The lecithin–cholesterol acyl transferase activity of rat intestinal lymph

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Abstract The lecithin–cholesterol acyl transferase (LCAT) activity in rat mesenteric lymph was examined as a possible source of chylomicron cholesteryl ester. Lymph activity was only 2–3% of rat serum activity. Removal of $d < 1.006$ lipoproteins increased lymph LCAT activity, but only to 6–8% of that of serum. Relative to total cholesterol in the $d > 1.08$ g/ml fractions, lymph LCAT activity in lymph from fasting rats was less than serum, but in lymph from nonfasting rats the ratio LCAT/HDL-cholesterol reached levels greater than serum, suggesting a contribution of enzyme from the gut. Both LCAT activity and HDL concentration in mesenteric lymph increased during feeding.

Subfractions of lymph that inhibited serum LCAT were: chylomicrons, VLDL, chylomicron lipid, VLDL apoprotein, and HDL apoprotein. In the rat, the low LCAT activity of mesenteric lymph was in part due to the low enzyme concentration present, and the activity was apparently lowered further by lipid-rich lipoproteins that inhibited the reaction. Enzyme inhibition due to the apoprotein fractions of lipoproteins is probably minor in the rat in vivo.

Supplementary key words cholesterol metabolism · intestinal enzymes · lipoproteins

All vertebrate plasma contains cholesterol (UC) and cholesteryl esters (CE). Their metabolism differs but, at present, many aspects of their origins and fates remain unknown. One major source of both UC and CE is the small intestine (1). Cholesterol present in the intestinal lumen is absorbed entirely as UC (2, 3) and is largely reesterified within mucosal cells (4). UC and CE are then secreted by the mucosal cells and enter lymphatic channels in the triglyceride-rich lipoproteins, chylomicrons, and very low density lipoproteins (VLDL) (5). The CE in mesenteric lymph thus has several potential sources. (*a*) Since lymph is a filtrate of plasma, some plasma lipoprotein CE may enter the lymph directly via interstitial spaces. (*b*) CE may originate within mucosal cells by reesterification of absorbed or newly synthesized UC. (*c*) CE may be formed from UC within lymphatic channels by the action of the enzyme lecithin–cholesterol acyl transferase (LCAT:EC 2.3.1.43).

In the present study, the LCAT activity of rat mesenteric lymph has been examined. The experimental results show that the lymphatic LCAT activity is very low compared with plasma, and that both the lipid and the protein components of lymph lipoproteins may exert inhibitory effects of LCAT activity.

MATERIALS AND METHODS

Animal methods

Mesenteric lymph, uncontaminated with hepatic lymph, was collected from male Wistar rats, 3–4 months of age. The animals were anesthetized with diethyl ether and the main mesenteric lymphatic vessels were exposed through a right paramedial incision. The inferior lymph duct was occluded and the superior duct was cannulated as described by Warshaw (6), using a polyvinyl cannula, ID 0.5 mm. A duodenal cannula of flanged polyvinyl tubing, ID 0.5 mm, was also implanted in the first portion of the duodenum. The animals were then placed in Bollman-type restraint cages for the duration of the experiment. Duodenal infusions of NaCl solution (0.9%) containing KCl (0.03%), or an emulsion of 3% corn oil (w/v) stabilized by vegetable lecithin (1.2%) and Pluronic F-68 (0.3%) in a 4.15% glucose solution, were administered as indicated in Results. In some experiments (see Results) water, or rat chow containing 5% fat,[2] was allowed ad libitum.

Abbreviations: LCAT, lecithin–cholesterol acyl transferase; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; UC, unesterified cholesterol; CE, cholesterol ester.

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[2] The diet contained 5% fat, 6% fiber, 20% protein, and 65% carbohydrate by weight (Peter Larsen & Co. A/S, Oslo, Norway).

Lymph collection and serum lipoprotein separations

Mesenteric lymph was collected in ice-cooled plastic containers without anticoagulant. Blood was collected without anticoagulant from the abdominal aorta and was allowed to clot at 0°C. Chylomicrons and VLDL were separated at 4°C by ultracentrifugation of defibrinated lymph or blood serum, either undiluted or, after dilution with 0.85% NaCl containing 0.1% EDTA, according to standard conditions (7). Low density lipoproteins (LDL) were removed after adjustment of the density to 1.063 g/ml with solid KBr and discarded. High density lipoproteins (HDL) were then collected by flotation after further addition of KBr to achieve a density of 1.21 g/ml. In some experiments all lipoproteins of density <1.08 g/ml were removed together by addition of KBr to whole lymph or serum followed by ultracentrifugation at 40,000 rpm for 20 hr in a 40.3 rotor; the whole infranatant, containing only HDL and higher density proteins (8), was then used in certain experiments as described.

Estimation of lecithin–cholesterol acyl transferase (LCAT)

Both the method of Stokke and Norum (9) and that of Glomset and Wright (10) were used, as indicated in Results. The Stokke–Norum method measures the rate of CE formation in a system such as lymph or blood serum and the value obtained is dependent both on the amount of LCAT enzyme and on the available substrates. [^{3}H]Cholesterol tracer was allowed to equilibrate with endogenous lipoprotein at 37°C during a 4-hr preincubation period during which LCAT was inhibited with a disulfide (5,5-dithiobis-2-nitrobenzoic acid, DTNB). The enzyme was then reactivated with mercaptoethanol. The reaction was allowed to proceed for 20 or 30 min and was then stopped by addition of chloroform–methanol 2:1. Lipids were extracted essentially according to Folch et al. (11) and were stored in hexane.

The Glomset–Wright method, on the other hand, reflects more closely the amount of LCAT enzyme present since reaction rates are determined in the presence of a large excess of substrate in the form of heat-inactivated serum. Preequilibration with [^{3}H]-cholesterol was omitted because the incubations continued for 4–8 hr. The samples were extracted and processed as above.

Preparation of apoproteins

VLDL from lymph and plasma were washed twice by recentrifugation through 0.85% NaCl containing 0.1% EDTA, density 1.006 g/ml. HDL was washed once through a KBr solution of density 1.21 g/ml containing 0.1% EDTA. The apoproteins were delipidated at 4°C with 50 volumes of absolute ethanol–diethyl ether 3:2 and were washed twice with diethyl ether.

Chemical methods

Protein was determined according to Lowry et al. (12) or by measurement of the optical density at 280 nm.

Cholesterol was measured by gas–liquid chromatography (Varian-Aerograph Model 1400 Varian Associates, Palo Alto, CA) on a 50 cm SE 30 column (3% on Chromosorb W, HP) at 250°C using stigmasterol as internal standard. CE was calculated by the difference between UC and total cholesterol, respectively determined before and after hydrolysis at 85°C for 1 hr in 1 N ethanolic KOH.

Glyceride–glycerol was determined enzymatically (13) and was assumed to represent triglyceride.

Phospholipid determinations were performed essentially according to the method of Bartlett (14) with minor modifications in the case of chylomicrons.

Materials

7α-[^{3}H]Cholesterol was from Radiochemical Center, Amersham, England, sp act 12.6 mCi/μmol; it was repurified by thin-layer chromatography. Final purity was better than 99.8%. Vegetable lecithin was from Eastman Kodak Co., Rochester, NY. Pluronic F-68 was from Wyandotte Chemical Co., Wyandotte, MI. 5,5-Dithiobis-2-nitrobenzoic acid (DTNB) was from Sigma Chemical Co., St. Louis, MO. SE 30, 3% on Chromosorb W (HP) was from Pierce Chemical Co., Rockford, IL. Stigmasterol standard was from Sigma Chemical Co., St. Louis, MO. Cholesterol standard was from British Drug Houses, Ltd., Poole, England. Permablend was from Packard Instrument Co., Downers Grove, IL. Reagents for glycerol determinations were obtained from Boehringer, Mannheim, GMBH, Germany. Intralipid was the generous gift of Vitrum, Stockholm, Sweden.

RESULTS

CE formation in mesenteric lymph

Esterification of [^{3}H]cholesterol by fresh lymph collected during the first 24 hr after cannulation from either fed or fasted rats is shown in **Table 1.** LCAT activity in fasting lymph was very low compared with blood serum and was reduced still further in lymph containing a high concentration of triglyceride-rich lipoproteins.

Since LCAT is reported to circulate with HDL in the plasma (15, 16) and since the HDL concentration

TABLE 1. Cholesterol esterification by rat mesenteric lymph

	No. of Animals	nmol CE/hr/ml
Fasting lymph	2	6.7, 4.8[a]
Nonfasting lymph	6	1.85 ± 0.41[b]
Nonfasting serum	5	102.2 ± 5.2[b]

Rats were duodenally infused with saline solution at a rate of 1.1 ml/hr during lymph collection and were allowed water ad libitum. Fasting rats were deprived of food for 48 hr. Nonfasting rats ate about 10 g of rat pellets per day. Lymph flow varied between 0.5 and 3 ml/hr. Lymph samples were collected at 0°C for one day. Cholesterol esterification was determined by the method of Stokke and Norum (9).

[a] Individual data.

[b] Mean ± SEM.

in rat mesenteric lymph was found to be approximately 15–20% of that in plasma[3], a LCAT activity several-fold higher than the one actually found in lymph might have been expected. Several possible explanations were considered. The low activity could have been caused by a low substrate concentration for the reaction, either UC or phosphatidylcholine, by a genuinely low level of enzyme which then might imply dissociation of LCAT from HDL, by a reduced concentration of activators for the LCAT reaction, and/or by the presence of inhibitors.

When heat-inactivated (56°C for 1 hr) blood serum containing activators and substrates was added in equal volume to nonfasting lymph from one rat, the LCAT activity determined by the Stokke–Norum method remained essentially unchanged (2.9 compared with a 3.3 nmol CE/hr per ml). This suggested that lack of substrates or activators was not the major reason for the low LCAT activity.

In order to determine whether the enzyme concentration per se was low, LCAT activity in the lymph of two nonfasting animals was also measured in the presence of a large excess of substrate (Glomset–Wright method). The results, shown in **Table 2**, indicated that the amount of LCAT in lymph was indeed low. However, the cholesterol esterification rate was nevertheless approximately doubled when measured by the Glomset–Wright method compared with the Stokke–Norum method. This suggested that an inhibitor for the reaction might be present in lymph, which was being diluted by the large volume of inactivated blood serum.

Triglyceride has been reported both to stimulate (17) and to inhibit (18) the LCAT reaction. LCAT activity therefore was measured in lymph from which the triglyceride-rich lipoproteins of density <1.006 g/ml had been removed by ultracentrifugation. In two fed animals the Stokke–Norum LCAT activity was 6.0 and 6.9 nmol CE formed/hr per ml after ultracentrifugation compared with 1.6 and 0.9 nmol CE/hr per ml in whole lymph, which suggested that chylomicrons and VLDL present in the lymph had inhibited the LCAT reaction. However, after their removal the reaction rate was still low compared with blood serum LCAT activity.

Comparison of lymph and blood serum HDL and LCAT

Lymph was collected continuously in 12- to 24-hr aliquots from two lymph fistula rats that were initially fasted with access to water only for two days, and then were refed a diet containing 5% fat ad libitum. All lipoproteins of density less than 1.08 g/ml were removed by ultracentrifugation from the lymph samples and also from blood serum collected from five normal rats, in order to retain only HDL (8). The total cholesterol concentration of the d > 1.08 g/ml fraction was used as a measure of HDL concentration in preference to lipoprotein protein estimations, because greater accuracy could be attained without the repeated ultracentrifugal washing necessary to remove albumin. The alternative immunological method for assaying HDL apoprotein may also be subject to significant error, since newly synthesized gut HDL apoprotein composition may differ from that of blood serum HDL.

The LCAT activity in the d 1.08 g/ml lymph infranatants determined by the Glomset–Wright method (**Table 3**) was found to be low compared with that of blood serum, especially during fasting. However, upon refeeding, the LCAT activity in lymph infranatant increased five- to sixfold.

In the fat-fed state, capillary filtration of plasma increases, leading to increases in both lymph flow and plasma protein secretion into lymph (19). In the present experiments, the lymph HDL-cholesterol concentration (total cholesterol per ml lymph in the d 1.08 g/ml infranatant) approximately doubled dur-

TABLE 2. Comparison of Stokke–Norum and Glomset–Wright determinations in nonfasting lymph

	nmol CE formed/hr/ml	
	Rat 1	Rat 2
Stokke–Norum	1.44	1.28
Glomset–Wright	2.02	3.00

Rats were duodenally infused with saline at a rate of 1.1 ml/hr and were allowed water ad libitum. Lymph was collected at 0°C on the third and fourth days after lymph cannulation. The animals were fasted for the first 48 hr and then refed ad libitum, ingesting about 10 g/day.

[3] Lymph was collected during the first 6–8 hr after lymph cannulation (Bennett Clark, S. unpublished experiments.)

TABLE 3. Relationship between LCAT activity and lipoprotein concentration in density > 1.08 g/ml fraction from mesenteric lymph and aortic serum

	LCAT Activity[a]	HDL-Cholesterol[b]	LCAT:HDL-Cholesterol Ratio
Rat 1 lymph fasting	0.94[c]	28	0.034
	0.15	24	0.006
	2.09	43	0.049
nonfasting	6.97	61	0.114
	8.56	53	0.162
	8.24	62	0.133
Rat 2 lymph fasting	1.93[c]	44	0.044
	1.17	36	0.033
	1.96	55	0.036
nonfasting	9.76	106	0.092
	7.04	83	0.085
	9.65	91	0.106
Nonfasting normal serum (n = 5)	39.4 ± 5.3[d]	552 ± 36	0.072 ± 0.008
Nonfasting normal serum preincubated with d < 1.08 g/ml lipoprotein	44, 53[e]	553, 662	0.079, 0.081

Lymph was collected at 0°C for 4 days. During fasting periods the rats received only water to drink ad libitum. Water and rat pellets containing 5% fat, 6% fiber, 20% protein, and 65% carbohydrate by weight were allowed during nonfasting periods.

[a] LCAT is expressed as nmol CE/hr per ml lymph or serum formed by the d 1.08 g/ml infranatant. For details see Methods (Glomset–Wright).

[b] HDL-cholesterol is expressed as nmol total cholesterol/ml lymph or serum in d 1.08 g/ml infranatant.

[c] Serial 12–24 hr lymph collections.

[d] Mean ± SEM.

[e] Individual data.

ing refeeding (Table 3). If all of this increase were due to HDL derived from blood plasma, and if no compositional change occurred in HDL when capillary filtration increased, the threefold increases in LCAT:HDL-cholesterol ratios found in the lymph of both rats upon refeeding could imply that about two-thirds of the LCAT activity in nonfasting lymph was derived from sources other than blood plasma. However, it was possible that large amounts of lipid-rich lipoproteins, such as those present in lymph, might have disturbed the distribution of LCAT between d < 1.08 g/ml and d > 1.08 g/ml fractions. To test this possibility, blood serum from two normal rats was mixed with aliquots of a pooled d < 1.08 g/ml fraction of lymph to achieve a lipid concentration in the serum approximating that of lipid-rich lymph. The mixture was allowed to stand for about 4 hr at 0°C. The d > 1.08 g/ml fractions of the serums were then prepared and the LCAT activity measured by the Glomset–Wright method. No change in LCAT:HDL-cholesterol ratio was observed compared with infranatants prepared from untreated sera (0.079 vs. 0.079 and 0.081 vs. 0.091 respectively). Furthermore, the d 1.08 g/ml infranatants from both the untreated and the lipid-treated aliquots of serum from one of these rats contained 90% of the LCAT activity of the whole blood serum.

The ratio of LCAT to HDL-cholesterol was less in lymph from fasting animals than in either lymph or blood serum from nonfasting rats (Table 3). Thus, under the present experimental conditions, there was no constant relationship between the LCAT activity and the HDL-cholesterol concentration. Moreover, in lymph of both animals during their nonfasting regimen, LCAT:HDL-cholesterol ratios reached values that were appreciably higher than those of normal rat serum ($P < 0.02$). In order for the LCAT:HDL-cholesterol ratio in lymph to exceed that of serum, either lymph HDL concentration must have decreased or lymph LCAT activity must have increased during feeding. Since lymph HDL actually increased during those collection periods where the ratios were highest, either LCAT enzyme or its activators must have increased appreciably in mesenteric lymph or inhibitors for the reaction decreased during the nonfasting state. Since triglyceride-rich lipoproteins also increased in lymph during feeding, there remains a strong possibility that the intestine may be a source of LCAT or LCAT activators. This is presently under investigation.

Inhibition of blood serum LCAT by whole lymph and components of lymph

Lymph from both fasting and nonfasting rats significantly depressed blood serum LCAT activity (**Fig. 1**). The effect was considerably greater with lipid-rich lymph from nonfasting rats than with lymph from fasting rats, which contained only 1.5–2.0 μmol triglyceride/ml (<5% of nonfasting triglyceride levels). This increased inhibition was probably due to the presence of triglyceride-rich lipoproteins. In order to determine which components were most inhibitory, chylomicrons and VLDL were isolated from lymph and examined separately.

Chylomicrons and lipid emulsions

LCAT inhibition by chylomicrons might be due to either the lipid or the apoprotein component. In order to separate these effects, an artificial lipid emulsion devoid of protein but containing triglyceride and egg phospholipids (Intralipid) was first examined. The marked reduction in serum LCAT activity in the presence of the lipid emulsion is illustrated in **Fig. 2.**

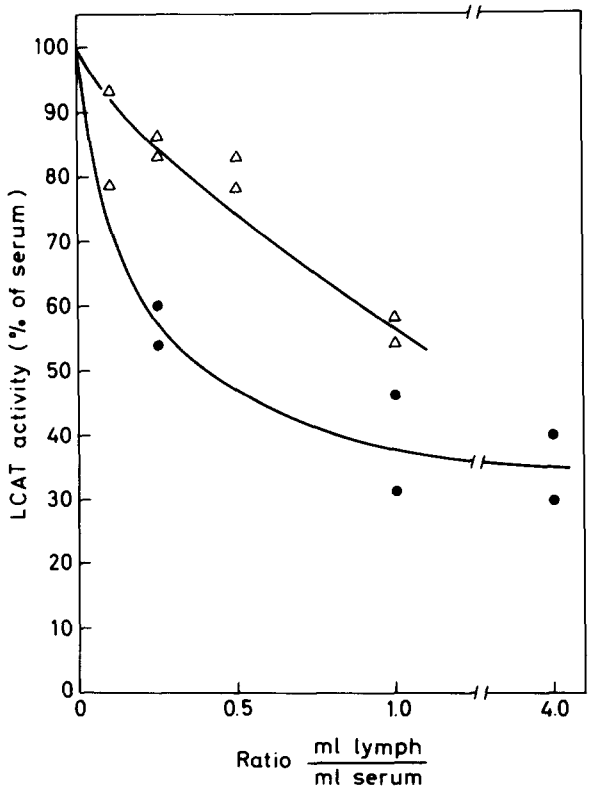

Fig. 1. Inhibition of serum LCAT by mesenteric lymph from two fasting (△) and two nonfasting (●) rats. LCAT activity is calculated as nmol CE formed/ml serum per hr for each vial, and is expressed as the percentage of activity of serum incubated without added lymph. Vials contained 40–160 μl of serum; the total volume was kept constant by variable addition of pH 7.2 buffer (Stokke–Norum method). Lymph was collected at 0°C 24–40 hr after cannulation.

Fifty percent inhibition was produced by the addition of 100 μl of Intralipid, containing 13 μmol of triglyceride and 1.2 μmol of phospholipid or a total of 14.2 μmol of lipid, to 1 ml of serum. Most of this inhibition was shown independently to be due to the phospholipid component of the Intralipid. The effects of Intralipid components were compared to those of nonfasting lymph. Chylomicrons of differing triglyceride and phospholipid content were isolated from the mesenteric lymph of two rats infused intraduodenally with a 3% corn oil emulsion. The triglyceride:phospholipid molar ratio in the chylomicrons varied between 5.7 and 11.2, depending on the time after the start of the fat infusion and on the rate of fat infusion. LCAT activity in the presence of chylomicrons was close to that obtained in the presence of Intralipid with a constant molar ratio of 9.3 (see Fig. 2). The data imply that, in contrast to Intralipid, both the triglyceride and the phospholipid components of chylomicrons contributed to the observed LCAT inhibition and their inhibitory effects were probably similar on a molar basis. Chylomicron CE and apoproteins were also considered as possible inhibitors. However, chylomicron CE added to the incubation vial was always <5% of the CE content of the serum already present so that no conclusions were possible. Apoproteins obtained from delipidated chylomicrons were slightly inhibitory in a concentration 20-fold higher than that present in the added chylomicrons themselves (17% inhibition when 0.6 mg of apoprotein was added to 1 ml of serum).

Lymph lipoproteins

Whole VLDL was strongly inhibitory (40% inhibition by VLDL equivalent to a lymph volume equal to the serum volume) and 30–50% of the inhibition remained after delipidation. Fifty percent inhibition was obtained by addition of 4.5 mg of Tris-soluble apoprotein (equivalent to 4 ml of lymph) per ml of serum.

When whole d 1.006 g/ml infranatant from lymph, which, in the rat, contains small amounts of LDL as well as all the high density lipoproteins, was mixed in equal volume with serum, LCAT activity was not depressed. Nevertheless, HDL apoproteins were tested separately because several apoprotein fractions of VLDL also occur in HDL. Delipidized HDL from lymph was strongly inhibitory in higher concentrations (**Fig. 3**).

Since the major source of lymph HDL probably is the blood plasma, HDL from plasma was also ex-

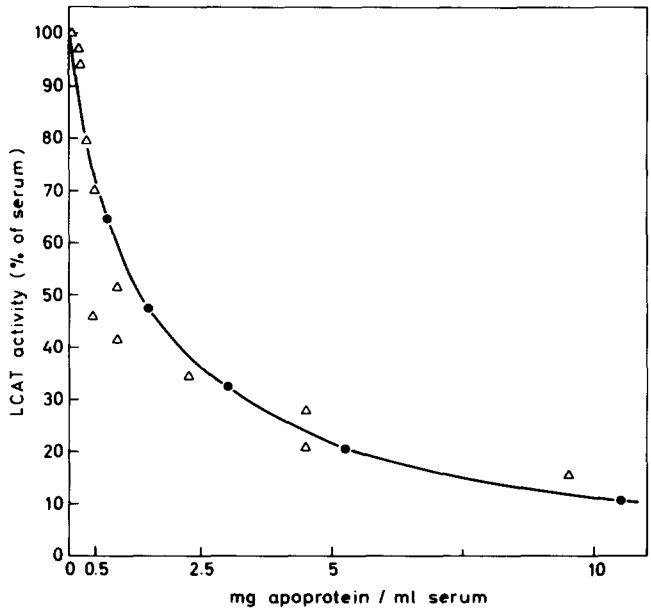

Fig. 2. Inhibition of serum LCAT by Intralipid (●) and chylomicrons (△). Chylomicrons were harvested from mesenteric lymph from nonfasting rats by ultracentrifugation at 4°C for 2×10^6 *g–min*. Triglyceride and phospholipid concentrations in Intralipid were 130 mM and 14 mM, respectively.

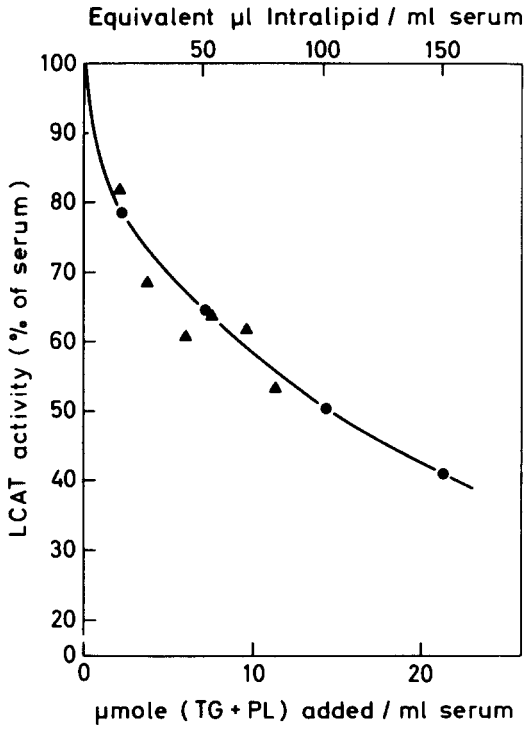

Fig. 3. Inhibition of serum LCAT, determined by the Stokke–Norum method, by serum apoHDL (●) and by lymph apoHDL (▲).

amined for possible serum LCAT inhibition. HDL was prepared from 40 ml of blood serum pooled from nonfasting rats, and the delipidized apoproteins were dissolved in 1 mM Tris HCl, pH 8.6. Inhibition of serum LCAT by whole serum apoHDL was similar to that produced by lymph HDL apoproteins (Fig. 3).

At least two mechanisms could be responsible for the inhibition of serum LCAT by HDL apoproteins. Competition between HDL and added apolipoproteins for the LCAT enzyme might occur. In this event, inhibition would be expected to occur very rapidly. Alternatively, apolipoproteins may remove lipid substrate from the HDL–LCAT complex. Such a process would occur more slowly (20). In order to determine which mechanism was operating in the present system, the time of preincubation with inhibitory apoprotein was varied between 0 and 4 hr. Vials containing all components except apoproteins were preincubated in the presence of DTNB for 4 hr at 37°C to allow the [^{3}H]cholesterol to equilibrate with endogenous substrate before the reaction was begun by the addition of mercaptoethanol. Apoproteins were added at different times during, or at the very end of, the preincubation period. No difference in the extent of LCAT inhibition was observed in any of the vials, suggesting that the inhibitory effect of the apoHDL could be due to competitive inhibition.

To test for competition between HDL and apolipoprotein for LCAT, the concentration of added apoprotein was kept constant and the HDL source (serum) was varied. The results, shown in **Fig. 4,** suggest competition between HDL and apolipoproteins for LCAT.

DISCUSSION

The present studies have demonstrated the presence of LCAT activity in rat mesenteric lymph. This result contrasts with an early report by Vahouny and Treadwell (21), who found no esterifying activity in lymph from the abdominal thoracic duct in rats. However, the enzyme has been reported in lymph from other organs in a few species. Reichl et al. (22) have demonstrated cholesterol esterifying activity in lymph collected from the foot in man. LCAT is also present in dog cardiac and thoracic duct lymph (23), but the activity is rather low (7% and 18% of serum activity, respectively). In rat mesenteric lymph, the activity compared with that of serum was also very low in the present experiments, but the ratio of LCAT to HDL-cholesterol was at times higher in lymph than in rat serum (see Table 3). This is in contrast to dog cardiac and thoracic duct lymph (23) where the ratio, LCAT activity (Glomset–Wright method):d > 1.063 g/ml total cholesterol, was similar to or less than that of serum (0.021 and 0.017 respectively, compared with 0.022).

When fasting, mesenteric lymph fistula rats were refed a diet containing 5% by weight of fat, the d 1.08 g/ml lymph infranatant LCAT activity increased. If LCAT is exclusively derived from plasma, the in-

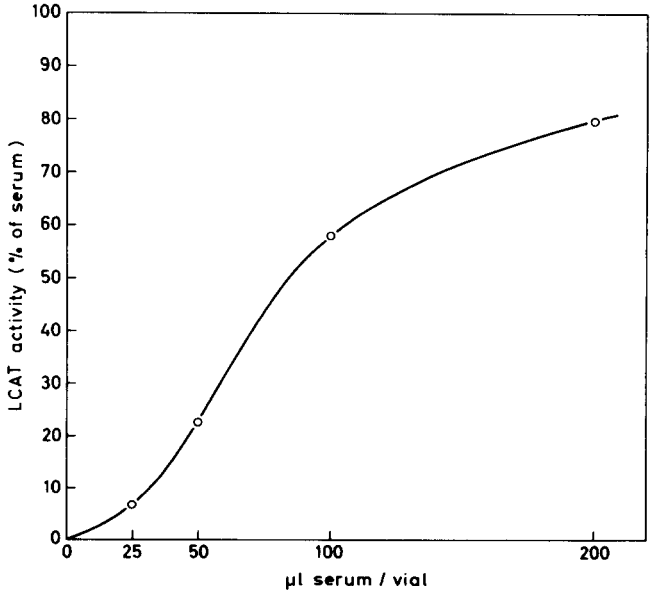

Fig. 4. Competitive inhibition of serum LCAT by HDL apoprotein. LCAT activity was determined by the Stokke–Norum method. Vials contained 255 µg of HDL apoprotein. The total volume was adjusted to 275 µl with pH 7.2 potassium phosphate buffer.

creased activity in lymph after feeding might then be due to increased capillary filtration of the LCAT enzyme or of the smaller HDL which offer a better substrate for the LCAT reaction (24). Recent studies in patients with LCAT deficiency have demonstrated that the small molecular weight fraction of plasma HDL increases when fat is added to the diet (25). Alternatively, LCAT in mesenteric lymph may be in part derived from the gut itself.

The d 1.08 g/ml lymph infranatant total cholesterol concentration also increased during feeding. This increase in lymph HDL-cholesterol may have been due to an increased transfer of all proteins from plasma to lymph during fat transport (19). However, direct synthesis of HDL by the intestine has also been demonstrated (26, 27) and this may have increased with fat absorption.

During feeding, the ratio of LCAT to HDL-cholesterol in mesenteric lymph was also increased, exceeding that of serum. It might be argued that during ultracentrifugation of lipid-rich lymph, HDL and/or LCAT might float at altered densities and thereby affect the LCAT:HDL ratios. However, in control experiments, addition of $d < 1.08$ g/ml lipoproteins to serum in concentrations similar to those in lipid-rich lymph did not affect the LCAT:HDL-cholesterol ratios. Direct synthesis of LCAT by the intestine would explain the findings, but this hypothesis requires further confirmation.

Although our experiments showed that LCAT was present in rat intestinal lymph, CE formation was always extremely low due, in part, to the presence of inhibitors. Other laboratories have reported several inhibitors of plasma LCAT, including both the protein and the lipid components of lipoproteins. Inhibition has been observed both after addition of whole human plasma apoHDL and of individual apolipoproteins (16, 20, 28, 29), both on partly purified LCAT (16, 20) and on crude enzyme preparations (28, 29). A similar inhibition of LCAT activity in the $d > 1.08$ g/ml fraction of rat serum by lymph lipoproteins has now been demonstrated. Lipoproteins rich in triglyceride, lecithin, and unesterified cholesterol have been reported to stimulate human LCAT at low concentrations (17) but were inhibitory at higher concentrations (18). Addition of a phospholipid-stabilized triglyceride emulsion, Intralipid, to guinea pig plasma stimulated LCAT at low concentrations and inhibited at higher concentrations (30). In one study of rat serum (31), addition of 2.5 mg of lecithin as polyene phosphatidylcholine to 1 ml of serum increased cholesterol esterification by about 50%. In the present experiments in rats, the lipid-rich lipoproteins from mesenteric lymph, chylomicrons, and VLDL inhibited both blood serum and lymph LCAT. The extent of LCAT inhibition by chylomicrons of widely differing triglyceride:phospholipid ratios paralleled that produced by differing amounts of Intralipid with a constant ratio of 9.3, suggesting that the triglyceride and the phospholipid of chylomicrons were equally inhibitory (see Fig. 2).

The apoproteins of chylomicrons had relatively little effect. Delipidized VLDL, however, inhibited serum LCAT moderately and the apoproteins of lymph and serum HDL were markedly inhibitory. Whether the same peptides were responsible in all three apoproteins is not certain. Peptide exchange between plasma VLDL and HDL has been documented in vitro in the presence of serum (32) and one study has shown that several electrophoretic protein bands of rat mesenteric lymph VLDL are antigenically similar to those of serum HDL (33). In the present study, similar elution volumes were observed for at least two inhibitory fractions obtained both from apoVLDL and apoHDL by Sephadex gel filtration of Tris-soluble apoproteins in the presence of 6M urea[4].

For the rat, the physiological significance of the inhibitory VLDL and HDL peptides in vivo nevertheless remains in doubt, despite their potency in vitro. Possibly, some regulation of the LCAT reaction by HDL inhibitory peptides may occur in blood serum, although even here the usual concentrations of lipid-rich lipoproteins are likely to be more important in controlling cholesterol esterification by LCAT. In mesenteric lymph it seems that the inhibitory apolipoproteins could in any event have little or no significant role in vivo. Even in whole lymph from fasting rats, the small amounts of $d < 1.006$ g/ml lipoproteins still present were sufficient to suppress LCAT activity to very low levels, since cholesterol esterification was increased in the $d > 1.006$ g/ml fraction. Most of the inhibition by $d < 1.006$ lipoproteins measured in vitro was due to the lipid component rather than the apoprotein. In lymph from nonfasting rats the net LCAT activity was even lower than that in the fasting state due to the presence of chylomicron lipids in high concentrations. In the rat, therefore, although inhibitory apoproteins can be demonstrated in both blood serum and lymph, they probably do not significantly regulate LCAT activity in vivo.

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[4] Bennett Clark, S., unpublished experiments.

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