Isolation and characterization of lipoprotein of density < 1.006 g/ml from rat hepatic lymph[1]

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Abstract The lipoprotein composition of rat hepatic lymph was studied using an animal model. The hepatic lymph duct of the adult male rat was cannulated and the hepatic lymph was collected. Hepatic lymph contains less than 1% of the total triglyceride output from the liver. Agarose gel electrophoresis of hepatic lymph showed the presence of two major lipoproteins bands, the α-migrating HDL and a band moving between plasma β and pre-β bands. Lipoprotein of density $\rho < 1.006$ g/ml was then isolated by ultracentrifugation and it was found to correspond to the slow-moving pre-β band. There was no difference in the mean diameter of hepatic lymph VLDL (64.4 nm) and that of plasma VLDL (64.6 nm). Compared with plasma VLDL, hepatic lymph VLDL has significantly more phospholipid (40% by weight), a higher cholesterol/cholesteryl ester ratio, and a marked reduction in triglyceride content (40% by weight). Although both plasma VLDL and hepatic lymph VLDL have apoE and apoB as the major apolipoproteins, there are other marked differences in apolipoprotein composition. Hepatic lymph VLDL has significantly less apoC and the apoB of hepatic lymph VLDL is predominantly the $apoB_{240K}$ (mol wt 240,000), with a small amount of the $apoB_{330K}$ (mol wt 335,000). On the other hand, plasma VLDL has an equal proportion of both $apoB_{240K}$ and $apoB_{330K}$. This study presents for the first time the lipid and protein composition of rat hepatic lymph VLDL. Furthermore it has provided evidence that the hepatic lymph duct-cannulated rat can be used as an *in vivo* model for studying the secretion of nascent hepatic lymph VLDL.—**Tso, P., J. B. Ragland, and S. M. Sabesin.** Isolation and characterization of lipoprotein of density < 1.006 g/ml from rat hepatic lymph. *J. Lipid Res.* 1983. **24:** 810–820.

Supplementary key words nascent VLDL • apolipoproteins • plasma • intestine

The liver is the major biosynthetic source of very low density lipoproteins (VLDL) and shares with the intestine the formation of high density lipoproteins (HDL). These lipoproteins are presumably secreted as precursor particles, nascent lipoproteins, which undergo rapid metabolic transformations after secretion from their cells of origin.

Most of our knowledge of nascent lipoprotein secretion by the liver is derived from studies with isolated perfused liver (1–6) and cultured hepatocytes (7–10). It has been demonstrated, using the isolated perfused liver, that VLDL and HDL are the major lipoproteins secreted by the liver (6, 11). Using cultured hepatocytes, Bell-Quint and Forte (9) have suggested that hepatocytes may also secrete low density lipoproteins (LDL). Thus far in vivo models have not afforded the opportunity to investigate directly the characteristics of nascent lipoproteins newly secreted by the liver. The secretion of nascent lipoproteins by the hepatocytes is thought to occur by direct secretion into the space of Disse (12–14). Ultrastructural studies of the liver have shown that VLDL-size particles, within Golgi-derived secretory vesicles, are secreted directly into the space of Disse.

Although no direct anatomical evidence is available to trace the origin of hepatic lymph, it is generally believed that hepatic lymph is formed at the space of Disse (15–17). Thus hepatic lymph may contain newly secreted lipoproteins. Therefore, an analysis of hepatic lymph lipoproteins may provide a unique means of investigating, in vivo, the factors that regulate the formation of nascent hepatic lipoproteins. Heretofore there have been no reports of the characterization of rat hepatic lymph lipoproteins, and only limited data are available on the lipid composition of rat hepatic lymph, such as a cholesterol concentration about two-thirds that of plasma cholesterol (18) and an ouput of total fatty acid of 2–3 μEq/hr (19). The cholesterol and the phospholipid concentration of rabbit hepatic lymph is about 75% that of the plasma and therefore quite similar to that of the rat (20). This paucity of data on rat hepatic lymph undoubtedly reflects the great diffi-

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Abbreviations: VLDL, very low density lipoprotein; HDL, high density lipoprotein; LDL, low density lipoprotein; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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culty involved in cannulating a fragile duct that measures only 0.75 mm diameter in the rat. We have succeeded in cannulating the rat hepatic duct and have isolated and characterized a lipoprotein of density < 1.006 g/ml. This report describes the techniques involved in isolating hepatic lymph VLDL and the characterization of this unique hepatic lymph lipoprotein that is clearly different in lipid and apolipoprotein composition from plasma VLDL.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (275–300 g) were used in all studies and the animals were fasted overnight before surgery.

Cannulation of the hepatic lymph duct

The cannulation of the hepatic lymph duct of the rat was performed under ether anesthesia by a modification of the method of Bollman, Cain, and Grindlay (21). After a midline abdominal incision, the stomach and the intestine were displaced to the left with a retractor over a wet gauze covering these organs and thereby exposing the hepatic artery. The anatomical arrangement of the hepatic lymph duct and its neighboring structures is illustrated in **Fig. 1.** Although no direct anatomical evidence is available, its seems reasonable to conclude that the hepatic lymph we collect is postnodal lymph. This is deduced from the position we cannulate and the anatomical arrangement of the hepatic lymphatic system depicted by Lee (22). There may be considerable difficulty in visualizing the hepatic lymph duct; however, its identification can be assisted by tieing the bile duct temporarily during the cannulation, a maneuver which causes the hepatic lymph duct to swell. The hepatic lymph duct was cannulated with a clear vinyl tubing (outer diameter 0.8 mm, Dural Plastic Engineering, Dural, Australia). After the cannula was bevelled, it was brought through the rat skin by an 18-gauge needle, and filled with heparinized saline (10 units/ml). The hepatic lymph duct was cut with ophthalmic iris scissors and the cannula was carefully inserted. A drop of Eastman Kodak 910 glue was then applied to secure the cannula. In more than 60% of animals we found a lymph vessel connecting the hepatic and the major intestinal lymph duct. This anastomotic vessel was visualized and ligated; however, in a few animals in which the connecting duct was not visualized, evidence of contamination of hepatic lymph with intestinal lymph lipoproteins was sought and if present those animals were discarded. Intestinal lymph contamination was detected in the following ways. A small dose of olive oil (0.1 ml) was infused via the duodenal tube. The subsequent appearance of turbidity in the hepatic lymph suggested the presence of intestinal lymph contamination. The other particularly useful marker for intestinal lymph contamination was the presence of apoA-I and apoA-IV in the hepatic lymph VLDL. These apolipoproteins of intestinal origin are not present in hepatic or plasma VLDL. Another useful reflection of intestinal lymph contamination was the increase in hepatic flow rate

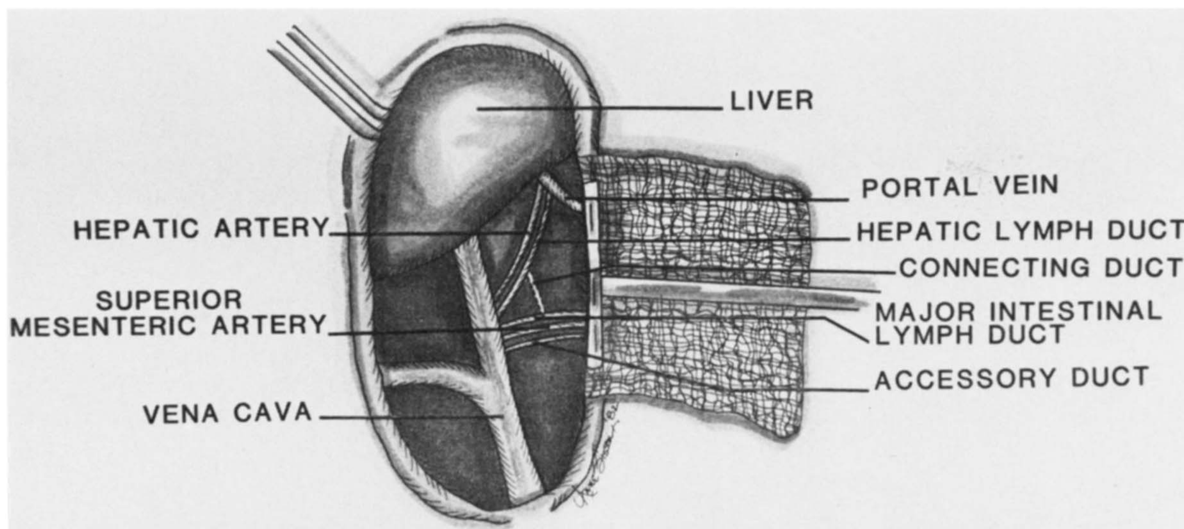

Fig. 1. The anatomical arrangement of the hepatic lymph duct, the connecting duct, and the intestinal lymph duct. The connecting duct connects the major intestinal and the hepatic lymph ducts. The hepatic artery we have labeled also carries blood to the stomach, spleen, omentum, and the upper duodenum, it has also been labeled as celiac artery by others (22). The presence of the connecting duct has been observed in more than 60% of the animals on which we have operated. (Figure was adapted from Tso and Simmonds (57)).

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(>0.5 ml/hr) with the sucrose infusion. The normal rate seldom exceeded 0.25 ml/hr.

Collection of intestinal lymph

The intestinal lymph was collected from intestinal lymph fistula rats. The major intestinal lymph duct as shown in Fig. 1 was cannulated according to the procedure described by Bollman et al. (21). The procedure used in the surgery is similar to that described for the cannulation of hepatic lymph duct.

Duodenal intubation and post-operative care

A soft silicone tubing (o.d. 2.2 mm, Dow Corning, Midland, Michigan) was introduced through the fundus of the stomach into the duodenum and secured with a transmural suture. Postoperatively the animals were allowed to recover in restraining cages kept in a warm box maintained at 30°C in order to prevent hypothermia. Throughout the recovery and experimental periods, the animals were infused continuously via the intraduodenal tube at a rate of 2.4 ml per hr with a saline solution containing 15% sucrose (154 mM NaCl, 4 mM KCl, 0.44 M sucrose). The saline solution was supplemented with sucrose in order to increase hepatic VLDL production (23). The sucrose infusion was calculated to be equivalent to 32 kcal/rat per day; the daily energy requirement of a 300-g rat is approximately 30 kcal (24–26). Therefore, feeding sucrose in the hepatic lymph duct-cannulated rats not only provides the daily energy requirement but it may also stimulate VLDL production. NaCl and KCl were in the infusate to replace electrolyte losses due to lymphatic drainage.

Collection of hepatic lymph

Hepatic lymph was collected into precooled conical centrifuge tubes containing 0.1 ml of a solution containing 25 mM EDTA, 2.5 mg/ml gentamycin sulfate, 1.25 mg/ml of chloramphenicol, 5 mg/ml sodium azide, and 1.5 mM phenylmethylsulfonyl fluoride. Collection tubes were changed every 12 hr and the ice bath was changed frequently to keep the tubes cool. After 12 hr of lymph collection, lymph volume was usually about 2.5 ml. After taking the dilution factor into account (25-fold), the final concentration of EDTA, gentamycin sulfate, chloramphenicol, and sodium azide in the lymph was similar to that used by Kane et al. (27) and should prevent hydroperoxidation and bacterial degradation of lymph lipoproteins. The phenylmethyl sulfonyl fluoride was added to prevent proteolysis (28). Hepatic lymph was usually collected from the rat for 2 days. At the end of the experiment, the rat was anesthetized with ether and blood was collected from the abdominal aorta. The blood was dispensed into evacuated blood-collecting tubes (B-D Company, Rutherford, NJ) containing ethylenediaminetetraacetic acid (EDTA) tripotassium salt as the anticoagulant. After centrifuging at 4,000 rpm 4°C for 15 min, the collected plasma was mixed in the same proportion with the preservatives as described above.

Isolation of lipoproteins of density < 1.006 g/ml

Lipoproteins of density < 1.006 g/ml were separated by ultracentrifugation in a Beckman L8-70 ultracentrifuge using a swinging bucket 50.1 rotor (Spinco Division, Palo Alto, CA). Three ml of either hepatic lymph or plasma was overlaid with 2 ml of saline at ρ = 1.006 g/ml (with preservatives added) and spun for 17 hr at 49,000 rpm at 17°C. The top 1.5 ml was removed by slicing the tube and lipoproteins with ρ < 1.006 g/ml were washed once by ultracentrifugation under identical conditions before lipoprotein and apolipoprotein analysis.

Negative staining of lipoproteins

To measure the distribution of lipoproteins with different diameters, the lipoprotein fraction was negatively stained. A formvar-coated grid was floated on a drop of the lipoprotein sample and the excess was removed by a tissue. The grid was floated on a drop of a 2% sodium phosphotungstate solution (pH 5.9) for about 40 sec and the grid was dried with a tissue. The negatively stained sample was examined with a Zeiss EM-10 electron microscope (Carl Zeiss Inc., Thornwood, NY). To measure the distribution of particles with different diameters, 100 particles on the enlarged prints of the electron micrographs were measured as the mean of the two estimates perpendicular to each other. The mean diameter was calculated from the above measurements.

Agarose gel electrophoresis

Small aliquots of the hepatic lymph and plasma were used for the separation of lipoproteins by agarose gel electrophoresis and stained with Fat Red 7B using the apparatus and method supplied by Corning-ACI, Palo Alto, CA.

Lipid extraction and analysis

One milliliter of the total hepatic lymph or the lipoprotein fraction of ρ < 1.006 g/ml from both lymph and plasma was extracted by the method of Folch, Lees, and Sloane Stanley (29). The lipid extract was then separated by thin-layer chromatography on Silica Gel G60 into various lipid classes. The solvent system was light petroleum ether–diethyl ether–acetic acid 75:15:0.6 (v/v/v). The appropriate spots as identified by the standards run on the same plate were scraped off and lipid was eluted with 3 × 5 ml of chloroform–methanol 2:1

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(v/v). Cholesterol and cholesteryl ester were determined by gas–liquid chromatography with a known amount of β-sitosterol as an internal standard (30). Triglyceride was determined by the colorimetric method of Biggs, Erickson, and Moorehead (31). Phospholipid phosphorus was determined by the method of Parker and Peterson (32).

Analysis of apolipoprotein composition

Lipoprotein samples were dialyzed against 0.01% disodium EDTA, pH 7.4. The dialyzing solution also contained preservatives at the same concentration as described previously. The protein concentration of the dialyzed hepatic lymph VLDL, intestinal lymph VLDL, and plasma VLDL fractions were determined by the modified Lowry procedure of Markwell et al. (33). The apolipoprotein composition of the VLDL from the hepatic lymph, intestinal lymph, and the plasma was then analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). The appropriate lipoprotein sample, containing 40 μg of protein was lyophilized and then partially delipidated with four washes of cold anhydrous diethyl ether. The procedure for PAGE was modified from the SDS system of Laemmli (34), using a vertical slab apparatus (LKB Scientific Instruments, Washington, DC). The separating gel was composed of a 3.5–27% gradient, and the sample buffer was twice the concentration of the Laemmli buffer. Utilizing this system, the two apoB bands ($apoB_{240K}$, mol wt, 240,000 and $apoB_{330K}$, mol wt 335,000) as described by Krishnaiah et al. (35) and Elovson et al. (36) were well separated. Gels were stained with 0.05% Coomassie Brilliant Blue R-250 and destained in the solutions of Weber and Osborn (37). All gels were scanned with an Isco Model 1310 gel scanner attachment to the model VA-5 absorbance monitor. The apolipoprotein bands were identified on the basis of molecular weights: *1*) as determined by reference to purified protein standards, and *2*) by comparison to published SDS gel electrophoretograms of rat apolipoproteins and also purified rat apolipoproteins (35, 38–42). The peaks were integrated by an electronic integrator Model MOP 3 (Georgia Instrument Inc., Atlanta, GA).

Statistics

Unless otherwise stated, the results from plasma and the lymph were tested by Student's *t*-test (43). Differences were considered significant if $P < 0.05$.

RESULTS

Flow rate and lipid composition of hepatic lymph

With the infusion of 2.4 ml/hr of the saline solution containing 15% sucrose (154 mM NaCl, 4 mM KCl, 0.44 M sucrose), the lymph flow rate for eight rats was 0.22 ± 0.01 ml/hr (mean ± SE) and the protein concentration was 3.4 ± 0.3 g/dl. The lipid concentrations in hepatic lymph (mg/dl) were: free cholesterol, 8.06 ± 0.73; cholesteryl ester, 39.70 ± 6.65; triglyceride, 23.67 ± 5.17; and phospholipid, 86.60 ± 8.99. The protein content of the plasma from the hepatic lymph duct-cannulated rat was 5.1 ± 0.1 g/dl (eight rats). The lipid concentrations in the plasma (mg/dl) were: free cholesterol, 14.53 ± 1.88; cholesteryl ester, 45.71 ± 1.97; triglyceride, 39.88 ± 5.03; and phospholipid, 151.91 ± 5.94.

Agarose gel electrophoresis of hepatic lymph

Fig. 2 shows the agarose electrophoretograms of hepatic lymph (A) and plasma (B). Two major lipoprotein bands were present in hepatic lymph, an α-migrating HDL and a band migrating between β- and pre-β. Also present in the hepatic lymph was a faint band with pre-β mobility. Unlike hepatic lymph, the plasma had three major lipoprotein bands corresponding to the β- (LDL), the pre-β (VLDL), and the α-band (HDL).

Electron microscopy of hepatic lymph lipoproteins

Electron microscopic examination of negatively stained hepatic lymph revealed lipoproteins primarily of VLDL size (30–70 nm) (**Fig. 3**). Although not readily

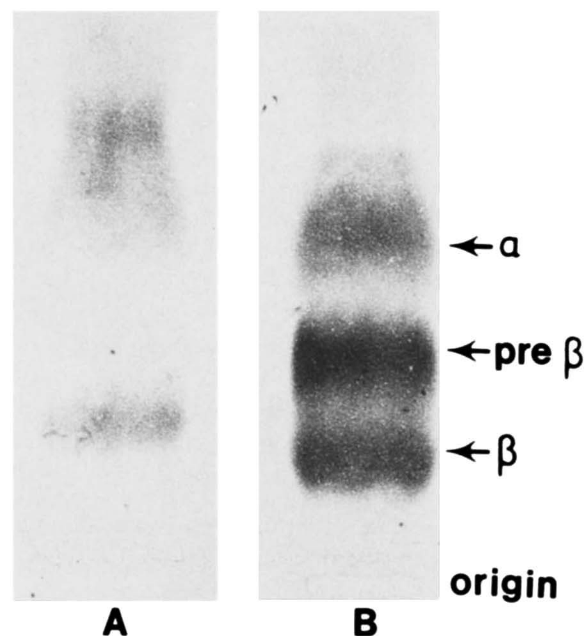

Fig. 2. The electrophoretic pattern of hepatic lymph (A) and plasma (B). The hepatic lymph has two major bands, an α band and a band moving between pre-β and β band. A faint band of pre-β mobility is also seen. Different from hepatic lymph, the plasma has three major bands, the α, the pre-β, and β. α Band, HDL; pre-β, VLDL; β band, LDL.

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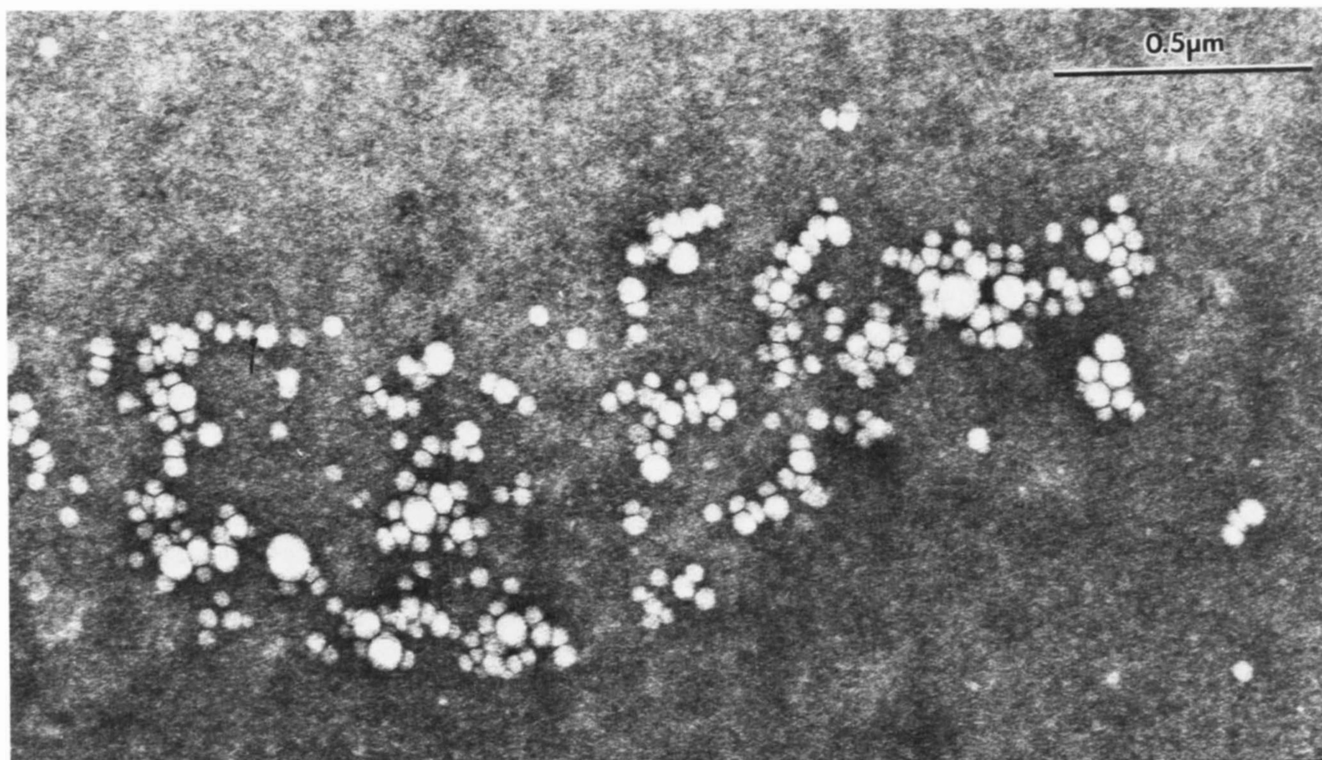

Fig. 3. Electron micrograph of rat hepatic lymph sample negatively stained with 2% phosphotungstate (pH 5.9). The magnification of the electron micrograph was ×70,000.

observable in **Fig. 3,** other particles of the HDL- and LDL-size were also present. After isolating the lipoproteins of $\rho < 1.006$ g/ml, they were examined by electron microscopy using negative staining (**Fig. 4**). The diameter of 100 VLDL particles, from each of the two samples (plasma and hepatic lymph), was measured using the mean of two measurements perpendicular to each other. A histogram showing the size distribution is shown in Fig. 4. The diameter of the hepatic lymph VLDL was 64.4 ± 2.4 nm (mean ± SE), whereas that of plasma VLDL was 64.6 ± 2.5 nm. To test whether there was a difference in the size distribution of the two VLDL populations, the nonparametric Kolmogoroz-Simirnov two-sample test (two-tailed test) was applied (44). No significant difference was detected between the two populations at a confidence level of 0.05.

Chemical composition of lipoproteins of $\rho < 1.006$ g/ml from hepatic lymph and plasma

The chemical composition of lipoproteins of $\rho < 1.006$ g/ml in hepatic lymph and plasma is summarized in **Table 1.** When plasma VLDL was compared to hepatic lymph VLDL, the following differences were observed. First, hepatic lymph VLDL was significantly enriched in phospholipid (40.8%) compared to plasma VLDL (18.6%) and this difference was statistically significant ($P < 0.001$). The high phospholipid content of hepatic lymph VLDL was accompanied by a significantly lower triglyceride content (40.5%) compared to plasma VLDL (61.9%) ($P < 0.001$). Furthermore, the cholesterol/cholesteryl ester ratio of hepatic lymph VLDL was significantly higher than that of plasma VLDL ($P < 0.001$). Since phospholipid has a higher density than triglyceride, one would expect hepatic lymph VLDL to have a higher density than plasma VLDL. To calculate the density of the hepatic lymph and plasma VLDL, we used the values reported by Sata, Havel, and Jones (45) for the density of the various lipid and protein components (phospholipid, 1.031 g/ml; cholesterol, 1.033 g/ml; cholesteryl ester, 0.958 g/ml; triglyceride, 0.915 g/ml; protein, 1.418 g/ml). The calculated density of hepatic lymph VLDL was approximately 1.003 g/ml and therefore the particles floated in a salt solution of $\rho = 1.006$. In contrast, density of the plasma VLDL was computed to be approximately 0.976 g/ml.

Apolipoprotein composition of hepatic lymph, plasma, and intestinal lymph VLDL

The apolipoproteins of the VLDL from hepatic lymph, intestinal lymph, and plasma were separated by SDS-PAGE using a 3.5–27% gradient gel as shown in **Fig. 5.**

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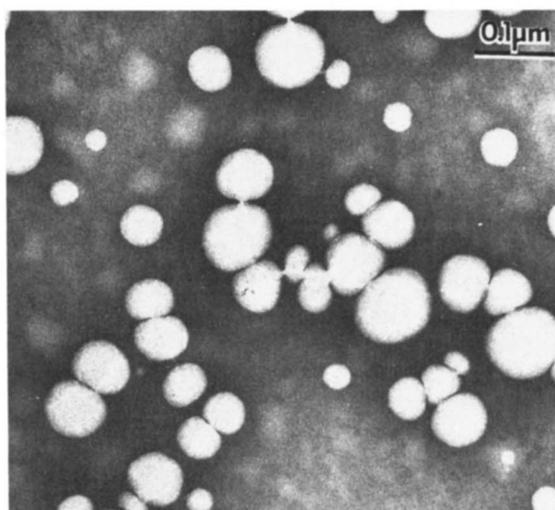

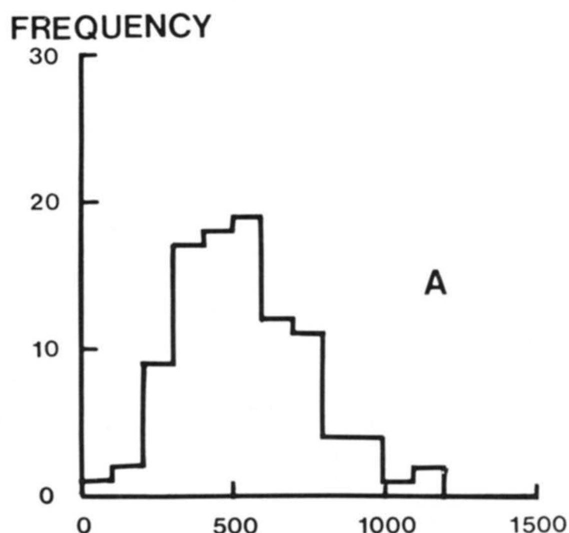

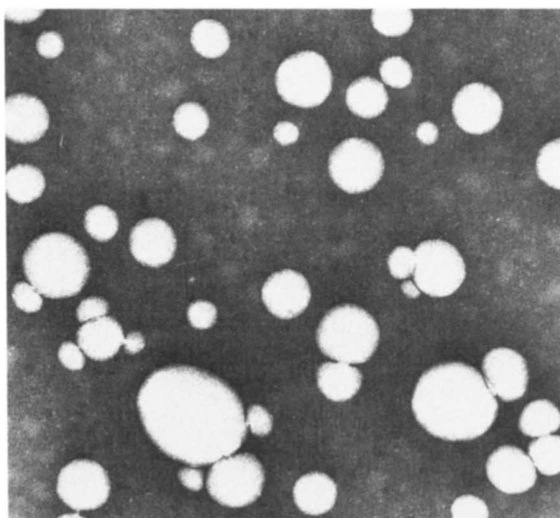

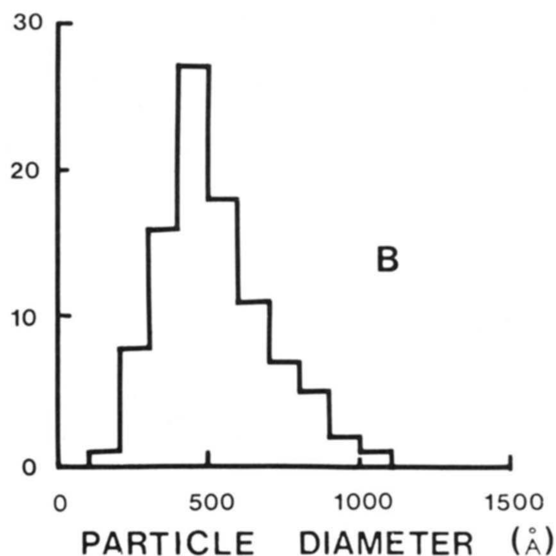

Fig. 4. Plasma VLDL (A) and hepatic lymph VLDL (B) were negatively stained with 2% phosphotungstate (pH 5.9) and examined with a Zeiss 10 electron microscope. The magnification of both micrographs was ×100,000 (slightly reduced in reproduction). The histogram on the right represents the distribution of the mean of two measurements perpendicular to each other of 100 particles.

The relative percentages of the various apolipoproteins derived from densitometric scans of SDS-PAGE are presented in **Table 2.** Both apoB and apoE were present as the major apolipoproteins in hepatic lymph and plasma VLDL. The ratio of apoE to apoB was about 3:2 in both types of lipoproteins. One of the major differences between the hepatic lymph VLDL and plasma VLDL was that the former had considerably less apoC than plasma VLDL and this difference was statistically significant ($P < 0.001$). The other major difference was in the relative proportions of the two apoB species ($apoB_{240K}$ and $apoB_{330K}$) in hepatic lymph VLDL and the plasma VLDL. Plasma VLDL had almost equal proportions of $apoB_{330K}$ and $apoB_{240K}$. Unlike plasma VLDL, hepatic lymph VLDL contained predominantly $apoB_{240K}$ (94% of the total apoB) with $apoB_{330K}$ present only in trace quantities. Unlike both plasma and hepatic lymph VLDL, intestinal lymph VLDL contained apoA-I, apoA-IV, $apoB_{240K}$, apoC, and small amounts of apoE. The only apoB present in intestinal lymph VLDL was $apoB_{240K}$. Since both apoA-I and apoA-IV were completely absent in hepatic lymph VLDL, contamination

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TABLE 1. Lipid and protein composition of hepatic lymph VLDL and plasma VLDL (% composition by weight)

	Hepatic Lymph VLDL	Plasma VLDL
Phospholipids	40.8 ± 4.9 (12)[a]	18.6 ± 1.5 (12)
Cholesterol	3.9 ± 0.4	3.5 ± 0.3
Cholesteryl ester	4.8 ± 0.8	6.3 ± 0.6
Triglyceride	40.5 ± 4.2	61.9 ± 1.7
Protein	10.1 ± 1.1	9.7 ± 0.7

[a] Mean ± SEM; number of animals used in parentheses.

of hepatic lymph by intestinal lymph could be detected by the presence of these apolipoproteins.

DISCUSSION

Nascent VLDL and HDL are thought to be secreted into the space of Disse (12–14). The nascent VLDL and HDL in the interstitial fluid of the space of Disse can then be transported in the hepatic lymph or the bloodstream (vascular pathway). Consequently, the hepatic lymph duct-cannulated rat model may prove to be an excellent in vivo model for studying nascent lipoprotein secretion by the liver. In this study we have described for the first time the various physical and chemical characteristics of rat hepatic lymph VLDL (lipoproteins of $\rho < 1.006$ g/ml). Furthermore, we have provided evidence to show that the composition of hepatic lymph VLDL is different from that of plasma VLDL and therefore is not derived from VLDL filtered from the blood.

Before explaining why hepatic lymph contains mainly the VLDL secreted by the liver, it is important to consider the anatomy of the endothelium separating the sinusoid and the space of Disse. Morphologically, it has been demonstrated that the sinusoidal endothelium, separating the sinusoid and the space of Disse, has fenestrations averaging 1,000 Å in diameter or smaller (46–48). Thus, there should be free exchange of VLDL particles between the sinusoid and the space of Disse. However, these observations should be interpreted carefully since it can be misleading to interpret a dynamic process from static electron micrographs. Furthermore, other evidence seemed to indicate considerable sieving of the endothelium. Consequently the exchange of VLDL between the space of Disse and the sinusoid may be limited. Dive, Nadalini and Heremans (49) studied the concentration of six plasma proteins in hepatic lymph and plasma. They showed that there are two components for the hepatic transfer of plasma proteins from serum to lymph. One component involves the bulk transfer and is independent of molecular size and the other is separated by molecular sieving. They estimated the limits of the semipermeable barrier to be 100 Å in diameter. The other evidence is that despite the high protein content of hepatic lymph, it never exceeds 80–90% of the plasma protein concentration (15, 16). The explanation for this difference has been that the peribiliary capillary system, which carries blood only from the hepatic artery, has a basement membrane and thus a different permeability than the sinusoidal membranes. As a result of the admixing of the filtrate from these arterial capillaries, there is a lower protein concentration in hepatic lymph (50). By obstructing the hepatic artery, Szabo and Magyar (50) could not demonstrate a change in hepatic lymph protein concentration and thus concluded that there might be some restriction to the passage of large protein molecules by the sinusoidal membrane. Third, when blue dextran 2000 (average mol wt 2,000,000) was injected intravenously, the lymph/plasma ratio equilibrated at 0.2–0.3 (19). Thus the author concluded that the passage of blue dextran from sinusoidal plasma to liver lymph was restricted. Lastly, by continuous intravenous infusion of spherical particles of polymethylmethacrylate up to 700 Å in diameter (of the same order of VLDL), Grotte, Juhlin, and Sandberg (46) found passage of the particles from plasma into hepatic lymph. However,

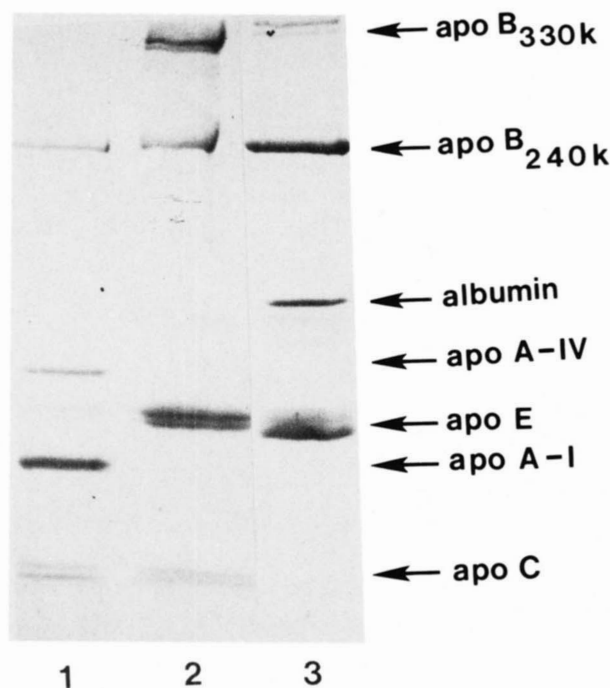

Fig. 5. Forty micrograms each of the hepatic lymph VLDL, intestinal lymph, and plasma VLDL were separated by gradient (3.5–27%) polyacrylamide gel electrophoresis. The two apoB species as described by Krishnaiah et al. (35) were well separated in our system. Gels 1, 2, and 3 correspond to intestinal lymph VLDL, plasma VLDL, and hepatic lymph VLDL, respectively.

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TABLE 2. Distribution of apolipoproteins in hepatic lymph VLDL, plasma VLDL, and intestinal VLDL

	Apolipoproteins (% of total dye uptake)[a]					
	$ApoB_{330K}$	$ApoB_{240K}$	ApoA-IV	ApoE	ApoA-I	ApoC
Hepatic lymph VLDL (n = 4)[b]	2.2 ± 1.0[c]	34.9 ± 2.5		61.4 ± 2.7		1.6 ± 0.6
Plasma VLDL (n = 4)	14.3 ± 1.1	18.6 ± 2.8		58.8 ± 2.9		8.3 ± 1.0
Intestinal lymph VLDL (n = 5)		24.9 ± 3.7	13.6 ± 1.0	5.5 ± 1.4	48.4 ± 5.8	7.6 ± 1.3

[a] The % distribution of total dye uptake by protein is calculated from the densitometric scanning of SDS-PAGE gradient gel (3.5–27% polyacrylamide) stained with Coomassie blue R-250.
[b] n, Number of observations.
[c] Mean ± SE.

there was considerable sieving, so that the lymph/plasma ratio equilibrated at 0.2, compared with 0.8 or more for albumin. The above observations suggest that the exchange of VLDL particles between the sinusoid and the space of Disse is limited. Consequently, the hepatic lymph should contain a significant concentration of nascent lipoproteins secreted directly by the hepatocytes.

Although the anatomy of the rat hepatic lymphatic system has not been well described, it is probably not too different from that of the dog. In the dog, approximately 80% of the hepatic lymph leaves the liver by the hilar lymph duct and empties into the cisterna chyli (51). The remaining 20% of the hepatic lymph in lymph vessels associated with the central vein does not empty into the thoracic duct. Instead, the lymph vessel passes in association with the hepatic veins into retrosternal lymphatics which eventually empty into large veins in the neck. The hepatic lymph duct that we have cannulated is the major hilar hepatic lymph duct. As described earlier, we have observed a lymph vessel connecting the hepatic and intestinal lymph duct. This connection has also been observed by others (22). Therefore it is extremely important to have this lymph vessel ligated for the collection of pure hepatic lymph or intestinal lymph.

The flow rate of the hepatic lymph and its lipid concentration determined in our studies is comparable to that observed by Friedman, Byers, and Omoto (18). The triglyceride output in hepatic lymph is small (approximately 1% of total hepatic lipid output) when compared with the triglyceride output in isolated perfused liver (5). Thus, the hepatic lymph is probably not the major route used for the transport of the newly secreted hepatic VLDL. However, it would still be a very useful model for studying the composition of the newly secreted hepatic VLDL. Currently we are studying the quantity and the composition of the newly secreted VLDL transported by the lymph and the bloodstream.

When hepatic lymph was analyzed by agarose gel electrophoresis, two major lipoprotein bands were observed: α-migrating HDL and the hepatic VLDL. The α-migrating HDL moved faster than the plasma HDL. There are at least two possible explanations for this. First, if the hepatic lymph is newly secreted by the liver, it may have a different electrophoretic mobility than plasma HDL. It is also possible that the HDL in hepatic lymph was derived from plasma through filtration and, therefore, the smaller HDL particles that have greater electrophoretic mobility were preferentially filtered. Further experiments are needed to test the two explanations. The hepatic lymph VLDL migrated between the plasma β and the pre-β lipoprotein. This observation is of interest since it has been shown that Golgi VLDL and VLDL recovered from liver perfusates migrated electrophoretically more slowly than plasma VLDL (11, 52). Thus, this electrophoretic pattern suggests that the major hepatic lymph VLDL (represented by the slow migrating pre-β band) could be nascent VLDL secreted by the liver. Also present in hepatic lymph was a faint band of pre-β mobility. This band may represent a small amount of VLDL filtered from the plasma of the sinusoidal space into the space of Disse.

By electron microscopy no differences were observed between the size of hepatic lymph and plasma VLDL. However, there were striking differences in their chemical composition. There was significantly more phospholipid in hepatic lymph VLDL (40% of mass) than in plasma VLDL or the VLDL isolated from recirculating liver perfusates (17% of mass) (11). It has been suggested by Chajek and Eisenberg (53) and Eisenberg and Olivecrona (54) that the lipolysis of triglyceride-rich lipoproteins (e.g., VLDL) results in the formation of HDL precursors. Thus, it is tempting to speculate that the conversion of the phospholipid-rich hepatic lymph VLDL to plasma VLDL probably involves the formation of HDL during lipolysis of the hepatic VLDL. This results in the loss of phospholipid from the hepatic

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VLDL and thereby makes the chemical composition resemble that of VLDL. Experiments are now being conducted to investigate this hypothesis. The high phospholipid content of hepatic lymph VLDL was associated with a significantly lower triglyceride content. Another difference between the two lipoprotein populations was the higher cholesterol/cholesteryl ester ratio in hepatic lymph VLDL. This marked difference between hepatic lymph and plasma VLDL seems to support the hypothesis that hepatic lymph VLDL is composed mainly of newly secreted hepatic VLDL and therefore is not derived from lipoprotein filtered from the plasma. This is also supported by the fact that the VLDL isolated during nonrecirculating liver perfusion studies is quite similar to the hepatic lymph VLDL, having about 40% by weight of the total lipid as phospholipid (55). This observation may reflect the true phospholipid composition of nascent hepatic VLDL since VLDL obtained from nonrecirculating perfusates and the hepatic lymph are both not susceptible to further uptake or modification by the hepatocytes once they have been secreted.

The apolipoprotein composition of hepatic lymph VLDL was also markedly different from plasma VLDL. The hepatic lymph contained considerably less apoC compared to plasma VLDL. Furthermore, it has predominantly the small molecular weight apoB ($apoB_{240K}$) whereas the plasma VLDL had almost equal proportions of both apoB ($apoB_{330K}$ and $apoB_{240K}$). The intestinal lymph VLDL was different in apolipoprotein composition from hepatic lymph VLDL and plasma VLDL in that it contained apoA-I and apoA-IV. There are considerable similarities between hepatic lymph VLDL apolipoprotein composition and those reported by others on newly secreted VLDL harvested from isolated liver perfusates and also hepatocyte cell cultures (10, 11). The markedly reduced apoC content of hepatic lymph VLDL as compared to plasma VLDL has also been observed in hepatic perfusate VLDL (11) and the Golgi VLDL (52). It has recently been reported by Bell-Quint, Forte, and Graham (10) that the major apoB component of VLDL recovered from hepatocyte cultures was $apoB_{240K}$. The fact that hepatic lymph VLDL has a markedly different apoB composition compared to plasma is of importance since it supports the hypothesis that the majority of hepatic lymph VLDL is newly secreted by the liver. This is because it has been shown that $apoB_{240K}$ has a higher turnover rate than $apoB_{330K}$ and therefore is removed from the circulation faster than $apoB_{330K}$ (28, 36). Since plasma VLDL has equal proportions of both apoB species, it would not be possible for the hepatic lymph VLDL to contain predominately the $apoB_{240K}$ if it originated from the filtration of plasma VLDL from the sinusoid into the space of Disse. Consequently, the best explanation for the apoB composition of hepatic lymph VLDL is that these particles are secreted directly by the liver into the lymph. It is interesting that hepatic lymph VLDL has a much higher $apoB_{240}/apoB_{330}$ ratio than VLDL obtained from isolated perfused liver (56). It is possible that a small fraction of the $apoB_{240}$-rich VLDL particles tends to be retained by the endothelial lining of the sinusoids and disproportionately populates the hepatic lymph. This observation would also seem to support the hypothesis that newly secreted hepatic VLDL particles contain either $apoB_{240}$ or $apoB_{330}$.

Thus, we have isolated and characterized for the first time lipoproteins of density < 1.006 g/ml from rat hepatic lymph. They differ from plasma VLDL by having: *1*) a high phospholipid content; *2*) a higher cholesterol/cholesteryl ester ratio; *3*) considerably less apoC; and *4*) predominantly $apoB_{240}$. The unique lipid and apolipoprotein composition would indicate their origin as nascent particles secreted into the lymph without undergoing metabolic transformation. Further studies using the hepatic lymph duct-cannulated rat model should provide important information about the factors regulating the secretion and composition of hepatic lipoproteins. They will also enhance our understanding of the metabolic events that occur when such lipoproteins enter the plasma compartment.

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