Distribution of apolipoproteins A-I and B among intestinal lipoproteins

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Abstract Chylomicrons and very low density lipoproteins (VLDL) are produced by the intestine and these nascent particles are thought to be similar to their counterparts in intestinal lymph. To study the relationship between these lipoproteins within the cell and those secreted into the lamina propria and lymph, we have isolated enterocytes, lamina propria, and mesenteric lymph from rats while fasted and after corn oil feeding. Apolipoprotein A-I and B content were measured by radioimmunoassay in cell, lamina propria, and lymph fractions separated by Sepharose 6B and 10% agarose chromatography, and by KBr isopycnic density centrifugation. ApoA-I in the cell and the underlying lamina propria was found partly in those fractions in which chylomicron and very low density lipoproteins (chylo-VLDL) and high density lipoproteins (HDL) elute, but more abundantly where unassociated ^{125}I-labeled apoA-I was eluted. In the lymph, however, 74% of apoA-I eluted in the HDL region and no peak of free apoA-I was found. ApoB and apoC-III within the enterocyte were found distributed in the position of particles eluting not only with chylomicrons and VLDL, but also in the regions corresponding to LDL and HDL. In the lamina propria and lymph, on the other hand, most of the apoB was found in the region of VLDL and chylomicrons. These results indicate that the patterns in lymph lipoproteins and the lamina propria do not exactly mirror the distribution of apoA-I and B among lipoproteins inside the cell. This may be because intracellular apoproteins may be unassociated with lipoproteins, or they could be associated with lipoproteins in various stages of assembly of protein with lipids. Furthermore, the apoprotein composition of intestinal lipoproteins is altered after secretion from the enterocyte. Finally, not all apoproteins seem to be secreted in association with identifiable lipoprotein particles from the enterocyte.—**Alpers, D. H., D. R. Lock, N. Lancaster, K. Poksay, and G. Schonfeld.** Distribution of apolipoproteins A-I and B among intestinal lipoproteins. *J. Lipid Res.* 1985. **26:** 1–10.

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The enterocyte is a significant source of plasma VLDL in the fasting rat (1–4) and of chylomicrons and VLDL after fat feeding (5, 6). In addition, a discoid nascent HDL particle presumably originating from the intestine has been found in mesenteric lymph (7, 8). Each of the lipoproteins isolated from intestinal lymph contains a characteristic complement of apoproteins, several of which are synthesized in enterocytes. For example, the production of apoA-I in rat intestinal cells has been demonstrated by direct immunofluorescence (9) and immune peroxidase techniques (10) and by synthesis in cell-free translation systems using intestinal mRNA (11). ApoB also has been demonstrated in the enterocyte by immunofluorescence (12, 13) and by synthetic studies (14).

Some proportion of apoproteins present in cells must be associated with intracellular particles inasmuch as nascent VLDL has been identified in and isolated from the Golgi apparatus of intestinal cells. Although no apoprotein characterizations were performed on the intestinal fractions (15), hepatic Golgi were found to contain VLDL which possessed some (16, 17) or all (18) of the apoproteins found in plasma VLDL. Presumably intestinal nascent chylomicrons and VLDL also contain apoproteins. However, it is not clear what proportion of intracellular apoproteins is associated with identifiable lipoprotein particles or whether this proportion is altered during fat absorption. The techniques available to isolate enterocytes and to determine quantitatively the intracellular concentration of apoproteins now make it possible to study the intracellular distribution of apolipoproteins and to assess any changes due to fat feeding.

Another unanswered question relates to the sites where association of some apoproteins with lipoproteins may occur. In the case of hepatic-derived lipoproteins, a comparison of the apoprotein compositions of

Abbreviations: apoA-I, apolipoprotein A-I; apoB, apolipoprotein B; apoC-III, apolipoprotein C-III; VLDL, very low density lipoprotein; HDL, high density lipoprotein; BSA, bovine serum albumin; LDL, low density lipoprotein; FFA, free fatty acids; CE, cholesteryl ester; PL, phospholipid.

VLDL isolated from hepatic Golgi apparatus and plasma suggests that some apoproteins (e.g., C-apoproteins) may be acquired after secretion of the particles from the cell (17, 18). In addition, newly secreted intestinal and hepatic high density lipoproteins (HDL) have a significantly different morphology and chemical composition from plasma HDL (8, 19). A comparison of apoprotein distribution inside the cell and intestinal lymph in the fasted and fed states makes it possible to draw some conclusions about lipoprotein assembly in the intestinal mucosa and to assess whether some apoproteins may be secreted relatively free of lipids.

METHODS

In vivo studies

Sprague-Dawley rats (150–180 g) obtained from Chappel Breeders (St. Louis, MO) were fasted overnight. They were fed by gastric tube either 1.5 ml of corn oil or normal saline. In some experiments 100 μCi of [^{3}H]palmitate (16.8 Ci/mmol, New England Nuclear Corp., Boston, MA) or 50 μCi of [^{3}H]cholesterol (54.8 Ci/mmol, New England Nuclear Corp.) were added to the corn oil and the mixture was sonicated. At 3 hr after feeding animals were decapitated and exsanguinated, and enterocytes were isolated from proximal jejunum as described previously (10, 20). Benzamidine (1 mM) and phenylmethylsulfonyl fluoride (0.1 mM) were included in the buffers. After removal of cells with the EDTA treatment for 60 min, no mucosal cells were seen on the underlying lamina propria by light microscopy (H & E stain), and no activity of a mature enterocyte marker (sucrase) was detected. This residual tissue in rat consists largely of lamina propria, and small amounts of muscularis mucosa, submucosa, and muscularis propria, and is referred to as lamina propria. Lipoproteins were obtained from both cells and lamina propria (see below).

In some animals the major mesenteric lymphatic vessel was cannulated (21) and the animals were allowed to recover from anesthesia. After gastric intubation of 1 ml of corn oil, lymph was collected at hourly intervals for up to 3 hr.

Tissue preparation

The isolated cells and lamina propria from jejunum were homogenized in 50 mM barbital buffer, pH 8.5, using three strokes with a Potter-Elvejhem homogenizer, followed by centrifugation at 100,000 *g* for 60 min. Total apoprotein content of the homogenate was determined by assaying the supernatant and pellet fraction individually. Aliquots of the supernatant fraction were removed for quantitation of apoprotein content by specific radioimmunoassays (10, 22, 23). The pellet was resuspended in buffer containing 1% Triton X-100 (Fisher Scientific, St. Louis, MO), rehomogenized and also assayed for apoprotein content. From six separate isolations, the mean percent of apoA-I in the supernatant fraction of jejunal cells was 68% of the content of total homogenate. The corresponding value for apoB was 45%. Recovery of apoA-I in the supernatant fraction of jejunal lamina propria was 78%, and 62% for apoB. Similar distributions were found following homogenization using a loose-fitting Dounce homogenizer. The number of strokes with either homogenizer (3 to 10) did not affect the partition of apoproteins between the soluble and particulate fraction or their elution positions and distribution on Sepharose columns. After fat feeding a lipid layer formed on top of the supernatant fraction. This was removed by careful aspiration in order to avoid altering the flow rate of the Sepharose column. This lipid layer was sonicated in barbital buffer containing 1% Triton X-100 and assayed for apoprotein content. It contained only 5% of the total apoB content, and none of the apoA-I. Less than 1% of fed fat was recovered in this lipid layer from jejunum.

To investigate the possible contamination of enterocyte or lamina propria preparation with serum, we measured the content of rat albumin in the supernatant fractions derived from these tissues by a rocket immunoassay using antiserum to rat albumin (24). In the enterocyte preparation taken from fat-fed rats, albumin levels measured 2.5 ng/ml, or a contamination of 0.0063% from serum containing 4 g/dl. If rat apoA-I levels in serum average 48 mg/dl, this degree of contamination would provide 3 ng of apoA-I per ml of enterocyte homogenate. Since the average apoA-I content of intestinal cells was 100 ng/ml (20), serum could only account for about 3% of the apoA-I measured in the cells. The degree of serum contamination was somewhat greater in the lamina propria fractions; but still accounted for only about 6.5% of the 500–600 ng/ml of apoA-I detected. The cells were also assayed for contamination of the intraluminal enzymes. Trypsin and lipase were assayed using *p*-toluene sulfonyl L-arginine methyl ester (TAME) and triolein emulsions (Dupont), respectively, as substrates. Hydrolysis of TAME was followed at 247 mμ, and lipase activity was followed by turbidimetric analysis in the autoanalyzer. No more than 0.2 units of trypsin and 0.05 units/ml of pancreatic lipase were detected in any final cell homogenate. When the assays were repeated in the presence of EDTA containing column buffer, no activity of either enzyme was detectable. Cathepsin activity was assayed at pH 7.4 using hemoglobin as substrate. No activity was detected.

Lipoprotein isolation

About 2 ml of each supernatant fraction from isolated enterocytes and lamina propria and mesenteric lymph were separated by molecular sieving chromatography on a 92 × 1 cm Sepharose 6B column (Pharmacia, Uppsala, Sweden), and a 50 × 0.8 cm Bio-Gel A-0.5 column (Bio-Rad, Richmond, CA) which were equilibrated with 0.15 M saline, containing 1 mM EDTA, pH 8.2, in the presence of 1 mM benzamidine and 0.3 mM phenylmethylsulfonyl fluoride (25). The same buffer was used to elute the columns at a flow rate of 4–5 ml/hr. Supernatant fractions were used either within 24 hr after preparation, or after remaining at 4°C for 1 or 2 weeks. The enterocyte columns were run at 4°C, while the lamina propria and lymph columns were eluted at 25°C, in order to prevent aggregation of the lipoprotein particles. Elution of enterocyte homogenates at 25°C gave results similar to those obtained at 4°C. In eight separate experiments the mean recovery of apoA-I from the column was 101%; recovery of apoB was 104%. Thus, no significant degradation of apoprotein occurred during chromatography. In addition, when fractions eluting in the VLDL and LDL positions were pooled and run again on the column, they appeared at their respective original elution volumes. Aliquots of column fractions were assayed either for apoprotein content by radioimmunoassays as described above, or analyzed for radioactivity by addition to 3a70 solution (RPI Corp., Mt. Prospect, IL) and counted in a Packard Tri Carb Spectrometer, model 3320. When [^{3}H]palmitate was used to label the cellular lipoproteins, the elution fractions from the Sepharose 6B column were lipid-extracted (26) and separated by thin-layer chromatography using petroleum ether–diethyl ether–acetic acid 84:15:1 (v/v/v) (27). The columns were standardized with lipoprotein particles obtained from human plasma by density ultracentrifugation (28). Elution patterns of the latter lipoproteins were detected by analyzing the fractions for absorbance at 260 nm. Bovine serum albumin was used for the albumin marker.

In a few experiments the enterocyte and lamina propria supernatant fractions were applied to a potassium bromide density gradient from d 1.005 to 1.21 g/ml and centrifuged 22 hr (or for isopycnic runs, 60 hr) at 15°C in an SW-40 rotor (29). Fractions were collected by puncturing the bottoms of the tubes, and their densities were measured (Mettler-Paar densitometer, model DMA-45, Gratz, Austria). Fractions were then dialyzed against 1 mM EDTA, 0.15 saline, and analyzed for apoprotein content as described above. Alternatively, ^{3}H-labeled lipids were extracted, separated by thin-layer chromatography, and counted. The flotation position of lipoproteins was determined by using human plasma and subjecting the resulting fractions to agarose electrophoresis (30). Flotation positions of lipoproteins were highly reproducible; i.e., LDL and HDL peaked at fractions 7.6 ± 0.5 and 4.1 ± 0.3, respectively (mean ± SD, n = 12 samples, see Figs. 2 and 6 for volumes of fractions).

RESULTS

Distribution of labeled lipids and demonstration of the presence of lipoproteins in homogenates

Fasted rats were given radiolabeled palmitate or cholesterol in corn oil. Two hours later they were killed and enterocytes were isolated and homogenized. **Fig. 1** demonstrates the elution patterns of radiolabeled palmitate and cholesterol in 100,000 *g* supernates of jejunal cell homogenates. The majority (86%) of the labeled lipids were distributed in the regions of chylomicron-VLDL (24–30 ml) and HDL-albumin (42–52 ml), although some eluted in the area of LDL (36 ml). The VLDL and HDL regions were pooled separately and the lipid extracts of the pools were separated by thin-layer chromatography. In the VLDL region, 74% of palmitate counts recovered were in triglycerides, 7% in PL, 7% in CE, and 12% in FFA, whereas in the HDL-albumin regions only 12% were esterified. When the chromatographic peak for VLDL (elution volume 20–30 ml) obtained from a fasted rat given [^{3}H]palmitate was subjected to density gradient ultracentrifugation (**Fig. 2**), 16% of the centrifuged [^{3}H]palmitate counts sedimented to the bottom of the tube; 85% of

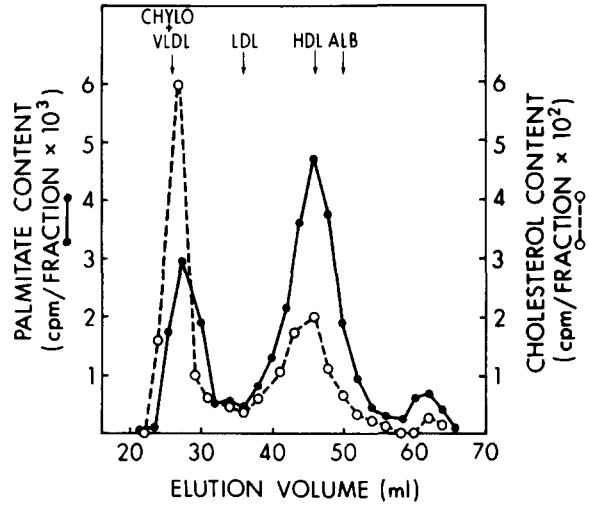

Fig. 1 Distribution on Sepharose 6B of absorbed cholesterol and fatty acids in jejunal enterocytes. Isolated jejunal cells were harvested 2 hr after corn oil feeding which contained either 50 μCi of [^{14}C]palmitate or 50 μCi of [^{3}H]cholesterol. Sepharose 6B columns were eluted at a flow rate of 4–5 ml/hr with 50 mM barbital buffer, pH 8.5. Aliquots of the elution fraction were counted for ^{14}C or ^{3}H. No difference in quenching was detected among column fractions.

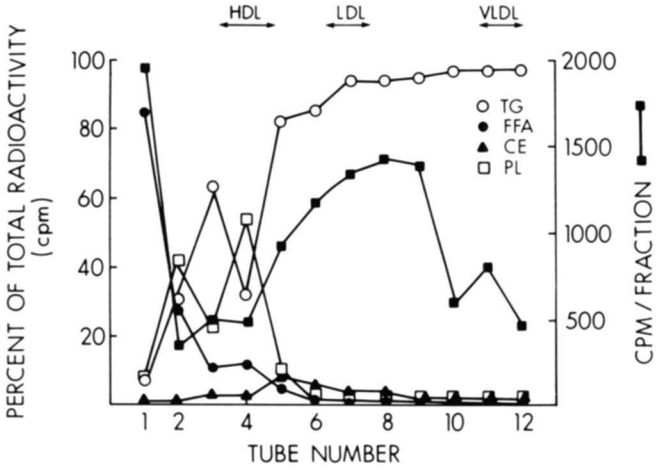

Fig. 2 Density gradient ultracentrifugation of the "chylomicron-VLDL" fraction (volume 20–30 ml from Fig. 1) of enterocyte homogenates. A 16-hr fasted rat was given [^{3}H]palmitate (100 μCi) in corn oil. Two hours later enterocytes were isolated, homogenized, and filtered on a Sepharose 6B column. The material eluting in the void volume was subjected to density gradient ultracentrifugation. Fractions were collected and their lipids were extracted and separated by thin-layer chromatography. The distribution of the [^{3}H]palmitate in major lipid classes is shown as a percentage of the total [^{3}H]palmitate in the density fraction.

these sedimented counts were in FFA and the rest were in esterified form. Seventy percent of the centrifuged counts floated in two broad peaks corresponding to LDL and VLDL. Less than 5% of this lipid was in the form of FFA, 90% was in triglycerides and the rest in other esterified fractions. These chylomicron-VLDL chromatographic peaks were also examined by electron microscopy (**Fig. 3**). A wide range of lipoprotein particle sizes was present (20–70 nm). Many of the particles contained membrane tabs. These intestinal lipoproteins resembled the particles isolated from rat liver Golgi apparatus in size, distribution, and appearance (18). In the material eluting in the HDL-albumin region, over 80% of the counts were in free fatty acids. Very similar results were found on chromatography and ultracentrifugation using extracts of lamina propria (not shown). It appears that the VLDL region of the column elution contains largely lipoprotein particles. However, the volume eluting at about 46 ml might contain not only HDL particles (Fig. 1), but also fatty acid-binding proteins that would bind free fatty acids (31).

Apoprotein distribution in jejunal enterocytes

The distribution of apolipoprotein on Sepharose 6B columns in the nonmembranous fractions of jejunal enterocytes is shown in **Fig. 4.** In the fasted state (Fig. 4A) about 10% of the intracellular apoA-I was eluted in the region of VLDL particles, whereas about 80% was found in fractions eluting after bovine serum albumin. A large peak of apoB was found in the VLDL region as expected. However, a larger peak of immunoreactivity was also found in the region corresponding to LDL, with some extension even into the HDL region. The peak of apoC-III was found in the VLDL region while lesser amounts of this protein were found in other fractions, including the LDL region.

Fig. 4B shows the results of a similar experiment, using a jejunal homogenate from a fasted rat after 1 week of storage at 4°C, instead of within the first 24 hr as in Fig. 4A. One major difference was apparent. Now virtually no apoA-I and much less apoB were found in the VLDL region. These changes were noted despite the fact that recovery of apoA-I and B from both columns was very similar, and proteolytic and lipolytic activity was very low (see Methods).

Acute fat feeding produced a noticable shift in the distribution of both apoA-I and apoB in freshly prepared extracts (Fig. 4C). ApoC-III was not examined

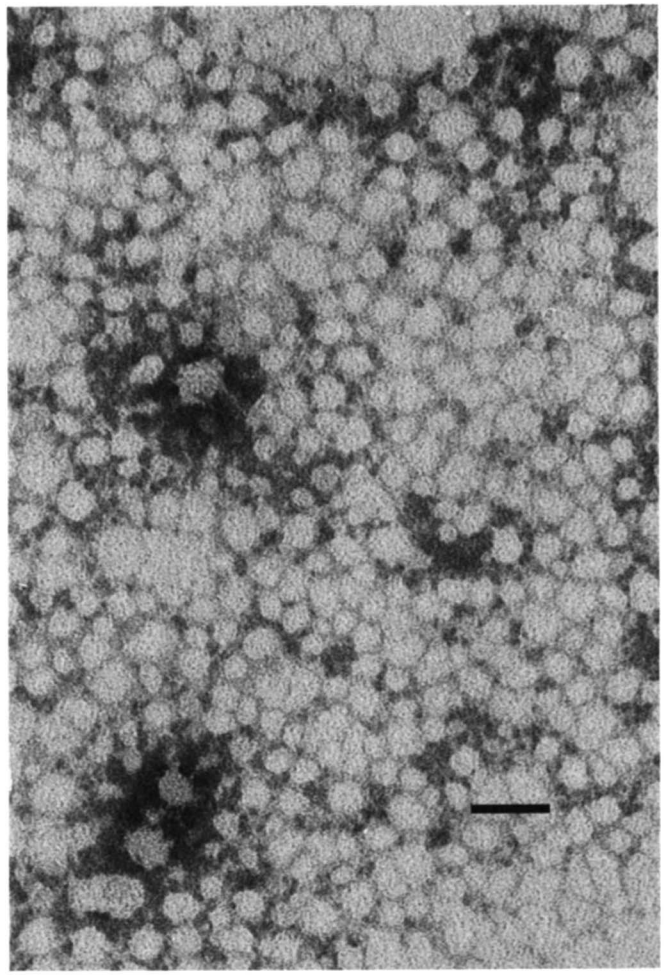

Fig. 3 Electron micrograph of the "chylomicron-VLDL" fractions of enterocyte homogenates. Animals and tissues were processed as in Fig. 1 and in the Methods section. The void volume materials of the Sepharose 6B column were examined on grids using 2% phosphotungstic acid, pH 7.2. Particle diameters ranged from 20 to 70 nm. The bar represents 1,000 A°.

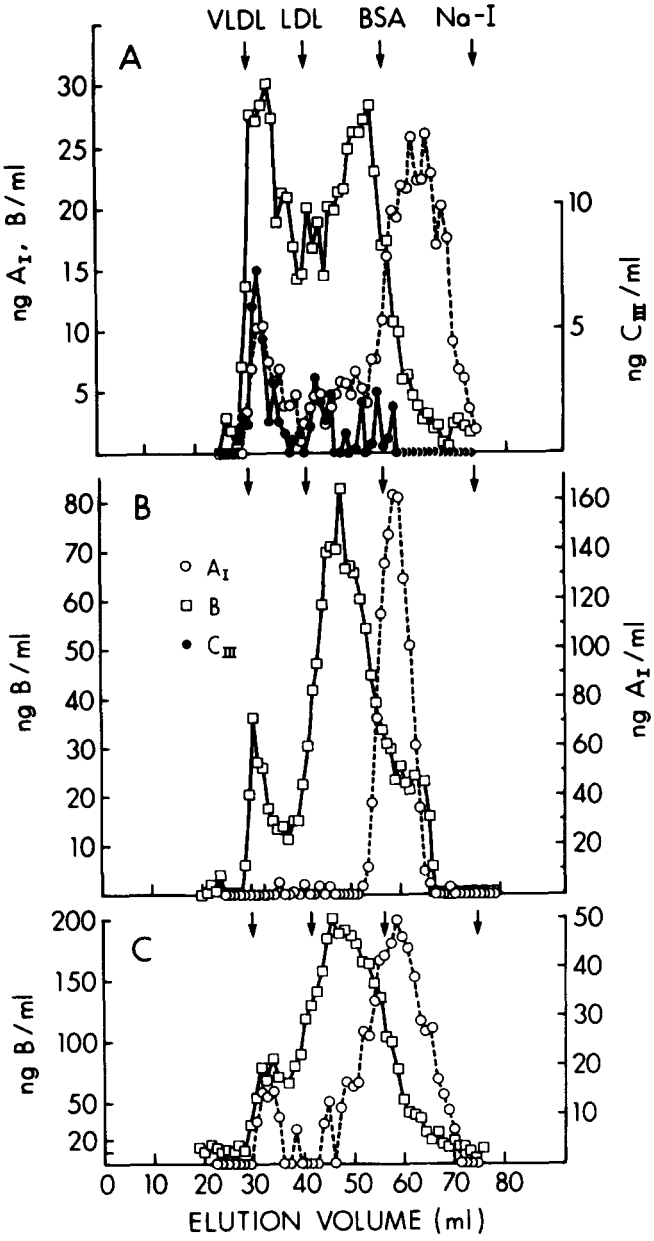

Fig. 4 Distribution on Sepharose 6B columns of apolipoproteins in jejunal enterocytes. Two ml of supernatant fractions was applied to the columns. Columns were developed as outlined in Methods and Fig. 1. Aliquots of fractions were assayed for apoA-I, B, and C-III content so that the amount detected ranged from 1 to 8 ng. The results shown are the mean of two separate determinations. Four animals were used for each experiment. A. Supernatant from fasting animals used within 12 hr of preparation. B. Supernatant from fasting animals used after 1 week storage at 4°C. C. Supernatant from animals 3 hr after fat feeding, used within 12 hr of preparation.

because of its limited distribution in the fasted state (Fig. 4A). Three hours after fat feeding the absolute amounts of apoA-I and B were greater in all peaks compared with the fasted profile (Fig. 4A). The major apoA-I peak had shifted from the region after BSA (60–70 ml) to overlap into the region of HDL (48–50 ml), although the peak of immunoreactivity still eluted in the position of BSA (58–60 ml). A small peak of apoA-I was still noted in the VLDL region, although its relative contribution had decreased. The peaks of apoB immunoreactivity now eluted in both the VLDL and LDL regions, although the separation of these two peaks was indistinct.

The relative concentrations of apoB to apoA-I in these column fractions are shown in **Table 1.** In the enterocytes from fasted rats, the ratio of apoB/apoA-I was fairly constant in the region of lipoproteins. With fat feeding, however, ratios more characteristic of plasma lipoproteins were achieved, with LDL very rich in apoB, and HDL in apoA-I.

Presumably a great portion of the apoA-I was not associated with discrete lipoprotein particles, inasmuch as the fraction in the elution volume beyond 50 ml contained little [^{3}H]palmitate (Fig. 1). However, the ability of Sepharose 6B columns to separate small particles is limited. For this reason, the experiments in Fig. 4 were repeated using 10% agarose columns to separate the intracellular particles (**Fig. 5**). In these columns VLDL and LDL eluted together at the void volume, but HDL, BSA, delipidated apoA-I, and free ^{125}I were easily distinguished.

The profiles elaborated on 10% agarose columns confirmed the results of the Sepharose 6B chromatography. When supernatant fractions from fresh jejunal enterocytes from fasted rats were used (Fig. 5A), the major portion (about 70%) of apoA-I immunoreactivity was eluted at the position of delipidated apoA-I (46–48 ml). The distribution of apoB, on the other hand, while largely in the excluded volume (VLDL and LDL), extended well into the eluted position of HDL (34–35 ml). ApoC-III was confined to the excluded volume, consistent with the results on Sepharose 6B.

The elution patterns using supernatant fractions from enterocytes from fed rats (Fig. 5B) also confirmed the Sepharose 6B patterns. ApoA-I immunoreactivity shifted somewhat in that peaks were seen corresponding to the elution volume of both BSA (42–44 ml) and delipidated apoA-I, and there was a hump extending

TABLE 1. Relative apoprotein concentrations in enterocyte lipoproteins

	Apo B/Apo A-I	
Particle	Fasted	Fed
Chylo-VLDL	3.0	6.5
LDL	3.6	16.6
HDL	3.4	0.61
A-I	0.08	0.1

The peak values for apoB and apo A-I concentration within the eluted volume for each lipoprotein particle were used. The Sepharose 6B column was used for chylo-VLDL and LDL, and the 10% agarose column was used for HDL and apoA-I.

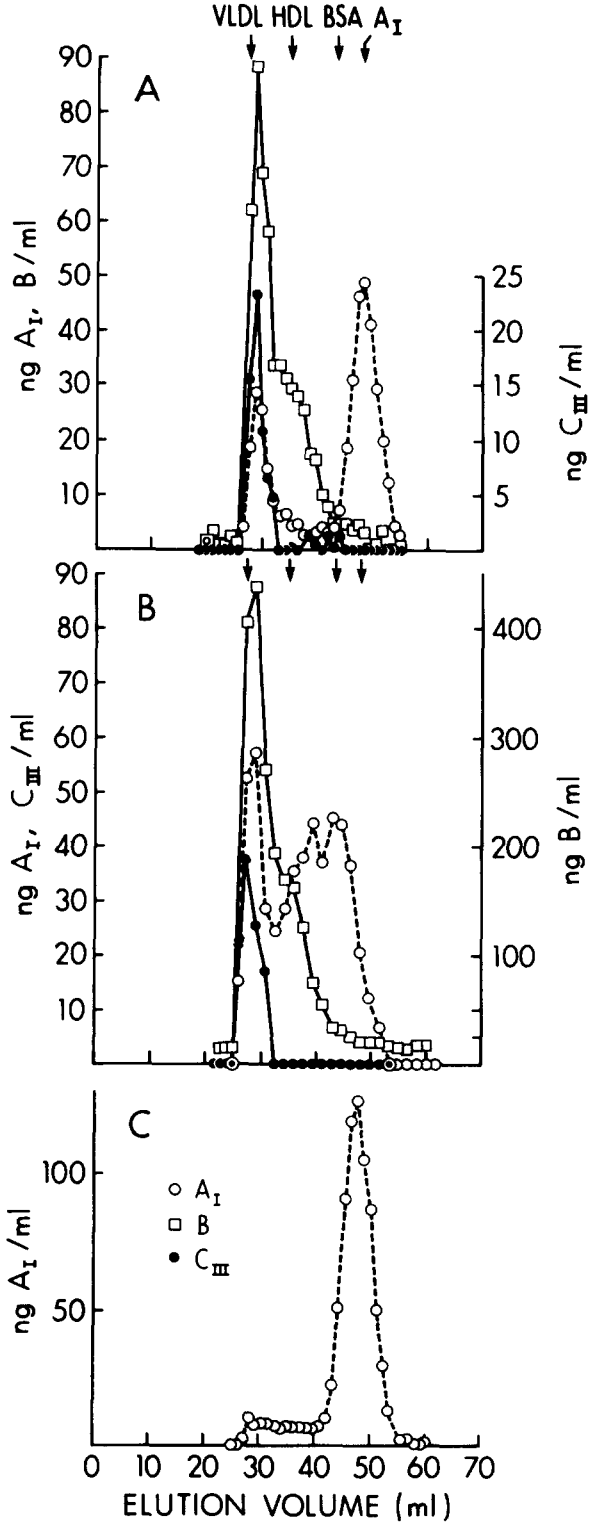

Fig. 5 Distribution on 10% agarose columns of apolipoproteins in jejunal enterocytes. Columns were developed and fractions were assayed as outlined in Fig. 4. A. Supernatant from fasting animals used within 12 hr of preparation. B. Supernatant from animals 3 hr after fat feeding used within 12 hr of preparation. C. Supernatant from animals 3 hr after fat feedings, used after 2 weeks storage at 4°C.

into the HDL region. The apoB and C-III patterns were unchanged. It would be expected that no change in apoB was noted, since only the distribution between VLDL and LDL was altered on Sepharose 6B. The loss of the 20–25% of apoA-I eluting with lipoprotein particles after prolonged incubation was confirmed (Fig. 5C). Supernatant fractions from enterocytes from fed rats were used in this experiment. Very little apoA-I was eluted in the position of either VLDL, LDL, or HDL.

The distribution of apolipoproteins in enterocyte supernatant fractions from fasted rats was assessed by a third technique, that of isopycnic density ultracentrifugation in KBr (**Fig. 6**). The results confirmed the observations obtained by column chromatography (Figs. 4 and 5). The bulk of apoA-I immunoreactivity banded at a density of 1.18 g/ml; apoB activity was widely distributed with a broad peak from density 1.06 to 1.18 g/ml. A small peak of both apoB and C-III remained on top of the gradient, presumably in very lipid-rich particles. Rat plasma HDL banded at density 1.12 g/ml. Once again apoA-I was found to be separate from and less lipid-rich than HDL. ApoB distribution crossed all lipoprotein positions, and was even found at densities greater than that associated with lipid-containing particles ($d > 1.20$ g/ml). ApoC-III, however, was only found at the density expected of lipid-rich particles.

Apoprotein distribution in lamina propria

The supernatant fraction derived fresh from the lamina propria after removal of enterocytes (see Methods) was examined by Sepharose 6B chromatography (**Fig. 7**). The fasting lamina propria contained small peaks of apoA-I and apoB which eluted in the regions of HDL and VLDL particles, respectively (Fig. 7A). Three hours after fat feeding the distribution of apoB in the lamina propria was unchanged, but the VLDL peak was more pronounced. A small peak of apoA-I was now apparent in the VLDL region. Moreover, the peaks were sharper and contained a larger proportion of total immunoreactivity than was the case in the HDL and VLDL regions of the corresponding enterocyte fractions (Figs. 4C and 5B).

Apoprotein distribution in mesenteric lymph

The distribution of apoA-I and apoB in lymph from fasting rats showed the majority of apoA-I in the HDL region with 26% eluting in the chylomicron-VLDL region (**Fig. 8A**). In contrast, nearly all of apoB appeared in the chylomicron-VLDL region or trailing behind (24–34 ml). The patterns in lymph collected 1 hr after fat feeding were similar (Fig. 8B). Treatment of the lymph with six strokes of a Dounce homogenizer

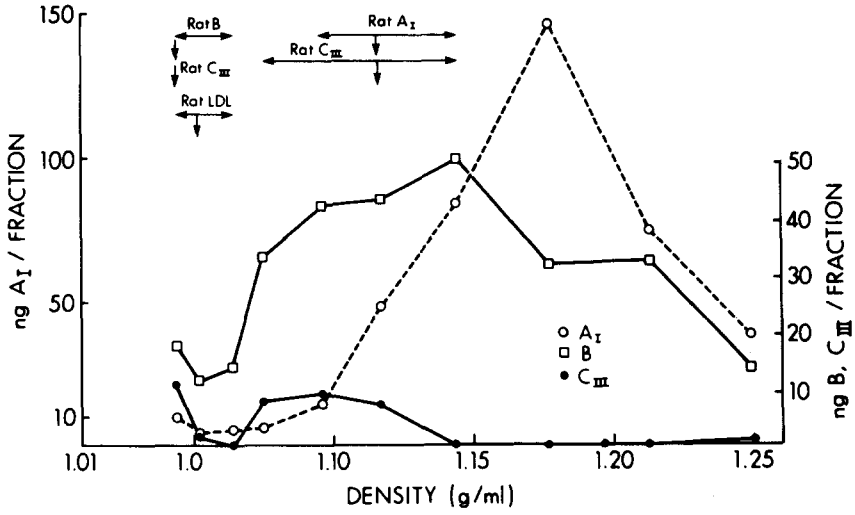

Fig. 6 Distribution on isopycnic centrifugation of apolipoproteins in jejunal enterocytes. One ml of supernatant fraction from fasted animals was applied to the gradient and ultracentrifugation was for 60 hr at 15°C in an SW 40 rotor. The supernatant was used within 12 hr of prepraration. Aliquots of extensively dialyzed fractions were assayed as described in Methods and Fig. 4. The rat apoprotein distribution indicated by the arrow corresponds to that found using rat plasma as the marker solution. Their distribution is demonstrated by the horizontal arrows, with the peak for each apolipoprotein or lipoprotein particle indicated by the vertical arrow.

(as used for tissue preparation) did not affect the distribution of apoproteins.

DISCUSSION

The data demonstrate that the distributions of apoA-I and apoB differ from each other in the enterocyte, and differ from those in the lamina propria and the mesenteric lymph. Whereas nearly all of intracellular apoB appeared to be associated either with triglyceride-rich lipoproteins or smaller LDL-sized lipoprotein or membrane particles, most of apoA-I in the cell was unassociated with any recognizable lipoproteins. Only a small minority of apoA-I eluted with VLDL-sized particles. In contrast to intracellular particles, the apoB in lamina propria and lymph was found mostly in very low density particles, while 75% of lamina propria apoA-I appeared to elute between albumin and HDL. Only the lymph showed apoA-I levels eluting with lipid-containing particles. Thus it appears that the larger apoB-containing particles are selectively secreted, while particles below a certain size tend to remain in the cell. It is likely that the smaller particles are partially assembled "nascent" precursors of the larger lipoproteins, and the latter represent a large proportion of the intracellular pool of apoB. Following a fat meal, the apoB content of the larger apoB particles rises. Increases of the smaller nascent apoB particles are even greater, suggesting that some step in assembly may be rate-limiting. The distribution of apoA-I is quite different. ApoA-I seems to be associated either with large particles or it remains at least partial-

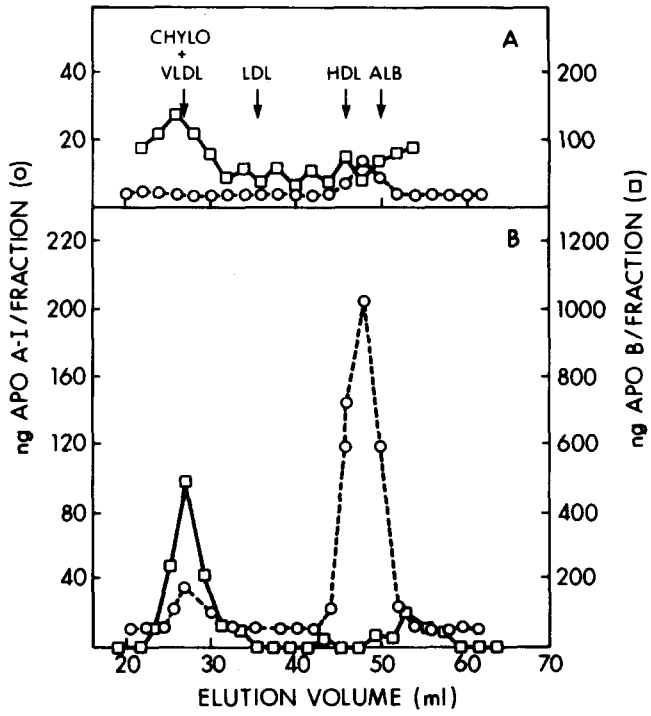

Fig. 7 Distribution on Sepharose 6B columns of apolipoprotein in lamina propria derived from jejunum. Columns were developed and aliquots were assayed as described in Fig. 4. Lamina propria supernatant fractions were used within 12 hr of preparation and were derived from fasting animals (A) or from animals 3 hr after fat feeding (B).

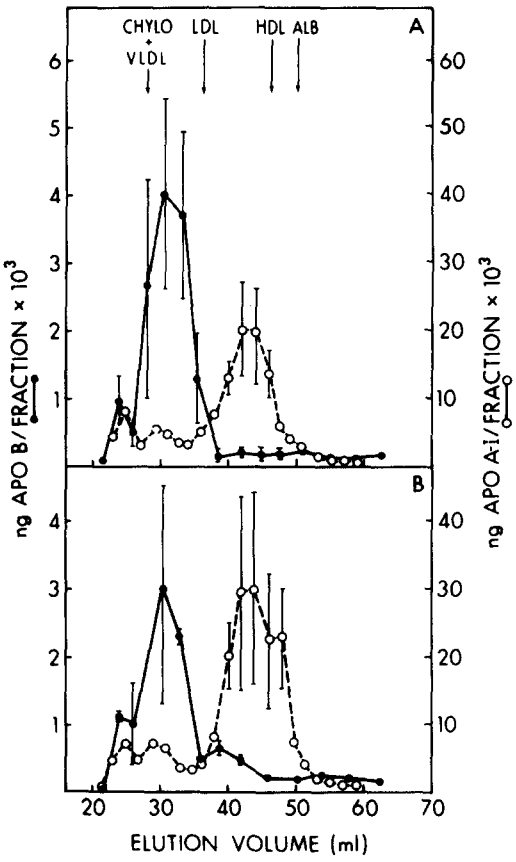

Fig. 8 Distribution on Sepharose 6B of apoA-I and apoB in mesenteric lymph. Assays were performed and data are displayed as in Fig. 4. Three separate lymph samples collected for an hour after fat feedings were fractionated and analyzed.

ly unassociated. Very little is found with LDL-sized particles. Fat feeding increases the apoA-I in both pools. Since the apoA-I in lamina propria is also distributed among two pools, similar to its intracellular distribution, it is likely that apoA-I secretion occurs from both pools.

The patterns of apoprotein distribution seen in enterocytes probably are reasonably accurate. We were not able to study the total apoproteins present in the enterocyte, as we could not use detergents to solubilize any membrane-bound apoproteins, a process which also may have disrupted lipoprotein structure. However, over 50% of the cell contents (including identifiable lipoprotein fractions) were studied. Moreover, the total protein recovered from cells obtained from fasting and fat-fed animals was the same, suggesting that no excessive loss of apoproteins occurred from cells of fat-fed rats (20). Additional losses were not experienced during chromatography, since apoprotein recovery from the columns was virtually complete. Presumably the extensive removal of pancreatic proteases by washing of cells and the use of serine protease inhibitors prevented significant proteolysis. Degradation of HDL protein by rat intestinal mucosal cells at 37°C has been reported to be due to lysosomal proteases (32). Since our experiments were performed at 4°C and pH 7.4, and were not accompanied by loss of immunoreactivity, it seems unlikely that proteolysis was responsible for the results obtained. Significant lipolysis during the process of cell preparation and chromatography also was unlikely inasmuch as no lipase activity was detected in the presence of prolonged exposure to EDTA (33). In the case of apoA-I, some immunoactivity was redistributed with prolonged incubation at 4°C. If the enterocyte preparation was used within 24 hr of preparation, however, elution patterns were quite consistent.

The intracellular chylo-VLDL fractions of the enterocyte preparation seemed to possess similarities to the plasma particles. They were rich in triglycerides (Fig. 2), contained particles of the proper size (Fig. 3), and contained the peaks of apoB and C-III immunoreactivity. The LDL-like fractions could have contained smaller lipoproteins or membrane associated particles. The apoA-I eluting with bovine serum albumin presumably represents apoproteins unassociated with known lipoproteins (Figs. 4–6). When fresh human plasma is filtered on Sephadex G-100 columns, a peak of apoA-I elutes with the albumin peak. This "50,000 mol wt" fraction represents 5% of apoA-I in most plasmas, but as much as 50% of apoA-I may be found in this fraction in extremely hypertriglyceridemic plasmas (34). Whether or not the rat intracellular and human plasma particles are analogous is not clear, but the resemblances are remarkable. The lipid composition of neither fraction is known. Thus the apoA-I peak at this position seen in jejunal supernatants from fasting rats may correspond to "lipid-free" apoA-I complexed with small amounts of lipid. The explanation of the shift of some of the apoA-I toward an earlier elution with fat feeding (Figs. 4C, 5B) may correspond to association of apoA-I with more lipid. Much of apoA-I synthesized in muscle has been shown to sediment at a density > 1.20 g/ml (35). These data suggest that apoA-I may be secreted from enterocytes either with very little or no lipid, as has been suggested for the apoE secreted from cultured macrophages (36) and for several apoproteins secreted by cultured hepatocytes (25). "Lipid-free" apoA-I also has been reported in the incubation medium of rat intestinal organ explants (37).

The distribution of apoA-I in intestinal cellular particles has not been previously reported. However, the distribution in mesenteric lymph has been documented by two separate groups, who found results that differ from each other. Imaizumi et al. (38) found over 80% of apoA-I in the $d < 1.006$ g/ml fraction of lymph in fasting animals and after continuous intraduodenal infusion of lipid. In contrast, Glickman and Green

found only 16% of apoA-I in the d < 1.006 g/ml fraction of lymph from glucose-perfused animals (9). When lipid was perfused intraduodenally, the percent rose to 46%. Our experiments were performed in intact animals and with only a single pulse of lipid, amounting to only 25% of the total dose administered in the other studies. Our data for fasting lymph (Fig. 8) agree with those of Glickman and Green (9), since 23% of apoA-I was in the chylomicron-VLDL fraction (24–32 ml). After fat feeding the percent remained unchanged (22%). The difference after fat feeding between our results and the earlier studies likely are related to the smaller and pulsed fat feed, since apoA-I output increases linearly in lymph during the time of the lipid infusion (9).

Because it is clear that rat apoA-I is made as a preproprotein (11), it is intriguing to consider whether the intracellular unassociated form of apoA-I is the proprotein. Further studies will be needed to determine whether this is indeed the case, and whether conversion to the mature protein is associated with the alteration in distribution among lipoproteins demonstrated in the studies.

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