A protein(s) which catalyzes the exchange of phosphatidylcholine and cholesteryl ester between plasma lipoproteins has been purified 10,000-fold from lipoprotein-free human plasma. The apparent molecular weight of the protein of the active fraction, designated lipid transfer complex (LTC), is approximately 61,000; when electrophoresed in 6 M urea, 0.1% sodium dodecyl sulfate on a 3–20% polyacrylamide gradient, the protein appears as a doublet of molecular weights 58,000 and 63,000. The active material is a glycoprotein which binds to concanavalin A. Human LTC is a lipid-protein complex with phospholipid, cholesterol, cholesteryl ester, and glyceride comprising 7% of the total mass. A similar glycoprotein (or glycoproteins) exists in rat plasma, although the fold-purification thus far achieved is low: about 500-fold. Moreover, the rat preparation enhances exchange of phosphatidylycerine, but does not appreciably enhance exchange of cholesteryl ester. Partially purified LTC (<3500-fold) exists in a complex with lecithin:cholesterol acyltransferase. Active lecithin:cholesterol acyltransferase is not, however, required for exchange of phosphatidylcholine or cholesteryl ester facilitated by human LTC. The rates of exchange of phosphatidylcholine and cholesteryl ester facilitated by human LTC are equal. Coupled lipid exchange occurs at all stages of LTC purification, at values of pH between 5 and 10, and at ionic strengths as great as 0.9. Moreover, phosphatidylcholine and cholesterol ester are exchanged with 1:1 stoichiometry in the presence of thiol group reagents such as 5,5'-dithiobis(2-nitrobenzoic acid). Both lipid exchange activities are relatively resistant to elevated temperatures.

Coupled exchange of phospholipid and neutral lipid is not dictated by the nature of the lipoprotein donor and acceptor substrates: bovine liver phospholipid exchange protein catalyzes exchange of phosphatidylcholine but not cholesteryl ester between low and high density lipoproteins under conditions identical with those in which human LTC facilitates exchange of both lipids.

Efforts to understand the processes of lipid transport and lipid metabolism, and recently to develop biodegradable en-

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folding purity of a triglyceride transfer protein from rabbit serum. The active fraction contained a glycoprotein with a molecular weight of 100,000-115,000 and an isoelectric point of 9.

The experiments reported herein were designed to purify further the human lipid exchange protein described initially by Glickman. Liposomes which simultaneously facilitate exchanges of cholesteryl ester and phosphatidylcholine, to assess the tightness of coupling between transfer of neutral lipid and phospholipid, and to probe the relationship between the lipid exchange protein(s) and lecithin:cholesterol acyltransferase. For comparison, a lipid exchange protein was isolated by similar procedures from rat plasma. The data suggest that lecithin:cholesterol acyltransferase activity is associated with the lipid exchange protein(s), lipid exchange and lecithin:cholesterol acyltransferase activity are uncoupled, indicating that active acyltransferase is not required for facilitated lipid exchange.

**EXPERIMENTAL PROCEDURES**

**Materials**—Cholesterol and Triglyceride Test Combination Kits were obtained from Boehringer Mannheim. [1,2-3H]Cholesterol (40-60 Ci/mmol), Lipidimolyl 1-14C phosphatidylcholine (60-100 mCi/mmol), and AquaSoil-2 were purchased from New England Nuclear. 1,α-Dipalmitoyl phosphatidylcholine and 1,α-dilinoleoyl phosphatidylcholine were obtained from Avanti Biochemicals; bovine serum albumin (Fraction V and essentially fatty acid-free) and human serum albumin from Sigma. Liquid chromatography supports were purchased as follows: phenyl-Sepharose and concanavalin A-Sepharose from Pharmacia Fine Chemicals; Bio-Gel P-4 and A-15 from Bio-Rad; and CM52 cellulose from Whatman. Ultradex was obtained from LKB. Silica Gel IB2 strips and Aquacide were obtained from J. T. Baker Chemical Co. and Calbiochem, respectively. Rat plasma was purchased from Pel-Freeze Biologicals. The phosphatidylcholine exchange protein was purified from beef liver by the method of Kamp and Wirtz (17). Purified apo-AI was obtained from Dr. Richard L. Jackson (University of Cincinnati).

**Isolation of Plasma Lipoproteins** and d > 1.21 g/ml Plasma Infranatant—Lipoproteins were isolated from the freshly collected plasma of normolipemic fasted human volunteers by sequential ultracentrifugation in KBr containing 4 × 10^{-3} M EDTA, pH 7.0 (18). A Beckman type 50.2 Ti rotor was used for all centrifugations steps. The purity of each lipid class was assessed by electrophoresis on agarose (15%, pH 8.6) and by immunodiffusion against antibodies raised against LDL, HDL, and apo-B, apo-E, apo-AI, apo-AII, and serum albumin. LDL were isolated between d 1.019 and 1.063 g/ml, HDL between d 1.063 and 1.21 g/ml, and the infranatant, excluding the clear zone immediately below the lipid exchange protein layer, termed the d > 1.21 g/ml plasma, was employed as the source of the lipid transfer complex for most of the experiments outlined. Lipoproteins and d > 1.21 g/ml plasma were stored at 4 °C in the KBr solution in which they were isolated; all lipoproteins were dialyzed immediately prior to use.

**Preparation of LDL and HDL Labeled with [1,2-3H]Dipalmitoyl Phosphatidylcholine and [1,2-3H]Cholesterol Ester**—To prepare double-labeled [1,2-3H]DPPC/[1,2-3H]CE-LDL or -HDL, the cholesteryl esters and the phospholipids were labeled sequentially. Freshly isolated plasma lipoproteins were diluted at 37 °C for 15 min with unlabeled human LDL. LDL cholesterol was impregnated on a Whatman No. 1 filter paper disc (19). Sodium azide (0.02%) was included in the incubation medium. At the conclusion of the incubation, the density of the solution was adjusted with KBr. LDL and HDL were obtained by sequential ultracentrifugation in KBr at salt densities 1.019, 1.063, and 1.21 g/ml, respectively, employing a Beckman 50 Ti rotor operating at 48,000 rpm for 18-24 h at 15 °C. About 65-75% of the radioactivity in the lipoproteins was cholesterol ester, the remainder being cholesterol. The radioabeled unesterified cholesterol was isolated with unlabeled human HDL. LDL were incubated with a 20-fold excess (cholesterol) of human HDL for 6 h at 37 °C. Similarly, the radiolabeled cholesterol in HDL was removed by incubation with a 5-fold excess of LDL (protein). The mixtures were then centrifuged at density 1.063 g/ml to isolate the [1,2-3H]CE-lipoproteins. The LDL typically had a radioactivity of 7,000 cpm/pg of total cholesterol, the HDL, about 6,000 cpm/pg of cholesteryl ester. In general, less than 4% of the radioactivity occurred as unesterified cholesterol.

[1,2-3H]CE-LDL were labeled with [1,2-3H]DPPC by incubation with [1,2-3H]DPPC-liposomes. To prepare [1,2-3H]DPPC-liposomes, 10 mg of DPPC and 10 μCi of [dipalmitoyl-1-14C]phosphatidylcholine were mixed in chloroform in a round-bottomed flask; the solvent was evaporated under N₂. The lipid was resuspended by addition of 5 ml of 10 mm Tris-HCl, pH 7.4, containing 0.15 M NaCl and 1 mm EDTA (Tris-buffered saline). The resulting suspension was incubated at 45 °C for 15 min (or until clear) with a Sonicator model W-225R (Heat Systems-Ultrasonics, Inc.) operating at an output of 50-70 watts. To prepare [1,2-3H]DPPC/[1,2-3H]CE-enriched LDL, [1,2-3H]DPPC-LDL (3.0 mg of phospholipid), dialyzed overnight against Tris-buffered saline, were incubated at 44 °C for 40 min with 2.5 mg of sonicated 1,α-dipalmitoyl phosphatidylcholine liposomes. The double-labeled bovine liver phospholipid exchange protein. Following incubation, the mixture was loaded onto Bio-Gel A-15m which had been packed to 1.6 × 110 cm and equilibrated with Tris-buffered saline. Elution was performed at room temperature in the same buffer at a flow rate of two ml/h. Of the total radioactivity added, 20-25% was recovered in LDL. Fractions containing [1,2-3H]DPPC/[1,2-3H]CE-LDL were pooled and concentrated by ultracentrifugation in KBr. [1,2-3H]DPPC/[1,2-3H]CE-HDL were prepared by incubation of [1,2-3H]CE-HDL (30 mg of phospholipid) with [1,2-3H]DPPC-LDL (1.1 mg of phospholipid, 1.58 × 10^6 cpm/μg of phospholipid) at 44 °C for 1 h in the presence of bovine liver phospholipid exchange protein (155 μg). The double-labeled HDL were separated from LDL and the exchange protein by gel filtration on Bio-Gel A-15m. Fractions containing HDL were pooled, adjusted to d 1.21 g/ml with KBr, and centrifuged for 24 h at 48,000 rpm in a Beckman 50.2 Ti rotor.

**Assay of the Lipid Transfer Complex**—The routine assay of column chromatographic fractions for transfer of phosphatidylcholine and cholesteryl ester was conducted in Tris-buffered saline at 44 °C with [1,2-3H]DPPC- and [1,2-3H]CE-labeled human LDL (usually 50-120 nmol of phospholipid per assay) and human HDL (500-1000 nmol of lipid donors) as acceptors. Experiments designed to probe stoichiometry of exchange of phosphatidylcholine and cholesteryl ester and to assess fold-purification utilized double-labeled [1,2-3H]DPPC/[1,2-3H]CE-LDL. In some experiments, lipid transfer was also monitored in the reverse reaction, i.e., from [1,2-3H]DPPC/[1,2-3H]CE-HDL (120 nmol of phospholipid per assay) to LDL (600 nmol of phospholipid per assay). After a 3-h incubation in the absence and presence of LDL, and HDL were resolated by ultracentrifugation. The density of the incubation mixture was raised to 1.21 g/ml by the addition of 0.32 g of KBr per ml of solution. The 1.21 g/ml mixture was layered under 0.683 g/ml KBr and the mixture was centrifuged at 48,000 rpm in a Beckman 50 Ti rotor. LDL floated as a thin yellow surface film and HDL sedimented to the bottom of the tube. The LDL and HDL were collected and 0.6 ml of each was added to 10 ml of Aquasol-2. The amount of radioactivity (H and 14C) in LDL and HDL was determined in a Beckman LS-230 liquid scintillation counter equipped with an adjustable discriminator. The discriminator was set so that H counts in the 14C channel were negligible. H counts detected in the 14C channel were 10-12% of 14C counts in the 14C channel. The percentage of overlap was determined in each experiment by counting standard 14C-containing solutions under the same conditions. The 14C counts in the 14C channel were subtracted to obtain H counts. To reduce the counting error, the ratio of H to 14C was usually maintained relatively high (between 7 and 10 to 1). The percentage of radioactivity recovered in the resolated LDL and HDL was usually 92-96% of the total amount added. LDL and HDL were isolated from the 14C channel of all assay conditions employed were analyzed by thin layer chromatography and it was determined that >98% of the transferred phospho-
lipid radioactivity and >99% of the transferred cholesteryl ester radioactivity was in fact d,labeled phosphatidylcholine and cholesteryl ester, respectively.

To analyze exchange kinetics, a rate expression, based on the assumption that there is lipid exchange only with no net transfer of lipid mass, was employed: In(C-C) / (C-C) = (1/1(A) + 1/1(B)) x f(t) where f(t) represents the rate of exchange. Since the rate of exchange is zero, time t, and time infinity, respectively. The concentration of either phosphatidylcholine or cholesteryl ester) in the donor lipoproteins is (A); the lipid concentration of acceptor lipoproteins is (B). The rate of exchange (nanomoles of lipid/h) or the rate of lipid flux between donor and acceptor lipoproteins, designated as ε, is determined by plotting In(C-C) / (C-C) vs. t. For ε = 0, f(t) is constant for a given experiment, ε is obtained by dividing the slope by (1/1(A) + 1/1(B)). To obtain the rate of LTC-facilitated lipid exchange, the rate of exchange in the absence of added LTC (background exchange) is subtracted from that in the presence of LTC (total exchange).

The maximum theoretical transfer of lipid from donor to acceptor lipoproteins was calculated from the formula ε [(B)/(A)] + (B), where (A) and (B) are the total phosphatidylcholine or cholesteryl ester pool of donor and acceptor, respectively, and ε is the lipid exchange velocity (in counts/min) in the donor lipoproteins. The extrapolated, equilibrium value for phosphatidylcholine and cholesteryl ester transfer was obtained by a double reciprocal plot of the counts/min transferred from donor to acceptor versus the incubation time.

**RESULTS**

The lipid transfer complex was isolated from the d > 1.21 g/ml infranatant of human and rat plasma by sequential chromatography on phenyl-Sepharose, CM-cellulose, and concanavalin A-Sepharose essentially as outlined by Ihm et al. (13) except that /β-mercaptoethanol was added to the d > 1.21 g/ml infranatant at a final concentration of 50 mM prior to the first purification step. This modification of the procedure alters the behavior of LTC on ConA-Sepharose so that the major active fraction is well-separated from the peak containing most of the ConA-bound proteins, resulting in an LTC preparation with high specific activity. The elution of human and rat LTC from ConA-Sepharose is illustrated in Fig. 1. In the case of human lipid transfer activities, cholesteryl ester and phosphatidylcholine exchange activities co-elute from ConA-Sepharose. Stoichiometry of exchange of neutral and polar lipid cannot be determined from the data of Fig. 1 since different assays were employed to assess exchange of the two lipids. Rat LTC has very little cholesteryl ester exchange activity.

Chromatographic fractions from ConA-Sepharose were combined to give two LTC preparations, one of low specific activity designated LTC-A (human, fractions 52-54; rat, fraction 47) and one of high specific activity designated LTC-B (human, fractions 58-67; rat, fractions 55-60). As is indicated in Fig. 1, lecithin:cholesterol acyltransferase activity is pronounced in human LTC-A; although the acyltransferase activity is measurable in LTC-B, its amount is considerably diminished. The fractions containing rat lecithin:cholesterol acyltransferase activity are concentrated between fractions designated LTC-A and -B. With respect to phosphatidylcholine exchange on a basis for comparison, the fold-purification from the d > 1.21 g/ml infranatant of human plasma LTC-B is 10,000; the amount of lipid transfer activity recovered is 30%. Rat LTC-B is purified only about 500-fold with recovery of 11% of the total activity. In an LDL to HDL assay, purified human and rat LTC transfer typically 1–2 mg/ml and 300–800 nmol of phospholipid, respectively, per mg of protein in 3 h at 37°C.

Electrophoresis of human and rat LTC-B in 7.5% polyacrylamide gels containing SDS produces a single broad stained band of an apparent molecular weight of 61,000; chromatography of human LTC-B on a calibrated Bio-Gel A-15m column results in one protein peak with an apparent molecular weight of 70,000 (data not shown). It was possible to obtain sufficient quantities of the human LTC-B to characterize it in more detail. The protein was subjected to gradient gel electrophoro...
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Fig. 1. Chromatography of the lipid transfer complex on ConA-Sepharose. A, human plasma LTC; B, rat plasma LTC. The active fractions obtained from CM-cellulose chromatography were dialyzed overnight against 10 mM Tris-HCl, pH 7.4, containing 1 mM CaCl₂ and 10 mg of protein was applied to the ConA-Sepharose column (2.4 x 40 cm). Step elution at 4 °C was performed at a flow rate of 35 ml/h with the initial buffer, and then with the initial buffer plus 150 mM a-methyl-D-mannoside. The arrow identifies the position where the eluant was changed to buffer containing a-methyl-D-mannoside. The volume of each collected fraction was 8 ml. Absorbance at 280 nm, phosphatidylcholine exchange activity, cholesteryl ester exchange activity, lecithin:cholesterol acyltransferase; (LCAT) activity.

Resins in 6 M urea, 0.1% SDS, and the resulting profile is illustrated in Fig. 2. Two major staining bands account for >95% of the staining intensity; these bands, present in almost equimolar amounts, have apparent molecular weights of 58,000 and 63,000. Minor bands visible on the gel have apparent molecular weights of 30,000, 42,000, 53,000, and 96,000. LTC-B contains no immunoassayable lecithin:cholesterol acyltransferase and less than 4% of the total protein is apo-D. The lipid composition of LTC-B was determined and is presented in Table I. By weight, LTC-B contains very little lipid—7% of the total mass. Calculated on a molar basis, however, the amount of lipid is more impressive, constituting 86% of the
Moreover, the amount of phospholipid and cholesterol-cholesteryl ester recovered in each lipoprotein class resisolated after incubation does not depend on the length of incubation time up to 6 h. As was reported previously (13), LTC purified about 5,000-fold from human d > 1.21 g/ml infranatant transferred phosphatidylcholine and cholesteryl esters with a 1:1 stoichiometry and at equal rates of flux. To determine if the more highly purified human lipid exchange complex, LTC-B, also catalyzes lipid exchange with a phosphatidylcholine:cholesteryl ester ratio of about 1:1, the flux rates for both lipids were calculated in an assay mixture consisting of [3H]DPPC/[3H]CE-LDL and HDL. The data, reported in Table III, indicate that the flux rate ratio is 0.9:1, phosphatidylcholine:cholesteryl ester. Moreover, it is evident that equimolar flux rates occur regardless of the extent of purification: addition to the assay of d > 1.21 g/ml infranatant or of LTC-B yield nearly identical stoichiometries. Moreover, varying the amount of transfer complex does not alter the exchange stoichiometry.

In contrast, the rat lip exchange complex does not catalyze exchange of the two lipids in equimolar amounts. Rat d > 1.21 g/ml infranatant has little cholesteryl ester transfer activity,
as is the case for the more purified rat LTC-B. The flux rate ratios vary from 5.8 to 26.7, phosphatidylcholine:cholesterol ester. The possibility exists that rat plasma contains an inhibitor of cholesteryl ester exchange. Rat d > 1.21 g/ml infranatant, and the unbound-inactive and partially purified bound-active preparations obtained from the phenyl-Sepharose chromatography step were added to an assay containing [3H]CE:HDL, LDL, and human d > 1.21 g/ml infranatant (Table IV). None of the fractions isolated from rat plasma inhibits exchange of cholesteryl ester catalyzed by human lipid exchange protein(s).

In the next series of experiments, the reaction conditions were varied in an effort to uncouple transfer of phosphatidylcholine and cholesteryl ester and to assess the importance of lecithin:cholesterol acyltransferase in lipid transfer. It was first established that the course of the reaction was lipid exchange and not net lipid transfer at all reaction conditions. The partially purified preparation of human LTC, LTC-A, was utilized in these studies since it contains sufficient lecithin:cholesterol acyltransferase activity to ensure accurate measurement (Fig. 1).

To determine the effect of ionic strength on the exchange of phosphatidylcholine and cholesteryl ester, the ionic strength of the reaction solutions was varied by addition of appropriate amounts of NaCl or CaCl2. As is shown in Fig. 3, phosphatidylcholine and cholesteryl ester exchange and lecithin:cholesterol acyltransferase activities are similarly influenced by ionic strength. In NaCl buffer, both phosphatidylcholine and cholesteryl ester exchange activities decrease by about 20% from μ = 0.3-0.9. The acyltransferase activity decreases steadily as the concentration of NaCl increases, and at an ionic strength of 0.9, 28% of the original lecithin:cholesterol acyltransferase activity is inhibited. When CaCl2 is used to adjust the solution ionic strength, the results are qualitatively similar although both lipid exchange activities and particularly lecithin:cholesterol acyltransferase activity are more sensitive to CaCl2 relative to NaCl. Both phosphatidylcholine and cholesteryl ester exchange activities decrease by approximately 40% between ionic strengths of slightly greater than 0 to 0.3 (which corresponds to 0.1 M CaCl2); no further decrease in lipid exchange activity occurs at higher ionic strengths. Lecithin:cholesterol acyltransferase activity decreases rather drastically as the CaCl2 ionic strength is increased to 0.9; only 50% of the original activity remains at an ionic strength 0.3, and only 24% remains at μ = 0.9. In these experiments, the mole ratio of phosphatidylcholine:cholesterol ester exchanged at the various ionic strengths ranges from 0.9 to 1.2.

To test the effect of pH on facilitated phosphatidylcholine or cholesteryl ester exchange, assays were performed at pH 5 through pH 10. As is shown in Fig. 4, rates of facilitated phosphatidylcholine and cholesteryl ester exchange do not vary markedly over the pH range 7 through 9. Decreases in both activities occur at pH 5-7 and 9-10, with facilitated exchange of both lipids considerably diminished at pH 5. Lecithin:cholesterol acyltransferase activity, on the other hand, has a fairly sharp pH optimum, pH 7. Relative to the optimum, lecithin:cholesterol acyltransferase is only 40% active at pH 6 and pH 9.

![FIG. 3. Influence of ionic strength on lipid exchange and lecithin:cholesterol acyltransferase (LCAT) activities.](http://www.jbc.org/)

**TABLE IV**

Influence of rat plasma fractions on cholesteryl ester exchange facilitated by human d > 1.21 g/ml infranatant

Human d > 1.21 g/ml infranatant, 4.5 mg of protein, was incubated with human [3H]CE-HDL (72 μg of cholesteryl ester) and human LDL (928 μg of cholesteryl ester) for 6 h at 37 °C. HDL and LDL were reisolated by centrifugation and the per cent of cholesteryl ester exchanged was calculated. At equilibrium (18 h), 97% of the cholesteryl ester exchanges in this assay system.

| Rat fractions (mg protein) | Cholesteryl ester exchange % |
|---------------------------|-----------------------------|
| None                      | 44.5                        |
| 1.21 g/ml                 | 44.1                        |
| Phenyl-Sepharose, unbound | 44.8                        |
| 0.4                       | 43.9                        |
| 0.5                       | 44.5                        |
| 1.0                       | 44.0                        |
| Phenyl-Sepharose, bound   | 44.4                        |

To investigate the heat stability of the lipid exchange activ-
ities and of lecithin:cholesterol acyltransferase, partially purified human LTC-A was heated at 37–72 °C for 15 min prior to the routine assay. Results are presented in Fig. 5. The phosphatidylcholine exchange activity is not influenced by temperatures as great as 55 °C for 15 min. Activity decreases only slightly at higher temperatures such that the complex heated at 72 °C retains 89% of the activity of the nonheated material. Cholesteryl ester exchange activity is similarly heat-stable. The complex heated at 62 °C retains 95% of its original activity; heating at 72 °C reduces the cholesteryl ester exchange to 84% of the original. In contrast to the lipid exchange activities, lecithin:cholesterol acyltransferase activity is very sensitive to elevated temperature. After 15 min at 55 °C, the acyltransferase loses 94% of the original activity. Incubation for 15 min at 62 °C or greater completely inactivates lecithin:cholesterol acyltransferase.

The acyltransferase is sensitive to thiol group blockers (26). In addition, Hopkins and Barter (10) report that p-chloromercuriphenyl sulfonate added to rabbit serum inhibited transfer of triglyceride; the transfer of cholesteryl ester was much less sensitive. It was therefore of interest to assess the effect of a thiol group reagent on phosphatidylcholine and cholesteryl ester exchange and on lecithin:cholesterol acyltransferase. Thus, the standard assays were performed in the absence and presence of DTNB. The results are presented in Fig. 6. Lecithin:cholesterol acyltransferase activity decreases dramatically in the presence of 0.4 mM DTNB. Further decreases occur at higher concentrations of DTNB such that only 10% of the original lecithin:cholesterol acyltransferase activity is expressed in 3 mM DTNB. These data confirm the report of Stokke and Norum (26) that DTNB inhibits lecithin:cholesterol acyltransferase.

![Fig. 5](http://www.jbc.org/) Sensitivity of phosphatidylcholine (••••) and cholesteryl ester (■■■■) transfer and lecithin:cholesterol acyltransferase (○○○○) activities to heat inactivation. The percent of original phosphatidylcholine and cholesteryl ester exchange and lecithin:cholesterol acyltransferase activities are plotted against the temperature at which transfer complex was heated for 15 min. The partially purified transfer complex (LTC-A) (1.1 mg) was heated at 37–72 °C for 15 min prior to assay. To assay phosphatidylcholine and cholesteryl ester transfer activity, [14C]DPPC/[3H]CE-labeled LDL (33 nmol of PC/29 nmol of CE) and HDL (280 nmol of PC/120 nmol of CE) were incubated with heat-treated LTC. After 3 h at 44 °C, LDL and HDL were reisolated by ultracentrifugation, and the 14C and 3H radioactivities recovered in each lipoprotein class were determined. The lecithin:cholesterol acyltransferase assay was performed for 10 h at 37 °C, as described under “Experimental Procedures.”
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cholesterol acyltransferase activity. In contrast, phosphatidylcholine and cholesteryl ester exchange activities are insensitive to DTNB. Only slight decreases (5–12%) in lipid exchange activities occur at DTNB concentrations greater than 3 mM (data not shown). In these experiments, exchange of phosphatidylcholine and cholesteryl ester occurs with a stoichiometry of 1:1.

Finally, LTC-A obtained from the ConA-Sepharose step was subjected to preparative isoelectric focusing. The gel was sliced in pieces (0.8 cm × 10.5 cm) and the protein was eluted with 2.0 ml of Tris-buffered saline. The protein mixtures were incubated with [14C]DPPC/[3H]CE and LDL to identify the isoelectric points of the phosphatidylcholine and cholesteryl ester exchange activities. The data presented in Fig. 7 indicate that both lipid exchange activities occur over the pH range 4.2–5.0. Over this range, the activities exist in equimolar stoichiometries.

To evaluate the possibility that coupled exchange of phosphatidylcholine and cholesteryl ester is dictated by the nature of lipoprotein substrates, phosphatidylcholine and cholesteryl ester exchange facilitated by LTC was compared to that facilitated by bovine liver phosphatidylcholine-specific exchange protein. The routine assay employing [14C]DPPC/[3H]CE-LDL and HDL was conducted at 37 °C and at 44 °C. As is shown in Fig. 8, the liver PLEP greatly facilitates the transfer of phosphatidylcholine both at 37 °C and 44 °C, while it does not enhance the transfer of cholesteryl ester even at the higher incubation temperature. Under the same conditions in the presence of human plasma lipid exchange complex, exchange of both lipids occurs with a mole ratio of phosphatidylcholine to cholesteryl ester about 1:1.

**DISCUSSION**

In the lipoprotein assay system employed, LTC clearly catalyzes lipid exchange rather than net lipid transfer. In three consecutive steps of chromatography of LTC through a hydrophobic column, a cation exchange column, and a carbohydrate affinity column, phosphatidylcholine and cholesteryl liver PLEP (0.5 mg/ml of protein). After incubation, LDL and HDL were separated and analyzed. A, the nanomoles of phosphatidylcholine and cholesteryl ester exchanged during a 40-min incubation at 37 °C. B, the nanomoles of phosphatidylcholine and cholesteryl ester exchanged during 3 h at 44 °C.
estor exchange activities are purified about 10,000-fold from human \( d > 1.21 \) g/ml infranatant. Based on behavior in these chromatography procedures, three features of the lipid exchange complex are revealed. Lipid transfer activity is retained on the phenyl-Sepharose column at a salt concentration of 0.15 M or higher and is eluted with water, suggesting that LTC possesses hydrophobic regions and/or it exists as a complex with lipid(s). On a CM52 cation exchange column, LTC is not eluted with acetate buffer, pH 4.5, but is eluted when the ionic strength of the elution buffer is raised to 0.1 M with NaCl. Therefore, LTC has a slight positive charge at pH 4.5. This conclusion is consistent with the isoelectric point range of 4.2-5.0. LTC binds to ConA-Sepharose and is eluted with 150 mM \( \alpha \)-methyl-d-mannoside, suggesting that LTC contains the carbohydrate residues mannose, glucose, or \( \alpha \)-acetylgalactosamine. In addition, LTC has associated lipid and must therefore be considered a lipid-protein complex. The lipid composition is surprisingly reproducible which suggests that it may be relevant to the biological function of this entity. LTC has an apparent molecular weight of 61,000 as judged by electrophoresis in 7.5% polyacrylamide-SDS. This value is consistent with that determined by chromatography of human LTC-B on Bio-Gel A-5m; the gel filtration molecular weight is approximately 70,000. In polyacrylamide gradient gels in urea/SDS, the 61,000 molecular weight band exists as two closely spaced bands of apparent molecular weights 58,000 and 63,000. LTC purified from \( d > 1.21 \) g/ml infranatant has an almost identical molecular weight based on electrophoresis in 7.5% acrylamide-SDS. It is not yet known whether the broad protein band of apparent molecular weight 61,000 resolves into a doublet when electrophoresis is performed in an acrylamide gradient. The question is an interesting one since the rat LTC does not appreciably facilitate exchange of cholesteryl ester. Lack of catalyzed exchange of the neutral lipid is probably not due to the presence of an inhibitor in rat plasma, although it is possible that an inhibitor is specific for the rat LTC.

Does LTC require lecithin:cholesterol acyltransferase to catalyze the transfer of cholesteryl esters? Since lecithin:cholesterol acyltransferase plays a role in both cholesteryl ester and phospholipid metabolism and is present in \( d > 1.21 \) g/ml plasma, the role of the acyltransferase in LTC-facilitated lipid exchange was investigated. Lecithin:cholesterol acyltransferase co-elutes with that of phosphatidylcholine and cholesteryl ester exchange activity through the first two steps of purification consistent with the report of Fielding and Fielding (27) that cholesteryl ester transfer activity is associated with lecithin:cholesterol acyltransferase. In the ConA-Sepharose step, however, lipid exchange activities separate from lecithin:cholesterol acyltransferase activity, although the fraction containing high specific activity LTC still has a small amount of acyltransferase activity. In any event, the data demonstrate conclusively that lecithin:cholesterol acyltransferase activity is not required for lipid exchange facilitated by LTC. First, lecithin:cholesterol acyltransferase is very sensitive to heat inactivation, whereas lipid exchange activities are stable to exposure to temperatures as great as 72 °C. Second, while lipid exchange activities do not vary markedly over the pH range 7 through 9, lecithin:cholesterol acyltransferase activity has a sharp pH optimum at pH 7. Finally, the sulfhydryl group reagent, DTNB, inhibits lecithin:cholesterol acyltransferase almost completely, while both lipid exchange activities are little influenced. Taken together, the data strongly indicate that active LCAT is not a prerequisite for phosphatidylcholine and cholesteryl ester exchange.

The most significant finding is that phosphatidylcholine and cholesteryl ester are exchanged with a 1:1 stoichiometry by human LTC. For example, facilitated exchange occurs with about a 1:1 stoichiometry in the presence of increasing amounts of \( d > 1.21 \) g/ml infranatant plasma protein and of LTC purified to high specific activity. Phosphatidylcholine and cholesteryl ester exchange is also stoichiometric at all values of ionic strength and pH tested. The activities are present in equimolar stoichiometries in protein-containing bands obtained by preparative isoelectric focusing. Furthermore, the activities are not uncoupled by elevated temperatures or by thiol group reagents. Under conditions in which human LTC facilitates exchange of phosphatidylcholine and cholesteryl ester equally between LDL and HDL, bovine liver phospholipid exchange protein catalyzes exchange of phosphatidylcholine only, suggesting that stoichiometric exchange is not dictated by the structure of the lipoprotein substrates. Available data may be interpreted to indicate that exchange/transfer of cholesteryl ester by LTC depends on the presence of phosphatidylcholine, but that facilitated exchange/transfer of phosphatidylcholine occurs independently of cholesteryl ester. In a liposome → mitochondria assay system, for example, LTC-facilitated phosphatidylcholine transfer occurs from liposomes to mitochondria; both assays substrates are deficient in cholesteryl ester (28). Furthermore, the rat LTC facilitates exchange of phosphatidylcholine under conditions in which little cholesteryl ester exchange occurs. Although cholesteryl ester transfer activity emerges as the fold-purification of LTC from \( d > 1.21 \) g/ml infranatant increases, it remains very low such that instead of the 1:1 stoichiometric exchange of phosphatidylcholine and cholesteryl ester facilitated by human LTC, the rat LTC catalyzes transfer in a mole ratio of about 17:1. Finally, human LTC removes cholesteryl oleate from a dioleoylphosphatidylcholine monolayer containing 1-6 mol % cholesteryl oleate. When a sphingomyelin monolayer contains the cholesteryl ester, the sterol is not removed by LTC. However, as increasing amounts of phosphatidylcholine are added to the sphingomyelin-cholesteryl ester monolayer, the rate of cholesteryl ester removal by LTC is markedly increased. Furthermore, phosphatidylcholine, as well as cholesteryl oleate, is removed from the monolayer by LTC, but removal of phosphatidylcholine does not require the presence of cholesteryl ester.

An important question which arises is: What is the biological role of the plasma LTC? Any hypothesis must accommodate the fact that human LTC facilitates equimolar exchange of phosphatidylcholine and cholesteryl ester while rat LTC does not. Resolution of this question must await the availability of additional information.

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