Metabolism of apoprotein B of plasma very low density lipoproteins in the rat.

O Faergeman, … , J P Kane, R J Havel

J Clin Invest. 1975;56(6):1396-1403. https://doi.org/10.1172/JCI108220.

Research Article

As an extension of metabolic studies of the cholesteryl ester component of rat very low density lipoproteins, we have studied the metabolism of the B apoprotein component labeled by intravenous injection of [3H]lysine. The B apoprotein separated from other apoproteins by delipidation and selective precipitation with tetramethylurea could not be distinguished from B apoprotein prepared by the conventional gel filtration technique. After injection of [3H]lysine, specific activity of B apoprotein was maximal in very low density and low density lipoproteins 1 and 11/2-h later, respectively, in a manner consistent with a precursor-product relationship. When protein-labeled very low density lipoproteins were injected into rats, the relationships of specific activity again indicated that B apoprotein of very low density lipoproteins may be the sole precursor of that of low density lipoproteins. However, less than 10% of the B apoprotein that disappeared from very low density lipoproteins appeared in density lipoproteins. To evaluate the sites of removal of B aproprotein of very low density lipoproteins from plasma, protein-labeled very low density lipoproteins were incubated with unlabeled high density lipoproteins to reduce radioactivity in non-B apoproteins selectively by molecular exchange. Most of the B apoprotein was rapidly removed by the liver. The extensive hepatic uptake of both the cholesteryl ester and B apoprotein components of rat very low density lipoproteins may explain the characteristically […]

Metabolism of Apoprotein B of Plasma Very Low Density Lipoproteins in the Rat

OLE FAERGEMAN, TSUNAKO SATA, JOHN P. KANE, and RICHARD J. HAVEL

From the Cardiovascular Research Institute and Department of Medicine, University of California, San Francisco, California 94143

ABSTRACT As an extension of metabolic studies of the cholesteryl ester component of rat very low density lipoproteins, we have studied the metabolism of the B apoprotein component labeled by intravenous injection of [^{3}H]lysine. The B apoprotein separated from other apoproteins by delipidation and selective precipitation with tetramethylurea could not be distinguished from B apoprotein prepared by the conventional gel filtration technique. After injection of [^{3}H]lysine, specific activity of B apoprotein was maximal in very low density and low density lipoproteins 1 and 1½-h later, respectively, in a manner consistent with a precursor-product relationship. When protein-labeled very low density lipoproteins were injected into rats, the relationships of specific activity again indicated that B apoprotein of very low density lipoproteins may be the sole precursor of that of low density lipoproteins. However, less than 10% of the B apoprotein that disappeared from very low density lipoproteins appeared in low density lipoproteins. To evaluate the sites of removal of B apoprotein of very low density lipoproteins from plasma, protein-labeled very low density lipoproteins were incubated with unlabeled high density lipoproteins to reduce radioactivity in non-B apoproteins selectively by molecular exchange. Most of the B apoprotein was rapidly removed by the liver. The extensive hepatic uptake of both the cholesteryl ester and B apoprotein components of rat very low density lipoproteins may explain the characteristically low concentrations of plasma low density lipoproteins in the rat.

A preliminary report of this work has been published (1).

Dr. Faergeman was the recipient of a U. S. Public Health Service International Research Fellowship (F05 TW 1939) and of stipends from the Danish Heart Association and the Danish Medical Research Council. Dr. Kane is an Established Investigator of the American Heart Association.

Received for publication 11 April 1975 and in revised form 29 July 1975.

INTRODUCTION

The plasma low density lipoprotein (LDL)[1] of both humans (2, 3) and rats (4, 5) contains as its major protein component the B apoprotein also found in very low density lipoproteins (VLDL). Early studies by Gitlin et al. (6) with radioiodine-labeled VLDL demonstrated transfer of protein from VLDL to LDL in normal as well as nephrotic humans. More recently, similar studies in humans (7, 8) and in rats (9, 10) have shown that the iodine-labeled B apoprotein of VLDL is transfered to that of LDL.

Although these studies indicate that some of the B apoprotein of LDL is derived from that of VLDL, the quantitative aspects of this conversion remain uncertain. Thus, the fraction of radioiodine-labeled B apoprotein of VLDL converted to LDL is considerably smaller in the rat than in man (6, 7, 11), but this could result from differing behavior of the modified protein in the two species. Also, these investigations have provided no information about the extent to which the B apoprotein of LDL arises from that of VLDL because specific activities of the protein were not measured. It is important to resolve these questions because of their bearing on the large species differences in concentration of LDL. Among mammals, rats have nearly the lowest and humans the highest levels.

In the present study, we have measured the specific activity of the B apoprotein of VLDL and LDL after injection of [^{3}H]lysine and also after injection [^{3}H]-lysine-labeled VLDL into recipient rats. Measurement of specific activities was greatly facilitated by separating the B apoprotein from other apolipoproteins on the basis of its insolubility in tetramethylurea (TMU). The results confirm that conversion of B apoprotein of

[1] *Abbreviations used in this paper:* LDL, low density lipoprotein(s) (d 1.019–1.063); TMU, tetramethylurea; VLDL, very low density lipoprotein(s) ($d < 1.006$).

VLDL to LDL is severely limited in this species and indicate that, like VLDL-cholesteryl esters (12), most of it is rapidly taken up by the liver. By contrast with the situation for LDL cholesteryl esters, our results indicate that virtually all of the B apoprotein of rat LDL may be derived from VLDL.

METHODS

Preparation of labeled lipoproteins. Experiments were conducted in the afternoon on nonfasting male Sprague-Dawley rats maintained on standard Purina rat chow (Ralston Purina Co., St. Louis, Mo.). 1–2 mCi of L-[4,5-^{3}H]-lysine (Amersham/Searle Corp., Arlington Heights, Ill.), sp act 90 mCi/mmol, was dissolved in 0.15 M NaCl solution (saline) and injected into a tail vein. At selected intervals the rats were anesthetized with diethyl ether and bled from the abdominal aorta. Serum was obtained and centrifuged in the 40.3 rotor of a preparative ultracentrifuge (Beckman Instruments, Inc., Fullerton, Calif.) at 10°C to separate the lipoprotein classes described below (13). All lipoproteins were recentrifuged at their upper density limit and then dialyzed against saline except $d < 1.006$ lipoproteins to be injected into recipient rats.

For experiments involving analysis of ^{3}H in tissue proteins, ^{3}H in apoproteins other than the B apoprotein was reduced by molecular exchange. This was accomplished by mixing unlabeled HDL with VLDL prepared from rats injected with [^{3}H]lysine 1 h before exsanguination. The ratio of protein in HDL to that in VLDL was about 10:1. After incubation for 30 min at 37°C, the mixture, containing 13–50 mg protein in a volume of less than 3 ml, was applied to a 1.2 × 90 cm column of 6% agarose gel (Bio-Rad Laboratories, Richmond, Calif.) that had been equilibrated with 0.2 M NaCl containing 1 mM EDTA at pH 7.0. The VLDL were eluted with this buffer in the void volume, uncontaminated by HDL, at a flow rate of 10 ml/h. VLDL prepared in this manner were injected into recipient rats within 42 h of the time that the donor rats were bled. These incubated VLDL had more cholesteryl esters than nonincubated VLDL (Table I), but their electrophoretic mobility and content of TMU-insoluble protein and of TMU-soluble proteins were not altered. A band corresponding to the major HDL apoprotein could not be detected in the incubated VLDL by polyacrylamide gel electrophoresis. However, the percentage of densitometric area owing to the major slowly migrating (arginine-rich) protein was reduced from 35 to 28. These VLDL contained 86.3% (SD 5.9) of the ^{3}H in TMU-insoluble protein.

Injection of labeled lipoproteins into recipient rats. Labeled VLDL, containing about 0.25 mg protein in a volume of 0.75–1.00 ml, were injected into tail veins of recipient rats. Six to eight rats were injected with each preparation of VLDL and bled at selected intervals under diethyl ether anesthesia. Blood was allowed to clot or was mixed with EDTA, 1 mg/ml, and placed on ice, and the serum or plasma was treated as described above to separate lipoprotein classes. Selected organs were removed promptly and rinsed in saline. The small intestine was flushed with saline and the mucosa expressed with a spatula. The organs were frozen at −20°C until they were analyzed 2–3 days later.

Preparation and analysis of apoproteins. Solutions of recentrifuged and dialyzed lipoproteins (200–400 μl containing less than 1 mg protein/ml) were rapidly mixed in a 12 ml conical centrifuge tube with an equal volume of TMU (Burdick & Jackson Laboratories, Inc., Muskegon, Mich., redistilled if necessary to give a pH of 6–7 when diluted 1:5 with distilled water) and allowed to stand for at least 24 h at room temperature (22–24°C) to form a flocculant precipitate of protein and lipid (14). In preparations of VLDL this precipitate usually formed a pellicle at the top of the tube after centrifugation at 1,000 *g* for 15 min at room temperature. Precipitates from LDL contained less lipid, and it was often impossible to separate the precipitate adequately from soluble components by centrifugation. In most cases, therefore, the TMU-lipoprotein mixture was filtered through glass wool packed into the neck of a disposable Pasteur pipette. Portions of the clear TMU-soluble material and of the untreated lipoprotein solution were taken for measurement of protein and ^{3}H. Content of protein and ^{3}H in the TMU-insoluble material was then calculated by difference. In some cases, ^{3}H in the TMU-insoluble material, isolated by centrifugation and washed with TMU-saline, 1:1 vol/vol, was also determined. For amino acid analysis of the total protein of VLDL and LDL and of the TMU-insoluble protein of VLDL, pooled material from 30 rats was delipidated overnight with ethanol-diethyl ether 3:1 (vol/vol). Additional ether was added to bring the ratio to 2:3 to ensure complete precipitation of protein. Delipidation was repeated and the protein was lyophilized and then hydrolyzed in vacuo in 5.9 M HCl in the presence of 10 mg of phenol at 110°C for 22 h. Amino acid content was determined by a dual column procedure on a Beckman Model 121M amino acid analyzer.

Polyacrylamide gel electrophoresis was performed in 8 M urea as described by Kane (14). Content of protein in bands stained with amido-Schwarz was estimated in the range in which each resolvable component of rat VLDL gave a linear response by densitometry at 550 nm with a Densicomp Model 445 (Clifford Instruments, Inc., Natick, Mass.).

To prepare apoproteins for separation by gel filtration, VLDL from rats injected with [^{3}H]lysine were dialyzed against saline to remove free [^{3}H]lysine, delipidated with 20 vol of ethanol-ether 2:1 vol/vol at room temperature, and redissolved in 0.1 M sodium decyl sulfate. TMU-insoluble material was washed with TMU-saline, 1:1 vol/vol and, after delipidation with ethanol-ether as above, partially redissolved in sodium decyl sulfate. Samples of TMU-soluble apoproteins, as well as sodium decyl sulfate-solubilized whole VLDL apoprotein and TMU-insoluble apoprotein, were separated by chromatography on a 0.9 × 90 cm column of G-150 Sephadex (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) essentially as described by Brown et al. (15). Fractions of 1.0 ml were collected. The column was calibrated with blue dextran.

TABLE I
Composition of VLDL

	Unincubated	Incubated with HDL
	% by weight	
Cholesteryl esters	2.9	4.7
Triglycerides	69.8	67.8
Cholesterol	3.2	3.8
Phospholipids	13.9	14.2
TMU-insoluble protein	3.0	2.8
TMU-soluble protein	7.4	6.8

In one experiment, the combined Sephadex fractions 2 and 3 (15) of the TMU-soluble material were subjected to ion exchange chromatography on a 0.9 × 4.5 cm column of DEAE cellulose (Whatman Chemicals, Div. W. & R. Balston, Maidstone, Kent, England) in 6 M urea, 0.15 Tris, pH 8.2. Elution was at an ionic strength corresponding to 0.15 M Tris followed by 0.15 M Tris containing 0.35 M NaCl.

Chemical analyses. Protein measurements were performed according to Lowry et al. (16) as modified by Kane for TMU-containing solutions (14). Chemical composition of lipoproteins was determined as described previously (12).

Analysis of ^3H. Lipoprotein solutions and samples of TMU-soluble and insoluble proteins were counted in either Protosol or Aquasol (New England Nuclear, Boston, Mass.). Efficiency of counting was determined with an internal standard of ^3H_2O.

Samples of wet tissue weighing about 1 g were minced and extracted with 25 parts acetone-alcohol, 1:1 (vol/vol), for 24 h at room temperature, and the insoluble material was collected on filter paper. This residue was dried and oxidized (17) in a Packard Model 305 Sample Oxidizer (Packard Instrument Co., Inc., Downers Grove, Ill.). The water of combustion was counted in Aquasol. Lyophilization of tissue samples and subsequent oxidation gave results identical to those obtained with the extraction and drying procedure but were more time-consuming.

All samples were counted in a Mark II liquid scintillation counter (Nuclear-Chicago Corp., Des Plaines, Ill.), equipped with a photon monitor to discriminate counts owing to chemiluminescence. When necessary, samples were acidified with a drop of glacial acetic acid to minimize chemiluminescence.

RESULTS

Distribution of TMU-insoluble protein in lipoprotein fractions. Although TMU-insoluble protein constituted about 30% of VLDL-protein and about 80% of LDL-protein, plasma concentrations of TMU-insoluble protein in VLDL and LDL were nearly equal (Table II). The amino acid composition of TMU-insoluble protein of VLDL closely resembled that of LDL-protein and differed substantially from that of total VLDL protein (Table III). The percent of protein that was TMU-insoluble in the small amount of intermediate density lipoprotein was intermediate between that of VLDL and LDL (Table II). Protein of HDL contained on the average less than 5% TMU-insoluble material, determination of which was subject to substantial variation because TMU-insolubility was determined by difference.

TABLE II

Protein Concentrations in Plasma Lipoprotein Fractions of the Rat

d	*n*	Total protein*	TMU-insoluble protein*	TMU-insoluble protein
		mg/ml	*mg/ml*	%
<1.006	16	0.101±0.049	0.034±0.024	30
1.006–1.019	12	0.0035±0.0010	—	40‡
1.019–1.063	16	0.043±0.012	0.034±0.012	79
1.063–1.21	16	0.587±0.056	0.028±0.115	4.8

* Mean values±SD.
‡ Determined on pooled samples

TABLE III

*Amino Acid Compositions of Whole Apoprotein of LDL and VLDL and of the TMU-Insoluble Protein of VLDL**

Amino acid	Whole apo-LDL‡	TMU-insoluble fraction from VLDL§	Whole apo-VLDL‖
Lys	66.1	62.9	70.2
His	14.7	15.1	9.9
Arg	45.5	47.7	51.4
Asp	111.1	107.4	101.6
Thr	64.3	65.9	70.9
Ser	83.5	86.8	81.3
Glu	134.5	124.4	162.1
Pro	51.9	56.4	53.5
Gly	58.3	62.9	80.3
Ala	63.0	64.4	72.2
Val	59.3	60.9	43.0
Ile	46.3	43.6	28.0
Leu	118.4	120.5	107.8
Tyr	32.0	31.8	27.3
Phe	50.3	49.0	40.3

* Because of lability of cysteine and methionine in presence of traces of TMU, these amino acids have been omitted from the calculation.
‡ Mean of two preparations of total protein component.
§ Mean of three preparations.
‖ Mean of two preparations.

Incorporation of [^3H]lysine into VLDL and LDL. Specific activity of TMU-insoluble protein of VLDL was maximal 1 h after injection of [^{3}H]lysine (Fig. 1). The specific activity of TMU-insoluble protein of LDL rose later and reached a maximal value about 1.5 h after injection near the point where its value crossed that of the descending curve for VLDL. By contrast, the specific activity of TMU-soluble proteins in both VLDL and LDL rose to reach a plateau 1 h after injection. The data shown in Fig. 1 are typical of two such experiments except that in the experiment not shown, the specific activity of TMU-soluble protein in VLDL slightly but consistently exceeded that of its counterpart in LDL.

Characterization of labeled VLDL. Less than 10% of the ^{3}H in VLDL obtained from rats 1 h after injection of [^{3}H]lysine was extracted by chloroform:methanol, 2:1 vol/vol. An average of 64.5% (SD 3.9) was insoluble in TMU. The proteins of nonincubated VLDL, delipidated in ethanol:ether and dissolved in sodium decyl sulfate, separated into three major peaks upon gel filtration on Sephadex G-150 (Fig. 2). 50% of the ^{3}H and 31% of the protein were recovered in the first peak that eluted in the void volume; 36% of the ^{3}H and 51%

of the protein eluted in the other peaks. Overall recovery was 86% for ^3H and 82% for protein. When TMU-soluble proteins were subjected to gel filtration, 92% eluted in peaks 2 and 3 (Fig. 2). Upon electrophoresis in polyacrylamide gel, the second peak contained the slowly migrating arginine-rich apoprotein of VLDL (18); the third contained the rapidly migrating C apoproteins. 100% of the TMU-soluble ^3H-apoprotein was eluted from a column of DEAE cellulose in 0.15 M Tris containing 0.35 M NaCl, indicating that contamination of TMU-soluble apoproteins with the B-apoprotein was negligible (19). When TMU-insoluble material, similarly delipidated with ethanol-ether and dispersed in sodium decyl sulfate, was applied to a column of Sephadex G-150, essentially all of the eluted ^3H ap-

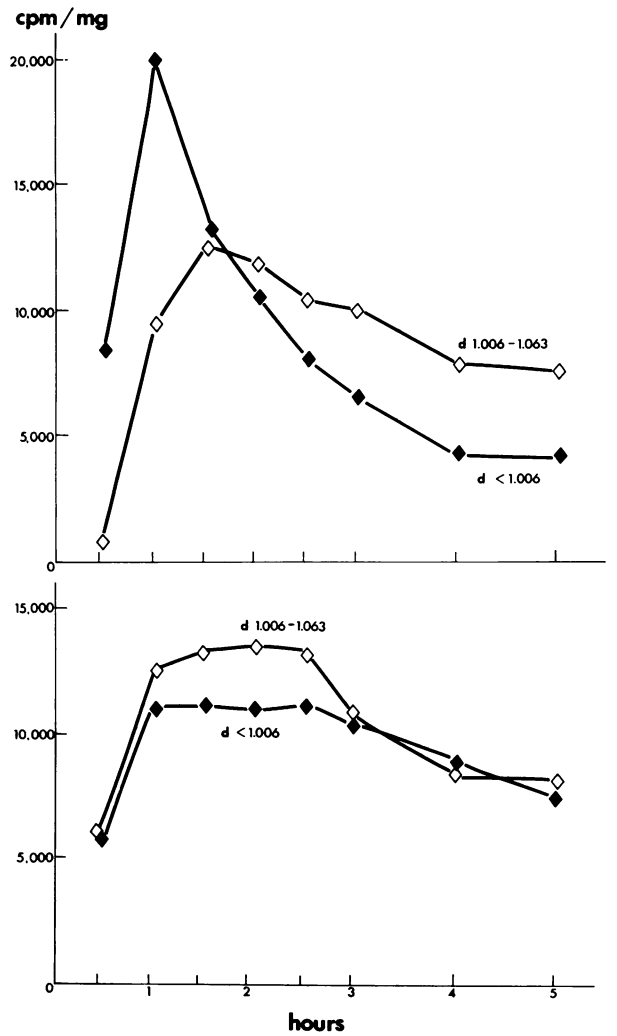

FIGURE 1 Specific activity of TMU-insoluble apoprotein (top) and TMU-soluble apoprotein (bottom) of VLDL and LDL of [^3H]lysine injected rats. Each point represents pooled lipoprotein from three animals.

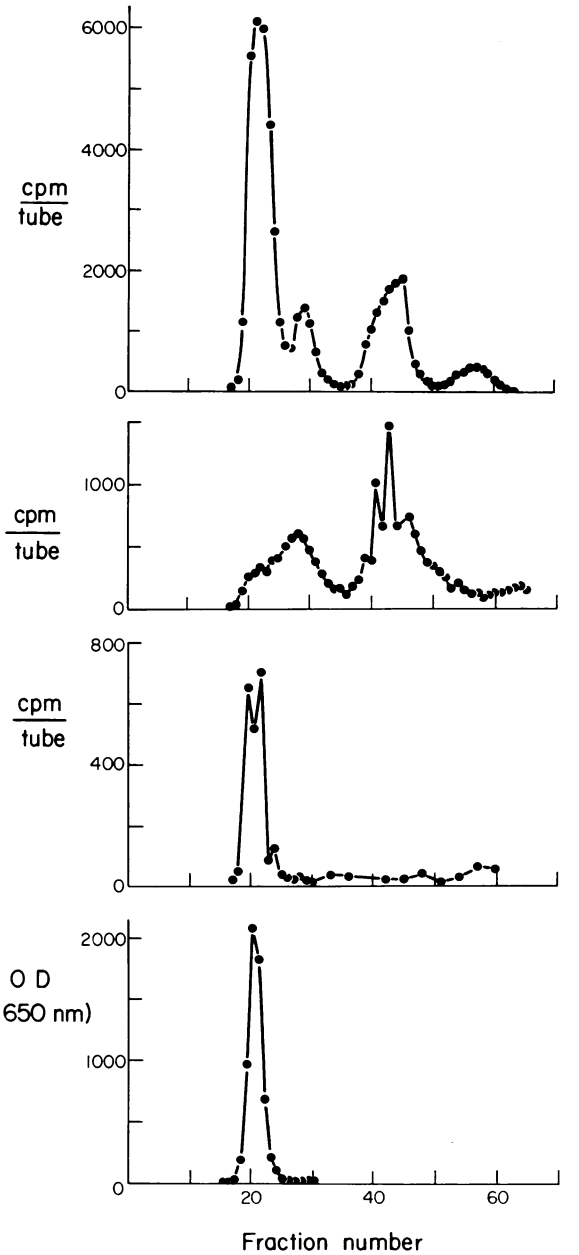

FIGURE 2 Gel filtration in G-150 Sephadex of delipidated, sodium decyl sulfate solubilized apoproteins of (from top): VLDL; TMU-soluble apoproteins of VLDL; and TMU-insoluble apoprotein of VLDL incubated with HDL (see text). VLDL were obtained from rats injected 1 h previously with [^3H]lysine. Bottom: elution of blue dextran.

peared in the void volume (Fig. 2), indicating that this material had the chromatographic properties of the B-apoprotein in human VLDL (2).

Fate of labeled VLDL apoproteins. After injection of labeled VLDL (nonincubated) into recipient rats, the behavior of TMU-insoluble and soluble proteins

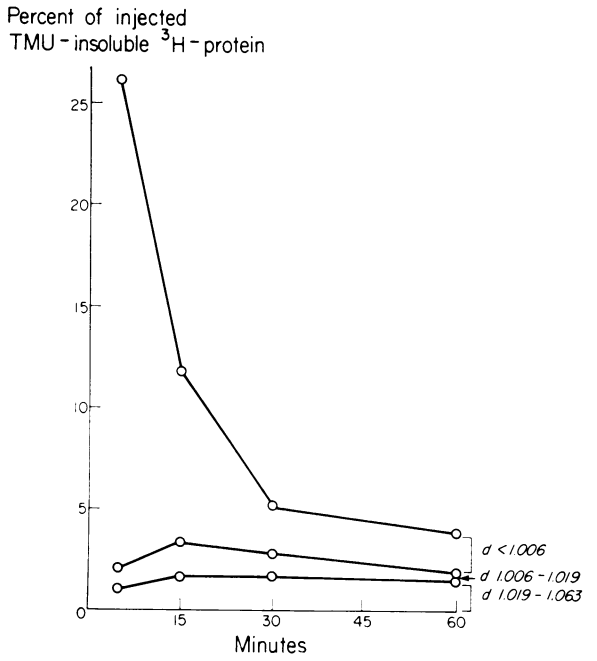

FIGURE 3 Distribution of TMU-insoluble ^{3}H-protein in plasma lipoprotein classes after intravenous injection of [^{3}H]lysine-labeled VLDL. Each point represents pooled lipoproteins from two rats. Values are calculated as total ^{3}H-protein less TMU-soluble ^{3}H-protein. Direct measurement of ^{3}H in the TMU-insoluble pellicle gave similar values.

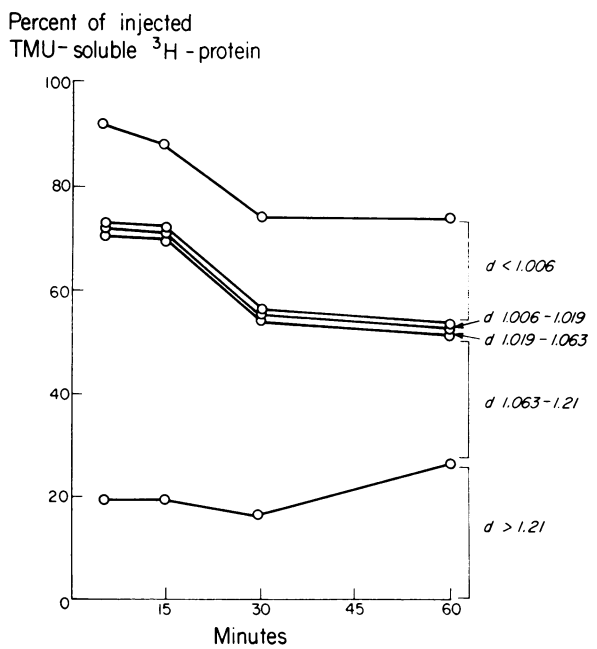

FIGURE 4 Distribution of TMU-soluble ^{3}H-protein in plasma lipoproteins. Same experiment as Fig. 3.

differed substantially. Removal of TMU-insoluble protein was rapid and little appeared in other lipoproteins (Fig. 3). ^{3}H in the intermediate fraction was maximal 15 min after injection and then declined, whereas that of LDL rose to a plateau value between 15 and 60 min. Removal of TMU-soluble proteins was slow, and most of the ^{3}H was found in HDL, falling from about 50% of that injected after 5 min to about 25% after 60 min (Fig. 4). From 15 to 20% of the injected TMU-soluble ^{3}H remained in VLDL and about 20% with other serum proteins ($d > 1.21$) at all times after injection. The amount of TMU-soluble ^{3}H-protein present in the plasma decreased from more than 90% of that injected after 5 min to 75% after 60 min.

To determine the site at which the TMU-insoluble protein was rapidly removed from the blood, incubated

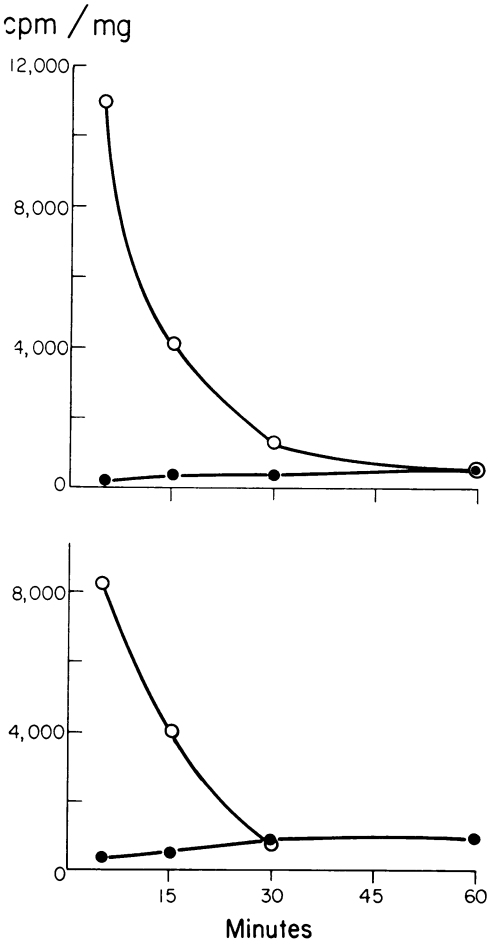

FIGURE 5 Specific activity of TMU-insoluble protein of VLDL and LDL after intravenous injection of [^{3}H]lysine-labeled VLDL. Each point represents pooled lipoproteins from two rats. Top: unincubated VLDL; bottom: VLDL incubated with HDL to reduce content of TMU-soluble ^{3}H-protein.

TABLE IV

Distribution of ^3H after Intravenous Injection of [^3H]Lysine-Labeled VLDL Incubated with HDL to Reduce ^3H in TMU-Soluble Proteins

	Number of rats	Time after injection			
		5 min	15 min	30 min	60 min
		*1% of injected ^3H**			
Lipoproteins					
d < 1.006	8	32.7±13.4‡	12.8±6.8	7.1±3.5	2.4±1.2
	(4)§	(34.3±14.4)	(11.9±7.7)	(4.9±2.3)	(1.0±1.0)
d 1.006–1.019	8	2.1±1.1	2.4±1.3	2.0±0.8	1.0±0.5
d 1.019–1.063	8	0.8±0.3	2.0±0.7	2.6±0.7	2.9±0.9
	(4)	(0.6±0.3)	(1.7±0.8)	(2.4±0.8)	(2.7±1.0)
d 1.063–1.21	8	3.8±1.8	4.1±2.0	2.9±1.8	4.1±2.4
d > 1.21	6	1.9±1.4	2.3±1.2	1.9±1.5	4.0±2.4
Organs					
Liver	8	36.3±11.5	50.5±5.3	48.5±8.3	40.9±10.4
Intestine	4	0.4±0.1	0.8±0.6	1.8±1.7	1.0±1.0
Spleen	2		0.4		0.3
Kidneys	2		0.1		1.0
Heart	2		0.3		0.2
Lungs	2		0.8		1.6
Total		78.0	77.5	66.8	59.4

* Based upon plasma volume of 4.5% of body weight (18).

‡ Mean values±SD.

§ Numbers in parenthesis refer to TMU-insoluble protein as percent of injected TMU-insoluble ^3H. 86% of the labeled protein injected was insoluble in TMU.

VLDL containing an average of 86% of the ^3H in TMU-insoluble fraction were used. Removal of this labeled protein from the plasma was rapid and the rate and extent of transfer of labeled TMU-insoluble protein to LDL were similar to those observed with VLDL not incubated with HDL (Table IV). Most of injected ^3H rapidly appeared in hepatic proteins. 15-min after injection, it amounted to 51% of the ^3H injected and 58% of that removed from plasma VLDL. The other organs analyzed contained very small amounts of ^3H. At early time points, recovery of ^3H in these tissues and in blood plasma was about 78% of the amount injected.

The specific activity of TMU-insoluble protein in VLDL and LDL was determined in two experiments in which labeled VLDL were injected; one with VLDL that had been incubated with HDL and one with unincubated VLDL. In both cases, the specific activity in LDL rose to equal that in VLDL after 30-60 min (Fig. 5), as in experiments in which [^3H]lysine was injected.

DISCUSSION

As in man (14), our results show that TMU selectively and quantitatively precipitates the B apoprotein in small samples of VLDL and LDL, greatly facilitating measurements of mass and radioactivity. Upon gel filtration in Sephadex G-150, the distribution of ^3H in TMU-insoluble protein of VLDL corresponded to peak VS-1 of Bersot et al. (4), and P1 of Koga et al. (5), identified by them as the rat apoprotein corresponding to the B apoprotein. The amino acid composition of the TMU-insoluble protein in VLDL also resembled that obtained for the P1 peak of both VLDL and LDL by Koga et al. (5). The biosynthetic tracer that we have used not only has theoretical advantages over tracers produced by derivatization, but it also labels VLDL-lipids to a lesser extent than the commonly used radioiodine.

Perfused livers of fed rats secrete substantial amounts of protein in VLDL (20, 21). Although such livers secrete only very small amounts of lipoprotein in the density range 1.019-1.063 (21),[2] it has been difficult to exclude significant direct secretion of LDL because their rate of catabolism is much lower than that of VLDL. Our measurements of specific activity of B apoprotein indicate that all of the B apoprotein of LDL could be derived from that of VLDL. A precursor-product relationship between the specific activity of B apoprotein in VLDL and LDL was observed not only after injection of [^3H]lysine but also after injection of

[2] Hamilton, R. L., M. C. Williams, R. J. Havel, and C. J. Fielding. Unpublished data.

VLDL containing lysine-labeled B apoprotein. In each case peak specific activity occurred in LDL about 1 h after the label was introduced into VLDL.

The rapid removal of lysine-labeled B apoprotein of VLDL from the blood and the persistence of the label in other (TMU-soluble) apoproteins is consistent with observations of Eisenberg and Rachmilewitz (10, 11) and Roheim et al., (22) with ^{125}I-labeled rat VLDL. This general agreement supports the usefulness of the derivatized proteins as tracers in metabolic studies. However, subtle changes in VLDL can substantially alter their metabolism (12) and it must be emphasized that precise comparisons cannot be made.

^{3}H-protein of incubated VLDL (86% B apoprotein) was rapidly removed by the liver and no evidence for appreciable uptake by other organs was obtained. This result substantiates the observations of Eisenberg and Rachmilewitz, who found that rat liver rapidly took up about half of ^{125}I-labeled VLDL-apoprotein after its intravenous injection (10). Since this corresponded to rapid removal of VS-1 apoprotein while most of the other apoproteins were retained in the blood, they concluded that the B apoprotein is rapidly removed by the liver. Roheim et al. have reported similar results (22).

The rate and extent of uptake of apoprotein B of VLDL by the liver closely resemble that of their cholesteryl esters (12). This process follows hydrolysis of component triglycerides of VLDL in extrahepatic tissues, which results in formation of a remnant particle that retains all of the component B apoprotein and cholesteryl esters of VLDL but which is substantially depleted not only of triglycerides but also of much of its phospholipids and C apoproteins (18). Thus, both a major apolar "core" component and a major polar "surface" component of remnant VLDL are rapidly removed by the liver. The hepatic uptake of component cholesteryl esters of VLDL is followed by gradual hydrolysis (12), and our results suggest that the B apoprotein is also slowly degraded (Table IV). ^{125}I-labeled VLDL apoprotein taken up by rat liver is initially concentrated at the cell surface, but gradually appears in the region of secondary lysosomes (23). Therefore, it seems likely that rapid hepatic uptake is followed by gradual enzymatic degradation of both lipid and protein components. The actual mechanism of uptake remains to be determined, but the comparable uptake of core and surface components is clearly consistent with endocytosis of the entire particle.

A small fraction of remnant VLDL evidently escapes hepatic degradation and appears as LDL, which contains very little triglyceride or protein other than the B apoprotein. Whether the liver participates in the formation of LDL remains uncertain because some labeled cholesteryl esters are found in *d* 1.019-1.063 lipoproteins after injection of cholesteryl ester-labeled VLDL into functionally eviscerated rats (18). Recent studies in pigs suggest that the liver may interact in some way with LDL but that catabolism of LDL-apoprotein occurs in extrahepatic tissues (24). This interpretation is supported by the recent demonstration of high affinity receptors for LDL in human fibroblasts, which evidently mediate the catabolism of both cholesteryl esters and the B apoprotein (25). The rapid uptake by the liver of these two components of remnant VLDL is in striking contrast to these observations and suggests that a highly specific mechanism operates in liver as well.

Approximately as much VLDL B apoprotein was converted to LDL as was observed previously for cholesteryl esters (12). However, in contrast to the B apoprotein, LDL derive their cholesteryl esters in part from sources other than VLDL, probably related to the action of lecithin:cholesteryl acyl transferase. The small fraction of B apoprotein of VLDL converted to LDL in the rat is consistent with their turnover times and pool sizes in the blood. Since the turnover times are on the order of 5.5 min[3] and 130 min (10), respectively, and we found that the pool sizes are approximately equal (Table II), it follows that transport of B apoprotein in LDL is only about 4% of that in VLDL.

The extent to which B apoprotein of VLDL is removed by the liver or converted to LDL may vary considerably among mammals. In adult humans, average intravascular pool sizes of apoprotein B in VLDL and LDL (for a plasma volume of 3,000 ml) are approximately 90 mg[4] and 2,500 mg (26) and their turnover times are about 2 h (7) and 100 h (27), respectively. This gives rates of transport of 45 and 25 mg/h for VLDL and LDL. If LDL are not secreted independently in man, it appears that a large fraction of the B protein in VLDL may be converted to LDL. This conclusion is supported by published data on the conversion of radioiodine-labeled apoprotein of VLDL to LDL (6, 7). Comparison of the approximately 15-fold difference in the fraction of B apoprotein of VLDL that is converted to LDL in rats and humans with the difference in ratio of B apoprotein concentration in the two lipoprotein classes (1:1 in the rat and 25-30:1 in man) indicates that the proportional use of the two pathways of catabolism of remnant VLDL evidently could be a major determinant of the gross differences in concentration of plasma LDL in the two species.

[3] Transport of B protein of VLDL in the two experiments shown in Fig. 5 was estimated from the relationship: cpm B apoprotein injected /$\int^{60}$ specific activity of B apoprotein. The turnover time was calculated from the value for transport and the plasma content of B apoprotein (for a plasma volume of 4.5%).

[4] Kane, J. P., and R. J. Havel. Unpublished data.

ACKNOWLEDGMENTS

We are grateful to Agnes Frank and Carol Lum for their expert technical assistance, and we wish to thank Dr. Chin-Tzu Peng for use of the sample oxidizer.

This work was supported by grants from the U. S. Public Health Service (HL 06285 and HL 14237).

REFERENCES

1. Faergeman, O., T. Sata, J. P. Kane, and R. J. Havel. 1974. Metabolism of apolipoprotein B of plasma very low density lipoproteins in the rat. *Circulation.* **50** (Suppl. 3): 114.
2. Brown, W. V., R. I. Levy, and D. S. Fredrickson. 1969. Studies of the proteins in human plasma very low density lipoproteins. *J. Biol. Chem.* **244**: 5687–5694.
3. Gotto, A. M., W. V. Brown, R. I. Levy, M. E. Birnbaumer, and D. S. Fredrickson. 1972. Evidence for the identity of the major apoprotein in low density and very low density lipoproteins in normal subjects and patients with familial hyperlipoproteinemia. *J. Clin. Invest.* **51**: 1486–1494.
4. Bersot, T. P., W. V. Brown, R. I. Levy, H. G. Windmueller, D. S. Fredrickson, and V. S. LeQuire. 1970. Further characterization of the apolipoproteins of rat plasma lipoproteins. *Biochemistry.* **9**: 3427–3433.
5. Koga, S., L. Bolis, and A. M. Scanu. 1971. Isolation and characterization of subunit polypeptides from apoproteins of rat serum lipoprotein. *Biochim. Biophys. Acta.* **236**: 416–430.
6. Gitlin, D., D. G. Cornwell, D. Nakasato, J. L. Oncley, W. L. Hughes, Jr., and C. A. Janeway. 1958. Studies on the metabolism of plasma proteins in the nephrotic syndrome. II. The lipoproteins. *J. Clin. Invest.* **37**: 172–184.
7. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoprotein proteins. I. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* **260**: 212–221.
8. Eisenberg, S., D. W. Bilheimer, R. I. Levy, and F. T. Lindgren. 1973. On the metabolic conversion of human plasma very low density lipoprotein to low density lipoprotein. *Biochim. Biophys. Acta.* **326**: 361–377.
9. Fidge, N. H., and C. J. Foxman. 1971. In vivo transformation of rat plasma very low density lipoprotein into higher density lipoproteins. *Aust. J. Exp. Biol. Med. Sci.* **49**: 581–593.
10. Eisenberg, S., and D. Rachmilewitz. 1973. Metabolism of rat plasma very low density lipoprotein. I. Fate in circulation of the whole lipoprotein. *Biochim. Biophys. Acta.* **326**: 378–390.
11. Eisenberg. S.. and D. Rachmilwitz. 1973. Metabolism of rat plasma very low density lipoprotein. II. Fate in circulation of apoprotein subunits. *Biochim. Biophys. Acta.* **326**: 391–405.
12. Faergeman, O., and R. J. Havel. 1975. Metabolism of cholesteryl esters of rat very low density lipoproteins. *J. Clin. Invest.* **55**: 1210–1218.
13. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34**: 1345–1353.
14. Kane, J. P. 1973. A rapid electrophoretic technique for identification of subunit species of apoproteins in serum lipoproteins. *Anal. Biochem.* **53**: 350–364.
15. Brown, W. V., R. I. Levy, and D. S. Fredrickson. 1970. Further separation of the apoproteins of the human plasma very low density lipoproteins. *Biochim. Biophys. Acta.* **200**: 573–575.
16. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265–275.
17. Sher, D. W., N. Kaartinen, L. J. Everett, and V. Justes, Jr. 1971. Preparing samples for liquid scintillation counting with the Packard sample oxidizer. *In* Organic Scintillators and Liquid Scintillation Counting. D. L. Horrocks and C. T. Peng, editors. Academic Press, Inc., New York. 849–868.
18. Mjøs, O. D., O. Faergeman, R. L. Hamilton, and R. J. Havel. 1975. Characterization of remnants produced during the metabolism of triglyceride-rich lipoproteins of blood plasma and intestinal lymph in the rat. *J. Clin. Invest.* **56**: 603–615.
19. Shore, V. G., and B. Shore. 1973. Heterogeneity of human plasma very low denstiy lipoproteins. Separation of species differing in protein components. *Biochemistry.* **12**: 502–507.
20. Hamilton, R. L., M. N. Berry, M. C. Williams, and E. M. Severinghaus. 1974. A simple and inexpensive membrane "lung" for small organ perfusion. *J. Lipid Res.* **15**: 182–186.
21. Noel, S.-P., and D. Rubinstein. 1974. Secretion of apolipoproteins in very low density and high density lipoproteins by perfused rat liver. *J. Lipid Res.* **15**: 301–308.
22. Roheim, P. S., D. I. Edelstein, G. Vega, and H. A. Eder. 1973. Metabolism of very low density apolipoproteins in sucrose-fed hyperlipemic rats. *Fed. Proc.* **32**: 672. (Abstr.).
23. Stein, O., D. Rachmilewitz, L. Sanger, S. Eisenberg, and Y. Stein. 1974. Metabolism of iodinated very low density lipoprotein in the rat. Autoradiographic localization in the liver. *Biochim. Biophys. Acta.* **360**: 205–216.
24. Sniderman, A. D., T. E. Carew, J. G. Chandler, and D. Steinberg. 1974. Paradoxical increase in rate of catabolism of low-density lipoproteins after hepatectomy. *Science (Wash. D. C.).* **183**: 526–528.
25. Brown, M. S., and J. L. Goldstein. 1974. Expression of the familial hypercholesterolemia gene in heterozygotes: mechanism for a dominant disorder in man. *Science (Wash. D. C.).* **185**: 61–63.
26. Schonfeld, G., R. S. Lees, P. K. George, and B. Pfleger. 1974. Assay of total plasma apolipoprotein B concentration in human subjects. *J. Clin. Invest.* **53**: 1458–1467.
27. Langer, T., W. Strober, and R. I. Levy. 1972. The metabolism of low density lipoprotein in familial type II hyperlipoproteinemia. *J. Clin. Invest.* **51**: 1528–1536.