Bile Salt and Micellar Fat Concentration in Proximal Small Bowel Contents of Ileectomy Patients

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ABSTRACT Studies were carried out to test the hypothesis that abnormal bile salt metabolism (interruption of the enterohepatic circulation) is responsible for steatorrhea in patients with ileal disease and (or) ileectomy.

Duodenal bile salt concentration after a single, standard meal eaten at 8 a.m. was measured in 8 patients with ileectomy steatorrhea and compared with 11 normal control subjects and 7 hospitalized patients without gastrointestinal disease. Mean bile salt concentration was approximately half normal in the ileectomy group, but some of the patients fell well within the normal range, even on repeat studies. However, it was shown that the second and third meals eaten during a single day were associated with a marked depression of duodenal bile salt concentration in ileectomy patients, which suggested that the first meals in these patients flush out a large fraction of the bile salt pool. Simultaneously measured turnover studies with taurocholate-^{14}C showed a $t_{\frac{1}{2}}$ of 3.1 hr in these patients compared with 29.5 and 32 hr in two control subjects, proving that the enterohepatic circulation had indeed been interrupted by ileectomy. Hepatic synthesis can apparently partially reconstitute the bile salt pool during the overnight period.

Additional studies were carried out to determine the relation between bile salt and micellar fat concentration in proximal small bowel contents after ingestion of the same standard meal. Below a bile salt concentration of 1.7 mg/ml, less than 0.8 mg/ml of lipid existed in the micellar phase of intestinal contents, whereas when bile salt concentration exceeded this level the amount of fat in the micellar phase rose progressively. Only 1 of 11 samples from three ileectomy patients had a micellar fat concentration > 0.8 mg/ml, whereas 33 of 42 samples from control subjects had micellar fat concentration > 0.8 mg/ml.

Thus, abnormally low duodenal bile salt concentration during at least a portion of the day, with the associated depression of micellar fat, appears to be a major cause of decreased fat absorption in patients with ileectomy steatorrhea.

INTRODUCTION

The etiology of steatorrhea in patients with ileal resection has been the source of much discussion and research during recent years. The original and logical hypothesis that ileectomy removed a quantitatively important fat-absorbing area of the intestine was made unlikely by the finding that absorption of dietary fats is normally almost entirely completed in the jejunum (1). Currently it is believed that abnormal bile salt metabolism is the most important cause of steatorrhea after ileectomy.

It is known that bile salts play an important role in normal fat absorption, probably by solubilizing

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fatty acids and monoglycerides in a micellar solution, and that intestinal reabsorption of secreted bile salts is important in maintaining an adequate amount of bile salts in the proximal small intestine. The total bile salt pool in man is only about 4 g, and it has been estimated that this entire amount is secreted and reabsorbed at least two times during the digestion and absorption of a single meal (2).

Recently a large amount of experimental data has confirmed older studies suggesting that the ileum is the primary site for bile salt absorption. Baker and Searle have been credited with first suggesting that interruption of the enterohepatic circulation of bile salts by ileectomy might be the cause of the severe steatorrhea seen after this operation (3).

Two basic assumptions of this current hypothesis are: (*a*) bile salts which escape absorption in the small bowel (after ileectomy) cannot be reabsorbed to a quantitatively significant degree in the colon. The fact that the turnover rate of isotopically labeled bile salts is greatly increased after ileal resection (4–7) seems to validate this assumption with reasonable certainty, in spite of the fact that significant colonic absorption of bile salts would have been predicted from the studies of Dietschy,[1] who has shown that unconjugated (by colon bacteria) bile salts can be absorbed rapidly in the colon by nonionic diffusion. (*b*) Hepatic synthesis of bile salts cannot increase enough to maintain a normal or near normal output of bile salts after the enterohepatic circulation has been interrupted by ileectomy. Support for this assumption has come from studies of bile salt pool size using cholic-^{14}C or taurocholic acid-^{14}C. It has been estimated that bile salt pool size after ileectomy is only one-sixth to one-fourth of that of normal (3, 6), and from this it has been deduced that hepatic bile salt synthesis cannot sustain even near normal bile salt concentration in intestinal contents when the enterohepatic circulation has been interrupted.

Before this hypothesis can be accepted as valid, it is necessary to demonstrate directly that bile salt and micellar lipid concentration in postcibal small bowel contents is actually reduced significantly by ileectomy. Two studies on this point have been reported. Hofmann and Grundy (4) made a preliminary report of a patient with gastrectomy, ileectomy, and colectomy with steatorrhea, who was found to have low intraluminal bile salt and micellar lipid concentrations. The type of meal and specific values were not given, however. Hardison and Rosenberg (7) recently reported similar studies in three patients with ileectomy and steatorrhea. They found maximum duodenal bile salt and micellar fatty acid concentrations to be low in two patients after the ingestion of 50 ml of corn oil. One other patient with ileectomy steatorrhea had values within the normal range, however. In none of the previous studies have the control experiments included a study of malnourished and chronically ill patients without ileectomy or ileal disease.

In order to test the hypothesis that abnormal bile salt metabolism is an important factor in the etiology of ileectomy steatorrhea, we measured bile salt concentration in duodenal and proximal jejunal contents in two types of experiments. First, bile salt concentration was determined in eight patients with ileectomy and in two control groups after the ingestion of a single standard meal, and second, the effect of four successive meals on intraluminal bile salt concentration was assessed in five ileectomy patients and four control subjects. In addition, the relation between micellar lipid and bile salt concentration was determined in these subjects.

METHODS

As noted in Table I, the ileectomy subjects were characterized by steatorrhea, decrease in vitamin B_{12} absorption, and normal or only slight depression of xylose absorption (except in patients H.A. and M.V., whose xylose tests were clearly abnormal). Disease of the jejunum was ruled out by X-ray studies, mucosal biopsies, and in two cases by subsequent autopsy (L.M. and B.J.).

Single meal experiments. Studies were made on four groups of adults: (*a*) 11 normal subjects who were either laboratory technicians or medical students; (*b*) seven hospital patients without steatorrhea (diagnosis on these patients given in Table II); (*c*) eight patients who had steatorrhea due to ileal resection which had been carried out for reasons given in Table I; and (*d*) a miscellaneous group of patients with steatorrhea (diagnosis given in Table II).

After an 8 hr fast, the small intestine was intubated with a double-lumen polyvinyl tube (8). The tube was positioned under fluoroscopy so that the proximal aspiration point was in the mid-duodenum and the distal aspiration point was at the ligament of Treitz. After placement of the tube, the subject ate a standard meal (described below), and aspiration of duodenal and proximal jejunal

[1] Personal communication.

TABLE I

Clinical Data on Ileectomy Patients

Subjects, age, and sex	Reason for resection	Approximate amount of small bowel resected	Steatorrhea*	D-xylose excretion	Schilling test
				g	*% excreted/ 24 hr*
F. R. 63 M	Regional enteritis	½	Severe, 67 g/24 hr	3.6	3.2
L. G. 61 M	Regional enteritis	¼	Severe	4.0	<1
J. O. 43 F	Adhesions	⅓	Moderate		
H. A. 60 F	Regional enteritis	½	Severe, 48 g/24 hr	2.3	‡
B. J. 26 M	Regional enteritis	½	Moderate	4.8	<1
L. S. 29 M	Volvulus	⅓	Moderate, 19 g/24 hr	10	2.7
L. M. 57 F	Gastrointestinal bleeding	⅔	Severe		
T. G. 35 F	Regional enteritis	⅓	Severe, 36 g/24 hr	4.3	<1
L. C. 50 F	Radiation damage	⅓	Slight, 14 g/24 hr	5.5	<1, 2.8
M. V. 47 F	Volvulus	§	Severe, 99 g/24 hr	1.4	3.2

* Severity of steatorrhea was judged clinically by the number and amount of bowel movements and by Sudan stain of at least three separate stool specimens. In six patients stool fat was measured chemically, while patients were on standard hospital food with an estimated fat content of 120 g.

‡ Macrocytic anemia with low serum B_{12} concentration was demonstrated on one occasion.

§ All of the small bowel from a few inches beyond the ligament of Treitz to approximately 2 in. proximal to the ileocecal valve was removed.

contents was carried out continuously for 2–2.5 hr and divided into 30-min samples. At times intestinal contents could be obtained from only one of the two tubes, and at times drainage from either tube was erratic, so that the actual number of samples obtained in the different subjects and patients varied.

The meal used in these studies consisted of a 6 oz sirloin steak, tossed salad with french dressing, bread with butter, and tea (8). The fat content of the meal was 42.5 g. 10 g of polyethylene glycol (PEG) was added to the tea to serve as an indicator of the dilution or concentration of the meal as it traversed the small bowel. Sips of tea were taken after eating small amounts of food to facilitate mixing of the solid and liquid portions of the meal.

Successive meal studies. In order to determine the bile salt concentration after the three successive meals eaten normally during a single day, we intubated normal and ileectomy subjects with a single-lumen polyvinyl tube. Subjects, who had fasted for 12 hr, swallowed the tube at approximately 7 a.m. The tube was positioned so that the aspirating holes were located in the mid-portion of the duodenum and was left in place as the subjects ate the same meal described previously on four occasions, at zero time (8–9 a.m.), 4, 8, and 24 hr. Aspiration of duodenal contents began as soon as each meal was completed, and was carried out for a 2 hr period at a rate of 0.5 ml/min. At the completion of this aspiration period the collected material was mixed, 10 ml was saved for analysis, and the rest injected into the patient's duodenum via the tube. It has been previously shown that the total volume of material passing this part of the duodenum after this same meal is of the order of 2 liters (8). Therefore, removal of only 10 ml of the material collected for analysis could not significantly reduce the bile salt pool and artifactually reduce intraluminal bile salt concentration of the second, third, and fourth meals.

This type of study was also used to determine taurocholate-^{14}C turnover as described below.

Bile salt concentration. The total bile salt concentration of each sample was determined by a modification of the spectrofluorometric method of Levin, Irvin, and Johnston (9). Intestinal samples were centrifuged at 1000 *g* for 10 min. A 1 ml sample was removed from the supernatant, placed in a 50 ml glass-stoppered centrifuge tube, and mixed with 1 ml of ethyl alcohol. The solution was

extracted three times with vigorous shaking using 25 ml of petroleum ether to remove the neutral sterols and triglycerides. The petroleum ether layer was discarded. After the extraction procedure, 4 ml of ethyl alcohol was added to the remaining sample of the centrifuge tube; it was again mixed carefully and refluxed for 20 min in a waterbath to precipitate the protein in the sample. The tubes were then cooled and centrifuged at 1000 *g* for 10 min. The supernate was removed, and the precipitant washed two times with 5 ml of ethyl alcohol. The super-

TABLE II

Concentration of Bile Salts in Duodenal and Proximal Jejunal Contents after Ingestion of a Standard Meal. Results are Given in mg/ml of Cholic Acid Equivalents

Subject	No. of separate times studied	No. of samples	Mean	Range
Normal control subjects				
S. M.	1	4	3.65	2.39–5.48
P. B.	1	7	3.00	2.11–3.61
T. D.	1	7	4.19	2.31–6.54
M. H.	1	8	1.90	1.25–2.53
J. H.	2	19	4.87	1.50–11.4
J. T.	1	4	3.40	2.50–3.80
B. F.	1	4	5.90	4.50–7.20
M. V.	1	4	3.60	0.90–5.70
D. L.	1	3	5.40	3.70–7.90
E. D.	1	7	4.20	3.20–5.20
J. J.	1	4	5.60	2.50–9.60
Average			4.15	
Hospital control subjects				
D. D., amebiasis	2	8	2.53	0.70–6.08
	No. 1	4	2.71	0.71–4.20
	No. 2	4	2.35	0.70–6.08
W. T., strongyloidosis	1	4	1.00	0.29–1.63
T. H., severe hypertension	1	8	2.95	1.56–3.76
B. I., congestive heart failure	1	6	2.54	1.27–3.55
N. M., rheumatoid arthritis	1	3	10.97	8.34–15.50
R. L., uremia	1	4	1.98	0.57–3.38
C. E., acute pancreatitis	1	1*	7.00	
Average			4.14	
Ileectomy patients				
F. R.	4	17	3.48	0.17–7.90
	No. 1	8	3.93	0.42–7.90
	No. 2	3	4.29	1.74–6.20
	No. 3	4	0.90	0.17–1.73
	No. 4	2	5.75	4.70–6.80
L. G.	1	4	0.68	0.08–0.70
J. O.	1	7	2.87	1.96–4.49
H. A.	1	3	1.97	1.21–3.40
B. J.	1	8	1.91	1.70–2.40
L. S.	1	3	1.83	1.42–2.34
L. M.	1	7	1.87	1.05–2.42
M. V.	1	4	1.99	0.81–3.20
Average			2.08	
Steatorrhea other than ileectomy				
O. B., complete bile duct obstruction	1	2	0.02	0.02–0.02
T. S., exocrine pancreatic insufficiency	1	7	2.81	1.26–4.50
G. C., sprue	1	4	3.06	0.47–5.70
W. G., sprue	1	12	1.53	0.22–3.68

* Slow drainage over a 2 hr period due to ball valve type obstruction at aspirating site.

nate and washings were then combined and taken to dryness by heating in a waterbath at 85–90°C. The residue was dissolved in 10 ml of ethyl alcohol and stirred carefully on a Vortex mixer. A 0.1 ml sample of this final mixture was placed in a test tube and taken to dryness in a waterbath. Standards of 0.050, 0.025, and 0.005 mg were prepared from a stock solution of cholic acid (purified by recrystallization from a saturated solution in ethyl acetate) and were then taken to dryness as described for the unknown sample. After the drying procedure, 5 ml of concentrated sulfuric acid was added to the unknowns and standards. A blank was prepared by adding 5 ml of concentrated sulfuric acid to an empty test tube. All tubes were then heated immediately for 1 hr at 65°C. After heating, the tubes were cooled to room temperature, and room temperature and fluorescence was read in an Aminco-Bowman spectrophotofluorometer (American Instrument Co., Silver Spring, Md.) using matched quartz crystal cuvettes, with the activation wave length set at 470 and the fluorescence at 490. All unknowns were bracketed by the reading of the standards. If the unknown sample did not give readings within the linear portion of the curve, the sample was diluted and reanalyzed. Recovery studies were performed using 0.025 mg of cholic acid added to 0.10 ml of intestinal contents that had been collected at 30-min intervals after subjects had eaten the previously described test meal. Average recovery in 14 determinations was 89.4% (range 81.9–100.0%). Eight additional studies with 5.00 mg of cholic acid added to 1 ml of intestinal contents revealed an average recovery of 98.4% (range 90.4–111.0%).

The type of bile salts present in intestinal contents was determined by thin-layer chromatography with the method described by Hofmann (10). 10 normal subjects and 4 ileectomy patients were studied in this manner, and in no instance was a measurable amount of unconjugated bile acid identified. Glycocholic and glycochenodeoxycholic or glycodeoxycholic bile acids made up greater than 80% of the total bile salts with small quantities of taurocholic and either taurochenodeoxycholic or taurodeoxycholic making up the rest. Absence of free bile salts suggests that bacterial flora is not abnormal in the jejunum of these ileectomy patients.

Bile salt turnover studies. In some subjects the turnover of taurocholate-24-^{14}C was studied. Sodium taurocholate-24-^{14}C (Tracerlab, Waltham, Mass.) was prepared in saline as described by Austad, Lack, and Tyor (6) and administered intravenously to subjects in the fasting state. Duodenal intubation was then performed. The subjects ate the test meal 1 hr after the isotope was injected, and duodenal contents were collected at a rate of 0.5 ml/min for 2 hr; 10 ml of the aspirate was saved for subsequent study and the remainder was reinjected into the duodenum. In the case of the patients, the tube was left in place for the duration of the study, and four collections were made during a 26-hr interval, each after the ingestion of the standard test meal. In the study of the normal control J.T., the duodenal tube was replaced on each of four successive mornings, and collections were again made after the ingestion of the standard meal. Taurocholate-^{14}C specific activity was determined on aliquots of bowel aspirate by a modification of the method described by Austad et al. (6). The samples were deproteinized in 50 ml of boiling methanol, filtered, taken to dryness, and dissolved in 0.3 ml methanol. Conjugated bile salts were separated on 20 × 40 cm plates of Silica Gel H, using the isoamyl alcohol-propionic acid–*n*-propional–water system (60 : 60 : 40 : 30 v/v) of Hofmann, as described in the article by Austad et al. (6). The taurocholate areas were identified by spraying with water, and then were scraped and eluted with ethanol. Taurocholate content was measured on one aliquot of the eluate as before, and one aliquot was added to 0.4% 2,5-diphenyloxazole in toluene and assayed for ^{14}C in a liquid scintillation counter.

Measurement of micellar lipid. Micellar lipid concentration was measured by the method of Hofmann and Borgström (11). After the standard meal, duodenal contents were collected at 1-min intervals by gentle hand suction with a syringe. The sample was immediately placed in a test tube, swirled rapidly by hand in a 70°C waterbath for 1 min, and left in the bath for an additional 9–10 min to inactivate pancreatic lipase. Samples were pooled at 0.5-hr intervals. An aliquot (10.4 ml to fill the plastic centrifuge tube) of the pooled 0.5-hr sample was centrifuged at 100,000 *g* at a temperature of 37–40°C for 18 hr using a Spinco ultracentrifuge. The centrifugation began within 2 hr after completion of the study. The tubes were removed from the rotor and head without agitation. A clear, transparent yellow-brown or green-brown solution was present in almost all tubes (termed the micellar phase). Rarely was the micellar phase turbid, and these samples were discarded. In all tubes there was a pellet of undigested food particles on the bottom and an oil layer above the micellar phase. Total, micellar, and oil phase volumes were recorded. A needle was then inserted through the wall of the tube and the entire micellar phase was aspirated and placed in a test tube. This phase was then mixed on a Vortex mixer, and a 1 ml aliquot was used to determine micellar phase lipid. The lipid was extracted with a liquid to liquid extraction system, developed by Blankenhorn and Ahrens (12), consisting of solutions of water-diethyl ether–heptane-ethanol. Solution 1 consisted of diethyl ether–heptane-ethanol (1 : 1 : 1, v/v). Solution 2 consisted of diethyl ether–heptane-ethanol-water (1 : 1 : 1 : 1, v/v). To 1 volume of intestinal contents was added 3 volumes of solution 1. This was shaken vigorously. The upper phase was aspirated with a pipette and the lower phase was extracted two additional times with solution 2. Such an extraction removes all the glycerides and fatty acids, some of the phospholipids, and none of the conjugated bile salts (11, 12). The pooled extracts of the micellar phase were taken to dryness under a nitrogen stream at room temperature and the residue was dissolved in 1 ml of petroleum ether. After drying with sodium sulfate, we transferred the petroleum ether solution with washing into weighing crucibles and evaporated them under a vacuum. The residue was dried in a desiccator for 24 hr. The lipids were determined gravimetri-

cally to give total lipid in milligrams per milliliter in the micellar phase of intestinal contents.

pH of intestinal contents was measured by glass electrode, and PEG concentration was determined by the method of Hyden (13).

RESULTS

Dilution of the meal. The volume of proximal small bowel contents can be measured indirectly, comparing different groups of patients or normal subjects, from the dilution or concentration of the nonabsorbable water-soluble marker PEG which was included in the test meal. As shown in Fig. 1, there was a wide scatter of PEG concentration, but the average value and range were approximately equal in the ileectomy patients and in the two control groups, which indicated that the ingested meal was diluted by digestive secretions to approximately the same extent in patients and control subjects. Therefore, the concentration of bile salts in duodenal contents can be used to assess the relative amount of bile salt available at the sites of fatty acid absorption in ileectomy patients and in the control subjects.

Single meal studies of bile salt concentration in patients with ileectomy and in control groups. Bile salt concentration was measured using a modification of the spectrofluorometric method of Levin et al. (9). This method proved valid because: (*a*) the only bile salts found in detectable quantities by thin-layer chromatography, performed on intestinal contents from 10 normal subjects and 4 patients with ileectomy, were conjugated derivatives of cholic and deoxycholic or chenodeoxycholic acids, and the fluorophores of these bile acids have the same extinction coefficients; (*b*) the recovery of bile salts added to intestinal contents was very good over a very wide range of internal standards (see Methods); and (*c*) there is apparently nothing in gut contents that gives a false positive reaction since the bile salt concentration in a patient with complete bile duct obstruction was virtually zero (Table II).

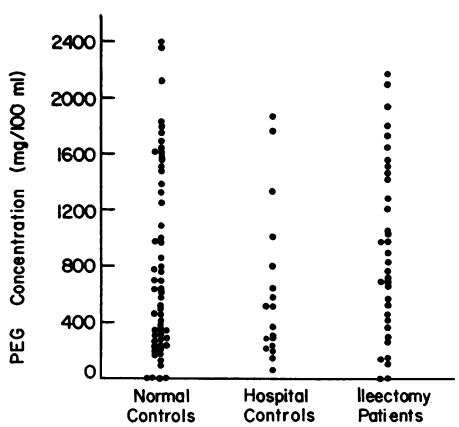

FIGURE 1 Concentration of polyethylene glycol (*PEG*) in proximal small bowel contents after the ingestion of the test meal.

In both control groups and in the patients with ileectomy steatorrhea, the average bile salt concentration of proximal small bowel contents rose slightly with time in the four 30-min samples. These differences were so slight that data from all collection periods will be presented together. Also, there was no consistent difference between bile salt concentration in fluid collected from the mid-duodenum and from the ligament of Treitz. The mean bile salt concentrations for all 30-min periods during which fluid was obtained are given in Table II. Mean bile salt concentration among the 11 normal subjects varied from 1.90 to 5.90 mg/ml, and the average for this entire group was 4.15 mg/ml. In the hospital control group, mean bile salt concentration varied from 1.00 to 10.97 mg/ml, and the average for the entire group of 7 patients was 4.14 mg/ml. This latter figure is somewhat misleading because one patient, N.M., had a very high concentration of bile salts. Were it not for this patient, the average for this group would have been approximately 3 mg/ml. The mean concentration of bile salts in the group with ileectomy steatorrhea varied from 0.68 to 3.48 mg/ml, and the average for the entire group was 2.08 mg/ml. This is approximately half the average concentration in the normal subjects, and all but two of the seven cases had mean concentrations less than 2 mg/ml.

Bile salt concentration was also studied in patients with steatorrhea due to causes other than ileectomy. Results in this group of patients are listed in the bottom of Table II. As expected, the patient with complete bile duct obstruction had virtually no bile salts in his proximal small bowel contents. A patient with exocrine pancreatic insufficiency had a mean of 2.81 mg/ml of bile salts, which is in the normal range. One patient with sprue had a mean of 1.53 mg/ml, while a second sprue patient was in the normal range with 3.06 mg/ml.

TABLE III

Effect of Successive Meals on the Concentration of Bile Salts in the Duodenal and Jejunal Contents

Diagnosis	Subjects	Total bile salt concentration in small bowel contents				Taurocholate-^{14}C	
		Meal . . . 1	2	3	4	Half-life	Estimated pool size
		mg/ml				*hr*	*mg*
Normal control subjects	P. B.	3.75	3.75	3.50	2.95		
	S. M.	*	2.84	2.02	3.60		
	T. D.	2.01	3.90	2.44	3.40		
	J. T.					29.5	555
Steatorrhea due to sprue	W. G.	4.82	2.21	4.13	3.42	32.0	556
Ileectomy patients	F. R.	2.04	0.54	0.25	1.18	1.3	43
	M. V.	0.96	0.62	1.11	0.49	5.5	50
	L. C.	4.17	0.09	1.59	1.50	3.8	104
	H. A.	1.05	0.97	0.48	1.05	2.5	147
	P. G.	1.91	1.38	1.01	5.00	2.5	191

* For first collection period, tube was in stomach instead of small bowel.

Successive meal studies. Two of the ileectomy patients described in Table II, F.R. and J.O., had bile salt concentrations that were clearly in the normal range, in spite of the fact that each had steatorrhea for which no other cause could be found. F.R. was studied on four separate occasions and on only one of these test days was his bile salt concentration abnormally low (Table II). It seemed possible that bile salt concentrations in patients F.R. and J.O. might be normal with the first meal of the day but be low after the second and third meals of the day, since the time for hepatic synthesis to replenish the bile salt pool is relatively short between breakfast and lunch and lunch and dinner. To check this possibility, total bile salt concentration and taurocholate-24-^{14}C turnover were measured in five ileectomy patients and four control subjects after four successive feedings of the same standard meal during a 26-hr period (Table III). In none of the control subjects (three normal controls and one patient with steatorrhea due to sprue) did the bile salt concentration fall below 2 mg/ml after any of the four meals. The results in the ileectomy patients were strikingly different. In three of the five patients the concentration after the first meal was within the normal range (4.17, 2.04, and 1.91 mg/ml). In each of these patients the concentration fell after the second and third meals to low values and tended to rise somewhat by the next morning (fourth meal). In the two subjects, H.A. and M.V., with low initial concentrations of bile salt the fall after subsequent meals was not so striking.

In order to establish that this fall in the intestinal bile salt concentration with successive meals in patients with ileectomy was not the consequence of varying dilution after different meals, we replotted the data for the ileectomy patients with normal first meal concentration (Table III) with and without correcting for dilution, using the PEG after the

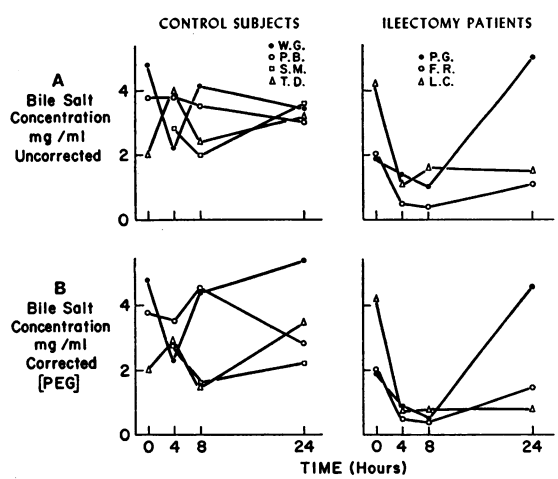

FIGURE 2 The effect of successive meals on the bile salt concentration of the proximal small bowel in four control subjects and three ileectomy patients who had normal bile salt concentrations after the first meal. A, bile salt concentration uncorrected for PEG concentration. B, bile salt concentration corrected for PEG concentration.

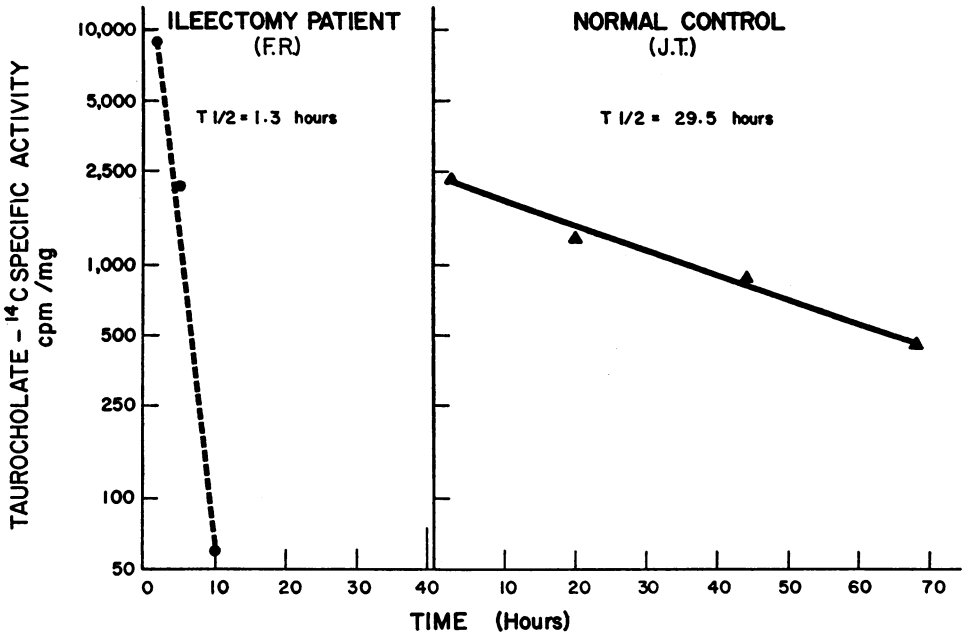

FIGURE 3 The rate of disappearance of sodium taurocholate-24-^{14}C from the duodenal contents of a normal subject (J.T.) and an ileectomy patient (F.R.). After the intravenous administration of taurocholate-24-^{14}C (5.6×10^6 cpm), duodenal samples were aspirated at 2, 6, 10, and 26 hr in F.R. and at 2, 20, 44, and 68 hr in J.T. and analyzed as described in the text. The specific activity of the 26 hr sample in F.R. was too low for assay. The bile acid concentrations from patient F.R. are shown in Table III.

first meal as the standard reference point (Fig. 2). Since the PEG concentration in ileectomy patients tended to rise progressively in the second and third meals, compared to the first and fourth, the corrected bile salt concentration after the second and third meals was even lower than the uncorrected concentration. This suggests that bile flow, as well as total bile salt secretion, was lower after the second and third meals than after the first meal in ileectomy patients.

The results of the studies of taurocholate-24-^{14}C turnover in these five ileectomy patients, in one normal control subject, and in one patient with sprue, are shown in Fig. 3 and Table III. By sampling four times in the 26 hr period after the administration of the taurocholate-24-^{14}C to the ileectomy patients, we were able to estimate half-life even when markedly abbreviated (Table III). The mean taurocholate-24-^{14}C turnover time in these ileectomy patients was markedly decreased ($t_{\frac{1}{2}} = 3.1$ hr) in comparison with the turnover time in the two control subjects (29.5 and 32.0 hr). The abbreviated half-time of this bile acid is at least as short as previously reported in patients with ileectomy (6, 7) and confirms the virtual absence of enterohepatic bile salt cycling in these patients.

Micellar lipid concentration. Micellar lipid concentration varied widely among all groups but showed a fair correlation with the bile salt con-

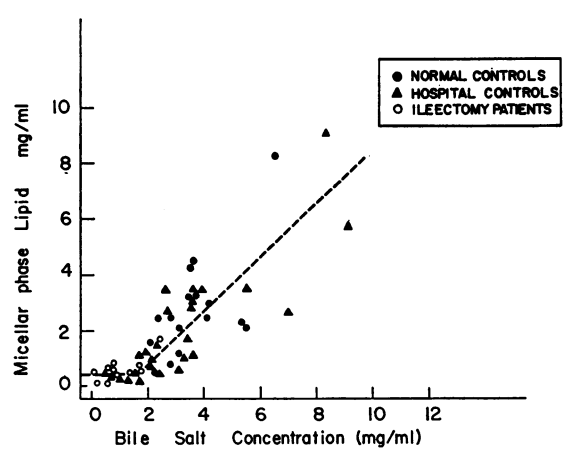

FIGURE 4 The relationship between the concentrations of bile salts and the micellar phase lipid in proximal small bowel contents after the ingestion of the test meal.

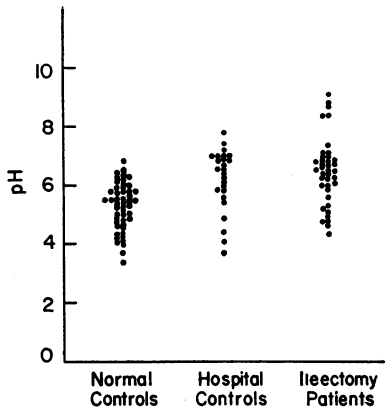

FIGURE 5 The pH of proximal small bowel contents after the ingestion of the test meal.

centration of gut contents. In Fig. 4 is shown the relationship between the concentration of lipid in the micellar phase and bile salt concentration in all control subjects in whom micellar fat was measured, and in three ileectomy patients. Micellar lipid was 0.8 mg/ml or less when bile salt concentration was less than 1.7 mg/ml but rose progressively thereafter with increasing levels of bile salt concentration. The values from the ileectomy patients fit reasonably well with those obtained in the control subjects, but since the bile salt concentration was lower in these subjects, the amount of micellar phase fat was also depressed. Of eleven 30-min samples obtained in three patients with ileectomy, only one had a micellar fat concentration greater than 0.8 mg/ml, whereas 33 of 42 samples obtained in the two control groups had micellar fat concentration greater than 0.8 mg/ml.

Since the amount of lipid in the micellar phase can be influenced by the pH of gut contents, pH was determined on most samples. The results are shown in Fig. 5. As can be seen, the pH was slightly higher in ileectomy subjects and in hospital control subjects than in normal subjects. Since micellar fat concentration would be expected to increase with alkalinity of gut contents (14), the micellar lipid content would be expected to be slightly higher after ileectomy than in normal subjects for any given concentration of bile salt.

DISCUSSION

The concentration of bile salts in duodenal and proximal jejunal contents after the ingestion of a single, standard meal has been measured in eight patients with steatorrhea due to ileectomy, in two control groups consisting of normal subjects and hospital patients without steatorrhea or small bowel or hepatobiliary disease, and in a group of patients with steatorrhea due to miscellaneous causes. The results of the present study are in general agreement with previous reports which have concluded that the steatorrhea of ileectomy is in large part the consequence of an inadequate bile salt pool for optimal fat absorption as the result of interruption of the normal mechanism of bile salt conservation via the enterohepatic circulation. Indeed, the mean duodenal bile salt concentration in the eight patients with ileectomy steatorrhea described in the present study was approximately half that found in each of two control groups after an 8 a.m. meal.

Several aspects of these findings deserve special comment. First, two ileectomy patients in this study had bile salt concentrations which fell (in four of five studies) clearly within the normal range when measured after the first meal of the day. However, it is still likely that bile salt availability is rate limiting for fat absorption in these patients, since in the subjects in whom this could be tested bile acid concentration fell to extremely low levels after three successive meals on a single day so that mean concentration during the entire day was very low. In addition, a bile acid concentration which is adequate for fat absorption in normal individuals may be insufficient under circumstances in which the small bowel is short.

Second, in no instance was the decrease in measured bile salt concentration as great as would have been anticipated on the basis of estimates of pool size with isotopically labeled taurocholic acid (6). A logical explanation for this apparent discrepancy can be deduced from the study in which the turnover of taurocholate-^{14}C and the concentration of bile salts in the lumen of proximal small bowel were studied simultaneously (Table III and Fig. 3). Such a situation in which there is massive shrinkage in the size of the pool within the day under circumstances in which synthesis is likely to remain unaffected would be expected to cause an underestimation of half-life and of pool size by the isotopic technique. Such was the case in this study in which the estimated pool size by this method was on an average about one-fifth that of the normal values, whereas the mean concentra-

tion of bile salt in the proximal intestinal contents after the first meal was on an average about half normal and after three successive meals, about one-third the normal level. In addition, taurocholate is a minor constituent of human bile and estimates of its pool size, even when determined under steady-state conditions, would not necessarily reflect the magnitude of alteration of the size of the entire bile acid pool under all conditions. Thus, on the basis of these studies it seems safe to conclude that the effective bile salt concentration at the sites of fatty acid absorption varies throughout the day and on an average lies somewhere between the half normal value measured early in the day and the low values measured at the end of the day.

The fact that ileectomy patients are able to maintain morning bile salt concentration at approximately half normal values, despite almost complete interruption of the enterohepatic circulation, is strong evidence for enhanced hepatic bile salt synthesis in these patients. Abundant evidence exists, both in experimental animals (15, 16) and in man (17, 18), that the hepatic synthesis of bile acids is regulated, at least in part, by the quantity of bile acids delivered to the liver via the enterohepatic circulation. After ileectomy in man, hepatic synthesis must rise appreciably to compensate in part for the accelerated bile acid loss in this condition.

However, the ability of some ileectomy patients to maintain normal or near normal first meal bile salt concentrations is due not solely to the ability of this group to sustain an increased hepatic synthesis of bile salts, but also to the ability of their gallbladders to function properly with regard to absorption and storage. Since the turnover time of ileectomy patients with normal first meal bile salt concentration was just as short as those ileectomy patients with very low first meal bile salt concentrations, it is apparent that variation in the degree to which the enterohepatic circulation is interrupted is not the reason why some patients do and some do not have normal first meal concentrations of bile salts.

Third, it should be emphasized that low duodenal bile salt concentration after a breakfast meal is not a specific finding in ileectomy steatorrhea. As shown in Table II, a patient with celiac sprue had bile salt concentrations that were approximately the same as in patients with ileectomy, and two of seven of the hospital control subjects without gastrointestinal disease had similarly low bile salt concentrations. However, low bile salt concentration in patients with a normal length of small bowel, such as the hospital control subjects and sprue patient, might not have as deleterious an effect as in patients with a short small bowel where a reserve area for fat absorption is not present. Furthermore, in patients with an intact ileum bile salt concentrations may not fall significantly with successive meals during a single day.

The final question is whether the bile salt concentration of duodenal and jejunal contents is low enough in ileectomy patients to significantly depress fat absorption. A pathophysiological interpretation of these results must take into account the amount of lipid existing in the micellar phase of gastrointestinal contents. When bile salt concentration was studied as a function of micellar lipid in duodenal and jejunal contents after the ingestion of the same standard meal, it was found that below a bile salt concentration of 1.7 mg/ml (4.0 μmoles/ml) less than 0.8 mg/ml of lipid existed in the micellar phase of intestinal contents, whereas when bile salt concentration was above this level, the amount of micellar lipid rose progressively. If this bile salt concentration of 1.7 mg/ml is used as a critical concentration below which very little lipid exists in the micellar phase, it is clear that 5 of our 7 ileectomy patients would be expected to have very low micellar fat concentrations in their proximal gut after a breakfast meal, and with the later meals of the day all of our ileectomy patients would be expected to have markedly depressed micellar fat concentration. This expectation was borne out in the three ileectomy subjects in whom micellar fat concentration was measured (Fig. 4). Since the *rate* of fat absorption within a given segment of intestine probably depends on the amount of fat in micellar phase, abnormal bile salt metabolism appears to play an important role in the pathogenesis of steatorrhea in patients who have had ileectomy.

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