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Mechanism of Cholesterol Exchange between Phospholipid Vesicles†

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ABSTRACT: The kinetics of cholesterol exchange between two populations of small unilamellar vesicles has been investigated. There is no change in the initial rate of this exchange process over a 100-fold change in the acceptor vesicle concentration at a constant donor concentration. These results are not consistent with a collision-dependent exchange mechanism. In support of transfer via the aqueous phase, the inclusion of a negatively charged lipid into the vesicles did not affect the exchange rate. Evidence for a water-soluble pool of cholesterol that had partitioned out of the vesicle was obtained. Finally, cholesterol exchange was observed when donor and acceptor membranes were separated by a barrier through which neither could pass. These data together support our contention that the exchange of cholesterol between these vesicles involves a water-soluble intermediate.

The spontaneous exchange of cholesterol between plasma and red blood cells was first reported by Hagerman & Gould (1951). Since then it has been demonstrated to occur in a variety of biological membranes, lipoproteins, and liposomes (Bruckdorfer & Graham, 1976; Bell, 1976). Although cholesterol exchange is well documented, the mechanism of this process is not well understood. Two possibilities have been proposed. The first, originally suggested by Hagerman & Gould (1951), involves the partitioning of cholesterol out of the membrane into the aqueous phase which then acts as an intermediate in the equilibration process. A second mechanism first outlined by Gurd (1960) suggests that cholesterol exchange between membranes takes place upon contact of the membranes as a result of collision. This latter proposal, which also applied to exchange of phospholipids, was based solely on the view that cholesterol and phospholipids are essentially insoluble in water.

Evidence in favor of the collision-dependent mechanism for cholesterol exchange has been presented in several studies (Bruckdorfer & Green, 1967; Lenard & Rothman, 1976; Haran & Shporer, 1977; Poznansky & Lange, 1978; Moore et al., 1978; Patzer et al., 1978; Jonas & Maine, 1979; Giraud & Claret, 1979; Gottlieb, 1980), although in some of these the data on which the conclusions were based is somewhat scant. In addition, Bruckdorfer & Graham (1976) argued that the involvement of an aqueous cholesterol intermediate could be dismissed, because Gould et al. (1955) had reported that they were unable to observe cholesterol exchange between membranes that were separated from each other by a dialysis membrane. The reference quoted by Bruckdorfer & Graham (1976) does not contain this information. Furthermore, in a search of Gould's publications we have been unable to find the source of the data cited.

In marked contrast to the above-mentioned studies are those in which the spontaneous exchange of phospholipids between small unilamellar vesicles (SUV's)[1] was examined (Martin & MacDonald, 1976; Duckwitz-Peterlein et al., 1977; Roseman & Thompson, 1980). These authors showed that the rate of this exchange process is independent of the acceptor membrane concentration, from which it was concluded that the spontaneous exchange of phospholipids between SUV's occurs via the aqueous phase and does not involve membrane collisions. Similar conclusions have been reached for the transfer of pyrene (Charlton et al., 1976), a fluorescent cholesterol analogue (Kao et al., 1977), and a diglyceride (Charlton et al., 1978) between high-density lipoproteins. Furthermore, evi-

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[1] Abbreviations used: SUV, small unilamellar vesicle; MLV, multilamellar vesicle; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane.

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dence in favor of transfer via the aqueous phase has been presented for the movement of fatty acids (Doody et al., 1980) and cytochrome b_5 (Leto et al., 1980) between phospholipid vesicles.

In a previous study, we developed a method for studying the exchange of cholesterol between two populations of SUV's (Backer & Dawidowicz, 1979). Our approach involved incorporation of a glycolipid into the donor vesicles, which enabled them to be precipitated out of solution by the addition of a lectin following the exchange process. We now report on our investigation of the kinetics of cholesterol exchange between two well-defined populations of SUV's, using this approach, in order to elucidate the mechanism of this process. A preliminary communication of this study has previously been presented (Backer & Dawidowicz, 1978).

Materials and Methods

Egg lecithin, cholesterol, and the glycolipid *N*-palmitoyl-DL-dihydrolactocerebroside were purchased and stored as previously described (Backer & Dawidowicz, 1979). Phosphatidic acid was prepared from egg lecithin by the action of phospholipase D (Holloway & Katz, 1975). The resulting phosphatidic acid which gave a single spot after thin-layer chromatography on silica gel H with chloroform/methanol/acetic acid/water (25:15:4:2 v/v) as the eluting solvent was stored in ethanol at −20 °C.

Cholesteryl [1-^{14}C]oleate (50 Ci/mol) and [1,2-$^{3}H_2$]-cholesterol (60 Ci/mmol) were obtained from New England Nuclear (Boston, MA). Greater than 98% of the radioactivity in each of these compounds comigrated with authentic samples after thin-layer chromatography on silica gel G with hexane/ether (80:20 v/v) as the eluting solvent. In addition, >98% of the ^{3}H counts in the [^{3}H]cholesterol comigrated with cholesterol with chloroform/methanol (100:2 v/v) as the eluting solvent.

Preparation of the lectin *Ricinus communis* and red cell ghosts together with the analytical determinations used was as described in our previous study (Backer & Dawidowicz, 1979).

Preparation of Vesicles. SUV's of the appropriate lipid composition were prepared by sonication followed by chromatography on Sepharose 4B (Backer & Dawidowicz, 1979). Only those fractions eluting on the descending portion of the included volume peak were pooled and are designated as SUV's throughout this study. Careful characterization of these fractions (Huang, 1969; Newman & Huang, 1975) has shown that they consist of unilamellar vesicles which are homogeneous with respect to both size and composition. When SUV's were prepared containing phosphatidic acid, the above procedure was modified to prepare them in deionized water. The dried lipid mixture was dispersed in 10 mM Na_2EDTA, sonicated, and chromatographed on Sepharose 4B in 10 mM Na_2EDTA. The SUV fractions described above were pooled and concentrated in an Amicon ultrafiltration cell using a PM-10 filter (Amicon Corp., Lexington, MA). These concentrated SUV's were then rechromatographed on Sepharose 4B in deionized water to remove the salt. The resulting SUV's were pooled and stored under nitrogen at 4 °C. Incorporation of phosphatidic acid was demonstrated by extraction of an aliquot from these SUV's with chloroform/methanol (2:1 v/v) followed by thin-layer chromatography on silica gel H with chloroform/methanol/acetic acid/water (25:15:4:2 v/v) as the eluting solvent. In order to prepare SUV's in deionized water, one must follow the above two-step procedure since the fractionation of vesicles on Sepharose 4B requires the presence of at least 10 mM salt. If the initial column is eluted with deionized water, no separation of the SUV's from the multilamellar vesicles (MLV) is achieved.

MLV's were prepared by collecting those fractions which eluted at the void volume of a Sepharose 4B column after chromatography of a sonicated lipid dispersion. The multilamellar nature of these fractions has been demonstrated by Huang (1969).

Exchange Experiments. The exchange of [^{3}H]cholesterol between two populations of SUV's was followed at 37 °C as previously described (Backer & Dawidowicz, 1979), with the exception that the serum albumin was omitted. Donor SUV's were composed of egg lecithin and cholesterol (molar ratio 1:0.7) containing 10% by weight glycolipid, [^{3}H]cholesterol, and cholesteryl [^{14}C]oleate. Acceptor SUV's were composed of egg lecithin and cholesterol (molar ratio 1:0.7). Due to the presence of the glycolipid, the donor vesicles have a slightly lower molar concentration of cholesterol than the acceptors. However, in our previous study (Backer & Dawidowicz, 1979) this small difference in vesicle composition was shown to have no detectable effect on both the rate and extent of cholesterol exchange between SUV's and erythrocyte ghosts.

Dialysis Experiments. SUV's were prepared from a mixture of egg lecithin and cholesterol (molar ratio 1:0.7) containing [^{3}H]cholesterol and cholesteryl [^{14}C]oleate. These SUV's were placed inside a dialysis tubing (average pore size 4.8 nm) and dialyzed against an aqueous solution which contained either buffer or egg lecithin SUV's. Incubations were conducted at 37 °C in a sealed bottle, to minimize evaporation, in the presence of 0.5 mM NaN_3. Aliquots were removed from the surrounding solution at various times, and the amount of radioactivity was determined. Appearance of ^{14}C counts outside the bag, $[^{14}C]_{out}$, indicated contamination or leakage of intact vesicles. The ^{3}H counts observed outside the bag, $[^{3}H]_{out}$, were therefore corrected by using the expression

$$[^{3}H]_{cor} = [^{3}H]_{out} - [^{14}C]_{out}([^{3}H]/[^{14}C])_{ves}$$

where $([^{3}H]/[^{14}C])_{ves}$ is the ratio in the original vesicles.

Lectin Precipitation. SUV's were prepared from egg lecithin and cholesterol (molar ratio 1:0.5) containing 10% by weight glycolipid, [^{3}H]cholesterol, and cholesteryl [^{14}C]oleate. One milliliter of these vesicles (0.2 mM lecithin) was incubated for 1 h at 37 °C under nitrogen. Five hundred microliters of *R. communis* (5 mg/mL) was then added at a final temperature of 24 °C. This mixture was spun for 5 min in a Brinkman 3200 centrifuge, and the radioactivity in the supernatant was determined. Correction of the [^{3}H]cholesterol for any unprecipitated vesicles was made in a manner similar to that described above for the dialysis experiments, using the ^{14}C counts remaining in the supernatant.

Results

There have been isolated reports in the literature (Hauser, 1971; Hauser & Irons, 1972) that sonication of egg lecithin containing dispersions for 1 h at 4 °C results in degradation of the phospholipid, which could give rise to artifacts in the exchange studies. However, under the carefully controlled preparative conditions used in this study, >99% of the egg lecithin migrated as a single spot on silica gel G with chloroform/methanol/water (65:25:4 v/v) as the eluting solvent, even after incubation of the fractionated lipid vesicles for 2 h at 37 °C under nitrogen. This result is in agreement with previous analyses of vesicles produced by sonication, where no phospholipid degradation was detected when the preparative procedure was conducted under carefully controlled conditions (Huang & Charlton, 1972; Litman, 1973; Newman & Huang, 1975; Backer & Dawidowicz, 1981).

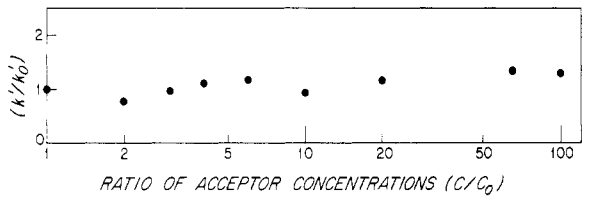

FIGURE 1: Effect of acceptor concentration on the initial apparent rate constant of cholesterol exchange between small unilamellar vesicles. 250 μL of donor SUV's (22 nmol of cholesterol) composed of egg lecithin and cholesterol (molar ratio 1:0.7) containing [^{3}H]-cholesterol, cholesteryl [^{14}C]oleate, and 10% by weight lactosylceramide was incubated with 1.2 mL of acceptor vesicles, with a range of 22–2200 nmol of cholesterol, at 37 °C in the presence of 0.5 mM NaN_3. Both populations of SUV were prepared in 100 mM KCl and 10 mM Tris-HCl, pH 7.4. At various times during the incubation, a 200-μL aliquot was removed, added to 400 μL of *R. communis* (2.5 mg/mL), at 24 °C, vortexed, and centrifuged in a Brinkman 3200 centrifuge for 5 min. 400 μL of the supernatant was removed, and the amount of radioactivity was determined. Each time point was taken in duplicate. The initial apparent rate constant for the exchange process was obtained as the initial least-squares slope from a plot of ln ([^{3}H]cholesterol remaining in donor) against time. This rate represents exchange of the initial 20% of the [^{3}H]cholesterol and was determined from six time points. The apparent rate constant k_0' is that obtained when the ratio of donor to acceptor is 1:1, at an acceptor concentration c_0. All other apparent rate constants k' at varying acceptor concentration c are expressed as a ratio with this initial value.

Kinetics. In both our present and previous study we have confirmed the finding originally reported by Hagerman & Gould (1951) that exchange of cholesterol between membranes exhibits overall first-order kinetics. The rate of cholesterol exchange from the donor membrane at a fixed donor–acceptor ratio can therefore be described by the rate equation

$$\frac{-\mathrm{d}[[^3\mathrm{H}]\text{cholesterol}]}{\mathrm{d}t} = k'[[^3\mathrm{H}]\text{cholesterol}]$$

which follows from the plot of ln (fraction of [^{3}H]cholesterol remaining in donors) against time, a straight line with slope k', the apparent rate constant.

To test one of the proposed mechanisms for cholesterol exchange, we have varied the acceptor vesicle concentration over a 100-fold range at a fixed concentration of donor vesicles. At low acceptor to donor ratios, there is a possible problem of back exchange of [^{3}H]cholesterol from the acceptor. In order to simplify our kinetic analysis, we have therefore measured only the initial rate of cholesterol exchange, which in all cases was linear for the exchange of at least the first 20% of the vesicular cholesterol. The apparent rate constant for the exchange was determined from the least-squares slope of the initial portion of the plot ln (fraction [^{3}H]cholesterol remaining in donors) against time. An example of this has been presented in our previous paper (Backer & Dawidowicz, 1979). A comparison of the apparent rate constants for cholesterol exchange at varying acceptor concentrations is shown in Figure 1. These data have been normalized to k_0' and c_0 which are the apparent rate constant and acceptor concentration measured at a 1:1 ratio of donor/acceptor. The value of k_0' corresponds to a half-time of 1.5 h for the exchange of [^{3}H]cholesterol from the donor vesicles, which is in excellent agreement with the result in our previous paper. It can be seen from Figure 1 that the apparent rate constant for cholesterol exchange between SUV's is essentially constant over a 100-fold range of acceptor to donor ratio.

If the rate of cholesterol exchange varies with changes in the concentration of the acceptor membrane, the apparent rate constant k', for a fixed donor/acceptor ratio, would be

$$k' = k''[\text{acceptor}]^n$$

at a constant donor concentration. The ratio of k' at differing acceptor concentrations would then be

$$\frac{k_1'}{k_2'} = \frac{k''}{k''}\left(\frac{[\text{acceptor}]_1}{[\text{acceptor}]_2}\right)^n$$

Our results show that k_1'/k_2' is close to 1 over a wide range of acceptor concentration, which implies $n = 0$, thus indicating that there is no dependence of the acceptor membrane concentration on the rate of cholesterol exchange between SUV's.

Lectin Precipitation. Since our kinetic data were not consistent with a collisional model for cholesterol exchange, we attempted to obtain evidence for the existence of a water-soluble pool of cholesterol that had partitioned out of lecithin–cholesterol vesicles. After incubation of SUV's containing 10% glycolipid for 1 h at 37 °C, the SUV's were precipitated with *R. communis* at 24 °C as described earlier. Greater than 98% of the vesicles were agglutinated under these conditions. Since the initial cholesterol concentration in the SUV's was 0.1 mM, the presence of the unprecipitated vesicles resulted in a cholesterol concentration of 2 μM. After corrections were made for the presence of these vesicles, as described earlier, a pool of solubilized cholesterol was indicated at a concentration of 190 nM. This cholesterol in solution (corresponding to 1.7×10^5 ^{3}H counts min^{-1} mL^{-1}) was extracted from 1 mL of the supernatant, following vesicle precipitation, with 4 mL of chloroform/methanol (2:1 v/v). The resulting lower phase was concentrated under a stream of nitrogen and analyzed by thin-layer chromatography on silica gel G with hexane/ether (80:20 v/v) as the eluting solvent. Greater than 98% of the ^{3}H counts comigrated with cholesterol, which demonstrates that the aqueous pool of cholesterol is not an artifact of cholesterol oxidation.

Haberland & Reynolds (1973) have reported that the critical micelle concentration of cholesterol in water at 25 °C is between 25 and 40 nM. However, this value must not be confused with the maximum solubility for cholesterol of 4.7 μM at 25 °C reported by the same workers. Our finding of a water-soluble pool of cholesterol with a concentration of 190 nM at 37 °C is clearly well below the maximum solubility of cholesterol at 25 °C.

Cholesterol Exchange from Multilamellar Vesicles. When multilamellar vesicles (MLV's) composed of egg lecithin, cholesterol, and glycolipid were incubated with red cell ghosts in our previous study (Backer & Dawidowicz, 1979), 75% of the cholesterol in the multilayers was exchanged with a single time constant. Similar findings have been reported by Bruckdorfer et al. (1968) and Bloj & Zilversmit (1977). This indicates that cholesterol present in the inner bilayers of the MLV is available for exchange. In order to reduce the possibility of contact between these inner bilayers, we prepared MLV's composed of cholesterol, egg lecithin, and phosphatidic acid (molar ratio 0.7:0.9:0.1) including [^{3}H]cholesterol and cholesteryl [^{14}C]oleate in 10 mM Na_2EDTA. The exchange of [^{3}H]cholesterol from these negatively charged MLV's into red cell ghosts is shown in Figure 2. Greater than 75% of the [^{3}H]cholesterol in the MLV's can be exchanged with a single time constant.

Cholesterol Exchange between Negatively Charged SUV's. We argued that if cholesterol exchange occurred by a collision-dependent mechanism, a change in the collision frequency between the membranes should have an effect on the exchange rate. To vary the collision frequency, we included phosphatidic acid, a negatively charged lipid, in the SUV's. In order to eliminate the suppression of the negatively charged vesicle surface by the presence of high salt, we prepared all vesicles

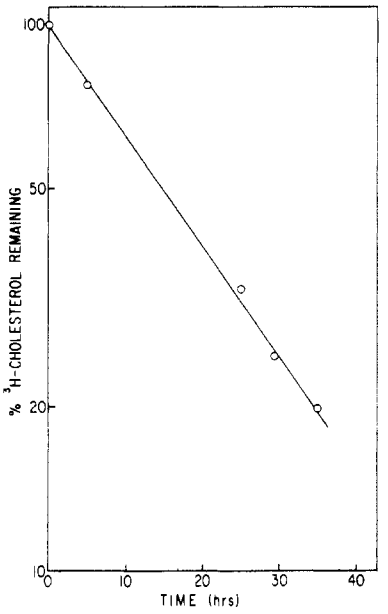

FIGURE 2: Exchange of [^{3}H]cholesterol between negatively charged multilamellar vesicles and erythrocyte ghosts. Multilamellar vesicles (MLV's) were prepared from a mixture of cholesterol/egg lecithin/phosphatidic acid (molar ratio 0.7:0.9:0.10) containing [^{3}H]-cholesterol and cholesteryl [^{14}C]oleate. 2 mL of negatively charged MLV's (0.5 mM cholesterol) was incubated with 7.7 mL of human erythrocyte ghosts (2.6 mM cholesterol) at 37 °C in the presence of 0.5 mM NaN_3. At various times, 1 mL was removed and spun for 5 min in a Brinkman 3200 centrifuge. The radioactivity in the supernatant was determined, from which the fraction of [^{3}H]cholesterol remaining in the MLV's was calculated.

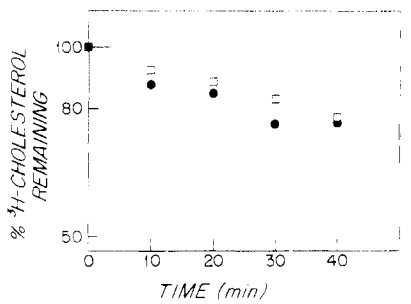

FIGURE 3: Exchange of [^{3}H]cholesterol between small unilamellar vesicles containing phosphatidic acid. Donor SUV's were prepared from a mixture of phospholipid and cholesterol (molar ratio 1:0.7) containing 10% by weight glycolipid, [^{3}H]cholesterol, and cholesteryl [^{14}C]oleate. The phospholipid was either egg lecithin (for uncharged donors) or egg lecithin/phosphatidic acid (molar ratio 3:1 for charged donors). Acceptor SUV's were prepared from egg lecithin, phosphatidic acid, and cholesterol (molar ratio 0.75:0.25:0.7). Exchange experiments were conducted at 37 °C between acceptor SUV's (0.55 mM cholesterol) and donor SUV's (0.025 mM cholesterol) which were either charged (●) or uncharged (□). The kinetics of the exchange of [^{3}H]cholesterol between these SUV's was determined as previously described.

used in studies involving exchange from negatively charged SUV's in deionized water as described earlier.

The rates of cholesterol exchange between charged and uncharged SUV's compared with that when both populations are charged are shown in Figure 3. Inclusion of the negative charge has no significant effect on the rate of cholesterol exchange between SUV's.

Dialysis Experiments. In order to prevent direct contact between donor and acceptor membranes, we separated them by a dialysis membrane through which neither could pass. Cholesterol is capable of partitioning out of SUV's and transferring across the dialysis membrane as shown in Figure 4. Transfer was observed when either buffer or SUV's com-

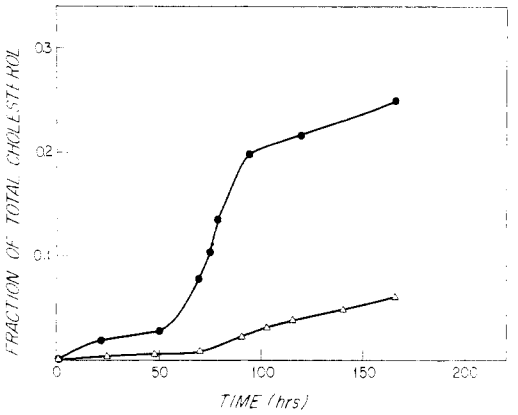

FIGURE 4: Transfer of [^{3}H]cholesterol from small unilamellar vesicles across a dialysis membrane. Donor SUV's composed of egg lecithin/cholesterol (molar ratio 1:0.7) containing [^{3}H]cholesterol and cholesteryl [^{14}C]oleate were prepared in 100 mM KCl and 10 mM Tris-HCl, pH 7.4. These fractionated vesicles (0.48 mM egg lecithin containing 9×10^4 ^{3}H counts min^{-1} mL^{-1}) were dialyzed at 37 °C under varying conditions. The fraction of [^{3}H]cholesterol that passed through the dialysis bag is depicted above. Similar results have been obtained in four separate experiments. (●) 1 mL of the donor SUV's was dialyzed against 15 mL of 100 mM KCl and 10 mM Tris-HCl, pH 7.4, containing SUV's composed of egg lecithin (0.5 mM lipid). Less than 5% of the donor SUV's, based on the presence of ^{14}C counts, was detected outside the dialysis bag at the end of the experiment. (△) 5 mL of the donor SUV's was dialyzed against 25 mL of the KCl–Tris buffer. Less than 2% of the donor SUV's was detected outside the dialysis bag at the end of this experiment. From the initial specific activity of the [^{3}H]cholesterol, it can be estimated that the cholesterol concentration outside the bag, at the end of this dialysis against KCl–Tris buffer, is 4.2 μM. In both experiments depicted above, thin-layer chromatographic analysis of an extract from an aliquot of the external aqueous phase (containing 1×10^3 ^{3}H counts/min) revealed no oxidation of the cholesterol.

posed of egg lecithin were placed outside the dialysis bag. An analysis by thin-layer chromatography (described earlier) of the [^{3}H]cholesterol outside the dialysis bag at the end of the experiment indicated that >95% of the ^{3}H counts comigrated with cholesterol. In addition, no degradation of the egg lecithin was detected by analysis on silica gel G with chloroform/methanol/water (65:25:4 v/v) as the eluting solvent.

Discussion

If cholesterol exchange between SUV's involved collision of the membranes, it would be predicted that the rate of this exchange process would increase in a manner directly proportional to the increase in the concentration of acceptor membranes at a constant donor concentration. Our finding that the initial rate of cholesterol exchange is essentially constant over a 100-fold range of acceptor to donor ratio is therefore incompatible with a collision-dependent exchange process. The kinetics are consistent with, but do not prove, cholesterol exchange occurring via a water-soluble intermediate. Interestingly, Quarfordt & Hilderman (1970) also concluded that their exchange kinetics were consistent with the involvement of a water-soluble cholesterol intermediate. However, they did not favor this interpretation, citing the low solubility of cholesterol in water as their objection. Solely on the basis of this latter argument, Gurd (1960) had originally proposed a collision-dependent cholesterol exchange mechanism, which has been restated by other workers (Haran & Shporer, 1977; Gottlieb, 1980). In addition, Gurd (1960) reached a similar conclusion for the spontaneous exchange of phospholipids, based only on their limited solubility in water. It has been shown that pyrene which has a maximum solubility in water of 0.4 μM (Charlton et al., 1976) and a fluorescent diglyceride with a solubility of <25 nM (Charlton et al., 1978)

both transfer between high-density lipoproteins by initially dissociating from the donor into the aqueous phase followed by rapid diffusion and subsequent uptake by the acceptor particle. For comparison, Haberland & Reynolds (1973) have reported a maximum solubility of 4.7 μM for cholesterol in water. Since this concentration is greater than for either pyrene or the fluorescent diglyceride, which undergoes exchange via the aqueous phase, it does not seem to be valid to rule out the involvement of a water-soluble cholesterol intermediate in the exchange process solely on the basis of the knowledge of the solubility of cholesterol in water. Indeed, recent experiments, described earlier, have also shown that the spontaneous exchange of phospholipids between SUV's occurs via the aqueous phase, in contrast to the prediction based on solubility arguments.

In our previous study (Backer & Dawidowicz, 1979) we were able to exchange >75% of the cholesterol present in MLV's. Since there are at least two concentric bilayers present in each MLV, the data indicate that cholesterol is traversing the inner interbilayer aqueous compartments. Nevertheless, the possibility of a transitory contact of the bilayers in the uncharged MLV's cannot be excluded. Inclusion of the negatively charged phosphatidic acid in the MLV to prevent such transitory contacts does not affect the amount of cholesterol that can be exchanged from an MLV.

To support our contention that cholesterol exchange does not involve collisions between membranes, we attempted to vary the collision frequency by including a negatively charged phospholipid in the vesicles. The rates of cholesterol exchange between two populations of SUV's, when either both contain a negative charge or only one is charged, are essentially identical. Gulik-Krzywicki et al. (1969), Shipley et al. (1969), and Bangham et al. (1967) have shown that the presence of negatively charged lipids increases the interbilayer spacing in smectic lipid–water systems and multilamellar vesicles, respectively. This would indicate that the negatively charged phospholipid should diminish or eliminate the probability of vesicle–vesicle collisions in deionized water. The fact that the inclusion of the negatively charged lipid is without effect on both the rate of cholesterol exchange between SUV's and the fraction of cholesterol that can be exchanged from an MLV further suggests that membrane–membrane contact is not an important aspect of the exchange process.

Studies on lamellar arrays of uncharged phospholipids provide additional evidence that collision is a most unlikely mechanism for cholesterol exchange. LeNeveu et al. (1976) have measured that a force of $20kT$ is necessary to bring two parallel bilayers of area (100 Å)^2 within ~15 Å of each other. This very strong repulsive force was suggested to have its origin in the work needed to remove the water of hydration from the lipid polar groups. Although these data were obtained for lamellar arrays of lipids, Cowley et al. (1978) and Parsegian et al. (1979) have indicated that these hydration forces would prevent phospholipid vesicles from approaching each other to allow fusion. Presumably, the existence of this large repulsive force would also suggest that collision between even uncharged SUV's is an unlikely event.

A necessary requirement for the involvement of a water-soluble cholesterol intermediate is the ability to demonstrate the existence of such an aqueous pool. We have demonstrated the presence of such a cholesterol pool at a concentration of 190 nM which is lower than the maximum solubility of cholesterol in water. Due to the incomplete precipitation of the SUV's by the lectin, detected by the cholesteryl [^{14}C]oleate left in the supernatant, we were unable to determine in this experiment whether there is any lecithin associated with this pool of cholesterol.

The attempt to observe cholesterol exchange between donor and acceptor membranes separated by a barrier is an experiment initially attributed to Gould et al. (1955). As mentioned earlier, we have been unable to find an account of this experiment in any of Gould's publications. Poznansky & Lange (1978) did however briefly indicate that they were unable to detect cholesterol exchange between membranes separated by a Millipore filter with 100-Å pores. Since no experimental details were presented, it is difficult to comment on the above-mentioned study. The only other reported attempt at such dialysis experiments, quoted as unpublished observations by Quarfordt & Hilderman (1970), indicates that cholesterol exchange did not occur when donor and acceptor were separated by a dialysis membrane. Since no details were presented, it is impossible to comment on these observations. In the present study we have been able to observe cholesterol transfer from SUV's across a dialysis membrane. Although this is a relatively slow process, which may be due to cholesterol and lipid sticking to the dialysis membrane, it provides strong support for cholesterol exchange occurring via the aqueous phase.

In conclusion, we have demonstrated that the kinetics of cholesterol exchange between SUV's does not support a collision-dependent exchange mechanism. We have, however, been able to provide several pieces of evidence to support a mechanism of transfer via a water-soluble cholesterol intermediate. A direct consequence of this study has been our ability to increase the cholesterol levels in cells grown in culture by incubation with a saturated aqueous solution of cholesterol (E. A. Dawidowicz and R. L. Hoover, unpublished results), in a manner similar to that described by Alivisatos et al. (1977). After this manuscript was completed, a preliminary report (Phillips et al., 1980) was brought to our attention which supports our conclusions concerning the mechanism of cholesterol exchange between phospholipid vesicles.

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Isolation and Structural Characterization of Human Lymphocyte Neutral Glycosphingolipids†

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ABSTRACT: The neutral glycosphingolipids of human peripheral blood lymphocytes and of the lymphoid cells from a patient with B cell chronic lymphocytic leukemia were chemically analyzed. Four neutral glycosphingolipids were isolated from each of these two sources and studied by gas chromatography, methylation analysis, and electron impact–desorption mass spectrometry. The results of these studies indicate that the compounds have the following structures:

Glc1→1Cer
Gal1→4Glc1→1Cer
Gal1→4Gal1→4Glc1→1Cer
GalNAc1→3Gal1→4Gal1→4Glc1→1Cer

These compounds, belonging to the globo series, were the only neutral glycosphingolipids found in the lymphoid cells. The ceramide (Cer) moiety of all these compounds contained 4-sphingenine with $C_{16:0}$, $C_{24:0}$, and $C_{24:1}$ as the major fatty acid species. There were no structural differences in the neutral glycosphingolipids of peripheral blood lymphocytes compared to those of chronic lymphocytic leukemia cells. Peripheral blood lymphocytes contained more di- than monohexosylceramide whereas the reverse was true of the chronic lymphocytic leukemia cells. The proportion of tri- and tetrahexosylceramide was <10% for both types of cells. The results of our analyses did not support the existence of any differences in the major neutral glycosphingolipids among T, B, and chronic lymphocytic leukemia cells.

Glycosphingolipids have been isolated from most types of human blood cells, and some of these have been chemically characterized. Human erythrocytes have neutral glycosphingolipids of both the globo and lactoneo series (Hakomori & Siddiqui, 1976), whereas human platelets contain only the globo analogues (Tao et al., 1973). Recently, we have characterized the neutral glycosphinoglipids of human neutrophils and have shown that they are predominantly of the lactoneo type with smaller amounts of the gala series (Macher & Klock, 1980). Thus far, the neutral glycosphingolipids of human lymphocytes have not been completely chemically analyzed. Partial chemical analysis of lymphocyte glycosphingolipids suggests the presence of only globo-type structures (Stein & Marcus, 1977). However, data obtained by

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