Rat High Density Lipoprotein Subfraction (HDL₃) Uptake and Catabolism by Isolated Rat Liver Parenchymal Cells*  
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Rat liver parenchymal cell binding, uptake, and proteolytic degradation of rat ¹²⁵I-labeled high density lipoprotein (HDL) subfraction, HDL₃ (1.110 < d < 1.210 g/ml), in which apo-A-I is the major polypeptide, were investigated. Structural and metabolic integrity of the isolated cells was verified by trypan blue exclusion, low lactic dehydrogenase leakage, expected morphology, and gluconeogenesis from lactate and pyruvate. ¹²⁵I-labeled HDL₃ was incubated with 10 x 10⁴ cells at 37° and 4° in albumin and Krebs-Henseleit bicarbonate buffer, pH 7.4. Binding and uptake were determined by radioactivity in washed cells. Proteolytic degradation was determined by trichloroacetic acid-soluble radioactivity in the incubation medium. At 37°, maximum HDL₃ binding (Bₘₐₓ) and uptake occurred at 30 min with a Bₘₐₓ of 31 ng/mg dry weight of cells. The apparent dissociation constant of the HDL₃ receptor system (Kₐ) was 60 x 10⁻⁹ M, based on Mₗ = 28,000 of apo-A-I, the predominant rat HDL₃ protein. Proteolytic degradation showed a 15-min lag and then constant proteolysis. After 2 hours 5.8% of incubated ¹²⁵I-labeled HDL₃ protein was degraded. Sixty per cent of cell radioactivity at 37° was trypsin releasable. At 37°, ¹²⁵I-labeled HDL₃ was incubated with cells in the presence of varying concentrations of native (cold) HDL₃, very low density lipoproteins, and low density lipoproteins. Incubation with native HDL₃ resulted in greatest inhibition of ¹²⁵I-labeled HDL₃ binding, uptake, and proteolytic degradation. When ¹²⁵I-labeled HDL₃ was preincubated with increasing amounts of HDL₃ antiserum, binding and uptake by cells were decreased to complete inhibition. Cell binding, uptake, and proteolytic degradation of ¹²⁵I-labeled HDL₃ were markedly diminished at 4°. Less than 1 mM chloroquine enhanced ¹²⁵I-labeled HDL₃ proteolysis but at 5 mM or greater, chloroquine inhibited proteolysis with ¹²⁵I-labeled HDL₃ accumulation in cells. L-[¹⁴C]lysine-labeled HDL₃ was bound, taken up, and degraded by cells as effectively as ¹²⁵I-labeled HDL₃. These data suggest that liver cell binding, uptake, and proteolytic degradation of rat HDL₃ are actively performed and linked in the sequence: binding, then uptake, and finally proteolytic degradation. Furthermore, there may be a specific HDL₃ (lipoprotein A) receptor or recognition site(s) on the plasma membrane. Finally, our data further support our previous reports of the important role of liver lysosomes in proteolytic degradation of HDL₃.

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The definite cellular and subcellular site(s) and basic mechanisms involved in lipoprotein and apolipoprotein catabolism have not been established. Langer et al. reported data indicating that the pathologic elevation of plasma low density lipoprotein (LDL) concentration in individuals with familial type IIa hyperlipoproteinaemia is due to decreased LDL¹ degradation rather than to an abnormality of LDL synthesis (1). However, little is known about the basic and regulative mechanisms for catabolism of apolipoproteins. Our previous studies indicate that liver and liver lysosomes are important organ and subcellular organelle sites for catabolism of apolipoprotein A (2, 3). It has been shown that human skin fibroblasts (4) and human and rat aortic smooth muscle cells in culture (5, 6) can take up and degrade various lipoproteins. Goldstein and Brown (4, 7-11) indicated that prerequisites for regulation of cholesterol synthesis by LDL and for cholesterol ester formation in cultured fibroblasts are: (a) the initial binding of low density lipoproteins (apolipoprotein B) to high affinity surface high density lipoprotein; VLDL, very low density lipoprotein; D₅₈-cAMP, dibutyryl adenosine 3',5'-monophosphoric acid.
As isolated rat liver parenchymal cells were prepared by liver perfusion with collagenase.

**MATERIALS AND METHODS**

**Isolation of Rat Liver Parenchymal Cells**—Male Holtzman rats weighing 250 to 300 g were given water and standard Purina Laboratory Chow (Ralston Purina Co., St. Louis, Mo.) ad libitum. Blood was obtained from healthy male Holtzman rats fasted 20 to 30 min. For electron microscopic study, cells were fixed with 4% glutaraldehyde in 0.01 M phosphate buffer, pH 7.2, and 0.154 M sodium chloride for 30 min at room temperature and postfixed at 4° for 30 min in 1% OsO₄ in the same buffer system. After fixation, cells were dehydrated quickly in graded ethanol and propylene oxide and embedded in Epon-Araldite. Thin sections were prepared on a Porter-Blum ultramicrotome MT 1 (Ivan Sorval, Newtown, Conn.) equipped with a diamond knife. The sections were picked up on naked copper grids and stained with uranyl acetate and Karnovsky’s lead hydroxide (23). The finished preparations were applied and photographed on a Hitachi HU-11B electron microscope (Hitachi Ltd., Tokyo, Japan) at original magnifications of 2,500 to 51,250.

To evaluate the integrity of the plasma membrane, the activity of lactate dehydrogenase, a soluble cytoplasmic enzyme, was determined in liver parenchymal cells and medium after incubation at 37° in Krebs-Henseleit bicarbonate buffer, pH 7.4, with and without 1.5% bovine serum albumin. Liver parenchymal cells were separated after incubation by centrifugation at 8,000 × g for 2 min in a Brinkmann centrifuge 3200 (Brinkmann Instruments, Westbury, N.Y.). Lactic dehydrogenase activity in cells after incubation in fresh medium with or without glucagon and CAMP was measured by sequential measurement of gluconeogenesis from L-lactate by isolated rat liver parenchymal cells and medium after incubation at 37° in Krebs-Henseleit bicarbonate buffer, pH 7.4, containing 1.5% fatty acid-free bovine serum albumin, 2.5 mM CaCl₂, and 10 mM lactate/1 mM pyruvate or 10 mM lactate alone. The flasks were gassed with 95% O₂/5% CO₂ for 30 s, capped and incubated at 37° with 90 to 100 oscillations per min in a Dubnoff metabolic shaker bath (Precision Scientific Co., Chicago, Ill.). The incubations were terminated by pouring the contents of the flasks into test tubes containing 0.5 ml of 30% perchloric acid. Rates of glucose production during the incubation was measured by an enzymatic method using hexokinase and glucose-6-phosphate dehydrogenase (Boehringer Mannheim Biochemicals, New York, N.Y.)(95) Gluconeogenesis was calculated as nmol of glucose produced per mg dry weight of cells per min.

To assess further the integrity of the plasma membrane and the metabolic integrity of the isolated liver parenchymal cells, the effects of glucagon and cAMP on gluconeogenesis from lactate and pyruvate were investigated. Aliquots of the cell suspension containing (6.3 ± 1.9) × 10⁶ cells (mean ± S.D., n = 12) in 1.0 ml were added to 25-ml Erlenmeyer flasks containing 1.0 ml of Krebs-Henseleit bicarbonate buffer, pH 7.4, containing 1.5% fatty acid-free bovine serum albumin, 2.5 mM CaCl₂, and 10 mM lactate/1 mM pyruvate or 10 mM lactate alone. The flasks were gassed with 95% O₂/5% CO₂ for 30 s, capped and incubated at 37° with 90 to 100 oscillations per min in a Dubnoff metabolic shaker bath (Precision Scientific Co., Chicago, Ill.). The incubations were terminated by pouring the contents of the flasks into test tubes containing 0.5 ml of 30% perchloric acid. Rates of glucose production during the incubation was measured by an enzymatic method using hexokinase and glucose-6-phosphate dehydrogenase (Boehringer Mannheim Biochemicals, New York, N.Y.)(95) Gluconeogenesis was calculated as nmol of glucose produced per mg dry weight of cells per min.
Characterization of HDL<sub>4</sub>-HDL<sub>3</sub> was characterized by column chromatography of delipidated HDL<sub>4</sub> (apo-HDL<sub>4</sub>) on Sephadex G-100 (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.). Delipidation was performed by the method of Glomset et al. (26). Characterization of HDL<sub>3</sub> and apo-HDL<sub>3</sub> and its Sephadex G-100 fractions was performed by polyacrylamide gel electrophoresis as previously described (2). Rabbit anti-rabbit HDL<sub>3</sub> antisera was prepared and characterized (2). Protein in HDL<sub>3</sub> was determined by the method of Lowry et al. (27) using bovine serum albumin as a standard.

Specific binding, uptake, and proteolytic degradation were defined as the sum of the '*I-labeled HDL<sub>3</sub> bound to the plasma membrane (binding) plus the '*I-labeled HDL<sub>3</sub> inside of the cells (uptake) plus the proteolytic degradation by cells was examined. Using our standard assay, '*I-labeled HDL<sub>3</sub> was incubated with isolated liver parenchymal cells in a medium containing different concentrations of VLDL, LDL, HLDL, and HDL<sub>3</sub>. Protein concentrations were estimated to be 0 to 500 μg/ml.

The effects of rabbit anti-rat HDL<sub>3</sub> serum on binding and uptake of '*I-labeled HDL<sub>3</sub> by isolated cells were investigated. Aliquots of '*I-labeled HDL<sub>3</sub> were preincubated with increasing quantities (0 to 500 μl) of rabbit anti-rat HDL<sub>3</sub> serum for 30 min at 37°C followed by the addition of isolated rat liver parenchymal cells and incubation for 30 min. The effects of chloroquine on binding, uptake, and proteolytic degradation of '*I-labeled HDL<sub>3</sub> by isolated liver parenchymal cells were studied. Chloroquine was purchased from Sigma Chemical Co., St. Louis, Mo. After preincubation, '*I-labeled HDL<sub>3</sub> alone or '*I-labeled LDL, and native HDL<sub>3</sub> were added and incubation was continued for another 2 hours. Chloroquine was dissolved in medium immediately before use and the pH was adjusted to 7.4. Binding, uptake, and proteolytic degradation of '*I-labeled HDL<sub>3</sub> by cells treated with chloroquine were investigated using our standard assay.
functionally intact during incubation, they should retain soluble cytoplasmic enzymes. The percentage of leakage of lactic dehydrogenase from cell to medium in Krebs-Henseleit bicarbonate buffer/1.5% bovine serum albumin, pH 7.4, was 0.49, 0.56, 0.31, 0.33, 0.66, 0.66, and 0.58% at 0, 5, 15, 30, 45, 60, 90, and 120 min, respectively, after incubation at 37°C (average values of two different experiments). In experiments without added bovine serum albumin, the percentage of loss of lactic dehydrogenase from cell to medium was 9.73, 9.25, 14.42, 12.65, 14.48, 12.57, 11.3, and 14.9% at 0, 5, 15, 30, 45, 60, 90, and 120 min, respectively, after incubation at 37°C. Total lactic dehydrogenase activity (the sum of activities in cells and medium) remained relatively constant. Total lactic dehydrogenase activities at 30, 60, and 120 min after incubation were 98.6, 93.5, and 91.1% of initial total lactic dehydrogenase activity. The differences in lactic dehydrogenase leakage between cells with and without added bovine serum albumin indicated a protective effect of albumin on the plasma membrane. Mean value of the percentage of leakage of lactic dehydrogenase from cell to medium in Krebs-Henseleit bicarbonate buffer/1.5% bovine serum albumin, pH 7.4, after approximately 30 min at 4°C in 23 separate isolated liver parenchymal cell preparations was 3.0 ± 0.9 (mean ± S.D.)%.

The highest glucose production rate (790 nmol of glucose produced/mg dry weight of cells/2 hours) was observed with the combination of 10 mM lactate/1 mM pyruvate as substrate. Other glucose production rates were as follows. 10 mM lactate alone, 580 nmol of glucose/mg dry weight of cells/2 hours; 10 mM pyruvate alone, 550 nmol of glucose/mg dry weight of cells/2 hours. Therefore, to investigate gluconeogenesis and the effect of glucagon, cAMP, and bovine serum albumin on gluconeogenesis, the combination of 10 mM lactate/1 mM pyruvate was used as substrate. Liver parenchymal cells isolated from rats fasted 24 hours produced approximately 4.05 nmol of glucose/mg dry weight of cells/min when incubated with 10 mM lactate/1 mM pyruvate at 37°C for 2 hours. After approximately a 15-min lag phase, the rate of gluconeogenesis was constant throughout the 2-hour incubation. In Fig. 1, the effects of 1.5% bovine serum albumin and 2.6 μM glucagon on gluconeogenesis are shown. By preparing and incubating the cells in Krebs-Henseleit bicarbonate buffer/1.5% bovine serum albumin, pH 7.4, the rate of gluconeogenesis was increased. Even though glucagon exhibited its effect on gluconeogenesis both in the cells with and without bovine serum albumin, the glucagon effect was much greater in the presence of albumin. At 120 min of incubation, the ratios of glucose produced with glucagon to glucose produced without glucagon were 1.36 and 1.22, with and without added serum albumin, respectively. This indicates that albumin has a protective effect on the structural and metabolic integrity of liver parenchymal cells, especially on the plasma membranes. BμCAMP, 10 μM, showed an effect on gluconeogenesis comparable to 2.6 μM glucagon. Glucose production through gluconeogenesis was always less than 10% of total glucose production during incubation.

**Characterization of Rat HDL**—Column chromatography of totally delipidated HDL₃ on Sephadex G-100 showed three peaks comparable with the column chromatographic pattern of canine apo-HDL₃ reported previously (2). Polyacrylamide gel electrophoresis of HDL₃, apo-HDL₃, and the column fractions indicated the presence of apo A-I as the major protein moiety and suggested apo-A-II, apo-C-II, apo-C-III, and apo-D as minor protein moieties. Immunodiffusion and immunoelectrophoresis confirmed this and ruled out contamination by apo-B. After iodination of HDL₃, approximately 97 to 98% of ¹²⁵I-labeled HDL₃ was precipitable by 15% trichloroacetic acid and less than 5% radioactivity could be extracted with chloroform/methanol (2/1, v/v) solution.

**Binding and Uptake of ¹²⁵I-labeled HDL₃ by Isolated Rat Liver Parenchymal Cells**—Radioactivity in cells reached a maximum at 30 min of incubation and this plateau or steady state persisted for at least 2 hours (Fig. 2). The amount of specific binding and uptake of ¹²⁵I-labeled HDL₃ at the plateau was 1.8% (mean of three experiments) of incubated ¹²⁵I-labeled HDL₃. The binding and uptake of ¹²⁵I-labeled HDL₃ by isolated liver parenchymal cells was decreased with increasing native HDL₃ as shown in Fig. 3. The apparent maximum HDL₃ binding and uptake capacity of rat liver parenchymal cells (Bₘₐₓ) and the apparent dissociation constant of the liver cell system (Kₐ) were estimated with one of the Woolf plots (30) commonly known as a Scatchard plot (31, 32). Bₘₐₓ was 31 ng of HDL₃ protein/mg dry weight of cells, and Kₐ was 17 μg of HDL₃ protein/ml liver cell system or Kₐ = 60 × 10⁻⁶ M based on M₉, 28,000 for apo-A-I as the predominant protein in HDL₃ (33). These data indicate a saturable or specific process for binding and uptake of rat HDL₃ by rat liver cells. Only a minimal amount of specific binding and uptake of ¹²⁵I-labeled HDL₃ occurred at 4°C. There were slight but constant increments in nonspecific binding and uptake of ¹²⁵I-labeled HDL₃ by cells, which were of similar magnitude at both 4°C and 37°C. Therefore, specific binding and uptake of ¹²⁵I-labeled HDL₃ by liver cells is temperature-dependent and is saturable in contrast to nonspecific processes. To measure the percentage of radioactivity on the surface of the plasma membrane, the cells were treated with trypsin at 60 min of incubation, which was after radioactivity in liver cells had reached a steady state plateau.
Approximately 60% of radioactivity in the cells was trypsin-releasable and 40% remained inside the cells after trypsin treatment. Thus, 60% of radioactivity was \(^{125}\text{I}\)-labeled HDL, bound on the plasma membrane surface (binding) and 40% of radioactivity was internalized \(^{125}\text{I}\)-labeled HDL, (uptake). To investigate further the specificity of the lipoprotein binding and uptake by cells, competition of native HDL, VLDL, and LDL with \(^{125}\text{I}\)-labeled HDL, for binding and uptake was studied. Native HDL, inhibited \(^{125}\text{I}\)-labeled HDL, binding and uptake much more effectively than did VLDL and LDL (Fig. 4). When \(^{125}\text{I}\)-labeled HDL, was preincubated with varying amounts of rabbit antiserum to rat HDL, binding and uptake by cells were decreased by increasing amounts of antisemur, and complete inhibition was observed with 500 µl of antiserum (Fig. 5). Both the antibody binding site and the receptor attachment site on the HDL, molecule may be in close proximity so that there can be an effect of steric hindrance. The

**Fig. 2.** Specific binding, uptake, and proteolytic degradation of \(^{125}\text{I}\)-labeled HDL, by isolated rat liver parenchymal cells at 37°. Specific binding, uptake, and proteolytic degradation were defined as the difference between the radioactivity in the absence and presence of an excess of native HDL, (200 µg of native HDL, protein/ml). Specific binding and uptake were determined by the appearance of radioactivity in cells, expressed as dpm/mg dry weight of cells accumulated over a 120-min incubation. Specific proteolytic degradation of \(^{125}\text{I}\)-labeled HDL, by isolated rat liver parenchymal cells is also shown. Specific proteolytic degradation was determined by the release of trichloroacetic acid-soluble radioactivity into the incubation medium as dpm/mg dry weight of cells over a 120-min incubation. \(^{125}\text{I}\)-labeled HDL, protein concentration was 100 µg/ml and specific radioactivity was 420 dpm/µg of HDL, protein. In each flask, there were 10.2 x 10⁶ cells with a total dry weight of 8.9 mg.

**Fig. 3.** Estimation of the maximum rat HDL, binding capacity (Bmax) and the dissociation constant (Kd) of the isolated rat liver cell system. Aliquots of rat liver cells were incubated with a fixed amount of \(^{125}\text{I}\)-labeled HDL, (100 ng/ml, specific radioactivity of 512 dpm/µg of HDL, protein). Increasing amounts of native HDL, were added to a maximum concentration of 200 µg/ml. In each flask, there were 5.4 x 10⁵ cells with a total dry weight of 7.0 mg. The binding and uptake of \(^{125}\text{I}\)-labeled HDL, by isolated rat liver parenchymal cells was decreased with increasing native HDL, and a straight line (r = -0.98) was obtained by a Scatchard plot. Bmax was 31 ng/mg dry weight of cells and Kd was 16.8 µg/ml or 60 x 10⁻⁹ M, based on M, 28,000, of apo-A-I, the predominant protein moiety of rat HDL,.

**Fig. 4 (left).** Effects of VLDL, LDL, and HDL, on \(^{125}\text{I}\)-labeled HDL, binding and uptake by isolated rat liver parenchymal cells. \(^{125}\text{I}\)-labeled HDL, was incubated with isolated rat liver parenchymal cells in a medium containing increasing concentrations of VLDL, LDL, and native HDL, at 37°. Lipoprotein protein concentrations ranged from 0 to 500 µg/ml. Incubation time was 60 min. In each flask, there were 7.0 x 10⁶ cells with a total dry weight of 6.1 mg.

**Fig. 5 (center).** Effect of rabbit anti-rat HDL, serum on binding and uptake of \(^{125}\text{I}\)-labeled HDL, by isolated rat liver parenchymal cells. \(^{125}\text{I}\)-labeled HDL, was preincubated with varying amounts of antibody to rat HDL, for 30 min at 37°, followed by a 30-min incubation with isolated rat liver parenchymal cells at 37°. \(^{125}\text{I}\)-labeled HDL, protein concentration was 100 ng/ml (specific radioactivity 458 dpm/µg of HDL, protein). In each flask, there were 6.4 x 10⁶ cells with a total dry weight of 6.4 mg.

**Fig. 6 (right).** Effect of increasing concentrations of chloroquine on specific binding, uptake, and proteolytic degradation of \(^{125}\text{I}\)-labeled HDL, by isolated rat liver parenchymal cells at 37°. Specific binding and uptake were determined by the appearance of radioactivity in cells, expressed as dpm/mg dry weight of cells accumulated during 120-min incubation. Specific proteolytic degradation was determined by the release of trichloroacetic acid-soluble radioactivity into the incubation medium, expressed as dpm/mg dry weight of cells during 120-min incubation. \(^{125}\text{I}\)-labeled HDL, protein concentration was 100 ng/ml and specific radioactivity was 463 dpm/µg of HDL, protein. In each flask, there were 10.0 x 10⁶ cells with a total dry weight of 10.4 mg.
mechanism of the initial increase in binding and uptake at very low amounts of rabbit anti-rat HDL₄ serum is not clear but may be due to an unspecified effect of serum factor(s) which promote binding (34, 35). These observations on lipoprotein competition for binding and uptake and on antiseraum inhibition of binding and uptake further support the specificity of binding and uptake for each lipoprotein.

Proteolytic Degradation of ¹²⁵I-labeled HDL₄ by Isolated Rat Liver Parenchymal Cells—The rate of ¹²⁵I-labeled HDL₄ proteolytic degradation was measured by counting radioactivity obtained from the incubation medium in the trichloroacetic acid-soluble fraction and in the chloroform phase and the resultant aqueous phase after extracting the trichloroacetic acid-soluble fraction with chloroform, as described under "Materials and Methods." After peroxidation, 72.6 ± 5.4% (mean ± S.D., n = 10) of radioactivity in the trichloroacetic acid-soluble fraction, aqueous phase (¹²⁵I-labeled tyrosine), and chloroform phase (oxidized free ¹²⁵I) were comparable to each other. There was no significant radioactivity increase observed in the trichloroacetic acid-soluble fraction, aqueous phase, or chloroform phase by incubating ¹²⁵I-labeled HDL₄ without cells, indicating that both ¹²⁵I-labeled tyrosine and free ¹²⁵I were formed through active catabolism by the liver cells. These data, and information on liver microsomal iodotyrosine deiodinase (36-38) suggest that radioactivity released from the cells to the medium following ¹²⁵I-labeled HDL₄ proteolytic degradation is not only in the form of ¹²⁵I-labeled tyrosine but also in the form of free ¹²⁵I. Therefore, we chose to express the rate of ¹²⁵I-labeled HDL₄ degradation by radioactivity change in the trichloroacetic acid-soluble fraction, since this represents the full liver cell catabolism of our ¹²⁵I-labeled HDL₄ used in these studies. At 37⁰, there was a lag phase for 15 min and after that a constant rate of degradation was observed up to 2 hours in which 5.8% (mean of three experiments) of radioactivity in the trichloroacetic acid-soluble fraction with chloroform, as described under "Results." Since Howard et al. (12) described an enzymatic method for preparation of isolated liver parenchymal cells from rat by shaking liver slices in a buffered medium containing hyaluronidase and collagenase, many modifications have been made in order to isolate large quantities of viable liver parenchymal cells that retain in vivo metabolic characteristics, maintain intact plasma membranes, and respond to hormones at physiological concentrations (41). Liver parenchymal cells isolated by this enzymatic digestion method have been used to investigate several aspects of protein synthesis and degradation (18, 42), gluconeogenesis (14, 15, 39, 40), fatty acid metabolism (16, 43), and chylomicron catabolism (43, 50). These reports indicate that metabolic activities by isolated liver parenchymal cells are comparable to liver function in vivo. The original method of Berdy and Friend (13) subjects the liver to 0.05% collagenase and 0.10% hyaluronidase for prolonged periods (60 to 90 min). By modifying these procedures to expose the liver to 0.05% collagenase without hyaluronidase, by perfusing for a shorter period (20 min) and by adding albumin to a concentration of 1.5% at the time the cells were washed, we were able to isolate large quantities of viable liver parenchymal cells with good morphologic and metabolic properties, as shown under "Results."

These well characterized cells were used in the present study to investigate binding, uptake, and proteolytic degradation of ¹¹¹I-labeled HDL₄. Our working hypothesis on the process of lipoprotein catabolism of liver parenchymal cells, shown in Fig. 7, was constructed from published data on: (a) lipoprotein binding, uptake, and proteolytic degradation by human skin fibroblasts (4, 7-11) and human and rat aortic smooth muscle cells (5, 6, 51) in culture, (b) glycoprotein uptake and catabolism by liver cells (38, 52, 53), and (c) endocytosis and catabolism of proteins (54-56). These steps may be divided into at least six major steps as described in the legend to Fig. 7. Both binding and uptake of ¹¹¹I-labeled HDL₄ by rat liver parenchymal cells were measured by counting radioactivities in cells washed and centrifuged three times at 4⁰. Following this procedure, radioactivity in cells was always constant. Radioactivity bound to the external surface of the plasma membran...
most studies of lipoprotein metabolism in vivo and in vitro
have been made with lipoproteins or apolipoproteins labeled with $^{125}\text{I}$ in vitro, or both. Labeling with $^{125}\text{I}$ is probably the only practical way to obtain high specific activities in complex lipoprotein molecules (64). Nevertheless, it is difficult to prove that $^{125}\text{I}$-labeled lipoproteins behave exactly like native lipoproteins when studied in biological systems. In our studies, binding, uptake, and proteolytic degradation of $^{125}\text{I}$-labeled HDL, were effective and were competitively inhibited by native HDL. Binding, uptake, and proteolytic degradation of $^{125}\text{I}$-labeled HDL, by rat liver parenchymal cells were as effective as binding, uptake, and proteolytic degradation of $^{125}\text{I}$-labeled HDL. These data suggest that $^{125}\text{I}$-labeled HDL behaves essentially as native HDL, in our in vitro isolated rat liver parenchymal cell system. This is fortunate because $^{125}\text{I}$-labeled HDL, or other $^\text{14}C$-aminoacid-labeled HDL production in vivo is not economically feasible due to the large amounts of costly $^\text{14}C$-aminoacid required.

In conclusion, our data suggest that binding, uptake, and proteolytic degradation of rat HDL, by isolated rat liver parenchymal cells are actively performed and linked in the sequence: binding, then uptake, and finally proteolytic degradation. Furthermore, there may be a specific HDL$_2$ (lipoprotein A) receptor or recognition site(s) on the plasma membrane. Finally, our data give further support to our previous reports (2, 3) of the important role of liver lysosomes in proteolytic degradation of HDL,.

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