Interrelationship between rat serum very low density and high density lipoproteins

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Abstract An exchange of phospholipids and certain peptides among various classes of rat serum lipoproteins has been demonstrated and its nature has been investigated. [^{32}P]-Phospholipid from isolated VLDL, prepared in vivo, was transferred to HDL, and to a much lesser extent to LDL, in vivo and in vitro. This difference between HDL and LDL can be abolished by ultracentrifugation of the serum at d 1.21. Unlabeled VLDL acquired [^{32}P]phospholipid from HDL of serum. Phospholipid associated with the α-lipoprotein component of VLDL exchanged more readily than that associated with the β-lipoprotein component of VLDL. Generally, the phospholipid species exchange in proportion to their distribution in the lipoproteins.

Radioactivity from ^{3}H-labeled protein of VLDL was transferred to HDL while HDL ^{3}H-labeled protein in serum was transferred to VLDL during a 20-min incubation. LDL was not involved in the transfer of protein. Protein associated with the α-lipoprotein component of VLDL exchanged more readily than that associated with β-lipoprotein component. Analysis of tritiated apoproteins of VLDL and HDL by polyacrylamide gel electrophoresis revealed that three of the six peptide bands of apo-VLDL exchanged between VLDL and HDL.

The data raise the possibility that intact subunits of the VLDL and HDL are being exchanged.

Supplementary key words phospholipids · apolipoproteins · low density lipoproteins · gel electrophoresis

PHOSPHOLIPIDS make up about 20% of each of the various classes of serum lipoproteins. It has been known for many years that the phospholipids exchange between the α- and β-lipoproteins in vivo (1) and in vitro (2–4). This has discouraged studies on the secretion and turnover of phospholipids in the various lipoprotein fractions. Eder, Bragdon, and Boyle (4) noted that the exchange is extremely rapid in vitro, but had not reached equilibrium after 21 hr. This observation suggested to us that the different classes of lipoproteins might exchange phospholipids at different rates. In view of the fact that the β-lipoproteins are now divided into VLDL and LDL fractions, and considerably more is known about the various apoproteins in these lipoproteins, the exchange of phospholipids among lipoproteins was reinvestigated. Preliminary studies have led us to the conclusion that, in addition to an exchange of phospholipids among each of the lipoprotein classes usually separated by ultracentrifugation, there is also a movement of both phospholipid and certain peptides of the apoproteins between VLDL and HDL (5). More complete data supporting these conclusions will be presented in this communication.

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; LP, lipoproteins.

METHODS

Serum was obtained from male hooded rats weighing between 250 and 350 g. The animals were fasted for 25 hr prior to being killed, but they were allowed water ad lib. Labeled serum phospholipids were obtained by injecting 8-hr-fasted rats intraperitoneally with 2 mCi of $Na_3{}^{32}PO_4$. The animals were exsanguinated 16 hr after the injection, when incorporation of ^{32}P into liver phospholipids is maximal (6). In order to prepare lipoproteins labeled in the protein moiety, [1-^{14}C]leucine or a mixture of ^{3}H-labeled amino acids was injected into the jugular bulbs of the fasting animals. The animals were bled 60 min later (7) by heart puncture, the blood was allowed to clot, and the serum was separated. Serum lipoproteins were isolated by a modification of the method of Havel, Eder, and Bragdon (8), using an IEC B-60 preparative ultracentrifuge with an SB-283 rotor at 14°C and 33,000 rpm. VLDL were centrifuged at a density of 1.006 for 18 hr, LDL at a density of 1.063 for 24

hr, and HDL at a density of 1.21 for 48 hr. In each case, the top 1.2 ml in the 12-ml centrifuge tube was taken for analysis. VLDL were separated into α- and β-lipoprotein-containing components by the heparin–Ca^{2+} precipitation technique of Kook, Eckhaus, and Rubinstein (9).

Lipoprotein lipid was extracted by the method of Folch, Lees, and Sloane Stanley (10). To remove contaminating inorganic [^{32}P]phosphate from the lipid extract, the lower (chloroform) phase was washed three times with upper phase solvents containing 1% inorganic phosphate and twice with upper phase without added phosphate. Lipid phosphorus was assayed by first digesting the purified lipid extract with sulfuric acid. The resulting inorganic phosphate was then determinated by the method of Fiske and SubbaRow (11). Phospholipids were separated by thin-layer chromatography on silica gel H, using chloroform–methanol–acetic acid–water 50:28:10:5 as a developing system. The phospholipids were eluted with chloroform–methanol–NH_4OH 1:3:1, giving a recovery of 96%. These phospholipids were estimated by the method of Bartlett (12). The radioactive lipid was counted in a scintillation counter using a toluene-based scintillation solution.

After incubation of serum or lipoprotein preparations containing isotopically labeled peptides in the experiments described in Results, the lipoproteins were isolated, washed by agitation with Krebs-Ringer solution at 37°C for 20 min, and then reisolated by ultracentrifugation. The purified lipoproteins were then dialyzed for 24 hr against isotonic saline containing 0.1% EDTA, with two changes of the dialysis fluid to remove any remaining radioactive amino acid contamination. The isolated lipoproteins containing the labeled protein moiety were dissolved in a dioxane-based scintillation fluid prior to estimation of the radioactivity. To determine the radioactivity in the apoproteins of the VLDL and HDL, the lipoproteins were delipidated by extraction with ethanol–ether 3:1 at −10°C for 18 hr. Less than 1% of the phospholipid remained associated with apoproteins after the delipidation. Several experiments were also carried out using the delipidation procedure of Bersot et al. (13), but the extra extractions had no effect on the patterns of electrophoresis or on the results. The precipitated peptides were then dissolved in a solution of 7 M urea in Tris–glycine buffer, pH 8.9, containing 25% sucrose, for at least 24 hr and separated by disc gel electrophoresis on a 13% polyacrylamide gel at 4°C according to the procedure of Reisfeld and Small (14). The gel was made up in 7 M urea in 0.12 M Tris buffer, pH 8.0. The gels were stained with 1% amido black in 7% acetic acid and destained by shaking in 5% acetic acid for 2–3 days with at least four changes of acetic acid. This procedure removes any remaining labeled free amino acids. The bands on the gel were cut out and combusted on a Packard sample oxidizer, model 300. The resulting water was collected and counted in a dioxane-based scintillation fluid. Approximately 70%[1] of the radioactivity present in the VLDL protein and 95% of that present in the HDL protein before delipidation was recovered by this procedure. All counting was corrected for quenching by the use of an external standard.

$Na_3{}^{32}PO_4$ was obtained from Merck and Co., Montreal, Que., and the labeled leucine or amino acid mixtures from New England Nuclear, Cambridge, Mass.

TABLE 1. Redistribution of VLDL [^{32}P]phospholipid in vivo

Lipoprotein Fraction	Control[a]	Time after Injection of ^{32}P-labeled VLDL	
		5 min	20 min
	% of total VLDL radioactivity		
VLDL	90 ± 4	16 ± 3	17 ± 2
LDL	4 ± 1	8 ± 1	7 ± 2
d > 1.063	1 ± 1	67 ± 5	65 ± 6

Each figure is the mean ± SEM of four animals. Each animal received between 80,000 and 180,000 cpm of ^{32}P-labeled VLDL containing 15 μg of lipid P. Each animal received ^{32}P-labeled VLDL individually prepared in a different donor animal.

[a] Control represents VLDL reisolated at the same time as the serum from the injected animal was separated into the lipoprotein fractions.

RESULTS

The exchange of phospholipid among serum lipoproteins was first studied by the injection of VLDL containing [^{32}P]phospholipid into rats. 5 or 20 min after the injection the rats were exsanguinated, and the serum was separated into the VLDL, LDL, and d > 1.063 fractions. The data are shown in Table 1. Recentrifugation of uninjected ^{32}P-labeled VLDL indicated that 90% of the radioactivity remained in the VLDL fraction. However, 5 or 20 min after intravenous injection only about 17% of the radioactivity remained in the VLDL, with the majority of the [^{32}P]phospholipid being found in the d > 1.063 fraction. It will be noted that there was only a slight movement of [^{32}P]phospholipid into the LDL fraction.

A similar series of experiments was carried out in vitro. The VLDL-bound [^{32}P]phospholipid was incubated for 20 min with 5 ml of rat serum or Krebs-Ringer phosphate buffer, pH 7.4, containing 4% albumin, which served as the control. As shown in Table 2, after incubation with serum the radioactivity of phospholipids of the VLDL was halved, with the phospholipid being transferred

[1] Losses in radioactivity occurred during delipidation; all of the radioactive peptides remaining after removal of the lipids dissolved in the urea–Tris buffer and were completely (95–102%) recovered from the polyacrylamide gel bands. The missing radioactivity could be recovered in the delipidating mixture.

TABLE 2. Redistribution of VLDL [^{32}P]phospholipid in vitro

Lipoprotein Fraction	Incubation Medium: Ringer–Albumin	Incubation Medium: Serum	Relative Specific Activity[a]
	% of VLDL radioactivity added		
VLDL	90 ± 3	46 ± 4	100
LDL	3 ± 1	6 ± 1	12 ± 3
d > 1.063	3 ± 1	45 ± 3	31 ± 4

Each figure is the mean ± SEM of three experiments. Each experiment involved incubation of ^{32}P-labeled VLDL from a different rat with serum from a separate donor. Incubation conditions: each flask contained 5 ml of serum or 4% albumin in Krebs-Ringer phosphate buffer, pH 7.4, and ^{32}P-labeled VLDL containing 15–30 × 10^3 cpm in 15 μg lipid P. Incubation time, 20 min; gas phase, air; temp, 37°C.

[a] The specific activity (cpm/mg lipid P) of VLDL after incubation is taken as 100.

TABLE 3. Effect d > 1.21 fraction of serum on the redistribution of VLDL [^{32}P]phospholipid

Lipoprotein Fraction	Control[a]	Incubation Medium: d > 1.21 Serum + d < 1.21 Lipoproteins	Incubation Medium: Ringer–Albumin + d < 1.21 Lipoproteins	Relative Specific Activity[b]
	% of added VLDL radioactivity			
VLDL	96 ± 3	74 ± 4	81 ± 5	100
LDL	2 ± 1	7 ± 2	6 ± 3	10 ± 3
d > 1.063	2 ± 1	18 ± 3	12 ± 1	9 ± 2

Each figure is the mean ± SEM of three experiments. Incubation conditions: as in Table 2.

[a] Control: ^{32}P-labeled VLDL not incubated, but reisolated with the incubated samples.

[b] Relative specific activities with the two incubation media were similar. The d > 1.21 fraction contains about one-third of the total phospholipids in the d > 1.063 fraction.

primarily to the d > 1.063 lipoproteins. As was the case in the in vivo experiments, there was only a slight increase in radioactivity in the serum LDL. There was no significant redistribution of the [^{32}P]phospholipid in the incubated control. Thus, the transfer of phospholipid from VLDL to LDL and HDL was not a result of ultracentrifugation or the incubation of VLDL. The specific activity of the phospholipids after the incubation was determined, with that of the VLDL taken as 100. The relative specific activity of the phospholipids in the d > 1.063 serum proteins was considerably higher than that of the LDL.

Attempts to demonstrate transfer of [^{32}P]phospholipid from isolated ^{32}P-labeled HDL to VLDL or LDL were generally unsuccessful. This raised the possibility that a fraction of the serum not in the usual lipoprotein spectrum might be involved in the exchange of phospholipids. To check this, rat serum was centrifuged at d 1.21 to separate the total lipoproteins. The lipoprotein-free serum and the lipoprotein preparations were then dialyzed to remove NaBr. The d < 1.21 lipoproteins were incubated with either the equivalent amount of d > 1.21 serum, thereby reconstituting the serum, or with a Ringer–albumin solution. ^{32}P-labeled VLDL was added to the incubation mixture and the redistribution of the VLDL phospholipid was studied. The ^{32}P-labeled VLDL retained 96% of the radioactivity upon recentrifugation at d 1.006. The data shown in Table 3 indicated that the presence of serum proteins of d > 1.21 had no significant effect on the redistribution of phospholipids of the VLDL. However, the amount of [^{32}P]phospholipid noted in the d > 1.063 fraction of the serum sample incubated with serum proteins of d > 1.21 was considerably less than that found when ^{32}P-labeled VLDL were incubated with serum which had not been centrifuged at d 1.21 in NaBr and then dialyzed (cf. Table 2). As a result, the relative specific activities of the phospholipids in the LDL and the d > 1.063 serum are identical. The relative specific activity of the ^{32}P-labeled LDL was not affected by ultracentrifugation in NaBr and dialysis of the serum.

It was therefore necessary to study the ability of VLDL to acquire phospholipids from other lipoproteins by using whole serum. VLDL isolated from 18 ml of unlabeled serum were incubated with 5 ml of the serum containing [^{32}P]phospholipids. Another 5 ml of the serum incubated without additional VLDL served as the control. The data, shown in Table 4, indicate that in the presence of added unlabeled VLDL, the [^{32}P]phospholipid in this lipoprotein was doubled, having originated from the d > 1.063 fraction of the serum. There was no transfer of phospholipids from LDL to the VLDL.

The possibility must be considered that the movement of phospholipid between lipoprotein fractions may favor one or more specific phospholipids. To this end, VLDL containing [^{32}P]phospholipid were incubated for 20 min with serum, and the distribution of the radioactivity among the various phospholipids of each of the lipoprotein fractions was studied. The results are shown in Table 5. As was noted in Table 2, about 50% of the

TABLE 4. Effect of added VLDL on the distribution of [^{32}P]phospholipid among serum lipoproteins

Lipoprotein Fraction	Serum with [^{32}P]Phospholipid	Serum with [^{32}P]Phospholipid + VLDL	*P*
	% of total radioactivity		
VLDL	14 ± 1	28 ± 2	<0.01
LDL	10 ± 1	10 ± 1	N.S.
HDL	51 ± 2	40 ± 2	<0.01
d > 1.21	25 ± 1	20 ± 1	<0.02

Each figure is the mean ± SEM of four experiments. Incubation conditions: 2.0 ml of unlabeled VLDL in an NaCl solution, d 1.006, containing 150 μg of lipid P, or 2.0 ml of the d 1.006 NaCl solution added to 5 ml of serum containing 4–7 × 10^6 cpm of [^{32}P]phospholipid. Incubation time, 20 min; gas phase, air; temp, 37°C.

TABLE 5. Distribution of radioactivity in phospholipid fractions after incubation of VLDL [^{32}P]phospholipid with serum

	Incubated with Ringer–Albumin	Incubated with Serum			
	VLDL	VLDL	LDL	HDL	d > 1.21
Radioactivity (cpm/fraction)	321,500	152,000	15,000	85,000	28,500
Distribution of radioactivity (%)					
Lecithin	81	85	78	82	32
Lysolecithin	7	2	5	4	63
Sphingomyelin	8	9	15	9	3
Phosphatidylserine + phosphatidylinositol	2	2	1	3	2

Incubation conditions: as in Table 2. The data are typical of three such experiments.

radioactivity in the VLDL is lost on incubation with serum and recovered primarily in the d > 1.063 serum fraction. However, the incubation of the VLDL with serum did not significantly alter the distribution of the radioactivity among the various phospholipid fractions of the VLDL with the exception of lysolecithin, which migrated mainly to the d > 1.21 fraction of the serum. The compositions of the various phospholipid fractions of the rat serum lipoproteins were determined simultaneously and are shown in Table 6. In each lipoprotein the major phospholipid is lecithin. Lysolecithin is found primarily in the d > 1.21 fraction of the serum. The lysolecithin found in the various lipoproteins represents 21% of the total phospholipids. This corresponds to the value reported by Switzer and Eder (15) for rat serum, and suggests that washing of the lipid extract to remove inorganic [^{32}P]phosphate did not result in a loss of lysolecithin. Two points emerge from the combined data in Tables 4 and 5. Firstly, the percentages of the various phospholipids are similar to the distribution of the radioactivity in the VLDL. Thus, the injection of $Na_3{}^{32}PO_4$ 16 hr before isolation of the VLDL results in a fairly even labeling of the various phospholipid fractions in this lipoprotein. In addition, there is little difference between the distribution of either the radioactivity or percentage of lipid phosphorus between the VLDL and HDL. The LDL have somewhat less lecithin and more lysolecithin and sphingomyelin, with corresponding changes in the acquisition of [^{32}P]phospholipid after incubation. The results in Tables 5 and 6 indicate that the movement of phospholipid from VLDL to LDL and HDL did not involve one specific phospholipid, but, with the exception of lysolecithin, all moved, with minor variations, according to their content in the corresponding lipoprotein.

The observations that after incubation with ^{32}P-labeled VLDL the relative specific activity of HDL was greater than that of the LDL (Table 2), and that this difference could be abolished after ultracentrifugation of the serum at a high salt concentration (Table 3), raised the possibility that both a specific and a nonspecific exchange of phospholipids could be occurring. Thus, phospholipids associated with some of the peptides of the VLDL might exchange more readily. To test this, ^{32}P-labeled VLDL were incubated with serum or with Ringer–albumin solution. At the end of the incubation an aliquot of the reisolated VLDL was extracted and the phospholipids were counted; the remainder of the VLDL was divided into heparin-precipitable and heparin-soluble fractions, representing the β- and α-lipoprotein-containing components, respectively, of the VLDL (9). These data are shown in Table 7. After incubation of the ^{32}P-labeled VLDL with the Ringer–albumin solution, 95% of the

TABLE 6. Phospholipid content of rat serum lipoproteins

	VLDL	LDL	HDL	d >1.21
Phospholipid (μg lipid P/ml serum)	15[a]	15	36	17
Lipid phosphorus (%)				
Lecithin	82	71	78	21
Lysolecithin	7	9	6	76
Sphingomyelin	8	18	13	2
Phosphatidylserine + phosphatidylinositol	2	1	3	1
Phosphatidylethanolamine	2	2	1	

[a] Includes ^{32}P-labeled VLDL containing 1.5 μg of lipid P/ml added to the serum prior to incubation.

TABLE 7. Effect of serum on the distribution of VLDL [^{32}P]phospholipids among α- and β-lipoprotein-containing components of VLDL

Lipoprotein Fraction	Incubation Medium: Ringer–Albumin	Incubation Medium: Serum	P
	% of VLDL radioactivity added		
Intact VLDL	95 ± 5	51 ± 4	<0.01
Heparin precipitate (containing β-LP)	74 ± 3	45 ± 4	<0.01
Heparin supernate (containing α-LP)	18 ± 2	6 ± 1	<0.01
% of ^{32}P-labeled VLDL in heparin supernate	20 ± 1	12 ± 1	<0.01

Each figure is the mean ± SEM of four experiments. Incubation conditions: as in Table 2.

total radioactivity remained in the VLDL, 75% being heparin-precipitable and 18% heparin-soluble. Incubation of the ^{32}P-labeled VLDL with serum resulted in the loss of almost half of the radioactivity of the VLDL; the heparin precipitate retained 45% of the radioactivity, and the supernatant solution had only 6% of the [^{32}P]-phospholipid. As a result, the heparin supernate of the VLDL retained only 12% of the labeled phospholipid, compared with 20% in the absence of an exchange. This suggests that the phospholipid associated with the heparin-soluble components of the VLDL exchanges to a greater extent.

The more extensive exchange of the phospholipids associated with one group of peptides of the VLDL raised the possibility that peptides may also be exchanging. Therefore, VLDL containing ^{3}H-labeled peptides were prepared in vivo using a mixture of ^{3}H-labeled amino acids. The isolated ^{3}H-labeled VLDL were then incubated for 20 min with serum or Ringer–albumin solution. The incubation mixture was then separated into VLDL, LDL, and HDL and purified as described in Methods. The distribution of the radioactivity is shown in Table 8. In the presence of serum, there is a significant movement of radioactivity from VLDL to HDL. The incubation with serum had no effect on the appearance of ^{3}H-labeled protein in the LDL. This pattern resembles that seen when the phospholipids are labeled. After incubation, the ^{3}H-labeled VLDL were separated into the α- and β-lipoprotein-containing components. In the presence of serum, the recovery of labeled lipoprotein in the heparin supernate was markedly decreased, but there was no significant effect on the radioactivity in the heparin precipitate. This follows the pattern noted when [^{32}P]phospholipid was studied.

If one or more peptides are exchanging between the VLDL and the HDL, it should be possible to demonstrate that labeled HDL peptides can be transferred to the VLDL. The use of isolated HDL was avoided, since phospholipid studies had suggested that the exchange is inhibited by the isolation procedure (see Table 3). Therefore, serum from animals which had been injected with [1-^{14}C]-leucine or a ^{3}H-labeled amino acid mixture was used. Serum containing the labeled proteins was incubated for 20 min with unlabeled rat VLDL or the d 1.006 saline solution used to suspend the VLDL. The VLDL, LDL, and HDL were then isolated. An amount of unlabeled VLDL equal to that added to the experimental flask before the incubation was added to the VLDL isolated from the control flask. The isolated lipoproteins were then washed and reisolated. The results of this series of experiments are shown in Table 9. It will be noted that in the presence of additional VLDL there was a significant increase in the radioactivity of this lipoprotein fraction with a concomitant decrease in the radioactivity of the HDL. There was no change in the radioactivity of the LDL.

Like human lipoproteins, rat VLDL (16) and HDL (17) are made up of a number of peptides. Since the exchange involves a larger proportion of the labeled phospholipid and protein moieties of the α-lipoprotein- than the β-lipoprotein-containing component of the VLDL, the exchange might be restricted to specific peptides of the VLDL. The apoproteins of VLDL and HDL in rat have not yet been isolated, nor have their terminal amino acids been determined. To sidestep this problem, rat lipoproteins, containing ^{3}H-labeled peptides prepared as previously described, were delipidated and separated by electrophoresis on 13% polyacrylamide gel. Fig. 1 illustrates the polyacrylamide gel patterns obtained for the VLDL and HDL. The VLDL pattern resembles that reported by Mahley et al. (16), except that the bands in Fig. 1 are more separated, probably due to a combination of the lighter gel and longer rur ning time used in our experiments. This has resulted in the separation from band 2 of an additional band, number 3. Similarly, the HDL pattern shown in Fig. 1 resembles that shown by Koga, Horwitz, and Scanu (17) when allowance is

TABLE 8. Redistribution of VLDL ^{3}H-labeled protein

Lipoprotein Fraction	Incubation Medium: Ringer–Albumin	Incubation Medium: Serum	*P*
	% of VLDL radioactivity added		
VLDL	88 ± 4	74 ± 3	<0.02
LDL	8 ± 2	8 ± 2	N.S.
HDL	0.2 ± 0.1	10 ± 1	<0.01
VLDL:			
Heparin precipitate (containing β-LP)	74 ± 4	68 ± 6	N.S.
Heparin supernate (containing α-LP)	16 ± 2	6 ± 1	<0.01
% of ^{3}H-labeled VLDL in heparin supernate	22 ± 2	9 ± 1	<0.01

Each figure is the mean ± SEM of five experiments. Incubation conditions: as in Table 2. The VLDL ^{3}H-labeled protein contained 6–10 × 10^{3} cpm.

TABLE 9. Effect of additional VLDL upon the distribution of ^{3}H- or ^{14}C-labeled serum protein among lipoproteins

Lipoprotein Fraction	Substrate for Protein Labeling	Incubation Medium: Serum	Incubation Medium: Serum + VLDL	*P*
		% of total radioactivity		
VLDL	^{3}H-labeled amino acids	30 ± 2	44 ± 2	<0.01
	[1-^{14}C]leucine	28 ± 1	42 ± 3	<0.01
LDL	^{3}H-labeled amino acids	24 ± 1	24 ± 2	N.S.
	[1-^{14}C]leucine	30 ± 3	28 ± 3	N.S.
HDL	^{3}H-labeled amino acids	40 ± 2	31 ± 1	<0.01
	[1-^{14}C]leucine	37 ± 2	29 ± 2	<0.03

Each figure is the mean ± SEM of three experiments. Incubation conditions: as in Table 4. The serum containing ^{3}H-labeled protein had 3–5 × 10^{3} cpm, and that containing ^{14}C-labeled protein had 5–10 × 10^{3} cpm.

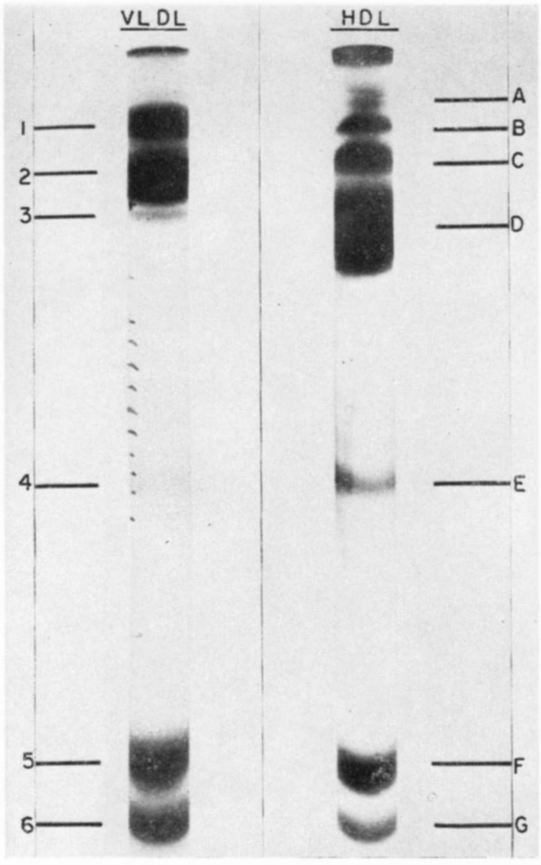

FIG. 1. Polyacrylamide gel electrophoretic patterns of rat apo-VLDL and rat apo-HDL.

made for the differences in percentage of polyacrylamide used. The VLDL and HDL peptide bands in Fig. 1 have been designated by numbers and letters, respectively. Inspection of the gel suggests that VLDL bands 1, 4, 5, and 6 have electrophoretic mobilities similar to HDL bands B, E, F, and G, respectively. Band 2 migrates to the same extent as C and D.

To determine which of the VLDL peptides exchange with HDL, purified VLDL containing peptides labeled with tritiated amino acids were incubated for 20 min with either serum or Ringer–albumin solution. After incubation, the VLDL and HDL were isolated, carrier VLDL were added, and the lipoproteins were purified as previously described, except that dialysis was unnecessary. The isolated lipoproteins were then delipidated, the apoproteins were separated by polyacrylamide gel electrophoresis, and the radioactivity of the peptide bands was determined. The results are shown in Table 10. In the absence of added serum, no radioactivity was found in the apo-HDL fractions. It will be noted that the incubation with serum resulted in a loss of radioactivity from bands 2, 5, and 6 and the appearance of ^{3}H-labeled peptides in apo-HDL bands of similar electrophoretic mobilities, C, F, and G. Apo-HDL band D showed only a small acquisition of radioactivity after incubation with labeled VLDL.

TABLE 10. Movement of VLDL ^{3}H-labeled peptides into HDL

VLDL				HDL	
	Incubation Medium				
Polyacrylamide Gel Band[a]	(1) Ringer–Albumin	(2) Serum	Ratio (2/1)	Polyacrylamide Gel Band[a]	Serum Incubation
	cpm/peptide				*cpm/peptide*
				A	—[b]
1	360	360	1.00	B	10
2	3600	3200	0.88	C	460
3	120	130	1.07	D	90
4	105	102	0.98	E	10
5	1090	750	0.69	F	400
6	1210	1050	0.86	G	360

Under HDL, only results of incubation with serum are shown, since no VLDL radioactivity is found in HDL in the absence of serum. Incubation conditions: as in Table 2. The pattern of results shown above is typical of three experiments.

[a] Band numbers or letters correspond to locations of the peptides shown in Fig. 1.

[b] Not significantly above background.

If the movement of peptides from VLDL to HDL represents an exchange, then it should be possible to demonstrate the transfer of the same HDL peptides into VLDL. To accomplish this, serum containing ^{3}H-labeled protein was incubated with unlabeled VLDL or an NaCl solution of d 1.006. The VLDL and HDL were isolated and carrier VLDL were added to the control; the lipoproteins were washed and delipidated, and the apoproteins were separated by polyacrylamide gel electrophoresis. The results are shown in Table 11. It will be noted that the presence of VLDL caused an increase in the radioactivity of the apo-VLDL peptide bands 2, 5, and 6. This resulted in a radioactivity ratio greater than unity. Simultaneously the apo-HDL peptide bands of corresponding mobilities, C, F, and G, showed decreases in radioactivity. Band D also showed a small but consistent decrease in radioactivity which corresponds to observations of this peptide noted in Table 10. On the other hand, there was little change in the radioactivities of apo-VLDL bands 1 and 4, which resemble the mobilities of bands B and E of the apo-HDL. Band 3 of apo-VLDL and band A of the apo-HDL also showed no change. The lower recovery of the ^{3}H-labeled apo-HDL peptides which have moved to VLDL (Table 11) and higher recovery of the apo-VLDL peptides found in HDL (Table 10) are due to the fact that protein recoveries are less in VLDL than in HDL (70% vs. 95%). Nevertheless, the data in Tables 10 and 11 clearly indicate that peptides can exchange between VLDL and HDL regardless of which lipoprotein is initially labeled.

TABLE 11. Movement of HDL ^{3}H-labeled peptides into VLDL

VLDL				HDL			
Polyacrylamide Gel Band[a]	(1) Serum	(2) Serum + VLDL	Ratio (2/1)	Polyacrylamide Gel Band[a]	(3) Serum	(4) Serum + VLDL	Ratio (4/3)
	cpm/peptide				*cpm/peptide*		
				A	103	99	0.97
1	190	200	1.05	B	495	555	1.10
2	1860	2260	1.22	C	1860	1170	0.62
3	110	112	1.01	D	1510	1300	0.86
4	100	97	0.97	E	120	120	1.00
5	225	340	1.50	F	320	200	0.63
6	500	595	1.19	G	500	340	0.68

Incubation conditions: as in Table 4. The pattern of results shown above is typical of three experiments.

[a] Band numbers or letters correspond to locations of the peptides shown in Fig. 1.

DISCUSSION

The data presented here indicate that at least some of the phospholipid and peptide moieties of the serum lipoproteins undergo exchange among the different lipoprotein classes. The exchange involves all the phospholipid fractions in the proportions in which they are found in each lipoprotein class and is not due to one phospholipid, except that lysolecithin moves from VLDL to the d > 1.21 fraction. The phospholipid exchange can be divided into two components. The first is a nonspecific exchange among the phospholipids in each lipoprotein fraction, while the second involves a specific exchange between the VLDL and HDL, and does not involve the LDL. Thus, if one starts with VLDL containing [^{32}P]-phospholipids, the relative specific activities of the HDL and LDL phospholipids are similar, provided the second, more specific, component of the exchange is inhibited (see Table 3). This apparently occurs when the serum is centrifuged in a high salt concentration and then dialyzed. We do not know the nature of the inhibition of the specific component of the phospholipid exchange, but it may be related to the observation that ultracentrifugation causes a dissociation of the subunits of HDL (18, 19). In the experiments carried out in vivo, the percentage of radioactivity from ^{32}P-labeled VLDL which appears in the LDL is low, duplicating the phenomenon seen in vitro, although the amounts of phospholipid are about equal in the VLDL and LDL (9). It is unlikely that lipoprotein lipase activity plays a role in the redistribution of VLDL phospholipids in vivo or in vitro since fasted rats were used in the present experiments, which were of short duration. The lower level of [^{32}P]phospholipid remaining in the VLDL after its intravenous administration, when compared with its incubation with serum (compare Tables 1 and 2), may be due to the larger amount of HDL in the total blood volume in vivo and the fact that plasma lipoprotein phospholipids can exchange with phospholipids in other tissues such as liver (20, 21).

In addition to phospholipids, peptides of VLDL exchange with those of HDL. It seems probable that this represents a true exchange, since peptides with the same electrophoretic mobility have been shown to move from VLDL to HDL or from HDL to VLDL. Recently, Bersot et al. (13) and Koga, Bolis, and Scanu (22) have isolated some of the peptides of rat VLDL and HDL seen on polyacrylamide gel electrophoresis. Inspection of the gel patterns suggests that our HDL bands F and G correspond to peptides H3-3 and H3-4 of Bersot et al. (13), while VLDL bands 5 and 6 may correspond to peptides V3-3 and V3-4. It would thus appear that the peptides which undergo exchange in our experiments are the ones which Bersot et al. (13) found to react with both anti-apo-VLDL and anti-apo-HDL sera. This is consistent with the suggestion that these peptides exchange between VLDL and HDL. Koga et al. (22) have shown that a third VLDL peptide band, PII, also cross-reacts with anti-apo-HDL serum. This is consistent with our observation of the exchange of a third VLDL peptide, band 2, which has approximately the same relative position on the gel as PII.

Scanu et al. (23) have postulated an exchange of peptides between human HDL and VLDL. The present data provide experimental evidence for this postulate in the rat. In a recent communication, Bilheimer, Eisenberg, and Levy (24), using human ^{125}I-labeled VLDL, have reported an exchange of apo-LP Glu and apo-LP Ala between VLDL and HDL. It appears likely that the exchange reported in humans (24) and in rats (5) represents a similar phenomenon, although there are apparently more than two peptides exchanging in the rat. However, because of the lack of knowledge of the precise nature of the various peptides found in rat apolipoproteins, a direct comparison between the two species is not feasible.

It is of interest to speculate whether the exchange of the phospholipid and peptide moieties of the lipoprotein represents the transfer of an intact lipoprotein subunit. Two observations lend support to this concept. Both the specific component of the phospholipid exchange and the exchange of peptides involve VLDL and HDL, but not LDL. In addition, the pattern of phospholipid and peptide exchange is similar when the VLDL is subdivided into α- and β-lipoprotein-containing components. On the other hand, since plasma contains a protein which can function as a phospholipid acceptor (20), it is possible that the apoprotein is transferred separately and may then combine with phospholipid. Unfortunately, no technique is presently available which separates the various peptides without dissociation of the lipid from the protein moiety of the lipoproteins. Studies on this point are currently under way.

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