The effect of pancreatic diversion on lymphatic absorption and esterification of cholesterol in the rat

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Abstract Lymph fistula rats with either biliary fistula or pancreaticobiliary fistula were used to measure coenzyme A-independent mucosal cholesterol esterifying activity in the presence and absence of pancreatic exocrine secretion. Efficiency of pancreatic diversion was verified to minimize contamination of mucosal homogenates with adherent luminal enzyme. The synthetic activity of cholesteryl ester hydrolase (E.C. 3.1.1.13) was measured directly in mucosal homogenates. Indirect evidence for mucosal esterifying activity was obtained from hourly cholesteryl ester output into lymph when other factors known to affect cholesterol absorption were controlled. Rats infused intraduodenally at a constant rate with different concentrations of bile salts, polar lipid, and [^{3}H]cholesterol showed that the infused [^{3}H]cholesterol was absorbed and esterified with equal efficiency in the presence and absence of pancreatic flow. Total lymph output of free and esterified endogenous cholesterol was slightly less efficient in the pancreaticobiliary fistula group (85% of bile fistula values) but percent esterification was the same for both groups. Infusion of lipid-free micellar bile salts separately from other bile components produced a highly significant increase in absorption and esterification of lymph cholesterol for both groups. No correlation was found between cholesterol esterifying activity in *a*) the lumen or *b*) the mucosa, and cholesteryl ester output into lymph. The present study suggests an alternative enzyme dependent directly or indirectly on the presence of micellar bile salts in the lumen to explain intestinal cholesterol esterifying activity during absorption.—**Watt, S. M., and W. J. Simmonds.** The effect of pancreatic diversion on lymphatic absorption and esterification of cholesterol in the rat. *J. Lipid Res.* 1981. **22:** 157–165.

Supplementary key words bile salts · cholesteryl ester · cholesteryl ester synthetase · intestinal absorption · lipid · lymph · mucosa · pancreatic secretion

During absorption, only free cholesterol (FC) is taken up by the mucosa yet the major fraction of cholesterol absorbed into lymph appears as cholesteryl ester (CE) (1).

For many years, esterification during absorption has been explained by the synthetic activity of an enzyme cholesteryl ester hydrolase (CEH) (E.C. 3.1.1.13) that is ATP-independent and requires bile salts as a cofactor (2). Many reports suggest that pancreatic secretion is either the source of the enzyme (3) or affects mucosal CEH-like activity. A link between bile and pancreatic juice in cholesterol absorption and esterification has been demonstrated (4). A recent study using isolated intestinal cells cites evidence for an essential role of pancreatic sterol ester hydrolase in the mucosal esterification of cholesterol (5). An alternative enzyme acyl-CoA: cholesterol acyltransferase (ACAT) (E.C. 2.3.1.26) that is inhibited by bile salts has now been reported in mucosa from rat (6), guinea pig (7), and man (8). The question of an effect of bile or pancreatic juice on cholesterol esterification during absorption remains unsettled. Much of the confusion arises because many of the studies were carried out before the luminal role of bile salts was fully understood. Often, total exclusion of pancreatic juice was not confirmed.

In the present study, the role of bile salts in modulating cholesterol esterification in the absorptive cells was studied separately from the effects of other biliary components, under conditions where other factors affecting mucosal uptake of cholesterol were carefully controlled (9). Constant rate intraduodenal infusions of different concentrations of bile salts, polar lipids, and [^{3}H]cholesterol were given to lymph fistula rats with either bile fistula (BF) or pancreatic and bile fistula (PBF). The experiments described in the present study do not support a rate-limiting effect of pancreatic or intestinal CEH in cholesterol esterification during absorption in the rat. Inasmuch as the source of the bile salt-dependent esterifying activity was not biochemically identified, it will be referred to hereafter as CEH-like activity or CES (cholesteryl ester synthetase).

Abbreviations: CES, cholesteryl ester synthetase; BF, bile fistula; PBF, pancreaticobiliary fistula; FC, free cholesterol; CE cholesteryl ester; NaTC, sodium taurocholate.

MATERIALS AND METHODS

In the main experiments, 33 intraduodenally infused lymph fistula rats with either bile diversion or bile plus pancreatic juice diversion were used to investigate lymph output of cholesterol and mucosal enzyme activity during steady rate infusions. A second series of 28 rats with intact lymph flow but otherwise similar to the first group was used to obtain additional data on the effect of pancreatic juice diversion on mucosal enzyme activity during constant rate infusions.

Animal methods

Operations were performed under ether anesthesia on overnight-fasted 200-g male rats of an inbred Wistar strain. The abdominal thoracic lymph duct was cannulated with a polyethylene cannula (i.d. 0.5 mm) in most of the rats used. Bile fistula (BF) rats had the bile duct cannulated with a silicone cannula tipped with polyethylene tubing (i.d. 0.28 mm). The same type of tubing was used to divert both bile and pancreatic juice (PBF rats) by cannulation of the common bile duct as close as possible to its entrance to the duodenum. For infusions, a silicone tube was passed through a small incision in the fundus of the stomach into the duodenum and its tip secured near the entrance of the common bile duct.

The rats were transferred post-operatively to restraining cages maintained at 30°C. A solution containing NaCl (0.9%) and KCl (0.03%) was continuously infused at 3 ml/hr through the duodenal cannula for 48 hr post-operatively. Eight-hour test infusions were then given at the same flow rate. Hourly lymph samples were collected over ice into heparinized graduated tubes for the hour before and 8 hr during the test infusion. After the 8-hr test infusion, rats were anesthetized with ether, the small intestine was tied off and carefully excised, and the rats were killed by cardiac puncture.

Preparation of infusions

Infusions were made up in phosphate-buffered saline (6.75 mM Na_2HPO_4, 16.5 mM NaH_2PO_4, 115 mM NaCl, 5 mM KCl, pH 6.4) containing 10 mM glucose and 1 mM $CaCl_2$. Where used, appropriate aliquots were taken from stock solutions of 100 mM oleic acid, 100 mM monoolein, and 2 mg/ml of [^{3}H]cholesterol made up in chloroform. Solvents were evaporated off under N_2 and the appropriate weight of sodium taurocholate, mixed in one-third of the final volume of warm buffer, was added to the evaporated lipid residue. The solution was mildly sonicated in a Branson sonifier. More warm buffer was gradually added with further sonication until the final volume was attained. In many experiments (see Results), bile salts were infused without other added lipid.

Preparation and assay of CE synthetase

After infusion and before cardiac puncture, the small intestine was tied off between the lower duodenum and about 6 cm proximal to the ileo-caecal junction; it was carefully removed from the anesthetized rat, blotted, and placed in isotonic saline at 4°C. Lumen contents were gently washed out with ice-cold phosphate buffer (pH 7.0) to give a total volume of 10 ml. All subsequent steps were performed in a cold room at 4°C. The small intestine was washed out further with five 20-ml volumes of saline, and the mucosa was scraped from the muscularis and added to four volumes of glycerol–water 1:1 (by vol). The mucosal suspension and lumen contents were homogenized for 2 min at 2500 rpm in a MSE homogenizer with further addition of glycerol–water to give a final volume of 10 ml.

The substrate mixture selected for the assay contained 5 μmoles ^{14}C-labeled cholesterol, 10 μmoles oleic acid, 20 μmoles sodium taurocholate in 3.5 ml of 0.154 M phosphate buffer, pH 6.2, for each sample. The required amount of substrate mixture was prepared with repeated sonication during addition of sodium taurocholate in phosphate buffer to the evaporated lipid residues as described for infusate preparation. To 3.5 ml of warm incubate was added either 1.0 ml of mucosal homogenate or 0.5 ml of lumen contents *plus* 0.5 ml of glycerol–water 1:1 and the samples were incubated, together with tissue-free blanks, in 10-ml round-bottomed flasks at 37°C for 4 hr with shaking. The reaction was stopped by adding 1 ml of the incubated enzyme mixture to 6 ml of diethyl ether–ethanol–petroleum ether 1:1:1 (by volume) plus 1 ml of acid saline. The upper phase was removed, solvents were evaporated, and FC and CE in the lipid residue were separated by thin-layer chromatography on large silica gel-coated plates developed in hexane–diethyl ether–glacial acetic acid 70:30:2. The FC and CE fractions, visualized by iodine vapor against standards run on the same plate, were scraped into counting vials, 0.5 ml of redistilled ethanol and then 10 ml of scintillant were added, and the samples were counted. Activity was expressed as μg of [^{14}C]cholesteryl ester formed/hr per rat since the mucosal protein content was not significantly different between BF and PBF rats (120 ± 13.3 mg for ten BF rats and 110.3 ± 6.3 mg for ten PBF rats, means ± SEM).

Considerable attention was given to the selection of the assay conditions for CES to detect maximum enzyme activity. The method of Vahouny and Treadwell (10) for radioassay was found to yield slightly higher

TABLE 1. Esterification of [^{14}C]cholesterol by homogenates of mucosa and lumen contents using two different assay procedures

	Method A		Method B	
	Mucosa	Lumen	Mucosa	Lumen
Rat 1	28.0[a]	706.5	20.8	720.0
	40.5	656.5	36.1	780.0
Rat 2	37.0	1000	64.0	966
	73.3[b]			

[a] μg of ^{14}C-labeled cholesteryl ester formed per hr per rat.
[b] 0.5 ml of mucosal homogenate was used.

For Method A which was used in the present study, duplicate aliquots of 1 ml of mucosal homogenate or 0.5 ml of lumen contents plus 0.5 ml glycerol–water 1:1 were incubated with 3.5 ml phosphate buffer, pH 6.2, containing 5 μmol [^{14}C]cholesterol, 10 μmol oleic acid, and 20 μmol NaTC. For Method B (11), 0.5 ml of mucosal homogenate or 0.25 ml of lumen contents plus 0.25 ml glycerol–water 1:1 were incubated with 2 ml phosphate buffer, pH 6.2, containing 2 mg [^{14}C]cholesterol, 4.4 mg oleic acid, 5.6 mg NaTC, and 4 mg bovine serum albumin. All samples were incubated at 37°C for 4 hr.

activity for luminal CES but even lower mucosal CES activity than shown by the present method, when duplicate samples from several rats were used to compare the two methods. The present assay method was also compared with the albumin-stabilized emulsion used by Vahouny, Weersing and Treadwell (11). A comparison of CES activity with the two procedures is shown in **Table 1.** Other modifications that failed to give higher CES activity than the presently adopted technique included *a*) using 0.25 M sucrose or phosphate buffer for suspension of mucosal homogenates; *b*) combining gentle sonication with homogenization for enzyme preparation; *c*) shaking or filtering the mucosal homogenate before incubation (4); or *d*) siliconizing glassware.

As shown in **Table 2,** enzyme activity was found to be linear with time (up to 4 hr for luminal enzyme) and with amount of enzyme added (up to 1 ml of mucosal homogenates). The conditions selected gave the most reproducible yield of CE. The possibility of simultaneous hydrolysis of CE during incubation was ruled out when substitution of ^{14}C-labeled cholesteryl oleate for ^{14}C-labeled cholesterol in the incubates resulted in recovery of total ^{14}C-label in the CE fraction after 4 hr.

Assay for proteolytic activity

Azocoll (Calbiochem) was used for rapid non-specific assay for luminal proteolytic activity for verification of pancreatic diversion. The substrate, an insoluble powdered cowhide to which a red dye is attached by peptide linkages, releases the dye when exposed to proteolytic enzymes under standard conditions. Aliquots of lumen contents selected from bile fistula rats showed a linear relationship between volume of enzyme and rate of dye release over the range used in the present experiments. To 25 mg of Azocoll suspended in 4.5 ml of phosphate buffer (pH 7.0) was added either *a*) 0.5 ml of luminal contents from pancreatic diverted rats, or *b*) 10 μl of luminal contents plus 0.5 ml phosphate buffer (pH 7.0) from bile fistula rats. The mixture, together with blanks, was incubated at 37°C with shaking for 15 min. The reaction was terminated by filtering off the substrate and the absorbance of released dye read at 520 nm. Values were expressed as arbitrary units of trypsin activity read from a calibration curve obtained under the same standard conditions and supplied by Calbiochem. One unit is stated to be equivalent to 1 mg of enzyme.

Analytical

Lipids were extracted from aliquots of lymph, intestinal contents, mucosal homogenates, and bile by the method of Blankenhorn and Ahrens (12). Aliquots of lipid extracts were taken for counting, assay of total cholesterol, and thin-layer chromatography.

TABLE 2. Effect of incubation time and enzyme volume on [^{14}C]cholesterol esterification by rat mucosa and lumen contents[a]

	Mucosa				Lumen			
		Incubation Time				Incubation time		
	Volume	60	120	240	Volume	60	120	240
	(ml)		*(min)*		*(ml)*		*(min)*	
Rat 1	0.25	40[b]	50	43.2	0.1			1784
	0.5	52.0	38.6	42.4	0.5	1672	1640	1852
	1.0	19.3	29.0	30.0	1.0			1293
Rat 2	0.25			82.9	0.1			1148
	0.5			73.3	0.5	1247	1098	1000
	1.0	40.5	36.7	36.7	1.0			973

[a] Assay procedure as for Method A in Table 1.
[b] μg of ^{14}C-labeled cholesteryl ester formed per hr per rat.

TABLE 3. The effect of pancreatic diversion on proteolytic activity in the lumen of lymph fistula rats

	[^{3}H]Cholesterol Infused	Proteolytic Activity
	μg/hr	*units/15 min*
Bile fistula	0	2.325 ± 0.276 (4)[a]
	150	1.853 ± 0.259 (3)
	300	2.345 ± 0.444 (4)
Pancreaticobiliary fistula	0	0.011 ± 0.008 (4)
	150	0.014 ± 0.006 (3)
	300	0.023 ± 0.017 (3)

[a] Mean ± SEM; number of rats is in parentheses.

Aliquots of lumen contents were taken immediately after 8-hr infusions and were incubated for 15 min using Azocoll as a substrate for nonspecific proteolytic activity (see Methods). The absorbance of the released dye was expressed as arbitrary units of trypsin activity where 1 unit/15 min = 1 mg enzyme.

Thin-layer chromatography was carried out on 0.25 mm layers of silica gel G (Merck, Darmstadt, Germany) on plates prewashed overnight in chloroform–methanol 2:1 (v/v). After activation and application of lipids, plates were developed in hexane–diethyl ether–glacial acetic acid 70:30:2 (v/v/v). Free and esterified cholesterol were identified with iodine vapor against standards run on the same plate, removed from the plate by suction (13), and eluted with chloroform–methanol 2:1 (v/v). Appropriate aliquots from each eluate were taken for saponification prior to cholesterol assay and also for counting.

Radioactivity was determined after evaporation of solvents and addition of a scintillant mixture containing 2,5-phenyloxazole, 4 g/liter, and 1,4-bis-2(4-methyl-5-phenyloxazolyl-benzene), 0.05 g/liter, in toluene. Counting was performed in a Nuclear Chicago Isocap 300 counter using the channels ratio method (14) to correct for quenching.

Cholesterol determinations were carried out by the method of Zlatkis and Zak (15). All standards, blanks, and lipid extracts were saponified (16) before cholesterol assays were carried out.

Protein in mucosal homogenates was determined according to Lowry et al. (17).

Materials

[4-^{14}C]Cholesterol and [7(n)-^{3}H]cholesterol (Radiochemical Centre, Amersham) were repurified by thin-layer chromatography before use. Unlabeled oleic acid, glycerol-1-mono-oleate, cholesterol, and cholesteryl oleate were all purchased as high purity grade (>99% pure) from Nu-Chek-Prep Inc., Elysian, MN and used as supplied. Sodium taurocholate was prepared by the method of Lack et al. (18) and moved as one spot on thin-layer plates developed in propionic acid–isoamylacetate–water–n-propanol 15:20:5:10 (v/v/v/v). o-Phthalaldehyde was from Sigma, St. Louis, MO. All other chemicals and solvents were of analytical grade, except ethanol which was redistilled.

RESULTS

The efficiency of pancreatic juice diversion

To verify the efficiency of eliminating pancreatic enzyme activity from the lumen, lumen contents from each rat were collected over ice immediately after each 8-hr infusion. Appropriate aliquots were assayed for both proteolytic enzyme activity and CES activity. **Table 3** shows that proteolytic enzyme activity was barely detectable in rats with pancreaticobiliary fistula (PBF) relative to those rats with only biliary fistula (BF). Occasionally a low but significant amount of proteolytic activity was found in the lumen of a PBF rat and these rats were excluded from the study. The advantage of using an assay for proteolytic activity as a marker for the presence of pancreatic juice was illustrated when rats with intact pancreatic flow were infused without micellar sodium taurocholate. CES activity was absent from the lumen of these rats (Table 5, first group) but high values for proteolytic enzyme activity (1.56 ± 0.2 units/15 min, mean ± SEM for four rats) verified the presence of pancreatic juice.

Lymphatic output of infused (exogenous) ^{3}H-labeled cholesterol

Groups of BF rats or PBF rats were infused intraduodenally for 8 hr at 3 ml/hr with micellar lipid solution. The infusates contained 3 mM oleic acid, 3 mM monoolein, [^{3}H]cholesterol in three different concentrations, (25, 50, or 100 μg/ml) with the lipids solubilized in 10 mM NaTC to form a clear micellar solution. **Fig. 1** shows the appearance in lymph of infused labeled cholesterol for BF and PBF rats, respectively. Groups PBF without pancreatic CES transported infused isotopic cholesterol through to lymph as efficiently as groups BF with intact pancreatic secretion. Increasing concentrations of [^{3}H]cholesterol were infused to investigate whether absence of pancreatic CES might be rate-limiting for cholesterol absorption with larger doses presented. The linear relationship between dose infused and lymph output at 8 hr of infusion as shown in Fig. 1B does not support a saturation of the absorptive capacity for either group of rats.

Since diversion of bile in both BF and PBF groups deprived the rats of an endogenous source of cholesterol, the hourly rate of biliary cholesterol output into the lumen was measured immediately after diversion

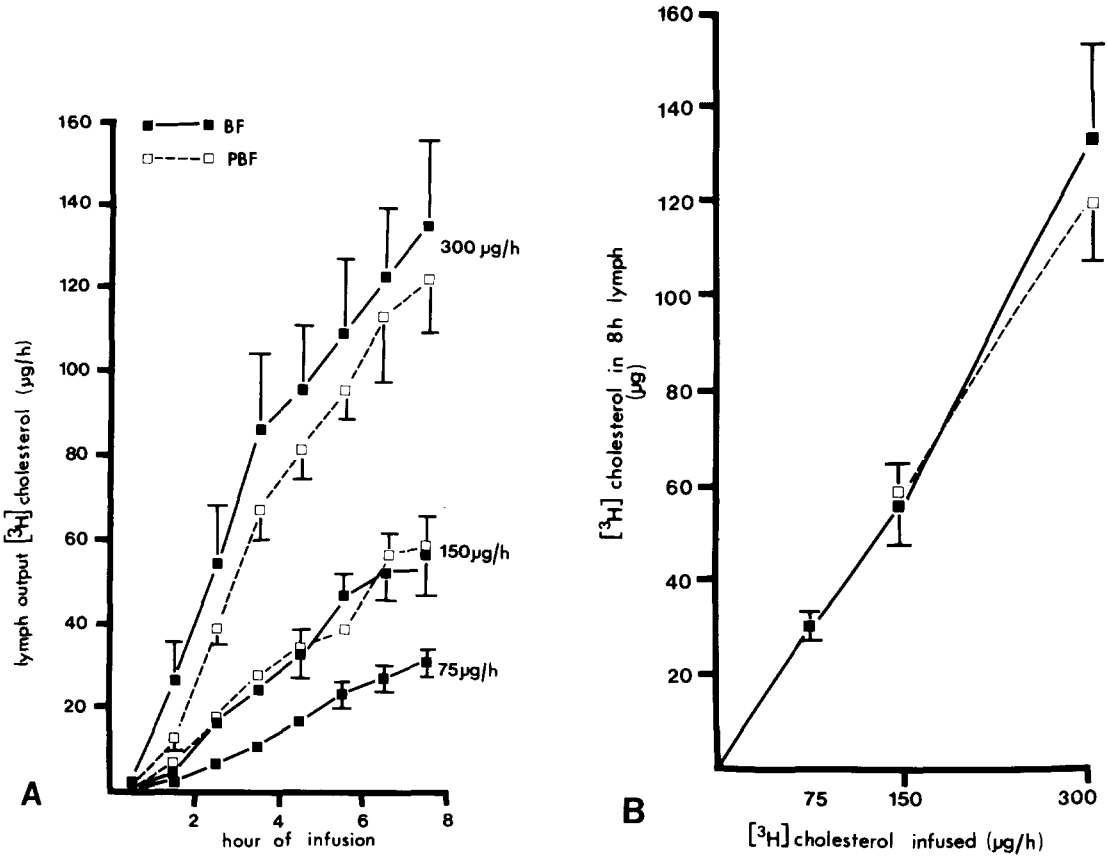

Fig. 1. A: Effect of pancreatic diversion on hourly lymph output of infused [^{3}H]cholesterol. Lymph fistula rats were infused intraduodenally at 3 ml/hr with 3 mM oleic acid, 3 mM monoolein, and 25, 50, or 100 µg/ml [^{3}H]cholesterol, respectively. Lipids were solubilized in 10 mM NaTC in phosphate buffer (pH 6.4). Lymph output of [^{3}H]cholesterol as µg/hr is shown for 11 BF rats (■——■) and 7 PBF rats (□– – –□) during the 8 hr infusion. Values are means ± S.E.M. At least three rats were used to obtain each curve. B: Effect of concentration of [^{3}H]cholesterol infused on lymph output of [^{3}H]cholesterol. Experimental procedure as in Fig. 1 for 11 BF rats (■) and 7 PBF rats (□). Values for lymph output of [^{3}H]cholesterol during 8th hour of infusion as µg/hr (means ± S.E.M.).

and also after prolonged diversion. Immediately after surgery 92.9 ± 0.7 µg/hr (means ± SEM for three rats) of cholesterol was recovered from diverted bile. It was therefore considered that infusion of 150–300 µg cholesterol/hr represented an appropriate condition for studying the absorption of exogenous cholesterol, i.e., the amount of infused cholesterol was considerably in excess of that normally contributed by bile.

Mass cholesterol output during exogenous infusion

During transport through the intestinal wall, exogenous ^{3}H-labeled cholesterol mixes with a large pool of endogenous mucosal cholesterol. Equilibration of newly absorbed cholesterol with the pool appears to be relatively slow. Isotopic cholesterol takes longer than mass cholesterol to reach a plateau in lymph. How much of the total mucosal cholesterol participates in transport into lymph is also debatable. It was therefore important to compare results for mass output of cholesterol and cholesteryl ester for those for labeled cholesterol.

Mass cholesterol output was increased by infusion of [^{3}H]cholesterol, more when 300 µg/hr was infused than when 150 µg/hr was given (**Fig. 2**). The output was less in PBF rats but the differences were small. It will be noted that with both groups the output of mass cholesterol approached a plateau value more rapidly (Fig. 2) than did [^{3}H]cholesterol output (Fig. 1). The curves for labeled cholesterol output therefore reflected mixing with the endogenous pool as well as increased mass turnover.

The effect of the [^{3}H]cholesterol infusion on the esterification of lymph cholesterol measured isotopically and chemically is compared in **Table 4.** The fraction esterified was higher for ^{3}H-labeled cholesterol than for mass cholesterol. However, mass cholesterol

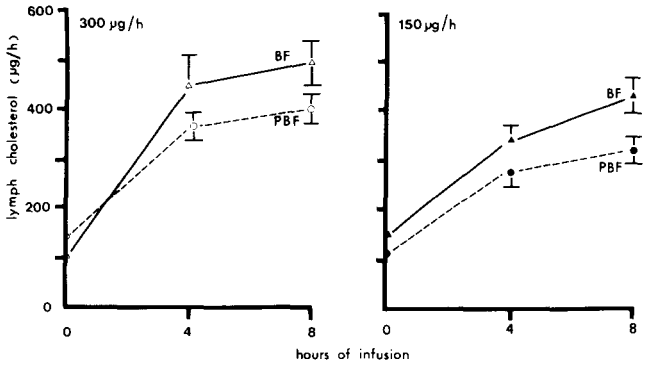

Fig. 2. Effect of pancreatic diversion on lymph output of mass cholesterol during infusion of [^{3}H]cholesterol. Lymph fistula rats infused at 3 ml/hr intraduodenally for 8 hr with 3 mM oleic acid, 3 mM monoolein, either 50 μg/ml [^{3}H]cholesterol (right panel) or 100 μg/ml [^{3}H]cholesterol (left panel). Lipids were solubilized in 10 mM NaTC in phosphate buffer (pH 6.4). Lymph output of mass cholesterol (μg/hr) is shown for eight BF rats (solid lines) and seven PBF rats (broken lines) during 0, 4, and 8 hr of the infusions. Values are mean ± S.E.M. for four rats in each group except for PBF rats given 150 μg cholesterol/hr, where three rats were used.

includes unlabeled "fasting" cholesterol, only 30% of which was esterified. If the fasting outputs of free and esterified cholesterol are subtracted (assuming this contribution is unchanged during absorption) the percent esterification of *excess* cholesterol in absorptive lymph, measured chemically, is the same as for labeled cholesterol. By the 8th hr of infusions, 80–84% of the *excess* mass cholesterol in lymph (i.e., 8 hr minus 0 hr output) was found as esterified cholesterol in both BF and PBF rats.

Cholesterol esterification in fasting lymph

Bile salts increased the output and esterification of cholesterol in lymph in the absence of exogenous lipid (**Fig. 3**). Infusion of glucose alone in buffered saline or infusion of glucose with bile salts below the critical micellar concentration had no effect. The effect of micellar bile salts was greater in BF rats than in rats with PBF, although the fasting output before bile salt infusion was the same in both groups. Volume of lymph flow was not significantly different between BF and PBF rats given micellar bile salts.

Endogenous biliary cholesterol was absent from the lumen in both BF and PBF groups. Fasting BF rats were found to have a higher ($P < 0.05$) lumen cholesterol content than PBF rats (430 ± 60 μg for ten BF rats compared with 229 ± 73.7 μg for six PBF rats, means ± SEM). Absence of pancreatic enzymes in the PBF rats might have rendered luminal cholesterol from other endogenous sources, e.g., cell debris, less available for solubilization and absorption when bile salts were infused. After an 8-hr infusion of micellar NaTC, total luminal cholesterol in six BF rats (204 ± 24 μg) was less than in ten saline-infused rats (430 ± 60 μg). Infusion of NaTC did not significantly reduce luminal cholesterol in PBF rats.

Mucosal cholesterol esterifying activity

So far the results have not supported a requirement for cholesterol esterifying activity of pancreatic origin in lymph output of esterified cholesterol.

In order to investigate mucosal activity of CES more directly, homogenates of mucosa taken from lymph fistula rats immediately after each 8-hr infusion were prepared at 4°C and aliquots were assayed for CEH activity. Four groups of BF rats and four groups of PBF rats given infusates which promoted lymph output of CE from 40 μg/hr to approximately 300 μg/hr were used. No correlation was found between mucosal CES activity and output of CE in lymph in either BF or PBF rats (**Fig. 4**). Nor was there any marked difference between the low levels of mucosal CES activity in the BF group (Fig. 4, left panel) compared with the group without pancreatic flow (right panel). Luminal levels of CES activity were about 200-fold higher in the BF group relative to the PBF rats (data not shown).

Having demonstrated an effect of cholesterol dose infused on lymph CE output in the rats described

TABLE 4. Effect of pancreatic diversion on esterification of rat lymph cholesterol

Experimental Animal	[^{3}H]Cholesterol Infused	Number of Rats	Mass			^{3}H-Labeled Cholesterol	
			0 hr	4 hr	8 hr	4 hr	8 hr
	μg/hr		*percent of lymph cholesterol as CE*				
Bile fistula	150	4	32.4 ± 0.7	59.2 ± 2.5	64.0 ± 2.3	70.8 ± 2.2	83.3 ± 1.8
Pancreaticobiliary fistula	150	3	31.1 ± 6.3	58.3 ± 1.5	60.4 ± 2.7	79.6 ± 0.8	82.7 ± 2.0
Bile fistula	300	4	35.3 ± 1.4	70.1 ± 1.5	69.2 ± 1.2	84.5 ± 1.0	84.2 ± 0.7
Pancreaticobiliary fistula	300	3	38.6 ± 5.0	73.0 ± 3.3	67.6 ± 1.7	82.7 ± 3.0	83.8 ± 1.3

Lymph fistula rats were intraduodenally infused at 3 ml/hr with 3 mM oleic acid, 3 mM monoolein, [^{3}H]cholesterol, 50 μg/ml or 100 μg/ml, with lipids solubilized in 10 mM NaTC in phosphate buffer (pH 6.4). Lymph samples collected over ice at 0 hr, 4 hr, and 8 hr of infusion were assayed for total and labeled cholesterol. FC and CE separated on TLC were saponified and cholesterol mass was determined by the method of Zlatkis and Zak (14). Values are mean ± SEM.

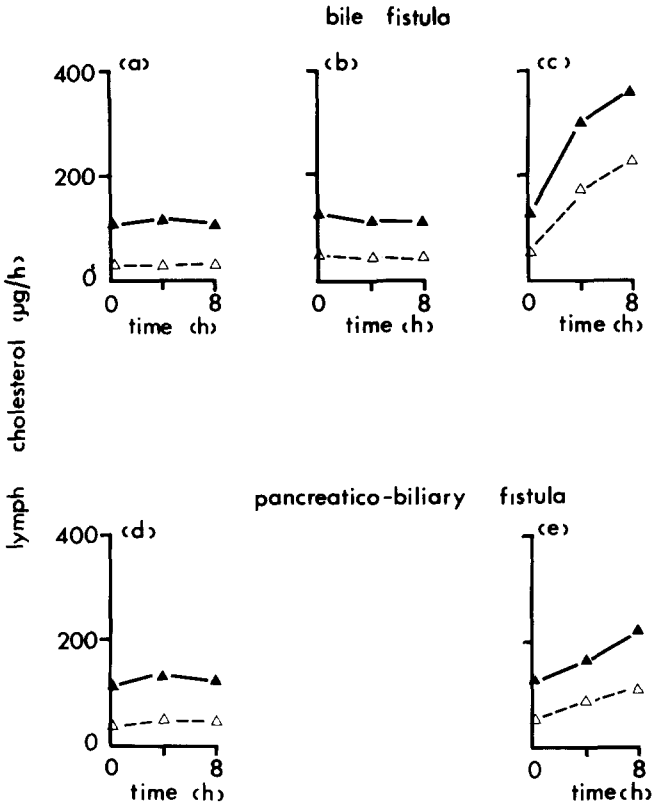

Fig. 3. Lymph output of cholesterol in fasting lymph. Lymph fistula rats with either biliary fistula (upper panel) or pancreaticobiliary fistula (lower panel) were infused intraduodenally at 3 ml/hr for 8 hr with glucose in buffered saline (a) and (d); glucose in buffered saline with 1 mM NaTC, (b); or glucose in buffered saline with 10 mM NaTC, (c) and (e). Hourly lymph output of total cholesterol (▲) and CE (△) is shown for 0, 4, and 8 hr of infusion for at least three rats in each experimental condition.

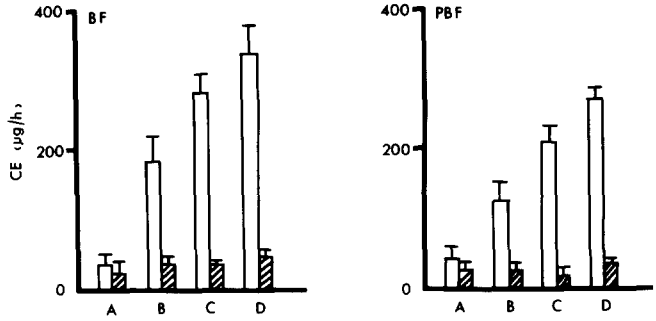

Fig. 4. Comparison between lymph output of CE and mucosal CES activity. Lymph fistula rats with either BF (left panel) or PBF (right panel) were infused intraduodenally at 3 ml/hr with buffered glucose solution, A; 10 mM NaTC, B; 3 mM oleic acid, 3 mM monoolein, 50 μg/ml cholesterol solubilized in 10 mM NaTC, C; and 3 mM oleic acid, 3 mM monoolein, 100 μg/ml cholesterol solubilized in 10 mM NaTC, D. Hourly lymph output of CE as μg/hr (open bars) is compared with mucosal CES activity expressed as μg CE formed/hr per rat (hatched bars) for each group of rats. CES assay is described in Methods. Values are mean ± S.E.M. for at least three rats in each experimental condition.

above, a second group of 27 rats without lymph fistulae were used to investigate more closely a relationship between *a*) mucosal CES and cholesterol dose given and *b*) mucosal and luminal CES activity. This second series of rats with either BF or PBF was infused intraduodenally at 1.5 ml/hr with cholesterol 0–600 μg/hr. The results in **Table 5** provide no evidence for an increase in mucosal CES with increased cholesterol infused for either BF or PBF rats. The highly significant differences in luminal CES activity ($P < 0.001$) between BF and PBF rats given NaTC were not accompanied by corresponding differences in mucosal CES activity between the two groups of rats. The absence of luminal CEH activity in the BF rats infused without micellar NaTC supports previous findings for a protective effect of trihydroxy bile salts on pancreatic CES (19).

DISCUSSION

The primary object of these experiments was to re-examine the effect of pancreatic fistula, with conse-

TABLE 5. Effect of pancreatic diversion and dose of cholesterol infused on CES activity in lumen and mucosa

	Infusage			Number of Rats	CES Activity	
	Cholesterol	Polar Lipid	NaTC		Mucosa	Lumen
	μg/hr	*μmol/hr*				
BF	0	0	0	4	17.6 ± 2.4[a]	0
BF	0	0	30	3	34.3 ± 8.3	1470 ± 488
BF	150	18	30	2	23.8,57.0[b]	1474,1147[b]
BF	300	18	30	3	45.8 ± 0.8	834 ± 314
BF	600	18	30	5	39.1 ± 7.2	1321 ± 285
PBF	0	0	0	3	20.1 ± 1.2	0
PBF	0	0	30	3	39.1 ± 4.5	0
PBF	600	18	30	5	32.7 ± 5.5	2.5 ± 2.8

[a] μg ^{14}C-Labeled cholesteryl ester formed per hr per rat; mean ± SEM.
[b] Individual values.

quent absence of CEH from the lumen, on the output and esterification of absorbed cholesterol in lymph. The design of the present experiments with constant-rate infusions of micellar lipid enabled a distinction to be drawn between effects of pancreatic diversion and other factors which have been shown to affect cholesterol absorption. Mucosal uptake of cholesterol has been shown to be linearly related to the luminal concentration of solubilized cholesterol (9). The efficiency of pancreatic diversion was verified by measurement of luminal proteolytic activity and CES activity. Diversion of bile in both BF and PBF groups excluded contributions by endogenous biliary cholesterol and phospholipid, while the replacement of bile salts as taurocholate ensured a standard load of micellar cholesterol (co-solubilized with fatty acid and monoolein) in both groups.

The transport of labeled cholesterol molecules from lumen to lymph at two dosage levels was not affected by the absence of pancreatic CEH, nor was the percentage esterification of labeled molecules. The output of chemically measured cholesterol in lymph was lower in PBF animals though the difference was small. The percent esterification measured chemically was the same in PBF and BF groups. When interpreting the chemical data, two points must be borne in mind. First, the fasting lymph, before infusion, contains cholesterol of which a considerable amount is derived from plasma lipoproteins, not from the intestine, and is esterified less than in absorptive lymph. It is likely, though unproven, that the amount of plasma-derived cholesterol and cholesteryl ester is not increased during cholesterol absorption. Second, the output of chemical cholesterol in lymph and its esterification is increased by taurocholate infusion in the absence of cholesterol. This effect was less in the PBF group, perhaps because non-biliary luminal cholesterol, e.g., from exfoliated cells, was less available for absorption in the absence of pancreatic digestive enzymes. Overall, the experiments do not support an effect of pancreatic juice on the efficiency of absorption and esterification of luminal, exogenous cholesterol.

Before discussing the discrepancy between these two findings and those in the classical experiments of Borja, Vahouny, and Treadwell (4), the present results of CES assay in lumen and mucosa require comment. Essentially, the mucosal CES activity was low and fairly uniform between all groups studied. There was no correlation between mucosal CES and CE output in lymph nor between activity in homogenates of well-washed mucosa and activity of luminal contents (Fig. 4 and Table 5). Thus no evidence was obtained that mucosal CES activity was modulated by the presence or absence of pancreatic CES in the lumen, nor did it seem to be rate-limiting for cholesterol esterification. While extrapolation from in vitro to in vivo is questionable, the total esterifying activity recovered per rat intestine in our experiments was about one order of magnitude lower than the output of absorbed cholesterol as cholesteryl ester (Fig. 4). There are reports that the Coenzyme A-dependent cholesterol esterifying mechanism has an activity sufficient to account for the cholesteryl ester output in lymph in rats and cholesterol absorption in man (6, 8) and that it may be rate-controlling (20).

No reason could be found for failure to produce the results of Borja et al.'s careful enzymatic measurements (4). There were differences in detail in the assay procedure but every effort was made to validate any departures from the protocol (see Methods). Discrepancies in the findings of lymph fistula experiments may be related to some differences in experimental design, steady perfusion of micellar cholesterol contrasted with an emulsified gastric test meal with or without duodenal infusion of whole bile, for example; of the use of animals 48 hr after operation compared with 24 hr later (21). Perhaps the most significant difference was the dose of cholesterol, about 5 mg in 8 hr was the highest dose given in the present experiments compared with 50 mg in the test meal of Borja et al. (4).

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