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Biosynthesis of lymph and plasma lipoprotein apoproteins by isolated perfused rat liver and intestine

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Abstract The ability of rat intestine and liver to synthesize the various apoproteins of plasma lipoproteins was investigated. After the individual isolated organs were perfused with blood containing [^{3}H]lysine, chylomicrons plus very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL) were isolated from the perfusates and the intestinal lymph. After lipoprotein delipidation, apoproteins were separated by polyacrylamide gel electrophoresis and the ^{3}H content was determined.

Livers incorporated [^{3}H]lysine into all apoprotein bands of VLDL and HDL. The ^{3}H content was greater in large proteins that remained in the stacking gel (group I, predominantly β-apoprotein) than in proteins with apparent molecular weights near 50,000 (group II) or in the smaller peptides (molecular weights near 10,000, group III).

In the intestine, ^{3}H was incorporated into group I and, in larger amounts, into group II apoproteins of lymph VLDL. No labeled VLDL appeared in the perfusate. ^{3}H was also incorporated into group II apoproteins of lymph and perfusate HDL. Significantly, no [^{3}H]lysine was found in the group III peptides of any lymph or intestinal perfusate lipoproteins. Since these peptides were always present in VLDL from mesenteric lymph collected in vivo, the results suggest that nascent VLDL of gut origin acquires group III peptides from other lipoproteins that penetrate lymph from plasma.

Supplementary key words chylomicrons · fat transport · very low density lipoproteins

RAT PLASMA LIPOPROTEINS, like those of the human, contain several different protein moieties, or apoproteins (2–5). Although liver and intestine have been identified as sites of lipoprotein production, very little is known concerning the synthetic sites of individual apoproteins, particularly concerning the role of the intestine.

An appropriate experimental preparation for answering the question of intestinal apoprotein synthesis might be one that permitted isolating the intestine from the animal while maintaining the integrity of the vascular and lymphatic network. This would allow the delivery of oxygen and nutrients, the disposal of metabolic end products and, most importantly, the release of the lipoproteins to proceed via the normal channels.

A viable preparation of vascularly perfused rat intestine was recently developed for this kind of study (6). It was demonstrated that, during a 5-hr period of extracorporeal perfusion, the preparation continuously produced lymph, transported fat, and incorporated [^{3}H]-lysine into lymph chylomicrons and VLDL (7). There was also incorporation of lysine into HDL recovered from the lymph as well as from the recycled perfusate (7).

We now report some information regarding intestinal synthesis of individual lipoprotein apoproteins, obtained by delipidation of the [^{3}H]lysine-labeled lipoprotein products and resolution of the resulting apoprotein mixture by polyacrylamide gel electrophoresis. For purposes of comparison and as a control, we have used similar techniques to examine lipoprotein apoprotein synthesis

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Abbreviations: VLDL, very low density lipoproteins, d < 1.006 g/ml; LDL, low density lipoproteins, d 1.006–1.035 g/ml; HDL, high density lipoproteins, d 1.063–1.21 g/ml.

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in a preparation of perfused rat liver. Earlier studies have shown that perfused rat livers can produce both low and high density lipoproteins (8–10) and have provided qualitative evidence for hepatic biosynthesis of most of the known plasma lipoprotein apoproteins (11, 12).

EXPERIMENTAL

Rats and diets

Rats used as blood donors were mature males of the Osborne-Mendel or Sprague-Dawley strains. They had free access to Purina chow and water until blood was drawn by aortic puncture under ether anesthesia. Rats used as intestine donors or liver donors were 260–280-g males from the National Institutes of Health pathogen-free Osborne-Mendel colony. For 10–20 days before perfusion, intestine donors had free access to a balanced semisynthetic diet (diet W-8); liver donors, in order to stimulate hepatic lipogenesis and lipoprotein production (13), were fed the same diet without fat (diet W-8FF) (7).

Intestinal perfusion

Three similar experiments were performed using the apparatus, perfusate composition, and procedure that have been described in detail (7). Briefly, the small bowel, cecum, and part of the colon, totally excised en bloc and submerged in a 37°C tissue bath, were vascularly perfused with about 120 ml of heparinized rat blood supplemented with dexamethasone and antibiotics. The recycling system included a membrane oxygenator. In the procedure, extracorporeal perfusion was begun without interrupting the flow of oxygenated blood through the tissue, and the initial 25–40 ml of the perfusing mixture that was flushed through the vasculature of the preparation was discarded before recycling was begun. Norepinephrine, 0.05–0.5 μg/min, was infused continuously into the perfusate to counteract the deleterious effects apparently due to sympathetic denervation (6). Lymph from the preparation was collected in ice from a catheter in the main mesenteric lymph channel. During the perfusion, the duodenum was infused continuously with the basic luminal infusate (7), a physiological salt solution supplemented with 220 mM glucose, 10 mM sodium taurocholate, and 2.5 mg of defatted bovine serum albumin per ml of infusate. During the first 4 hr of perfusion, the duodenum was also infused at 0.128 ml/hr with a triglyceride–phospholipid emulsion (Intralipid; Vitrum AB, Stockholm, Sweden), containing 243 μmoles of fatty acid/ml, to provide a small compensatory amount of luminal lipid in the absence of bile flow. 20 min after perfusion was begun, 1.4 mCi of L-[G-^{3}H]lysine (3.91 Ci/mmole, New England Nuclear Corp.) was added to the perfusate. Perfusion was continued for 5 hr. Lymph collected throughout each experiment (average volume, 2.7 ml) and plasma separated from the recycled blood by centrifugation (subsequently referred to as perfusate) were stored at 4°C after adding sodium EDTA, pH 7.4, to a final concentration of 3 mM.

Liver perfusion

Two experiments were performed, differing in perfusate composition and perfusion apparatus.

In one experiment (LP-118), the recycled perfusate consisted of 50 ml of defibrinated rat blood plus 50 ml of rat serum, the mixture being supplemented with 100 mg of glucose, 100,000 units of penicillin G, and 5 mg of streptomycin sulfate. Perfusion in situ was carried out by the method of Mortimore (14, 15), with oxygenation of the perfusate in a rotating flask that also served as the perfusate reservoir.

In a second experiment (LP-119), the rotating flask was replaced with a silicone rubber reservoir and a membrane oxygenator, as used for intestinal perfusion (7). Thus, blood–air interfaces were eliminated from the perfusion circuit in an effort to minimize possible structural alterations of the perfusate lipoproteins (7). In this experiment, the perfusate consisted of 50 ml of whole rat blood and 50 ml of rat plasma plus 25 mg of sodium heparin, 100 mg of glucose, and antibiotics as used in experiment LP-118.

The liver was flushed with a 60–85-ml portion of the perfusing mixture in both experiments. This was discarded before recycling of an additional 100 ml of perfusate was begun. As with intestine, 1.4 mCi of L-[G-^{3}H]-lysine (3.91 Ci/mmole) was added to the recycling perfusate, and the perfusion was continued for 5 hr. EDTA was added to the collected perfusate as described above. Results from the two experiments were similar and are reported together.

Isolation of VLDL and HDL

Because the amount of mesenteric lymph lipoprotein from a single perfused intestine is small (7), it was necessary to add carrier to improve recovery of protein in the delipidation step (see below). In two intestine experiments, this was done before lymph lipoprotein isolation was begun by adding 13 vol of carrier mesenteric lymph freshly collected in vivo (16) from rats fed the fat-free diet (W-8FF). In the other experiment, the lymph lipoproteins collected during perfusion were first isolated by ultracentrifugation, and carrier mesenteric lymph lipoproteins were added just before delipidation. The two procedures gave similar results.

Lipoprotein isolation was begun within 72 hr of sample collection. Lymph and perfusates were centrifuged in a Beckman Ti-60 rotor for 12 hr (1.83×10^8 *g*-min, aver-

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age) at 5°C. The top 8–10 ml (VLDL)[1] was resuspended in 0.85 M NaCl, and the centrifugation was repeated twice more.

The density of the infranatant from the first centrifugation was adjusted to 1.063 g/ml with solid KBr (18) and centrifuged in a Beckman Ti-60 rotor for 18 hr (2.75 $\times$ 10^8 g-min, average). The top 6 ml was removed by tube slicing, and the density of the infranatant was adjusted to 1.21 g/ml with solid KBr. Pycnometry was used to monitor density adjustments. Centrifugation of this fraction was performed first in a Beckman Ti-60 rotor for 24 hr (3.66 $\times$ 10^8 g-min, average). The top 6 ml (HDL) was removed, adjusted to a density of 1.22 g/ml with solid KBr, overlayered with an equal volume of aqueous KBr, d 1.21 g/ml, and centrifuged for 48 hr in a Beckman 40 rotor (3.04 $\times$ 10^8 g-min, average).

The isolated lipoprotein fractions were dialyzed in no. 18 cellulose casing (Union Carbide) for 72 hr against 50 vol of 5 mM ammonium bicarbonate, pH 8.0, containing 3 mM sodium EDTA, with dialysate changes every 12 hr.

Similar procedures were used for isolation of lipoproteins from rat plasma and from mesenteric lymph collected in vivo.

Lipoprotein delipidation

Dialyzed lipoprotein fractions were lyophilized and suspended in 15 ml of methanol at 0°C with shaking to obtain a fine dispersion of the lipoprotein, and then 30 ml chloroform was added and the samples were extracted with shaking for 1 hr. The flocculated protein was sedimented by low-speed centrifugation after adding 15 ml of anhydrous diethyl ether to lower the density of the organic phase. The organic phase was decanted and the extraction was repeated twice more with 45 ml of chloroform–methanol 2:1 (v/v). Finally, the protein was washed three times with anhydrous diethyl ether and dried under a stream of N_2, and the remaining ether was removed under vacuum in a desiccator.

The delipidated apoproteins were solubilized in 0.2 M Tris chloride, pH 8.2, containing 6 M urea and 0.1 M decylsulfate. Prior to polyacrylamide gel electrophoresis, the samples were dialyzed against six changes of 5 mM ammonium bicarbonate, pH 8.0, for 72 hr.

Recovery of protein following delipidation, resolubilization, and dialysis ranged from 55 to 83% (average, 62%) for all VLDL and HDL preparations. The specific radioactivity of the total lipoprotein protein after these procedures, however, never increased or decreased more than 15% and averaged −4%.

Isolation of LDL

In one experiment, the d 1.063 centrifugation step (see above) was circumvented by heparin–manganese precipitation of the LDL from the d 1.006 infranatant fraction (19). The resulting precipitate was removed by centrifugation, and the supernatant solution was dialyzed against aqueous KBr, d 1.21 g/ml, and then centrifuged, as above, to recover the HDL. The heparin–manganese precipitate was washed with 0.85 M NaCl containing 200 units of heparin sulfate and 50 mmoles of $MnCl_2$ per ml and then solubilized in 10% sodium citrate. After dialysis against 5 mM ammonium bicarbonate, 3 mM EDTA, this material was lyophilized, delipidated, and solubilized, as described above. This procedure was considered suboptimal because of the difficulty in completely solubilizing the precipitate obtained.

In another set of experiments, the d 1.006–1.063 fraction was adjusted to a density of 1.035 g/ml by dialysis against a KBr solution of that density. The top (d 1.006–1.035) and bottom (d 1.035–1.063) fractions recovered after ultracentrifugation were lyophilized, delipidated, and solubilized (see above).

Polyacrylamide gel electrophoresis

10% acrylamide gels (0.6 $\times$ 11.5 cm) were prepared in 8 M urea at pH 8.9 using Tris–HCl buffer (20). The gels were loaded with 15–80 μg of protein, containing 10^3–10^5 cpm when the sample was radioactive.

Determination of radioactivity

Radioactivity of total protein and of lipoprotein fractions before and after delipidation was determined as described previously (7).

The radioactivity of lipoprotein apoprotein bands after polyacrylamide gel electrophoresis was determined by a modification of the procedure of Tishler and Epstein (21). Gels fixed in trichloroacetic acid and stained with Coomassie blue were sliced transversely with a razor blade, giving slices 1–3 mm thick depending on the width of the protein bands. The slices were cut as the gel column was extruded from the end of a length of glass tubing with a bore slightly larger than the gel and illuminated from below. Individual gel slices were transferred to the bottom rim of open counting vials and dried for 6 hr at 55°C. After adding 0.2 ml of 30% H_2O_2, the vials were capped and heated for 10 hr at 50°C with the vials positioned to ensure contact between the slice and the H_2O_2. After adding 1 ml of NCS (Amersham/Searle) to the

[1] Previous work with rat plasma and lymph has shown that, although the fraction of d < 1.006 g/ml contains a continuous spectrum of lipoprotein particles with flotation rates ranging from S_f 20 to S_f > 3200, most of the particles would be in the range S_f 20–400 under the conditions of the present perfusion experiments (17.) Since any separation of large particles (chylomicrons) from smaller particles (VLDL) would be rather arbitrary, and since the large particles account for very little of the total protein of the d < 1.006 fraction (17), no subfractionation was attempted and the entire d < 1.006 fraction is referred to as VLDL.

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TABLE 1. Incorporation of [³H]lysine into secreted proteins by isolated perfused liver and intestine

Perfused Tissue	Route of Secretion	Total Protein	Lipoproteins[a] VLDL	HDL
		% of administered ³H	*% of ³H in total protein*	
Liver (2)[b]	Perfusate	16.8 (13.5–20.0)	1.9 (1.8–2.0)	2.0 (1.9–2.1)
Intestine (3)	Perfusate	0.2 (0.16–0.23)	0.0 (0.0–0.1)	10.5 (6.3–14.0)
	Lymph	0.3 (0.28–0.41)	24.5 (20.0–30.7)	6.5 (5.6–9.8)

Organs from rats were perfused for 5 hr with blood containing a tracer dose of L-[³H]lysine and perfusate and lymph lipoproteins were isolated (see Experimental section). Values are the means and ranges for the number of experiments indicated.

[a] Data show incorporation into the protein portion of the lipoprotein complexes.

[b] Number of experiments.

resulting clear solutions, the uncapped vials were placed on a mechanical shaker and flushed repeatedly with a stream of N_2 for 15 min to facilitate removal of dissolved O_2. 9 ml of Liquifluor (Packard Instrument Co.) was added and the vials were counted in a Packard model 3003 Tri-Carb scintillation spectrometer. Counting efficiency was monitored with the instrument's external standard, and all vials showing excessive quenching were shaken vigorously and again flushed with N_2. Absolute counting efficiency, determined by adding internal standard, was 32% and recovery of radioactivity loaded on the gels averaged 87%.

Identification of apoprotein bands after polyacrylamide gel electrophoresis

For clarity of discussion, it is useful to divide the apoproteins into three groups corresponding to the three protein peaks obtained when VLDL or HDL apoprotein is fractionated on a column of Sephadex G-150 (4) or G-200 (5). Group I contains predominantly a large protein or protein aggregate that is unique to VLDL and LDL and has been referred to as the β-apoprotein (16) or apo-LDL (4). After polyacrylamide gel electrophoresis (Fig. 1), this protein remains in the stacking gel or at the interface between the stacking and separation gels (zone I). Group II contains several proteins migrating near the top of the separation gel (zone II) and having apparent molecular weights near 50,000, based on their elution from Sephadex columns. Group III comprises a group of peptides with apparent molecular weights near 10,000. 90% of the protein of this latter group is accounted for by the two prominent farthest-migrating bands on the gels (zone III) (Fig. 1). The apoproteins in these two bands are also found in HDL (4, 5) and readily exchange between VLDL and HDL (22). The remaining 10% of the group III peptides is accounted for by two minor bands. One migrates in the upper part of zone II and the other in the region between zones II and III (4). These bands are barely visible when gels are run with the protein loads used in these experiments.

Protein determination

The procedure of Lowry et al. (23) was used, with bovine serum albumin as standard. When turbidity was present, it was removed by extraction with diethyl ether before determining absorbancy (24).

RESULTS

Total incorporation of [³H]lysine into secreted proteins

During the course of the 5-hr perfusions, the livers incorporated 16.8% of the [³H]lysine dose into secreted proteins, of which 1.9% was recovered in the protein of VLDL and 2.0% in HDL[2] (Table 1). With intestine, 0.2% of the radioactive dose appeared in perfusate protein and 0.3% in lymph protein. 10% of the gut perfusate protein radioactivity was in lipoproteins, and it was all in HDL. In the intestinal lymph, VLDL and HDL together accounted for 31% of the protein radioactivity, and most of the label was in VLDL (Table 1). Thus, HDL protein synthesized by the gut appeared in both lymph and perfusate while VLDL protein synthesized by the gut appeared only in the lymph. It is known from previous labeling studies in the isolated rat intestine that the specific radioactivity of the newly synthesized lymph lipoproteins rises to a maximum during the first or second

[2] We did not observe a rapid (10 min) incorporation of [³H]-lysine into perfusate lipoproteins, as reported by Wilcox, Fried, and Heimberg (25). The specific activities of perfusate VLDL and HDL protein measured at 10 min in one experiment were less than 0.2% of the values observed after 5 hr of perfusion.

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TABLE 2. Distribution of radioactivity among lipoprotein fractions after perfusion of liver and intestine with [³H]lysine

Lipoprotein Density	Source of Lipoproteins		
	Liver	Intestine	
	Perfusate	Perfusate	Lymph
g/ml	*% of total lipoprotein radioactivity*		
<1.006	44.2	0.0	73.3
1.006–1.035	1.4	0.5	4.1
1.035–1.063	9.5	13.3	9.4
1.063–1.21	44.9	86.2	13.2
Total	(100.0)	(100.0)	(100.0)

The lipoproteins from one intestinal perfusion and one liver perfusion experiment (LP-119) were fractionated and counted as described in the Experimental section.

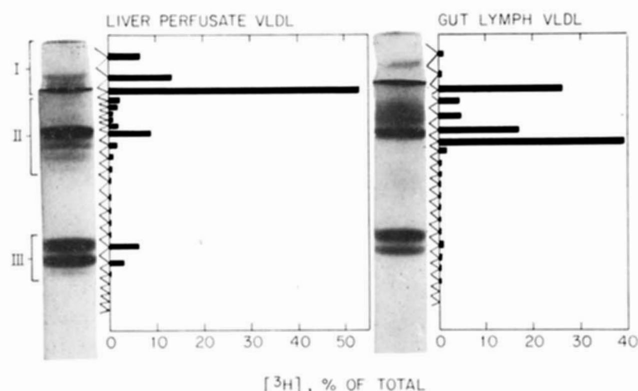

FIG. 1. Incorporation of [³H]lysine into VLDL apoproteins by isolated liver and intestine. After perfusion of the organs with blood containing [³H]lysine for 5 hr, VLDL isolated from the liver perfusate and the gut lymph were delipidated, and the resulting apoprotein mixture was subjected to electrophoresis on polyacrylamide gels. The direction of migration in the gels was from top (cathode) to bottom (anode). The three zones of each gel, identified by the roman numerals, correspond approximately to three groups of apoproteins; see Experimental section for details. The bars indicate the percentage of total recovered radioactivity that was found in the corresponding region of the gel. The data are mean results from two liver perfusion and three gut perfusion experiments. The quantitative incorporation into VLDL in these experiments is given in Table 1.

hour of perfusion, with little subsequent change (7). Also, the specific radioactivity of lymph HDL is always 400–800 times as high as that of the perfusate HDL, indicating that at least some of the newly synthesized HDL protein enters the lymph directly from the intestine without first passing through the plasma (perfusate) compartment (7).

The lipoproteins in one series of experiments were more completely fractionated, as indicated in Table 2. Most of rat plasma HDL has a density between 1.063 and 1.21 g/ml, but some was recovered in the fraction between 1.035 and 1.063 g/ml. LDL was recovered largely in the fraction of d 1.006–1.035 g/ml (11). Only 1.4% of the liver perfusate lipoprotein radioactivity was recovered in this LDL fraction, and only 4.1% of the mesenteric lymph lipoprotein radioactivity was found there. Moreover, when the LDL fractions (d 1.006–1.035 g/ml) were delipidated and examined by polyacrylamide gel electrophoresis and immunoelectrophoresis using previously described antisera (4), the presence of HDL peptides was always detected, further reducing the likelihood that significant amounts of LDL were produced.

[³H]Lysine incorporation into VLDL apoproteins

Characteristic polyacrylamide gel patterns were obtained with VLDL apoproteins from liver perfusate and from intestinal lymph (Fig. 1).

The liver perfusate VLDL protein on the gel (Fig. 1) is a mixture of VLDL produced by the perfused liver (~60%) and VLDL already present in the perfusate at the start of the perfusion (~40%). The above estimates of relative contribution are based on the observation that during the course of the liver perfusions there was a 2.5-fold (approximately) increase in the concentration of VLDL protein in the perfusate. The pattern of apoprotein bands observed (Fig. 1) was very similar to that seen previously with VLDL from rat plasma (3, 4). After perfusion with [³H]lysine, there was radioactivity associated with all visible apoprotein bands, indicating that the perfused liver was able to synthesize proteins associated with all of these bands. More than 70% of the radioactivity remained in the stacking gel, suggesting extensive incorporation into the β-apoprotein.

VLDL from intestinal lymph, after delipidation and electrophoresis on polyacrylamide gels (Fig. 1), gave a reproducible pattern of apoprotein bands that was similar to, though not identical with, the pattern observed with VLDL from plasma or liver perfusate. A marked difference was the more rapid migration rate of the most prominent zone II band of the lymph VLDL. The gel pattern, of course, reflects largely the protein distribution in lymph VLDL in vivo, since the radioactive lymph produced by the isolated intestine was diluted with about 13 vol of carrier lymph collected in vivo before electrophoresis of the apoprotein mixture. From Fig. 1 it is seen that the isolated gut incorporated [³H]lysine into zone I protein and into all visible zone II protein bands, but that little or no radioactivity was found in the zone III peptide bands. The radioactivity associated with the zone III bands was so low that it could not be reliably distinguished from background, and this was true for all three intestinal perfusion experiments. Noteworthy also is the relatively large incorporation of [³H]lysine into the zone II apoprotein bands by the intestine.

[³H]Lysine incorporation into HDL apoproteins

The polyacrylamide gel patterns of HDL apoproteins from liver perfusate and from gut lymph were indistin-

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guishable from each other (Fig. 2) and were similar to the pattern observed previously with HDL from rat plasma (3, 4). Approximately 45% of the liver perfusate HDL was produced by the isolated liver and 55% was contributed by the perfused blood, these figures being determined, as before, from the increase in perfusate HDL protein concentration during the 5-hr experiment.

The isolated liver incorporated [^{3}H]lysine into all visible zone II and zone III apoprotein bands, the bulk of the radioactivity being associated with the two most prominent zone II bands (Fig. 2).

The isolated intestine also incorporated [^{3}H]lysine into all visible zone II HDL apoprotein bands, about 75% of the counts being associated with the major band in zone II (Fig. 2). There was, again, no detectable radioactivity in the two major zone III peptides or in the faint band migrating between zones II and III of the gels.

The small amount of radioactivity found in zone I of the HDL gels may have been due to the presence of some incompletely delipidated or aggregated protein (4).

HDL isolated from the gut perfusate gave a polyacrylamide gel pattern essentially identical with that of gut lymph HDL and is not shown. The distribution of radioactivity was also similar to that of lymph HDL. Zone II apoprotein bands contained, on the average, 87% of the counts, with about 60% being in the two major bands of this group. Only traces of radioactivity were found with the zone III peptides.

DISCUSSION

Lipoprotein apoprotein synthesis by intestine

Earlier biochemical studies had provided evidence that intestine could incorporate radioactive amino acids into lymph and plasma lipoproteins, although the specific proteins synthesized were not identified (7, 26–30). Evidence for β-apoprotein synthesis came from the observation that mesenteric lymph of orotic acid-fed rats had normal amounts of this protein, detected immunochemically, while plasma levels were near zero, the result of an orotic acid-induced block in hepatic but not intestinal β-apoprotein secretion (16). Subsequently, Kessler et al. (31) found evidence for β-apoprotein synthesis by a cell-free preparation of rat intestinal mucosa, and Mahley et al. (32) have detected the β-apoprotein immunochemically in Golgi vesicles isolated from rat intestinal mucosa. In the present study, the extensive incorporation of [^{3}H]lysine into VLDL apoprotein that remained in the stacking gel (Fig. 1) is also consistent with β-apoprotein synthesis by the intestine. However, present techniques cannot permit the identification of all zone I proteins as β-apoprotein.

What the data in Figs. 1 and 2 and Table 1 do show very clearly, however, is that β-apoprotein is not the only lipoprotein apoprotein synthesized by gut. Most of the incorporated [^{3}H]lysine, in fact, appeared in the group II apoproteins of VLDL and HDL. From the present study it cannot be determined whether the group II proteins found in the lymph VLDL were present in the nascent VLDL particles released from the intestinal cell or whether they were acquired from lymph HDL. Preliminary biochemical and immunochemical analyses have suggested close similarities between the major group II protein in lymph VLDL and that in lymph and plasma HDL. Moreover, they indicate that the major group II apoprotein in plasma VLDL, which comes predominantly from the liver, is not identical with that found in lymph VLDL and plasma and lymph HDL (33). The gels in Fig. 1 also provide evidence for this difference; the major group II apoprotein of lymph VLDL migrated more rapidly than the major group II apoprotein of liver perfusate VLDL. Rather, it migrated like the major group II HDL protein (Fig. 2).

Quite unexpected was the finding that the gut incorporated little or no radioactive lysine into the major group III peptides of lymph or perfusate lipoproteins. These peptides constitute about 35% of normal lymph VLDL protein, similar to their contribution to plasma VLDL protein (Table 3), and they contain about 6 mole % lysine (see Table 3). Several unlikely possibilities might be considered to explain this finding: (*a*) Possibly the gut did synthesize the group III peptides, but the pool of these peptides in the gut cells is very large so that newly synthesized peptides become greatly diluted before being released into the lymph. This cannot be ruled out at present. However, it may be noted that Mahley et al. (32), in their examination of intestinal Golgi vesicles, found evidence for the presence of β-apoprotein, some zone II proteins, but no proteins that migrated in the zone III region of polyacrylamide gels. (*b*) Possibly, these peptides were lost during delipidation and dialysis of the VLDL. If so, one would have to postulate preferential loss of the newly synthesized peptides since it is clear from Figs. 1 and 2 and Table 3 that these procedures do not result in a general loss of the group III apoproteins. Furthermore, newly synthesized peptides were not lost when liver perfusate VLDL was carried through the same analytical procedures. It therefore seems most likely that under the present perfusion conditions the gut released nascent lipoprotein particles that contained newly synthesized group I and group II apoproteins only. There is no reason to believe that this could not also occur in vivo. All plasma proteins, including lipoproteins, are continuously being filtered from the circulation into tissue spaces, from which they gain entrance to the lymph (34). As these lipoproteins, particularly the HDL, pass

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through the intestinal lymphatics, they may give up some of their group III peptides to nascent lipoproteins entering the lymph from the intestinal mucosal cells. Equilibration of group III peptides between HDL and VLDL in rat plasma has been previously demonstrated (22). Furthermore, evidence has been obtained for a net movement of these peptides from HDL to synthetic fat emulsions in vitro, and from HDL to d < 1.006 lipoproteins in vivo in man during alimentary lipemia (35, 36). As VLDL triglyceride is cleared from plasma, these peptides are found to shift back to HDL (37, 38).

Under the conditions of the present experiments, the gut lumen was infused with only a small amount of lipid, an amount equivalent to the normal flow of bile lipid into the gut in vivo. Thus, few chylomicrons were produced. It should be pointed out that the relationship of intestinal VLDL particles to chylomicrons remains unclear. There is some evidence to suggest that VLDL and chylomicrons are produced in intestine by a process that can yield a continuous spectrum of particles differing in size and triglyceride content (16, 17, 32, 39).

Heparin was routinely present in the gut perfusate. The initial 25–40 ml of perfusing mixture that was passed through the preparation, however, was discarded and was not recycled, and it is likely that this contained the bulk of the lipoprotein lipase released by the perfused tissue. Absence of significant lipolytic activity in the recycled perfusate is indicated by the recovery of nearly 90% of the perfusate triglyceride after 5 hr of perfusion (7). Lymph samples collected from the perfused gut have been stored for several days without evidence of lipolysis.

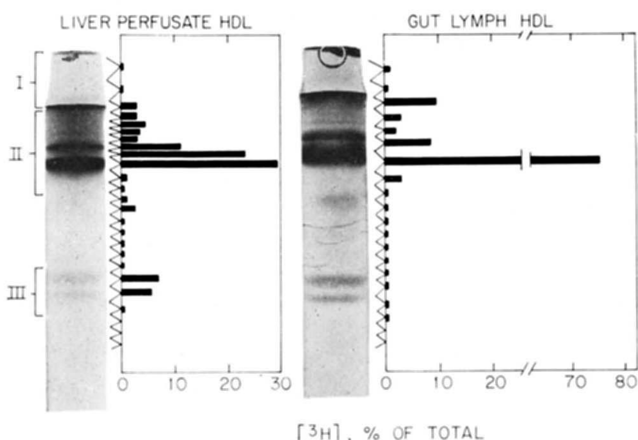

Fig. 2. Incorporation of [³H]lysine into HDL apoproteins by isolated liver and intestine. Data are from the same experiments as in Fig. 1 and Table 1.

Lipoprotein apoprotein synthesis by liver

Liver perfusion was undertaken as part of this study primarily as a control for the experiments with intestine, since it was anticipated that the liver could synthesize all or most of the lipoprotein apoproteins (11, 12). Figs. 1 and 2 show that the perfused livers did, indeed, incorporate [³H]lysine into all the distinguishable polyacrylamide apoprotein bands of both VLDL and HDL. Again, there is no assurance that newly synthesized apoproteins entered the perfusate as part of the same lipoprotein complex from which they were later recovered. The results do, however, show that the liver can syn-

TABLE 3. Protein distribution and [³H]lysine recovery in groups of VLDL apoproteins

Apoprotein Group[a]	Protein Distribution[b] in VLDL Isolated from: Plasma[c]	Mesenteric Lymph[d]	Relative Amount of [³H]Lysine in VLDL Apoproteins[e] From Isolated Liver	From Isolated Intestine
	% of total protein		*% of total ³H*	
I	30	35	67 (62–72)	28 (22–34)
II	25	30	23 (16–30)	71 (69–74)
III	45	35	10 (8–12)	<1
(Total)	(100)	(100)	(100)	(100)

[a] See Experimental section.
[b] Determined from the distribution of protein among peaks I, II, and III after chromatography of apoproteins on columns of Sephadex G-150 (4). Values are from a single analysis of a pooled sample collected from 10 (lymph) or 200 (plasma) rats.
[c] From adult male rats (Sprague-Dawley) fed Purina chow.
[d] Collected from rats on a fat-free diet (see Experimental section).
[e] Calculated from the [³H]lysine incorporation shown in Fig. 1, corrected to reflect the differences in lysine content for each apoprotein group. The lysine content, determined after acid hydrolysis of the Sephadex fractions above, was as follows (mole %): groups I, II, and III of plasma VLDL, 7.5, 5.0, and 6.0, respectively; groups I, II, and III of lymph VLDL, 7.5, 7.0, and 6.0, respectively. Values in the table are the means and ranges for two liver and three gut perfusion experiments.

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the size all the main constituent apoproteins that can be separated from VLDL and HDL by the procedures used.

The relative extent of incorporation of radioactivity into the three apoprotein groups of VLDL by the liver is summarized in Table 3. Data for intestine is included for comparison. The relative pool sizes for the various apoproteins in liver and gut are unknown, nor is it known whether some newly synthesized apoproteins may have been catabolized upon recycling through the liver. This limits any attempt to strictly interpret the relative incorporations as the relative amounts of the various groups of apoproteins that were synthesized and secreted under the present conditions. However, with these limitations in mind, the data do suggest that liver and gut did not produce the same proportion of group I to group II VLDL apoproteins. Also, the livers released newly synthesized group III peptides and the perfused intestine did not. It therefore appears that chylomicrons and VLDL entering the circulation from the gut must acquire group III peptides synthesized in the liver, and possibly other tissues, before they are further metabolized, since one of the major group III peptides in the rat,[3] as in man (40–42), functions as an activator of the triglyceride-clearing lipoprotein lipase. Finally, these data suggest that these perfused livers did not release newly synthesized VLDL apoproteins into the perfusate in the same proportions as found in VLDL isolated from plasma. This is not surprising, since plasma VLDL is a heterogeneous mixture of particles, composed of gut-derived lipoproteins and liver-derived lipoproteins that have undergone changes in protein content by acquisition from the plasma and by the differential loss of apoproteins (37) as metabolism of VLDL proceeds. Furthermore, the relative amounts of apoproteins produced may be influenced by the physiological state of the animal.

The hepatic perfusate contained heparin in one experiment of the present series, but the initial 85 ml of perfusate flushed through the tissue was discarded. The other experiment, in which defibrinated blood and no heparin was used, gave results similar to those of the first, so it is unlikely that the presence of lipase in the perfusate influenced the results significantly.

The present results demonstrate the usefulness of perfused organ preparations in elucidating aspects of lipoprotein synthesis and metabolism. Their utility can be expected to increase as improved methods for isolation and purification of small quantities of apoproteins become available.

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[3] Bersot, T. P., and R. I. Levy. Unpublished results.

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