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Research Article

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Bile Salt Regulation of Fatty Acid Absorption and Esterification in Rat Everted Jejunal Sacs In Vitro and into Thoracic Duct Lymph In Vivo

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ABSTRACT This study was performed to investigate whether the malabsorption of fat in the blind loop syndrome is due to the presence of free bile acids or to a deficiency of conjugated bile salts produced by bacterial degradation of normal bile salts, as well as to learn something of the mechanisms by which bile salts might regulate fat absorption. In the everted gut sac of the rat in vitro, conjugated bile salts were necessary for maximal rates of fatty acid esterification to triglycerides, whereas free bile acids inhibited this process even in the presence of physiologically normal or higher concentrations of conjugated bile salts. In contrast, in the living animal the addition of similar or higher concentrations of free bile acids to infusions of fatty acids in taurocholate micellar solutions produced no reduction in the amount of fatty acid absorbed into lymph or the amount of fatty acid esterified into lymph triglyceride. Both in vitro and in the living animal, reduction in the conjugated bile salt concentration reduced both the rate of fatty acid uptake by the intestine and the esterification into triglycerides. It is concluded that the steatorrhea of the blind loop syndrome or other conditions in which upper intestinal stasis allows bacterial proliferation is not due to presence of increased gut luminal concentrations of free bile acids, but rather is a consequence of lowered concentrations of conjugated bile salts.

INTRODUCTION

The importance of conjugated bile salts in facilitating fat absorption has been well established (1), and steatorrhea has been recognized in a number of disease states associated with deconjugation of bile salts in the proximal gut or reduction of the bile acid pool size. It has been unclear, however, whether malabsorption of fats associated with bacterially-altered bile salts is due to excessive amounts of unconjugated bile salts or deficiency of conjugated bile salts. That certain bacteria which may be present in the small intestine and which can produce free bile acids [1] from conjugated bile salts has been known for many years (2) but recently has received much additional attention (3–5). Tabaqchali and Booth (6), and also Rosenberg, Hardison, and Bull (7), demonstrated free bile acids in the upper small gut of patients with steatorrhea and proximal gut bacterial overgrowth. These workers attributed the steatorrhea to the presence of these free bile acids, since Dawson and Isselbacher (8) and Donaldson (9) had shown that deoxycholate markedly inhibited fatty acid esterification by intestinal segments in vitro. However, Tabaqchali, Hatzioannou, and Booth (10) have reported also that decreased concentrations of conjugated bile acids were found in the proximal small gut in nine patients with steatorrhea and bacterial overgrowth in the upper small gut; the steatorrhea was lessened in one patient by feeding supplemental taurocholate. These results supported the previous work in dogs by Kim, Spritz, Blum, Terz, and Sherlock (11), who from similar findings suggested that deficient concentrations of conjugated bile salts were more important in producing fat malabsorption than the accumulation of free bile acids.

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[1] Systemic names of bile acids referred to are: cholic acid, 3α, 7α, 12α-trihydroxy-5β-cholanoic acid; deoxycholic acid, 3α, 12α-dihydroxy-5β-cholanoic acid; chenodeoxycholic acid, 3α, 7α-dihydroxy-5β-cholanoic acid; taurocholic acid, 3α, 7α, 12α-trihydroxy-5β-cholanoyl taurine, taurochenodeoxycholic acid, 3α, 7α-dihydroxy-5β-cholanoyl taurine; taurodeoxycholic acid, 3α, 12α-dihydroxy-5β-cholanoyl taurine.

We have studied the effect of different conjugated and unconjugated bile salts on fatty acid esterification in vitro by the small gut mucosa of the rat, as well as the role of the different bile salts in fatty acid absorption and esterification into rat lymph in vivo. It is the purpose of this report to show that free bile salts produce no inhibition of total fat absorption or esterification of fatty acids in vivo, despite the inhibitory effects of free bile acids in vitro. Both in vivo and in vitro, the concentration of conjugated bile salts seems to be the critical factor in fatty acid absorption and esterification by the intestine.

METHODS

Radioactive palmitic acid-1-^{14}C and oleic acid-1-^{14}C were greater than 98% pure as supplied,[2] which was confirmed by thin-layer chromatography. The fatty acids were kept in hexane at 5°C; unlabeled palmitic acid[3] (greater than 99% purity) or oleic acid[4] (greater than 99% purity) were added to give a final specific activity of 0.1 μc/1.0 μmole. All bile salts were of the highest purity commercially available,[5, 6] and were compared with pure bile salts graciously supplied.[7] All bile salts were tested by thin-layer chromatography (12), and the amount of contamination was estimated by the use of an enzymatic method for determination of bile acids, modified from Iwata and Yamasaki (13). The enzyme used in this estimation was obtained initially as a crude cell preparation of *Pseudomonas testosteroni*, although recently a more purified preparation from the same source has been generously provided.[8] The commercial preparations of sodium taurocholate contained no deoxycholate, but both contained cholic acid in varying amounts up to 3%.

Albumin fraction V powder and glycerol-1-monopalmitate (1-monopalmitin) were obtained commercially,[3] and the albumin subsequently was washed with chloroform or hexane to reduce the free fatty acid content. Glycerol-2-monoolein (2-monoolein)[9] and the 2-hexadecyl ether of glycerol[7] were generously donated. All other chemicals were analytical grade obtained from commercial sources.

In vitro. Fasted female Sprague-Dawley rats weighing approximately 200 g were killed by a blow on the neck. Intestinal everted sacs of 5 cm length were prepared in a fashion similar to the technique described by Wilson and Wiseman (14), everting the intestine over a glass rod. The sacs were incubated in micellar solutions of palmitic or oleic acids made up as follows: the fatty acids in hexane were evaporated to dryness under N_2 in tissue homogenizers, and then solubilized with ether, which was allowed to evaporate, leaving a thin film of lipid on the wall of the homogenizers. The bile salts were then homogenized at 40°C in an isotonic mixture of M/15 phosphate buffer pH 6.5 in 0.08 M NaCl containing 2 mg/ml of glucose; this process produced a clear micellar solution with an optical density of < 0.025, compared with phosphate buffer at 650 mμ. Monoglycerides were added as indicated at the same time as the fatty acids. The sacs were incubated under 95% O_2 and 5% CO_2 at 37°C, using an oscillating water bath. At the end of the experiments the sacs were rinsed serially in saline and 1% albumin solutions; the serosal fluid was removed and the empty sac weighed. The sac was placed immediately in 3 ml of methanol, homogenized, and 3 ml of chloroform was added; after further homogenization and acidification with 3 ml of 0.1 M HCl in 0.1 M KCl, the mixture was frozen. A 1-ml sample of the medium was extracted in a similar fashion. A fraction of the chloroform layer was removed and evaporated, lipids redissolved, and a portion spotted and run on thin-layer chromatograms according to the method of Brown and Johnston (15), using a solvent system composed of hexane: diethyl ether: methanol: acetic acid (80:20:3:2). The separate lipid fractions were identified by relating mobility to pure standards; the silica gel containing the bands of the various lipid classes was scraped into separate vials and counted using a mixture of 6 g of 2,6-diphenyloxazole and 100 mg of 1-4-bis-[2-(5-phenyloxazolyl)]benzene/liter of toluene. The overall efficiency of the counting system using a liquid scintillation spectrometer[10] was about 60%; quenching was corrected by using internal standards of toluene-^{14}C. The results are expressed as micromoles of fatty acid esterified into triglyceride per hour per gram of tissue wet weight. Esterification of fatty acid into mono- or diglycerides using this technique was only a small fraction of that into triglyceride, and for conciseness the results reported here show only triglyceride formation. After incubation of the everted sacs, portions of the tissue were fixed in formalin for light microscopy.

In vivo. Female Sprague-Dawley rats were anesthetized with ether and a polyethylene catheter (PE 50; I.D., 0.023 inch; O.D., 0.038 inch)[11] inserted into the thoracic lymph duct using the technique described by Bollman, Cain, and Grindlay (16). For purposes of infusion, a Transflex[12] catheter (I.D., 0.020 inch; O.D., 0.027 inch) was placed into the stomach and through to the duodenum, and was secured by a stitch just proximal to the entrance of the bile duct. In addition to the lymph duct catheter, the bile duct was catheterized with a polyethylene catheter (PE 10; I.D., 0.011 inch; O.D., 0.024 inch)[11] and the bile drained externally. After operation animals were placed in a transparent plastic cage (30 × 5 × 8 cm) which allowed free movement forward and backward but prevented turning. Under these conditions the animals were under minimal restraint and survived up to 20 days. Animals were allowed to recover from operative trauma usually until the second postoperative day before infusions were begun. In early experiments during the test infusions the animals were allowed to drink normal saline ad lib.; however, this produced some variability in lymph flow, and in later experiments no oral fluids were given during the period of test infusion. At all other times the animals were given free access to 5% glucose in saline. Standard rat pellets[13] were given overnight, but no food given during the period of the test infusion. Overnight infusion of Ringer's lactate containing 5% glucose or test solutions was introduced at 2.3 ml/hr in most experiments

[2] New England Nuclear Corp., Boston, Mass.
[3] Sigma Chemical Co., St. Louis, Mo.
[4] Applied Science Laboratories, Inc., State College, Pa.
[5] Maybridge Ltd., Cornwall, U. K.
[6] Calbiochem, Los Angeles, Calif.
[7] Dr. Alan F. Hofmann, Rochester, Minn.
[8] Mr. J. D. Teller, Worthington Biochemical Corp., Freehold, N. J.
[9] Dr. F. H. Mattson, Procter & Gamble Co., Cincinnati, Ohio.
[10] Model 314A. Packard Instrument Co., Inc., Downers Grove, Ill.
[11] Clay-Adams, Inc., New York.
[12] Minnesota Mining & Mfg. Co., Mico Div., St. Paul. Minn.
[13] Purina Chow Co., St. Louis, Mo.

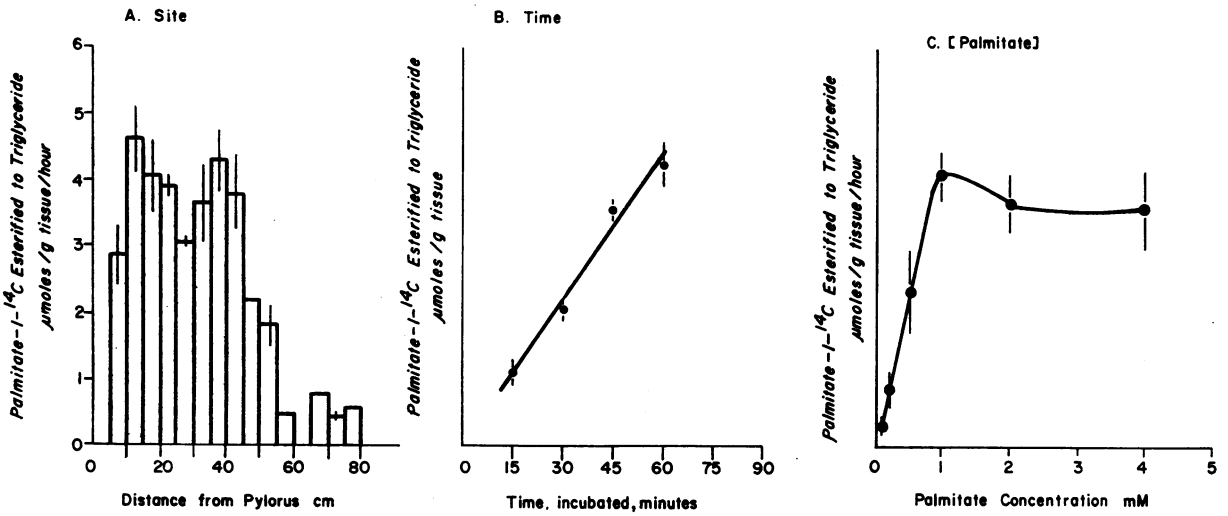

FIGURE 1 Conditions tested to establish standard conditions for esterification of palmitate-1-^{14}C in rat everted jejunal sacs. (A) Site, distance from pylorus; (B) Rate, over one hour; (C) Palmitate concentration. Values shown are the means and SEM.

with an infusion pump.[14] The test solutions were prepared freshly each day as described in the in vitro study. Change to a different composition of infused solution was effected after no less than 5 hr and the different test infusions were then given in reverse order on successive days (17), so that each animal might act as its own control. In all the studies involving low concentrations of taurocholate below 10 mmoles/liter, infusions were maintained at 37°C by keeping the syringe heated with a coil of tubing through which warm water circulated. Samples of lymph were collected hourly into heparinized graduated tubes, and kept at 0–4°C until extraction the same day. All animals were healthy at the time of the infusions; poor lymph flow (< 1.0 ml/hr) or generally poor condition of the animals led to rejection of the experiments.

1 ml of the lymph and of each infusate was extracted with methanol (3 ml), chloroform (3 ml), and 0.1 M HCl/0.1 M KCl (3 ml); after centrifugation a fraction of the chloroform layer was removed. This chloroform was evaporated under nitrogen, and the lipids were redissolved in a smaller known volume of chloroform. An aliquot of this solution was placed in a counting vial, the chloroform evaporated, and the lipid residue was counted directly as described above to quantitate total radioactivity. An additional aliquot was run on thin-layer silica gel plates, and subsequent steps were carried out as described for the in vitro study. The results were expressed as μmoles of total lipid in the lymph per hour and μmoles of fatty acid esterified to triglyceride per hour. In other in vivo experiments, infusions of micellar 1 mM palmitate in 15 mM taurocholate–polyethylene glycol (PEG) solution, with or without added deoxycholate, were carried out in rats with jejunal cannulae to recover all unabsorbed fluid infused. The sites of jejunal cannulation were at the duodenal-jejunal junction, and at 10 cm and 20 cm distal to that point; either the deoxycholate or the palmitate were labeled with ^{14}C to permit estimation of their absorption. In some of the animals perfused with labeled palmitate, assay of incorporation into triglycerides in the intestinal wall was also carried out. In some of the long-term perfusion experiments, the animals were killed with ether after the infusion, and segments of the intestine were fixed in formalin for light microscopy. Other animals infused with similar micellar solutions were killed after infusing 3% glutaraldehyde solution through the duodenal cannula, and the excised mucosa further fixed in glutaraldehyde and osmium tetroxide before embedding the tissue in Epon Resin for thin sectioning and electron microscopy.

[14] Harvard Apparatus Co., Inc., Dover, Mass.

RESULTS

In vitro. In Fig. 1 are shown the variables that were tested to achieve optimal esterification of fatty acids and standardization of conditions in vitro using rat everted jejunal sacs, namely, site in the intestine, time, and fatty acid concentration. In Fig. 1 A, with 1 mM palmitic acid in micellar solution in 15 mM sodium taurocholate at pH 6.5, it can be seen that there was considerable decrease in esterification in the ileum, as the jejunum showed maximal activity of approximately 4–5 μmoles/g of wet tissue per hour. Fig. 1 B shows that the esterification of palmitic acid was linear with time up to 60 min under similar incubation conditions as described for Fig. 1 A. Fig. 1 C shows no great increase in esterification with increasing the concentration of palmitate above 1 mmole/liter in micellar solution in 15 mM taurocholate. The optimal pH for esterification of palmitate under conditions as given for Fig. 1 A was 6.5–7.0. There was considerable decrease in the esterification of fatty acids below pH 6, but these solutions were not clear, indicating incomplete micellar solubilization and probable reduction in availability of substrate. At pH values from 7.5 to 8.5 the rate of esterification fell from about 50% to 20% of that in the

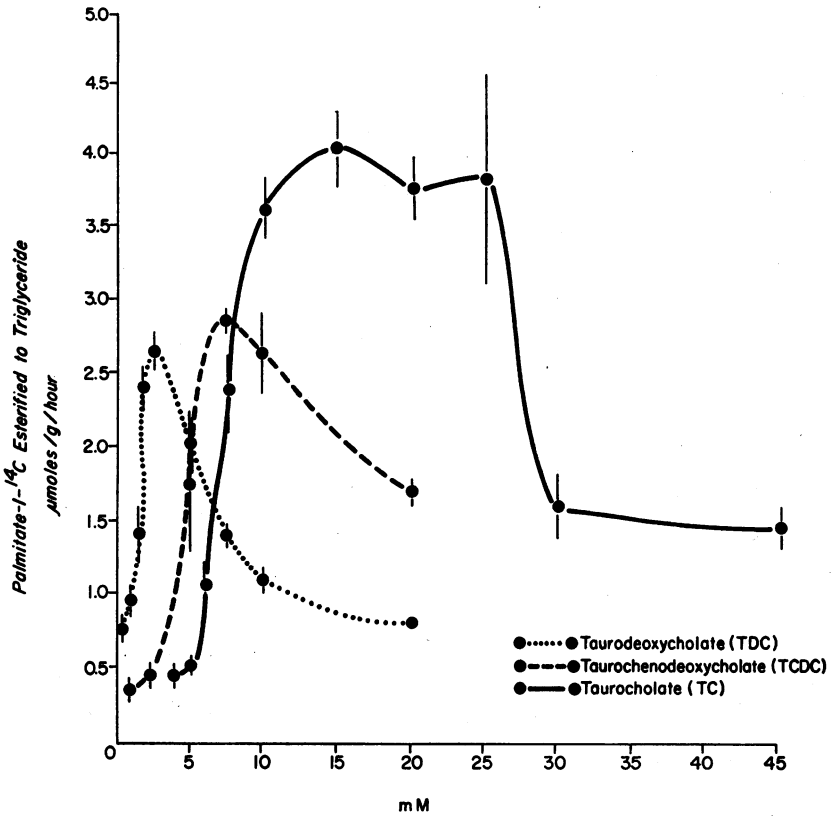

FIGURE 2 Effect of increasing concentrations of taurine-conjugated bile salts on the esterification of palmitate-1-^{14}C to triglycerides in mucosa of everted jejunal sacs. The means and SEM are shown.

6.5–7.0 range. Thus in all subsequent experiments jejunal sacs obtained 15–45 cm from the pylorus were incubated for 30 min at pH 6.5 with 1 mM palmitic acid in 15 mM sodium taurocholate. This set of conditions was taken as a standard against which were compared subsequent experiments using different bile salt concentrations.

Conjugated bile salts. The esterification of palmitic acid into triglyceride with increasing concentrations of sodium taurocholate, sodium taurochenodeoxycholate, and sodium taurodeoxycholate is shown in Fig. 2. There was a broad peak of high palmitate esterification rates from 10 to 25 mM sodium taurocholate; the value at 5 mM was similar to that obtained without bile salts, with palmitic acid solubilized in 5% albumin solution. Palmitate in sodium taurodeoxycholate and taurochenodeoxycholate showed somewhat lower maximal rates of esterification than with sodium taurocholate; both of the dihydroxy bile salts produced optimal rates of palmitate esterification of approximately 3 μmoles/g per hour. However, optimal esterification occurred at 2.5 mM taurodeoxycholate, and at 7.5 mM taurochenodeoxycholate concentrations. Further increase in the concentrations of the taurine-conjugated bile salts decreased the rates of palmitate esterification to triglycerides. Addition of suboptimal amounts of different taurine-conjugated bile acids produced effects approaching those of optimal

TABLE I

Effects of Addition of Suboptimal Amounts of Taurine-Conjugated Bile Salts on Palmitic Acid Esterification by Intestinal Sacs

Taurocholate	Taurodeoxycholate	Palmitate-1-^{14}C incorporation into triglycerides
mmoles/liter	*mmoles/liter*	*μmoles/g per hr*
4	0.0	0.65*
0	1.0	0.34
4	1.0	2.10
5	1.5	3.50
15	0.0	3.70

* Mean values of paired experiments.

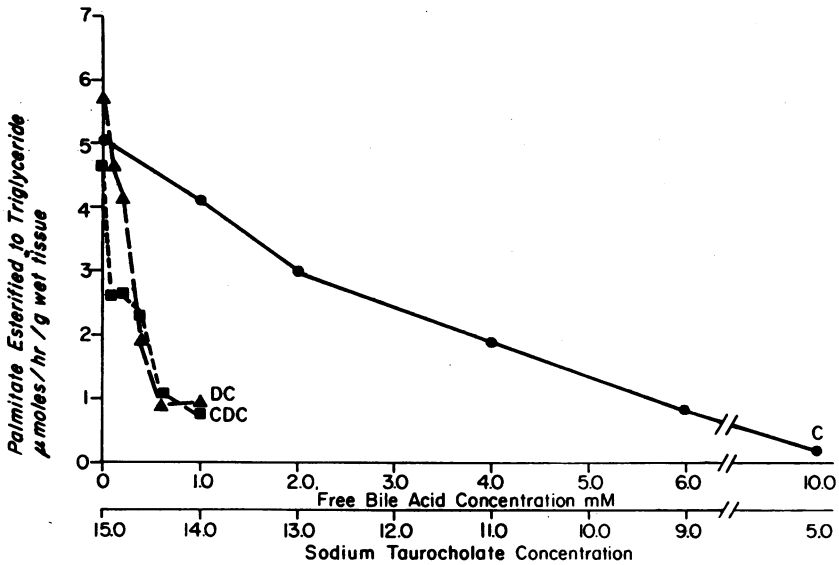

FIGURE 3 Inhibitory effect of free bile acids on esterification of fatty acid to triglycerides in rat everted jejunal sac mucosa, keeping total bile acid/salt concentration at 15 mM and substituting free bile acids for taurocholate. Deoxycholic acid (DC), ▲···▲; chenodeoxycholic acid (CDC), ■---■; cholic acid (C), ●——●.

amounts of a single conjugated acid (Table I). Under the conditions of these in vitro experiments, addition of 0.5 mM concentrations of 1-monoolein, 2-monoolein, or 2-glycerol-hexadecyl ether did not produce any increase in the amount of oleate esterified to triglyceride.

Free bile acids. Intestinal sacs were incubated in micellar solutions of fatty acid and sodium taurocholate in which the free bile acids were either substituted for or added to the sodium taurocholate. In Fig. 3 are shown the results of replacing taurocholate with progressively larger amounts of cholic, deoxycholic, or chenodeoxycholic acids, keeping the total bile salt concentrations constant at 15 mmoles/liter. It can be seen that substitution of even small amounts of deoxycholic or chenodeoxycholic acid for taurocholate reduced esterification of palmitic acid so that at 0.6–1.0 mM concentrations almost complete inhibition of esterification was found, and there was severe histological damage seen by light microscopy. Cholic acid also produced inhibition in fatty acid esterification rates, but the amount required was about 10 times that required for inhibition with the free dihydroxy bile acids. This inhibitory effect of free bile acids on esterification was seen whether the amount of taurocholate was kept at 15 mmoles/liter or increased to 20 mmoles/liter, i.e., the inhibition could not be overcome by additional conjugated bile salt. The inhibition occurred under these conditions even though micellar solubilization of fatty acid was excellent. The bile acid effects on rates of esterification of oleate were approximately similar to those found using palmitate.

Since it is now known that bile salts are absorbed in varying degrees even in the proximal intestine (18, 19), we questioned the effects of bile salts placed on the serosal side of the sacs, although it is not known whether bile salts can enter intestinal cells from the

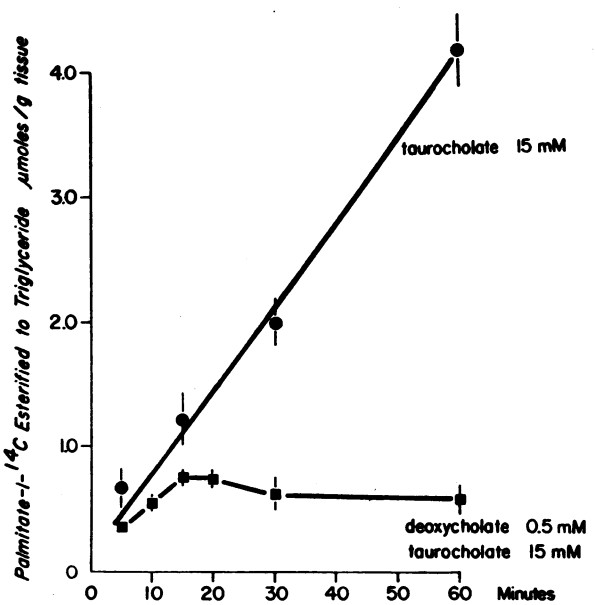

FIGURE 4 Effect of 0.5 mM deoxycholic acid added to 15 mM taurocholate upon the esterification of palmitate-1-^{14}C into triglyceride, over the course of one hour. Values are the means and standard errors of the means.

TABLE II

Effect upon Palmitate Esterification of Preincubation of Intestinal Sacs in Deoxycholic Acid Followed by Incubation with 1 mM Palmitate in 15 mM Taurocholate

Deoxycholic acid concentration	Preincubation temperature	
	4°C	37°C
mmoles/liter	*μmoles/g per hr*	
0.0	*2.0 ±0.34	*1.84 ±0.37
0.1	1.95 ±0.43	1.29 ±0.18
0.2	1.4 ±0.66	0.96 ±0.13
0.4	1.5 ±0.48	0.61 ±0.01
0.6	1.54 ±0.91	0.52 ±0.20
1.0	1.80 ±0.36	0.46 ±0.19

* Mean and SD; four experiments.

serosal side. Using 1 mM palmitic acid in micellar solution with 15 mM taurocholate on the mucosal side, and various concentrations of deoxycholate ranging from 0.2 to 3.0 mmoles/liter at pH values of 6.5 and 7.4 on the serosal side, no apparent inhibition of esterification occurred.

To study how free bile acids cause inhibition of cellular function in vitro, time course and preincubation experiments with deoxycholate were carried out. Fig. 4 shows the effect of 0.5 mM deoxycholate in 15 mM taurocholate on the esterification of 1 mM palmitate into triglycerides, compared with experiments done at the same time using 15 mM sodium taurocholate alone. Inhibition in esterification began at approximately 10 min, and further esterification ceased after 15 min. Table II shows the results of preincubating the sacs in solutions of buffer containing various concentrations of deoxycholate for 15 min at 4° or 37°C, followed by careful washing in saline and in 15 mM taurocholate solutions. After incubation for 30 min at 37°C with 1 mM palmitate in 15 mM taurocholate, inhibition of fatty acid esterification occurred in sacs preincubated with deoxycholate at 37°C, whereas preincubation in deoxycholate solution at 4°C very much reduced this effect.

In vivo. Micellar solutions of 1 mM palmitate in 15 mM sodium taurocholate were infused intraduodenally over 10-hr periods, or for as long as several days. Bile fistula produced slight reduction in lymph lipids which was always noted to some degree and has been commented on also by Simmonds, Redgrave, and Willix (20).

In Table III are shown the results of infusing 1 mM palmitate with either 15 mM taurocholate alone or 15 mM taurocholate and three different concentrations of deoxycholate into three rats with intact biliary systems. In contrast to the data given in Fig. 3, it can be seen that concentrations of deoxycholate several fold greater than those which had inhibited severely the esterification of fatty acids in the in vitro system, produced no in vivo inhibition in the rate of absorption of fatty acid nor any consistent decrease in the amount of esterification of palmitic acid into triglycerides. This is shown even more dramatically in the reverse-order and long-term, steady-state studies, a typical example of which is illustrated by Fig. 5, in which it is quite evident that 2 mM concentrations of deoxycholic acid produced no inhibition in fatty acid absorption and esterification in vivo.

TABLE III

Rate of Incorporation of Infused Palmitate into Lymph Total Lipids and Triglycerides

Solution*		Hours 1	2	3	4	5	Solution*		Hours 6	7	8	9	10
		mEq/hr							*mEq/hr*				
15 mM TC	TL‡	(0.07)	0.56	0.52	0.76	0.67	15 mM TC+1 mM DC	TL	0.75	0.54	0.72	0.61	0.55
	TG‡	(0.04)	0.39	0.34	0.47	0.39		TG	0.37	0.30	0.35	0.27	0.23
15 mM TC	TL	(0.13)	0.43	0.54	0.57	0.64	15 mM TC+2 mM DC	TL	0.71	0.73	0.90	0.83	0.78
	TG	(0.10)	(0.29)	0.44	0.45	0.52		TG	0.42	0.54	0.63	0.58	0.56
15 mM TC	TL	(0.02)	(0.21)	0.64	0.63	0.67	15 mM TC+3 mM DC	TL	0.77	0.46	0.84	1.10	1.30
	TG	(0.01)	(0.10)	0.42	0.26	0.32		TG	0.68	0.36	0.76	1.00	1.10

TC = taurocholate; DC = deoxycholate.

* Infusion rate, 1.4 ml of 1 mM palmitate-1-^{14}C per hour.

‡ TL = total lipids; TG = triglycerides. Transient low values obtained before approaching steady state given in parentheses.

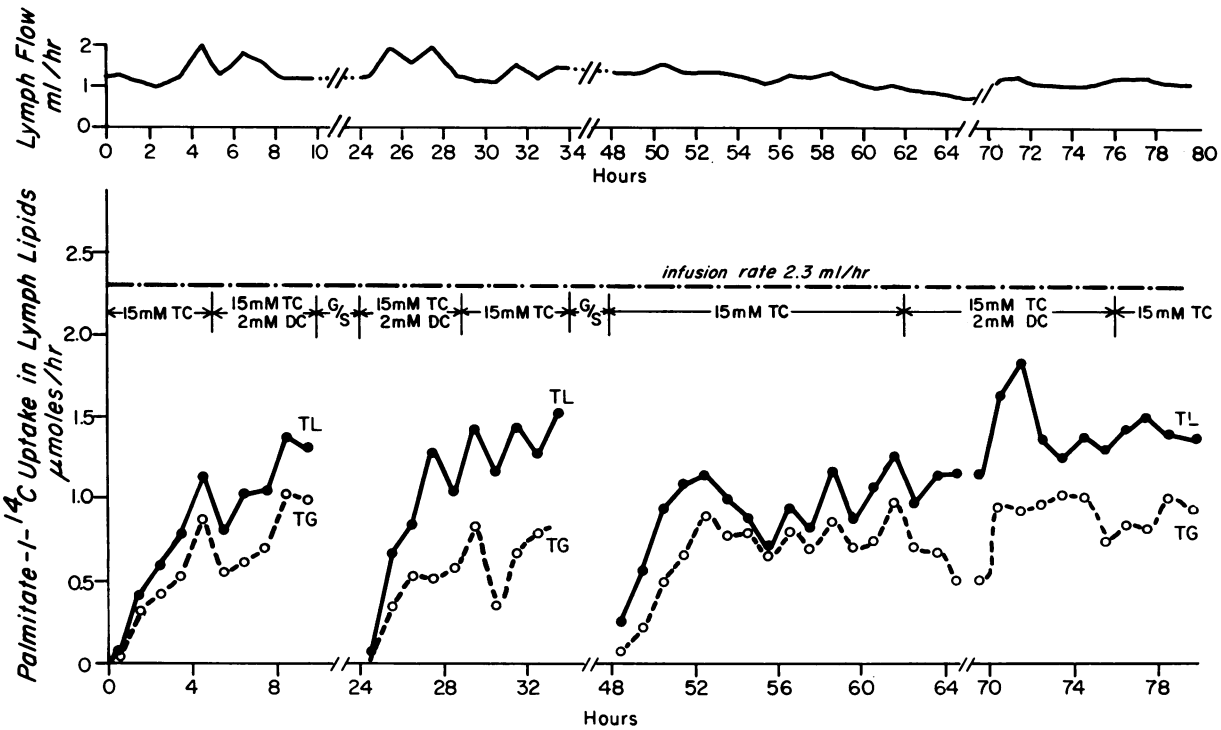

FIGURE 5 The obvious lack of inhibitory effect in vivo of 2 mM deoxycholic acid upon absorption-esterification of 1 mM palmitate-1-^{14}C in 15 mM taurocholate micellar solutions, as shown by reverse-order and long-term, steady-state intraduodenal infusions, begun on the second postoperative day. Taurocholate (TC); deoxycholic acid (DC); glucose (G); saline (S); total lipids (TL), ●——●; triglycerides (TG), ○---○.

Tissue from the jejunum after 10 hr of infusion with 2 mM deoxycholate in 15 mM taurocholate was studied histologically by light and electron microscopy (Fig. 6). No damage to the villi was seen and the fine structure of the mucosal cells was also normal in appearance.

In some preliminary experiments on the absorption from perfused loops of proximal small gut of either 1 mM palmitic acid-1-^{14}C or 2 mM deoxycholate-^{14}C in the presence of the other unlabeled compound and 15 mM taurocholate in 0.1% PEG, it was found that deoxycholate was still in relatively high concentration (1.5 mmoles/liter) in the luminal fluid, although 80–90% of the fatty acid had been absorbed. This indicated that the difference in the in vivo and in vitro data was not due to rapid selective absorption of the deoxycholate in the proximal intestine, followed by more distal absorption of the fatty acid.

The results of experiments in which lower concentrations of bile salt were used are shown in Fig. 7. The over-all rate of fatty acid absorption into lymph was markedly reduced at lower concentrations of taurocholate than 10 mmoles/liter, and at 5 and 3 mM taurocholate no increase in absorption of palmitate into lymph was seen over that observed when the fatty acid was suspended in albumin solution. At both these latter concentrations of taurocholate, 3 and 5 mmoles/liter, the mixtures of fatty acid and bile salts were cloudy and hence a suspension rather than a micellar solution was probably present, since the critical micellar concentration of taurocholate is in the range of about 7–8 mmoles/liter under these conditions (21). At these low levels of absorption, the small amount of lipid absorbed in lymph still was predominantly esterified to triglyceride, although the amounts involved were small. Even more striking were the reverse-order data (Fig. 8) obtained when 1 mM palmitate was infused in either 15 mM or 7.5 mM taurocholate solutions.

Regulation of the rate of fatty acid absorption by the concentration of taurocholate is shown (Fig. 9) to extend beyond the range indicated by the previous in vitro data. In the living animal with bile and thoracic duct fistulas receiving constant intraduodenal infusions of 1 mM palmitate-1-^{14}C in varying concentrations of taurocholate, no reduction in absorption-esterification rates was noted as taurocholate concentrations were increased above 25 mmoles/liter. These findings also contrasted with the data obtained in the everted sac preparations. Below the critical micellar concentration of the bile salt,

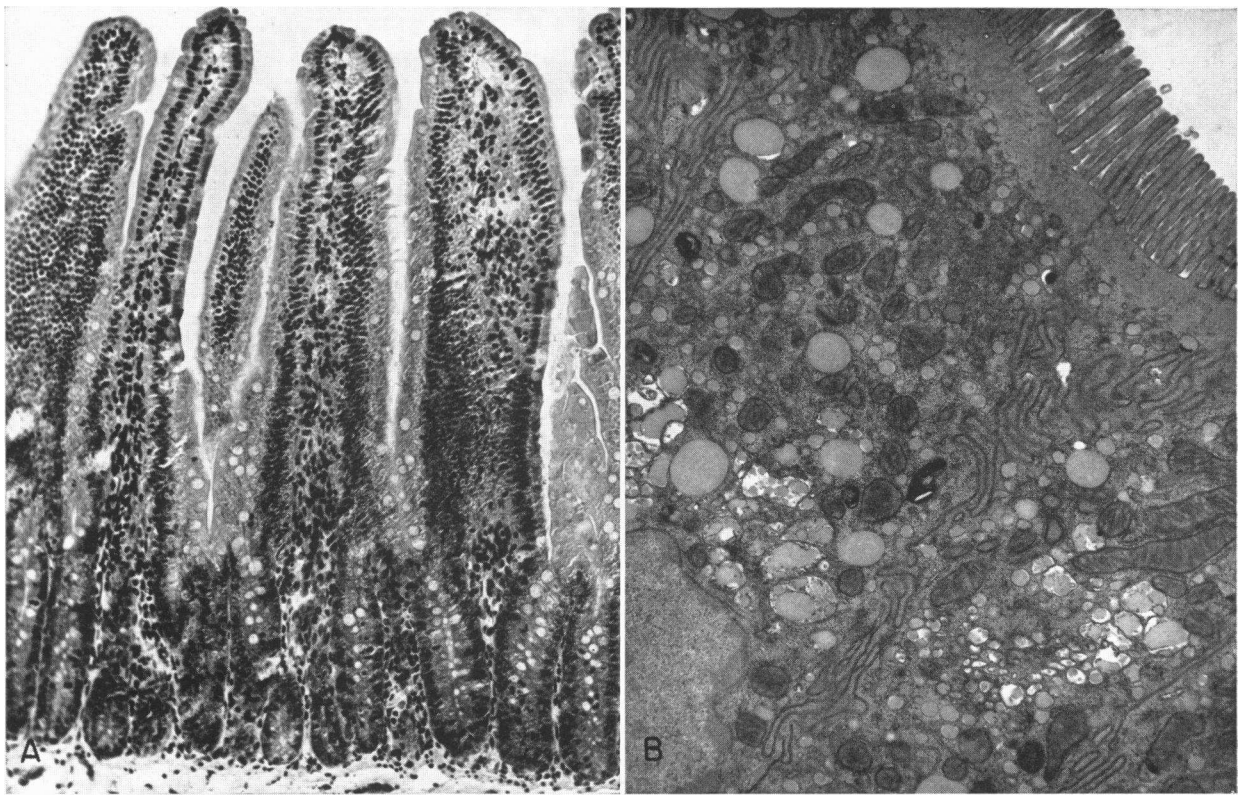

FIGURE 6 Appearance of the rat jejunal mucosa after 10 hr of infusion of 2 mM deoxycholic acid in 15 mM sodium taurocholate, buffered to pH 6.5 with 1/15 M phosphate in 0.08 M sodium chloride and 2 mg/ml glucose. (A) Light microscopy (×108); (B) electron microscopy (×9000). No damage to the structure or ultrastructure of the mucosal cells is seen.

the rate of fatty acid absorption was low also in the living animal, but above that bile salt concentration fatty acid absorption and esterification rates increased without passing through a maximum, approaching asymptotically the infusion rate.

The specific activity of lymph triglycerides (μc per μmole) was noted to approach the specific activity of the infused palmitate (0.1 μc/μmole) as the taurocholate concentration was increased above 30 mmoles/liter. At lower cencentrations of taurocholate in the 10–20 mM range both the lymph total fatty acid absorption rate (μmoles per hour) and triglyceride specific activity (μc per μmole) were always observed to be less than corresponding values for the infused palmitate. Intestinal mucosal retention of a portion of the infused fatty acid was found consistently, part as free palmitate and part as glycerides, but the sum was not, in general, enough to account quantitatively for the discrepancy between fatty acid infused and that recovered in the lymph.

It was further observed that infusion of bile salts alone, without fatty acid, stimulated release of intestinal mucosal triglycerides into thoracic duct lymph. As shown in Fig. 10, there was a marked release of labeled triglycerides into lymph chylomicrons after infusion of 15 mM sodium taurocholate in a rat labeled the previous day with palmitate-^{14}C. When glucose-saline solution was infused instead, the output of triglycerides decreased to nearly zero, only to rise sharply again when taurocholate was reinfused.

DISCUSSION

Our in vitro studies using the rat intestinal everted gut sac show that deoxycholic acid inhibits esterification in rat intestinal tissue and appears to accelerate the histological damage which occurs ordinarily in this preparation. We found that this inhibition of esterification by deoxycholate occurred rapidly, but was dependent upon temperature; preincubation in deoxycholate at 4°C produced considerably less inhibition of subsequent palmitate esterification than did deoxycholate preincubation at 37°C. These data are consistent with those of earlier workers (8, 9), who suggested that such inhibition might explain the steatorrhea observed in the blind loop syndrome, and the finding that appreciable concentrations of free bile acids were indeed demonstrable (6, 7) in the intestinal lumens of patients with the syndrome

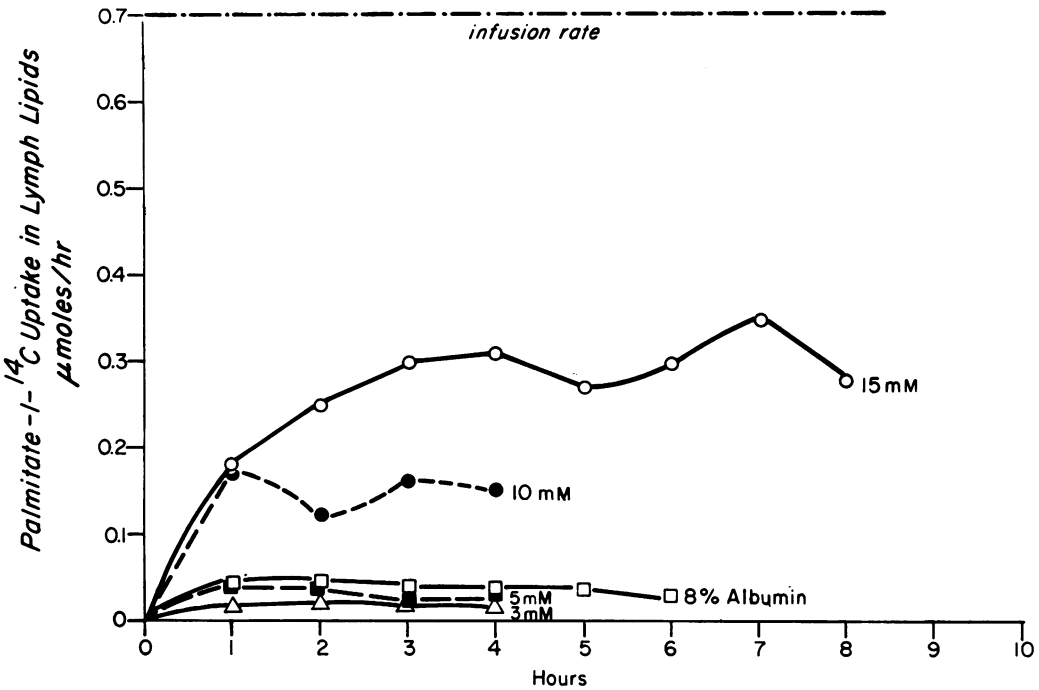

FIGURE 7 Effect of lowering the concentration of sodium taurocholate infused with 1 mM palmitate-1-^{14}C upon rate of uptake into lymph lipids, compared with infusing 8% bovine albumin suspensions of the palmitate.

added support to this view. The ability of certain enteric bacteria to dehydroxylate (22) as well as deconjugate (2) normal bile salts had been proved earlier.

It should be pointed out that measurement of palmitate incorporation into triglycerides represents the resultant of several steps; the uptake of fatty acid at the cell surface, penetration, fatty acid activation, and glyceride synthesis. Attempts to estimate fatty acid uptake by tissue rinsed after incubation produced unreliable results since it could not be determined whether the radioactive

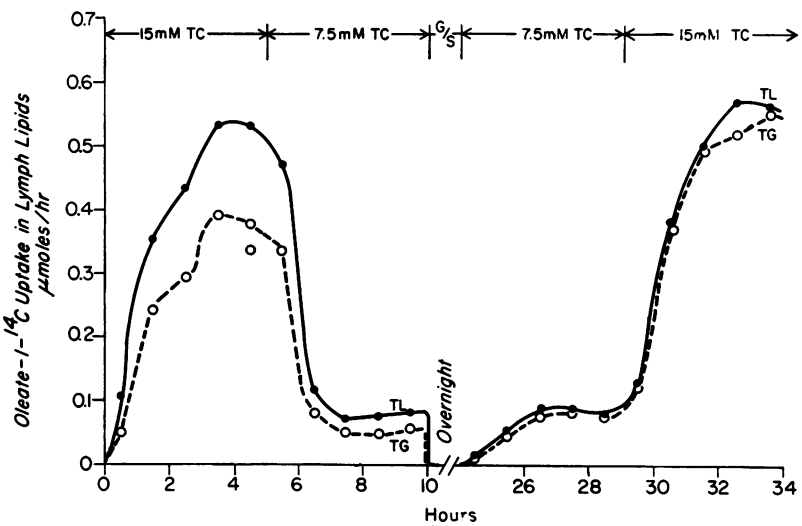

FIGURE 8 Rate of uptake of 1 mM oleate-1-^{14}C into lymph total lipids (TL), ●——●, and triglycerides (TG), ○- - -○, during intraduodenal infusions of 15 mM or 7.5 mM taurocholate micellar solutions of the oleate, or of glucose-saline (G/S).

fatty acid was actually in the cells or merely adherent to the fuzzy external surface coating of the cells.

The marked difference in effective concentrations of cholic and deoxycholic acids is notable in view of their very similar chemical structures, differing by only one oxygen atom at position 7 on the steroid nucleus. There are no data available on the concentration of bile salts at the intracellular site of glyceride synthesis. While neither an intracellular inhibitory effect of free bile acids on glyceride synthesis nor an altered micellar form, which is less efficient for fatty acid delivery, can be excluded entirely, it seems likely from the known effects of deoxycholate on membrane dissolution that at least an initial inhibitory effect would be expected at the cell surface. Pope, Parkinson, and Olson (23) have demonstrated that deoxycholate inhibits intestinal transport of both amino acids and glucose, neither of which are dependent upon micellar solution or intracellular synthesis. They commented also upon inhibition of other cell functions such as protein synthesis, oxygen uptake, and glucose incorporation into lipids, suggesting general cell damage induced by deoxycholate acting destructively upon the cells. Although the interference by free bile acids with micelle formation can be overcome by additional conjugated bile salts (24), our observations on fatty acid esterification to triglycerides in everted sacs indicated that the inhibition induced by 1 mM deoxycholate was not overcome by up to 20 mM taurocholate. These data were taken to imply that impaired micelle formation by free bile acids was not the entire explanation for decreased esterification, but that inhibition at the cell surface or within the cell might be occurring, possibly owing to accelerated disintegration of the cells in various in vitro preparations. Sodium taurocholate, the prevalent natural bile salt of the rat, seemed to produce optimal fatty acid esterification at about 15–20 mmoles/liter, the normal total bile salt concentration in the upper small gut area used for our sacs being 10–20 mmoles/liter (25). It cannot be resolved by these experiments whether the higher rates of esterification observed could have been entirely due to enhanced delivery of fatty acids by micellar solubilization, to improved penetration of the cell surface due to bile salt effects on the membrane, or to bile salt effects at the site of activation and glyceride formation. Reduction below 10 mM taurocholate in our in vitro system produced lower rates of fatty acid esterification, and below 7.5 mmoles/liter this effect was marked. Decreased solubilization of fatty acid in micellar solution reasonably could account for decreased availability of fatty acid for delivery and uptake, and secondarily, of esterification. In this system addition of monoolein did not alter the rate of esterification of fatty acids, but the actual amount of fatty acid supplied in micellar solution was not rate limiting for the esterifying system. We concluded from our in vitro study that free bile salts generally are inhibitory regard-

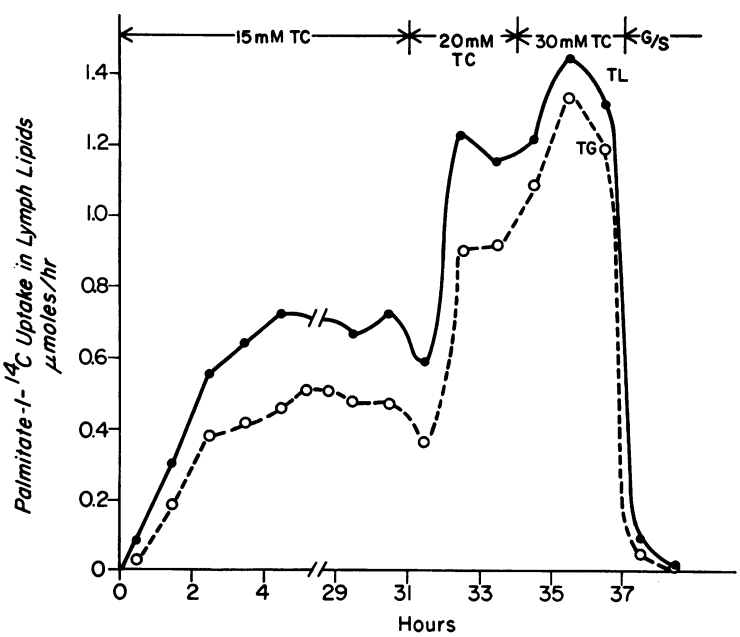

FIGURE 9 Effects of increasing concentrations of taurocholate (TC) infused with 1 mM palmitate-1-^{14}C upon rate of uptake of the palmitate into lymph total lipids (TL), ●——●, and triglycerides (TG), ○- - -○, and the effect of glucose-saline (G/S) infusion.

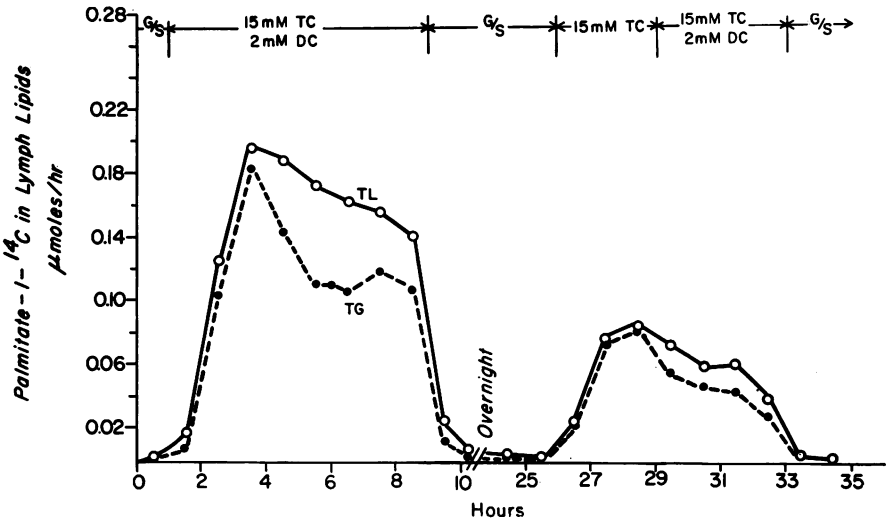

FIGURE 10 Release of labeled palmitate in lymph total lipids (TL), O——O, and triglycerides (TG), ●---●, during intraduodenal infusion of taurocholate (TC) with and without deoxycholic acid (DC) or glucose-saline (G/S), all solutions containing no palmitate-1-^{14}C. The animal had been prelabeled by an infusion of 1 mM palmitate-1-^{14}C in 15 mM taurocholate during the previous day, and had then been infused during the intervening night with glucose-saline solution.

less of the conjugated bile salt concentration, and secondly, that reduced concentration of conjugated bile salt decreases the amount of fatty acid esterified.

However, in the living animal, findings had been made (11) which cast some doubt on the actual importance of free bile acids in the pathogenesis of the steatorrhea observed in the blind loop syndrome. Dogs with surgically-created blind jejunal segments and proximal gut bacterial overgrowth had steatorrhea which could be decreased by feeding additional amounts of conjugated bile salts; this latter finding has been recently confirmed in man (10). Kim et al. (11) suggested that perhaps bacterial deconjugation of bile salts caused a deficiency of these necessary detergents for micelle formation, which was critical in producing the fat malabsorption, and implied that the presence of free bile acids might not be so important. Since we had found that extra conjugated bile salts did not overcome the inhibition in fatty acid esterification by rat intestinal tissue in vitro despite clear micelle formation, this discrepancy indicated that in vitro data perhaps could not be extrapolated to the situation in the live animal and that resolution of the question was required.

Therefore, we began the series of infusions into unanesthetized rats of micellar solutions of similar composition to those which had been used in the sac experiments. The results of these studies showed that no inhibition by deoxycholate of fatty acid uptake and esterification into lymph triglycerides occurred. No inhibition of fat absorption occurred even at high concentrations of deoxycholate, as long as sufficient (8–10 mmoles/liter) levels of taurocholate were present in the infusate. Microscopic study of the upper and lower jejunum confirmed other workers' data (26) that no histological damage is noted in the mucosa after the deoxycholate infusion. The differences between in vitro and in vivo results suggest that some compensatory factors act in the whole animal. Initially, we postulated that fatty acids were absorbed more distally after deoxycholate was absorbed rapidly and selectively in the proximal gut. Dietschy, Salomon, and Siperstein (18) had shown that in the upper small gut of rats free bile acids can be absorbed rapidly by passive nonionic diffusion, and this was confirmed in man by Hislop, Hofmann, and Schoenfield (19). However, our experiments in which the jejunum of the unanesthetized rat was perfused with labeled deoxycholate or palmitic acid showed that most of the fatty acid is absorbed even though the concentration of deoxycholate remains well above the level which produces inhibition of esterification in the everted gut sac. We have recently demonstrated that after 30 min exposure to 2 mM deoxycholic acid in 15 mM taurocholate the mucosal concentration of deoxycholate in the everted sac is approximately tenfold higher than in a similar area of jejunum perfused during life, presumably due to the intact circulation which can remove free bile acids via the mesenteric blood in the living animal.

The concentration of conjugated bile salts both in vitro and in the living animal is critical to the amount of

fatty acid esterified to triglyceride by the intestinal tissue. In rats with intact biliary systems we have not found appreciable differences in the rates of fatty acid absorption into lymph using concentrations of taurocholate ranging from 10 to 20 mmoles/liter to solubilize the fatty acid infused. However, clear differences have emerged below 7.5 mmoles/liter, especially in rats with biliary fistulae, presumably due to inadequate micelle formation. In our studies with 1 mM palmitate, the amount of fat absorbed, or the proportion esterified when the palmitate was suspended in an 8% albumin solution was at least as great as when the palmitate was suspended in 5 mM taurocholate. Simmonds et al. (20), in contrast, have shown good absorption in bile fistula rats when oleic acid was presented as a sonicated emulsion in a bile salt mixture at its critical micellar concentration (CMC) in phosphate buffer using a synthetic detergent. In all our in vivo studies we used palmitic acid, which is considerably less soluble than oleic acid. Both Simmonds et al (20) and Morgan (17) have shown good fat absorption from emulsions produced by ultrasonification; these fine emulsions of less than 0.5 μ mean particle diameter were stable for up to 1 wk.

From our in vivo studies it appears that the steatorrhea of the blind loop syndrome is not due to the intraluminal production of free bile acids by bacterial action on normal bile salts. We believe that this malabsorption of fat is caused by inadequate micelle formation and reduced penetration of the fatty acids into the gut cells due to a decrease in conjugated bile salt concentration after bacterial hydrolysis of the taurine or glycine bound in peptide linkage to the side-chain. A possible additional effect of conjugated bile salts may be to facilitate release of triglycerides from the intestinal mucosa, although absorption-esterification of luminal fatty acids resulting from cell shedding has not been excluded as an explanation for this phenomenon. Our studies are relevant to the conditions seen clinically (10) in the blind loop syndrome where the levels of conjugated bile salts may be reduced well below the CMC and unstable emulsions would be expected to occur. Although feeding extra taurocholate reduced steatorrhea of the blind loop syndrome (10, 11), a more practical approach to the problem in man seems to be elimination of the excess bacteria through relieving the intestinal stasis by appropriate surgical therapy, or reducing the bacterial population by suitable antibiotic treatment.

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