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Rat Intestinal Acyl Coenzyme A: Cholesterol Acyl Transferase Properties and Localization (41931)

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Abstract. Microsomal acyl coenzyme A:cholesterol acyltransferase activity of rat intestinal mucosa was measured as the incorporation of [1-^{14}C]oleic acid into cholesterol esters. Exogenous cholesterol and sterol carrier protein singly and together extended the time over which the reaction rate was linear. Cholesterol esterification was suppressed by 2-monooleoyl glyceryl ether a 2-monoglyceride analog and potential substrate, but was unaltered by α-glycerophosphate or lysolecithin. Esterifying activity was lowest in microsomes from the intestinal segment 0–15 cm distal to the pylorus and highest in microsomes from the 30- to 45-cm segment. The total and specific activities of acyl coenzyme A:cholesterol acyltransferase were highest in cells isolated from the crypt zones. The location and level of activity were unaffected by diversion of either pancreatic juice or pancreatic juice and bile from the intestinal lumen. These findings question the physiological significance of acyl coenzyme A:cholesterol acyl transferase in the regulation of the absorption of exogenous cholesterol.

Intracellular esterification is considered a regulatory step in the absorption of luminal cholesterol. Seventy to ninety percent of the cholesterol taken up by absorptive cells from exogenous sources is secreted into the lymph as cholesterol ester (1). Though untested, it is probable that biliary cholesterol is esterified similarly. Three cholesterol esterifying systems have been described in intestinal mucosa: cholesterol esterase (CEase) (2) which is taken up from the intestinal lumen and whose source is pancreatic juice (3–5); acyl CoA: cholesterol acyltransferase (ACAT) (6–10); and cholesterol ester synthetase (CES) (11). Each has been implicated in the esterification of exogenous cholesterol.

CEase is a soluble fraction enzyme which catalyzes the esterification of cholesterol with free fatty acid at an optimum pH of 6.2. The enzymatic activity in mucosa is dependent upon pancreatic juice as the source of the enzyme (3–5) and upon normal enterohepatic circulation of bile to supply the obligatory requirement for taurocholic acid (12).

ACAT is a microsomal enzyme which catalyzes the esterification of cholesterol with acyl-CoA over the broad pH optimum of 6.4–7.2. Bile salts are reported to inhibit ACAT activity *in vitro* (8, 13).

CES catalyzes the esterification of cholesterol with free fatty acid at a pH of 6.2. The enzymatic activity in mucosa is independent of pancreatic juice but is dependent upon bile salts (11).

The purpose of the present study was to determine properties of rat intestinal ACAT relevant to its role in the esterification of exogenous cholesterol.

Materials and Methods. *Chemicals.* Isotopes which include [1-^{14}C]oleic acid (57.4 mCi/mmole) and [2-^{14}C]thymidine (55 mCi/mmole) were obtained from Amersham/Searle, Arlington Heights, Illinois. [^{3}H]Cholesterol oleate was prepared and purified as described by others (14, 15). Lipid standards for thin-layer chromatography were purchased from Nu-Chek Prep, Inc., Elysian, Minnesota. ATP, coenzyme A, fatty acid poor human serum albumin (HSA), oleic acid, and sodium taurocholate were obtained from Sigma Chemical Company, St. Louis, Missouri. Lysolecithin (lyso PC), α-glycerophosphate (α-GP), 2-monooleoyl glyceryl ether (2-ME), and cholesterol were obtained from Serdary Research Laboratories, London, Ontario. Betafluor, liquid scintillant, was obtained from National Diagnostics, Somerville, New Jersey. Uniplates were obtained from Analtech, Newark, Delaware.

Other chemicals and all solvents were reagent grade and were supplied by Fisher Scientific, Fair Lawn, New Jersey.

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Animals. Male Wistar rats weighing 200 to 250 g were obtained from Charles River Breeding Laboratories. The animals were housed in a room maintained at 22°C and were exposed to a 12-hr light–dark cycle. The animals had free access to standard laboratory chow (Ralston–Purina Co.) and water until the time of sacrifice except for those with pancreatic or pancreobiliary fistulas. These animals were fasted from the time of surgery but received water and electrolytes by duodenal infusion as described below.

Preparation of microsomes and assay of ACAT. Rats were sacrificed; the entire small intestine was removed, rinsed with 100 ml of iced 0.9% NaCl solution, and everted. The mucosa from the entire intestine or defined segments was scraped with a microscope slide onto the surface of an ice-filled Petri dish. Wet-weight mucosa was added (1 g/10 ml) to 0.278 *M* mannitol/3 m*M* imidazole, pH 7.4, for the preparation of a whole homogenate. The microsomal fraction was prepared from the homogenate as previously described (16), and the microsomal pellet was resuspended in 0.1 *M* potassium phosphate buffer, pH 7.4. In experiments employing isolated intestinal epithelial cells from intestinal segments, the microsomal fraction was prepared, in the same manner, from a homogenate of the cell pellet (1 g cells/10 ml). In these studies, which are consistent with those reported earlier (16), recovery of the microsomal fraction from the total homogenate was routinely high (~80%). Of the total ACAT, 77–83% was recovered in the microsomes, and the sum of the activity in the several organelles equaled that in the whole homogenate. The activity of ACAT in microsomes was determined by the rate of incorporation of [1-^{14}C]oleate into cholesterol ester as described by Balasubramaniam *et al.* (17) with minor modifications to extend the time over which the rate of cholesterol ester formation was linear. In the standard assay, 100–300 μg of microsomal protein was added to an incubation mixture in which the final volume was 0.5 ml and contained 0.1 *M* potassium phosphate, pH 7.4, 1.2 mg HSA, 2 m*M* ATP, 0.2 m*M* coenzyme A, 4 m*M* $MgCl_2 \cdot 5H_2O$, 250 μM [1-^{14}C]oleic acid (650,000 dpm), and 334 μM cholesterol. The cholesterol was added in 5 μl dioxane (freshly distilled):propylene glycol, (2:1 v/v) (18). These solvents alone had no effect on ACAT activity. Microsome-free reagent blanks were prepared. These and replicate assay mixtures containing microsomes were incubated in a shaker bath at 37°C. The reactions were terminated at 2, 3, and 4 min by the addition of 4 ml of chloroform:methanol (2:1 v/v). [^{3}H]Cholesterol oleate (20,000 dpm) was added to the extraction mixture as an internal standard to monitor and correct for procedural losses. To establish these standard conditions, the concentrations of cholesterol, oleic acid, and microsomal protein were varied within the 0.5-ml reaction volume as described later, and reactions were terminated at intervals from 2 to 15 min. In addition to a limited number of assays, sterol carrier protein, SCP_2, was added (30 μg). The lipids were extracted according to the procedure of Folch *et al.* (19), the solvent evaporated under nitrogen, and the lipid residue redissolved in 5 ml of hexane. Aliquots of the lipid extract were spotted on silica gel G precoated uniplates. The routine lipid classes were resolved in a solvent system of hexane, acetone, acetic acid (89:11:3 v/v), and lipid mixtures containing glyceryl ethers, specifically, ether analogs of di- and triglycerides were resolved in chloroform as reported earlier (20). Lipids were visualized with iodine vapor and identified with authentic standards. The silicic acid area corresponding to each lipid was scraped into a counting vial, 1 ml methanol and 10 ml Betafluor were added, and radioactivity was measured in a Beckman LS-250 liquid scintillation system. The quenching in the dual labeled samples was corrected by automatic quench compensation function in conjunction with the external standard–channels ratio method of quench calibration. Values for cholesterol [1-^{14}C]-oleate formed in the assays containing microsomes were corrected for background dpm measured in the microsome-free controls.

Cell preparation and purity. Cells were isolated from crypt and villus zones of proximal, middle, and distal thirds of small intestine as described by Weiser (21). Briefly, with buffers A and B as defined (21), the intestinal segments were incubated with buffer A at

37°C for 15 min and drained. Then the segments, with minimal handling, were filled with buffer B and incubated at 37°C for 4 min, drained and flushed with 80 ml buffer B, and the dislodged cells were designated fraction 1. The buffer B cycle was repeated eight times for time periods of 2, 2, 3, 4, 5, 7, 10, and 15 min and eight additional populations of cells were collected and designated fractions 2–9. Cells were pelleted by centrifugation (900*g*, 5 min). The cross-contamination of cells from the two zones was estimated with the crypt zone marker, thymidine kinase (22), and villus zone marker, alkaline phosphate (21). The marker enzymes were assayed in a 10% homogenate of each packed cell pellet. Based upon this estimate, a pool of fractions 1–5 was called "villus zone" epithelial cells and a pool of fractions 6–9 was called "crypt zone" epithelial cells. Marker enzyme assay on each pool of cells revealed an average of 20% contamination of villus cells with crypt cells, and an average of 17% contamination of crypt cells with villus cells. These marker enzymes were stable during cell isolation based upon a comparison of their total activity in combined cell pool homogenates with that in homogenates of fresh whole mucosa. Specifically, the marker enzyme activity in the cell pool homogenates ranged from 92 to 101% of that in the fresh mucosal homogenates. This was determined by two approaches. In the first, the entire small intestine was split lengthwise. The mucosa was obtained from one-half and the other half was subjected to the cell isolation procedure (the lengthwise segments were incubated in the buffers for the times indicated). In the second, alternating 15-cm segments of intestine were employed for cell isolation or for preparation of fresh whole mucosal homogenates. In both cases, a comparison was made between the total level of marker enzyme activity in the homogenates of combined cells and the homogenates of fresh whole mucosa. Additionally, the homogenate prepared from the submucosa, after epithelial cell removal, was devoid of marker enzyme activity.

Animal techniques. Surgery was performed on rats under pentobarbital anesthesia. Animals were sham operated (laparotomy) or prepared with either a pancreatic fistula or a combination bile and pancreatic fistula (3, 23). Bile and pancreatic juice were diverted to the outside by insertion of a cannula into the common duct at its entrance to the duodenum. To divert only pancreatic juice to the outside while maintaining normal bile flow to the intestine, an additional cannula was inserted into the common duct at the point of bifurcation close to the liver. This cannula was inserted through the intestinal wall and returned bile to the duodenum while the former cannula drained pancreatic juice to the outside. In all animals subjected to surgery, an infusion tube was inserted into the duodenum for fluid replacement. An aqueous solution of NaCl (0.9%) and KCl (0.03%) was infused at a rate of 3 ml/hr from surgery to sacrifice 48 hr later.

Other methods. Cholesterol mass in the microsomal fraction was determined enzymatically (24). Protein was determined by the method of Bradford (25).

Results. *Assay conditions.* To obtain maximum ACAT activity, the optimum assay conditions reported for liver (17) were tested in intestine as illlustrated in Fig. 1. With the concentrations of ATP, coenzyme A, Mg^{2+}, and albumin constant at levels described above, the concentrations of exogenous cholesterol, oleic acid, and microsomal protein were individually varied. In the presence of the optimum concentration of exogenous cholesterol, 334 μM (Panel A), the optimum concentration of oleic acid was 250 μM (Panel B), and the reaction rate was linear over the range of 50–450 μg of microsomal protein (Panel C). The addition of exogenous cholesterol had no effect upon the initial reaction rate but approximately doubled the time over which the reaction was linear (Panel D). Specifically, in the experiment shown, linearity was extended an additional 2 min by the addition of exogenous cholesterol. In other experiments, linearity was extended as much as an additional 7 min. This finding in intestine differs from that in hepatic microsomes where added exogenous cholesterol increased the initial reaction rate (18) and suggests that endogenous cholesterol is not saturating in the liver system. The addition of SCP_2 (30 μg) extended the linearity of the reaction both in the presence and absence of exogenous cholesterol. These results suggest

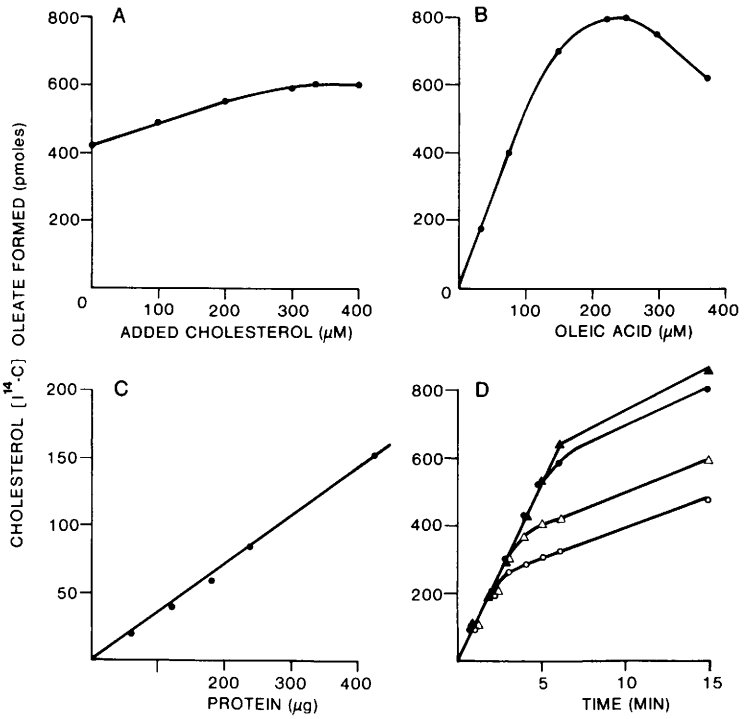

FIG. 1. Determination of optimum conditions for assay of acyl-CoA:cholesterol acyltransferase in intestinal microsomes. Each incubation flask contained in a total volume of 0.5 ml: 0.1 *M* potassium phosphate, pH 7.4; 2 m*M* ATP; 0.2 m*M* coenzyme A; 4 m*M* $MgCl_2 \cdot 5H_2O$; 1.2 mg HSA; and unless otherwise indicated 334 μM exogenous cholesterol, 250 μM [1-^{14}C]oleic acid, and 200 μg microsomal protein. (A) Exogenous cholesterol varied, each point represents the total cholesterol ester formed in the reaction, incubation time was 7.0 min. (rates not linear for 7.0 min at suboptimal concentrations of exogenous cholesterol); (B) oleic acid varied, incubation time was 3.5 min; (C) microsomal protein varied, incubation time was 1.0 min; (D) no exogenous cholesterol, O; 334 μM exogenous cholesterol, Δ; no exogenous cholesterol + 30 μg SCP_2, ●; 334 μM exogenous cholesterol + 30 μg SCP_2, ▲, incubation time as indicated.

that the available endogenous cholesterol is rate limiting, the exogenous cholesterol can provide additional substrate, and the SCP_2, consistent with its reported carrier functions (18), can increase the availability of both endogenous and exogenous cholesterol to the enzyme.

Substrate specificity. The effect of several lipid substrates on cholesterol esterification was studied. The selected substrates like cholesterol are esterified by microsomal enzyme systems which have a requirement for acyl coenzyme A. As shown in Table I cholesterol esterification decreased in a dose-dependent manner upon addition of 2-ME, the stable ether analog of 2-monoolein. Neither α-GP nor lyso PC (not shown) inhibited cholesterol esterification (ester formed at rates of 242 and 271 pmole/min/reaction, respectively) when added to the incubation mixture at these same levels (0.025–0.15 μmole). The data suggest that the 2-ME may compete with cholesterol for effective esterification in microsomes.

Localization. The activity of ACAT was measured in microsomes prepared from homogenates of mucosa obtained from six successive 15-cm segments of intestine removed in series from pylorus to cecum. These measurements, shown in Table II, reveal that ACAT total activity and specific activity were lowest in the first 15-cm segment and were highest in the third segment, 30–45 cm below the stomach.

The activity of ACAT was measured also in microsomes prepared from homogenates

TABLE I. EFFECT OF 2-ME ON CHOLESTEROL ESTERIFICATION

2-ME[a] (μmole)	[1-^{14}C]Oleate incorporation[b] into CE (pmole/min)	[1-^{14}C]Oleate incorporation[b] into DG_a + TG_a (nmole/min)
0	150 ± 15	0
0.025	123 ± 8	1.8 ± 0.4
0.050	86 ± 10	3.2 ± 0.5
0.10	55 ± 10	2.8 ± 0.3
0.15	32 ± 5	3.0 ± 0.2

Note. Animals were offered chow and water *ad libitum* until sacrifice. Each incubation flask contained in a total volume of 0.5 ml: 0.1 *M* potassium phosphate, pH 7.4; 250 μ*M* [1-^{14}C]oleic acid; 334 μ*M* exogenous cholesterol; 2 m*M* ATP; 0.2 m*M* coenzyme A; 4 m*M* $MgCl_2 \cdot 5H_2O$; 1.2 mg HSA; the indicated level of 2-ME added in 5 μl diethyl ether; and 178–210 μg microsomal protein. The level of endogenous cholesterol ranged from 14.4 to 15.1 μg per reaction. The ACAT initial reaction rate was determined by a 3-point assay run in duplicate.

[a] Abbreviations: 2-ME = 2-monooleoyl glyceryl ether; DG_a = 1,2-diglyceride analog; TG_a = triglyceride analog; CE = cholesterol ester.

[b] The results represent the means of four experiments ±SEM.

these conditions, the properties and localization of ACAT were studied in relationship to its suggested role in the esterification and absorption of exogenous cholesterol.

First, lipids that undergo fatty acyl-CoA-dependent esterification in the microsomes and which are present simultaneously with cholesterol during lipid absorption were tested as potential competing substrates with cholesterol in the ACAT assay. The data demonstrate that 2-monoether, the stable ether analog of 2-monoglyceride, inhibits cholesterol esterification (Table I) whereas α-GP and lyso PC do not (not shown). The observed 2-ME inhibition may be explained in several ways: (a) the detergent properties of 2-ME may be deleterious to ACAT which is known to be inhibited by bile salts (9) possibly through detergent action; (b) the 2-ME may compete with cholesterol for the fatty acyl-CoA generated in the system; and (c) the 2-ME may compete with cholesterol for or affect its binding to a site on the esterification enzyme. A choice cannot be made among

of cells obtained from villus and crypt zones of proximal, middle, and distal thirds of intestine. These measurements shown in Table III reveal a higher specific and markedly higher total activity in the crypt zone cells from each third of intestine (crypt cells contained higher levels of microsomal protein).

Effect of pancreatic juice and pancreobiliary diversion on ACAT activity. The effect of the diversion of pancreatic juice or of bile and pancreatic juice on ACAT activity was determined. ACAT activity was measured in microsomes prepared from the mucosa taken from the proximal, middle, and distal thirds of intestine 48 hr after surgical diversions. As shown in Table IV, neither pancreatic juice diversion nor pancreobiliary diversion affected ACAT activity at any intestinal site.

Discussion. In the present study the conditions were optimized for the measurement of ACAT activity (incorporation of [1-^{14}C]oleic acid into cholesterol esters) in microsomes prepared from homogenates of rat intestinal mucosa. In the standard assay, ACAT specific activity was approximately 500 pmole/min/mg protein and comparable to that reported by others (7, 31). Under

TABLE II. DISTRIBUTION OF ACAT ACTIVITY IN INTESTINAL SEGMENTS

Segment (cm)	ACAT activity: Total (nmole/min/segment)	ACAT activity: Specific (nmole/min/mg)
0–15	4.3	0.21
15–30	11.9	0.71
30–45	19.4	0.83
45–60	10.0	0.60
60–75	6.4	0.45
75–90	4.6	0.39

Note. Animals were offered chow and water *ad libitum* until sacrifice. Each incubation flask contained in a total volume of 0.5 ml: 0.1 *M* potassium phosphate buffer, pH 7.4; 250 μ*M* [1-^{14}C]oleic acid; 334 μ*M* exogenous cholesterol; 2 m*M* ATP; 0.2 m*M* coenzyme A; 4 m*M* $MgCl_2 \cdot 5H_2O$; 1.2 mg HSA; and 129–196 μg microsomal protein. The level of endogenous cholesterol varied from 11.4 to 12.9 μg per reaction. Each segment is identified by a measured distance from the pylorus. Total activity is expressed as the nmole cholesteryl ester formed/min/segment. Specific activity is expressed as nmole cholesteryl ester formed/min/mg microsomal protein. The distribution of ACAT activity is typical of three separate experiments. The average percentage distributions of ACAT activity in each successive segment are 6.1 ± 1.9, 22.6 ± 4.6, 35.0 ± 5.1, 17.0 ± 4.0, 11.1 ± 3.4, and 8.3 ± 2.4 (n = 3).

TABLE III. DISTRIBUTION OF ACAT ACTIVITY IN CRYPT AND VILLUS CELLS

Intestinal segment[a]	Cell type	ACAT activity Total (nmole/min)	ACAT activity Specific (nmole/min/mg)	Enzyme distribution (%)[b] ACAT	T.K.	A.P.
Proximal	Crypt	4.0	0.33	84	89	20
	Villus	1.2	0.27	16	11	80
Middle	Crypt	8.7	0.72	88	79	12
	Villus	1.2	0.48	12	21	88
Distal	Crypt	1.6	0.16	93	71	20
	Villus	0.16	0.11	7	29	80

Note. Animals were offered chow and water *ad libitum* until sacrifice. Each incubation flask contained in a total volume of 0.5 ml: 0.1 *M* potassium phosphate buffer, pH 7.4; 250 μM [1-^{14}C]oleic acid; 334 μM exogenous cholesterol; 2 m*M* ATP; 0.2 m*M* coenzyme A; 4 m*M* $MgCl_2 \cdot 5H_2O$; 1.2 mg HSA; and 168–190 μg microsomal protein. The level of endogenous cholesterol ranged from 13.9 to 14.7 μg per reaction. Total activity is expressed as the nmole cholesteryl ester formed/min/microsomes from the total crypt or villus cell population in segment. Specific activity is expressed as nmole/min/mg microsomal protein. The localization of ACAT is typical of three separate experiments. The average percentage distributions of ACAT activity in crypt cells from proximal, middle, and distal segments are 85 ± 10, 86 ± 9, and 88 ± 10, respectively, and in villus cells from proximal, middle, and distal segments are 15 ± 3, 13 ± 5, and 8 ± 4, respectively (n = 3).

[a] Segment = ~32 cm.

[b] Abbreviations: T.K. = thymidine kinase; A.P. = alkaline phosphatase.

these possibilities. Moreover, kinetic studies which could explain the results are difficult since ACAT activity depends upon a pool of endogenous substrate which is of indefinite size and not easily manipulated. However, the data indirectly suggest that 2-ME inhibition of cholesterol esterification either by detergent action or through competition for

TABLE IV. EFFECT OF PANCREATIC JUICE OR PANCREOBILIARY DIVERSION ON ACAT ACTIVITY

Animal treatment	Intestinal segment[a]	ACAT activity[b] Total (nmole/min/segment)	ACAT activity[b] Specific (nmole/min/mg)
Control-sham operated	Proximal	9.7 ± 0.2	0.46 ± 0.01
	Middle	15.8 ± 0.7	0.72 ± 0.08
	Distal	8.0 ± 0.3	0.42 ± 0.03
Pancreatic juice diversion	Proximal	10.1 ± 0.2	0.54 ± 0.02
	Middle	15.0 ± 0.4	0.75 ± 0.07
	Distal	8.1 ± 0.2	0.39 ± 0.01
Pancreatic juice and bile diversion	Proximal	9.8 ± 0.3	0.51 ± 0.02
	Middle	14.3 ± 0.8	0.83 ± 0.08
	Distal	7.7 ± 0.4	0.42 ± 0.03

Note. Rats were offered chow and water *ad libitum* until surgery. From surgery to sacrifice (48 hr) rats were infused duodenally (3 ml/hr) with a maintenance solution containing NaCl (0.9%) and KCl (0.03%). Each incubation flask contained in a total volume of 0.5 ml: 0.1 *M* potassium phosphate buffer, pH 7.4; 250 μM [1-^{14}C]oleic acid; 334 μM exogenous cholesterol; 2 m*M* ATP; 0.2 m*M* coenzyme A; 4 m*M* $MgCl_2 \cdot 5H_2O$; 1.2 mg HSA; and 137–178 μg microsomal protein. The level of endogenous cholesterol ranged from 11.7 to 12.8 μg per reaction. Total activity is expressed as nmole cholesterol ester formed/min/segment. Specific activity is expressed as nmole cholesterol ester formed/min/mg microsomal protein.

[a] Segment = ~32 cm.

[b] The results represent the means of 4 or 5 experiments ± SEM.

acyl-CoA is unlikely. Specifically, comparable concentrations of lyso PC, also a detergent, had no inhibitory effect on cholesterol esterification, and α-GP which incorporated 77% as much fatty acid into glycerides as did 2-ME (3.0 nmole vs 2.3 nmole) was, likewise, an ineffective inhibitor of cholesterol esterification. From a physiological viewpoint, exogenous cholesterol presented to the intestine for absorption is generally accompanied by a large excess of monoglycerides. Whether the monoglycerides compete for the cholesterol esterification enzyme (ACAT) available in absorptive cells and/or for the activated fatty acid, they are potential inhibitors of ACAT catalyzed cholesterol esterification *in vivo*. In fact, this finding may explain, in part, the 50% decrease in ACAT activity observed in rat intestine following the perfusion of moderately high levels of triolein (28).

Second, the localization of ACAT in regions of intestine from proximal to distal end and in cells from the villus and crypt zones in these regions was determined. The region of intestine from 15 to 60 cm below the pylorus contained 73% of the total intestinal ACAT. This intestinal region coincides broadly with that reported active in cholesterol absorption despite variation in the absorptive site with the mode of administration and physical form of the cholesterol meal. In particular, the proximal and distal halves of rat intestine are reported (27) to absorb exogenous cholesterol delivered by constant perfusion equally well with major absorption occurring in the 50–75-cm region. While in rats given a single bolus of emulsified exogenous cholesterol as in the present study, the 30- to 45-cm segment is reported (26) most active in absorption. Similarly, in rats given a single bolus of micellar cholesterol, only the proximal half of intestine absorbs (29). Not only does ACAT distribution in the intestine overlap with the reported sites of cholesterol absorption but also agrees broadly with the ACAT-rich regions reported by others in chow-fed rats. That is, of the total ACAT about 80% compared to 86% (6) is localized in the proximal two-thirds of intestine.

Further localization of ACAT to cell type within the proximal, middle, and distal thirds of intestine reveals a small percentage (10–20%) of the total ACAT in villus cells. Our finding in the chow-fed rat agrees with that reported for the chow-fed rabbit (7) but is in disagreement with two studies in chow-fed rats. In one such study (30) 62% of the total intestinal ACAT was present in villus cells with 38% in crypts and in the same report with a different set of chow-fed rats the villus cell ACAT reached 90% of the total intestinal ACAT. In another study (31) the average ACAT specific activity determined for pooled cell preparations from several rats was approximately 8-fold higher in villus than in crypt cells, and although total ACAT activity for the two cell populations was not given, villus cell microsomes contained 20-fold more protein than the crypt cell microsomes when studied in a single rat intestine.

The cell preparations in the present study and from the differing study (30) were carefully characterized. Moreover, in the present report, marker enzyme recovery studies allowed for reliable calculation of total cell recovery and cross-contamination of cell types. Therefore, reasons for differences in the cellular distribution of ACAT are not readily apparent. Possibly the larger percentage of total cellular protein associated with crypt zone cells and the dependence of the ACAT assay upon a fatty acyl-CoA generating system in this study influence the results. Further, cholesterol plus corn-oil feeding is reported to increase ACAT specific activity in rabbit villus cells (7), in rat villus cells, (31) and in guinea pig intestinal microsomes (9, 10). Thus, the villus cell ACAT activity may represent a minimum level in the present study.

Finally, the surgical diversion of pancreatic juice or of pancreatic juice and bile from the intestine has no effect upon intestinal ACAT activity. Both secretions are required for efficient esterification and substantial absorption of cholesterol from a lipid meal in the lymph fistula rat (1, 3, 32). In this respect ACAT differs from CES which requires bile salt (11), and CEase which requires bile salt for activation and pancreatic juice as the enzyme source (1, 3, 32).

Cholesterol absorption studies in the rat are not consistent with respect to the importance of ACAT. In one study, the inhibition

of intestinal ACAT reduced the absorption of exogenous cholesterol (33) and in another, the absorption of exogenous cholesterol was reduced in intestine deficient in CEase even though ACAT levels were normal (32). In addition, studies in the rat and rabbit are not consistent with respect to the relationship of diet to the ACAT activity–cholesterol absorption correlate. Inhibitors of ACAT reduce cholesterol absorption in the chow-fed rat (33) and the cholesterol-fed (1%) rabbit (34) but are ineffective in the chow-fed rabbit (34). Thus further study is necessary to clarify the relationship of the esterification enzymes to the absorption process. This remains an important problem since the evidence is strong that dietary cholesterol plays a causal role in the pathogenesis of coronary heart disease in man. In the present study the location of ACAT in the intestine and its susceptibility to 2-ME inhibition raise the question of its quantitative role in the absorption of exogenous cholesterol.

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