Specific binding of high density lipoprotein (HDL_3) is not related to sterol synthesis in rat intestinal mucosa

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Abstract There is good evidence that high density lipoprotein (HDL) is involved in the flux of cholesterol into the cells of some organs and out of the cells of other tissues. Because we have previously found that HDL is bound specifically by mucosal cells of the small intestine, we have examined the possibility that this was associated with regulation of cholesterol flux. We have, therefore, compared the specific binding of ^{125}I-labeled HDL_3 with cholesterol synthesis in mucosal cells obtained from rats that had been treated to alter intestinal cholesterol metabolism. The rate of sterol synthesis measured in tissue slices, by the incorporation of [^{3}H]water into sterols, was altered up to fivefold by treatment with cholestyramine (to induce bile salt loss), by surformer treatment (to reduce absorption of cholesterol), and by biliary diversion. Yet the capacity of mucosal cells to bind, internalize, and degrade ^{125}I-labeled HDL_3 was unchanged. Cholesterol feeding influenced neither the interaction of ^{125}I-labeled HDL_3 with cells nor the rate of sterol synthesis. Furthermore, the interactions of ^{125}I-labeled HDL_3 with mucosal cells isolated from the proximal and distal halves of the intestine or between the upper and lower villus cells were similar, despite differences in sterol synthesis. These data suggest that, in rat intestine, the specific binding of HDL is not related to sterol synthesis. However, since the cellular cholesterol level was also unchanged by the above manipulations, through appropriate increases in synthesis, it does not exclude the possibility that HDL binding might be affected by changes in cholesterol concentration. —**Kagami, A., N. H. Fidge, and P. J. Nestel.** Specific binding of high density lipoprotein (HDL_3) is not related to sterol synthesis in rat intestinal mucosa. *J. Lipid Res.* 1985. **26:** 705–712.

Supplementary key words proximal intestine • distal intestine • villus cells • cholestyramine • surformer • bile diversion

The presence of specific binding sites for HDL devoid of apolipoprotein E have now been demonstrated in cultured human fibroblasts and arterial smooth muscle cells (1), human lymphoblastoid cells (2), liver (3), and steroidogenic tissues (4, 5) from several laboratories. However, the physiological role associated with HDL binding may differ, with some tissues (such as steroidogenic tissues) accepting cholesterol and others donating excess cholesterol for eventual excretion from the body.

Recent studies from our laboratory have shown that rat intestinal mucosal cells possess high affinity HDL binding sites (6), which differ from LDL binding sites (B/E receptor) and are located only on basolateral plasma membranes (7). The intestine is an important organ with respect to formation and secretion of lipoproteins, although there are quantitative differences among species. In the rat, the intestine is responsible for approximately 19% of synthesized apolipoprotein (8). It is possible, therefore, that the metabolism of a specific lipoprotein is closely linked to sterol synthesis (9). To determine whether the specific interaction between HDL and mucosal cells is involved in regulating intestinal sterol metabolism, studies were carried out to measure HDL binding by mucosal cells obtained from rats treated by various dietary and drug treatments or by surgical manipulations that affected sterol synthesis. Because the morphology and function of the intestine varies lengthwise and crosswise, cells from the proximal and distal segments as well as from the upper and lower villus portions were obtained to compare HDL binding and sterol synthesis.

METHODS

Experimental animals

Male Sprague-Dawley rats, with initial body weights of 140–160 g, were fed ad libitum with ground commercial rat chow, or with ground chow containing added 2% (w/w) cholesterol, 2% (w/w) cholestyramine, or 2% (w/w) surfomer for 14 days. The animals were studied in the non-fasting state and, to exclude diurnal changes of sterol and bile acid synthesis, each experiment was begun at a constant time.

Abbreviations: HDL, high density lipoproteins; BME, Eagle's basal medium; DPS, digitonin-precipitable sterols.

Under light ether anesthesia, biliary diversion was performed by ligating the common bile duct near the duodenum and cannulating a polyethylene tube (O.D. 0.96 mm, I.D. 0.58 mm) into the duct. The abdomen was closed and the rat was placed in a restraining cage. Sham-operated rats underwent an abdominal incision. All animals were allowed 5% glucose in 0.45% saline and commercial food pellets ad libitum and were studied 16–20 hr after the surgery.

Isolation and radioiodination of human and rat lipoproteins

Human HDL_3 (d 1.12–1.21 g/ml) and rat HDL (d 1.07–1.12 g/ml) were prepared as described previously (7). Lipoproteins were fractionated by sequential flotation according to the method of Havel, Eder, and Bragdon (10). All lipoproteins were recentrifuged at the appropriate higher density, and were homogeneous by agarose gel electrophoresis.

Since apolipoprotein E binds to the B/E receptor in the intestinal mucosal cells, we checked the apolipoprotein composition of human HDL_3 devoid of apolipoprotein E as well as rat HDL, which contained apolipoprotein E, by 15% SDS-polyacrylamide gel electrophoresis (11).

Lipoproteins were iodinated with ^{125}I by the iodine monochloride method modified for lipoproteins (12) as described previously (13).

Preparation of intestinal mucosal cells

The small intestine was divided into proximal and distal portions and 10 cm of proximal duodenum was discarded. These were cut into segments (20 cm) that were then rinsed extensively with ice-cold 0.9% saline to wash out luminal contents. Mucosal cells were isolated by the citrate-EDTA chelation technique as described by Stange and Dietschy (14) with some modifications. Briefly, the segments were tied at one end and filled with gassed buffer A (95% O_2:5% CO_2) containing KCl (1.5 mM), NaCl (96 mM), sodium citrate (27 mM), KH_2PO_4 (8 mM), Na_2HPO_4 (5.6 mM), glucose (11.1 mM), dithiothreitol (1 mM), and gentamycin (10 μg/ml), pH 7.3. The ends were closed and the segment was incubated at 37°C in a shaking water bath at 80 oscillations per min for 15 min. The buffer was then decanted and the segments were distended with gassed buffer B containing EDTA (1.5 mM), plus KCl (2.7 mM), NaCl (137 mM), KH_2PO_4 (1.5 mM), Na_2HPO_4 (4.3 mM), glucose (11.1 mM), dithiothreitol (1 mM), and gentamycin (10 μg/ml), pH 7.2, and incubated at 37°C for a further 60 min.

To separate upper villus cells, incubation with buffer B was carried out for 40 min and a further 40-min incubation was carried out to obtain lower villus cells. Alkaline phosphatase activity in buffer B was assayed as a marker of brush border membrane-rich cells. The released cells were washed with buffer A three times by centrifugation at 350 *g* and filtered through a layer of polyethylene mesh (100 μm). Aliquots were dissolved in 0.2 M NaOH for determination of protein (15) and the remaining cells were immediately suspended in culture medium (10% human LDS in Eagle's basal medium (BME) phosphate buffer, pH 7.4). The viability of cells was checked by the trypan blue (0.05%) exclusion test (16), and cells with at least 90% exclusion of dye were used for experiments.

Determination of binding, internalization, and degradation of ^{125}I-labeled HDL

Mucosal cells (0.4–0.5 mg of protein) were incubated in 1 ml of BME containing human lipoprotein-deficient serum (final concentration 10%) and the indicated concentrations of ^{125}I-labeled lipoprotein in a scintillation vial at 4°C or 37°C for 3 hr. The viability of cells remained >80%. Determination of binding, internalization, and degradation were carried out as described previously (6) with some modifications. Briefly, at the end of incubation, the cells were transferred to plastic centrifuge tubes and centrifuged at 350 *g* for 3 min. The cells were washed once with 0.2% albumin in 0.9% saline, pH 7.4, and sedimented by centrifugation. Then cells were gently transferred to another tube to minimize contamination of tubes with ^{125}I-labeled HDL_3 (and thus an overestimate of binding activity) and washed a further three times.

The cell pellets from the 4°C-incubations were radioassayed directly to determine binding of labeled lipoproteins. To determine binding following the 37°C-incubations each cell pellet was initially treated with 0.05% trypsin to release bound lipoprotein. The cell pellets were subsequently radioassayed for the determination of internalized lipoprotein. To determine cell association at 37°C, the cell pellet was radioassayed without trypsin treatment. Degradation of lipoprotein by cells was determined in the medium (6) and corrected for degradation in the absence of cells.

Specific binding, internalization, and degradation are defined as the differences between the amounts of ^{125}I-labeled lipoprotein bound, internalized, and degraded in the absence and presence of 50-times excess unlabeled lipoprotein.

Determination of sterol synthesis rates in vitro

Rats were killed and the intestines were immediately removed, chilled, and sliced. Aliquots of these slices were then incubated in Krebs-Ringer bicarbonate buffer containing 25 mCi of [^3H]water as previously described by Stange and Dietschy (14). The digitonin-precipitable sterols were isolated and assayed for ^3H content. The data are expressed as nmol of [^3H]water incorporated into digitonin-precipitable sterols per hr per g of intestine (nmol/g · hr).

Determination of enzyme and cellular cholesterol

Alkaline phosphatase activity was assayed by the method described by Forstner, Sabesin, and Isselbacher (17). Five to 10 mg of protein of homogenized mucosal cells was extracted according to the procedure of Folch et al. (18) and saponified for measurement of total cholesterol by gas–liquid chromatography (19) using 5α-cholestane as the internal standard.

Materials

Sodium (^{125}I)iodine (carrier-free) and [^{3}H]water were obtained from Amersham International Ltd. (U.K.). Cholestyramine was purchased from Bristol-Myers Company (N.S.W. Australia). Surfomer was generously supplied by the Monsanto Company (St. Louis, MO). Fetal calf serum and Eagle's basal medium were from the Commonwealth Serum Laboratories (Melbourne, Australia).

RESULTS

An initial study was undertaken to determine the time course of ^{125}I-labeled human HDL_3 binding to control rat intestinal mucosal cells at 4°C (**Fig. 1**). Mucosal cells were isolated by the chelating technique to avoid the effect of scraping on HMG-CoA reductase (20) and unknown effects of hyaluronidase. Specific binding increased rapidly during the first 0.5–1 hr and began to plateau between 2–3 hr. **Fig. 2** shows the concentration-dependent binding of ^{125}I-labeled HDL_3 to intestinal mucosal cells at 4°C which was characterized by a high affinity, saturable process with specific binding representing 80% of the total binding. From double reciprocal plots, the K_d value for ^{125}I-labeled HDL_3 was calculated at 31 μg/ml. This is similar to the results obtained when binding experiments were carried out with cells isolated by an enzymatic method (35 μg/ml) (6), but slightly lower than that found with basolateral plasma membrane (21 μg/ml) (7).

The interaction of ^{125}I-labeled HDL_3 was compared in cells in which cholesterol synthesis had been altered by various treatments. **Table 1** shows that the body weights of the treated rats did not differ significantly from those of control and sham-operated rats.

To compare the effect of each treatment on total cholesterol balance in the mucosal cells, cellular cholesterol levels were also measured. Cholestyramine (which interrupts the enterohepatic circulation (21)), surfomer (which blocks the uptake of cholesterol from intestinal lumen without effect on bile acid uptake (22)), dietary cholesterol, and biliary diversion did not result in significant alterations of cellular cholesterol content (Table 1).

The rates of sterol synthesis were determined by measuring the rates of incorporation of [^{3}H]water into DPS by intestinal slices incubated in Krebs bicarbonate buffer

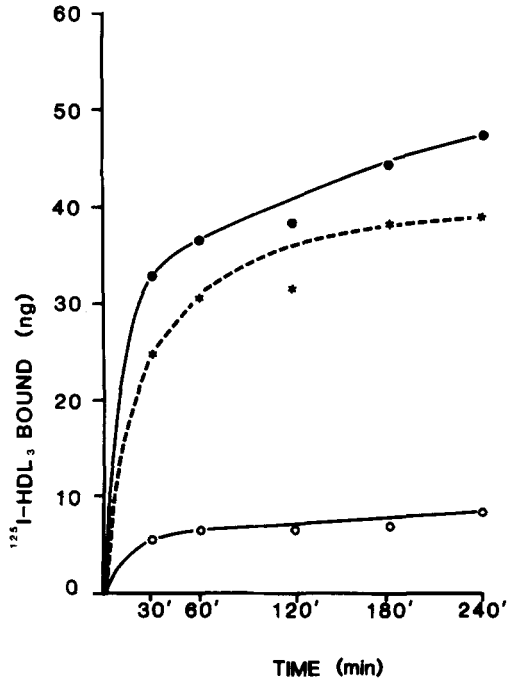

Fig. 1. The time-course of ^{125}I-labeled human HDL_3 binding to rat mucosal cells. Incubation was carried out with 20 μg/ml of ^{125}I-labeled HDL_3 at 4°C for 3 hr in the absence (●—●) and presence (○–○) of 50–100 times excess unlabeled HDL_3. Specific binding (•–•) was obtained by subtracting the ^{125}I-labeled HDL_3 bound in the presence of excess unlabeled HDL_3 from that bound in the absence of unlabeled HDL_3. Each point represents mean data of duplicate incubations.

(**Fig. 3**). In the control group, the rate of sterol synthesis in the distal intestine was about twice that in the proximal intestine. This regional difference in the rate of sterol

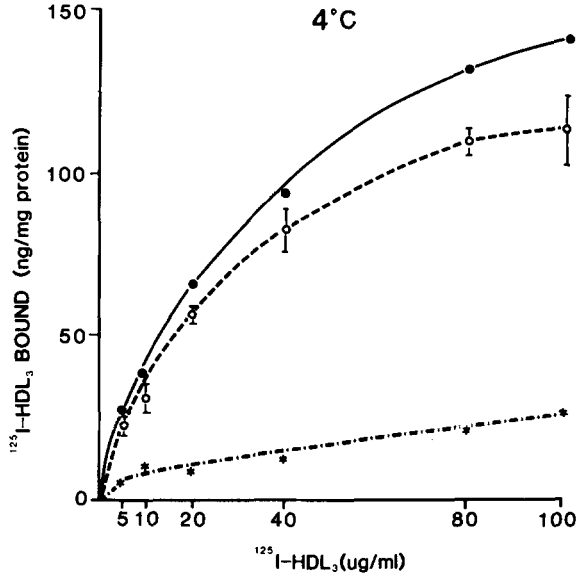

Fig. 2. Binding of ^{125}I-labeled human HDL_3 to rat mucosal cells (0.5 mg) at 4°C for 3 hr (total (●—●), nonspecific (•–•), and specific binding (○--○)). The data represent the means ± SD (n = 4).

TABLE 1. Effect of cholesterol feeding, cholestyramine, or surfomer treatment and removal of bile acids on plasma and intestinal cholesterol levels

Treatment	Weight Gain[a]	Plasma (Total) Cholesterol[a]	Cellular Cholesterol[b]
	g ± SD	*mg/dl ± SD*	*μg/mg protein ± SD*
Control	65 ± 6	76.2 ± 6.4	28.4 ± 2.6
Cholesterol	62 ± 8	78.5 ± 4.5	30.0 ± 5.6
Cholestyramine	61 ± 9	71.5 ± 5.2	26.1 ± 4.7
Surfomer	59 ± 7	70.3 ± 7.8	26.7 ± 3.0
Sham-operated		67.7 ± 5.0	27.6 ± 2.9
Biliary diversion		66.6 ± 4.6	26.4 ± 4.5

[a]Eight rats.
[b]Five rats.

synthesis was also evident in all the other groups.

Compared with control studies, both cholestyramine and surfomer treatment stimulated the rates of sterol synthesis in the intestine approximately two- to fivefold. However, cholesterol feeding did not significantly affect sterol synthesis in either the proximal or distal intestine (Fig. 3A). As seen in Fig. 3B, there was a three- to four-fold increase in the rate of sterol synthesis in the rats subjected to biliary diversion when compared with sham-operated rats.

We next compared the level of specific binding of ^{125}I-labeled HDL_3 at 4°C in mucosal cells obtained from the proximal and distal intestine (**Fig. 4**). In contrast to the different rates of sterol synthesis, specific binding of ^{125}I-

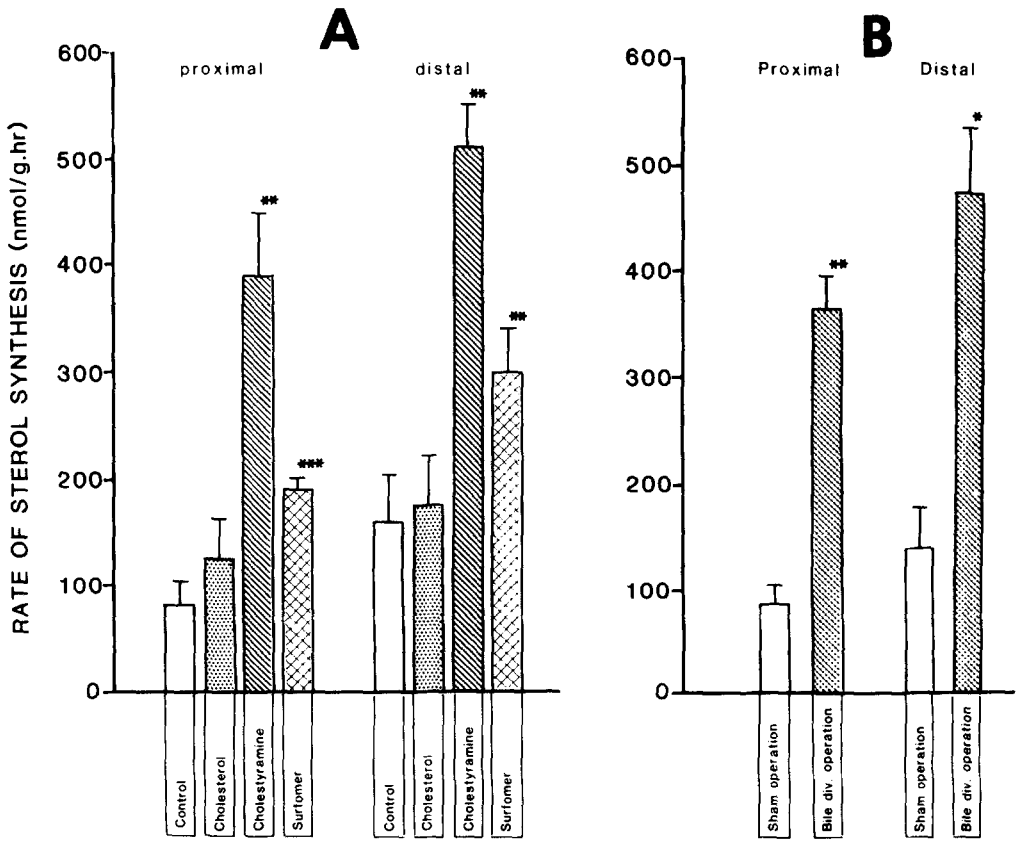

Fig. 3. The effect of feeding cholesterol, cholestyramine, and surfomer (panel A) and removal of bile acids (panel B) on the rate of sterol synthesis in whole gut slices obtained from rat proximal and distal intestine. Sterol synthesis was determined with [^{3}H]water as substrate in vitro as described in the text. The data represent the means ± SD obtained from four to six animals. The asterisk indicates that the value is significantly different from the corresponding value for control or sham-operated group. ($^*P < 0.01$, $^{**}P < 0.005$, $^{***}P < 0.001$).

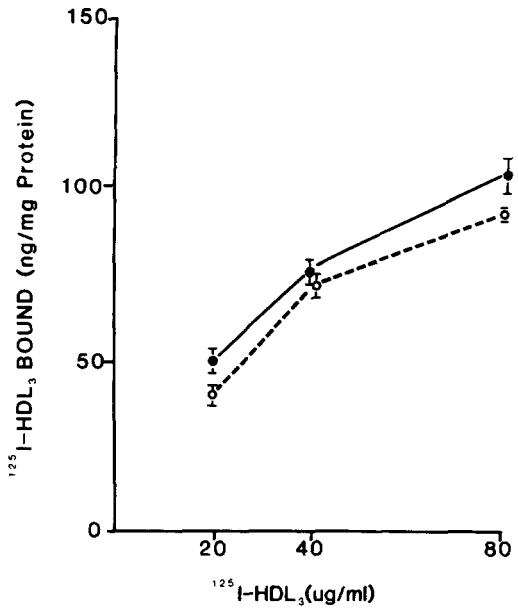

Fig. 4. Specific binding of ^{125}I-labeled human HDL$_3$ to rat mucosal cells obtained from proximal (●—●) and distal (O--O) intestine. Mucosal cells were incubated with increasing concentrations of ^{125}I-labeled human HDL$_3$ at 4°C for 3 hr. The data represent the means ± SD obtained from three different incubations.

labeled HDL$_3$ was not significantly different.

As shown in **Fig. 5,** we next examined whether the administration of cholesterol, cholestyramine, or surfomer affected the interaction of ^{125}I-labeled HDL$_3$ with mucosal cells. In spite of the opposing effects of cholesterol feeding and cholestyramine treatment on sterol synthesis, specific binding of ^{125}I-labeled HDL$_3$ at 4°C was similar to that in the control group (Fig. 5A). Following incubations at 37°C, specific cell-associated HDL$_3$ and degraded HDL$_3$ were similar in the treated and control groups (Fig. 5B, C). Specific binding, internalization, and degradation of ^{125}I-labeled HDL$_3$ at 37°C were also not significantly different in control and surfomer-treated groups (Fig. 5D, E, F). In order to avoid overestimation of degradation (expressed in cpm per mg cell protein), which might be caused by protein loss during copious washing procedures after incubation, the cell protein content was measured before incubation. As a result, the assessment of degraded ^{125}I-labeled HDL$_3$ was lower than that reported previously (6). As shown in a previous report from our laboratory (6), this method reliably measures cellular degradation alone and not that produced by contaminating enzymes (since none were

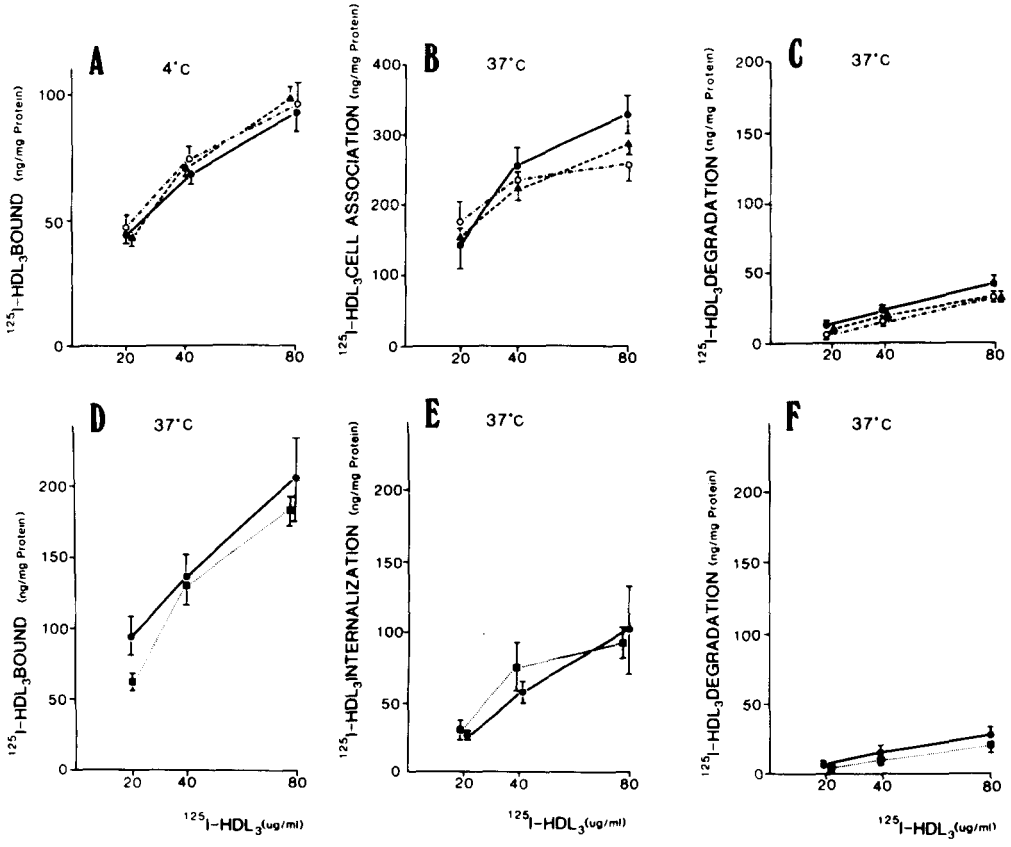

Fig. 5. The effect of feeding cholesterol, cholestyramine, and surfomer on specific binding, cell association (binding plus internalization), internalization, and degradation of ^{125}I-labeled human HDL$_3$ in isolated mucosal cells. Rats were treated as described in the Methods section and mucosal cells were incubated with increasing concentrations of ^{125}I-labeled human HDL$_3$ at 4°C or 37°C for 3 hr. Each point represents the means ± SD obtained from three different incubations. Panel A), B), C), (●—●) control; (▲---▲) cholesterol-fed; (O-·-O) cholestyramine-treated. Panel D), E), F), (●—●) control; (■····■) surfomer-treated.

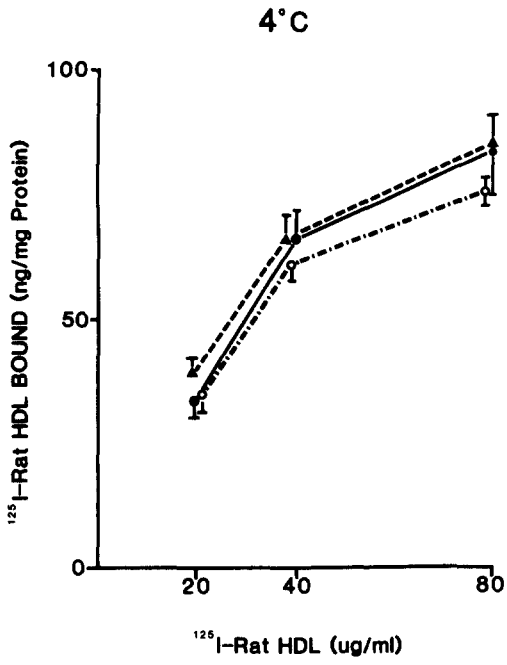

Fig. 6. Specific binding of ^{125}I-labeled rat HDL to mucosal cells isolated from control, cholesterol-fed, and cholestyramine-treated rats. Mucosal cells were incubated with increasing concentrations of ^{125}I-labeled rat HDL at 4°C for 3 hr. (●—●) Control; (▲---▲) cholesterol; (○--○) cholestyramine-treated. Each point represents the means ± SD obtained from three different incubations.

used here) or by extracellular enzymes released into the medium.

Since rat HDL, which contains apoE, may bind to both the LDL receptor and to the HDL receptor, we tested whether specific binding of ^{125}I-labeled rat HDL was affected by cholesterol feeding or cholestyramine treatment. **Fig. 6** demonstrates that the binding of ^{125}I-labeled rat HDL was similar in these groups, which suggests that neither LDL nor HDL_3 receptor activity in the mucosal cells was affected by these treatments.

Since cholestyramine and surfomer treatment produces a long term inhibitory effect on cholesterol absorption which consequently influences sterol synthesis, we next compared the acute effect on cholesterol absorption caused by biliary diversion. We isolated mucosal cells from rats that had undergone biliary diversion for about 16–20 hr which eliminates more than 80% of the total bile acid pool (23). There was, however, no change in the capacity of cells to bind ^{125}I-labeled HDL_3 (**Fig. 7**), even though this manipulation had caused a dramatic increase in the rate of intestinal sterol synthesis.

As the intestinal epithelium is composed of upper villus cells where lipid absorption takes place (24) and lower villus to crypt cells where sterol synthesis mostly occurs (14), we tested ^{125}I-labeled HDL_3 interaction to these two groups of cells (**Fig. 8**). However, specific binding, internalization, and degradation of ^{125}I-labeled HDL_3 were similar along the axis of the villus.

DISCUSSION

The present experiments suggest that HDL binding to rat intestinal cells, which has the characteristics of a specific, saturable process, is not affected by changes in cellular cholesterol synthesis. In previous studies from this laboratory, high affinity binding sites for HDL_3 were shown in rat intestinal mucosal cells isolated by scraping and dispersion with hyaluronidase (6), and in the basolateral plasma membrane of rat small intestine (7). However, the mechanism of regulation of this HDL binding site and possible receptor is not clear.

In other tissues, correlations between HDL binding and cellular events have been demonstrated. Oram, Brinton, and Bierman (25) found enhancement of HDL binding to cultured human fibroblasts and arterial smooth muscle cells that had been preloaded with cholesterol, presumably to facilitate cholesterol efflux. A previous study from this laboratory has reported increased HDL_3 receptor activity in rat adrenal cells incubated with ACTH (26). This was presumably also independent of any change in sterol synthesis which is not altered by

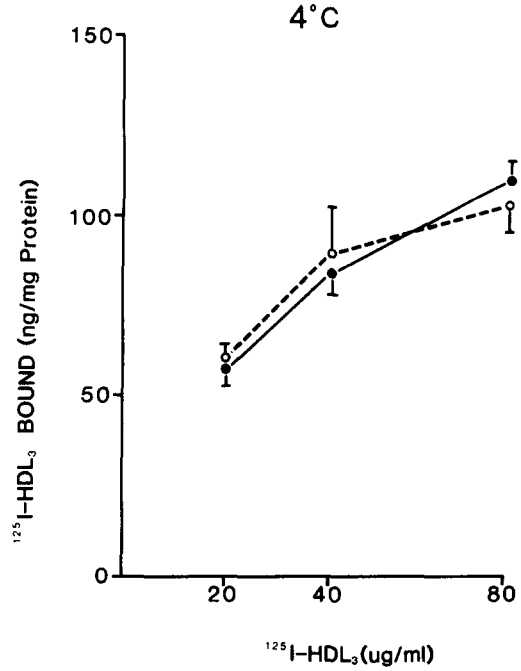

Fig. 7. The effect of biliary diversion on specific binding of ^{125}I-labeled human HDL_3 to rat mucosal cells. Mucosal cells were isolated 16–20 hr after surgery and incubated with increasing concentrations of ^{125}I-labeled human HDL_3 at 4°C for 3 hr. (●—●) Sham operated; (○--○) biliary diversion. The data represent the means ± SD obtained from three different incubations.

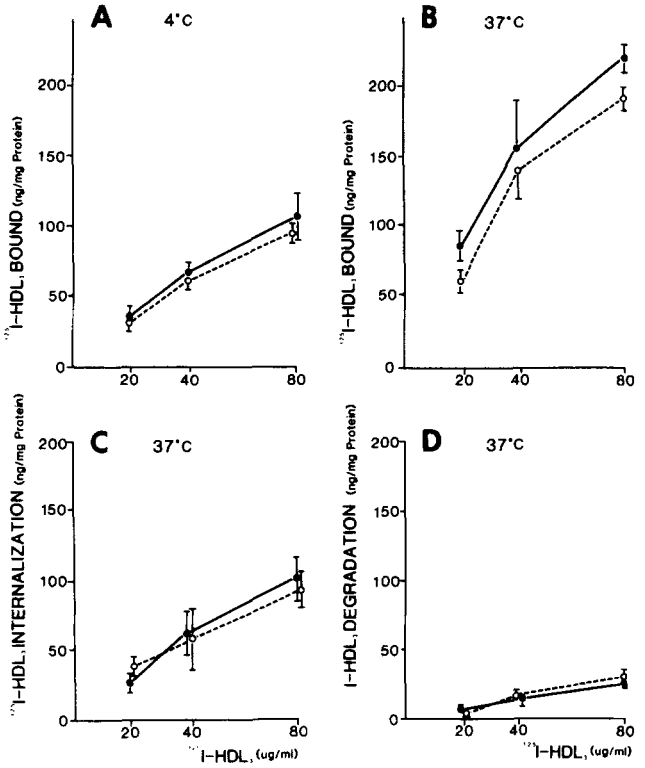

Fig. 8. Comparison of specific binding, internalization, and degradation of ^{125}I-labeled human HDL_3 in upper villus (●—●) and lower villus cells (○--○) isolated from control rat intestine. Mucosal cells were incubated with increasing concentrations of ^{125}I-labeled human HDL_3 at 4°C (A) and 37°C (B, C, D). Each point represents the means ± SD obtained from three different incubations.

ACTH administration (27). The in vivo clearance of ^{125}I-labeled HDL or ^{125}I-labeled LDL is also unaffected by changes in sterol synthesis in rat liver (28), although specific binding sites for both lipoproteins are present (29). In human lymphoblastoid cell, HMG-CoA reductase activity is stimulated by HDL_3 at and below saturation level but not at higher concentrations of HDL_3 (2). Why the relationship between HDL_3 and sterol synthesis is different in these tissues remains to be elucidated.

Since the small intestine plays an important role in cholesterol metabolism and since a major function of HDL appears to be the control of cholesterol flux both into and out of cells, we had anticipated that the activity of the HDL binding site or possible receptor would be subject to regulation by changes in sterol synthesis. This was, however, not borne out by the experiments. Twofold differences in the rates of sterol synthesis in the distal and proximal intestine and up to fivefold changes brought about by cholestyramine and surfomer treatment failed to influence the capacity of mucosal cells to bind, internalize, or degrade ^{125}I-labeled HDL_3. Biliary diversion, which has been shown to increase lymph apoA-I and HDL output by Bearnot et al. (30) as well as sterol synthesis, had no quantitative effect on the interaction of ^{125}I-labeled HDL_3 with mucosal cells. However, it should be noted that the cellular cholesterol concentration was also unaltered by these manipulations (Table 1). It is, therefore, possible that intestinal cells preferentially replete cholesterol through rapid changes in synthesis rather than through up-regulation of lipoprotein binding sites.

Recently Stange and Dietschy (22) have demonstrated that, in the rat intestine, about 60% of LDL uptake was apparently receptor (B/E receptor)-dependent. However, rat LDL uptake was also independent of changes in cholesterol synthesis within the cells. The absence of a relationship between intestinal sterol synthesis and lipoprotein binding and uptake therefore exists for rat HDL, human HDL_3, and for rat LDL.

The lack of correlation between HDL_3 receptor activity and the rate of sterol synthesis still leaves the question of the physiological significance of specific binding sites for HDL in the intestine unanswered. It is possible that alteration of other components involved in intestinal lipid transport may influence the binding of HDL to the mucosal cells. Cholesterol concentrations remained unchanged by the various treatments used in these experiments and it is conceivable that HDL binding is more likely to be influenced by sterol content than by synthesis rates, as mentioned above (25-27). A substantial fall in intestinal cholesterol concentration may result in delivery of HDL cholesterol to the intestine, particularly in the rat in which plasma sterol is transported principally within the HDL class. Reverse cholesterol transport, that is, efflux of sterol from an overloaded mucosal cells, may also occur via HDL, such as has been demonstrated with cholesterol-loaded fibroblasts (25). Manipulation of sterol content of the intestine followed by HDL binding studies may thus provide useful information regarding a physiological role for HDL in intestinal metabolism.

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