Cholesterol absorption in rat intestine: role of cholesterol esterase and acyl coenzyme A:cholesterol acyltransferase

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Abstract Cholesterol esterase of pancreatic juice origin and acyl coenzyme A:cholesterol acyltransferase, both associated with the intestinal mucosa, are implicated in the extensive esterification of exogenous cholesterol during absorption. To assess the role of each enzyme, [4^{14}-C]cholesterol absorption into mesenteric lymph of rats with normal mucosal levels of both esterification enzymes was compared with that of rats with normal acyl coenzyme A:cholesterol acyltransferase activity but deficient cholesterol esterase activity. The cholesterol esterase deficiency was accomplished by either surgical diversion of the pancreatic juice from the intestinal lumen or removal by specific immunoprecipitation of cholesterol esterase from the otherwise complete pancreatic juice. In the rats that were transferase-complete and esterase-deficient, cholesterol absorption into lymph and esterase activity in the mucosa were decreased an average of 83% and 75%, respectively, compared with rats complete with both esterification enzymes. Of the absorbed [4-^{14}C]cholesterol in all rats, 82–90% was esterified and the mucosal levels of cholesterol esterase, even in the esterase-deficient rats, could readily account for this esterification. Because transferase activity was normal in rat intestine in which cholesterol esterase was deficient and cholesterol absorption was inhibited, transferase alone does not support the absorption of exogenous cholesterol in the absence of esterase. These results reconfirm the importance of esterification in the absorption of exogenous cholesterol and demonstrate that cholesterol esterase plays an essential role in the regulation of the absorption process.—**Gallo, L. L., S. Bennett Clark, S. Myers, and G. V. Vahouny.** Cholesterol absorption in rat intestine: role of cholesterol esterase and acyl coenzyme A:cholesterol acyltransferase. *J. Lipid Res.* 1984. **25:** 604–612.

Supplementary key words cholesterol esterification • dietary cholesterol • lymph

Cholesterol absorbed from the intestinal lumen undergoes an extensive esterification in the intestinal wall and then appears largely as cholesteryl ester in the lymph lipoproteins (1). This esterification is thought to regulate the absorption of luminal sources of cholesterol. There are two major cholesterol esterification enzymes in the small intestine, cholesterol esterase (CE'ase) (2) and acyl coenzyme A:cholesterol acyltransferase (ACAT) (3). Each has been implicated in the esterification and absorption of exogenous cholesterol. An early study (4) demonstrated that mucosal CE'ase activity and cholesterol absorption are both decreased in pancreatic fistula rats, and it was suggested that mucosal CE'ase is derived from the CE'ase of pancreatic juice (4). This observation supported and extended earlier reports of a stimulatory role for pancreatic juice or pancreatic tissue components in cholesterol absorption (5–8). In subsequent studies, the pancreatic juice origin of mucosal CE'ase and the uptake of the CE'ase into the absorptive villus (but not crypt) cells have been shown (9, 10). Recently, in addition to this suspected intracellular role of CE'ase in cholesterol esterification, it has been suggested (11) that pancreatic CE'ase, present on the cell surface membranes, may also facilitate cholesterol uptake by the intestine.

ACAT is endogenous to the intestine and its presence is reported in cells isolated from both the villus (absorptive cells) and crypt zones (12, 13). ACAT activity is responsive to cholesterol and fat feeding (12, 14, 15). Both CE'ase and ACAT are found in the intestine of many species including humans (1, 3, 16–18). The purpose of the present research was to assess the relative role of each enzyme, CE'ase and ACAT, in the intestinal esterification and lymphatic absorption of exogenous cholesterol in the rat.

MATERIALS AND METHODS

Experimental protocol

Male rats of Wistar strain (Charles River Breeding Laboratories, Wilmington, MA) weighing 220–310 g at

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

surgery were housed in cages for 2 weeks after receipt and allowed free access to laboratory chow (Ralston-Purina Co., St. Louis, MO) and water. The temperature of the animal room was maintained at 22°C and the lights were operated on a 12-hr light-dark cycle.

A general account of the protocol follows and a precise description of each stage is given under the appropriate headings. At time zero, rats under pentobarbital anesthesia were surgically equipped with: a common bile duct cannula which diverted bile (in particular, biliary cholesterol and bile salts) and pancreatic juice (in particular CE'ase) from the intestinal lumen; a duodenal infusion tube for both constant infusion of a maintenance mixture containing bile salt, glucose, electrolytes, and water and for administration of the [4-^{14}C]cholesterol meal; and a mesenteric lymph cannula for collection of lymph to be monitored for cholesterol absorption. After surgery, the rats were placed in Bollman-type restraint cages (19). Thirty-six hours after surgical diversion of the pancreatic juice, the intestinal mucosa is deficient in CE'ase (4, 7). At this time, the following additions were made to the maintenance infusion: Group 1 received untreated pancreatic juice from donor rats, thus repleting the intestine with CE'ase; Group 2 received no additions to the infusate and the intestine was maintained CE'ase-deficient. Group 3 received donor pancreatic juice from which CE'ase had been immunoprecipitated by treatment with anti-CE'ase; this exposes the intestine to pancreatic juice deficient only in CE'ase. Group 4 received donor pancreatic juice treated with control IgG; this repletes the intestine with CE'ase and provides a control for Group 3. Twelve hours after infusing the maintenance mixture with these additions, the infusion was interrupted briefly (15 min) to administer, via the duodenal infusion tube, a lipid meal containing [4-^{14}C]cholesterol. During the next 6 hr the respective duodenal infusions were continued and lymph was collected in tubes on ice. The animals were killed by cervical dislocation, the small intestine was removed, and the mucosa was collected for assay of the esterification enzymes, CE'ase and ACAT.

Surgical procedures

Pancreatic juice donor rats were prepared with separate bile and pancreatic juice fistulas and a duodenal infusion tube. Rats in the experimental groups were prepared with a common bile and pancreatic juice fistula, a duodenal infusion tube, and a mesenteric lymph fistula. Procedurally, as described in detail elsewhere (4), bile and pancreatic juice together were diverted to the outside by insertion of a cannula into the common duct at its entrance into the duodenum. To collect pancreatic juice separate from bile, an additional cannula to divert bile was inserted into the common duct at the point of bifurcation close to the liver. The duodenal infusion tube and mesenteric lymph fistula were inserted in the manner and with the materials previously described (4, 20).

Maintenance infusion

The infusion mixture contained: sodium chloride, 0.9%; potassium chloride, 0.03%; glucose, 5.0%; and sodium taurocholate (Sigma Chemical Co., St. Louis, MO) 10 mM. The components were dissolved in distilled water. All animals were infused (Harvard infusion pump, Model 940, Harvard Apparatus Co., Inc., South Natick, MA) from the time of surgery to killing at a rate of 3.0 ml/hr.

Pancreatic juice treatment

Pancreatic juice from donor rats was collected in iced tubes (15 ml capacity) containing soybean trypsin inhibitor (Washington Biochemical Corp, Freehold, NJ), 1 mg/tube, and stored at 4°C. CE'ase activity was assayed as described below. A pool of juice containing CE'ase with an activity of 24 units/ml was subdivided: 84 ml was reserved as untreated juice and two additional 84-ml volumes were treated with 195 mg of either control or immune IgG. (One unit = 1 μmol of cholesterol converted to cholesteryl ester/hr.) The immune IgG was raised in rabbits against purified rat pancreas CE'ase. The control IgG was isolated from the sera obtained from the same rabbits prior to immunization (21). Both immune and control IgG were purified from rabbit sera as previously described (21). The quantity of immune IgG required to immunoprecipitate the CE'ase was determined by immunotitration as reported earlier (21). To insure the complete precipitation of the antigen-antibody complex, 488 mg of second antibody, i.e., goat anti-rabbit IgG (Cappel Laboratories, Lutherville, MD) were added, mixed, and incubated with the CE'ase-anti CE'ase digest for 6 hr at 4°C. A similar addition of second antibody was made to the pancreatic juice treated with control IgG. In both cases, the immune precipitates (CE'ase + immune IgG + 2nd antibody and control IgG + 2nd antibody) were removed by centrifugation. The CE'ase activity remaining in the pancreatic juice treated with immune IgG was 1 unit/ml and that in the juice treated with control IgG was 22 units/ml. The untreated or the treated juice was added to the maintenance infusion in place of an equivalent volume of distilled water. Specifically, each 3.0 ml of infusate delivered 0.8 ml of juice. Thus the quantity of maintenance infusion components delivered to the lumen per hour for the duration of the experiment was constant. Physiological levels of CE'ase activity were delivered to the lumen based upon an average juice flow rate of 0.4 ml/hr with an average CE'ase

activity of 50 units/ml in the fresh juice collected from a pancreatic fistula rat.[1]

Lipid meal

A lipid meal was prepared in emulsion form; it contained 1 μCi of [4-^{14}C]cholesterol (sp act 54 mCi/mmol; Amersham Searle, Arlington Heights, IL); 25 mg of cholesterol (Serdary Research Laboratories, London, Ontario); 145 mg of oleic acid; 144 mg of sodium taurocholate; and 25 mg of BSA (each from Sigma Chemical Co., St. Louis, MO) per 3.0 ml of isotonic NaCl solution. The meal was infused intraduodenally over a 15-min period.

Lymph analyses

Lymph was collected in iced graduated cylinders which contained the LCAT inhibitor, DTNB, 14.3 mg (Eastman Kodak Co., Rochester, NY) and heparin, 6.3 mg (Sigma Chemical Co.). 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 (22). 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 used as 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 a Silica Gel G precoated thin-layer chromatography plate (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 (23). 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). 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; it was rinsed with 100 ml of iced 0.9% NaCl, everted, and the mucosa was scraped with a glass slide onto the surface of an ice-filled Petri dish. Wet weight mucosa was diluted 5-fold (w/v) with 0.278 M mannitol–3 mM imidazole, pH 6.2, and homogenized in a Potter-Elvehjem homogenizing tube to yield a 20% homogenate. One aliquot was extracted directly with chloroform–methanol (22) to monitor the level of labeled test meal cholesterol associated with the mucosa; another aliquot was assayed for CE'ase activity as described below; and the remainder was diluted in 0.278 M mannitol to yield a 10% homogenate from which the microsomal fraction was prepared as described previously (24). The microsomal pellet was resuspended in 0.1 M potassium phosphate buffer, pH 7.4, to yield a 30% microsomal suspension based upon the original wet weight mucosa. In some experiments as indicated, the nuclei and cell debris fraction and mitochondria fraction were similarly resuspended, and the soluble fraction was retained. In these cases, all fractions were assayed for ACAT activity, and ACAT distribution among the fractions was calculated.

The activity of ACAT in microsomes, and in the other subcellular fractions when performed, was determined by the rate of incorporation of [1-^{14}C]oleic acid, (sp act, 57.4 mCi/mmol; Amersham Searle) into cholesteryl ester as described by others (25), except that exogenous cholesterol was added to extend the incubation time over which the rate of cholesteryl ester formation was linear. In the standard assay, 25 μl of a 30% microsomal or other subcellular organelle suspension 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 of BSA, 2 mM ATP (Sigma Chemical Co.), 4 mM $MgCl_2 \cdot 5H_2O$, 250 μM [1-^{14}C]oleic acid (650,000 dpm), and 334 μM cholesterol added in 5 μl of dioxane (freshly distilled)–propylene glycol 2:1 (v/v) (26). These solvents alone had no effect on ACAT activity. The assay mixture was in-

[1] Gallo, L. L., and G. V. Vahouny. Unpublished observations.

cubated in a shaker bath at 37°C and the reaction was terminated by addition of 4 ml of chloroform–methanol 2:1 (v/v). Replicate incubations were stopped at 2, 3.5, and 5 min. These timed points fell on the linear region of the rate curve. [^{3}H]Cholesteryl oleate (20,000 dpm), prepared and purified by thin-layer chromatography just prior to use as described by others (27, 28), was added to the extraction mixture as the internal standard to correct for losses (found to be <10%). All glassware employed in the ACAT assay and in lipid extraction was siliconized to maximize cholesteryl ester recovery. Zero-time controls were included and also incubations containing buffer but no microsomes. ACAT specific activity obtained with this oleoyl CoA-generating system was 538 ± 38 pmol/min per mg (mean ± SEM, n = 14) and is consistent with that reported by others (12, 15).

The activity of CE'ase in whole homogenates of mucosa was assayed as previously described (29), i.e., by determining the rate of incorporation of [4-^{14}C]cholesterol into cholesteryl ester. Briefly, 1.0 ml of a 20% mucosal homogenate was added to an incubation mixture in which the final volume was 2.0 ml and contained 7.75 mM [4-^{14}C]cholesterol (500,000 dpm, 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. The assay mixture was incubated in a shaker bath at 37°C for 5 hr and the reaction was stopped by the addition of 40 ml of chloroform–methanol 2:1 (v/v). In both enzyme assays, the lipids were extracted, the cholesterol and cholesteryl ester were separated by thin-layer chromatography, and their radioactivity was monitored as described above. For the dual-labeled samples from the ACAT assay, the automatic quench compensation function was used in conjunction with the external standard–channels ratio method of quench calibration.

In addition, a control experiment was performed to determine the contribution to cholesteryl ester formation of the [4-^{14}C]cholesterol from the lipid meal which remained associated with either the whole homogenate (CE'ase source) or the microsomal fraction (ACAT source) at the end of the absorption period. Standard assays for both enzymes were performed as described above, except that in the ACAT assay the oleic acid substrate was unlabeled and in the CE'ase assay the cholesterol in the substrate emulsion was unlabeled. The tissue-associated [4-^{14}C]cholesterol contributed less than 1% to the total cholesteryl ester formed in either assay. Thus, no corrections were applied when the cholesteryl ester product was calculated.

Other assays

Cholesterol (30) and protein (31) in the microsomal fraction were determined colorimetrically.

Calculations

Statistical significance of the data between the control and experimental groups was determined by the Student's *t*-test (Group 1 vs. Group 2 and Group 4 vs. Group 3).

RESULTS

Characteristics of mucosal microsomes

The key study was designed to determine the relative importance of mucosal CE'ase of pancreatic juice origin and ACAT in the esterification and absorption of exogenous cholesterol. A component of this comparison was the assay of ACAT activity in mucosal microsomes, the organelle traditionally monitored. Microsomes from the four experimental groups were characterized for consistency in their content of endogenous cholesterol, protein, and residual [4-^{14}C]cholesterol derived from the lipid meal. In addition, the percentage of the whole homogenate ACAT activity recovered in the microsomal fractions was determined as shown in **Table 1.** The cholesterol

TABLE 1. Characterization of microsomal preparations

Group[a]	Addition to Maintenance Infusion	Free Cholesterol[b]	Protein[b]	[4-^{14}C]Cholesterol Content[b]	Fraction[c] of Total ACAT
		μg	*μg*	*dpm*	%
1	Pancreatic juice	3.3 ± 0.2	95.4 ± 8.9	75 ± 19	80
2	None	3.4 ± 0.3	97.9 ± 4.2	72 ± 18	79
3	Immune IgG-treated pancreatic juice	3.3 ± 0.3	97.5 ± 11.3	40 ± 10	77
4	Control IgG-treated pancreatic juice	2.8 ± 0.6	103.9 ± 7.2	42 ± 3	81

[a] Intestines surgically deprived (36 hr) of pancreatic juice (CE'ase) were repleted with CE'ase in Groups 1 and 4 and maintained deficient in CE'ase in Groups 2 and 3. All groups were administered intraduodenally a lipid meal containing 25 mg of [4-^{14}C]cholesterol. Microsomes were prepared as described under Materials and Methods. Cholesterol, protein, and [4-^{14}C]cholesterol content are given for 25 μl of a 30% microsomal suspension, the volume added to each ACAT assay. ACAT activity was assayed as described under Materials and Methods.

[b] Values are means ± standard error for four animals, except Group 4 with two animals.

[c] Represents the fraction of ACAT activity in the microsomal preparation relative to the ACAT activity of the whole homogenate. This determination was made for one intestine per group.

(isotope and mass) and protein content did not differ significantly among the control and experimental groups and the percentage recovery of ACAT activity in the microsomes consistently ranged from 77 to 81%. These results demonstrate that comparable microsomal preparations were employed in the study.

Cholesterol absorption in CE'ase-deficient intestine

The lymphatic absorption of a duodenal bolus of exogenous [4-^{14}C]cholesterol under conditions where both esterification enzymes displayed normal in vitro activity and under conditions where ACAT alone displayed normal activity and CE'ase was deficient is shown in **Table 2.** A comparison of Group 1 rat intestines, repleted with CE'ase by infusion of pancreatic juice, with Group 2 intestines, deficient in CE'ase and receiving no juice, revealed a substantial decrease in [4-^{14}C]cholesterol absorption into lymph during the 6-hr period (5.2 ± 1.6 vs. 0.9 ± 0.1 μmol; $P < 0.025$) with concomitant decreases in CE'ase activity (8.3 ± 1.8 vs. 2.2 ± 0.2 μmol of CE/intestine per 6 hr, $P < 0.005$) and lymph [4-^{14}C]cholesteryl ester (4.6 ± 1.5 vs. 0.8 ± 0.1 μmol; $P < 0.05$). In these same intestines, ACAT activity was unaltered (3.1 ± 0.3 vs. 2.6 ± 0.3 μmol of CE/intestine per 6 hr). Similarly, a comparison of Group 4 intestines, which were repleted with CE'ase by influsion of control IgG-treated pancreatic juice, with Group 3 intestines, deficient in CE'ase and receiving immune IgG-treated pancreatic juice (CE'ase-deficient juice), revealed comparable decreases in [4-^{14}C]cholesterol absorption into lymph during the 6-hr period (6.8 ± 1.4 vs. 1.1 ± 0.2 μmol; $P < 0.005$), CE'ase activity (6.0 ± 1.3 vs. 1.5 ± 0.04; $P < 0.005$), and lymph [4-^{14}C]cholesteryl ester (6.0 ± 1.5 vs. 0.9 ± 0.2; $P < 0.005$). Again ACAT activity was unchanged (2.6 ± 0.5 vs. 2.9 ± 0.8). In other words, [4-^{14}C]cholesterol absorption was decreased a minimum of 83% in rat intestine which was ACAT-complete and CE'ase-deficient, i.e., where CE'ase was decreased an average of 75%. This decrease in [4-^{14}C]cholesterol absorption is accounted for by a decrease of between 80 and 90% in the mass of esterified [4-^{14}C]cholesterol. Clearly, the decrease in cholesterol absorption is related to the decrease in mucosal CE'ase activity.

Moreover, the activity of total mucosal CE'ase can account potentially for the total [4-^{14}C]cholesteryl ester transported into lymph of individual rats (**Table 3**). The validity of this comparison is based upon the report (32) that exogenous cholesterol is absorbed equally well by proximal and distal halves of rat intestine, a finding supported in the present study where mucosal homogenates prepared from proximal, middle, and distal thirds of intestine contain nearly equivalent amounts of [4-^{14}C]cholesterol from the lipid meal. Even if the proximal three-fourths of intestine is the most active in cholesterol uptake as reported by others (33), the conclusion is the same inasmuch as CE'ase activity derived from only the proximal and middle thirds of intestine (80% of the total intestine CE'ase) can account for the [4-^{14}C]cholesteryl ester transported into lymph. This conclusion is true both for rat intestines (Groups 1 and 4) which have normal in vitro levels of CE'ase and ACAT and which are transporting substantial amounts of cholesteryl ester, and for intestines (Groups 2 and 3) which are deficient in CE'ase and transporting greatly reduced amounts of cholesteryl ester. For example, in Group 1-rat #1, complete with ACAT and CE'ase, 4.2 μmol of [4-^{14}C]cholesteryl ester is absorbed and the CE'ase activity in the intestine of that animal could potentially esterify 5.8 μmol of cholesterol. Similarly, in Group 3-rat #1, complete with

TABLE 2. Relationship between [4-^{14}C]cholesterol absorption and esterification enzyme activities (CE'ase and ACAT)

Group[a]	Additions to Maintenance Infusion	Cholesterol [4-^{14}C][b]		Esterification Enzyme Activity[b]	
		Absorbed	Esterified	CE'ase	ACAT
		μmol[c]		*μmol CE/intestine per 6 hr*[d]	
1	Pancreatic juice	5.2 ± 1.6	4.6 ± 1.5	8.3 ± 1.8	3.1 ± 0.3
2	None	0.9 ± 0.1	0.8 ± 0.1	2.2 ± 0.2	2.6 ± 0.3
3	Immune IgG-treated pancreatic juice	1.1 ± 0.2	0.9 ± 0.2	1.5 ± 0.04	2.9 ± 0.8
4	Control IgG-treated pancreatic juice	6.8 ± 1.4	6.0 ± 1.5	6.0 ± 1.3	2.6 ± 0.5

[a] Intestines surgically deprived (36 hr) of pancreatic juice (CE'ase) were repleted with CE'ase in Groups 1 and 4 and maintained deficient in CE'ase in Groups 2 and 3. All groups were administered intraduodenally a lipid meal containing 25 mg of [4-^{14}C] cholesterol. Intestinal lymph, 6-hr pool, was analyzed for cholesterol absorbed and esterified. CE'ase activity was assayed in 20% mucosal homogenates and ACAT activity was assayed in 30% microsomal suspensions.
[b] Values are means ± SEM for four animals, except Group 4 with two animals.
[c] Micromoles of cholesterol absorbed/intestine per 6 hr.
[d] Micromoles of cholesterol esterified/intestine per 6 hr.

TABLE 3. Potential of intestinal CE'ase and ACAT to esterify exogenous cholesterol[a]

Group[b]	Additions to Maintenance Infusion	Rat #	Lymph [4-^{14}C]Cholesteryl Ester	Esterification Enzyme Activity	
				CE'ase	ACAT
			μmol[c]	*μmol CE/intestine per 6 hr*[d]	
1	Pancreatic juice	1	4.2	5.8	2.7
		2	8.9	11.9	3.5
		3	3.3	10.8	3.8
		4	2.0	4.8	2.3
2	None	1	0.8	2.4	3.1
		2	1.1	1.6	3.1
		3	0.6	1.9	2.3
		4	0.7	2.3	1.9
3	Immune IgG-treated pancreatic juice	1	1.0	1.6	2.5
		2	1.1	1.5	1.1
		3	0.5	1.4	3.0
		4	—	—	4.9
4	Control IgG-treated pancreatic juice	1	7.4	7.3	3.1
		2	4.5	4.7	2.1

[a] Correlation coefficients between [4-^{14}C]CE transport into lymph and CE'ase and ACAT activities are 0.83 and 0.42, respectively.
[b] Intestines surgically deprived (36 hr) of pancreatic juice (CE'ase) were repleted with CE'ase in Groups 1 and 4, and maintained deficient in CE'ase in Groups 2 and 3. All groups were administered intraduodenally a lipid meal containing 25 mg of [4-^{14}C]cholesterol. Intestinal lymph (6-hr pool) was analyzed for cholesterol, absorbed, and esterified. CE'ase activity was assayed in 20% mucosal homogenates and ACAT activity was assayed in 30% microsomal suspensions.
[c] Micromoles of [4-^{14}C]cholesteryl ester absorbed/intestine per 6 hr.
[d] Micromoles of cholesterol esterified/intestine per 6 hr.

ACAT and deficient (↓ ~ 75%) in CE'ase, 1.0 μmol of [4-^{14}c]cholesteryl ester is absorbed and the "residual" CE'ase activity could have esterified 1.6 μmol of cholesterol. Further, intestine from rats in groups 2 and 3 with a normal ACAT activity did not support cholesterol esterification and absorption. For example, in Group 3-rat #1, only 1.0 μmol of [4-^{14}C]cholesteryl ester was absorbed, even though microsomal ACAT, if involved, could have potentially esterified 2.5 μmol of cholesterol (3.0 μmol when microsomal ACAT recovery is considered).

When lymph was analyzed for total and free cholesterol mass after the cholesterol meal, the absorption results (**Table 4**) were in agreement with the isotopic data (Table 2). Total cholesterol mass was higher in the lymph of rats whose intestines had been repleted with CE'ase and were actively absorbing exogenous cholesterol (Group 1, 9.0 ± 1.7 μmol; Group 4, 13.1 ± 4.1 μmol) than in the CE'ase-deficient intestines (Group 2, 3.3 ± 0.7 μmol; Group 3, 2.8 ± 0.1 μmol). These decreases were accountable largely to decreases in the cholesteryl ester mass (Table 4). The

TABLE 4. Lymph cholesterol mass and volume

Group[a]	Additions to Maintenance Infusion	Cholesterol[b]		Lymph Volume[b]
		Free	Esterified	
		μmol/6 hr		*ml/6 hr*
1	Pancreatic juice	2.2 ± 0.2	6.8 ± 1.5	23.2 ± 4.0
2	None	1.5 ± 0.3	1.5 ± 0.2	15.6 ± 2.4
3	Immune IgG-treated pancreatic juice	1.3 ± 0.1	1.5 ± 0.1	8.2 ± 1.2
4	Control IgG-treated pancreatic juice	2.1 ± 0.6	11.0 ± 3.5	27.0 ± 17.0

[a] Intestines surgically deprived (36 hr) of pancreatic juice (CE'ase) were repleted with CE'ase in Groups 1 and 4 and maintained deficient in CE'ase in Groups 2 and 3. All groups were intraduodenally administered a lipid meal containing 25 mg of [4-^{14}C]cholesterol. Intestinal lymph (6-hr pool) was analyzed for total and free cholesterol mass. Cholesteryl ester mass was derived by difference.
[b] Values are mean ± SEM for four animals, except Group 4 with two animals.

esterified fraction of lymph total cholesterol also was less (49.0 ± 7.8% and 52.5 ± 0.8%) in rats not actively absorbing exogenous cholesterol compared to those that were (74.7 ± 2.4% and 83.9 ± 0.6%). In these nonabsorbing groups, the total lymph cholesterol is largely endogenous.

Lymph flow rate was also monitored prior to and during the absorption period. Animals with a lymph flow rate of at least 1.0 ml/hr in the preabsorptive period were given the cholesterol meal. During the absorption period, lymph flow rates were variable among the experimental groups (Table 4) and also among animals within each group. In general, lymph flow rate was greater in those animals (Groups 1 and 4) most actively absorbing cholesterol. However, among animals within an experimental group, there was no direct relationship between lymph flow rate and the amount of cholesterol absorbed.

DISCUSSION

The present study provides convincing evidence of the requirement of mucosal CE'ase of pancreatic juice origin in the absorption of exogenous cholesterol (see Table 2). Under conditions in which the intestine is rendered specifically deficient in CE'ase, the lymphatic absorption of cholesterol is greatly decreased. Intestinal ACAT levels in rats deficient in CE'ase are unaltered, showing that the ACAT enzyme cannot support a normal rate of cholesterol absorption in the absence of CE'ase.

In the present study while the intestine was rendered deficient in CE'ase, the enzyme was not eliminated (↓75%) nor was cholesterol absorption (↓83%; see Table 2). CE'ase activity in the CE'ase-deficient intestine was 25% (Groups 2 and 3) of the levels in their respective control groups (Groups 1 and 4). This activity is comparable to the "residual" levels of CE'ase in juice-deficient intestine reported earlier (4). There are several possible explanations for this finding. First, pancreatic juice diversion may have been incomplete. Since the luminal content was not analyzed for CE'ase, this possibility was untested. However, in earlier experiments (20), identical surgical techniques produced complete pancreatic juice diversion. Second, immunoprecipitation of CE'ase activity from pancreatic juice was not entirely complete and CE'ase in the treated juice (1 unit/ml) could have contributed to mucosal activity. Third, the "residual" esterifying activity considered to be CE'ase may, in fact, represent the intestinal enzyme, cholesteryl ester synthetase (CES) which has recently been described (34). CES is similar to CE'ase in its bile salt requirement and pH optima but is pancreatic juice-independent. The two enzymes are not distinguished by the CE'ase assay system. The level of CES activity reported for intestine (34) is equivalent to the "residual" activity measured in this study. Whatever the origin of this esterifying activity, "residual" CE'ase and/or CES, it can account nevertheless for the small amount of cholesteryl ester transported into the lymph by CE'ase-deficient intestine. Therefore, it is unnecessary to assume a role for ACAT in the esterification of the absorbed cholesterol in these animals.

It has been the position of the first author's laboratory that the function of CE'ase in cholesterol absorption is to promote the synthesis of cholesteryl ester, the form in which 70–90% of the exogenous cholesterol enters the lymph (33). The current study supports this view since the mucosal CE'ase activities in individual rats (except in rat #1-Group 4; see Table 3) can account for appreciably more than the total cholesterol ester absorbed by each (Table 3). Hence, it may be postulated that CE'ase can meet the esterification demands even during peak cholesterol absorption which occurs 2–4 hr after the lipid meal (35).

These results, considered solely by themselves, indicate that CE'ase is the primary regulator in the esterification and absorption of exogenous cholesterol and leads to the speculation that ACAT may esterify other sources of mucosal cholesterol such as that from de novo synthesis and/or plasma lipoproteins (36). The latter role is clearly analogous with ACAT function in other cells and tissues (37). However, a recent study in rats challenges this conclusion (38). In this study, rats were dosed with an ACAT inhibitor which reduced microsomal ACAT specific activity and cholesterol absorption. All functional aspects of the ACAT inhibitor have not been studied, but it appears nontoxic to the tissue, and neither affects triglyceride transport nor inhibits CE'ase (38).

The combination of both sets of results suggests that CE'ase and ACAT each play a role in the absorption of exogenous cholesterol in the rat and that ACAT function is dependent upon CE'ase. Such a concept has been proposed (11) in which it is hypothesized that CE'ase promotes cholesterol uptake by mucosal cells via cholesteryl ester synthesis on the luminal facing surface and hydrolysis on the cytoplasmic surface while ACAT esterifies the entering cholesterol, i.e., the two enzymes work in series. Such organization would give function to both enzymes and explain the conflicting results.

Another possibility is that the contribution of these two enzymes in cholesterol absorption depends upon exogenous cholesterol load. In a recent study in the rabbit (39), the administration of ACAT inhibitor decreased cholesterol absorption in the cholesterol-fed but not chow-fed animals. On the other hand, in the chow-fed rats, the administration of ACAT inhibitor reduced the lymphatic

absorption of both exogenous and endogenous cholesterol (38). Thus, while the exact role of CE'ase and ACAT in cholesterol absorption is subject to further study, the current results demonstrate a requirement for mucosal CE'ase of pancreatic juice origin in the absorption of exogenous cholesterol. Others (4–8), in earlier studies, had suggested a role for pancreatic juice in the normal absorption of cholesterol in several species. This study identifies CE'ase as the specific component in juice which regulates cholesterol absorption in the rat.

This research was supported by PHS grants AM-26346 and HL-26335.

Manuscript received 28 November 1983.

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