Metabolic fate of rat and human lipoprotein apoproteins in the rat

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Abstract The fate of ^{125}I-labeled apolipoproteins was studied in vivo in rats that had received intravenous injections of ^{125}I-labeled rat HDL and ^{125}I-labeled human HDL, LDL, and VLDL.

Plasma decay curves of rat and human HDL were exponential with similar half-lives in the circulation (11–12 hr). After injection, low molecular weight apolipoproteins (apoLP-alanine of human HDL and fraction HS-3 of rat HDL) were found to redistribute to other lipoproteins, predominantly VLDL. Decay curves of individual HDL proteins were constructed after lipoprotein fractionation, delipidation, and polyacrylamide gel electrophoresis. It was found that the half-lives of the different HDL apoproteins were not identical. A major rat HDL protein (52% of total counts) had a circulating half-life ($t_{1/2}$) of 12.5 hr. Two others had a $t_{1/2}$ of 8–9 hr while the $t_{1/2}$ of several others was 11–12 hr. The $t_{1/2}$ of three well-characterized human HDL apoproteins, apoLP-glutamine I, apoLP-glutamine II, and apoLP-alanine, were 13.5, 9.0, and 15.0 hr, respectively.

The fate of ^{125}I-labeled human VLDL and LDL apoproteins in rats was similar to that described previously in humans. After injection of ^{125}I-labeled human VLDL into rats, apoLP-glutamic acid and apoLP-alanine rapidly transferred to rat HDL and were lost thereafter from the circulation from both VLDL and HDL. The apoLDL moiety of human VLDL moved metabolically to the LDL density range (d = 1.019–1.063) through a lipoprotein of intermediate density (d = 1.006–1.019).

Analysis of tissue radioactivity at time intervals after the injection of labeled lipoproteins suggests that the liver may play an important role in the clearance of all lipoprotein preparations from plasma.

Supplementary key words lipoprotein turnover · lipoprotein catabolism · hepatic lipoprotein catabolism · iodinated lipoproteins

The rat has been used extensively in the past as an experimental animal to study the metabolism of plasma lipoproteins. These studies, however, were concerned primarily with the lipoprotein lipids and not with the protein moiety of the lipoproteins. Recently, it has been shown that rat lipoproteins contain a number of different apoproteins (1, 2). It has also been shown that the same apolipoprotein is present in rat VLDL and LDL and that several other apoproteins are shared by VLDL and HDL (1, 2). This distribution of apoproteins among plasma lipoproteins is very similar to that described for the human (3–6).

Some interrelationships among the apoproteins of human plasma lipoproteins have been recently described (7, 8). It was found that apoLP-glutamic acid[4] and apoLP-alanine are readily transferred between VLDL and HDL in vitro and in vivo, and that the apoLDL moiety of VLDL is a precursor of the apoprotein of

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; apoLP, apolipoprotein; apoLDL, the apoprotein moiety of low density lipoprotein; EDTA, ethylenediaminetetraacetate.

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[4] The nomenclature of apolipoproteins of human origin is at present somewhat arbitrary. We have adopted the suggestion of Shore and Shore (9) to refer to human apolipoproteins according to their carboxyl-terminal amino acid when feasible. The following names are used: apoLDL, the protein moiety of low density lipoprotein (4); apoLP-serine, apoLP-glutamic acid, and apoLP-alanine, small molecular weight VLDL apoproteins (10); apoLP-glutamine I and apoLP-glutamine II, the major apoproteins present in HDL (11). With regard to rat apolipoproteins, the nomenclature of Bersot et al. (1) was followed when possible.

plasma low density lipoprotein (7, 8). Thus, in the human, the fate of the VLDL apoproteins in the circulation is heterogeneous.

Several studies have been published in the last decade on the turnover in vivo of rat lipoproteins, and two recent reports indicate that both rat LDL and HDL may be catabolized in the liver (12, 13). However, the fate of the individual rat plasma lipoprotein apoproteins is not known. In the present study we have investigated in the rat the metabolism and catabolic sites of the protein moieties of rat and human lipoproteins.

MATERIALS AND METHODS

Rats and diet

The rats used were 250–300 g males of the Osborne-Mendel strain from the National Institutes of Health pathogen-free colony. From 3–6 days prior to injection until the end of the experiments they were fed a fat-free but otherwise balanced semisynthetic diet containing 69% corn starch, 20% casein, and 0.3% DL-methionine, plus adequate amounts of vitamins and minerals. The drinking water was supplemented with 0.45% NaCl and 0.05% NaI.

Preparation of labeled lipoproteins

Human lipoproteins were isolated from normal fasting human plasma in 0.1% EDTA with Beckman Ti-60 and -65 rotors and the L2-65B Beckman ultracentrifuge, as previously described (7). Human VLDL was isolated at plasma density ($d < 1.006$), human LDL at a density of 1.019–1.063, and human HDL at a density of 1.085–1.21 g/ml. Each lipoprotein fraction was recentrifuged twice at its respective density. Rat HDL was similarly isolated from rat plasma by centrifugation at a density of 1.085–1.21 (14, 15). The protein/cholesterol ratio (w/w) of rat HDL was characteristically 1.85, similar to values reported by others (9, 12). Lipoproteins were shown to be free of contamination with other plasma proteins or lipoproteins by immunoelectrophoresis and immunodiffusion techniques, employing specific antisera (16).

Lipoproteins were labeled by a modification of the iodine monochloride method of McFarlane (17), as described previously (7). Labeled lipoprotein lipids were determined in the chloroform phase after lipid extraction as described by Folch, Lees, and Sloane Stanley (18).

The efficiency of iodination averaged 6.3% for human VLDL, 28.9% for human LDL, 41.5% for rat HDL, and 61.0% for human HDL.[5] Labeled lipids comprised 14.3% of the total ^{125}I bound to human VLDL, 3.5% of human LDL, 4.6% of rat HDL, and 1.3% of human HDL.

^{125}I-labeled lipoproteins were indistinguishable from nonlabeled lipoproteins with respect to their immunological and electrophoretic properties (16). $Na^{125}I$, carrier free, was purchased from New England Nuclear, Boston, Mass.

Distribution of tissue and plasma radioactivity

Rats, under light ether anesthesia, were injected intravenously, via an exposed saphenous vein, with labeled lipoprotein suspended in 0.3–0.5 ml of 0.85% NaCl and 1% bovine serum albumin. The rats were kept in metabolism cages and urine and feces were collected. With some rats, 0.1 ml of blood was sampled periodically from the tip of the tail for determining the decay of total circulating radioactivity. Other rats were killed by exsanguination under ether anesthesia and 8–10 ml of blood was collected from the left ventricle of the heart with a 19-gauge needle and syringe containing 0.4 ml of 4% sodium EDTA, pH 7.4. Whole body perfusion with 0.9% NaCl and 0.01% EDTA (saline–EDTA) was started immediately after blood collection through the needle in the heart, the outlet being the inferior vena cava above the diaphragm. Perfusion was continued at room temperature for 10–15 min with a total volume of about 150 ml. The perfusate was sampled serially for radioactivity. The last 10 ml contained less than 2% of the radioactivity found in the rat's plasma. Liver, kidneys, testes, lungs, heart, spleen, aorta, and samples of the psoas muscle and paraortic adipose tissue were removed, cleaned of adhering connective tissue, and rinsed in ice cold saline–EDTA. With the exception of the spleen, which always contained some blood, all other tissues were either visibly devoid of blood (liver, testes, heart, intestine, muscle, aorta, adipose tissue) or contained large areas that were free from blood (kidney and lung). The entire small intestine was removed and opened, the intestinal contents were collected, and the small intestinal wall was first washed in cold saline–EDTA (the washings were added to the intestinal content material) and then rinsed under running tap water. Colonic material was likewise collected, and the colon was washed under tap water before determination of radioactivity.

[5] In the labeling of proteins with radioactive iodine, the number of atoms of iodide introduced per mole of protein becomes important (19). However, lipoproteins contain several different proteins, with different molecular weights. Moreover, the molecular weights of some apolipoproteins are not known. Yet, with lipoproteins this ratio is of operational value and is important for comparison of different studies. Assuming apoprotein molecular weights of 300,000 for human VLDL, 100,000 for human LDL, and 50,000 for human and rat HDL, the following number of atoms of iodide were introduced per "mole" of apoprotein: human VLDL, 0.50; human LDL, 0.90; human HDL, 0.90; and rat HDL, 0.50. The assumed molecular weights are higher than the best reported estimates, where available, in order not to underestimate the molar ratio of iodide to protein (Refs. 1–6).

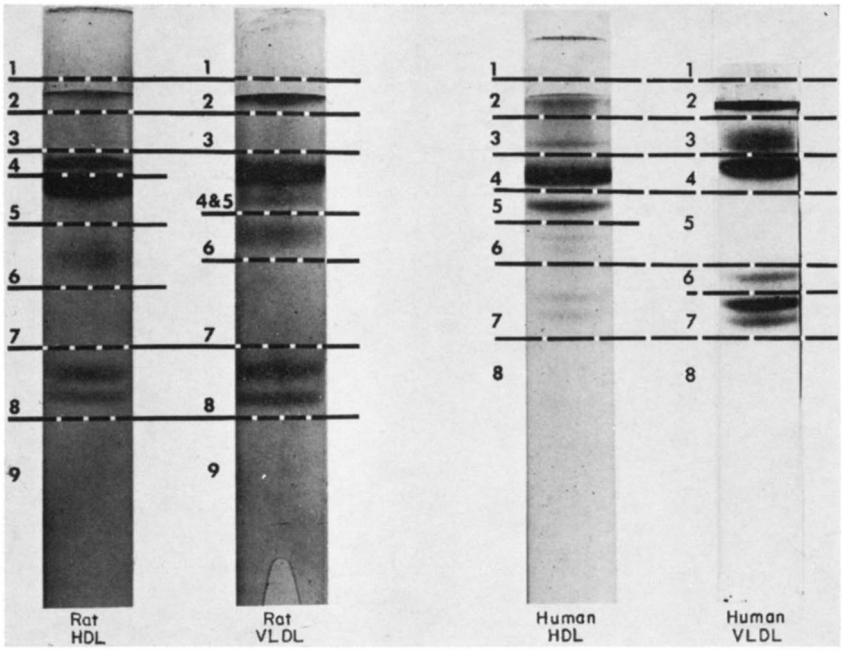

FIG. 1. Stained polyacrylamide gels of apoproteins isolated from rat VLDL, rat HDL, and human HDL. To determine the distribution of radioactivity among apoproteins, the gels were sliced to zones, as shown by horizontal lines, and counted. The following apoproteins can be identified: rat HDL, zone 5 = HS-2, zone 8 = H3-3 and H3-4 (Ref. 1); rat VLDL, zone 2 = VS-1, zone 8 = V3-3 and V3-4 (Ref. 1); human HDL, zone 4 = apoLP-glutamine I, zone 5 = apoLP-glutamine II, zone 7 = apoLP-glutamic acid + apoLP-alanine$_{1+2}$; human VLDL, zone 2 = apoLDL, zone 3 = apoLP-serine, zone 5 = apoLP-glutamic acid, zone 6 = apoLP-alanine$_{1+2}$ (Ref. 7).

To determine the distribution of radioactivity among the plasma lipoproteins, 3–5-ml plasma samples from the injected animals were diluted to 11 ml with either unlabeled rat plasma or unlabeled human plasma as indicated in the table legends. One 5-ml aliquot was centrifuged at plasma density to obtain the VLDL ($d < 1.006$). The infranate was adjusted to $d = 1.040$, and after centrifugation the top 2.5 ml was recovered ($d = 1.006$–1.040). A second 5-ml aliquot of the plasma was centrifuged after the density was adjusted to $d = 1.063$, and the top 2.5 ml was recovered ($d < 1.063$). The infranate was recentrifuged at a density of 1.21 to isolate the HDL ($d = 1.063$–1.21).

The density was adjusted with either NaCl–KBr solu-

TABLE 1. Distribution of radioactivity in plasma after injection of ^{125}I-labeled rat HDL[a]

Time after Injection	Plasma Radioactivity	Distribution of Radioactivity among Lipoproteins[b]				
		($d < 1.006$)	($d = 1.006$–1.040)	($d = 1.040$–1.063)	($d = 1.063$–1.21)	($d > 1.21$)
hr	*% of injected dose*	*% of plasma radioactivity*				
1/12	100[c]	4.1; 4.3	1.2; 1.2	7.4; 3.0	82.7; 85.5	4.6; 6.0
1	92.6 ± 2.8	4.0; 6.0	1.0; 1.0	10.6; 11.2	82.0; 77.2	2.4; 4.6
4	75.7 ± 2.9	4.0; 5.2	1.5; 2.5	9.5; 10.7	83.0; 77.4	2.0; 4.2
8	60.3 ± 2.9	4.5; 5.1	1.9; 2.5	9.1; 4.1	82.1; 83.5	2.4; 4.8
24	21.8 ± 1.5	4.6; 6.0	1.4; 3.0	11.3; 5.1	80.3; 81.7	2.4; 5.8
48	5.5 ± 0.4	4.4; 2.6	1.2; 2.6	9.6; 9.4	82.5; 81.1	2.3; 4.3
72	1.6 ± 0.1	5.3;	1.4;	10.0;	78.9;	4.4;

[a] Rats were injected with 0.26 mg of HDL protein containing 2.2×10^7 cpm.

[b] Data are from individual rats in two experiments. In each experiment, 3–5 ml of plasma from a single rat was diluted to 11 ml with unlabeled rat plasma (experiment 1, first set of data) or unlabeled human plasma (experiment 2, second set of data) before ultracentrifugation to isolate the lipoproteins as described in Materials and Methods.

[c] Plasma radioactivity 5 min after the injection was taken as 100% of the injected dose in this and subsequent tables. This value is somewhat arbitrary because even at 5 min after the injection some of the radioactivity has disappeared from the circulation. At 5 min, each milliliter of plasma contained 8.0% of the injected dose, and 280-g rats have about 11.8 ml of plasma (4.2% of body weight). Data in this column are means ± SE for a total of six determinations in two experiments. In each experiment, two rats were bled sequentially from the tip of the tail (0.1 ml) and one rat was exsanguinated at each time interval and the plasma was used for lipoprotein isolation.

tions of known density or solid KBr, and lipoproteins were separated in the Beckman L-1 ultracentrifuge as described previously (7). The distribution of radioactivity among plasma lipoproteins was independent of the source of unlabeled plasma (rat or human) used to dilute plasma obtained from rats injected with ^{125}I-labeled lipoproteins.

Delipidation and polyacrylamide gel electrophoresis

After dialysis against 0.85% NaCl, 0.1% EDTA, lipoproteins were freeze-dried and delipidated with anhydrous ethanol–diethyl ether 3:1 (v/v) and ether at 4°C (4–6). Apoproteins were separated by polyacrylamide gel electrophoresis using 10% polyacrylamide gels (7). To determine the distribution of radioactivity in the lipoprotein apoproteins, protein bands were stained with 0.05% Coomassie blue (20) and sliced from the gels. Rat VLDL, rat HDL, human VLDL, and human HDL apoproteins were sliced from the gels as shown in Fig. 1. In this way several previously characterized rat and human apoproteins (1–6, 9–11) were sliced free from other proteins (see legend to Fig. 1), whereas other gel slices contained more than one apoprotein.

After injection of human lipoproteins, unlabeled human apoproteins were added to each sample prior to polyacrylamide gel electrophoresis, and the gels were sliced according to the apoprotein pattern of the injected lipoprotein. From Fig. 1 it is seen that, although the general patterns of VLDL and HDL apoprotein bands in the rat and human are similar, the relative migration of most of the analogous apoproteins are sufficiently different that individual human apoproteins could be identified on composite gels.

The gels were loaded with 20–100 μg of protein containing 2×10^3–2×10^4 cpm. Total recovery of counts in all gel slices was always more than 90% of that applied. Of this 90%, more than 85% was associated with visibly stained protein bands.

Radioactivity was determined using a Packard Auto-Gamma spectrometer, model 5022 or 3375. All counts in the ultracentrifugal fractions were corrected for quenching due to high salt concentrations by using Na^{125}I as internal standard.

RESULTS

Metabolism of rat HDL

Within 5 min of the injection of ^{125}I-labeled rat HDL into rats, some radioactivity was found on other plasma lipoproteins (Table 1). Of the label present in plasma, 4.1–4.3% was associated with lipoproteins of density less than 1.006 g/ml, 1.2% with lipoproteins of density 1.006–1.040, 3.0–7.4% with lipoproteins of density 1.040–1.063, 4.6–6.0% remained in the plasma fraction with density greater than 1.21 g/ml, while 82.7–85.5% remained in the lipoprotein fraction with density 1.063–1.21 g/ml. Only small changes occurred in this distribution during the next 72 hr.

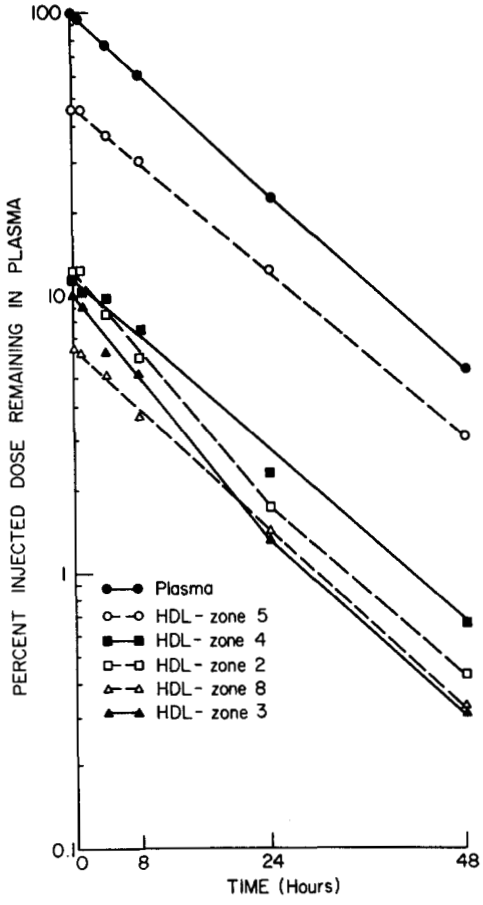

FIG. 2. Loss of radioactivity from plasma and from plasma HDL apoproteins after injection of ^{125}I-labeled rat HDL. Individual points were calculated from the data shown in Tables 1 and 2 and are the means of the two experiments. The loss of radioactivity of the HDL apoproteins was calculated after polyacrylamide gel electrophoresis, as described in Materials and Methods.

The disappearance of total plasma radioactivity and HDL radioactivity (d = 1.063–1.21) followed an exponential course with a half-life ($t_{1/2}$) of 11 hr (Fig. 2).

The distribution of radioactivity among the HDL and VLDL apoproteins is shown in Table 2. In the injected HDL, 50–55% of the label was localized in zone 5 of the polyacrylamide gel (as described in Fig. 1), corresponding to the major HDL protein previously designated HS-2 by Bersot et al. (1). Appreciable amounts of radioactivity were also associated with proteins in zones 2, 3, 4, and 8 (12.5%, 10.2%, 9.1%, and 11.8% of the label, respectively). The two protein bands sliced together in zone 6 contained only 1–2% of the total HDL radioactivity.

The distribution of radioactive proteins in VLDL was markedly different from that of the injected HDL (Table

TABLE 2. Labeled apoproteins of plasma lipoproteins after injection of ^{125}I-labeled rat HDL

Lipoprotein	Time after Injection	Distribution of Radioactivity among Apoproteins: Zone 2	3	4	5	8	Zone 5/ Zone 2+3
	hr	*% of total radioactivity*					
HDL, injected		12.5	10.2	9.1	52.3	11.8	
		Experiment 1					
HDL (d = 1.063–1.21)	1/12	13.0	11.1	12.5	50.8	7.1	2.11
	1	13.8	10.5	11.9	52.5	7.2	2.16
	4	12.6	8.7	14.3	53.2	7.3	2.45
	8	10.7	9.5	13.1	56.0	6.6	2.77
	24	8.8	6.8	11.4	62.3	7.3	4.00
	48	8.6	6.1	13.6	60.9	7.7	4.75
VLDL (d < 1.006)	1/12	3.0	1.6	7.0[a]		77.7	
	1	2.7	1.2	6.6		79.3	
	4	2.0	1.1	7.4		82.3	
	8	2.3	2.1	6.6		83.7	
	24	2.6	1.8	4.9		79.3	
		Experiment 2					
HDL (d = 1.063–1.21)	1/12	14.0	12.5	12.5	52.8	4.9	2.00
	1	15.3	12.0	10.5	53.8	5.4	1.96
	4	10.0	8.5	13.2	55.9	7.4	3.00
	8	8.0	8.5	9.5	59.3	9.3	3.59
	24	7.4	6.0	13.7	60.4	8.8	4.51
	48	5.0	5.2	12.2	66.6	6.9	6.53
VLDL (d < 1.006)	1/12	3.0	1.0	6.5[a]		86.7	
	1	4.2	3.8	9.8		77.5	
	4	3.0	1.7	12.6		75.0	
	8	2.8	3.7	4.7		86.6	
	24	3.5	1.5	10.0		78.0	

HDL and VLDL from the two experiments shown in Table 1 were delipidated, and the resulting apoproteins were fractionated on polyacrylamide gels. 2–20 × 10^3 cpm was applied per gel, and apoprotein bands were sliced as shown in Fig. 1 and counted.

[a] Apoproteins of zones 4 and 5 were sliced together.

2). At all time intervals, 85–90% of the radioactivity associated with VLDL was found in zones 8 and 9, corresponding to the low molecular weight proteins shared by both VLDL and HDL (V3-3, V3-4, and V3-5 [1]). Zones 2–5 contained only small amounts of radioactivity.

Although the decay of total HDL protein in the plasma was exponential, the decay of all HDL apoproteins was

TABLE 3. Distribution of radioactivity in plasma after injection of ^{125}I-labeled human HDL[a]

Time after Injection	Plasma Radioactivity	Distribution of Radioactivity among Lipoproteins[b]: (d < 1.006)	(d = 1.006–1.040)	(d = 1.040–1.063)	(d = 1.063–1.21)	(d > 1.21)
hr	*% of injected dose*	*% of plasma radioactivity*				
1/12	100[c]	0.7; 1.1	0.3; 0.4	3.3; 3.3	91.3; 88.2	4.4; 6.8
1	87.0 ± 1.3	0.9; 0.9	0.4; 0.4	3.1; 4.4	91.9; 88.4	3.7; 5.9
2	83.2 ± 1.6	1.0; 1.0	0.6; 0.6	6.3; 6.3	87.6; 87.6	4.5; 4.5
4	75.6 ± 1.7	1.0; 1.0	0.4; 0.6	4.3; 5.6	91.4; 89.9	2.9; 2.9
8	64.3 ± 1.0	1.3; 1.7	0.8; 1.0	6.8; 7.3	88.8; 87.3	2.3; 2.7
24	24.5 ± 0.8	1.2; 1.5	0.9; 1.0	4.4; 6.4	91.5; 88.9	2.0; 2.2
48	7.1 ± 0.5	1.7; 1.7	1.9; 1.9	9.5; 9.5	84.9; 84.8	2.0; 2.1
72	1.9 ± 0.1	1.9; 2.1	0.9; 0.9	9.1; 12.6	84.4; 80.2	3.7; 4.2

[a] Rats were injected with 0.45 mg of HDL protein containing 4.2 × 10^7 cpm.

[b] Data are from individual rats in two experiments. In each experiment, 3–5 ml of plasma from a single rat was diluted to 11 ml with unlabeled rat plasma (experiment 1, first set of data) or unlabeled human plasma (experiment 2, second set of data) before ultracentrifugation to isolate the lipoproteins as described in Materials and Methods.

[c] See footnote *c* to Table 1. 5 min after injection, each milliliter of plasma contained 9.2% of the injected dose. Data in this column are means ± SE for a total of six rats in two experiments. In each experiment, two rats were bled sequentially from the tip of the tail (0.1 ml) and one rat was exsanguinated at each time interval and the plasma was used for lipoprotein isolation.

TABLE 4. Labeled apoproteins of plasma lipoproteins after injection of ^{125}I-labeled human HDL

Lipoprotein	Time	Distribution of Radioactivity among Apoproteins				
		Zone 4 (ApoLP-Gln I)[a]	Zone 5 (ApoLP-Gln II)[a]	Zone 6	Zone 7 (ApoLP-Glu + Ala)	Gln I/Gln II
	hr	*% of total radioactivity*				
HDL, injected		49.9	32.6	8.1	4.4	1.53
				Experiment 1		
HDL (d = 1.063–1.21)	1/12	51.9	27.9	7.3	3.9	1.86
	1	53.0	25.5	5.3	5.3	2.08
	2	53.2	23.3	7.6	4.9	2.29
	4	54.7	21.2	7.3	4.5	2.58
	8	56.6	20.7	7.1	5.2	2.74
	24	58.2	15.4	9.0	6.5	3.78
	48	59.5	8.2	11.7	8.1	7.26
	72	63.5	5.4	8.7	9.2	11.76
VLDL (d < 1.006)	1/12	40.2	23.1	9.4	19.7	
	1	42.9	15.4	7.4	23.9	
	2	38.9	18.6	8.9	25.3	
	4	40.9	15.2	11.7	28.7	
	8	31.0	14.5	13.0	36.3	
				Experiment 2		
HDL (d = 1.063–1.21)	1/12	45.1	36.3	6.0	1.3	1.24
	24	53.7	24.3	11.3	1.3	2.21

HDL and VLDL from the two experiments shown in Table 3 were delipidated, and the resulting apoproteins were fractionated on polyacrylamide gels. 2–20 × 10^3 cpm was applied per gel, and apoprotein bands were sliced as shown in Fig. 1 and counted. Data for only two of the seven time points is shown for experiment 2.

[a] In HDL.

not parallel (Table 2 and Fig. 2). The apoprotein(s) of zone 4 had a circulating half-life of 11.5 hr. Radioactivity associated with zones 2 and 3 proteins disappeared from plasma more rapidly ($t_{1/2}$ = 8–9 hr) and that of zone 5 protein less rapidly ($t_{1/2}$ = 12.5 hr) than the whole HDL ($t_{1/2}$ = 11 hr). There was a progressive change with time in the relative distribution of radioactivity among the various proteins, resulting in a progressive change in the ratio of label in zone 5 to zones 2 plus 3 apoproteins (Table 2). The half-life of the peptides in zone 8 was similar to that of whole HDL despite their distribution between HDL and VLDL. They disappeared from plasma VLDL and HDL at similar rates.

Metabolism of human HDL

Groups of rats were injected with ^{125}I-labeled human HDL, and the distribution of radioactivity among the plasma lipoproteins and lipoprotein apoproteins was determined at intervals from 5 min to 72 hr after injection (Tables 3 and 4). Within 5 min, about 1% of the radioactivity injected as HDL was recovered in VLDL (Table 3). The peptides of zone 7, which accounted for only about 4% of the radioactivity of the injected HDL, accounted for about 20–25% of the radioactivity transferred to VLDL (Table 4). These peptides have been referred to previously as apoLP-glutamic acid and apoLP-alanine$_{1+2}$ on the basis of their carboxyl-terminal amino acid residues (10). As indicated earlier, these peptides can be distinguished from the analogous rat peptides when the two are coelectrophoresed (Fig. 1). There was also some transfer of other apoproteins to the VLDL, but relative enrichment of none was observed in the VLDL except for the apoLP-glutamic acid and apoLP-alanine$_{1+2}$.

The rate of disappearance of radioactivity from plasma HDL was not the same for all the human HDL apoproteins (Fig. 3). The half-life in plasma HDL was 13 hr for apoLP-glutamine I, 9 hr for apoLP-glutamine II, 15 hr for apoLP-alanine$_{1+2}$, and 14 hr for the protein(s) in zone 6 (Fig. 3). These differences resulted in large changes in the relative distribution of labeled HDL apoproteins with time; e.g., in 72 hr apoLP-glutamine II decreased from 27.9% to 5.4% of the remaining HDL radioactivity, while apoLP-glutamine I, apoLP-alanine$_{1+2}$, and zone 6 proteins increased from 51.9%, 3.9%, and 7.3% to 63.5%, 9.2%, and 11.7%, respectively (Table 4).

Metabolism of human LDL

At all time intervals after injection of ^{125}I-labeled human LDL into rats, more than 90% of the radioactivity was recovered in the plasma lipoprotein fraction with density 1.019–1.063 g/ml. The disappearance of radioactivity from plasma was exponential, with a $t_{1/2}$ of about 15 hr (Fig. 4).

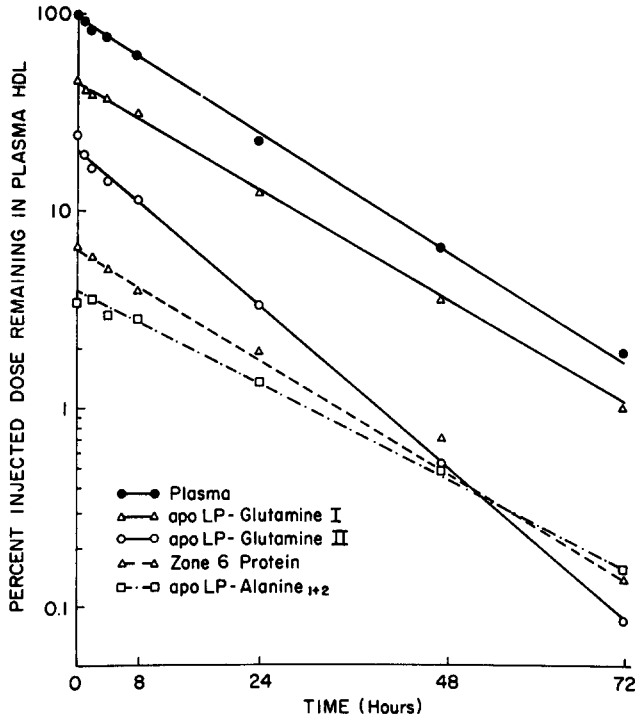

FIG. 3. Loss of radioactivity from plasma and from plasma HDL apoproteins after injection of ^{125}I-labeled human HDL. Individual points were calculated from data shown in Tables 3 and 4 and are the means of the two experiments. The loss of radioactivity of the HDL apoproteins was calculated after polyacrylamide gel electrophoresis, as described in Materials and Methods.

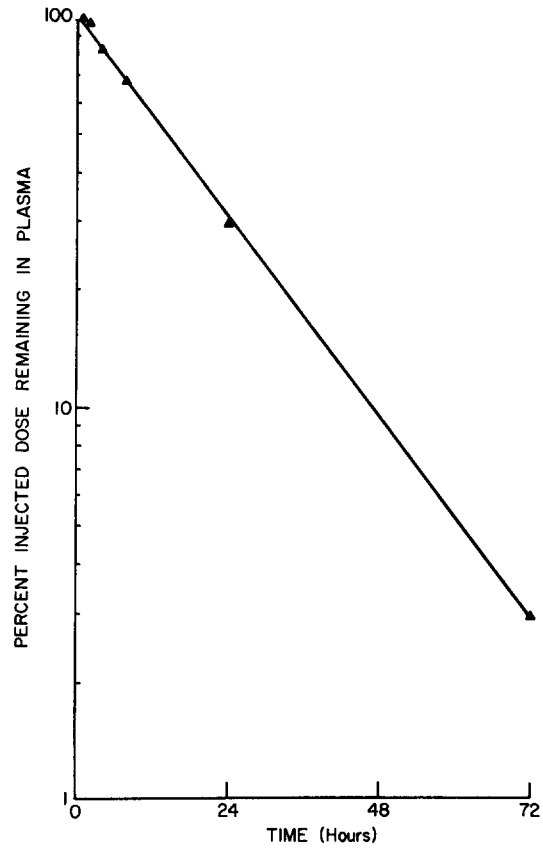

FIG. 4. Loss of plasma radioactivity after injection of ^{125}I-labeled human LDL. Rats were injected with 0.3 mg of LDL protein containing 1.4×10^7 cpm. Each point is the mean of results obtained from two rats bled sequentially from the tip of the tail (0.1 ml) and two rats exsanguinated at each time interval. The standard error at each point was always less than 10% and usually less than 5% of the mean.

Metabolism of human VLDL

The disappearance from plasma of ^{125}I-labeled human VLDL in the rat did not follow a single exponential curve (Fig. 5). About 40% of the VLDL radioactivity disappeared from plasma at a far more rapid rate than the remaining 60%.

Injection of the VLDL led to a redistribution of radioactivity similar to that previously observed in humans (7). 5 min after injection, only 62.5% of the plasma

TABLE 5. Distribution of radioactivity in plasma after injection of ^{125}I-labeled human VLDL[a]

Time after Injection	Plasma Radioactivity	Distribution of Radioactivity among Lipoproteins				
		(d < 1.006)	(d = 1.006–1.019)	(d = 1.019–1.063)	(d = 1.063–1.21)	(d > 1.21)
hr	*% of injected dose*	*% of plasma radioactivity*				
1/12	100[b]	62.5[c]	5.2	3.3	26.0	3.0
1/2	86.0	31.2	22.7	6.0	33.2	6.9
1	60.8 ± 4.5	30.1	14.8	9.6	37.5	8.0
4	50.1 ± 1.0	29.1	7.3	20.2	34.5	8.9
8	37.8 ± 1.1	26.7	1.9	24.7	38.1	8.6
24	13.2 ± 0.9	27.0	5.9	27.2	34.5	5.4
48	4.5 ± 0.2	24.7	7.1	25.0	34.2	9.0

[a] Rats were injected with 0.4 mg of VLDL protein containing 1.4×10^7 cpm.
[b] See footnote *c* to Table 1. 5 min after the injection, each milliliter of plasma contained 5.6% of the injected dose. Values are means ± SE for a total of seven rats in three experiments.
[c] Values at each time interval were obtained from one experiment in which 3–5 ml of plasma obtained from a single rat was diluted to 11 ml with unlabeled rat plasma before ultracentrifugation to isolate the lipoproteins as described in Materials and Methods. Very similar results were obtained in two other experiments when human plasma was used to dilute the [^{125}I]VLDL-containing rat plasma.

radioactivity was found in VLDL (d < 1.006) and 26% was found in the HDL (Table 5). 30 min after injection, there was further loss of radioactivity from VLDL but an increase in radioactivity in the intermediate density lipoprotein (d = 1.006–1.019). Between 1 and 4 hr, there was a decline in the radioactivity of the intermediate density lipoprotein and a relative increase in that of the LDL (d = 1.019–1.063). The relative distribution of radioactivity changed little after 4 hr (Table 5). These two types of movement of radioactive apoproteins from VLDL to other lipoproteins were observed irrespective of the source of unlabeled plasma used for the lipoprotein isolation (human or rat) and therefore cannot be ascribed to exchange of proteins during dilution of labeled with unlabeled plasma.

Examination of the radioactivity on the apoproteins of VLDL revealed a striking difference between the metabolism of the apoLDL and the smaller peptides, apoLP-glutamic acid and apoLP-alanine$_{1+2}$ (Table 6 and Fig. 5). During the first 5 min after injection, about half of the labeled apoLP-glutamic acid and apoLP-alanine were lost from the VLDL. These lost apolipoproteins were recovered with HDL (Table 6). During the next hour, there was a rapid loss of apoLDL from VLDL ($t_{1/2}$ of about 10 min), whereas the loss of the smaller peptides proceeded at a much slower rate. This difference in rate of disappearance of apoproteins from

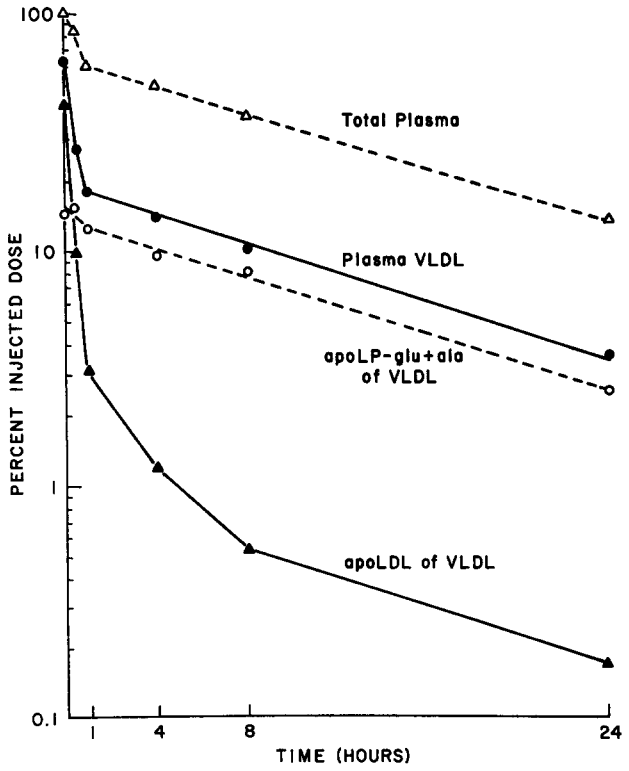

FIG. 5. Loss of radioactivity from plasma VLDL and from plasma VLDL apoproteins after injection of ^{125}I-labeled human VLDL. The values were calculated from data shown in Tables 5 and 6.

TABLE 6. Labeled apoproteins of plasma lipoproteins after injection of ^{125}I-labeled human VLDL

Lipoprotein	Time after Injection	Radioactivity in Lipoprotein	Distribution of Radioactivity among Apoproteins[a]		
			Zone 1 (ApoLDL)	Zones 5 and 6 (ApoLP-Glu + Ala)	Zone 1/ Zones 5 and 6
	hr	*% of injected dose*	*% of lipoprotein radioactivity*		
VLDL, injected			44.5	41.0	1.09
d < 1.006 (VLDL)	1/12	62.5[b]	66.4	22.5	2.95
	1/2	27.3	36.4	57.2	0.64
	1	18.2	16.8	70.2	0.24
	4	14.5	8.1	66.8	0.12
	8	10.1	6.5	83.0	0.08
d < 1.019[c]	1/2	46.3	51.3	39.5	1.30
d < 1.063[d]	1/12	71.0	67.4	24.7	2.73
	1/2	51.4	51.4	40.0	1.28
	1	33.1	32.8	48.4	0.68
	4	28.4	45.4	47.7	0.95
	8	19.5	39.8	51.0	0.78
d = 1.063–1.21 (HDL)	1/12	26.0	5.6	76.7	
	1	22.8	8.0	75.1	
	4	17.3	11.2	71.1	
	8	14.4	4.7	80.5	

[a] Lipoproteins from the experiment shown in detail in Table 5 were delipidated, and the resulting apoproteins were fractionated on polyacrylamide gels. 2–20 × 10^3 cpm was applied per gel, and apoprotein bands were sliced as shown in Fig. 1 and counted. Very similar results were obtained in the two other experiments.

[b] Values obtained in the experiment described in Table 5.

[c] VLDL plus intermediate density lipoprotein (d = 1.006–1.019).

[d] VLDL plus intermediate plus LDL.

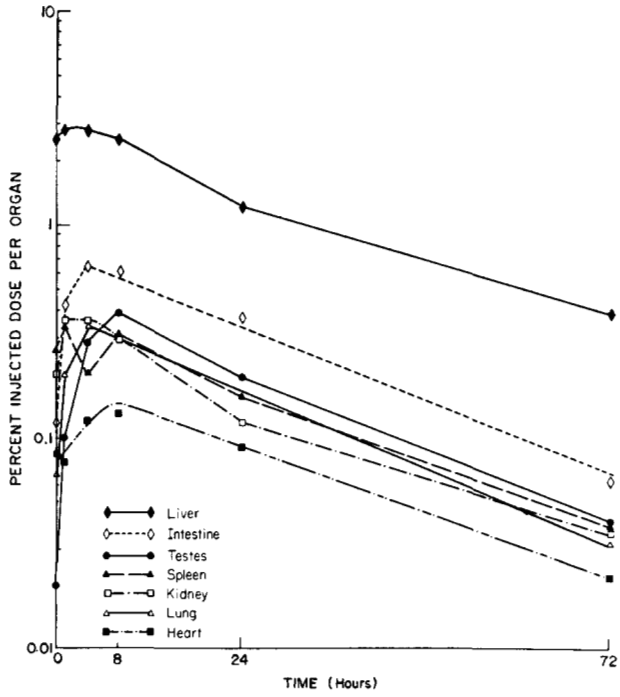

FIG. 6. Tissue radioactivity at time intervals after injection of ^{125}I-labeled rat HDL.

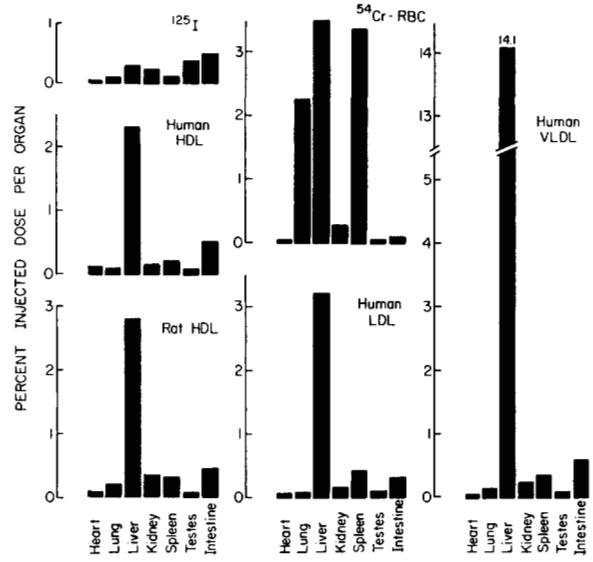

FIG. 7. Tissue radioactivity 1 hr after injection of Na^{125}I, ^{54}Cr-labeled rat erythrocytes, and ^{125}I-labeled lipoproteins.

VLDL resulted in a change in the ratio of radioactive apoLDL to radioactive apoLP-glutamic acid + apoLP-alanine in the VLDL from 3.0 at 5 min to 0.2 at 1 hr (Fig. 5 and Table 6). The sequence observed was similar to that described in humans (7, 8); it occurred, however, at a much faster rate. When the nature of radioactive proteins present in other lipoproteins was investigated, it was found that at all time intervals apoLP-glutamic acid and apoLP-alanine were the predominant labeled proteins of HDL, whereas apoLDL accounted for the bulk of labeled protein of the intermediate density lipoprotein and LDL (Table 6).

Tissue radioactivity

A preliminary experiment was conducted to characterize the solubility of the ^{125}I found in the tissues. 5 min to 8 hr after rats were injected with ^{125}I-labeled rat HDL, 5% tissue homogenates were prepared in 0.85% NaCl–0.1% EDTA–0.05% NaI. One aliquot was dialyzed against three changes of the NaCl–EDTA–NaI solution for 1 hr, and to another aliquot trichloroacetic acid was added to a final concentration of 8.3%. The amount of dialyzable and trichloroacetic acid-soluble radioactivity did not exceed 20% of the total radioactivity in the tissues examined (heart, liver, kidney, testes, muscle, and intestine) and was below 10% in most determinations.

In all experiments, tissue radioactivity was determined at 5-min to 48- or 72-hr intervals after the injection of the labeled lipoprotein preparation. The amount of radioactivity found in the tissues after injection of ^{125}I-labeled rat HDL is shown in Fig. 6. It can be seen that the radioactivity recovered in liver far exceeded that in other tissues. Not shown are data for muscle, aorta, and adipose tissue. Muscle and adipose tissue contained relatively low levels of radioactivity and their total mass could not be determined with certainty. 1 hr after the injection of rat ^{125}I-labeled HDL, muscle contained 0.03% and adipose tissue contained 0.04% of the injected dose per gram wet weight. The corresponding values for liver, kidney, small intestine, and heart were 0.22%, 0.13%, 0.11%, and 0.07%, respectively. Aortic radioactivity was of the same order of magnitude as that of muscle and adipose tissue when expressed on a per gram wet weight basis.

The distribution of tissue radioactivity 1 hr after the injection of labeled lipoprotein preparations or ^{54}Cr-labeled rat erythrocytes and [^{125}I]iodide is seen in Fig. 7. The tissue distribution after labeled erythrocyte or labeled iodide injection is clearly different from that seen after administration of labeled lipoproteins. With lipoproteins, the radioactivity of liver was 5–15 times higher than that of other tissues. This predominant concentration of radioactivity in the liver was also evident when tissue radioactivity was expressed as the fraction of the injected dose per gram wet weight of tissue. Regardless of source (rat or human) or density of the lipoprotein preparation, the distribution of radioactivity in the tissues was always similar. The similarity was particularly striking when rat and human HDL were compared. With these two preparations, the amount of radioactivity as well as the relative distribution among

the tissues was very similar (Fig. 7). There was little change at later time intervals from the distribution of tissue radioactivity observed at 1 hr. It should be specifically noted that the rapid initial clearance of plasma radioactivity after injection of ^{125}I-labeled human VLDL was associated with a high content of radioactivity in liver but not in other tissues.

Radioactivity of urine and intestinal contents

In all experiments, urine collected for 48–72 hr after injection contained more than 80% of the label that disappeared from the plasma. Feces collected from the metabolism cage contained 3–6% of the label. Since the feces may have been contaminated with urine in the cage, the radioactivity of colonic feces and the contents of the small intestine were determined. Both contained a substantial amount of radioactivity, together accounting for 20–30% of the radioactivity that disappeared from plasma at the early time intervals (1–4 hr). More than 70% of the radioactivity of the intestinal contents was dialyzable and was not precipitated by trichloroacetic acid.

In order to determine the source of the intestinal content radioactivity, external bile fistulas were established in two groups of rats. The rats were injected with either ^{125}I-labeled human VLDL or [^{125}I]iodide (as Na^{125}I). During 24 hr of bile drainage, 5–15% of the radioactivity injected as ^{125}I-labeled VLDL and 6.1–6.5% of that injected as [^{125}I]iodide appeared in bile. More than 70% of the bile radioactivity of the animals injected with lipoproteins was dialyzable, and 15% was associated with lipids (extractable with chloroform–methanol 2:1 [v/v]). Intestinal content radioactivity of bile fistula animals was reduced to about 10% of that found in animals without bile fistulas. The recovery of radioactivity in the liver and in the intestinal wall, however, was not different from that of normal animals.

DISCUSSION

Several reports have appeared in the literature regarding the fate of radiolabeled HDL in humans and animals (12, 21–23). However, in none of these studies was the metabolism of the individual apoproteins (1, 2, 9, 11, 24, 25) considered. As part of the present study, the fate of the apoproteins of rat and human HDL was investigated in the rat. With both preparations the loss of total plasma radioactivity and HDL radioactivity was exponential, with a half-life of 11–12 hr. A similar value for rat HDL has been reported recently for biologically screened lipoprotein (12).

To determine the half-lives of individual apoproteins in the circulation, lipoproteins were isolated and delipidated, and the labeled apoproteins were separated by polyacrylamide gel electrophoresis. Prior to delipidation, lipoproteins were lyophilized, and delipidation was performed with anhydrous ethanol and diethyl ether. It has recently been shown that under these conditions no loss of apoproteins occurs during delipidation (26). The recovery of radioactivity after delipidation was more than 90% in most instances. To ascertain the reliability of polyacrylamide gel electrophoresis for separation of lipoprotein apoproteins, several gels of the same sample were prepared, and apoprotein radioactivity was assayed by the slicing technique (Fig. 1). The variation of distribution of radioactivity among the analogous apoprotein bands sliced off different gels was less than 2%. Thus, highly reliable apoprotein decay curves could be constructed from data obtained by lipoprotein fractionation and polyacrylamide gel electrophoresis.

Using this method, it was found that the various HDL apoproteins did not disappear from the HDL at the same rates. 5 min after the injection of rat HDL, about one-third of the peptides of zone 8 of the polyacrylamide gel (Fig. 1) was recovered with the plasma VLDL. These proteins, previously referred to as fraction HS-3 of HDL (1), are common to both rat plasma VLDL and HDL (1, 2) and can exchange between these lipoproteins (27). The apoproteins of zones 2 and 3 had shorter half-lives in the circulation than those of zones 4 and 5.

This conclusion is based on analysis of plasma from one rat at each time point in each of two separate experiments. In one experiment, unlabeled human plasma was used as diluent prior to plasma fractionation and delipidation; in the other study the diluent used was rat plasma. From the data presented in Tables 1 and 2, it is clear that the results of the two experiments are in essential agreement. Despite the small number of observations at each time point, the finding that there was a progressive change in the relative contribution made by some of the apoprotein bands to the total HDL radioactivity lends strong support to the conclusion of heterogeneous metabolism. The ratio of zone 5 radioactivity to zone 2 plus 3 radioactivity, for example, changed progressively from 2.11 at 5 min to 4.75 at 48 hr (Table 2, experiment 1). A similar progressive change was observed in experiment 2 (Table 2).

After injection of human HDL into rats, labeled apoLP-alanine$_{1+2}$, analogous to the zone 8 proteins of rat HDL, were found in all lipoprotein fractions, suggesting that the metabolism of these proteins is considerably different from that of the other labeled human HDL apoproteins. The apoproteins that were not appreciably transferred from HDL showed different rates of clearance from the plasma. ApoLP-glutamine II disappeared from HDL about 50% faster than apoLP-glutamine I (Fig. 3).

This difference in decay rates of HDL apoproteins in the circulation would seem to suggest that these apoproteins are not metabolized as a group but rather that they are metabolized independently. We cannot rule out, however, that molecules exist within the HDL density range with different proportions of apoproteins, as demonstrated recently for human HDL (28, 29), and that they may be cleared from the circulation at a different rate. Such molecules can also be prepared artifactually from human HDL (30, 31).

The metabolism of rat and human HDL in the rat showed a number of similarities. Both remained mostly in the plasma fraction of density 1.063–1.21, and with neither preparation was there much radioactivity associated with the proteins of $d > 1.21$ at any time interval. They had similar half-lives in the circulation, and with both there was a preferential transfer of certain apoproteins to lipoproteins of lower density. With both preparations there was a divergence in the metabolism of the individual apoproteins, and their metabolism led to a very similar distribution of radioactivity in the tissues.

The fates of human VLDL and LDL in the rat were similar to those observed previously in humans (7, 8, 32). The decay of LDL was primarily from the density of the injected material ($d = 1.019–1.063$). Disappearance from the plasma followed an exponential course, with a $t_{1/2}$ of about 15 hr. Three separate and comparable experiments were carried out in which labeled human VLDL was injected into groups of rats, and plasma from individual animals was analyzed at various time intervals. The results of the three experiments were in substantial agreement, but, because the relative labeling among the apoproteins in the VLDL injected in the separate studies did not correspond precisely, the data could not be readily combined and are presented in detail for only one experiment (Tables 5 and 6).

As in humans (7, 8), the metabolism of human VLDL in rats was heterogeneous, with wide differences in the behavior of the various apoproteins. The smaller apoproteins, apoLP-glutamic acid and apoLP-alanine$_{1+2}$, were readily transferred from VLDL to HDL. The half-life of those remaining in the VLDL was much longer than that of the apoLDL. In contrast, the apoLDL moiety of the VLDL was not transferred to other lipoproteins immediately after injection but disappeared more rapidly from VLDL at later time intervals. It was recovered first in lipoproteins of intermediate density ($d = 1.006–1.019$) and later in LDL.

These results encourage us to believe that human lipoproteins may be metabolized in the rat in a manner analogous to the corresponding rat lipoproteins. Furthermore, similar to human VLDL in squirrel monkeys (33) and human HDL in dogs and mice (22), all three human lipoprotein families seemed to be handled in rats as lipoproteins rather than as foreign molecules. Thus, like human ceruloplasmin (34) and other proteins (35), lipoproteins may be another example of a human plasma protein constituent whose metabolism can be studied in experimental animals. However, it should be emphasized that in spite of the marked similarities of the fate of human lipoproteins in humans and rats, additional or different mechanisms may operate in the catabolism of human lipoproteins in humans.

In an attempt to identify the site(s) of catabolism of the injected lipoproteins, the radioactivity of tissues was determined after copious vascular perfusion with saline–EDTA to remove plasma radioactivity. All tissues except the spleen were visibly devoid of blood. More than 80% of the tissue radioactivity was nondialyzable and could be precipitated by trichloroacetic acid. It therefore probably represents lipoproteins entrapped in the tissue and may indicate the relative uptake of the labeled lipoproteins by these tissues.

After injection of ^{125}I as $Na^{125}I$, no preferential concentration of the label was found in any one tissue, and the radioactivity was excreted in urine and bile. After injection of labeled erythrocytes, radioactivity was found primarily in the spleen, lung, and liver, probably reflecting the activity of the reticuloendothelial system. The distribution of radioactivity after injection of lipoproteins was markedly different, the liver in every case accumulating more radioactivity than any other organ. The small intestinal wall usually contained about 20–30% as much radioactivity as liver. Counts were also found in other organs, and also in muscle and adipose tissue, but these generally accounted for less than 0.1% of the injected dose. Although we cannot exclude the possibility that some catabolism of lipoproteins is taking place in all tissues, it seems that the liver is a major site for catabolism of lipoproteins. A similar conclusion was recently reported by Roheim et al. for HDL in the rat (12) and by Hay et al. for LDL in the rat (13).

A considerable amount of radioactivity was found in the present study to be associated with intestinal and colonic contents, and some was excreted with feces. Since the bulk of this radioactivity was dialyzable, nonprecipitable with trichloroacetic acid, and not extractable in chloroform, we assume that it represents iodide liberated from the tyrosine molecules after degradation of apoproteins. Most of the iodide entered the intestinal lumen via the bile duct, and some probably through the intestinal wall. However, the bulk of the radioactive iodide was probably reabsorbed and ultimately excreted in the urine. Since urine radioactivity is used to calculate fractional catabolic rates (36), our results point to a need to determine fecal radioactivity after in vivo in-

jection of ^{125}I-labeled proteins, especially when recoveries of radioactivity are incomplete or when subjects with possible defective gastrointestinal functions are studied.

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