These vesicles were centrifuged at 45,000 rpm for 15 min, the supernatant was completely removed, and finally the pellet was resuspended in the same buffer.

The small vesicles were centrifuged at 45,000 rpm for 45 min, and the top two-thirds of the supernatant was harvested. The bottom one-third of the supernatant and any pellet were discarded. By ultracentrifugation of both preparations, contamination from small vesicles in the large vesicle preparation and large vesicles in the small vesicle preparation could be avoided. The success of these procedures was verified by gel filtration of the vesicles in Sepharose 4B (see below).

Vesicles were incubated at 37 °C (unless otherwise specified) in a shaking water bath during the exchange reaction. Aliquots were removed at indicated times, and the large and small vesicles were separated by differential centrifugation. The vesicles were centrifuged at 45,000 rpm for 15 min in a 50 rotor using a Beckman L5-50 ultracentrifuge. The supernatant was harvested for the small vesicles, and the pellet was harvested for the large vesicles. About one-fourth of the supernatant of the fraction directly on top of the pellet was discarded to reduce the chance of cross-contamination of the two fractions. All buffers were thoroughly purged with nitrogen gas, and vessels were flooded with argon before incubations. Furthermore, all buffers contained 1 mM EDTA to inhibit oxidation. The extent of oxidation was determined by gas chromatography. Sonication, centrifugation, and dialysis were performed at 4 °C for most preparations.

Exceptions to the above procedure occurred in the case of vesicles formed from sphingomyelin and dimyristoylphosphatidylcholine. All vesicle preparations for sphingomyelin were carried out at 45 °C or above the phase transition temperature of bovine brain sphingomyelin. Incubations involving this lipid were also performed at 45 °C rather than 37 °C. Vesicles of the fully saturated phospholipid were prepared at 30 °C, which is above the phase transition temperature of this phospholipid, and were maintained at this temperature or higher to prevent fusion of the vesicles. Fusion of vesicles can occur at or near the phase transition temperature. Incubations were then carried out at 37 °C, as for the other experiments.

Sepharose 4B column chromatography was carried out using a 1 x 30-cm column equilibrated with buffer consisting of 100 mM histidine at pH 7 and 1 mM EDTA. The eluent was pumped with a Gilson D-271 pump, monitored with a Gilson UV monitor at 280 nm, and fractions were collected using a Gilson fraction collector. 2-ml fractions were collected. The concentration of phospholipid in fractions of interest was determined by phosphate analysis.

Labeling with trinitrobenzene sulfonate was performed as described previously (12). Electron microscopy was performed on a JEOL JEM100B transmission microscope. 1% uranyl acetate was used to stain the preparations.

RESULTS

Our approach in these experiments was to use two vesicle populations that could be readily and rapidly separated. Because of the nature of the question asked and the results, a serious constraint was placed on the system. No foreign lipids could be included in the vesicles since their presence would obscure the role of the vesicle lipid content in the equilibrium distribution of cholesterol. Therefore, glycolipids could not be used with lectin columns to facilitate separation of the two vesicle populations. Neither could negatively charged lipids be added that would allow separation by ion exchange column chromatography. The only feasible technique remaining was to form vesicles of a significantly different size that could be separated rapidly by differential centrifugation. Characterization of these vesicle populations and the ability of centrifugation to separate them without significant cross-contamination is now described.

Characterization of Vesicle Preparations—Both small unilamellar vesicles and large unilamellar vesicles were made of 16:0,18:1 phosphatidylcholine, as described under “Experimental Procedures.” Chromatography on Sepharose 4B demonstrated that almost no small vesicle contamination occurred in the large vesicles, and correspondingly little large vesicle contamination occurred in the small vesicle preparation (an example is shown in Fig. 1). This was important to determine because contamination could affect the effectiveness of the separation methods used during the experiment.

For many of the experiments, separation of the vesicle populations was achieved by differential centrifugation. As described under “Experimental Procedures,” both kinds of vesicle populations were centrifuged before incubation. By this means, it was possible to select small vesicles that would not pellet under the centrifugation conditions used for separation. Correspondingly, it was also possible to select large vesicles that contained no unseparable small vesicles for the incubation experiments. These preparations were then rechecked for vesicle size by column chromatography with Sepharose 4B.

The adequacy of this separation procedure was checked as follows. In the case where vesicle populations of different phospholipid composition were incubated, thin layer chromatography of the total lipid extract of the separated populations demonstrated no significant cross-contamination. For example, in the case where the acceptor vesicles contained phosphatidylethanolamine, thin layer chromatography of the donor vesicles, after incubation for 12 h and separation from

![Fig. 1. Sepharose 4B chromatography of vesicles in 100 mM NaCl, 10 mM histidine, 1 mM EDTA. The apparent absorbance (ABS) (light scattering) at 280 nm is graphed as a function of fraction number. This represents the readout of the UV monitor and is not proportional to the phospholipid content, since light scattering is much greater per amount of phospholipid in large vesicles compared to small vesicles. A, small unilamellar vesicles, prepared as described in the text; B, large unilamellar vesicles, prepared as described in the text; C, mixture of large and small unilamellar vesicles after 6 h of incubation. Two peaks can be seen; one corresponds to the position of the large vesicles (void volume), and the other corresponds to the position of small vesicles (about fraction 35). Flow rates of about 30 ml/h were used.](image-url)
the acceptor vesicles, showed no spot in the position of phosphatidylethanolamine. Considering the loading on the plates, the acceptor vesicles, showed no spot in the position of phosphatidylethanolamine. Contamination of less than a 5% contamination of the donor vesicles by the acceptor vesicles. Contamination of the donor vesicles by the acceptor vesicles. Contamination of this extent is not significant in the analysis that follows. Negative stain electron micrographs also showed no significant cross-contamination. Total phospholipid in the donor and acceptor fractions after separation did not change significantly throughout all the incubations, consistent with little cross-contamination. Finally, in those cases where vesicles were separated by chromatography, the same answer (as in the cases of separation by centrifugation) was obtained. This also indicates no significant cross-contamination in the experiments performed using differential centrifugation.

Of considerable concern to these studies was the nature of the vesicle surfaces in these exchange experiments from which cholesterol would exit or the surface into which cholesterol would enter. Unilamellar vesicles are required to avoid the complication of migration of cholesterol between lamellae in a multilamellar vesicle. Negative stain electron microscopy revealed that the vesicles prepared by dialysis of octylglucoside-solubilized phospholipid were predominantly unilamellar. The size ranged from 1200 to 2500 Å in diameter (data not shown). This is in agreement with our previous results using egg phosphatidylcholine, where the nature of the vesicles was examined by freeze fracture electron microscopy (11). Thus, both electron microscopic techniques indicated that the vesicles were unilamellar. These results agree with the original report of this preparation method (13).

Control of Oxidation—Samples were prepared in nitrogen-purged buffers containing EDTA and were stored under argon during incubation. This precaution was designed to prevent lipid oxidation. At the start of the incubations, the sample was split into premeasured aliquots, each of which was sealed separately. By so doing, it was possible to harvest an aliquot without exposing the remainder of the incubation mixture to oxygen, even transiently. Thus, oxidation was again minimized.

Because of the long incubation times employed, there was concern whether or not any appreciable oxidation had occurred. If so, it might affect the final equilibrium of the process being measured. Therefore, the extent of oxidation of the hydrocarbon chains was measured by gas chromatography. The methyl esters of the fatty acids that were derived from the phospholipids in the vesicles actually used in the incubation experiments were analyzed. Within the uncertainty of the measurement, no significant change occurred in the oleic acid content of the phospholipids during 48 h of incubation. In most of these experiments, the phospholipid contained palmitic acid at position 1 and oleic acid at position 2. Since oleic acid is the only oxidizable fatty acid in this experiment, these results indicated that no significant oxidation occurred during the course of the incubation. These data also verified that an equimolar ratio between palmitic acid and oleic acid was observed for this synthetic phospholipid.

Cholesterol Distribution between Phosphatidylcholine Vesicles of Different Size—The first experiments were designed to assess the effect of radii of curvature on movement and distribution of cholesterol among vesicle membranes. For these experiments, only a single cholesterol/phospholipid mol ratio was used in the donor vesicles. Previous studies showed that changes in starting cholesterol content had little effect on the trends characterizing movement of cholesterol (6). Furthermore, the rate of exchange of cholesterol between vesicles is not very dependent upon vesicle cholesterol content (14). Finally, it should be noted that the main thrust of this work was to study the role of phospholipid composition in cholesterol distribution, and the best way to understand that role was to keep variables, other than the phospholipid content, constant.

Two vesicle populations with equal amounts of phospholipid were incubated for these experiments. One population contained cholesterol at approximately 22 mol per cent. The second population did not contain cholesterol. The two vesicle populations were separated by two different methods. The first separation method was Sepharose 4B chromatography. An example can be seen in Fig. 1C. The void volume contained the large vesicles. The small vesicles produced a small peak at a position corresponding to the position at which they appeared when chromatographed separately. The profile represents light scattering. Therefore, even though equimolar amounts of each of the two vesicle populations were applied to the column, the large vesicles produced a much larger change in light scattering because of their larger size. After chromatography, the fractions corresponding to the center of the respective peaks were analyzed for phosphate and cholesterol. Approximately 3–5 h were required for a complete column run. Therefore, early time points were not possible by this method.

The second separation method was differential centrifugation as described under "Experimental Procedures." This procedure required about 15 min. Because of the much shorter time required for vesicle separation, this method was employed for most of the experiments reported here. However, when the time resolution problem was taken into account, the two techniques produced the same result when used on the same system.

The results for the first experiment employing small unilamellar vesicles and large unilamellar vesicles of 16:0,18:1 phosphatidylcholine appear in Fig. 2. Here the cholesterol/phospholipid mol ratio of each of the two vesicle populations is plotted as a function of time. The experiments were designed so that the donor vesicles initially contained membrane cholesterol, whereas the acceptor vesicles initially contained no cholesterol. Therefore, these are mass transfer experiments.

Fig. 24 indicates that when the cholesterol donors are the small vesicles, a monotonic decrease in the cholesterol content of the small vesicles was observed during incubation with large unilamellar vesicles containing no cholesterol. A corresponding increase in the cholesterol content of the large vesicles occurred on the same time scale. Therefore a net flux of cholesterol from the donors to the acceptors was apparent.

At approximately 30 h, the cholesterol content of the two vesicle populations was equal, and no further change took place. Therefore, at equilibrium, cholesterol was likely distributed between these two vesicle membranes such that the cholesterol/phospholipid ratio in each was the same. These data represent the average of three independent experiments. The kinetics of cholesterol movement were not the subject of study here, but the time course was similar to that observed previously (14–16).

Since the nature of the vesicle surface has been implicated in the kinetics of cholesterol exchange rates between vesicles, it was important to determine whether the same result would be obtained if cholesterol originally resided in the large vesicles. Fig. 2B shows the result. These data represent the average of three independent experiments. Clearly, here the rate of cholesterol movement was much slower than when the donor vesicles were the small unilamellar vesicles. Equilib-
Cholesterol Distribution

Effect of Lipid Hydrocarbon Chain Composition on Cholesterol Distribution between Phospholipid Vesicles—To test the role of phospholipid hydrocarbon chain composition in cholesterol distribution, three different phosphatidylcholines were used in the donor and acceptor vesicles: 16:0,18:1 PC, 14:0,14:0 PC, and egg PC. Thus a constant phospholipid headgroup composition was maintained in these experiments, eliminating complicating factors due to different headgroup structures. Small unilamellar vesicles were chosen as cholesterol donors because the above experiments indicated that with such donors net cholesterol movement proceeded to equilibrium in a readily measurable manner.

Two kinds of experiments were performed. In both cases, small unilamellar vesicles of 16:0,18:1 phosphatidylcholine were used as cholesterol donors. In the first of these experiments, the acceptor vesicles were large unilamellar vesicles of egg phosphatidylcholine. In the second of these experiments, large unilamellar dimyristoylphosphatidylcholine vesicles were used as acceptors. The incubation experiments were performed as a function of temperature. The results are summarized by a "partition coefficient," reported in Table I. This table represents the cholesterol/phospholipid ratio in the donor vesicles divided by the cholesterol/phospholipid ratio in the acceptor vesicles at equilibrium. Small vesicles of dimyristoylphosphatidylcholine were not used because of their propensity to fuse when the vesicles encounter surfaces at temperatures near or below the gel to liquid crystalline phase transition. These results indicate that at temperatures where DMPC is in the gel state (i.e. at 20 °C), it clearly is a poor cholesterol acceptor. However, even in the liquid crystal state, DMPC is apparently a less favorable environment for cholesterol than 16:0,18:1 phosphatidylcholine. No effect of temperature was observed in the distribution of cholesterol between SUV and LUV of 16:0,18:1 phosphatidylcholine.

The Effect of Phospholipid Headgroups on Cholesterol Distribution: Phosphatidylserine and Phosphatidylinositol—The effect of different phospholipid headgroups was examined

![Diagram](image)

**Fig. 2.** Cholesterol/phospholipid (Chol/PL) mol ratio in large and small vesicles of 16:0,18:1 phosphatidylcholine (LUV and SUV, respectively) as a function of incubation time. Incubations were carried out in a shaking water bath at 37 °C, in 100 mM NaCl, 10 mM histidine, 1 mM EDTA, pH 7. Vesicles were separated by either Sepharose 4B column chromatography or by differential centrifugation (see text). Similar results were obtained by each method. This graph represents the average of three independent experiments. A, SUVs as the cholesterol donors; B, LUVs as cholesterol donors.

<table>
<thead>
<tr>
<th>System</th>
<th>Values</th>
<th>K*</th>
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<tbody>
<tr>
<td>SUV PC—LUV PC</td>
<td>20 °C</td>
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</tr>
<tr>
<td>SUV PC—LUV PC</td>
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<td>1.0</td>
</tr>
<tr>
<td>SUV PC—LUV PC</td>
<td>37 °C</td>
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<td>SUV PC—LUV DMPC</td>
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<td>SUV PC—LUV DMPC</td>
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<tr>
<td>SUV PC—LUC DMPC</td>
<td>37 °C</td>
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</tr>
<tr>
<td>SUV PC—LUV egg PC</td>
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<tr>
<td>SUV PC/PS—LUV PC</td>
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</tr>
<tr>
<td>SUV PC—LUV PE*</td>
<td>5 M guanidine HCl</td>
<td>1</td>
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* Calculated as the quotient of the cholesterol/phospholipid mol ratio in the donor vesicles and the cholesterol/phospholipid mol ratio in the acceptor vesicles, at apparent equilibrium. The latter is defined as an unchanging value for K at long incubation times.

* PE, 16:0,18:1 PE.

* PE, transphosphatidylated from egg PC.
of cholesterol is observed. The apparent partition coefficient is similar to the inverse of the partition coefficient of the first of these two experiments with phosphatidylethanolamine. A lower limit to the apparent partition coefficient is given in Table I.

Subsequently, analogous experiments were performed as a function of dioleoylphosphatidylethanolamine content. The results are summarized in Table I. The effects are not quite as dramatic as with 16:0,18:1 phosphatidylethanolamine, but a similar effect is observed on the cholesterol partitioning.

These experiments were designed to test the effect of various phosphatidylethanolamine contents on the distribution of cholesterol. Previous studies of SUV with varying amounts of phosphatidylethanolamine indicated that this amino phospholipid tended to be distributed preferentially toward the inside of the vesicle bilayers as the amino phospholipid content increased (17). It was therefore important to characterize the LUV containing phosphatidylethanolamine in a similar manner. This was done by chemically labeling the free amino groups on the outside of the vesicle with trinitrobenzene sulfonate as described previously (12, 17).

Measurements were made on LUV with overall phosphatidylcholine/phosphatidylethanolamine mol ratios of 2:1, 1:1, and 1:2. The results showed that the phosphatidylcholine/phosphatidylethanolamine ratio on the outside of the LUV increased proportionately with the increase in overall phosphatidylcholine/phosphatidylethanolamine ratio within experimental error. Thus, there is no preferential partitioning of phosphatidylethanolamine toward the inside of the LUV with increasing content of amino phospholipid. The difference between this result and the results for SUV (17) is expected. Because of the much larger radius of curvature of the LUV, there are no packing constraints driving the phosphatidylethanolamine to the inside, as there are in the case of the SUV.

Transphosphatidylated (from egg PC) PE is stable in the lamellar phase over the temperature range employed in these experiments. Therefore, pure PE LUV were formed as described under "Experimental Procedures." When the PE LUV were used as acceptors, very little cholesterol transferred from the SUV PC donor vesicles in good agreement with the results obtained from mixed PE/PC membranes described earlier (see Table I). Therefore, the influence on cholesterol transfer apparently arises predominantly from phosphatidylethanolamine.

The one question remaining is why PE has this effect on cholesterol distribution. PE headgroups are poorly hydrated relative to PC headgroups, and PE headgroups engage in intermolecular hydrogen bonding (25). This leads to the suggestion that the surface of a PE bilayer interacts with the aqueous phase less favorably than does the surface of a PC bilayer. Phase contrast light microscopy revealed that the pure PE LUV were aggregated. Light microscopy of the other vesicles used in this study showed little or no evidence for aggregation. These results are consistent with the hypothesis that the surface of the PE bilayer interacts with water less favorably than a PC bilayer.

Results like these further suggest that changes in the nature of the aqueous phase might change the behavior of the PE membrane surface. Therefore, experiments were performed in which the nature of the aqueous phase was altered by the addition of the chaotropic agent, guanidine HCl. Phase contrast light microscopy indicated that the PE LUV no longer aggregated in 5 M guanidine HCl. Incubation experiments in 5 M guanidine HCl were then carried out with PC SUV and PE LUV. As can be seen in Table I, the distinction between cholesterol environments in PC and PE membranes disap-
Duration of membrane composition is evident in a recent study in which biological membranes of different composition and different cholesterol content were incubated (6). The data indicated that membranes high in cholesterol tended to stay high in cholesterol, while membranes low in cholesterol tended to stay low in cholesterol. These results suggested that membrane composition plays at least some role in cholesterol distribution among membranes, although it is probably not fully responsible (6).

The present study examines the potential role of lipid composition in determining cholesterol distribution among membranes. To do so, care was taken to control as many of the variables as possible. Therefore, only vesicles of defined structure and lipid composition were used. Care was also taken to separate the question of variation in headgroup composition from variation in hydrocarbon chain composition.

Vesicles with phosphatidylethanolamine proved to be poor acceptors of cholesterol from donor vesicles of phosphatidylcholine. The effect is dependent upon the content of phosphatidylethanolamine in the membrane. A similar conclusion was offered several years ago on the basis of an entirely different manner of investigation, differential scanning calorimetry (2). However, this previous study strictly applied only to membranes undergoing a phase transition. Therefore, application of the results to understanding biological membranes was much more difficult than for the results reported here. A more recent study reports no such preference of cholesterol for phosphatidylethanolamine in a dipalmitoylphosphatidylcholine/dipalmitoylphosphatidylethanolamine system (3). However, again the role of unsaturation in the phospholipid hydrocarbon chains must be considered. It would appear that the studies reported here, which do not depend upon a phase transition, are easier to interpret in a manner relevant to biological membranes.

These conclusions are intriguing in the context of the rod outer segment disc membranes where cholesterol content differs in old and new membranes. These disc membranes pinch off from the plasma membrane during formation, and thus new discs are expected to be higher in cholesterol than old discs (since the plasma membrane is high in cholesterol). It has been suggested that, during aging of the discs, they may lose some of their cholesterol as their age, relative to the time of formation from the plasma membrane, increases because the high cholesterol content creates a thermodynamically unfavorable environment for cholesterol.

Preliminary measurements analogous to those reported here reveal that the disc membrane cannot be readily enriched in cholesterol and that it is the lipid content which is the cause of this observation. Why phosphatidylethanolamine exerts this effect must be addressed. One must look for significant differences in the properties of membranes of phosphatidylcholine and phosphatidylethanolamine. Since in some of these experiments the hydrocarbon chains are identical, the region of importance would appear to be the phospholipid headgroups. As reviewed previously (25), the headgroups of both phospholipids are oriented approximately parallel to the membrane surface. A crystal structure of phosphatidylethanolamine shows that the amino group is hydrogen-bonded to the phosphate of a neighboring molecule (26). While the N-methyl hydrogens of phosphatidylcholine also interact with neighboring phosphates (25), hydrogen bonding is not possible. Therefore, the interaction would be expected to be weaker than in the case of phosphatidylethanolamine. For cholesterol to be inserted between the phospholipids, it must break that intermolecular...
headgroup interaction as has been observed previously (27).

Therefore, one might predict that insertion of cholesterol into phosphatidylethanolamine bilayers would be thermodynamically less favorable than insertion of cholesterol into phosphatidylcholine bilayers. Since this is what is observed, these arguments constitute a reasonable but not unique explanation for the phosphatidylethanolamine effect.

Results presented here with pure phosphatidylethanolamine strongly support another explanation, however. The interaction of the surface of PE membranes with the aqueous phase is less favorable than the interaction of the surface of PC membranes with the aqueous phase. This is manifest in the aggregation of PE LUV which leads to at least a partial exclusion of water from the bilayer surface. The presence of the chaotrophic agent, guanidine HCl, changes the aqueous phase, reducing the unfavorable nature of the interaction of the PE surface with the aqueous phase. The vesicles then no longer aggregate.

These effects likely result from the lesser capability of PE than PC to bind water and from the intermolecular hydrogen bonding of the PE headgroups (25). Cholesterol can be expected to bind even fewer water molecules than PE. Insertion of cholesterol into a PE membrane would make the resulting surface one that interacts with the aqueous phase even less favorably than the PE surface. Hence, cholesterol transfer into such a membrane is not favored. The chaotropic agent, guanidine HCl, reduces the importance of these factors by changing the nature of the aqueous phase. Therefore, in the presence of guanidine HCl, cholesterol readily enters the PE membrane.

Why does 14:0,14:0 phosphatidylcholine, as indicated in Table I, prove to be a relatively poor acceptor of cholesterol? At 20 °C, the answer lies at least partially in the fact that the DMPC membrane is in the gel state. But what about when both donor and DMPC acceptor membranes are in the liquid crystal state?

One could suggest that it is because cholesterol prefers the unsaturated phosphatidylcholine to the saturated phosphatidylcholine (28). However, previous work has shown that cholesterol prefers a 16:0,16:0 phosphatidylcholine membrane to an egg phosphatidylcholine membrane (29). Therefore, the protonation explanation for the effect with 14:0,14:0 phosphatidylcholine likely lies in the chain length. The length of the cholesterol is better matched to the 16-carbon chain than to the 14-carbon chain (30), thereby favoring the longer chain phospholipid in the present case.

Radius of curvature appears to be important kinetically. It was not the point of this study to determine the kinetics of cholesterol transfer. However, the observation of a common transfer of cholesterol from SUV to LUV at high temperatures suggests there is a kinetic barrier to cholesterol exit from the SUV. The surface of the SUV is more poorly packed than the surface of the LUV because of the small radius of curvature of the SUV. The rate-limiting step of cholesterol transfer between phosphatidylcholine vesicles has been suggested to be exit from the donor vesicle (14–16). Exit from the poorly packed surface of the SUV should be easier than from the LUV (31). Therefore, the greater kinetic barrier to cholesterol exit from the LUV can be understood.

Charged lipids, at the low contents used here, have no observable effect. This is presumably because the membrane properties are still largely determined by the major membrane component, phosphatidylcholine. However, it is interesting that vesicles with negatively charged phospholipids behave similarly to those without the negatively charged phospholipids. This result is consistent with previous suggestions that cholesterol transfer occurs through the aqueous phase rather than by vesicle collision (14–16).

Sphingomyelin apparently produced a kinetic barrier to cholesterol exit from, and entrance to, the sphingomyelin bilayer. In fact, as in the case of SUV sphatidylcholine donors and LUV sphingomyelin acceptors, sphingomyelin can apparently make the entrance to the acceptor vesicle the rate-limiting step of the cholesterol transfer. However, it is beyond the scope of this work to determine the molecular mechanism of this effect.

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REFERENCES


