Effect of liposomal phospholipid composition on cholesterol transfer between microsomal and liposomal vesicles

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Preincubation of rat liver microsomal vesicles at 37 °C in the presence of [³H]cholesterol/phospholipid liposomes results in a net transfer of cholesterol from liposomes to microsomal vesicles. This transfer follows first-order kinetics. For similar concentrations of the donor vesicles, rates of transfer are about 6–8 times lower with cholesterol/sphingomyelin liposomes compared with cholesterol/phosphatidylcholine liposomes. Also, transfer of cholesterol from cholesterol/sphingomyelin liposomes to microsomal vesicles reveals a larger activation energy than for the process from cholesterol/phosphatidylcholine liposomes. There is a significant correlation between the amount of liposomal cholesterol transferred to microsomal vesicles during preincubation and the increase found with acyl-CoA:cholesterol acyltransferase activity in these microsomes over their corresponding controls. If, however, liposomes made solely of phospholipids are substituted for the cholesterol/phospholipid liposomes in the preincubation system containing microsomal vesicles, then the acyl-CoA:cholesterol acyltransferase activity is decreased compared with the corresponding control system. Both sphingomyelin and phosphatidylcholine liposomes are equally effective in decreasing the enzyme activity. These results offer direct kinetic evidence for the positive correlation between cholesterol and sphingomyelin found *in vivo* in biological membranes.

INTRODUCTION

It has been recognized that there is a correlation between the concentration of cholesterol and of sphingomyelin in biological membranes (Patton, 1970; Demel *et al.*, 1977). High sphingomyelin and high cholesterol contents are, for instance, found in the central or the peripheral nervous system and the erythrocyte membranes. In contrast, low cholesterol and sphingomyelin concentrations are encountered in nuclear, mitochondrial and endoplasmic-reticular membranes (Rouser *et al.*, 1972). In the lens cortex membrane, increased cholesterol content associated with aging is also associated with an increase in the ratio of sphingomyelin to total phospholipid (Broekhuysen, 1973). The lysosomal sphingolipidoses in humans (Fredrickson & Sloan, 1972; Brady, 1983) or lysosomal storage of sphingomyelin in experimental animal models (Sakuragawa *et al.*, 1976; Bhuvaneshwaran *et al.*, 1982, 1985) is associated with increased concentration of non-esterified cholesterol in these organelles. Although the association between cholesterol and sphingomyelin is evident, it is not yet clear as to the reason for such a correlation *in vivo*. Several studies, based on equilibrium partitioning of cholesterol between various classes of phospholipids, have indicated that sphingomyelin has a higher affinity for cholesterol among the varied phospholipids evaluated (Bloj & Zilversmit, 1977; Demel *et al.*, 1977; Nakagawa *et al.*, 1979; Lange *et al.*, 1979; Wattenberg & Silbert, 1983). However, in the situation *in vivo*, transfer of cholesterol among membranes is not an equilibrium

process, but rather reflects the steady-state kinetic process.

In the present paper we have evaluated the kinetics of cholesterol transfer between rat liver microsomal vesicles and sphingomyelin liposomes and compared this with those found with phosphatidylcholine liposomes. The data show that sphingomyelin accepts the sterol from microsomal vesicles at the same rate as with phosphatidylcholine, whereas it gives up the sterol at a very much slower rate when compared with phosphatidylcholine. These results could explain the positive correlation between sphingomyelin and cholesterol found *in vivo* under varied conditions.

MATERIALS AND METHODS

Materials

[1 α ,2 α -³H₂]cholesterol, [1 α ,2 α -³H₂]cholesteryl oleate and [1-¹⁴C]oleoyl-CoA were obtained from Amersham International (Amersham, Bucks., U.K.). Oleoyl-CoA, dithiothreitol, human serum albumin (essentially free of fatty acids), egg-yolk phosphatidylcholine, bovine brain sphingomyelin and cholesteryl oleate were from Sigma (Poole, Dorset, U.K.). No impurities could be detected in the phosphatidylcholine or sphingomyelin by t.l.c. using standard solvent systems. [³H]Cholesterol was purified before use by t.l.c. on Kieselgel H at 5 °C, with diethyl ether as solvent. Cholesterol was of commercial source and recrystallized three times before use.

Abbreviation used: ACAT, acyl-CoA:cholesterol acyltransferase (EC 2.3.1.26).

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Preparation of liposomes

Phosphatidylcholine, sphingomyelin and cholesterol/phosphatidylcholine and cholesterol/sphingomyelin liposomes were prepared by using procedures described by Batzri & Korn (1973) and Kremer *et al.* (1977) for preparation of single-bilayer vesicles of relatively narrow range of size. The dilute suspension of vesicles in 0.15 M-NaCl was concentrated and washed free of ethanol by ultrafiltration on an Amicon (Woking, Surrey, U.K.) ultrafiltration device with an XM-100A membrane with rapid stirring under N_2 pressure (103 kPa). These liposomes could not be sedimented by centrifugation at 104000 g for 60 min (Kirby *et al.*, 1980; Schwendener *et al.*, 1984). This suggests that they are small unilamellar vesicles. The liposomes derived from both sphingomyelin and phosphatidylcholine have been assumed to be of similar size, on the basis of the observations of Shipley *et al.* (1974) and Guiot & Baudhuin (1984) that, at maximum hydration, both of these phospholipids give similar values for lipid thickness and surface area per molecule.

Preparation of rat liver microsomal fraction and its treatment

Male Wistar rats weighing 200–250 g were used for all experiments. The animals were kept under controlled conditions of lighting and feeding as described previously (Mitropoulos *et al.*, 1973). The rats were killed by cervical dislocation at about 07:00 h on the day of the experiment. The livers were removed immediately, chilled on ice and perfused with ice-cold 0.25 M-sucrose to remove contaminating blood. The liver homogenate and microsomal fraction therefrom, containing about 15 mg of protein/ml, was obtained as described previously (Venkatesan *et al.*, 1980).

Portions of the microsomal fraction (2.3 mg of microsomal protein/ml of incubation mixture) were incubated in a medium consisting of 12.3 mM-NaCl, 230 mM-sucrose, and 3 mM-imidazole/HCl buffer, pH 7.4, in the presence of either [3H]cholesterol/phosphatidylcholine (1:1, mol/mol) or [3H]cholesterol/sphingomyelin (1:1, mol/mol) liposomes at the concentrations indicated for various time periods and at the temperature indicated. For the buffer-treated controls, the volume of liposomes was substituted with an equal volume of 0.15 M-NaCl. All such incubations contained the same amount of microsomal protein. At the end of the incubation period, the mixture was cooled on ice. Portions of this mixture were centrifuged at 104000 g for 60 min to isolate the treated microsomal vesicles from the supernatant containing the liposomal fraction. Previous studies (Synouri-Vrettakou & Mitropoulos, 1983) showed that, under such conditions, the microsomes can be cleanly separated from the liposomes. The transfer of liposomal cholesterol to the microsomal vesicles was found to be unidirectional. Also, the re-isolated microsomal vesicles were enriched with respect to *cholesterol alone* without any change in the phospholipid concentration, indicating that the enrichment of cholesterol was due to net transport and not due to sticking, fusion or co-sedimentation of the liposomes. The microsomal pellets were resuspended and portions were removed to measure the radioactivity associated with the re-isolated microsomal vesicles and to assay ACAT activity. The mass of cholesterol transferred to the microsomes was calculated from the radioactivity

incorporated and the specific radioactivity of liposomal cholesterol and equalled the increment of cholesterol determined in treated microsomal vesicles by g.l.c. (Synouri-Vrettakou & Mitropoulos, 1983).

Reverse transfer of cholesterol from microsomal to liposomal vesicles was mediated by incubation of microsomal vesicles with liposomes made solely of either phosphatidylcholine or sphingomyelin (Mitropoulos *et al.*, 1984). The rest of the procedures and methods for separation of the treated microsomal vesicles were identical with the ones described above.

Assays

The activity of ACAT in the microsomal fraction or the treated and re-isolated microsomal vesicles was assayed by using [$1-^{14}C$]oleoyl-CoA (specific radioactivity 5 Ci/mol) as described by Synouri-Vrettakou & Mitropoulos (1983). Correction for losses during the extraction and isolation of cholesteryl [^{14}C]oleate as well as evaluation of zero-time controls and reagent blanks was by the procedure outlined by Synouri-Vrettakou & Mitropoulos (1983).

Total lipids were extracted by the method of Folch *et al.* (1957). Phospholipids in the total lipid extracts were assayed by the method of Bartlett (1959), and protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. Cholesterol was quantified by g.l.c. (Mitropoulos *et al.*, 1978).

RESULTS

Comparison of cholesterol/sphingomyelin and cholesterol/phosphatidylcholine liposomes as cholesterol donors to microsomal vesicles

The liver microsomal fraction was incubated at 37 °C for various periods of time (0–50 min) in the presence of various concentrations of [3H]cholesterol/sphingomyelin or [3H]cholesterol/phosphatidylcholine liposomes and then re-isolated in order to assess the extent of cholesterol transfer. We have previously shown that, under the present conditions, transfer of liposomal cholesterol to the microsomal vesicles is unidirectional and that the mass of cholesterol transferred (calculated from the radioactivity incorporated and the specific radioactivity of liposomal cholesterol) is equal to the increment of cholesterol determined in treated microsomal vesicles by g.l.c. (Synouri-Vrettakou & Mitropoulos, 1983). The above studies further suggest that the exchange of cholesterol between the two vesicles occurs at a considerably lower rate than the rate for net transport, such that, within the preincubation times used (0–50 min.), the impact of exchange on the final result is insignificant. The liposomal/microsomal cholesterol ratio (R) present at the beginning of preincubation is identified for each system. For both cholesterol donors, the time-dependent transfer of cholesterol followed first-order kinetics (Fig. 1). The slope of each of these lines represent the first-order rate constant (k) for that system. Half-times ($t_{1/2}$) were calculated using the equation:

$$t_{1/2} = \ln 2/k$$

Table 1 shows these values for various ratios of liposomal to microsomal cholesterol. Rates of cholesterol transfer to microsomal vesicles are about 8–10 times lower from cholesterol/sphingomyelin than from chole-

Fig. 1. Time-dependent transfer of cholesterol from cholesterol/phosphatidylcholine or cholesterol/sphingomyelin liposomes to the microsomal vesicles

Portions of the microsomal fraction (2.3 mg of microsomal protein; 109 nmol of non-esterified cholesterol and 1350 nmol of microsomal phospholipid per ml of mixture) were preincubated at 37 °C as described in the Materials and methods section in the presence of [^3H]cholesterol/sphingomyelin (C/Sph; 1:1, mol/mol; specific radioactivity 7.46 d.p.m./pmol) or [^3H]cholesterol/phosphatidylcholine (C/PC; 1:1, mol/mol; specific radioactivity 7.46 d.p.m./pmol) liposomes. The ratio of liposomal to microsomal cholesterol (R) present at the beginning of pre-incubation is given. Portions of the mixtures were removed at various time intervals (0–50 min) and centrifuged to separate the treated microsomal vesicles from the liposomal vesicles. The results are expressed as the fraction of total radioactivity remaining in the cholesterol/sphingomyelin (----) or the cholesterol/phosphatidylcholine (—) vesicles. The data were analysed by a least-squares method and the best-fitting regression lines drawn. The correlation coefficients for all the lines were better than -0.991 ($P < 0.001$). The slopes of the lines give the first-order rate constants (k) for transfer of cholesterol to microsomal vesicles from each of the liposomal donors.

sterol/phosphatidylcholine liposomes. In other experiments of similar design involving four ratios of liposomal to microsomal cholesterol for each of the liposomal preparations, rates of cholesterol transfer to microsomal vesicles were 4–6 times lower from cholesterol/sphingomyelin than from cholesterol/phosphatidylcholine liposomes.

Effect of liposomal donor on the temperature-induced kinetics of cholesterol transfer to the microsomal vesicles

The liver microsomal fraction was incubated at various temperatures (25–37 °C) for various times (0–50 min) in the presence of [^3H]cholesterol/sphingomyelin or [^3H]cholesterol/phosphatidylcholine liposomes. At the end of the incubation period, portions of the mixture were centrifuged to isolate the treated microsomal vesicles. The extent of cholesterol transfer was then assessed.

The first-order rate constant (k) for transfer of liposomal cholesterol to the microsomal vesicles for each of the temperatures evaluated was determined as in Fig. 1. Activation energy for transfer of cholesterol from liposomes to microsomal vesicles was obtained from an Arrhenius plot of these rate constants against the incubation temperature (Fig. 2). For any one cholesterol donor, activation energies were constant between 25 and 37 °C. However, the activation energy was higher with cholesterol/sphingomyelin liposomes [69.4 kJ (16.6 kcal)/mol] than with cholesterol/phosphatidylcholine liposomes [47.2 kJ (11.3 kcal)/mol].

Reverse cholesterol transport and modulation of ACAT activity

There is a significant correlation of ACAT activity in the treated microsomal vesicles with the amount of cholesterol transferred from liposomal to microsomal vesicles (Fig. 3). By this criterion, it should be possible to deplete microsomal vesicles of cholesterol and thereby decrease ACAT activity. Recent studies (Mitropoulos *et al.*, 1984) show that depletion of microsomal cholesterol can be achieved by preincubation of microsomal fraction in the presence of liposomes made solely of phosphatidylcholine. These studies also revealed that the progressive loss of microsomal cholesterol is reflected by progressive decrease in ACAT activity. We have used a similar system to compare the abilities of sphingomyelin

Table 1. Comparison of cholesterol/sphingomyelin and cholesterol/phosphatidylcholine liposomes as cholesterol donors to rat liver microsomal vesicles

The first-order rate constant (k) for each of the systems was calculated from the slope $\pm$ S.E.M. of the slope of the best-fitting regression lines as shown for Fig. 1. The correlation coefficient for each of the lines was better than -0.991 with $P < 0.001$. Half-times ($t_{1/2}$) were calculated using the equation $t_{1/2} = \ln 2/k$. Other experimental details are given in the legend to Fig. 1.

Composition of liposomes	Ratio (R) of liposomal to microsomal cholesterol	$10^3 \times$ First-order rate constant (k) (min^{-1})	$t_{1/2}$ (h)
Cholesterol/sphingomyelin	1.09	0.41 ± 0.03	28.18
	2.18	0.48 ± 0.07	24.07
Cholesterol/phosphatidylcholine	0.93	4.49 ± 0.25	2.57
	1.86	3.43 ± 0.18	3.37
	3.71	2.57 ± 0.12	4.50

Fig. 2. Activation energy for the transfer of cholesterol from cholesterol/sphingomyelin (C/Sph) or cholesterol/phosphatidylcholine (C/PC) liposomes to microsomal vesicles

Portions of the microsomal fraction (2.0 mg of protein/ml of preincubation mixture) were incubated at the temperatures indicated for various periods of time (0–50 min) in the presence of [^3H]cholesterol/sphingomyelin (1:1, mol/mol; specific radioactivity 7.20 d.p.m./pmol) or [^3H]cholesterol/phosphatidylcholine (1:1, mol/mol; specific radioactivity 7.28 d.p.m./pmol) liposomes. The ratio of liposomal to microsomal cholesterol at the beginning of the incubation was in all cases 2. Aliquots were removed at each time interval, the treated microsomal vesicles were re-isolated and the fraction of radioactivity remaining in the liposomal fraction was determined. The first-order rate constant (k) for each temperature was obtained from plots similar to those indicated for Fig. 1. Activation energies [kJ (kcal)/mol] were calculated from the slope of the Arrhenius plot and are given beside each line. The lines shown are the best fit for the points determined. The correlation coefficients are -0.999 ($P < 0.001$).

and phosphatidylcholine liposomes to accept cholesterol from microsomal vesicles and thereby alter ACAT activity. In agreement with previous results (Mitropoulos *et al.*, 1984), the phospholipid and protein content of the re-isolated microsomal vesicles remained unchanged, whereas the content of non-esterified cholesterol decreased as a function of both the preincubation time and the concentration of liposomal phospholipid. The loss of sterol in the microsomal fraction was accounted for by its recovery in the re-isolated liposomal fraction. Fig. 4 describes the data on ACAT activity of re-isolated microsomal vesicles after preincubation at 37°C for various periods of time (0–50 min) in the presence of various concentrations of either sphingomyelin liposomes or phosphatidylcholine liposomes. The amount of liposomal phospholipid present in the preincubation mixture is expressed in terms of liposomal/microsomal phospholipid ratio (R'). Data in Fig. 4 reveal that control microsomal vesicles preincubated in buffer with no added liposomes (top line, $R' = 0$) and subsequently re-isolated show an increase in ACAT activity with preincubation time. This has been attributed to a time-dependent transfer of cholesterol during preincubation from plasma-membrane vesicles to the vesicles that contain ACAT (Mitropoulos *et al.*, 1984); the slope of the line is thereby a reflection of the rate at which intervesicular transport of cholesterol had been achieved. With both sphingomyelin ($R' = 0.63$; $R' = 1.25$) and

Fig. 3. Correlation between cholesterol transfer and microsomal vesicles from liposomes and the increment in ACAT activity

Portions of the microsomal fraction (2.3 mg of microsomal protein/ml) were incubated at 37°C in presence of various concentrations of [^3H]cholesterol/phosphatidylcholine liposomes for various times as detailed for Fig. 1. Isolation of treated microsomal vesicles, assessment of cholesterol transfer and assay for ACAT activity are described in the Materials and methods section. The increment in ACAT activity was calculated by subtracting the corresponding ACAT activity for microsomal fraction treated identically, but in the absence of cholesterol/phosphatidylcholine liposomes in the incubation mixture, from the values for ACAT activity in microsomal fraction incubated in the presence of these liposomes. The data from three experiments were analysed by a least-squares method and the best-fitting regression line was drawn. The correlation coefficient is 0.961 ($n = 18$, $P < 0.001$).

phosphatidylcholine ($R' = 1.88$; $R' = 3.75$) as acceptors of cholesterol during preincubation, these rates diminish progressively with increasing liposomal/microsomal phospholipid ratios. A semilogarithmic plot of these rates against the concentration of phospholipid used for these pretreatments yielded straight lines for both acceptors (Fig. 5). The slopes for both the lines in Fig. 5 are similar, suggesting that sphingomyelin liposomes and phosphatidylcholine liposomes are equally efficient as acceptors of microsomal cholesterol. The arrows in Fig. 5 identify the ratio of liposomal to microsomal phospholipid needed to change the rate of the control system (Fig. 4, $R' = 0$) by one-half. The values are 1.17 and 1.18 for sphingomyelin and phosphatidylcholine liposomes respectively. Two additional experiments of the same design gave similar results.

DISCUSSION

The present studies evaluate the kinetics of unidirectional forward and reverse transfer of cholesterol between rat liver microsomal and artificial membrane vesicles. We chose liver microsomal fraction as a component here, since microsomal ACAT is involved in

Fig. 4. Effect of liposomal phospholipid concentration and period of preincubation on ACAT activity in the microsomal fraction

The microsomal fraction (1.8 mg of microsomal protein; 103 nmol of non-esterified cholesterol and 1032 nmol of microsomal phospholipid per ml of mixture) was pre-incubated at 37 °C with various concentrations of liposomes made solely of either sphingomyelin (Sph) or phosphatidylcholine (PC). The liposomal/microsomal phospholipid ratios (R') were as shown. At various time intervals (0–50 min) portions of the mixture were removed and centrifuged to isolate the treated microsomal vesicles. ACAT activities were assayed in portions of the re-isolated microsomal vesicles as described in the Materials and methods section. The ACAT activity is plotted against time of preincubation. The best-fitting regression lines are drawn for each system. The correlation coefficients are greater than 0.962 ($P < 0.001$).

the esterification of cholesterol made available to it via transport out of the lysosomal compartment. The microsomal ACAT enzyme operates below substrate saturation and the activity responds linearly to substrate availability over a large range (Synouri-Vrettakou & Mitropoulos, 1983; see also Fig. 3 of the present paper). Hence the rate at which this activity changes can be useful as an index of the rate at which cholesterol gets transferred in the immediate vicinity of the enzyme.

A change in the phospholipid content of the lysosomal membrane could affect the above process significantly. Normal lysosomes contain phosphatidylcholine as the major phospholipid component (Hostetler *et al.*, 1985). On the other hand, lysosomes isolated from the livers of rats and NCTR-BALB/c mice that store excessive amounts of non-esterified cholesterol (Bhuvaneshwaran *et al.*, 1982, 1985) have sphingomyelin as the predominant phospholipid.

Our choice of liposomes consisting of phosphatidylcholine or of sphingomyelin to yield model systems that reflect the extreme limits of normal liver lysosomes and the liver lysosomes that store non-esterified cholesterol is based on the finding of Wattenberg & Silbert (1983) that sterol partitioning of a given membrane is reflected closely by liposomes that have similar lipid composition to that of the biological membrane. Understanding the ability of these two model systems to donate or to accept cholesterol to and from microsomal membranes should be useful to delineate the changes in kinetic parameters of cholesterol movement between cellular membranes

Fig. 5. Correlation of the rate at which ACAT activity changes with the liposomal/microsomal phospholipid ratio present during preincubation

The conditions were as described for Fig. 4. The rates at which ACAT activity changes due to preincubation at various liposomal-to-microsomal phospholipid ratios were evaluated as shown for Fig. 4. Both sphingomyelin (Sph) and phosphatidylcholine (PC) liposomes were used for this study. The data for the semilogarithmic plot were analysed by a least-squares method and the best-fitting regression lines drawn. For PC: $y = -0.57x + 0.98$ [correlation coefficient is -0.999 ($n = 5$, $P < 0.001$)]; and for Sph: $y = -0.69x + 1.11$ [correlation coefficient is -0.98 ($n = 6$, $P < 0.001$)].

in vivo as a consequence of enrichment with one of these two phospholipids in the membranes.

The mechanism of cholesterol transfer under our conditions has been shown to involve desorption of cholesterol from the donor vesicles, diffusion of the sterol molecule through the aqueous phase and absorption by the acceptor vesicles (Mitropoulos *et al.*, 1984; Bruckdorfer *et al.*, 1984; Backer & Dawidowicz, 1981). Desorption of cholesterol from the donor vesicles into the aqueous phase seems to be the rate-determining step in the transfer of cholesterol to an acceptor (McLean & Phillips, 1981). The present results (Fig. 1, Table 1) showing that, at similar donor-to-acceptor ratios, cholesterol/sphingomyelin liposomes transfer cholesterol to microsomal vesicles at a considerably lower rate than do cholesterol/phosphatidylcholine liposomes, suggest that the desorption rate of cholesterol from the former vesicles is lower than from the latter. Consistent with this observation is an increase in the activation energy for transfer of cholesterol to microsomal vesicles found with sphingomyelin vesicles (Fig. 2). Since the activation energy is a reflection of the energy barrier for desorption of cholesterol, it suggests that interaction of cholesterol with sphingomyelin is higher than that with phosphatidylcholine, resulting in decreased rate of cholesterol transfer from sphingomyelin-containing liposomes.

The liver microsomal fraction, isolated by standard techniques, consists mainly of two types of vesicles: those

derived from the plasma membrane and those derived from the endoplasmic-reticular membranes (rough and smooth). The major part of non-esterified cholesterol present in the microsomal fraction is associated with the former vesicles, whereas the ACAT enzyme is associated with vesicles derived from rough-endoplasmic-reticular membranes (Balasubramaniam *et al.*, 1978; Venkatesan *et al.*, 1980). Cholesterol can be transferred from liposomal to plasma membrane vesicles and/or from plasma-membrane vesicles to those that contain ACAT (Synouri-Vrettakou & Mitropoulos, 1983; Mitropoulos *et al.*, 1984). This would explain the stimulation of ACAT activity when microsomal vesicles are preincubated in buffer (Fig. 4, ●) and even greater stimulation of ACAT activity associated with net cholesterol transfer to microsomal vesicles from an artificial membrane donor (Fig. 3).

As with the forward transfer of cholesterol (Fig. 3), the change in ACAT activity with preincubation time observed with reverse transfer of cholesterol (slopes of lines in Fig. 4) is a reflection of the differential substrate availability. Thus decreases in the slopes for ACAT activity compared with the control found in Fig. 4 with microsomal vesicles that had been preincubated with liposomes indicate a decreased availability of cholesterol to the ACAT enzyme. The decreased availability of the sterol can be attributed to a competition by the liposomes with the ACAT-containing membranes for the transferred cholesterol from the plasma-membrane vesicles (Mitropoulos *et al.*, 1984). The similar effect of both sphingomyelin and phosphatidylcholine liposomes (Fig. 5) indicate that these liposomes compete equally and accept the sterol at about the same rate.

The present results offer a kinetic explanation for the positive correlation found *in vivo* between the concentration of cholesterol and that of sphingomyelin in biological membranes. Assuming that all membranes accept cholesterol from common donors, the membranes with high sphingomyelin content, although accepting cholesterol at the same rate as for phosphatidylcholine-containing membranes, would exhibit a lower desorption rate when serving as a donor and therefore at steady state would accommodate a higher concentration of cholesterol.

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