Intravascular metabolism of the cholesteryl ester moiety of rat plasma lipoproteins

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Abstract The intravascular metabolism of the cholesteryl ester moiety of rat plasma LDL, HDL_1, and HDL_2 was determined in intact male rats. Biosynthetically labeled lipoproteins were prepared by zonal ultracentrifugation from the plasma of rats injected with [^{3}H]cholesterol. The lipoproteins were concentrated by vacuum ultrafiltration as other procedures were found to alter the biological properties of the lipoproteins. After injection of labeled LDL, [^{3}H]cholesteryl esters remained with the injected lipoprotein and decayed from plasma with a $t_{1/2}$ of 7–8 hours. [^{3}H]Cholesteryl esters in HDL_1 behaved similarly and decayed with a $t_{1/2}$ of 10.5 hours. With HDL_2, however, a different metabolic pattern was observed with intraplasma conversion of some [^{3}H]cholesteryl ester HDL_2 particles to HDL_1. Since such conversion of HDL_2 to HDL_1 was not observed after in vitro incubations of rat plasma, this process seems to depend on metabolic events that occur in vivo. [^{3}H]Cholesteryl esters disappeared from HDL_2 with a $t_{1/2}$ of 6–7 hours, while the esters that were transferred to HDL_1 decayed with a $t_{1/2}$ of 10–11 hours, similar to labeled cholesteryl esters injected with HDL_1. The study demonstrated that the high apoE content of rat plasma HDL_1 is not associated with rapid catabolism of the lipoprotein and that a major source of HDL_1 in the rat is the intraplasma conversion of HDL_2 particles to HDL_1.—**Eisenberg, S., Y. Oschry, and J. Zimmerman.** Intravascular metabolism of the cholesteryl ester moiety of rat plasma lipoproteins. *J. Lipid Res.* 1984. **25:** 121–128.

Supplementary key words low density lipoproteins • high density lipoprotein-1 • high density lipoprotein-2 • cholesteryl ester exchange • zonal ultracentrifugation

Studies on the intravascular metabolism of rat plasma lipoproteins are usually carried out with methods developed for similar experiments in humans (1–5). For example, lipoproteins are isolated at salt density intervals that define human plasma lipoproteins, but are inadequate in rats (6). Another example is the use of iodinated apolipoproteins to determine the fate of lipoprotein particles. As most apolipoproteins exchange among lipoproteins (the only exception being apoB), it is uncertain to what extent the plasma decay of a labeled apoprotein does represent that of the whole particle (7).

The present investigation takes advantage of newly developed techniques to prepare rat plasma lipoproteins (6) and of the absence (or near absence) of core-lipid transfer in this species (6, 8–10) for studies of lipoprotein metabolism in rats. Centrifugation in a zonal rotor allows isolation of homogeneous lipoprotein preparations in rats. When rats are injected with radioactive cholesterol, this method yields biosynthetically labeled lipoproteins suitable for metabolic study. With these procedures, we have investigated the intraplasma metabolism of the cholesteryl ester moiety of LDL, HDL_1, and HDL_2 in the rats. The results of these investigations are reported here.

METHODS

Preparation of lipoproteins and of [^{3}H]cholesterol-labeled lipoproteins

Cholesterol-labeled lipoproteins were prepared from pooled plasma of two male rats exsanguinated 6 hr after intravenous injection of 100–200 μCi of [7(N)-^{3}H]cholesterol (Radiochemical Centre, Amersham, England). Plasma was first separated to three density fractions of d < 1.006 g/ml, d 1.006–1.085 g/ml and d > 1.085 g/ml by salt density centrifugation in a 50 Ti rotor at densities of 1.006 g/ml and 1.085 g/ml (6). Labeled LDL and HDL_1 were isolated by zonal ultracentrifugation from the plasma fraction of d 1.006–1.085 g/ml using a 1.0–1.3 g/ml NaBr gradient at 42,000 rpm for 170 min; HDL_2 was prepared from the plasma fraction of d > 1.085 g/ml after similar 22-hr centrifugation at 41,000 rpm and a 1.0–1.4 g/ml gradient, as previously described (6). Radioactivity and absorbance (at 280 nm) were monitored across the zonal eluent; lipoproteins were identified, collected, and dialyzed extensively against 0.9% NaCl, 0.01% EDTA, pH 7.4. Lipoproteins were concentrated to desirable volumes by vacuum ultrafiltration (also see Results, first paragraph). Chemical composition and

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; LCAT, lecithin:cholesterol acyltransferase; [^{3}H]FC, free [^{3}H]cholesterol; [^{3}H]CE, ^{3}H-labeled cholesteryl esters.

apoprotein profiles of lipoproteins thus obtained were similar to those recently described (6). The radiochemical composition of different lipoproteins is shown in Table 1 (see Results).

Zonal ultracentrifugation was carried out in a Beckman L-5 ultracentrifuge and a 14 Ti zonal rotor. NaBr gradients were formed with a Beckman model 141 piston gradient pump. During elution of the zonal rotor, effluent absorbance was continuously monitored at 280 nm by an ISCO model UA-5 Absorbance Monitor (6).

To allow separation of all major rat plasma lipoproteins with a single zonal spin, the following procedure was developed. Rat plasma lipoproteins were isolated at density less than 1.21 g/ml after a 48-hr centrifugation in a 50 Ti rotor at 45,000 rpm. The lipoprotein fraction ($d < 1.21$ g/ml) was then applied to the bottom of a 1.0–1.3 g/ml NaBr gradient and lipoproteins were separated after 170 min centrifugation at 42,000 rpm. **Fig. 1** shows the elution profile of rat plasma lipoproteins isolated by this procedure. Chemical composition and polyacrylamide gel electrophoresis of apoproteins (not shown) confirmed the identity of VLDL, LDL, HDL_1, and HDL_2 with lipoproteins obtained by multiple centrifugations (6). Lipoprotein fractions 1 through 4, which correspond to VLDL, LDL, HDL_1, and HDL_2, were pooled, dialyzed, and concentrated as described above.

Analytic procedures

Lipoprotein protein, triglyceride, cholesterol, and phospholipid were determined by standard procedures (11–13). Polyacrylamide gel electrophoresis (15% acrylamide) of apoproteins was performed as described by Weber and Osborne (14). Plasma and lipoprotein lipids were extracted by the procedure of Folch, Lees, and Sloane Stanley (15). Free [^{3}H]cholesterol ([^{3}H]FC) and [^{3}H]cholesteryl esters ([^{3}H]CE) were separated by thin-layer chromatography using pre-coated silica gel plates (Schleicher and Schull, F-1500) and a solvent system of petroleum ether–diethyl ether–acetic acid 160:40:2 (v/v/v). Lipids were visualized with iodine vapor and identified with the help of purified lipid standards. The plates were then cut accordingly and the amount of radioactivity in each lipid class was determined in a Tricarb liquid scintillation spectrometer, No. 2660.

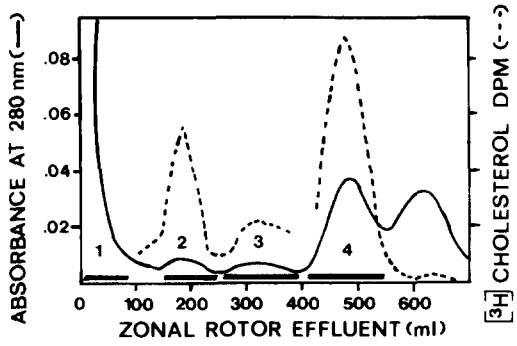

Fig. 1. Rate zonal ultracentrifugation of [^{3}H]cholesterol-labeled rat plasma lipoproteins of $d < 1.21$ g/ml separated in a 1.0–1.3 g/ml NaBr gradient. Lipoproteins were isolated from the plasma of two rats 6 hr after being injected with 0.2–0.4 mCi [^{3}H]cholesterol. After 48 hr of centrifugation at d 1.21 g/ml, in a fixed angle 50 Ti rotor, the biosynthetically labeled lipoprotein fraction was applied to the bottom of a 14 Ti zonal rotor and eluted after 170 min centrifugation at 42,000 rpm. Solid line, absorbance at 280 nm; broken line, [^{3}H]cholesterol radioactivity in dpm/ml. Four lipoprotein fractions were isolated (1 through 4), and they correspond to rat plasma VLDL, LDL, HDL_1, and HDL_2. The fifth peak is residual plasma protein fraction. Radioactivity scale for HDL_2 is one-eighth that of LDL and HDL_1. Specific radioactivity of [^{3}H]CE in LDL, HDL_1, and HDL_2 ranged between 1.0–2.0, 1.5–3.0, and 1.5–3.0 millions dpm/mg, respectively.

In vivo turnover of [^{3}H]cholesterol-labeled lipoproteins

Male rats of the Hebrew University strain (16, 17) (250–300 g) were used. Lipoproteins were injected to ether-anesthetized rats through an exposed saphenous vein. Each rat received $1–2 \times 10^5$ dpm, 0.2–0.4 mg protein of [^{3}H]cholesterol-labeled LDL, HDL_1, or HDL_2 in a volume of 0.25–0.6 ml, and blood was collected at time intervals (2 min to 24 hr) from the abdominal aorta. The blood was allowed to clot on ice and serum was separated at 4°C by 20 min centrifugation at 3000 rpm. A 0.5-ml serum aliquot was taken for radioactivity determination, and another 0.5 ml for thin-layer chromatography of lipids. Five ml of the remaining serum was taken for sequential separation of lipoprotein fractions of $d < 1.006$ g/ml, d 1.006–1.085 g/ml, and d 1.085–1.21 g/ml in a 40.3 rotor. Lipoproteins were isolated by the tube-slicing technique and aliquots were taken to determine total fraction radioactivity and for thin-layer chromatography of lipids. All procedures were carried out at 4°C.

Lipoproteins from rats injected with [^{3}H]cholesterol-labeled lipoproteins were also separated by centrifugation in the zonal rotor, using the procedure described above. The rotor eluent was collected in 25-ml fractions, and radioactivity was determined in each tube. [^{3}H]FC and [^{3}H]CE radioactivity in either individual tubes or in pooled fractions (as shown in Fig. 1) was determined by thin-layer chromatography.

Lipoprotein-[^{3}H]CE plasma decay was determined as follows. Plasma radioactivity 2 min after the injection of a labeled lipoprotein ([^{3}H]FC and [^{3}H]CE) was considered as the injected dose (100% radioactivity). Lipoprotein-[^{3}H]CE as percent of injected dose was then obtained from the percent radioactivity remaining in plasma at time intervals multiplied by the percent of radioactivity in a lipoprotein fraction ($d < 1.006$ g/ml, d 1.006–1.085 g/ml, and d 1.085–1.21 g/ml) and by the contribution of [^{3}H]CE to the lipoprotein fraction radioactivity.

In some experiments, [^{3}H]cholesterol-labeled lipopro-

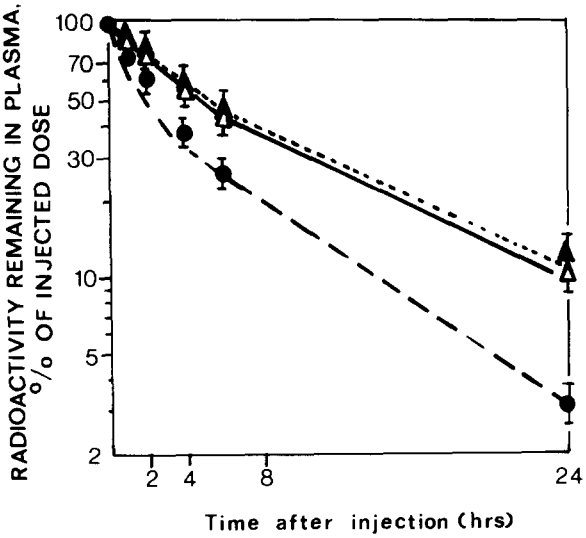

Fig. 2. Plasma decay of ^{125}I-labeled human LDL diluted in NaBr solutions and concentrated by two different techniques: vacuum ultrafiltration (▲ - - - ▲) and Amicon ultrafiltration system (● - - - ●). The results are compared to the decay of undiluted ^{125}I-LDL (△ —— △). Data are mean ± SEM of three experiments.

teins were first injected into a rat and allowed to circulate for 1 hr. At the end of this period, the rat was exsanguinated and aliquots of the plasma containing the remaining injected lipoprotein (biologically "screened" preparations) were injected to other rats. The plasma decay of "screened" lipoproteins was then determined as above.

RESULTS

In a preliminary study we searched for a method to prepare lipoproteins separated by zonal centrifugation that are suitable for metabolic experiments. To that end, rat plasma lipoproteins isolated in the zonal rotor, and ^{125}I-labeled human LDL diluted in concentrated NaBr solutions were used. Lipoproteins were dialyzed against 0.9% NaCl and concentrated by two techniques. **Fig. 2** presents data with human ^{125}I-LDL. Concentration of the ^{125}I-LDL in Amicon ultrafiltration systems yielded preparations that were unsatisfactory and decayed from plasma much faster than untreated LDL; vacuum ultrafiltration ("reverse dialysis") in contrast, did not change the biological properties of the lipoprotein. Similar results were obtained with rat plasma lipoproteins. Hence, in all subsequent experiments the vacuum ultrafiltration method was used.

Biosynthetically labeled LDL, HDL_1, and HDL_2 contained both [^{3}H]FC and [^{3}H]CE (**Table 1**). During the first 2 hr after the injection, [^{3}H]FC disappeared from plasma faster than [^{3}H]CE and by the end of that period, 86–94% of plasma radioactivity was in [^{3}H]CE. This probably reflects exchange of lipoprotein [^{3}H]FC with unlabeled free cholesterol in cell membranes as well as plasma cholesterol esterification in HDL.

Distribution of [^{3}H]cholesterol between lipoproteins was investigated first by centrifugation in a zonal rotor. One, 4, and 8 hr after the injection of LDL or HDL_1, the bulk of plasma [^{3}H]cholesteryl esters separated with the injected lipoprotein (**Fig. 3A and B**). Tritiated free cholesterol initially distributed to VLDL and HDL_2 and [^{3}H]cholesterol in HDL_2 became rapidly esterified. One hour after the injection, 70–75% of plasma [^{3}H]CE separated with either LDL or HDL_1 (according to the injected lipoprotein) and 15–25%, with HDL_2. At 4 and 8 hr, no change in the distribution of [^{3}H]CE between LDL or HDL_1 and HDL_2 was observed, indicating that [^{3}H]CE molecules do not exchange between the lipoproteins, and that the [^{3}H]CE in HDL_2 represented esterification of free [^{3}H]cholesterol. With [^{3}H]CE-labeled HDL_2, a different metabolic behavior was observed. One hour after the injection about 90% of plasma radioactivity was in HDL_2 and only a few percent in other lipoproteins, mainly VLDL (Fig. 3C). In HDL_2, more than 90% of the [^{3}H]cholesterol was in esterified form, whereas in VLDL, only 57.2% was esterified. With time, however, increasing amounts of radioactive cholesteryl esters sep-

TABLE 1. Plasma [^{3}H]free cholesterol and [^{3}H]cholesteryl esters at time intervals after the injection of labeled LDL, HDL_1, or HDL_2

Time after Injection	[^{3}H]-LDL		[^{3}H]-HDL_1		[^{3}H]-HDL_2	
	[^{3}H]FC	[^{3}H]CE	[^{3}H]FC	[^{3}H]CE	[^{3}H]FC	[^{3}H]CE
min	*% of total radioactivity*					
0[a]	46.2 ± 6.3	52.6 ± 6.0	55.0 ± 5.8	43.5 ± 5.6	21.2 ± 4.8	77.7 ± 4.6
2	35.3 ± 6.8	63.3 ± 7.0	44.3 ± 8.6	55.1 ± 8.8	12.0 ± 2.8	87.4 ± 2.8
120	12.7 ± 2.4	86.6 ± 2.3	8.3 ± 1.8	91.3 ± 1.7	5.5 ± 1.3	93.9 ± 1.2

Data are mean ± SEM of four experiments. [^{3}H]-LDL, [^{3}H]-HDL_1, and [^{3}H]-HDL_2 refer to the injected labeled lipoprotein.
[a] [^{3}H]FC and [^{3}H]CE of the injected lipoprotein.

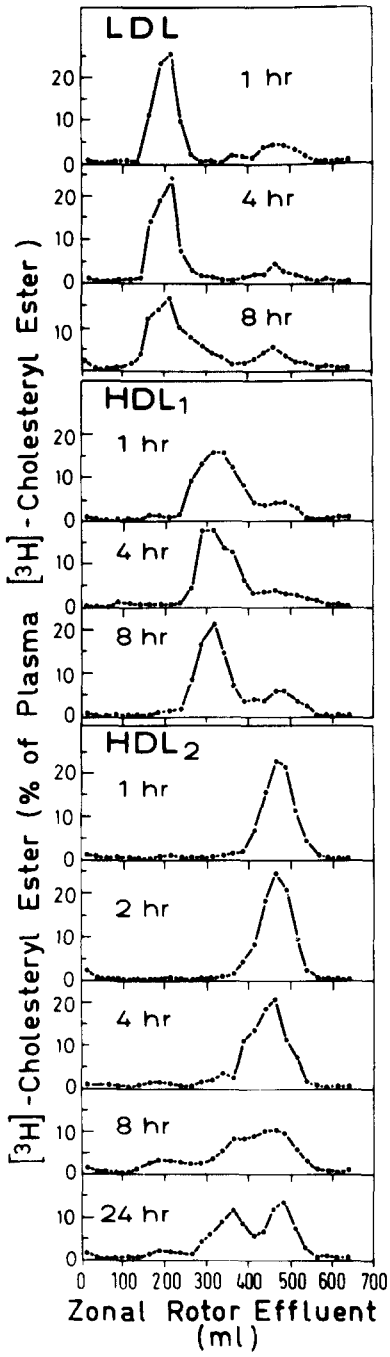

Fig. 3. Rate zonal ultracentrifugal separation of rat plasma lipoproteins isolated at time intervals after injection of [^{3}H]cholesterol-labeled LDL (3A), HDL_1 (3B), or HDL_2 (3C). Rats were exsanguinated 2–24 hr after the injection of a labeled lipoprotein. Plasma lipoproteins were first isolated at d < 1.21 g/ml (in a fixed angle 50 Ti rotor) and then applied to the bottom of a zonal gradient as described in legend to Fig. 1. [^{3}H]Cholesteryl ester was determined in individual effluent fractions (25 ml) and is presented as percent of total plasma radioactivity. Absorbance (at 280 nm) across the rotor effluent was identical to that shown in Fig. 1.

arated with lipoproteins of lower densities, predominantly HDL_1. One hour after the injection, 86.4% of total plasma [^{3}H]CE was associated with HDL_2 (400–550 ml of the rotor effluent) and only 3.5% with HDL_1 (250–400 ml of the rotor effluent). At 2, 4, 8 and 24 hr after the injection the following values were found for [^{3}H]CE in HDL_2: 79.3%, 66.0%; 42.6%, and 44.1% of total plasma [^{3}H]CE, respectively; in HDL_1, the corresponding values for [^{3}H]CE were 11.7%, 25.8%, 35.6%, and 42.3%, respectively. At 8 and 24 hr, small amounts of plasma [^{3}H]CE were also found with LDL (9.5% and 6.3%, respectively), presumably reflecting extension of HDL_1 to the LDL density range.

Table 2 presents data on the distribution of [^{3}H]FC and [^{3}H]CE between lipoproteins separated by centrifugation at densities of 1.006 g/ml, 1.085 g/ml, and 1.21 g/ml. With this procedure, LDL and HDL_1 are separated at the density interval of d 1.006–1.085 g/ml; some HDL_2 (10–20%) is also present at that density but the bulk of this lipoprotein separates at d 1.085–1.21 g/ml (6). Two minutes after the injection of ^{3}H-labeled LDL, 66.9% of plasma radioactivity was in [^{3}H]CE, and 65.5% was associated with the fraction of d 1.006–1.085 g/ml. Thus, 97.9% of plasma [^{3}H]CE was in the density fraction of the injected lipoproteins. At that time, 32.8% of plasma radioactivity was in [^{3}H]FC and the labeled free cholesterol distributed among all density fractions. At later time intervals, [^{3}H]CE remained predominant at the fraction of d 1.006–1.085 g/ml, and cholesterol esterification in HDL_2 was apparent. The findings after the injection of HDL_1 were qualitatively and quantitatively similar to those observed with LDL except for separation of somewhat higher proportion of plasma [^{3}H]CE with HDL_2. With HDL_2, a different metabolic sequence was again observed. With time, increasing proportions of [^{3}H]CE separated with the plasma fraction of d 1.006–1.085 g/ml. Eight hours after the injection the amount of radioactivity at d 1.006–1.085 g/ml was similar to that in HDL_2, and by 16 and 24 hr, it was higher.

The data included in Table 2 was used to construct plasma decay curves of [^{3}H]cholesteryl esters in LDL, HDL_1, and HDL_2 (**Fig. 4**). After a 2–4 hr equilibration period, the decay curves of LDL-[^{3}H]CE and HDL_1-[^{3}H]CE were linear, with circulating half lives ($t_{1/2}$) of 7.5 and 10.5 hours, respectively (Fig. 4). For HDL_2, [^{3}H]CE decay was calculated at two density fractions: d 1.006–1.085 g/ml and d 1.085–1.21 g/ml (Fig. 4). Accumulation of [^{3}H]CE in the d 1.006–1.085 g/ml fraction is apparent, with subsequent different decay of [^{3}H]CE from the two fractions: $t_{1/2}$ of 10–11 hr at density 1.006–1.085 g/ml and of 6–7 hr at density 1.085–1.21 g/ml.

The plasma decay of [^{3}H]cholesterol-labeled LDL, HDL_1, and HDL_2 after a 1-hr "screen" is shown in **Fig. 5.** In these experiments [^{3}H]CE in screened LDL, HDL_1, and HDL_2 contributed 86.7%, 77.8%, and 95.3% of total lipoprotein radioactivity, respectively. The metabolic behavior of the "screened" lipoproteins was very similar to

TABLE 2. [^{3}H]Cholesteryl ester and [^{3}H]free cholesterol in lipoprotein fractions at time intervals after the injection of labeled LDL, HDL_1, and HDL_2

Injected Lipoprotein	Time after Injection	[^{3}H]Cholesteryl Ester			Free [^{3}H]Cholesterol		
		d < 1.006	d 1.006–1.085	d 1.085–1.21	d < 1.006	d 1.006–1.085	d 1.085–1.21
	hr	*percent of injected dose*					
LDL	2/60	0.2 ± 0.1	65.5 ± 7.7	1.2 ± 0.6	7.3 ± 2.6	12.7 ± 1.5	12.8 ± 6.2
	1	0.2 ± 0.1	36.9 ± 2.0	1.3 ± 0.5	1.1 ± 0.2	1.3 ± 0.2	2.4 ± 0.8
	2	0.2 ± 0.1	32.3 ± 1.5	2.5 ± 0.8	1.5 ± 0.3	1.4 ± 0.3	0.9 ± 0.4
	4		21.3 ± 1.4	2.0 ± 0.6		1.3 ± 0.3	0.5 ± 0.2
	8		13.2 ± 0.2	2.4 ± 0.7		2.6 ± 0.4	0.5 ± 0.2
	24		3.6 ± 0.5	1.8 ± 0.4		0.9 ± 0.2	0.5 ± 0.2
HDL_1	2/60	0.5 ± 0.2	45.5 ± 5.7	6.1 ± 1.5	16.0 ± 2.9	15.7 ± 2.0	15.7 ± 3.9
	1	0.3 ± 0.1	29.5 ± 2.6	4.9 ± 1.8	1.1 ± 0.3	4.9 ± 0.6	3.0 ± 1.1
	2	0.3 ± 0.1	28.8 ± 2.5	5.7 ± 1.8	1.1 ± 0.3	2.5 ± 0.4	3.6 ± 1.1
	4		25.5 ± 1.7	4.9 ± 1.6		3.3 ± 0.6	1.1 ± 0.4
	8		21.0 ± 1.5	3.3 ± 1.0		1.9 ± 0.6	1.1 ± 0.2
	24		6.4 ± 1.0	2.3 ± 0.5		1.0 ± 0.4	1.0 ± 0.2
HDL_2	2/60	1.4 ± 0.2	18.5 ± 4.6	67.3 ± 4.4	2.4 ± 0.4	2.6 ± 0.6	2.3 ± 0.2
	1	0.8 ± 0.2	11.6 ± 3.0	52.4 ± 2.4	2.2	0.8 ± 0.3	1.3 ± 0.1
	2	1.4 ± 0.2	13.4 ± 3.9	38.1 ± 3.5	0.6	0.6 ± 0.2	0.8 ± 0.1
	4		13.8 ± 2.6	26.7 ± 1.7		0.9 ± 0.2	1.1 ± 0.1
	8		9.3 ± 1.3	11.1 ± 1.0		1.4 ± 0.4	1.3 ± 0.1
	16		6.3 ± 0.8	5.7 ± 0.6		1.1 ± 0.3	0.6 ± 0.1
	24		3.6 ± 0.3	2.1 ± 0.3		0.7 ± 0.2	0.4 ± 0.1

Data are mean ± SEM of four to six experiments. Values are derived from plasma radioactivity (percent of injected dose, the 2 min taken as 100%), distribution of radioactivity among lipoproteins, and radiochemical composition of each lipoprotein.

that of "unscreened" preparations (compare Figs. 4 and 5), i.e., after the injection of LDL or HDL_1, 80–95% of plasma radioactivity separated at density less than 1.085 g/ml, while re-distribution of HDL_2-[^{3}H]CE to HDL_1 (d < 1.085 g/ml) was observed.

The finding that some HDL_2-[^{3}H]CE distributes to HDL_1 raises the possibility that a similar phenomenon might take place during in vitro incubation of rat plasma. **Fig. 6** shows the lipoprotein and radioactivity profiles of rat plasma containing [^{3}H]cholesterol-labeled HDL_2 incubated in vitro for 1 and 24 hr. During the 24-hr incubation about 50% of the plasma free cholesterol became esterified, and a shift of the HDL_2 towards lighter density was observed. Yet, there was no transfer of HDL_2 to HDL_1, and only minimal amounts of radioactivity separated with LDL.

DISCUSSION

The experiments contained in the present report describe the intravascular metabolism of the cholesteryl ester moiety of rat plasma LDL, HDL_1, and HDL_2. Because the rat is an animal species whose plasma lacks (6, 8), or nearly lacks (9) the cholesteryl ester transfer reaction, these experiments also reflect the fate in plasma of lipoprotein particles. The study demonstrates as well the usefulness of rate zonal centrifugation to prepare labeled lipoproteins suitable for metabolic studies, and to determine their intraplasma metabolism. For comparison, data from studies using iodination techniques are included (**Table 3**).

After the injection of labeled LDL, cholesteryl esters remain predominantly with the injected lipoprotein. The circulating half-life time of LDL cholesteryl esters, 7–8 hr, is 10–20% shorter than that reported for ^{125}I-LDL (1, 5, 20). Since ^{125}I-LDL isolated at density intervals of 1.006–1.040 g/ml (1) or 1.030–1.055 g/ml (5) probably contains labeled apoproteins other than apoB and these apoproteins are cleared from plasma slower than LDL (17), we consider our results similar to those reported with ^{125}I-LDL. We therefore suggest that in rats LDL is catabolized with the amounts of apoB and cholesteryl ester originally associated with the lipoprotein particle.

Previous results on the circulating life time of protein-labeled HDL_1 are controversial. One report from Dr. Mahley's laboratory indicates that after injection of iodinated HDL_1 to rats, labeled apoE distributes to other lipoproteins and the clearance of the apoprotein from plasma is rapid (19). In another report, ^{125}I-HDL_1 decayed from the plasma with a $t_{1/2}$ of about 6 hr (20). ApoE-HDL, prepared by affinity chromatography on heparin-Sepharose was reported to be catabolized in a liver perfusion system 10 times faster than apoA-I-HDL (26). Even faster decay of an analogous lipoprotein, HDL_c, was found in dogs (27). Van't Hooft and Havel, in contrast, reported

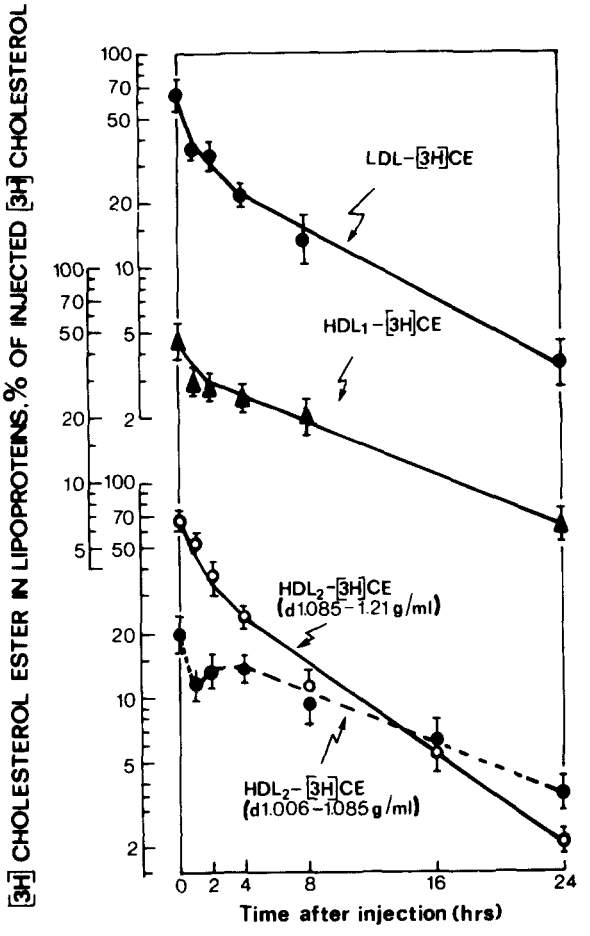

Fig. 4. Plasma decay of [^{3}H]cholesteryl esters injected with biosynthetically labeled LDL, HDL_1, and HDL_2. Decay curves were constructed as described in Methods. LDL-[^{3}H]CE decay and HDL_1-[^{3}H]CE decay are presented for the fraction of d 1.006–1.085 g/ml. HDL_2-[^{3}H]CE decay is presented at two density fractions: 1.006–1.085 g/ml and 1.085–1.21 g/ml. Data are means ± SEM of four experiments described in legend to Table 2.

that HDL labeled with either apoE or apoA-I and separated by gel filtration have similar metabolic behavior in rats and are removed rather slowly from the plasma (21). In that experiment, however, about 50% of the labeled apoprotein (A-I or E) disappeared from the plasma within 2 hr of the injection. Injection of a labeled apoprotein fraction enriched with apoE to rats, reported by us in 1973, also demonstrated a relatively slow plasma decay with a $t_{1/2}$ of about 7–8 hr (17). While some of the discrepancies between studies are undoubtedly due to different methods used to prepare labeled HDL_1, other differences reflect transfer of apoE between VLDL and HDL and insufficient knowledge of all catabolic pathways. The use of HDL_1 labeled with cholesteryl esters eliminates some of these uncertainties. With this method we found that HDL_1 particles are cleared from the circulation with a relatively long $t_{1/2}$ of 8–10 hr, in spite of their high apoE content. These observations support the conclusions of Van't Hooft and Havel that apoE present in HDL_1 does not cause rapid catabolism of the lipoprotein (21).

The results recorded here on the metabolic fate of HDL_2-cholesteryl esters are different from those obtained with labeled proteins and demonstrate the usefulness of the methods employed by us. With iodination techniques, labeled apoA-I remains at the density of the injected lipoprotein, and decays from plasma with a $t_{1/2}$ of 9–11 hr (see Table 3). After injection of HDL_2 labeled biosynthetically with [^{3}H]CE, however, a heterogenous metabolism of the lipoprotein is observed. With time, some of the HDL_2 cholesteryl esters (and therefore some of the HDL_2 particles) appear at a lighter density and gradually form HDL_1. Thus, [^{3}H]cholesteryl ester disappears from HDL_2 by two routes: catabolism of the lipoprotein and conversion to HDL_1. As a result, the $t_{1/2}$ of [^{3}H]CE in HDL_2 is only 6 hr, much faster than apoA-I. Appar-

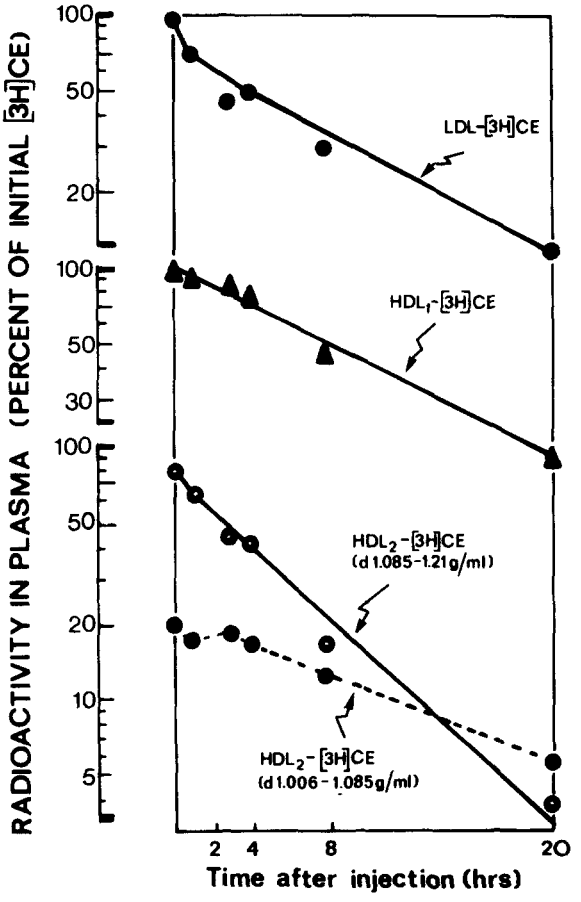

Fig. 5. Plasma decay of [^{3}H]cholesteryl ester injected with biologically screened, biosynthetically labeled LDL, HDL_1, and HDL_2. Decay curves were constructed as described in Methods. LDL-[^{3}H]CE decay and HDL_1-[^{3}H]CE decay are presented for the fraction of d 1.006–1.085 g/ml. HDL_2-[^{3}H]CE decay is presented at two fractions: 1.006–1.085 g/ml and 1.085–1.21 g/ml. Data are mean of two experiments.

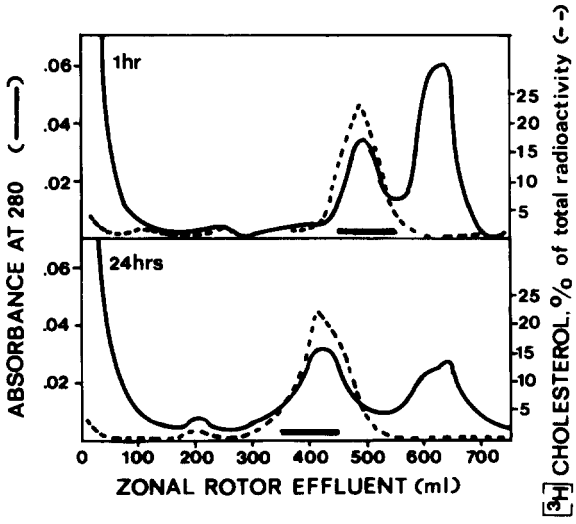

Fig. 6. Effect of in vitro incubation of [^{3}H]cholesterol-labeled HDL_2 with rat plasma on the lipoprotein elution profile and radioactivity distribution. Absorbance at 280 nm (solid line) and [^{3}H]cholesterol as percent of total radioactivity (broken line) were monitored along the rotor effluent. The solid bar denotes the position of HDL_2. [^{3}H]Cholesteryl ester contributed more than 96% of total radioactivity of the incubation mixtures and of the HDL_2 peak. Free [^{3}H]cholesterol was abundant in VLDL (the first 25 ml of the rotor effluent) and contributed 76.8% and 35.4% of total fraction radioactivity after 1 and 24 hr, respectively. Data are means of two experiments.

ently, apoA-I leaves HDL_2 during conversion of the particle to HDL_1 and re-distributes to newly formed HDL_2.

The data discussed above establish metabolic relationships between rat plasma HDL_1 and HDL_2 and indicate that at least part of the HDL_1 is formed in the plasma compartment. This probably reflects accumulation of LCAT-derived cholesteryl esters in HDL_2 and conversion of small HDL_2 to large HDL_1 particles. A plasma origin of HDL_1 was recently suggested by us when we found that HDL_1 cholesteryl esters contained a high proportion of arachidonate, indicating LCAT origin of these esters (6). Conversions of small and dense HDL to large and light particles (i.e., HDL_3 to HDL_2) was originally demonstrated during the course of VLDL lipolysis (28). More recent reports established the role of LCAT in HDL conversion (29). Yet, in the present investigation we were not able to form HDL_1 in vitro, even after 24 hr incubation of rat plasma when about 50% of plasma free cholesterol became esterified. This probably reflects paucity of free cholesterol in rat plasma and the necessity for extra-plasma sources of free cholesterol for $HDL_2 \rightarrow HDL_1$ conversion. Such sources are nascent lipoproteins and cell membranes. Even in the whole animal, however, only a small part of the HDL_2 is converted to HDL_1, as can be inferred from the low HDL_1 levels in rats (about one-fifth that of HDL_2 (6)) and the slow plasma decay of HDL_1. A similar situation exists in humans where HDL_2 levels are considerably lower than HDL_3 (30) in spite of the presumed $HDL_3 \rightarrow HDL_2$ conversion. Yet, the conversion of a small HDL particle to a larger lipoprotein (HDL_3 to HDL_2 in humans and HDL_2 to HDL_1 in rats) allows for accommodation of excess cholesterol in this lipoprotein, under appropriate conditions. We suggest that the abundance of HDL_c lipoprotein during cholesterol feeding in rats (31) represents a "cholesterol overload" situation and formation in plasma of the larger HDL population. The metabolic consequences of HDL conversions, however, are unclear and should be investigated in future studies.

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TABLE 3. Circulating half-life times of protein-labeled rat plasma lipoproteins

LDL			HDL_1			HDL_2		
Density	Half-life	Ref.	Density	Half-life	Ref.	Density	Half-life	Ref.
g/ml	*hr*		*g/ml*	*hr*		*g/ml*	*hr*	
1.006–1.04	9.4	(1)	[a]	Short	(19)	1.09–1.21	10.5	(2)
1.035–1.055	9.0	(5)	[a]	6–7	(20)	1.085–1.21	11–12	(4)
1.019–1.050	4.3	(18)	[b]	10	(21)	[b]	10	(21)
[a]	7–8	(20)	[c]	7–8	(17)	[c]	10	(17)
						1.05–1.21	6.2	(22)
						1.09–1.21	10.6	(23)
						1.09–1.21	8–9	(5)
						1.085–1.21	11–12	(24)
						1.06–1.21	7–8	(25)

Data are from 13 published studies.
[a] Prepared by electrophoresis on Geon-Pevikon.
[b] Prepared by chromatography on agarose columns.
[c] Iodine-labeled apoproteins. Prepared by chromatography on Sephadex columns: HDL_1, fraction VS-I (predominantly apoE); HDL_2, fraction HS-II (predominantly apoA-I).

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