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Kinetics and mechanism of phosphatidylcholine and cholesterol exchange between chylomicrons and high-density lipoproteins

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The exchange of phosphatidylcholine and unesterified cholesterol between rat mesenteric lymph chylomicrons and human high-density lipoproteins was studied *in vitro* by incubation of radiolabelled chylomicrons (with [*N-methyl*-^{14}C]phosphatidylcholine and [7(n)-^{3}H]cholesterol) with unlabelled high-density lipoproteins. The kinetic analysis was based on the extent of radioisotope exchange, which was determined by the proportion of label appearing in the high-density lipoprotein elution peak after rapid fractionation on analytical agarose columns. Under our experimental conditions, no net transfer of either phosphatidylcholine or cholesterol is observed. The kinetics of exchange of both phosphatidylcholine and cholesterol are biphasic. Over the first 30 min a maximum of 25% of the phosphatidylcholine and 33% of the cholesterol in chylomicrons exchanges rapidly into the high-density-lipoprotein fraction. Thereafter both lipids continue to exchange for up to 3 h at a much lower rate. For the rapid exchange process the calculated exchange rates for phosphatidylcholine and cholesterol are proportional to the concentrations of both chylomicrons and high-density lipoproteins. The second-order rate constants are $(10.5 \pm 0.5) \times 10^{-5} \mu M^{-1} \cdot min^{-1}$ for phosphatidylcholine and $(32.1 \pm 4.5) \times 10^{-5} \mu M^{-1} \cdot min^{-1}$ for cholesterol. The kinetics of the exchange process thus suggest that a significant proportion of both phosphatidylcholine and unesterified cholesterol is rapidly exchangeable between these lipoproteins, and that this exchange is mediated by a 'bimolecular', or collisional, mechanism.

The ability of lipid components to exchange spontaneously between various lipid–protein structures such as membranes, cells and lipoproteins is well known (for review, see Bell, 1978). However, the mechanisms by which many of these exchange processes occur have not been completely defined. The serum lipoproteins, which mediate lipid transport in blood, provide a convenient system for studying lipid exchange in more detail. It has in fact been demonstrated that all classes of lipoproteins can readily exchange 'structural' lipids such as phospholipids and cholesterol with cell membranes (for review, see Jackson *et al.*, 1976). In addition, lipid exchange is known to occur between individual lipoprotein classes (Minari & Zilversmit, 1963; Redgrave & Small, 1979; Tall *et al.*, 1979). For those lipids that have been studied, exchange appears to constitute the major mode of lipid movement between lipoproteins *in vitro* (Redgrave & Small, 1979; Tall *et al.*, 1979). However, the factors that govern inter-lipoprotein exchange have only recently been explored. There is some evidence from model studies utilizing phospholipid vesicles and HDL that suggests that the exchange of both PtdCho and cholesterol occurs by a collisional process (Jonas & Maine, 1979). However, this mechanism may not be generally applicable to all inter-lipoprotein exchange processes, since the exchange of cholesterol between HDL and low-density lipoproteins by an aqueous transfer mechanism has also been reported (Lund-Katz *et al.*, 1981). Presumably, exchange characteristics will in general be affected by a number of factors, such as the size, source, chemical composition and physical proper-

Abbreviations used: HDL, high-density lipoproteins; PtdCho, phosphatidylcholine.

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ties of the particles as well as the incubation conditions and methods of separation employed.

Preliminary studies in our laboratory revealed a rapid initial exchange of both PtdCho and cholesterol between chylomicrons and HDL *in vitro* (Lippiello, 1980). In the present studies we have probed the mechanism of this exchange process by monitoring the kinetics of isotope exchange after incubation of radiolabelled chylomicrons with unlabelled HDL. Because of the considerable difference in the size of these particles, it is possible to achieve rapid and complete separation on analytical agarose columns. Since this minimizes any compositional changes or inaccuracies that may result from more lengthy isolation procedures, kinetic analysis of the initial interactions between these particles has been facilitated. On the basis of this method, the present studies have revealed a previously unobserved rapid initial exchange of both PtdCho and cholesterol between chylomicrons and HDL. The kinetics of this rapid exchange process are consistent with a collisional mechanism.

Experimental

Materials

Egg PtdCho (type V-E) was purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. [^{14}C]Methyl iodide was obtained from Amersham/Searle Corp., Arlington Heights, IL, U.S.A. [*N-methyl*-^{14}C]PtdCho was synthesized by the method of Stoffel (1975). [7(n)-^{3}H]Cholesterol was purchased from New England Nuclear, Boston, MA, U.S.A., and 5,5-dithiobis-(2-nitrobenzoic acid) was also obtained from Sigma. All other chemicals and solvents utilized were of reagent grade.

Analytical methods

Total lipoprotein lipids were extracted by the method of Bligh & Dyer (1959). The major lipid classes were separated by t.l.c. on silica gel H, utilizing a hexane/diethyl ether/formic acid (20:10:1, by vol.) solvent system. PtdCho was separated from total phospholipid utilizing chloroform/methanol/water (65:25:4, by vol.) as solvent system. Triacylglycerol was quantified by the method of Sardesai & Manning (1968). Phospholipid was determined by the method of Chalvardjian & Rudnicki (1970). Total, unesterified and esterified cholesterol were quantified by the procedure of Rudel *et al.* (1974). Protein was determined by the method of Lowry *et al.* (1951), utilizing bovine serum albumin as standard.

Isolation and characterization of lipoproteins

Male Sprague–Dawley rats were maintained *ad libitum* on standard rat chow. Chylomicrons were prepared as described by Imaizumi *et al.* (1978). Chylomicrons were labelled *in vivo* by duodenal infusion of 250μCi each of [*N-methyl*-^{14}C]PtdCho and [7(n)-^{3}H]cholesterol, sonicated together with an emulsion of soyabean oil and phospholipid. Lymph was collected over a period of 12–15h in a vessel containing 1ml of a 2% 5,5-dithiobis-(2-nitrobenzoic acid)/5% EDTA solution per 50ml of lymph, and maintained at 0–4°C. Chylomicrons were isolated by centrifugal flotation, as described by Imaizumi *et al.* (1978). Chylomicrons were further purified by gel chromatography on columns (2.5cm × 90cm) of 4% agarose. Purity was assessed on the basis of lipid composition and apoprotein electrophoretic patterns obtained on 10% polyacrylamide slab gels (Imaizumi *et al.*, 1978). Chylomicrons utilized in the present studies contained, by weight, 93% triacylglycerol, 5% phospholipid, 1% total cholesterol (50% unesterified) and 1% protein. More than 95% of the recovered ^{14}C label was in the PtdCho fraction. The ^{3}H label was equally distributed between unesterified cholesterol and cholesteryl ester. The apoprotein composition agreed well with that previously reported for chylomicrons isolated by this procedure (Imaizumi *et al.*, 1978). HDL were isolated from the plasma of a normal, fasted male subject by density-gradient centrifugation (density 1.225) followed by passage down 6% agarose columns as previously described by Rudel *et al.* (1974). PtdCho:cholesterol acyltransferase activity was inhibited with 5,5-dithiobis-(2-nitrobenzoic acid) by the method of Stokke & Norum (1971). The composition of the HDL utilized in the present studies was, by weight, 48% protein, 22% phospholipid, 5% triacylglycerol, 20% cholesteryl ester and 5% unesterified cholesterol. The content and relative proportions of apoproteins, determined by densitometric scans of 10% polyacrylamide slab gels, compared well with profiles reported for normal human HDL (Rubenstein, 1979).

Incubation procedures

Purified chylomicrons and HDL were incubated in various concentrations in a total volume of 80μl of 0.15M-NaCl, at 37°C. After appropriate incubation periods, samples were immediately applied to 4% agarose mini-columns (Bio-Gel A-15m), of dimensions 0.5cm × 12cm, and eluted with 0.15M-NaCl. Columns were operated at 24°C. Typically, chylomicrons and HDL were visibly separated as distinct bands within 2min and total elution times ranged from 12 to 15min. Recovery of total lipid from columns was 90% or better for both chylomicrons and HDL. Five-drop fractions were collected in counting vials containing a toluene/Triton X-100-based scintillation fluid, and subjected to double-label counting. For kinetic analyses, elution profiles were determined for both

[^{14}C]PtdCho and [^{3}H]cholesterol, and the radiolabel recovered in the HDL peak was expressed as a percentage of the total counts eluted from the column. In the case of [^{3}H]cholesterol, only radiolabelled unesterified cholesterol was found to elute with the HDL peak, even after 3 h incubations. The total unesterified [^{3}H]cholesterol eluted from the column was determined as the sum of the chylomicron and HDL-associated radiolabel, minus the known proportion of labelled cholesteryl ester (i.e., 50% of the total).

Kinetic analysis

The kinetic analysis of phospholipid and cholesterol exchange was based on isotope exchange theory, as described by Frost & Pearson (1961) and applied to lipid exchange by Jonas & Maine (1979). To summarize, the process of equilibration of an isotope between two lipid pools, one labelled and one unlabelled, can be described by the following equation:

$$-\ln(1-F) = k't = (R/ab)(a+b)t \qquad (1)$$

F is the fraction of the radiolabel exchange that has occurred (i.e., the percentage of chylomicron label transferred to the HDL fraction at time t divided by the percentage transferred at equilibrium, $t = \infty$). The parameters a and b, determined experimentally, are the concentrations of exchangeable lipid in the labelled (chylomicron) and unlabelled (HDL) pools respectively. R is the constant rate of exchange of total lipid, labelled plus unlabelled, and has the units concentration · time^{-1}. Its value is a constant under a given set of experimental conditions, and can be calculated from eqn. (1). R is itself a function of both a and b, of the form $R = ka^m b^n$. This relationship can be determined empirically, by holding either a or b constant. Although the overall equilibration process is described by a first-order rate expression (eqn. 1), the mechanism of exchange will be related to the dependence of R on a and b. For example, if $R = kab$, where k has the dimensions of a second-order rate constant, then the mechanism of exchange is inferred to be 'bimolecular', or collisional.

Finally, since some exchange occurs during the time required for separation of chylomicrons and HDL on the column, the value of F becomes $(\bar{F}-\bar{F}_0)/(1-\bar{F}_0)$, where $\bar{F}$ is the fractional exchange measured at (incubation) time t and F_0 is the fractional exchange at time zero (equal to the separation time). In the present experiments $\bar{F}_0$ varied with concentration and was determined separately for each set of incubation conditions. The only assumption required in this analysis, that the system be at mass equilibrium, is valid since no net lipid transfer was observed under our experimental conditions (see the Results section).

Results

The use of agarose gel chromatography for preparative isolation of the major lipoprotein classes is well established, and has been discussed in detail previously (Rudel *et al.*, 1974). For the present studies, we modified this technique by utilizing analytical agarose columns (0.5 cm × 12 cm) to achieve rapid and essentially complete separation of chylomicrons from HDL after incubations *in vitro*. Total elution times were of the order of 15 min. Variations in elution profiles obtained from different columns were negligible and the values for radiolabel transfer, determined from eluted material, were reproducible to within ±1%. Overlap of the chylomicron elution peak with the HDL peak varied, depending on relative concentrations. This value was determined at all concentrations by separate elution of the individual lipoproteins, and was always less than 5% of the total chylomicron radiolabel.

To determine whether the transfer of radiolabelled lipids into the HDL fraction resulted from net transfer of lipid mass or from lipid exchange, the total PtdCho and unesterified cholesterol contents of chylomicrons and HDL were quantified after separation from several incubation mixtures. Within experimental error there was no evidence for mass transfer of either PtdCho or cholesterol. For example, the following results were obtained for pooled chylomicrons and HDL after various incubation times: zero time, 1.40 mM chylomicron PtdCho, 0.27 mM chylomicron cholesterol, 4.50 mM HDL PtdCho, 2.25 mM HDL cholesterol; after 30 min incubation time, 1.45 mM chylomicron PtdCho, 0.28 mM chylomicron cholesterol, 4.60 mM HDL PtdCho, 2.30 mM HDL cholesterol; after 180 min incubation time, 1.39 mM chylomicron PtdCho, 0.26 mM chylomicron cholesterol, 4.45 mM HDL PtdCho, 2.19 mM HDL cholesterol. The standard error for all mass determinations was within ±5%.

The time courses for transfer of radiolabelled PtdCho and cholesterol into the HDL fraction for one set of incubation conditions are presented in Figs. 1 and 2 respectively. The zero time values of 4% for PtdCho and 16% for cholesterol represent the transfer of labelled lipid that occurred during separation, following simultaneous application of chylomicrons and HDL to the agarose columns. The amount of exchange at zero time was less pronounced, particularly for cholesterol, at lower incubation concentrations. The data indicate that there was a rapid initial exchange of radiolabelled lipid into HDL followed by a slower equilibration, which continued for up to several hours. This biphasic equilibration of both PtdCho and cholesterol was observed at all concentrations tested.

The kinetics of exchange for both PtdCho and cholesterol can be described as bi-exponential. The theoretical curves of Figs. 1 and 2 represent the best

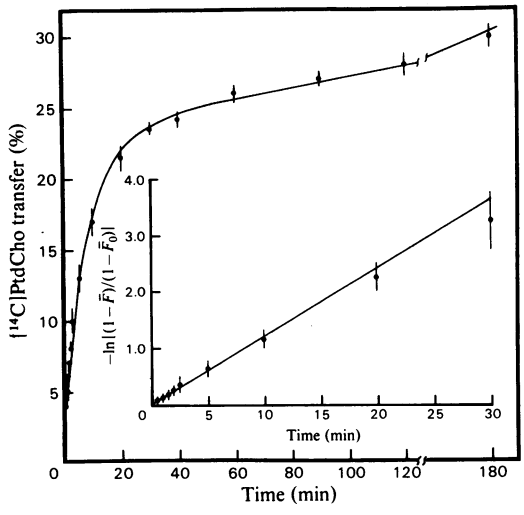

Fig. 1. *Kinetics of [14C]PtdCho exchange between chylomicrons and HDL*
Chylomicrons (0.39 mM total PtdCho) and HDL (5.0 mM total PtdCho) were incubated for appropriate time intervals, applied to analytical agarose columns, and the fraction of the total [14C]PtdCho that eluted with HDL fraction was determined. Incubation conditions and separation procedure are described in the Experimental section. Each point represents the mean of at least three experiments.

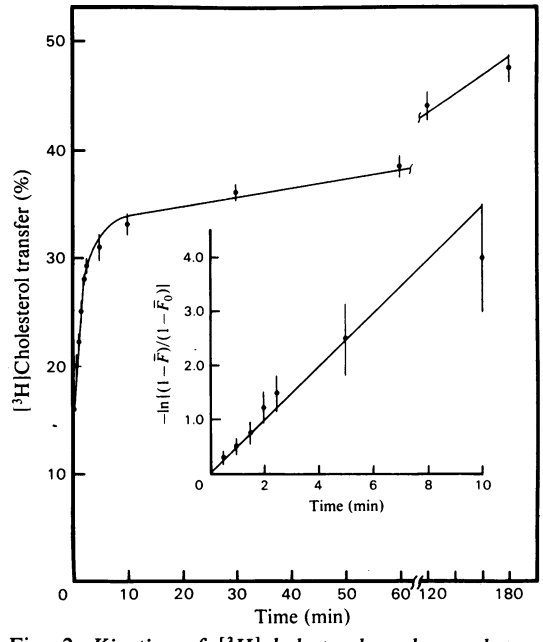

Fig. 2. *Kinetics of [3H]cholesterol exchange between chylomicrons and HDL*
Chylomicrons (0.075 mM unesterified cholesterol) and HDL (5.0 mM unesterified cholesterol) were incubated for appropriate time intervals, applied to analytical agarose columns and the fraction of the total eluted unesterified [3H]cholesterol associated with the HDL fraction was determined. Incubation and separation procedures are described in the Experimental section. Each point represents the mean of at least three experiments.

fit of the combined fast- and slow-phase exchange components to the data, determined by the iterative curve-fitting method described by Goodman *et al.* (1973). Additional exponentials did not significantly improve the fit. The kinetic analysis to be presented applies only to the fast-phase exchange contribution, with the assumption that it represents a discrete and independent exchange process.

Since there was no evidence for mass transfer of either PtdCho or cholesterol, the kinetics of the fast-phase exchange process can be described by rearrangement of the isotope exchange expression (eqn. 1) as follows:

$$\%\ \text{Transfer}_t = [(1-\bar{F}_0)(1-e^{-k't}) + F_0] \cdot \%\ \text{transfer}_{t=\infty}$$

where $k' = (R/ab)(a+b)$.

For the data of Figs. 1 and 2 the values of k', calculated from the fitted exponential curves for the fast-phase exchange components, were $0.12\,s^{-1}$ for PtdCho and $0.54\,s^{-1}$ for cholesterol. Similarly, the extrapolated maximum values for the fast-phase components were determined to be 23% for PtdCho and 33% for cholesterol. In general, these parameters, as well as the half time for radiolabel equilibration, varied with chylomicron and HDL concentrations. However, regardless of the excess of HDL in the incubation mixtures, the maximum amount of radiolabelled lipid transferred to HDL by the rapid exchange process did not exceed 25% for PtdCho and 33% for cholesterol.

The kinetic analysis of the fast-phase exchange process was based on the radioisotope exchange method, utilizing eqn. (1). The results for PtdCho and cholesterol are shown in Tables 1 and 2 respectively. The percentage of radiolabel transferred to HDL was determined for several concentrations of chylomicrons and HDL at a fixed time of 5 min. The overall lipid exchange rates, R, were determined at each concentration from $\ln(1-F)$ and the exchangeable lipid pool sizes, according to eqn. (1). Equilibrium values for the fast-phase exchange process (Tables 1 and 2) were taken to be equal to the extrapolated maxima of the fitted kinetic curves, after subtraction of the slow-phase contribution. Kinetic curves were based on at least 10 time points at each concentration. The concentrations listed in Tables 1 and 2 represent exchangeable lipid only. For chylomicrons, the fractions of exchange-

Table 1. *Concentration-dependence of phosphatidylcholine exchange between chylomicrons and HDL*
Chylomicrons and HDL were incubated in various concentrations for 5 min in a total volume of 80 μl of 0.15 M-NaCl, at 37°C. The percentage of total radiolabel in the HDL elution peak was determined after rapid separation on analytical agarose columns. All values are means ± S.D. for four incubations.

[PtdCho] (mM)*		[^{14}C]PtdCho transferred to HDL (%)			
Chylomicrons	HDL	$t = 5$ min	Fast-phase equilibrium†	F‡	R (μM/min)§
0.390	0.075	1.8 ± 0.5	4.0 ± 0.5	0.20	2.81 ± 0.51
0.390	0.150	3.1 ± 0.6	6.9 ± 0.6	0.25	6.23 ± 1.20
0.390	0.300	6.0 ± 0.8	10.9 ± 0.4	0.31	12.58 ± 0.78
0.390	0.600	8.5 ± 0.7	15.2 ± 0.9	0.43	26.57 ± 1.80
0.390	1.200	12.3 ± 0.9	18.9 ± 0.7	0.55	47.00 ± 3.20
0.097	1.200	13.1 ± 0.5	23.1 ± 1.1	0.48	11.74 ± 1.80
0.195	1.200	12.5 ± 0.7	21.5 ± 0.9	0.50	23.20 ± 2.50
0.097	0.075	2.5 ± 0.5	11.2 ± 0.8	0.10	0.89 ± 0.21
0.195	0.075	2.0 ± 0.3	7.3 ± 0.6	0.16	1.89 ± 0.43

* Values represent exchangeable PtdCho concentrations, taken as 25% of the total PtdCho for chylomicrons; HDL concentrations were calculated from the relative proportion of exchangeable [^{14}C]PtdCho in the HDL fraction at fast-phase equilibrium and the exchangeable chylomicron concentrations (see the Results section).
† Values were determined by extrapolation of the fast-phase exponential component of kinetic curves (see the Results section).
‡ F values have been corrected for time-zero exchange, which ranged from 1 to 4%.
§ R, the overall lipid exchange rate, was determined from $\ln(1-F)$ and the exchangeable PtdCho concentrations.

Table 2. *Concentration-dependence of cholesterol exchange between chylomicrons and HDL*
Chylomicrons and HDL were incubated in a total volume of 80 μl of 0.15 M-NaCl, at 37°C, and rapidly separated by agarose gel chromatography. Fractions corresponding to chylomicron and HDL elution peaks were pooled and analysed for total (unesterified) cholesterol content and radiolabel distribution. Values represent means ± S.D. for three incubations, and are corrected for peak overlap.

[Cholesterol] (mM)*		[^{3}H]Cholesterol transferred to HDL (%)			
Chylomicrons	HDL	$t = 5$ min	Fast phase equilibrium†	F‡	R (μM/min)§
0.100	0.050	5.2 ± 0.8	11.0 ± 0.9	0.21	1.90 ± 0.38
0.100	0.100	9.3 ± 0.6	16.3 ± 0.8	0.31	3.71 ± 0.75
0.100	0.200	13.8 ± 0.7	23.1 ± 0.8	0.43	7.49 ± 1.62
0.100	0.400	19.0 ± 0.7	27.0 ± 0.6	0.52	11.74 ± 0.76
0.100	0.800	24.5 ± 1.2	30.0 ± 1.2	0.71	22.00 ± 2.21
0.025	0.800	26.2 ± 0.9	32.2 ± 0.7	0.73	6.35 ± 0.84
0.050	0.800	25.4 ± 0.8	31.1 ± 0.5	0.70	11.33 ± 1.50
0.025	0.200	15.1 ± 1.1	29.5 ± 1.0	0.32	1.71 ± 0.32
0.050	0.200	13.9 ± 0.8	26.8 ± 0.9	0.32	3.09 ± 0.98

* Exchangeable unesterified cholesterol concentrations were taken as 33% of the total for chylomicrons; HDL concentrations were calculated from the relative proportion of exchangeable [^{3}H]cholesterol in the HDL fraction at fast-phase equilibrium and the exchangeable chylomicron concentrations (see the Results section).
† See the equivalent footnote symbol to Table 1.
‡ F values have been corrected for zero-time exchange, which ranged from 3 to 12% in this concentration range.
§ R, the overall lipid exchange rate, was determined from $\ln(1-F)$ and the exchangeable cholesterol concentrations.

able lipid were determined from the maximum radiolabel transfer observed in the presence of excess HDL (Jonas & Maine, 1979). For PtdCho, this was 25% of the total and for cholesterol, 33% of the total. The amounts of exchangeable PtdCho and cholesterol in the HDL fraction were calculated, knowing the chylomicron concentrations and the relative proportion of exchangeable label associated with HDL at equilibrium.

In order to determine the mechanism of the fast-phase exchange process, values of R were plotted as a function of lipid concentrations, with either the chylomicron or HDL concentration held constant (Figs. 3 and 4). It can be seen that R is proportional to the exchangeable chylomicron and HDL concentrations for PtdCho and cholesterol. Therefore $R = kab$, where k has the units of a second-order rate constant, and the mechanism of

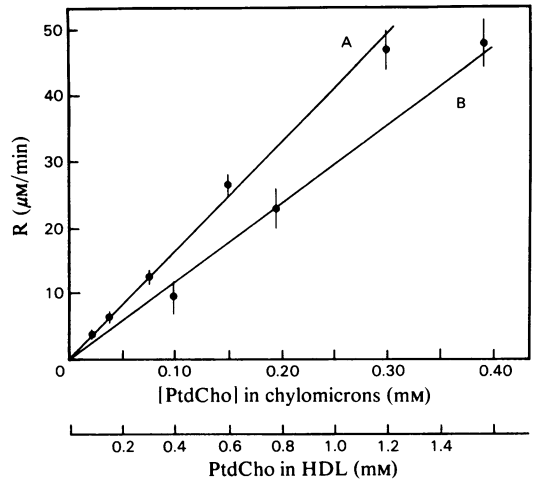

Fig. 3. *Concentration-dependence of phosphatidylcholine exchange rates (R)*

(A) Exchangeable chylomicron PtdCho concentration maintained constant at 0.39 mM; (B) exchangeable HDL PtdCho concentration maintained constant at 1.20 mM.

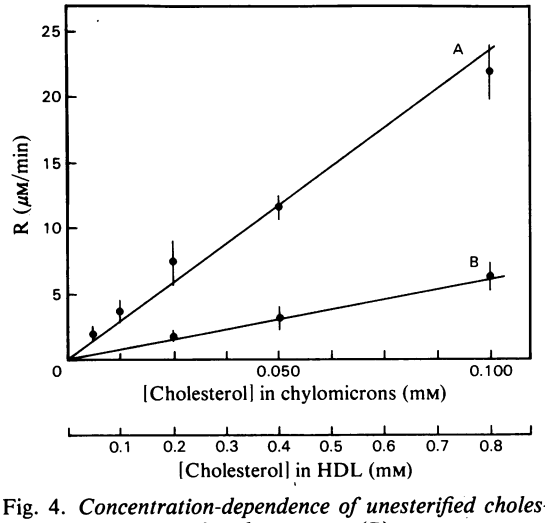

Fig. 4. *Concentration-dependence of unesterified cholesterol exchange rates (R)*

(A) Exchangeable chylomicron cholesterol concentration maintained constant at 0.1 mM; (B) exchangeable HDL cholesterol concentration maintained constant at 0.2 mM.

exchange can be described as 'bimolecular' or collisional. The calculated second-order constants for the fast-phase exchange component, from the data of Tables 1 and 2, are $(10.5 \pm 0.5) \times 10^{-5} \mu M^{-1} \cdot min^{-1}$ for PtdCho and $(32.1 \pm 4.5) \times 10^{-5} \mu M^{-1} \cdot min^{-1}$ for unesterified cholesterol.

Discussion

The results of previous studies suggest that, in general, 'structural' lipids tend to exchange more rapidly between individual lipoproteins than they do between lipoproteins and cell membranes (Bell, 1978; Minari & Zilversmit, 1963; Illingworth & Portman, 1972). Preliminary experiments in our laboratory revealed that limited pools of PtdCho and cholesterol could exchange quite rapidly between chylomicrons and HDL, with concentration-dependent half times of the order of minutes. The purpose of the present studies was to probe in more detail the kinetics and mechanism(s) of these rapid exchange processes.

Initially, since the movement of lipids between lipid–protein structures can involve net transfer as well as exchange (Bell, 1978; Minari & Zilversmit, 1963) it was necessary to establish that conditions of mass equilibrium were maintained during the time course of the experiments. Mass analysis at several different times and relative concentrations of chylomicrons and HDL confirmed that there was no mass transfer of either PtdCho or cholesterol. This agrees with the results of studies *in vitro* with chylomicrons and serum, which indicated that the equilibration of 'structural' lipids between lipoproteins occurs primarily by exchange (Redgrave & Small, 1979; Tall *et al.*, 1979). In addition, there was no detectable change in the total protein content or relative apoprotein composition of the particles, as judged by polyacrylamide-gel electrophoresis (results not shown). Imaizumi *et al.* (1978) have observed net transfer of apoproteins C and E to chylomicrons in the presence of rat HDL. The present results probably reflect the relatively low content of these peptides in human HDL (Rubenstein, 1979) compared with rat HDL (Chapman, 1980).

At the highest concentrations of HDL used in these studies (5 mM-PtdCho) the total radiolabel transfer (i.e., fast plus slow) in 3 h was 33% for PtdCho and 50% for cholesterol. Both lipids continued to equilibrate slowly beyond this point, but there was some difficulty in maintaining the integrity of the particles over longer periods of time. The rapid initial transfer of radiolabelled lipid was essentially complete in 30 min, and approached limiting values of 25% for PtdCho and 33% for cholesterol, regardless of the excess of HDL used. In previous exchange studies utilizing radiolabelled vesicles and HDL it was suggested that the maximum percentage of transfer observed was indicative of the amount of exchangeable lipid in the donor particle (Jonas & Maine, 1979). On the basis of this interpretation, the maximum values that we observed for fast-phase exchange represent the

amounts of PtdCho and cholesterol in chylomicrons that are present in rapidly exchangeable pools.

The exchangeable pool sizes in HDL were not directly determined by the present methods. However, assuming that the rapidly exchangeable lipid partitions between the particles in proportion to their relative concentrations, the equilibrium values of Tables 1 and 2 can be used to estimate the HDL pool sizes. The values obtained are 24% for PtdCho and 32% for cholesterol. This method appears to be generally valid. For instance, the exchangeable HDL pool sizes experimentally determined in the 'reverse' exchange studies of Jonas & Maine (1979) are consistent with what would be predicted on the basis of 'forward' (i.e., vesicle to HDL) exchange alone. In general, the sizes of exchangeable pools appear to vary for different exchanging species. For example, 62% of HDL cholesterol is exchangeable with erythrocytes (Ashworth & Green, 1964), but 100% is exchangeable with lipid vesicles (Jonas & Maine, 1979). In addition to the physicochemical properties of the acceptor particles, the source and/or preparative procedures involved may also have some bearing on the observed variations.

At all concentrations of chylomicrons and HDL used, the kinetics of PtdCho and cholesterol exchange were biphasic. There are several possible explanations for this phenomenon. For example, it has been suggested by Gottlieb (1980) that the two rates of cholesterol exchange that he observed between erythrocytes and HDL resulted from a slow diffusion of lipid molecules within the membrane to specific 'sites' that facilitated exchange. Relevant to this Illingworth & Portman (1972) have in fact suggested the involvement of collisional 'protein complexes' in mediating rapid lipid exchange between low-density lipoproteins and HDL. An analogous situation may provide a plausible explanation for the present results, with specific apoproteins acting as rapid-exchange 'sites' for an associated pool of structural lipids. Conversely, it is possible that the slow-phase exchange of the chylomicron lipid components reflects the rate-limiting dissociation of lipid molecules, which are tightly associated with protein, into a more readily exchangeable bulk lipid pool. In the case of cholesterol there is the additional possibility that there is a rate-limiting partitioning between the lipoprotein core and the surface monolayer of phospholipid and protein. The existence of two such pools of cholesterol has in fact been previously postulated (Smith *et al.*, 1978).

The linear dependence of exchange rates on the concentration of both lipids in chylomicrons and HDL suggests that the rapid initial exchange of both PtdCho and cholesterol requires a collisional interaction between the lipoprotein particles. This is in agreement with the data of others, which suggest that collisional mechanisms are operative in a number of related lipid exchange systems (Bell, 1978; Jonas & Maine, 1979; Gottlieb, 1980). In general, the inference of a collisional interaction in this and other systems is based on the assumption that second-order kinetics (i.e., exchange-rate dependence on the concentrations of both species) are indicative of diffusion-limited, collision-frequency-dependent, particle interactions. In some heterologous exchange systems (e.g., serum/red cells) it had been suggested that aqueous transfer can also exhibit second-order kinetics (Bojesen, 1982). However, such a model requires the assumptions that the dissolution rate of lipids from the donor particle surface be significantly different from that of the acceptor and that these rates be low relative to the diffusional equilibration of the lipids in the bulk water phase. These suppositions are not compatible with our system. First, the exchange rates that we have observed are several orders of magnitude faster than those reported by Bojesen (1982). This would necessitate dissolution rates which would be incompatible with the known solubility properties of cholesterol. Secondly, the data from the red-cell system indicate that serum lipoproteins do not have significantly different lipid dissolution rates. This predicts, according to the aqueous transfer model, no dependence of exchange rate on the acceptor species concentration (see Bojesen, 1982). Our data clearly indicate that this is not the case (see Tables 1 and 2).

Finally, the calculated second-order rate constants indicate that, for the fast-phase exchange process, cholesterol exchange is approximately 3-fold faster than PtdCho. This is less than the relative exchange rates reported by Jonas & Maine (1979) for vesicle/HDL exchange (9-fold) or by Bloj & Zilversmit (1977) for erythrocyte-vesicle exchange. However, the absolute rates of exchange of both lipids are severalfold higher than those observed in other systems.

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