Intestinal Fatty Acid Absorption and Esterification from Luminal Micellar Solutions Containing Deoxycholic Acid[1] (34442)

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(Introduced by J. J. Spitzer)
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Deoxycholic acid has been shown *in vitro* to inhibit fatty acid esterification by jejunal segments and to produce severe histological damage to the mucosa (1). However, in patients with steatorrhea due to overgrowth of bacteria in the upper intestinal tract, luminal deoxycholic acid may be found (2), but no mucosal histological damage is seen (3). In experimental dogs (4) and in patients (5) shown to have deoxycholic acid in the upper small-gut lumen, the amelioration of steatorrhea by the administration of supplemental taurocholate has raised the question of whether deoxycholic acid is inhibitory at all to fat absorption in the living state.

Dietschy, Salmon, and Siperstein (6) demonstrated absorption of unconjugated bile acids in the upper gut, but much less absorption of conjugated bile salts from the lumen at that site. We questioned whether deoxycholic acid might be absorbed proximally, leaving adequate luminal taurocholate concentration to facilitate fatty acid absorption further down the intestinal tract. In the living animal this whole process might mask deoxycholic inhibition of fat absorption in the more proximal areas of the intestine. On the other hand, proximal fatty acid absorption might proceed normally despite the presence of luminal deoxycholic acid in the living animals (7), as distinct from what had been found by *in vitro* studies (1, 3).

As a result of investigating these two possibilities, we have found that deoxycholic acid does not appreciably prevent fatty acid absorption *in vivo*, even at the same site where deoxycholic acid is absorbed.

Procedure. A. *Perfusion experiments.* Female rats of the Sprague-Dawley strain, weighing 200–250 g were anesthetized with intraperitoneal 50% aqueous ethyl carbamate, 1 g/kg body weight. The technique of perfusion was as described by Burgen and Goldberg (8), with the addition of bile duct ligation just proximal to its entrance into the duodenum. Palmitic-1-^{14}C acid (New England Nuclear Corporation), with added unlabeled palmitic acid (Applied Sciences Laboratories) to make specific activity 0.1 μCi/μmole, in benzene solution was evaporated to dryness in a tissue homogenizer; isotonic M/15 phosphate buffer (pH 6.5 containing 0.08 M NaCl), 15 mM taurocholate, and 1 mg/ml of polyethylene glycol (PEG) was added, and the mixture homogenized at 40 to obtain a clear micellar solution containing 1 mM palmitic acid. Deoxycholic acid was added to yield concentrations of 1 or 2 mM in some cases and all solutions were infused into the duodenum at 2.3 ml/hr. After a 90-min equilibration period, collections were made at 20 and 10 cm below the duodenojejunal juncture, and at the juncture itself, defined by the ligament of Treitz. The collection periods were normally 1 hr in length, after which the samples were kept at 4° until assays were performed. Duplicate samples were assayed for PEG according to the method of Hydén (9). For palmitic acid-^{14}C estimates, 1 ml of each collected specimen or 1 ml of the infusate were extracted with 3 ml each of methanol, chloroform, and 0.1 M HCl–0.1 M KCl. The palmitic acid contained in the chloroform was dried for counting in a liquid

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scintillation spectrometer (Packard 314A), using 10 ml of a toluene solution containing 6 g of 2, 5-diphenyloxazole and 100 mg of 1, 4-bis-[2-(5-phenyloxazolyl)]-benzene per liter.

In two experiments 24-^{14}C-deoxycholic acid (Tracerlab) was added to the infusate. The palmitic acid and the deoxycholic acid were separated by thin-layer chromatography using the solvent system; hexane:diethyl ether:methanol:acetic acid (80:20:3:2). The silicic acid spots were then scraped into counting bottles and counted in a 2:1 vol mixture of Triton X-100 (Rohm and Haas) and toluene-containing scintillation solutes as described previously.

The fractional absorption of fatty acid was calculated from the equation:

Fractional absorption $= 1 - f_2P_1/f_1P_2$, where f_1 is the fatty acid concentration in the infusate, f_2 the fatty acid concentration in the effluent, P_1 the PEG concentration in the infusate, and P_2 the PEG concentration in the effluent.

B. *Incubation of intestinal segments.* Overnight-fasted rats were anesthetized with ether. The incubation technique in isolated intestinal segments was similar to that used by Greenberger, Rodgers, and Isselbacher (10). Micellar solutions were prepared as in the preceding experiments and were injected into 10-cm segments of the intestine in the upper gut, isolated between ties. The animals were kept under light anesthesia for a 30-min incubation period. The individual segments were removed from the animal, the mucosa was then scraped from each segment, and the lipids extracted from the mucosa into chloroform as before with methanol-chloroform–acidic saline solution. The palmitic acid and triglycerides were separated by silicic acid thin-layer chromatography using the same solvent system as was used before. The silicic acid spots were then scraped into counting bottles and counted in 10-ml toluene-scintillation solution. Results were calculated as micromoles of palmitic acid incorporated into triglycerides/hour/gram of wet mucosa.

C. *Deoxycholic acid concentration in mucosa* of small intestine. *In vivo.* Perfusion experiments similar to those mentioned previously were performed. The concentrations of the bile acid and palmitic acid were the same as in several of the previous perfusions, though the radioactive isotopes used were changed. Unlabeled 15 m*M* taurocholic acid sodium salt, unlabeled 2 m*M* deoxycholic acid with added deoxycholic carboxyl-^{14}C acid (Tracerlab, sp act 2.07 mCi/mmole, unlabeled 1 m*M* palmitic acid, and inulin methoxy-^{3}H (New England Nuclear Corp., sp act 641 mCi/mmole) were homogenized in the phosphate buffer used previously to form a clear micellar solution.

The rats were infused for 1 hr at a relatively high rate (11.5 ml/hr) to attempt to keep the concentration of deoxycholic acid in the lumen more nearly constant throughout the entire perfused section of intestine. Effluent collections were made at a sampling site 20 cm from the ligament of Treitz. After 1 hr the animal was sacrificed and the proximal intestine was removed from the animal and divided into 10-cm segments. The mucosa was then scraped from the intestine, extracted in 4 ml of 0.01 *N* NaOH, homogenized, and boiled for 5 min. The supernatant fluid was then extracted and counted in Triton-toluene mixture mentioned earlier. As two radioactive isotopes were present, they were counted on separate channels, corrected by using simultaneous equations which gave the counts resulting from each isotope.

In vitro. Incubation experiments with everted sacs, using the techniques of Dawson and Isselbacher (1) were also performed. The first 30 cm of jejunum of a fasted rat was divided into three 10-cm segments, which were made into everted sacs and incubated at 37° for 1 hr in a micellar solution, identical to that used in the perfusion experiments. The mucosa was extracted and the concentrations of deoxycholic acid were determined as in the perfusion experiments in this section.

Results. From the fractional absorption values shown in Fig. 1, it can be seen that in all cases, as expected, there was progressive absorption of fatty acid at the more distal sites of sampling compared to that at the duodenjejunal juncture. There was a very slight reduction, not statistically significant, in absorption of fatty acid in the presence of

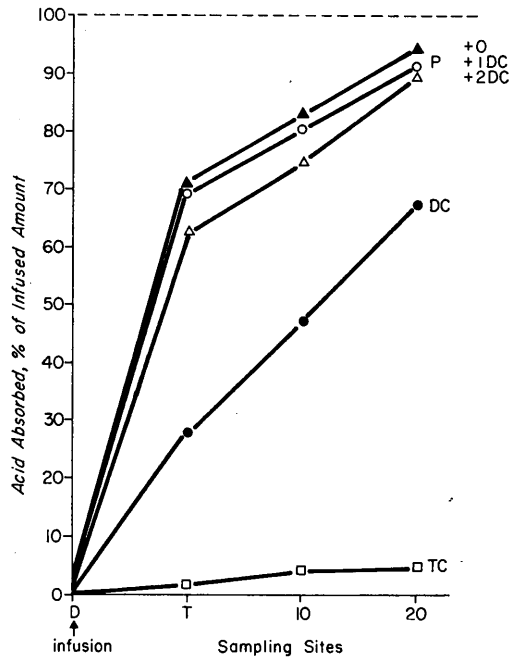

FIG. 1. Absorption of infused acids at sequential intestinal sampling sites. P, palmitic acid-^{14}C; DC, deoxycholic acid-^{14}C, ●; + 0, no added deoxycholic acid, ▲; + 1 DC, added 1 m*M* deoxycholic acid, ○; +2DC, added 2 m*M* deoxycholic acid, △; TC, taurocholic acid-^{14}C, □. Infusions into duodenum (D) at 2.3 ml/hr of labeled acid in isotonic, buffered, 15 m*M* taurocholate micellar solution. Sampling by cannula at ligament of Treitz (T), 10 cm (10) or 20 cm (20) distal thereto. Points represent mean values of four experiments each.

1 m*M* or 2 m*M* deoxycholic acid. The deoxycholic acid absorption was less rapid than that of the palmitic acid at comparable sites. Above all it is evident that fatty acid was well absorbed despite presence of residual deoxycholic acid concentrations in the lumen far in excess of those which caused 10-fold reduction in esterification rates *in vitro* (1, 3, 7).

From the results in Table I it can be seen that esterification of fatty acid taken up by the intestinal mucosa occurred about as rapidly in the presence of deoxycholic acid as when 15 m*M* taurocholate was used alone. Again a minimal trend toward reduction of esterification rates was seen with added deoxycholic acid, but the differences were not significant. These findings demonstrated that the fatty acid absorbed was esterified in the mucosa of the intestinal wall, not simply taken up as free fatty acid.

From the bar graph in Fig. 2 it can be seen that the concentration of deoxycholic acid in the mucosa of the everted sacs was six to ten times as great as it was in the mucosa of the perfused rats. Results were expressed in terms of deoxycholic acid per gram of wet mucosa, since interpretation of the intracellular water content arrived at by calculation from the ^{3}H-inulin data led to additional unresolved questions.

Discussion. These experiments show that in the living animal, contrary to results obtained *in vitro*, deoxycholic acid does not inhibit absorption of fatty acid from the lumen into the mucosa of the proximal small intestine. They show further that in the same proximal area of intestine the fraction of fatty acid absorbed is greater than the fraction of deoxycholic acid absorbed, so that the fatty acid is actually absorbed along with but more rapidly than the deoxycholic acid.

Further, the data show in the *in vivo* situation that a high concentration of deoxycholic acid in the lumen does not inhibit the esterification of fatty acid into triglycerides in the upper small gut. Thus the living animal can handle concentrations of deoxycholic acid in

TABLE I. Esterification of Triglycerides in Rat Jejunal Mucosal Sacs.[a]

Deoxycholic acid added	Micromoles of triglycerides/g of wet mucosa/hr	Mean ± SD[b]
None	2.8 2.4 2.6 2.6 2.5 2.0 3.7 1.6 2.4 1.5 0.9 1.3 1.8 1.6	2.1 ± 0.71
1 m*M*	1.8 1.8 2.2 2.3 1.5 1.6 2.7	2.0 ± 0.40
2 m*M*	0.8 2.1 2.5 2.2 1.9 1.7 1.1 2.1	1.9 ± 0.48

[a] Incubated 30 min with 15 m*M* taurocholate, 1 m*M* palmitic acid ± deoxycholic acid.
[b] SD = standard deviation.

the lumen which *in vitro* cause both extensive damage to the mucosa and inhibitory effects on the esterification of fatty acids to triglycerides. The high concentration of deoxycholic acid found in the mucosa of the everted sacs compared to that of the perfused rat indicates a very rapid removal of deoxycholic acid from the mucosa of the living animal. The earlier work of Sjövall and Åkesson (11) had shown the quick recovery from bile fistulas of labeled deoxycholic acid absorbed from the intestinal lumen, and negligible recovery of it from thoracic duct drainage. This was confirmed under our experimental conditions and indicates removal of deoxycholic acid from mucosa *in vivo* via intact mesenteric circulation, maintaining mucosal concentrations below levels found *in vitro* which are damaging to intestinal epithelial cells.

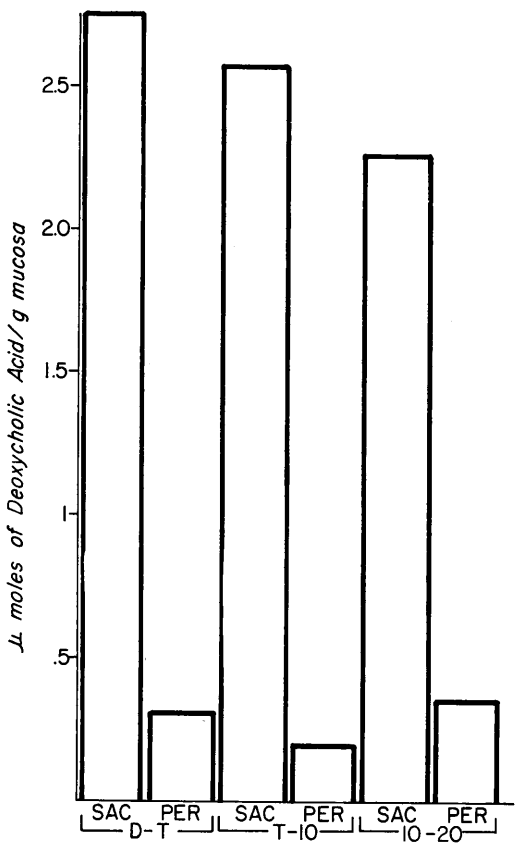

FIG. 2. Concentrations of deoxycholic acid remaining in the mucosa after 1 hr *in vitro* incubation of everted sacs (Sac) or *in vivo* perfusion (Per) under conditions described in text. Mucosa from three different 10-cm segments of intestine was extracted: (1) duodenum to ligament of Treitz (D-T); (2) the ligament of Treitz to 10 cm distal thereto (T-10; (3) from 10 cm to 20 cm distal to the ligament of Treitz (10-20).

In summary, these experiments indicate that the toxic effects shown by deoxycholic acid *in vitro* do not occur *in vivo,* apparently because transfer of free bile acids into the mesenteric venous system prevents them from reaching dangerous intracellular concentrations. This adds further evidence to the concept that in the blind loop syndrome, unconjugated bile salts are not responsible for the production of steatorrhea, but that decrease in the concentration of conjugated bile salts is the more likely mechanism. The importance of an intact mesenteric circulation in keeping intestinal mucosal free bile acid concentrations from reaching damaging levels is emphasized by these experimental findings.

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