Catabolism of the apoprotein of low density lipoproteins by the isolated perfused rat liver

Gunnar Sigurdsson,[1] Simon-Pierre Noel, and Richard J. Havel

Cardiovascular Research Institute and Department of Medicine, University of California, San Francisco, CA 94143

Abstract Isolated rat livers were perfused for 4 hours in a recirculating system containing washed rat erythrocytes. Biologically screened, radioiodinated low density lipoproteins ($1.030 < d < 1.055$ g/ml) were added to the perfusate with different amounts of whole serum to supply unlabeled rat low density lipoproteins. Apolipoprotein B contained 90% of the bound ^{131}I, other apolipoproteins contained 4%, and lipids contained the remainder. 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 fractional catabolic rate ranged from 0.3 to 1.7%/hr in seven experiments and was inversely related to the size of perfusate pool of low density apolipoprotein. The catabolic rate of low density apolipoprotein (fractional catabolic rate × pool size) in four livers, in which the concentration of rat low density lipoproteins was 50–100% of that present in intact rats, was 5.3 ± 2.7 μg hr^{-1} (mean ± SD). Similar results were obtained with human low density lipoproteins. These rates were compared with catabolic rates for the apoprotein of rat low density lipoproteins in intact animals. Fractional catabolic rate in vivo, obtained by multicompartmental analysis of the disappearance curve of ^{131}I-labeled low density apolipoprotein from blood plasma, was $15.2 \pm 3.1\%$ hr^{-1} (mean ± SD). Total catabolic rate in vivo (fractional catabolic rate × intravascular pool of low density apolipoprotein) was 76 ± 14 μg hr^{-1} (mean ± SD). The results suggest that only a small fraction of low density apolipoprotein mass in rats is degraded by the liver.

Supplementary key words fractional catabolic rate · apoB

Cholesterol is excreted from the body primarily in the bile and to a lesser extent with desquamated skin. For this reason, it has been postulated that the cholesterol-rich lipoproteins, low density lipoproteins (LDL), and high density lipoproteins (HDL) are catabolized by the liver. In the case of LDL, this view has been supported by measurements of the distribution of protein-bound radioiodine in tissues after injection of radioiodinated LDL, which showed that the liver contained the highest concentration of radioactivity (1, 2). However, the radioiodine recovered in the liver in these studies represented less than 3% of that injected (1, 2).

The only direct evidence that the liver is capable of taking up and degrading LDL comes from the study of Hay et al. (3). They perfused isolated rat livers with radioiodinated rat LDL and concluded that the liver can account for most of the LDL catabolized in the intact rat. In these studies rat LDL was isolated by preparative ultracentrifugation between densities of 1.006 and 1.040 g/ml. The density of some very low density lipoprotein (VLDL) remnants may be as high as 1.019 g/ml or more (5) so that uptake of VLDL remnants by the liver (4, 5) may have contributed to the apparent uptake of LDL. Heparin, which has been shown to affect both VLDL and LDL metabolism (6, 7), was also used by Hay et al. (3).

In recent years evidence has been accumulating that several cells, including human skin fibroblasts, aortic smooth muscle cells, and blood lymphocytes, are capable of taking up and degrading LDL in tissue culture (7). These and related observations have led to the hypothesis that uptake of LDL by cells in human extrahepatic tissues normally provides the cholesterol needed for membrane synthesis and, in the case of the adrenal cortex and gonads, synthesis of steroid hormones (8). Sniderman et al. (9) have found degradation of apoLDL to be increased rather than decreased in hepatectomized pigs, which suggests that in this species LDL is primarily degraded in extrahepatic tissues.

In the present research the rate of catabolism of radioiodinated rat LDL was measured in intact rats and in isolated perfused rat livers. LDL was isolated within a narrow density interval ($1.030 < d < 1.055$

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; EDTA, disodium ethylenediaminetetraacetate; TMU, tetramethylurea; TCA, trichloroacetic acid; FCR, fractional catabolic rate; ^{131}I-LDL, ^{131}I-labeled LDL.

[1] Present address: Icelandic Heart Association, Lagmuli 9, Reykjavik, Iceland.

g/ml) and no heparin was added to the perfusates. A preliminary report of this work has appeared (10).

MATERIALS AND METHODS

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

Preparation of radioiodinated LDL

To minimize incorporation of labeled iodine into the lipid moiety of LDL, donor rats were fed a saturated fat diet (butterfat 50%, casein 30%, cellulose 10%, salt mixture 6%, a complete vitamin mixture 3%) for 3 days before they were exsanguinated under diethyl ether anesthesia. The blood was mixed with disodium ethylene-diaminetetraacetate (EDTA), 1 mg/ml, and chilled on ice. LDL, $1.030 < d < 1.055$ g/ml (4), was isolated by sequential ultracentrifugations (11) at 4°C for 1.4×10^8 *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 ($1.030 < d < 1.055$ g/ml) were recovered by tube-slicing and dialyzed against 0.15 M NaCl and 0.04% disodium EDTA. The purified LDL was 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 mole of iodine was attached per 100,000 mol wt units of apoLDL. Unbound iodine was removed by dialysis against 0.15 M NaCl–0.04% EDTA. The radiolabeled preparation of LDL was then screened by injecting it into a tail vein of a rat. The recipient rats were bled 3 hr later. The density of the serum obtained was adjusted to 1.055 g/ml (10) and the sample was centrifuged for 20 hr at 105,000 *g*. The supernatant lipoproteins were recovered by tube-slicing and dialyzed against Krebs-Ringer–bicarbonate buffer. This preparation was used for in vivo studies or for liver perfusions on the same day. Human LDL ($1.019 < d < 1.063$ g/ml) was isolated from plasma of normolipidemic individuals; it was then iodinated and screened as described for rat LDL.

Characterization of radioiodinated LDL

After biological screening of rat ^{131}I-LDL for 3 hr, about 90% of the radioactivity in LDL was in apolipoprotein B as determined by its insolubility in tetramethylurea (TMU) (14). Four percent of the ^{131}I was in the TMU-soluble apolipoproteins (14) and the remaining 6% was in the lipoprotein lipids as determined by extraction into ethanol–ether 3:1 (v/v) (15). Upon agarose gel electrophoresis (4) more than 95% of total ^{131}I-LDL migrated with beta lipoprotein. No discrete bands of ^{131}I were found in other gel segments. By radioimmunoassay (16, 17) apoprotein A-I comprised 0.04% and arginine-rich apoprotein 3.6% of the total apoprotein.

Feeding the saturated fat diet to donor rats for 3 days reduced labeling of the lipid moiety from 20–30% to about 6%. The rate of appearance of nonprotein-bound ^{131}I into the perfusate with ^{131}I-LDL prepared from rats fed a standard Purina chow (Ralston Purina Co., St. Louis, MO) (0.29% hr^{-1}) was closely similar to that obtained with ^{131}I-LDL prepared from rats fed the saturated fat diet (0.31% hr^{-1}) and the disappearance rates of the two preparations from blood plasma after intravenous injection into intact rats were indistinguishable.

More than 90% of circulating ^{131}I remained within the range $1.030 < d < 1.055$ g/ml in the perfusate as well as in the plasma of recipient rats, as determined by sequential ultracentrifugation. Lipid-soluble ^{131}I was about 6% at the beginning of perfusion and about 5% at the end of a 4-hr perfusion.

Technique of liver perfusion

The rats used as donors for livers and erythrocytes were fed the standard Purina chow ad libitum. To obtain erythrocytes, blood drawn from the abdominal aorta of rats under light diethyl ether anesthesia was mixed with 0.2 ml of a solution containing 1.32% sodium citrate, 0.44% citric acid, and 1.32% glucose (18) and then chilled on ice. The procedure for isolation and perfusion of livers was adapted from Miller et al. (19). Fed liver donors were anesthetized by intraperitoneal injection of sodium pentobarbital (60 mg/kg body wt) between 10 AM and noon. The peritoneal cavity was opened and the common bile duct was cannulated with polyethylene tubing (PE10, Clay Adams, Division of Becton Dickinson and Co., Parsippany, NJ). The portal vein was then cannulated with a Teflon catheter (16 gauge, Angiocath Intravenous Placement Unit, Deseret Pharmaceutical Co., Sandy, UT) and the flow of perfusate (10% hematocrit) was started 10–20 sec after blood flow into the liver had ceased. The liver was flushed with 100 ml of this perfusate to remove blood trapped within the liver. Perfusion was then continued with fresh perfusate at 25% hematocrit for 4 hr in a recirculating system. Membrane oxygenation was with a "lung" of silastic tubing as described by Hamilton et al. (20). The perfusate contained washed (four times) rat erythrocytes in Krebs–Ringer–calcium bicarbonate buffer with 100 mg/dl glucose, equilibrated with 95% O_2–5% CO_2 at pH 7.4

(21). Monoiodotyrosine, 100 mg, was added to reduce nonspecific deiodination owing to deiodinase in rat serum (22). The volume of perfusate was 70 ml and the flow rate was 13 ml/min. The concentration of LDL in the perfusate ranged from 3% to 100% of the normal concentration in rat blood plasma. To obtain the different concentrations of LDL in the perfusate, measured volumes of 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. The perfused liver was thus exposed to all the lipoproteins and other colloidal components of rat serum present under in vivo conditions. The radioiodinated preparation was added to the perfusate after a 15-min "recovery" period. In those experiments in which the liver was perfused with radioiodinated human LDL, unlabeled human LDL ($1.019 < d < 1.063$ g/ml) was added to the perfusate.

A control experiment was carried out for each liver perfusion in which the same preparation of iodinated LDL in the standard perfusion mixture was circulated in the perfusion apparatus for 4 hr in the absence of a liver.

Viability of the perfused livers was evaluated by the rates of O_2 consumption, CO_2 production, bile secretion, and secretion of triglycerides (20, 21). The O_2 tension of the perfusate was >400 mm Hg on the arterial side and <100 mm Hg on the venous side of the perfusate circuit. The CO_2 tension was about 50 mm Hg on the arterial side and about 60 mm Hg on the venous side. The rate of bile flow was 0.4–0.6 ml/hr. Secretion of triglycerides was 0.2–0.4 mg/ml of perfusate in 4 hr. Only livers fulfilling these criteria, which are equal or better than those described previously (20, 21), were accepted for analysis.

Estimation of catabolic rates

Every 30 min during the perfusion 1.5 ml of perfusate was removed from the circuit and chilled on ice. After removal of erythrocytes by centrifugation, a portion of the "plasma", mixed with 0.05 vol of 20% bovine serum albumin, was added to 2 vol of 20% trichloroacetic acid (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 counter. The results for each were expressed as percentage of total radioactivity (TCA precipitate + TCA-soluble). The TCA-soluble ^{131}I in the supernatant fluid was subsequently mixed with chloroform (1:2 v/v), after peroxidation as described by Bierman, Stein, and Stein (22) to remove radioiodide not bound to iodotyrosine, and chloroform-soluble and water-soluble ^{131}I were determined.

The fractional catabolic rate (FCR) of apoLDL 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 catabolic rate of apoLDL was determined as the product of the FCR and the amount of apoLDL in the perfusate.

A fraction of the radioiodinated LDL 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 24 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 and during the experiment in order to block uptake of iodine by the thyroid gland. FCR was estimated by the method of Mathews (23), with the assumption of a two-compartmental model for the metabolism of apoLDL. The catabolic rate was determined as FCR × intravascular pool of apoLDL. Plasma volume was taken as 4.5% of body weight (24). The mean concentration of TMU-insoluble apoLDL in rat plasma (0.034 mg/ml) as determined by Faergeman et al. (4) in the same strain of rats fed the same standard Purina chow was used for the calculation.

RESULTS

Appearance of TCA-soluble ^{131}I in liver perfusates

Any significant accumulation of iodide in the liver during the perfusion would invalidate calculations of catabolic rate based upon the level of non-protein-bound ^{131}I released into the perfusate. Control experiments were therefore carried out in which a tracer amount of carrier-free ^{131}I was added to the perfusate and the volume of distribution was determined at different times of perfusion. This volume increased about 5% during the first 15 min but remained constant thereafter for 4 hr. These findings are similar to those of Cohen and Gordon (25). On the basis of these observations the baseline sample was taken 15 min after adding the radioiodinated LDL to the perfusion circuit. Non-protein-bound ^{131}I increased linearly with time in the perfusate after a 30–60 min "latent" period (**Fig. 1**).[2] This latent period is similar to that observed in cell culture studies (26).

[2] In preliminary experiments with unscreened ^{131}I-LDL, the rate of appearance of non-protein-bound ^{131}I was greater during the first hr of perfusion (0.7%) than with screened ^{131}I-LDL (~0.45%). Thereafter the rates were the same for the two preparations.

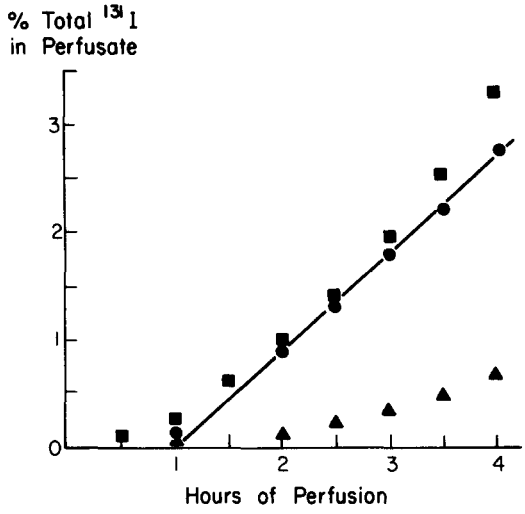

Fig. 1. Production of non-protein-bound ^{131}I during perfusion of a rat liver with labeled and screened rat LDL. Total pool size of apoLDL was 1100 μg. ■ —— ■, Increment of TCA-soluble ^{131}I in perfusate; ▲ —— ▲, increment of TCA-soluble ^{131}I without liver; ● —— ●, corrected rate of accumulation of TCA-soluble ^{131}I.

The rates of appearance of water-soluble ^{131}I (presumably ^{131}I-tyrosine) and chloroform-soluble ^{131}I (radioiodide) were, respectively, 55 ± 5% and 45 ± 4% of total TCA-soluble ^{131}I (mean values ± SD). The disappearance of protein-bound ^{131}I from the perfusate was accounted for by the increase in non-protein-bound ^{131}I in the perfusate and the ^{131}I remaining the liver (after flushing 50 ml of Krebs–Ringer–calcium bicarbonate buffer through the liver via the portal vein) at the end of 4 hr of perfusion. Total recovery was 100 ± 2%. The amount remaining in the liver was less than 3% of the ^{131}I-LDL added to the perfusate. More than 80% of this ^{131}I radioactivity was precipitated by TCA. A similar but smaller increment of non-protein-bound ^{131}I was observed during control perfusions in the absence of a liver (Fig. 1); 70–80% of this increment was chloroform-soluble. The total increment of ^{131}I radioactivity in the chloroform phase was consistently greater during liver perfusions than in the control circuit. This suggests that ^{131}I released when ^{131}I-LDL 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.[3] Therefore we chose to express the rate of ^{131}I-LDL 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 a liver. It should be noted that the actual degradation by the liver could be overestimated by as much as 45% as the extent to which deiodination by the liver reflects actual proteolysis is unknown.

[3] This is consistent with the finding of Nakai et al. (27), who measured the uptake of radioiodinated HDL by isolated rat liver parenchymal cells. Iodotyrosine deiodinase activity has also been found in liver microsomes (28).

TABLE 1. Catabolism of screened ^{131}I-labeled LDL in isolated, perfused rat livers

	FCR	Perfusate Pool Size	Catabolic Rate
	% hr^{-1}	μg	μg hr^{-1}
Rat LDL[a]	1.70	50	0.90
	1.19	200	2.40
	1.30	680	8.80
	0.31	1000	3.10
	0.44	1000	4.40
	0.75	1100	8.30
	0.31	1700	5.30
Human LDL[b]	1.28	450	2.50
	0.22	2000	4.40

[a] $1.030 < d < 1.055$ g/ml.
[b] $1.019 < d < 1.063$ g/ml.

The small amount of ^{131}I secreted into the bile during the perfusion was also measured and this value was added in the calculation of FCR, although it accounted for less than 10% of the total non-protein-bound ^{131}I produced.[4] The calculated FCR ranged from 0.3 to 1.7% per hr (**Table 1**) and was inversely related to the mass of apoLDL in the perfusate (**Fig. 2**).

[4] For comparison, the bile duct was cannulated in intact rats under diethyl ether anesthesia and the bile was collected for 4 hr after injection of ^{131}I-LDL into a tail vein. The results obtained in two experiments were closely similar to those obtained during liver perfusions. During this 4-hr period less than 0.5% of the amount of the ^{131}I injected was recovered in the bile.

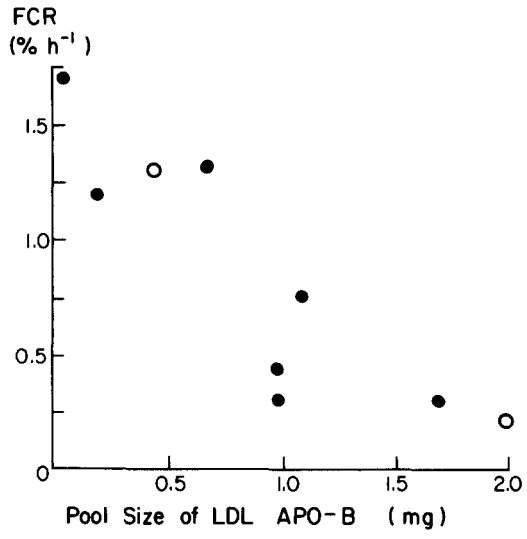

Fig. 2. Relationship between pool size of B apoprotein of LDL and its fractional catabolic rate, measured from production of TCA-soluble ^{131}I during perfusion of rat livers with labeled and screened rat or human LDL. ● —— ●, Rat LDL; ○ —— ○, human LDL.

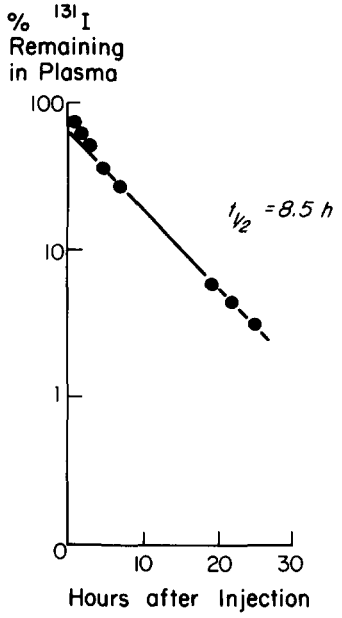

Fig. 3. Disappearance of ^{131}I-labeled and screened rat LDL injected intravenously into an intact rat.

No correction was made for the small amount of ^{131}I-LDL removed by sampling during the perfusion. This correction would slightly decrease the observed rate of degradation of apoLDL.

The catabolic rate of apoLDL in four livers, in which the concentration of rat LDL was 50–100% of that present in intact rats, was 5.3 ± 2.7 μg hr^{-1}. (Table 1). The catabolic rate of human LDL was in the same range as for rat LDL (Table 1).

Catabolism of ^{131}I-apoLDL in vivo

In intact rats, the rate of removal of labeled LDL from blood plasma was biexponential (**Fig. 3**), as described in humans (29). The mean t½ of the second exponential was 8.5 hr by linear regression analysis. Results for seven rats are summarized in **Table 2.** The average catabolic rate was 76 ± 14 μg hr^{-1}. No difference between the behavior of screened and unscreened ^{131}I-LDL was detected in vivo. The rate of appearance of non-protein-bound ^{131}I in bile was similar (<0.05% hr^{-1}) to that observed in experiments with perfused livers.

DISCUSSION

Our results showing rates of catabolism of ^{131}I apoLDL in the perfused liver of 5.3 ± 2.7 μg hr^{-1}, as compared with 76 ± 14 μg hr^{-1} in intact rats, suggest that only about 7% of apolipoprotein B of rat LDL is catabolized by the liver. It is of course possible that the rat liver catabolizes LDL more rapidly in vivo than we have observed in perfused livers. In the experiments with rat lipoproteins, we added whole rat serum to the perfusate to reproduce physiological conditions with respect to exposure of the liver to other lipoproteins that might influence uptake and catabolism of LDL. The similar rate of appearance of non-protein-bound ^{131}I in bile in the intact rat and perfused liver provides some support for the use of this approach to the study of LDL catabolism. Our findings are consistent with those of Sniderman and associates (9) in pigs which also suggested that apoLDL is removed mainly by extrahepatic tissues.

Whether the LDL particle is taken up intact by liver cells by the receptor-dependent pinocytotic mechanism described by Goldstein and Brown (7) and others for some extrahepatic cells is not known. The inverse relationship found in the present study between the FCR and the mass of apoLDL in the perfusate is consistent with a pinocytotic mechanism of uptake that is readily saturated. This inverse relationship contrasts with the constant fractional removal rate found for albumin, transferrin, and fibrinogen in isolated perfused rat livers (30).

The rate of apoLDL catabolism by the perfused liver in the present study was somewhat greater at higher concentrations and it is possible that two mechanisms, with "low" and "high" affinities, exist, as described for uptake of apoLDL by human fibroblasts.

Hay and associates (3) concluded from their studies of the uptake of radioiodinated LDL by isolated perfused rat livers that the liver may account for a major part of the disappearance of radioiodinated LDL in vivo. These investigators calculated the rate of disappearance of protein-bound ^{125}I-LDL from the perfusate to estimate degradation by the liver. This fall in protein-bound ^{125}I may reflect only uptake from the perfusate rather than actual degradation. The rate of accumulation of non-protein-bound radioiodine in the perfusate in their studies, however, was very similar to that observed by us.

TABLE 2. Catabolism of screened ^{131}I-labeled rat LDL in intact rats

FCR	Intravascular Pool Size	Catabolic Rate
% hr^{-1}	μg	$\mu g\ hr^{-1}$
13.4	500	67
18.0	535	93
19.6	470	98
13.8	480	69
12.5	475	60
15.1	490	74
14.3	505	72
15.2 ± 2.6[a]	494 ± 22[a]	76.1 ± 14[a]

[a] Mean ± SD.

Stein and associates (31) have recently reported that intraperitoneal injection of chloroquine, a known inhibitor of the lysosomal proteases that have been implicated in the degradation of apoLDL by cultured human fibroblasts (32), does not cause accumulation of TCA-precipitable ^{125}I derived from radioiodinated LDL in livers of rats. Administration of this compound led to a substantial increase in hepatic ^{125}I when radioiodinated VLDL was injected. From these indirect studies, they also concluded that degradation of LDL occurs mainly in extrahepatic tissues.

Our results and those of others are all consistent with the view that LDL serves to transport cholesterol mainly to extrahepatic tissues. In the rat, most of the cholesteryl esters of LDL are derived from the action of LCAT (33) and therefore do not originate in the liver. The fate of cholesteryl esters of rat HDL, which are also derived from LCAT, is unknown.

The rate at which cholesteryl esters of rat LDL are removed from the blood can be calculated from the ratio of esterified cholesterol to B apoprotein in this lipoprotein (1.8:1), if it is assumed that LDL are removed by pinocytosis (7). This rate is about 140 $\mu g\ hr^{-1}$, which can be compared with the catabolic rate of esterified cholesterol in rat VLDL of about 2400 $\mu g\ hr^{-1}$ (33). Esterified cholesterol in rat VLDL is largely taken up by the liver and it is therefore evident that hepatic uptake of esterified cholesterol in LDL is likely to account for very little of the total hepatic uptake of esterified cholesterol in these two lipoproteins. It should not be concluded, however, that hepatic uptake of LDL cholesterol is physiologically insignificant. In homozygous familial hypercholesterolemia, in which no functional high-affinity receptors for LDL are present, the rate of synthesis of B apoprotein is considerably increased (34). Receptor-dependent hepatic uptake of LDL could regulate synthesis of B apoprotein in the liver. Hepatic uptake of LDL may be of more importance in this regulation in humans than in rats, because little B apoprotein enters the human liver in the form of VLDL remnants (35).

We are grateful to Esther Chou and Anneliese Merlino for expert technical assistance. Dr. Noel was a Fellow of the Canadian Medical Research Council. This research was supported by a grant from the United States Public Health Service (HL-06285).

Manuscript received 16 August 1977; accepted 1 February 1978.

REFERENCES

1. Eisenberg, A., H. G. Windmueller, and R. I. Levy. 1973. Metabolic fate of rat and human lipoprotein apoproteins in the rat. *J. Lipid Res.* **14:** 446–458.
2. Calvert, G. D., P. J. Scott, and D. N. Sharpe. 1975. The plasma and tissue turnover and distribution of two radio-iodine-labelled pig plasma low density lipoproteins. *Atherosclerosis* **22:** 601–628.
3. Hay, R. V., L. A. Pottenger, A. L. Reingold, G. S. Getz, and R. W. Wissler. 1971. Degradation of ^{125}I-labelled serum low density lipoprotein in normal and estrogen-treated male rats. *Biochem. Biophys. Res. Commun.* **44:** 1471–1477.
4. Faergeman, O., T. Sata, J. P. Kane, and R. J. Havel. 1975. Metabolism of apoprotein B of plasma very low density lipoproteins in the rat. *J. Clin. Invest.* **56:** 1396–1403.
5. Eisenberg, S., and D. Rachmilewitz. 1973. Metabolism of rat plasma very low density lipoprotein I. Fate in circulation of the whole lipoprotein. *Biochim. Biophys. Acta* **326:** 378–390.
6. Bragdon, J. H., and R. J. Havel. 1954. In vivo effects of antiheparin agents on serum lipids and lipoproteins. *Am. J. Physiol.* **177:** 128–133.
7. Goldstein, J. L., and M. S. Brown. 1976. The LDL pathway in human fibroblasts: a receptor-mediated mechanism for the regulation of cholesterol metabolism. *In* Current Topics in Cellular Regulation. Vol. II. B. L. Horecker and E. R. Stadtman, editors. Academic Press, Inc., New York. 147–181.
8. Havel, R. J., J. L. Goldstein, and M. S. Brown. 1978. Lipoproteins and lipid transport. *In* Diseases of Metabolism. P. K. Bondy and L. E. Rosenberg, editors. W. B. Saunders Co., Philadelphia, PA. In press.
9. Sniderman, A. D., T. E. Carew, J. G. Chandler, and D. Steinberg. 1974. *Science* **183:** 526–528.
10. Sigurdsson, G., S.-P. Noel, and R. J. Havel. 1977. Degradation of low density lipoprotein (LDL) by isolated perfused rat livers. *Clin. Res.* **25:** 500A.
11. Havel, R. J., H. A. Eder, and J. H. Bradgon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
12. McFarlane, A. S. 1948. Efficient trace-labeling of proteins with iodine. *Nature (Lond.)* **182:** 53.
13. Langer, T., W. Strober, and R. I. Levy. 1972. The metabolism of low density lipoprotein in familial type II hyperlipoproteinemia. *J. Clin. Invest.* **51:** 1528–1536.
14. Kane, J. P. 1973. A rapid electrophoretic technique for identification of subunit species of apoproteins in serum lipoproteins. *Anal. Biochem.* **53:** 350–364.
15. Scanu, A. M., and C. Edelstein. 1971. Solubility in aqueous solution of ethanol of the small molecular weight peptides of the serum very low density and high density lipoproteins: relevance to the recovery problem during delipidation of serum lipoproteins. *Anal. Biochem.* **44:** 576–588.
16. Fainaru, M., R. J. Havel, and T. Felker. 1976. Radioimmunoassay of apolipoprotein A-1 of rat serum. *Biochim. Biophys. Acta* **446:** 56–68.
17. Fainaru, M., R. J. Havel, and K. Imaizumi. 1977. Radioimmunoassay of arginine-rich apolipoprotein of rat serum. *Biochim. Biophys. Acta* **490:** 144–155.
18. Exton, J. H., and C. R. Park. 1967. Control of gluconeogenesis in liver. I. General features of gluconeogenesis in the perfused livers of rats. *J. Biol. Chem.* **242:** 2622–2636.
19. Miller, L. L., C. G. Bly, M. L. Watson, and W. F. Bale.

1951. The dominant role of the liver in plasma protein synthesis. A direct study of isolated perfused rat liver with the aid of lysine-ε-^{14}C. *J. Exp. Med.* **94:** 431–453.
20. Hamilton, R. L., M. N. Berry, M. C. Williams, and E. M. Severinghaus. 1974. A simple and inexpensive membrane "lung" for small organ perfusion. *J. Lipid Res.* **15:** 182–186.
21. Hamilton, R. L., M. C. Williams, C. J. Fielding, and R. J. Havel. 1976. Discoidal bilayer structure of nascent high density lipoproteins from perfused rat liver. *J. Clin. Invest.* **58:** 667–680.
22. Bierman, E. L., O. Stein, and Y. Stein. 1974. Lipoprotein uptake and metabolism by rat aortic smooth muscle cells in tissue culture. *Circ. Res.* **35:** 136–150.
23. Mathews, C. M. E. 1957. The theory of tracer experiments with ^{131}I-labelled plasma proteins. *Phys. Med. Biol.* **2:** 36–53.
24. Mjøs, O. D., O. Faergemen, R. L. Hamilton, and R. J. Havel. 1975. Characterization of remnants produced during the metabolism of triglyceride-rich lipoprotein of blood plasma and intestinal lymph in the rat. *J. Clin. Invest.* **56:** 603–615.
25. Cohen, S., and A. H. Gordon. 1958. Catabolism of plasma albumin by the perfused rat liver. *Biochemistry* **70:** 544–551.
26. Goldstein, J., and M. Brown. 1974. Binding and degradation of low density lipoprotein by cultured human fibroblasts. *J. Biol. Chem.* **249:** 5153–5162.
27. Nakai, T., P. S. Otto, D. L. Kennedy, and T. F. Whayne. 1976. Rat high density lipoprotein subfraction (HDL_3) uptake and catabolism by isolated rat liver parenchymal cells. *J. Biol. Chem.* **251:** 4914–4921.
28. Dumas, P., B. Maziere, N. Antissier, and R. Michel. 1973. Specificité de l'iodotyrosine désiodase des microsomes thyroidiens et hépatiques. *Biochim. Biophys. Acta* **293:** 36–47.
29. Sigurdsson, G., A. Nicoll, and B. Lewis. 1975. The metabolism of low density lipoprotein in endogenous hypertriglyceridaemia. *Eur. J. Clin. Invest.* **6:** 151–158.
30. Hoffenberg, R., A. H. Gordon, E. G. Black, and L. N. Louis. 1970. Plasma protein catabolism by the perfused rat liver. The effect of alteration of albumin concentration and dietary protein depletion. *Biochem. J.* **118:** 401–404.
31. Stein, Y., V. Ebin, H. Bar-On, and O. Stein. 1977. Chloroquine-induced interference with degradation of serum lipoproteins in rat liver, studied in vivo and in vitro. *Biochim. Biophys. Acta* **486:** 286–297.
32. Goldstein, J. L., G. Y. Brunschede and M. S. Brown. 1975. Inhibition of the proteolytic degradation of low density lipoprotein in human fibroblasts by chloroquine, concanavalin A, and Triton WR 1339. *J. Biol. Chem.* **250:** 7854–7862.
33. Faergeman, O., and R. J. Havel. 1975. Metabolism of cholesteryl esters of rat very low density lipoproteins. *J. Clin. Invest.* **55:** 1210–1218.
34. Simons, L. A., D. Reichl, N. B. Myant, and M. Mancini. 1975. The metabolism of the apoprotein of plasma low density lipoproteins in familial hyperbetalipoproteinaemia in the homozygous form. *Atherosclerosis* **21:** 283–298.
35. Sigurdsson, G., A. Nicoll, and B. Lewis. 1975. Conversion of very low density lipoprotein to low density lipoproteins. A metabolic study of apolipoprotein B kinetics in human subjects. *J. Clin. Invest.* **56:** 1481–1490.