Normal cholesterol absorption in rats deficient in intestinal acyl coenzyme A:cholesterol acyltransferase activity

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Abstract Acyl coenzyme A:cholesterol acyl transferase and/or cholesterol esterase may regulate the esterification and absorption of exogenous cholesterol. To assess this, mucosal acyl coenzyme A:cholesterol acyl transferase activity was inhibited selectively with three different drugs [Sandoz #58-035, inhibitor 1; Lederle inhibitor 2 and inhibitor 3] and the effect upon the absorption of a [4-^{14}C]cholesterol meal was studied in the lymph fistula rat. Compared to control rats, ACAT activity measured in mucosal homogenates from the drug-treated rats was reduced 80–90%, 40%, and 30%, respectively, during the predicted timeframe for maximum mucosal esterification of cholesterol (i.e., after cholesterol is fed and before it appears in lymph). In contrast, [^{14}C]cholesterol absorption in the drug-treated animals was unchanged from controls [5.7 ± 1.2 (inhibitor 1) vs. 5.4 ± 1.6 μmol/6 hr (control); 6.1 ± 2.1 (inhibitor 2) and 5.2 ± 1.5 (inhibitor 3) vs. 4.1 ± 1.3 μmol/6 hr (control)]. Of the absorbed [^{14}C]cholesterol, approximately 75% was esterified in all groups. Cholesterol esterase activity measured in the drug-treated rats was unchanged compared to controls nor did the drugs inhibit this enzyme in vitro. Under the conditions of this study, drugs causing substantial inhibition of acyl coenzyme A:cholesterol acyl transferase activity had no effect on the absorption of exogenous cholesterol.—**Gallo, L. L., J. A. Wadsworth, and G. V. Vahouny.** Normal cholesterol absorption in rats deficient in intestinal acyl coenzyme A:cholesterol acyltransferase activity. *J. Lipid Res.* 1987. **28:** 381—387.

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Esterification occuring in the intestinal wall may regulate the absorption of luminal sources of cholesterol. Two major esterification enzymes, acyl coenzyme A:cholesterol acyltransferase (ACAT) and cholesterol esterase, found in the small intestine of many species have been implicated in this regulation. ACAT (1), endogenous to the intestine and present in cells isolated from both villus and crypt zones (2–4), is responsive to cholesterol and fat feeding (2, 5, 6). However, results of inhibitor studies designed to assess the role of ACAT in the absorption of luminal cholesterol, when taken together, are indecisive. For example, it has been determined that ACAT inhibition interferes with cholesterol absorption in the cholesterol-fed rabbit (7) and rat (Heider, J., personal communication), but has no effect on absorption or serum cholesterol levels (Heider, J., personal communication; Schaffer, S., personal communication) in either normal fed species. In another study, ACAT inhibition decreased the lymphatic secretion of esterified cholesterol in the normal fed rat (8).

Cholesterol esterase, present in the mucosa, is derived from the cholesterol esterase of pancreatic juice as demonstrated by several lines of evidence. In early studies it was shown that mucosal cholesterol esterase and cholesterol absorption both decreased in pancreatic fistula rats (9) and that pancreatic juice or replacement tissue feeding stimulated cholesterol absorption (10–13). Subsequently, the uptake of pancreatic cholesterol esterase by intestinal cells was demonstrated (14, 15). Recently, we have reported a reduction of approximately 80% in cholesterol absorption and esterification in the lymph fistula rat with normal mucosal ACAT and deficient cholesterol esterase activities (16). Thus, the available data lend support to a role for each enzyme in the process of cholesterol absorption. The purpose of the present study was to assess the effect of a series of structurally distinct ACAT inhibitors on the intestinal esterification and lymphatic absorption of exogenous cholesterol in the normal fed rat. The effects of three inhibitors were studied.

Abbreviations: ACAT, acyl coenzyme A:cholesterol acyltransferase; BSA, bovine serum albumin; DTNB, 5,5'-dithiobis(2-nitrobenzoic acid); ATP, adenosine triphosphate; CES, cholesteryl ester synthetase; CE, cholesteryl ester; LCAT, lecithin:cholesterol acyltransferase.

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MATERIALS AND METHODS

Experimental protocol

Male rats of the Wistar strain (Charles River Breeding Laboratories, Wilmington, MA) were acclimated under a normal light cycle for 2 weeks after receipt with free access to laboratory chow (Ralston-Purina Co., St. Louis, MO) and water. At the end of this period, rats were divided into groups for administration of the individual ACAT inhibitors or to serve as controls.

Inhibitor 1 (I-1). At −20 hr (9–12 AM) nonfasted rats weighing 200–250 g were anesthetized with pentobarbital and surgically equipped with a mesenteric lymph cannula and a duodenal infusion tube in a manner and with the materials previously described (9, 17). After surgery, the rats were placed in restraint cages, moved to a warm room, and infused intraduodenally, at a rate of 3.0 ml/hr, with a maintenance mixture containing 0.9% NaCl, 0.03% KCl, and 5.0% glucose dissolved in distilled water. This infusion was maintained during the entire experiment (26 hr). Lymph flow was monitored in the postoperative period. Rats with lymph flow rates less than 2.0 ml/hr were excluded from further study. Those qualifying were divided into two groups matched as closely as possible by lymph flow rate. At −3 hr, a gastric dose of I-1 (20 mg/kg body weight) in suspending vehicle or suspending vehicle alone (described below) was administered. Three hours later, at zero time, the constant duodenal infusion was stopped briefly (15 min) and each rat received via the duodenal infusion tube an emulsified lipid meal containing [4-^{14}C]cholesterol (1 μCi, sp act 54 mCi/mmol; Amersham Searle, Arlington Heights, IL); cholesterol, 25 mg (Serdary Research Laboratories, London, Ontario); oleic acid, 145 mg; sodium taurocholate, 144 mg; and bovine serum albumin (BSA), 25 mg (each from Sigma Chemical Co., St. Louis, MO) in 3.0 ml of isotonic NaCl solution. During the next 6 hr the maintenance infusion was continued and lymph was collected in iced graduated cylinders that contained the LCAT inhibitor, DTNB, 14.3 mg (Eastman Kodak Co. Rochester, NY) and heparin, 6.3 mg (Sigma Chemical Co.). The animals were killed by cervical dislocation, the entire small intestine was removed, and the mucosa was collected for immediate assay of the esterification enzymes, ACAT and cholesterol esterase. Lymph lipids were extracted immediately for analysis of cholesterol mass and isotope.

Inhibitors 2 and 3 (I-2, I-3). At −2 weeks, acclimated rats were given free access to ground laboratory chow (Ralston-Purina Co.) that contained either I-2 or I-3 at final concentrations of 0.003% by weight (prepared as described below) or ground chow with no additions. The diets were consumed equally well and body weight gains among groups were comparable. After 2 weeks on diet, at −20 hr, nonfasted rats weighing 200–250 g were subjected to the identical surgical treatment, maintenance infusion, lymph-flow monitoring, lipid meal (at zero time), lymph collection, and killing as described above for I-1-treated animals. Also the mucosa from the entire small intestine and the 6-hr lymph pool collected after the lipid meal were processed in the same way.

Lymph analysis

At the end of the 6-hr absorption period the lymph volume was recorded, the lymph was well mixed, and the lipids in an aliquot were extracted according to the procedure of Folch, Lees, and Sloane Stanley (18). The solvent was evaporated to dryness under nitrogen and the lipid residue was redissolved in hexane. The hexane solution of lymph lipids was analyzed to determine total and free cholesterol mass and radioactivity. The radioactivity in an aliquot of the hexane extract was measured in a Beckman liquid scintillation counter (Model LS-250, Beckman Instruments, Fullerton, CA). Quench corrections were performed by external standardization. The radioactivity calculated for total lymph volume is a measure of exogenous cholesterol absorption.

To determine the percentage esterification of the lymph [4-^{14}C]cholesterol, the lipids in an aliquot of the hexane extract were separated on Silica Gel G precoated thin-layer chromatography plates (Uniplates, Analtech, Newark, DE). The silicic acid areas corresponding to cholesterol and cholesteryl ester, as identified with authentic standards (Nu-Chek-Prep, Inc., Elysian, MN), were scraped into counting vials, and the radioactivity was monitored as described above.

To determine free cholesterol and total cholesterol mass in lymph, two separate aliquots of the hexane extract were evaporated to dryness under nitrogen. For determination of free cholesterol mass, the lipid residue in the tube was redissolved in 0.4 ml of hexane containing 0.5 μg/ml of cholestane (Applied Science Laboratories, Inc., State College, PA) as the internal standard. For total cholesterol mass, the lipid residue was treated with alcoholic potassium hydroxide to hydrolyze the cholesteryl esters as described by others (19). After hydrolysis, the digest was extracted three times with 4-ml portions of hexane. The extract was evaporated to dryness under nitrogen and the residue was redissolved in hexane containing the cholestane internal standard as described above. Free and total cholesterol were determined by gas–liquid chromatography (Varian Associates, Model 3700 GC; Walnut Creek, CA) on a 2-ft column (4 mm o.d.; 2 mm i.d.) packed with 3% OV-17 on 100/120 Gas Chrom Q (Applied Science Laboratories, State College, PA). Columns were operated at 250°C with a carrier gas flow of 25 ml/min.

Corrections for losses were based on recoveries of the cholestane standard. Esterified cholesterol mass was calculated by difference (total minus free).

Esterification enzyme assays

The small intestine from pylorus to ileocecal junction was removed immediately after the rat was killed, flushed with cold isotonic saline, and blotted according to the procedure described by others (7) for eliminating drug contamination of the luminal surface of the intestine. The mucosa was scraped with a glass slide onto the surface of an ice-filled Petri dish. Wet weight mucosa was diluted fivefold (w/v) with isotonic saline, pH 6.2, and homogenized in a Potter-Elvehjem homogenizing tube to yield a 20% homogenate. One aliquot was lipid extracted with chloroform–methanol (18) to monitor the level of test meal cholesterol associated with the mucosa; another aliquot was assayed for cholesterol esterase activity; and the remainder was diluted twofold with 0.2 M potassium phosphate buffer, pH 7.4, and gently homogenized, and the 10% homogenate was assayed for ACAT activity.

The activity of ACAT in homogenates was determined by the rate of incorporation of [1^{14}C]oleic acid into cholesteryl ester (20, 21). Siliconized 50-ml glass conical tubes containing 5.1 mM ATP, 512 μM coenzyme A, 5.0 mg of BSA (each from Sigma Chemical Co.), 10.5 mM $MgCl_2 \cdot 6\ H_2O$, 0.2 M potassium phosphate, pH 7.4, in a volume of 0.47 ml, and 227 μM [1^{14}C]oleic acid (0.4 μCi, sp act, 57.4 mCi/mmol, Amersham Searle), added in 10 μl of acetone, were preincubated at 37°C for 15 min. The ACAT-catalyzed reaction was initiated by the addition of 80 μl of freshly prepared 10% mucosal homogenate. After 3 min in a 37°C oscillating Dubnoff metabolic shaker, reactions were terminated by the addition of 20 volumes of chloroform–methanol (18). The 3-min reaction time was determined previously to fall on the linear region of the rate curve. [1,2-^{3}H]Cholesterol oleate (20,000 dpm), prepared and purified by thin-layer chromatography just prior to use as described by others (22, 23), was added to the extraction mixture as the internal standard to correct for procedural losses (not > than 10%).

The activity of cholesterol esterase in homogenates was assayed by determining the rate of incorporation of [4-^{14}C]cholesterol into cholesteryl ester as previously described (24). Twenty five-ml Erlenmeyer flasks containing 7.75 mM [4-^{14}C]cholesterol (0.5 μCi, sp act 54 mCi/mmol), 23.15 mM oleic acid, 10 mM sodium taurocholate, 65 mM $(NH_4)_2SO_4$, 4 mg of BSA, and 0.154 M sodium phosphate, pH 6.2, were preincubated at 37°C for 15 min. The cholesterol esterase-catalyzed reaction was initiated by the addition of 1.0 ml of 20% mucosal homogenate which gave a final incubation volume of 2.0 ml. After 5 hr (on linear region of rate curve) in a 37°C oscillating shaker, reactions were terminated by the addition of chloroform–methanol (18).

For both enzyme assays, zero-time and buffer (homogenization buffer minus tissue) controls were included; each assay was run in triplicate, and in some experiments, where indicated, ACAT inhibitors in 10 μl of acetone were added during the preincubation period. In these cases control flasks received the same volume of acetone; and mucosa-associated [4-^{14}C]cholesterol from the lipid meal contributed < 1% to the total cholesteryl ester formed in either assay as reported previously (16). In the ACAT assay the extent of cholesterol esterase-catalyzed hydrolysis of ACAT-generated cholesteryl esters (the activity of cholesterol esterase in mucosal homogenates under ACAT assay conditions favors cholesteryl ester hydrolysis) was confirmed (4) as insignificant during the time-frame of the incubation. Thus, based on the above determinations, no corrections were applied when the amount of cholesteryl ester product was calculated. For both assays the lipids were extracted, separated by thin-layer chromatography, and monitored for radioactivity as described above for lymph. For the dual-labeled samples (^{14}C and ^{3}H) from the ACAT assay, the automatic quench compensation function was used in conjunction with the external standard-channels ratio method of quench calibration.

Preparation and administration of ACAT inhibitors

I-1(#58035). I-1 (mol wt 460, 3-decyldimethylsilyl-N-[2-(4-methylphenyl)-1-phenylethyl] propanide) was suspended on CM-cellulose (Bio-Rad Laboratories, Richmond, CA) as described by others (8). Briefly, a 1.5% aqueous suspension of CM-cellulose containing 0.2% Tween-80 (Fisher Scientific Co., Fairlawn, NJ) was stirred vigorously for 3 hr. Then I-1 was added to yield a 2% (w/v) mixture and stirring was continued for 3 days at room temperature and a stable suspension resulted. The drug (20 mg/kg body weight) in vehicle or vehicle alone was administered intragastrically with a syringe fitted with a ball-tipped intubation tube.

I-2, I-3. Thirty milligrams of either I-2 (mol wt 581,N-[1(4-benzyloxyphenyl)-2-phenylethyl]-N-benzyl-N-(3-trifluorotolyl) urea or I-3 (mol wt 347,1-benzylidene-4,4-diphenyl thiosemicarbozone) were dissolved in 100 ml of chloroform and mixed with 1 kg of ground rat chow to yield a final inhibitor concentration of 0.003% (w/w) (Schaffer, S., personal communication). Diets for control animals were made by addition of solvent alone to the chow. After solvent evaporation, the diets were mixed again to insure even inhibitor distribution. Diets were fed ad libitum.

Calculations

Statistical significance of the data between each drug-treated group and its respective control group was determined by Student's *t*-test.

RESULTS

Effect of ACAT inhibitors on ACAT and cholesterol esterase in vitro

The in vitro potency and specificity of the ACAT inhibitors with respect to both cholesterol esterification enzymes were determined. The results are shown in **Table 1.** Increasing concentrations of I-1 (52 nM–52 μM) reduced ACAT activity linearly with the log concentration of inhibitor. An inhibitor concentration of 0.4 μM produced 50% inhibition of ACAT activity. I-2 (80 nM–400 μM) and I-3 (2 nM–21 μM) were less potent inhibitors of intestinal ACAT with concentrations of 80 μM and 2 μM, respectively, required to produce 50% inhibition of activity. Each of the ACAT inhibitors was without effect on cholesterol esterase activity both at the concentration that produced maximum inhibition of ACAT activity and at higher concentrations (700 μM, 610 μM, and 30 μM of I-1, I-2, and I-3 respectively).

TABLE 1. Effect of ACAT inhibitors on the enzymatic activity of ACAT in vitro

Inhibitor	Concentration	ACAT Activity
	μM	*% of control*
I-1	0.052	97
I-1	0.52	48
I-1	5.2	17
I-1	52.0	5
I-2	0.08	88
I-2	0.80	75
I-2	8.0	60
I-2	80.0	53
I-2	400.0	42
I-3	0.0021	95
I-3	0.021	78
I-3	0.21	63
I-3	2.1	48
I-3	21.0	27

Each ACAT assay contained in a total volume of 0.55 ml: 5.1 mM ATP, 512 μM coenzyme A, 5.0 mg BSA, 10.5 mM $MgCl_2 \cdot 6H_20$, 0.2 M potassium phosphate, pH 7.4, 227 μM [1-^{14}C]oleic acid, 80 μl of 10% mucosal homogenate, and either the indicated concentration of ACAT inhibitor added in 10 μl of acetone or acetone alone. Mean ACAT activity ± SEM in the controls (n = 14) was 689 ± 10 pmol of cholesteryl ester formed/3 min per 80 μl of 10% mucosal homogenate.

Time course of ACAT inhibition in vivo

Groups of animals were treated exactly as described above in the experimental protocol but were killed at time intervals after administration of the ACAT inhibitors. ACAT activity was measured in the mucosal homogenates prepared from each group. The results are shown in **Fig. 1.** With I-1 the inhibition of ACAT was greatest in the period 3–5 hr after the gastric bolus, reaching 87 ± 2% in 3 hr, peaking at 90 ± 2% in 4 hr, and diminishing to 80 ± 5% in 5 hr. Thus, the administration of the cholesterol meal 3 hr after the gastric bolus of I-1 closely coordinated maximum ACAT inhibition with the predicted cholesterol esterification period in the absorption process, i.e., the time lapse between free cholesterol uptake by and cholesteryl ester exit from the absorptive cells.

In the case of I-2 and I-3, ACAT inhibition occurred only after the cholesterol meal with 40% and 30% inhibition, respectively, measured at the conclusion of the absorption period (6 hr after the meal). There was no significant inhibition of mucosal ACAT activity by either inhibitor in rats that had received chow containing inhibitor for 2 weeks and killed in either the fed state or just prior to the cholesterol meal (20 hr-fasted).

Effect of ACAT inhibitors on the lymphatic absorption and esterification of [4-^{14}C]cholesterol

The effects of the three ACAT inhibitors on the absorption and esterification of [4-^{14}C]cholesterol from the intraduodenally administered lipid meal are shown in **Table 2.** In study 1 with I-1, neither absorption nor esterification of cholesterol (measured in the 6-hr lymph pool collected after the lipid meal) differed between the experimental and the control groups (5.7 ± 1.2 vs. 5.4 ± 1.6 μmol of cholesterol absorbed per 6 hr; 4.5 ± 1.1 vs. 4.2 ± 1.3 μmol of cholesterol esterified per 6 hr, respectively). In addition, cholesterol esterase activity (not shown), measured at the end of the absorption period, was unchanged (103 ± 3% of control, n = 6) in I-1- treated rats. However, in contrast, ACAT activity was markedly inhibited, 80–90% (Fig. 1), during the 2-hr span after the meal and when the greatest flux of cholesterol through the absorptive cells and its esterification are expected based upon the time and rate of cholesterol appearance in the lymph observed by us and others (8). Even at the end of the absorption period, 6 hr after the meal, ACAT activity had recovered to just half of the control level.

Similarly, in study 2 using I-2 and I-3, the absorption and esterification of cholesterol did not differ significantly in the presence or absence of inhibitor (5.2 ± 1.5 and 6.1 ± 2.1 vs. 4.1 ± 1.3 μmol of cholesterol absorbed per 6 hr; 3.7 ± 1.0 and 4.4 ± 1.7 vs. 3.2 ± 1.1 μmol of cholesterol esterified per 6 hr with I-2, I-3, and controls, respectively). As with I-1, cholesterol esterase activity measured in the mucosa at the end of the absorption period, was unchanged (104 ± 5% of control and 98 ± 7% of control with I-2 and I-3, respectively, n = 5–7). In the same homogenates, ACAT activity was reduced 40% and 30% by I-2 and I-3, respectively.

When lymph from these same 6-hr pools was analyzed for total and free cholesterol mass, the absorption and esterification results **(Table 3)** were in agreement with the isotopic data (Table 2), i.e., there were no significant differences in lymph free and esterified cholesterol between the control and ACAT-inhibited groups (compare,

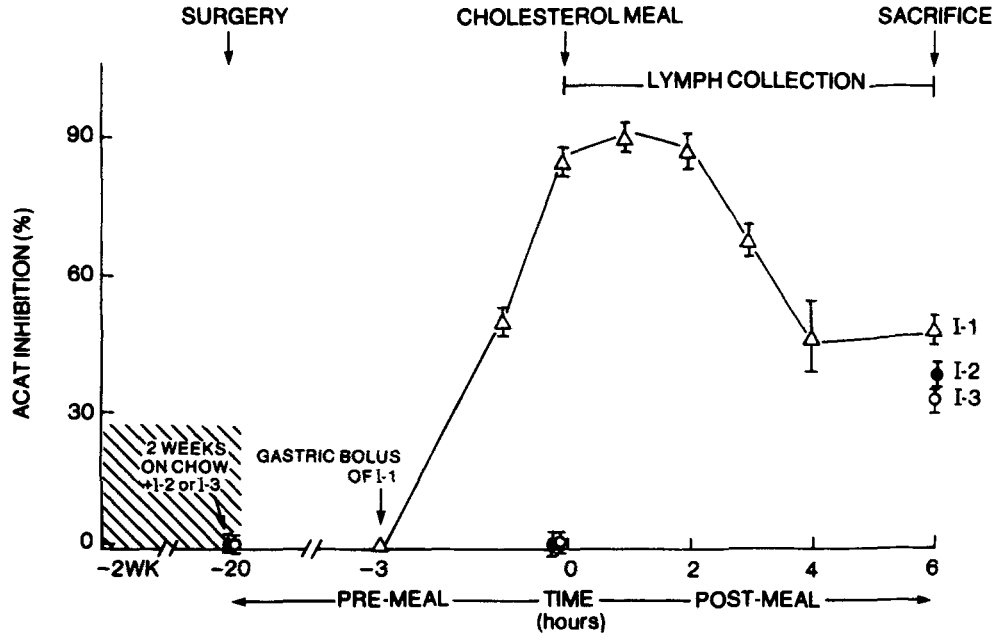

Fig. 1. Time course of ACAT inhibition. Rats were subjected to the experimental protocol described in the text. Those administered I-1 (△—△) received the gastric bolus 3 hr prior to the cholesterol meal and were killed at the indicated times. Each point is the mean ± SEM for n = 4. Rats administered I-2 (●—●) and I-3 (○—○) received chow containing the drug for 2 weeks. Groups of these rats were killed in the fed state at the end of the 2-week period; after a 20-hr fast, pre-cholesterol meal; and at the end of the experimental period, 6 hr post-meal. Each point is the mean ± SEM for n = 5. ACAT activity was assayed in 10% mucosal homogenates as described in Table 1.

e.g., in Table 3 the control vs. I-1: 3.1 ± 0.6 vs. 4.1 ± 0.7 μmol of free cholesterol absorbed per 6 hr and 10.5 ± 2.4 vs. 10.5 ± 2.0 μmol of esterified cholesterol per 6 hr). In addition, the average total lymph volumes collected during the 6-hr absorption period were relatively constant among all groups (Table 3). However, flow rates were variable among animals within each group, but these variations showed no direct relationship to the amount of cholesterol absorbed.

DISCUSSION

Three structurally distinct drugs, known to function as inhibitors of ACAT activity, have been employed in rats to study the relationship between intestinal ACAT activity and exogenous cholesterol absorption and esterification. I-1 has been reported to inhibit rat (7, 8) and rabbit (Heider, J., personal communication) intestinal ACAT. In each study ACAT activity was assayed in a system employing preformed acyl CoA. Thus the observed inhibition of ACAT activity was independent of fatty acyl CoA generation. Similarly, I-2 and I-3 inhibited ACAT activity in monkey aortic smooth muscle microsomes and rat adrenal cells (Schaffer, S., personal communication) in an assay system independent of fatty acid activation. Moreover, in the current study, no effect on the lymphatic absorption of glycerides was observed, which provides additional evidence that ACAT itself is the target of the drugs.

The drugs produced no overt signs of toxicity in either the intestine or the intact animals. Rats receiving the single bolus of I-1, 20 mg/kg body weight, absorbed cholesterol normally and the appearance of the intestine was normal. The absence of I-1 toxicity is consistent with an earlier report (8). Rats fed a diet containing 0.003% I-2 or I-3 for 2 weeks consumed the same amount of food and gained the same weight as controls (data not shown), showed no obvious histological alterations in the intestine, and also absorbed cholesterol normally.

The mechanism of action of the three drugs in the reduction of ACAT activity has not been determined. It was observed in the present studies that the drugs are effective inhibitors at concentrations that approach those of the substrates in the ACAT assay. Frequently, this finding is consistent with competitive inhibition. However, we do not think that the drugs compete with cholesterol, since the addition of exogenous cholesterol (300 μM, solubilized in dioxane–propylene glycol 2:1(v/v) to the assay did not reverse the inhibition (data not shown). We have shown previously that exogenous cholesterol added in this way is available to mucosal ACAT (21).

TABLE 2. Effect of ACAT inhibitors on the [4-^{14}C]cholesterol absorption

Study	Inhibitor	Cholesterol [4-^{14}C] Absorbed	Esterified
		μmol/6 hr	
1	none	5.4 ± 1.6	4.2 ± 1.3
1	I-1	5.7 ± 1.2	4.5 ± 1.1
2	none	4.1 ± 1.3	3.2 ± 1.1
2	I-2	5.2 ± 1.5	3.7 ± 1.0
2	I-3	6.1 ± 2.1	4.4 ± 1.7

In study 1, rats were given a gastric dose of I-1 in vehicle or vechicle alone. In study 2, rats were fed chow containing I-2 or I-3 or chow alone for 2 weeks. Three hr after receiving I-1 and 20 hr after last access to I-2 or I-3 (the post-surgery recovery period), all rats were administered, intraduodenally, a lipid meal containing 25 mg of [4-^{14}C]cholesterol. Mesenteric lymph (6 hr pool) was analyzed for cholesterol, absorbed and esterified. Mucosal homogenates prepared at the end of the absorption period were assayed for ACAT and cholesterol esterase activity as described in the text. Values are means ± SEM for n = 5–7 animals. At the end of the absorption period ACAT activity in the drug-treated rats remained inhibited to the extent of 45%, 40%, and 30% with I-1, I-2, and I-3, respectively, compared to controls ($P < 0.05$) while cholesterol esterase in the drug-treated groups did not differ from controls.

Utilization of these three inhibitors has revealed that the intestinal absorption of cholesterol from a lipid meal is normal when mucosal ACAT activity is reduced substantially, while mucosal cholesterol esterase activity is unchanged. In fact, correlation coefficients, between the appearance of [4-^{14}]cholesteryl ester in lymph after a cholesterol meal and the activities of the two esterification enzymes measured in intestine at the conclusion of the absorption period were 0.71 and −0.19 for cholesterol esterase and ACAT, respectively. The correlation, determined by linear regression analysis, between the appearance of ester in lymph and cholesterol esterase activity in intestine was positive and significant. These results complement those of our earlier study in the lymph-fistula rat in which cholesterol absorption from a lipid meal was decreased markedly in intestine deficient in cholesterol esterase but with normal levels of ACAT (16).

Individual consideration of the ACAT inhibitors reveals that I-1 is the most potent, reducing ACAT activity to 5% and 10% of control levels in vitro and in vivo, respectively. The in vivo level of inhibition agrees with that attained by constant infusion of the drug (8). Since ACAT activity occurs in both villus and crypt zone cells (2, 3, 21) and predominates in the crypts in the chow-fed rat (21), it is apparent that the inhibitor has access to both cell types to produce such complete inhibition in vivo. With I-2 and I-3, ACAT activity in vitro was inhibited 58% and 73%, respectively, at the highest level of drug. I-3 the most effective in vitro was least effective in vivo, i.e., I-2 produced a 40% inhibition of ACAT activity compared to 30% for I-3. Curiously, the prolonged feeding regimen in the case of both drugs did not attain the level of ACAT inhibition measured in vitro. This would suggest poor absorbability of both drugs and a difference between the two drugs in accessibility to the interior of the epithelial cells in general, and perhaps also to specific cell type (villus vs. crypt). In other words, if the drug is taken up preferentially by villus absorptive cells or crypt cells, then ACAT activity to the extent present in that cell type in 20-hr fasted rats, as in this study, may be inhibited maximally. No experiments were conducted to determine the distribution of the drugs between cell types. Further, although both drugs were fed for two weeks, ACAT inhibition did not occur prior to administration of the lipid meal and presumably was dependent upon lipid uptake. It is unlikely that the exogenous lipids promoted inhibitor uptake since rats in the study were without access to chow with drug for 20 hr prior to the lipid meal, and the intestine appeared chow-free at the time of killing. Possibly, the lipids, after uptake, facilitated the movement of the lipophilic drugs to microsomal ACAT.

The identity of the esterification enzyme that regulates cholesterol absorption continues to be debated. The preponderance of studies (7; Heider, J., personal communication; Schaffer, S., personal communication) that project a role for ACAT in cholesterol absorption have been carried out in animals chronically administered dietary cholesterol (1% cholesterol or 1% cholesterol and 0.5% cholic acid) for 2 weeks. In these studies ACAT inhibition is reported to reduce cholesterol absorption (ACAT inhibitors have no effect in chow-fed animals). Studies from our laboratory that project cholesterol esterase as the regulatory enzyme have been carried out in animals administered cholesterol in a single lipid meal. In these studies, cholesterol esterase deficiency is reported to reduce cholesterol absorption or, as in the current study, ACAT inhibition has no effect upon cholesterol absorption. The

TABLE 3. Effect of ACAT inhibitors on lymph cholesterol mass and volume

Study	Inhibitor	Cholesterol Mass Free	Esterified	Volume
		μmol/6 hr		*ml/6 hr*
1	None	3.1 ± 0.6	10.5 ± 2.4	29.1 ± 7.5
1	I-1	4.1 ± 0.7	10.5 ± 2.0	35.1 ± 5.7
2	None	2.9 ± 0.6	8.0 ± 2.2	18.4 ± 4.4
2	I-2	3.1 ± 0.5	10.8 ± 2.3	28.9 ± 4.5
2	I-3	3.6 ± 0.5	8.1 ± 1.4	28.9 ± 11.7

In study 1, rats were given a gastric dose of I-1 in vehicle or vechicle alone. In study 2, rats were fed chow containing I-2 or I-3 or chow alone for 2 weeks. Three hr after receiving I-1 and 20 hr after last access to I-2 or I-3 (the post-surgery recovery period), all rats were administered, intraduodenally, a lipid meal containing 25 mg of [4-^{14}C]cholesterol. Mesenteric lymph (6 hr pool) was analyzed for total and free cholesterol mass. Values are means ± SEM for n = 5–7 animals.

combined data have led to the suggestion that cholesterol load dictates the regulatory enzyme (7). Only one study is outside this hypothesis (8).

An alternative hypothesis, which attempts to explain the dual cholesterol esterase and ACAT requirements in cholesterol absorption (25), suggests that cholesterol esterase associated with the plasma membrane of absorptive cells supports cholesterol uptake which ACAT subsequently esterifies. However, isolated intestinal cells from intestine deprived of pancreatic juice (i.e., cholesterol esterase) for 72 hr take up cholesterol from mixed micelles as well as normal intestinal cells (14). Further, the hypothesized plasma membrane localization of cholesterol esterase does not agree with its localization as determined by immunocytochemistry (15). Moreover, this hypothesis does not fit the several observations (7; Heider, J., personal communication; Schaffer, S., personal communication) that suggest a role for ACAT only in cholesterol-fed animals or the present report which concludes that ACAT inhibition is unrelated to the absorption of exogenous cholesterol in the normal chow-fed rat.

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