Turnover and tissue distribution of ^{125}I-labeled low density lipoprotein in swine and dogs

Allan D. Sniderman,[1] Thomas E. Carew, and Daniel Steinberg[2]

Division of Metabolic Disease, Department of Medicine, University of California, San Diego School of Medicine, La Jolla, California 92037

Abstract Swine plasma low density lipoprotein (LDL) isolated ultracentrifugally (d 1.019–1.063) was labeled with ^{125}I, dialyzed, and reisolated by centrifugation at d 1.063. Over 96% of the radioactivity was shown to be associated with the apoprotein. After reinjection into the donor animal, disappearance of ^{125}I was followed for up to 122 hr. At all time intervals examined, over 95% of the total plasma ^{125}I was recovered in LDL (d 1.006–1.063), i.e., there was apparently no transfer of radioactivity to high density or very low density lipoproteins. The disappearance curve was biexponential, with half-lives of 0.83 ± 0.06 and 22.5 ± 1.7 hr for the first and second phases, respectively (13 studies). The mean calculated fractional catabolic rate was 0.041 ± 0.003 hr^{-1}. Similar results were obtained in three dogs using autologous LDL of density 1.020–1.050; fractional catabolic rates were 0.031, 0.031, and 0.029 hr^{-1}. Tissue distribution of ^{125}I was determined in swine killed at various time intervals after [^{125}I]LDL injection with corrections for radioactivity in trapped plasma. Of the tissues examined, the liver showed by far the highest concentration. Total hepatic radioactivity, expressed as a percentage of total plasma radioactivity, was rather constant and independent of the time of killing from 3 to 122 hr (15.8 ± 1.9%). The total extravascular LDL pool calculated from analysis of the plasma disappearance curves was about 20–30% of the size of the plasma LDL pool. These data are consistent with the conclusion that the liver accounts for a very large fraction of the total extravascular LDL pool and that it is in fairly rapid equilibrium with the plasma pool. To what extent the liver is involved in irreversible degradation cannot be inferred from these findings.

Supplementary key words lipoprotein turnover · lipoprotein catabolism · iodinated lipoproteins

In view of the established correlation between plasma levels of LDL and the risk of ischemic heart disease, it is important to understand the metabolism of this lipoprotein fraction. Elevated levels of plasma LDL could arise (*1*) by an increase in the production rate of LDL, (*2*) by a reduction in the rate of removal of LDL from plasma, or (*3*) by a combination of these mechanisms. Studies in man suggest that some, perhaps all, of the plasma LDL arises from degradation of plasma VLDL (1–4). If this is the case, overproduction of LDL implies overproduction of VLDL or a more efficient conversion of VLDL to LDL. However, the mechanism(s) and site(s) of LDL removal from plasma have not been firmly established. In the present study, the kinetics of the disappearance of [^{125}I]LDL from plasma of swine and dogs were investigated. Tissue distribution of ^{125}I was also determined in swine killed at various time intervals after injection of [^{125}I]LDL. Preliminary reports of some of these results have appeared elsewhere (5).

MATERIALS AND METHODS

Hormel white or miniature swine, 30–50 kg in weight, were maintained on a standard chow diet (Ralston Purina Co., St. Louis, Mo.) but were fasted overnight prior to kinetic studies.

Preparation and iodination of LDL

Lipoproteins with density between 1.019 and 1.063 were isolated from plasma of donor animals essentially as described by Havel, Eder, and Bragdon (6), using a Ti-60 rotor (Beckman Instruments, Inc., Fullerton, Calif.). This material was diluted with a salt solution of density 1.063 and centrifuged again in a Ti-50 rotor for 20 hr at 40,000 rpm. Agarose gel immunoelectrophoresis showed that the final preparation reacted with antiserum against swine LDL but not with antiserum against swine albumin, HDL, or the apoproteins in VLDL other than apo-LDL.

The purified LDL fraction was labeled with ^{125}I by a modification of the iodine monochloride method of McFarlane (7) using glycine–NaOH buffer, pH 9.6. 10 moles of

Abbreviations: LDL, low density lipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein; FCR, fractional catabolic rate; TCA, trichloroacetic acid; EDTA, (ethylene dinitrilo)tetraacetate (disodium salt); [^{125}I]LDL, ^{125}I-labeled low density lipoprotein.

[1] Dr. Sniderman was the recipient of a Canadian Medical Research Council Fellowship. Present address: Department of Cardiology, Royal Victoria Hospital, Montreal 112, Quebec, Canada.

[2] To whom reprint requests should be addressed.

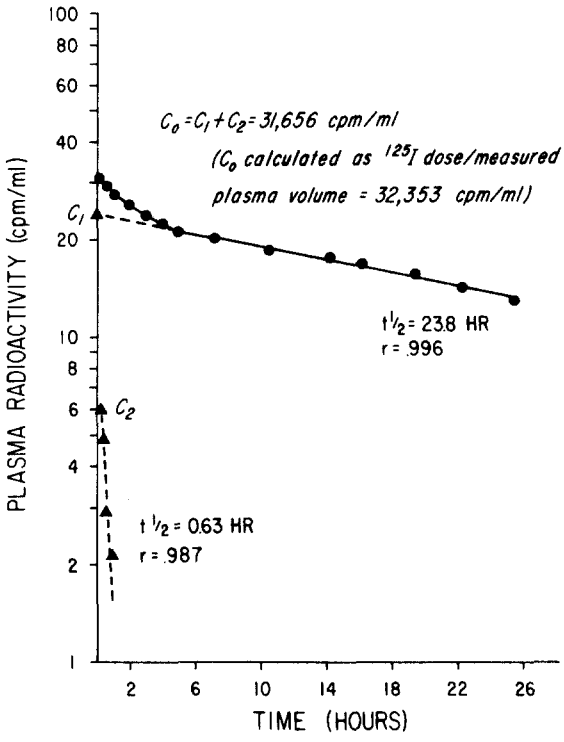

Fig. 1. Representative semilogarithmic decay curve for plasma ^{125}I after injection of [^{125}I]LDL into a pig. The analysis of the curve into two monoexponential functions with slopes b_1 and b_2 and zero-time intercepts C_1 and C_2 is shown. The plasma concentration of ^{125}I at zero time calculated on the assumption that the injected dose was initially distributed in the measured total plasma volume ([^{14}C]dextran space) agreed to within less than 3% with the zero-time concentration indicated by the sum of the ordinate intercepts of the two exponentials.

ICl were added per mole of LDL protein (assumed mol wt, 200,000), yielding labeled preparations with 0.5–1.5 moles of iodine per mole of LDL. Unbound iodide was removed by dialysis against 0.01% EDTA–0.9% NaCl. After dialysis, less than 1% of the ^{125}I remained unbound, as determined by precipitation with 10% trichloroacetic acid (TCA). Less than 3% of the ^{125}I radioactivity could be extracted with chloroform–methanol 2:1. The ^{125}I comigrated with freshly prepared, unlabeled swine LDL on agarose gel electrophoresis.

After the iodination procedure, the [^{125}I]LDL was reisolated by centrifugation at d 1.063. In all preparations, over 93% of the radioactivity was recovered in the d < 1.063 supernatant fraction, which was then passed through a 0.45-μm Millipore filter (Millipore Corp., Bedford, Mass.). The distribution of [^{125}I]LDL in an equilibrium sucrose density gradient ranged smoothly and essentially symmetrically from d = 1.020 to d = 1.066 with a peak at d = 1.038.

Study protocol

Under general anesthesia (sodium thiopental–halothane), a polyvinyl catheter was inserted into each external jugular vein, and the distal end was brought out through a separate stab incision in the lateral aspect of the neck. The animal was allowed to recover from the anesthetic and was given free access to water containing 3 drops of Lugol's solution per liter. The following day, [^{125}I]LDL (20–40 μCi) was administered intravenously to the fasting animal. Plasma samples drawn from the contralateral catheter were obtained at appropriate time intervals for periods up to 5 days. Total plasma radioactivity at each time point was determined using a Nuclear-Chicago gamma counter.

Kinetic analysis

The plasma radioactivity decay curve was satisfactorily fitted to a biexponential function using curve-peeling techniques and least square fits with an EAI model 640 digital computer. Using the three-compartment mammillary model described by Matthews (8), the slopes (b_1 and b_2) and the normalized ordinate intercepts (C_1 and C_2; $C_1 + C_2 = 1.0$) of the first and second exponentials were used to calculate the fractional rate of irreversible removal of LDL from plasma (fractional catabolic rate, FCR). The model consists of a plasma compartment connecting reversibly with an extravascular compartment and irreversibly with an "excretion compartment" (irreversible degradation). The equation for FCR is

$$\text{FCR} = (C_1/b_1 + C_2/b_2)^{-1}$$

The ratio of the radioactivity in the extravascular compartment to that in the plasma compartment was determined from the following equation (8):

$$\text{activity distribution ratio} = \frac{\text{FCR} - b_1}{b_1}$$

Tissue distribution studies in swine

Animals were killed at various time intervals from 3 to 122 hr after injection of [^{125}I]LDL. 10 min prior to killing, [^{14}C]dextran (mean mol wt, 60,000) was administered intravenously for measurement of plasma volume. The animals were then rapidly exsanguinated, and the lungs, liver, heart, small intestine, aorta, spleen, kidneys, thyroid, and pancreas were removed and weighed. Aliquots of each tissue were homogenized in 20 vol of 0.15 M NaCl, and total ^{125}I was determined. Additional samples were digested with Protosol (New England Nuclear Corp.) for scintillation counting of ^{14}C in Bray's scintillation fluid (9). All samples were counted to an error of ± 2% and counting efficiency was determined using an external standard. The plasma "trapped" in each organ was calculated from the [^{14}C]dextran content of the tissue sample, and the extravascular ^{125}I was determined by subtracting the ^{125}I attributable to "trapped plasma" from the total organ ^{125}I content. This value was then expressed as a percentage of the total ^{125}I radioactivity in plasma at the time of killing. More

TABLE 1. Kinetic parameters for [^{125}I] LDL turnover in swine

	Time-zero Intercept, Rapid Phase C_2	Slope of Rapid Phase b_2	Time-zero Intercept, Slow Phase C_1	Slope of Slow Phase b_1	Fractional Catabolic Rate FCR
	fraction of injected dose	hr^{-1}	*fraction of injected dose*	hr^{-1}	hr^{-1}
	0.18	1.019	0.82	0.0291	0.0354
	0.25	0.835	0.75	0.0359	0.0471
	0.16	0.603	0.84	0.0197	0.0233
	0.27	0.680	0.73	0.0325	0.0437
	0.25	0.950	0.75	0.0359	0.0496
	0.14	0.745	0.86	0.0236	0.0274
	0.18	1.873	0.82	0.0219	0.0269
	0.18	1.019	0.82	0.0361	0.0440
	0.20	0.797	0.80	0.0354	0.0439
	0.14	0.654	0.86	0.0405	0.0466
	0.24	0.707	0.76	0.0392	0.0509
	0.16	0.770	0.84	0.0304	0.0358
	0.33	1.155	0.67	0.0444	0.0652
Mean	0.21	0.908	0.793	0.0326	0.0415
SEM	±0.015	±0.092	±0.015	±0.0021	±0.0032

than 93% of the ^{125}I radioactivity in the tissue homogenates was precipitable with 10% TCA.

RESULTS

Disappearance of [^{125}I]LDL from plasma in swine

After injection of [^{125}I]LDL, total plasma radioactivity disappeared rapidly during the first 2 hr and then more slowly in a first-order manner for the remainder of the study. A representative result is shown in **Fig. 1.** The plasma decay curve for total ^{125}I in every case was described adequately as the sum of two exponential components. Listed in **Table 1** are the key parameters describing the decay curves in 13 studies (C_2, b_2, C_1, b_1, and FCR). The mean half-life for the rapid phase of disappearance was 0.83 ± 0.06 hr ($0.693/b_2$), and that for the slow phase was 22.5 ± 1.7 hr ($0.693/b_1$). The fractional catabolic rates were calculated as described under Materials and Methods; the mean value was 0.0415 ± 0.0032 hr^{-1} (SEM).

In seven experiments, repeated plasma samples taken 10 min to 50 hr after injection of [^{125}I]LDL were subfractionated to determine the distribution of radioactivity among the several lipoprotein subfractions (6). Over 95% of the plasma ^{125}I (95.5–97.5%) was recovered in the fraction with density 1.006–1.063, and this was true at all time intervals. Thus, there was little or no conversion to HDL or VLDL, and total plasma ^{125}I closely approximated plasma LDL ^{125}I. In two of these experiments each plasma sample was extracted with chloroform–methanol 2:1 (v/v); less than 4% of the total LDL radioactivity was found in the lipid fraction. In several experiments the density distribution of the [^{125}I]LDL injected and of the [^{125}I]LDL reisolated from the plasma of the recipient pig was determined by sucrose density gradient centrifugation. The distribution at later times (4–24 hr) was slightly skewed but was smooth and without any evident buildup of radioactivity at either extreme of the density range.

Two types of evidence tend to rule out the possibility that the labeled LDL preparations contained significant amounts of labile (denatured or degraded) material that might be rapidly removed or sequestered from the plasma compartment. First, the expected zero-time plasma concentration of [^{125}I]LDL (total ^{125}I administered divided by the [^{14}C]dextran space measured just before killing) was shown to differ by less than 5% from that calculated as the sum of the zero-time intercepts of the two exponentials (see Fig. 1). Thus, no significant amounts of labeled [^{125}I]LDL were being rapidly sequestered during the first few minutes after injection. Second, biological screening studies were done to test whether the first rapid phase of disappearance might reflect a more rapid disappearance of damaged [^{125}I]LDL. Plasma was removed from two animals 4–6 hr after they had received [^{125}I]LDL. At this time, disappearance is first-order at the rate characteristic for the slow phase. In other words, any material with an intrinsically short half-life would already have been cleared from the plasma compartment. When this labeled donor plasma was injected into recipient animals (three studies), the decay curves again showed a rapid initial decay (b_2 = 0.88; C_2 = 0.24) followed by a slower first-order decay (b_1 = 0.038; C_1 = 0.76). Mean FCR was 0.0506, not significantly different from the value seen after injection into primary recipients.

Disappearance of [^{125}I]LDL from plasma in dogs

Three mongrel dogs were studied using similar methods. Biexponential decay was again observed. The half-life for

TABLE 2. Organ distribution of ^{125}I in swine killed at intervals after injection of [^{125}I] LDL[a]

Time after LDL Injection	Liver	Spleen	Pancreas	Heart	Small Intestine	Lung	Aorta
hr							
3	10.1	0.18	<0.001	0.17		<0.001	<0.001
4	25	0.41	<0.001	<0.001		0.08	
8	11.7	<0.41	<0.001	<0.001		<0.001	<0.001
17	19	0.16	<0.001	<0.001	<0.001	<0.001	<0.001
17	15	0.09	<0.001	<0.001	<0.001	<0.001	<0.001
52	14	0.07	0.06	<0.001	0.10	1.46	0.06
99	14	0.06		<0.001		0.6	<0.001
122	17.9	0.8	<0.001	<0.001		0.03	<0.001
Mean ± SEM	15.8 ±1.9	0.22 ±0.05					

[a] (Total organ ^{125}I)/(Total plasma ^{125}I) × 100.

the rapid phase of disappearance was somewhat longer (0.6–3.3 hr), as was the half-life for the second phase of disappearance (26.2–28.7 hr). Fractional catabolic rates were remarkably similar in the three studies, 0.029–0.031 hr^{-1}, about three-fourths that found in swine. From the slopes and intercepts of the two phases of disappearance, the amount of LDL in the extravascular pool was calculated to be 20% of that in the plasma pool.

Tissue distribution studies

Table 2 lists the organ-to-plasma ^{125}I ratios obtained in pigs killed 3–122 hr after injection of [^{125}I]LDL. All values were corrected for [^{125}I]LDL attributable to trapped plasma as described under Materials and Methods. Only the liver and spleen contained appreciable amounts of radioactivity relative to that remaining in plasma at the time of killing. The amount of radioactivity in the liver (relative to that in plasma) showed a progressive increase in three animals killed within 2–4 hr after injection of [^{125}I]LDL. From 8 hr on, the extravascular radioactivity in the liver relative to that in the plasma at killing remained relatively constant, approximately 16%, with no obvious trend to increase or decrease as a function of time. This was true even at 122 hr, by which time more than 99% of the injected material had already been irreversibly removed from the plasma compartment. The fact that the radioactivities in the liver and plasma compartments paralleled each other over several half-lives suggests that these LDL pools are in a reversible and fairly rapid equilibrium with one another.

A comparison was then made between the directly measured size of the hepatic extravascular pool, estimated as described above, and the calculated size of the *total* extravascular pool, derived from analysis of the plasma biexponential decay curves (8).[3] As shown in **Table 3**, the pool in the liver accounted for 41–94% of the theoretical total extravascular pool. Small differences in the estimated values for the fitted constants (C_1, C_2, b_1, b_2) describing the biexponential decay curve may cause significant differences in the calculated size of the total extravascular pool. When an alternative method of curve fitting was employed (a nonlinear least squares program yielding the best fit for all data points without curve peeling), the half-lives of the first and second phases were in close agreement with the values obtained by curve peeling, as were the values for FCR (0.0389 ± 0.0035 SEM). However, the calculated size of the total extravascular LDL pool (as a percentage of the plasma pool) was significantly greater, 31% (22–38%) instead of 20.8% (16–29%). This sensitivity of the calculated extravascular pool size to the method of data fitting makes it difficult to be certain of the exact percentage of the total extravascular pool represented in the liver. However, even using the larger values it appears that the hepatic pool accounts for at least 50% of the total extravascular LDL pool.

DISCUSSION

The present results suggest that the ^{125}I-labeled protein in the density class studied (d 1.019–1.063) is kinetically homogeneous. After the initial disappearance phase, representing equilibration with extravascular pools, the decay of radioactivity was first-order over the time intervals studied. Janado, Martin, and Cook (10) have reported the occurrence of two subclasses of LDL in swine (LDL_1 and LDL_2). These were differentiated and partially resolved physically on the basis of their different rates of flotation in solutions of high density even though their mean hydrated densities differed very little, 1.034 and 1.05, respectively. Jackson et al.[4] concluded that the apoproteins of swine

[3] The relative sizes of the extravascular and intravascular pools are cited in terms of activity distribution ratios (see Materials and Methods). Because true isotopic equilibrium is never reached, these values differ slightly from the mass ratios of the two pools (8).

[4] Jackson, R. L. Personal communication.

TABLE 3. Comparison of the size of the intrahepatic LDL pool, determined directly, with the size of the total extravascular LDL pool, calculated from disappearance curves

Time of Killing	Total ^{125}I Liver/Total ^{125}I Plasma × 100	Total Extravascular [^{125}I]LDL Pool/Plasma-[^{125}I]LDL Pool × 100[a]	% of Extravascular Pool Accounted for by Liver Pool
hr			
8	12	29	41
17	19	22	86
17	15	16	94
52	14	18	78
99	14	18	78
122	18	22	82
Mean ± SEM	15.3 ± 1.1	20.8 ± 1.9	76.5 ± 7.5

[a] Determined from plasma decay curve.

LDL_1 and LDL_2 were identical by their criteria. The d 1.019–1.063 fractions used in the present studies might be expected to include some of the LDL_2 component. However, the protein content determined in three of our LDL preparations was 21.3%, 20.1%, and 21.8%; the protein content of the LDL_1 fraction of Janado et al. (10) was 20.4% and that of their LDL_2 was 27.3%. Evidently, the fractions used in the present studies correspond most closely to LDL_1. Furthermore, there was no significant transfer of label to fractions of density greater than 1.063, such as might be expected if LDL_1 were converted to LDL_2 prior to irreversible degradation.

Our interpretation of the initial, more rapid disappearance as reflecting exchange with an extravascular pool rather than heterogeneity of the labeled LDL preparations is supported by several lines of evidence. Some of these have been discussed above and need only be listed here: (*1*) evidence that the [^{125}I]LDL was indistinguishable from freshly prepared plasma LDL with regard to immunochemical, ultracentrifugal, and electrophoretic properties; (*2*) evidence from "biological screening" studies that the phase I disappearance is *not* due to the more rapid disappearance of a subset of modified molecules in the labeled preparation injected; and (*3*) evidence from direct postmortem tissue analyses that the liver represents the major extravascular pool of LDL and that the kinetics of the radioactivity in that pool are those expected for a large pool in relatively rapid equilibrium with the plasma pool. Additional validation comes from studies of [^{125}I]LDL injected into hepatectomized swine, reported elsewhere (11, 12), in which total plasma LDL concentrations fell monoexponentially and *without change in measured LDL specific radioactivity*. Finally, Murthy, Manchesky, and Steiner (13) recently reported preliminary results with swine LDL labeled by a quite different method, reductive alkylation. They also observed a biphasic disappearance that persisted after biological screening, and the apparent half-lives for the first and second phases were in general agreement with those observed by us.

A biexponential decay of LDL has been reported in man by Walton et al. (14, 15), Scott and Hurley (16), and Langer, Strober, and Levy (17) and interpreted to reflect exchange with an extravascular pool or pools. Their estimates of the size of the equilibrating extravascular pool, 25–40% of that of the intravascular pool, are not greatly different from that estimated here for swine. Our dog studies also showed biexponential LDL decay, and the extravascular pool size was estimated to be 20% of that of the intravascular pool. On the other hand, Eisenberg, Windmueller, and Levy (18) observed a monoexponential decay after injection of human [^{125}I]LDL into rats. Whether this represents a true species difference, an artifact due to the use of heterologous lipoprotein, or the result of plasma–tissue equilibration occurring too rapidly to be readily detected is not clear. Although the plasma decay curve did not appear to be biexponential, it is of interest that the liver was shown to contain the largest concentration of protein radioactivity at the time of killing, in agreement with the present results in swine.

The mean FCR for [^{125}I]LDL isolated in the density range 1.020–1.050 in the dog was 0.030 hr^{-1}. As mentioned previously, the disappearance from plasma could be adequately described by a biexponential function, and thus this lipoprotein fraction appears to behave kinetically as a single species. However, Mahley, Weisgraber, and Fry (19) report that lipoproteins isolated from dog plasma in this density range are not homogeneous, as they show both alpha and beta electrophoretic mobility. The lipoprotein with beta mobility contains one major apoprotein and is analogous to LDL in other species. The lipoprotein with alpha mobility, which they call HDL_1, appears to have an apoprotein pattern similar to that of HDL and extends into the density range > 1.050 g/ml.

The rapidity of equilibration between intravascular and extravascular LDL deserves comment. The half-life for equilibration in swine was 3.34 hr, the rate constant for transport between the intravascular and extravascular compartments (8) being 0.207 hr^{-1}. Movement of such a large molecule across ordinary capillary beds would not be expected to occur this rapidly. For example, the half-life for equilibration of serum albumin with extravascular pools of albumin in man is at least 6 hr (8). To the extent that this point has any merit, it is consonant with the finding that tissues other than the liver contained very little extravascular radioactive protein. The rapid equilibration with the hepatic pool may reflect the special anatomy of the liver, where large lacunae connect the vascular and interstitial spaces (20). To what extent the estimated extravascular pool is interstitial and to what extent it is membrane-bound or intracellular remains to be established.

The mean half-life for irreversible degradation of plas-

ma LDL in swine (0.693/FCR) was 16.7 hr, to be compared with 35.4 hr in man (17). In the human studies the appearance of labeled iodide in the urine closely paralleled the disappearance of labeled LDL from the plasma compartment, indicating that there was little accumulation of degradation products of LDL in tissues.

The site of the irreversible LDL degradation and the nature of the process remains to be determined. The fact that there is a large extravascular pool of LDL in the liver and that there is a large flux of LDL through that pool certainly might suggest that the liver may be an important site of LDL degradation, as has been proposed (18, 19, 21). Yet, there need not be any close correlation between the steady state pool size in a tissue and the rate of irreversible degradation in that tissue. Indeed, tissues catabolizing LDL at a higher rate might conceivably show lower concentrations of [^{125}I]LDL (rapid degradation with rapid removal of products). A kinetic argument can also be made against irreversible degradation occurring predominantly from the exchangeable LDL pool. Alternative models in which irreversible LDL degradation takes place from the extravascular exchangeable LDL pool have also been considered. These models predict a larger extravascular pool and a larger activity distribution ratio than the model used here. Since our preliminary results in hepatectomized animals yield monoexponential decay curves, it seems that the extrahepatic, extravascular pool of exchangeable LDL is small (11). Because the present results show that the major extravascular pool is in the liver (suggested also by the results of Eisenberg et al. [18]) and because the observed ratio of liver to plasma radioactivity in the present studies was lower than the activity distribution ratio predicted by the model used, the alternative models that predict still higher activity ratios fit the data less well but cannot be ruled out. From 25 to 50% of the extravascular LDL pool may be extrahepatic, and so cells in the periphery certainly have access to LDL at some rate. Recent studies by Brown and Goldstein (22), by Bierman, Stein, and Stein (23), and from this laboratory (24) show that fibroblasts and arterial smooth muscle cells in culture do take up and catabolize LDL. The relative importance of various other tissues in LDL catabolism remains to be determined. We have published preliminary results suggesting that removal by extrahepatic tissues may be quantitatively important in vivo (11, 12).

The devoted technical assistance of Mrs. Susan Hayes and Mr. Joseph Mullen is gratefully acknowledged. This research was supported by PHS grant HL-14197 from the National Heart and Lung Institute.

Manuscript received 6 November 1974; accepted 21 March 1975.

REFERENCES

1. Gitlin, D., D. G. Cornwell, D. Nakasato, J. L. Oncley, W. L. Hughes, and C. A. Janeway. 1958. Studies on the metabolism of the plasma proteins in the nephrotic syndrome. II. The lipoproteins. *J. Clin. Invest.* **37:** 172–184.
2. Havel, R. J. 1961. Conversion of plasma free fatty acids into triglycerides of plasma lipoprotein fractions in man. *Metabolism.* **10:** 1031–1034.
3. Quarfordt, S. H., A. Frank, D. M. Shames, B. Berman, and D. Steinberg. 1970. Very low density lipoprotein triglyceride transport in type IV hyperlipoproteinemia and the effects of carbohydrate-rich diet. *J. Clin. Invest.* **49:** 2281–2297.
4. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoprotein proteins. I. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* **260:** 212–221.
5. Sniderman, A. D., T. E. Carew, and D. Steinberg. 1972. Metabolism of low density lipoprotein apoprotein in the pig. *Circulation.* **46(Suppl. 2):** 11. (Abstr.)
6. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
7. McFarlane, A. S. 1958. Efficient trace-labelling of proteins with iodine. *Nature.* **182:** 53.
8. Matthews, C. M. E. 1957. The theory of tracer experiments with ^{131}I-labelled plasma proteins. *Phys. Med. Biol.* **2:** 36–53.
9. Bray, G. A. 1960. A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Anal. Biochem.* **1:** 279–285.
10. Janado, M., W. G. Martin, and W. H. Cook. 1966. Separation and properties of pig-serum lipoproteins. *Can. J. Biochem.* **44:** 1201–1209.
11. Sniderman, A. D., T. E. Carew, J. G. Chandler, and D. Steinberg. 1974. Paradoxical increase in rate of catabolism of low-density lipoproteins after hepatectomy. *Science.* **183:** 526–528.
12. Steinberg, D., T. E. Carew, J. G. Chandler, and A. D. Sniderman. 1974. The role of the liver in the metabolism of plasma lipoproteins. *In* Regulation of Hepatic Metabolism. F. Lundquist and N. Tygstrup, editors. Munksgaard, Copenhagen. 144–154.
13. Murthy, V. K., T. C. Monchesky, and G. Steiner. 1973. Turnover of β-apolipoprotein labelled in vitro with ^{3}H or ^{14}C. *Federation Proc.* **32:** 363Abs.
14. Walton, K. W., P. J. Scott, J. V. Jones, R. F. Fletcher, and T. W. Whitehead. 1963. Studies on low-density lipoprotein turnover in relation to Atromid therapy. *J. Atheroscler. Res.* **3:** 396–414.
15. Walton, K. W., P. J. Scott, P. W. Dykes, and J. W. L. Davies. 1965. The significance of alterations in serum lipids in thyroid dysfunction. II. Alterations of the metabolism and turnover of ^{131}I-low-density lipoproteins in hypothyroidism and thyrotoxicosis. *Clin. Sci.* **29:** 217–238.
16. Scott, P. J., and P. J. Hurley. 1969. Effect of clofibrate on low-density lipoprotein turnover in essential hypercholesterolaemia. *J. Atheroscler. Res.* **9:** 25–34.
17. 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.
18. Eisenberg, S., 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.
19. Mahley, R. W., K. H. Weisgraber, and D. L. Fry. 1973. Canine lipoproteins and atherosclerosis. *Federation Proc.* **32:** 294Abs.

20. Bradley, S. E. 1963. The hepatic circulation. *In* Handbook of Physiology. Section 2: Circulation. Vol. 2. W. F. Hamilton and P. Dow, editors. American Physiological Society, Washington, D.C. 1387–1438.
21. Hay, R. V., L. A. Pottenger, A. L. Reingold, G. S. Getz, and R. W. Wissler. 1971. Degradation of ^{125}I-labeled serum low density lipoprotein in normal and estrogen-treated male rats. *Biochem. Biophys. Res. Commun.* **44:** 1471–1477.
22. Brown, M. S., and J. L. Goldstein. 1974. Expression of the familial hypercholesterolemia gene in heterozygotes: mechanism for a dominant disorder in man. *Science.* **185:** 61–62.
23. 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.
24. Weinstein, D., T. E. Carew, and D. Steinberg. 1974. Uptake of low density lipoprotein by porcine aortic smooth muscle cells with inhibition of cholesterol synthesis. *Circulation.* **50:** 70. (Abstr.)