Very-Low-Density Lipoprotein in Intestinal Lymph: Evidence for Presence of the A Protein

Abstract. *Rat mesenteric lymph very-low-density lipoproteins of intestinal origin contain components which are antigenically identical to plasma high-density lipoprotein. Because the antigenicity of the latter most likely resides in its apoprotein (the A protein), it is concluded that intestinal very-low-density lipoproteins contain the A protein. This and other evidence supports the concept that the intestine is a source of plasma very-low-density lipoprotein.*

Very-low-density lipoproteins (VLDL) are the major means of transport of endogenous triglyceride in plasma (*1*). These particles are lighter than plasma (density less than 1.006, S_f 20 to 400) and on paper electrophoresis they have the mobility of an α_2 ("pre-beta") globulin. There is ample evidence for the hepatic production of plasma VLDL, but there have been only limited studies suggesting that the intestine may be a source of these particles (*2*).

In studies of the lipoproteins of rat mesenteric lymph, we observed that VLDL of *intestinal* origin enter the circulation of rats in the fasting state (*3*, *4*). These particles were similar to plasma VLDL in percentage of lipid and protein composition and showed α_2 electrophoretic mobility in agarose gel. Although these findings provided support for the concept that the intestine is a source of endogenous plasma VLDL, it was important to characterize the apoproteins of intestinal VLDL.

Plasma VLDL contain at least two proteins (A and B) (*5*), which appear to be similar to the apoproteins of the high-density (α_1 or HDL) and low-density (β or LDL) lipoproteins, respectively (*6*). There is direct and indirect evidence for the presence of B protein in *intestinal* VLDL and chylomicrons as well (*7*). However, the presence of A protein in the intestinal particles has been less conclusively established. A peptide similar to A protein has been demonstrated in chylomicrons ($S_f > 600$) of human chylothorax fluid and dog thoracic duct lymph (*8*), and in those ($S_f > 10^4$) of human plasma (*9*). However, in these experiments it is possible that the A protein may have reflected the presence of lipoproteins of hepatic origin.

In this report we present immunochemical evidence for the presence of A protein in VLDL of intestinal origin. This finding, together with our previous observations, lends strong support to the concept that the intestine is a source of plasma VLDL.

We isolated VLDL (*10*, *11*) from the mesenteric lymph of fasting rats, and emulsified them in complete Freud's adjuvant. Three milliliters of emulsion, containing 270 μg of lipoprotein protein, were injected into the hind footpads of rabbits. After 10 days, a second intradermal injection (1 ml, 90 μg) was given at multiple sites. Four weeks after the initial injection, rabbits were bled, and the antiserum to lymph VLDL was separated.

Immunoelecrophoresis was performed in 1 percent agar (*12*), by using the apparatus of Wieme (*13*), with pentane as coolant. Similar results were obtained by using standard apparatus, and ice water as coolant. Dried gels were stained for lipid with oil red O in methanol–acetic acid–water. Partial delipidation of VLDL was done as described by Levy *et al.* (*5*).

Following electrophoresis of fresh rat serum in agar gel, two parallel linear streaks emanating a short distance from the origin toward the anode were produced by the addition of antiserum to lymph VLDL to the troughs (Fig. 1A). A longer precipitin arc, which characteristically had two components differing slightly in electrophoretic mobility (Fig. 1A), formed in the gel between the origin and the anodal region (*14*). Both the arc and the streaks nearer the origin stained with oil red O. In addition, some oil red O staining material remained at the periphery of the well.

On immunoelectrophoresis of the density 1.006 infranatant of rat serum, the anodal end of the lipid-staining material near the origin was diminished (Fig. 1B), suggesting that a portion of this material consisted of VLDL. Following additional centrifugation at densities 1.035 and 1.063, the remaining lipid-staining zones near the origin decreased, then disappeared (Fig. 1, C and D), suggesting that this material was LDL. The persistence of the major precipitin arcs in the density 1.063 infranatant indicates that the antigens involved in the formation of these arcs were HDL. These arcs were not seen with unconcentrated density 1.21 infranatant.

Immunoelectrophoresis of lymph and purified lymph VLDL also revealed oil red O staining streaks on the anodal side of the origin (Fig. 1, E and F). This zone was markedly diminished with the density 1.006 infranatant, suggesting that most of this material was due to VLDL (Fig. 1G). The precipitin arcs attributed to HDL in serum were not detected in fresh lymph. When purified lymph VLDL were stored at 4°C for several days, however, a faint precipitin arc resembling that of serum HDL was observed.

Following delipidation of lymph VLDL, two precipitin arcs were formed (Fig. 2). On comparing delipidated VLDL with whole serum by the interrupted trough technique, we found these two precipitin arcs merged with those of serum HDL. This reaction indicates antigenic identity between the two components of intestinal lymph VLDL and those of serum HDL. At the end nearer the origin in Fig. 2, only one reaction of identity is seen, with an unmatched HDL precipitin line extending toward the VLDL side. This pattern was most likely due to inadequate concentrations of the corresponding component of delipidated VLDL in this region of the gel.

In additional studies a comparison of

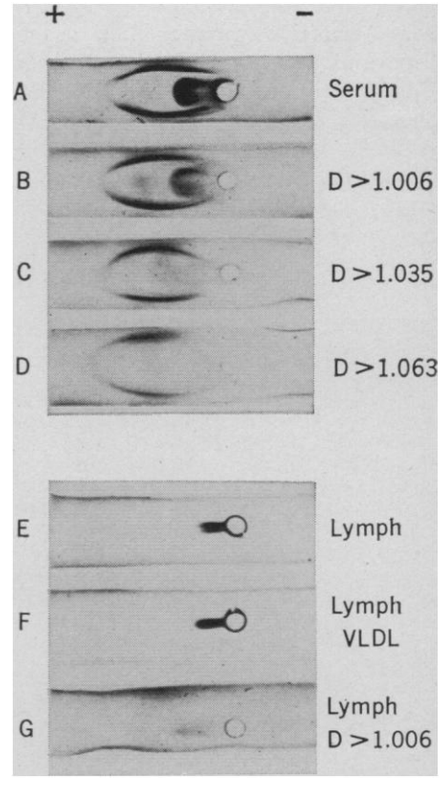

Fig. 1. Immunoelectrophoresis of rat serum and intestinal lymph, as well as subfractions obtained by preparative ultracentrifugation (10^5 *g*-min). Antiserum to lymph VLDL (0.05 ml) was applied to all troughs; slides were stained with oil red O.

serum with delipidated intestinal lymph chylomicrons ($S_f > 400$) showed results similar to those obtained with delipidated VLDL.

These studies demonstrate that delipidated rat intestinal lymph VLDL contain two components which are antigenically identical to two components of native serum HDL. In all probability the antigenicity of these components resides in the apoprotein. Since the only apoprotein known to be associated with the mammalian HDL is the A protein, it is concluded that this protein is present in VLDL of intestinal origin.

Although these studies do not directly demonstrate B protein in intestinal lymph VLDL, the fact that antiserum to lymph VLDL reacts with serum LDL is indirect evidence for its presence. Moreover, Windmueller and Levy have previously detected B protein in rat intestinal lymph by using specific antiserum to LDL (*15*). The available evidence, however, suggests that B protein is quantitatively a minor peptide of plasma and lymph VLDL, and chylomicrons, in the rat as well as in dog and man (*8, 16, 17*).

In this light, it seems paradoxical that only reductions in the availability of B protein have been associated with impaired glyceride transport, either in the liver or the intestine. Thus, patients with β-lipoprotein deficiency (abetalipoproteinemia) have steatorrhea (*18*), and inhibition of hepatic β-lipoprotein production in rats leads to impaired triglyceride release from the liver (*19*). However, patients with hereditary α-lipoprotein (A protein) deficiency have no impairment in their ability to transport either exogenous or endogenous triglycerides (*1*).

The presence of a third peptide with *N*-terminal serine and threonine residues was noted earlier by Rodbell (*17*) and has also been studied recently by Gustafson *et al.* (*20*). Our studies of intestinal VLDL failed to detect the presence of this so-called C protein. Its precise role in lipid metabolism and transport is unclear (*1*).

The immunoelectrophoretic demonstration of two components of HDL in native serum was somewhat unexpected. Levy and Fredrickson (*21*) have shown that delipidation and ultracentrifugation cause heterogeneity of HDL which in fresh plasma or serum are essentially homogeneous. While this mechanism could account for our demonstration of two components of delipidated VLDL, it is noteworthy that antiserum to lymph VLDL revealed two components of HDL in serum (and in plasma) which had not been subjected to treatments known to introduce heterogeneity. Such heterogeneity does not necessarily indicate the presence of different peptides, but may reflect differences in the aggregation of peptide subunits, or in the degree of association with lipid.

Although we have not definitely identified the source of the A protein, the lipids in intestinal lymph VLDL are derived from the intestinal lumen and mucosa (*3*), and the final lipoprotein "package" therefore appears to be assembled within the absorptive cell or as the lipid leaves the cell. Demonstration of the A protein in VLDL of intestinal origin, together with other evidence for the similarity between intestinal lymph VLDL and plasma VLDL, supports the concept that the intestine is one of the sources of endogenous plasma VLDL. The quantitative significance of this source remains to be determined.

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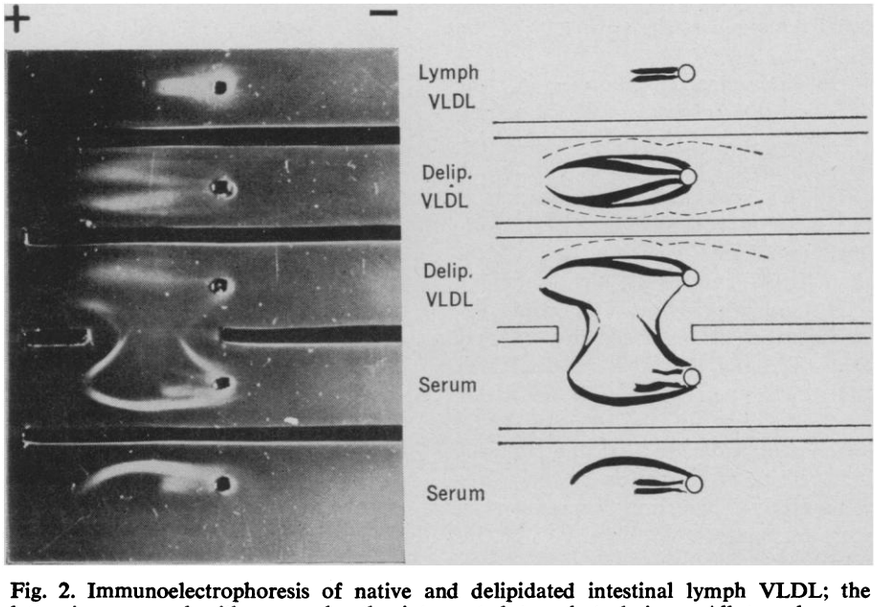

Fig. 2. Immunoelectrophoresis of native and delipidated intestinal lymph VLDL; the latter is compared with serum by the interrupted trough technique. All troughs contained antiserum to lymph VLDL. Broken lines on the schematic drawing indicate the presence of albumin and β-globulin precipitin arcs.

References and Notes

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4. Definitions, unless otherwise indicated: "chylomicron," a triglyceride-rich lipoprotein particle of intestinal origin having an S_f value of >400 [D. B. Zilversmit, *Fed. Proc.* **26**, 1599 (1967)]; "VLDL," particles of S_f 20 to 400 regardless of origin. The latter are considered by some to be chylomicrons if they originate in the intestine; however, studies of chylomicrons have for the most part dealt with the lighter particles ($S_f > 400$) and there has been little information on the properties of the heavier group of intestinal triglyceride-rich particles.
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10. Chylomicrons ($S_f > 400$) were separated by preparative ultracentrifugation (Spinco SW 25.1 rotor; 3×10^6 *g*-min), and VLDL (S_f 20 to 400) subsequently separated from the lymph subnatant by recentrifugation at 10^8 *g*-min. The VLDL were then washed twice, each time by layering them under normal saline, and centrifuging at 10^8 *g*-min. A saline suspension of this purified VLDL (protein concentration 0.37 mg/ml) was examined by immunoelectrophoresis by using rabbit antiserum to rat serum proteins; no precipitin lines were observed.
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