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The Effect of Lipids on Taurocholate Absorption from the Rat Small Intestine[1] (40500)

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Children with cystic fibrosis (CF) lose large amounts of bile acids in their stools (1, 2). The extent of this loss is comparable to that observed following an ileal resection (1). It occurs regardless of age, is dependent on the presence of steatorrhea, varies with its severity and responds to pancreatic enzymes (3). It leads to decrease bile acid duodenal concentrations during a meal (4), a reduced bile acid pool (5) and lithogenic bile (6).

The pathogenesis of altered bile acid metabolism remains unknown. The present experiments were designed to explore the possibility that unabsorbed fat and particularly unhydrolyzed triglycerides could interfere with bile acid absorption.

Materials and methods. Ninety five % pancreatectomy. Male Sprague-Dawley rats weighing 130–170 g underwent either a 95% pancreatectomy by the technique of Scow (7) or a sham operation. The weight gain ($\bar{X} \pm$ SE) on rat chow during the following 10 days was 63 ± 7 g in the pancreatectomized animals compared to 49 ± 5 g in the sham. Their respective blood sugars were 137 ± 5 mg/dl and 110 ± 8 mg/dl. Four days after increasing the 7% lipid content of the diet to 40% with butter, 1.4 μCi of Na cholate-24-[^{14}C] (New England Nuclear, sp act 45 mCi/m*M*) was administered iv. Over a period of 10 days, stools were collected daily for fat (8), nitrogen (9) and measurement of the decay constant of bile acids. After extraction and enzymatic determination of bile acids (10), the ^{14}C activity was determined in a scintillation spectrometer. An automatic external standard was used to correct for quenching. The decay constant was calculated by plotting the DPM per mg of bile acids (11).

Pancreatic fistula. In another group of experiments, a pancreatic fistula was created by ligating the common duct at its entrance point in the duodenum. Drainage of pancreatic secretions was effected through a common bile duct cannula directed caudally and immobilized at a distance of 3 mm from the take-off point of the right and left hepatic ducts. A second catheter was introduced through the same incision in the common duct and placed at the bifurcation of the right and left hepatic ducts (Fig. 1). It permitted continuous drainage of bile and measurement of bile acid (10) biliary output and pool size. A duodenal tube (5F feeding tube) was placed through a gastrostomy. Chymotrypsin (12) could not be detected in the small bowel effluent of a few rats with an ileostomy. Serum amylase determinations (13) carried out in a few animals remained normal.

The seven rats were given a continuous 24 hr duodenal infusion of each of three solutions at a speed of 3.3 ml/hr. The duodenal perfusates were freshly prepared daily and were made up of a 10 m*M* phosphate buffer (14). Na taurocholate 15 m*M* (Maybridge Research Chemicals U.K.) purified to more than 98% by thin layer chromatography (15), [^{3}H]taurocholic acid (New England Nuclear, sp act 2.3 mCi/m*M*) 7.5 μCi/dl, amigen 5 g/dl (Baxter) and sucrose 13.5 m*M*. NaCl and KCl were added to increase the Na^+ and K^+ concentrations to 150 m*M* and 35 m*M* respectively. The pH of this lipid-free solution infused for the first 24 hr was 6.9. The order in which the other two solutions were administered was randomized. They were identical in composition and pH except for their fat content which provided the animals with a daily fat intake exceeding that of adult rats (16). These two infusates contained either a 1:1 molar ratio of oleic acid and 1-monoolein (Sigma Chemical Co., Purity grade 98–99%)

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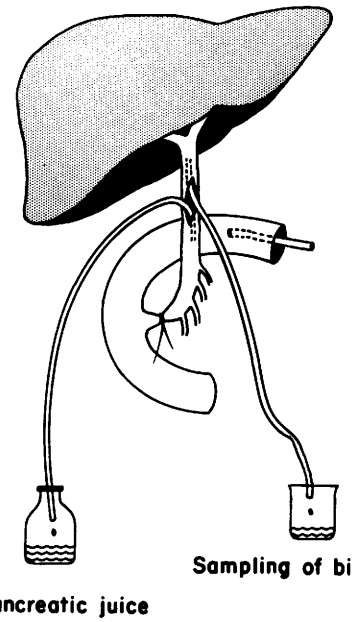

FIG. 1. Pancreatic and biliary fistulae permitting separate and complete drainage of pancreatic and biliary secretions. At autopsy, the common bile duct was not dilated on either its hepatic or pancreatic portion. The liver and pancreas appeared histologically normal.

with a total lipid concentration of 7 m*M* or an emulsion of 7 m*M* of triolein (Sigma Chemical Co., Purity grade 95–99%) stabilized with gum acacia 1%. The micellar solution was prepared by adding the Na taurocholate and phosphate buffer mixture to the oleic acid and 1-monoolein evaporated of their solvent followed by stirring with the rest of the solution by means of a magnetic bar at 40°. It remained optically clear for several days at room temperature. The emulsion of triolein was prepared in the same fashion. Under a phase microscope it showed an average fat droplet size of 2 μm. However it did not remain stable for more than 6 hr and required sonication for periods of 15 min. In two animals, the lipid content of both the micellar solution and of the emulsion was doubled to 14 m*M*.

After 16 hr of steady infusion of each solution, bile was collected for four periods of 2 hr for measurement of taurocholate absorption and averaged. Radioactivity was measured using Biosolv (Beckman) as solubilizer. Toluene omnifluor was used as scintillation fluid. Counting efficiency for tritium was 28%. Counts were corrected for quenching by the double channel ratio method. The % taurocholate absorption was calculated as follows:

$$\text{dpm/hr in bile/dpm/hr in perfusate} \times 100$$

Ileal perfusions. Perfusions were carried out in 40 cm segments of terminal ileum of rats maintained under light pentobarbital anesthesia. Outflow catheters located 2 cm proximal to the ileocecal valve and inflow catheters (Polyethylene 8F) were implanted in male Sprague-Dawley rats weighing 190–210 g with a biliary fistula (Clay Adams PE-10) created 48 hr previously. The ileal segment was maintained out of the abdomen at a temperature of 35–39° with warm saline compresses and an overhanging lamp. It was infused at a rate of 8.8 ml/hr with a pH 8.0 lipid-free solution containing 280 mOsm/kg. Its composition per liter was as follows: Na^+ 120 m*M*, K^+ 20 m*M*, Cl^- 50 m*M*, HCO_3^- 90 m*M*, D-xylose 10 m*M*, polyethylene glycol, mol wt 4000, (PEG) 5 g and Na taurocholate 5 m*M* to which [^{3}H]taurocholic acid 18 μCi was added. The lipid-free infusate was given for a period of 45 min and followed by the same solution made into either a micellar solution by the addition of oleic acid and monoolein (2:1, v/v) 5 m*M* or into a fine emulsion of triolein 5 m*M* stabilized with gum acacia 1% given for a second period of 45 min. The last 15 min of each of the two infusion periods were experimental periods during which intestinal absorption of water, Na^+ and D-xylose was monitored by measurement of differences between perfusate and effluent concentrations relative to changes in the concentration of PEG using standard formulas. The rate of appearance of the radiolabeled bile acid in the biliary drainage was used as a measure of the rate of bile acid absorption (17). Na^+ was determined by flame photometry, D-xylose and PEG by modifications of the method of Roe and Rice (18) and of the one described by Hyden (19).

Further ileal perfusion experiments were carried out in 20 cm segments. The oleic-monoolein and triolein infusates were each given for a 45-min period in the same animal and their order of administration was randomized. They were of the same composition as the one described above except for the fact that they were prepared with .05, 1, 2, 3 and 4 m*M* concentrations of Na taurocholate.

The rate of appearance of the labeled bile acid used as a measure of Na taurocholate absorption was assessed by sampling bile every 2 min for the last 16 min of each infusion period. Partitioning of the fatty acid–monoglyceride mixture between the micellar and the emulsion phase (20) at various Na taurocholate concentrations was carried out by passing the mixture through two filters (2200 and 1000 Å) separated by Dacron mesh (21) (Millipore Corp., Bedford) after adding a trace amount of [^{14}C]oleic acid (New England Nuclear). The % of radioactivity recovered in the filtrate was minimal at Na taurocholate concentrations of .05, 1 and 2 m*M* while most of the labeled oleic acid was present in the filtrate at concentrations of 3 and 4 m*M* taurocholate.

Results. The 95% resection of the pancreas was well tolerated by the animals. The caloric intake and weight gain recorded in the eight-pancreatectomized animals during their 10 days on a diet containing 40% lipids did not differ from those recorded in the eight sham rats. Table I shows that the daily fat excretion (mg/24 hr) and the decay constant of bile acid sp act were not affected by excision of the splenic, gastric and of most of the duodenal portion of the gland. However, nitrogen excretion (Table I) was increased ($P < .025$) and this correlated with some degree of proteolytic enzyme deficiency since fecal chymotrypsin in two experimental and in two sham rats averaged 6.9 mg/72 hr/kg and 25.6 mg/72 hr/kg respectively.

During the 24-hr infusion of each of the three solutions (lipid-free, oleic–monoolein, triolein), the seven animals with a biliary and pancreatic fistula lost 10–15% of their initial weight. Urinary volume did not change. Diarrhea was never observed and there was no evidence of dehydration. The average amount of pancreatic juice collected was .6 ml/hr. The hourly excretion of bile acids during the first 2 hr following bile duct cannulation is representative of the normal bile acid output (20). The output doubled to 42 μM/hr and was steady in two control animals in which bile acid secretion was monitored hourly following bile duct cannulation and from the 10th to the 16th hr after beginning the infusion of Na taurocholate (15 m*M*). This concentration of Na taurocholate is known to be sufficient to suppress endogenous bile acid synthesis (22). Figure 2 shows that the average ($\bar{X} \pm$ SE) % of bile acid absorption during the three perfusion periods did not differ. The figure of 94 ± 4 achieved with Na taurocholate alone decreased to 86 ± 5 when a micellar solution of oleic–monoolein was infused and to 90 ± 4 during the infusion of the fine emulsion of triolein. Results were identical to two animals in which the lipid content of the duodenal infusates was 14 m*M* instead of 7 m*M*.

Ileal perfusions carried out in 40 cm segments with Na taurocholate 15 m*M* and a micellar solution of oleic–monoolein in six rats led to a decrease in water, Na^+, D-xylose and Na taurocholate absorption (Table II). In a few animals studied less than 1 hr after creation of the bile fistula, the same results were obtained. In contrast to the results shown in Table II, the six animals infused with Na taurocholate 5 m*M* and a fine emul-

TABLE I. EFFECT OF PANCREATECTOMY ON FAT AND NITROGEN EXCRETION AND ON THE DECAY CONSTANT OF BILE ACIDS.

		Fat (mg/24 hr)	Nitrogen (mg/24 hr)	Decay constant
Pancreatectomy	$\bar{X}$	398	85*	.093
(8)	SE	46	5	.015
Sham	$\bar{X}$	345	57	.096
(8)	SE	15	2	.021

* $P < .025$.

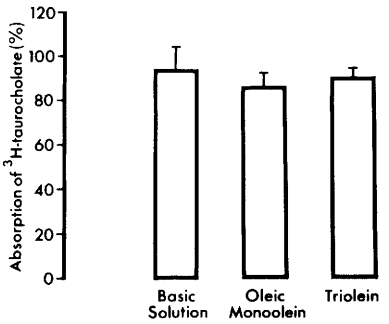

FIG. 2. The % absorption of taurocholate ($\bar{X} \pm$ SE) was determined two hourly during the final 8 hr of the 24 hr steady duodenal infusion of each of three solutions. The lipid-free solution (basic solution) was given initially in seven rats with pancreatic and biliary fistulae. The order of administration of the two other solutions, oleic–monoolein and triolein was randomized. A one-way analysis of variance revealed no significant difference.

sion of triolein did not show a drop in the intestinal absorption of water, Na^+, D-xylose and bile acids. In fact, there was some increase ($P < .01$) for both water and Na taurocholate absorption (Table III).

When 20 cm lower segments of ileum were infused with the fatty acid–monoglyceride mixture followed or preceded by the triglyceride emulsion containing varying concentrations of Na taurocholate, the rate of appearance of the radiolabeled bile was lower in the oleic–monoolein experiments at all concentrations of the bile acid (Fig. 3). It is apparent that the difference in the rate of absorption of Na taurocholate was larger at Na taurocholate concentrations of 3 and 4 m*M* than at .05, 1 and 2 m*M* (Fig. 3). This relationship appeared to correspond with the observation that under the experimental conditions used only a negligible fraction of the fatty acid–monoglyceride mixture was in micellar solution at these three lowest bile acid concentrations.

Discussion. The data show that despite a five- to sixfold increase of daily fat intake in the 95% pancreatectomized rat, fat assimilation remained essentially normal at close to 95%. In the human, steatorrhea is not observed until lipase output is reduced to less than 10% of normal (23). The large reserve capacity of the exocrine pancreas was observed in rats with a 95% pancreatectomy who developed steatorrhea only when fed 20% tripalmitin–tristearin (24). This is in contrast to the present results obtained with a 40% saturated fat diet. A modest degree of creatorrhea was found in conjunction with evidence of decreased proteolytic activity but it did not modify the rate of disappearance of bile acids from the enterohepatic circulation. These observations tend to rule out the possibility that undigested nutrients such as proteins could prevent bile acid absorption (1).

In the second part of this study, the creation of pancreatic fistulas insured that the experimental animals were completely deprived of pancreatic exocrine secretions. They were given approximately twice their usual intake of triglycerides (triolein emulsion). The % Na taurocholate absorption during the infusion period of triolein did not differ from the % observed with the continuous administration of its lipolytic products (oleic acid–monoolein). Thus impaired lipolysis of neutral fat was not associated with a decrease in the absorption of Na taurocholate in the rat.

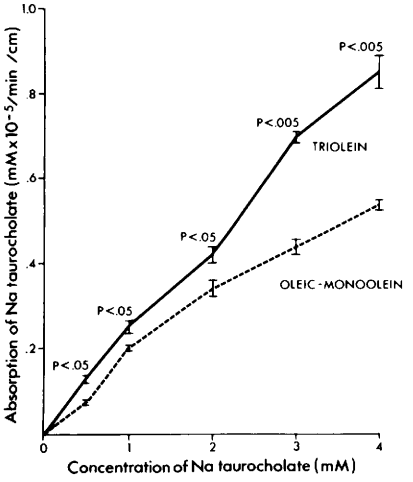

FIG. 3. Ileal perfusion experiments in 20 cm segments carried out with an infusate of oleic–monoolein followed or preceded by an emulsion of triolein. Progressively higher concentrations of Na taurocholate were used to prepare the infusates given for 45-min periods. Each point on the two curves represents experiments in one animal. The $\bar{X} \pm SE$ was calculated from the rate of appearance of taurocholate in eight specimens obtained every 2 min during the last part of each infusion period.

TABLE II. EFFECT OF OLEIC–MONOOLEIN ON ILEAL ABSORPTION.

N = 6[b]	H_2O (ml/hr)	Na (m*M*/hr)	D-xylose (μ*M*/hr)	NaTc in bile[a] (μ*M*/hr)
Lipid-free SOLN	2.6 ±.6	258 ±24	51.2 ±8.2	15.4 ±1.3
Micellar SOLN	1.2* ±.3	185* ±24	28.8* ±6.0	10.9* ±1.8

* $P < .05$.
[a] NaTc: Na taurocholate.
[b] N: number of animals.

TABLE III. EFFECT OF TRIOLEIN ON ILEAL ABSORPTION.

N = 6[b]	H_2O (ml/hr)	Na (m*M*/hr)	D-xylose (μ*M*/hr)	NaTc in bile[a] (μ*M*/hr)
Lipid-free SOLN	1.7 ±.3	178 ±30	44.2 ±4.1	10.6 ±.6
Emulsion	2.6* ±.4	232 ±60	40.4 ±6.3	12.8* ±.5

* $P < .01$.
[a] NaTc: Na taurocholate.
[b] N: number of animals.

Although these experiments would appear to invalidate the working hypothesis that in CF unhydrolyzed triglycerides might interfere with bile acid absorption (1, 3), they have to be interpreted in the context of the experimental conditions. At a pH of 6.9, the perfused taurocholate was essentially present only in its ionized form and therefore was actively transported since passive ionic diffusion is negligible. Had glycocholate been used, the same results could be anticipated since its Km is only slightly lower and its pKa is not high enough (17) for one to worry about non ionic diffusion.

The ileum has long been considered to represent the main site of bile acid absorption. However, recent reports in man (25) and in the rat (26) have indicated particularly in the latter that the jejunum plays an important role in bile acid absorption. Since micellar lipids in the rat are absorbed from the proximal small intestine (27) it can be presumed that in the experiments carried out in animals with pancreatic fistulas both oleic acid and 1-monoolein were absorbed from the upper jejunum almost as fast as they were infused. Lipids such as oleic acid and monoolein have been reported to enhance the jejunal uptake of Na taurocholate (28). In contrast, the present findings are compatible with a previous report (17) since they show that the bile acid absorption during the infusion of the micellar solution of oleic–monoolein was slightly lower than during that of the lipid-free and of the triolein solutions.

In the absence of a digestive or of an absorptive phase defect, the site of fat absorption is limited to the proximal part of the small intestine (29). However both the distal part of the jejunum and the ileum constitute a vast anatomical reserve for fat absorption in conditions where digestive and absorptive functions are impaired. It was elected to carry out ileal perfusions with micellar solutions and emulsions to explore the role of unabsorbed lipids on bile acid absorption.

Experiments carried out in 40 cm segments of ileum infused with a 5 m*M* micellar solution of oleic–monoolein confirm a previous paper showing that oleic acid induces malabsorption of water in dog ileum (30) and extend them to Na^+, D-xylose and taurocholate. In that study, malabsorption of these substrates was also found but had been tested only with two hydroxy fatty acids (30). The ileal perfusion results also provide confirmation for the work in rats showing a slight depression of ileal transport rates of taurocholate by oleic acid and low concentrations of monoolein (28). The absence of any adverse effect of a triolein emulsion on the ileal absorption of water, Na^+, D-xylose and taurocholate is of interest and suggests that triglycerides reaching the ileum do not interfere with bile acid absorption.

Having shown that taurocholate is not absorbed as readily in the ileum from a micellar solution with a mixture of oleic–monoolein as from a molecular phase within an emulsion of triolein, the role of micelles was investigated by perfusions of 20 cm segments of ileum using progressively lower bile acid concentrations. Fatty acids and monoglycerides in the small intestine exist as an oil phase in emulsified droplets, as a monomolecular solution in the aqueous phase and as mixed micelles solubilized within molecular aggregates of bile salts. Micelle formation accelerates the diffusion of lipolytic products and their absorption by overcoming the resistance offered by the unstirred water layer (31). However there may be an optimal size of micelles for bile acid absorption because of a slower diffusion to the absorptive surface. Expansion of micelles by added lipids (32) inhibits the jejunal absorption of bile acids (33). At all taurocholate concentrations, the ileal segments of animals infused with the fatty acid–monoglyceride mixture absorbed less bile acid. However, the difference became particularly striking when the infusates were made up of the two higher taurocholate concentrations permitting the formation of mixed micelles with the fatty acid–monoglyceride mixture. The present findings are therefore consistent with the interpretation that bile acids are not as effectively absorbed in the ileum from mixed micelles and add a further dimension to the recent demonstration that unhydrolyzed dietary and biliary lecithin interferes with the ileal absorption of taurocholate (34).

Summary. The effect of lipids on taurocholate absorption was studied in the rat small intestine. It was found that: (1) experimental pancreatic insufficiency induced by a 95% pancreatectomy or by a pancreatic fistula did not lead to impairment of bile acid transport

in response to an increase in fat intake; (2) the ileal absorption of water, Na^+, D-xylose and taurocholate was normal in response to an emulsion of triolein and impaired by a micellar solution of oleic acid–monoolein; (3) fatty acids and monoglycerides interfere with ileal bile acid absorption particularly if they form mixed micelles with bile acids. These data do not support the hypothesis that unhydrolyzed triglycerides account for the increased loss of bile acids in CF and suggest that unabsorbed lipolytic products reaching the ileum can adversely affect bile acid transport.

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