Comparison of the metabolic behavior in vitro of the apoproteins of rat serum high density lipoprotein$_2$ and high density lipoprotein$_3$

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Abstract Rat serum high density lipoproteins were divided into two fractions, HDL_2 (d 1.063–1.12) and HDL_3 (d 1.12–1.21). These fractions were compared on the basis of (*a*) the pattern of the apolipoprotein peptides obtained on polyacrylamide gel electrophoresis in 7 M urea, (*b*) the exchange of some of the peptides with those in very low density lipoproteins (VLDL), and (*c*) the incorporation by perfused rat liver of [^{3}H]leucine into the peptides of the HDL_2 and HDL_3 secreted into the perfusate. Among the peptide bands of HDL_3, one is absent and another present only in trace amounts in HDL_2. After electrophoresis on polyacrylamide gel for 24 hr, a major peptide band of HDL_2 is split into three distinct areas, whereas it remains as a single area in HDL_3. Both HDL_2 and HDL_3 exchange prelabeled protein with VLDL. However, the exchange is much more limited in HDL_3, even though it contains most of the protein found in circulating rat HDL. Analysis of the individual peptides, separated by polyacrylamide gel electrophoresis after incubation with VLDL, reveals that in HDL_3 the exchange is limited to two peptides, whereas a third, although present in both subfractions of rat HDL, exchanges only when found in HDL_2. This peptide represents most of the exchange with VLDL. Perfused rat liver incorporates [^{3}H]leucine into HDL of the perfusate, primarily into HDL_2. Most of the radioactivity is found in those peptides that do not take part in the exchange with VLDL. These data lead to the conclusions that there are functional and structural differences between HDL_2 and HDL_3 and that some of the peptides of HDL may be derived from exchange with, and breakdown of, VLDL. Others are secreted, at least in part, directly into the circulation by the liver.

Supplementary key words very low density lipoproteins · apolipoproteins · perfused liver

Abbreviations: HDL, high density lipoprotein (d 1.063–1.21); LDL, low density lipoprotein (d 1.006–1.063); VLDL, very low density lipoprotein (d < 1.006).

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It has recently been observed that there is an exchange of phospholipids and peptides between rat very low density lipoproteins and high density lipoproteins. This exchange is restricted to three of the seven peptides of HDL or VLDL that can be separated by polyacrylamide gel electrophoresis (1). A similar exchange involving the apolipoprotein-glutamic acid, apolipoprotein-alanine$_1$, and apolipoprotein-alanine$_2$ of human VLDL has also been reported (2). Further preliminary studies of the exchange in rat serum lipoproteins revealed that it occurred predominantly with that fraction of rat HDL having a density of 1.063–1.12, while more of the HDL protein was found in the fraction of d 1.12–1.21 (3). This raised the possibility that in the rat the fraction corresponding in density to human HDL_3 might differ in its metabolic activity from the rat HDL_2. Although it has been postulated that human HDL_3 is an artifact of the ultracentrifugal procedure used to isolate the lipoproteins and consists to a large extent of products of the breakdown of HDL_2 (4), Scanu and Wisdom (5) have recently suggested that HDL_2 and HDL_3 may not be merely operational classes, but may be different metabolic entities separable in the ultracentrifuge. In view of the apparent differences in the metabolic behavior of the corresponding rat HDL_2 and HDL_3, the relationship between these two fractions was further investigated using the rat lipoproteins as a model. The data to be presented here, based on the exchange of peptides, the synthesis of the peptides of apoHDL by the perfused rat liver, and the patterns of the peptides seen with polyacrylamide gel electrophoresis, suggest that there are differences in the metabolic as well as compositional characteristics of the two subgroups of rat HDL.

METHODS

Serum was obtained from male hooded rats weighing 250–300 g that had been fasted for 24 hr but allowed water ad lib. In order to prepare lipoproteins labeled in the protein moiety, 30 μCi of [1-^{14}C]leucine or 60 μCi of [4,5-^{3}H]leucine was injected into the jugular bulb of each fasting animal. The animals were bled 60 min later (6) by heart puncture, and serum lipoproteins were isolated by a modification of the method of Havel, Eder, and Bragdon (7), using an IEC B-60 preparative ultracentrifuge with an SB-283 rotor at 14°C and 33,000 rpm (100,000 *g*). VLDL was centrifuged at d 1.006 for 18 hr, LDL at d 1.063 for 24 hr, and HDL_2 at d 1.12 for 48 hr. In each case the top 1.2 ml in the 12-ml centrifuge tube was taken for analysis. To separate HDL_3, the top 1.5 ml remaining in the centrifuge tube after removal of the HDL_2 was discarded to avoid any possible contamination of HDL_3 by HDL_2; the density was then adjusted to 1.21 and HDL_3 was isolated by centrifugation for 48 hr. Each lipoprotein fraction was washed by recentrifugation under conditions identical with those used in the original isolation. On several occasions the infranate from the isolated labeled HDL_2 was recentrifuged at d 1.21. No labeled lipoprotein (HDL_3) could be detected at the top of the centrifuge tube. In addition, in a number of control experiments the isolated, washed total HDL was again centrifuged for 48 hr. 90–95% of the radioactivity was recovered in the top 1.2 ml of the centrifuge tube. The lipoprotein fractions were free from contaminants when checked with anti-rat whole serum by immunoelectrophoresis.

After incubation of the isolated lipoprotein or serum samples containing the labeled proteins, the lipoproteins were reisolated by ultracentrifugation. Before determination of the radioactivity of the intact isolated lipoproteins, they were dialyzed for 24 hr against isotonic saline containing 0.1% EDTA, with two changes of the dialysis fluid, to remove any residual radioactive amino acid contamination. The dialysis was unnecessary when the lipoproteins were delipidated and separated by electrophoresis on polyacrylamide gel prior to determination of radioactivity, since tests revealed that no free amino acid remained after delipidation, electrophoresis, and destaining. Delipidation of the lipoproteins, solubilization of the peptides in 7 M urea, separation on polyacrylamide gel electrophoresis, and estimation of the ^{3}H of the stained peptide bands were carried out as previously described (1). The urea was purified by passage through a DEAE-cellulose column. We have noted that the lipoprotein peptides can remain dissolved in 7 M urea for at least 1 wk without alteration of the electrophoretic pattern or distribution of the radioactivity among the bands. Polyacrylamide gel bands containing ^{14}C-labeled peptides were counted by cutting out the stained bands and dissolving the peptides in Protosol (New England Nuclear Corp., Boston, Mass.). Total radioactivity recovered in the bands represented over 70% and 90% of that present in the protein moieties of the VLDL and HDL, respectively, before delipidation. Protein was determined by the method of Lowry et al. (8). Liver perfusions were carried out for 2 hr by the method of Miller et al. (9), using a perfusate made of 30 ml of packed washed rat erythrocytes and 100 ml of Krebs-Ringer bicarbonate solution, pH 7.4. The perfusate also contained 3% bovine serum albumin, 500 mg of glucose, and 40 mg of an amino acid mixture (10) per 100 ml, 100 μCi of [1-^{14}C]leucine, and 10 mg of palmitic acid bound to albumin (11). The flow rate of the perfusate was 10–13 ml/min. The lipoproteins were isolated from the perfusate as described above. However, relatively small amounts of HDL_2 and HDL_3 were secreted by the perfused liver in 2 hr compared with those found in vivo. To minimize losses and facilitate washing, delipidation, and visualization of the bands on the gels, isolated carrier HDL_2 and HDL_3 containing about 500 μg of protein each were added to the corresponding lipoproteins isolated from the perfusate prior to washing the fractions by ultracentrifugation.

RESULTS

The exchange of labeled protein moieties of the lipoproteins is illustrated in Table 1. Isolated ^{3}H-labeled VLDL was incubated for 20 min with either rat serum or Ringer solution. In the presence of serum, approximately 12% of the radioactivity was found in the HDL, whereas none could be found in its absence. When serum containing ^{3}H-labeled protein was incubated with unlabeled isolated VLDL, the VLDL fraction increased its share of the total lipoprotein radioactivity by 15%, while that of the HDL fell by 12%. The elevation of the VLDL levels in these incubations may increase the degree of exchange of HDL peptides above that seen in vivo. These experiments utilized labeled serum rather than isolated ^{3}H-labeled HDL in order to avoid excessive ultracentrifugation prior to the exchange of the protein which, as has been suggested for human HDL, may result in structural changes with the loss of lipid (12). The use of whole serum for the experiments involving labeled HDL was also adopted because earlier experiments in our laboratory (1) had suggested that the in vitro exchange was inhibited by prior isolation of rat HDL. Roheim et al. (13) observed that, in vivo, virtually none of the isolated rat HDL protein injected into the animal was detected in other lipoprotein classes. Similar observations have been made using isolated human HDL (14–16). It will be noted that incubation with serum or unlabeled VLDL had no effect on the amount of radioactivity found in the LDL. These data

TABLE 1. Exchange of protein among rat serum lipoproteins

	VLDL ^{3}H-Containing Peptide		Serum ^{3}H-Containing Peptide	
	Ringer	Serum	Control[a]	Unlabeled VLDL
	% of radioactivity[b]			
VLDL	90 ± 5	70 ± 4	30 ± 2	45 ± 4
LDL	7 ± 1[c]	8 ± 2[c]	23 ± 3	24 ± 2
HDL	0	12 ± 1	39 ± 3	27 ± 2

Incubation conditions: In one series of experiments, 3 mg of VLDL containing 6–10 × 10^3 cpm of ^{3}H was added to the 5 ml of serum or Ringer solution containing 3% bovine serum albumin. In the other series, 30 mg of unlabeled VLDL in 2 ml of NaCl (d 1.006) was added to 5 ml of serum with protein containing 10–15 × 10^2 cpm of ^{3}H. Incubation time, 20 min at 37°C; gas phase, air.

[a] 2.0 ml of NaCl solution (d 1.006) added to the serum.

[b] The data for the experiments in the two columns on the left represent the percentage of total radioactivity added as ^{3}H-labeled VLDL, while the data in the two columns on the right represent the percentage of radioactivity distributed among the three lipoprotein fractions. The radioactivity (cpm) of all lipoprotein fractions recovered in the control and in the presence of unlabeled VLDL agreed within 6% in each experiment.

[c] The radioactivity found as LDL probably represents some LDL of lower density contaminating the VLDL preparations. No isolated LDL was added.

TABLE 2. Distribution of VLDL ^{3}H-containing proteins among HDL_2 and HDL_3

	Radioactivity	Protein	Relative Specific Activity
	% of total HDL		
HDL_2	69 ± 2	39 ± 4	100
HDL_3	31 ± 2	61 ± 4	32 ± 1

3 mg of VLDL, containing 5–15 × 10^3 cpm of ^{3}H, was added to the serum. Radioactivity obtained in the various experiments: HDL_2, 400–1600 cpm; HDL_3, 200–600 cpm. Total protein: HDL_2, 0.9–1.7 mg; HDL_3, 1.5–3.0 mg. Each figure is the mean of three experiments ± SE. Incubation conditions as in Table I.

are consistent with the exchange of peptides between VLDL and HDL as previously reported (1). The distribution of labeled peptides from VLDL between rat HDL_2 and HDL_3 was then studied. The data in Table 2 show that HDL_2 acquired from VLDL approximately 70% of the total radioactivity of HDL but contained only 40% of the protein of unfractionated HDL.

If the acquisition of radioactive protein from VLDL by HDL_2 and HDL_3 was due to an exchange, the loss of labeled protein of HDL_2 and HDL_3 due to the addition of unlabeled VLDL should be demonstrable. Therefore, whole serum containing labeled protein was incubated with or without additional unlabeled VLDL, and the radioactivity in the isolated HDL_2 and HDL_3 was estimated after the incubation. These data are shown in Table 3. HDL_2, containing 54% of the radioactivity of HDL, lost 14% upon incubation with VLDL. The HDL_3 did not lose a statistically significant portion of its radioactivity to VLDL, although the small loss noted in the table occurred in each of the individual experiments. Incubation with VLDL had no effect on the amount of protein present in either HDL_3 or HDL_2, consistent with the concept of exchange of protein. Calculation of the relative specific activity from these data indicates that HDL_2 lost about 25% of its radioactive protein to VLDL, whereas the loss in HDL_3 was only 10%. Thus, the data in Tables 2 and 3 suggest that HDL_2 and HDL_3 differ in their ability to exchange protein with VLDL. This raises the possibility that rat HDL_3 may have less of the specific peptide(s) involved in the exchange with VLDL, or that some peptide(s) may have been altered in the circulation, making it impossible for it to undergo exchange.

The relationship of specific peptides of HDL to the difference in the extent of exchange between VLDL and either HDL_3 or HDL_2 was then investigated. The radioactivity acquired by the various peptides of HDL during the exchange with ^{3}H-labeled VLDL was determined after delipidation and separation of the peptides by polyacrylamide gel electrophoresis. Fig. 1 shows the resulting pattern of the peptides of HDL, HDL_2, and HDL_3. It will be noted that the pattern of HDL_3 resembles that of unfractionated HDL. However, HDL_2 does not contain peptide band A, and B is present in trace quantities only. In other respects HDL_2 resembles unfractionated HDL and HDL_3. To study the exchange of the specific peptides, serum was incubated with ^{3}H-labeled VLDL, and the acquisition of radioactivity

TABLE 3. Loss of serum HDL ^{3}H-containing protein in the presence of VLDL

	Radioactivity			Protein			Relative Specific Activity		
	Serum	Serum + VLDL	*P*	Serum	Serum + VLDL	*P*	Serum	Serum + VLDL	*P*
	% of total			*% of total*					
HDL_2	54 ± 3	40 ± 2	<0.01	41 ± 1	40 ± 1	NS[a]	100	77 ± 1	<0.01
HDL_3	46 ± 2	42 ± 2	NS	59 ± 2	62 ± 3	NS	100	90 ± 1	<0.01

30 mg of unlabeled VLDL was added. Total cpm in the various experiments: HDL_2, 5,000–19,000; HDL_3, 6,000–15,000. Each value is the average of three experiments ± SE. Incubation conditions as in Table I.

[a] NS, not statistically significant.

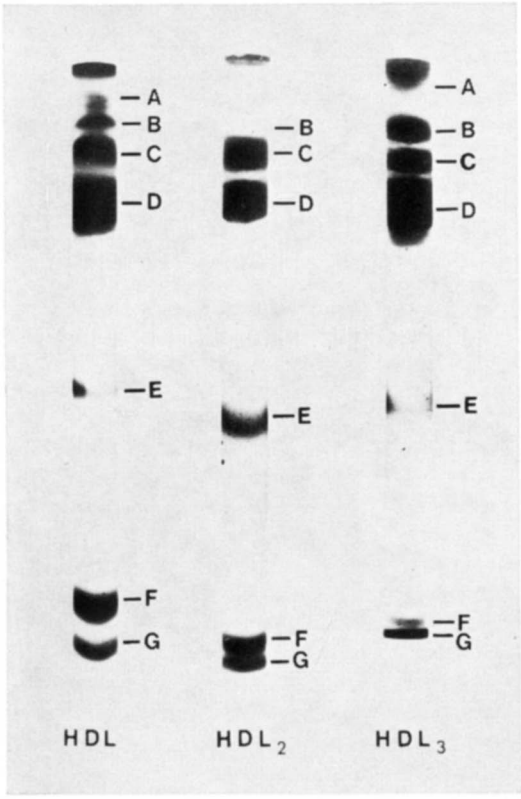

FIG. 1. Polyacrylamide gel electrophoretic patterns of unfractionated HDL, HDL_2, and HDL_3. Band B is not seen in the photograph of HDL_2, but inspection of the original gel revealed a very light band at this spot. The gels were run in tubes of 7 mm I.D. loaded with 500–700 μg of protein in order to obtain adequate radioactivity. Similar, but lighter and thinner, bands were obtained at lower protein concentrations.

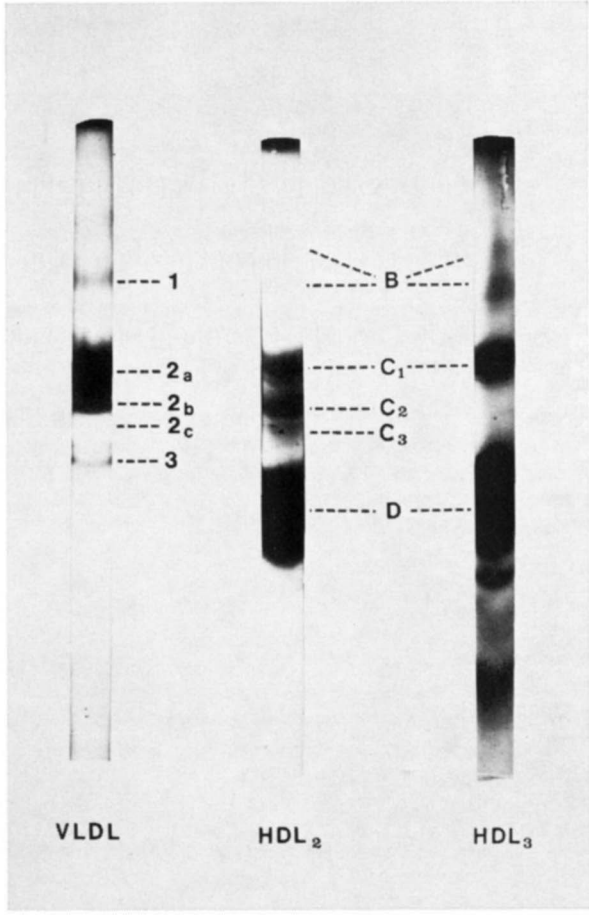

FIG. 2. Polyacrylamide gel patterns after simultaneous electrophoresis of VLDL, HDL_2, and HDL_3 for 24 hr. The protein load and conditions of electrophoresis were identical with those used for the gels shown in Fig. 1, except for the longer duration. The bands seen below D in HDL_3 run with D in the regular 5-hr electrophoresis and probably represent the material seen at the bottom of band D in Fig. 1.

by the various peptides making up the HDL_2 and HDL_3 of the serum was investigated (Table 4). As previously noted in Table 2, radioactivity was acquired primarily by the peptides of HDL_2. Bands C, D,[2] F, and G of HDL_2, but only bands F and G of HDL_3, obtained ^{3}H-labeled peptide from VLDL. Peptide band C picked up most of the radioactivity from VLDL, but a band of similar mobility in HDL_3 did not acquire a significant amount of radioactivity, although both contained considerable quantities of protein. The ability of HDL_2 and HDL_3 to provide labeled peptides for VLDL was also investigated. As in the previous experiments, labeled whole serum was incubated with additional VLDL; HDL_2 and HDL_3 were isolated and delipidated, and the peptides were separated by electrophoresis on polyacrylamide gels. The resultant radioactivity found in the peptide bands is shown in Table 5. It will be noted that after incubation with VLDL, there was a significant loss in radioactivity from bands C, F, and G of HDL_2 as reflected in the ratio between the radioactivity remaining in the presence and absence of added VLDL. However, HDL_3 lost radioactivity only from bands F and G. Thus, these studies indicate that HDL_2, in addition to lacking one and possibly two peptides present in HDL_3, contains a specific peptide that readily exchanges with VLDL, while one of similar electrophoretic mobility in HDL_3 does not undergo exchange.

In view of the difference in the pattern of exchange between the peptides of band C in HDL_2 and HDL_3, this area of the gel was studied in greater detail by electrophoresis of the apoproteins on polyacrylamide gel for 24 hr instead of 5 hr. It will be seen in Fig. 2 that under these conditions band C of HDL_2 was split into three peptide groups designated C_1, C_2, and C_3. The possibility that these bands represent microheterogeneity of the carbohydrate moiety of a single peptide cannot

[2] It is likely that the radioactivity in band D is a contaminant from band C. When the two bands are further separated by electrophoresis for 24 hr (see Fig. 2 and Table 6), no radioactivity is acquired from VLDL by the peptides in band D, indicating that essentially none of the apoprotein characterized by mobility as band D truly exchanges.

be ruled out at this time. However, it is significant that C_2 and C_3 are absent from HDL_3. A comparison of the 24-hr gel electrophoresis pattern of HDL_2 with one obtained from VLDL indicates that the latter also contains bands corresponding in mobility to C_1, C_2, and C_3, which are designated 2a, 2b, and 2c in Fig. 2, although 2c is present in very small quantities.

Attempts were made to determine if bands C_1, C_2, and C_3 of HDL_2 were all involved in the exchange of peptides with VLDL. VLDL containing peptides labeled with [1-^{14}C]leucine or [4,5-^{3}H]leucine was incubated with serum. The loss of radioactivity from VLDL peptide bands 2a and 2b and gain by HDL_2 peptide bands C_1 and C_2 as a result of the incubation is shown in Table 6. It will be noted that the greatest exchange occurred between band 2a of VLDL and C_1 of HDL_2. Band 2b lost a smaller amount of radioactivity, which was found in band C_2. However, band 2c of VLDL and C_3 of HDL_2 did not appear to take any part in the exchange.

The evidence cited above is consistent with the concept that rat HDL_2 and HDL_3 are separate metabolic entities. If this is the case, it is possible that the patterns of synthesis of the various peptides represented by the electrophoretic bands might differ. In order to investigate this possibility, livers were perfused with [1-^{14}C]-leucine. The perfusate contained an albumin–Ringer solution instead of serum to minimize the exchange between HDL and VLDL. After 2 hr of perfusion the HDL_2 and HDL_3[3] were isolated by ultracentrifugation, unlabeled serum HDL_2 or HDL_3 was added as carrier, and the lipoproteins were washed by recentrifugation. The incorporation of leucine into the peptide bands of the polyacrylamide gels was then determined. The appearance of radioactivity in the various peptide bands of HDL_2 and HDL_3 is shown in Table 7. Most of the isotope was incorporated into HDL_2, primarily into bands C and D, with some additional incorporation in band B. On the other hand, there was relatively little incorporation of [1-^{14}C]leucine into HDL_3, with only band D being significantly radioactive. It should be noted that although the perfused liver incorporated more of the amino acid precursor into HDL_2, in the circulation the greatest portion of the protein of HDL was found in HDL_3 (cf. Table 2).

In view of the fact that much of the leucine incorporated into HDL by the perfused liver was found in peptide band C of HDL_2, the distribution of the newly synthesized peptide among its three subfractions separated by 24-hr polyacrylamide gel electrophoresis was studied. The results, shown in Table 8, indicate that the greatest incorporation of leucine occurred in band

[3] The radioactivity found in the HDL of the perfusate represented 10–25% of that found in VLDL in the various experiments. LDL contained negligible radioactivity (17).

TABLE 4. Movement of VLDL ^{3}H-containing peptides into HDL

Band[a]	HDL_2	HDL_3
	cpm/band[b]	
A		0
B	20	4
C	740	20
D	150	30
E	0	0
F	200	130
G	250	150

The results are typical of three experiments. In the experiment cited above, 560 μg of protein was placed on each gel. The recovery of radioactivity was 90% for HDL_2 and 96% for HDL_3. Incubation conditions as in Table 1.

[a] Letters correspond to the location of the peptide bands shown in Fig. 1.

[b] The gels used to separate the HDL_2 and HDL_3 peptides were loaded with equal amounts of apolipoprotein dissolved in 7 M urea. The HDL_3 data were then corrected to account for the relatively higher concentration of protein in this fraction in serum (cf. Table 2).

C_3, which apparently does not undergo exchange with VLDL and contains the smallest amount of protein among the subfractions of band C; the smallest incorporation was noted in band C_1, which showed the greatest exchange with VLDL. These studies suggest that the circulating HDL may be made up of some components that are derived, at least in part, by direct synthesis and secretion and others that may be the result of exchange or breakdown of VLDL.

DISCUSSION

One of the problems in dealing with rat apolipoproteins is the relative paucity of analytical data about each of the peptides represented by the bands of the polyacrylamide gel electrophoresis patterns. This is to a large degree due to the difficulty of isolating and purifying reasonable quantities of the various peptides. Attempts in our laboratory to separate rat apoHDL peptides by ion exchange chromatography resulted in losses of 60% or greater.[4] Similar problems have been reported with rat apoHDL by Bersot et al. (19). Thus, a standard nomenclature for the rat peptides has not as yet been adopted (18, 19). Therefore, we have tentatively designated the HDL peptides by letters and the VLDL peptides by numbers. These should not be compared with the "ABC" nomenclature suggested by Alaupovic (20) for human lipoproteins.

[4] Attempts were also made to separate apoHDL by gel filtration on Sephadex G-200. Although a pattern of peaks similar to that reported by Koga, Bolis, and Scanu (18) was obtained, electrophoretic bands A, B, and C appeared in the void volume. The other peaks each contained more than one peptide when monitored by polyacrylamide gel electrophoresis.

TABLE 5. Movement of HDL peptides in the presence of VLDL

Band	HDL_2 (1) Serum ^{3}H-Containing Protein	HDL_2 (2) Serum ^{3}H-Containing Protein + VLDL	HDL_2 2/1	HDL_3 (1) Serum ^{3}H-Containing Protein	HDL_3 (2) Serum ^{3}H-Containing Protein + VLDL	HDL_3 2/1
	cpm/band[a]		%[b]	*cpm/band*[a]		%[b]
A				400	360	93 ± 3
B	1250	1140	93 ± 3	1120	1050	100 ± 5
C	4000	2850	67 ± 3	980	900	95 ± 3
D	2260	1950	90 ± 4	3400	3800	104 ± 5
E	400	400	103 ± 4	650	670	96 ± 4
F	970	650	73 ± 3	900	600	67 ± 1
G	1470	800	61 ± 5	920	560	65 ± 3

Incubation conditions as in Table I.
[a] These data are from a typical experiment.
[b] The percentages are averages of three experiments ± SE.

The data presented in this communication provide evidence that when rat HDL is arbitrarily divided into HDL_2 and HDL_3 using densities similar to those established for human HDL subfractions (21), the two subfractions differ in biosynthesis and in structural and functional aspects. Comparison of the polyacrylamide gel electrophoresis patterns of peptides derived from delipidated rat HDL_2 and HDL_3 revealed apparent compositional differences. Rat HDL_3 contains one peptide (A) that is not found in HDL_2 and another (B) that is found only in trace amounts in HDL_2. On the other hand, HDL_2 contains two peptides, C_2 and C_3, separable only after 24 hr of electrophoresis, that are not present in HDL_3 but that correspond to two peptide bands of similar mobility in rat VLDL.

Rat HDL_2 also differs from HDL_3 in the pattern of exchange of its peptides with VLDL. Although the protein content of HDL_3 is greater than that of HDL_2, it is the latter that undergoes the greatest exchange with VLDL, due primarily to the peptide present in electrophoretic band C. Although this peptide band is also present in HDL_3, it plays no role in the exchange with VLDL. There are several possible explanations for this. Band C of HDL_2 may contain several peptides, only one of which exchanges with VLDL. It is also possible that the same peptide is present in HDL_2 and HDL_3 but in the latter does not undergo exchange due to a difference in configuration or lipid content. A second peptide band of HDL_2, C_2, which is not present in HDL_3, also takes part in the exchange with VLDL. It is unlikely that a specific peptide is being lost from HDL_3 during the additional ultracentrifugation required to isolate this fraction, because less than 10% of the radioactivity of HDL is lost on recentrifugation. Furthermore, the difference in exchange is due to the fact that band C_1 in HDL_3 does not exchange, although it is present in both HDL_2 and HDL_3.

A difference between the synthesis of the peptides of HDL_2 and that of HDL_3 by perfused rat liver is also apparent. Relatively little HDL_3 peptide is synthesized, and the synthesis is restricted almost entirely to the peptide of band D. On the other hand, the peptides of bands B, C, and D of the HDL_2 are synthesized. It is noteworthy that the greatest incorporation of leucine among the peptides of band C occurs in C_3, which is apparently present in the lowest concentration in the circulating

TABLE 6. Movement of ^{14}C-containing peptides of band 2 of VLDL into band C of HDL_2

Band[a]	VLDL Ringer	VLDL Serum	HDL_2 Band	HDL_2 Serum
	cpm/peptide			*cpm/peptide*
2a	3000	2400	C_1	480
2b	820	620	C_2	140
2c	240	255	C_3	10
			D	0

The experiment is typical of four such experiments, including two in which [4,5-^{3}H]leucine was utilized as precursor for VLDL peptides. Incubation conditions as in Table 1.
[a] Band letters and numbers correspond to the location of the peptides illustrated in Fig. 2.

TABLE 7. Synthesis of HDL_2 and HDL_3 peptides by perfused rat liver

Band	HDL_2	HDL_3
	cpm/band	
A		0
B	140	20
C	1000	50
D	650	100
E	10	20
F	30	10
G	60	10

The data are typical of three such experiments. The lipoproteins were isolated from the perfusate after 2 hr, and the values represent the total radioactivity present in each of the two fractions.

TABLE 8. Synthesis of peptides of band C by perfused rat liver

Peptide Fraction	cpm/ Fraction[a]	% Total cpm in Band C
C_1	250	20 ± 3
C_2	310	33 ± 3
C_3	500	47 ± 1

[a] These data represent a typical experiment while the % total cpm is the average of three experiments.

lipoproteins and does not appear to undergo exchange with VLDL peptides.

It thus seems likely that, in the rat, HDL_3 is not an artifact of centrifugation derived from HDL_2, but rather a separate lipoprotein entity that differs in peptide composition, metabolic function, and synthesis. If these observations were extended to human HDL, they would provide experimental evidence for the suggestion of Scanu and Wisdom (5) that HDL_2 and HDL_3 are not merely operational classes but are metabolic entities separable in the ultracentrifuge. However, our data do not rule out the possibility that HDL_3 is derived from HDL_2 as a result of metabolic events occurring in the circulation.

There appears to be an inverse relationship between the peptides of rat HDL synthesized by the perfused rat liver and those undergoing exchange. Thus C_3, which shows the greatest incorporation of the precursor amino acids during synthesis by the perfused liver, apparently does not undergo exchange, whereas C_2 and C_1, which exchange in progressively increased amounts, show progressively less incorporation of leucine after perfusion of the liver. In addition, there is no apparent synthesis of peptides F and G in HDL_3 and only slight incorporation of [^{14}C]leucine into these peptides in HDL_2. However, both apoHDL subfractions take part in considerable exchange with VLDL. In contrast, the peptides of bands B and D in HDL_2 do not undergo exchange but are synthesized by the perfused liver. These observations suggest that the exchanging peptides are derived in the circulation primarily from VLDL, whereas those that do not exchange may be directly secreted, at least in part, into the circulation. However, the possibility that the latter group of peptides may also be derived in part from the breakdown of VLDL has not been ruled out.

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