Characterization of Remnants Produced during the Metabolism of Triglyceride-Rich Lipoproteins of Blood Plasma and Intestinal Lymph in the Rat

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ABSTRACT The metabolism of intravenously injected large and small chylomicrons from intestinal lymph and of very low density lipoproteins from blood plasma was studied in functionally eviscerated "supradiaphragmatic" rats. For studies with lymph lipoproteins, recipient animals were injected with 4-aminopyrazolopyrimidine 18 h before injection of lipoprotein to prevent secretion of very low density lipoproteins into their blood plasma. In all cases, most of the triglycerides (labeled with ^{14}C) were rapidly metabolized, whereas cholesteryl esters (labeled with ^{3}H) persisted in the blood. Most of the cholesteryl esters remained in smaller "remnant" lipoproteins, less dense than 1.006, which retained an apparently spherical shape, as determined by electron microscopy of negatively stained preparations. Whereas the diameters and chemical compositions of large chylomicrons were substantially different from those of small chylomicrons and very low density lipoproteins, all remnants were similar in these respects. Average remnant diameters were 400–600 Å and remnants were enriched in cholesteryl esters and in protein insoluble in tetramethylurea. In addition to triglycerides, remnants were depleted of phospholipids and tetramethylurea-soluble proteins with rapid electrophoretic mobility. When related to particle size, the composition of remnants, like that of their precursors, was consistent with the "pseudomicellar" model of lipoproteins, in which a core of nonpolar lipids is covered by a monolayer of polar lipids and protein. These results demonstrate the fundamental similarity of the initial step in the metabolism of triglyceride-rich lipoproteins from intestinal mucosa and liver and show that loss of triglycerides from the core of the particles is accompanied by removal of polar components from the surface.

INTRODUCTION

The two nonpolar lipid components of chylomicrons (1) and very low density lipoproteins (VLDL) [1] (2), triglycerides and cholesteryl esters, are thought to be transported in the core of these particles, surrounded by a monolayer of polar lipids and protein. Studies in four mammalian species have shown that the triglycerides of chylomicrons are largely removed in extrahepatic tissues, whereas the cholesteryl esters are taken up almost entirely in the liver (3–5). Nestel, Havel, and Bezman found that component cholesteryl esters remained in the blood when chylomicrons were injected into functionally hepatectomized dogs, while triglycerides were removed rapidly, as in intact dogs (6). They suggested that the triglycerides are normally removed first through the action of lipoprotein lipase in extrahepatic tissue and that the cholesteryl esters, contained in a

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[1] *Abbreviations used in this paper:* 4-APP, 4-aminopyrazolopyrimidine; HDL, high density lipoproteins; TMU, tetramethylurea; VLDL, very low density lipoproteins.

partially degraded particle ("skeleton") are subsequently removed by the liver (7). This interpretation was supported by Redgrave, who found that cholesteryl ester-rich particles ("remnants") accumulated in the blood of functionally hepatectomized rats given chylomicrons intravenously (8).

Two populations of triglyceride-rich lipoproteins, rich in cholesteryl esters, accumulate in blood plasma of patients with primary dysbetalipoproteinemia (type III hyperlipoproteinemia), and it has been suggested that they represent remnants of the metabolism of normal chylomicrons secreted from the intestinal mucosa and VLDL secreted primarily from the liver, respectively (2, 9, 10). The surface proteins of these particles also differ characteristically from their putative precursors: "B" apoprotein and an arginine-rich protein are increased at the expense of "C" apoproteins (11). The present investigation was undertaken to characterize chylomicron remnants in the rat more completely and also to determine whether similar remnants are formed from VLDL secreted by the liver.

METHODS

Preparation of triglyceride-rich lipoproteins. Male, Sprague-Dawley rats, weighing 350–400 g, were anesthetized with diethyl ether. The main intestinal lymph duct was cannulated by the method of Bollman, Cain, and Grindlay (12), and the animals were placed in restraining cages. 12–48 h later, the rats were given a meal of 1–2 ml of corn oil or safflower oil containing 2% cholesterol, 2–3 ml cows' milk (8% butterfat), 50 μCi [1,2-^{3}H]cholesterol and 5–10 μCi [1-^{14}C]palmitic acid. Samples of lymph were collected from about 2–12 h later in a flask kept in ice, and were allowed to clot. After removal of fibrin by filtration through glass wool, chylomicrons were separated by centrifuging samples of the lymph beneath 0.15 M sodium chloride solution containing 0.04% disodium EDTA (saline) in the 40.3 rotor of a Beckman ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) for 3.0×10^6 g·av min at 12°C. The material floating into the layer of saline was mixed with rat serum high density lipoprotein (HDL) (prepared at d 1.063–1.21 [13], reisolated by recentrifugation, and extensively dialyzed against saline) in a ratio of about 250 mg chylomicron triglyceride to 30 mg HDL protein and incubated for 30 min at 37°C. This procedure was carried out to ensure that the material to be injected into recipient rats contained a substantial complement of C apoproteins. The mixture was then centrifuged at 1×10^8 g·av min at 12°C and the supernatant triglyceride-rich lipoproteins were subjected to chromatography on 2% agarose gel (14) to separate particles exceeding 800 Å in diameter in the void volume (large chylomicrons) from smaller particles (small chylomicrons). The large chylomicrons were concentrated by centrifugation at 6×10^6 g·av min at 12°C and the small chylomicrons were concentrated in an ultrafiltration apparatus (Amicon Corp., Lexington, Mass.) with the UM 50 membrane. Protein content of large chylomicrons incubated with HDL was increased by about 50% and that of small chylomicrons was increased by about 25% without changing their composition in other respects.

Plasma from blood treated with 1 mg disodium EDTA/ml, or blood serum was obtained from male rats maintained on Purina rat chow (Ralston Purina Co., St. Louis, Mo.) and VLDL were separated by centrifugation at 1×10^8 g·av min in the 40.3 rotor at 12°C. In some experiments, these rats were injected intravenously with 1–2 μCi [1,2-^{3}H]cholesterol and 15 μCi [1-^{14}C]palmitate-albumin complex 6 h and 30 min, respectively, before they were bled, under diethyl ether anesthesia, from the abdominal aorta (15).

Preparation of functionally hepatectomized ("supradiaphragmatic") rats. Male rats, weighing 300–350 g, were prepared as described by Bezman-Tarcher and Robinson (16). Rats fed ad libitum were anesthetized with diethyl ether. The abdominal aorta cephalad to the celiac artery, the vena cava cephalad to the renal veins, and the portal vein were ligated, and the intestines were removed. Polyethylene catheters (PE 20, Clay Adams, Div. of Becton, Dickinson & Co., Parsippany, N. J.) were inserted into the aorta just above the ligature for sampling blood and into the vena cava as a route for injection of lipoproteins and for injection of 0.5 ml of 5% glucose in water every 20 min to avoid hypoglycemia. In preliminary experiments, rats so treated maintained constant aortic blood pressure and Po_2 near 100 mm Hg for at least 90 min.

Preparation of remnants of triglyceride-rich lipoproteins. In preliminary experiments, chylomicrons (labeled with [^{3}H]cholesterol and [^{14}C]triglyceride fatty acid), obtained by centrifugation and incubated with HDL, but not fractionated by gel chromatography, were injected into supradiaphragmatic rats and blood was obtained from individual animals 5, 15, 30, 45, and 60 min later. The triglyceride fatty acids disappeared rapidly until the ratio of ^{14}C to ^{3}H in lipids of $d < 1.006$ lipoproteins was 10–20% of that in the injected material. This occurred by 30 min or less in some animals and in almost all animals by 60 min. In subsequent experiments, all animals were bled 60 min after injection of preparations of triglyceride-rich lipoproteins.

In our early experiments, when labeled chylomicrons were injected into supradiaphragmatic rats, the remnants formed were derived both from the injected lipoproteins and from the recipient's own VLDL. To eliminate the latter, secretion of VLDL from the liver was inhibited in recipient animals by injecting 4-aminopyrazolopyrimidine (4-APP) (17) in a dose of 30 mg/kg body wt intraperitoneally 18 h before the supradiaphragmatic preparation was produced. This amount of the drug was found to reduce the concentration of VLDL by about 95%, while lipolytic activity in post-heparin plasma, estimated by the method of Boberg and Carlson (18), was reduced by 40–50%. Formation of remnants in such treated animals was somewhat less rapid than in untreated ones. Accordingly, the amount of lipoprotein-triglyceride injected was reduced by about half in treated animals.

In each supradiaphragmatic rat, lipoproteins were injected in a volume of less than 1 ml. In each experiment, blood from 6 to 30 rats was pooled to obtain sufficient material for analysis. Four groups of experiments were performed. In the first group, no lipoprotein was injected, and formation of remnants from the supradiaphragmatic rats' own VLDL was studied. In the second group, remnants were produced after the injection of VLDL from blood serum or plasma into 4-APP-treated animals. In the third group, remnants were produced from injected large chylomicrons (mean diameter 1,050–1,460 Å) in 4-APP-treated as well as untreated rats. In the fourth group, remnants

were produced similarly from small chylomicrons (mean diameter 480–600 Å).

Analytical techniques. Lipoproteins were separated sequentially from plasma or serum at d 1.006, 1.019, 1.063, and 1.21 (13). Samples to be analyzed chemically were recentrifuged under the same conditions in the presence of 0.04% disodium EDTA. Cholesteryl esters, cholesterol, triglycerides, and phospholipids were quantified by standard methods, as described elsewhere (15). Content of total protein in lipoprotein fractions was determined by the method of Lowry, Rosebrough, Farr, and Randall (19); turbidity in the reaction mixture owing to lipid was removed by extraction with diethyl ether. Content of tetramethylurea (TMU)-soluble and TMU-insoluble protein was determined by the method of Kane (20). Content of FFA was measured in extracts prepared by Dole's method (21) by semiautomatic titration in a single phase system (22). Agarose gel electrophoresis of lipoprotein fractions was performed by the method of Noble (23) and polyacrylamide gel electrophoresis of TMU-soluble proteins was performed as described by Kane (20). The cofactor property of lipoproteins as an activator of substrate for lipoprotein lipase was assayed as described by Bier and Havel (24). ^{3}H and ^{14}C in lipid fractions were measured by liquid scintillation spectrometry (15). For measurement of plasma volume of intact and supradiaphragmatic rats, a measured amount (approximately 0.3 μCi) of ^{125}I-labeled human serum albumin was injected intravenously in a volume of less than 0.5 ml and the animals were bled 10 min later from the aorta. Content of ^{125}I in plasma was measured by scintillation spectrometry.

Electron microscopy. Negatively stained lipoprotein fractions, prepared as described previously (25), were examined in a Siemens 101 electron microscope (Siemens Corp., Medical/Industrial Groups, Iselin, N. J.) and photographed at initial magnifications of 20,000 and 60,000. The photographic plates were further enlarged to a magnification of 200,000 in a Nikon model 6C profile projector (Nippon Kogaku K.K., Tokyo, Japan) and the diameters of 100–400 particles in each plate were measured directly.

Materials. [1-^{14}C]Palmitate (17.9 mCi/mmol) was obtained from New England Nuclear, Boston, Mass. [1,2-^{3}H]-Cholesterol (50 Ci/mmol), obtained from the same source, was almost completely precipitated by digitonin (>97%). ^{125}I-labeled albumin was obtained from Nuclear Chicago Corp., Des Plaines, Ill., TMU from Burdick & Jackson Laboratories, Inc., Muskegon, Mich., and 4-APP from Aldrich Chemical Co., Inc., Milwaukee, Wis.

RESULTS

Formation of remnants from VLDL of blood plasma. A tracer amount of [^{14}C]triglyceride fatty acid and [^{3}H]cholesterol-labeled VLDL was injected into two supradiaphragmatic rats. 30–40% of the [^{3}H]cholesterol was in cholesteryl esters. The ratio of [^{14}C]triglyceride to esterified [^{3}H]cholesterol in the $d < 1.006$ lipoproteins, prepared from blood obtained 1 h later, was 0.21 and 0.30 of that in the injected VLDL (Table I). This indicated that removal of VLDL triglyceride fatty acids was reasonably efficient in these animals and suggested that remnants had been produced. Lipoproteins of greater density also accumulated in such animals. The results of one experiment, in which four lipoprotein classes were separated from individual rats killed at various time intervals after injection of doubly labeled VLDL, is shown in Table II. At all times up to 30 min, an amount of esterified [^{3}H]cholesterol equivalent to 72–82% of the esterified [^{3}H]cholesterol injected was recovered in lipoproteins less dense than 1.063. The amount recovered in d 1.006–1.019 and 1.019–1.063 lipoproteins varied inversely with the amount recovered in $d < 1.006$ lipoproteins. There was a tendency for the amount present in the two denser fractions to increase with time and with increasing efficiency of removal of triglyceride relative to cholesteryl ester in $d < 1.006$ lipoproteins (see last column of Table II). ^{14}C accumulated rapidly in FFA of $d > 1.063$ lipoproteins during the first few minutes after injection and then rapidly disappeared. ^{3}H accumulated progressively in cholesteryl esters of $d > 1.063$ lipoproteins. The ratio of esterified [^{3}H]cholesterol to free [^{3}H]cholesterol in $d > 1.063$ lipoproteins increased progressively from 0.02 at 2.5 min after injection of labeled VLDL to 0.09 after 30 min. Similar results in all respects were obtained in another experiment. Evidently, some particles resulting from VLDL catabolism accumulated at $d > 1.006$. However, most remained in the VLDL range ($d < 1.006$) and only in this class was it possible to evaluate the properties of the catabolic products unambiguously.

The chemical composition of the $d < 1.006$ lipoproteins isolated from supradiaphragmatic rats is shown in Table III. Compared with VLDL from intact rats, these lipoproteins were depleted of triglycerides and enriched in cholesteryl esters, cholesterol, and proteins

TABLE I

Removal of Triglycerides and Cholesteryl Esters from $d < 1.006$ Lipoproteins of Untreated and 4-APP-Treated Supradiaphragmatic Rats

Source and mass of injecte triglyceride	Number of rats injected	Recovery ratio*
Untreated rats		
Plasma VLDL (<0.5 mg)	2	0.21 0.30
Large chylomicrons (9–15 mg)	42	0.13 (0.01)
Small chylomicrons (6–10 mg)	18	0.26 (0.10)
Treated rats		
Plasma VLDL (3–5 mg)	1	0.35
Large chylomicrons (4–9 mg)	115	0.23 (0.06)
Small chylomicrons (4–6 mg)	44	0.26 (0.11)

* Ratio of [^{14}C]triglyceride fatty acid to esterified [^{3}H]cholesterol in d <1.006 lipoproteins 1 h after injection of labeled lipoprotein (ratio in injected material normalized to unity); values in parenthesis are 1 SD of the mean.

TABLE II

Distribution of Radioactivity in Lipids of Lipoprotein Fractions after Injection of Doubly Labeled VLDL into Supradiaphragmatic Rats

Time after injection	Esterified [^{3}H]cholesterol as percent of that injected*					Injected ^{14}C recovered in:		
	$d < 1.006$	d 1.006–1.019	d 1.019–1.063	Total in $d < 1.063$	$d > 1.063$	$d < 1.006$ TGFA	$d > 1.063$ FFA	Recovery ratio‡
min			%				%	
2.5	66.9	4.2	1.2	72.3	1.6	62.8	3.0	0.92
2.5	64.3	4.9	3.7	72.9	2.2	37.6	18.6	0.58
5	78.5	2.0	1.4	81.9	2.9	60.7	8.8	0.78
5	61.4	5.1	5.1	71.6	2.7	29.2	10.3	0.48
15	63.0	7.4	10.2	80.6	3.7	26.6	6.2	0.42
15	33.6	14.0	27.7	75.3	4.5	4.4	1.4	0.13
30	53.7	8.6	11.2	73.5	5.5	13.2	0.8	0.25
30	64.3	5.8	5.4	75.5	6.6	17.7	1.1	0.28

* Calculated from radioactivity contained in blood plasma. Plasma volume was determined for each rat from dilution of ^{125}I-labeled human serum albumin (see Methods).

‡ Ratio of [^{14}C]triglyceride fatty acid to esterified [^{3}H]cholesterol in $d < 1.006$ lipoproteins (ratio in injected material normalized to unity).

TABLE III

Chemical Composition of $d < 1.006$ Lipoproteins in Untreated and Treated Supradiaphragmatic Rats

Experimental group	Composition		
	Injected lipoproteins	Remnants: Untreated rats	Remnants: Treated* rats
		% *by wt*	
Serum or plasma VLDL		(n = 26)‡	(n = 32)
CE§	1.9 (0.3)‖	6.7 (2.4)	4.2 (1.5)
UC	2.7 (0.8)	5.9 (1.2)	5.7 (0.5)
PL	12.6 (0.5)	14.4 (2.2)	11.1 (2.5)
TG	73.6 (0.5)	62.9 (6.7)	63.4 (1.3)
Insoluble protein	1.0 (0.7)	2.4 (1.0)	2.5 (1.1)
Soluble protein	8.4 (1.6)	7.7 (0.3)	13.2 (0.8)
Large chylomicrons		(n = 42)	(n = 71)
CE	0.6 (0.1)	6.3 (2.3)	3.4 (0.7)
UC	0.5 (0.1)	5.0 (1.5)	4.5 (0.6)
PL	4.8 (0.8)	10.4 (3.4)	9.0 (3.5)
TG	92.7 (0.6)	70.0 (7.7)	68.6 (6.2)
Insoluble protein	0.1 (0.1)	2.1 (0.8)	2.7 (1.5)
Soluble protein	1.4 (0.3)	6.8 (1.7)	11.9 (4.4)
Small chylomicrons		(n = 18)	(n = 26)
CE	2.5 (0.8)	9.8 (2.0)	5.6 (0.5)
UC	1.1 (0.3)	4.9 (2.1)	7.2 (1.6)
PL	14.4 (3.0)	11.6 (3.0)	11.2 (5.2)
TG	77.0 (2.7)	60.8 (8.0)	60.2 (7.4)
Insoluble protein	1.3 (0.4)	3.8 (1.6)	4.1 (2.2)
Soluble protein	3.8 (0.8)	9.2 (0.8)	11.9 (4.0)

* Treated rats received 4-APP intraperitoneally the day before the study (see Methods).

‡ No lipoprotein was injected in this group; all others received injection of triglyceride-rich lipoproteins into the caval stump 5–10 min after the abdominal aorta and vena cava were ligated; all rats were bled from the aortic stump after 1 h.

§ Abbreviations: CE, cholesteryl esters; UC, unesterified cholesterol; PL, phospholipids; TG, triglycerides; insoluble protein, protein insoluble in 4.2 M TMU; soluble protein, protein soluble in 4.2 M TMU.

‖ Numbers in parentheses represent SD.

insoluble in TMU. Weight percent of phospholipids and of TMU-soluble proteins was similar to that of normal VLDL. The change in content of protein was accompanied by reduced electrophoretic mobility (Fig. 1) and by a systematic alteration in the composition of the TMU-soluble proteins (Fig. 2). The C proteins that migrate rapidly in polyacrylamide gel were substantially reduced relative to the arginine-rich protein that migrates more slowly. Lipoprotein lipase cofactor activity of the $d < 1.006$ lipoproteins was reduced, as evidenced by the increased quantity of protein required to produce half-maximal activation of triglyceride substrate for lipoprotein lipase in cows' milk ($K_m = 2.8$ μg/ml for native VLDL and 10 μg/ml for VLDL from supradiaphragmatic rats). The altered VLDL from the supradiaphragmatic rats were smaller than VLDL from intact rats but they retained the same spherical shape and appearance when examined by electron microscopy (Fig. 3).

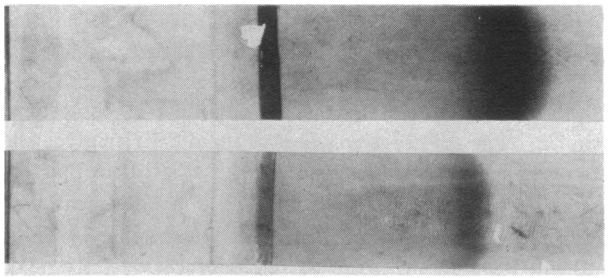

FIGURE 1 Agarose gel electropherograms of native plasma VLDL (above) and plasma VLDL remnants (below) from supradiaphragmatic rats exsanguinated 1 h after the inferior vena cava and aorta were tied off just below the diaphragm.

Characterization of 4-APP-treated rats. The results presented above indicated that altered VLDL (remnants) are formed in supradiaphragmatic rats from VLDL normally present in their plasma. It was therefore necessary to exclude these particles to characterize particles formed when triglyceride-rich lipoproteins from lymph are injected. This was accomplished by injecting a compound (4-APP) that inhibits hepatic secretion of VLDL, but it was necessary first to compare the formation of remnants from injected plasma VLDL in animals given 4-APP with their formation in untreated animals. As discussed under Methods, a smaller amount of lipoprotein was injected into 4-APP-treated animals because they cleared triglyceride less rapidly than untreated animals. The concentration of plasma FFA in intact 4-APP-treated animals was the same (0.3–0.4 mM) as in untreated animals and was also similar 1 h after the supradiaphragmatic preparation was made (about 0.2 mM). The concentrations of all major lipoprotein classes were reduced in treated animals. Mean values for protein in $d < 1.006$, 1.006–1.063, and 1.063–1.21 lipoproteins were 0.7, 0.8, and 6.8 mg/dl in 16 treated animals and 13.0, 3.8, and 36.0 mg/dl in 4 untreated ones. The composition of the d 1.063–1.21 proteins was also altered, with reduction in the proportion of rapidly migrating components by polyacrylamide gel electrophoresis (Fig. 4). Lipoprotein lipase cofactor activity in serum of treated rats was only 10–20% that of untreated ones. Treated rats lost weight (average 14 g) during the 18-h period after injection of 4-APP. The plasma volume of intact, treated rats was lower (3.96±0.35% [SD] of body weight) than that of untreated ones (4.51±0.30). The plasma volume of the supradiaphragmatic preparation of treated rats (2.02±0.15) was also lower than that of untreated ones (2.35±0.25). Sections of the livers of two 4-APP-treated rats were examined by both light and electron microscopy. Apart from depletion of glycogen and absence of secretory vesicles containing nascent VLDL, cellular architecture appeared to be normal.

Formation of remnants after injection of plasma VLDL into 4-APP-treated rats. Doubly labeled VLDL were injected into a single supradiaphragmatic preparation. The lipids of $d < 1.006$ lipoproteins, obtained 1 h later, contained 35% as much of the injected [^{14}C]triglyceride fatty acid as [^{3}H]cholesterol (Table I), indicating that remnants were formed as in untreated animals. These lipoproteins were also depleted of triglycerides and enriched in cholesteryl esters, cholesterol, and protein insoluble in TMU (Table III). However, by contrast with untreated animals, weight percent of TMU-soluble protein was also increased ($P < 0.05$ for difference between treated and untreated animals). Weight percent of "surface" components (cholesterol, phospholipids, and proteins) in $d < 1.006$ remnants from untreated animals (30.4) was slightly lower than that of treated animals (32.5), and a greater fraction was composed of phospholipids. Electron microscopic appearance of these lipoproteins was similar in the two groups (Fig. 3). The content of fast-migrating protein components by polyacrylamide gel electrophoresis appeared to be reduced to a greater extent in treated animals and cofactor activity for lipoprotein lipase was lower ($K_m = 30$ μg/ml) than in untreated animals ($K_m = 10$ μg/ml).

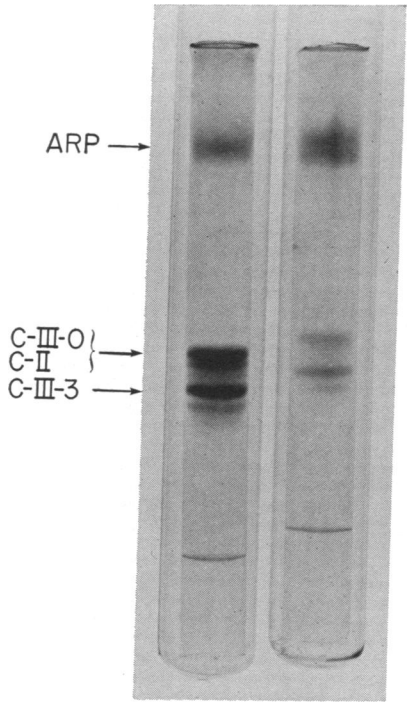

FIGURE 2 Polyacrylamide disk gel electropherograms (7.5% gels containing 8 M urea, run at pH 8.9, and stained with amido-Schwartz 10B) of TMU-soluble proteins of native plasma VLDL (left, 117 μg protein applied) and plasma VLDL remnants from supradiaphragmatic rats (right, 56 μg protein applied). The slowly migrating protein was identified as an arginine-rich protein (ARP) from amino acid analysis of the band eluted from gels (unpublished data) and the rapidly migrating C proteins were identified on the basis of data reported by Herbert, Windmueller, Bersot, and Shulman (26). The rapidly migrating bands account for 83% of total densitometric area for native VLDL and 37% for VLDL remnants.

Formation of remnants from large chylomicrons. Triglyceride-depleted particles were formed from injected chylomicrons in both untreated and 4-APP-treated supradiaphragmatic preparations, but the extent of depletion was somewhat greater in untreated animals (Table I). The chemical composition of these par-

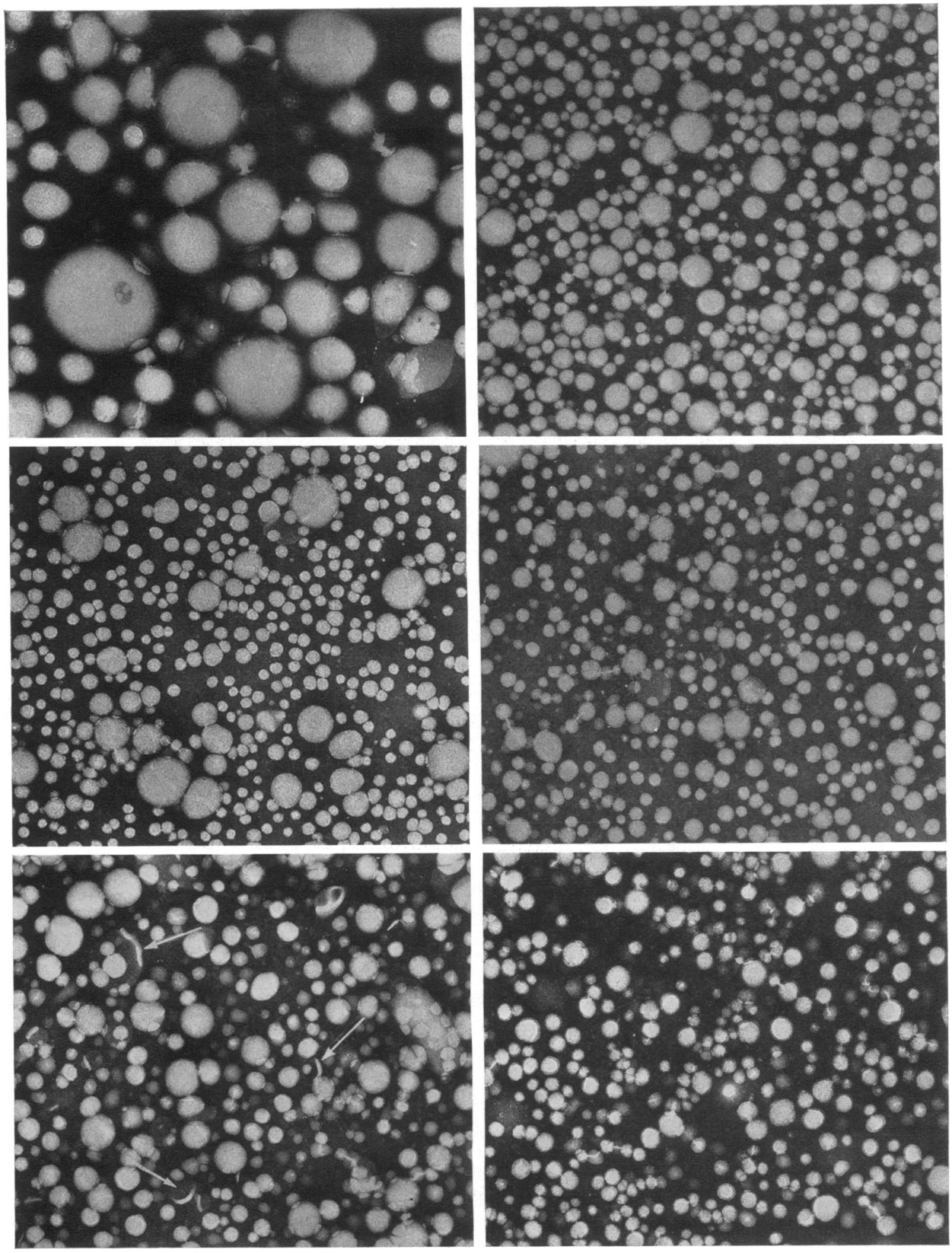

FIGURE 3 Negatively stained large chylomicrons and VLDL and their remnants (× 60,000). Left side: top, large chylomicrons; middle, large chylomicron plus plasma VLDL remnants from untreated supradiaphragmatic rats injected with chylomicrons 1 h before exsanguination; bottom, uncontaminated chylomicron remnants from supradiaphragmatic rats treated with 4-APP and similarly injected with chylomicrons. Arrows indicate flattened structures. Right side: top, native plasma VLDL; middle, plasma VLDL remnants from untreated supradiaphragmatic rats; bottom, plasma VLDL remnants from supradiaphragmatic rats treated with 4-APP and injected with native plasma VLDL 1 h before exsanguination.

ticles in treated animals was in all respects quite similar to that observed after injection of plasma VLDL (Table III) but the weight percent of surface components (28.1) was somewhat lower, suggesting a larger size. It was difficult to obtain sufficient protein for polyacrylamide gel electrophoresis of the proteins. However, by pooling samples from 30 animals, it was possible to show that the content of fast-migrating components was greatly reduced relative to those of lower mobility. The electron microscopic appearance of the remnants formed from large chylomicrons resembled that of remnants formed from plasma VLDL (Fig. 3). However, in 4-APP-treated animals, atypical discoid and flattened particles, resembling those seen in plasma of patients with lecithin-cholesterol acyltransferase deficiency and cholesterol-fed guinea pigs (28), were more prevalent (Figs. 3 and 5). Composition of $d < 1.006$ remnants from untreated animals also resembled that of animals that received no injection of lipoprotein; however, these particles were composed of a mixture of remnants from the recipient animals' own VLDL and the injected chylomicrons.

Formation of remnants from small chylomicrons. Depletion of triglycerides in untreated and 4-APP-treated supradiaphragmatic rats was similar to that observed with plasma VLDL and large chylomicrons (Table I). In one experiment with untreated animals, the distribution of esterified [^{3}H]cholesterol 1 h after injection was 76% in $d < 1.006$, 6% in d 1.006–1.019, and 17% in d 1.019–1.063 lipoproteins. Although the small chylomicrons contained less cholesterol and TMU-soluble protein and were somewhat larger than plasma VLDL, as indicated by a lower content of surface components (20.6% and 24.7% by weight for small chylomicrons and plasma VLDL, respectively [Table III]), the remnants formed from small chylomicrons in 4-APP-treated animals appeared on this basis to be slightly smaller than those formed from plasma VLDL; their composition was otherwise similar. The pattern of TMU-soluble proteins of small chylomicrons by polyacrylamide gel electrophoresis resembled that of large chylomicrons (Fig. 6) and differed from that of plasma VLDL by the presence of a major component that had the same mobility as the major apoprotein of HDL. The pattern in remnants formed from small chylomicrons resembled that of remnants formed from large chylomicrons; rapidly migrating components were reduced relative to the major slowly migrating component. As in the case of large chylomicrons, the composition of $d < 1.006$ remnants from untreated animals also resembled that of animals that received no injection of lipoprotein. The electron microscope images of the remnants formed from small chylomicrons could not be distinguished from those of rats that received an injection of plasma VLDL.

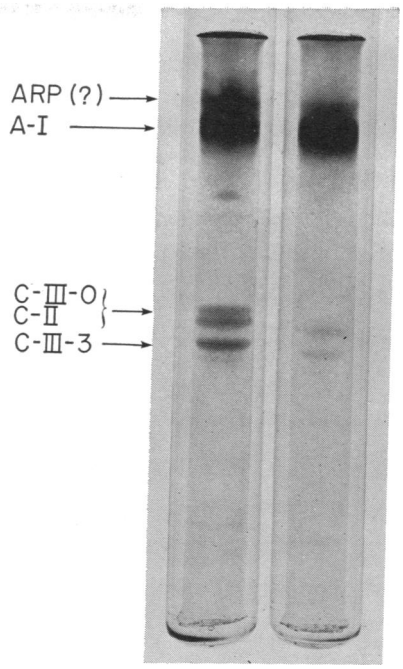

FIGURE 4 Polyacrylamide gel electropherograms of TMU-soluble proteins of HDL from untreated rats (left, 338 μg applied) and rats given 4-APP intraperitoneally 18 h before exsanguination (right, 358 μg applied). The major band is identified as the A-1 protein and the band above it is tentatively identified as the arginine-rich protein (27). The content of rapidly migrating proteins is reduced in HDL from treated animals.

Relationship between size and chemical composition of native lipoproteins and their remnants. The distribution of particle diameters, determined from negatively stained preparations photographed at a magnification of 20,000, was skewed, with a preponderance of large particles. The skewness was less pronounced for remnants than for their precursors; this was particularly true for large chylomicrons. Diameters of large chylomicrons ranged from 700 to about 3,000 Å, with a mean of about 1,300 Å, whereas their remnants seldom exceeded 1,200 Å in diameter. Typical distributions for the three types of particles and their remnants are shown in Fig. 7. Although large chylomicrons were much larger than small chylomicrons or plasma VLDL, the mean diameter of their remnants generally exceeded that of small chylomicrons and VLDL by only about 100 Å.

In man, the diameter of relatively homogeneous subfractions of VLDL bears a predictable relationship to chemical composition (2). To explore this relationship for the various particles observed in this study, the diameter of particles having the average volume of each

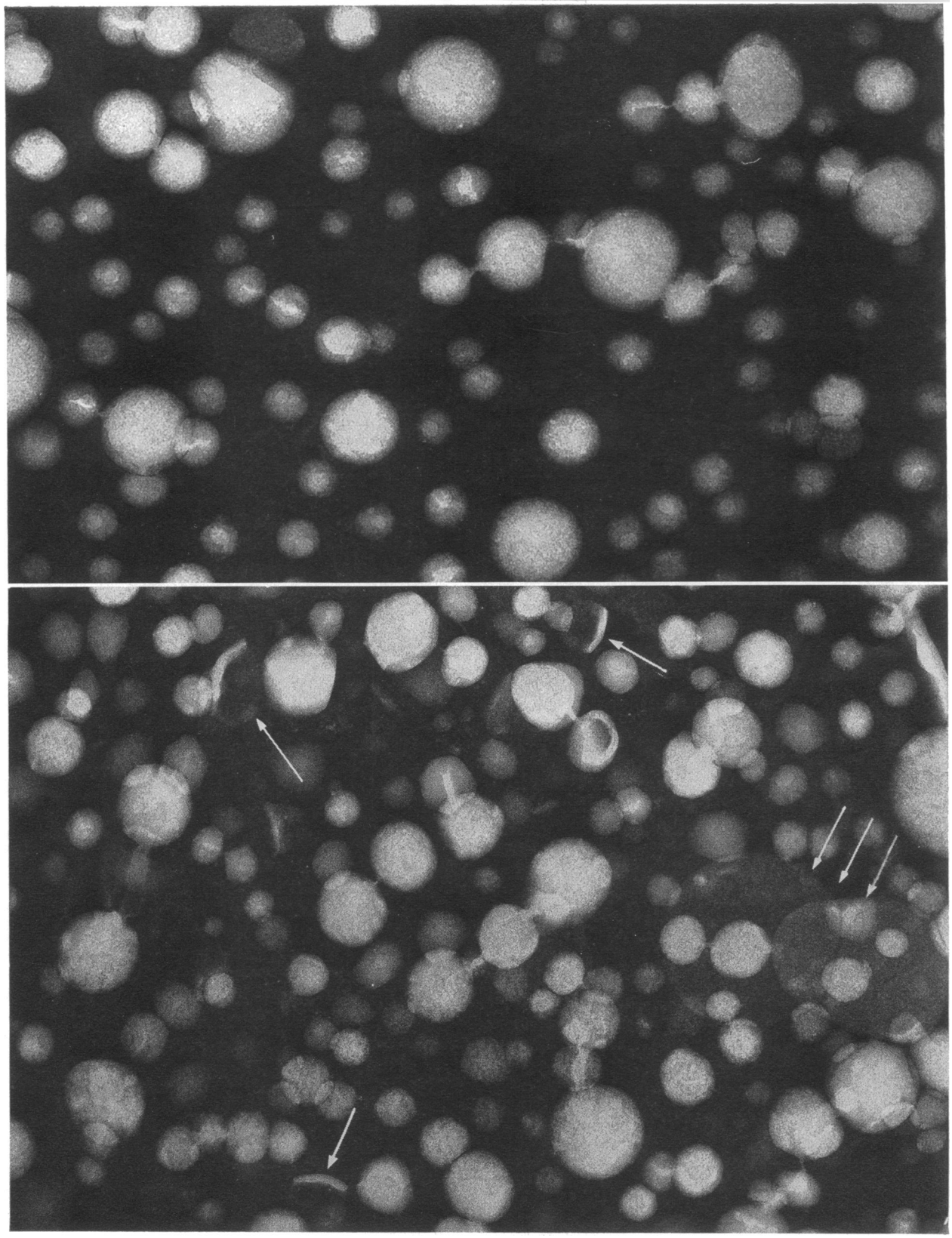

FIGURE 5 Negatively stained large chylomicron remnants (× 180,000). Top, from untreated rats; bottom, from 4-APP-treated rats. Arrows indicate discoid and flattened structures, more prevalent in remnants from 4-APP treated rats. Small electron-translucent "cores," evident in many of the smaller particles, are commonly seen in remnants from all sources.

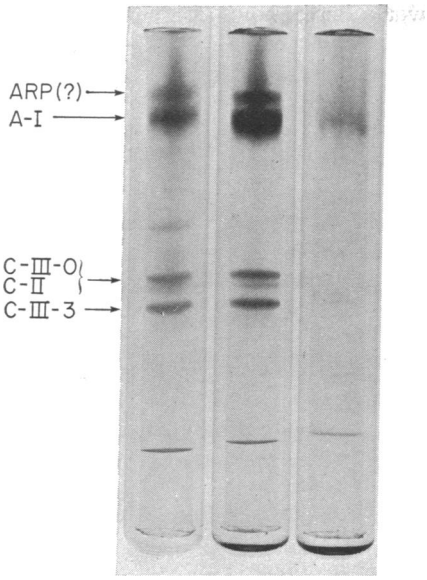

FIGURE 6 Polyacrylamide disc gel electropherograms of TMU-soluble proteins of triglyceride-rich lipoproteins from lymph. From left: large chylomicrons (272 μg applied); small chylomicrons (320 μg applied); small chylomicron remnants from supradiaphragmatic rats treated with 4-APP and injected with small chylomicrons 1 h before exsanguination (86 μg applied). Bands are identified on the basis of their mobilities relative to those of plasma HDL.

population was calculated rather than the number-average, in order to take the skewed size distribution into account. The relationship between these values and the volumes percent of presumed surface components (protein, cholesterol, and phospholipids) was analyzed as described by Sata, Havel, and Jones (2). As shown in Fig. 8, the relationship for small chylomicrons and plasma VLDL was generally similar to that observed with human VLDL subfractions. The same was true for remnants formed from chylomicrons or plasma VLDL in untreated animals. However, remnants formed in animals given 4-APP appeared to be slightly larger than predicted from their composition. The predicted diameters of large chylomicrons substantially exceeded the observed volume-average diameters. From 4 to 6 vol/100 vol of large chylomicrons was composed of measured polar components, whereas about 8 vol/100 vol would have satisfied the relationship, based upon a surface monolayer 21.5 Å thick. The presence of a small amount of additional polar lipid, such as a partial glyceride, could explain the discrepancy. In one experiment, the distribution of [^{14}C]palmitate in tri-, di-, and monoglycerides of plasma VLDL, determined after separation of neutral lipids by thin layer chromatography on silicic acid, was 95, 5, and $< 0.5\%$, respectively. A similar distribution was found in remnants produced from these VLDL in untreated rats.

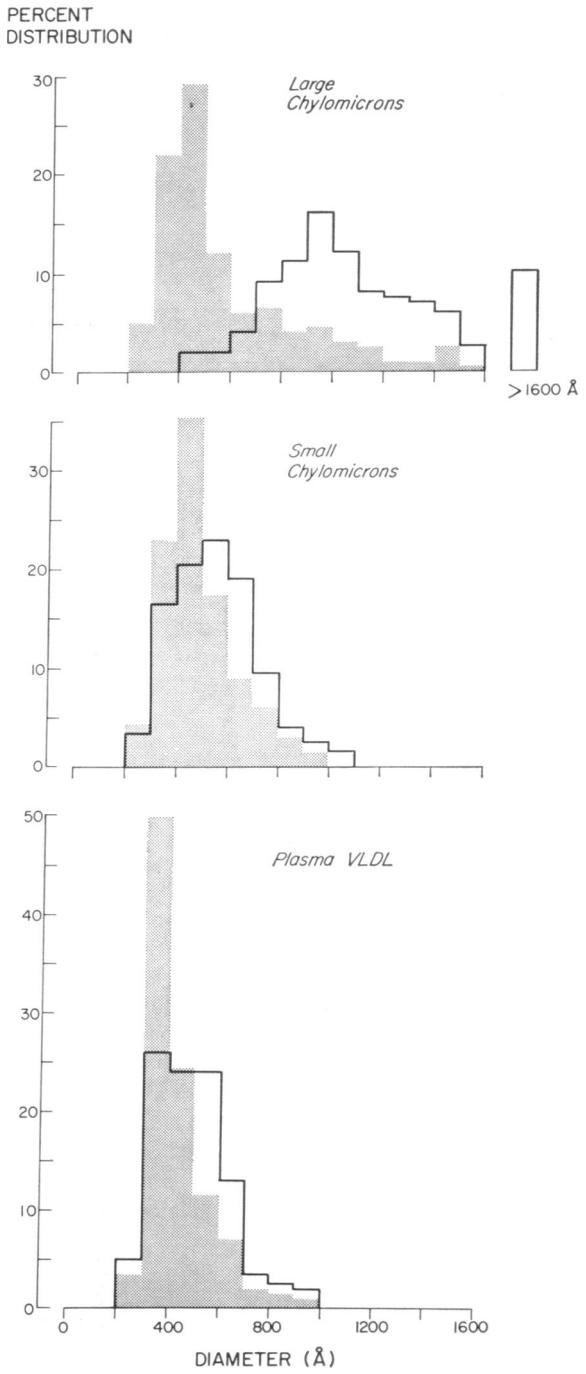

FIGURE 7 Distribution of particle diameters, measured on electron photomicrographs of negatively stained triglyceride-rich lipoproteins and their remnants. In each case, the remnants (stippled area) are smaller and the bulk of the remnant particles are more homogeneous in size than their native precursors.

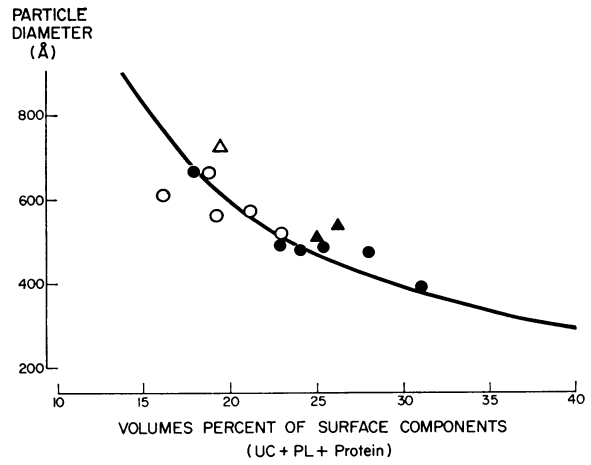

FIGURE 8 Relationship between volume-average diameter of triglyceride-rich lipoproteins and their remnants (as determined from negatively stained electron photomicrographs) and the percent of particle volume occupied by polar lipids and protein. The curved line defines this relationship for spherical particles covered by a surface shell 21.5 Å thick (2). ○, native plasma VLDL or small lymph chylomicrons; ●, remnants of chylomicrons and VLDL from untreated rats; △ and ▲, remnants from the same precursors in 4-APP-treated rats.

DISCUSSION

In this study we have confirmed that triglycerides, the major core component of $d < 1.006$ lipoproteins derived from lymph or blood plasma, are efficiently removed from the blood of functionally eviscerated rats (8, 29) whereas the other core component, cholesteryl esters, remains (4–6, 8). Depletion of triglycerides relative to cholesteryl esters in $d < 1.006$ lipoproteins was somewhat less efficient for plasma VLDL and small chylomicrons than it was for large chylomicrons. In all cases, however, it was possible to harvest particles from which at least 70% of the labeled triglycerides had been removed.

Our results with chylomicrons confirm and extend those of Redgrave (8), who first isolated partially degraded particles (remnants) from plasma of functionally eviscerated rats. The existence of such particles had been postulated earlier from studies of the removal of triglycerides and cholesteryl esters from doubly labeled chylomicrons injected into hepatectomized dogs (6, 7). Redgrave calculated that the median particle diameter of the remnants that accumulated in his rats was about 900 Å and that they were derived from chylomicrons with a median diameter of about 2,000 Å (8). Remnant particles larger than about 800 Å ($S_f > 400$), which he separated on discontinuous sucrose gradients, contained 13% cholesteryl esters and 79% triglycerides. He did not analyze smaller particles, which would have been contaminated with material derived from plasma VLDL.

In the present study, remnants from all sources contained a spectrum of particles whose diameter overlapped that of the injected precursor. In this connection, it should be pointed out that the diameter of the core of a spherical particle will be reduced by only 37% when 75% of the core components are removed. We observed that remnants from plasma VLDL extended into the density range of LDL. These particles, which contained very little labeled triglyceride in relation to cholesteryl esters, must have been smaller than those whose density was less than 1.006; possibly, they were derived from the smaller injected VLDL, which contained a higher than average ratio of cholesteryl esters to triglycerides in their cores. The efficiency with which remnants were formed was clearly a determinant of the reduction in particle diameter (and increased proportion of surface components) since, as shown in Table II, the fraction of injected esterified [^{3}H]cholesterol found in particles of $d > 1.006$ increased as the ratio of [^{14}C]triglyceride fatty acid to esterified [^{3}H]cholesterol in $d < 1.006$ lipoproteins fell. These results allow us to conclude that in the rat remnant particles are formed from plasma VLDL, which are derived mainly from the liver, as well as from chylomicrons. Bierman, Eisenberg, Stein, and Stein have shown that exposure of rat VLDL to lipase-rich plasma obtained after injection of heparin results in formation of particles of $d < 1.019$, enriched in cholesterol and protein relative to triglycerides (30). Their observations in vitro are consistent with ours in functionally eviscerated rats that received no heparin. Our findings are consistent with a large body of evidence that indicates that the pathways of transport of exogenous and endogenous triglycerides are qualitatively similar (7, 31).

When $d < 1.006$ lipoproteins from lymph are injected into functionally eviscerated (supradiaphragmatic) rats, the remnant particles formed are derived from both lymph and plasma, and our preliminary experiments indicated that they could not be separated on the basis of differences in density or size. Characterization of the remnants formed when lymph lipoproteins were injected into supradiaphragmatic rats therefore required elimination of plasma VLDL. This was accomplished by preventing secretion of VLDL with the adenine derivative, 4-APP. In confirmation of Shiff, Roheim, and Eder (17), we found that 4-APP-treated animals also had almost no LDL and greatly reduced levels of HDL in their blood plasma. Lower doses of 4-APP, which resulted in higher concentrations of LDL and HDL, failed to eliminate VLDL. Since HDL participate in the metabolism of both polar lipid and

protein components of triglyceride-rich lipoproteins (28, 31), the question arose whether such animals provide a suitable model for the study of remnant formation. We therefore compared the remnants formed from plasma VLDL in rats given 4-APP and in untreated animals. No significant differences in size, electron microscope appearance, or content of triglycerides were observed. Furthermore, the weight percent of TMU-insoluble protein and the polyacrylamide gel electrophoretic pattern of the TMU-soluble proteins were similar. Weight percent of cholesteryl esters was lower in preparations from 4-APP-treated animals, and that of TMU-soluble proteins was higher.

These results seemed to justify comparison of remnants formed from lymph and plasma lipoproteins in treated animals. The remnants formed from small chylomicrons closely resembled those formed from plasma VLDL, although small chylomicrons contained less cholesterol and TMU-soluble protein than plasma VLDL (Table III). Also, as with plasma VLDL, remnants formed from small chylomicrons extended into the density range of LDL. Large chylomicron remnants contained relatively more core components, but the differences were minor, presumably because formation of remnants from chylomicrons was more efficient (Table I). In all cases, the percentage content of protein, both insoluble and soluble in TMU, increased substantially. As in man, TMU-insoluble protein of rat VLDL represents almost entirely the B-apoprotein (32), and it seems likely that this is also the case for chylomicrons. The fast-migrating electrophoretic components of the soluble proteins were consistently reduced relative to the components that migrated slowly, and no differences in the pattern of the fast-migrating components were observed among the three lipoprotein classes injected. However, the major slowly migrating component in chylomicrons differed from that of VLDL, as observed previously by Windmueller, Herbert, and Levy (33). Unfortunately, the amounts of protein obtained from remnants in treated rats were insufficient to determine whether the mobilities of the slowly migrating components differed systematically from those of plasma VLDL.

Remnants derived from all of the three forms of triglyceride-rich lipoproteins injected appeared to retain the fundamental structure characteristic of their precursors. Except in animals given 4-APP, there was no evidence for the formation of $d < 1.006$ lipoproteins with excessive polar components. Thus, it appears that removal of triglycerides from these lipoproteins in extrahepatic tissues is accompanied by removal of sufficient surface components that their diminished core remains covered by a surface monolayer. It is not possible to deduce whether removal of polar components

TABLE IV

Physical and Chemical Properties of Individual VLDL and Their Remnants

	Plasma VLDL	Remnants	
		Untreated rats	4-APP treated rats
Physical properties			
Diameter, Å*	570	445	430
Volume, $cm^3 \times 10^{18}$	97.3	45.6	41.7
Density, g/cm^3*	0.965	0.976	0.991
Weight, $g \times 10^{18}$	94.0	44.5	41.3
Mol wt, *daltons* $\times 10^{-6}$	56.3	26.6	24.7
Chemical composition,‡ *daltons* $\times 10^{-6}$			
CE	1.1	1.8 (1.6)	1.0 (0.9)
TG	41.5	16.7 (0.40)	15.6 (0.38)
Total core	42.6	18.5 (0.43)	16.6 (0.39)
UC	1.5	1.6 (1.1)	1.4 (0.9)
PL	7.1	3.8 (0.54)	2.7 (0.38)
Insoluble protein	0.56	0.64 (1.1)	0.62 (1.1)
Soluble protein	4.7	2.0 (0.43)	3.3 (0.70)
Total surface	13.9	8.0 (0.57)	8.0 (0.57)

* Values calculated as described in reference 2 for particles with average composition given in Table III.

‡ Calculated from molecular weights and average composition given in Table III. Values in parenthesis are values for remnant divided by value or plasma VLDL.

is simultaneous with removal of triglyceride or whether it occurs subsequently. From the changes in chemical composition and in protein distribution, it seems clear that both phospholipids and certain soluble proteins leave the surface of the particles. To evaluate this phenomenon more precisely, we have calculated the mass of each component for the average plasma VLDL particle and for the remnants produced from them (Table IV). The values for untreated rats suggest that approximately half of the phospholipids and soluble proteins (principally the C proteins) left the surface when remnants were formed, whereas the quantity of cholesterol and insoluble protein changed little. Mass of cholesteryl esters in the core appeared to increase. As in intact rats (15), cholesteryl esters produced from labeled VLDL-free cholesterol by the action of lecithin-cholesterol acyltransferase also accumulated in HDL during the metabolism of VLDL (Table II). Although the amount so formed cannot be determined without knowledge of the specific activity of the precursor cholesterol, our results suggest that any labeled cholesterol transferred from VLDL is replaced by unlabeled cholesterol from another source. By contrast, a substantial quantity of lecithin, the other substrate for lecithin-cholesterol acyltransferase, may be transferred to HDL. Removal of phospholipid from VLDL was not impaired in 4-APP-treated rats and mass of cholesterol remained constant in remnants, as in intact rats (Table IV).

Thus, the reduced content of HDL (and possible associated reduction in activity of lecithin-cholesterol acyltransferase) evidently did not substantially impede removal of polar lipids associated with formation of remnants from plasma VLDL. Loss of TMU-soluble proteins was reduced in 4-APP-treated animals, however. In addition, mass of cholesteryl esters did not increase as in untreated animals, perhaps because of the paucity of cholesteryl esters available for transfer from LDL and HDL. Generally similar relationships were obtained for small chylomicrons and their remnants in 4-APP-treated rats. However, phospholipids accounted for 70% of the surface of the small chylomicrons and for about 90% of the loss of surface material when remnants were formed. Also, mass of B-apoprotein was higher in individual small chylomicrons and their remnants ($\sim 1.2 \times 10^6$ daltons) than in plasma VLDL. Estimates for large chylomicrons were not considered accurate because of uncertainty about their average size. Individual large chylomicron remnants also contained about 1.2×10^6 daltons of B-apoprotein. Phospholipids accounted for about 70% of the surface mass of large chylomicrons and for 80–90% of the loss of surface material that accompanied formation of remnants. The presence of discoid and flattened particles among remnants formed from large chylomicrons in 4-APP-treated animals suggests that in this case the reduced levels of HDL (and possibly of lecithin-cholesterol acyltransferase) led to formation of some particles with excessive polar lipids, similar to those observed in lecithin-cholesterol acyltransferase deficiency (28).

Almost all of the cholesteryl esters of rat lymph chylomicrons (3) and plasma VLDL (15) are taken up very rapidly by the liver, whereas most of the triglycerides are removed by extrahepatic tissues (15, 34), indicating that the core components of these two particles are metabolized similarly. The present studies suggest that the polar components of both classes of triglyceride-rich lipoproteins are also metabolized by similar pathways. In particular, our results are consistent with the concept (31, 35–37) that the group of small C apoproteins with rapid electrophoretic mobility, which transfer to rat chylomicrons (33) and VLDL (38) from HDL after they are secreted, return to HDL from these lipoproteins as triglycerides are taken up in extrahepatic tissues. Phospholipids also leave the surface and may be transferred similarly, as suggested by the substantial increase in HDL phospholipids during alimentary lipemia in man (35). By contrast, the B-apoprotein, represented by the protein insoluble in TMU, is retained in remnants. In the case of plasma VLDL, almost all of this protein, like cholesteryl esters, is rapidly and efficiently removed by the liver in the rat (32, 39).

These observations have relevance for the pathogenesis of at least one form of hyperlipoproteinemia in man. Two abnormal populations of particles, about 800 and 300 Å in diameter, accumulate in blood plasma of individuals with the disorder, primary dysbetalipoproteinemia (2). These particles resemble the remnants isolated in the present study in a number of respects. Compared with normal particles of equivalent diameters, their content of triglycerides is reduced and that of B-apoprotein and the arginine-rich apoprotein is increased. Patients with dysbetalipoproteinemia also have plasma VLDL of normal composition (10, 11) and, during alimentary lipemia, apparently normal chylomicrons as well (9, 11). The size and chemical composition of the two abnormal populations of lipoproteins suggests that they are remnants of chylomicrons and VLDL.

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