Lecithin:cholesterol acyltransferase and cholesteryl ester transfer activity from the isolated perfused rabbit liver

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Abstract Lecithin:cholesterol acyltransferase (LCAT) and cholesteryl ester (CE) transfer activity accumulate linearly in the recirculated medium of the isolated perfused rabbit liver. The appearance of both activities in the perfusate was blocked by the addition of 10 μM colchicine, indicating that these two proteins are synthesized and secreted by the liver. CE transfer activity catalyzed the net transport of cholesteryl ester from high density lipoprotein (donor) to very low and low density lipoproteins (acceptor), both in the perfusate medium and in whole rabbit blood plasma. The activity of LCAT in the perfusate was dependent on the presence of the major protein of the high density lipoprotein class, apoA-I. The similar properties of LCAT and CE transfer activity in rabbit liver perfusate and plasma, compared to these same activities in human blood, suggest that the rabbit is an appropriate model for the study of the cholesterol transport system in man.—**De Parscau, L., and P. E. Fielding.** Lecithin:cholesterol acyltransferase and cholesteryl ester transfer activity from the isolated perfused rabbit liver. *J. Lipid Res.* 1984. **25:** 721–728.

Supplementary key words apolipoprotein A-I • high density lipoprotein • low density lipoprotein

The major part of the cholesteryl ester (CE) of human plasma lipoproteins is generated within the circulation by the action of lecithin:cholesterol acyltransferase (LCAT) (1). However, the direct lipoprotein substrate of this enzyme is limited to a subfraction of only one of the major lipoprotein classes, high density lipoprotein (HDL) (2). This enzyme activity is completely dependent upon the presence of apoA-I, the major protein of HDL (3). The distribution of LCAT-derived CE is a function of a cholesteryl ester transfer protein reactive with all the major lipoprotein classes (4).

A comparable situation appears to exist in rabbit plasma, where levels of LCAT similar to those in human plasma have been reported (5, 6), and where cholesteryl esters distribute between plasma lipoproteins by a reaction distinct from the esterification process (7, 8).

While the activities of both LCAT and CE transfer activity are clearly coordinated in the generation of CE-rich lipoprotein particles (9), the factors affecting the levels of these activities in plasma have remained almost totally unknown. Several studies have reported the secretion of LCAT activity from perfused rat liver or dissociated rat hepatocytes (10–12); however, the rat lacks the CE transfer activity (13), together with appreciable levels of low density lipoprotein (LDL) which is the major acceptor of LCAT-derived CE in humans (14). There have been no reports of the origin of CE transfer activity. The present study demonstrates the secretion of both LCAT and CE transfer activity from the isolated perfused rabbit liver, and characterizes some properties of these activities.

METHODS AND MATERIALS

Animals

Male New Zealand White rabbits maintained on Purina Rabbit Laboratory Chow and weighing between 1.6–2.2 kg were used for liver perfusion. Blood from similar rabbits weighing 2.5–3.5 kg was taken into sodium citrate (0.01 M final concentration) and used as a source of both erythrocytes and plasma. In some cases, rabbits were fasted for 6 days, the blood was taken as described above, and this plasma was used for the preparation of LDL ("fasted LDL").

Perfusion of the isolated liver

Rabbits were anesthetized with ketamine (Vetalar^{R} Parke-Davis, Morris Plains, NJ), 80 mg/kg, and the liver perfusion was carried out essentially as described by Hamilton et al. (15) and by Hornick et al. (16). The liver was initially washed in a non-recirculating system with 500 ml of perfusion medium containing Krebs-Henseleit buffer, pH 7.4, with 1 g of glucose/l, and washed erythrocytes at a hematocrit of 10%. A recirculating system was then established with a flow rate of 1 ml $min^{-1}g^{-1}$

Abbreviations: FC, free cholesterol; CE, cholesteryl ester; LCAT, lecithin:cholesterol acyltransferase; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; DTNB, 5,5'-dithiobis(2-nitrobenzoic acid); EDTA, ethylenediaminetetraacetic acid; ww, wet weight of liver.

of liver and a total perfusion volume of 60–90 ml. The perfusion experiments were carried out at 37°C for 3–4 hr at a hematocrit of 20%. At intervals, samples were taken for determination of pH and blood gases. The pH of the perfusate decreased slightly over the 4-hr time course of the experiment from pH 7.4 to 7.1. Blood gases were monitored in the inflow and the outflow tubing by direct determination and remained essentially constant over the perfusion period. Bile flow was 0.45 ± 0.2 μl $min^{-1}g^{-1}$, and urea production was linear over the time course of the perfusion (3–4 hr).

Isolation of lipoproteins

Lipoproteins were isolated from perfusion medium and from rabbit plasma. In the case of the perfusion medium, the erythrocytes were first separated by low speed centrifugation (900 *g*) at 4°C for 15 min. This supernatant medium and whole rabbit plasma were adjusted to a density of 1.019 g/ml with KBr and centrifuged for 18–20 hr at 4°C in a 50.2 rotor (48,000 rpm) or a 40.3 rotor at 38,000 rpm (Beckman Instruments Inc., Palo Alto, CA) to isolate VLDL. For the plasma, the density was then adjusted to 1.063 g/ml to separate the LDL fraction, and finally to density 1.21 g/ml for HDL. The isolated lipoprotein fractions were then recentrifuged under the original conditions.

Chemical analyses

Free cholesterol (FC) and CE mass of isolated lipoproteins, and of relevant fractions in the cholesterol metabolic assays discussed below, were determined with a fluorometric assay utilizing cholesterol oxidase and esterase as previously described (17). Lipoprotein-phospholipid was determined as lipid phosphorus (18) and triglyceride by the method of Carlson (19). Protein was measured according to Lowry et al. (20). Urea was determined by the method of Crocker (21) after deproteinization of the perfusate samples by precipitation with trichloracetic acid.

Activity of lecithin:cholesterol acyltransferase

LCAT was assayed by two methods. *1*) Activity with endogenous substrate was determined as the loss of free cholesterol mass in the medium during a 4-hr incubation at 37°C in samples of perfusate. In the case of rabbit plasma, samples were diluted 4-fold with cold 0.15 M NaCl brought to 0.01 M with Tris-HCl buffer, pH 7.4, and to 1 mM with disodium EDTA, and then incubated for 60 min at 37°C as previously described (2). Quintuplicate 100-μl samples, taken initially and then after the incubation at 37°C, were extracted with chloroform and methanol (22). Portions of the bottom phase were taken for determination of cholesterol mass.

2) LCAT activity was also measured with an assay supplying exogenous substrate. Free cholesterol phospholipid liposomes with a tritium label in the cholesterol moiety were prepared as previously described (2), and then activated (for LCAT activity) by incubation with pure apoA-I isolated from rabbit or human plasma. Standard assays contained 5 μg of apoA-I, 13 nmol cholesterol, and human serum albumin at a final concentration of 2.5% in a total volume of 200 μl. The specific activity of cholesterol in these liposomes was 0.39–0.58 × 10^5 cpm/nmol. Assays were carried out with aliquots of perfusate or plasma for 10–60 min at 37°C. Production of labeled CE was linear under the assay conditions described. The reaction was terminated by the addition of methanol and chloroform. Portions of the bottom phase were separated by thin-layer chromatography and the CE spot was scraped and counted by liquid scintillation spectrometry (9).

Determination of cholesteryl ester transfer rates

CE transfer was also measured in two ways: *1*) The unidirectional flux of exogenous labeled CE between HDL and LDL or VLDL, and *2*) the net transport of CE mass from HDL to LDL and/or VLDL, utilizing endogenous substrates.

The transfer of radioactive CE was measured with centrifugally isolated HDL, labeled with ^{3}H-labeled CE, as donor. This lipoprotein fraction was prepared as described previously (4). Briefly, fresh rabbit plasma was labeled with [^{3}H]cholesterol in the presence of 1.5 mM DTNB to inhibit LCAT, and then incubated at 37°C for 60 min. Betamercaptoethanol was then added to reverse the inhibition of the enzyme and the plasma was incubated for a further 60 min. HDL was then isolated by centrifugation as described above. After dialysis against 0.15 M NaCl–1 mM EDTA, pH 7.4, the preparation of HDL was adjusted to a final concentration of 1 mM Tris-HCl, pH 7.4, containing 1 mM $CaCl_2$, $MgCl_2$, and $MnCl_2$. This material was passed through a Concanavalin A column (Pharmacia, Uppsala, Sweden) equilibrated in the same buffer as the HDL, in order to remove endogenous transfer activity (2). The unretained HDL peak was pooled, and a portion of this HDL was extracted and the content of CE mass and radioactivity, separated from free cholesterol by thin-layer chromatography, was measured. The specific activity of the HDL varied between 1–6 × 10^3 cpm/nmol HDL-CE.

Several acceptor lipoproteins were used in this system and are described under individual experiments in the Results section. In addition to donor and acceptor lipoproteins, the assay medium also contained human serum albumin, pH 7.4, at a final concentration of 1%, and DTNB at a final concentration of 1.5 mM in 0.01 M sodium phosphate buffer, pH 7.4. These assays were incubated in 2-ml tubes adapted to the 40.3 rotor at 37°C for 3 hr, then chilled and brought to a final density of

1.063 g/ml. They were centrifuged at 4°C for 18–20 hr at 38,000 rpm. The LDL/VLDL floating fraction was collected and extracted and the labeled CE was determined after a portion of the bottom phase was separated by thin-layer chromatography. In some experiments, the donor (HDL) and acceptor (VLDL, LDL) lipoprotein species were separated by precipitation (as described below for the net transport assay with endogenous substrate) instead of by centrifugation. The supernatant fraction resulting from the removal of the VLDL and LDL by precipitation was analyzed for loss of labeled CE and for loss of CE mass.

To determine net transport of CE in rabbit plasma or perfusate, LCAT was inhibited by DTNB and transport was measured as the amount of HDL-CE mass transferred to VLDL and LDL. Tris-HCl buffer and disodium EDTA were added as described for the LCAT assay. An initial sample was taken and the VLDL and/or LDL were precipitated by the addition of 0.05 vol of dextran sulfate (20 mg/ml; Pharmacia, Uppsala, Sweden) and 0.05 vol of 2 M $MgCl_2$. After 15 min on ice, the samples were centrifuged (1000 *g* at 4°C) for 30 min. Quintuplicate samples of the supernatant fraction were extracted as described above. Portions of the bottom phase were taken to determine cholesterol and CE mass. After incubation at 37°C for 1 hr for plasma samples and 4 hr for perfusate samples, a final sample was taken and the analysis was repeated.

Preparation of rabbit and human apoA-I

ApoA-I was isolated both from rabbit and human plasma. The method used for the isolation of the human protein was also used for rabbit apoA-I (23). The HDL fraction was purified by centrifugation as described above. Delipidation was carried out with ethanol–diethyl ether 1:2 (v/v) at −20°C, and the proteins were fractionated by chromatography on Sephadex G-150 (equilibrated in 0.015 M Tris-HCl and 6 M urea, pH 8.2) followed by DEAE-cellulose chromatography with a NaCl gradient (0.04–0.15 M) in the same buffer. This purified protein was homogeneous, as judged by electrophoresis in anionic urea gels (24) and SDS gels (25). ApoA-I from rabbit plasma has an apparent molecular weight of 27,900 ± 600 daltons (three determinations), as determined by SDS gel electrophoresis in the presence or absence of reducing agent. As shown in **Table 1,** the only major differences observed between the amino acid analysis of human and rabbit apoA-I were the presence of isoleucine in rabbit apoA-I and its absence in the human protein, and the lower content of methionine in the rabbit protein compared to the human. The apoA-I was used to activate rabbit LCAT as described under Results. Rabbit apoA-I was injected into guinea pigs to prepare apoA-I antiserum. The resulting antibody gave a single precipitin line against plasma, against the pure antigen, and showed no reaction with other isolated proteins. Radial immunoassays for rabbit apoA-I and for rabbit albumin were established under exactly the same conditions as for human apoA-I (9).

TABLE 1. Amino acid composition of rabbit apoA-I

Amino Acid	Rabbit	Human[a]
	mol/ 10^3 mol of amino acid	
Lys	87.1 ± 3.8	79
His	12.3 ± 4.2	22
Arg	73.5 ± 1.6	67
Asp	85.0 ± 3.5	95
Thr	45.7 ± 1.5	38
Ser	75.6 ± 3.9	62
Glu	201.0 ± 3.5	183
Pro	34.1 ± 1.3	38
Gly	41.5 ± 4.0	45
Ala	79.5 ± 0.8	76
Cys/2[b]	0.0	0
Val	58.8 ± 3.4	52
Met[b]	4.3	15
Ileu	9.3 ± 1.9	0
Leu	141.4 ± 5.4	149
Tyr	23.2 ± 0.4	28
Phe	30.9 ± 3.5	23
Trp		29

Values are means ± SD for three determinations of separate preparations. Trp was not determined.
[a] Data from reference 23.
[b] The methionine (4.23; 4.41) and cys/2 values are the average of two determinations carried out as described in reference 35.

Partial purification of perfusate medium

Samples of perfusate were centrifuged at a density of 1.019 g/ml to remove VLDL particles. The infranatant fraction was then applied to a 1 × 14 cm phenyl sepharose column (Pharmacia, Uppsala, Sweden) equilibrated with 0.15 M NaCl–1.5 mM EDTA. The column was then washed with the same buffer (10 volumes), and finally eluted with distilled water. Fractions containing protein were pooled. The major part of the LCAT and transfer activity recovered from the column co-migrated with this protein peak.

Amino acid analysis

Samples for amino acid analysis were hydrolyzed under vacuum in 1 ml of 6 N HCl and 0.005% phenol for 22 hr. After lyophilization, the hydrolysates were dissolved in 0.2 M sodium citrate, pH 2.2, and analyzed on a Beckman model 121 M amino acid analyzer (26).

RESULTS

Secretion of LCAT and CE transfer activity from perfused rabbit liver

The activities of LCAT and CE transfer accumulated at linear rates in rabbit liver perfusates over a 4-hr period.

Results of a representative experiment are shown in **Fig. 1.** The rate of accumulation of LCAT activity measured with the synthetic liposome assay, was 2.5 ± 1.1 nmol h^{-1} perfusion g^{-1} of liver (n = 12). CE transfer activity, also measured with the exogenous substrate assay, accumulated at a rate of 2.9 ± 1.2 nmol h^{-1} perfusion g^{-1} of liver (n = 11). These results indicate that, in the presence of optimal concentrations of exogenous substrates, both processes proceed at similar rates.

Synthesis of LCAT and CE transfer activity by the liver

Fig. 1 shows that LCAT and CE transfer activities were low at the beginning of the perfusion, and that the increase with time was linear. Additional stronger evidence that the liver synthesizes and secretes these activities is presented in **Fig. 2.** The addition of colchicine to the perfusate, at a final concentration of 10 μM, 60 min after the initiation of the recirculating perfusion, essentially completely blocked further accumulation of both LCAT and CE transfer activity in the medium. That the colchicine did not exert a nonspecific toxic effect on liver function is indicated by the continued linear increase in urea production (Fig. 2). When colchicine was added at a concentration of 85 μM, a similar inhibition was also observed (data not shown). Colchicine blocked the appearance of albumin, another protein secreted by the liver, to a similar extent (data not shown). Appearance of apoA-I in the perfusion medium was also measured. A very low level could be detected in the medium during the first hour of the perfusion (11 ± 3 μg/ml; n = 5), which did not increase over the period of the experiment. These values were not affected by the addition of either concentration of colchicine. Colchicine did not act by influencing the activity or stability of LCAT or CE transfer activity in the perfusate. Samples of the medium, taken before and after the addition of colchicine, were incubated at 37°C for 2 hr. Subsequent assay of LCAT and CE transfer activity showed that there was no loss of either activity (±10%) during the incubation period.

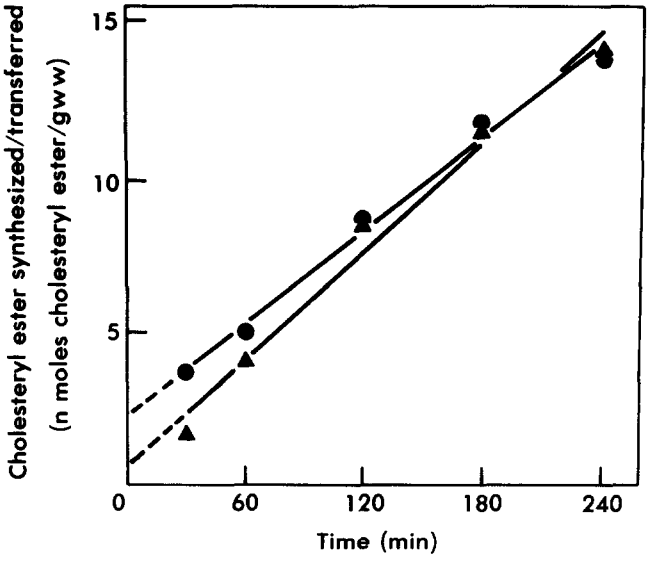

Fig. 1. Time course of accumulation of LCAT (▲) and CE transfer activity (●) in the recirculating medium of an isolated perfused rabbit liver. Samples were taken at the indicated intervals and centrifuged to remove red cells. Data are normalized for 1-hr incubation. LCAT was assayed with exogenous substrate (13 nmol of free cholesterol/assay) in a 200-μl assay volume as desribed under Methods. CE transfer activity was assayed in a 500-μl assay containing ^{3}H-labeled HDL (25 nmol CE) and LDL (100 μg of protein). Values are means of duplicate determinations.

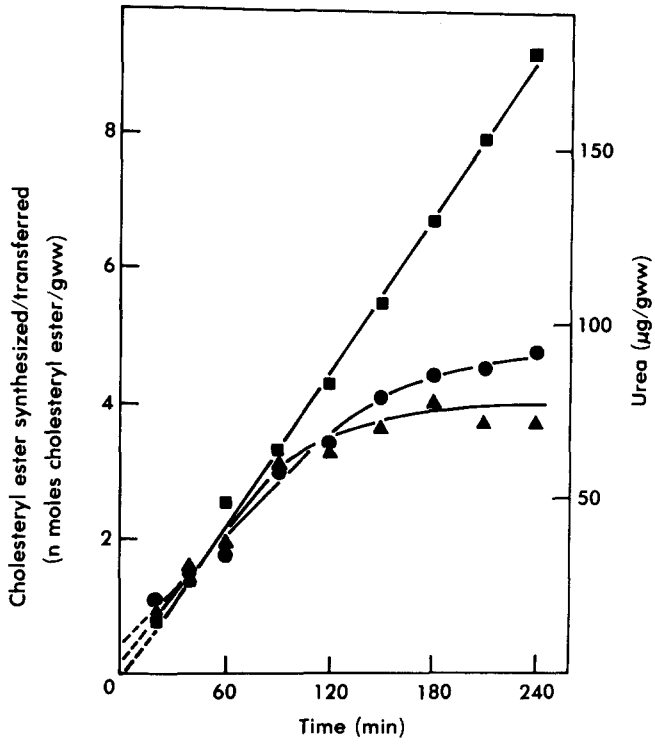

Fig. 2. Effect of colchicine on the accumulation of LCAT (▲) and CE transfer activity (●) in the recirculating medium of an isolated perfused rabbit liver. Colchicine was added to the reservoir 60 min after the initiation of the recirculating perfusion at a final concentration of 10 μM. LCAT and CE transfer activity are measured as described under Fig. 1. Urea secretion (■) was also measured. Values are means of duplicate determinations.

Properties of perfusate and plasma LCAT

The rate of esterification of LCAT was measured by incubating erythrocyte-free, complete or partially purified perfusate with free cholesterol-phospholipid liposomes. The representative experiment shown in **Fig. 3** demonstrates that in the presence of substrate, but without addition of apoA-I, there was little (<5%) detectable activity, relative to that measured in the presence of optimal concentrations of added apoA-I. ApoA-I isolated from rabbit or human HDL stimulated LCAT to a similar ex-

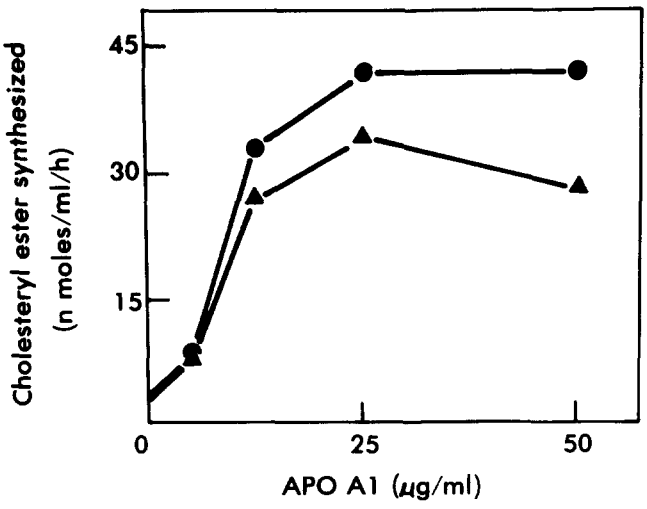

Fig. 3. Activation of LCAT by human (●) or rabbit (▲) apoprotein A-I. The LCAT activity of the perfusate, partially purified by phenyl sepharose chromatography (see Methods), was measured with the liposome assay as described under Fig. 1 and in Methods. The liposomes were preincubated for 60 min with increasing concentrations of pure human apoA-I or rabbit apoA-I, as indicated. Values are means of duplicate determinations.

tent, although activation by the human protein was somewhat higher at all concentrations of apoA-I (Fig. 3).

These data show that LCAT, secreted by the perfused rabbit liver, was readily detected under the conditions described. However, when LCAT was assayed in whole perfusate by measurement of loss of free cholesterol, little or no activity could be detected (**Table 2**).

The endogenous and exogenous (liposome) assays were also carried out with whole rabbit plasma (Table 2). The rate of LCAT activity with endogenous substrate was about one-half of that obtained with exogenous substrate.

CE transfer activity in rabbit liver perfusate

Characteristics of CE transfer activity from perfusate were investigated on samples of medium from which endogenous VLDL secreted by the liver during the experiment was first removed by centrifugation at a density of 1.019 g/ml for 18 hr at 4°C. The infranatant fraction, further purified and concentrated by phenyl-Sepharose chromatography (see Methods section), was used as a source of CE transfer activity to determine the kinetic properties of plasma and perfusate lipoprotein acceptors. The representative result shown in **Fig. 4A** indicates that, in the presence of increasing concentrations of plasma HDL as the CE donor, transfer activity showed saturation kinetics with a half-maximal transport rate of about 21 nmol HDL-CE/ml. Increasing the acceptor lipoprotein in this assay (either VLDL or LDL) also showed saturation (Fig. 4B) with a half-maximal rate of about 85 nmol CE/ml for plasma VLDL and about 70 nmol CE/ml for normal plasma LDL.

Perfusate VLDL showed similar kinetic properties in the transfer assay. As compared to plasma VLDL, these particles are CE-poor (**Table 3**), contain more triglyceride, and have similar amounts of phospholipid and free cholesterol. When these two fractions of VLDL were compared at the same protein concentration, they were similar in their ability to accept CE (Fig. 4B).

A final lipoprotein evaluated as an acceptor particle was LDL from fasted rabbits. This lipoprotein has a greater CE content than normal LDL (Table 3). However, when LDL from normal animals was compared with LDL from fasted rabbits in the ability to accept CE under the conditions of this transfer assay, the two fractions were similar (Fig. 4B).

CE transfer activity and net transport of CE

The transfer assay described in the preceding section measures the unidirectional flux of radiolabeled CE from HDL to VLDL or LDL. To determine whether this flux was accompanied by an equivalent back-transfer (exchange) of CE, or whether it represented an uncompensated transfer (net transport) of CE, the assay was carried out with ^{3}H-labeled HDL as donor and with plasma VLDL as acceptor. Initial samples were taken before and after incubation at 37°C. The acceptor particles were precipitated with dextran sulfate and $MgCl_2$, and the supernatant was assayed both in terms of CE mass and radioactivity. Unidirectional flux from HDL to VLDL was then calculated from the specific activity of the original material. The data in **Table 4** show that transfer measured as the movement of labeled CE out of HDL and into

TABLE 2. Measurement of LCAT activity in rabbit plasma and liver perfusion medium

	Plasma	Perfusion Medium
	nmol cholesterol esterified $ml^{-1}h^{-1}$	
Liposome assay	92.9[a] (range 55.6–126.5, n = 6)	8.0 (range 6.1–10.8, n = 3)
Endogenous assay	49.3 (range 39.8–66.2, n = 4)	0.7 (range 0–1.9, n = 3)

[a] Values are means of measurements from individual animals.

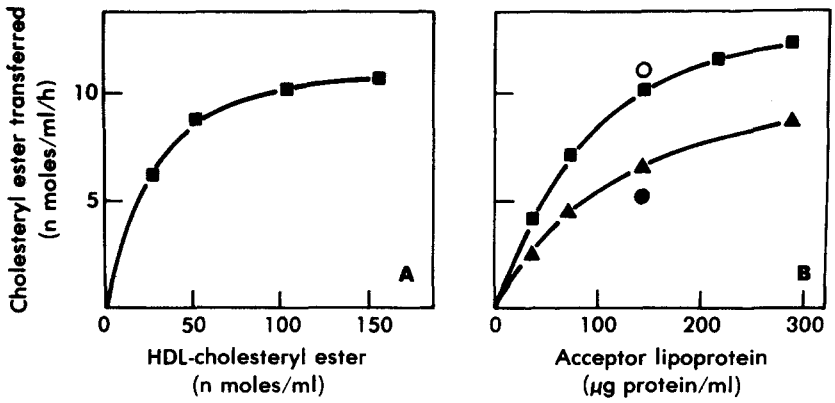

Fig. 4. Dependence of CE transfer activity on the concentrations of donor (HDL) and acceptor (VLDL, LDL) lipoproteins. Source of transfer activity was the phenyl sepharose eluate from hepatic perfusate which had been centrifuged for 18 hr at a density of 1.019 g/ml. A, Effect of medium HDL concentration. The effect of increasing concentrations of ^{3}H-labeled HDL as the CE donor in the transfer assay was determined at a constant concentration of LDL acceptor lipoprotein of 200 μg protein/ml. Other conditions of the assay are described under Methods. Values are means of duplicate assays. B, Effect of medium VLDL or LDL concentration. Effects of increasing concentrations of LDL (▲) or VLDL (■) acceptor lipoproteins at a constant concentration of ^{3}H-labeled HDL (50 nmol/ml). Endogenous perfusate VLDL (○) and LDL from fasting rabbit plasma (●) were also tested as acceptors at a single concentration. Values are means of duplicate determinations.

VLDL was 1.4–2.0 relative to the transfer measured in the same assay as the movement of CE mass from HDL to VLDL.

CE transfer activity was also determined in whole rabbit plasma (Table 4). Net transport of CE through the action of CE transfer protein with endogenous substrates was measured in terms of the change in CE mass in HDL during incubation at 37°C in the presence of the LCAT inhibitor, DNTB. In rabbit plasma, as in the liver perfusate, net transport of CE mass from HDL to acceptor lipoproteins could be demonstrated. Rabbit plasma was also assayed in the transfer system using labeled HDL and exogenous acceptor lipoproteins, in this case VLDL (Table 4). Again, as in the perfusate, the movement of ^{3}H-labeled CE was greater than the transport of CE mass.

DISCUSSION

This research documents the linear accumulation of LCAT and CE transfer activity in the medium of the perfused rabbit liver and shows that the appearance of both of these activities is controlled by a colchicine-sensitive mechanism. A similar inhibition has been reported for other hepatic proteins including those of VLDL (27–29). Present evidence indicates that cholchicine acts at a post-Golgi stage which may involve disruption of the microtubular system (29, 30). LCAT and CE transfer protein therefore appear to be hepatic secretory products. The similar rates of accumulation of LCAT and CE transfer activity are consistent with the close biochemical relationship previously proposed for these activities (31). The rates of net secretion reported in this research indicate that the perfused rabbit liver produces approximately 4–5% of total plasma LCAT and transfer activity per hr.

Rabbit LCAT, like that of other species (3, 32), is dependent for activity essentially completely on the presence of the major HDL apolipoprotein (apoA-I). The analysis of apoA-I in rabbit indicates that its amino acid composition (except for the presence of isoleucine) is similar to that of the analogous human polypeptide (23). The low level of apoA-I measured in the perfusion medium

TABLE 3. Composition of rabbit lipoproteins

Component	Fasted LDL[a]	Normal LDL	Normal VLDL	Endogenous VLDL[b]
		Weight %		
Phospholipids	21.7 ± 1.8	29.6 ± 3.5	14.1 ± 4.3	11.9 ± 2.7
Triglycerides	9.5 ± 3.1	21.7 ± 6.0	58.7 ± 10.9	67.5 ± 3.5
Cholesterol	10.7 ± 0.9	8.5 ± 0.7	5.7 ± 1.0	5.0 ± 0.6
Cholesteryl esters	34.9 ± 2.6	22.7 ± 5.3	11.2 ± 4.0	4.0 ± 2.1
Total protein	23.3 ± 1.2	26.6 ± 3.6	11.3 ± 1.9	8.6 ± 1.4

Values are means ± SD of preparations from four individual animal donors.
[a] LDL isolated from rabbit fasted for 6 days.
[b] VLDL isolated from the perfusion medium.

TABLE 4. Determinations of CE transfer activity in rabbit plasma and liver perfusion medium

	Plasma	Perfusion Medium
	nmol CE transferred $ml^{-1}h^{-1}$	
Net transport of CE mass from HDL to LDL + VLDL	32.6[a] (range 24.8–45.0; n = 3)	10.3 (range 6.9–17.3; n = 4)
Movement of ^{3}H-labeled CE from HDL to LDL	70.6 (range 51.2–88.5; n = 4)	17.0 (range 8.7–31.3; n = 4)

[a] Values are means of individual animals.

which does not increase with time probably represents a washout from plasma trapped within the tissue. Because of the low concentration of apoA-I required to fully activate LCAT, this level should have been sufficient to support a measurable rate of endogenous enzyme activity if bound to a suitable substrate in the perfusion medium. However, no detectable endogenous LCAT was found. This result supports the concept that much of the rabbit perfusate apoA-I does not represent de novo secretion inasmuch as newly secreted HDL of hepatic origin, at least in the rat (33, 34), appears to be an excellent substrate for LCAT. The necessity of a suitable substrate for LCAT activity, and not simply the presence of apoA-I and substrate lipids, is underlined by published data demonstrating that the addition of purified LCAT to human plasma yielded no increase in activity (2).

The data obtained in this study indicate as well that CE transfer activity secreted by the liver has all of the major properties required for either the unproductive exchange or net transfer of cholesteryl esters in the plasma. As in the case of the analogous human activity (31), transfer of preformed cholesteryl esters to VLDL was more rapid than to LDL, although both lipoproteins were effective acceptors and there was less differential between VLDL and LDL than is the case in human plasma. Transfer to the triglyceride-rich VLDL of perfusate was at a similar rate to that found with the VLDL of plasma, which is more cholesteryl ester-rich. These results indicate that, in normal rabbit plasma (as in normal, although not pathological, human plasma) (14), the lipid composition of acceptor lipoproteins is not rate-limiting for transfer. This study also demonstrates that, as with native human plasma (4), the CE transfer activities of rabbit hepatic perfusate and rabbit plasma efficiently promote the net transfer of cholesteryl ester from HDL to the non-substrate acceptors VLDL and LDL.

In summary, these data indicate that the perfused rabbit liver secretes both LCAT and CE transfer activity in proportions, and with properties, that are consistent with the integrated functions of these proteins active in the major cholesterol transport system in the plasma. The present results suggest that, as in other aspects of plasma cholesterol and lipoprotein metabolism, the rabbit may represent an appropriate model for human cholesterol metabolism.

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