Facilitation of phosphatidylcholine transfer into high density lipoproteins by an apolipoprotein in the density 1.20–1.26 g/ml fraction of plasma

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Abstract Incubation of unilamellar vesicles of egg phosphatidylcholine (PC) in human plasma results in rapid transfer of phospholipid into high density lipoproteins (HDL). A similar but much slower transfer occurs upon incubation of PC vesicles with centrifugally isolated HDL. Experiments were conducted to characterize a plasma factor that might facilitate PC transfer into HDL. Addition of the d > 1.21 g/ml fraction of plasma to isolated HDL caused a marked increase in the rate of transfer of PC from vesicles to HDL. Fractionation of plasma by vertical rotor density gradient ultracentrifugation revealed that the factor that facilitated transfer of PC into HDL resided in the density 1.20–1.26 g/ml fraction, associated with a lipoprotein particle of apparent Stokes' diameter 10.2 nm. This fraction caused facilitated transfer of PC mass from vesicles into HDL_3, resulting in formation of larger, less dense "HDL_{2a}"-like particles. A partially purified preparation of phospholipid transfer activity was obtained from the d 1.20–1.26 g/ml fraction by a sequence of phenyl-Sepharose, heparin-Sepharose, and carboxymethylcellulose chromatography. The most purified fraction facilitated transfer of 4 μg of ^{14}C-labeled PC/μg protein per 60 min and also promoted transfer of radioactive cholesteryl esters from HDL to LDL. The results suggest that during lipoprotein metabolism the insertion of PC molecules into HDL may be facilitated by a plasma lipid transfer protein.—**Tall, A. R., L. R. Forester, and G. L. Bongiovanni.** Facilitation of phosphatidylcholine transfer into high density lipoproteins by an apolipoprotein in the 1.20–1.26 g/ml fraction of plasma. *J. Lipid Res.* 1983. **24:** 277–289.

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During lipolysis of the triglyceride-transporting lipoproteins, chylomicrons and very low density lipoproteins, there is rapid transfer of phosphatidylcholine into plasma high density lipoproteins (HDL) (1–3). This process can be simulated in vitro by incubation of phospholipid liposomes with HDL or with plasma (4). For both isolated HDL and plasma, the major mechanism of phospholipid uptake by HDL appears to be insertion of phospholipid molecules into pre-existing spherical HDL particles (4, 5). However, during a 4-hr incubation, the uptake of phospholipid by isolated HDL was only about one-third that of HDL in whole plasma (4). The ability of the plasma lipoprotein fraction to incorporate phospholipid into HDL was reduced in proportion to the period of preparative ultracentrifugation, suggesting centrifugal separation from HDL of a factor that promotes phospholipid uptake (4). In an attempt to isolate such a factor, we have tested various plasma fractions for their ability to enhance phospholipid uptake by centrifugally isolated HDL. Following our initial identification and characterization of phospholipid transfer activity in the d 1.20–1.26 g/ml fraction, we found this activity could be further purified by a scheme based on that described by Pattnaik and Zilversmit (6) for purification of cholesteryl ester exchange protein. The most purified material stimulated mass transfer of PC from vesicles into HDL, as well as transfer of radioactive cholesteryl esters from HDL to LDL.

MATERIALS AND METHODS

Materials

HDL was prepared from plasma of fasting donors by centrifugation at 40,000 rpm and 5°C in a Beckman 40.3 rotor, for 24 hr at d 1.063 g/ml and 36 hr at 1.21 g/ml. The d > 1.21 g/ml fraction was obtained from the bottom 2 ml of the centrifuge tubes. The supernatant HDL was removed and centrifuged for a further 36 hr through d 1.19 g/ml solution. HDL containing [^3H]cholesteryl esters was prepared by the procedure of Ihm et al. (7), using partially purified lecithin:cholesterol acyltransferase; radioactive cholesterol was removed from the HDL by incubation with an excess of LDL. As determined by thin-layer chromatography, 96% of ^3H radioactivity was in cholesteryl esters

Abbreviations: PC, phosphatidylcholine; HDL, high density lipoproteins.

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and 4% in cholesterol. The specific activity of cholesteryl esters was 206,000 dpm/mg. Phosphatidylcholine was prepared from egg yolks by the method of Singleton (8) and purified by silicic acid chromatography. The phosphatidylcholine was eluted in chloroform–methanol–water 65:25:4 (v/v/v) and found to be >99% pure, as judged by thin-layer chromatography. Phosphatidylcholine (L-α-1-palmitoyl-2-linoleoyl-{linoleoyl-1-^{14}C}) was purchased from New England Nuclear, mixed with egg phosphatidylcholine to give a specific activity of approximately 43,000 dpm/mg, and lyophilized from cyclohexane. The lyophilized phosphatidylcholine was taken up in 5.0 ml of 0.15 M Tris-phosphate, 0.01% NaN_3, pH 8.0 (buffer A), and sonicated for 30–45 min in a water-cooled cell at 5–10°C. The sonicated mixture was centrifuged to remove Ti particles and then chromatographed on a 40 × 2.5 cm column of Sepharose CL-4B. Small unilamellar vesicles were obtained from the included volume peak and their specific activity was determined by liquid scintillation counting, after extraction (9) and phosphorus assay (10). The specific activity was found to be identical to the stock phosphatidylcholine. Vesicles were stored at 4°C and used within 5 days.

Incubations

A standardized assay procedure was used to determine transfer of phosphatidylcholine into HDL. Various fractions and HDL were dialyzed into buffer A. The incubations contained 0.25–1.0 mg of phosphatidylcholine vesicles (15–60,000 cpm) and the fraction to be studied, with or without HDL (0.125–0.25 mg of protein), diluted to a final volume of 4.0 ml with buffer A. Incubation mixtures were prepared in Pyrex test tubes immersed in ice, with addition of components in order: the fraction, HDL, diluent, vesicles. The mixtures were vortexed for 15 sec, then incubated for 60–120 min at 37°C in a metabolic shaker. Incubations were stopped by immersion of test tubes in ice and the mixtures were transferred to pre-chilled polyallomer tubes containing NaBr solution to give a final solution density of 1.063 g/ml. The tubes were centrifuged at 10°C for 14–16 hr at 36,000 rpm. From each tube the bottom 2.8 ml was taken for liquid scintillation counting. Prolongation of centrifugation time to 24 hr did not result in further transfer of PC radioactivity into the d > 1.063 g/ml fraction. When fractions at different stages of purity were assayed for PC transfer activity, recentrifugation of the d > 1.063 g/ml fraction at 1.21 g/ml resulted in >95% of radioactivity in the top 2.8 ml of the centrifuge tube, showing that for all preparations the assay was measuring transfer of PC radioactivity into the HDL density range.

Vertical rotor ultracentrifugation

In initial experiments the distribution of phospholipid transfer activity was determined by density gradient ultracentrifugation of plasma in a Sorvall TV865B vertical rotor. Subsequently, in order to prepare larger amounts of material, the TV850 rotor was used. Ten ml of plasma was raised to density 1.37 g/ml by addition of solid NaBr and placed in the bottom of each centrifuge tube. This was overlaid with 5 ml of plasma at 1.30 g/ml, then with 19 ml of density 1.060 g/ml NaBr solution. The rotor was centrifuged for 5 hr at 50,000 rpm, using slow acceleration and deceleration with the brake off. Following centrifugation, VLDL and LDL were found at the top of the tubes and HDL was seen as a broad band one-third to halfway down the tube. The fraction containing facilitation activity was found in the clear zone below the HDL and extended into the top 1–2 ml of plasma proteins.

Chromatography

Agarose gel chromatography was conducted on a 1.25 × 100 cm column of Sepharose CL 6B, eluted with 0.15 M NaCl, adjusted to pH 8.0 by addition of NH_4OH. The column was calibrated with standards of apoferritin, LDH, catalase, albumin, LDL, and HDL. The void and total column volumes were determined from the elution of chylomicrons and dithionitrobenzoic acid (DTNB), respectively. ConA-Sepharose (Pharmacia) chromatography was performed at 10°C on a 15 × 1.25 cm column, using 0.05 M Tris, 1.0 M NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.0 (buffer B). Bound fractions were eluted with buffer B containing 0.2 M methylglucopyranose. Phenyl-Sepharose chromatography was performed on a 60 × 2.0 cm column, by the procedure described by Morton and Zilversmit (11). Heparin-Sepharose (Pharmacia) chromatography was performed at 10°C on a 15-cm column; bound fractions were eluted by batch elution or with a linear NaCl gradient. Cation exchange chromatography was performed on a 15 × 1.25 cm column containing carboxymethyl cellulose (Whatman CM 52), following the procedure of Pattnaik et al. (12).

Electrophoresis

Polyacrylamide gradient gel electrophoresis of intact HDL was performed as described (13). Lipoproteins were delipidated in 20 volumes of ethanol–ether 2:1 at 0°C and the protein precipitate was washed twice with diethyl ether. Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis was performed in 0.1% SDS, 5.8% acrylamide gels as described previously (3). Samples were preincubated at 37°C for 30 min in 1% SDS,

0.6% dithiothreitol. The gels were stained for protein with Coomassie Blue and for carbohydrate by the periodic acid-Schiff technique, as described by Segrest and Jackson (14). Phosphorylase B, albumin, ovalbumin, and chymotrypsinogen were used as molecular weight standards. ApoA-I, apoA-IV, and albumin were identified by comparison of their mobilities with purified standards of these proteins. Protein was estimated by the method of Lowry et al. (15).

RESULTS

Time course of facilitated phospholipid transfer

Unilamellar vesicles of egg phosphatidylcholine (PC) were incubated with plasma, with HDL plus the d > 1.21 g/ml fraction, with the d > 1.21 g/ml fraction alone, and with HDL alone. The time course of transfer of PC radioactivity into the d 1.063–1.210 g/ml fraction (HDL) is shown in **Fig. 1**. Addition of the d > 1.21 g/ml fraction to HDL greatly increased the rate of PC transfer into HDL, apparently reconstituting the activity of whole plasma. The percentage ^{14}C-labeled PC transfer effected by HDL plus the d > 1.21 g/ml fraction (A) was greater than the sum of ^{14}C-labeled PC transfer by HDL alone (B) + ^{14}C-labeled PC transfer by the d > 1.21 g/ml fraction alone (C). The difference A − (B + C) quantitates the amount of facilitated PC transfer resulting from interaction of the d > 1.21 g/ml fraction with HDL. This facilitation activity, shown as a dashed line in Fig. 1, reached a maximum at the 30-min time point. The effect of the d > 1.21 g/ml fraction on ^{14}C-labeled PC transfer into HDL was identical in incubations conducted with and without 2 mM DTNB, showing independence of lecithin: cholesterol acyltransferase (LCAT).

Further incubations using the same conditions as those of Fig. 1, were performed for 0, 2, 4, 6, and 8 hr. A plateau of ^{14}C-labeled PC transfer into HDL was attained at 2 hr for plasma, for HDL + the d > 1.21 g/ml fraction, and for the d > 1.21 g/ml fraction alone; however, when PC vesicles were incubated with HDL alone there was a continuous linear increase in ^{14}C-labeled PC transfer between 0 and 8 hr. In a 24-hr incubation, similar transfer of PC radioactivity was obtained for HDL alone and HDL + the d > 1.21 g/ml fraction. Thus, the d > 1.21 g/ml fraction contains a factor that interacts with isolated HDL to increase its initial rate of uptake of PC radioactivity. In experiments where HDL_2 (1.063–1.110 g/ml) and HDL_3 (1.110–1.210 g/ml) were compared as acceptors of PC, the time course and amount of facilitated PC transfer were similar for incubations containing the same amount of HDL protein.

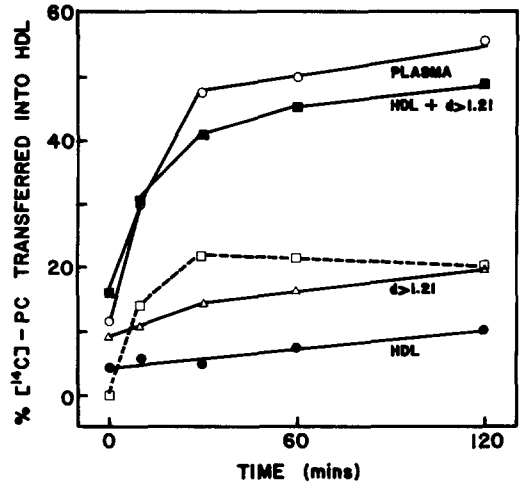

Fig. 1. Facilitation of transfer of PC radioactivity into HDL by the d > 1.21 g/ml fraction of plasma. Unilamellar vesicles of egg PC (1.5 mg PC, containing 45,000 cpm) were incubated with (A) fresh plasma (1.0 ml); (B) d > 1.21 g/ml fraction (from 1.5 ml plasma) + HDL (1.5 mg protein); (C) d > 1.21 g/ml fraction (from 1.5 ml plasma); and (D) HDL (1.5 mg protein) in a metabolic shaker at 37°C. At each time point, the incubation was stopped by chilling on ice and the % of PC radioactivity transferred into the d 1.063–1.210 g/ml fraction was determined by preparative ultracentrifugation in a Beckman 40.3 rotor. Facilitated PC transfer (dashed line) was determined from the difference B − (C + D).

Density distribution, size, and composition of particles bearing phospholipid transfer activity

To analyze the distribution of this facilitation activity, plasma was subjected to density gradient ultracentrifugation for 3 hr in a vertical rotor and equal volume aliquots from each gradient fraction were incubated with PC vesicles or PC vesicles plus isolated HDL. The content of phospholipid and cholesterol and the densities of the gradient fractions are shown in the top panel of **Fig. 2** and the PC uptake and facilitation activities are shown in the bottom panel. The greatest transfer of PC radioactivity into HDL (solid line) occurred upon incubation of vesicles with fractions 8 (d 1.12 g/ml), 11 (d 1.16 g/ml), and the bottom fraction of the gradient. By contrast, the facilitation activity (dashed line) was found in a peak of density 1.20 to 1.26 g/ml, corresponding to a relatively clear zone just above and extending into the plasma protein fraction. In 12 different preparations from 5 plasma donors, the d 1.20–1.26 g/ml fraction was found to contain all the facilitation activity of plasma.

Analysis of fractions of plasma centrifuged for 48 hr in the SW 50.1 rotor also showed a peak of facilitation in the d 1.20–1.26 g/ml fraction, but some activity was present in the bottom gradient fraction. Following centrifugation of plasma in fixed angle rotors (such as the 40.3 or Ti 50.2 rotor), most of the activity was found

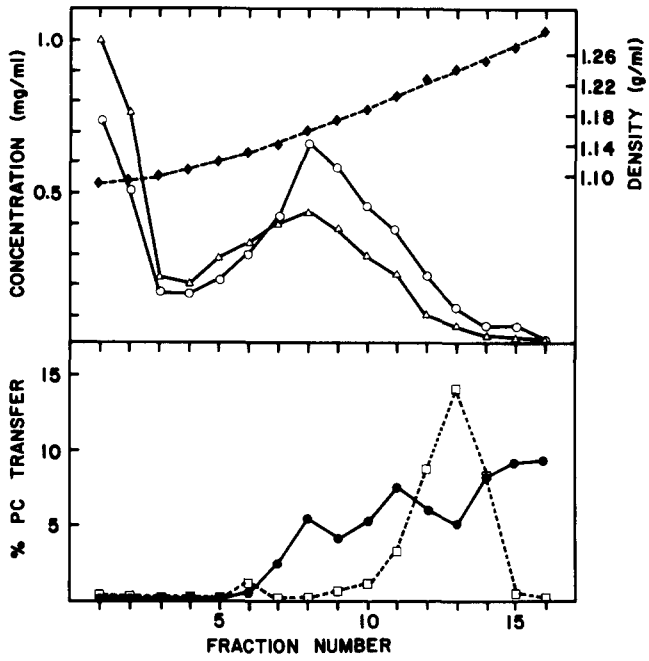

Fig. 2. Vertical rotor density gradient ultracentrifugation of plasma, showing distribution of the activity that facilitates transfer of PC radioactivity into HDL. In the top panel are shown the concentrations of total cholesterol (Δ), phospholipids (○), and the densities (♦) of individual gradient fractions. In the bottom panel are shown the amounts of PC transfer (●) and facilitated PC transfer (□) into HDL. Five ml of plasma were raised to density 1.35 g/ml, overlaid with 12 ml of density 1.063 g/ml NaBr solution in a Sorvall TV 865B vertical rotor, and centrifuged for 3 hr at 5°C and 65,000 rpm in a Beckman L5-75 centrifuge, using slow acceleration and deceleration with the brake off, as described in greater detail in (17). Seventeen fractions of 1.0 ml were removed by pipetting. The densities of fractions were determined from duplicate blank gradients in the same rotor, using an Abbe 3L refractometer. An aliquot of each fraction (0.05 ml) was incubated with (A) PC vesicles (0.25 mg) or (B) PC vesicles (0.25 mg) + HDL (0.125 mg protein); also, in (C) PC vesicles (0.25 mg) were incubated with HDL (0.125 mg protein). Incubations were conducted in 2 mM DTNB for 2 hr at 37°C. The % PC transfer was determined from incubation A and facilitated PC transfer from the difference B − (A + C).

in the plasma protein fraction. For example, incubations conducted with the very high density lipoprotein fraction (VHDL, d 1.19–1.27 g/ml) obtained by prolonged preparative ultracentrifugation in the 40.3 rotor (total of 144 hr at 40,000 rpm) showed only one-third of the facilitation activity of the same density fraction prepared by vertical rotor ultracentrifugation of an equivalent amount of plasma; following the VHDL preparation about two-thirds of the facilitation activity was present in the d > 1.27 g/ml fraction. Recovery of facilitation activity in the vertical rotor d 1.20–1.26 g/ml fraction suggests that the activity resides in a protein-rich lipoprotein. The redistribution of the activity in fixed angle rotors points to dependence on an apolipoprotein that may dissociate from the lipoprotein during prolonged ultracentrifugation.

To determine the size of the particles bearing the facilitation activity, plasma was subjected to vertical rotor ultracentrifugation and the fraction containing highest facilitation activity (d ~ 1.24 g/ml) was analyzed by chromatography on 6% agarose. Equal volume aliquots of each fraction of column eluate were incubated with PC vesicles or PC vesicles + HDL (**Fig. 3**). When incubated with vesicles alone, fractions eluting just ahead of the peak of plasma proteins (circles) showed greatest PC transfer into HDL (triangles). By contrast, incubation with vesicles plus HDL showed that the greatest facilitated PC transfer (squares) occurred in fractions eluting considerably further ahead of the plasma proteins. The elution volume of the fraction showing maximum PC uptake from vesicles alone was consistent with a particle diameter of 7.3 ± 0.3 nm (mean ± SEM, n = 4), while the elution volume of the peak of facilitation activity indicated particles of mean diameter 10.2 ± 0.5 nm. The average composition of the fractions eluting under the peak of facilitation activity (fractions 26–28) was 78% protein, 16% phospholipid, 5% cholesteryl ester, and 1% cholesterol, with no detectable triglycerides. Thus, the facilitation activity resides in a protein-rich, relatively dense lipoprotein. The differential elution of PC uptake and facilitation activity (Fig. 3) suggests that the d 1.20–1.26 g/ml fraction contains a heterogeneous population of particles with different properties.

Similar experiments were conducted to determine the distribution of facilitation activity in plasma. Chromatography of plasma showed that peak facilitation activity was present at an identical elution volume to that of the d 1.20–1.26 g/ml fraction. However, in plasma this was coincident with the peak of HDL cholesterol. Thus, the active fraction can be separated from HDL by centrifugation of plasma but not by agarose chromatography of plasma.

Characterization of HDL following facilitated phospholipid transfer

To determine if facilitated transfer of PC radioactivity into HDL was due to exchange or transfer of PC molecules, the time course of PC mass transfer into HDL was compared to that of PC radioactivity. HDL_3 (4 mg of protein) was incubated with 8 mg of PC vesicles, with or without the d 1.20–1.26 g/ml fraction (0.5 ml of fractions 12–13 from a gradient identical to that of Fig. 2). The percentages of vesicle PC radioactivity or mass showing *facilitated* transfer into HDL were, respectively, 0 min (−0.5%, 0%), 30 min (−0.3, 0.4), 60 min (1.2, 1.3), 90 min (2.5, 2.8), 120 min (3.3, 3.9), and 180 min (4.6, 3.0). Except for the final time point,

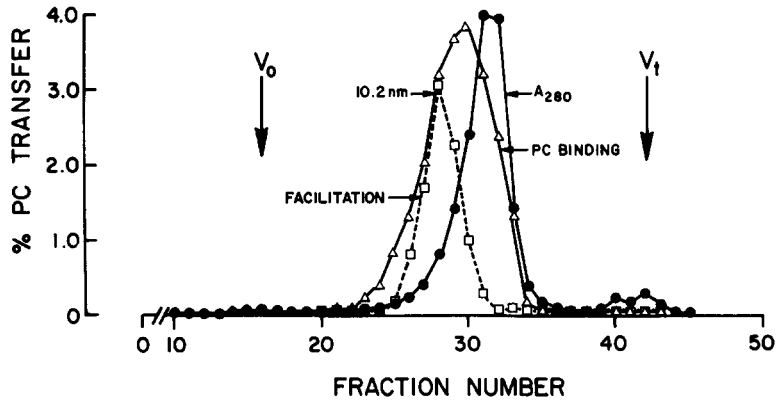

Fig. 3. Agarose gel chromatography of the d 1.24 g/ml plasma fraction showing PC transfer (△) and facilitated PC transfer (□) into HDL and the absorbance at 280 nm of each column fraction (●). Plasma was fractionated as described in Fig. 2, and 0.8 ml of the fraction showing greatest facilitated PC transfer was chromatographed on a 1.25 × 100 cm column of Sepharose CL-6B. The absorbance is shown on a linear scale with peak absorbance of 2.0 and lowest absorbance of 0. Two-ml fractions were collected and the % of PC radioactivity transferred into HDL was determined following incubation in 2 mM DTNB for 2 hr at 37°C of (A) 0.3 ml of each fraction + 0.4 mg PC; (B) 0.3 ml of each fraction + 0.4 mg PC + 0.25 mg HDL; or (C) 0.4 mg PC + 0.25 mg HDL. The % PC transfer was determined from (A) and facilitated PC transfer from (B) − (A + C). V_o and V_t show the void and total column volumes.

the time course of facilitated PC mass transfer was similar to that of facilitated transfer of PC radioactivity, indicating facilitation of net transfer of PC molecules by the d 1.20–1.26 g/ml fraction.

In further experiments the composition of HDL was determined following incubation with PC vesicles or PC vesicles plus the d > 1.19 g/ml fraction (**Table 1**). Facilitated PC transfer was associated with a markedly increased content of phospholipid in HDL, with a small decrease in total protein mass. SDS polyacrylamide gels of the HDL proteins showed no discernible change in protein composition associated with facilitated PC transfer (not shown).

To assess size changes in HDL, polyacrylamide gradient gel electrophoresis was performed on holo-HDL (d 1.063–1.190 g/ml) after incubation with PC vesicles or PC vesicles plus an aliquot of the d > 1.19 g/ml fraction. The scans of the gradient gels are shown in **Fig. 4**, where the control HDL preparation is shown as a solid line. The control HDL showed major peaks of diameter 9.2 nm (HDL_{3a}) and 10.8 nm (HDL_{2b}) and lesser peaks of diameters 8.1 (HDL_{3b}) and 7.6 nm (HDL_{3c}) (see reference 13 for a detailed description of these HDL subclasses). Incubation of holo-HDL with PC vesicles resulted in an increase in diameter of the major HDL peak to 9.5 nm, with smaller increases in size of all of the other HDL subfractions. In the presence of the d > 1.19 g/ml fraction there was a pronounced further increase in size of all of the HDL subfractions, with or without DTNB in the incubation. The size of the major peak of HDL after facilitated PC transfer was 10.0 to 10.2 nm, resembling that reported previously for HDL_{2a} (13). It is evident that facilitated PC transfer resulted in a major increase in mass of both HDL_2 subfractions, with loss of mass of HDL_3 subfractions (Fig. 4).

To determine the density changes resulting from fa-

TABLE 1. Composition of HDL after PC enrichment[a]

Incubation	Pro[b]	PL[b]	Chol[b]	Chol Esters[b]	TG[b]
HDL	0.43 (53)	0.17 (21)	0.016 (2)	0.16 (20)	0.03 (4)
PC + HDL_3	0.31 (40)	0.25 (34)	0.002 (0.3)	0.16 (22)	0.03 (4)
PC + HDL_3 + d > 1.19	0.30 (31)	0.49 (49)	0.013 (1.3)	0.14 (16)	0.03 (3)
PC + d > 1.19	0.03	0.10	N.D.	0.01	N.D.
PC	N.D.[c]	0.03	N.D.	N.D.	N.D.

[a] Results are presented in mg/dl; the values in parentheses are % compositions. The incubations contained HDL_3 (1.5 mg); HDL_3 (1.5 mg) + PC vesicles (5 mg); HDL_3 (1.5 mg) + PC vesicles (5 mg) + d > 1.19 g/ml fraction (0.5 ml); PC vesicles (5 mg) + d > 1.19 g/ml fraction (0.5 ml); PC vesicles (5 mg). Incubations were for 2 hr at 37°C in 2 mM DTNB, 0.15 M Tris, pH 8.0, in a final volume of 4 ml. Following the incubation, HDL (1.063–1.190 g/ml) was reisolated by preparative ultracentrifugation. The values shown are the mean results obtained by quadruplicate analysis of HDL samples, which were present in a total volume of 3 ml.

[b] Pro, protein (15); PL, phosholipids (10); Chol, cholesterol, determined by gas–liquid chromatography; Chol Esters, cholesteryl esters, measured by difference after hydrolysis and gas–liquid chromatography; TG, triglycerides (16).

[c] N.D., not detected.

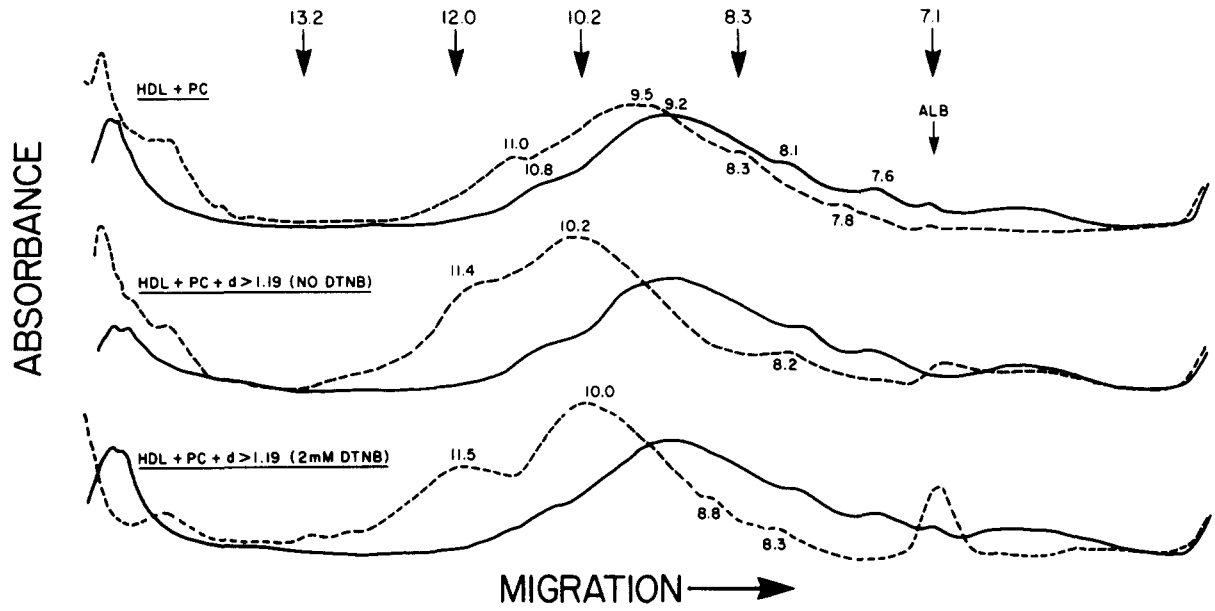

Fig. 4. Gradient gel scans of HDL showing changes associated with facilitated and nonfacilitated PC transfer. The profile of control HDL (4 mg) is shown as a solid line in all three sections. The dashed lines show the profiles resulting from incubation of HDL with PC vesicles (6 mg), PC vesicles (6 mg) + d > 1.19 fraction (1.5 ml), and PC vesicles (6 mg) + d > 1.19 fraction (1.5 ml) in 2 mM DTNB. The arrows indicate the positions of the protein standards (thyroglobulin, apoferritin, catalase, LDH, and albumin). Lipoprotein diameters (nm) are shown above their corresponding peaks. Samples were incubated for 2 hr at 37°C. Subsequently the samples were centrifuged for 48 hr at density 1.21 g/ml in a 40.3 rotor. The top 2 ml was removed, dialyzed, and an aliquot containing 75 μg protein was analyzed by polyacrylamide gradient gel electrophoresis on 4/30% acrylamide gels. Samples were electrophoresed for 3,000 volt-hours. Gels were stained in Coomassie Blue and scanned at 590 nm in a Transidyne reflectance photodensitometer.

cilitated PC transfer, PC vesicles were incubated with HDL_3, with or without an aliquot of the d 1.20–1.26 g/ml fraction, then analyzed by density gradient ultracentrifugation in the SW50.1 rotor. In **Fig. 5** are shown the densities, phospholipid radioactivity, and cholesterol concentrations of the individual density gradient fractions. Incubation with PC vesicles resulted in uptake of PC radioactivity and a movement of the peak of HDL cholesterol from d 1.130 to 1.105 g/ml. In the presence of the d 1.20–1.26 g/ml fraction, there was about twice as much PC radioactivity incorporated into HDL and the peak of HDL cholesterol was moved to even lower density (about 1.095 g/ml). Similar results were obtained in four different experiments, with a significantly lower mean density of the HDL peak resulting from facilitated PC transfer (1.101 ± 0.003 g/ml) compared to nonfacilitated PC transfer (d 1.112 ± 0.002 g/ml, $P < 0.01$ by paired t test). Incubation of vesicles with the d 1.20–1.26 g/ml fraction resulted in transfer of a small amount of PC radioactivity into the HDL density region. The percentage of PC radioactivity incorporated into HDL (fractions 6–13) was 5.4% for HDL alone, 2.1% for the 1.20–1.26 g/ml fraction alone, and 11.6% for HDL + the d 1.20–1.26 g/ml fraction (Fig. 5). Thus, interaction of HDL and the d 1.20–1.26 g/ml fraction resulted in facilitated transfer of 4.1% of PC radioactivity. Determination of phospholipid mass of fractions 6–13 indicated that incubation with HDL + the d 1.20–1.26 g/ml fraction was associated with facilitated transfer of 3.2% of the phospholipid vesicle mass. These results indicate that facilitated PC mass transfer resulted in a shift of the HDL peak to lower density.

Partial purification of phospholipid transfer activity

Incubation of the vertical rotor d 1.20–1.26 g/ml fraction with egg PC vesicles resulted in transfer of activity to the vesicles. Vesicles were incubated with the vertical rotor fraction showing greatest facilitation activity, then were reisolated by chromatography on Sepharose CL6B and examined for facilitation activity (**Table 2**). When incubated with HDL, these vesicles showed approximately twice the transfer of PC radioactivity into HDL (4.5%) compared to control vesicles that had been incubated with saline (2.1%), showing transfer of facilitation activity from the d 1.20–1.26 g/ml fraction to the vesicles. Delipidation of the vesicles resulted in a mixture of proteins that retained the ability to facilitate PC transfer into HDL (Table 2). Under identical conditions the delipidated protein of HDL (d 1.063–1.210 g/ml) or purified apoA-I, apoA-II, apoA-IV, apoE, or albumin showed no facilitation activity.

In order to characterize the active component further, the d 1.20–1.26 g/ml fraction was subjected to chromatography on ConA-Sepharose (**Fig. 6**). Most of the protein eluted in the void volume. A small peak of retained material eluted after application of buffer containing 0.2 M 3-O-methyl-α-D-glucopyranose. Material eluting in the void volume showed greater PC binding

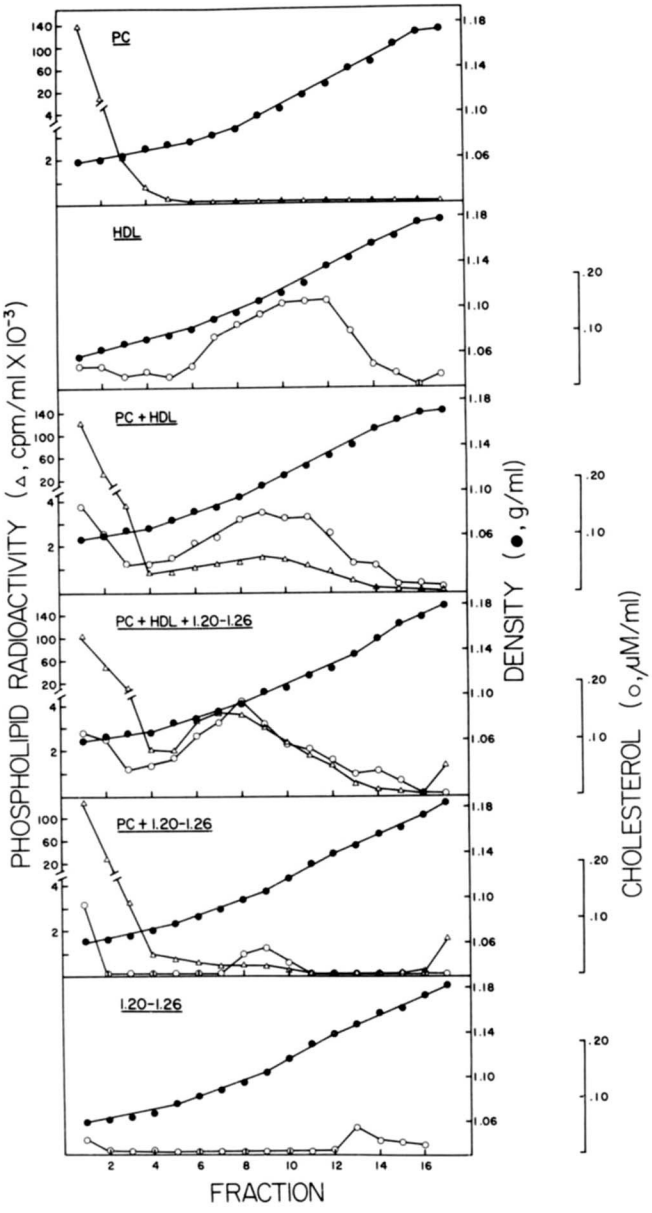

Fig. 5. Density gradient ultracentrifugation of HDL showing facilitated PC transfer as a result of incubation with the 1.20–1.26 g/ml fraction. From the top to the bottom panel, the incubations contained: 2.0 mg PC vesicles; 0.5 mg HDL_3 (d 1.100–1.210 g/ml); 2.0 mg PC vesicles + 0.5 mg HDL_3; 2.0 mg PC vesicles + 0.5 mg HDL_3 + 100 μl of d 1.20–1.26 g/ml fraction (obtained from a gradient identical to that shown in Fig. 2); 2.0 mg PC vesicles + 100 μl of d 1.20–1.26 g/ml fraction; 100 μl of d 1.20–1.26 g/ml fraction. Incubations were conducted in 3.0 ml of 0.15 M Tris-phosphate, pH 8.0, 2 mM DTNB, for 1 hr at 37°C in a metabolic shaker. Gradients were constructed by layering 1 ml each of solutions of densities 1.18, 1.14, 1.10, 1.06, and 1.03 g/ml; the sample was included in the middle three fractions, raised to appropriate density by addition of solid NaBr. Gradients were centrifuged for 72 hr at 49,000 rpm in a Beckman SW 50.1 rotor. Eighteen fractions of equal volume were removed from the tubes by pipetting and their density (4), radioactivity, and cholesterol (22) concentrations were determined.

activity (triangles) than that eluting after application of methylglucopyranose. However, facilitated PC transfer (open squares) was slightly greater for the retained fraction. Expressed as a specific activity, the retained fraction showed a 50- to 100-fold (n = 3) increase in facilitation activity (closed squares). The delipidated protein prepared from the retained and void volume fractions

TABLE 2. Effects of protein adsorbed to vesicles on transfer of ^{14}C-labeled PC into HDL

Incubation	% PC → HDL[a] A	B	C	Facilitation C − (A + B)
Vesicles with adsorbed protein (VP)	0.3	2.1	4.5	2.1
Delipidated protein (P)	0.2	1.8	5.4	3.4

[a] Percentage of PC radioactivity transferred into HDL after incubation for 2 hr at 37°C (means of triplicate determinations). All values are corrected for PC transfer of PC vesicles alone (0.1%).

Plasma was subjected to vertical rotor density gradient ultracentrifugation, and an aliquot of the fraction containing peak facilitation activity was incubated with PC vesicles, then chromatographed on Sepharose CL 6B. Fractions containing vesicles were pooled and used in incubations, or delipidated then used in incubations. Control PC vesicles (PC) were incubated with saline, chromatographed, and pooled. Incubations of vesicles with adsorbed protein (VP) contained: A) VP (1.0 mg phospholipid); B) 1.0 mg PC + 0.3 mg HDL; C) VP (1.0 mg) + 0.3 mg HDL. Incubations of delipidated protein (P) contained A) P (20 μg) + 1.0 mg PC; B) 1.0 mg PC + 0.3 mg HDL; C) P (20 μg) + 0.3 mg HDL + 1.0 mg PC.

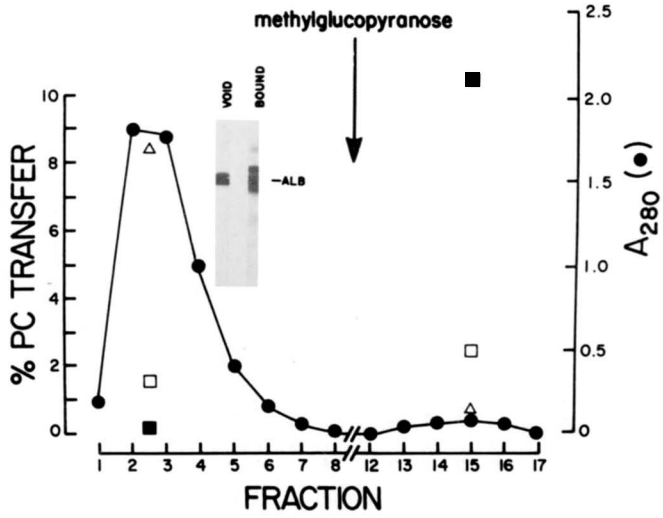

Fig. 6. Con A-Sepharose chromatography of the d ~ 1.24 g/ml plasma fraction, showing the absorbance at 280 nm of individual column fractions (●) and PC transfer (△) and facilitated PC transfer (□) into HDL and specific activity of facilitated PC transfer (■) (% PC transfer/0.1 mg protein) displayed by pools of void and retained fractions. Plasma was analyzed by density gradient ultracentrifugation in the SW 50.1 rotor as reported (4), and 0.3 ml of the fraction showing peak facilitation activity was dialyzed into buffer A (see Methods) and applied to ConA-Sepharose. After washing with approximately five bed volumes, buffer A containing 0.2 M methylglucopyranose was applied. Transfer of PC radioactivity and facilitated transfer of PC radioactivity into HDL were determined using one-sixth of the volume of fractions 2–5 (void) and one-sixth of fractions 12–17 (retained). Incubations were conducted as indicated in the legends to Fig. 2 and 3, using 0.5 mg of PC and 0.3 mg of HDL. The inset shows SDS polyacrylamide gels of 10 μg of the delipidated protein of the void and bound fractions. ALB shows the mobility of an albumin standard.

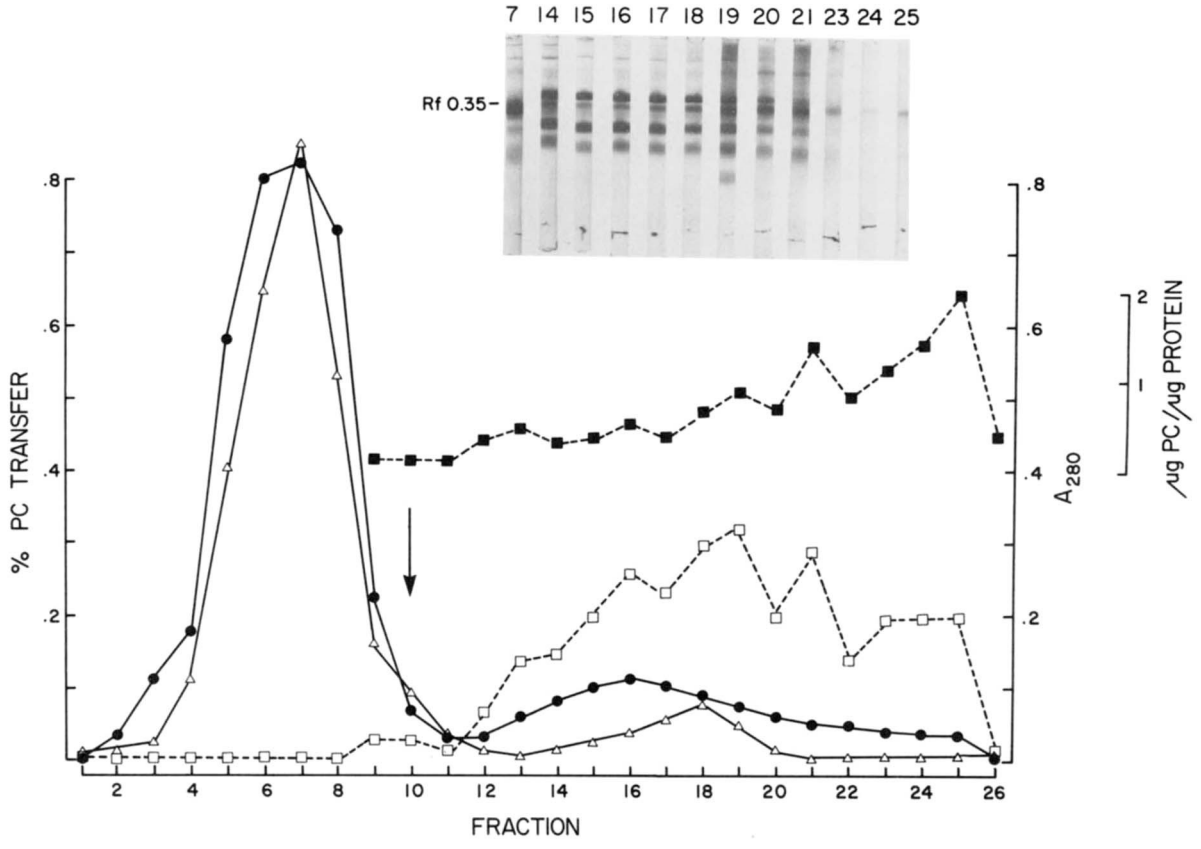

Fig. 7. Heparin-Sepharose chromatography of the fraction eluting from the phenyl Sepharose column with application of water, showing A_{280} (●), PC transfer into HDL (△), facilitated PC transfer into HDL (□), specific activity of facilitated PC transfer into HDL (■), and SDS polyacrylamide gels of some individual column fractions. The phenyl Sepharose fraction was dialyzed for 2 hr against distilled water, pH raised to 8.0 by addition of NH_4OH, then applied to the heparin-Sepharose column. After elution of the void volume peak a linear gradient was begun using 110 ml of distilled water, pH 8.0, as start buffer and 110 ml of 100 mM NaCl, pH 8.0, as the end buffer. The PC transfer and facilitated PC transfer were determined as described in Methods, using 0.5 ml of each fraction. The fraction volume was 7 ml and the flow rate 30 ml/hr. A total of 40 fractions was collected; only fractions containing protein are shown.

also showed facilitation activity, with about 100-fold greater specific activity of the former. The proteins present in the ConA fractions were analyzed by SDS polyacrylamide gel electrophoresis (inset to Fig. 6). The high specific activity fraction retained by the ConA column showed three major bands of apparent molecular weight 97,000, 80,000, and 63,000; all stained positively with periodic acid-Schiff. These results are similar to those described for cholesterol ester exchange protein (12).

During attempts to purify the protein responsible for facilitation activity, it was found that several steps (ConA Sepharose chromatography and adsorption of protein to vesicles) were associated with poor recovery of activity (less than 50%). By omission of low recovery steps and by reference to the earlier experience with purification of the cholesteryl ester exchange protein (6, 7), the following purification scheme was devised: vertical rotor ultracentrifugation, phenyl Sepharose (6), heparin Sepharose, and carboxymethylcellulose chromatography (6). Since dialysis and storage were found to be associated with loss of activity, this scheme employed minimum dialysis and storage and was completed in 48–72 hr. The vertical rotor d 1.20–1.26 g/ml fraction was prepared from 80–320 ml of plasma using the TV 850 vertical rotor; a scaled-up procedure was used to increase the amount of plasma that could be processed (see Methods). This fraction was applied directly to a phenyl Sepharose column, eluted with 0.15 M NaCl and then with distilled water. About half of the protein and almost all of the facilitation activity eluted following application of distilled water. However, if elution with 0.15 M NaCl was prolonged (12 hr), it was necessary to apply 5% ethanol–95% water to elute facilitation activity. The fraction showing facilitation activity also displayed intrinsic ability to transfer PC into HDL.

The fraction eluted from the phenyl Sepharose column with distilled water was applied to a heparin-Sepharose column. Following elution of the void volume peak, a linear gradient was begun (arrow in **Fig. 7**) from

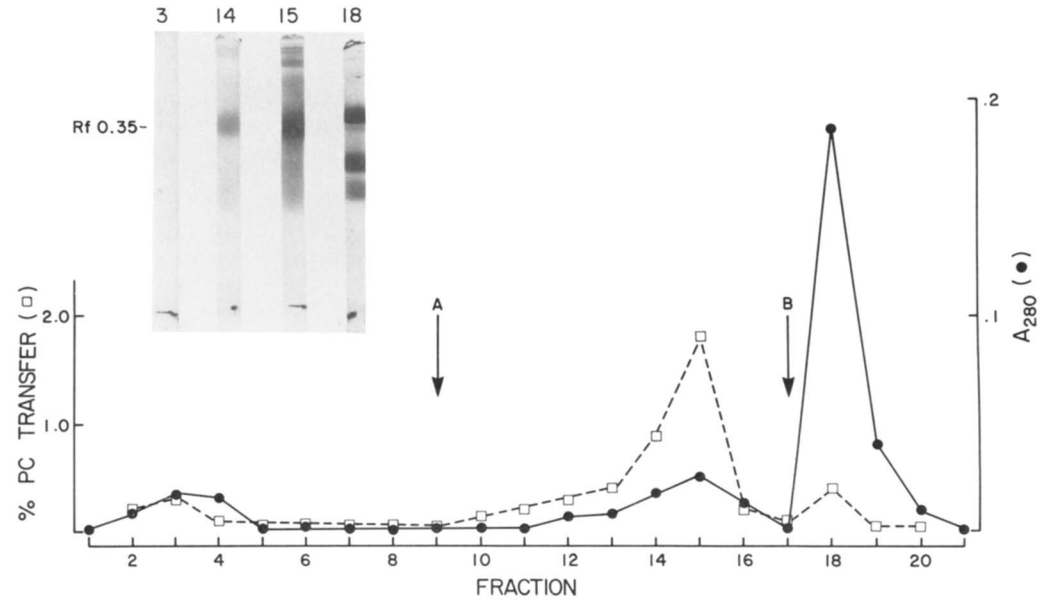

Fig. 8. Carboxymethyl cellulose chromatography of the retained heparin-Sepharose fraction (Fig. 7), showing A_{280} (●), facilitated PC transfer into HDL (□), and SDS gels of some individual column fractions. The retained fraction from the heparin-Sepharose column was dialyzed against 50 mM Na acetate, pH 4.5, and applied to the carboxymethylcellulose column. After the A_{280} had returned to baseline, 90 mM NaCl, 50 nM Na acetate, pH 4.5, was applied (at A) then 400 mM NaCl, 50 mM Na acetate, pH 4.5 (at B). Facilitated PC transfer was determined as described in Methods using 1.0 ml per fraction. SDS gels are designated by their fraction number; gels 15 and 18 contained approximately 20 μg and 30 μg protein, respectively.

distilled H_2O to 100 mM NaCl, pH 8.0. Most of the protein eluted in the void volume. A small peak was eluted with the gradient, between about 10–80 mM NaCl. Following completion of the gradient, 1.5 M NaCl, pH 8.0, was applied, without elution of further protein. Assays of individual gradient fractions showed that most of the PC binding activity was present in the void volume fraction, while the peak of facilitation activity eluted slightly after the peak of retained protein. The specific activity of facilitated PC transfer (closed squares in Fig. 7) was greatest in fractions 21–25, reaching peak values of about 1–2 μg ^{14}C-labeled PC/μg protein. SDS-polyacrylamide gels are shown for individual fractions of the heparin-Sepharose profile (Fig. 7). Fraction 16, with highest protein content in the retained peak, showed major bands of proteins of R_f 0.32, 0.35, 0.46, and 0.57. Proceeding to fractions of higher specific activity of facilitation, the band of R_f 0.35 appeared more prominent.

Fractions of the retained peak of the heparin-Sepharose column (fractions 12–25) were pooled and dialyzed into 50 mM Na acetate, pH 4.5, and then applied to a carboxymethylcellulose column which had been equilibrated with the same buffer. A small peak eluted in the void volume (**Fig. 8**). When the A_{280} had returned to baseline, 50 mM Na acetate, 90 mM NaCl was applied (Fig. 8 at A); the same buffer containing 400 mM NaCl was then applied (Fig. 8 at B). Almost all of the facilitation activity (dashed line, Fig. 8) was eluted following application of buffer containing 90 mM NaCl. SDS gels of individual fractions showed that the fractions with highest facilitation activity contained a major band of protein of R_f 0.35, and several other broad, poorly defined bands of lower and higher molecular weights. Scans of gels 14 and 15 showed peaks of chromogenicity corresponding to R_f values of 0.35, 0.46, and 0.57. The molecular weight calibration curve for Figs. 7 and 8 is shown in **Fig. 9**. The mobilities of the standard proteins (closed circles) and of the major bands of the most active fractions (open circles) are shown. The latter corresponded to apparent molecular weights of 81,000 (R_f 0.35), 56,000 (R_f 0.46), and 42,000 (R_f 0.57).

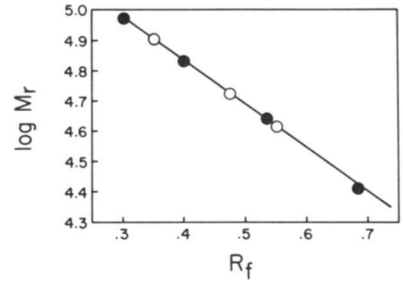

Fig. 9. Molecular weight calibration curve for gels shown in Figs. 7 and 8. The closed circles show the mobilities of the standards (phosphorylase B, albumin, ovalbumin, and chymostrypsinogen), while the open circles show the positions of the three major peaks present on a scan of gel 15 (Fig. 8).

TABLE 3. Purification scheme for phospholipid transfer activity[a]

	Protein	Facilitation Activity	Specific Activity	Purification	Recovery
	mg	*μg [^{14}C]PC*	*μg [^{14}C]PC/μg protein per hr*	×	%
Plasma	5000	7143[b]	0.00143		100
1.20–1.26	115	9300	0.081	57	130
Phenyl Sepharose	48	5732	0.12	84	80
Heparin Sepharose	4.2	4242	0.14–2.1[c]	98–1469[c]	59
CM Cellulose	0.2	1200	4.0	2800	17

[a] Results shown are for a typical preparation.
[b] Estimated from data in (4).
[c] Range of values for active fractions of the NaCl gradient.

The purification scheme is summarized in **Table 3.** The specific activity of the most purified fraction was about 4 μg ^{14}C-labeled PC/μg protein per 60 min, with recovery of about 0.2 mg of protein. This represented approximately 2,800-fold purification. However, this estimate must be considered semiquantitative since linearity of the assay with respect to time and amount of material was not established at each stage of purification.

Characterization of partially purified phospholipid transfer protein

Using material purified by the method shown in Table 3, the products of facilitated transfer into HDL_3 were analyzed by density gradient ultracentrifugation (**Fig. 10**). These experiments showed that facilitated PC transfer into HDL was associated with a more pronounced shift of the HDL peak to lower density. The peak of PC radioactivity was at d 1.102 g/ml, compared to d 1.118 g/ml for nonfacilitated PC transfer. Also the mass of HDL protein and cholesterol (not shown) were moved towards lower density. Incubation of HDL with PC vesicles resulted in an increase of 55 μg PC (2.2% of vesicle mass) in the HDL peak. In the presence of HDL + transfer protein the increase was 125 μg (5% of vesicle mass); for PC vesicles and transfer protein, 6 μg of PC was transferred into the HDL density region. Thus, transfer protein caused facilitated transfer of 64 μg (2.7%) of vesicle PC into HDL. By comparison, there was 2% facilitated transfer of PC radioactivity. In other experiments (not shown), incubation of HDL with transfer protein alone resulted in no change in density or phospholipid mass of the HDL peak.

To determine if facilitated mass transfer of PC into HDL was associated with reciprocal transfer of other HDL lipids into vesicles or discs, the lipids of fractions 1–6 (Fig. 10) were analyzed by quantitative thin-layer chromatography (16). Using 200 μg of total lipid, less than 0.5 μg each of cholesteryl esters, triglycerides, and fatty acids were found in fractions 1–6 of all gradients. Incubation of PC vesicles with HDL resulted in transfer of 30 μg of cholesterol from HDL into fractions 1–6; in the presence of transfer protein only 15 μg of cholesterol was transferred into fractions 1–6. Thus, facilitated PC transfer into HDL was not associated with reciprocal transfer of HDL lipids to vesicles.

In further experiments the most purified fraction (Table 3) was examined for its ability to stimulate transfer of cholesteryl esters from HDL to LDL. HDL (0.13 mg of protein) and LDL (0.25 mg of protein) were incubated for 3 hr at 37°C in the presence of 28 μg of transfer protein. This resulted in facilitated transfer of 28 μg of cholesteryl esters from HDL to LDL, giving a specific activity of 1 μg of cholesteryl ester transferred/μg protein per 3 hr. When ^{14}C-labeled PC vesicles were incubated with HDL containing ^{3}H-labeled cholesteryl esters in the presence of transfer protein, there was facilitated transfer of PC into d > 1.063 g/ml fraction, as expected. However, in these experiments the ^{3}H/^{14}C ratio of the d < 1.063 g/ml fraction was unaltered by facilitated PC transfer, indicating lack of reciprocal transfer of cholesteryl esters from HDL to vesicles.

DISCUSSION

We have shown an increased rate of transfer of PC from vesicles into HDL, resulting from addition of the d > 1.21 g/ml fraction. Importantly, this activity was shown to enhance transfer of PC mass into HDL, resulting in formation of larger, less dense particles (Figs. 4, 5, 10). The major lipoprotein product of facilitated PC transfer into HDL_3 had similar size and density to "HDL_{2a}" (13). However, gradient gel electrophoresis (Fig. 4) showed that facilitated PC uptake by holo-HDL was associated with size increases of all of the subclasses of HDL. There were major increases in amounts of larger HDL_{2b} and HDL_{2a} and decreases in HDL_3 subclasses. A very similar change in distribution of HDL

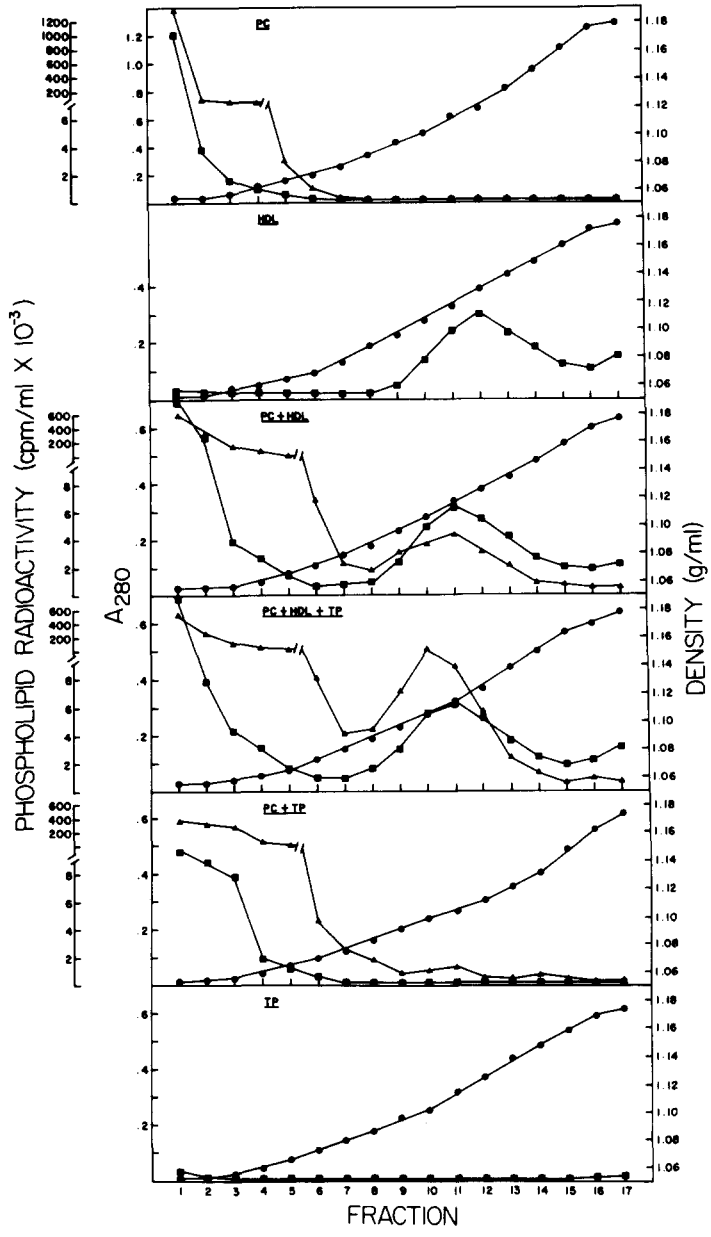

Fig. 10. Density gradient ultracentrifugation of HDL showing facilitated PC transfer as a result of incubation with partially purified transfer proteins. The symbols indicate PC radioactivity (▲, cpm/ml gradient × 10^{-3}); A_{280} (■); and densities (●). From the top to the bottom panels the incubations contained: 2.2 mg PC vesicles; 1.0 mg HDL_3 (1.100–1.190 g/ml); 2.2 mg PC vesicles + 1.0 mg HDL_3; 2.2 mg PC vesicles + 1.0 mg HDL_3 + 25 μg transfer protein; 2.2 mg PC vesicles + 25 μg transfer protein; 25 μg transfer protein. Transfer protein was isolated as described in Table 3. Incubations were conducted in 3.0 ml of 0.15 M Tris-phosphate, pH 8.0, for 1 hr at 37°C. Gradients were constructed by layering 1 ml each of solutions of densities 1.18, 1.14, 1.06, and 1.02 g/ml, then centrifuging for 72 hr at 49,000 rpm in a Beckman SW 50.1 rotor.

subclasses occurs during alimentary lipemia (17). These changes are thought to result from transfer of chylomicron PC into HDL (17). Thus, by analogy, the physiological transfer of PC into HDL may be facilitated by a lipid transfer activity.

The density distribution of the facilitation activity (d 1.20–1.26 g/ml) suggests its association with a lipoprotein particle. Paradoxically, although the activity resided in protein-rich, dense particles, their elution volumes indicated a relatively large apparent Stoke's diameter (10.2 nm), similar to that of centrifugally isolated HDL_2. Thus, the structure of these particles may deviate from the paradigm of lipoprotein structure, which on the basis of density and composition would

predict a smaller particle size (18). Alternatively, the particles may have anomalous elution on agarose gel, perhaps as a result of high glycoprotein content. The particles bearing facilitation activity were isolated with a minimum of ultracentrifugation (3 hr) and were found at an identical elution volume upon agarose chromatography of plasma or the d 1.20–1.26 g/ml fraction. Thus, it seems unlikely that they were formed as an ultracentrifugal artefact.

Protein mixtures derived from the d 1.20–1.26 g/ml fraction were able to facilitate PC transfer into HDL. Omitting steps that were associated with substantial loss of activity, we developed a relatively rapid and simple purification scheme that was associated with a progressive increase in specific activity of facilitated ^{14}C-labeled PC transfer (Table 3). The most purified preparation had a specific activity of about 4 μg of ^{14}C-labeled PC/μg protein per hr, representing approximately 2800-fold purification relative to plasma, with about 17% recovery at the final step of purification (Table 3). The most purified preparation contained a major component of apparent molecular weight 81,000 and several other components of lower molecular weight. However, in view of the presence of several bands on SDS gels of the most pure fraction (Fig. 8), phospholipid transfer activity cannot be unambiguously assigned to a particular protein at this stage of purification.

Several steps in our purification scheme were based on the earlier purification of a plasma cholesteryl ester exchange protein (7, 12). Pattnaik et al. (12) have isolated a protein mixture containing cholesteryl ester exchange activity by phenyl Sepharose, carboxymethylcellulose, and ConA Sepharose chromatography of the plasma d > 1.25 g/ml fraction. Using a similar procedure, Ihm et al. (7) obtained a fraction that caused equimolar exchange of PC and cholesteryl esters between LDL and HDL. Recently, this group (19) has shown that both lipid exchange activities show parallel salt inhibition, temperature inactivation, and pH dependence. These activities were associated with two major bands on SDS gels, of apparent molecular weights 63,000 and 58,000. Points of similarity between our observations on PC transfer activity and the earlier reports are association of the activity with a lipoprotein complex of similar apparent size to HDL (6, 7), binding to ConA-Sepharose, (7, 12), and elution from phenyl Sepharose and carboxymethylcellulose by similar buffers (7, 12). Furthermore, our most pure fraction was found to stimulate cholesteryl ester exchange between HDL and LDL. Thus, there is a possibility that cholesteryl ester and PC exchange/transfer are mediated by the same protein. However, rat plasma shows high exchange activity for PC, but little for cholesterol esters (19). We have also found that fractions prepared from the d > 1.21 g/ml fraction of rat plasma are potent in stimulating facilitated PC transfer from vesicles to HDL, but do not facilitate cholesteryl ester exchange.[2] Thus, the rat transfer protein may have different specificity to its human counterpart, or cholesteryl ester and PC exchange may be mediated by different proteins. It is also possible that PC exchange and transfer are enhanced by different plasma proteins.

There are several potential mechanisms of protein-facilitated lipid transfer. Wirtz and co-workers (20) have isolated a phosphatidylcholine-specific exchange protein from bovine liver. This protein promotes PC exchange between lipid surfaces by shuttling single PC molecules through the aqueous compartment, exchanging a bound PC molecule for one in the lipid surface. Since the protein's hydrophobic binding site is always occupied by a PC molecule, net mass transfer does not occur. In our experiments the facilitated net transfer of PC molecules from vesicles to HDL was not associated with reciprocal transfer of other lipids. Thus it appears that the plasma PC transfer protein does not operate by a shuttle-exchange mechanism. In view of the binding of transfer protein to PC vesicles (Table 1, Fig. 10) it is possible that the mechanism of action involves alteration of the properties of the vesicle surface, promoting fusion of vesicles with HDL or desorption of individual PC molecules into the aqueous phase with subsequent transfer to HDL. In the absence of transfer protein, PC exchange between vesicles and HDL shows second order kinetics, suggesting a bimolecular process involving collision between HDL and vesicles (21). Thus, we suggest that the plasma PC transfer protein alters the vesicle surface in a way that promotes PC uptake during collision with HDL. Similar alterations in the surface properties of chylomicrons or VLDL may be involved in promoting transfer of surface lipids into HDL during lipolysis.

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