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Metabolism of native and of lactosylated human low density lipoprotein: Evidence for two pathways for catabolism of exogenous proteins in rat hepatocytes

(lysosomes/glycoproteins/cholesterol/protein degradation/colchicine)

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ABSTRACT **Human low density lipoprotein (LDL) covalently conjugated with 200–250 residues of lactose per LDL particle (Lac-LDL) was bound and rapidly taken up by the galactose-specific receptor of rat hepatocytes. Uptake of Lac-LDL was associated with inhibition of 3-hydroxy-3-methylglutaryl-CoA reductase and stimulation of cholesterol esterification. Uptake of native human LDL had no significant effects on these enzyme activities even when the rates of LDL uptake equaled those of Lac-LDL. When injected into rats, Lac-LDL was selectively removed by the liver (98% of injected dose). The hepatic subcellular distribution of simultaneously injected native ^{125}I-labeled LDL and ^{131}I-labeled Lac-LDL differed significantly. Lac-LDL was associated with fractions enriched in lysosomal hydrolases whereas native LDL was found predominantly in the supernatant fraction enriched in lactate dehydrogenase. Chloroquine (0.1 mM) markedly suppressed uptake of Lac-LDL by cultured rat hepatocytes (>80%) but had only a small effect on uptake of native LDL. Leupeptin (0.625 mM) inhibited degradation of Lac-LDL more than it did degradation of native LDL. Colchicine (0.25 μM) dramatically suppressed uptake of Lac-LDL (>70%) but did not affect native LDL uptake even at concentrations as high as 10 μM. Uptake of human LDL by rat hepatocytes occurs largely by nonspecific mechanisms, including fluid endocytosis, whereas Lac-LDL, as shown here, is taken up by a specific receptor-mediated mechanism. The results show further that native human LDL, representing an example of a protein taken up nonspecifically, is processed intracellularly by a pathway qualitatively distinct from that for Lac-LDL, an example of a protein taken up by a specific mechanism. Lac-LDL may serve as a vehicle for specifically delivering drugs, hormones, or radioactive compounds to hepatocytes for therapeutic or diagnostic purposes.**

A lysosomal pathway for the catabolism of low density lipoprotein (LDL) has been well characterized in cultured human fibroblasts (1). The pathway involves high-affinity binding of LDL to cell surface receptors, endocytosis of the bound lipoprotein, and lysosomal hydrolysis of both the lipid and protein moieties. This process results in suppression of cholesterol synthesis, stimulation of cholesterol esterification, and regulation of the number of cell surface LDL receptors. All of these metabolic effects of LDL are suppressed by inhibitors of lysosomal hydrolases (2) and are absent or much reduced in cells from patients with deficiencies of lysosomal cholesterol ester hydrolase (3). A similar LDL uptake mechanism has been described in several cell types of extrahepatic origin, but there is still uncertainty as to whether or not LDL uptake by liver cells is mediated by an analogous high-affinity mechanism.

Recent studies in this laboratory, using a new technique for determining tissue sites of degradation of plasma proteins (4), have established that in pigs the liver is highly active in LDL degradation *in vivo* (5). The method exploits the fact that sucrose, once introduced into a cell, escapes at a very slow rate (6). [^{14}C]Sucrose is covalently linked to the LDL apoprotein and is thus carried along with it into the cell. There the LDL lipid and protein are degraded and the degradation products leave quite rapidly. The [^{14}C]sucrose "marker," however, remains trapped and serves as a cumulative indicator of the number of labeled LDL molecules catabolized. In swine, 40% of LDL degradation occurred in the liver, and over 90% of this occurred in the hepatic parenchymal cells. Using the same approach we have found that human or rat LDL injected intravenously into rats is again degraded to a large extent in the liver [about 50% of total LDL degradation (7)]. During these studies in rats it was noted that some of the ^{14}C from [^{14}C]sucrose-LDL was lost in the bile. When [^{14}C]sucrose-asialofetuin was injected, the fraction of hepatic ^{14}C excreted in bile per unit time was greater than in the case of human [^{14}C]sucrose-LDL. Cellular catabolism of asialofetuin by hepatocytes occurs by receptor-mediated endocytosis followed by lysosomal degradation (8, 9), that is, essentially the same way LDL is catabolized by human fibroblasts after specific binding and uptake (1). Recently, it was reported that lysosomal hydrolases are coordinately secreted into bile (10). Thus, the difference in the hepatic handling of human LDL and asialofetuin suggested that the former might *not* be processed, at least not exclusively, by the same lysosomal pathway.

In the present studies human LDL was derivatized with 200–250 lactose residues per LDL molecule (Lac-LDL) so that it was recognized by the galactose-specific receptor described by Ashwell and Morell (8). Thus, we were able to compare catabolism of heterologous native LDL to that of an altered LDL particle that was catabolized by a known lysosomal mechanism. It was previously demonstrated that the addition of galactose residues in this manner caused ribonuclease A dimer, a protein not normally destined for hepatic clearance, to be rapidly taken into the liver (11).

Degradation of the two forms of LDL was compared in terms of their rate of clearance from plasma, their tissue sites of degradation, the subcellular distribution of the two lipoproteins after uptake by liver, the effects of inhibitors on their uptake and degradation by rat hepatocytes, and their effects on hepatic cholesterol synthesis and esterification.

METHODS

Preparation and Radiolabeling of Proteins. Human LDL (density, 1.02–1.06 g/ml) was isolated from normal human plasma by preparative ultracentrifugation by using KBr to adjust solution densities (12). The product showed one band of β mobility on agarose gel electrophoresis (13). Asialofetuin was

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Abbreviations: LDL, low density lipoprotein; Lac-LDL, lactosylated LDL; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl-CoA reductase.

prepared from fetal bovine fetuin (Sigma type IV) by enzymatic desialylation with agarose-bound neuraminidase (14).

Lac-LDL was prepared by reductive amination (11). Lac-LDL was in every case prepared from the same batch of LDL to which it was experimentally compared. [^{14}C]Sucrose-labeled Lac-LDL was prepared by coupling [^{14}C]sucrose to LDL prior to its lactosylation. LDL or [^{14}C]sucrose-LDL (10–15 mg of protein in 1 ml of 50 mM sodium phosphate, pH 7.0) was incubated at room temperature with lactose (100 mg/ml) and sodium cyanoborohydride (50 mg/ml, Aldrich) (15), including a tracer amount of radiolabeled lactose (Amersham) to assess the extent of derivatization. The reaction mixture was incubated sterilely at room temperature for 1–10 days before separation of the derivatized LDL from reactants and side products by gel filtration on 10% agarose and subsequent exhaustive dialysis. The extent of derivatization was 200–250 lactose residues per LDL particle.

[^{14}C]Sucrose-labeled LDL was prepared by coupling carrier-free [*U*-^{14}C]sucrose (370–390 Ci/mol; 1 Ci = 3.7×10^{10} becquerels; Amersham) to LDL by reaction with cyanuric chloride (1,3,5-trichloro-2,4,6-triazine) as described (4).

Proteins were radioiodinated by a modification (16) of the iodine monochloride method (17).

Studies *in Vivo*. Under ether anesthesia, rats were injected with the radiolabeled proteins by intracardiac injection. For turnover studies, periodic blood samples were withdrawn through a cannula that had been inserted into an external jugular vein of the ether-anesthetized animal. To assess the tissue distribution of ^{14}C from [^{14}C]sucrose-labeled proteins, we exsanguinated rats through the aorta and then perfused them through the inferior vena cava for 20 min with isotonic saline containing 0.1 mM EDTA (pH 7.4). Organs were then removed and homogenized, and their ^{14}C content was determined as described (4, 5).

Studies *in Vitro*. Rat hepatocytes were isolated by a modification (14) of the method of Berry and Friend (18). Cells were plated and experimental protocols were carried out in serum-free, arginine-deficient Dulbecco's modified Eagle's medium (GIBCO). Studies of protein catabolism using radioiodinated proteins were initiated 3–24 hr after cells were plated and were carried out as reported (14). Protein degradation was calculated from ^{125}I appearing in the medium as products soluble in trichloroacetic acid (10% wt/vol) for LDL and Lac-LDL and from ^{125}I in the medium as products soluble in 10% trichloroacetic acid and 2% phosphotungstic acid for asialofetuin. Uptake of [^{14}C]sucrose-labeled proteins was measured as total ^{14}C in washed cells at the end of the incubation. In some studies, undegraded intracellular protein was separated from degradation products by precipitation with 5% trichloroacetic acid.

Subcellular Fractionation. Fractionation was carried out by differential sedimentation. The freshly excised and perfused livers were homogenized in 5 vol of ice-cold 0.25 M sucrose and homogenized by four strokes of a tight-fitting Potter–Elvehjem homogenizer. The Teflon pestle was motor driven, and its speed was manipulated to maintain a rather constant rotation of 200–250 rpm. Fractions were sequentially sedimented by centrifugation at 4°C as follows: $750 \times g$ for 10 min, $5000 \times g$ for 10 min, $20{,}000 \times g$ for 30 min, $40{,}000 \times g$ for 30 min, and $100{,}000 \times g$ for 30 min.

Assay of Enzyme Activities. 3-Hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase) activity in hepatocyte microsomes was measured 5 hr after the cells were plated and 3 hr after introduction of test lipoproteins. Activity in microsomal fractions was determined in terms of [^{14}C]mevalonate formation from [3-^{14}C]HMG-CoA, prepared by the method of Hilz *et al.* (19), as described in detail elsewhere (20).

Cholesterol esterification was measured in terms of the incorporation of [^{3}H]oleic acid into cholesterol esters by the intact cells, as described elsewhere (20). Cells were preincubated with the indicated lipoproteins for 3 hr; then [^{3}H]oleic acid bound to bovine serum albumin was added (final concentrations: 50 μM oleic acid, 25 Ci/mol; 5 mg of albumin per ml). After 2 hr, the cells were harvested and the cholesterol esters were isolated for radioassay by thin-layer chromatography of a lipid extract.

Acid phosphatase activity was measured in terms of the appearance of *p*-nitrophenol from *p*-nitrophenylphosphate (21). *N*-Acetylglucosaminidase activity was measured in terms of the formation of *p*-nitrophenol from *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide at pH 5.0 (22). 5′-Nucleotidase activity was measured in terms of the release of inorganic phosphate from adenosine 5′-monophosphate at pH 9.1 (23). Inorganic phosphate was determined by the method of Ames and Dublin (24). Lactate dehydrogenase was measured at pH 7.5 according to Bergmeyer *et al.* (25).

RESULTS

Lac-LDL injected intravenously into rats disappeared from the plasma compartment at a rate 8 times that of native LDL (Fig. 1). The contribution of hepatic and extrahepatic tissues to degradation of the two forms of LDL was determined by the [^{14}C]sucrose-labeling method (5). The ^{14}C recovered in bile is presumed to represent [^{14}C]protein taken up by the liver. Twenty-four hours after injection of [^{14}C]sucrose-LDL, 46% of the ^{14}C recovered in tissues was found in the liver and 8% in bile. In contrast, essentially all of the ^{14}C from [^{14}C]sucrose-Lac-LDL (98%) was found in the liver and bile (74% and 24%, respectively). In both cases, more than 90% of the hepatic ^{14}C was accounted for in parenchymal cells. Biliary recovery of ^{14}C from [^{14}C]sucrose-Lac-LDL was 2.5-fold greater than that from native [^{14}C]sucrose-LDL, expressed as a fraction of irreversibly cleared lipoprotein. Even when the greater absolute hepatic uptake of Lac-LDL is taken into account, biliary output of ^{14}C from [^{14}C]sucrose-Lac-LDL was greater (30–50% greater, expressed as a fraction of hepatic ^{14}C content lost per unit time).

The effects of increasing concentrations of unlabeled asialofetuin on the degradation of native ^{125}I-labeled LDL and of ^{125}I-labeled Lac-LDL by cultured rat hepatocytes were compared (Fig. 2). Asialofetuin competed very effectively with ^{125}I-labeled Lac-LDL, as well as it did with ^{125}I-labeled asialofetuin, but did not compete with native ^{125}I-labeled LDL.

The effects of the two forms of LDL on HMG-CoA reductase, generally accepted as the rate-determining step in cho-

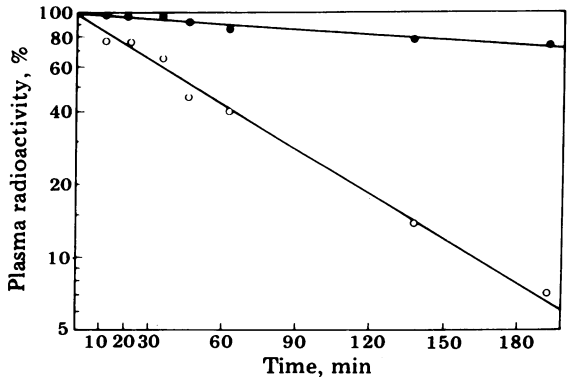

FIG. 1. *In vivo* disappearance from rat plasma of simultaneously injected human ^{125}I-labeled LDL (●) and ^{131}I-labeled Lac-LDL (○). Data are normalized to the initial plasma radioactivity (933,300 cpm/ml for ^{125}I and 188,330 cpm/ml for ^{131}I).

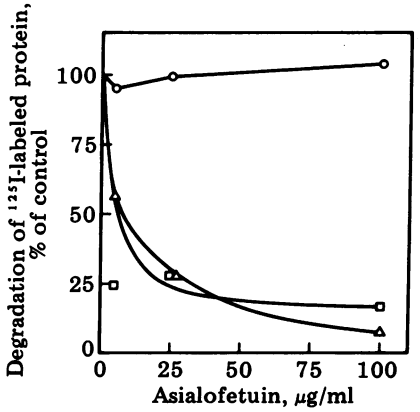

FIG. 2. Degradation of ^{125}I-labeled LDL (○), ^{125}I-labeled Lac-LDL (□), and ^{125}I-labeled asialofetuin (△) by cultured rat hepatocytes: inhibition by asialofetuin. One-day-old hepatocyte cultures were incubated for 3 hr at 39°C in 2 ml of serum-free medium containing the indicated radioiodinated proteins at 5 μg/ml and the indicated concentrations of competing asialofetuin. Each value represents the mean of triplicate determinations. Trichloroacetic acid-soluble ^{125}I in dishes without cells was 4256 ± 309, 8071 ± 265, and 2244 ± 113 (±SEM) for native LDL, Lac-LDL, and asialofetuin, respectively. In the absence of competing unlabeled asialofetuin, the values in dishes with cells were 6525 ± 174, 28,169 ± 740, and 119,335 ± 603 (±SEM), respectively. Absolute degradation rates in the absence of competing asialofetuin were: 27, 419, and 293 ng/mg of protein for LDL, Lac-LDL, and asialofetuin, respectively.

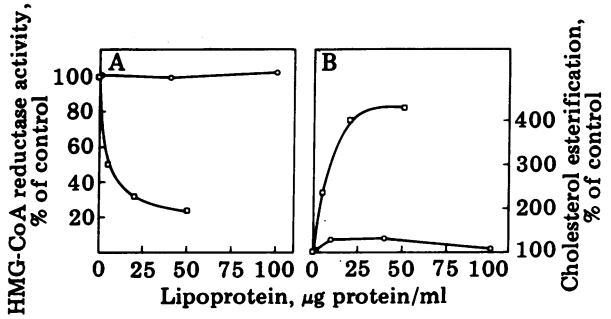

FIG. 3. Effect of LDL (○) and Lac-LDL (□) on HMG-CoA reductase activity (*A*) and cholesterol esterification (*B*) in cultured rat hepatocytes. Cells were plated 3 hr before study. LDL or Lac-LDL was added, and the two activities were determined 3 hr later. Each value represents the mean of triplicate determinations. Activities in the absence of added lipoproteins were 55.3 ± 7.9 pmol/min per mg of protein for HMG-CoA reductase activity and 87 ± 13 pmol/hr per mg of protein for cholesterol esterification.

lesterogenesis (26), and on cholesterol esterification were examined in cultured hepatocytes (Fig. 3). Lac-LDL, even at very low concentrations in the medium, suppressed HMG-CoA reductase and stimulated cholesterol esterification. Similar concentrations of native LDL in the medium failed to significantly alter either activity. Because Lac-LDL at equal concentrations in the medium was more rapidly catabolized than native LDL, the effects of the two lipoproteins were compared under conditions that yielded equal rates of catabolism of the two. To reduce the rate of catabolism of Lac-LDL, we added unlabeled asialofetuin at different concentrations to inhibit uptake in cells exposed to [^{14}C]sucrose-Lac-LDL. As shown in Table 1, very high levels of native LDL failed to suppress HMG-CoA reductase or to stimulate cholesterol esterification, whereas Lac-LDL was effective at equivalent rates of cellular uptake. At lower levels of LDL the effects were variable.

In view of these indications that the cellular processing of the two forms of LDL was different, a comparison was made of their subcellular localization in liver 1 hr after simultaneous intravenous injection into rats. As shown in Fig. 4, radioactivity from ^{131}I-labeled Lac-LDL precipitable by trichloroacetic acid (undegraded or only partially degraded) was found predominantly in fractions enriched in lysosomal hydrolases, whereas radioactivity from ^{125}I-labeled native LDL was found predominantly in the 100,000 × *g* supernate. This higher concentration of LDL in the supernatant fraction was shown not to simply reflect extracellular LDL trapped in the liver. At 1 hr after injection 82% of the ^{14}C, and at 24 hr essentially all of the ^{14}C, from [^{14}C]sucrose-LDL found in the liver was shown to be within hepatocytes (isolated from companion lobes by collagenase digestion). In other experiments, sucrose-labeled lipoproteins ([^{3}H]sucrose-Lac-LDL and [^{14}C]sucrose-native LDL) were used to determine the subcellular distribution of accumulated degradation products 24 hr after injection. In these cases, the sucrose-labeled degradation products (soluble in 5% trichloroacetic acid) from both forms of LDL were predominantly associated with the fractions enriched in lysosomal enzymes (results not shown).

To explore further the role of lysosomes in catabolism of the two forms of LDL, we studied the effects of leupeptin, a competitive inhibitor of cathepsin B_1 and some other proteases, by using [^{14}C]sucrose-labeled preparations and measuring intracellular accumulation of ^{14}C-labeled degradation products soluble in 5% trichloroacetic acid. In a 6-hr incubation (1 hr of preincubation with inhibitor), leupeptin (0.625 mM) inhibited the degradation of Lac-LDL by 62% and the degradation of native LDL by 34%. Total cell uptake was unchanged. The effect of chloroquine, a more general lysosomal inhibitor, was similarly examined. Surprisingly, chloroquine (0.1 mM) inhibited not only the rate of degradation of Lac-LDL taken up but also the *uptake* of Lac-LDL (84%). Uptake of native LDL was decreased by only 15%. The fraction of the lipoprotein taken up that appeared as degradation products was decreased by chloroquine more in the case of Lac-LDL (92%) than in the case of native LDL (58%).

Colchicine blocks high-affinity uptake of normal LDL by human fibroblasts but does not interfere with uptake by receptor-deficient cells (27). The effects of colchicine on cellular uptake of [^{14}C]sucrose-labeled native LDL and Lac-LDL are shown in Fig. 5. Hepatocyte uptake of Lac-LDL was suppressed 73% at 0.25 μM colchicine whereas uptake of native LDL was virtually unaffected even at concentrations as high as 10 μM.

Table 1. Effects of uptake of LDL and of Lac-LDL on cholesterol metabolism in rat hepatocytes

Test proteins	Uptake of [^{14}C]lipoprotein, ng/mg cell protein in 4 hr	HMG-CoA reductase activity, % of control	Cholesterol esterification, % of control
[^{14}C]Sucrose-LDL, 400 μg/ml	456	89	102
[^{14}C]Sucrose-LDL, 600 μg/ml	646	110	73
[^{14}C]Sucrose-Lac-LDL, 10 μg/ml	2057	17	374
+ Asialofetuin, 5 μg/ml	643	30	161
+ Asialofetuin, 10 μg/ml	351	44	112

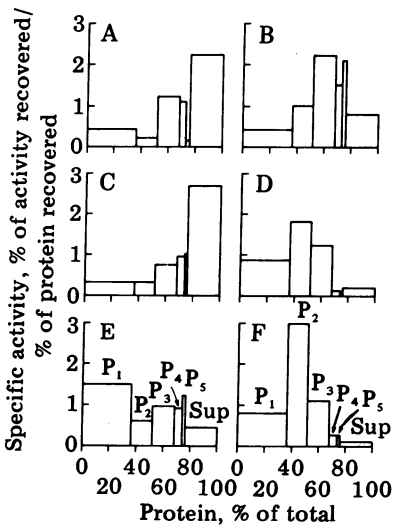

FIG. 4. Subcellular distributions of ^{131}I-labeled LDL (*A*) and ^{125}I-labeled Lac-LDL (*B*) in rat livers. Rats were killed 1 hr after the simultaneous intravascular injection of the radioiodinated lipoproteins, and subcellular fractions were prepared. Fraction designations: P_1, the 750 × *g* pellet; P_2, the 750–5000 × *g* pellet; P_3, the 5,000–20,000 × *g* pellet; P_4, the 20,000–40,000 × *g* pellet; P_5, the 40,000–100,000 × *g* pellet; Sup, the 100,000 × *g* supernate. The cytosolic marker was lactate dehydrogenase (*C*); lysosomal markers were acid phosphatase (*D*) and *N*-acetylglucosaminidase (*F*); and the plasma membrane marker was 5′-nucleotidase (*E*). The data are the means of two experiments.

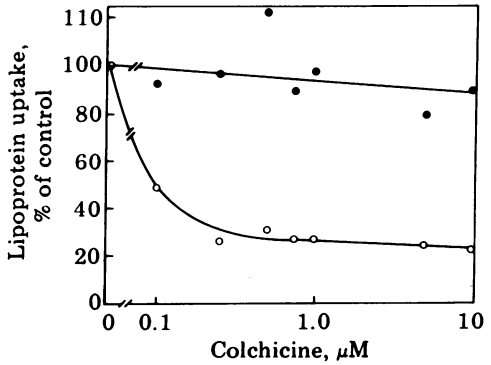

FIG. 5. Effect of colchicine on hepatocyte uptake of [^{14}C]sucrose-labeled LDL (●) and Lac-LDL (○). Hepatocyte cultures were incubated for 2 hr in the presence of the indicated concentrations of colchicine before addition of the test lipoproteins (native LDL, 100 μg/ml; Lac-LDL, 5 μg/ml). Uptake was measured 18 hr later as the total cell-associated radioactivity in the trypsin-treated cells. Uptake in the absence of colchicine was 395 ± 2 ng/mg of cell protein for native LDL and 6.05 ± 1.13 μg/mg of cell protein for Lac-LDL.

DISCUSSION

Several lines of evidence support the conclusion that the Lac-LDL used in these studies was taken up by the galactose-specific receptor: (*i*) the rate of removal from the plasma compartment was markedly increased (7); (*ii*) uptake was essentially confined to the liver; (*iii*) uptake was predominantly into parenchymal cells; and (*iv*) asialofetuin at low concentrations competed very effectively with Lac-LDL for degradation by hepatocytes in culture. In order to convert native LDL to a form recognized by the galactose-specific receptor, it was necessary to lactosylate it extensively. In previous studies (14) it was shown that removal of 70% of the sialic acid residues from swine LDL did not alter its rate of disappearance from plasma. A similar extent of desialylation of human LDL did not alter its uptake and degradation by cultured rat hepatocytes. In both cases, the estimated total surface density of exposed galactose residues was about one residue per 62 nm^2. In the present studies Lac-LDL with 113 residues of lactose added (one residue per 9.8 nm^2) showed no increase in degradation rate. This is well above the surface density sufficient for rapid clearance of ceruloplasmin (28) and transferrin (29). Thus, factors in addition to an apparent minimal density of exposed galactose residues on plasma proteins appear to affect their recognition and rapid clearance. The heavily lactosylated LDL used in all subsequent studies, which was rapidly cleared, contained 200–250 lactose residues per LDL particle (one residue per 4.4–5.6 nm^2).

In the present studies we compare one protein (lactosylated human LDL) that is taken up by a highly specific, saturable mechanism with another protein (native human LDL) that is taken up mostly, if not exclusively, by a nonspecific, nonsaturable mechanism. Studies by Pangburn and Weinstein (30) in this laboratory showed that uptake and degradation of human ^{125}I-labeled LDL by rat hepatocytes is a linear function of LDL concentration up to 200 μg/ml; degradation even at low concentrations is not significantly affected by addition of a large excess of unlabeled LDL (unpublished observations). Treatment of rats with large doses of estrogenic hormone induces the appearance of high-affinity hepatic receptors recognizing both human and rat LDL, but normal rat liver shows few if any such receptors (31, 32). Under physiological conditions, much of the LDL catabolism in rat liver may occur independently of a specific receptor (32). It should be stressed, however, that the rat may be atypical in this respect. Cultured neonatal pig hepatocytes have a readily demonstrable high-affinity receptor for LDL (33), and high-affinity receptors have been reported on plasma membranes from porcine and bovine liver (34, 35). In any case, the native human LDL in the present studies serves as a model for proteins taken up nonspecifically.

Our data show that the mechanisms of internalization of LDL and Lac-LDL differed. Lac-LDL was taken up at a rapid rate by the high-affinity, saturable, galactose-specific receptor whereas LDL was cleared more slowly by a nonsaturable, low-affinity mechanism. Chloroquine effectively inhibited the internalization of Lac-LDL but did not substantially affect that of LDL. Internalization of Lac-LDL, but not of native LDL, was strongly inhibited by low concentrations of colchicine. The last finding implies that the microtubular system is involved in endocytosis from specialized areas of the plasma membrane, perhaps coated pits (36), but not from nonspecialized areas.

The data also imply a difference in intracellular processing of the two forms of LDL studied. The hepatic subcellular distribution of the two forms of LDL shortly after injections was found to differ; a much greater fraction of Lac-LDL was associated with lysosomes. At later times, however, degradation products from both forms were predominantly in lysosomal fractions, suggesting a cytoplasmic "delay pool" in the processing of native LDL. We suggest that pinosomes formed from areas of the membrane containing specific receptors fuse more rapidly with lysosomes, possibly because they contain integral proteins, the inner portions of which are recognized by receptors on the lysosomal surface. Pinosomes involved in uptake of native LDL, in part by fluid endocytosis and perhaps in part by nonspecific adsorptive endocytosis, might lack such recognition markers. The degradation of native LDL was less sensitive to lysosomal inhibitors, raising the possibility that it is degraded in part extralysosomally, a process demonstrated for at least partial proteolysis of some endogenous proteins (37–39), or in lysosomes with properties different from those that degrade Lac-LDL. The eventual accumulation of degradation products in lysosomes may not be inconsistent with such an extralysosomal process. Dean (40) has observed that [^{14}C]sucrose introduced into the cytoplasm of 3T3 cells in microvesicles is progressively transferred to a sedimentable fraction, apparently lysosomal. The sucrose, once introduced into the cytoplasm, is

trapped and does not readily escape from the cell. Thus it appears that, contrary to our original supposition (4), intracellular trapping of ^{14}C from [^{14}C]sucrose-labeled proteins does not necessarily denote lysosomal catabolism.

Some investigators have reported that rat LDL does not affect HMG-CoA reductase activity in rat hepatocytes (41, 42). Similar reports have appeared concerning human LDL (41), but suppression has also been reported (43–45), usually at high concentrations of LDL. In our studies, incubation with Lac-LDL sharply reduced HMG-CoA reductase activity and increased cholesterol esterification; native LDL at much higher concentrations, which were chosen to yield equal rates of lipoprotein uptake, did neither. Thus, uptake and degradation of the Lac-LDL, occurring via a receptor not normally involved in lipoprotein uptake but delivering its ligand rapidly to lysosomes, regulates cholesterol metabolism in the hepatocytes in a manner analogous to that demonstrated for native LDL in fibroblasts. These findings provide additional evidence that LDL regulation of HMG-CoA reductase activity and of cholesterol esterification requires the participation of the specific LDL receptor only or primarily as a means of gaining rapid entry of lipoprotein cholesterol into the lysosomal compartment. In the hepatocytes, cholesterol from native LDL does not appear to reach the appropriate site for suppression of HMG-CoA reductase or activation of cholesterol esterification, whereas cholesterol from lysosomally catabolized Lac-LDL does. Whether this reflects qualitative differences in the intracellular handling of the cholesterol moieties of the two forms of LDL or some other difference remains to be established. It is possible, for example, that at high concentrations of LDL the efflux of cholesterol from cells is great enough that there is no net change in cell cholesterol content and thus no effect on HMG-CoA reductase or cholesterol esterification.

The highly selective uptake of Lac-LDL into hepatocytes should find application as a stratagem for targeting drugs, hormones, radiolabeled compounds, or other materials of interest to the liver. Any compound that can be covalently linked to the protein moiety or incorporated into the lipid core of LDL, as described by Krieger *et al.* (46), could be delivered almost exclusively to the liver for research, diagnostic, or therapeutic purposes.

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