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Research Article

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Effects of Controlled Interruption of the Enterohepatic Circulation of Bile Salts by Biliary Diversion and by Ileal Resection on Bile Salt Secretion, Synthesis, and Pool Size in the Rhesus Monkey

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ABSTRACT The effects of controlled interruption of the enterohepatic circulation (EHC) of bile salts by biliary diversion on bile volume, bile salt secretion and synthesis rates, bile salt pool size, and the relationship to fecal fat excretion were studied in 16 rhesus monkeys.

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The effects of interruption of the EHC by the stream-splitter were compared with those produced by resection of the distal one-third or two-thirds of small bowel. While ileal resection appreciably reduced bile salt secretion, the EHC was by no means abolished. Bile salt reabsorption from the residual intestine was greater after one-third than after two-thirds small bowel resection. These observations suggest that jejunal reabsorption of bile salts occurs and may well contribute to the normal EHC.

INTRODUCTION

Bile salts are synthesized in the liver from cholesterol and after conjugation with glycine or taurine are secreted in the bile and pass into the duodenum. Having contributed to fat absorption in the upper small bowel, they then pass along the intestine and are largely reabsorbed by an active transport mechanism in the ileum (1, 2) to return to the liver via the portal vein (3–5). The efficiency of this enterohepatic circulation (EHC) means that only a small percentage (less than 5% each day) (6–8) of the circulating bile salts escape reabsorption to appear in the feces. To maintain a steady state, therefore, the liver is normally required to synthesize only enough bile salts to replace the fecal loss.

It is now well-known that ileal disease or resection may cause bile salt malabsorption (9–16), but it is not known to what extent the liver may compensate for such interruption of the EHC by increasing its bile salt synthesis. Furthermore, the net effect of ileal dysfunction on bile salt secretion clearly depends not only on the adaptive increase in hepatic synthesis, but also upon

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the amount of bile salts which may be reabsorbed from other areas of the intestine. Since bile salt secretion cannot be studied directly in man, we designed an experimental model to study the rhesus monkey (17) in which the composition of the bile closely resembles that of man (18, 19).

The model has been used to study various aspects of the enterohepatic circulation of bile, and this paper is the first of a series of articles describing our findings.

The purpose of the present communication is fourfold: (*a*) to measure the changes in bile volume and bile salt secretion (total bile salt output by the liver, which includes both newly synthesized and recycled bile salts) in response to varying degrees of interruption of the EHC; (*b*) to examine the adaptive increase in *de novo* hepatic bile salt synthesis in relation to varying degrees of interruption of the EHC and to show how this is related to the effective circulating bile salt pool size; (*c*) to show how bile salt secretion, synthesis, and pool size are related to fecal fat excretion; and (*d*) to compare the effects of interruption of the EHC produced by the stream-splitter with that produced by ileal resection.

A subsequent publication[1] will deal with the effect of interruption of the EHC on the composition of bile in respect to bile salt, phospholipid, and cholesterol with emphasis on cholesterol solubility (20) in bile and gallstone formation (21).

METHODS

The general design of the experimental model used in these studies, together with details of the surgery, care and management of the experimental animals, the type of restraining chair and electronic equipment used, and the experimental procedure followed, has already been published in detail (17) but may be summarized as follows:

Experimental model. Large female rhesus monkeys were trained to sit in restraining chairs before surgical implantation of biliary and upper intestinal fistulae. The biliary cannula drained the total bile output which was then returned to the intestine[2] through an electronic stream-splitter which diverted different percentages of the bile to a collecting system. The model thus provided a varied and controlled degree of interruption of the EHC, a representative sample of bile for analysis, and a measure of the volume of bile produced during any given period of time.

In each monkey, 5% of the bile was collected (the minimum level of biliary diversion which consistently provided sufficient volume for analysis) and since 95% of the bile was still being returned to the intestine, this level of interruption of the EHC was taken as the "base line" for the experimental model. Bile composition was then studied in the steady state for 1 wk intervals at 10, 20, 33, 66, and 100% biliary diversion.

Laboratory procedures (collection of samples). The volume of bile produced at the various levels of biliary diversion was recorded at 12- or 24-hr intervals and samples of bile were then quickly frozen at −20°C until analyzed. For analysis, bile samples were brought to room temperature, diluted in spectranalyzed methanol (usually 1:20 dilution), and the bile acid concentration measured.

Biliary bile acids. Total bile acid concentration in bile was measured enzymatically with hydroxysteroid dehydrogenase using a modification (20)[3] of Talalay's method (22). This enzyme, by oxidizing the 3-OH group of the bile acid molecules, converted diphosphopyridine nucleotide (DPN) to its reduced form DPNH which was then measured in a Beckman DU2 spectrophotometer at 340 mμ. With each series of bile acid estimations, three solutions containing 0.06, 0.12, and 0.24 μmole/0.1 of pure sodium taurocholate in methanol were also measured enzymatically to obtain a standard curve.

Bile acid synthesis. Since, at each level of interruption of the EHC, steady-state conditions were reached for bile salt secretion (and for bile volume), bile salt synthesis must equal loss, and in turn bile salt loss is the sum of that removed by the stream-splitter plus that excreted in the feces.[4] To determine bile salt synthesis, therefore, fecal bile acid excretion was measured in 3-day stool collections from six animals having small biliary diversions (5, 10 and 20% diversions) and six animals having large biliary diversions (33 and 66%). The stools for bile acid determination were collected during the last 3 days of the steady-state period at each of the different levels of biliary diversion. Normal bile salt synthesis was also obtained in eight control monkeys by measuring fecal bile acid excretion before abdominal surgery.

Fecal bile acids. Fecal bile acids were extracted with modifications of the method described by Grundy, Ahrens, and Miettinen (8), and the 3-OH bile acids were then measured with essentially the same enzymatic method described above for biliary bile acids.

The steps of homogenization, mild saponification, and extraction of neutral sterols were followed as previously described (8). Rigorous saponification was carried out in steel bombs overnight at 110°C, rather than in a pressure cooker. After column chromatography with Florisil (in an attempt to separate bile acids from fatty acids) and extraction with ethyl acetate and water, the residue in the flask usually still contained an oily material which had the same R_f as long-chain fatty acids on thin-layer chromatography (TLC). When this residue (containing the bile acids) was dissolved in methanol and added to the mixture for enzy-

[1] Presented in part to the American Federation for Clinical Research and the American Society for Clinical Investigation, 4 May 1969. (Dowling, R. H., E. Mack, and D. M. Small. 1969. Cholesterol and bile composition after interruption of the enterohepatic circulation of the Rhesus monkey. *Clin. Res.* 17: 301.)

[2] The bile was returned either to the proximal duodenum or in some cases to the distal stomach. Studies in one monkey showed no significant difference in the levels of bile volume and bile salt secretion when these were measured, first with bile returned to the stomach and later with return to the duodenum.

[3] Details of the method may be obtained on request from D. M. Small, Boston University Medical School, Boston, Mass. 02118.

[4] Small quantities of bile salts are also lost each day in the urine and through the skin, but since these routes of excretion are responsible for less than 2% of the total bile salt loss (8), only fecal and biliary bile salt loss were measured in this study.

matic determination of bile acids, an emulsion frequently developed so that the initial background reading with the spectrophotometer, before the addition of enzyme, was often so high as to make bile acid determination impossible.

For this reason, in addition to Florisil column chromatography, we found it necessary to separate further the fatty acids from the bile acid containing residue by TLC using a hexane : ether : acetic acid (60 : 40 : 1) solvent system. With this system, the bile acids remained at or near the point of application and were clearly separated from the contaminating fatty acids, as demonstrated by iodine, phosphomolybdic, and sulphuric acid identification.

The bile acids were eluted from the silica with methanol and then estimated enzymatically. Deoxycholic acid-24-^{14}C (Tracerlab, Waltham, Mass.) was added to each fecal homogenate as an internal standard to check the recovery of bile acids. Usually > 90% of the internal standard was recovered. Radioactivity was counted in Bray's solution using a Packard Tri-Carb liquid scintillation counter (model 314E), and the samples were then corrected for quenching by adding standards of ^{14}C in toluene.

It should be noted that this method measured only 3-OH bile acids and does not measure keto forms of the different bile acids which might be present in feces. However, since Danielsson, Eneroth, Hellström, Lindstedt, and Sjövall (23), Eneroth, Gordon, Ryhage and Sjövall (24), and Eneroth, Gordon, and Sjövall (25) have shown by gas-liquid chromatography (GLC) and mass spectrometry that, in man, the predominant fecal bile acids are nonketonic and that most of the fecal bile acids contain the 3-OH group, the enzymatic bile acid determination used here was considered adequate for the present investigation.

Bile salt pool size. By plotting the changes in bile salt secretion at short time intervals after an "intact" EHC (5% biliary diversion) is interrupted with a complete bile fistula (100% biliary diversion), a "washout curve" is obtained (26–28) from which several measurements may be made (refer to Fig. 1). For the first 1–3 hr after the acute change from 5 to 100% biliary diversion, bile salt secretion is maintained at the same level as that observed during the preceding 24-hr estimations in the base line state. Then during the next 3 hr, secretion falls rapidly to a low point,

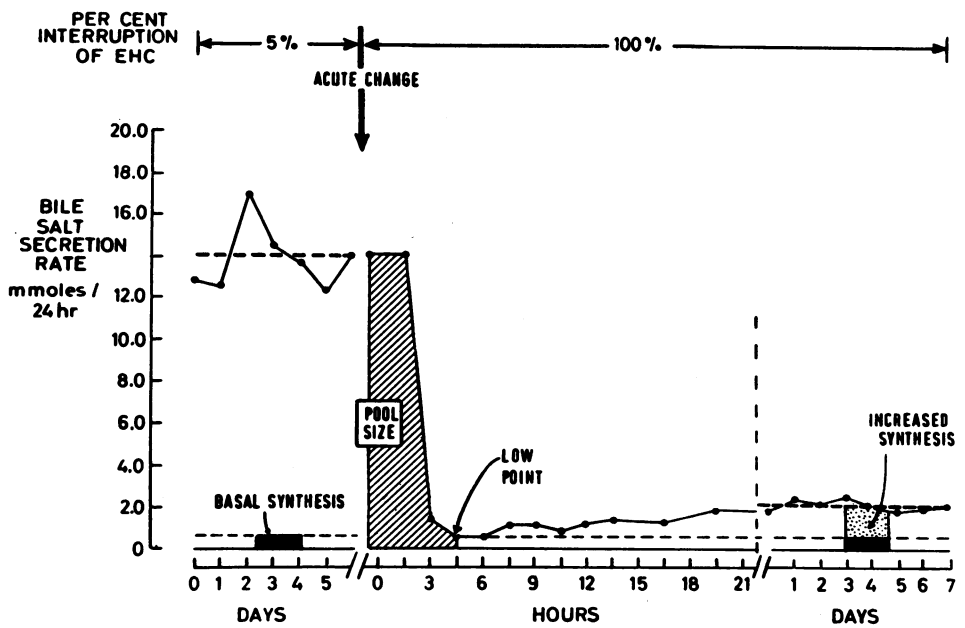

FIGURE 1 Changes in bile salt secretion rate in monkey 18 seen immediately after breaking the enterohepatic circulation (EHC). The daily base line secretion rate (5% interruption of the EHC) is plotted on the left, the upper broken horizontal line being the mean value over 6 days (14.01 mmoles/24 hr). In the center portion of the figure, the changes in bile salt secretion rate are followed at 90-min intervals for a total of 21 hr after changing abruptly from 5 to 100% interruption. For the first 90 min, bile salt secretion is similar to that seen at 5% interruption. Then, as the bile salts which remain in the EHC are "washed out," the secretion rate falls and a low point is reached in 4½ hr. The bile salts secreted from the time of interruption until the low point is reached give a measure of the circulating bile salt pool size (hatched area) which here was 1.01 mmoles. An estimate of basal synthesis (the solid bar on the left, 0.58 mmole/24 hr) may be obtained by extrapolating (the lower broken horizontal line) from the low point (26, 28, 29). This estimate of synthesis is similar to that calculated by the fecal excretion method (0.70 mmole/24 hr). Bile salt secretion after the low point is due exclusively to bile salt synthesis by the liver, which rises gradually over the next 24 hr to reach a new steady state. The 24 hr secretion rate over the subsequent 7 days and the mean value in the new steady state are shown on the right. The increase in hepatic synthesis at 100% interruption of the EHC, over the basal synthesis, is shown by the stippled bar.

and finally over the ensuing 12–24 hr, bile salt secretion increases to levels severalfold greater than the low point (Fig. 1). The amount of bile salts secreted from the time of changeover until the low point is reached, represents the effective bile salt pool which is "washed out" during one circulation.

In previous studies in which an intact EHC was abruptly interrupted by a complete bile fistula, it was suggested that the low point in the washout curve represented the basal rate of bile salt synthesis in the normal state, and the subsequent rise in the bile salt secretion represented the compensatory increase in hepatic bile salt synthesis in response to the interrupted EHC (26, 28, 29).

The technique of changing abruptly from steady-state conditions with an intact EHC to a complete bile fistula was used in four monkeys to measure the bile salt pool size. The same technique was also applied to measure pool size at 5, 20, 33, and 66% interruption of the EHC in one monkey. The total quantity of bile salts obtained in the washout includes not only the circulating pool, but a contribution from endogenous synthesis occurring during collection.

Fecal fat determination. The dietary intake of Purina monkey chow in healthy animals (120–180 g/day) was approximately equal to 4% of the monkeys' body weights. Periodical analyses of the chow showed that its mean lipid content was 7% (±SEM 0.34) by weight, thus giving a dietary fat intake of 8.4–12.6 g/day. On this dietary regime, fecal fat excretion was measured in pooled 3-day collections of feces using the method of Bowers, Lund, and Mathies (30). In this method, an aliquot of homogenized stool is acidified to pH 4, extracted with chloroform : methanol (2 : 1), and the lipid residue from the chloroform phase is then measured gravimetrically. This technique gives very comparable results with the more widely used method of Van de Kamer, ten Bokkel Huininck, and Weyers (31) as paired estimations with the two methods on 14 stool samples gave a correlation coefficient (r) of 0.975 ($P < 0.001$).

Distal small bowel resection. After studying bile salt secretion at the different levels of biliary diversion in five monkeys, distal bowel resections were performed. Thus, the effects of interruption of the EHC produced by the stream-splitter could be compared with that produced by small bowel resection in the same animals.

In three monkeys, the distal one-third of the combined lengths of jejunum and ileum was resected (26–34 inches or 66–86 cm); in two other animals, approximately two-thirds of the distal small bowel was removed. In each case, the cecum was also resected and the proximal intestinal remnant anastomosed end-to-end with the ascending colon. When the animals recovered from this second operation and were again eating normally (some 2 wk later), bile salt secretion was measured when 95% of the biliary output was returned to the intestine. The interruption of the EHC by ileectomy was also compared with that produced by a complete bile fistula when no bile was returned to the shortened intestine.

RESULTS

Bile volume and bile salt secretion in response to controlled interruption of the EHC. The results of bile volume and bile salt secretion when steady-state conditions were reached at the different levels of interruption of the EHC are given in Table I.

TABLE I

Effect of Graded Interruption of the Enterohepatic Circulation (EHC) of Bile on Bile Voluem and Bile Salt Secretion

Per cent interruption of the EHC	No. of observations	No. of monkeys	Bile volume, Mean ±SEM	Bile salt secretion Mean ±SEM
%			*ml/24 hr*	*mmoles/24 hr*
5	22	13	178 ± 9.5	9.98 ±0.62
10	6	5	185 ± 7.5	9.45 ±0.88
20	5	5	176 ± 7.8	8.74 ±1.51
33	8	8	134* ± 9.8	5.71‡ ±0.53
66	7	7	105* ±10.7	3.21‡ ±0.33
100	11	9	94* ± 7.2	1.91‡ ±0.13

* Significantly less than "base line" value (5% interruption) $P < 0.005$–0.001.

‡ Significantly less than base line value (5% interruption) $P < 0.001$.

At 5% diversion, the mean bile volume for 13 monkeys was 178 ml/24 hr (±SEM 9.5), while bile salt secretion averaged 9.98 mmoles/24 hr (±SEM 0.62) (Fig. 3). There was no significant difference in the daily levels of bile volume and bile salt secretion at 10 and 20% interruption of the EHC when compared with the base line (5%) values.

However, as the percentage of bile diverted increased from 33% to 66% to 100%, there was a progressive fall both in bile volume and in bile salt secretion and all these values were less than base line values by statistically significant amounts. (Table I).

The values for bile volume and bile salt secretion in individual monkeys did not appear to be related to body weight. For example, when measurements of bile volume in 13 monkeys at 5% interruption were normalized per 5 kg body weight, the mean 24 hr bile volume was 170.6 ml/5 kg per 24 hr (±SEM 9.6). The scatter of results, therefore, as indicated by the standard error of the mean in these corrected measurements was the same as that observed in the raw data.

Relationship to fecal fat excretion. The relationship between fecal fat excretion and bile salt secretion in 5–16 monkeys is shown graphically in Fig. 2. The overall results in all the monkeys studied show that with diversion of 33% or more of bile to the exterior, there was a progressive increase in fecal fat to reciprocate with the decreasing levels of bile salt secretion. However, in individual monkeys, this relationship between bile salt secretion and the degree of steatorrhea did not always correlate well.

The mean control fecal fat excretion in seven monkeys with an intact EHC before surgery was 2.35 g/day per monkey (±SEM 0.35) which was 19–28% of the dietary fat intake. There was no statistically significant difference between this normal level of fat excretion and that

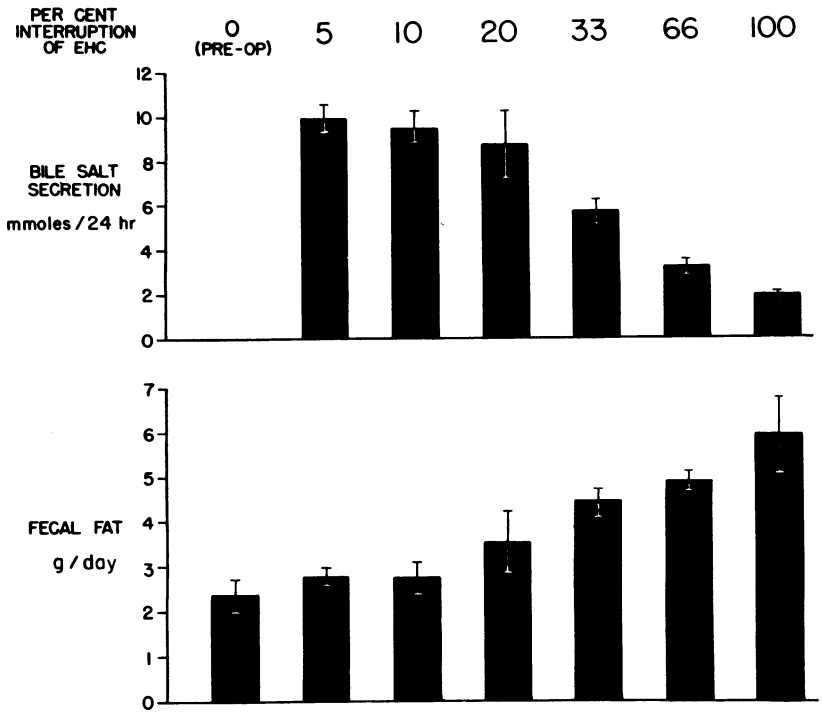

FIGURE 2 The results of bile salt secretion in 5–16 monkeys at various levels of interruption of the enterohepatic circulation and their relationship to fecal fat excretion. The bars represent mean values and the vertical lines, the SEM.

associated with diversion of 5, 10, or 20% of the bile to the exterior.

However, with interruption of 33, 66, and 100% of the EHC, the average fecal fat excretion increased progressively and these values were significantly greater than the normal level of fat excretion ($t = 3.95–6.03$; $P < 0.01–0.001$).

Bile salt synthesis. The results of *de novo* bile salt synthesis when steady-state conditions were reached at the various levels of interruption of the EHC are shown in Fig. 3.

The normal bile salt synthesis in animals before surgery (which of course is simply the normal fecal bile salt excretion) was 0.21 mmoles/day. There was a rapid

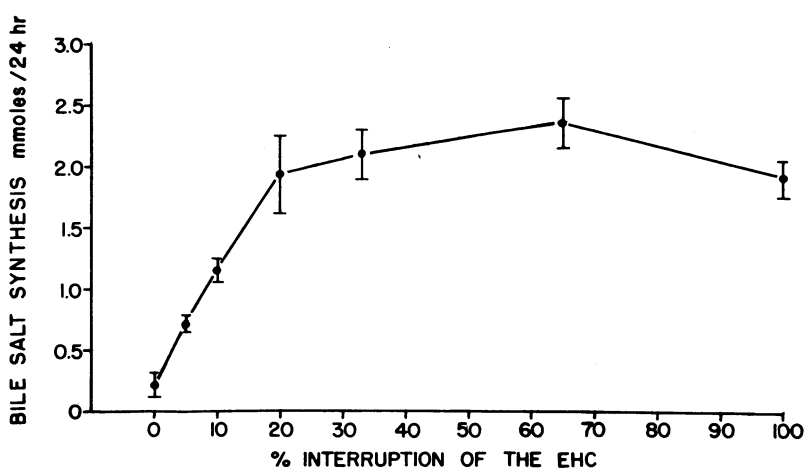

FIGURE 3 Bile salt synthesis in 5–16 monkeys at different levels of interruption of the enterohepatic circulation (mean values ±SEM).

TABLE II

Bile Salt Pool Size and Circulation Frequency at Different Levels of Interruption of the Enterohepatic Circulation

Monkey No.	Per cent interruption of the EHC	Bile secretion	Bile salt pool size	No. pool circulations per day
	%	*mmoles/24 hr*	*mmoles*	
15	5	9.96	1.59	6.3
18	5	14.01	1.01	13.8
19	5	17.21	1.60	10.8
21	5	7.10	0.88	8.1
21	20	6.90	0.73	9.5
21	33	4.23	0.53	7.7
21	66	2.27	0.22	10.0

and progressive increase in bile salt synthesis as the per cent of bile diverted increased from 5% to 10% to 20%, the actual values being 0.60, 1.06, and 2.27 mmoles/day. Synthesis was almost maximal at 20% interruption of the EHC, and there was little further increase in bile salt synthesis with greater degrees of biliary diversion. The maximal rate of synthesis, therefore, represents approximately a 10-fold increase above the normal rate of synthesis.

The values for bile salt synthesis were based not only on bile salt diversion with the stream-splitter, but also on fecal bile salt excretion levels. The normal fecal levels of bile salt excretion, 0.21 mmole/25 hr (±SEM 0.09), did not differ significantly from those either with small interruptions (5, 10, and 20% diversion), 0.26 mmole/24 hr (±SEM 0.03), or with large interruptions (33 and 66%), 0.25 mmoles/24 hr (±SEM 0.01).

Bile salt pool size. The normal bile salt pool size in four monkeys, as measured from washout curves at 5% biliary diversion (see Fig. 1), ranged from 0.88–1.60 mmoles (mean = 1.27 mmoles). Since the corresponding values of bile salt secretion at 5% interruption of the EHC are known, one may calculate the number of times the pool must circulate each day by dividing the pool size into the 24 hr bile secretion. These calculations show that, on the average, the bile salt pool must circulate 9.8 (range 6.3–13.8) times each day (Table II).

In monkey 21 in which bile salt pool size was measured at different levels of interruption of the EHC, the base line pool was 0.88 mmole. As suggested by the studies of bile salt secretion and synthesis, the increased synthesis was almost able to maintain the bile salt pool size at the base line level with 20% bile diversion (0.73 mmole). However, although the increased synthesis was maximal at 33 and 66%, it was no longer able to maintain the pool size which fell to 0.53 mmoles at 33% and 0.22 mmole at 66%. At 20, 33, and 66% interruption of the EHC, once the low point of the washout curve (refer to Fig. 1) was reached, there was no subsequent rise in bile salt secretion, confirming that synthesis was already maximal.

Ileal resection. The effects of ileal resection on bile salt secretion are illustrated by the results from one animal (monkey 18) in Fig. 4, and the over-all results in five monkeys are given in Table III.

After resection of the distal one-third of the small bowel (Fig. 4), bile secretion, as measured by the stream-splitter when all but 5% of the bile was returned to the duodenum, was 4.84 mmoles/day (±SEM 0.29) and while this represents considerable reduction in bile salt secretion as compared with the base line state (14.01 mmoles/day ±SEM 0.61), the enterohepatic circulation was by no means abolished. In fact, this postresection value for bile salt secretion was more than double that found with a complete interruption of the EHC pro-

TABLE III

Effect of One-Third or Two-Thirds Distal Small Bowel Resection on Bile Salt Secretion and Reabsorption

Monkey No.	Length of small bowel resected	Bile salt secretion after resection		Bile salt reabsorption after resection	% Reabsorbed
		5% collection	100% collection		
		mmoles/24 hr		*mmoles/24 hr*	%
9	Distal one-third	4.46	1.89	2.57	57.7
18	Distal one-third	4.84	2.30	2.53	52.2
21	Distal one-third	4.86	1.84	3.02	62.1
19	Distal two-thirds	2.65	1.09	1.56	58.6
20	Distal two-thirds	2.59	1.61	0.98	37.9
Mean results from all normal intact monkeys	None	—	—	—	97.8

duced by 100% bile diversion (2.30 mmoles/day ±SEM 0.14) and comparing bile salt secretion after ileectomy with that found with controlled interruption of the EHC before resection, this level was approximately equivalent to 50% interruption of the EHC (Fig. 4). Similar findings were true for the two other monkeys with a distal one-third small bowel resection (Table III). However, in the two animals in which two-thirds of the small bowel was removed, there was a more drastic reduction in bile salt secretion, 2.59 and 2.65 mmoles/24 hr respectively, and while these values are still more than those with complete bile fistula (1.61 and 1.09 mmoles/24 hr), the greater loss of absorptive surface after two-thirds distal resection was associated with a more marked effect on the enterohepatic circulation of bile salts (Table III).

It is worth emphasizing, perhaps, that a certain percentage interruption of biliary flow by the stream-splitter, for example 33%, does not imply a comparable interruption in bile acid absorption from the ileum. This is clearly illustrated in Fig. 4.

Assuming that bile salt synthesis is maximal both with a complete bile fistula and after ileal resection, the difference between bile salt secretion after resction and that found with 100% bile diversion must represent bile salt reabsorption by the residual intestine. After distal

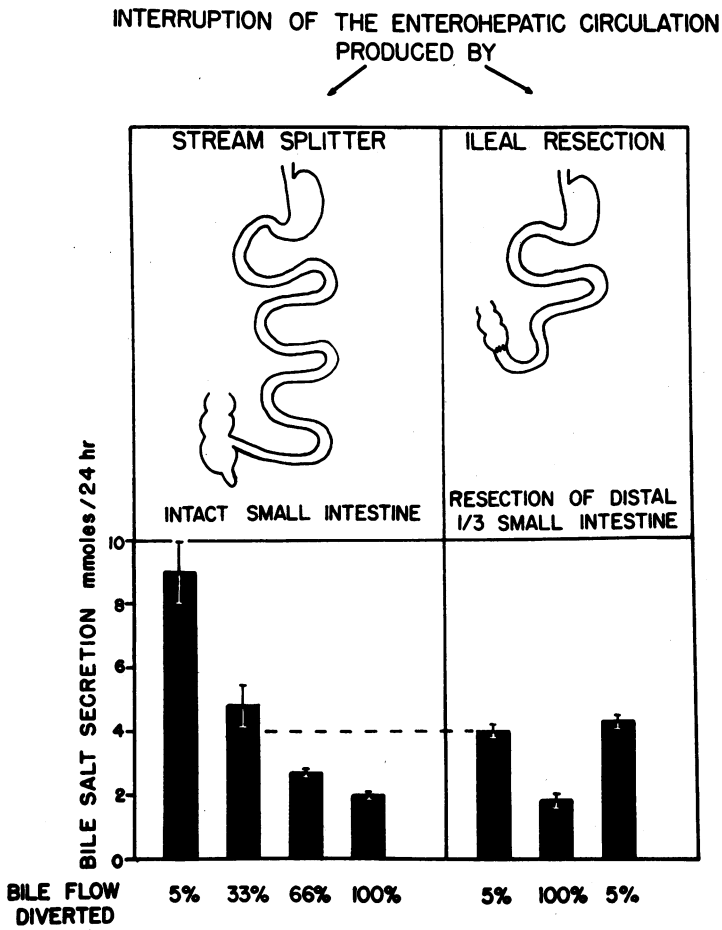

FIGURE 4 Example of bile salt secretion values in one monkey, when the enterohepatic circulation was interrupted mechanically by the electronic stream-splitter compared with that produced by distal one-third small bowel resection. The postresection bile salt secretion was measured by sampling 5% of the biliary output with the stream-splitter and compared with bile salt secretion with a complete bile fistula (100% interruption of the enterohepatic circulation). As is shown by the broken horizontal line, the postresection value for bile salt secretion was approximately equivalent to 50% interruption of the EHC as measured by the stream-splitter.

one-third resection, the estimated bile salt reabsorption was 2.53, 2.57, and 3.02 mmoles, while the two monkeys with more extensive resections reabsorbed only 0.98 and 1.56 mmoles (Table III). Since the normal monkeys excrete only 0.21 mmole bile salts/day and secrete a total of 9.98 mmoles into their intestine, they reabsorb about 98% of all secreted bile salts. The animals with small bowel resections secrete less total bile salt but only reabsorb 38–58% of total bile salt entering the intestine.

DISCUSSION

These results have shown by direct measurement that, in in the rhesus monkey, the adaptive increase in hepatic bile salt synthesis is limited and is almost maximal at 20% interruption of the EHC. With diversion of 33% or more of the bile, the increased synthesis, although maximal, is inadequate to compensate for bile salt loss produced by the stream-splitter. As a result, there is a progressive fall in bile salt secretion as the per cent of bile diverted increases from 33% to 66% to 100%. Thus, although the liver is capable of increasing its bile salt synthesis, because the normal level of synthesis is so small, even the 10-fold increase in hepatic bile salt synthesis found in this study could not compensate for more than 20% biliary diversion. The measurements of bile salt pool size (in one animal, monkey 21) at different degrees of interruption of the EHC also confirmed this pattern. In this monkey, although a modest reduction in the pool size occurred at 20% interruption, marked reduction in bile salt pool size occurred at 33 and 66% interruption of the EHC.

Although in the past few years it has often been shown that ileal disease or resection may cause bile salt malabsorption (9–16, 32, 33), the extent of the resultant interruption of the EHC has not been directly quantitated. Using the dog, Playoust, Lack, and Weiner (9) showed that ileectomy virtually abolished the EHC of ^{14}C-labeled taurocholate, while jejunal resection had no such effect. Subject studies in man confirmed these results (10, 12–14, 16, 32, 33), but most of these investigations suffer the disadvantage that the fate of a single bile salt radioisotope was followed either by duodenal drainage or by fecal analysis. Since the rates of absorption and excretion of the different bile acids may vary considerably (34–36), it is difficult to extrapolate the findings with a single isotope to the total bile salt pool.

In the present investigation, the use of the stream-splitter provides sampling access to bile for direct measurement of bile salt concentrations. The model also provided a means of varying and controlling the degree of interruption of the EHC in the steady state long after the effects of anesthesia and surgery (17). By collecting only 5% of the bile produced and returning the remaining 95% to the intestine, the obvious problems created by interruption of the EHC with simple bile fistulae were largely avoided. However, although 95% of the bile was being returned to the intestine, the liver was still required to synthesize the amount of bile salts being diverted by 5% interruption of the EHC so that even this level of biliary diversion does not represent a truly "normal" or "intact" EHC.

Several previous investigators have also attempted to avoid the artifacts produced by surgical operation or by simple bile fistula preparations. In small laboratory animals, by using both intestinal and biliary cannulae, it has been possible to replace bile salts lost through external drainage (7) or to maintain the EHC until the time of the experiment (37).

In man, various ingenious methods have been used to occlude the distal limb of T-tubes draining the common bile ducts of patients who had undergone biliary surgery (38–40). After sampling, the bile was returned to the intestine either by bypassing the occluded limb of the T-tube or by transnasal duodenal intubation. These methods were reviewed by Thureborn (38) whose bile sampling technique was modified in the development of the present model.

The bile of the rhesus monkey has ratios of glycine: taurine conjugates and of trihydroxy: dihydroxy bile acids comparable with those found in man (18, 19). In this respect, the monkey is a more suitable experimental animal than the more widely used rat in which the taurine-conjugated bile acids predominate. Since taurine-conjugated bile acids are strong acids with low pKa values (41–44), they largely remain in the ionized form in the intestinal lumen. As such, they cannot be reabsorbed from the jejunum by passive nonionic diffusion (1) and are largely dependent on the active transport system of an intact ileum for their reabsorption. This being the case, studies of ileal resection in the rat might be expected to yield very different results from studies in the rhesus monky or in man where the glycine-conjugated bile acids predominate.

The compensatory increase in bile salt synthesis by the liver after ileal resection was suggested by similar adaptive responses to complete interruption of the EHC by a bile fistula. As long ago as 1928, Whipple and Smith (45) noted that with time, the levels of bile salt secretion tended to rise in bile fistula dogs. Similar observations were made by Thompson and Vars (46) who thought that the gradual increase in bile salt secretion was due to recovery from liver damage inflicted at the time of the operation to insert the bile cannula.

Washout curves for bile salt secretion after acute bile fistulation, similar to those described in this study, have also been noted in rabbits (47) and in man (48). Eriksson (26) first suggested that the rise in bile salt secretion which follows the low point of the washout

curve was due to increased bile salt synthesis by the liver. From acute studies in the bile fistula rat, he suggested that the liver was capable of a 10 to 20-fold increase in bile salt synthesis. Kay and Entenman (29) found similar results in the rat, but Myant and Eder (28) thought that the adaptive increase in bile salt synthesis was more limited (4- to 5-fold increase).

Furthermore evidence that the liver is capable of an adaptive increase in bile salt synthesis comes from cholestyramine feeding experiments in mice (49). Cholestyramine is an anionic binding resin which combines with bile acids in the small bowel lumen to form a complex which cannot be absorbed. The increased fecal bile acid excretion which results produces an interrupted EHC functionally similar to that caused by a bile fistula, and as in the bile fistula rat, these animals also develop a compensatory increase in hepatic bile salt synthesis (49).

The fecal fat measurements in control animals showed that a surprisingly high percentage of the dietary fat intake was excreted each day in the stools. This may be related to the crude nature of the dietary fat in the commercial monkey chow. Lipid extracts of both monkey chow and feces were solid at room temperature and while some of this lipid melted at or below body temperature, examination by hot-stage polarizing microscopy showed that much of the lipid remained in a crystalline state until 57°–58°C. This high melting point suggests that much of the dietary lipid would be unavailable for intestinal absorption at body temperature.

In spite of this, the fecal fat excretion showed the expected inverse relationship to bile salt secretion and pool size, and although the fecal fat excretion in the complete absence of bile was less than three times the normal fecal fat, it is well-recognized that the steatorrhea of biliary insufficiency is usually modest. The origin of the fecal fat and the relative concentrations from dietary and endogenous sources were not studied here, although it has been suggested that endogenous fat excretion may be increased in animals with biliary diversion (50, 51).

The findings of an incomplete interruption of the EHC by ileal resection in the present study conflicts with previous investigations where it was suggested that disease or resection of the distal small intestine virtually abolished the EHC (9, 12).

As indicated previously, this may be related to the use of a nonrepresentative isotope such as the sodium salt of taurocholic acid-^{14}C which, at intestinal pH, would remain in the ionized form and would therefore require the active transport system of the ileum for its reabsorption. Using this isotope to study the effects of ileal disease might suggest that there was almost complete interruption of the EHC. However, it is known that bile salts may be absorbed by diffusion from many segments of the intestinal tract (1). Small and Dietschy (36) defined the kinetics of bile salt diffusion from the jejunum of the rat and showed that glycine-conjugated bile salts were readily absorbed from the upper small bowel while Hislop, Hofmann, and Schoenfield (35) showed by intestinal perfusion in man that the weaker bile acids with higher pKa values, such as the glycine-conjugated acids, were also absorbed in the jejunum. Since the glycine-conjugated bile salts in fact predominate in man in a ratio of about 3:1 (52), absorption by diffusion may well be an important component of the normal EHC. This becomes of even greater significance after ileal resection, since the ratio of glycine-: taurine-conjugated bile acids is greatly increased after ileectomy (32, 53).

Bile salts may also be reabsorbed from the colon. Norman and Sjövall (54) showed that in the rat more than 50% of cholic acid-^{14}C was recovered from the bile when the radioisotope was placed in the cecum. Similarly in patients undergoing biliary surgery, Samuel, Saypol, Meilman, Mosbach, and Chafizedeh (55) recovered an average 58.9% of administered radioactivity in T-tube bile when cholic acid-^{14}C was injected into the large bowel.

The quantitative significance of the bile salt reabsorption from different sites along the intestinal tract remains to be evaluated, but the limited data from the present experiments would suggest that, in ileal resection at least, absorption from the residual small bowel may be an important factor in maintaining the EHC.

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