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Kinetics and Mechanism of Phosphatidylcholine and Cholesterol Exchange between Single Bilayer Vesicles and Bovine Serum High-Density Lipoprotein†

Ana Jonas* and Gregory T. Maine

ABSTRACT: We investigated the exchange of lipids between sonicated, single bilayer vesicles containing egg phosphatidylcholine and cholesterol in a molar ratio of 2:1 and bovine serum high density lipoprotein, free of lecithin:cholesterol acyltransferase activity. Lipid exchange was followed by incubating radiolabeled vesicles ([^{14}C]cholesterol and [^{3}H]-phosphatidylcholine) with the lipoprotein (preequilibrated with excess unlabeled vesicles) at a constant temperature of 37 °C and by fractionating the reaction mixtures on Sepharose CL-4B columns at 5 °C. The proportion of radiolabeled lipid appearing in the lipoprotein elution peak, with time, was used in the kinetic analysis based on the radioisotope exchange treatment. Results indicate that there is no net transfer of lipids under our experimental conditions. All of the vesicle cholesterol is exchangeable, with a radiolabel exchange half-life of 1 h 8 min. In contrast, only 69% of the vesicle phosphatidylcholine exchanges with the high density lipoprotein, suggesting that only the outer half of the vesicle bilayer participates in the exchange; the half-life for [^{3}H]phosphatidylcholine exchange is 6 h. Reverse exchange from radiolabeled, preequilibrated high density lipoprotein to unlabeled vesicles was also observed. In the reverse exchange experiments, all of the lipoprotein cholesterol participates in the exchange, but only 49% of the lipoprotein phosphatidylcholine is involved. The exchange of both lipids is temperature dependent and has activation energies of 17.0 ± 2.8 kcal/mol for cholesterol and 12.5 ± 2.0 kcal/mol for phosphatidylcholine. A linear concentration dependence of the exchange rates indicates that both lipids exchange between vesicles and high density lipoprotein by a "bimolecular" process with second-order rate constants of 1.44 ± 0.56 mM^{-1} h^{-1} for cholesterol and 0.164 ± 0.045 mM^{-1} h^{-1} for phosphatidylcholine.

Serum lipoproteins, the lipid carriers in blood, are capable not only of solubilizing lipids but also of exchanging lipids with various membranes, cells, and tissues of the organism. A number of studies over the years have demonstrated that all classes of lipoproteins can exchange "structural" lipids, i.e., phospholipids and cholesterol with cell membranes (reviewed by Jackson et al., 1976; Bruckdorfer & Graham, 1976; Bell, 1976). Reed (1968) showed that phosphatidylcholine (PC)[1] and sphingomyelin of erythrocytes exchange only partially with the corresponding phospholipids in serum and that phos-

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[1] Abbreviations used: PC, phosphatidylcholine; HDL, high-density serum lipoprotein; TLC, thin-layer chromatography; Ans, 8-anilino-1-naphthalenesulfonate.

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phatidylethanolamine and phosphatidylserine do not exchange at all. Cholesterol of low density lipoproteins is completely exchangeable with cholesterol from erythrocyte ghosts (Bruckdorfer & Green, 1967), whereas only 62% of the cholesterol of high density lipoproteins (HDL) is exchangeable in an analogous system (Ashworth & Green, 1964). More recently, cholesterol exchange was shown to occur between fat cells and serum lipoproteins (Kovanen & Nikkilä, 1976). Apparently, these exchange processes do not involve net transfer of lipids, do not require the expenditure of energy, are temperature dependent, and have exchange half-lives of the order of hours. In contrast to the latter type of interactions between membranes and lipoproteins, which are not mediated by transfer proteins or cell membrane receptors, some peripheral tissues have specific receptors for low density lipoproteins. The interaction between the receptors and low density lipoproteins triggers the internalization of the entire lipoprotein and the subsequent events of lipoprotein hydrolysis, depression of cholesterol synthesis, and stimulation of cholesteryl ester synthesis (Goldstein & Brown, 1974; Balasubramaniam et al., 1977). Similar specific receptors have not been found for high density lipoproteins with the possible exception of rat liver cells (Drevon et al., 1977). The exchange of lipids between high density lipoproteins and cell membranes seems to be a physicochemical process, whose kinetics and mechanism are largely unknown. In this work we chose a simple system consisting of artificial membranes of sonicated egg PC and cholesterol (2:1 molar ratio) with well-defined physical properties (Huang, 1969; Newman & Huang, 1975) and bovine high density lipoprotein (Jonas, 1972a,b) in order to investigate quantitatively the exchange of lipids between bilayer structures and lipoproteins.

Experimental Procedures

Materials

Egg PC of type III-E and crystalline cholesterol were obtained from Sigma Chemical Co. The egg PC was purified by chromatography on a silicic acid column according to the procedure of Rouser et al. (1963). Cholesterol was used without further purification. [4-^{14}C]Cholesterol was purchased from New England Nuclear Co. and [9,10-^{3}H]dipalmitoyl-PC from Applied Science Laboratories, Inc. The radiolabeled lipids were purified by thin-layer chromatography (TLC) on Eastman Kodak silica gel plates followed by scraping of the plates at the appropriate position and extraction of the silica gel with chloroform:methanol, 2:1 (v/v). The following solvent systems were used for the development of TLC plates: petroleum ether:diethyl ether:acetic acid, 90:10:1 (v/v), or hexane:diethyl ether:acetic acid, 70:30:1 (v/v), for cholesterol and chloroform:methanol:water, 65:25:4 (v/v), for PC. Unlabeled lipids were detected by exposure to I_2 vapors and by spraying (glass plates) with 6 N H_2SO_4 and charring. Radiolabeled cholesterol was detected by autoradiography and the tritiated PC by scraping sections of TLC plates into a naphthalene–dioxane-based scintillation fluid and counting tritium in a Beckman LS-100 scintillation counter. All the lipids used in this study gave single spots by the TLC detection methods outlined above. Similar TLC analysis of lipids extracted with chloroform:methanol, 2:1 (v/v), after the sonication procedure, or collected under the vesicle peak at the end of kinetic experiments, gave identical results to the starting lipids.

Organic solvents of reagent grade were distilled prior to use. The buffer used in these studies was 0.1 M Tris-HCl, pH 8.0, 0.001% EDTA, 10^{-3} M NaN_3; it was prepared with reagent grade materials and deionized water. The gel-filtration media, Sepharose CL-4B and Sepharose 6B, were obtained from Pharmacia Fine Chemicals. 8-Anilino-1-naphthalenesulfonate magnesium salt (Ans) was obtained from Eastman Kodak Co., and 5,5-dithiobis(2-nitrobenzoic acid) was purchased from Sigma Chemical Co.

Preparations

Bovine HDL was prepared and analyzed for purity as described previously (Jonas, 1972a). Preparations of bovine HDL, passed through a Sepharose 6B column (1.8 × 40 cm) and analyzed for protein and lipid content and composition, gave similar results (±15%) across the entire elution peak, indicating a low degree of chemical heterogeneity. Previous molecular weight, sedimentation velocity coefficient, and rotational relaxation time determinations (Jonas, 1972a,b) and electron micrographs (Jonas, 1976, unpublished results) suggested particles of fairly uniform size with spherical shapes, around 100 Å in diameter. The chemical composition varies somewhat from preparation to preparation of bovine HDL. The average weight composition for the two preparations used in this study is 30% protein and 70% lipid. Of the total lipids, 46% are phospholipids, including 35% PC, and 54% are nonpolar lipids, including 4.4% free cholesterol and 39% cholesteryl esters. The remaining nonpolar lipids are mostly triglycerides. Prior to use, the HDL preparation was heated at 58 °C for 30 min in order to remove any traces of lecithin:cholesterol acyltransferase activity that may be associated with the lipoprotein (Glomset, 1972). Heated and original lipoprotein preparations had identical lipid and protein compositions after passage through a Sepharose 6B column (1.8 × 40 cm). HDL treated with 5,5-dithiobis(2-nitrobenzoic acid) according to the procedure of Stokke & Norum (1971) gave similar kinetic results to heat-treated HDL in forward exchange experiments for 1, 5, and 16 h. All the reported results were obtained with heat-treated HDL. In order to obviate subsequent problems due to possible net lipid transfer during incubation with radiolabeled vesicles, we preequilibrated all the heated lipoprotein preparations with an excess of unlabeled vesicles of PC:cholesterol, 2:1 (mol/mol), for 6 h at 37 °C. Saturation of HDL with vesicle lipids was determined by incubating a constant HDL sample with increasing concentrations of unlabeled vesicles. After fractionation of the reaction mixture, the protein and phospholipid composition of the HDL was determined, and it was established that a ratio of vesicle PC to HDL PC of 1/2 is sufficient for saturation. In subsequent preequilibration experiments a one- to twofold excess of vesicle PC over HDL PC was employed (A. Jonas, 1979, unpublished experiments). The preequilibration mixtures were fractionated on a Sepharose CL-4B column (2.4 × 40 cm) at 5 °C; the fractions corresponding to the lipoprotein elution were pooled and concentrated with Amicon conical filters. Two HDL preparations treated in this manner had very similar final compositions: 24% protein and 76% lipid. Of the total lipids, 55% were phospholipids, including 44% PC, and 45% were nonpolar lipids, including 4.4% free cholesterol and 33% cholesteryl esters. Protein was determined by the method of Lowry et al. (1951) by using bovine serum albumin as the standard. Total lipid was determined by weight after quantitative extraction of lipid by the procedure of Folch et al. (1957). Phospholipids were determined by the method of Chen et al. (1956), and free and total cholesterol was determined by the procedure of Sperry & Webb (1950). Triglycerides were quantitated by the method of Van Handel & Zilversmit (1957).

Electron micrographs of "heated" and "preequilibrated" HDL preparations were obtained at instrument magnifications of 40 000–80 000 using a Siemens 102 electron microscope operating at 80 kV. The negative staining procedure used in this work was taken from Anderson et al. (1977), but the lipoprotein solutions contained only 0.2 mg of the total lipoprotein/mL.

Vesicle Preparations. Typically, 15.0 mg of egg PC and 3.8 mg of cholesterol (i.e., 2:1, mol/mol) and 1–2 × 10^6 cpm of each radiolabeled lipid were mixed in 3–5 mL of chloroform:methanol, 2:1 (v/v), and were dried under N_2. The lipid mixture was suspended in 3.5 mL of buffer by vortexing for 5 min in a tightly capped tube flushed with N_2. The tube was then placed in a sonic bath from Heat Systems Ultrasonics Inc. at 0–10 °C for 3 h, in the dark. Following sonication, known aliquots of the lipid suspension were diluted to 1.0 mL with buffer and were used in ^3H and ^{14}C cpm determinations. From the total counts and the lipid weights in the dispersion, working specific activities in terms of cpm/mg of lipid were determined. These specific activities include the effects of counting efficiency and quenching by buffer. After centrifugation of the lipid dispersion at 18 000 rpm, 5 °C, for 1 h, the supernatant contained about two-thirds of all the lipid in the same PC to cholesterol ratio as the starting mixture. The supernatant was placed on a Sepharose CL-4B column (2.4 × 40 cm) at 5 °C and was eluted with buffer. Scintillation counting of the column fractions showed two peaks: one corresponding to multilamellar liposomes or large vesicles at the excluded volume and another corresponding to Huang type vesicles (Huang, 1969; Newman & Huang, 1975). The fractions in the middle (toward the trailing edge) of the latter peak were pooled and were used in the exchange experiments. These vesicles represented about 20% of the starting lipid and had the same mole ratios of PC to cholesterol. Typically the lipid concentrations were around 0.1–0.5 mM PC and 0.1–0.2 mM cholesterol with specific activities around 4 × 10^5 cpm/mg and 2 × 10^5 cpm/mg, respectively. Kinetic experiments, performed with vesicles that had been prepared by probe sonication for 0.5 h, gave results identical with those reported here.

Incubation and Separation of Vesicles from Bovine HDL. Incubations of preequilibrated HDL and vesicles were carried out in a covered shaking water bath (40 oscillations/min) at 37 °C, unless stated otherwise. The experimental results reported in this paper correspond to incubation mixtures of 1.32 mL containing 0.388 mM HDL cholesterol, 0.089 mM vesicle cholesterol, 1.72 mM HDL PC, and 0.174 mM vesicle PC. Throughout this paper the concentrations of PC and cholesterol are given in molar terms as though they were homogeneously distributed in the solution. This was done to facilitate analysis and, at the same time, to give an indication of the relative molar concentrations of the lipoprotein and vesicle particles. After the appropriate incubation periods, samples were immediately placed on ice and, within 2 min, were transferred to the analytical Sepharose CL-4B columns (1.8 × 30 cm) which were operated at 5 °C. Elution times were around 3 h; 1.6-mL fractions were collected and were analyzed by measuring absorbance at 280 nm and by double-label scintillation counting of ^{14}C- and ^3H-labeled lipids in 1.0-mL aliquots added to a naphthalene–dioxane-based scintillation fluid. The resulting elution profiles, with partially separated peaks for vesicles and HDL, were analyzed with a Curve Resolver, Model 310, manufactured by Du Pont, by assuming Gaussian shapes for the peaks. The areas under the peaks were expressed as a percent of the total eluted counts. Lipid recoveries from the column were at least 90%.

The fate of vesicles during incubation was determined by measuring the fluorescence of 0.20 mL of Ans (10^{-3} M) added to 1.5 mL of column fractions corresponding to the vesicle elution. At these concentrations, Ans is present in excess and gives a linear fluorescence increase with increasing vesicle concentrations. Exciting wavelength was 400 nm, emission wavelength was set at 493 nm, and slits were 6–7 nm on the MPF-III Perkin-Elmer recording spectrofluorometer. Vesicle integrity was also tested by careful chemical analysis of column fractions for several incubation mixtures scaled up to allow colorimetric determination of lipids.

For the "reverse" exchange experiments, preequilibrated radiolabeled HDL was prepared by the same procedure that was used to make the unlabeled, preequilibrated HDL, except that radiolabeled vesicles were substituted for unlabeled vesicles during the preincubation step. The kinetic experiments were carried out by using radiolabeled HDL and unlabeled vesicles as the acceptor particles. For the "reverse" exchange experiments reported in this work, the incubation mixture was 1.06 mL and the lipid concentrations were: HDL cholesterol 0.077 mM; vesicle cholesterol 0.636 mM; HDL PC 0.199 mM; and vesicle PC 1.20 mM.

Methods

Kinetic Analysis. The treatment of isotope exchange kinetics summarized by Myers & Prestwood (1951) and Frost & Pearson (1961) was applied in this work. The result is a first-order rate expression:

$$-\ln (1 - F) = (R/ab)(a + b)t$$

where F is the fraction of radiolabel exchange that has occurred (i.e., percent of counts in the acceptor divided by the number of counts in acceptor at infinite time), R is the constant rate of exchange of the lipid, labeled and unlabeled, a is the total concentration of the lipid in the acceptor species (i.e., PC or cholesterol in bovine HDL), b is the total concentration of the lipid in the donor species (i.e., PC or cholesterol in vesicles), and t is time. The only assumption made in the derivation of the rate expression is that the system is at mass equilibrium. R, although a constant in any given experiment, can be any function of a and b and depends on the mechanism of the exchange reaction. Generally, R can be expressed as

$$R = ka^m b^n$$

and its dependence on a and b can be determined empirically by carrying out exchange reactions at constant a and at constant b.

When reactants are only incompletely separated, and there is some exchange during the separation procedure

$$F = (\bar{F} - \bar{F}_0)/(1 - \bar{F}_0)$$

where $\bar{F}$ is the fraction of exchange at a certain time and $\bar{F}_0$ is the fraction of exchange at time zero.

Results

Electron micrographs of preequilibrated HDL showed spherical particles at least as homogeneous as the original HDL preparation. From hexagonally close-packed regions of the electron micrographs, a center-to-center distance of 111 ± 5 Å was determined. No disk-like particles could be detected. The original HDL had an average center-to-center distance of 105 ± 5 Å. The increase in HDL volume of 18% upon preequilibration coincides with a weight increase of 19% due to incorporation of PC and cholesterol from vesicles. The increase in size and relatively homogeneous distribution of

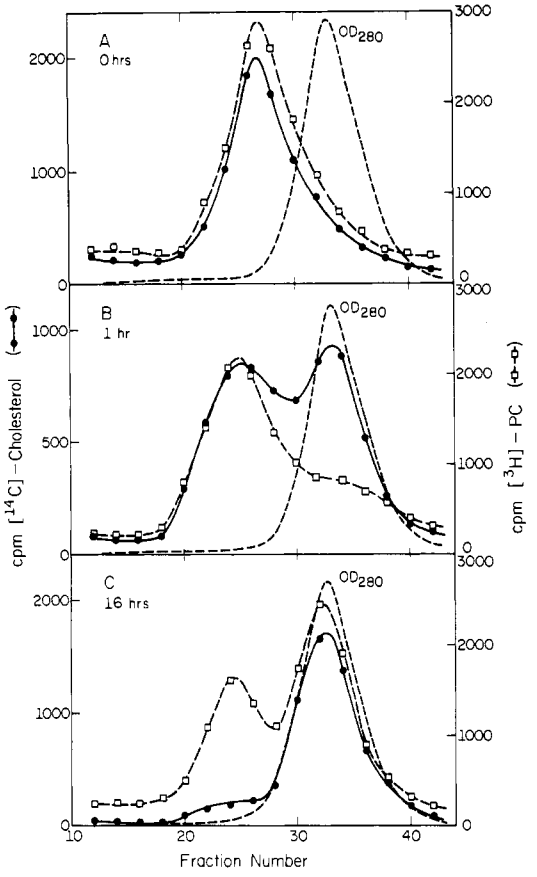

FIGURE 1: Gel filtration of reaction mixtures on a Sepharose CL-4B column (1.8 × 30 cm). Incubations were carried out at 37 °C for 0 (A), 1 (B), and 16 h (C), and fractionation was performed at 5 °C. The reaction mixtures of 1.32 mL contained 0.841 mM exchangeable PC and 0.388 mM cholesterol in HDL and 0.120 mM exchangeable PC and 0.089 mM cholesterol in vesicles. The specific activity of the radiolabeled lipids was of the order of 5×10^5 cpm/mg. The OD at 280 nm was 0.3 at the maximum. Fraction volume was 1.6 mL.

HDL in the preequilibrated samples was confirmed by gel filtration through a Sepharose CL-4B column and chemical analysis of the column fractions. Preequilibrated HDL eluted in a narrower peak, a few fractions ahead of the original HDL, indicating a larger Stokes radius. A more detailed description of the preequilibrated HDL properties will be published elsewhere (A. Jonas, 1979, unpublished experiments).

Figure 1 shows typical column elution profiles of radiolabeled vesicle mixtures with HDL, incubated at 37 °C for various time periods. At zero time there is an apparent 3–5% radiolabel transfer to the HDL peak; after 1 h the transfer is about 43% for cholesterol and 21% for PC; after 16 h the transfer of cholesterol is 83% and PC is 54%. During lipid exchange, the absorbance at 280 nm due to the HDL proteins remains associated with the HDL peak, indicating that there is no detectable protein transfer nor HDL incorporation into vesicle structures. The fate of the vesicles during incubation was followed by measuring the fluorescence intensity of Ans added to column fractions corresponding to the vesicle elution. At all times, the fluorescence in the vesicle fractions was similar and two- to threefold greater than the background fluorescence, suggesting that the vesicle concentration remained essentially the same throughout the incubation period. Lipid analysis of pooled vesicle or HDL fractions, at various incubation times (0, 3, 10, 24 h for the forward and reverse exchange experiments), gave similar results within experimental error, indicating the absence of mass transfer. For example, a scaled-up preparation gave the following results

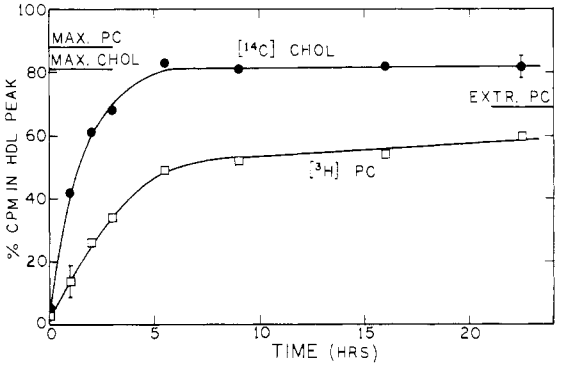

FIGURE 2: Radiolabeled lipid transfer from vesicles to HDL with time. The maximum theoretical transfer for cholesterol (●—●) is 82% and for PC (□—□) is 88%. The extrapolated, equilibrium PC transfer of 69% was obtained from double-reciprocal plots. The same experimental conditions were used as described in the legend to Figure 1.

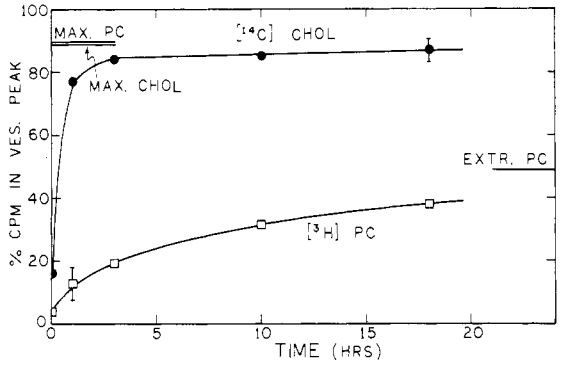

FIGURE 3: Radiolabeled lipid transfer from HDL to vesicles with time. The maximum theoretical transfer for cholesterol (●—●) is 89% and for PC (□—□) is 90%. The extrapolated, equilibrium PC transfer of 49% was obtained from double-reciprocal plots. The conditions for this experiment are described in the text.

for pooled top fractions (three fractions from the vesicle peak and three fractions from the HDL peak): after zero time incubation, 0.036 mM vesicle cholesterol, 0.068 mM vesicle PC, 0.150 mM HDL cholesterol, and 0.701 mM HDL PC; after 24 h incubation, 0.034 mM vesicle cholesterol, 0.067 mM vesicle PC, 0.154 mM HDL cholesterol, and 0.695 mM HDL PC. Vesicle preparations were tested for stability by passage through Sepharose CL-4B columns. Up to 2 weeks storage of vesicles at 5 °C had no effect on their elution position; incubation of vesicles at 37 °C for 10 h caused a 7% transformation to multilamellar vesicles.

The percent of radiolabel transfer from vesicles to HDL, with time, is shown in Figure 2, which also indicates that the experimental error in area determinations was of the order of ±3% for large areas and near ±5% for small areas. For a one-to-one exchange, the maximum expected [^{14}C]cholesterol transfer to HDL is 82%, in close agreement with the equilibrium value of 83%. For [^{3}H]PC the expected maximum transfer is 88%; the extrapolated equilibrium value of 69% probably indicates that only this proportion of vesicle PC can exchange with HDL.

The time dependence of percent radiolabel transfer from HDL to vesicles, or "reverse exchange", is shown in Figure 3. The maximum expected [^{14}C]cholesterol transfer to vesicles is 89%; the observed value is 87%. For [^{3}H]PC, the expected transfer is 90%, whereas the extrapolated, experimental value at equilibrium is only 49%. This information indicates that all of the cholesterol in vesicles and in HDL can be exchanged but that only 69% of the vesicle PC and 49% of the pree-

Table I: Temperature and Concentration Dependence of Cholesterol Exchange between Vesicles and HDL

[cholesterol] (mM)		incubation	% cpm appearing in HDL		R^b
vesicles	HDL	temp (°C)	peak in 1 h	$-\ln(1-F)^a$	(μM/h)
0.089	0.388	37	42	0.663	48.1
		25	19	0.203	14.7
		15	11	0.083	6.04
		5	8	0.040	1.39
0.045	0.388	37	56	0.922	18.4
0.027	0.388	37	59	0.934	11.7
0.089	0.194	37	43	0.912	27.8
0.089	0.121	37	35	0.845	21.7

[a] F, the fraction of radiolabel exchange, was corrected for exchange at time zero (5% cpm in HDL peak). [b] The rate of exchange, R, was calculated from $-\ln(1-F)$ and the corresponding cholesterol concentrations in vesicles and HDL.

Table II: Temperature and Concentration Dependence of Phosphatidylcholine Exchange between Vesicles and HDL

[PC] (mM)		incuba- tion temp	% cpm appearing in HDL peak	$-\ln$	R^b
vesicles	HDL	(°C)	in 2 h	$(1-F)^a$	(μM/h)
0.120[c]	0.841[d]	37	24	0.286	15.1
		25	18	0.197	10.4
		15	9	0.067	3.55
		5	6	0.031	1.65
0.060	0.841	37	25	0.268	7.62
0.036	0.841	37	25	0.268	4.64
0.120	0.420	37	16	0.188	8.82
0.120	0.263	37	12	0.144	5.96

[a] F, the fraction of radiolabel exchange, was corrected for exchange at time zero (3% cpm in HDL peak). [b] The rate of exchange, R, was calculated from $-\ln(1-F)$ and the corresponding PC concentrations in vesicles and HDL. [c] The exchangeable PC in vesicles was 69% of the total PC. [d] The exchangeable PC in pre-equilibrated HDL was 49% of the total PC in HDL, as determined by the "reverse" exchange experiments.

quilibrated HDL PC can be exchanged.

In the kinetic analysis, only the exchangeable PC concentrations were used, i.e., 69% of the vesicle PC concentration and 49% of the HDL PC concentration. The results shown in Figure 2 were analyzed by the radioisotope exchange method to give plots of $-\ln(1-F)$ vs. time. F is the fraction of radiolabel exchange corrected for exchange at time zero. The semilog plot for cholesterol was linear over 3 h; for PC, it was linear for 5 h. The overall rates of exchange (R) calculated from the slopes of the semilog plots and from the lipid concentrations in vesicles and HDL were 46.3 μM/h for cholesterol and 15.0 μM/h for PC; the corresponding half-lives for radioisotope exchange were 1 h 8 min and 6 h, respectively.

Temperature and concentration dependence experiments were performed at fixed incubation times of 1 and 2 h. The original data and calculated $-\ln(1-F)$ and R values are summarized in Tables I and II.

Arrhenius plots of $-\ln R$ vs. $1/T$ are shown in Figure 4. The activation energies for cholesterol and for PC exchange are 17.0 ± 2.8 and 12.5 ± 2.0 kcal/mol, respectively.

Concentration-dependence experiments were performed at constant HDL or constant vesicle concentrations, while the concentration of the other particle was varied. Data for 1-h incubations were used in calculating the exchange rates (R). The results are presented graphically in Figure 5. Within experimental error, the rate of exchange is a linear function of both lipid concentrations in HDL and in vesicles. Therefore, $R = kab$, and the calculated second-order rate constants are 1.44 and 0.164 mM^{-1} h^{-1} for cholesterol and PC exchange,

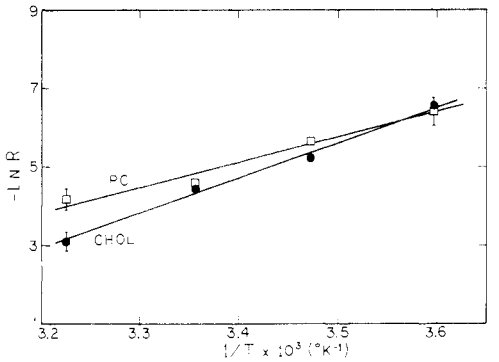

FIGURE 4: Arrhenius plots of the exchange rates (R) for cholesterol (●—●) and PC (□—□) between vesicles and HDL.

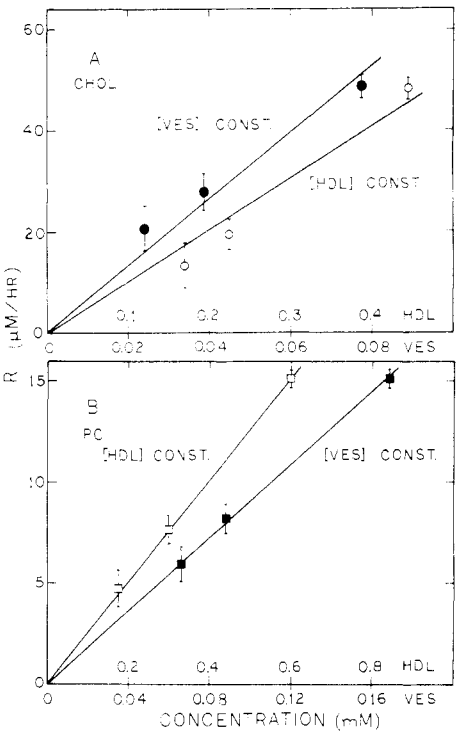

FIGURE 5: Concentration dependence of the exchange rates (R) for cholesterol (A) and for PC (B). In panel A, ●—● corresponds to an experiment where the vesicle cholesterol was held constant at 0.089 mM and HDL cholesterol was varied from 0.121 to 0.388 mM; ○—○ corresponds to constant HDL cholesterol (0.388 mM) and variable vesicle cholesterol (from 0.027 to 0.089 mM). In panel B, ■—■ corresponds to constant vesicle PC (0.120 mM) and variable HDL PC (0.263–0.841 mM); □—□ corresponds to constant HDL PC (0.841 mM) and variable vesicle PC (0.036–0.120). These experiments were conducted at 37 °C, and R's were calculated from $-\ln(1-F)$ and lipid concentrations.

Table III: Results of Cholesterol and Phosphatidylcholine Exchange between Vesicles and HDL

	exchange of	
	cholesterol	PC
extent of exchange		
Ves to HDL	complete[a]	69%[b]
HDL to Ves	complete[a]	49%[b]
activation energy (E_a)		
Ves to HDL	17.0 ± 2.8 kcal/mol	12.5 ± 2.0 kcal/mol
second-order constant (k)		
Ves to HDL	1.44 ± 0.56 mM^{-1} h^{-1}	0.164 ± 0.045 mM^{-1} h^{-1}
HDL to Ves	1.75 mM^{-1} h^{-1}	0.132 mM^{-1} h^{-1}

[a] The experimental and the predicted maximum % of radiolabel in the acceptor particles were identical (see Figures 2 and 3). [b] The maximum experimental percent of radiolabel appearing in the acceptor particles was obtained by extrapolation to infinite time (double-reciprocal plots) of the data shown in Figures 2 and 3.

respectively. Table III summarizes the results of the exchange experiments and includes the second-order rate constants for the "reverse" exchange experiment. The rate constants are very similar for both lipids in the "forward" and "reverse" directions, confirming previous evidence that we are dealing with an exchange situation.

Discussion

Numerous incubation experiments of native bovine HDL preparations (freed of lecithin–cholesterol–acyltransferase activity) with radiolabeled egg PC:cholesterol vesicles (2:1, mol/mol) indicated to us that some mass transfer can occur from vesicles into particles in the size range of HDL. There are two possible routes for this transfer of lipid: one is the incorporation of vesicle lipid into HDL, and the other is the formation of new complexes between the vesicle lipids and apolipoprotein A-I which may come off the HDL. Examples of analogous situations have been described in the literature. We reported that up to 20% by weight of cholesterol can be incorporated into human and bovine HDL without major effects on the lipoprotein structure (Jonas et al., 1978). Tall et al. (1978) and Tall & Small (1977) showed that human HDL incubated with dimyristoylphosphatidylcholine liposomes led to the formation of disk-like complexes containing liposome lipids and apolipoprotein A-I plus fused particles from the remaining HDL components.

Since we used bovine HDL preequilibrated with vesicles in all of the kinetic experiments described in this work, we had to characterize this lipoprotein preparation before a meaningful interpretation of the exchange results could be attempted. Electron micrographs of preequilibrated HDL indicated a homogeneous preparation of spherical particles, 111 ± 5 Å in diameter, with no evidence of disk-like species. Since the original HDL had diameters of 105 ± 5 Å, the volume increase in forming the preequilibrated lipoprotein was 18%. The increase in mass of the preequilibrated preparations of 19% was consistent with the increase in volume observed by electron microscopy. In gel-filtration experiments, the preequilibrated HDL eluted ahead of the original lipoprotein, in a relatively narrow and symmetrical peak, which had as uniform a composition across as did the original lipoprotein. These results, to be reported elsewhere in detail (A. Jonas, 1979, unpublished experiments), indicate that, under the conditions of our experiments, the preequilibrated HDL preparation is a relatively homogeneous lipoprotein sample enriched with vesicle lipids.

Since we used the same vesicle preparations in the preequilibration of HDL and in the kinetic experiments, we assumed that mass equilibrium conditions prevailed. Several lines of evidence confirmed this assumption. Chemical analysis and Ans fluorescence indicated that, at zero time, as well as after 24 h of incubation, the vesicle concentration and composition remained the same. The "reverse" exchange experiments, giving second-order rate constants similar to those for the "forward" exchange, further support our contention that mass equilibrium conditions are met in our kinetic experiments.

Figures 2 and 3 report the results of percent radiolabel transfer from vesicles into HDL ("forward") and from HDL into vesicles ("reverse"), respectively. Such data have been used traditionally to give an indication of the extent of exchange and of the existence of nonexchangeable pools. Both the "forward" and the "reverse" exchange experiments indicate that radiolabeled cholesterol is completely exchanged within a few hours. For the vesicles, this means that the cholesterol distributed on both halves of the bilayer can exchange during a period of a few hours. This result is in agreement with the observations made by Bloj & Zilversmit (1977) using radiolabeled vesicles and erythrocyte membranes as acceptors. Other workers (Poznansky & Lange, 1976) report only partial exchange of cholesterol from vesicles into erythrocyte membranes. The extent of cholesterol exchange from HDL donors is also complete; the discrepancy between our results and those of Ashworth & Green (1964) may be due to the different properties of human and bovine HDL and the different methods used for the introduction of radiolabeled lipids into the lipoproteins.

The "forward" PC exchange, regardless of the excess of HDL used, approached 69% of all [^{3}H]PC counts in HDL. The most plausible explanation for this observation is that only the outer half of the vesicle bilayer participates in the exchange. Our vesicle preparation was taken from the middle to the trailing edge of the Sepharose CL-4B peak, which corresponds to Huang (1969) and Newman & Huang (1975) vesicles with an outside:inside distribution of PC from 68:32 to 74:26. The "flip-flop" rates of PC in vesicles were determined first by Kornberg & McConnell (1971). Their half-lives of several hours were subsequently extended to days and weeks by other workers using different methods (Johnson et al., 1975; Rothman & Davidowicz, 1975). In our longest experiments (24 h), we see no indications of more vesicle PC becoming available for exchange. PC exchange from HDL into vesicles is only 49% complete at equilibrium. Since the content of PC in preequilibrated HDL is almost twice that in the origina! HDL, it may be that the added phospholipid behaves in a manner different from the intrinsic phospholipid; yet, the radiolabeled PC appears to mix with both pools of lipid. At this point, we have no explanation for this observation.

The temperature dependencies of the exchange rates for cholesterol and PC are similar—a two- to threefold change in rate for a 10 °C change in temperature (see Tables I and II and Figure 4). The activation energy for cholesterol exchange (17.0 ± 2.8 kcal/mol) is somewhat higher than the activation energy for PC exchange (12.5 ± 2.0 kcal/mol); the difference may reflect the temperature dependence of cholesterol movement through the viscous lipid domains of the interior of vesicles and lipoproteins. Activation energies expected for lipid exchange through monomeric lipids in solution would possibly reflect the solubility properties of each

lipid in water. Since the solubility of long-chain phosphatidylcholines in water is less than 10^{-10} M (Smith & Tanford, 1972) and for cholesterol is about 10^{-7} to 10^{-8} (Saad & Higuchi, 1965), the activation energy for PC exchange would be expected to be higher than for cholesterol exchange. Our results indicate the opposite relationship.

Furthermore, the exchange rates for cholesterol and PC are linearly dependent on the concentration of both lipids in vesicles and in HDL (Figure 5), indicating that the exchange is a "bimolecular" second-order process. This process probably involves collisions of HDL and vesicles that result in the exchange of cholesterol and PC between both particles. If the exchange of lipids were to occur through solution, the rate of exchange would be independent of the concentration of donor and acceptor particles and would only depend on the "critical micellar concentration" of the lipid. The second-order rate constants determined in our experiments (Table III) indicate that the exchange of cholesterol is about ninefold faster than the exchange of PC, in reasonable agreement with the observed relative rates of cholesterol and PC exchange (13/1) between vesicles and erythrocyte membranes (Bloj & Zilversmit, 1977).

The implications of such an exchange process to the exchange of lipids between membranes and HDL in vivo are very important. This means that, for exchange to occur, the membrane lipids and the lipoprotein would have to come into contact; however, contact may not be possible at all sites on the cell surface, in particular the ones where protein concentration is high.

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