Quantification of the hepatic contribution to the catabolism of high density lipoproteins in rats

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Abstract Isolated rat livers were perfused for four hours in a recirculating system containing washed rat erythrocytes. Biologically screened radioiodinated rat high density lipoproteins ($1.090 < d < 1.21$ g/ml) were added to the perfusate with different amounts of whole serum to supply unlabeled rat high density lipoproteins. The protein moiety of the lipoprotein contained more than 95% of the radioiodine. The fraction of apolipoprotein mass degraded during the perfusion was quantified by the linear increment of non-protein-bound radioiodine in the perfusate, corrected for the increment observed during recirculation of the perfusate in the absence of a liver. The small amount of ^{131}I secreted into bile was added to calculate the fractional catabolic rate. The fractional catabolic rate ranged from 0.22 to 0.63% per hour in 12 experiments and was inversely related to the size of the perfusate pool of high density apolipoprotein. The absolute catabolic rate of high density apolipoprotein (fractional catabolic rate × pool size) in three livers in which the concentration of rat HDL in the perfusate approximated that in intact rats was 69.5 ± 10.4 $\mu g\ hr^{-1}$ (mean ± SD). The rate of disappearance of cholesteryl esters of rat high density lipoproteins (labeled biologically by injecting donor rats with [5-^{3}H]mevalonic acid) from the liver perfusate did not exceed that of the apoprotein component. These rates were compared with catabolic rates for rat high density lipoproteins in intact rats. Fractional catabolic rate in vivo, obtained by multicompartmental analysis of the disappearance curve of ^{131}I-high density apolipoprotein from blood plasma, was 11.9 ± 1.3% hr^{-1} (mean ± SD). Total catabolic rate in vivo (fractional catabolic rate × intravascular pool of high density apolipoprotein) was 986 ± 145 $\mu g\ hr^{-1}$ (mean ± SD). The results suggest that only a small fraction of high density lipoproteins in blood plasma of rats is degraded directly by the liver.—**Sigurdsson, G., S-P. Noel, and R. J. Havel.** Quantification of the hepatic contribution to the catabolism of high density lipoproteins in rats. *J. Lipid Res.* 1979. **20:** 316–324.

Supplementary key words HDL apoproteins · cholesteryl esters · fractional catabolic rate

Glomset (1) has suggested that the high density lipoproteins (HDL) of blood plasma, whose component lipids comprise the substrate of lecithin-cholesterol acyltransferase, take up unesterified cholesterol from extrahepatic tissues and transport it as cholesteryl esters to the liver for excretion in the bile. Recent observations suggest that the liver secretes a nascent lamellar HDL, poor in cholesteryl esters (2). The major component lipids of the nascent HDL are rapidly acted on by lecithin-cholesterol acyltransferase to yield cholesteryl esters that are stored in the core of the particle, consistent with a role for HDL in removal of cholesterol from cells. A role for HDL in the delivery of cholesteryl esters to the liver is supported by several reports which indicate that both isolated, perfused rat livers (3) and rat hepatocytes (4, 5) can take up and degrade radiolabeled HDL. Results of studies in which radioiodinated HDL has been injected into intact rats have led to the conclusion that the liver is a major site of removal of this plasma lipoprotein (6–8), but none of the studies reported to date has quantified the participation of various organs and tissues in HDL catabolism.

In the present research we have measured the rate of uptake of the major surface and core components of plasma HDL (apoproteins and cholesteryl esters) as well as the rate of degradation of the protein components of HDL by the isolated perfused rat liver. The values obtained in this system, when compared with the absolute catabolic rate of apoHDL in intact rats, suggest that only about 7% of HDL mass is degraded directly by the liver in the rat. A preliminary report of this work has appeared (9).

MATERIALS AND METHODS

Male Sprague–Dawley rats weighing 300–350 g were used in all experiments.

Abbreviations: VLDL, LDL, and HDL are very low density, low density, and high density lipoproteins, respectively; DTNB, 5,5-dithionitrobenzoic acid; EDTA, ethylenediaminetetraacetate; FCR, fractional catabolic rate; TCA, trichloroacetic acid; ^{131}I-HDL, ^{131}I-labeled HDL.

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Preparation of ^{131}I-HDL

To minimize incorporation of ^{131}I into the lipid moiety of HDL, the rats were fed a saturated fat diet (10) for 3 days before they were exsanguinated under diethyl ether anesthesia. The blood was mixed with disodium ethylenediaminetetraacetate (EDTA), 1 mg/ml, and chilled on ice. HDL, $1.090 < d < 1.21$ g/ml, were isolated by sequential preparative ultracentrifugation (11) at 4°C for 3×10^8 g-min in the 40.3 rotor of a Beckman preparative ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, CA) and recentrifuged once under the same conditions. The supernatant lipoproteins ($1.090 < d < 1.21$ g/ml) were recovered by tube slicing and dialyzed against 0.15 M NaCl and 0.04% disodium EDTA. The purified HDL were labeled with ^{131}I (carrier-free, Amersham/Searle Corp., Arlington Heights, IL) by the iodine monochloride method of McFarlane (12) as modified for iodination of apolipoproteins by Langer, Strober, and Levy (13). The amount of ICl added was such that less than 1 mol of iodine was attached per 100,000 molecular weight units of apoHDL. The protein concentration was measured by the method of Lowry et al. (14) using bovine albumin as a standard. The protein concentration in the preparations used for iodination was 10–15 mg/ml. Unbound iodine was removed by dialysis against 0.15 M NaCl, 0.04% EDTA. Specific activity of ^{131}I-HDL was 3.5–7.0 μCi/mg protein. Less than 4% of the ^{131}I-HDL was bound to lipids as estimated by extractability into ethanol–ether 3:1 (v/v) (15), when HDL were obtained from rats fed the saturated fat diet. When rats were fed a standard chow, 15–20% of the ^{131}I was lipid-bound.

The radiolabeled preparation of HDL was screened by injecting it into a tail vein of a rat. The recipient rats were exsanguinated 3 hr later under diethyl ether anesthesia. The blood serum was dialyzed overnight against Krebs–Ringer bicarbonate buffer (1000 volumes) and used the next day for in vivo studies or for liver perfusion. More than 99% of the ^{131}I in the serum was precipitated by 20% trichloroacetic acid (TCA) and less than 4% was extractable into ethanol–ether.

Preparation of [^{3}H]cholesteryl ester-labeled HDL

DL-[5-^{3}H]Mevalonic acid (dibenzylethylenediamine salt, New England Nuclear Corp., Boston, MA) was converted to the free acid (16) and 0.5 mCi was injected into two rats through a tail vein. The rats were anesthetized with diethyl ether and exsanguinated 5–6 hr later. The non-protein solvent density of the serum obtained was raised to 1.090 g/ml by addition of KBr (11) and the preparation was centrifuged at 4°C in the 40.3 rotor of a Beckman preparative ultracentrifuge for 2.3×10^8 g-min. The supernatant HDL ($1.090 < d < 1.21$ g/ml) were then isolated as described above except that centrifugation at a density of 1.21 g/ml was performed only once and dialysis was against Krebs–Ringer bicarbonate buffer instead of 0.15 M NaCl.

Technique of liver perfusion

The technique of liver perfusion with an erythrocyte-containing medium in a recirculating system with a membrane oxygenator was as described previously (2, 10). In experiments with ^{131}I-HDL, the concentration of HDL in the perfusate "plasma" ranged from 6 to 180% of the normal concentration in rat blood plasma. To obtain the different concentrations of HDL, measured volumes on Krebs–Ringer calcium bicarbonate buffer were replaced with serum from rats fed Purina chow ad libitum. This serum was dialyzed overnight against 100 volumes of Krebs–Ringer calcium bicarbonate buffer. To obtain a higher concentration of HDL in the perfusate than in rat plasma, a measured quantity of rat HDL ($1.090 < d < 1.21$ g/ml) was also added to the perfusate. The radioiodinated preparations were added to the perfusate after a 15 min recovery period. A control experiment was carried out for each liver perfusion in which the same preparation of ^{131}I-HDL in the standard perfusion mixture was circulated in the perfusion apparatus in the absence of a liver for 4 hr. In one experiment a comparison was made between the uptake and degradation of biologically screened rat ^{131}I-HDL and heat-denatured rat ^{125}I-HDL (iodinated as described above for ^{131}I). After exhaustive dialysis against 0.15 M NaCl and 0.04% disodium EDTA the ^{125}I-HDL preparation was incubated at 80°C for 1 hr before it was added to the perfusate.

In the experiments with [^{3}H]cholesteryl ester-labeled HDL, whole rat serum dialyzed against bicarbonate buffer constituted the perfusate plasma. In two of these experiments the labeled HDL (800,000 cpm in 1.5 ml of buffer) were incubated with 15 ml of dialyzed rat serum at 37°C for 2 hr before addition to the liver perfusate in an attempt to increase the content of [^{3}H]cholesteryl esters through the action of lecithin-cholesterol acyltransferase in the serum. In these two experiments the action of lecithin-cholesterol acyltransferase produced by the liver was inhibited by addition of 0.4 ml of a 0.02 M solution of 5,5-dithionitrobenzoic acid (DTNB) in 0.975 M phosphate buffer, pH 7.4, to the perfusate at intervals of 30 min during perfusion (2). In the third experiment, labeled HDL were added directly to the perfusate without incubation and no DTNB was added during the per-

fusion. In all experiments with [^{3}H]cholesteryl ester-labeled HDL, samples of perfusate taken for analysis were mixed with additional DTNB to give a final concentration of 1.0–1.2 mM.

Criteria of the viability of perfused livers were as described previously (10) and only livers fulfilling these criteria of rates of gas exchange, bile flow, and triglyceride secretion were accepted for analysis.

Estimation of catabolic rates in the perfused liver

A 1.5-ml portion of perfusate was removed from the circuit every 30 min during the perfusion and chilled on ice. Erythrocytes were removed by centrifugation. In experiments with ^{131}I-HDL, a portion of the "plasma", mixed with 0.05 vol of 20% bovine serum albumin, was added to 2 vol of 20% TCA. After centrifugation for 30 min at 2000 rpm, ^{131}I in the precipitated proteins and the supernatant fluid was determined in a gamma scintillation spectrometer. The results for each were expressed as percentage of total radioactivity (TCA precipitate + TCA-soluble). The supernatant fluid was subsequently mixed with chloroform (1:2 v/v), after peroxidation as described by Bierman, Stein, and Stein (17) to remove radioiodide not bound to iodotyrosine, and chloroform-soluble and water-soluble ^{131}I were determined (10).

The fractional catabolic rate (FCR) of apoHDL during the perfusion was calculated from the sum of the linear increment of total non-protein-bound ^{131}I in the perfusate, plus ^{131}I secreted into the bile, less the increment of ^{131}I in the perfusate during circulation of an identical perfusate in the absence of a liver. The total catabolic rate of apoHDL was determined as the product of the FCR and the amount of apoHDL in the perfusate. In two experiments, HDL ($1.090 < d < 1.21$ g/ml) were isolated from portions of the perfusate at the beginning and end of the perfusion by sequential ultracentrifugation and recentrifuged at the upper density limit. After dialysis against 0.15 M NaCl, a portion was subjected to electrophoresis in 10% polyacrylamide gels (pH 7.1) containing 0.034 M sodium decyl sulfate (SDS) (18). The gels were stained in 0.25% Coomassie brilliant blue in 50% methanol and 9.2% glacial acetic acid at 70°C for 45 min and destained overnight in methanol–acetic acid–water 5:5:90. The gels were cut with a razor blade according to the position of the protein bands (apoprotein A-IV, arginine-rich apoprotein, apoprotein A-I, and the combined C and A-II apoproteins (18)) and the segments were assayed for ^{131}I.[3]

In experiments with [^{3}H]cholesteryl ester-labeled HDL, 0.5-ml portions of perfusate plasma were extracted in isopropanol–heptane–1 N sulfuric acid 40:10:1 as described by Dole (19). After separation of the extract into two phases by addition of water, the lipids contained in the heptane phase were separated by thin-layer chromatography on silicic acid (silica gel G, Analtech, Inc., Newark, DE). The plates were developed with hexane–diethyl ether–methanol–acetic acid 90:20:3:2 and ^{3}H was measured in the free and esterified cholesterol fractions (16). Recovery of applied ^{3}H exceeded 90%. In two experiments, portions of the perfusate were subjected to ultracentrifugation in the 40.3 rotor for 2.3×10^6 g-min at a density of 1.090 g/ml. The supernatant and infranatant fractions were recovered after tube slicing and ^{3}H was estimated in a portion of each fraction. Quenching was corrected by use of an internal standard. A separate portion of the infranatant fraction ($d > 1.090$ g/ml) was extracted in Dole's mixture (19) and ^{3}H in free and esterified cholesterol was determined as described above.

Estimation of catabolic rates in vivo

A fraction of the ^{131}I-HDL used for the liver perfusions was injected into fed rats between 9 AM and noon through a femoral vein during brief diethyl ether anesthesia. Blood samples were obtained from a tail vein at intervals during the next 28–48 hr. ^{131}I was determined in 100-μl samples of serum and the results were expressed as percentage of the ^{131}I in the sample taken 2–3 min after the injection. Three drops of saturated KI was added to the rats' drinking water 24 hr before the experiment to block uptake of iodine by the thyroid gland. In two experiments, rats were anesthetized with ether and exsanguinated from the abdominal aorta at different times after injection of the ^{131}I-HDL. HDL were separated from the plasma and ^{131}I in individual apoproteins was determined as described for the liver perfusion experiments. FCR was estimated by the method of Mathews (20), with the assumption of a two-compartmental model for the metabolism of apoHDL. The catabolic rate was determined as FCR × intravascular pool of apoHDL. Plasma volume was taken as 4.5% of body weight (21). The mean concentration of apoHDL in rat plasma (0.587 mg/ml), as determined by Faergeman et al. (22) in the same strain of rats fed the same standard Purina chow, was used for the calculation.

RESULTS

Composition of HDL

The apoprotein of the HDL used in these experiments was mainly apoA-I (**Table 1**). Most of the re-

[3] No attempt was made to measure changes in mass of apoHDL during perfusion because perfused livers secrete HDL that contain all of these aproproteins (18).

mainder was apoA-IV as judged from the intensity of staining of protein bands in gel electropherograms. The arginine-rich apoprotein comprised about 7% of the protein mass, indicating that only a small fraction of particles rich in this apoprotein were present.

Catabolism of ^{131}I-HDL in perfused livers

The baseline sample was taken 15 min after adding the radioiodinated HDL to the perfusion circuit in order to allow for any uptake of non-protein-bound ^{131}I present in the ^{131}I-HDL preparation into the liver (10, 23). Non-protein-bound ^{131}I increased linearly with time in the perfusate after a 0.5–1 hr latent period (**Fig. 1**). Rate of appearance of water-soluble ^{131}I (presumably ^{131}I-tyrosine) and chloroform-soluble ^{131}I (radioiodide) were, respectively, 55 ± 6% and 45 ± 6% of total TCA-soluble ^{131}I (mean values ± SD). The fall in lipoprotein-bound ^{131}I in the perfusate with time was accounted for by the increase in non-protein-bound ^{131}I in the perfusate plus the ^{131}I radioactivity remaining in the liver at the end of the 4-hr perfusion, after flushing 50 ml of Krebs–Ringer calcium bicarbonate buffer through the liver via the portal vein (total recovery 99 ± 2%). The proportion of lipoprotein-bound ^{131}I that was soluble in ethanol–ether was unchanged (about 2.5%) after 4 hr of perfusion and more than 92% of the lipoprotein-bound ^{131}I remained in the density range >1.090 g/ml, as determined by ultracentrifugation. The amount remaining in the liver was less than 3% of the ^{131}I-HDL added to the perfusate. More than 80% of this ^{131}I was precipitated by TCA. ^{131}I-apoHDL, isolated in two experiments from samples of perfusates 15 min as well as 4 hr after addition of biologically screened ^{131}I-HDL, contained 60–65% of ^{131}I in apoprotein A-I, 4–6% in apoprotein A-IV, 5–10% in arginine-rich apoprotein, and about 15% in the combined A-II and C apoproteins.

A smaller increment of non-protein-bound ^{131}I was observed during control perfusions in the absence of liver (Fig. 1). Seventy to eighty percent of this increment was chloroform-soluble. As in our experiments with ^{131}I-low density lipoproteins (LDL) (10), the total increment of ^{131}I in the chloroform phase was consistently greater during liver perfusions than in the control circuit, which suggests that ^{131}I released when ^{131}I-HDL is degraded by liver cells is not only in the form of ^{131}I-labeled tyrosine but also in the form of free ^{131}I. Therefore we chose to express the rate of ^{131}I-HDL degradation by the linear increment of total non-protein-bound ^{131}I in the perfusate corrected for the increment observed during circulation of the perfusate through the perfusion apparatus in the absence of liver (Fig. 1).

TABLE 1. Composition of rat serum HDL (1.09 < d < 1.21 g/ml)

Component	% Mass (mean ± SD)[a]
Cholesteryl esters	14.6 ± 1.1
Triglycerides	3.6 ± 2.2
Cholesterol	4.4 ± 0.7
Phospholipids	17.1 ± 2.0
Total protein	60.4 ± 1.4
Apoprotein A-I	38.5 ± 1.9
Arginine-rich apoprotein	5.1 ± 1.6

[a] $n = 4$. Analyses were performed as described in references 2, 18, and 24.

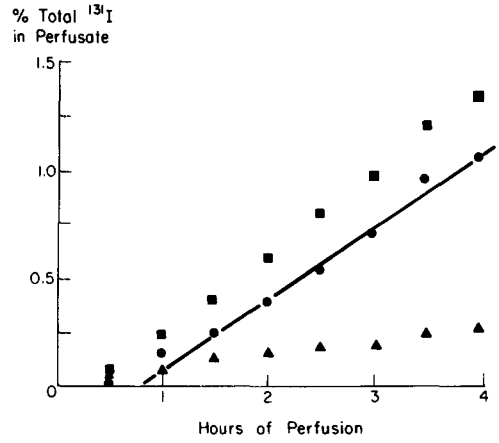

Fig. 1. Production of non-protein-bound ^{131}I during perfusion of a rat liver with labeled and screened rat HDL. Pool size of apoHDL in perfusate in this experiment was 17.1 mg. Results shown here are typical of those obtained in other experiments. ■——■, Increment of TCA-soluble ^{131}I in perfusate; ▲——▲, increment of TCA-soluble ^{131}I without liver; ●——●, corrected rate of accumulation of TCA-soluble ^{131}I.

The rate of appearance of non-protein-bound radioiodine in the perfusate was the same for radioiodinated HDL, biologically screened for either 3 hr or 14 hr, as for nonscreened radioiodinated HDL. This observation makes it unlikely that the screening removed a population of rapidly catabolized molecules. We chose to use biologically screened radioiodinated HDL routinely to obviate effects of any denaturation that might occur during some iodinations (10).

The small amount of ^{131}I secreted into the bile during the perfusion was also measured and this value (percent of added ^{131}I) was added in the calculation of FCR although it accounted for less than 10% of the total non-protein-bound ^{131}I produced. The calculated FCR ranged from 0.22 to 0.63% hr^{-1} (**Table 2**) and, at low concentrations of HDL and other serum proteins, was inversely related to the mass of apoHDL in the perfusate (**Fig. 2**). No correction was made for the small amount of ^{131}I-HDL removed by sampling during the perfusion. This correction would slightly decrease the observed rate of degradation of apoHDL.

The catabolic rate of apoHDL was directly related to the concentration of HDL in the perfusate (**Fig. 3**). Catabolism of apoHDL evidently was not saturated at

TABLE 2. Catabolism of screened ^{131}I-labeled HDL in isolated, perfused rat livers

	FCR % hr^{-1}	Perfusate apoHDL mg (total)	Perfusate apoHDL μg/ml	Catabolic Rate μg hr^{-1}
Rat HDL (1.090 < d < 1.21 g/ml)	0.56	1.3	25	7
	0.63	1.8	34	11
	0.63	2.2	42	14
	0.51	5.0	95	26
	0.29	9.1	174	26
	0.36	12.3	236	43
	0.30	16.4	315	50
	0.23	27.5	590	63
	0.23	27.8	534	64
	0.25	32.1	617	82
	0.30	37.0	740	111
	0.22	52.7	1054	116

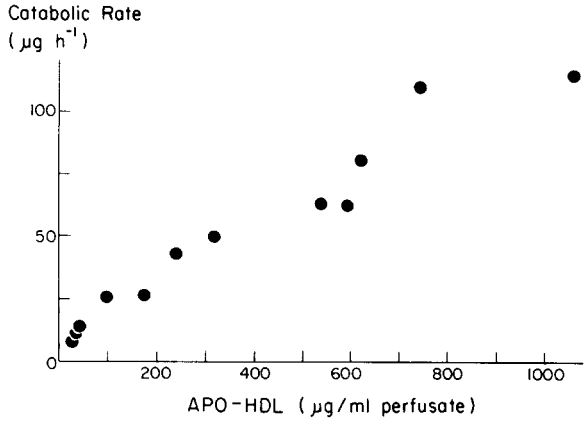

Fig. 3. Relationship between the concentration of apoHDL in liver perfusates and the catabolic rate of apoHDL. Each point represents one experiment.

approximately double the physiological concentration. The mean catabolic rate of apoHDL in three livers perfused with HDL at a concentration similar to that of intact rats was 69.5 μg hr^{-1} (Table 2).

In two additional experiments radiolabeled rat HDL were isolated in the range 1.063 < d < 1.21 g/ml and in one additional experiment in the range 1.110 < d < 1.21 g/ml. The catabolic rate obtained in these experiments was in the same range as shown in Table 2 at comparable HDL concentrations.[4] The rate of appearance of non-protein-bound ^{131}I in the perfusate with ^{131}I-HDL prepared from rats fed a standard Purina chow was closely similar to that obtained with ^{131}I-HDL prepared from rats fed the saturated fat diet. In two liver perfusions in which the concentration of HDL was held constant but the perfusate volume was reduced from 70 ml to 35 ml, a closely similar catabolic rate of apoHDL was obtained (25.5 vs. 26.3 μg hr^{-1}) whereas the FCR differed (0.29 vs. 0.52% hr^{-1}). These results indicate that the uptake and degradation of HDL by the liver are mainly dependent on the concentration of HDL in the perfusate rather than the total pool size.

Fig. 4 shows the appearance of non-protein-bound radioiodine in the liver perfusate when a liver was perfused simultaneously with biologically screened ^{131}I-HDL and nonscreened, heat-denatured ^{125}I-HDL. The denatured apolipoprotein was catabolized much more rapidly.

[4] In one experiment with human ^{131}I-HDL, isolated at 1.063 < d < 1.21 g/ml, the catabolic rate was 17 μg hr^{-1} at a concentration of 100 μg ml^{-1} (i.e., in the same range found for rat HDL). In this experiment the iodinated HDL was screened as for rat HDL, and unlabeled human HDL was added to the perfusate.

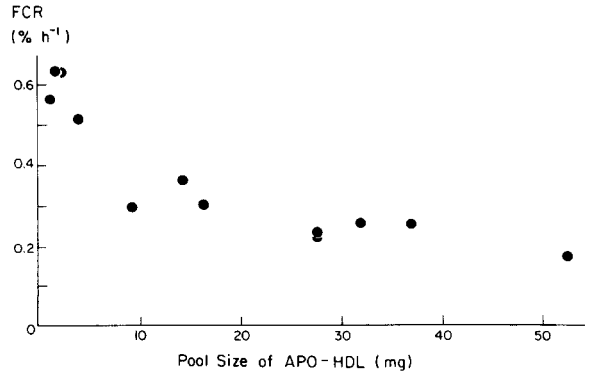

Fig. 2. Relationship between pool size of apoHDL and its fractional catabolic rate (FCR), measured from production of TCA-soluble ^{131}I during perfusion of rat livers with labeled and screened rat HDL. Each point represents one experiment.

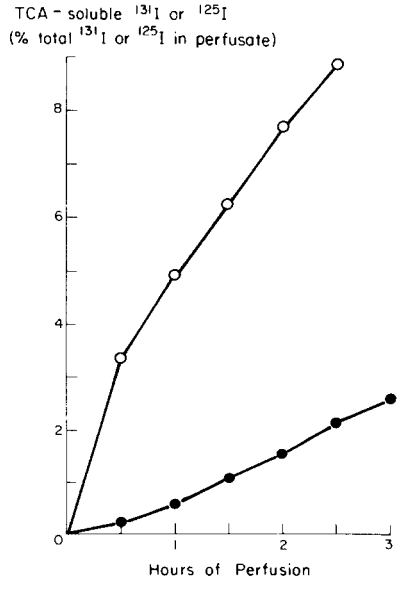

Fig. 4. Production of non-protein-bound (TCA-soluble) ^{131}I (closed circles) and ^{125}I (open circles) during perfusion of one liver with both screened rat ^{131}I-HDL and unscreened, heat-denatured ^{125}I-HDL.

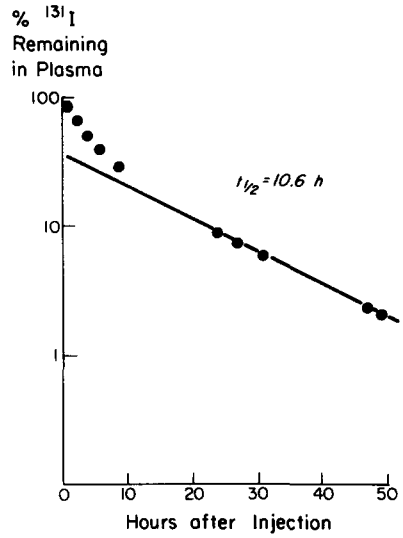

Fig. 5. Disappearance of ^{131}I-labeled and screened rat HDL injected intravenously into an intact rat.

Catabolism of ^{131}I-HDL in vivo

In intact rats, the rate of removal of the labeled HDL from blood plasma was biexponential (**Fig. 5**). The disappearance from plasma was identical when nonscreened HDL or HDL screened for 3 hr or 14 hr was injected. This suggests that the rapid early phase reflects mixing of plasma HDL with extravascular pools rather than preferential removal of denatured lipoprotein. The mean half-time of the second exponential was 10.6 hr which is closely similar to the findings of Roheim et al. (6). More than 92% of the ^{131}I remained in the density range >1.090 g/ml at all times after injection and less than 5% was in lipoproteins of d < 1.055 g/ml. The disappearance of ^{131}I from individual apoproteins of HDL, determined in two experiments, was closely similar to that of total ^{131}I-HDL in plasma, indicating that the fractional removal rates for the different apoproteins are nearly the same. The disappearance rate of total ^{131}I-HDL from plasma was therefore taken to represent the turnover rate of apoHDL. Insufficient radioactivity was found in apoproteins of lipoproteins of lower density to permit reliable analysis of its distribution among apoproteins. Results for seven rats are summarized in **Table 3.** The mean catabolic rate in these seven rats was 986 ± 145 μg hr^{-1}.

Removal of [^{3}H]cholesteryl esters from liver perfusates

The rate at which [^{3}H]cholesteryl esters of rat HDL were removed from liver perfusates did not exceed that of ^{131}I-HDL (**Table 4**). The amount of ^{3}H secreted into the bile during the perfusion was less than 0.5% of the ^{3}H added to perfusates, mostly in the form of free cholesterol.

TABLE 3. Catabolism of screened ^{131}I-labeled rat HDL in intact rats

FCR % hr^{-1}	Intravascular Pool Size mg apoprotein	Catabolic Rate μg apoprotein hr^{-1}
12.6	9.2	1160
11.6	7.9	920
13.9	8.0	1120
11.2	7.92	887
13.1	8.45	1107
10.0	7.66	766
11.0	8.59	944
11.9 ± 1.3[a]	8.25 ± 0.53[a]	986 ± 145[a]

[a] Mean ± SD.

More than 85% of ^{3}H was in the d > 1.090 g/ml ultracentrifugal fraction of a sample taken from the liver perfusate at the beginning of the perfusion (i.e., 15 min after adding the ^{3}H-labeled HDL) and also at the end of a 4-hr perfusion. Eighty-five to ninety percent of the added ^{3}H was in cholesteryl esters. At the end of the perfusion this value had risen to 92–94%. This increase could reflect the action of lecithin-cholesterol acyltransferase. However, in two of the three experiments, lecithin-cholesterol acyltransferase secreted by the liver was inhibited by addition of DTNB and incubation at 37°C of ^{3}H-labeled HDL with 15 ml of perfusate for 3 hr prior to the liver perfusion did not alter the percentage of [^{3}H]cholesteryl esters. Hence the increase during liver perfusion probably reflects uptake of ^{3}H-labeled free cholesterol by molecular exchange with free cholesterol in the liver rather than esterification.

To compare the metabolism of the apoprotein and cholesteryl ester components of HDL in vivo with their metabolism in the isolated perfused liver, the bile duct was cannulated in intact rats under diethyl ether anesthesia and the bile was collected for 4 hr

TABLE 4. Uptake of [^{3}H]cholesteryl esters of rat HDL by perfused rat livers

Hours of Perfusion	Percent of Initial Radioactivity Remaining in Perfusate					
	[^{3}H]Cholesteryl Esters in Total Perfusate			[^{3}H]Cholesteryl Esters in Perfusate HDL		^{131}I-HDL in Total Perfusate[a]
	Exp. 1	*Exp. 2*	*Exp. 3*	*Exp. 2*	*Exp. 3*	
1	97	100	93	101	100	98.8
2	98	96	100	101	101	98.3
4	96	96	98	97	99	96.0

DTNB was added to perfusate at intervals of 30 min in experiments 2 and 3 to inhibit lecithin-cholesterol acyltransferase (see Methods).

[a] Mean values for three experiments.

after the injection of ^{3}H-labeled HDL or ^{131}I-HDL into a tail vein. The results obtained in two experiments with each form of labeled HDL were closely similar to those obtained during liver perfusions: less than 0.5% of the injected radioactivity was recovered in the bile. In a single in vivo experiment, bile was collected for 18 hr after intravenous injection of ^{131}I-HDL. The rate of excretion of ^{131}I in the bile did not increase with time which suggests that the added HDL was well mixed with the extravascular pool of HDL in the liver within the 4-hr period used for the perfusion experiments.

DISCUSSION

This research has confirmed that the liver is capable of taking up and degrading apoHDL (3, 4). However, the catabolic rate of ^{131}I-apoHDL in the perfused liver at physiological concentrations of HDL (~70 μg hr^{-1}), when compared with a value near 1000 μg hr^{-1} in intact rats, suggests that only about 7% of rat apoHDL is catabolized by the liver. These results resemble those we have obtained for rat LDL (10) and, as in that study, it is not certain that the rates observed in perfused livers mimic those occurring in vivo. However, we adhered to strict criteria for viability of the perfused livers and no more ^{131}I or ^{3}H appeared in the bile when ^{131}I-HDL or [^{3}H]cholesteryl ester-labeled HDL were injected into intact rats than with perfusion of ^{131}I-HDL through the isolated liver. The rates observed in the perfused liver could overestimate catabolism of HDL by as much as 45% inasmuch as deiodination of ^{131}I-tyrosine (reflected by chloroform-soluble ^{131}I) may occur by action of a hepatic deiodinase without actual proteolysis.

Studies of the distribution of radioactivity in tissues at intervals after the injection of radioiodinated HDL into rats have shown the highest concentration of radioactivity to be in the liver. Although less than 10% of the injected radioiodine was in the liver in one study (6) and less than 3% in another (7), the conclusion drawn from these results has been that the liver is a major catabolic site for HDL. We found less than 3% of ^{131}I-HDL added to the liver perfusate to be in the liver at the end of perfusions lasting 4 hr. Measurements of radioactivity in tissues do not distinguish between actual cellular uptake and distribution of the radiolabeled lipoprotein into an extravascular pool. The liver could contain a sizeable part of the extravascular pool of HDL as reflected in the studies on tissue distribution of labeled HDL in vivo.

The rate of uptake of [^{3}H]cholesteryl esters of rat HDL by the perfused liver was low and comparable to that of ^{131}I-apoHDL (Table 4), consistent with uptake of HDL by pinocytosis or phagocytosis. Our data apply to the predominant HDL in rat blood plasma ($1.090 < d < 1.21$ g/ml) in which apoA-I is the major apoprotein. It is possible that HDL rich in the arginine-rich apoprotein, which are mainly larger particles of lower density (24), are taken up by the liver to a greater extent. However, the similarity of distribution of ^{131}I in individual HDL-apoproteins at the beginning and end of the liver perfusions suggests that HDL particles that contain relatively more arginine-rich apoprotein and less apoprotein A-I are not removed at a substantially different rate than apoprotein A-I-rich HDL.

Drevon, Berg, and Norum (5) have recently reported a substantial uptake of HDL-cholesteryl esters by isolated liver cells. By extrapolation, they calculated that the half-life of plasma HDL in intact rats should be only 30 min. We found the half-time of removal of apoHDL in intact rats to be 10.6 hr, which is very similar to the values found by Roheim et al. (6) and Eisenberg, Windmueller, and Levy (7). Drevon, Berg, and Norum (5) labeled cholesteryl esters of HDL in vitro by the action of lecithin-cholesterol acyltransferase. Although this method may be more "physiological" than incorporation into plasma lipoproteins of cholesteryl esters dissolved in dimethylsulfoxide (25) or absorbed on coated particles (26), the labeled cholesteryl esters produced enzymatically may be associated with HDL that are enriched in this lipid component. In these studies with isolated liver cells, uptake of HDL-cholesteryl esters was saturated at about 170 μM, whereas uptake by nonparenchymal hepatic cells was nonsaturable (5). Rachmilewitz et al. (3) found by radioautography that both parenchymal and epithelial cells incorporated radiolabeled HDL. Uptake by parenchymal cells predominated. Uptake by Küpffer cells was considered possibly to reflect removal of denatured lipoprotein. Our experiment with heat-denatured HDL (Fig. 4) is consistent with this proposal.

We found the rate of removal of the different apoproteins of HDL from plasma to be similar. Roheim et al. (27) have reported comparable results, whereas distinct differences for the major apoproteins of human and rat HDL were reported by Eisenberg et al. (7). For those apoproteins that readily exchange between VLDL and HDL particles, and exist in substantial amounts in both, differences in removal rates from HDL, as observed by Eisenberg et al. (7), are difficult to interpret. Our results for intact rats, those of Roheim et al. (27) and the recently reported results of Blum et al. (28) in humans are consistent with removal of HDL by pinocytosis or phagocytosis, as the present study also suggests for liver cells. Miller, Weinstein,

and Steinberg (29) have recently reported that cultured human fibroblasts bind and take up HDL in a manner consistent with adsorptive pinocytosis. They calculated the rate of uptake, if representative of all body cells, to be more than sufficient to account for the known catabolic rate of apoHDL in humans. Carew et al. (30) found the fractional catabolic rate of apoHDL to be unaffected by side-to-side portacaval shunting of blood in swine, an observation that is also consistent with a major role of extrahepatic tissues in catabolism of HDL. Our results, which provide the first quantitative estimates of hepatic catabolism of HDL, are in accord with this possibility and further suggest that the liver is not the preferred site of HDL catabolism.

On the assumption that HDL components are taken up by cells as a unit, the rate at which cholesteryl esters of rat HDL are removed from the blood can be calculated from the ratio of cholesteryl ester to apoHDL mass (1:2) (2). This rate is about 500 μg hr^{-1}, as compared with the catabolic rate of cholesteryl esters in rat LDL, estimated similarly, of 140 μg hr^{-1} (10). The catabolic rate of cholesteryl esters in rat very low density lipoproteins (VLDL) is about 2000 μg hr^{-1} (31). Owing to the large size of VLDL, transcapillary transport of VLDL-cholesteryl esters is probably negligible in most extrahepatic tissues. In the rat, VLDL remnants transport cholesteryl esters mainly to the liver (31), whereas LDL seem to transport cholesterol mainly to extrahepatic tissues (10). It is thus evident that HDL may supply a major fraction of the cholesteryl esters delivered to extrahepatic tissues in this species. In relating these values to the situation in humans, it should be kept in mind that HDL are the major cholesterol-bearing lipoproteins in rats whereas LDL have that distinction in humans.

Cholesteryl esters of rat HDL and LDL are produced mainly by the action of lecithin-cholesterol acyltransferase whereas those of VLDL are predominantly synthesized in the liver (31). The results of the present study and our similar research on the metabolism of rat LDL (10) therefore are consistent with the concept that HDL and LDL in this species participate mainly in an extrahepatic cycle whereby cholesterol is delivered to and taken up from tissues (**Fig. 6**). However, a small fraction of the cholesteryl esters leaving the blood may be taken up by the liver as a component of these lipoproteins. In addition, HDL-cholesteryl esters may cross over into the largely separate cycle of cholesterol transport in triglyceride-rich lipoproteins by transfer to VLDL or chylomicrons (21, 32, 33), perhaps mainly from nascent HDL during the formation of cholesteryl esters by lecithin-cholesterol acyltransferase (2). These esters will then be taken up by the liver as a component of VLDL or chylomicron remnants. In this manner HDL may participate in a "centripetal" transport of cholesterol. As discussed elsewhere (33), the extent to which cholesteryl esters of HDL are transferred to other lipoproteins may differ substantially among mammals.

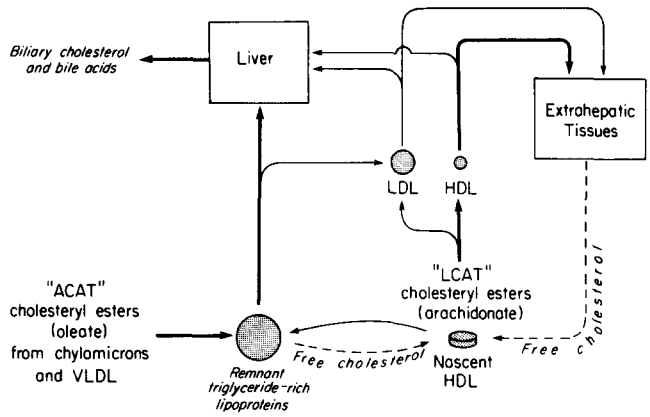

Fig. 6. Concept of two cycles of cholesteryl ester transport in rat blood plasma, derived in part from this and other studies (2, 10, 21, 22, 31). Unless noted otherwise, all arrows refer to movement of cholesteryl esters. Nascent HDL (components of which originate in liver and small intestine), poor in cholesteryl esters but containing apoprotein A-I cofactor, are acted upon by lecithin-cholesterol acyltransferase (LCAT) to yield mainly cholesteryl arachidonate. The cholesterol used in this reaction may come from the surface of remnant triglyceride-rich lipoproteins or surface membranes of cells (dashed lines). Cholesteryl esters in triglyceride-rich lipoproteins (in which cholesteryl oleate predominates) are largely preformed in the small intestine and liver by acyl cholesterol acyltransferases (ACAT). LCAT-derived esters make a minor contribution to cholesteryl esters in remnants, but a major contribution to those of LDL, and they constitute almost all of the cholesteryl esters of plasma HDL. Esters produced by ACAT are mainly taken up by the liver in remnant lipoproteins whereas LCAT-derived esters are predominantly transported to extrahepatic tissues. The extrahepatic cycle may serve in part to deliver cholesterol from dying cells to proliferating cells or cells synthesizing steroid hormones. The hepatic cycle is largely enterohepatic and, in this species, normally contributes little to the steady-state concentration of plasma cholesteryl esters. Small but significant cross-over between the two cycles can occur during the formation of LDL from remnant lipoproteins, by transfer of LCAT-derived esters to remnant lipoproteins, and by hepatic uptake of LDL or HDL (or extrahepatic uptake of remnant lipoproteins).

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