Removal of lipid-rich lipoproteins by the liver

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Abstract Studies were performed to determine the mechanism of hepatic removal of a cholesterol-rich β-migrating lipoprotein. This fraction, designated IDL_c, was isolated from the serum of cholesterol-fed diabetic rats by ultracentrifugation at d 1.006–1.03 g/ml and contained apoproteins B, E,.C, and A-I. When ^{125}I-IDL_c (^{125}I-labeled IDL_c) was injected into normal chow-fed rats, 40% of the radioactivity was cleared from the plasma within 5 minutes with slight additional removal during the next 25 minutes. The rapid removal phase was due to the clearance of apoB-containing lipoproteins. The slow removal phase was due to transfer of apoA-I and C-apoproteins to HDL which has a considerably slower rate of turnover. The in vivo clearance of total ^{125}I-IDL_c radioactivity was enhanced by pretreatment of normal rats with 17α-ethinyl estradiol. This appeared to be associated with lack of transfer of apoA-I and C-apoproteins to HDL, and the removal of these apoproteins along with the apoB-containing lipoproteins. Treatment of rats with 17α-ethinyl estradiol did not result in an increased rate of removal of ^{125}I-IDL_c when their livers were perfused and this suggests that the removal of IDL_c is not mediated by the LDL (B, E) receptor whose activity is stimulated by estradiol administration—**Arbeeny, C. M., and H. A. Eder.** Removal of lipid-rich lipoproteins by the liver. *J. Lipid Res.* 1983. **24:** 1457–1467.

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Feeding a diet enriched in cholesterol and cholic acid to diabetic rats results in marked hypercholesterolemia (1) characterized by high concentrations of VLDL (d < 1.006 g/ml) and appearance of an IDL fraction (d 1.006–1.03 g/ml). This IDL has a mean particle diameter of 350 Å and contains cholesteryl esters as its primary core lipid. It contains both $apoB_H$ and B_L, apoE and C and, in addition, apoA-I. To differentiate this fraction from the IDL resulting from the metabolism of VLDL in normocholesterolemic rats, we propose to designate the d 1.006–1.03 g/ml fraction of the cholesterol-fed diabetic rats as IDL_c.

Studies by Breslow et al. (2) using primary hepatocyte cultures demonstrated that a similar fraction prepared from hypothyroid rats fed cholesterol and cholic acid strongly inhibited HMG-CoA reductase activity whereas fractions of d 1.006–1.04 g/ml lipoproteins prepared from serum of chow-fed rats were not inhibitory. They hypothesized that the inhibition of HMG-CoA reductase was due to increased uptake of the d < 1.04 g/ml lipoproteins of the hypercholesterolemic rats; these lipoproteins behaved like chylomicron remnants whose rapid removal increased hepatic cholesteryl ester content, thus inhibiting hepatic cholesterol synthesis.

Subsequent studies have shown that liver cells have two distinct receptor systems for the removal of lipoproteins from the circulation. One is the LDL receptor described by Brown and Goldstein (3) and the other is the chylomicron remnant receptor whose presence was suggested by perfusion studies of Cooper (4) and Sherrill and Dietschy (5). In addition to binding LDL, the LDL receptor binds other lipoproteins containing both apoB and apoE (3). The activity of this receptor is suppressed by cholesterol feeding (6, 7) and augmented by treatment with 17α-ethinyl estradiol (8). Studies by Mahley, Hui, and Innerarity (9) reported that the livers of mature dogs, swine, and humans do not have the LDL (apoB, E) receptor in their livers but have a specific high affinity receptor (the apoE receptor) that interacts with HDL_c and chylomicron remnants. Cooper et al. (10) prepared hepatocyte membranes that bind chylomicron remnants and found that this binding was competitively inhibited by DMPC vesicles containing apoE. However this receptor differed from the apoE receptor of Hui, Innerarity, and Mahley (11) in that it was not EDTA-sensitive.

Recent studies (12–14) have indicated that apoB is heterogeneous and consists of proteins of high molecular weight, B_H, and of lower molecular weight, B_L. In most species the B_H is produced by the liver and the B_L by the intestine (12, 15) and the type of apoB appears to play an important role in determining the receptor pathway involved. Thus in the rabbit, lipoproteins con-

Abbreviations: VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; DMPC, dimyristoyl phosphatidylcholine; CHD, cyclohexanedione; SDS, sodium dodecyl sulfate; TCA, trichloroacetic acid; TMU, tetramethylurea, NaPhT, sodium phosphotungstate; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; IDL_c, cholesterol-rich β-migrating lipoprotein isolated at d 1.006–1.03 g/ml.

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taining apoB_H are removed by the LDL pathway and lipoproteins containing apoB_L are removed by the chylomicron receptor pathway (15, 16). However, in the rat, VLDL secreted by the liver contains both B_H and B_L (17–19) and it could, therefore, be removed by both or either of the pathways. Rat chylomicrons contain predominantly B_L (13, 18, 20) and it could be expected that their remnants are removed by the chylomicron remnant receptor pathway.

Since IDL_c contains apoB_H, apoB_L, and apoE, it could be removed by the LDL receptor or by the chylomicron remnant receptor. We have, therefore, studied the removal of IDL_c in intact animals and in perfused livers in order to determine which of the receptor pathways mediates its removal.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 200–250 g were used in all studies. Unless otherwise noted, all rats had free access to water and standard Purina rat chow. In both in vivo and perfusion studies some chow-fed rats were injected subcutaneously with 17α-ethinyl estradiol dissolved in propylene glycol at a dose of 5 mg/kg of body weight (21). Rats were made hypercholesterolemic by feeding a diet containing 2% cholesterol and 1% cholic acid to streptozotocin-diabetic animals as previously described (1). One group of non-diabetic rats was fed a sucrose-enriched diet (22) in order to increase the levels of serum VLDL for the preparation of VLDL remnants. Animals were bled from the abdominal aorta under ether anesthesia and serum was obtained by low speed centrifugation.

Lipoproteins

Serum was fractionated by sequential ultracentrifugation (23) into the following density classes: VLDL, d < 1.006 g/ml; IDL_c, d 1.006–1.03 g/ml; LDL, d 1.03–1.063 g/ml; and HDL, d 1.063–1.21 g/ml. Human LDL was isolated from plasma at d 1.019–1.063 g/ml. Lipoproteins were purified by recentrifugation at the higher density limit. All density adjustments were made by the addition of solid KBr.

In one study the apoB-containing lipoproteins were removed from the serum of rats injected with ^{125}I-IDL_c by precipitation with sodium phosphotungstate (NaPhT) and $MgCl_2$ by a modification of the method described by Burstein, Scholnick, and Morfin (24). To 5 ml of serum, 0.5 ml of 4% NaPhT and 0.125 ml of 2 M $MgCl_2$ were added and the mixture was centrifuged for 1 hr at 1500 *g*. The supernatant was decanted and dialyzed against 0.85% NaCl and 0.01% EDTA. The density of the supernatant was then adjusted to 1.21 g/ml by the addition of solid KBr and centrifuged in the SW 41 rotor at 100,000 *g* for 48 hr at 15°C. The HDL and d > 1.21 g/ml fraction were separated by slicing the centrifuge tubes and the radioactivity in each fraction was determined. The precipitate was washed in 5 ml of 0.85% NaCl, 0.4% NaPhT, and 0.05 M $MgCl_2$, and recovered by centrifugation at 1500 *g* for 1 hr. The radioactivity in the precipitate was then determined. In control experiments using ^{125}I-IDL_c in 5 ml saline, 98% of the radioactivity could be precipitated by NaPhT and $MgCl_2$. A similar experiment was done with ^{125}I-HDL in saline and only 4% of the radioactivity was precipitated.

The VLDL and IDL_c were isolated from the serum of cholesterol-fed diabetic rats by ultracentrifugation and further characterized by gel chromatography (25) with 2% agarose (Bio Gel A 50 m). The VLDL eluted as two peaks: the first peak consisted of particles with a mean diameter of 1100 Å (950 to 1600 Å); the second peak consisted of particles with a mean diameter of 350 Å (300 to 500 Å). The IDL_c eluted with the second peak. Electrophoresis of the VLDL and IDL_c was performed by the method of Noble (26). The VLDL appeared as a broad band extending from the origin to the β region; the IDL_c exhibited β mobility.

Lymph was collected according to the method of Bollmann and Van Hook (27). Normal chow-fed rats were anesthetized with pentobarbital and the main mesenteric lymph duct and duodenum were cannulated. The rats were then placed in restraining cages and a sonicated lipid emulsion (28) was infused through the duodenal tube. Lymph was collected at room temperature over a 24-hr period in flasks containing 2 mg of dipotassium EDTA and 4 mg of gentamycin as described by Cooper et al. (10). Chylomicrons were isolated from lymph by ultracentrifugation at 15,000 rpm for 30 min at 15°C (10^6 *g* avg-min).

Remnants were produced by intravenous injection of lymph chylomicrons, VLDL, or IDL_c into fasted rats, functionally hepatectomized by ligation of the celiac axis, superior mesenteric artery, and the portal vein as described by Redgrave (29). After 40 min, the rats were exsanguinated. Remnants were prepared from chylomicrons or VLDL by ultracentrifugation of serum at 10^8 *g* avg-min at a density of d 1.019 g/ml and recentrifugated under the same conditions. Remnants prepared from IDL_c were centrifuged at d 1.03 g/ml at 10^8 *g* avg-min and washed by recentrifugation.

Lipoproteins were dialyzed against saline containing 0.01% EDTA. Protein was determined by a modification of the method of Lowry et al. (30). After delipi-

dation of the lipoproteins with ethanol–ether (31), the apoproteins were separated by SDS slab gel electrophoresis utilizing gels with a gradient of 3–27% acrylamide by the method of Swaney and Kuehl (32).

Lipoproteins were labeled with ^{125}I by the iodine monochloride method (33). The percentage of total radioactivity in chloroform–methanol extracts was 1.4%, 39.8%, 19.7%, and 2.1% in the IDL_c, chylomicron remnants, VLDL remnants, and human LDL, respectively. Cyclohexanedione modification of the arginyl residues of radioiodinated IDL_c was performed by the method described by Mahley et al. (34).

In vivo studies

Chow-fed rats were injected intravenously with ^{125}I-IDL_c containing 1 mg of protein. A 100-μl aliquot of blood was taken from the tail vein at frequent intervals over a 30-min period. The total radioactivity in the plasma at each time point was expressed as the percentage of administered radioactivity in the trichloroacetic acid-precipitable fraction, adjusted for plasma volume (4.5% of body weight). In the experiments shown in Fig. 1, two rats were killed at each time point and, in addition to the determination of radioactivity in plasma, the radioactivity in the liver was measured. Triplicate samples (ca. 250 mg) were removed and weighed, and the radioactivity was determined. The total radioactivity in the liver was calculated after determination of the total weight of the liver. Liver radioactivity was expressed as percent of injected radioactivity.

Removal of injected ^{125}I-IDL_c from the serum was also measured in functionally hepatectomized rats as well as in intact rats. Both groups were divided into two subgroups, one treated with 17α-ethinyl estradiol and the other untreated. At the 30 min time point, the rats were exsanguinated, the serum was fractionated into VLDL, IDL, LDL, and HDL by sequential ultracentrifugation, and the lipoproteins were washed by recentrifugation. The lipoprotein fractions were dialyzed, and the radioactivity in each fraction was determined. The apoproteins in each lipoprotein fraction were separated by SDS slab gel electrophoresis. The recovery in each apoprotein of the injected ^{125}I-IDL_c was determined as follows. The percentage of radioactivity associated with a specific apoprotein of each lipoprotein was determined by slicing the stained gels, as described by Weisgraber, Mahley, and Assmann (35). The recovery of radioactivity from the gel was 85%. The apoprotein radioactivity in the injected IDL_c was determined by multiplying the percentage of radioactivity associated with each apoprotein in the injected IDL_c by the total radioactivity injected. The apoprotein radioactivity in each lipoprotein isolated from the serum was determined by multiplying the percentage of radioactivity in that apoprotein by the total radioactivity recovered in the lipoprotein after adjusting for plasma volume (36). This value was then divided by the total radioactivity of the apoprotein in the injected IDL_c to give the percentage of the injected apoprotein recovered in each of the lipoprotein fractions.

Liver perfusions

Livers from chow-fed rats and from chow-fed rats treated with 17α-ethinyl estradiol were perfused with human LDL, VLDL remnants, IDL_c, IDL_c remnants, and chylomicron remnants in situ with 80 ml of perfusate as previously described (37). The perfusate consisted of washed bovine erythrocytes with a hematocrit of 22% in Krebs-Ringer bicarbonate buffer with 0.1% glucose and 1% bovine serum albumin (fraction V), equilibrated with 95% O_2 and 5% CO_2 at pH 7.4. For uptake studies, 1 mg of radiolabeled lipoprotein protein was added to the perfusate reservoir and recirculated for 60 min. At specific time intervals after addition of the lipoprotein, 250 μl of perfusate was removed from the reservoir, the erythrocytes were removed by centrifugation, and TCA-insoluble and soluble radioactivity was determined. The results were expressed as percent of initial radioactivity remaining in the perfusate. In a control experiment, when ^{125}I-labeled lipoproteins were circulated in the perfusion apparatus for 60 min in the absence of the liver, greater than 95% of the radioactivity could be recovered in the perfusate. The redistribution of IDL_c apoprotein radioactivity after 30 min of perfusion was also assayed. The perfusate was recovered, the red blood cells were removed by centrifugation, and the lipoproteins were separated by ultracentrifugation. Radioactivity in the lipoproteins and their constituent apoproteins was determined as described above.

In one study, ^{125}I-IDL_c, cyclohexanedione-modified ^{125}I-IDL_c, and ^{125}I-IDL_c remnants were added to the perfusate and the disappearance of TMU-precipitable radioactivity from the perfusate was measured. A 250-μl aliquot of perfusate was removed from the reservoir at each time point and cleared of red blood cells. Unlabeled human LDL (1 mg of protein/ml) was then added to each aliquot and precipitation was performed by adding an equal volume of TMU (38). The mixture was then allowed to stand for 24 hr at room temperature. The precipitate was pelleted by centrifugation at 2,000 *g* for 30 min at 4°C. The supernatant and precipitate were then counted. The results were expressed as the percent of initial TMU-precipitable radioactivity remaining in the perfusate.

RESULTS

IDL_c uptake

In vivo. When ^{125}I-IDL_c was injected into chow-fed rats and radioactivity in the serum and liver was measured at 1, 2, 5, 10, 15, and 30 min, it was found that more than 30% of the radioactivity disappeared from the plasma during the first 5 min, and after 30 min half of the administered radioactivity was removed from the plasma (**Fig. 1**). Corresponding to the decrease in plasma radioactivity was the accumulation of radioactivity in the liver. Within 15 min all the radioactivity removed from the plasma was recovered in the liver. To further demonstrate the role of the liver in the removal of ^{125}I-IDL_c, we measured its removal in functionally hepatectomized rats (**Fig. 2**). The removal was markedly decreased with over 80% of the administered radioactivity remaining in the plasma after 30 min. These findings indicate that the liver is the primary site of removal of IDL_c.

To study the time course of removal of ^{125}I-IDL_c, we compared the removal of apoB-containing lipoproteins separated by precipitation with NaPhT with the removal of radioactivity from the whole serum (**Fig. 3**). We also measured the appearance of radioactivity in the NaPhT supernatant. Radioactivity in the apoB-containing lipoproteins disappeared from the serum at a rate considerably more rapid than that of total radioactivity. After 5 min only 29% of the administered radioactivity was recovered in the NaPhT precipitate as compared to 58% in the unfractionated serum. The soluble fraction obtained after precipitation of the apoB-containing lipoproteins consisted of HDL and the $d > 1.21$ g/ml lipoprotein free fraction. Within 5 min 26% of the injected radioactivity was recovered in this fraction. This supernatant was subjected to ultracentrifugation and we found that at each time point 75% of the radioactivity recovered was in HDL ($d < 1.21$ g/ml) and the remainder was in the $d > 1.21$ g/ml fraction.

To determine the effect of estradiol on the removal of IDL_c, we administered ^{125}I-IDL_c to estrogen-treated rats. In Fig. 2 is shown the disappearance of radioactivity from the plasma of chow-fed rats and chow-fed rats treated with estradiol. The initial rates of disappearance were similar but the total radioactivity recovered after 30 min was 36% of the injected dose in the untreated rats and 12% in the estradiol-treated rats. Pretreatment of the functionally hepatectomized rats with estradiol did not result in augmented removal of radioactivity, indicating that estradiol has no effect on the uptake of IDL_c by extrahepatic tissues.

After the injection of ^{125}I-IDL_c into intact rats (**Table 1**), very little radioactivity was recovered in VLDL, IDL_c, and LDL but 30% was present in HDL. The data in **Table 2** demonstrate that this was due to removal of $apoB_H$ and $apoB_L$ from the plasma and transfer of apoA-I and C-apoproteins to HDL. In the hepatectomized rat 90% of the administered radioactivity was recovered in the plasma and this was due in part to the virtual absence of removal of $apoB_H$ and B_L and transfer of apoA-I and C-apoproteins to HDL.

Estradiol-treated rats behaved differently in that removal of all apoproteins was augmented. The removal

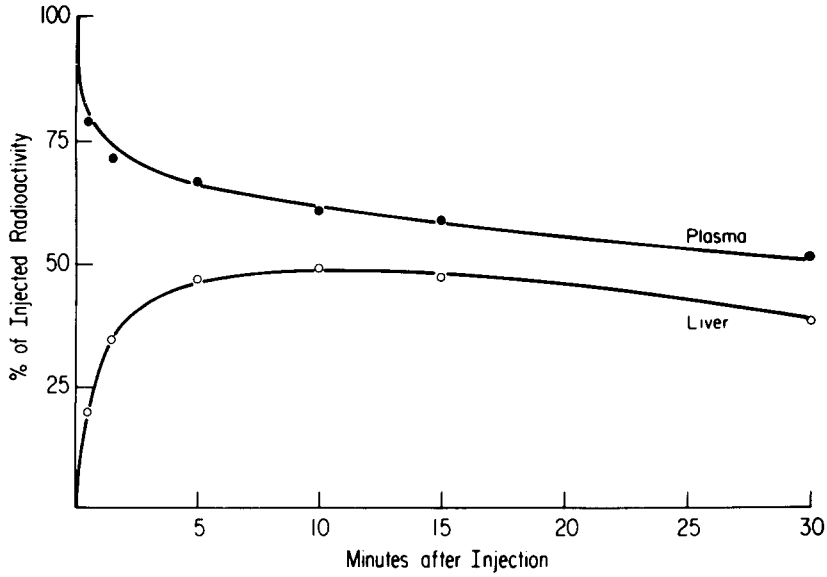

Fig. 1. Percentage of radioactivity recovered in the plasma and in the liver of chow-fed rats after intravenous injection of 1 mg of ^{125}I-IDL protein. Each point is the mean of values from three rats.

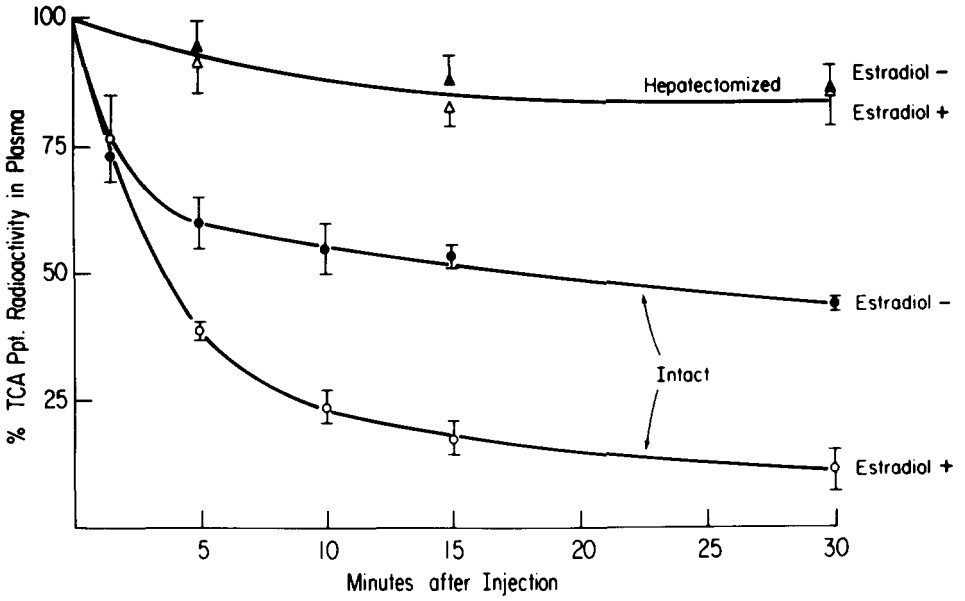

Fig. 2. Percentage of radioactivity in the plasma after injection of ^{125}I-IDL into intact or hepatectomized rats. Four animals in each of the two groups had been treated with estradiol (estradiol +) while four were not treated (estradiol −). Blood samples were obtained at each time point and TCA-precipitable radioactivity was determined in 100-μl aliquots of serum. Each point represents the mean values and the bars indicate standard deviation.

of apoB_H, B_L, and apoE was very slightly increased, but the major difference was the absence of accumulation of apoA-I and C-apoproteins in HDL. In the absence of transfer to HDL, it appears that apoA-I and C-apoproteins are removed to the same extent as apoB. In the hepatectomized rats given estradiol, no appreciable transfer of apoA-I and C-apoproteins to HDL occurred and considerably more of these apoproteins remained in the IDL_c fraction. However, in the hepatectomized rats treated with estrogen, less apoA-I and C-apoproteins were recovered in the plasma, suggesting extrahepatic removal of these apoproteins.

The transfer of apoA-I, C-apoproteins, and apoE was found to occur during in vitro incubation at 37°C of ^{125}I-IDL_c with normocholesterolemic serum from chow-fed rats (**Table 3**). Over 80% of the apoA-I recovered after incubation was present in HDL, as were almost half of the C-apoproteins. In studies where in vitro transfer of radioactivity to HDL was measured after precipitation of the apoB-containing lipoproteins with NaPhT, it was found that the transfer was rapid, with

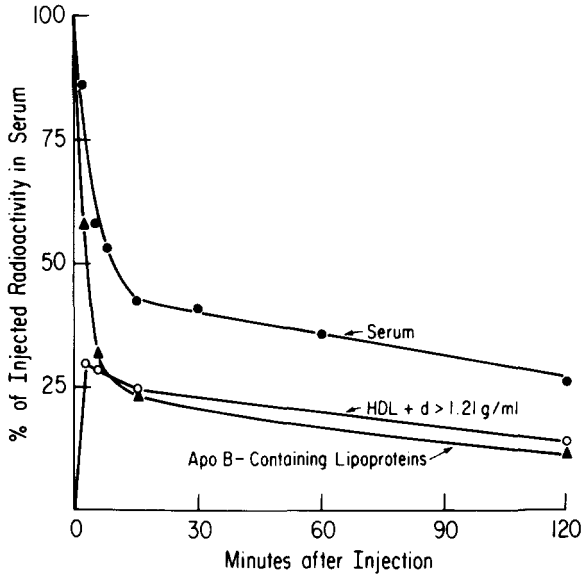

Fig. 3. Redistribution of radioactivity among NaPhT-soluble and -insoluble lipoproteins after injection of 1 mg of ^{125}I-IDL protein into chow-fed rats. At each time point the serum from two rats was pooled. The NaPhT-soluble (○ —— ○) and -insoluble (▲ —— ▲) lipoproteins were separated and radioactivity was determined.

TABLE 1. Recovery of radioactivity 30 min after injection of ^{125}I-IDL into rats[a]

	% of Injected Radioactivity[b]					
Recipients	VLDL	IDL	LDL	HDL	d > 1.21	Total
Untreated	4.6	2.4	2.6	30.5	3.2	43.3
Estradiol-treated	1.4	1.4	1.3	4.9	2.5	11.5
Hepatectomized	12.4	41.4	4.8	27.8	3.0	89.4
EE[c] hepatect.	10.0	61.4	4.2	5.5	10.5	91.6

[a] Equal aliquots of serum from four rats were pooled.
[b] The plasma volume was assumed to be 4.5% of the body weight except for the hepatectomized rats where it was assumed to be 2.35% of the body weight (36).
[c] Estradiol-treated.

TABLE 2. Recovery of apoproteins in the serum lipoprotein fractions 30 min after injection of ^{125}I-IDL[a]

Recipient Group	Apoprotein	% of Injected Apoprotein Radioactivity				
		VLDL	IDL	LDL	HDL	Total
Untreated[b]	B_H	1.7	5.7	2.4	0.8	10.6
	B_L	1.8	3.4	0.9	1.0	7.1
	E	4.5	1.5	2.1	17.2	25.3
	A-I	3.1	0.4	0.9	62.8	67.2
	C	11.0	0.9	7.5	62.0	81.4
Estradiol-treated[b]	B_H	0.7	2.8	1.5	0.1	5.1
	B_L	0.7	1.3	0.9	0.2	3.1
	E	1.4	1.1	0.6	2.8	5.9
	A-I	0.4	0.3	0.5	3.1	4.3
	C	3.7	2.6	1.0	11.6	18.9
Hepatectomized[c]	B_H	14.6	71.6	6.0	0.4	92.6
	B_L	16.2	62.9	7.2	0.0	86.3
	E	7.1	39.2	3.0	22.6	71.9
	A-I	1.6	8.6	1.8	70.4	82.4
	C	2.4	11.4	4.4	59.8	78.0
Estradiol hepatectomized[c]	B_H	8.0	74.9	5.5	0.5	88.9
	B_L	11.9	70.9	6.8	0.5	90.1
	E	9.1	70.4	3.0	3.0	85.5
	A-I	6.2	38.1	1.5	8.7	54.5
	C	7.4	45.5	4.0	4.7	61.6

[a] Equal aliquots of sera from four rats were pooled.
[b] The distribution of radioactivity among the apoproteins of the injected IDL was: B_H, 19.4%; B_L, 17.6%; A-IV, 1.4%; E, 5.5%; A-I, 23.9%; and C, 19.2%.
[c] The distribution of radioactivity among the apoproteins of the injected IDL was: B_H, 20.5%; B_L, 20.0%; A-IV, 1.9%; E, 7.5%; A-I, 20.3%; and C, 17.4%.

25% occurring in the first 5 min. It would thus appear that much of the transfer of apoA-I and C-apoproteins observed in vivo is due to exchange.

Liver perfusion. We have also studied the removal of IDL_c by the perfused liver (**Fig. 4**). Of the ^{125}I-IDL_c added to the perfusate, 50% was removed during the 60-min perfusion and over half of the radioactivity removed was taken up during the first 5 min of perfusion. When CHD-^{125}I-IDL_c was added to the perfusate, appreciably less was removed and 77% of the radioactivity remained in the perfusate after 60 min. Livers from estradiol-treated rats were perfused and no augmentation of removal of IDL_c was found, whereas the removal of human LDL and rat VLDL remnants was considerably increased (shown below). Livers from cholesterol-fed diabetic rats were also perfused. These livers were fatty and had cholesterol concentrations of 127 mg/g of liver as compared to those of chow-fed rats with cholesterol concentrations of 1.54 mg/g; triglyceride concentrations were 26 mg/g vs. 9 mg/g for the control rats. Uptake of IDL_c by the livers of cholesterol-fed diabetic rats did not differ from that of chow-fed untreated or estradiol-treated rats. It thus appears that the removal of IDL_c is not down-regulated by increasing the cholesterol content of the liver.

The recovery of labeled apoproteins present in the IDL_c added to the perfusate is shown in **Table 4.** As previously noted, 51% of the added radioactivity was recovered in the perfusate. The VLDL + IDL fraction contained 55% of the radioactivity recovered; HDL contained only 6% of the radioactivity recovered. Consistent with this is the finding of 66% of the administered B_H and 29% of the administered B_L in VLDL, IDL, and LDL. The ratio of B_H to B_L in the added ^{125}I-IDL_c was 1.2, whereas the ratio in the VLDL + IDL recovered was 2.1 and in the LDL it was 2.5. LDL contained 25% of the administered B_H but only 10% of the B_L. Thus

TABLE 3. Percentage of each apoprotein recovered in the apoproteins of the lipoproteins after incubation of ^{125}I-IDL with whole rat serum for 30 min at 37°C[a,b]

Apoprotein	% of Added Apoprotein Radioactivity				
	VLDL	IDL	LDL	HDL	Total
B	8.4	79.6	9.2	0.1	97.3
E	7.5	49.6	5.2	25.8	88.1
A-I	1.0	12.2	2.5	64.5	80.2
C	35.6	13.1	4.3	40.6	93.5

[a] The recovery of injected label in the lipoproteins after incubation of 10 ml of serum from chow-fed rats with 1 mg of IDL protein was: VLDL, 7.6%; IDL, 43.7%; LDL, 5.9%; HDL, 31.4%; and the d > 1.21 g/ml fraction, 4.0%; total recovery was 92.6%.
[b] The distribution of radioactivity among the apoproteins of the added IDL was: B, 35.7%; A-IV, 1.6%; E, 4.7%; A-I, 34.3%; and C, 14.7%.

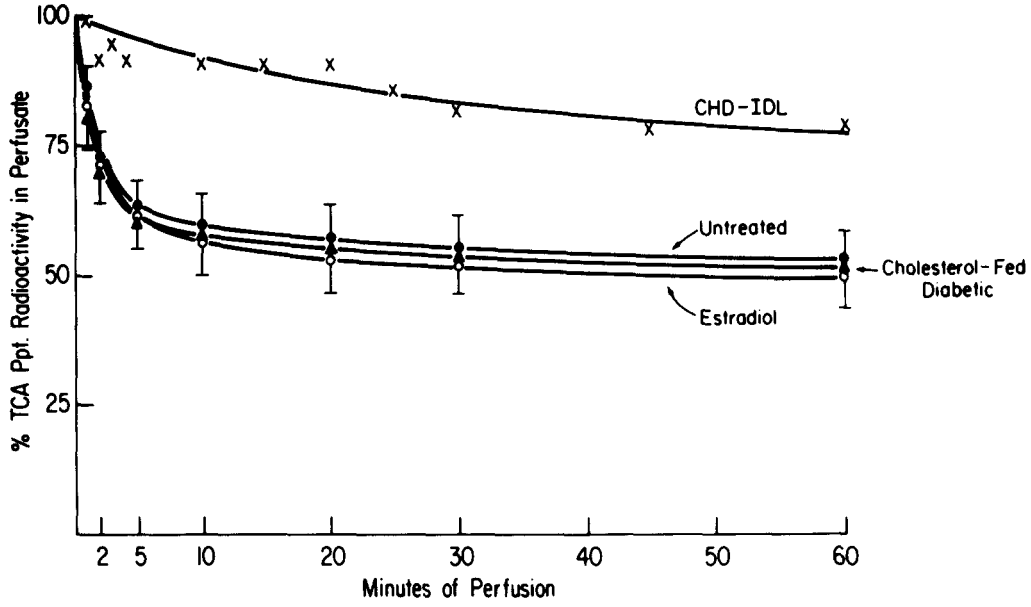

Fig. 4. Percentage of radioactivity recovered in the perfusate after addition of ^{125}I-IDL. Livers of untreated chow-fed rats (● —— ●, n = 4), chow-fed rats treated with estradiol (○ —— ○, n = 4), and cholesterol-fed diabetic rats (▲ —— ▲, n = 2) were perfused with ^{125}I-IDL (1 mg of protein) in a recirculating perfusion. In one experiment, the liver from a chow-fed rat (× —— ×) was perfused with cyclohexanedione-modified ^{125}I-IDL. Aliquots of perfusate were taken at the times indicated and the TCA-precipitable radioactivity was measured. Each point represents the mean values; bars indicate standard deviation.

a considerably greater proportion of B_L than B_H was removed and an appreciable amount of the B_H was recovered in LDL. These findings indicate that there is selective removal of B_L by the liver and formation of LDL enriched in B_H. A considerably greater proportion of apoE was removed, and of that remaining, more than 60% was in the VLDL + LDL fraction. Virtually all of the apoA-I was removed during the perfusion. On the other hand, the C-apoproteins, which in the intact animal also transfer to HDL, were not removed to any appreciable extent and were recovered in the VLDL + IDL fraction. The distribution of radioactivity in the apoproteins of the perfusates from the estradiol-treated liver donors was identical to that of the untreated animals (Table 4).

To test the possibility that the presence of apoA-I may affect the uptake of lipoproteins by the liver, we prepared IDL_c "remnants" by injecting IDL_c into functionally hepatectomized rats and harvesting the IDL_c "remnants" after 40 min. In **Fig. 5** are shown the remnants obtained after injection of IDL_c into functionally hepatectomized rats (lane 3) and into similar rats that had been treated with estradiol (lane 5). The principal difference was the absence of apoA-I in the remnants obtained from the rats not treated with estradiol and the presence of apoA-I in the hepatectomized rats treated with estradiol. There were no differences in the other apoproteins. We compared the removal of apoB from apoA-I-deficient IDL_c remnants and the removal of the apoB in remnants containing apoA-I. **Fig. 6** shows the removal of the TMU-precipitable radioactivity of ^{125}I-IDL_c, CHD-^{125}I-IDL_c, and both types of ^{125}I-IDL_c remnants during 60 min of liver perfusion. It is evident that the removal of the apoB from the remnants was more complete than apoB removal from IDL_c. The removal of CHD-^{125}I-IDL_c apoB was greatly impaired. However, the removal of both types of remnant particles was identical. This experiment suggests that the loss of apoA-I does not explain the difference between the

TABLE 4. Percentage of injected apoproteins recovered after 30 min perfusion of livers from untreated and estradiol-treated chow-fed rats

	% of Injected Apoprotein Radioactivity[a]		
Apoprotein	VLDL + IDL	LDL	HDL
B_H	41.0[b] (38.6)[c]	25.1 (22.0)	3.4 (2.7)
B_L	19.4 (20.0)	10.0 (9.2)	
E	17.7 (16.3)	5.5 (4.7)	3.5 (2.5)
A-I	1.8 (2.0)	1.3 (0.9)	1.5 (1.0)
C	54.3 (52.6)	5.4 (4.2)	5.3 (4.8)

[a] The distribution of radioactivity in the injected IDL was: B_H, 19.2%; B_L, 16.0%; A-IV, 3.5%; E, 5.5%; A-I, 36%; and C, 15%.
[b] With livers from untreated rats, the recovery of radioactivity in the perfusate was as follows: VLDL + IDL, 28%; LDL, 10%; HDL, 3.1%; and d > 1.21 g/ml fraction, 9.8%; total recovery was 51%.
[c] With livers from estradiol-treated rats, the recovery of radioactivity in the perfusate of estradiol-treated rats was as follows: VLDL + IDL, 25%; LDL, 9.5%; HDL, 2.9%; and d > 1.21 g/ml fraction; 10.3%; total recovery was 47.7%.

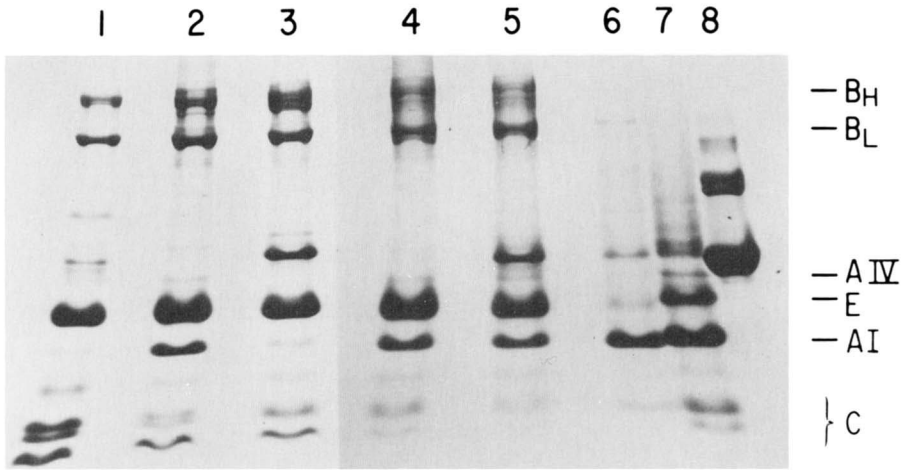

Fig. 5. SDS polyacrylamide gel electrophoresis, utilizing a gradient gel of 3 to 27% acrylamide. An aliquot containing 50 μg of apoprotein was applied to each position. Position 1, VLDL from chow-fed rats; positions 2 and 4, the ^{125}I-IDL injected; positions 3 and 7, IDL and HDL recovered from the plasma of functionally hepatectomized rats; positions 5 and 6, IDL and HDL recovered from the plasma of functionally hepatectomized rats treated with estradiol; position 8, bovine serum albumin. This figure is a composite of two gels.

removal of IDL_c and the IDL_c remnants, and that apoA-I does not modulate the uptake of apoB-containing lipoproteins by the liver. In an experiment (not shown) we found that the removal of the IDL_c remnants was not stimulated by estradiol pretreatment of the liver donor rats.

Removal of LDL, VLDL remnants, and chylomicron remnants

In order to compare removal of IDL_c with removal of lipoproteins whose pathways of removal have been defined more clearly, we have studied the removal of human LDL, rat VLDL remnants, and rat chylomicron remnants by perfused rat livers (**Fig. 7**).

When livers were perfused with human ^{125}I-LDL, 20% of the added radioactivity was removed after 60 min, with most of this occurring within the first 10 min. Estradiol treatment of the liver donor rats resulted in greatly increased uptake, with 40% of the radioactivity removed from the perfusate after 60 min. When ^{125}I-VLDL remnants were perfused through livers of normal chow-fed rats, 50% of the injected radioactivity

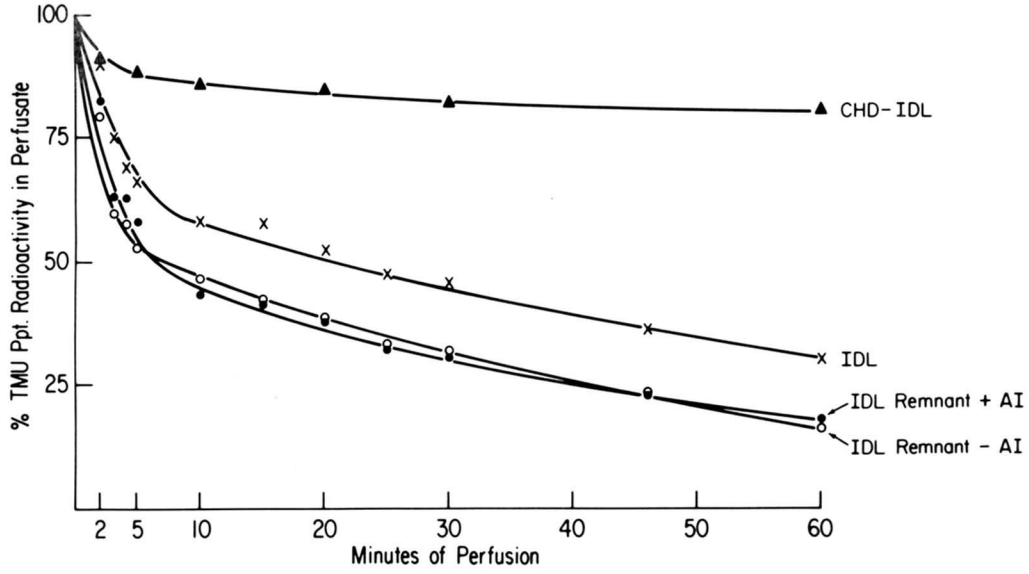

Fig. 6. Recovery of TMU-precipitable radioactivity in the perfusate after the addition of ^{125}I-IDL (500 μg of B-apoprotein). Livers of chow-fed rats were perfused with CHD-^{125}I-IDL (▲ —— ▲), ^{125}I-IDL (× —— ×), IDL remnant containing apoA-I isolated from functionally hepatectomized rats (● —— ●), and IDL remnant lacking apoA-I isolated from functionally hepatectomized rats pretreated with estradiol (○ —— ○). Data from a single representative experiment are shown.

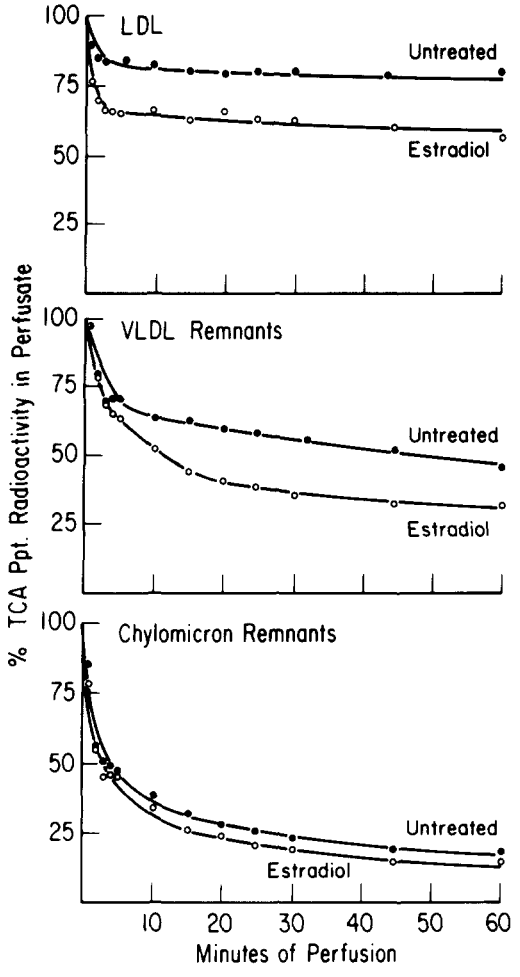

Fig. 7. Recovery of radioactivity in the perfusate after addition of human ^{125}I-LDL, ^{125}I-VLDL remnants, and ^{125}I-chylomicron remnants. Livers from chow-fed rats (● —— ●) and chow-fed rats treated with estradiol (○ —— ○) were perfused with radiolabeled lipoprotein in a recirculating system. Each point represents the mean of two paired experiments.

was removed within 60 min. However, when VLDL remnants were perfused through livers of estradiol-treated rats, there was a marked augmentation of removal and 65% of the radioactivity was removed after 60 min. The removal of chylomicron remnants prepared from lymph chylomicrons was compared by perfusing livers from normal and estradiol-treated rats. In both preparations 85% of the administered radioactivity was removed during the 60-min perfusion and there was no significant augmentation of chylomicron remnant removal by estradiol administration.

DISCUSSION

These studies have shown that IDL_c prepared from serum of cholesterol-fed diabetic rats is removed almost entirely by the liver. This was demonstrated in Fig. 1, showing that after 10 min almost 50% of the radioactivity in the injected ^{125}I-IDL_c was recovered in the liver at a time when 40% was removed from the plasma. Also, in functionally hepatectomized rats receiving ^{125}I-IDL_c, there was very little removal of radioactivity from the plasma. The marked impairment in removal of CHD-IDL_c suggests that this lipoprotein is removed by a receptor-mediated process and this was confirmed by the findings of Rifici, Arbeeny, and Eder (39) showing that isolated liver cells exhibit saturable binding of ^{125}I-IDL_c and that this binding is competitively inhibited by excess unlabeled IDL_c.

In parallel studies we have compared the removal of LDL, VLDL remnants, and chylomicron remnants and have found that the removal of LDL (containing B_H) and VLDL remnants (containing B_H and B_L) by perfused livers was stimulated by treatment of the liver donor rats with estradiol. Chylomicron remnants contain apoB_L and their removal is not stimulated by estradiol. The demonstration by Cooper, Shrewsbury, and Erickson (40) of competition between VLDL remnants and chylomicron remnants for hepatic binding suggests that VLDL remnants are also recognized by the chylomicron remnant receptor. It appears that response to estradiol treatment may serve to identify involvement of the LDL receptor pathway. The finding that receptor-mediated removal of IDL_c by the perfused liver is not stimulated by estradiol treatment of liver donor rats suggests that IDL_c is removed by the chylomicron remnant receptor despite its containing B_H. Further evidence that IDL_c is removed by the chylomicron remnant receptor was the finding that uptake of IDL_c was not down-regulated when livers of cholesterol-fed diabetic rats were perfused, since the activity of the chylomicron remnant receptor is not down-regulated by increasing the cholesterol content of the liver (41). The data in Table 4, demonstrating greater removal of B_L than B_H as well as transfer of considerable B_H radioactivity to LDL, are in accord with the findings of others (14, 17, 19). This observation raises the question whether the IDL_c fraction contains two different particles, one with B_L and the other with B_H, or whether during the metabolism of a particle containing both forms, there is preferential removal of the B_L, leaving B_H in the plasma where it becomes converted to LDL.

In the acute studies in the whole animal where IDL_c was administered, the removal of the apoB was minimally, if at all, affected by estrogen administration. This suggests that in the whole animal, as well as the perfused liver, removal of injected ^{125}I-IDL_c is mediated by the chylomicron remnant receptor. However, since this lipoprotein contains B_H, there is the potentiality for its removal by the LDL receptor. We had previously shown (31) that when hypercholesterolemic cholesterol-fed diabetic rats were injected with 17α-ethinyl estradiol for 5 days, the

VLDL, IDL_c, and LDL concentrations decreased significantly and this suggests involvement of the LDL receptor. It appears probable that in the short term experiments, lasting less than an hour, the removal of IDL_c by the chylomicron remnant receptor is so rapid that the activity of the LDL receptor is not apparent. However, during the 60-min perfusion, removal of VLDL remnants which have a very similar apoprotein composition to IDL_c is stimulated by estrogen administration. The reason for this difference may be related to the differences in the lipid composition of these lipoproteins: the core lipid in VLDL remnants consists largely of triglyceride while in the IDL_c it is largely cholesteryl ester and in this respect resembles β-VLDL.

In addition to uptake of apoB when ^{125}I-IDL_c was administered, transfer of labeled apoA-I, apoE, and C-apoproteins to HDL occurred. Since the rate of turnover of HDL is slow, radioactivity from these apoproteins remains in the plasma. Sparks and Marsh (19) also found that there was considerably less removal of apoC and apoE than apoB after administration of VLDL. Transfer occurred both in intact animals and in functionally hepatectomized animals, but not when these groups were treated with estradiol. Since estradiol-treated rats have greatly reduced concentrations of HDL (31), there is virtually no HDL pool to which apoA-I, apoC, and apoE can transfer. Similarly, in the perfusion studies where no HDL was present, apoA-I, apoE, and C-apoprotein did not transfer and apoA-I and apoE were removed to an even greater extent than $apoB_H$. In vitro studies where ^{125}I-IDL_c was added to serum also demonstrated transfer of apoA-I, apoE, and C-apoproteins to HDL. This transfer was shown to occur within 2 min after addition of IDL_c in a study where the HDL was separated by NaPhT precipitation. Since the amount of radioactivity transferred corresponded to the relative pool size of these apoproteins in IDL_c and HDL, it would appear that this transfer occurs by exchange and probably not by net transfer as suggested by Suri, Targ, and Robinson (42). In hepatectomized rats treated with estradiol, almost half of the apoA-I was lost from the plasma and this suggests that apoA-I is removed by extrahepatic tissues as shown by Glass, Pittman, and Steinberg (43).

Previous studies have shown that apoC can modulate the removal of triglyceride-rich lipoproteins (44) and it has been suggested that apoA-I may play a similar role. Since IDL_c contains apoA-I we have been able to study this possible role of apoA-I by injecting ^{125}I-IDL_c into functionally hepatectomized rats. This results in loss of apoA-I from the IDL_c. However, when the same experiment is done with functionally hepatectomized rats treated with estradiol, apoA-I is not lost from the IDL_c. When livers were perfused with IDL_c remnants containing apoA-I and deficient in apoA-I, no differences in the rate or amount of removal of the two lipoproteins was found. This experiment suggests that apoA-I does not modulate the removal of IDL_c. Although the apoprotein composition of IDL_c was not altered after it was injected into estradiol-treated hepatectomized rats, its removal was increased and this provides additional evidence that factors other than apoprotein composition affect lipoprotein removal by the liver. Thus, because IDL_c contains apoA-I, it provides a unique opportunity to study the behavior of apoA-I present in lipid-rich lipoproteins.

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