Effect of Biliary Diversion on Rat Mesenteric Lymph Apolipoprotein-I and High Density Lipoprotein

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ABSTRACT The effect of biliary diversion on intestinal apolipoprotein (apoA)-I and high density lipoprotein formation was studied in mesenteric lymph fistula rats. Bile diversion was produced by an exteriorized catheter that allowed interruption and reconstitution of the enterohepatic circulation. Bile diversion reduced lymph cholesterol output from 0.47 ± 0.05 μmol/h to 0.17 ± 0.03 μmol/h ($P < 0.025$), and lymph triglyceride output from 3.6 ± 0.3 μmol/h to 0.6 ± 0.05 μmol/h ($P < 0.025$) after 24 h. This was due to depletion of lymph chylomicrons and very low density lipoprotein (VLDL). Despite the reduced lipid outputs, lymph apoA-I output was maintained during biliary diversion (basal: 119 ± 15 μg/h; diverted 140 ± 20 μg/h, $n = 12$). During biliary diversion, high density lipoprotein (HDL) were maintained in mesenteric lymph as shown by lipoprotein and immunoelectrophoresis. Bile diversion altered the lipid composition of lymph HDL. Bile-diverted lymph HDL was depleted in total cholesterol and has a greater phospholipid/cholesterol ester ratio than basal lymph HDL. Lymph HDL contained discoidal particles when examined by negative stain electron microscopy. Bile diversion was associated with a reduction in the size of discoidal HDL particles (basal, nondiverted, 165 ± 7 Å ($n = 112$) compared with diverted 126 ± 5 Å ($n = 98$, $P < 0.025$). Experiments were then carried out to determine the source of the apoA-I and HDL found in lymph from bile-diverted animals. The transfer of HDL from plasma into lymph was determined by the intravenous infusion of ^{125}I-apoA-I labeled HDL into lymph fistula rats. In both nonbile-diverted and diverted rats, the specific activity of apoA-I in the HDL fraction of lymph was 23% of the specific activity of apoA-I in plasma HDL, indicating that the major portion (75%) of mesenteric lymph apoA-I did not come from plasma filtration. In other experiments the intraduodenal infusion of [^{3}H]leucine to bile fistula, lymph fistula rats resulted in relative fivefold increase in the specific activity in apoA-I in lymph HDL when compared with the specific activity of apoA-I in plasma HDL from the same animal. We conclude that intestinal apoA-I secretion is maintained during biliary diversion and that synthesis of this apoprotein occurs in the absence of chylomicron formation. We also conclude that discoidal HDL are present in mesenteric lymph despite reduced triglyceride absorption and secretion into lymph.

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INTRODUCTION

The role of the intestine in the synthesis and secretion of chylomicrons and very low density lipoproteins (VLDL)[1] has been well described (1–3). Much less is known concerning high density lipoprotein (HDL) metabolism, however, several studies have suggested that HDL may be synthesized by the intestine (4, 5).

ApoA-I, the principal apoprotein of plasma HDL, is the major apoprotein of mesenteric lymph lipoproteins (chlyomicrons, VLDL, and HDL) and is actively synthesized by rat small intestine during triglyceride absorption (6–8). Studies have also demonstrated that in the fasting state, 50–85% of lymph apoA-I was present in the $d > 1.006$-g/ml infranate suggesting that this apoprotein was associated with mesenteric lymph HDL (6, 8). More recent studies demonstrated that rat mesenteric lymph HDL was heterogenous and contained a spherical particle resembling plasma HDL and discoid, phospholipid-rich particle structurally similar to nascent rat hepatic HDL (9, 10). This suggested that the intestine also secreted a nascent form of HDL. The relationship, however, between the syn-

[1] *Abbreviations used in this paper:* ApoA-I, E etc., apolipoprotein A-I, etc.; DTNB, dithionitrobenzoic acid; HDL, high-density lipoprotein; LCAT, lecithin cholesterol acyltransferase; VLDL, very low-density lipoprotein.

thesis and secretion of triglyceride-rich lipoproteins and HDL is not known.

Ockner and others (1, 11, 12) have demonstrated that triglyceride absorption and chylomicron and VLDL secretion into lymph are markedly reduced by bile diversion. The model of biliary diversion permits an examination of lymph HDL secretion and composition in the setting of markedly reduced triglyceride absorption and chylomicron production. Therefore, we decided to study, in rats, the effect of bile diversion on the secretion and composition of mesenteric lymph HDL and the intestinal secretion of apoA-I. The present studies indicate that apoA-I and HDL secretion into mesenteric lymph are maintained despite markedly reduced triglyceride secretion into lymph.

METHODS

Animals and operative technique. Male rats (200–250 g) of the CD strain (Charles River Laboratories, Inc., Wilmington, Mass.) were used for all studies. All animals were fed commercial rat chow (Ziegler Brothers, Inc., Gardners, Pa.) to which they had free access. Cannulation of the main mesenteric lymphatic duct and duodenum was performed as described (13). An exteriorized catheter connecting the common duct to the duodenum was placed as described by Ockner et al. (1) and diverted pancreato-biliary secretions. All animals were studied 16–24 h after surgery. They were maintained in restraining cages and infused intraduodenally with 1–2 cm^3/h of 0.9% (wt/vol) saline and 5% (wt/vol) dextrose.

Lymph was collected continuously in the presence of 5',5' dithionitrobenzoic acid (DTNB) added to a final concentration of 1 mM. DTNB was added to inhibit the action of LCAT (10). After 12–16 h, the bile duct catheter was divided resulting in acute bile diversion without further operative risk. Subsequent collection periods (bile diverted) were 12 h. After 24 h of diversion, the two divided ends were reconnected in three animals, reconstituting the enterohepatic circulation.

Isolation of HDL. Lymph samples were defibrinated with wooden applicator sticks and HDL was separated by sequential ultracentrifugation in a Beckman L5-75 ultracentrifuge (Beckman Instruments Inc., Fullerton, Calif.). The $d < 1.063$ fraction was isolated by ultracentrifugation for 1×10^8 *g* min. The $d = 1.063$–1.21-g/ml fraction was centrifuged in a 40.3 Ti rotor at 39,000 rpm for 44 h and washed by recentrifugation for another 44 h (9). The lipoproteins were dialyzed for 24–48 h at 4°C against deionized water adjusted to pH 8 with NH_4OH, and then lyophilized (Virtis Co. Inc., Gardiner, N. Y.). The dried samples were delipidated by the addition of 5–10 ml of chloroform/methanol (2:1 vol/vol) (14), and the apoproteins were solubilized in 0.2 M Tris buffer, pH 7.2, containing 0.1 M decyl sulphate, and analyzed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (15).

Chemical methods. Lipoprotein lipids of the 1.063–1.21-fraction were extracted in chloroform/methanol (2:1 vol/vol), according to the methods of Folch et al. (16). Lipid composition was determined by the methods of Downing (17) as modified by Katz et al. (18). Cholesterol and triglyceride values of whole lymph were determined by the autoanalyzer technique (Auto analyser I, Technicon Instruments Corp., Tarrytown, N. Y.).

Lipoprotein electrophoresis. Lipoprotein electrophoresis of whole plasma and lymph lipoproteins was performed on 1% agarose (Seachem, Bio. Products, Rockland, Mass.) containing 12% bovine serum albumin (BSA) in an immunoelectrophoresis apparatus (M.R.A. Corp., Clearwater, Fla.). A current of 30 mA was applied with a dye marker in BSA, and electrophoresis was stopped when the dye had progressed two-thirds of the plate. Samples were fixed in 5% acetic acid, washed with water, and dried and stained with Oil Red 0.

Immunoelectrophoresis. Serum and lymph were subjected to immunoelectrophoresis against antibody to apoA-I. The characterization of this antibody has been reported (6) and was found to be monospecific (Fig. 2).

Apoprotein analyses. Apoproteins were analyzed by SDS polyacrylamide gel electrophoresis in 5.6% polyacrylamide gels, and the protein bands were stained with Coomassie Blue (15). The protein bands were identified by comparison of their Rf values with those of purified rat apoA-IV, apoE, and apoA-I (19). Purified apoproteins (apoA-I, A-IV, E) were obtained from rat plasma HDL by preparative electrophoresis (polyprep 100, Buchler Instruments Inc., Fort Lee, N. J.) by using 5.6% polyacrylamide in SDS. Delipidated HDL apoproteins were solubilized in 0,2 M tris buffer, pH 7.2, containing 0.1 M decyl sulfate, and 40 mM dithiothreitol. Purified apoproteins gave single bands on SDS gels and had characteristic mobilities. ApoA-I was also prepared by gel filtration chromatography (see below).

Apoproteins were quantitated by densitometric scanning of stained gels at 550 nm with a Gilford linear gel scanner (Gilford Instrument Laboratories, Inc., Oberlin, Ohio, see below). Protein concentration was determined using the method of Lowry et al. (20).

Quantitative electrophoresis of ApoA-I. Quantitation of apoA-I was performed by the rocket immunoelectrophoresis technique of Laurell (21) as described (6). The value for male rat plasma apoA-I in this assay was 54.4 ± 1.6 mg/dl ($n = 12$ animals). The intraassay and interassay coefficients of variation were 2.1 and 3.6%, respectively.

Electron microscopy. Lymph and plasma HDL fraction were negatively stained with 2% sodium phosphotungstate, pH 7.4, on Formvar-coated copper grids (Monsanto Co., St. Louis, Mo.). Electron micrographs were obtained with an AEI-6B electron microscope, calibrated with a catalase standard, at $\cong \times 100{,}000$. Each sample was examined on three different grids. Five photographs of each grid were randomly taken from areas of the grid where particles were not obviously confluent and particle sizes determined by direct measurement.

^{125}I-apoA-I (HDL) infusion studies. To determine the contribution of apoA-I in plasma HDL to apoA-I in lymph HDL in controls and bile-diverted animals, the specific activity of apoA-I in lymph and plasma HDL was determined after the intravenous infusion of HDL labeled with ^{125}I-apoA-I. Plasma HDL was isolated by ultracentrifugation between densities of 1.070 and 1.21 g/ml. After exhaustive dialysis, HDL was delipidated in ethanol/ether as described by Brown et al. (22). Apo HDL was solubilized in 0.01 M tris, pH 8.6, 6 M urea, and chromatographed on a 2.5×90-cm column containing Sephadex G 150, (Pharmacia, Div Pharmacia Fine Chemicals, Piscataway, N. J.) equilibrated with the tris-urea. Column fractions were monitored at 280 nm using a LKB recorder (LKB Instruments, Inc., Rockville, Md.). Three peaks were obtained and the descending portion of the second peak was found to contain pure apoA-I by

SDS-polyacrylamide gel electrophoresis. Purity of apoA-I obtained by this method was also assessed by immunodiffusion against monospecific antiserum to apoA-I and gave a single immunoprecipitation arc (Fig. 2) while giving no arcs against antisera to apoE and apoA-IV (not shown).

Iodination of apoA-I. 15 μg of apoA-I was labeled with 1 mCi ^{125}I (New England Nuclear, Boston, Mass.). 30 μg chloramine T was added, and the reaction was stopped with 100 μg sodium metabisulfate. Free iodine was removed by chromatography on a 0.8 × 30-cm column of Sephadex G-25, preequilibrated with buffer containing 1% BSA, 100 mM NaCl, 50 mM dibasic sodium phosphate, and 0.02% sodium azide, pH 7.4. The efficiency of labeling was between 40 and 50%. A modified method described by Shepard et al. (23) was used to label HDL. 10 mg of HDL (~0.08 μmol) was incubated with 10 μg of ^{125}I-labeled A-I (~0.0004 μmol) for 20 min at 25°C in 3 cm^3 of 0.05 M PO_4 buffer at pH 8.5. After incubation, the mixture was raised to a density of 1.21 g/ml and ultracentrifuged for 44 h in a Ti 40.3 rotor (100,000 *g*, 4°C). The top 1 ml was removed by aspiration. The material was dialyzed for 24 h against 0.05 M PO_4 buffer at pH = 8.5 for 24 h. The resulting radioactive HDL was 100% TCA precipitable (sp act ~ 7.7×10^6 cpm/μg HDL). SDS gels of HDL revealed 90% of the label to be in apoA-I.

The biological activity of the ^{125}I HDL was determined. Approximately 6×10^6 cpm were injected into the femoral veins of two male rats. Blood samples were obtained from tail veins at several hour intervals over a 24-h period. The $t_{1/2}$ of the infused HDL was ~10 h in both rats agreeing with published values (24).

HDL infusion experiments were then performed. KI (0.1 gm/liter) was added to the drinking water of all animals 3 d before the infusion. Control animals with indwelling femoral vein and duodenal catheters were lymph diverted for ~20 h. 2.3×10^6 cpm of ^{125}I HDL was injected as a bolus via the femoral vein and followed by a continuous hourly infusion of 0.56×10^6 cpm/h (vol = 0.15 cm^3/h). Lymph and tail vein blood samples (300 μl) were collected at hourly intervals. Infusions were then performed on lymph-diverted animals that had also been bile diverted for 24 h. Infusions of ^{125}I HDL identical to those of the control animals were then performed. After 6 h of infusion, both groups of rats were anesthetized with pentobarbital and exsanguinated from the aorta. ApoA-I was quantitated by quantitative immunoelectrophoresis. TCA precipitable counts were used to determine specific activity of apoA-I in plasma and lymph. Both plasma and lymph HDL were subjected to SDS gel electrophoresis and 90% of the counts on the gel was associated with the apoA-I band.

[^3H] leucine incorporation studies. Two lymph- and bile-diverted rats were studied 24 h after biliary diversion. 300 μCi[^3H]leucine was administered intraduodenally in 1 cm^3 saline to each animal. Lymph was collected on ice in 1 mM DTNB for a 90-min collection period. The animals were then killed and exsanguinated from the abdominal aorta. Lymph and plasma HDL (d = 1.063–1.21 g/ml) were prepared as described above. HDL samples were lyophilized, delipidated, and the apoproteins were separated by SDS gel polyacrylamide electrophoresis. Gels were stained with Coomassie Blue and scanned in a Guilford linear gel scanner as reported (2). We have previously reported that densitometric scanning of individual proteins is directly proportional to the amount of protein applied and is a valid method for comparing the relative amounts of a given protein (2, 19). Because the same protein (i.e., apoA-I) is being compared in plasma and lymph, differences in the chromogenicity of different apoproteins do not influence the results. After scanning, gels were sliced into 1-mm slices using a lateral gel slicer and the radioactivity in each slice determined as reported from this laboratory (2). The relative specific activities for apoproteins were then determined for lymph and plasma apoA-I and apoE.

RESULTS

Mesenteric lymph analysis

Lymph triglyceride and cholesterol output. With biliary diversion, a marked reduction in mesenteric lymph cholesterol and triglyceride was noted (Table I). After 24 h of bile diversion, cholesterol output had fallen to 36% of basal (control) production and triglyceride output to 17% of basal values. With reconstitution of the enterohepatic circulation, cholesterol output returned to basal levels (Table I).

Lipoprotein electrophoresis of whole lymph (Fig. 1) demonstrated progressive loss of the chylomicron and pre-β band during biliary diversion that reappeared upon restoration of the enterohepatic circulation. The α-migrating band of HDL persisted throughout the period of biliary diversion, suggesting a preservation of this lipoprotein in lymph. Because apoA-I is the major apoprotein of mesenteric lymph HDL, it was next of interest to quantitate the apoA-I content in lymph from bile-diverted animals.

Lymph apoA-I content. The basal mesenteric lymph output of apoA-I in rats being perfused intraduodenally with 5% dextrose/normal saline was 119±15μg/h (n = 12, Table I). The secretion rate is similar to that found by other investigators (8), but lower than previous results reported from this laboratory (6). After 24 h of biliary diversion, A-I output

TABLE I
Effect of Biliary Diversion of Mesenteric Lymph Cholesterol, Triglyceride, and apoA-I Secretion

	Cholesterol	Triglyceride	ApoA-I
	μmol/h		μg/h
Basal (12 h)			
n = 12	0.47±0.05	3.6±0.3	119±15
Diverted (24 h)			
n = 12	0.17±0.03*	0.6±0.05*	140±20‡
Reconnected (12 h)			
n = 3	0.45±0.05	Not Determined	119±20‡

Cholesterol and triglyceride were quantitated in whole fresh lymph during the designated collection periods. ApoA-I was quantitated by quantitative immunoelectrophoresis (Methods). Three animals had their enterohepatic circulation reconnected after 24 h of biliary diversion.

* P = 0.025 compared with basal period.

‡ No significant difference from control period $P > 0.2$.

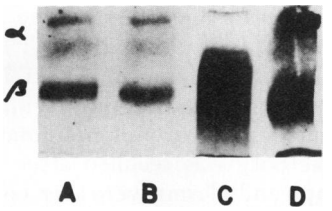

FIGURE 1 Effect of biliary diversion on the lipoprotein electrophoresis of rat mesenteric lymph. Rat mesenteric lymph from two bile-diverted animals (A, B, 24-h biliary diversion) basal lymph (nonbile diverted, C), and rat plasma (D) were subjected to lipoprotein electrophoresis (Methods). The plate is stained for lipid with Oil Red 0. Note the loss of lipid staining material in the bile-diverted animals associated with chylomicrons and very low density lipoproteins when compared with nonbile-diverted lymph (C). Note also the preservation of lipoproteins in the α-migrating (HDL) region of bile-diverted lymph. Because HDL contain less lipid than the other lipoproteins, they stain less intensely with Oil Red 0. Similar amounts of lymph were applied in all wells.

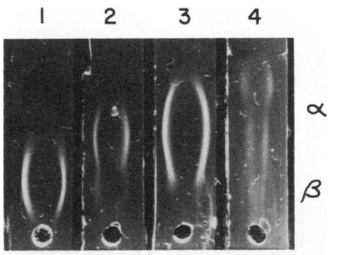

FIGURE 2 Immunoelectrophoresis of lymph and plasma. Antiserum in all troughs: anti-apoA-I. 1-delipidated apoA-I; 2-bile-diverted lymph; 3-rat plasma 1; 4-basal (nonbile-diverted lymph). Note the loss of A-I containing lipoproteins in the chylomicron and VLDL regions after biliary diversion and a preservation of A-I in the HDL region (compare lanes 2 and 4). All arcs with the exception of lane 1 stained with Oil Red 0.

was $140 \pm 20 \mu g/h$, $n = 12$. Thus, the content of mesenteric lymph apoA-I was maintained during a period of biliary diversion despite a marked reduction in lymph triglyceride secretion. Lymph flow rates did not fluctuate significantly from one collection period to another (mean $1.4 \pm 0.3 ml/h$). In three animals apoA-I output was maintained ($119 \pm 20 \mu g/h$, $n = 3$) after bile reconnection. We next determined the distribution of apoA-I in mesenteric lymph from bile-diverted animals. In nonbile-diverted lymph, apoA-I in the $d < 1.063$ fraction represented 25.4% ($n = 4$) of total lymph apoA-I. In contrast, the $d < 1.063$ fraction of lymph from bile-diverted animals represented 3.6% ($n = 3$) of total lymph A-I. This finding is consistent with the marked reduction in triglyceride-rich lipoproteins in the lymph of bile-diverted animals. The finding of >90% of lymph apoA-I from bile-diverted animals in the $d > 1.063$ g/ml fraction of lymph prompted further studies to determine whether this apoA-I was present within a lipoprotein fraction (i.e., HDL). Further ultracentrifugal separation revealed that 50% of the apoA-I in the $d > 1.063$ g/ml of lymph resided in the HDL fraction of lymph ($d = 1.063 - 1.21$ g/ml) and 50% was recovered in the $d > 1.21$ g/ml fraction of lymph in both bile-diverted and nondiverted animals.

Because it is known that prolonged ultracentrifugation may dissociate apoA-I from lipoproteins, we wished to localize apoA-I in lymph without ultracentrifugation. We therefore carried out immunoelectrophoresis of whole lymph from control lymph fistula as well as bile-diverted lymph fistula animals. As shown in Fig. 2, immunoelectrophoresis of whole lymph from bile-diverted animals demonstrated a loss of the apoA-I immunoprecipitation arc associated with triglyceride-rich lipoproteins. A strong immunoprecipitation arc was preserved in the α-migrating (HDL) region. A-I immunoprecipitation arcs in control and bile-diverted lymph stained with Oil Red 0 indicating that these arcs were lipoproteins. In contrast (Fig. 2), delipidated (free) apoA-I gave an immunoprecipitation arc with slower mobility than seen in bile-diverted lymph. This arc did not stain with Oil Red 0. These findings indicate a preservation of apoA-I in mesenteric lymph HDL, which were further characterized.

Characterization of mesenteric and serum HDL during bile diversion. Serum and lymph HDL isolated in the presence of DTNB were isolated and purified by recentrifugation, delipidated, and the apoproteins were subjected to SDS gel electrophoresis. Two major apoproteins were seen in all samples (Fig. 3) as previously reported from this laboratory (9). In lymph the major apoprotein was apoA-I (mol wt 25,000). The other prominent apoprotein band was apoE (mol wt ~35,000). Serum HDL contained both apoA-I, apoE, and apoA-IV. Lipid analysis of mesenteric lymph HDL from bile-diverted lymph was compared with nondiverted lymph and serum HDL. Bile diversion resulted in a significant reduction in the percentage of total cholesterol in HDL and an increase in the phospholipid/cholesterol ester ratio in lymph (Table II) suggesting the presence of phospholipid-rich discoidal particles. Reconstitution of the enterohepatic circulation resulted in an increased contribution of cholesterol to the HDL mass (Table II).

Electronmicroscopy. Negative staining electron microscopy of lymph HDL confirmed the presence of discoidal HDL. The morphological appearance was similar to previously reported data from this laboratory (9). Discoidal particles that formed rouleaux were noted in all lymph samples examined (Fig. 4). Discoidal particles were only rarely seen in plasma. It was also noted that bile diversion was associated with a significant reduction in the mean size of discoidal HDL

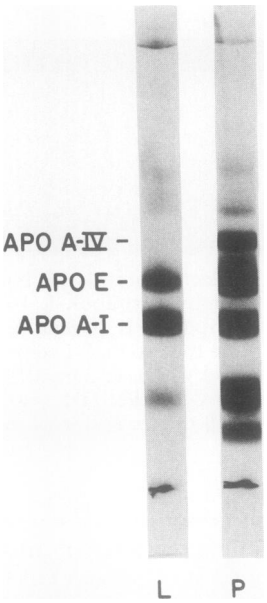

FIGURE 3 SDS polyacrylamide gel electrophoresis of lymph (L) and plasma (P) HDL from a bile-diverted animal. Note that lymph HDL are enriched in apoA-I when compared with plasma HDL from the same animal.

particles (basal, nondiverted: 165±7; Å (n = 112) compared with bile-diverted lymph HDL: 126±5 Å (n = 98, $P < 0.025$).

Infusion studies. We next wished to explore the relative contribution of plasma HDL to mesenteric lymph HDL in control and bile-diverted animals. An intravenous bolus of HDL labeled with ^{125}I apoA-I followed by a constant infusion of ^{125}I HDL was given to four lymph-diverted rats and three lymph-diverted, bile-diverted rats. In both groups of animals a constant level of radioactivity was reached after 2 h. Hourly samples of lymph and plasma were then collected. The specific activity of apoA-I was determined hourly for simultaneous plasma and lymph HDL samples (Table III). The specific activity of apoA-I in the lymph HDL was consistently less than the specific activity of apoA-I in plasma in both groups of animals. In both groups there was approximately a three- to fourfold decrease in apoA-I specific activity in lymph HDL when compared with plasma HDL. Because significant amounts of apoA-I were recovered in the $d > 1.21$ g/ml fraction of lymph, the specific activity of apoA-I was determined in this fraction as well. In each animal, it was similar to lymph HDL (apoA-I) specific activity. These data indicate that apoA-I is actively secreted by the intestine during biliary diversion, causing a dilution of its specific activity in lymph when compared with the plasma specific activity of apoA-I. Further evidence that apoA-I in mesenteric lymph HDL is synthesized during biliary diversion was obtained by studying the incorporation of [^{3}H]leucine into lymph HDL apoA-I.

[^3H] *Leucine incorporation studies*

Two lymph- and bile-diverted rats were studied 24 h after biliary diversion. 300 μCi [^{3}H] leucine was ad-

TABLE II
Effect of Biliary Diversion on Lipid Composition of Mesenteric Lymph and Serum HDL

	Total cholesterol	CE	Phospholipid	PL/CE	PL/total cholesterol
			% Total lipid		
Lymph					
Basal (n = 5)	27.7±1.2	15.9±1.3	65.7±2.1	4.1	2.4
Bile diverted 12–24 h (n = 5)	22.8±1.5*	13.2±1.1*	68.4±3.7	5.2*	3.0*
Bile reconnected 12 h (n = 3)	37.1±2.3‡	22.0±2.3*	55.6±4.9‡	2.5‡	1.8*
Serum					
Bile diverted 24 h (n = 3)	34.5±2.5	26.7±1.7	63.0±3.0	2.4	1.8
Bile reconnected 12 h (n = 3)	25.6±1.0*	21.4±.9*	69.2±2.3	3.2*	2.7*

* = $P < 0.025$, ‡ = $P < 0.005$ } compared with basal lymph.

Lipid composition of lymph and serum HDL was determined by quantitative TLC (Methods).

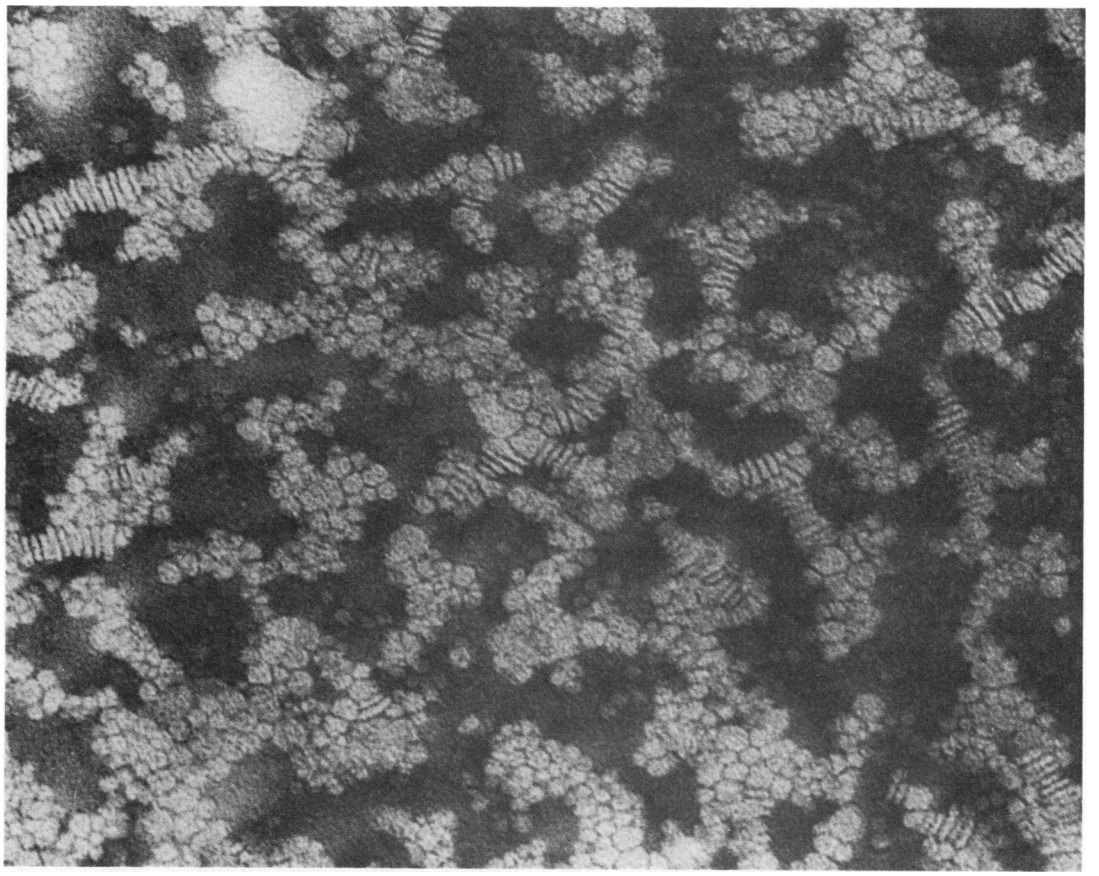

FIGURE 4 Negative stain electron microscopy of mesenteric lymph HDL during bile diversion. Lymph was collected in presence of DTNB and HDL (d = 1.063 − 1.21 g/ml) isolated by ultracentrifugation. (×64,000).

ministered i.d. to each animal. Lymph was collected for 90 min, at which time the animal was exsanguinated from the abdominal aorta. HDL was prepared from the plasma and lymph of each animal, delipidated, and the apoproteins separated on SDS acrylamide gels (Methods). Gels were stained, scanned, and then sliced into 1-mm segments for radioactivity determination (Methods). The specific activity of apoA-I was determined for lymph and plasma HDL. In both animals the ratio of lymph to plasma A-I sp act was >5 (6.6 and 5.4), while lymph to plasma apoE sp act were ~1.5. These results demonstrate synthesis of apoA-I in lymph HDL from bile-diverted animals.

DISCUSSION

Several laboratories have established that the rat intestine is a source of apoA-I secreted on VLDL and chylomicrons (6–8) and that apoA-I is actively synthesized during triglyceride absorption. Recent evidence suggests that rat intestine also synthesizes a discoidal HDL particle rich in apoA-I (7). We hypothesized that the experimental model of biliary diversion would permit an examination of lymph HDL secretion and composition in the absence of significant triglyceride absorption.

The output of mesenteric lymph cholesterol and triglyceride was markedly reduced during biliary diversion compared with the nondiverted periods (Table I). This is consistent with the loss of chylomicrons and VLDL. The results are in agreement with the findings of Ockner and others (1, 11, 12) and confirm that biliary components are the source of the lipid constituents of triglyceride-rich lipoproteins formed by the intestine during fasting periods.

ApoA-I output was maintained during biliary diversion (Table 1). Despite reduction in chylomicrons and VLDL, which are known to transport up to 80% of fasting lymph apoA-I (6, 8), there was no reduction in apoA-I output during biliary diversion. These results indicate that intestinal apoA-I secretion into lymph is maintained despite low levels of triglyceride secretion

TABLE III
Specific Activity of Lymph HDL (apoA-I) after Intravenous Infusion of ^{125}I HDL (apoA-I)

	cpm/μg AI		
	Lymph	Serum	L/P
Lymph and bile diverted			
1	341±53	1126±92	0.303
2	80±3	626±12	0.128
3	240±7	940±28	0.256
			Mean = 0.229
Lymph diverted			
1	344±9	945±40	0.360
2	174±28	953±48	0.183
3	104±14	335±23	0.310
4	177±17	597±7	0.280
			Mean = 0.285

Animals were infused intravenously with ^{125}I HDL. Plasma radioactivity achieved a constant specific activity after 2 h. The specific activity of apoA-I in lymph and plasma was then determined hourly for the next 4 h. Values shown are the means±SEM of four hourly specific activities for lymph and plasma. There was no significant difference between the lymph/plasma specific activities in both groups of animals ($P > 0.15$).

and suggests that factors other than triglyceride resynthesis may regulate intestinal apoA-I secretion. The recent findings of Windmueller and Wu (25) also support this concept. The preservation of lymph apoA-I secretion during biliary diversion was largely due to its presence in lymph HDL. This was demonstrated by immunochemical measurement of apoA-I distribution in lymph. In addition, both lipoprotein electrophoresis and immunoelectrophoresis (Figs. 1, 2) demonstrated that HDL was the major lipoprotein in lymph from bile-diverted animals and that HDL was maintained in lymph after bile diversion.

The finding of discoidal HDL in mesenteric lymph from bile-diverted animals lends further support to earlier observations from this laboratory describing a discoidal fraction in rat mesenteric lymph (9). In the bile-diverted animal, these particles could not arise from less dense lipoproteins either due to lipolysis or as an artifact of centrifugation because chylomicrons and VLDL were virtually eliminated from lymph by bile diversion. In addition, discoidal HDL, easily demonstrable in lymph from bile-diverted animals (Fig. 4), were only rarely seen in plasma from these animals and argue against these particles being filtered from plasma. Additional evidence indicating an intestinal origin for lymph HDL derives from infusion studies of HDL labeled with ^{125}I apoA-I (Table III). In both control and lymph-diverted as well as in bile- and lymph- diverted animals, there was a relative decrease in the specific activity of lymph HDL apoA-I when compared with plasma. These results are compatible with newly secreted intestinal apoA-I in the HDL fraction of mesenteric lymph and suggest that the intestine synthesizes about 70% of HDL apoA-I. Further support for apoA-I synthesis during biliary diversion is provided by [^{3}H]leucine incorporation into lymph apoA-I in HDL where a relative increase in lymph A-I specific activity compared with plasma A-I was found.

The major finding of this study was the preservation of intestinal apoA-I secretion and lymph HDL in bile-diverted animals. In addition, biliary diversion was associated with alteration in lymph HDL composition and morphology. Lymph HDL from bile-diverted animals were enriched in phospholipid with respect to cholesterol and cholesterol ester (Table III). This composition is compatible with at least a portion of the lymph HDL fraction being discoidal, as was demonstrated. The present studies have not defined which feature of biliary diversion results in apoA-I and HDL secretion, as well as the observed morphological changes in lymph HDL. Whether the deficiency of bile salts, cholesterol or phospholipid produced by biliary diversion, or the increase in intestinal cholesterol synthesis that results (26) are responsible for the observed changes remains to be determined. The present studies do, however, demonstrate a secretion of lymph HDL and apoA-I under conditions of minimal triglyceride absorption and reduced chylomicron formation.

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