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Effect of surface curvature on the rate of cholesterol transfer between lipid vesicles

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The effect of surface curvature on the spontaneous movement of cholesterol between membranes was investigated by measuring the rates of cholesterol transfer from donor vesicles of various sizes to a common acceptor vesicle. Donor vesicles of size in the range 40–240 nm were prepared by extruding multilamellar dispersions through polycarbonate filters of different pore sizes under pressure. The smallest donor vesicle and the acceptor vesicles were obtained by the normal sonication procedures. The rate of cholesterol transfer, as measured by the movement of [^{3}H]cholesterol, decreases with increasing size of the donor vesicle in an almost linear fashion. The extrapolation of the results gave a half-time ($t_{\frac{1}{2}}$) of 16–20 h of the desorption of cholesterol from a planar bilayer, and this can be considered as a reference value for most cellular membranes which are characterized by very low curvatures. Our earlier studies have shown that the $t_{\frac{1}{2}}$ for cholesterol efflux is influenced by the presence of gangliosides and phosphatidylethanolamine, and the asymmetric distribution of these lipids in the plasma membrane could partially account for the large difference in the rates of cholesterol movement from the two sides of the plasma membrane. The small differences in rates arising from asymmetric distribution will be magnified by the longer $t_{\frac{1}{2}}$ obtained here for membranes of low curvatures, so that the large difference in rates might be a coupled effect of lipid asymmetry and low curvature of the plasma membrane. This, in turn, may have a role in maintaining the large differences in cholesterol/phospholipid molar ratios observed between plasma membrane and intracellular membranes.

INTRODUCTION

Cholesterol moves spontaneously between membranes, and between membranes and lipoproteins, by an aqueous diffusion mechanism probably involving monomers (Green, 1983; Magot *et al.*, 1985; Yeagle, 1985). Model-membrane studies have shown that both exchange and net transfer of cholesterol can occur, the difference in the cholesterol/phospholipid (C/P) molar ratio of donor and acceptor being the driving force for the latter. Despite the apparent rapid movement of cholesterol between membranes, the plasma membrane and intracellular membranes maintain widely different C/P ratios. Efforts to explain this in terms of a difference in affinity of cholesterol for these membranes or their matrix lipids have not been entirely satisfactory (Wattenburg & Silbert, 1983). Another possibility is a steady-state distribution determined by the rates of cholesterol movement in different directions. The plasma membrane is likely to play a crucial role in the steady-state distribution, because it can exchange cholesterol with the intracellular membranes and extracellular components and yet possesses the highest C/P values.

The movement of cholesterol from the plasma membrane (cell surfaces) to plasma lipoproteins or phospholipid vesicles is a relatively fast process, with a half-time ($t_{\frac{1}{2}}$) in the range of 1–4 h (Lange *et al.*, 1983; Poznansky & Czekanski, 1982), whereas the movement from the plasma membrane to the intracellular membranes is a much slower process (Poznansky & Czekanski, 1982; Slotte & Lundberg, 1983; Robertson & Poznansky, 1985; Lundberg & Suominen, 1985). Three factors likely to be responsible for the large difference in cholesterol efflux rates from the two sides of the plasma membrane are: (i) the asymmetric disposition of membrane proteins; (ii) asymmetric dispositions of lipids; and (iii) the curvature of the surface from which cholesterol is leaving. Recently we have observed that phosphatidylethanolamine and sphingomyelin decrease, and gangliosides increase, the rate of cholesterol efflux from lipid vesicles, and if the effect of their asymmetric distribution in plasma membrane is taken into consideration, the observed rate differences can partially account for the large difference in cholesterol efflux rates from the two sides of the plasma membrane and varied cholesterol distribution (Thomas & Poznansky, 1988). In the present paper an attempt is made to evaluate the role of membrane curvature by measuring the rate of cholesterol efflux from lipid vesicles of various sizes. The results indicate a continuous increase in $t_{\frac{1}{2}}$ for cholesterol efflux with increasing size of the donor vesicle, and this curvature effect could be the major contributor to the very slow movement of cholesterol from the plasma membrane to intracellular membranes.

MATERIALS AND METHODS

Materials

Cholesterol, egg phosphatidylcholine (PC) (Type V), bovine brain phosphatidylserine (PS) and DEAE-cellulose were obtained from Sigma. [^{3}H]Cholesterol (sp.

Abbreviations used: PC, phosphatidylcholine (egg): DOPC, dioleoyl phosphatidylcholine; PS, phosphatidylserine; PLEP, phospholipid-exchange protein; C/P, cholesterol/phospholipid molar ratio; SUV, small unilamellar vesicle; LUV, large unilamellar vesicle.

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radioactivity 60 Ci/mmol), [^{14}C]cholesteryl oleate (sp. radioactivity 56.6 mCi/mmol) and D-[^{3}H]glucose (sp. radioactivity 4.5 Ci/mmol) were products of New England Nuclear. [^{3}H]Dioleoyl phosphatidylcholine ([^{3}H]-DOPC) and phospholipid-exchange protein (PLEP) were prepared in our laboratory by standard procedures (Davis & Keough, 1983; Westerman *et al.*, 1983). Cholesterol was recrystallized from ethanol. [^{3}H]Cholesterol was purified by t.l.c. on silica gel, with diethyl ether/benzene/ethanol/acetic acid (200:250:10:1, by vol.) as the solvent system. All other chemicals were used without further purification. (PC and PS were found to be pure by t.l.c.). Polycarbonate membranes were from Nuclepore, Toronto, Ontario, Canada.

Preparation of vesicles

Donor vesicles (C/P = 1/0) of various sizes were prepared by the extrusion technique (Hope *et al.*, 1985). Briefly, 4.8 μmol of PC, 1.2 μmol of PS, 6 μmol of cholesterol, 30 μCi of [^{3}H]cholesterol and 3 μCi of [^{14}C]-cholesterol oleate (added as a non-exchangeable marker) were mixed in chloroform; solvent was evaporated under an N_2 stream to form a thin film, and the mixture was dried under vacuum for 2 h. A 4 ml portion of 20 mM-Tris/acetate buffer, pH 7.4, containing 100 mM-NaCl, was added to the dried lipids and vortex-mixed thoroughly. The multilamellar dispersion was transferred to the central cavity of the extrusion apparatus with one, or a stacked pair of, polycarbonate filters of the desired pore size (50–400 nm) at the bottom of the cavity. N_2 pressures of 345–1725 kPa (50–250 lbf·in^{-2}) were applied and the extruded dispersion was recycled. After ten cycles of extrusion, the dispersions obtained with 100, 200 and 400 nm-pore-size filters were frozen in liquid N_2 and thawed in 15–20 min. The dispersions were extruded through the same filters twice more. After extrusion, all the dispersions were centrifuged at 15000 *g* for 15 min to remove large particles. The supernatant was used as donor vesicles for pore sizes of 50 and 80 nm. For the larger pore sizes, the supernatant was spun again at 160000 *g* for 1 h at 20 °C and the pellet was suspended in 2–3 ml of the buffer and used as donor vesicles.

Acceptor vesicles containing PC and cholesterol (C/P = 0.27) and one of the donor vesicles were prepared by sonicating the multilamellar dispersion, prepared as above, for 6 min at a temperature of 0–5 °C using a broad-tip probe in a Heat Systems model W185 sonifier. The dispersion was centrifuged at 15000 *g* for 20 min to remove the titanium particles and multilamellar liposomes. The supernatant was centrifuged again at 160000 *g* for 1 h at 20 °C. The supernatant from this spin was used as small unilamellar vesicles (SUVs).

Characterization of vesicles

The vesicle preparations were characterized by measuring the size, the trapped volume and the transbilayer distribution of PC. The vesicle size was measured by negative-stain electron microscopy (Enoch & Strittmatter, 1979). The vesicle preparations were diluted to 0.25 mM in PC and a drop of this was placed on a formvar-coated grid. After 30 s, one drop of 1% uranyl acetate solution was added to it and the whole solution was removed after another 30 s with a filter paper. Pictures were taken with a Phillips 300 electron microscope operating at 80 kV.

Trapped volume was measured by equilibrating the vesicles with externally added [^{3}H]glucose (Wong *et al.*, 1982). A 20–40 μCi portion of [^{3}H]glucose was added and allowed to equilibrate at room temperature for more than 50 h. The vesicles were then passed through a Sephadex G-50 (medium grade) column (1 cm × 25 cm) and eluted with Tris buffer at a flow rate of 20 ml/h at 4 °C. Four 1 ml of fractions eluted 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 in larger vesicles were determined from the extent of PLEP-mediated transfer of [^{3}H]DOPC from these vesicles to SUVs of the same C/P ratio (1.0). For this, vesicles were prepared by the extrusion technique as described above except that [^{3}H]cholesterol were replaced by [^{3}H]DOPC and the buffer was 10 mM-Tris/HCl, pH 7.4, containing 50 mM-NaCl and 1 mM-EDTA. Incubations were carried out at 37 °C at final PC concentrations of 0.2 and 2 mM of donor and acceptor (SUV) vesicles respectively and a PLEP concentration of 0.14 mg/ml. At different time intervals, 100 μl aliquots were withdrawn and spun in an Airfuge at 160000 *g* for 10 min. A 50 μl portion of the supernatant and the pellet with the remaining supernatant were counted for ^{3}H and ^{14}C ([^{14}C]cholesteryl oleate added as a non-exchangeable marker) radioactivity after adding 15 ml of aqueous counting scintillant (Amersham) to each. The ^{3}H radioactivity was corrected for ^{14}C cross-over, and the supernatant ^{3}H radioactivity was also corrected for a small fraction of non-pelleted donor vesicles by using the ^{14}C radioactivity in the supernatant. From the equilibrium ^{3}H radioactivity, the percentage of PC present in the outer surface of vesicles was calculated.

Rate measurement

The rate of appearance of [^{3}H]cholesterol in acceptor SUVs was monitored by separating the acceptor (neutral) and donor (charged) vesicles by DEAE-cellulose ion-exchange chromatography after incubation and counting the radioactivity in the eluate (SUVs) in a Beckman LS3133 liquid-scintillation counter (Nakagawa *et al.*, 1979). Incubations were carried out at 37 °C in a shaker bath, usually in a total volume of 2 ml consisting of donor and acceptor (SUVs) vesicles at final concentrations of 0.2 mM and 2 mM of phospholipid respectively in 20 mM-Tris/acetate buffer, pH 7.4, containing 100 mM-NaCl. At specific time intervals (0.25–12 h) 100 μl aliquots were withdrawn and applied to a DEAE-cellulose column (5.5 mm diameter Pasteur pipettes packed to a height of 45–55 mm) at room temperature and eluted with 1 ml of the Tris/acetate buffer and collected in a scintillation vial. The column was eluted again with 1 ml of the buffer and the eluate collected in a separate vial. To each vial 15 ml of aqueous counting scintillant was added and counted for ^{3}H and ^{14}C radioactivity. The ^{3}H radioactivity corrected for ^{14}C cross-over, differential quenching and the very small fraction of donor vesicles eluted by using the ^{14}C radioactivity. In order to minimize non-specific adsorption to the column, DEAE-cellulose was pretreated with a sonicated dispersion of PC and washed a few times with the buffer after packing the column. Under these

experimental conditions nearly 99% of the charged vesicles were retained on the column and nearly 99% of the neutral vesicles were eluted.

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 by the method of Fiske & SubbaRow (1925), with Elon (*p*-methylaminophenol sulphate) as the reducing agent.

RESULTS

The size determined from electron micrographs, the trapped volume, and the percentage of PC on the outer surface of donor vesicles prepared by sonication and extrusion technique are given in Table 1. The vesicle size increases with increasing pore size of the polycarbonate filter used, in agreement with the results reported for vesicles made up of PC alone (Mayer *et al.*, 1986). Freeze–thawing of vesicles during the extrusion procedure has a dramatic effect on the size of the vesicle, as is evident from the trapped volumes of vesicles prepared by using filters of 200 nm pore size with and without freeze–thawing. This also agrees with previous reports (Hope *et al.*, 1985; Mayer *et al.*, 1986). In fact larger vesicles cannot be prepared without freeze–thawing. The transbilayer distribution of PC suggests the presence of multilamellar structures in vesicle preparations made using 100, 200 and 400 nm-pore-size filters. As much as 25–30% of the total lipid might be forming interior bilayers. This phenomenon does not interfere with the rate determinations as a function of radius.

Fig. 1 gives some typical rate curves for the transfer of [^{3}H]cholesterol from donor vesicles of different sizes to acceptor SUVs. Obviously the rate decreases with increasing size of the donor vesicle. Since cholesterol movement between vesicles follows first-order kinetics (Backer & Dawidowicz, 1981*a*; McLean & Phillips, 1981), further quantification of the rate data was made by fitting the rate curves to the first-order rate equation:

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

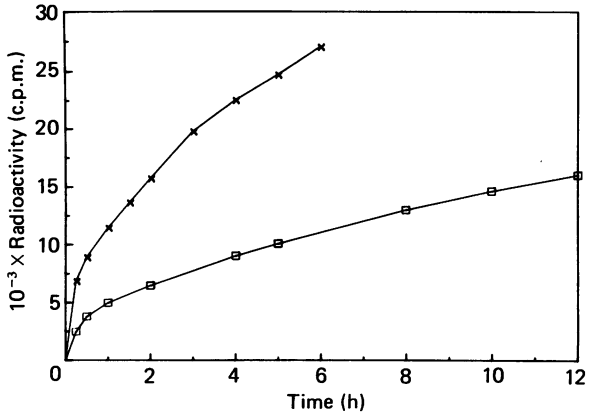

Fig. 1. Rate curves for the transfer of [^{3}H]cholesterol from PC/cholesterol donor vesicles of diameters 97±27 nm (×) and 231±49 nm (□) to acceptor SUVs at 37 °C from typical single experiments

The donor vesicles were prepared by using polycarbonate filters of pore sizes 50 and 400 nm respectively and found to have C/P ratios of 0.905 and 1.078 respectively. C/P ratios for the corresponding acceptors were 0.282 and 0.283. The donor vesicle and the acceptor SUV were mixed to final concentrations of 0.2 mM and 2 mM of phospholipid respectively and 100 μl aliquots were withdrawn at indicated time intervals, the donor and acceptor vesicles were separated on a DEAE-cellulose column and the radioactivity in the eluate (acceptor) counted (see the Materials and methods section for details). Radioactivities in 100 μl aliquots for the two incubation mixtures were 47816 and 47358 c.p.m. respectively.

where H_0, $H_{eq.}$ and H_t are the ^{3}H counts initially, at equilibrium and at any time t during the incubation. $H_{eq.}$ values were determined experimentally by measuring H_t after 30–75 h of incubation. (In most cases, these $H_{eq.}$ values were found to be very close to the values calculated by assuming the same C/P ratios for donor and acceptor vesicles at equilibrium.) The linear fitting was done by the method of least squares on a Hewlett–Packard 85 microcomputer using the General

Table 1. Characteristics of donor vesicles (C/P = 1) and the half-times for the transfer of cholesterol to acceptor SUVs (C/P = 0.27) at 37 °C

'Sup' represents the supernatant from a 160000 *g* spin for 1 h. 'FT' indicates that the dispersion was freeze–thawed during the extrusion procedure. 'PC exposed' is the average of two readings from two different preparations differing by less than 1%. The values in parentheses are the numbers of independent determinations from a minimum of three different preparations for each size.

Pore size of the filter (nm)	Vesicle diameter from electron micrograph (nm) (mean±S.D.)	Trapped volume (litre/mol)	PC exposed on the outer surface of vesicle (%)	$t_{\frac{1}{2}}$ (h) (mean±S.E.M.)
Sonication	46±17	0.412		1.76±0.03 (10)
50	97±27	0.665		3.00±0.19 (6)
80		0.672		4.67±0.11 (6)
80 (Sup)	100±35	0.588		4.29±0.33 (6)
200		1.089	38.1	6.72±0.21 (6)
100 (FT)	145±35	1.697	38.4	8.16±0.31 (8)
200 (FT)	181±43	2.041	33.3	9.43±0.36 (6)
400 (FT)	231±49	3.536	37.0	13.54±0.30 (8)

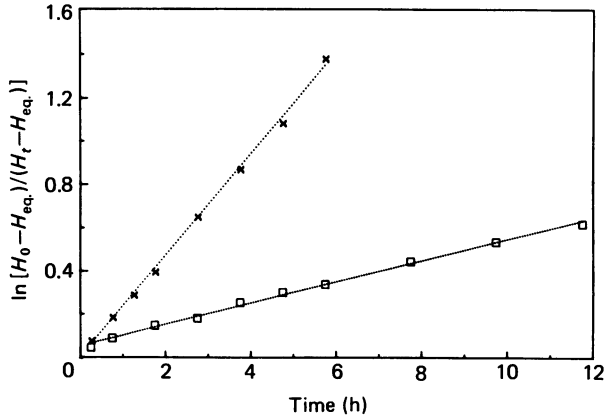

Fig. 2. First-order plots for the same rate data given in Fig. 1

Donor vesicles of diameter 97±27 nm (×) and 231±49 nm (□). The data were fitted to straight lines with r^2 values of 0.998 and 0.996 respectively.

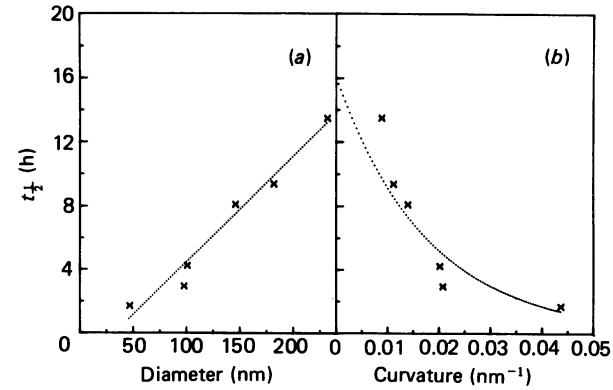

Fig. 3. Effect of donor vesicle size on $t_{\frac{1}{2}}$ for the transfer of cholesterol to a common SUV acceptor (from Table 1)

(*a*) $t_{\frac{1}{2}}$ as a function of the diameter of the donor vesicles (as measured from electron micrographs). The linear fitting has an r^2 value of 0.968. (*b*) $t_{\frac{1}{2}}$ as a function of the curvature (reciprocal of radius) of the donor vesicles. The exponential fitting shown gives an r^2 value of 0.838, whereas linear fitting (not shown) gives an r^2 value of 0.954.

Statistics Pac supplied by the manufacturer. The data fitted the equation with correlation coefficients (r^2) in the range of 0.960–0.999 (most of them > 0.99), but gave a small intercept in almost all cases, indicating an initial much faster phase comprising 10–15% of the total reaction, as we have noticed in other systems as well (Thomas & Poznansky, 1988). A similar rapid initial phase had also been reported previously (McLean & Phillips, 1982; Lange *et al.*, 1983). Refitting the data with the 15 min point as $t = 0$ also gave a good fit (r^2 = 0.970–0.999, most of them > 0.99), but with intercepts very close to zero. Typical plots are given in Fig. 2. The half-times ($t_{\frac{1}{2}}$) for the transfer calculated from the relation: $t_{\frac{1}{2}} = 0.693/k$, where k is the slope from the latter fittings (first-order rate constant), are also given in Table 1.

There is an apparent linear relationship between $t_{\frac{1}{2}}$ and the diameter of the donor vesicle (r^2 = 0.968), as shown in Fig. 3(*a*). Since the interaction of cholesterol with its neighbouring molecules and water on the surface of a membrane is likely to be influenced by the curvature of the surface rather than the size of the particle, a more meaningful analysis will be the correlation between $t_{\frac{1}{2}}$ and the curvature (geometrically defined as the reciprocal of radius) of the vesicle as shown in Fig. 3(*b*). An exponential extrapolation (r^2 = 0.838) of the data in Fig. 3(*b*) gives a $t_{\frac{1}{2}}$ of 16 h and a linear extrapolation (omitting the smallest vesicle) (r^2 = 0.954) gives a $t_{\frac{1}{2}}$ of 19 h for planar membrane. Fig. 3(*b*) describes only the exponential extrapolation.

DISCUSSION

The role of lipid composition in cholesterol exchange between membranes has been studied by several laboratories. However, a possible role for membrane curvature in this phenomenon has largely been ignored, mainly because of the lack of suitable methods to prepare vesicles of various sizes. Even though a few previous reports have indicated an influence of vesicle size on the rate of cholesterol efflux, these data were not obtained rigorously enough to generalize about effect of curvature and were not sufficient to enable one to extrapolate to cellular membranes (McLean & Phillips, 1984; Fugler *et al.*, 1985; Yeagle & Young, 1986). In those studies, sonicated vesicles were compared with vesicles prepared by detergent dialysis or reverse-phase evaporation. This renders their conclusions questionable, because properties of vesicles prepared by different techniques are likely to be different. This is evident from the inconsistencies among these data. According to those studies, $t_{\frac{1}{2}}$ for cholesterol exchange is expected to increase with increasing size of the vesicle. But vesicles of diameter 80 nm prepared by detergent dialysis gave a $t_{\frac{1}{2}}$ of 10.2 h, whereas much larger vesicles (160 nm diameter) prepared by reverse-phase evaporation gave a $t_{\frac{1}{2}}$ of 5.5 h (McLean & Phillips, 1984; Fugler *et al.*, 1985). Contaminants such as detergents and organic solvents have in fact been shown to influence cholesterol exchange, phospholipid exchange and phospholipid 'Flip Flop' (Kramer *et al.*, 1981; Clejan & Bittman, 1984; Nichols, 1986). Thus, in order to ascertain the effect of curvature, a reinvestigation using vesicles of various sizes prepared by a single and fairly reproducible method was necessary. The extrusion technique is ideally suited for this, and gives vesicles free of detergents and organic solvents. Further, the broad range of vesicle size obtained by this method enables us to establish a functional correlation between $t_{\frac{1}{2}}$ and curvature.

The transfer of cholesterol from SUV to SUV proceeds with a $t_{\frac{1}{2}}$ of 1.76 h (Table 1). Previously we and others have observed that the $t_{\frac{1}{2}}$ for the transfer of cholesterol from SUV to LUV (of corresponding compositions) is 2.12 h (Thomas & Poznansky, 1988). This suggests that the size of the acceptor vesicle has no effect on the rate, a conclusion reported by Fugler *et al.* (1985). However, an increase in the size of the donor vesicle results in a significant increase in $t_{\frac{1}{2}}$: a doubling of size leads to a doubling of $t_{\frac{1}{2}}$ (Table 1; Fig. 3). There is an apparent similar relationship in the limited data reported by McLean & Phillips (1984) for 23 and 80 nm-size vesicles prepared by two different methods. However, there are significant quantitative differences. Those authors obtained a $t_{\frac{1}{2}}$ of 10.2 h for 80 nm vesicles, whereas the present results suggest a value of 3.1 h (from the linear relationship between $t_{\frac{1}{2}}$ and size) for the same size.

Similar quantitative differences exist between the present data and those of Fugler *et al.* (1985). Those authors noticed a continuous increase in $t_{\frac{1}{2}}$ with increasing size of the vesicle [3,4 and 5.5 h for 25, 60–160, and 160 nm vesicles respectively]. But the dependence of $t_{\frac{1}{2}}$ on size seems to be much less than that obtained in the present studies. The discrepancy is probably due to the fact that those authors are comparing vesicles prepared by three different methods. A possible argument against the greater dependence of $t_{\frac{1}{2}}$ on the size of donor vesicle observed in the present studies is that the vesicle preparations made using 100, 200 and 400 nm-pore-size membranes contain multilamellar structures. The presence of multilamellar structures is not likely to influence $t_{\frac{1}{2}}$, as is evident from the following observations. There is a continuous and significant increase in $t_{\frac{1}{2}}$ as the pore size increases, through the percentage of lipid present as multilamellar vesicles is more or less constant for 100, 200 and 400 nm pore sizes. The results with sonicated vesicles and vesicles prepared using 50 and 80 nm-pore-size membranes also show an increase in $t_{\frac{1}{2}}$ with increasing size. The previous reports also indicate qualitatively the same relationship (McLean & Phillips, 1984; Fugler *et al.*, 1985; Yeagle & Young, 1986). Hence, the rate-limiting step in a multilamellar vesicle is also likely to be the desorption of cholesterol from the outermost layer if one assumes rapid cholesterol Flip Flop (Backer & Davidowicz, 1981*b*).

The mechanism responsible for the curvature-dependence of the rate of cholesterol efflux from vesicles is not clear. A slight increase in the concentration of monomeric cholesterol in dispersions of smaller particles could be a minor factor contributing to this (McLean & Phillips, 1984). Altered interactions of cholesterol with its nearest neighbours in the lipid/water interface arising from differences in molecular packing in the bilayer have been suggested as the major cause (McLean & Phillips, 1984; Fugler *et al.*, 1985). Our observation that the larger vesicles reach the calculated equilibrium in 50–75 h suggests that there is no difference in the stability of cholesterol in vesicles of various curvatures. This implies that cholesterol in highly curved membrane is closer to the transition state without any significant change in its overall interaction energy with the environment in vesicles of different curvatures. Increased hydration of cholesterol molecules and decreased cholesterol–phospholipid interactions due to the greater surface area per molecule on a highly curved surface could be a possibility. It may be mentioned here that a mechanism involving hydrogen-bonding between cholesterol and phospholipid, which apparently is influenced by the curvature of the surface, has been suggested (Brockerhoff, 1974).

The extrapolation of the present data gives a $t_{\frac{1}{2}}$ of 16–20 h for cholesterol desorption from planar membranes (Fig. 3). This means cell membranes, which are characterized by very low curvatures, will also have similar cholesterol desorption rates. This may explain the slow movement of cholesterol from the plasma membrane to the intracellular membranes. The implication here may be that cholesterol movement within the cell may be as likely to be as a result of membrane or vesicle movement as it is the movement of monomeric cholesterol. The movement of cholesterol from cells to extracellular particles, however, is much faster (Poznansky & Czekanski, 1982; Lange *et al.*, 1983). An explanation for this may lie in our observation that gangliosides increase the rate of cholesterol desorption from SUVs and may do so in spite of low curvatures (Thomas & Poznansky, 1988). On the other hand, the presence of phosphatidylethanolamine on the inner surface of plasma membrane could lead to a further slowing down of cholesterol movement from the plasma membrane to the intracellular membranes (Thomas & Poznansky, 1988). Thus the very low curvature of plasma membrane, coupled with the asymmetric distribution of glycoconjugates and phosphatidylethanolamine on it, in principle, may account for the widely different rates of cholesterol efflux from the two sides of the plasma membrane. This, in turn, may have a role in the steady-state distribution of cholesterol between plasma membrane and intracellular membranes.

Even though plasma membrane may be considered as planar bilayer for practical purposes there are some localized regions of high curvature, such as villi and sites of vesiculation. In such regions, the large difference in curvature between the inner and outer layers (concave and convex surfaces) might contribute to a further difference in the rates of cholesterol efflux from the two sides of the plasma membrane, in accordance with the present results.

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