Cholesterol transfer between lipid vesicles

Effect of phospholipids and gangliosides

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The effect of lipid composition on the rate of cholesterol movement between cellular membranes is investigated using lipid vesicles. The separation of donor and acceptor vesicles required for rate measurement is achieved by differential centrifugation so that the lipid effect can be quantified in the absence of a charged lipid generally used for ion-exchange-based separation. The rate of cholesterol transfer from small unilamellar vesicles (SUVs) containing 50 mol % cholesterol to a common large unilamellar vesicle (LUV) acceptor containing 20 mol % cholesterol decreases with increasing mol % of sphingomyelin in the SUVs, while phosphatidylethanolamine and phosphatidylserine have no appreciable effect at physiologically relevant levels. There is a large decrease in rate when phosphatidylethanolamine constitutes 50 mol % of donor phospholipids. Interestingly, gangliosides which have the same hydrocarbon moiety as sphingomyelin exert an opposite effect. The effect of sphingomyelin seems to be mediated by its ability to decrease the fluidity of the lipid matrix, while that of gangliosides may arise from a weakening of phosphatidylcholine–cholesterol interactions or from a more favourable (less polar) microenvironment for the desorption of cholesterol provided by the head-group interactions involving sugar residues. If the effect of asymmetric transbilayer distribution of lipids is taken into consideration, the observed composition-dependent rate changes could partly account for the large difference in the rates of cholesterol desorption from the inner and outer layers of plasma membrane. Such rate differences may be responsible for an unequal steady-state distribution of cholesterol among various cellular membranes and lipoproteins.

INTRODUCTION

Cholesterol is the most mobile lipid component of membranes, yet it is the major lipid component most unevenly distributed among cellular membranes. Plasma membrane has a cholesterol/phospholipid (C/P) ratio as high as 0.8–0.9 whereas the intracellular membranes including the endoplasmic reticulum, have C/P ratios of less than 0.2 (Colbeau *et al.*, 1971; Houslay & Stanley, 1982; Kaplan & Simoni, 1985). The reason for this uneven distribution is not yet clear. Thermodynamic equilibrium and steady-state distribution have been suggested as possible mechanisms responsible for this uneven distribution (Wattenberg & Silbert, 1983; Yeagle, 1985). The available data on the equilibrium partitioning of cholesterol are not sufficient to explain this in terms of thermodynamic stability alone (Wattenberg & Silbert, 1983). Further, since cholesterol synthesis is a continuous process and since plasma-membrane cholesterol can exchange with plasma lipoproteins, a circulating component, a steady-state distribution is more likely. Several studies from our laboratory as well as others have shown that the rate of cholesterol movement from the plasma membrane to the intracellular membranes is a very slow process (Poznansky & Czekanski, 1982; Slotte & Lundberg, 1983; Robertson & Poznansky, 1985). At the same time, the available data suggest that the movement of cholesterol from the cell surface to plasma or to phospholipid vesicles is considerably faster, with a half-life ($t_{1/2}$) of 1–4 h (Poznansky & Czekanski, 1982; Lange

et al., 1983). The large difference in the rates of cholesterol movement from the two sides of the plasma membrane could be an important factor contributing to the steady-state distribution of cholesterol. It was interesting to investigate the molecular mechanism responsible for the large differences in the rates of cholesterol movement from the two sides of the plasma membrane. Three factors likely to contribute to this are (i) the asymmetric disposition of lipids of the plasma membrane, (ii) the asymmetric disposition of membrane proteins, and (iii) the curvature of the membrane surface from which cholesterol is leaving. The present study examines the possible role of lipid asymmetry by investigating the effect of lipid composition on the rate of cholesterol movement using model-membrane systems.

The spontaneous movement of cholesterol between membranes, both exchange and flux (transfer), follows first-order kinetics, thereby implying that the transport mechanism involves the diffusion of cholesterol through the aqueous phase, probably as monomers (Backer & Dawidowicz, 1981; McLean & Phillips, 1981, 1982; Wharton & Green, 1982; Bruckdorfer *et al.*, 1984). The desorption of cholesterol from the donor membrane into the aqueous phase seems to be the rate-limiting step in the process. The effect of lipid composition on the kinetics of cholesterol movement has been studied, suggesting that the rate depends on the nature of the fatty acid chains (length and unsaturation) of the phosphatidylcholine (PC) in the donor vesicle (Bloj & Zilversmit, 1977; Poznansky & Lange, 1978; Nakagawa

Abbreviations used: SUV, small unilamellar vesicle; LUV, large unilamellar vesicle; C/P, cholesterol/phospholipid molar ratio; SM, sphingomyelin (bovine brain); PC, phosphatidylcholine (egg); PE, phosphatidylethanolamine; PS, phosphatidylserine (bovine brain); DPPC, dipalmitoyl phosphatidylcholine; $t_{1/2}$, half-life.

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et al., 1979) and the presence of sphingomyelin (SM) in the donor vesicle (Nakagawa *et al.*, 1979; Fugler *et al.*, 1985). Most of these results were obtained using vesicles containing a considerable amount of a negatively charged lipid (to effect the separation of donor and acceptor vesicles) (Nakagawa *et al.*, 1979) or using whole cells and membrane preparations as the donor (Clejan & Bittman, 1984a,b; Davis *et al.*, 1984). These variations make it difficult to assess the real contribution of various lipids. Hence a detailed investigation of the effect of the type and content of phospholipids and gangliosides on the rate of cholesterol transfer from SUVs to LUVs was undertaken. Differential centrifugation was used to separate the donor and acceptor vesicles. The results indicate that SM decreases the rate of cholesterol transfer, while phosphatidylethanolamine (PE) and phosphatidylserine (PS) have no effect at physiologically significant levels. Gangliosides, on the other hand, increase the rate of cholesterol transfer. The effects of SM and gangliosides are found to depend on the amount of these lipids in the donor vesicle. The increase in rate of cholesterol transfer in the presence of gangliosides and a dramatic decrease at high PE content may contribute to the large difference in the rates of cholesterol desorption from the inner and outer layers of plasma membrane.

MATERIALS AND METHODS

Materials

Cholesterol, egg phosphatidylcholine (PC) (type V), egg PE, bovine brain PS, bovine brain SM and sodium deoxycholate were obtained from Sigma Chemicals. Bovine brain mixed gangliosides was a product of Supelco, Oakville, Ontario, Canada. [1,2(n)-³H]-cholesterol (specific activity 46.5 Ci/mmol), [¹⁴C]-cholesterol oleate (specific activity 56.6 mCi/mmol) and D-[³H]glucose (specific activity 4.5 Ci/mmol) were obtained from New England Nuclear. Cholesterol was recrystallized from ethanol. All other chemicals were used without further purification.

Preparation of small unilamellar vesicles (SUVs)

SUVs used as donor vesicles in these studies, were prepared by sonication with a C/P ratio of 1.0. Typically, 6 μ mol of cholesterol, 6 μ mol of other lipids in the required ratio, 10 μ Ci of [³H]cholesterol and 2 μ Ci of [¹⁴C]cholesterol oleate (added as a non-exchangeable marker) were mixed in chloroform or a chloroform/methanol mixture. Solvent was evaporated under a N₂ stream to form a thin film and the mixture was dried under vacuum for 2 h. Buffer (4 ml of 20 mM-Tris/acetate, pH 7.4, containing 100 mM-NaCl) was added to the dried lipids and vortexed thoroughly. The multilamellar dispersion was sonicated for 6 min at a temperature of 20–25 °C using a broad-tip probe in a Heat Systems Model W 185 sonifier. The dispersion was centrifuged at 15000 *g* for 20 min to remove the Ti particles and multilamellar liposomes. The supernatant was centrifuged again at 170000 *g* for 1 h at 20 °C. The supernatant from this spin was used as SUVs immediately for rate measurements.

Preparation of large unilamellar vesicles (LUVs)

PC-cholesterol LUVs (4:1), used as acceptor vesicles in these studies, were prepared by a deoxycholate dialysis

procedure, based on the method of Enoch & Strittmatter (1979). Briefly, a sonicated dispersion was made as above deleting the 170000 *g* spin. A small part (1 ml) of the 15000 *g* supernatant was titrated with an 0.1 M-sodium deoxycholate solution by monitoring the turbidity changes at 450 nm. (*A*₄₅₀ initially decreases, then increases, reaches a maximum and finally decreases with increasing addition of deoxycholate.) To the remaining supernatant, deoxycholate solution was added in the correct proportion to give maximum turbidity. [Usually, turbidity is at a maximum at a lipid (PC+cholesterol)/deoxycholate ratio of nearly 2:1. However, a titration is carried out every time LUVs are prepared.] The mixture was then dialysed against Tris buffer (4 litres changed four times) over a period of 72 h. The suspension was then centrifuged at 15000 *g* for 20 min to remove multilamellar liposomes. The supernatant was spun again at 145000 *g* for 1 h. The pellet was washed once with Tris buffer by spinning at 145000 *g* for 30 min. The pellet was finally suspended in Tris buffer to give a PC concentration of 4–6 mM and used as LUVs. The LUVs were stored under N₂ at 4 °C and used as acceptor vesicles for rate studies for a maximum of 10 days. No changes were seen in rate measurements when the acceptor LUVs were used fresh or after 10 days of storage.

Characterization of LUVs

The LUV preparation was characterized by measuring the trapped volume and the transbilayer distribution of PC. Trapped volume was measured by equilibrating with externally added radioactive glucose (Wong *et al.*, 1982). To 1 ml of LUVs, 10 μ Ci of [³H]glucose was added and allowed to equilibrate at room temperature for more than 50 h. LUVs were then passed through a Sephadex G-50 (medium) column (25 cm $\times$ 1 cm) and eluted with Tris buffer at a flow rate of 20 ml/h at 4 °C. Four 1 ml fractions immediately after the void volume were analysed for radioactivity and phospholipid content. From these values and the radioactivity in the equilibrated mixture, trapped volume was calculated.

The transbilayer distribution of PC was determined by measuring the ³¹P n.m.r. signal intensity in the presence and absence of Mn²⁺ (Hope *et al.*, 1985). First, the spectrum of LUVs (3 ml, 10.6 mM-PC) was taken. Then 10 μ l of MnCl₂ solution was added to give a final concentration of 5 mM and two more spectra were recorded under the same conditions. (The second spectrum in the presence of Mn²⁺ was necessary to see whether Mn²⁺ penetrates LUVs.) The spectra were recorded with a Nicolet NT 300 NMR spectrometer operating at 121.5 MHz. Transients (5000) were collected using a 20 μ s pulse, gated proton decoupling and a 62.5 kHz sweep width. The signal intensities were measured by cutting out and weighing the spectra.

Rate measurements

The rates of removal of [³H]cholesterol from SUVs and of incorporation into LUVs were monitored simultaneously by separating the two after incubation by centrifugation and counting the supernatant and pellet in a Beckman LS 3133 scintillation counter. Incubations were carried out at 37 °C in a shaker bath, usually in a total volume of 2.2 ml consisting of SUVs and LUVs at final concentrations of 0.2 mM and 2 mM of phospholipid respectively in Tris buffer. At specific time intervals

(0.25–10 h) 100 μ l portions of the incubation mixture in duplicate were transferred to pre-cooled (in ice) tubes and immediately centrifuged in a Beckman Airfuge using a pre-cooled rotor at 158 000 g for 15 min. Two 20 μ l portions of the supernatant, the tube containing the pellet, and the remaining supernatant were counted for ^3H and ^{14}C radioactivity after adding 15 ml of aqueous counting scintillant (Amersham) to each. The ^3H counts were corrected for ^{14}C cross-over and differential quenching (though marginal). Control experiments show that centrifugation at 158 000 g for 15 min pellets the LUVs completely while the SUVs remain in the supernatant.

Analytical methods

Cholesterol in vesicle preparations was determined by the method of Rudel & Morris (1973). The phospholipid concentration in vesicles was estimated by measuring the total lipid phosphorus according to the method of Fiske & Subbarow (1925) using Elon (*p*-methylaminophenol sulphate) as the reducing agent. Concentration of gangliosides in vesicles was calculated assuming 2 mol of sialic acid/mol of ganglioside and estimating sialic acid by the method of Svennerholm (1957).

Isolation of erythrocyte lipids

Human erythrocytes, washed three times with phosphate-buffered saline, were haemolysed by stirring with 40 vol. of 5 mM-sodium phosphate buffer, pH 8.0, and centrifuged at 22 000 g for 10 min. The pellet (unsealed ghosts) was washed with the same buffer twice and the total lipids were extracted by the method of Bligh & Dyer (1959). SUVs were prepared with erythrocyte lipids by the sonication procedure described above after adding [^3H]cholesterol and [^{14}C]cholesterol oleate.

RESULTS

The LUV preparation used as acceptor vesicle is likely to be a fairly homogeneous population of vesicles, as it is obtained as a fraction sedimenting between 15 000 g and 145 000 g . Characterization of LUVs was done in terms of transbilayer distribution of PC and trapped volume. If the vesicles are large and unilamellar, one would expect nearly 50% of the ^{31}P n.m.r. signal intensity to remain after the addition of Mn^{2+} corresponding to the 50% PC molecules forming the inner layer of the LUVs. The spectra recorded immediately and 3 h after the addition of Mn^{2+} were found to retain 54% and 52.5% of the original signal intensity respectively. These results indicate that Mn^{2+} does not penetrate the vesicle and our LUV preparation is indeed large and unilamellar. Trapped volume measurements gave a value of 4.91 ± 0.54 litres/mol of total lipids (i.e. PC + cholesterol) from three different LUV preparations. The LUVs prepared by Enoch & Strittmatter (1979) are reported to have a trapped volume of 2.4 litres/mol. The difference could arise from (i) the fractionation of vesicles in our procedure since 70–80% of the lipid is removed as multilamellar vesicles and smaller vesicles, and (ii) the slower rate of detergent removal in the dialysis procedure we used compared with gel filtration used by these authors. Since our vesicle preparation is unilamellar as indicated by ^{31}P n.m.r. measurements, a trapped volume of 4.91 litres/mol corresponds to a vesicle size of 160 nm (Mimms *et al.*, 1981). Even though fractionation resulted

Fig. 1. Rate curves for the transfer of [^3H]cholesterol from PC-cholesterol SUV (C/P = 0.96) to PC-cholesterol LUV (C/P = 0.26) at 37 °C from a typical single experiment

The donor SUV and the acceptor LUV were mixed to a final concentration of 0.2 mM and 2 mM of phospholipid respectively. Portions were withdrawn at indicated time intervals, centrifuged and the radioactivity in the supernatant (SUV) and pellet (LUV) measured (see Materials and methods section for details). O, ^3H counts in the supernatant; ●, ^3H counts in the pellet.

in a poor yield of LUVs, it gave vesicles which could be sedimented in a short time, a requirement for rate measurement.

Fig. 1 gives some typical rate curves for the transfer of [^3H]cholesterol from SUVs to LUVs. At the end of incubation (6–10 h), 80–95% of [^3H]cholesterol had been found to be transferred from SUV to LUV. The rate curves differ for SUVs of different composition. Since cholesterol movement between vesicles follows first-order kinetics (Backer & Dawidowicz, 1981; McLean & Phillips, 1981, 1982, 1984), further quantification of rate data was made by fitting the rate curves to the first-order rate equation:

$$\ln[(H_0 - H_{eq.})/(H_t - H_{eq.})] = kt \quad (1)$$

where H_0 , $H_{eq.}$ and H_t are the ^3H counts initially, at equilibrium, and at any time t during incubation respectively. $H_{eq.}$ was calculated assuming the same C/P ratios for SUVs and LUVs at equilibrium. (A reading taken after 30 h of incubation showed that 4:1 PC/PS SUVs attained the calculated equilibrium very closely.) The linear fitting was done by the method of least squares on a Hewlett Packard 85 microcomputer using the General Statistics Pac supplied by the manufacturer. The data fitted to the equation with correlation coefficients (r^2) in the range 0.980–0.999. The first-order rate constant k , obtained as the slope, was used to calculate the $t_{1/2}$ of the process from the relation: $t_{1/2} = 0.693/k$. The

Table 1. Parameters of linear fitting of rate data (as in Fig. 1) to first-order kinetics equation (described in the Results section) for the transfer of [³H]cholesterol from donor SUVs to acceptor LUVs for typical individual kinetic runs

For each rate data point, the linear fitting was done with the time of mixing of SUV and LUV as $t = 0$ and the 15 min time point as $t = 0$. Considerable intercepts for fitting with mixing time as $t = 0$ indicate the presence of a fast phase. C/P is the cholesterol to phospholipid molar ratio determined by chemical methods. r^2 is the coefficient of correlation for the linear fitting. The values in parentheses give the % reaction corresponding to the intercept.

Composition of SUV			Rate data					
Lipids	C/P ratio	Rate measurement source	Mixing time as $t = 0$			15 min point as $t = 0$		
			Intercept	Slope	r^2	Intercept	Slope	r^2
PC	0.96	Supernatant	0.177 (16)	0.323	0.987	-0.065	0.328	0.986
PC	0.96	Pellet	0.244 (22)	0.304	0.991	-0.013	0.275	0.994
1:1 PC/SM	0.75	Supernatant	0.291 (25)	0.219	0.997	0.018	0.218	0.997
1:1 PC/SM	0.75	Pellet	0.323 (28)	0.210	0.996	0.034	0.200	0.996
12:1 PC/ganglioside	0.98	Supernatant	0.237 (21)	0.341	0.996	-0.032	0.403	0.980
12:1 PC/ganglioside	0.98	Pellet	0.268 (24)	0.412	0.989	-0.024	0.349	0.994
Erythrocyte lipids	0.81	Supernatant	0.217 (20)	0.276	0.963	-0.029	0.278	0.957
Erythrocyte lipids	0.81	Pellet	0.228 (20)	0.233	0.997	0.012	0.245	0.952

Fig. 2. First-order plots of rate data from typical single experiments (as in Fig. 1) for the transfer of [³H]-cholesterol from two different donors

For each donor, the same rate data are plotted with different initial conditions. (a) PC-cholesterol SUV (C/P = 0.96) as donor with the mixing times as $t = 0$ (○) and with the 15 min point as $t = 0$ (●). (b) 1:1 PC-SM-cholesterol (C/P = 0.75) as donor with the mixing time as $t = 0$ (△) and with the 15 min point as $t = 0$ (▲).

Fig. 3. Variation of the mean $t_{1/2}$ (from Table 2) for the transfer of [³H]cholesterol from PC-SM-cholesterol donor SUVs (C/P = 0.84–1.05) to acceptor LUVs (C/P = 0.27) as a function of phospholipid composition of the donor vesicles

Mol % SM is based on total phospholipids (PC + SM).

k values (representing the slopes) obtained from either supernatant or pellet data for a given system agreed closely (Table 1; Fig. 2), but the linear fitting gave a small intercept for both supernatant and pellet in almost all cases, indicating an initial, much faster, phase comprising 15–25% of the total reaction. Refitting the data with the 15 min point as $t = 0$ also gave a good fit ($r^2 = 0.960$ – 0.999), but with intercepts very close to zero. Obviously $t_{1/2}$ values obtained this way represent a better approximation of the rate of the predominant slower phase and are used in discussing the results. The $t_{1/2}$ values for donors of different composition are tabulated in Table 2. There are significant differences in $t_{1/2}$ as different lipids are incorporated into SUVs though the C/P ratio is held roughly constant.

The rate of cholesterol transfer depends not only on

Table 2. Effect of the lipid composition of donor SUVs on the $t_{1/2}$ for [^3H]cholesterol transfer to a common LUV acceptor having a C/P of 0.27 ± 0.01 at 37 °C

$t_{1/2}$ was calculated from the slope of the linear fittings of rate data with the 15 min time point as $t = 0$ (as in Table 1) using the relation $t_{1/2} = 0.693/\text{slope}$. All the values are given as mean $\pm$ S.E.M. The numbers in parentheses give the number of $t_{1/2}$ values obtained from independent kinetic runs using a minimum of two different SUV preparations. Each run gives two $t_{1/2}$ values, one each from the supernatant and the pellet. The P values obtained by pairing the indicated SUV with the first (PC) SUV except for 5:5:1 PC/SM/gangliosides which is paired with 1:1 PC/SM SUV. Gangliosides are included in phospholipids when calculating C/P. 1:1 PC/PE system may not exist as vesicles (see the Discussion section).

Composition of SUV			
Lipids	C/P ratio	$t_{1/2}$ (h)	P
PC	0.87 ± 0.04	1.94 ± 0.12 (8)	—
9:1 PC/SM	1.05 ± 0.02	2.65 ± 0.27 (8)	0.015
3:1 PC/SM	0.99 ± 0.06	2.49 ± 0.11 (8)	0.002
1:1 PC/SM	0.84 ± 0.06	3.53 ± 0.17 (8)	0.000
1:3 PC/SM	0.99 ± 0.01	4.49 ± 0.23 (8)	0.000
1:9 PC/SM	1.01 ± 0.08	5.50 ± 0.26 (8)	0.000
4:1 PC/PE	0.97 ± 0.01	2.19 ± 0.17 (12)	0.146
1:1 PC/PE	1.02 ± 0.02	9.58 ± 1.58 (10)	0.000
1:1:1 PC/PE/SM	1.03 ± 0.00	2.81 ± 0.17 (8)	0.001
4:1 PC/PS	1.04 ± 0.01	2.12 ± 0.10 (8)	0.131
12:1 PC/ganglioside	0.95 ± 0.02	1.68 ± 0.07 (8)	0.047
6.5:1 PC/ganglioside	0.73 ± 0.01	1.31 ± 0.12 (8)	0.001
5:5:1 PC/SM/ganglioside	0.80 ± 0.00	2.74 ± 0.10 (8)	0.001
Erythrocyte lipids	0.79 ± 0.02	2.48 ± 0.15 (8)	0.007

the nature of the lipid, but also on its content. This is more evident in the case of PC–SM vesicles, for which the $t_{1/2}$ values are plotted as a function of the mol % of SM (based on total phospholipids) in Fig. 3. $t_{1/2}$ increases almost linearly ($r^2 = 0.955$) with increasing mol % of SM. PE and PS have no effect on $t_{1/2}$ when they form 20 mol % of the total phospholipids (Table 2). When PE constitutes 50 mol % of the total phospholipids there is a dramatic increase in $t_{1/2}$ (from 1.94 to 9.58 h). We believe that this reflects a change in the lipid structure at high PE levels (see the Discussion section). Of all the lipids tested, only gangliosides were able to facilitate the transfer of cholesterol from SUVs to LUVs and the effect is stronger at a higher mol % of ganglioside.

DISCUSSION

The role of membrane components in cholesterol exchange between membranes is not well understood. Proteins (Bellini *et al.*, 1984; Clejan & Bittman, 1984a), phospholipids (Nakagawa *et al.*, 1979; Fugler *et al.*, 1985; Yeagle, 1985), fatty acyl chains (Bloj & Zilversmit, 1977; Poznansky & Lange, 1978) and glycolipids (Davis *et al.*, 1984) have been shown to influence the rate of cholesterol movement between membranes. Since these studies have used intact cells as either donor or acceptor, the interpretation of results is not so straightforward. Moreover, the presence of bovine serum albumin in some systems may influence the rate by interacting preferentially with certain lipids such as gangliosides (Tomasi *et al.*, 1980). Thus, further studies with well-defined donor and acceptor membranes, such as the SUV–LUV system used in the present study, are necessary to quantify the contribution of lipids to this phenomenon.

The rate, in all cases measured, follows first-order

kinetics in agreement with earlier studies (Backer & Dawidowicz, 1981; McLean & Phillips, 1981, 1982, 1984). However, the kinetics are not as simple as suggested by the presence of a small, but significant, initial fast component in all systems studied. An initial fast phase of 10–15 % was observed when the ion-exchange method was used to separate the donor and acceptor vesicles and also when purified (> 99 %) [^3H]cholesterol was used. McLean & Phillips (1982) have observed a similar fast component accounting for 9 % of the cholesterol transferred when a 1:3.4 DPPC/cholesterol dispersion was incubated with PC vesicles containing 15 mol % dicetyl phosphate. The authors have suggested that the rapid initial phase may be due to loosely absorbed cholesterol molecules on the vesicle surface. This seems to be unlikely in our case as the donor vesicles have a C/P ratio of 1.0 or less when all of the cholesterol is expected to be in the bilayer (Collins & Phillips, 1982). A similar fast component (15 %) has been reported in the transfer of cholesterol from erythrocytes to plasma (Lange *et al.*, 1983).

The incorporation of SM into PC donor vesicles decreases the rate of cholesterol transfer, the effect being apparent at levels as low as 10 mol % of the phospholipid. A similar effect of SM on kinetics has been reported for vesicles containing SM as well as cells enriched with SM (Nakagawa *et al.*, 1979; Clejan & Bittman, 1984b; Fugler *et al.*, 1985). The retarding effect of SM on cholesterol transfer rate can be explained in two ways. (1) SM rigidifies the PC bilayer matrix (Barenholz & Thompson, 1980) thereby rendering it more difficult for the cholesterol molecule to leave the bilayer. (2) Stronger interactions in the polar region of the SM–cholesterol system compared with the PC–cholesterol system retard the movement of cholesterol out of the membrane. The second possibility is not consistent with the results of

Lange *et al.* (1979) who have shown that there is no preferential partitioning of cholesterol between SM and DPPC and there is a 2-fold preference for DPPC over PC (egg) at the same temperature. If there is a significant difference in the interaction of cholesterol with PC and SM, one would expect a lateral phase separation (formation of SM-enriched regions) in the PC-SM-cholesterol bilayer, which in turn is expected to give rise to biphasic kinetics as reported in some cases (Davis *et al.*, 1984). But the rate curves are monophasic (ignoring the initial rapid phase) at all compositions studied for at least 60–70 % of the reaction (excluding the initial rapid phase). The linear increase in $t_{1/2}$ with increasing mol % of SM (Fig. 3) also argues against a possible lateral phase separation. On the other hand, the linear increase in $t_{1/2}$ parallels the increase in apparent microviscosity of the PC-SM system as a function of the mol % of SM (Untracht & Shipley, 1977; Barenholz & Thompson, 1980). At 37 °C, the microviscosity of SM vesicles is 3 times greater than that of PC vesicles (Shinitzky & Barenholz, 1974) which correlates with the nearly 3-fold increase in $t_{1/2}$ from PC to 1:9 PC-SM vesicles (Table 2; Fig. 3) and from PC to DPPC (Poznansky & Lange, 1978). Thus the present kinetic data are more consistent with a fluidity effect of SM, though contribution from head-group interactions cannot be ruled out entirely.

PE and PS seem to have no effect on cholesterol efflux at a concentration of 20 mol % of the total phospholipid. PE seems to have no effect even at 30 mol % as is evident from the $t_{1/2}$ values for an equimolar PC-PE-SM system and erythrocyte lipids. However, there is an apparent drastic slowing down of the rate at 50 mol % PE. Alterations in yield and sedimentation of SUVs at these PE values are seen and we believe that these abnormalities may be a result of the tendency of PE to form a hexagonal phase which is enhanced by a high cholesterol content (Cullis & de Kruijff, 1979). This result is not in agreement with the results of Yeagle & Young (1986) who showed that cholesterol partitioning is reduced significantly in membranes with substantial PE content. The matrix lipids are different and the equilibrium partitioning method used by these authors, in general, has the disadvantage of long incubation periods which are comparable to the $t_{1/2}$ for the movement of phospholipids between membranes.

Perhaps the most interesting observation is the opposing effect of SM and gangliosides on the rate of cholesterol transfer (Table 2). Gangliosides seem to enhance the rate marginally at concentrations as low as 7.7 mol %, but the effect is very significant at 13.5 mol %. The effect of gangliosides is not likely to be due simply to the surface charge provided by sialic acid residues because PS has no effect on $t_{1/2}$ even at 20 mol %. The fact that both SM and gangliosides have the same hydrocarbon tail and rigidify the PC matrix (Untracht & Shipley, 1977; Bertoli *et al.*, 1981), but have opposing effects on the cholesterol transfer rate, suggests the importance of sugar residues in cholesterol desorption from the bilayer. Preliminary studies have shown that the initial rate of cholesterol exchange between human erythrocyte ghosts and SUVs decreases significantly when the ghosts are treated with neuraminidase, again suggesting a role for sugar residues in the phenomenon.

It is reasonable to assume that the rate of cholesterol desorption from a membrane is determined mainly by the interaction of cholesterol with its nearest neighbour

molecules on the same monolayer. If so, the lipid composition effect on the rate of cholesterol transfer reported here could partially account for the large difference in the rates of cholesterol efflux from the two sides of the plasma membrane. Several studies on biological membranes have indicated that PC and SM prefer the outer layer while PE and PS are more asymmetrically distributed in favour of the inner layer (Houslay & Stanley, 1982). Gangliosides are located almost exclusively on the outer layer of biomembranes even though they are less asymmetrically distributed in lipid vesicles (Svennerholm, 1980; Thomas & Podder, 1982). Since gangliosides are located on the outer layer, the rate of cholesterol desorption from the outer layer of the plasma membrane is likely to be greater than that from the inner layer in accordance with the present results. This effect may be amplified by the presence of glycoproteins on the outer layer because the ganglioside effect appears to be mediated by the sugar residues. Since erythrocytes and many other plasma membranes contain 25–35 mol % PE of which 60–100 % is located in the inner layer (Houslay & Stanley, 1982), the PE content of the inner layer of the plasma membrane could be as high as 60–70 mol %. This could lead to a drastic decrease in the rate of cholesterol efflux from the inner layer of the plasma membrane if the structure of the PE-enriched regions is similar to that of the dispersion containing 50 mol % PE in our studies. Thus the asymmetric disposition of PE and glycoconjugates can, in principle, give rise to the widely different rates of cholesterol efflux from the two sides of the plasma membrane and this might be an important factor controlling cholesterol distribution. Some of the above considerations might be applicable to the movement of cholesterol between microsomes and plasma membrane since they also differ in their lipid composition.

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