Studies on the metabolism of rat serum very low density apolipoprotein

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Abstract There was a rapid transfer of radioactive peptides to other lipoprotein fractions during the first 30 min after the intravenous injection of ^{125}I-labeled rat very low density lipoprotein (VLDL) into rats. After this initial redistribution of radioactivity, label disappeared slowly from all lipoprotein fractions. The disappearance of ^{125}I-labeled human VLDL injected into rats was the same as that of rat VLDL. Most of the radioactivity transferred from VLDL to low density (LDL) and high density (HDL) lipoproteins was associated with two peptides, identified in these studies by polyacrylamide gel electrophoresis as zone IVa and IVb peptides (fast-migrating peptides, possibly analogous to some human C apolipoproteins), although radioactivity initially associated with zone I (analogous to human apolipoprotein B) and zone III (not characterized) was also transferred to LDL and HDL. That the transfer of label from VLDL to LDL and HDL primarily involved small molecular weight peptides was confirmed in studies using VLDL predominantly labeled in these peptides by in vitro transfer from ^{125}I-labeled HDL. Both zone I and zone IV radioactivity was rapidly removed from VLDL during the first 5 min after injection. However, although most of the zone IV radioactivity was recovered in LDL and HDL, only 12% of the label lost from zone I of VLDL was recovered in other lipoproteins, with the remainder presumably having been cleared from the plasma compartment. We have concluded that, during catabolism of rat VLDL apoprotein, there is a rapid transfer of small molecular weight peptides to both LDL and HDL. During the catabolic process, most of the VLDL is rapidly removed from the circulation, with only a small portion being transformed into LDL molecules.

Supplementary key words turnover · iodinated lipoprotein · apoproteins · polyacrylamide gel electrophoresis

It has now been established by several groups that there is a metabolic as well as a structural relationship between several apoproteins of the major serum lipoproteins (1–7). Such an interrelationship may possibly be connected with the secretion of triglyceride-rich VLDL molecules from the liver into the plasma and the subsequent series of rapid catabolic events. Among these events (some of which are as yet not clearly defined) are the clearance of triglyceride from the plasma, presumably attributable to the action of various lipases (8–11), a rapid exchange or transfer of the small molecular weight peptides from VLDL to other lipoproteins, notably HDL, and possibly the formation of VLDL remnants (1–7, 12) some or all of which are eventually further transformed to LDL molecules.

Our interest in this problem has been to investigate the suitability of the rat as an experimental model for studying human VLDL apoprotein metabolism. The rat has been used to provide much useful information about fat transport, and it seemed reasonable that it might be of further use in an investigation of the functional relationship that may exist between apolipoprotein and lipid metabolism. A previous study from this laboratory (1) has established an interrelationship between the apoprotein moieties of rat lipoproteins, and recent reports by Eisenberg and Rachmilewitz (13, 14) clearly demonstrated the existence of metabolic heterogeneity between apoprotein moieties of rat lipoproteins. The present paper describes experiments that also confirm the existence of metabolic heterogeneity among rat apolipoproteins. This was accomplished by labeling all the rat VLDL protein with ^{125}I in some experiments, while in other experiments only the small molecular weight peptides of the lipoprotein were labeled. The iodinated VLDL was then administered to recipient animals in order to study the in vivo metabolism of VLDL apoprotein. Using similar techniques we have also studied the fate of iodinated human VLDL in the rat.

MATERIALS AND METHODS

Lipoprotein preparation

In order to prepare rat VLDL for labeling and reinjection, blood was obtained from 20–40 donor rats (250–350

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; apo-LDL, B protein, the major protein of LDL; C peptides, the small molecular weight peptides present in VLDL, LDL, and HDL.

g) for each preparation. The animals were fasted overnight, lightly anesthetized with ether, and bled from the abdominal aorta. The blood was allowed to clot for 30 min, and the serum was obtained by centrifugation at 2500 rpm for 30 min in an MSE Mistral refrigerated centrifuge at 5°C. Disodium ethylenediaminetetraacetic acid was added to the serum to a final concentration of 0.1%. In some preparations, VLDL of d < 1.006 was prepared by ultracentrifugation of the chylomicron-free serum for 16 hr at 40,000 rpm (40.3 rotor) in a Spinco model L3-50 ultracentrifuge at 12°C. Rat VLDL prepared in this manner contained approximately 7% protein, 68% triglyceride, 16% phospholipid, and 9% cholesterol. In other preparations, VLDL subfractions of S_f > 400, 100–400, and 20–100 were isolated from rat serum using the method described by Gustafson, Alaupovic, and Furman (15). An SW 50.1 rotor was used for this purpose in order to achieve a more efficient separation of VLDL subclasses. All VLDL preparations were washed twice by flotation through saline (d 1.006) under the same conditions used for their isolation, and they were checked for purity by agarose gel electrophoresis or immunoelectrophoresis. No other plasma protein was detected by these methods.

Human VLDL (d < 1.006) was similarly isolated from pooled serum obtained from four or five normal male donors who had fasted overnight. The triglyceride, cholesterol, phospholipid, and protein content of this preparation was similar to that reported elsewhere (15).

Radioiodination of VLDL

Human VLDL was iodinated as reported previously (16), using a modification of the method of McFarlane (17). With this technique we consistently achieved preparations containing 85–93% of the label attached to protein, with most of the remainder present in the lipid moiety. Assuming a molecular weight of 250,000 for the protein moiety of VLDL, but recognizing that this was a considerable overestimate of the actual functional apoprotein molecular weights (7), all preparations were calculated to contain between 0.5 and 1.0 atom of iodine per mole of protein. It should be noted that this technique requires only 1 mg of VLDL protein/ml of iodination mixture to achieve labeling characteristics similar to those reported by others (2) using concentrations of 10 mg of protein/ml.

The radioiodination of rat VLDL presented many more problems. Under conditions identical with those used for labeling human VLDL, a greater proportion (30–60%) of the total ^{125}I was associated with the lipid moiety. This observation has been reported in detail elsewhere (16) and, as was suggested, the labeling characteristics were greatly improved by increasing the saturated:polyunsaturated fatty acid ratio in the plasma lipids of the rat by dietary means. Briefly, rats were fed a synthetic diet containing 5% lard for 7–10 days prior to their use as VLDL donors. The labeling characteristics of rat VLDL were studied as the blood lipids became progressively more saturated, and it was found that a decreasing incorporation of ^{125}I into VLDL lipid was closely correlated with a lowering of the polyunsaturated fatty acid content (particularly linoleic acid)[1] of the serum lipids.[2] As indicated in the Results section, no significant alterations of VLDL apoprotein composition were detected as a result of the diet. VLDL from animals fed saturated fat was then consistently labeled at 8–12% efficiency, with 94.0 ± 2.7% (SD) of the label in the protein moiety, 5.2 ± 2.9% associated with lipid, and 0.8 ± 0.76% in the free state. The method for determining this distribution has been described in detail previously (16). The molar ratio of iodine to protein was 0.5–1.5 in all such preparations.

In vivo experiments

Studies were carried out on 200-g (range 195–200 g) male rats of the John Curtin School strain that were fed a commercial rat chow containing 18% protein, 5% fat, 6% fiber, and the usual trace elements and vitamins. In some experiments, duplicate (two animals for each time point) rather than triplicate series (three animals) of animals were chosen because the subsequent operational procedures were dependent on the rotor and centrifuge space available; consequently, experiments were repeated two or three times. Animals were injected via the tail vein with 0.5 ml of the iodinated VLDL preparations in 0.85% NaCl, pH 7.4. The amount of VLDL protein and radioactivity injected was between 25 and 90 μg and between 2 and 5 × 10^6 cpm/animal, respectively, in different experiments. At various times thereafter, the rats were lightly anesthetized and exsanguinated by collecting 7–8 ml of blood from the abdominal aorta. After clotting, the serum was obtained by centrifugation, and 100-μl samples were taken for determination of the disappearance rates of ^{125}I-labeled protein from the circulation. Protein-bound radioactivity was determined after chloroform–methanol extraction and trichloroacetic acid precipitation of the aqueous phase as described elsewhere (16). The distribution of radioactivity among serum lipoproteins was determined by the sequential ultracentrifugation of 3-ml samples of serum from injected animals in a 40.3 rotor, as described by Havel, Eder, and Bragdon (18). However, because there is evidence to

[1] Of the various parameters measured, the most significant changes accompanying the saturated fat diet were (*a*) a decrease in rat serum and VLDL linoleic acid content from 24.5 to 10.5% and from 27.1 to 9.8%, respectively, after 10 days on the diet; this was associated with (*b*) a decrease in labeling of VLDL lipid from 40 to 10%; and (*c*) separation, followed by radioassay, of the major lipid fractions of radioiodinated VLDL revealed a decrease in the ^{125}I content of phospholipid. Thus, the degree of lipid labeling is probably related to the content and accessibility of the linoleic acid molecules of rat VLDL phospholipid.

[2] Poulis, P., and N. H. Fidge. Unpublished observations.

suggest different density limits for delineation of rat lipoprotein classes, the following steps were considered to be more appropriate in the present study.

After VLDL isolation, the infranate was adjusted to d 1.019 g/ml because we found that in all experiments a considerable proportion of the radioactivity was present in the d 1.006–1.019 fraction. LDL was then first isolated between 1.019 and 1.063 g/ml despite the slight contamination with HDL. However, when lipoproteins were repurified for apoprotein analysis, LDL was recentrifuged at d 1.055 g/ml to eliminate traces of HDL. Less than 5% of the total radioactivity was lost between d 1.055 and 1.063. Rat HDL was isolated between densities 1.063 and 1.21 g/ml and purified by one further centrifugation at d 1.21. VLDL was also washed once at d 1.006 for 16 hr as described above.

Labeling of VLDL by in vitro incubation with ^{125}I-labeled HDL

During the incubation of protein-labeled VLDL with rat serum in vitro, there is an exchange of small molecular weight peptides between VLDL and HDL (4). In order to label the small molecular weight C proteins and not the apo-LDL (B protein) moiety of VLDL, purified rat HDL (d 1.063–1.21) was iodinated with ^{125}I using the iodine monochloride method described previously (16). HDL thus labeled contained 80–85% of the label in the protein moiety, with an iodine:protein substitution ratio of 0.5:1. Iodinated HDL was then mixed with 20 ml of rat serum to which was added rat VLDL isolated from 30–40 ml of rat serum. After incubation for 1 hr at 37°C, rat VLDL was isolated by ultracentrifugation at d 1.006 g/ml and washed twice. The distribution of label (^{125}I) among the apoproteins of in vitro labeled rat VLDL was determined by polyacrylamide gel electrophoresis after delipidation and resolubilization of a portion of the labeled VLDL.

Delipidation of lipoproteins and apoprotein analysis

Lipoprotein fractions were dialyzed against 5 mM NH_4HCO_3, pH 7.4, and lyophilized in 5-ml stoppered delipidation tubes. Delipidation was achieved by the addition of 1 ml of methanol and, after mixing, 1 ml of chloroform. The resultant protein suspension was then mixed on a Vortex mixer; the tubes were filled with diethyl ether and then centrifuged at 2000 rpm at 4°C for 10 min in an MSE Mistral centrifuge. Protein precipitates were reextracted twice in the same manner and then washed twice with diethyl ether. After removal of the ether with a gentle stream of N_2, the proteins were resolubilized in 100 μl of 0.05 M sodium decyl sulfate in 0.05 M Tris–HCl containing 8 M urea. This solvent was chosen after comparing various solutions for their ability to dissolve the B (apo-LDL) protein of rat VLDL and LDL. After addition of the solubilizing buffer, 300 μl of 8 M urea in 0.05 M Tris–HCl, pH 8.3, was added to the mixture in order to reduce the concentration of decyl sulfate to approximately 16 mM. These solutions remained at room temperature overnight prior to electrophoretic separation. Although rat HDL apoprotein was readily soluble in aqueous solutions, the same detergent–buffer system described above for VLDL and LDL was used to maintain standard conditions for all apoproteins fractionated by electrophoresis. Calculations based on protein determinations before and after delipidation showed that 80–90% of VLDL, 88–95% of HDL, and 70–75% of LDL apoprotein was recovered after solubilization with the buffer described above.

Urea used in these procedures was purified by passage through Amberlite MB or Rexyn I-300 columns. It was deionized immediately prior to use in order to minimize the formation of cyanate and subsequent carbamylation of the apoproteins.

Electrophoresis was carried out on 7.5% polyacrylamide gels containing 8 M urea in tubes measuring 7 × 150 mm. The same discontinuous buffer system described by Kane (19) was used in the upper and lower reservoirs, and electrophoresis was carried out at 2.5 mA/tube for 4–5 hr. During operation of the system, the gels were cooled by running tap water through a glass coil incorporated into the apparatus. Between 80 and 150 μg of apoprotein was loaded onto the gels, and after electrophoresis the gels were stained with 1% amido black for 1 hr and destained in 7% acetic acid for 2.5 days. The stained bands were then separated by slicing the gels with a razor blade, and after being transferred to small tubes the slices were dried at 50°C for 12–20 hr prior to radioassay. More than 90% of the total radioactivity applied to the gels was recovered in all slices, with approximately 85–90% of the radioactivity associated with stained bands.

Radioactivity was measured in a Packard Auto-Gamma spectrometer. Quenching caused by high salt concentrations in lipoprotein fractions was corrected by reference to appropriate quenching curves. The efficiency of counting gel slices was checked by counting them before and after dehydration and after solubilization in 30% H_2O_2. No differences were observed between these methods. Na^{125}I, carrier free, was obtained from the Radiochemical Centre, Amersham, England.

RESULTS

Metabolism of rat VLDL

In order to detect possible changes in the VLDL apoprotein composition due to an increased content of saturated fatty acids in the diet, rats were killed at various times from 1 to 9 days after commencement of the above diet. No dif-

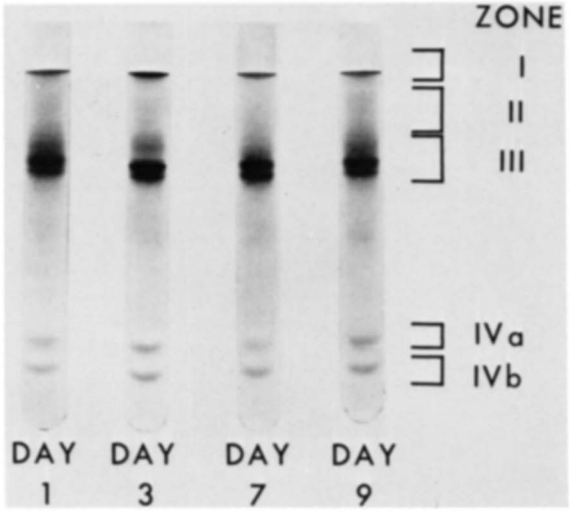

Fig. 1. Disc electrophoresis patterns of VLDL apolipoproteins from rats fed a saturated diet for up to 9 days. No changes were seen in the proportion of apoprotein constituents either visually or after scanning the gels.

ferences in the VLDL protein composition were observed (**Fig. 1**) or detected by scanning the gels. The possibility of such a diet causing changes in the biological behavior of VLDL was also investigated by injecting ^{125}I-labeled VLDL, obtained from donor rats fed a saturated diet for 1, 3, 7, or 9 days, into normal rats. Each preparation showed similar biological behavior as determined from VLDL disappearance curves (**Fig. 2**)[3] and from the rate of transfer of label to other lipoproteins (to be reported in detail elsewhere). All data reported below were obtained using VLDL isolated from rats fed the saturated diet for 8–10 days.

The redistribution of radioactivity among the serum lipoproteins after administration of ^{125}I-labeled VLDL is shown in **Table 1.** These values are the means ± SEM from seven rats and are representative of one triplicate and two duplicate experiments. After 5 min, 32.7% of the serum radioactivity was associated with HDL, and the proportion of radioactivity in HDL increased with time while the proportion of label in VLDL decreased rapidly. There was also an increase in the amount of radioactivity in the d 1.006–1.019 (LDL_1) and 1.019–1.063 (LDL_2) fractions although it was less marked than in the HDL fraction. Radioactivity increased in the LDL_1 fraction to a peak of 7.8% at 1 hr whereas the label in the LDL_2 fraction steadily increased for the duration of the experiment. Using the data from Table 1, it was possible to determine the redistribution of radioactivity as percentages of the total injected dose. Thus, **Fig. 3** illustrates the relative changes of radioactivity in LDL_1, LDL_2, and HDL that accompany the disappearance of label from VLDL after administration of ^{125}I-labeled VLDL. Although most of the radioactivity was associated with the protein moiety of VLDL, the clearance rate of the ^{125}I-labeled lipid moiety from the serum was also determined in some experiments. The results showed that lipid radioactivity was cleared more rapidly from the blood than protein label, similar to the results described by Eisenberg and Rachmilewitz (13). Extraction of the lipid fraction of all lipoproteins showed that most (92–95%) of the label was bound to the protein moiety.

Table 2 shows the redistribution of radioactivity among rat serum lipoproteins after the injection of iodinated human VLDL into rats. It is apparent that human VLDL, like rat VLDL, is rapidly catabolized in the rat. Although a higher proportion of the injected dose remained in the circulation after 5 min (81% compared with 69%), the subsequent rapid metabolism was associated with a similar redistribution of radioactivity among the other serum lipoproteins. These results are also expressed as percentages of the injected dose, as shown in **Fig. 4.** The disappearance of label from the serum of human VLDL was characterized by a multiphasic clearance curve similar to that observed after administration of rat VLDL. Radioactivity was rapidly transferred to HDL, which contained 23.8% of the total serum radioactivity 5 min after injection (Table 2), whereas approximately 7 and 11% of the serum radioactivity was associated with LDL_1 and LDL_2, respectively.

Metabolism of individual apoproteins

After staining and isolating the protein bands separated by electrophoresis on polyacrylamide gels, most of the radioactivity was found to be associated with three major apoprotein groups in VLDL and LDL (I, III, IV) and with four groups in HDL (I, II, III, IV). These zones are shown in **Fig. 5.** The distribution of radioactivity among apoproteins after injection of ^{125}I-labeled VLDL into rats is shown in **Table 3.** It can be seen that a significant change in the distribution of radioactivity occurred among VLDL apoproteins during circulation in vivo for 6 hr, with zones I and IV accounting for most of the changes. At 5 min, zone I protein contained 42.8% of the label but only 11.1% at 6 hr. During this time the percentage radioactivity in zone IV increased from 41% to 83% of the total counts.

The LDL fraction contained a smaller percentage (8.2%) of the total radioactivity after administration of ^{125}I-labeled VLDL, and a large proportion (approximately 80%) of this label was associated with the zone IV peptides. This distribution remained fairly constant over the time period studied. A similar although more pronounced

[3] The data in Fig. 2 represent only apoprotein and not the total VLDL radioactivity. Separation of the protein was necessary because the VLDL was obtained from donor rats fed saturated fat for different times and therefore varied in lipid ^{125}I content. The percentage of total injected radioactivity in this fraction is comparable with, although slightly less than, that shown for VLDL in Fig. 3.

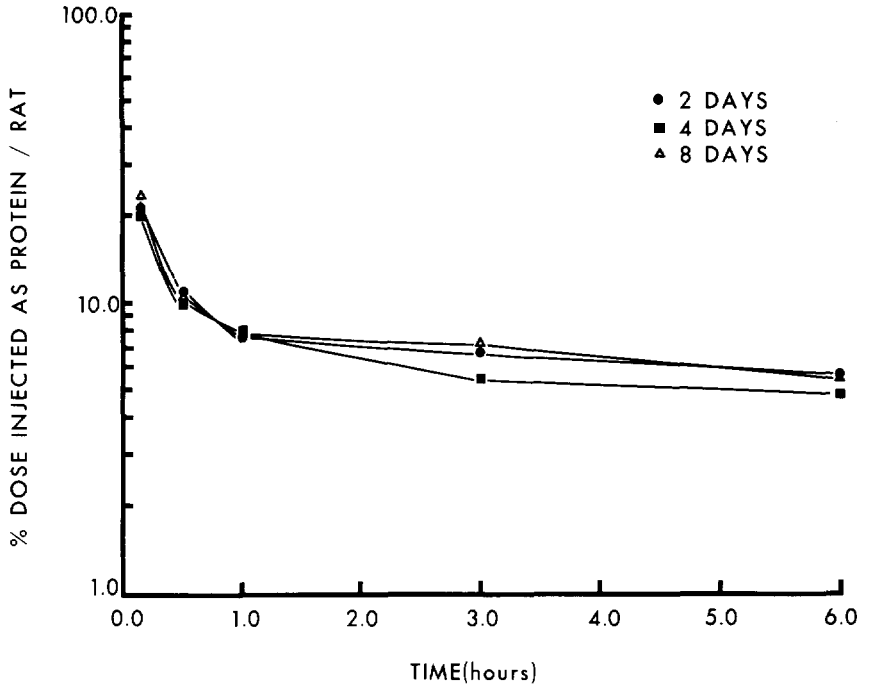

Fig. 2. Disappearance of ^{125}I-labeled VLDL from rats. Each point is the mean from two rats. Variation between rats was small and similar to that shown in Fig. 3 for VLDL disappearance. VLDL for injection was isolated from rats fed saturated fat for 2, 4, and 8 days and injected into animals fed the normal rat chow.

effect was seen after electrophoretic analysis of the HDL apoprotein. Most of the label in this fraction was associated with the zone IV peptides, although some radioactivity was associated with the other gel zones.

Fig. 6 demonstrates the redistribution of label among the three lipoprotein classes, for each apoprotein group, when expressed as a percentage of the total injected dose. Initially, the label disappeared at similar rates from the zone I and IV peptides of VLDL, and at 5 min most of the zone IV radioactivity removed from VLDL (27%) was present in LDL and HDL. However, only 5–6% of the label originally present in zone I of VLDL was present in LDL and HDL at 5 min, suggesting that most of the remainder (33%) was removed from the plasma lipoprotein pool. Fig. 6 (bottom panel) also demonstrates that from 10 min to 60 min there was a much more rapid disappearance of radioactivity from the zone I apoprotein than from the zone III or IV apoproteins. After 60 min, the label in all apoproteins of VLDL was removed at similar rates, although there was an apparent increase in the proportion of label in group IV apoprotein between 3 and 6 hr. The center panel of Fig. 6 shows the distribution of label among LDL apoproteins. An initial rapid accumulation of radioactivity in the zone IV apoprotein was seen to precede the appearance

TABLE 1. Distribution of radioactivity in serum after injection of ^{125}I-labeled rat VLDL[a] into rats

Time after Injection	Distribution of Radioactivity among Lipoproteins[b]					
	VLDL (d < 1.006)	LDL_1 (d = 1.006–1.019	LDL_2 (d = 1.019–1.063	HDL (d = 1.063–1.21)	Infranate (d > 1.21)	Serum Radioactivity
min	*% of serum radioactivity*					*% of injected dose/9 ml of serum*[c]
5	50.6 ± 6.0	4.0 ± 0.9	6.4 ± 2.6	32.7 ± 3.9	6.3 ± 2.6	69.2 ± 9.4
10	41.5 ± 3.1	5.5 ± 0.6	8.4 ± 2.7	38.3 ± 3.1	6.3 ± 2.2	69.2 ± 8.1
30	33.0 ± 8.2	7.0 ± 1.3	10.1 ± 3.8	42.0 ± 3.2	7.9 ± 2.8	54.0 ± 2.7
60	29.4 ± 4.5	7.8 ± 2.3	10.6 ± 3.3	44.1 ± 2.1	8.1 ± 2.7	44.8 ± 3.2
180	21.2 ± 2.4	7.2 ± 1.7	12.9 ± 4.6	48.6 ± 8.6	10.1 ± 4.6	28.7 ± 6.8
360	14.1 ± 4.0	4.3 ± 1.2	18.7 ± 8.5	50.0 ± 11.5	12.9 ± 6.3	16.8 ± 7.8

[a] Rats were injected with 60–80 μg of VLDL protein containing 5–10 × 10^6 cpm.
[b] Values are means ±SEM of seven rats from two duplicate and one triplicate experiment.
[c] Calculated on the basis that 200-g rats have 9 ml of serum (4.5% of body weight).

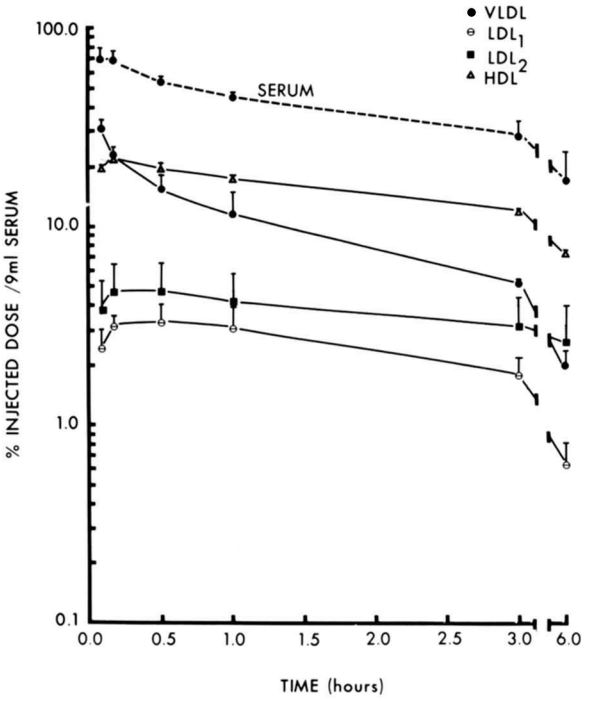

Fig. 3. Redistribution of radioactivity among serum lipoproteins after injection of ^{125}I-labeled rat VLDL into rats. Values are means ± SE from seven rats from three different experiments as described in the text.

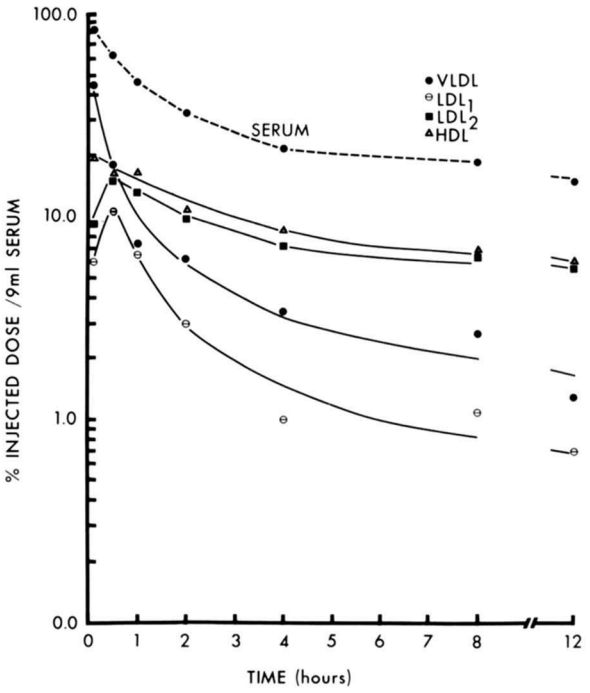

Fig. 4. Redistribution of radioactivity among serum lipoproteins after injection of ^{125}I-labeled human VLDL into rats. Data shown are means from two rats in one experiment.

of label in zone I peptides, which peaked at 30 min and remained fairly constant in this apoprotein up to 3 hr. The radioactivity present in zone III apoprotein showed a catabolic fate similar to that in the zone I apoprotein. As shown in the top panel, zone IV apoproteins of HDL contained most of the label and reached a peak of activity at 10 min. There was also evidence for the rapid transfer of zone I and III peptides to HDL 5 min after the injection of labeled VLDL although radioactivity present in those zones decreased rapidly during the subsequent 5 min and fell more slowly thereafter.

Fig. 5 demonstrates the clear separation of the zone IV apoproteins, and the actual distance between these bands on the gels (approximately 1 cm) was sufficient to allow the easy separation of these bands by the slicing technique. As shown in **Fig. 7**, zone IVb (the fastest-migrating apoprotein) contained approximately 30–40% more label than zone IVa apoprotein, and the relationship between these apoproteins in VLDL and HDL remained approximately the same after injection of ^{125}I-labeled VLDL. These data suggest that a considerable portion of the label that disappeared rapidly from VLDL zone IV peptides is accounted for by their transfer to HDL. The data also suggest that, between 3 and 6 hr, radioactivity that is lost from zone IV apoprotein of HDL returns to the same apoprotein group of VLDL. A similar comparison of the zone III apoprotein illustrates that after an initial rapid exchange the label disappears from VLDL and HDL at a similar rate.

Studies with VLDL labeled in vitro from ^{125}I-labeled rat HDL

In order to study the metabolism of VLDL labeled only with zone III and IV peptides, rat VLDL was labeled from iodinated rat HDL in an in vitro system as described in the Methods section. This system was based on the results of a

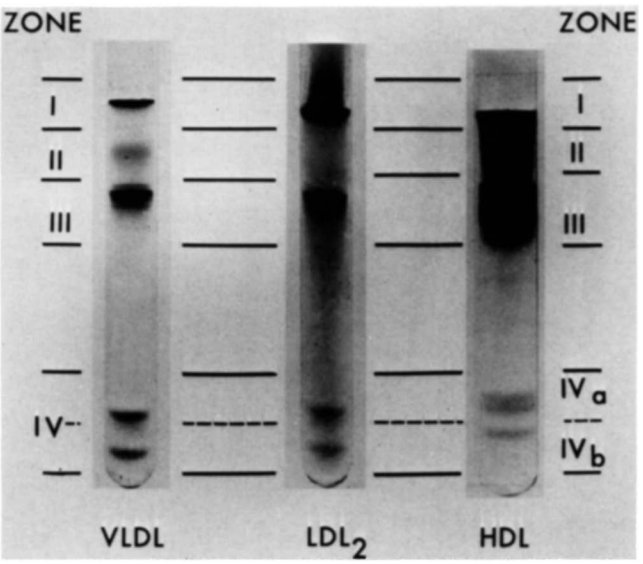

Fig. 5. Identification of gel zones after electrophoresis and staining of rat VLDL, LDL, and HDL apolipoproteins on 7.5% polyacrylamide gels. Most of the radioactivity was associated with the stained bands, as described in the text.

TABLE 2. Distribution of radioactivity in serum after injection of ^{125}I-labeled human VLDL into rats[a]

Time after Injection	Distribution of Radioactivity among Lipoproteins[b]					Serum Radioactivity
	VLDL (d < 1.006)	LDL_1 (d = 1.006–1.019)	LDL_2 (d = 1.019–1.063)	HDL (d = 1.063–1.21)	Infranate (d > 1.21)	
	% of serum radioactivity					*% of injected dose/9 ml of serum*[c]
5 min	55.3	7.4	11.4	23.8	2.1	81.7
30 min	28.3	16.5	25.8	25.7	3.7	62.9
1 hr	15.6	14.1	30.6	35.4	4.3	46.5
2 hr	19.2	8.8	30.1	36.3	5.6	32.6
4 hr	16.5	4.3	33.4	39.8	6.0	21.4
8 hr	14.3	5.7	35.7	35.8	8.5	19.0
12 hr	8.8	4.9	38.3	39.9	8.1	15.1

[a] Rats were injected with 70–80 μg of human VLDL protein containing 5 × 10^6 cpm.
[b] Values are means from two rats exsanguinated at each time interval. Similar results were obtained in two other experiments.
[c] See Table 1.

preliminary experiment carried out to determine the optimum ratio of VLDL protein per milliliter of serum required for maximum labeling of VLDL by exchange from ^{125}I-labeled HDL. In Table 4 it can be seen that the percentage of total radioactivity transferred to VLDL increased approximately fourfold when 2.0 mg of VLDL protein was added to the incubation system that contained 4.5 ml of serum and ^{125}I-labeled HDL. This ratio was maintained in the "scaled-up" system described in the Methods section.

After the isolation of the in vitro labeled VLDL, the lipoprotein was fractionated into three subclasses: S_f > 400, S_f 100–400, and S_f 20–100. After washing twice, the lipoproteins were delipidated and the distribution of radioactivity among the apoproteins was determined. Approximately 80–85% of the radioactivity of all VLDL subfractions was associated with the faster-migrating zone III and IV peptides (Table 5).

The results obtained after injecting VLDL subfractions of S_f > 400 and S_f 100–400 labeled in vitro from ^{125}I-la-

TABLE 3. Distribution of radioactivity among rat apolipoproteins after injection of ^{125}I-labeled rat VLDL

Lipoprotein Fraction	Time	% of Injected Dose of Apoprotein/ 9 ml of Serum	Distribution of Radioactivity among Apoproteins[a]			
			Zone I	Zone II	Zone III	Zone IV
	min		*% of total radioactivity*[b]			
VLDL injected			43.8		12.4	43.8
VLDL (d = 1.006)	5	29.7	42.8 ± 0.47		15.9 ± 0.33	41.4 ± 3.3
	10	23.9	44.9 ± 0.75		13.6 ± 0.15	41.6 ± 3.5
	30	8.7	23.2 ± 0.80		30.7 ± 0.10	46.1 ± 3.7
	60	7.7	7.6 ± 0.34		10.8 ± 0.90	81.5 ± 5.0
	180	6.2	14.2 ± 1.90		6.5 ± 0.17	79.2 ± 17.5
	360	6.8	11.1 ± 0.82		5.8 ± 0.15	83.1 ± 4.2
LDL (1.019–1.055)	5	8.2	14.2 ± 1.90		6.5 ± 0.17	79.2 ± 17.3
	10	9.5	11.1 ± 0.82		5.8 ± 0.15	83.1 ± 4.2
	30	9.8	13.6 ± 1.30		6.2 ± 0.46	82.2 ± 6.6
	60	7.9	11.3 ± 1.00		4.8 ± 0.31	83.9 ± 8.3
	180	6.5	14.6 ± 0.56		6.2 ± 0.35	79.1 ± 3.2
	360	6.0	12.1 ± 1.10		3.0 ± 0.35	84.9 ± 6.8
HDL (1.063–1.21)	5	32.1	14.4 ± 0.78	12.7 ± 2.00	12.9 ± 0.90	60.0 ± 5.4
	10	31.9	6.4 ± 0.47	5.1 ± 0.56	9.1 ± 0.54	79.4 ± 3.9
	30	29.9	7.1 ± 0.50	8.0 ± 1.10	11.7 ± 0.72	73.2 ± 5.8
	60	24.7	6.2 ± 0.36	4.8 ± 0.41	8.8 ± 0.40	80.1 ± 4.5
	180	21.2	10.8 ± 0.55	7.5 ± 0.18	10.1 ± 0.45	71.6 ± 1.1
	360	14.2	8.9 ± 0.70	5.3 ± 0.40	8.4 ± 0.44	77.4 ± 6.5

[a] Results obtained from three separate gels from the lipoproteins obtained from three rats; values are means ± SEM of three rats at each time point.
[b] Obtained after electrophoresis of apoprotein on 7.5% polyacrylamide gels as described in the text.

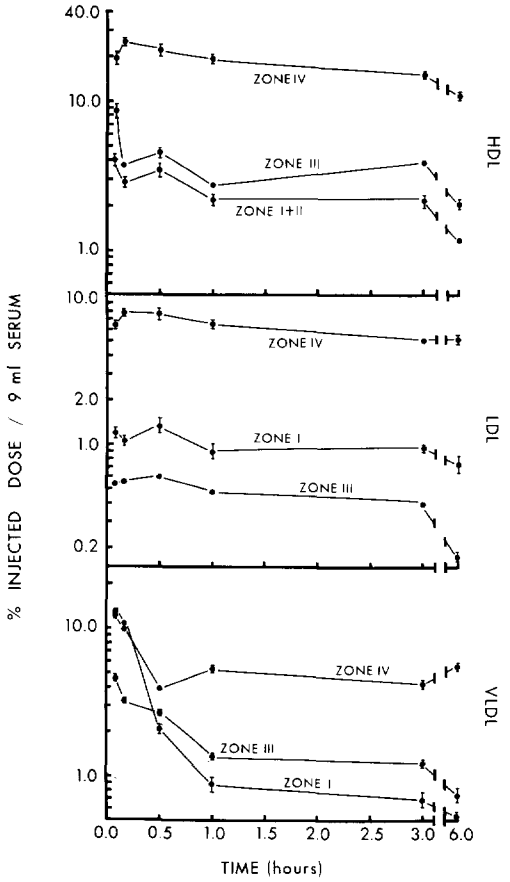

Fig. 6. Redistribution of label among apoproteins of VLDL, LDL, and HDL after injection of ^{125}I-labeled rat VLDL into rats. Each point is the mean ± SD from three rats. SD bars are omitted when SD < 0.1.

beled HDL are shown in **Tables 6** and **7** and **Fig. 8.** There was insufficient radioactivity in the S_f 20–100 subfractions for in vivo investigation. The data suggest that the redistribution of radioactivity between lipoproteins of S_f 100–400 and HDL was faster than that between VLDL of $S_f > 400$ and HDL. 2 min after injection of the smaller VLDL molecules, HDL contained twice as much label as VLDL, whereas 2 min after injection of the VLDL subclass $S_f >$ 400, there was an approximately equal amount of label in VLDL and HDL.

TABLE 4. In vitro transfer of peptide from ^{125}I-labeled HDL to VLDL

Rat Serum	Unlabeled VLDL Added[a]	Radioactivity in VLDL[b]
ml	*mg protein*	*% of total*
4.5		0.8
4.5	0.5	1.2
4.5	1.0	2.7
4.5	2.0	4.1

[a] Incubation at 37°C as described in text.
[b] VLDL radioactivity calculated after isolation and washing by ultracentrifugation.

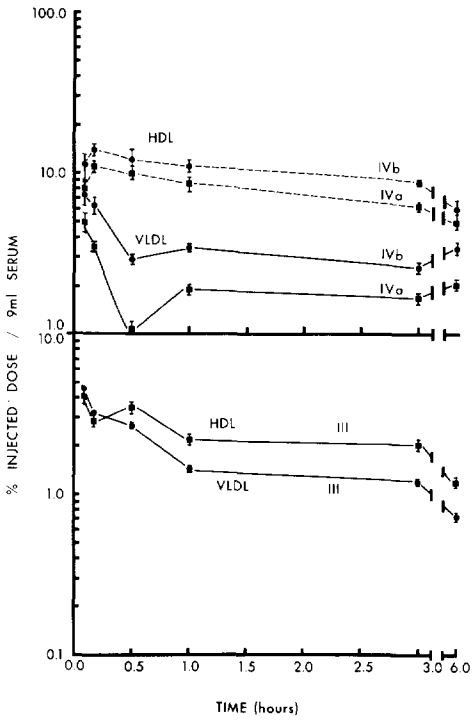

Fig. 7. Redistribution of radioactivity among zone III and zone IV apoproteins of rat VLDL and HDL after injection of ^{125}I-labeled rat VLDL into rats. IVa and IVb were separated as described in the text. Each point is the mean ± SD from three rats. SD bars are omitted when SD < 0.1.

It was interesting to note that a significant proportion of the radioactive peptide was transferred to LDL_1 and LDL_2 fractions after injection of both subclasses of zone IV labeled VLDL. In both cases, there was an absolute increase in radioactivity in LDL_1 and LDL_2 for 5 min and up to 15 min, respectively. Electrophoresis of the LDL_2 and HDL apoproteins confirmed that the fastest-migrating peptides were involved in the transfer of label.

DISCUSSION

In a previous communication from this laboratory (1), it was shown that after the administration of ^{125}I-labeled VLDL to rats a considerable proportion of the labeled peptide was redistributed among the other higher density lipoproteins, suggesting that they were metabolically related to VLDL. The present study was undertaken in order to ex-

TABLE 5. Distribution of radioactivity in VLDL apoproteins labeled from ^{125}I-labeled HDL

	Zones I and II	Zone III	Zone IV
	% of total radioactivity		
VLDL $S_f > 400$	17.9	22.4	59.7
VLDL S_f 100–400	14.2	22.3	63.5
VLDL S_f 20–100	15.8	20.4	63.0

TABLE 6. Metabolism of ^{125}I-labeled S_f 100–400 subfraction of VLDL labeled by in vitro incubation with ^{125}I-labeled HDL[a]

Time after Injection	Distribution of Radioactivity among Lipoproteins[b]					
	VLDL (d < 1.006)	LDL_1 (d = 1.006–1.019)	LDL_2 (d = 1.019–1.063)	HDL (d = 1.063–1.21)	Infranate (d > 1.21)	Serum Radioactivity
min	*% of serum radioactivity*					*% of injected dose/9 ml of serum*[c]
2	28.7	6.1	10.6	47.7	6.9	81.4
5	26.6	7.1	11.3	48.9	6.1	80.3
10	27.1	5.3	12.2	50.9	4.5	68.0
15	19.8	5.8	16.0	52.0	6.4	66.3
30	24.4	5.4	14.1	50.9	5.2	52.0
45	21.8	5.8	13.3	53.2	5.9	49.3
60	17.6	6.6	16.5	54.2	5.1	40.8

[a] 81 μg of VLDL protein and approximately 4 × 10^6 cpm injected per rat.
[b] Values are means from two rats at each time point.
[c] As in Table 1.

amine in more detail the catabolic events involved in this interrelationship and to identify, as far as possible, the apoproteins involved in the process. During the course of these experiments, the results of a similar study were reported by Eisenberg and Rachmilewitz (13, 14) and, where comparable, their findings were similar to those described in this paper.

The disappearance of total serum radioactivity after the administration of ^{125}I-labeled VLDL was characterized by a curve different from that described by a plot of the radioactivity remaining in the VLDL fraction. This difference is accounted for by a transfer or exchange of labeled protein between VLDL and the other lipoprotein fractions. Attempts to define accurately the early kinetic processes involved in the clearance of VLDL from the circulation are hindered by various factors. Firstly, it is recognized that the whole VLDL used in these experiments (Fig. 1) is not homogeneous with regard to its composition (15), size (20, 21), heterogeneity of apoproteins (22), or origin (23, 24), so there can be no assumption of uniform catabolism within this fraction. Secondly, the metabolism of VLDL is associated with an exchange or transfer of several components (25, 26) to other lipoproteins while there is evidence to suggest that there is a reciprocal net transfer of protein from HDL to triglyceride-rich lipoproteins (27). For these reasons we have not attempted to assign turnover rates for apo-VLDL metabolism, although it can be seen that, when expressed as total serum or VLDL disappearance, broadly two phases of clearance are associated with its catabolism. From the time of injection to the earliest sample at 5 min, there is a very rapid loss of radioactivity from the serum that continues and is still apparent between 5 and 30 min. The multiexponential nature of this rapid phase was revealed in subsequent studies (not shown) in which a greater number of samples were obtained between 5 and 60 min, demonstrating that the first phase could not be de-

TABLE 7. Metabolism of ^{125}I-labeled S_f > 400 subfraction of VLDL labeled by in vitro incubation with ^{125}I-labeled HDL[a]

Time after Injection	Distribution of Radioactivity among Lipoproteins[b]					
	VLDL (d < 1.006)	LDL_1 (d = 1.006–1.019)	LDL_2 (d = 1.019–1.063)	HDL (d = 1.063–1.21)	Infranate (d > 1.21)	Serum Radioactivity
min	*% of serum radioactivity*					*% of injected dose/9 ml of serum*[c]
2	36.1	4.1	11.1	42.0	6.7	75.0
5	24.8	9.0	11.7	46.4	8.1	82.6
10	19.1	7.6	14.7	50.8	7.8	63.3
15	14.4	9.1	12.7	58.8	5.0	54.0
30	18.4	9.0	14.4	50.8	7.4	47.8
45	14.7	9.9	14.1	56.0	5.4	46.2
60	14.5	18.1	11.9	50.6	4.9	42.8

[a] 62.5 μg of VLDL protein and 5 × 10^6 cpm injected per rat.
[b] Values are means from two rats at each time period.
[c] As in Table 1.

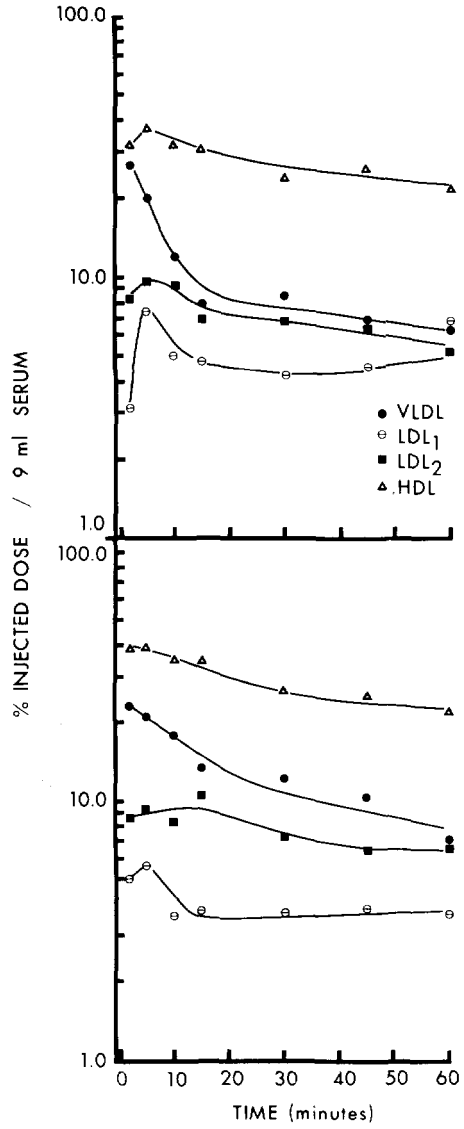

Fig. 8. Redistribution of radioactivity after injection of predominantly zone III and IV ^{125}I-labeled VLDL from rats. Rat VLDL was labeled as described in the text. *Top panel,* results after injection of VLDL of $S_f >$ 400; *bottom panel,* redistribution after injection of S_f 100–400 fraction.

fined by one component only. This early rapid clearance is followed by a slower phase of removal that approaches linearity after 3 hr.

These studies clearly demonstrated the occurrence of rapid exchange or transfer of radioactivity to other lipoproteins, because 5 min after ^{125}I-labeled VLDL injection only 50.6% of the total serum radioactivity remained in VLDL and 32.7% was already present in HDL. The kinetics of the redistribution in the rat is much faster than in the human (12). In the human (12), LDL radioactivity peaked at 12–24 hr and HDL at 9 hr, whereas in the rat the transfer of label to HDL and to LDL peaked at only 30 min when expressed as a percentage of the injected dose. This discrepancy can possibly be attributed to the difference in size of the two species and consequent differences in standard metabolic rate and cardiac output that result in a more rapid catabolism of the VLDL molecule in the rat.

Our results are similar to those of Eisenberg and Rachmilewitz (13, 14), who found that, 2 min after injection of ^{125}I-labeled rat VLDL into rats, only 77.4% of total serum ^{125}I was left in VLDL, with 5.5% in LDL and 12.2% in HDL. By 15 min these values had changed to 44.6% of serum radioactivity for VLDL, 8.7% for LDL, and 36.1% for HDL. It is extremely unlikely that the rapid removal of VLDL in our studies was due to the presence and removal of denatured lipoprotein because the VLDL had been reisolated by ultracentrifugation immediately prior to injection. Also, in other experiments, VLDL that had been filtered through 0.22-nm cellulose nitrate Millipore filters showed essentially the same turnover rate as unfiltered preparations.[2] Furthermore, the half-life of the VLDL preparations used in these studies was similar to that reported (28) for native VLDL labeled biologically with [^{14}C]leucine in which an initial rapid clearance phase (half-life approximately 40 min) was followed by a later slower disappearance phase. In this laboratory, VLDL labeled biologically with [^{3}H]lysine exhibited initial rapid and later slower clearance rates similar to the iodinated lipoproteins.

In our studies, the LDL fraction was subfractionated into two subclasses, LDL_1 (d 1.006–1.019) and LDL_2 (d 1.019–1.063). LDL_1 contained at least 56% of the LDL_2 radioactivity and did not exceed 74% up to 3 hr after VLDL injection, although the percentage decreased to 23% at 6 hr. It has been suggested that the LDL_1 fraction is an intermediate lipoprotein formed from the degraded VLDL molecule and could possibly be a precursor of LDL_2 molecules. However, there is no direct evidence of a precursor–product crossover relationship between LDL_1 and LDL_2 after injection of VLDL of $d < 1.006$. After 3–6 hr, the amount of radioactivity in LDL_2 is approximately equal to that present in VLDL, and although this may indicate the existence of a precursor–product relationship between VLDL and LDL, specific radioactivity data, not obtained in these studies, are required before any quantitative interpretation can be considered.

To investigate further the transfer of apoproteins, the lipoproteins were delipidated and resolved using polyacrylamide gel electrophoresis as described in the Methods section. The gel studies showed that most of the label transferred to both LDL and HDL was due to zone III and IV apoproteins. Due to its poor solubility, some selective loss of apo-LDL (B protein) may have occurred during delipidation and resolubilization of rat LDL. Even taking this into account, the results still show that a considerable proportion of the radioactivity was associated with the faster-migrating peptides, which are present in small amounts in rat LDL, as shown in the gel pattern in Fig. 5 and re-

ported by others (29). The significance of this exchange is not known, although the transfer of zone IV peptides from VLDL to LDL and HDL is roughly proportional to the mass of zone IV peptides present in each lipoprotein fraction. Unfortunately, the quantitative significance of this exchange between VLDL and LDL in our studies cannot be compared with the one other reported study (13, 14) of ^{125}I-labeled rat VLDL metabolism because these authors did not separately isolate LDL but based their information on a $d < 1.063$ fraction that contained both VLDL and LDL.

A close examination of the kinetics of apoprotein transfer by examination of the distribution of radioactivity in gel zones I, III, and IV of VLDL and LDL and in zones I–IV of HDL revealed considerable metabolic heterogeneity among these zones. The disappearance of label from zone IV and zone III in VLDL and LDL is different from that in zone I. In VLDL zone I, radioactivity decreases more rapidly than in zones III and IV. This result is in accordance with the human situation as described by Eisenberg, Windmueller, and Levy (7), zones III and IV being equivalent to the human C proteins and zone I to apo-LDL, or B protein (29). In LDL, zones I and III follow the same kinetics, suggesting a parallel clearance of these components from the circulation. These zones may therefore be parts of a subunit or component of apo-LDL that are metabolically related. Zone IV radioactivity, however, increased then decreased as did zone IV of HDL, which suggests that C protein exchange is occurring.

Gel zone IV was further divided into zones IVa and IVb, which were labeled to different extents, IVb containing more radioactivity than IVa. However, both IVa and IVb in VLDL, LDL, and HDL were metabolized at similar rates. It has been suggested by Herbert et al. (30) that a rat C protein (C-II) associated with gel zone IV acts as a cofactor of lipoprotein lipase. According to their nomenclature, rat C-II peptide comigrates with C-III (our C-IVa) on polyacrylamide gels at pH 8 and, like human C-II peptide, is a potent activator of lipoprotein lipase. We are also presently investigating the involvement of rat VLDL apoproteins in lipolytic activity.

The similarity in metabolism of rat and human VLDL in the rat suggests that this animal is a suitable model for studying human VLDL metabolism. The results of our studies with human VLDL in rats are in accord with those described by Eisenberg et al. (7) in which the fates of human VLDL and LDL in the rat were similar to those observed previously in humans. Like these authors, we are encouraged to continue further investigations with this animal in an attempt to explain the involvement of some apolipoproteins in the metabolism of serum lipoproteins.

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