

Interaction of serum lipoproteins with the intestine. Evidence for specific high density lipoprotein-binding sites on isolated rat intestinal mucosal cells

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Abstract To determine if plasma lipoproteins interact and therefore possibly regulate intestinal lipoprotein metabolism, we investigated the binding, internalization, and degradation of ¹²⁵I-labeled low density lipoprotein (LDL) and high density lipoprotein (HDL) by enzyme-dispersed rat intestinal mucosal cells. Both human and rat LDL and HDL were bound, internalized, and degraded in a concentration-dependent manner with calculated half-saturation occurring at approximately 30, 35, 35, and 15 $\mu\text{g}/\text{ml}$ for human LDL, rat LDL, human HDL, and rat HDL, respectively. Isolated brush border membranes had no saturable or specific binding sites for ¹²⁵I-labeled HDL or LDL, suggesting that lipoproteins may be bound to receptors on lateral or basal membranes of mucosal cells. Compared with HDL, LDL binding was characterized by a large non-specific component. LDL of human and the rat were not only displaced by excess LDL but at least as effectively by excess HDL of their own species. Labeled HDL was displaced by corresponding unlabeled lipoproteins, but human LDL could produce only minor displacement of human HDL₃. ApoE-deficient rat HDL, separated by heparin-Sepharose affinity chromatography also showed highly specific saturable binding to intestinal cells. Thus, apparently two different lipoprotein binding sites exist in intestinal plasma membranes, one recognizing B and/or E apoproteins present in human and rat LDL and rat HDL while another binds human HDL₃ and apoE-deficient rat HDL which contain A apoproteins as major components.—Suzuki, N., N. Fidge, P. Nestel, and J. Yin. Interaction of serum lipoproteins with the intestine. Evidence for specific high density lipoprotein-binding sites on isolated rat intestinal mucosal cells. *J. Lipid Res.* 1983. **24**: 253–264.

Supplementary key words apoE-deficient rat HDL • human LDL • rat LDL • human HDL • rat HDL • brush border membranes

Circulating plasma lipoproteins exert a major influence on cellular lipid metabolism. These regulatory processes include interaction of lipoproteins with receptors on cell membranes that leads to the control of lipid homeostasis within the cells and that of cholesterol in particular. The processes whereby the apoproteins of lipoproteins are recognized by receptors have been characterized largely in cultured human fibroblasts (1), although they occur in many other cell types including smooth muscle cells, endothelial cells, lymphocytes, and

various propagated cells (2). So far only receptors that specifically recognize lipoproteins carrying the B (1, 2) or E (3) apoproteins have been defined, though cultured cells of steroidogenic tissues such as adrenocortical cells (4–6) and testicular cells (7), which are dependent on lipoprotein cholesterol for the production of steroid hormones, also appear to have binding sites that recognize other apoproteins present in high density lipoproteins. The presence of high affinity binding sites for LDL and HDL has also been demonstrated in rat liver cells (8–10).

Roheim et al. (11) found 10% of the ¹²⁵I-labeled HDL injected into rats in the liver and 5% in the small intestine. Recently, Stein, Halperin, and Stein (12) reported that human LDL is catabolized in the rat by both liver and extrahepatic tissues, including the small intestine which catabolized 6% of the injected LDL. However, the mechanisms involved in taking up lipoproteins by rat intestinal cells remain unknown. We have therefore sought, in rat intestinal mucosal cells dispersed by treatment with hyaluronidase, evidence for receptor-mediated uptake of low density and high density lipoprotein.

Since cell-associated, species-specific factors plus differences in apoprotein composition have been shown to influence the interaction of rat and human lipoproteins with fibroblasts (13, 14), we have compared the binding of lipoproteins from both species with rat enterocytes in this study.

METHODS

Materials

Na¹²⁵I, carrier-free, was obtained from Radiochemicals, Amersham Australia Pty. Ltd.; hyaluronidase

Abbreviations: HDL, high density lipoprotein; LDL, low density lipoprotein; LDS, lipoprotein-deficient serum; FCS, fetal calf serum; BME, Eagle's basal medium.

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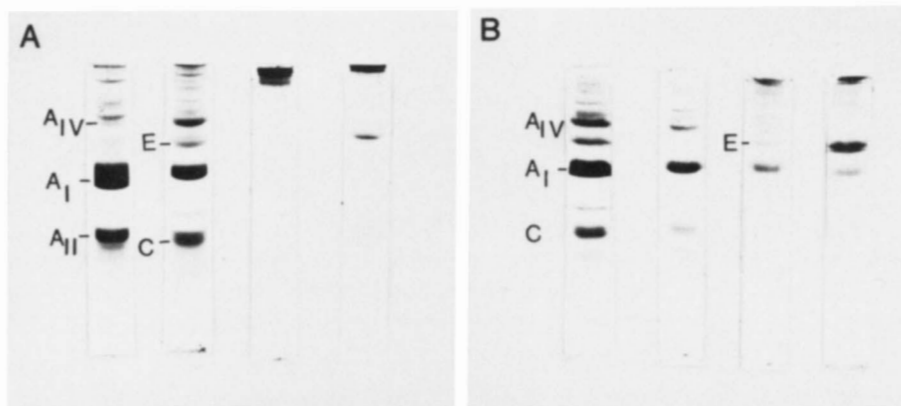


Fig. 1. Polyacrylamide gel electrophoresis of A), left to right, human HDL, rat HDL, human LDL, and rat LDL and B), rat HDL subfractionated by heparin-Sepharose affinity column chromatography (from Fig. 8); left to right, rat HDL, rat HDL A peak, rat HDL B peak, and rat HDL C peak.

Type I (bovine testes), deoxyribonuclease I (bovine pancreas), trypsin inhibitor Type I-P (bovine pancreas), and HEPES buffer (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) were purchased from the Sigma Chemical Company (St. Louis, MO). Hanks balanced salt solution (Hanks BSS), Eagle's basal medium (BME), and fetal calf serum were obtained from the Commonwealth Serum Laboratories (CSL), Parkville, Victoria. Lipoprotein-deficient serum (LDS) was prepared from fetal calf serum by ultracentrifugation at density 1.25 g/ml (see below). After recentrifugation at the same density, the infranatant fraction was dialyzed against buffer A (0.15 M NaCl, 1 mM EDTA, pH 7.4) and finally against phosphate-buffered saline without EDTA. The LDS was sterilized by filtration through 0.45 μ m Millipore filters. Heparin was obtained from the Commonwealth Serum Laboratories, Melbourne, Australia, and CNBr-activated Sepharose-4B was from Pharmacia.

Isolation of lipoproteins

Human LDL (d 1.030–1.050 g/ml) and HDL₃ (1.12–1.21 g/ml) were prepared from 200 ml of plasma collected from healthy subjects who had fasted overnight. Lipoproteins were fractionated by sequential flotation in a Beckman L5-65 ultracentrifuge using either 40 or 50 Ti rotors (Beckman Instruments Inc., Palo Alto, CA) at 210,000 *g* at 4°C for 24 hr according to the method of Havel, Eder, and Bragdon (15). Rat lipoproteins were isolated from the serum of male Sprague-Dawley rats, 300–400 g, that had been deprived of food overnight. Rat LDL was isolated between d 1.030–1.055 g/ml and rat HDL was isolated between 1.070–1.21 g/ml. All lipoproteins were recentrifuged at the appropriate higher density, or alternatively respun until each fraction was homogeneous as judged by agarose gel electrophoresis. The isolated lipoprotein fractions were dialyzed against three changes of 100 volumes of

0.15 M NaCl, pH 7.4, containing 1 mM EDTA and finally dialyzed against PBS without EDTA. After dialysis the lipoproteins were sterilized by filtration through 0.45 μ m Millipore filters.

The apoprotein pattern of delipidated human and rat lipoproteins obtained after electrophoresis on 15% polyacrylamide gels containing SDS (16) is shown in Fig 1(A).

Heparin-Sepharose affinity column chromatography

Rat HDL was subfractionated into apoE-rich and apoE-poor fractions by affinity chromatography through heparin-Sepharose columns. Heparin-Sepharose was prepared by covalent linkage of heparin with CNBr-activated Sepharose-4B (Pharmacia) as described by Iverius (17). Affinity chromatography was carried out at 4°C in 1.0 \times 30 cm glass columns at a flow rate of 24 ml/hr.

The heparin-Sepharose was equilibrated with NaCl-Tris buffer (0.005 M NaCl, 0.005 M Tris, pH 7.4) containing 0.025 M MnCl₂ according to the method of Weisgraber and Mahley (18). Rat HDL (5–8 mg of protein) was dialyzed against NaCl-Tris buffer and then mixed with solid MnCl₂ at a final concentration of 0.025 M immediately before applying to the column. The sample was allowed to equilibrate with the column overnight.

The unbound lipoprotein was eluted in the column void volume with Tris-NaCl-Mn²⁺ buffer. The column was then eluted in a stepwise manner into 2-ml fractions. As fraction 15 was being collected, eluting buffer was changed to a buffer containing 0.095 M NaCl, 5 mM Tris-Cl, pH 7.4, but no Mn²⁺.

Elution with this second buffer was continued until tube 30; then NaCl concentration in the eluting buffer was finally increased to 1 M. On the basis of their absorption at 280 nm, the fractions were combined into

appropriate pools and dialyzed against NaCl-EDTA (0.15 M NaCl, 1 mM EDTA). Recoveries of protein applied to the column averaged 92%. Apoprotein compositions of column fractions are shown in Fig. 1(B) (see Results).

Radioiodination of lipoproteins

All lipoproteins were iodinated with ^{125}I by the iodine monochloride method, modified for lipoproteins (19) as described previously (20). After iodination the labeled lipoproteins were dialyzed against 0.15 M NaCl, pH 7.4, and sterilized by filtration as described above. All labeled preparations contained less than 1 g-atom of iodine per mole of protein. After extraction with chloroform-methanol according to the method of Folch, Lees, and Sloane Stanley (21), 92–95% of the ^{125}I was found attached to the protein moiety of human lipoproteins and 75–82% was bound to the protein moiety of rat lipoproteins. Specific radioactivities of each preparation were between 200–350 cpm per ng of protein.²

Isolation of intestinal mucosal cells

Cell isolation was based on the improved procedure reported by Hoffman and Kuksis (22) using male Sprague-Dawley rats weighing between 90–130 g, which had been fed on commercial rat chow. Animals were stunned and decapitated and segments of small intestine, approximately 50 cm in length, were immediately removed into ice-cold Hank's solution containing 10 $\mu\text{g}/\text{ml}$ of gentamycin. These were cut into three segments of 15 cm each that were then flushed twice with 20 ml of buffer using light pressure from a plastic bottle fitted with a pointed tube. The segments were cut longitudinally, blotted dry, and flattened, with the mucosal surface up, onto a glass plate. Upper and middle section villus cells were obtained by two light passes over each 15-cm segment with a Teflon-coated spatula and the scrapings were transferred to 10 ml of modified Hank's BBS³ containing 1 mg/ml hyaluronidase, 1 $\mu\text{g}/\text{ml}$ deoxyribonuclease, and 10 inhibitory BAEE units/ml pancreatic trypsin inhibitor. The scrapings were incubated in 10 ml of buffer in silicone-treated scintillation vials for 20–30 min. The incubations were carried out in

a metabolic shaker, under an atmosphere of 95% $\text{O}_2/5\%$ CO_2 at 30°C, set at approximately 100 cycles per min. The scrapings were then gently dispersed by alternatively withdrawing and expelling the cell suspension with a plastic disposable syringe fitted with a 10-cm length of soft polyethylene tubing. The isolated dispersed cells plus cell clusters were filtered through a double layer of polyethylene mesh (100 μm) and transferred to plastic tubes. The cell pellet was washed three or four times with ice-cold buffer by centrifugation at 400 g for 2 min at 4°C and finally suspended in culture medium (10% LDS in BME, pH 7.4). Cells were then counted and viability was checked by the Trypan blue exclusion test. The viability of cells in our procedure was 95–99%. Cell yield was 75–100 $\times 10^6$ per segment and most of the preparations consisted of 90% single dispersed villus cells with some doublets. After 3 hr incubation at 37°C, viability was 85–90%.

The incorporation of [^{14}C]leucine into total cell trichloroacetic acid-precipitable protein proceeded linearly from 20 min to 3 hr (see legend to Fig. 2) suggesting that dispersed mucosal cells remained metabolically active without significant loss of viability throughout the incubation periods used in these studies.

Preparation of intestinal brush border membrane and binding of ^{125}I -labeled HDL and LDL to membrane

Preparation of rat intestinal brush border membrane was based on the procedure by Forstner, Sabesin, and Isselbacher (23) with some modifications. The entire small intestine was removed and irrigated with cold 0.9% NaCl. The mucosal cells were removed by scraping (see above), pooled in 5 mM EDTA adjusted to pH 7.4, and homogenized (Polytron, Kinematica, Switzerland) for 25 sec. The mucosal homogenates were overlaid onto a gradient consisting of a bottom layer of 44% sucrose (v/w) (3 ml), a middle layer of 33% sucrose (3 ml), and a top layer of 10% sucrose (3 ml) in 5 mM EDTA, pH 7.4. After centrifugation for 20 min at 1400 rpm, sediments between top and middle layers and between the middle and bottom layers were separated and mixed. This membrane fraction was suspended in 20 volumes of 5 mM EDTA buffer and centrifuged at 4000 rpm for 8 min; the precipitate was resuspended with 20 volumes of EDTA buffer, recentrifuged at 2000 rpm for 1 min, and once more at 1400 rpm for 30 sec.

Membrane pellets were finally suspended in 30 volumes of EDTA buffer and filtered through 90- μm double-layered nylon mesh. Before binding experiments, brush border membranes were washed twice with buffer B (100 mM NaCl in 50 mM Tris-HCl, pH 7.4) to remove EDTA. All the above procedures were carried out at 4°C.

² The integrity of the labeled apolipoproteins was assessed by experiments in which various amounts of corresponding lipoproteins were added to incubation tubes. Binding and internalization parameters were then calculated for cells that had been incubated with lipoproteins at different specific activities but in which the total lipoprotein concentration remained unchanged. The calculated values for total lipoprotein binding and internalization were unchanged, which suggests that there is no preferential uptake or internalization of labeled and unlabeled lipoprotein.

³ Modified Hanks balanced salt solution (BSS) used in the experiments was modified to contain HEPES, final concentration 15 mM and MgCl_2 , final concentration 0.231 mM. The pH was adjusted to 7.35 with either 0.1 M NaOH or 0.1 M HCl.

Binding assays were conducted at 4°C in a total volume of 250 μ l, containing 50 μ l (100–200 μ g of protein) of brush border membrane, 25 μ l of concentrated human albumin (CSL 25% w/v), different amounts of ¹²⁵I-labeled LDL or ¹²⁵I-labeled HDL as described later, and buffer B. After incubation, the medium was layered onto 150 μ l of 100% fetal calf serum in Beckman microtubes according to the method of Goldstein and Brown (24). The tubes were centrifuged at 100,000 *g* for 20 min at 4°C and the supernatant was removed by aspiration. Two hundred μ l of 100% fetal calf serum was added to the membrane pellet and then centrifuged at 100,000 *g* for 5 min at 4°C. After removing the supernatant, the bottom portion of the tube containing the membrane pellet was sliced and radioassayed. The pellet was dissolved in 0.2 M NaOH and its protein content was determined by the method of Lowry et al. (25).

Binding, internalization, and degradation experiments

Approximately 3–5 $\times 10^6$ washed intestinal mucosal cells were incubated in 2 ml of Eagle's basal medium (BME) containing 10% LDS (fetal calf serum) in siliconized scintillation vials at 37°C or 4°C under conditions described in the appropriate Results section.

Fresh cells were used in each experiment. They were resuspended to a concentration of 7–10 $\times 10^6$ per ml of BME. Cell suspension (0.5 ml) was then added to 1.5 ml of BME containing 10% LDS. Labeled or unlabeled lipoproteins were added according to the details provided in the Results section. In studies carried out at 0°C, the cell suspension and medium were placed on crushed ice for 20 min before the start of the experiment and then held on ice in a 4°C coldroom. Studies carried out at 37°C were performed in a warmroom maintained at this temperature. All incubations were carried out by placing the scintillation vials on the platform of an orbital shaker. At the end of the incubation period (generally 3 hr) the cells were transferred to plastic centrifuge tubes and centrifuged at 800 *g* for 2 min. Cells were washed four times with 0.2% albumin in 0.9% saline, pH 7.4, and sedimented by centrifugation at the above conditions after each washing procedure. The cells were further washed twice with 0.9% saline, pH 7.4.

Binding

Cells incubated at 4°C were separated from the medium by centrifugation and washed as described above. The final cell pellet was radioassayed in order to determine binding of labeled lipoproteins. Cells that had been incubated at 37°C were washed as described above. Preliminary experiments showed that the HDL

surface-bound material was not released by heparin but required the addition of trypsin for complete release of surface-bound HDL. Two ml of 0.5% trypsin in 0.54 mM Versene buffer (pH 6.4) was added to each cell suspension incubated with HDL and incubated at 37°C for 3–4 min. The trypsin was inhibited by adding 1 ml of 5% FCS in 0.9% saline, pH 7.4, and the cells were respun at 800 *g* for 2 min. The supernatant, which contained trypsin-releasable lipoprotein, was radioassayed to determine binding as described previously (26). In order to determine binding of ¹²⁵I-labeled LDL, cells were incubated with heparin as described previously (27). This method releases surface bound LDL. The cell pellets obtained after removal of trypsin or heparin-releasable radioactivity were washed once with PBS and the pellet was radioassayed for determination of internalized lipoprotein. The supernatant from various centrifugations was radioassayed to determine the efficiency of the washing procedure. This showed that less than 0.5% of the bound radioactivity was present in the final supernatant. The cell pellet was finally dissolved in 1 ml of 0.1 M NaOH and kept at 37°C overnight. Aliquots were removed for protein determination (25) and radioassay.

Degradation

At the end of the incubation with ¹²⁵I-labeled lipoprotein, the medium was removed and an aliquot was assayed for total radioactivity. A portion of the remaining medium was treated as follows to determine the amount of degraded material released from the cells into the medium. Trichloroacetic acid was added to a final concentration of 10% and the mixture was centrifuged at 6000 *g* for 10 min. A portion of the supernatant was treated to remove free iodine by oxidation with H₂O₂ and extraction of I₂ into chloroform (27). Thus medium degradation refers to noniodide, TCA-soluble radioactivity. Additional no-cell control incubations were performed at similar concentrations of labeled lipoproteins in the absence of cells, to determine the extent of substrate degradation in medium alone.

RESULTS

A typical preparation of intestinal mucosal cells, dispersed with hyaluronidase, is illustrated in **Fig. 2** which shows the morphology of single and occasional doublet cells. The cells were reasonably homogeneous in both size and shape. Observations before and after incubations indicated that cells remained viable and morphologically unchanged during this period. Initial experiments were carried out to determine the nature of the time course of interaction between ¹²⁵I-labeled human

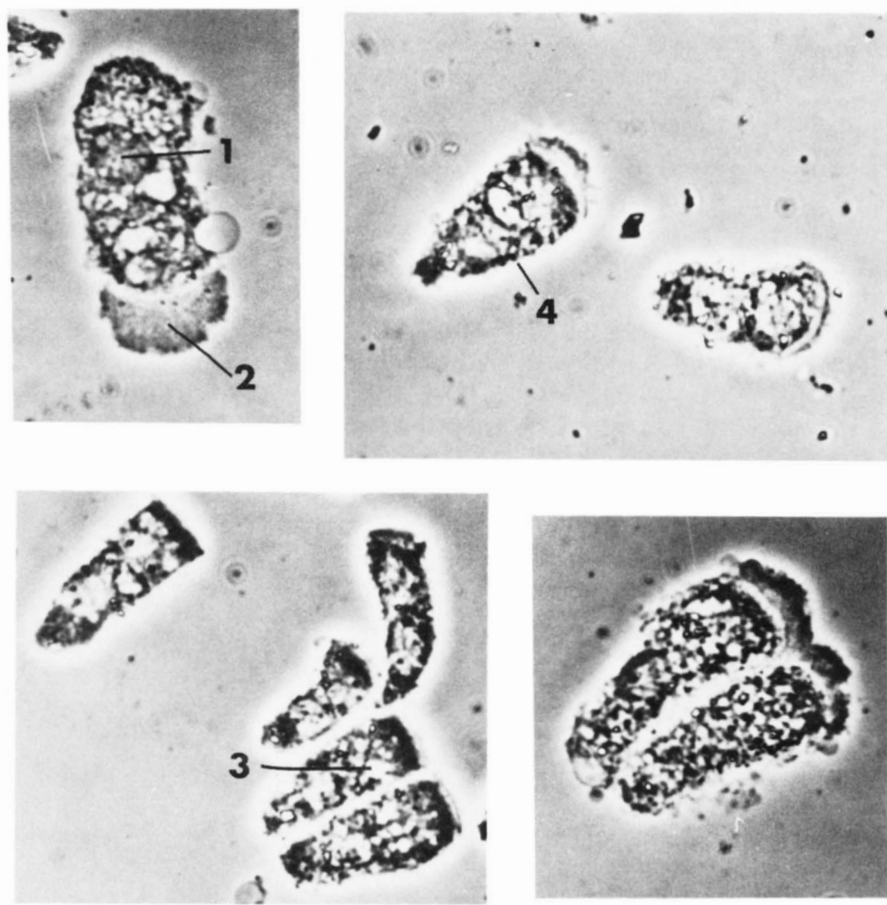


Fig. 2. Illustration of a typical preparation of rat intestinal mucosal cells after dispersion with hyaluronidase as described in the text. Cells were tested for viability and metabolic activity, also described in text. 1, Nucleus; 2, brush border membrane; 3, lipid droplet; 4, lateral membrane. Actual incorporation of [14 C]leucine into TCA-precipitable cell-associated protein was at 20 min, 280 ± 45 ; 40 min, 410 ± 28 ; 1 hr, 710 ± 45 ; 2 hr, 1598 ± 93 ; 3 hr, 2020 ± 150 cpm per mg cell protein.

LDL and intestinal mucosal cells. Cells were incubated at 4°C and 37°C with LDL for varying periods ranging from 5 min to 8 hr and the results are shown in **Fig. 3**. Since at 4°C internalization and degradation are assumed to be minimal, the data predominantly represent cell surface binding. Binding rapidly reached plateau values at about 1 hr. At 37°C total cell-associated LDL (which comprises both surface binding and internalization) rose linearly for about 8 hr.

Fig. 4 compares the saturability of binding at 4°C of human LDL and HDL with rat LDL and HDL. A precise determination of saturation was made using double reciprocal plots of specific binding curves shown in **Fig. 4**. The calculated half-saturation values were 30, 35, 35, and 15 $\mu\text{g}/\text{ml}$ for human LDL, rat LDL, human HDL, and rat HDL, respectively. To compare the characteristics of binding, we calculated the dissociation constants from Scatchard plots that were 2.9×10^{-8} M, 15.8×10^{-8} M, 3.09×10^{-8} M, and 8.9×10^{-8} M for

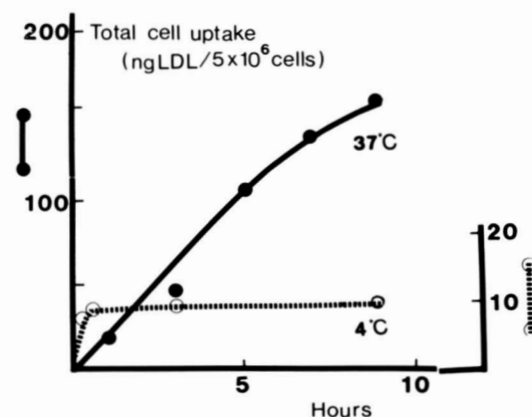


Fig. 3. Time course for binding at 4°C and 37°C of human ^{125}I -labeled LDL ($5 \mu\text{g}/\text{ml}$ medium) by suspensions of rat intestinal mucosal cells (5×10^6 per ml medium). Data (that is representative of three separate experiments) is total cell-associated radioactivity (μg LDL protein/ 5×10^6 cells, calculated from specific activity of LDL) after subtraction of nonspecific binding which was carried out in the presence of excess ($500 \mu\text{g}$) unlabeled LDL. Note different scales for 4°C and 37°C plots.

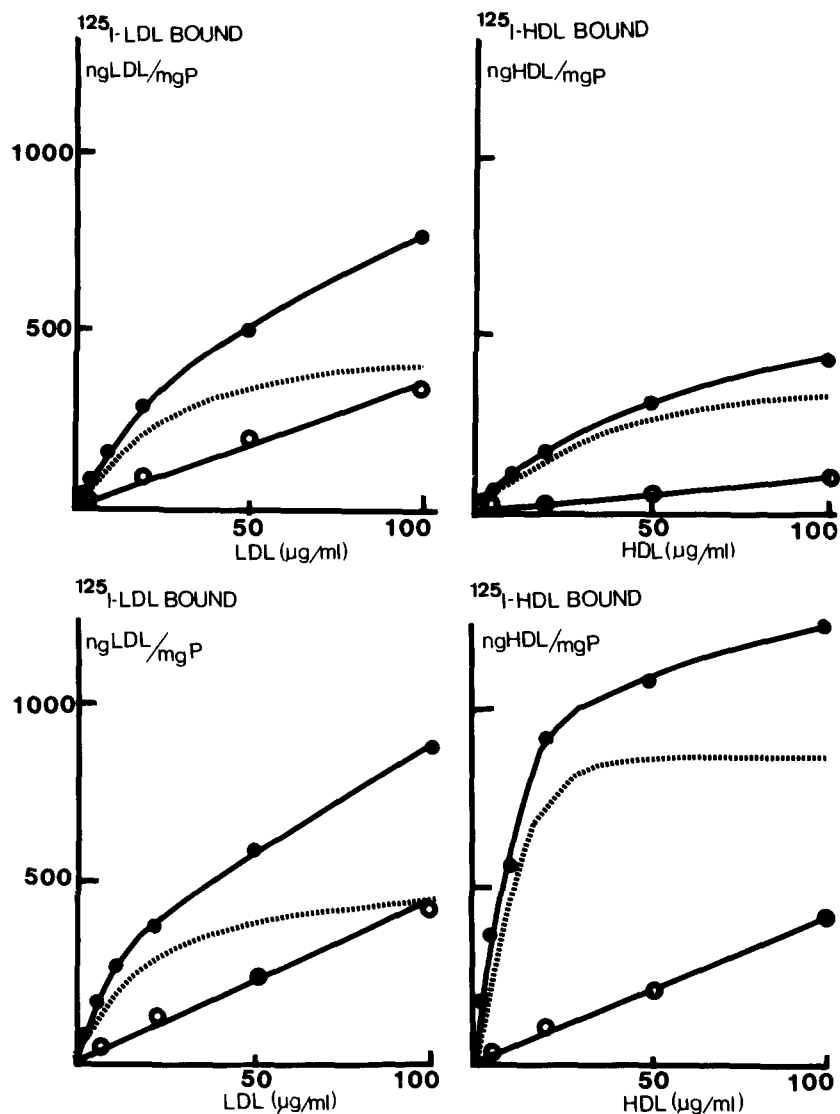


Fig. 4. Saturation data for human (top panels) and rat (bottom panels) lipoprotein binding to rat intestinal mucosal cells. ●—● represents total binding; ○—○ represents binding data in presence of 500 µg excess unlabeled lipoprotein (nonspecific binding); and the dotted line shows specific binding (total minus nonspecific). Each point represents the mean data of duplicate experiments, except points of nonspecific binding (single experiment). The binding characteristics of each (four) lipoprotein class were compared using the same batch of cells. This experiment was repeated three times with similar results.

human LDL (mol wt, 2.5×10^6 ; 20% protein), human HDL₃ (mol wt, 1.7×10^5 ; 50% protein), rat LDL (mol wt, 2.5×10^6 ; 20% protein), and rat HDL (mol wt, 6×10^5 ; 50% protein), respectively. Thus human LDL and rat LDL showed similar binding affinities, but rat HDL showed a twofold higher affinity than human HDL. About half of total bound LDL of both human and rat derivation appeared to be nonspecifically bound. By contrast, less than 20% of rat or human HDL binding was nonspecific at the saturating concentration.

In order to determine whether lipoproteins bind to the brush border membrane of villus cells with char-

acteristics that may give false subsequent interpretations of interaction with expected physiological sites, e.g., lateral or basal membrane receptors, we investigated the binding of human LDL and HDL₃ to intestinal brush border membranes. As seen in **Fig. 5**, the binding of both lipoproteins is characterized by a highly nonspecific, nonsaturable process that does not resemble the interaction with whole intestinal cells. In addition, the brush border membrane has been concentrated several-fold higher than that present in the usual cell preparation, so the amount of total lipoprotein bound to brush border membrane in whole cell preparations is

much lower than that which is shown for the pure brush border alone.

In order to determine if human LDL and HDL₃ were degraded by rat intestinal cells, ¹²⁵I-labeled lipoproteins were incubated for 3 hr at 37°C in the presence and absence of excess unlabeled LDL or HDL. Concentration of labeled lipoproteins was increased in individual incubation tubes from 5 μg to 100 μg/ml. The data in Fig. 6A and B show that the accumulation of degradation products in the medium increased rapidly and linearly at concentrations between 2–50 μg ¹²⁵I-labeled lipoprotein/ml; while in the presence of excess unlabeled lipoprotein, substantially lesser amounts of both lipoproteins were degraded reflecting internalization of lipoprotein by nonspecific processes. The dotted line represents degradation attributable to specific binding alone (total minus nonspecific values). When lipoproteins were incubated with “conditioned medium” (medium obtained after 3 hr incubation with cells and subsequent removal of cells), only a small proportion (less than 5%) of lipoprotein was degraded compared with incubations carried out in the presence of cells.

That degradation was associated with internalization of the HDL₃ particle is suggested by the data presented in Fig. 7. When increasing concentrations of the lysosomal inhibitor chloroquine were added to the medium, inhibition of degradation was associated with an increase in cell-associated label, most of which was resistant to trypsin treatment. These observations suggest that internalization of HDL₃ is required before degradation by lysosomes can occur. Binding was not inhibited by chloroquine. In other experiments at 37°C (not shown), internalization paralleled and exceeded the amount of HDL₃ bound by mucosal cells.

Competitive displacement data are described in Fig. 8. ¹²⁵I-Labeled rat LDL binding was displaced by both rat LDL and HDL (Fig. 8A). Fifty percent displacement occurred at about 25 μg/ml of rat HDL and 50 μg/ml of rat LDL. Much stronger and more specific competition was evident for rat HDL; 20 μg/ml of unlabeled rat HDL displaced 50% of ¹²⁵I-labeled rat HDL (Fig. 8B). There was much less displacement by rat LDL with only 50% displacement of ¹²⁵I-labeled rat HDL in the presence of 100 μg/ml of unlabeled rat LDL.

¹²⁵I-Labeled human LDL was similarly displaced by unlabeled human LDL and HDL₃ (Fig. 8C); 50% displacement occurred in the presence of 50 μg/ml of the unlabeled lipoproteins. However ¹²⁵I-labeled human HDL₃ binding was not displaced by human LDL (Fig. 8D), whereas 30 μg of unlabeled human HDL₃ displaced approximately 50% of bound ¹²⁵I-labeled human HDL₃.

These data strongly suggest that human HDL₃ was bound by a specific lipoprotein binding site unrelated to that for LDL, whereas rat HDL binding sites appear

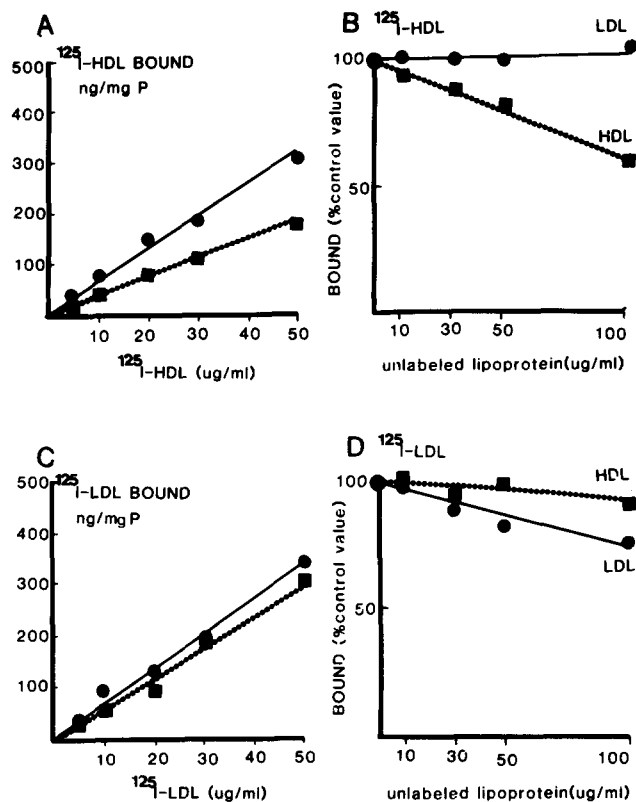


Fig. 5. Saturation data for human HDL (A) and human LDL (C) binding to rat intestinal brush border membranes and competitive displacement of HDL (B) and LDL (D) when incubated with brush border membranes and unlabeled lipoproteins. Approximately 200 μg and 120 μg of brush border membrane were used in experiments A and B and C and D, respectively. ¹²⁵I-Labeled HDL or LDL were incubated in the absence (●—●) or presence (■—■) of each corresponding unlabeled lipoprotein at 4°C for 3 hr (A and C). In B and D panels, ● and ■ refer to unlabeled LDL and HDL, respectively. Each point represents mean data of duplicate experiments. Mean 100% of control values were 73 and 110 ng lipoprotein bound per mg protein for B and D, respectively.

to be partly shared by rat LDL. When rat HDL and human HDL₃ were compared in cross-competition experiments (Fig. 9), ¹²⁵I-labeled human HDL₃ binding was displaced similarly by both human and rat HDL. On the other hand, ¹²⁵I-labeled rat HDL binding was displaced by cold human HDL₃ to a lesser extent than by rat HDL. These observations suggest that rat and human HDL₃ possibly shared a common binding site, but that some additional binding sites were involved in the interaction between intestinal cells and rat HDL. This situation might possibly explain the moderate cross-competition displayed between unlabeled rat LDL and ¹²⁵I-labeled HDL shown in Fig. 8B. The reason for enhanced binding upon addition of low concentrations of LDL (Fig. 8D) is not known, but suggests cooperative binding caused by either lipoprotein-lipoprotein or cell surface-lipoprotein interaction.

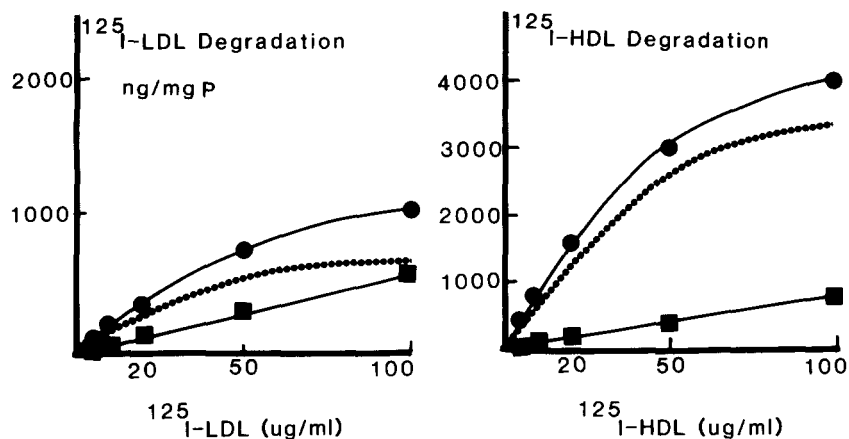


Fig. 6. Degradation of ^{125}I -labeled human LDL (left) and ^{125}I -labeled human HDL (right) by rat intestinal cells. Increasing amounts of labeled ^{125}I -labeled LDL and HDL were added to incubation flasks containing no unlabeled (\bullet — \bullet) or $500\ \mu\text{g}/\text{ml}$ excess unlabeled lipoproteins (\blacksquare — \blacksquare). Dotted line represents specific degradation (total minus nonspecific degradation). Points represent mean of duplicate incubations and similar data was obtained in three experiments. Nonspecific degradation was a single experiment. All data points were obtained after subtraction of corresponding no-cell control incubations (see text).

In order to confirm the indirect observation that rat HDL binds to an additional site that specifically recognizes human HDL₃, we prepared a rat HDL subfraction devoid of E apoprotein and rich in A peptides similar to human HDL₃. The apoprotein composition of three subfractions obtained from heparin-Sepharose columns (Fig. 10A) is shown in Fig. 1B. Fraction A (R-HDL_A) contains mainly apoA-I, apoA-IV, and C apoproteins but no E. After iodination, R-HDL_A was added to intestinal cells in increasing concentrations and, as shown in Fig. 10B, bound to cells with specific binding reaching saturable levels between 80–100 μg of protein.

Specificity of binding was investigated in competition experiments and the results are shown in Fig. 10C and D. Labeled R-HDL_A was displaced similarly by unlabeled R-HDL_A and R-HDL, but minimally by human LDL. When whole iodinated R-HDL was incubated with cells, ^{125}I -labeled R-HDL was displaced more effectively by E- or B-containing lipoprotein, R-HDL, and H-LDL than by R-HDL_A confirming the involvement of E peptide in R-HDL binding with intestinal cells.

DISCUSSION

Radioiodinated LDL and HDL were bound, internalized, and degraded by rat intestinal mucosal cells in a concentration-dependent manner that appeared to be saturable processes with saturation occurring at different concentrations for the various lipoproteins studied (Figs. 4 and 6). According to calculated dissociation constants (K_d), rat LDL and human LDL bound to the intestinal cell with the same affinity, suggesting that rat intestinal cell might not distinguish between human and rat LDL. Innerarity, Pitas, and Mahley (14) and Drevon et al. (13) found species differences in lipoprotein receptors between rat and human fibroblasts, which suggested that rat fibroblast surface receptors interact with rat LDL but not with human LDL. However, our data suggest that this is not so for rat intestinal cells, which bound both rat and human LDL with similar affinity. Despite the failure of rat fibroblasts and rat liver to metabolize human LDL in vitro (13, 28), it is interesting that both human and rat LDL are catabolized at similar rates in vivo (28). Our present studies suggest either that the rat intestine is an organ responsible for de-

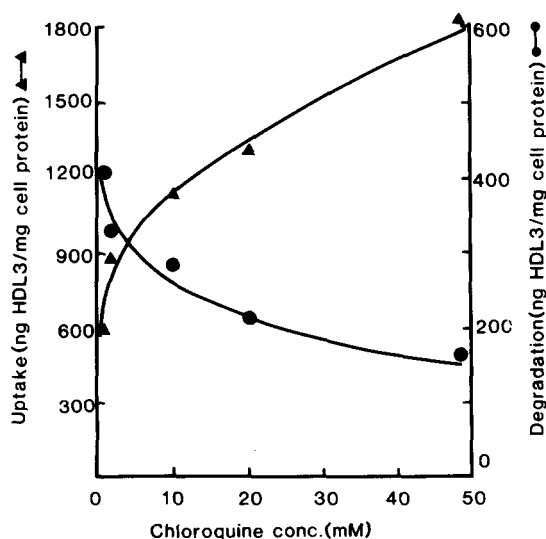


Fig. 7. The effect of chloroquine on uptake and degradation of human HDL₃ by rat intestinal mucosal cells. Cells were incubated with $5\ \mu\text{g}$ of ^{125}I -labeled HDL₃ for 3 hr and cell-associated and degraded material was determined as described in Methods.

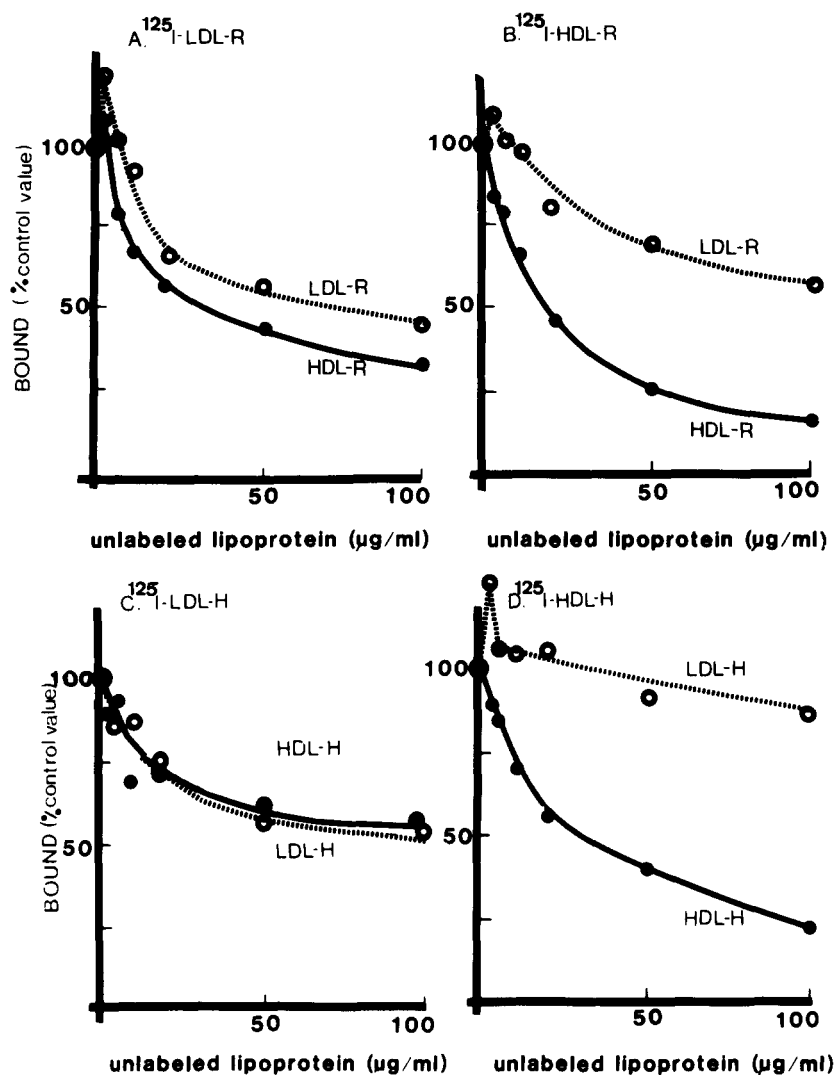


Fig. 8. Competitive displacement of ^{125}I -labeled rat (top panels) lipoproteins by unlabeled rat lipoproteins and of ^{125}I -labeled human (bottom panels) lipoproteins by unlabeled human lipoproteins. Experiments were carried out at 4°C for 3 hr with $5\ \mu\text{g}$ of labeled HDL and $8\ \mu\text{g}$ of LDL. Each point represents mean data of duplicate incubations. Mean 100% control values were 265, 330, 298, and 237 ng lipoprotein bound per mg cell protein for A, B, C, and D, respectively.

grading substantial amounts of LDL or that several additional rat tissues do not distinguish between rat and human LDL.

The presence of the brush border membranes on intestinal cells may have contributed to the interaction between cells and lipoproteins, perhaps providing spurious information regarding specificity of the binding. The data shown in Fig. 5 exclude this possibility. The saturation kinetic (Figs. 5A and C) and competitive study (Figs. 5B and D) showed that the binding of human HDL₃ and LDL was characterized by highly non-specific and nonsaturable processes that do not resemble the interaction with whole intestinal cells. This observation implies that HDL and LDL interact with intestinal cell plasma membranes and not with brush border

membranes, at least under our experimental conditions.

Competitive displacement data (Fig. 6) demonstrated the degree of specificity of binding of individual lipoproteins and additionally provided further information about the lipoprotein species specific component involved in the interaction. Both human and rat LDL were displaced, not only by excess LDL but at least as effectively by excess HDL of their own species. However, HDL of both species was strongly displaced by corresponding excess lipoprotein and, in the case of human HDL₃, only minor displacement was observed by adding cold LDL.

These data suggest the presence of different lipoprotein binding sites on rat mucosal cells and it is interesting to speculate about the possible nature of these inter-

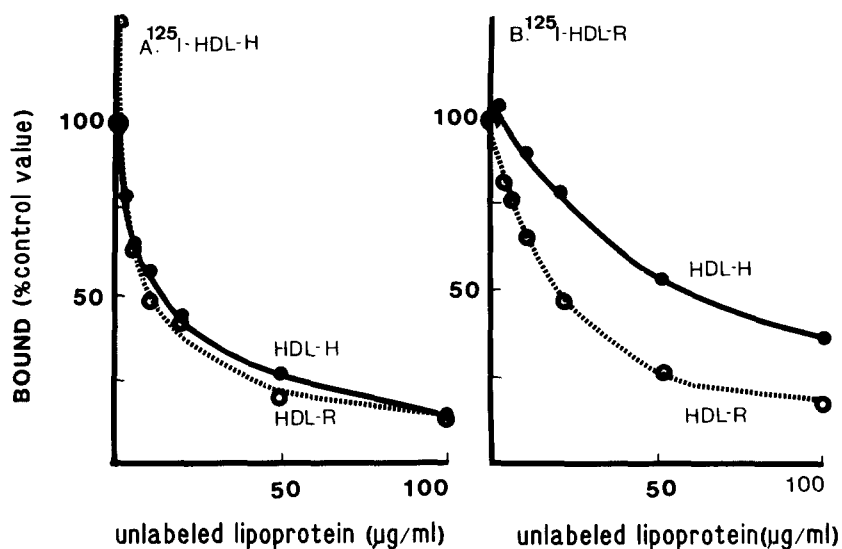


Fig. 9. Cross-competition experiments with human and rat HDL. Data on the left show displacement of ^{125}I -labeled human HDL with human (●—●) and rat (○—○) HDL. Right panel shows displacement of ^{125}I -labeled rat HDL with human and rat HDL. Each point represents mean data of duplicate experiments. Mean 100% control values were 105 and 260 ng lipoprotein bound per mg cell protein for A and B, respectively.

actions. The different displacement characteristics displayed by HDL of the two species point to the involvement of at least two binding interactions. Human HDL₃, which was shown to contain no detectable E apoprotein by gel electrophoresis (Fig. 1), strongly displaced labeled HDL₃ whereas human LDL had little effect. This shows that the receptor pathway of LDL is not involved in this association and therefore an alternative apoprotein of HDL₃, such as one of the A peptides, may participate in the interaction. However, since partial displacement of ^{125}I -labeled rat LDL occurred (which contained B and E but no A apoproteins (Fig. 1)), it is likely that rat HDL binding may also involve the E binding site.

Heparin affinity column-subfractionated R-HDL_A (devoid of E peptides) in fact bound to specific and saturable binding sites of the cell (Fig. 9). R-HDL_A binding was displaced similarly by unlabeled R-HDL_A and R-HDL but minimally by human LDL, suggesting similar characteristics between rat HDL_A and human HDL₃ binding. Unlabeled HDL₃ also displaced R-HDL_A (data not shown). These observations also suggested that rat HDL shared two binding sites, one for lipoproteins that include apoE and/or apoB, the other for lipoproteins that include A apoproteins without apoB and E. Although LDL binding to intestinal cells was characterized by a large nonspecific component and lacked lipoprotein class specificity, the amount of LDL bound, internalized, and degraded was substantial. It is interesting that substantial LDL binding to human adipocytes also occurs through nonspecific processes (29). It is therefore possible that in tissues which functionally store chole-

sterol, such as the gut and adipose tissue, LDL uptake is regulated by mechanisms that are less specific than through the receptor system described in other cells, e.g., fibroblasts (1).

Binding of HDL at specific sites has now been demonstrated in many tissues. Wu, Butler, and Bailey (30) found evidence of HDL binding in normal and virus-transformed human living fibroblasts. Miller et al. (31) found an interaction between HDL and LDL during uptake of labeled lipoproteins by fibroblasts and suggested that HDL was mostly bound to sites other than the high affinity receptor of LDL, although their preparation of HDL may have contained apoE. Several examples of specific binding of HDL have been reported recently for cells derived from steroidogenic tissue and the liver. Kovanen et al. (6) demonstrated that mouse adrenal gland binds LDL and HDL by two distinct mechanisms, while Gwynne and Hess (32) found evidence of a specific saturable and reversible binding site for human HDL on rat adrenocortical cells. The presence of HDL binding sites on rat ovarian tissue has also been reported (33) and Chen, Kraemer, and Reaven (7) recently identified specific HDL binding in rat testis that was regulated by gonadotropin. Although the authors (7) were unable to demonstrate high affinity, saturable binding of HDL to other nonsteroidogenic tissues, their investigations did not include a study of HDL with intestinal cells. The liver plays an important role in lipoprotein catabolism and Ose et al. (34) recently reported the uptake and degradation of ^{125}I -labeled HDL by rat liver cells that appeared to proceed via a saturable high affinity binding process. Van Berkel et

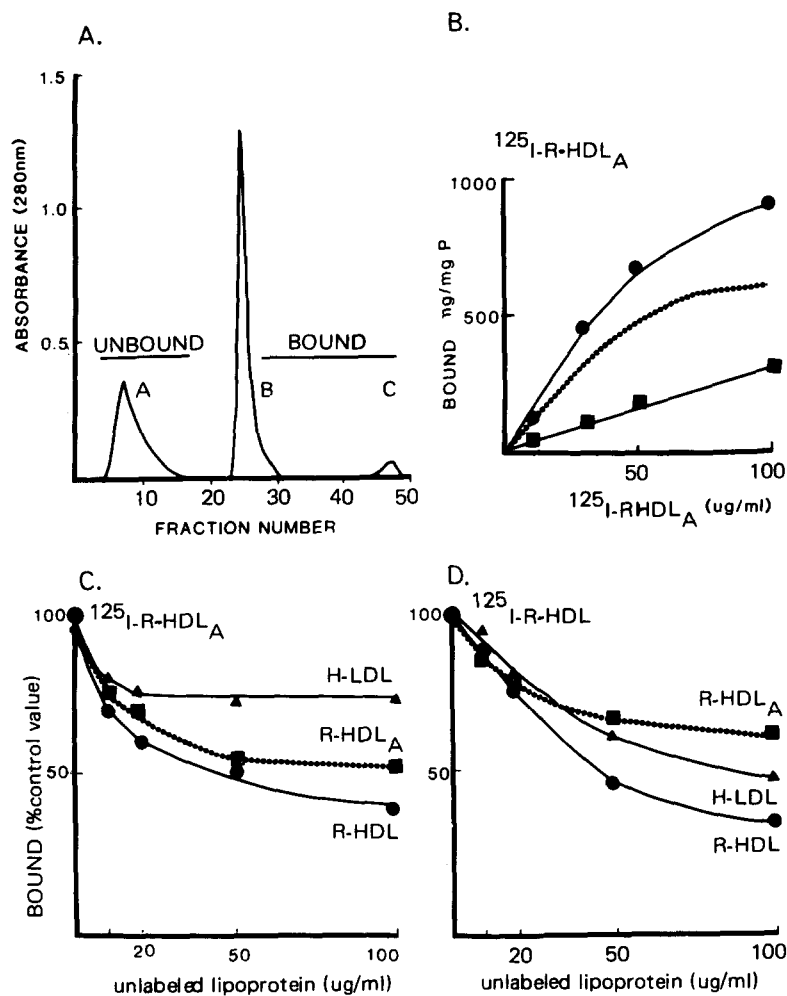


Fig. 10. A: Rat HDL subfractionation by heparin-Sepharose affinity column chromatography. Eight mg of rat HDL was applied to the column. Protein content was 43% for unbound fraction A (R-HDL_A), 46% for bound fraction B, and 11% for bound fraction C. B: Saturation data for ^{125}I -labeled R-HDL_A binding to intestinal cells. (●—●) represents total binding; (■—■) represents binding data in the presence of 500 $\mu\text{g}/\text{ml}$ excess unlabeled R-HDL (nonspecific binding); dotted line shows specific binding (total minus nonspecific). C and D: Cross-competition experiments with R-HDL_A and R-HDL. Data show displacement of ^{125}I -labeled R-HDL_A (5 $\mu\text{g}/\text{ml}$) and ^{125}I -labeled R-HDL (5 $\mu\text{g}/\text{ml}$) by unlabeled human LDL (▲—▲), R-HDL (●—●), and R-HDL_A (■—■). Each point represents the mean data of duplicate incubations and similar results were obtained in three separate experiments. Mean 100% control values were 180 and 310 ng lipoprotein bound per mg cell protein for C and D, respectively.

al. (10) found similar mechanisms operating in both isolated parenchymal and nonparenchymal cells from rat liver that displayed saturable, high affinity binding of LDL and HDL. This recent evidence suggests that several organs in the rat possess HDL binding sites in addition to LDL receptors. In this context it is important to realize that HDL in the rat, unlike the human, is the major lipoprotein responsible for plasma cholesterol transport and metabolically may carry out different functions than HDL in human subjects. It is therefore possible that the binding and subsequent metabolism of HDL by intestinal cells is also receptor-mediated. Since human HDL₃ also bound to rat intestinal cells by a pro-

cess analogous to that of rat HDL, it is possible that the human intestine also metabolizes HDL through receptor-like regulation. The intestine may therefore play a significant role in lipoprotein metabolism that may be related to cholesterol transport and apolipoprotein secretion. ■■

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