Rapid transient absorption and biliary secretion of enantiomeric cholesterol in hamsters

Emily J. Westover,†,‡ Xiaobo Lin,§ Terrence E. Riehl,‖ Lina Ma,‖ William F. Stenson,‖ Douglas F. Covey,∗ and Richard E. Ostlund, Jr.†

Department of Molecular Biology and Pharmacology,† Division of Endocrinology, Metabolism, and Lipid Research,‡ and Division of Gastroenterology,§ Department of Medicine, Washington University School of Medicine, St. Louis, MO 63110

Abstract  To probe the pathway and specificity of cholesterol absorption, the synthetic enantiomer of cholesterol (ent-cholesterol) and cholesterol were labeled with deuterium, gavaged into hamsters, and measured by negative ion mass spectrometry. Initial uptake of both tracers into the intestinal mucosa at 30 min was similar but cholesterol was temporarily retained there, whereas mucosal ent-cholesterol declined rapidly with concomitantly increased enrichment in both the systemic circulation and the gut lumen. In a 3 day fecal recovery study, ent-cholesterol was quantitatively recovered in the stool, whereas cholesterol absorption was 53.2%. ent-Cholesterol given by intracardiac injection was selectively secreted into bile, and the ratio of ent-cholesterol to cholesterol tracers in the gut lumen increased down the length of the small bowel, with the largest value being found in stool. ent-Cholesterol is efficiently taken up by the intestinal mucosa and undergoes transient enterohepatic recirculation, but it is quantitatively eliminated over 3 days as a result of selective secretion into bile and selective enrichment within the lumen of the intestine. These findings suggest that cholesterol absorption is structurally specific and likely to be mediated by enantiospecific cellular proteins.—Westover, E. J., X. Lin, T. E. Riehl, L. Ma, W. F. Stenson, D. F. Covey, and R. E. Ostlund, Jr. Rapid transient absorption and biliary secretion of enantiomeric cholesterol in hamsters. J. Lipid Res. 2006. 47: 2374–2381.

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The intestinal absorption of dietary and endogenous biliary cholesterol is a key regulatory control point for cholesterol homeostasis. In contrast to other nutrients, only approximately half of intestinal cholesterol is normally absorbed, and excretion of the unabsorbed fraction constitutes the principal pathway for cholesterol catabolism (1). Reducing cholesterol absorption is a potentially effective way to reduce LDL-cholesterol levels and cardiovascular risk. However, the molecular mechanisms underlying cholesterol absorption are complex and not yet fully understood.

Although cholesterol absorption can be completely blocked by biliary diversion or bile salt deficiency, attempts to disrupt cholesterol absorption selectively while maintaining the absorption of other nutrients have been only moderately successful. Phytosterols reduce cholesterol absorption by ~35% when added to the diet (2), whereas the new drug ezetimibe displays maximum inhibition of 54% (3). Many of the proteins that may play a role in cholesterol absorption have been identified, including ABCA1, ABCG5, and ABCG8 (sterol efflux pumps), Niemann-Pick C1-Like 1 (NPC1L1) (a target of ezetimibe), and ACAT-2 (the intestinal form). However, single-gene knockout of each of these candidates has produced incomplete reduction in cholesterol absorption efficiency (4–8). Likewise, overexpression of NPC1L1 in nonenterocyte cells failed to generate active cholesterol transport, suggesting that additional subunits or cofactors may be required (9). Therefore, although the hypothesis that cholesterol is absorbed by a specific protein or proteins is attractive, the evidence to support it is still incomplete.

Cholesterol absorption is inherently a low-affinity process acting on millimolar amounts of cholesterol in the intestine. Thus, the specificity of cholesterol absorption cannot be studied effectively by traditional kinetic methods using a tracer and its competing unlabeled ligand. An alternative approach is to use the unnatural enantiomer of cholesterol (ent-cholesterol) in metabolic studies. As shown in Fig. 1, ent-cholesterol is the mirror image of cholesterol and is chemically synthesized with the opposite configuration at each of the eight chiral centers. ent-Cholesterol has physical properties identical to those of cholesterol with a different three-dimensional configuration, making it likely to be recognized differently by cell
Moreover, despite rapid transient absorption into the systemic circulation, cholesterol was recognized differently from cholesterol labeled with two deuterium atoms and measured by negative ion mass spectrometry. Cholesterol labeled with seven deuterium atoms and ent-cholesterol were analyzed in an Agilent Technologies 5972 quadrupole mass spectrometer (Palo Alto, CA) using negative ion chemical ionization (CI) mass spectrometry. The use of differential deuterium labeling because enantiomers are not separable by conventional, nonchiral chromatographic methods and have identical mass spectra, the use of differential deuterium labeling and mass spectrometry for their detection is required. In this work, we gavaged hamsters with a mixture of cholesterol labeled with seven deuterium atoms and ent-cholesterol labeled with two deuterium atoms and measured tracer enrichment with negative ion mass spectrometry. Ent-cholesterol was recognized differently from cholesterol and, despite rapid transient absorption into the systemic circulation, was quantitatively excreted in the stool over 3 days. Moreover, ent-cholesterol tracer given by intracardiac injection was selectively secreted into bile, enriched in the intestinal lumen, and eliminated in the stool.

Materials and Methods

Materials

[25,26,26,27,27,27-2H]cholesterol was synthesized by CDN Isotopes (Montreal, Quebec, Canada). Deuterated ent-cholesterol was synthesized by reduction of ent-desmosterol (ent-Δ5-cholesterol) using a platinum/carbon catalyst and deuterium gas as described (12). The actual distribution of deuterium incorporation was 38.2% none, 35.7% one, 16.2% two, 6.4% three, 2.3% four, 0.9% five, and 0.3% six, similar to previous reports of sterol side chain labeling reactions (13). [5,6,22,23-2H4]sitostanol was purchased from Medical Isotopes (Pelham, NH).

Animals

FIB male hamsters weighing ~120 g were obtained from Charles River Breeders (Boston, MA) and housed individually in a room maintained at 24°C with a 12 h light/12 h dark cycle. Animals were fed Purina Rodent Chow 5053 containing 4.5% fat, 20.0% protein, and 54.8% carbohydrate. Procedures were approved by the Washington University Animal Studies Committee.

Animal protocols

Ent-cholesterol and cholesterol tracers were mixed in ethanol, dried under vacuum, and redissolved in soybean oil. For time-course experiments, food was removed at the beginning of the light cycle at 6 AM; the animals were fasted for 2 h and then gavaged with 0.6 ml of soybean oil only (for time 0 controls) or containing 0.34 mg of cholesterol-d2 and 1.06 mg of ent-cholesterol-d2. Hamsters (three for each time point) were euthanized by carbon dioxide asphyxia at 0, 0.5, 1, 2, and 4 h afterward. Blood was collected by heart puncture, and the liver and small intestine were removed. The small intestine was divided into four equal segments that were analyzed separately. Lumenal contents were collected by washing with ice-cold PBS, and then the mucosa was separated from the bowel wall and extruded by exerting pressure with a microscope slide. Lipids were extracted by the method of Bligh and Dyer (14) and saponified. The neutral sterols were extracted into petroleum ether, dried, and converted to pentafluorobenzoyl esters (15).

In some experiments, mucosal free and esterified cholesterol were analyzed separately. To aliquots of the unsaponified total lipid extracts were added 3 μg of sitostanol oleate and 3 μg of sitostanol-d9 as internal standards. The mixture was taken up in 0.5 ml of hexane and applied to 3 ml silica solid-phase extraction columns (LC-Si; Sigma, St. Louis, MO) preequilibrated with hexane. Sterol esters were eluted with 6 ml of 2% ethyl acetate in hexane, and free sterols were then collected in 6 ml of 25% ethyl acetate in hexane. The fractions were then saponified and prepared for GC-MS.

For 3 day cholesterol absorption measurements, five hamsters were gavaged with 0.9 ml of soybean oil containing 2.390 mg of ent-cholesterol-d2, 0.787 mg of cholesterol-d2, and 0.0787 mg of sitostanol-d9. Three control hamsters received 0.9 ml of soybean oil alone. Stools were collected from wire-bottomed cages for a 3 day period, after which blood was obtained by cardiac puncture. Serum and stool samples were processed as described above.

For the intracardiac injection experiment, ent-cholesterol and cholesterol tracers were dissolved in ethanol and then mixed with Intralipid (16). Either Intralipid only (0.25 ml for time 0 controls) or Intralipid containing 0.160 mg of cholesterol-d2 and 0.490 mg of ent-cholesterol-d2 was administered to hamsters by intracardiac injection after 2 h of fasting, and animals (n = 3 for each time point) were euthanized at time 0 and after 4 or 24 h. Tissues were collected as for the gavage experiment. Food was only returned to animals for the 24 h time point.

GC-MS

Steryl pentafluorobenzoates, which give intense molecular anions (15), were separated on a 15 m × 0.25 mm inner diameter RTX-200 trifluoropropylmethyl polysiloxane column with 0.5 μm film thickness (Restek, Bellefonte, PA). The oven temperature was kept at 250°C for 2 min, increased at a rate of 20°C/min to a final temperature of 320°C, and held for 4.5 min. The effluent was analyzed in an Agilent Technologies 5972 quadrupole mass spectrometer (Palo Alto, CA) using negative ion chemical ionization mode. Selected ion monitoring was performed at m/z 580 (M⁺ for cholesteryl pentafluorobenzoate), 581, 584, and 587.
Calculations

The enrichment of deuterated tracers was determined from ion areas measured at m/z 587 (cholesterol) and 584 (ent-cholesterol). The latter mass was chosen because it resulted in optimum discrimination between ent-cholesterol tracer and the large background of unlabeled natural cholesterol. Using a system of simultaneous equations, the tracer peaks at m/z 587 and 584 were corrected for the contribution of unlabeled natural cholesterol and for contributions from each other. Note that more ent-cholesterol than cholesterol tracer was given to improve its detection but that enrichments are reported as the mole ratio of tracer with respect to natural cholesterol per milligram of administered tracer [(mol tracer/mol cholesterol-d0) × 100/mg tracer administered]. The percentages of ent-cholesterol-d2 and cholesterol-d7 in the liver and stool were calculated from each tracer recovered relative to its respective total amount administered. Results presented are means ± SEM. The independent effects of time, intestinal quarter, and type of tracer were analyzed using a general linear statistical model (SAS Institute, Cary, NC). A two-sample t-test was used to compare tracer enrichments whenever appropriate.

RESULTS

Oral time-course study

Cholesterol absorption is a multistep process that includes uptake into the mucosa from the intestinal lumen, transit across the enterocyte, and delivery into the systemic circulation. To examine the initial phase of absorption, hamsters were gavaged with a mixture of cholesterol-d7 and ent-cholesterol-d2. Tracer levels in the intestinal lumen and mucosa, serum, and liver were then determined by GC-MS at 0.5, 1, 2, and 4 h. Each quarter of the small bowel was analyzed separately. Data are presented as tracer enrichment (defined in Materials and Methods), values analogous to specific activity or cpm/mg cholesterol in experiments using radioactivity. Figure 2A, B show that mucosal uptake of both tracers was similar at early time
forms). By ANOVA, there were significant overall effects of time (P < 0.001) and tracer type (P < 0.007). Values are means ± SEM.

| Tracer Administered | 1 h     | 2 h     | 4 h     |
|---------------------|---------|---------|---------|
| Cholesterol-d7      | 4.3 ± 1.5 | 13.2 ± 1.0 | 7.4 ± 2.9 |
| ent-Cholesterol-d2   | 1.3 ± 0.3 | 6.8 ± 0.6  | 4.8 ± 1.3  |

Male hamsters (n = 3) were gavaged with a mixture of cholesterol-d7 and ent-cholesterol-d2 as described in Materials and Methods. Mucosa was collected and analyzed for free and esterified sterol content by mass spectrometry. Esterification of each of the cholesterol enantiomers was calculated as a percentage of the total (esterified and free forms). By ANOVA, there were significant overall effects of time (P = 0.001) and tracer type (P = 0.007). Values are means ± SEM.

points. The cholesterol tracer level increased in general over time across the intestinal segments (Fig. 2A). In contrast, the level of ent-cholesterol tracer peaked at ~1 h and then declined; this pattern was similar for all four quarters of the small intestine (Fig. 2B). These data suggest that ent-cholesterol is rapidly taken up and then eliminated from the enterocyte, either by efflux back into the intestinal lumen or by delivery across the basolateral side of the enterocyte, or both.

The potential effect of sterol esterification was studied by determining the percentage of each tracer that was esterified at 1, 2, and 4 h after tracer administration in the first quarter of the intestinal mucosa (Table 1). By ANOVA, there were independent statistically significant effects of both time (P = 0.001) and tracer type (P = 0.007). However, the esterification of both tracers was relatively small.

Upon harvesting the small intestine, the contents of the lumen were collected using a PBS wash. Sterols were subsequently extracted from the lumenal contents and analyzed by GC-MS. Figure 2C shows that the level of cholesterol tracer was relatively steady over 4 h in each region of the intestinal lumen. However, for any given time, there was more cholesterol tracer in more distal sections of the bowel. Similarly, ent-cholesterol tracer levels increased with distance down the bowel (Fig. 2D). Furthermore, the ratio of ent-cholesterol to cholesterol tracer increased with distance down the intestine (P = 0.01); for example, the ratio was 2.2 in quarter 4 at 4 h, indicating some differential recognition of the sterol tracers by the mucosa.

To assess transit across the basolateral side of the enterocyte, the tracer content of the serum was measured. Only small amounts of the cholesterol tracer were found in the serum over the 4 h time course (Fig. 3A, open circles). The accumulation of the cholesterol tracer in the mucosa and the small enrichment in serum indicated a delay in the systemic absorption of cholesterol tracer. In contrast, significant amounts of ent-cholesterol tracer (Fig. 3A, closed circles) were found in the serum as early as 30 min. By 4 h, there was a 48.3-fold enrichment of ent-cholesterol tracer compared with cholesterol tracer in the serum.

Only small amounts of the cholesterol tracer were found in the liver (Fig. 3B); for example, <1% of the administered cholesterol tracer was in the liver at 4 h. By contrast, a significant portion of the ent-cholesterol tracer (14% of the administered tracer) was detected in the liver at 4 h after administration. The enrichment of the ent-cholesterol tracer was 21.2-fold that of the cholesterol tracer in the liver at 4 h. Gallbladder bile was aspirated and analyzed for sterol tracers, but neither cholesterol nor ent-cholesterol tracer was found in significant amounts within the time frame of these experiments (data not shown).

**Cholesterol and ent-cholesterol absorption over a 3 day period**

A traditional measurement of sterol absorption was made by gavaging the tracers and collecting stools from the animals for 72 h. At the end of the experiment, serum samples were also analyzed for tracer levels. Based on anal-

![Fig. 3. Oral time-course study: enrichment of cholesterol-d7 (C-d7) and ent-cholesterol-d2 (ent-C-d2) in serum (A) and liver (B). Hamsters (n = 3 for each time point) were fasted for 2 h before gavage of cholesterol-d7 and ent-cholesterol-d2 in soybean oil. The levels of cholesterol and ent-cholesterol tracers in serum and liver were determined as described in Materials and Methods. The enrichment of each tracer was calculated relative to cholesterol-d0 in the sample and normalized to its respective amount administered. Values are means ± SEM.](image)
Analysis of the 72 h stool samples, cholesterol absorption was calculated as 53.2% (Table 2), similar to the mean of 56% found in humans (16). In contrast, all of the administered ent-cholesterol was recovered in the stool. At 72 h, cholesterol-d7 was readily detectable in serum but ent-cholesterol tracer was not (data not shown).

**Table 2. Three day study: absorption of cholesterol and ent-cholesterol by fecal recovery**

| Tracer Administered | Amount Administered | Amount in Stool | Absorption |
|---------------------|---------------------|-----------------|------------|
| Cholesterol         | 0.79 mg             | 0.37 ± 0.03 mg  | 53.2 ± 3.9%|
| ent-Cholesterol      | 2.39 mg             | 2.57 ± 0.17 mg  | N.D.       |

Male hamsters (n = 5) were gavaged with a mixture of cholesterol-d7 and ent-cholesterol-d2 as described in Materials and Methods. Stools were collected for 72 h and analyzed for sterol content by mass spectrometry. Absorption of each of the cholesterol enantiomers was calculated by reference to unabsorbed sitostanol-d4. N.D., not detected.

**Intracardiac injection of cholesterol enantiomers**

To explore the mechanism(s) by which ent-cholesterol-d2 was differentiated from cholesterol and excreted, a mixture of cholesterol and ent-cholesterol tracers was delivered directly to the systemic circulation by intracardiac injection. Tracer levels in various compartments were then determined by GC-MS at 4 or 24 h.

The serum levels of cholesterol and ent-cholesterol were similar at 4 h after intracardiac injection (Fig. 4A). However, serum enrichment of both tracers decreased at 24 h (overall time effect; P = 0.0004), with the cholesterol-d7 being 2.5-fold higher relative to ent-cholesterol-d2 (P = 0.025), suggesting more rapid clearance of ent-cholesterol-d2. Compared with cholesterol-d7, more ent-cholesterol-d2 was found in the liver (P < 0.0001), and liver enrichment of both tracers decreased over time (P < 0.0001) (Fig. 4B). Approximately 82.9% of the injected ent-cholesterol-d2 was found in the liver at 4 h versus 51.1% of cholesterol-d7; at 24 h, the corresponding values were 28.8% versus 18.4%. Figure 4C shows even higher ent-cholesterol-d2 enrichment relative to cholesterol-d7 in the bile (P = 0.0002 for the tracer effect), indicating a selective excretion of ent-cholesterol-d2 through the bile.

The enrichment of ent-cholesterol-d2 in the stool was 26-fold that of cholesterol-d7 at 4 h after injection (P = 0.03) and 27-fold at 24 h (P = 0.02) (Fig. 4D). As a percent-

![Fig. 4. Intracardiac injection study: enrichment of cholesterol-d7 (C-d7) and ent-cholesterol-d2 (ent-C-d2) in serum (A), liver (B), bile (C), and stool (D). Hamsters (n = 3 for each time point) were fasted for 2 h before intracardiac administration of cholesterol-d7 and ent-cholesterol-d2 in Intralipid. The levels of cholesterol and ent-cholesterol tracers were determined as described in Materials and Methods. The enrichment of each tracer was calculated relative to cholesterol-d0 in the sample and normalized to its respective amount administered. Values are means ± SEM.](image-url)
age of the amount injected, the amounts of ent-cholesterol-d$_2$ increased from 2.7% at 4 h to 24.1% at 24 h, whereas the amounts of cholesterol-d$_7$ were 0.11% and 0.97%, respectively. These results indicated that ent-cholesterol-d$_2$ was selectively excreted over the 24 h of this experiment.

After intracardiac injection, there were significant effects of time ($P < 0.0001$) and quarter ($P = 0.0009$) in the enrichment of cholesterol-d$_7$ (Fig. 5A) but none in ent-cholesterol-d$_2$ in the mucosa (Fig. 5B). The ratio of ent-cholesterol to cholesterol tracers in the mucosa changed across all quarters ($P = 0.0007$) and over time ($P < 0.001$). In the intestinal lumen, there were significant effects of time ($P = 0.0009$ for cholesterol-d$_7$ enrichment and $P < 0.0001$ for ent-cholesterol-d$_2$) and quarter ($P = 0.0181$ for cholesterol-d$_7$ and $P < 0.0001$ for ent-cholesterol-d$_2$) (Fig. 5C, D). Significantly, the ratio of ent-cholesterol to cholesterol tracers in the lumen increased across all quarters ($P < 0.0001$) but remained unchanged over time.

**DISCUSSION**

Approximately 53% of the deuterated cholesterol tracer administered as a single oral dose was absorbed over 3 days. This result is consistent with previous reports in humans (16). In contrast, after 3 days, the ent-cholesterol tracer was not detectable in the serum. Rather, it was quantitatively recovered in the stool. ent-Cholesterol was thus considered not “absorbed” in this classical cholesterol absorption experiment.

Previous studies of orally administered tracers have shown that <2% of phytosterols are absorbed over several days (17). Because phytosterols were not included in this study for direct comparison, whether ent-cholesterol was handled similarly to phytosterols by the absorptive process remains unknown. Nonetheless, it is clear that the overall process of cholesterol absorption not only distinguishes cholesterol from phytosterols, which differ from cholesterol physically and structurally, but also distinguishes cholesterol from its enantiomer, which differs only in absolute configuration. Because cholesterol and ent-cholesterol differ in three-dimensional configuration, chiral molecules such as proteins have the potential to distinguish between the two sterols. The finding that hamsters discriminate completely between intragastrically delivered cholesterol and ent-cholesterol provides firm evidence that overall cholesterol absorption is highly steroispecific and likely to be protein-mediated, with little reliance on diffusion or nonspecific pathways.
Despite the lack of systemic retention after several days, the intragastric time-course studies showed that ent-cholesterol was taken up by the enterocyte and transiently absorbed. The mucosal levels of cholesterol and ent-cholesterol tracers were similar through the first 30 min across all quarters. Over the first 4 h after administration, cholesterol tended to accumulate in the mucosa. However, this tracer was found only in small amounts in the serum or liver by 4 h after oral administration, indicating slow systemic absorption of cholesterol. We cannot exclude the possibility that cholesterol could be delivered efficiently to peripheral tissues. Yet, our results are consistent with other reports that cholesterol tracers given orally may take several days to reach a peak in plasma (16, 18, 19). By contrast, mucosal levels of ent-cholesterol peaked across all quarters at 1 h and then declined rapidly afterward, indicating a rapid uptake of ent-cholesterol across all quarters of the small intestine at 1 h followed by export.

Other studies have shown that phytosterols also show rapid but transient accumulation in enterocytes (20). Although its exact role is still unclear, NPC1L1 appears to play an important role in the uptake of both cholesterol and phytosterols from the intestinal lumen into the enterocyte brush border (5, 9), and our results suggest that NPC1L1 may not discriminate between enantiomers.

In the serum and liver, a significant enrichment of ent-cholesterol over cholesterol was observed as early as 30 min after oral administration. The ratio of ent-cholesterol to cholesterol enrichment reached 48.3- and 21.2-fold in the serum and liver, respectively, at 4 h after oral administration. These results indicated that ent-cholesterol was indeed rapidly transported across the basolateral membrane of enterocytes, whereas part of it may have been resecreted back into the intestinal lumen. It is likely that the majority of ent-cholesterol was transported in its unesterified form, because only a small portion was found to be esterified in the mucosa (Table 1). Because free cholesterol is transported by apolipoprotein B-independent pathways, such as efflux mediated by ABCA1 or HDL assembly (19), these pathways may be responsible for the rapid absorption of ent-cholesterol from the enterocyte to the systemic circulation (19, 21). Alternatively, other pathways may be involved.

Despite rapid uptake and delivery to the systemic circulation, the net absorption of ent-cholesterol is apparently incomplete. Of the ent-cholesterol-d2 given by intracardiac injection, 2.7% of this tracer was lost in the stool at 4 h, and this loss increased to 24.1% at 24 h (Fig. 4D). In contrast, loss of cholesterol-d2 accounted for <1% of the intracardiac administered tracer at both time points. Consistent with the selective resecretion of ent-cholesterol tracer, its serum enrichment in the intracardiac injection study was similar to that of cholesterol tracer at 4 h but was lower than that of cholesterol at 24 h (Fig. 4A). The intracardiac injection study directly showed a preferential accumulation of ent-cholesterol-d2 over cholesterol-d1 in liver and bile at 4 h (Fig. 4B, C). The enrichment of both tracers in liver and bile decreased at 24 h, which is consistent with continued clearance over time. Nonetheless, at 24 h, ent-cholesterol tracer was still 1.6- and 3.8-fold that of cholesterol tracer in liver and bile, respectively. These results indicate that the liver contributes to the specificity of cholesterol absorption by selectively secreting ent-cholesterol-d2 into bile for elimination.

Luminal levels of both ent-cholesterol and cholesterol in the intragastric experiment increased with distance down the small intestine. There was, however, a 2.2-fold enrichment of ent-cholesterol compared with cholesterol in quarter 4 at 4 h. Because only negligible amounts of ent-cholesterol were found in the bile within 4 h of oral tracer administration, there should be no significant biliary ent-cholesterol contribution to its luminal enrichment within this short time in the oral study. Similarly, ent-cholesterol enrichment in the lumen increased steadily across quarters at both 4 and 24 h after intracardiac injection (Fig. 5D). In fact, the ratio of ent-cholesterol to cholesterol tracers increased 2.2-fold from quarters 1 to 4 at 4 h and 4.3-fold at 24 h. In contrast, the ratio of ent-cholesterol to cholesterol tracers in the mucosa was reduced at 24 h compared with 4 h. The sustained increase of ent-cholesterol-to-cholesterol ratio in the lumen is consistent with the preferential elimination of ent-cholesterol. Therefore, the enterocyte may confer some specificity in the luminal enrichment of ent-cholesterol, possibly through efflux mediated by ABCG5/8 (22) or other transporters in the brush border.

Rapid intestinal transport and preferential biliary secretion of ent-cholesterol strongly suggest that ent-cholesterol may have undergone enterohepatic recirculation multiple times. Unexpectedly, a significant amount of this tracer was found in the circulation at 4 h after oral administration. Detailed time-course quantification of biliary tracer excretion using bile fistula hamsters would help clarify the enterohepatic cycling of ent-cholesterol, but such studies are currently limited by tracer availability.

ACAT-2 plays a role in cholesterol absorption when intestinal cholesterol levels are high (7). In our studies, only a small fraction of either tracer was esterified by 1 h. Our finding that the mucosal pool of cholesterol was mostly in the unesterified form is consistent with the existence of a metabolic pool of free cholesterol in the mucosa described many years ago (23). In fact, cholesterol was selectively directed into this pool, and its absorption into the systemic circulation was delayed compared with that of ent-cholesterol. Little is known of the possible role of this free cholesterol pool in regulating cholesterol absorption, but it seems possible that it may represent a physiological measure of the amount of recently absorbed cholesterol that could regulate the proteins involved in the absorption process.

Noncholesterol sterols can have profound effects on cholesterol metabolism. Active exclusion of phytosterols appears to be critical for the maintenance of normal cholesterol homeostasis (24). The results of this study demonstrate that overall cholesterol absorption is enantioselective. Our data indicate that ent-cholesterol is efficiently recognized and resecreted by both the mucosa and the liver. An understanding of the source of this enantioselect-
tivity may provide a rationale for developing better pharmacological agents aimed at reducing the intestinal absorption of both cholesterol and noncholesterol sterols.\footnote{Reference}

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