The economy of the enterohepatic circulation of bile acids in the baboon. 1. Studies of controlled enterohepatic circulation of bile acids[1]

Richard N. Redinger,[2] James W. Hawkins, and D. Michael Grace

Department of Medicine, University Hospital, Boston University School of Medicine, Evans Memorial Department of Clinical Research, Boston, MA, and University Hospital, Faculties of Medicine and Surgery, University of Western Ontario, London, Ontario, Canada

Abstract A primate model with controlled enterohepatic circulation has been developed which allowed short-term evaluation of bile salt pool size, cycling frequency, and fecal losses. We found that when bile salt secretion into the small bowel was increased above 700 μmol/hr bile acid pool cycling frequency was also increased. The latter allowed short-term regulation of bile salt pool size by affecting increased fecal losses. These compensatory mechanisms were associated with a return of bile acid secretion and cycling frequency to normal usually within 12–24 hours. Conversely, during decreased bile salt secretion, regulation of bile salt pool size was accomplished by decreased pool cycling, which decreased fecal losses. Bile acid secretion into the intestine was therefore a major determinant of bile salt cycling frequency. The latter affected bile salt fecal loss to provide short-term regulation of bile salt pool when feedback regulation of bile salt synthesis was nonoperative.—**Redinger, R. N., J. W. Hawkins, and D. M. Grace.** The economy of the enterohepatic circulation of bile acids in the baboon. 1. Studies of controlled enterohepatic circulation of bile acids. *J. Lipid Res.* 1984. **25:** 428–436.

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Normal bile flow in mammals is largely dependent upon bile salts which require an enterohepatic circulation (EHC) between intestine and liver for their preservation and function (3, 4). Bile salt economy within the EHC is accomplished by efficient bile acid absorption from the small intestine, since only a small percent of secreted bile salts is lost from the intestine per bile salt pool cycle (5). The hepatic component of the EHC is characterized by rapid hepatic bile salt uptake and secretion (6) so that when bile salts are not sequestered in the gallbladder the EHC becomes rate-limited by bile salt transit down the small intestine. The clinical importance of the intestinal component of the EHC on bile salt metabolism in man was highlighted by Duane and Bond (7) and Duane (8) when they demonstrated that an experimentally induced delay in intestinal transit resulted in enhancement of the bile salt pool, while the converse occurred when bile salt intestinal transit was increased. In their studies pool size changes were found to be better correlated with fractional bile salt turnover than with bile salt synthesis. Since fractional bile salt turnover is affected by fecal loss of bile acids, regulation of bile salt pool size may be affected by factors that directly influence intestinal bile salt losses rather than by feedback regulation of synthesis by bile salts absorbed from the small intestine and returned to the liver. Further studies of the intestinal component of the EHC are, however, necessary to better define how fecal losses might play a role in the regulation of bile salt metabolism.

The baboon model with exteriorized EHC allows detailed study of selective components of EHC in primates (9). Previous studies with a similar rhesus monkey model were mainly designed for the study of acute and chronic interruption of the EHC in the fed state (10–12) and focused mainly on biliary lipid secretion and composition rather than on alterations of specific components of the EHC of bile salts on bile salt turnover.

To determine the short-term effect of alterations of the intestinal component of the EHC of bile salts on the economy of bile acids we varied bile salt input into the intestine in the baboon model with exteriorized EHC (9) and concomitantly examined bile salt pool size, cycling frequency, and fecal losses in both fed and fasting animals. During short-term experiments of less than 24-hr duration we found that the rate of bile salt input into the intestine in these animals affected bile salt pool size by influencing

Abbreviations: BA, bile acid; EHC, enterohepatic circulation; NS, not significant; PL, phospholipid; SR, secretion rate; CF, cycling frequency.

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[2] Present address: Department of Medicine, University of Louisville Health Sciences Center, Louisville, KY 40292.

both bile salt cycling frequency and intestinal bile salt losses. In fed animals enhanced bile salt input into the intestine resulted in increased bile salt pool cycling, which increased fecal losses to help maintain bile salt pool size. In fasting animals with diminished bile salt synthetic activity, secretion and input into the intestine, decreased bile salt pool cycling, and fecal losses helped preserve pool size.

METHODS AND PROCEDURES

Materials

Chromic oxide was purchased from VWR Scientific, Inc. (Columbus, OH), α-tocopherol from ICN Pharmaceuticals, Inc. (Life Sciences Group, Cleveland, OH), and dextran from Sigma Chemical Company, (St. Louis, MO).

Diet preparation and intake

Diet was prepared from ground monkey chow (Ralston Purina, St. Louis, MO) which was mixed in a rotary blender with a fecal flow marker (chromium oxide 0.375 mg/g), an anti-oxidant (1% α-tocopherol), and binder (0.1% dextran). This mixture was compressed into biscuits which were hardened by gently heating to dryness (60°C). Animals were fed at 8 AM and consumed their daily ration of 300 g of fresh monkey chow within several hours. In "fasting" experiments, food was removed at 12:00 noon of the day prior to experimentation and withheld until completion of the experiment; water was allowed ad libitum.

Animal model

The objective was to study the influence of the intestinal component of the EHC on bile salt metabolism by varying bile salt input into the intestine in both fed and short-term fasting states.

The primate baboon with exteriorized EHC was surgically prepared as described previously (9). No experiments were begun until the animals recovered from both cholecystectomy and T-tube placement into the common bile duct and duodenum. Recovery was assured when dietary intake, weight, and bile salt pool size stabilized 2–3 weeks postoperatively. Bile salt analyses were performed on bile sampled continuously at regular intervals (usually 1–2 hr) during experiments, including an immediate 6-hr pre-experimental control period. Daily steady state secretion rates were also required for 2–3 days before the start of each experiment.

Experimental design and definitions (Fig. 1)

Bile salt pool size. Pool size was determined at the beginning (Pool A) and end of each experiment (Pool B or

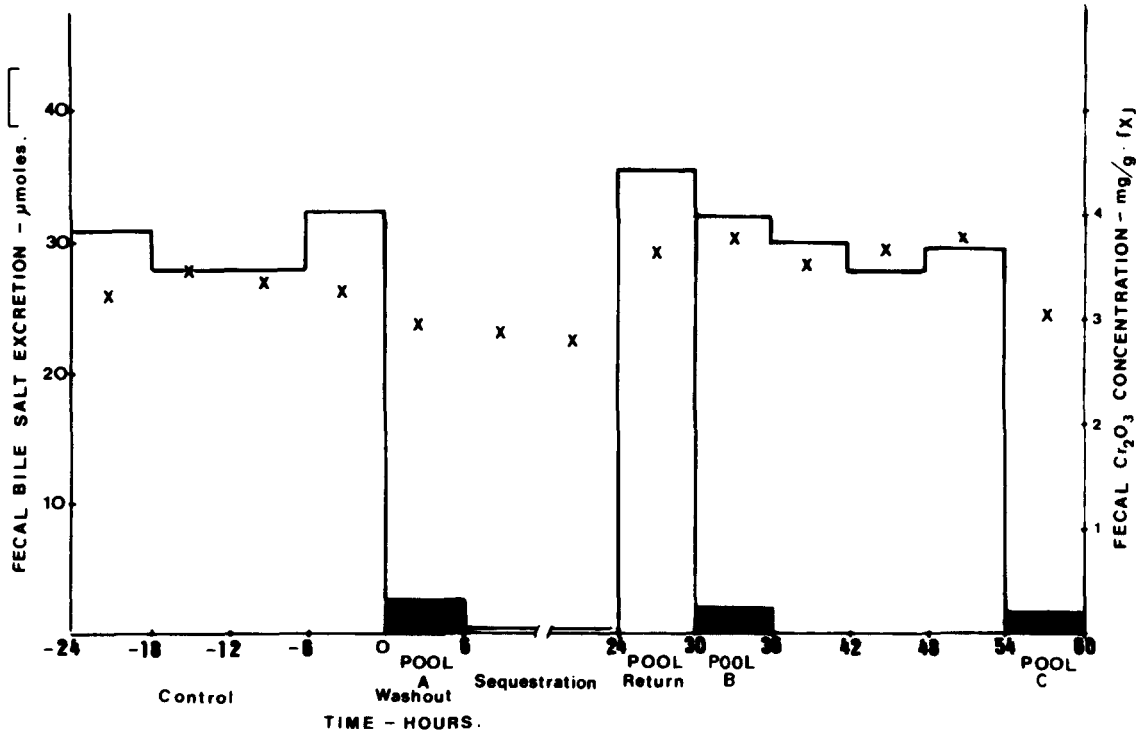

Fig. 1. Fecal flow (X) and bile salt fecal excretion are shown for 6-hr intervals for a representative animal during a control period and following pool return. Bile salt fecal loss between Pool B and C washout was evaluated between the low points of excretion following each pool washout, since excretion decreased to less than 10% of control levels in each instance. All animals had steady-state fecal flow as determined by chromium oxide marker in stool.

C) by draining all bile from the EHC (10) at these times into a 100-ml sterile plastic reservoir which substituted for the gallbladder. Bile drainage was carried out for 6–8 hr to achieve the secretion nadir denoting pool removal. A small aliquot of less than 2% of the measured pool was used for analysis of its bile salt content to determine pool size.

Bile salt pool sequestration. After initial pool drainage (designated Pool A), the bile salt pool was withheld for 6 or 18 hr to simulate short- or long-term interrupted EHC, respectively. All bile secreted during pool sequestration was kept separate from the pool and was not returned to the EHC. Since the bile salt pool had been drained and no bile salt was returned to the intestine during pool sequestration, bile salt secretion equalled bile salt synthesis during both 6- and 18-hr pool sequestration.

Bile salt pool return to intestine. After either 6- or 18-hr pool sequestration, bile salt input into the intestine was varied by infusing in random order the sequestered pool at rates of 600, 200, or 100 μmol of bile salt per hr so that the total pool was returned within approximately 2, 6, or 12 hr, respectively. Bile salt input into the intestine included bile salt secreted by the liver during pool return in animals with intact EHC.

To assess changes in pool size consequent to pool return, pool drainage was repeated either immediately after pool return (designated Pool B) or 12–24 hr later when biliary lipid secretion rates had returned to steady state control levels (designated Pool C). Pools B or C (minus a small aliquot amounting to less than 2% of the volume used for analysis) were returned to their donors over a 3-hr period immediately after pool drainage to preserve the pool and its EHC. Steady state biliary lipid secretion for at least 72 hr was a prerequisite prior to repeat experimentation.

Bile salt pool cycling frequency. Pool cycling rates were assessed at 2-hr intervals during pool return by dividing bile salt secretion during these intervals by the accumulated pool in the EHC. The accumulated pool in the EHC included the accumulated infused pool plus bile salt added to the pool by the liver during the interval studied. This contribution from liver was calculated from the basal bile salt secretion rate observed at beginning of pool infusion. The accumulated pool was drained and measured at the end of pool infusion in one set of experiments. In other experiments in which the EHC remained intact and pool drainage was not repeated until secretion rates returned to control levels, cycling rates following pool return were determined by dividing 2-hr bile salt secretion rates by Pool C. For the determination of this quotient in each 2-hr interval, the values used for Pool C were adjusted prior to the last 2-hr interval, when it was measured to account for changes in bile salt synthesis or fecal losses occurring following pool return. We assumed that such changes were constant throughout all intervals studied. The expansion or depletion of the pool was determined from the difference between Pool B and C. To do this, Pool B was normalized to the same control as Pool C (see Table 2).

Fecal bile salt losses. Fecal losses during pool return were assessed in each experiment by analyzing fecal samples every 6 hr between pool drainage A and C. Such frequent fecal analysis was extended for 48 hr beyond final pool (C) drainage to allow the detection of the low point of BA excretion in stool after pool washout (Fig. 1). Fecal losses between these two low points of excretion following pool A and pool C were then compared to bile acid losses in fecal samples collected for 48 hr in the control period immediately prior to pool drainage. Steady state fecal flows were assured from constant chromium oxide concentrations in stool (13). Fecal losses were not routinely evaluated in those animals in which the pool was removed immediately after pool return (i.e., pool B).

Special studies

Diurnal variation of bile salt cycling frequency and correlation with cholesterol 7α-hydroxylase activity. Bile salt pool size was assessed the day prior to the study and at the end of the studies by the method described above. Since pool sizes did not change significantly (See Table 3), diurnal variation of bile salt cycling frequency was determined from the quotient of bile salt secretion rate over mean pool size (pre + post pool ÷ 2) from each of 3-hr sequential bile samples secreted over 24 hr in four fed and three fasting animals with intact EHC (i.e., 5% bile collection). Bile salt secretion rates were similarly studied in these animals after complete bile diversion for 48 hr in fed and fasting states to assess diurnal variation of bile salt synthesis. Fecal bile salt losses were assessed in both fed and fasting animals as described above.

Percutaneous liver biopsies were performed in both fed and fasting animals with intact or interrupted EHC at 8:00 to 10:00 AM and 6:00 to 8:00 PM for evaluation of cholesterol 7α-hydroxylase activity, the rate-limiting enzyme involved in BA synthesis. During interrupted EHC, liver biopsies were done at the end of 6- and 18-hr pool sequestrations.

Chemical analyses

Bile salt analyses in bile were carried out by the enzymatic method of Talalay (14). Activity of cholesterol 7α-hydroxylase was measured by the method of Shefer, Nicolau, and Mosbach (15) in 30–50 mg of liver biopsy tissue. We found that optimal conditions for enzyme activity in the baboon were achieved by incubating 1 mg of microsomal protein at pH 7.4 for 20 min (**Fig. 2**). Fecal BA and BA pool size were determined as described previously in baboons (9) and fecal chromium oxide concentrations were determined by the method of Bolin, King, and Klosierman (16).

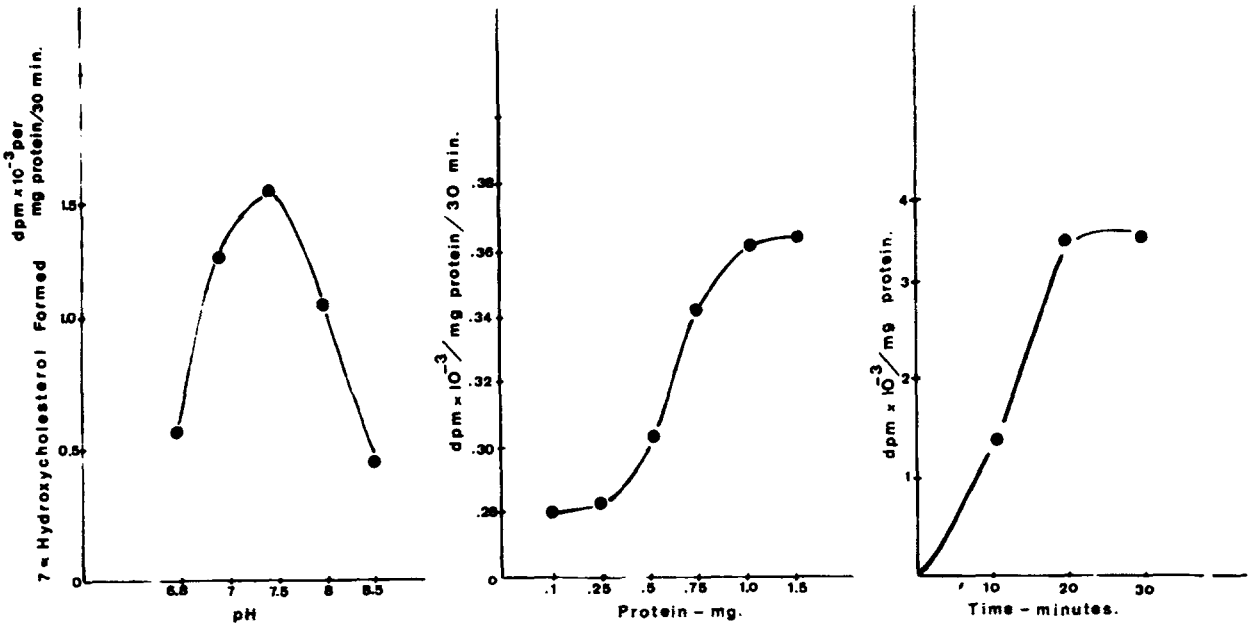

Fig. 2. Effect of pH, microsomal protein, and time of incubation on rate of 7α-hydroxycholesterol formation. Assay conditions are as described by Shefer, Nicolau, and Mosbach (15). Optimal conditions were: pH, 7.5; microsomal protein, 1 mg/tube; incubation time, 20 min, for baboon microsomal preparations.

Statistical analysis

Variance was reported as mean ± SEM. Student's *t* tests for paired and unpaired experiments and analysis of variance were applied to grouped data for statistical analysis (17).

RESULTS

Effects of bile salt input into the intestine

Bile salt secretion and pool size. In experiments in which bile salt synthesis was not enhanced during or following pool return (i.e., after 6 hr pool sequestration), bile salt input into the intestine remained consistently below 600 μmol/hr. Pool size did not change following pool return in any of these experiments when measured either immediately or 12 hr after pool return (when secretion rates normalized) (**Table 1A** and **Fig. 3A**).

Bile salt input into the intestine was greater following 18-hr pool sequestration since bile salt synthesis was enhanced more than twofold at the beginning of pool return in these experiments. During 600-μmol/hr pool return, bile salt input in these animals approached 1000 μmol/hr which returned to control levels within 6 hr. Bile salt input consistently exceeded 1000 μmol/hr for 12 hr following 200-μmol/hr pool infusion but remained below 600 μmol/hr during and following 100-μmol/hr pool infusion (Table 1B). Bile salt pool size was found to be enhanced over control when measured immediately (Pool B) after pool return at both these rates of pool infusion, i.e., 14.9% ($P < 0.05$) after 200-μmol/hr and 29.3% ($P < 0.01$) after 100-μmol/hr pool infusion. Similar results were found when the pool was measured after bile salt secretion rates had returned to control levels (Pool C), i.e., 17.3% ($P < 0.05$) and 31.6% ($P < 0.01$) for 200- and 100-μmol/hr infusions, respectively. However, pool size B or C was not significantly increased following rapid (600 μmol/hr) pool return (**Table 2**).

Fecal bile salt losses. Fecal bile salt losses did not increase in any experiments in which bile salt input into the intestine remained consistently below 600 μmmol/hr, such as after 100-μmol/hr pool infusion. On the other hand, there was enhancement of fecal bile acid loss in experiments in which bile salt input into the intestine exceeded 1000 μmol/hr such as during and following 600- and 200-μmol/hr pool infusion in animals with enhanced bile salt synthesis (Table 2). Enhanced bile salt synthetic rate, however, was not the factor that enhanced fecal bile salt losses since these losses were not enhanced during 100-μmol/hr pool return experiments following 18-hr pool sequestration. In these experiments bile salt input into the intestine remained below 600 μmol/hr during most of the experiment (Table 1B).

Bile salt pool cycling frequency. As bile salt secretion rate increased from 0.1 to 1.4 mmol/day, cycling frequency increased from 3.5 to 22 cycles per day ($r = 0.87$, $y = 0.01X + 3.0$) (**Fig. 4**). A positive correlation also existed between fecal bile salt losses and the maximal pool cycling rate attained in these experiments ($r = 0.77$, $y = 0.05X + 4.5$).

TABLE 1. Bile salt secretion rate (SR), pool size, and cycling frequency (CF) following 6-hr and 18-hr pool sequestration and return

Infusion Rate (n)	Control	End Pool	Time Following Start of Pool Return					
			0	3	4	6	12	24
μmol/hr			*hr*					
A. Following 6-hr pool sequestration and return								
600 (6)								
SR	439.7 ± 38[a]	58.4 ± 19	80.9 ± 17	450 ± 42	412 ± 37	436 ± 27	440 ± 20	
Pool	1240	0	0	1262[b]	1262	1262	1262	
CF	8.5	0	0	8.5	7.8	8.3	8.4	
200 (6)								
SR	528.5 ± 8	70.7 ± 17	78.5 ± 13	310 ± 31	392 ± 90	441 ± 19	436 ± 20	
Pool	1062	0	0	794	1059	1118[c]	1108	
CF	11.9	0	0	9.4	8.9	9.5	9.4	
100 (6)								
SR	485.3 ± 36	66.1 ± 10	72.6 ± 11	96 ± 15	96 ± 41	210 ± 77	347 ± 57	
Pool	1115	0	0	499	665	998	1124[d]	
CF	10.4	0	0	4.6	3.5	5.1	7.4	
B. Following 18-hr pool sequestration and return								
600 (5)								
SR	473 ± 80	61.4 ± 18	150.1 ± 6	750 ± 156	807 ± 176	736 ± 205	593 ± 83	450 ± 135
Pool	836	0	0	882	882	882	829[e]	829
CF	13.6	0	0	20.4	21.9	20.0	17.2	13.0
200 (5)								
SR	459 ± 56	73.5 ± 3	139.5 ± 26	801 ± 428	924 ± 428	1351 ± 548	1166 ± 190	1070 ± 155
Pool	1265	0	0	1144	1527	1453[f]	1484	1484
CF	8.7	0	0	16.8	14.5	22.3	18.9	17.3
100 (5)								
SR	400.6 ± 56	59.7 ± 13	140 ± 6	350 ± 50	453 ± 18	511 ± 29	627 ± 44	580 ± 84
Pool	1144	0	0	790	1053	1580	1479[g]	1506
CF	8.4	0	0	10.6	10.3	7.8	10.1	9.2

[a] Values are means ± SEM (SR, μmol/hr; Pool, μmol; CF, cycles/day).

[b] Pool C as measured at 12 hr was also used in calculating cycling frequency for all intervals since no changes in pool size or fecal losses were found following pool return.

[c] Pool B measured at end of 6-hr infusion was adjusted to allow comparisons to Pool C, i.e., Pool B $\times \frac{\text{Control C}}{\text{Control B}}$. Pool size determination prior to completion of pool infusion was calculated from the fraction of pool infused plus basal secretion rate (i.e., rate at 0 time).

[d] Pools B and C were similar. Pool size prior to end of pool infusion was calculated as above for *c*.

[e] Pool C as measured at 12 hr. Pool size used to calculate cycling frequency during intervals prior to this was adjusted to accommodate fecal losses in this experiment.

[f] Pool B was adjusted to allow comparison to Pool C as in *c* above, and values shown prior to 6 hr included fraction of pool infused plus basal secretion rate.

[g] Pool B at 12 hr and Pool C at 24 hr were similar. Values shown prior to 12 hr were calculated as above for *f*.

Special studies

We examined the diurnal variation of bile salt secretion and pool cycling frequency and the effects of fasting and correlation with cholesterol 7α-hydroxylase activity with intact and interrupted EHC.

In fed animals with physiologically intact EHC, bile salt secretion rates exhibited a distinct diurnal variation with zeniths between 8 AM and 2 PM and nadirs between 8 PM and 2 AM (**Table 3**). Since bile salt pool sizes remained constant during these experiments, a diurnal pattern also existed for pool cycling frequency which decreased as secretion rates decreased. During fasting this same diurnal pattern persisted in animals with intact EHC although both secretion and pool cycling rates were significantly lower than in the fed state. A high correlation between cycling frequency and secretion rates existed in all of these experiments ($r = 0.99$, $y = 0.02X + 0.7$). After 48 hr interruption of the EHC, the diurnal pattern for bile salt secretion (and therefore synthesis) persisted in fed states but was less obvious in fasting animals in which bile salt secretion rates (i.e., synthesis) were lower than in the fed state.

Cholesterol 7α-hydroxylase activity was distinctly lower in fasted animals at both 8 AM and 6 PM (**Table 4**). Enzymatic activity was also lower at 6 PM than at 8 AM in all animals, suggesting a diurnal pattern similar to that found for bile salt secretion. Following pool drainage, enzymatic activity increased significantly ($P < 0.001$) in

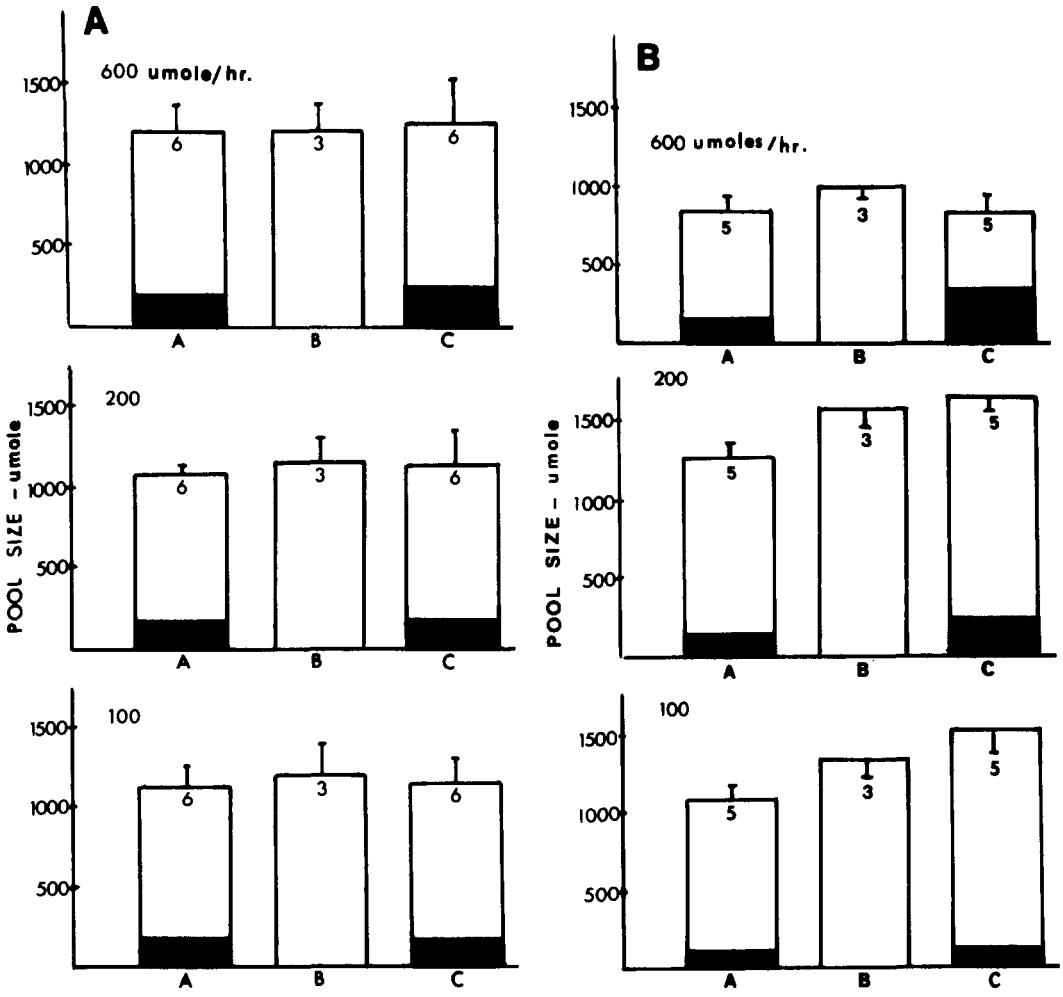

Fig. 3. Bile salt pool sizes are shown for three different infusion rates in animals with unstimulated (3A) and stimulated (3B) bile acid synthesis. Bile salt pool sizes are shown by height of open bars + 1 SEM with number of studies denoted at top during control (A), immediately following pool infusion (B), and after normalization of bile salt secretion rates (C). The closed bars represent fecal losses in control and pool return experiments.

fed animals after 18 hr pool sequestration. Hydroxylase activity did not increase after either 6 or 18 hr pool sequestration in fasting animals.

DISCUSSION

The distinction between fecal loss and feedback inhibition of BA synthesis as the major contributor to regulation of BA pool size is difficult to make when hour-to-hour changes in either cannot be measured. Previous investigators have therefore had to make a number of assumptions during studies of BA pool turnover when BA isotope dilution methodology was used to assess pool size (18, 19). These assumptions included: insignificant isotope loss prior to complete mixing of administered isotope and pool; complete mixing of isotope with the endogenous bile acid; and little new synthesis of BA during collection compared to pool size (18, 19). In order to evaluate BA fecal losses in the past, it was necessary to assess BA mass in pooled fecal collections or to administer oral isotope and assess consequent fecal recovery of isotopic bile acids (20, 21). Mok, Von Bergmann, and Grundy (22), employing both isotopic and mass BA analyses, came to the conclusion that alterations in feedback inhibition of BA synthesis were necessary to explain differences between subjects with small versus normal pool sizes, since they found that BA fecal losses were similar in these two groups. Furthermore, it may be considered moot to make the distinction between BA fecal losses and feedback inhibition of BA synthesis, since feedback inhibition inevitably results from absorption of BA from the intestine while fecal losses are the consequences of unabsorbed bile salts. We, however, have known for some time (9, 10) that there is a lag of up to 24 hr in the primate liver's ability to enhance BA synthesis consequent to decreased bile salt feedback from the intestine. In our current studies bile salt secretion did not become enhanced for at least 12 hr following drainage of the BA

TABLE 2. Bile salt pool size and fecal loss[a]

Infusion Rate	Immediate Pool Removal			Delayed Pool Removal			Fecal Loss		
	Pool A	No. of Exp.	A–B[b]	Pool A	No. of Exp.	A–C[b]	Control	Experimental	
μmol/hr	*μmol*			*μmol*			*μmol/day*		
Post 6S[c]									
600	1251 ± 119	3	12, NS	1240 ± 165	6	22, NS	117 ± 8	128 ± 10	NS
200	1102 ± 39	3	58, NS	1062 ± 22	6	46, NS	107 ± 11	110 ± 10	NS
100	1095 ± 73	3	9, NS	1115 ± 81	6	8, NS	115 ± 8	99 ± 8	NS
Post 18S[c]									
600	963 ± 51	3	90	836 ± 98	5	−7, NS	134 ± 11	320 ± 21	$P < 0.001$
200	1168 ± 90	3	174, $P < 0.01$	1265 ± 132	5	219, $P < 0.01$	104 ± 8	196 ± 13	$P < 0.01$
100	1199 ± 74	3	351, $P < 0.05$	1144 ± 92	5	362, $P < 0.01$	85 ± 5	90 ± 7	NS

[a] Values are means ± SEM.

[b] A–B and A–C refer to changes in pool size after immediate (B) or delayed (C) pool return, and statistical significance differences are noted as nonsignificant (NS) or otherwise.

[c] Following 6-hr pool sequestration (6S), bile salt synthesis remained unstimulated and was <90 μmol/hr; after 18-hr pool sequestration (18S), synthesis was stimulated to >135 μmol/hr.

pool and continued interruption of EHC (Table 1A and B). We speculated, therefore, that fecal losses might alter pool size during this lag period and evaluation of such BA losses in short-term experiments were needed to evaluate the economy of the EHC more critically. It was also important in the design of our experiments to examine which factors might influence fecal losses, in order to determine how homeostasis might be maintained at a time when feedback inhibition of BA synthesis was not responsive to the need to preserve BA pool size. We therefore altered bile salt input into the intestine of the baboon during both stimulated and unstimulated states of BA synthesis, and examined the enterohepatic circulatory response to this stimulus including the effect on BA fecal losses. Several assumptions were made which allowed us to calculate short-term changes in BA input or secretion into the intestine. We first assumed that BA synthesis would remain at preinfusion levels during BA pool return. In experiments in which synthesis was not enhanced, this assumption was completely valid. In other experiments in which synthesis was enhanced, feedback inhibition due to reinstitution of the EHC could have decreased BA synthesis. Since our infusions were administered intraduodenally, feedback inhibition should not have become effective for at least 4 hr since we had previously shown that in the baboon at least 4–6 hr were necessary for feedback inhibition to occur after intraduodenal infusion (9). Therefore, feedback inhibition of BA synthesis would be unlikely in 2- or 6-hr pool return experiments. It is, however, possible that in the second half of 12-hr pool infusion experiments feedback inhibition had repressed BA synthesis. We may, therefore, have overestimated the hepatic contribution to bile salt input into the intestine in long-term experiments. Since secretion rate was always accurately measured, we used it in the numerator of the quotient to calculate BA cycling frequency.

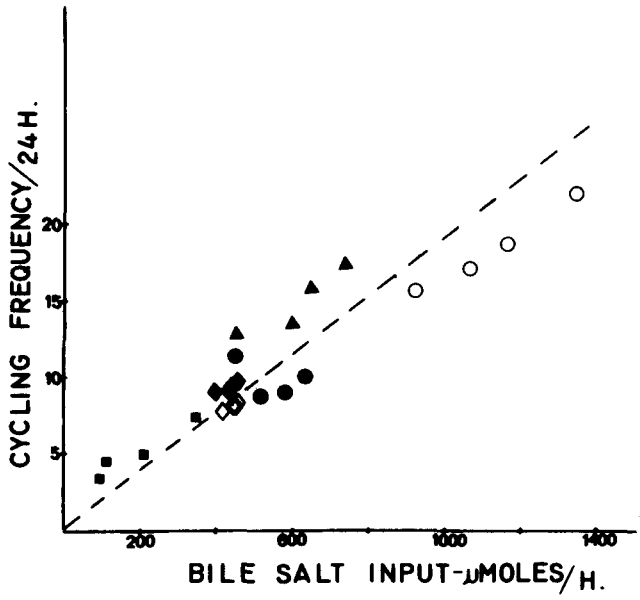

Fig. 4. The relationship between mean daily bile salt cycling frequency and bile salt input into intestine (μmol/hr) is shown for all studies at different rates of bile salt infusion in animals with stimulated bile salt synthesis 600 (▲), 200 (○), and 100 (●) μmol/hr; or unstimulated synthesis 600 (◇), 200 (◆), and 100 (■) μmol/hr. Note: bile salt input into the intestine includes total secretion from the liver resulting from bile salt infusion and recycled bile salt in enterohepatic circulation.

The second assumption we made was that changes in BA fecal losses were constant throughout pool return. Since no changes in fecal BA losses occurred unless BA input into the intestine exceeeded 700 μmol/hr (which occurred only with 2- and 6-hr pool return after 18 hr pool sequestration, i.e., when bile acid synthesis was enhanced) we feel that any errors made due to this assumption were minimal. In fact, when one uses the cycling frequency rates we derived, absorption efficiency of 98%

TABLE 3. Diurnal variation of bile salt secretion rates and pool cycling frequency

	BA Secretion Rate				Pool Size		# EHC/Day	
	Intact EHC		Interrupted (100%)					
	Fed (n = 4)	Fasting (n = 3)	Fed	Fasting	Fed	Fasting	Fed	Fasting
	μmol/hr				*μmol*			
Control					1237 ± 93	1179 ± 90		
8–11 AM	650 ± 86[a]	416 ± 76[b]	73 ± 35	49 ± 9 NS			12.7 ± 2	8.6 ± 1
11–2 PM	646 ± 74	427 ± 74[b]	52 ± 13	52 ± 8 NS			12.6 ± 1	8.8 ± 1
2–5 PM	529 ± 69	330 ± 35[c]	35 ± 10	46 ± 10 NS			10.3 ± 1	6.8 ± 1
5–8 PM	538 ± 65	346 ± 40[c]	40 ± 11	45 ± 9 NS			10.5 ± 1	7.1 ± 1
8–11 PM	442 ± 93	238 ± 40[b]	63 ± 30	43 ± 7 NS			8.6 ± 2	4.9 ± 1
11–2 AM	435 ± 101[d]	281 ± 66 NS	NA	62 ± 17			8.5 ± 2	5.8 ± 1
2–5 AM	488 ± 108	352 ± 72 NS	69 ± 18	57 ± 11 NS			9.5 ± 2	7.3 ± 1
5–8 AM	542 ± 95[a]	348 ± 70[b]	60 ± 20	51 ± 20			10.6 ± 2	7.2 ± 1
					1229 ± 126	1135 ± 238		
Fecal loss	121 ± 12	70 ± 11			X 1223[e]	1157		

[a] Values are means ± SEM; NS, not significant; NA, not available for analysis.
[b] Fed vs fasting, $P < 0.05$.
[c] Fed vs fasting, $P < 0.01$.
[d] Nadir 11–2 AM vs zenith 8–11 AM, $P < 0.01$.
[e] X, mean of pre- and post-pool sizes used in calculation of # EHC/day.

or better could account for all the BA losses found in our experiments. Other investigators have concluded that BA absorption efficiencies greater than 90% had to exist in normals to explain the small amount of BA lost per BA pool cycle (23). It was therefore not surprising to us that, as BA cycling frequency was enhanced more than twofold in our animals, a proportional loss in fecal BA followed. We found that this response was beneficial in maintaining BA pool size such that when BA input into the intestine exceeded 700 μmol/hr, pool cycling frequency was concomitantly enhanced. This resulted in increased fecal BA losses so that pool size enlargement was prevented. Conversely, when only BA synthesis was enhanced without a concomitant increase in BA input into the intestine, neither cycling frequency nor BA pool size increased. We therefore concluded that bile salt input into intestine, and not changes in BA synthesis, were responsible for changes in fecal losses in our experiments.

The evidence that fasting inhibited bile salt synthesis in our animals came from several observations: *1*) fasting inhibited cholesterol 7α-hydroxylase during interruption of EHC; *2*) fasting dampened the secretion and diurnal variation of BA secretion; and *3*) fasting prevented enhancement of BA secretion by the liver following interruption of the EHC. Employing in vitro techniques, Bergstrom and Danielsson (24) also observed inhibition of the diurnal variation of cholesterol 7α-hydroxylase during fasting in rats, while Duane, Gilberstadt, and Wiegand (25) observed physiologic evidence for diurnal rhythms in this species. Similar to our observations in the baboon, Duane, Ginsberg, and Bennion (26) have also shown in man that BA pool size did not decrease during fasting. We also showed that fasting had other effects upon the EHC in the baboon. In addition to decreased BA secretion rate, cycling frequency was also significantly decreased during short-term fasting. The cumulative effect of these changes was to decrease fecal BA losses and preserve BA pool size. Since BA synthesis could not be enhanced during a period of time analagous to an overnight fast (these conditions were met by ensuring that

TABLE 4. Cholesterol 7α-hydroxylase activity

	Intact EHC			Interrupted EHC		
Time	Fed	Fasting		Fed	Fasting	
	pmol per min per mg protein					
8 AM	18.8 ± 4[a]	7.4 ± 1	$P < 0.05$[b]	52.1 ± 5[c]	7.8 ± 1	$P < 0.001$
6 PM	13.0 ± 3	6.1 ± 1	$P < 0.05$	12.6 ± 1[d]	6.8 ± 1[d]	$P < 0.05$

[a] Values are means ± SEM.
[b] P value for statistical significance, fed versus fasting state.
[c] Performed after 18-hr pool sequestration; $P < 0.001$ compared to intact EHC fed state or interrupted EHC fasting state.
[d] Performed after 6-hr pool sequestration.

no food was eaten during pool sequestration for up to 18 hr in the baboon), decreased BA fecal losses were the only way in which the baboon could preserve BA pool size. The baboon was able to accomplish this by reducing BA pool cycling fivefold during fasting so that fecal BA losses were decreased to maintain BA pool size. However, alterations in liver function during fasting could also have contributed to decreased BA cycling, as for example by decreasing hepatic bile salt uptake. Such effects, however, have not been described as yet (27). A better explanation from our experiments would be that the intestine responds to changes in both diurnal variation and decreased BA secretion during fasting to affect parallel changes in BA cycling frequency.

Since enhancement of bile salt synthesis was prohibited by fasting and occurred 12 or more hours after interruption of EHC even in fed animals, a simple homeostatic mechanism involving the intestine's direct response to decreased BA secretion maintained bile salt pool size in the short-term. The economy of the EHC in the baboon is therefore subserved by an intricate interrelation between BA input into the intestine, pool cycling frequency, and intestinal loss of BA from the pool. Thus, intestinal rather than hepatic alterations of the EHC help maintain BA pool size within narrow limits when feedback regulation of BA synthesis by itself cannot satisfy the need for BA pool preservation in the baboon.

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REFERENCES

1. Redinger, R. N., and D. M. Grace. 1978. Biliary lipid metabolism in fed and fasting states in the baboon model with controlled enterohepatic circulation. *Gastroenterology.* **74:** 1141 (Abstract).
2. Redinger, R. N. 1983. Evidence that bile acid (BA) secretion rate regulates BA pool size by altering cycling frequency. *Gastroenterology.* **82:** 1157 (Abstract).
3. Erlinger, S. 1981. Hepatocyte bile secretion: current views and controversies. *Hepatology.* **1:** 352–359.
4. Small, D. M., R. H. Dowling, and R. N. Redinger. 1972. The enterohepatic circulation of bile salts. *Arch. Intern. Med.* **130:** 552–573.
5. Dietschy, J. M., H. S. Solomon, and M. D. Siperstein. 1966. Bile acid metabolism in studies on the mechanism of intestinal transport. *J. Clin. Invest.* **45:** 832–846.
6. Hoffman, N. E., D. E. Donald, and A. F. Hofmann. 1975. The effect of primary bile acids on bile lipid secretion from the perfused dog liver. *Am. J. Physiol.* **229:** 714–720.
7. Duane, W. C., and J. H. Bond. 1980. Prolongation of intestinal transit and expansion of bile acid pools by propantheline bromide. *Gastroenterology.* **78:** 226–230.
8. Duane, W. C. 1978. Simulation of the defect of bile acid metabolism associated with cholesterol cholelithiasis by sorbital ingestion in man. *J. Lab. Clin. Med.* **91:** 969–978.
9. Redinger, R. N., L. Chow, and D. M. Grace. 1978. Cholesterol oxidation in primates by simultaneous sterol balance and breath analysis. *Am. J. Physiol.* **235:** R55–63.
10. Dowling, R. H., E. Mack, and D. M. Small. 1970. 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. *J. Clin. Invest.* **49:** 232–242.
11. Dowling, R. H., E. Mack, and D. M. Small. 1971. Biliary lipid secretion and bile composition after acute and chronic interruption of the enterohepatic circulation in the rhesus monkey. *J. Clin. Invest.* **50:** 1917–1926.
12. Redinger, R. N., and D. M. Small. 1973. Primate biliary physiology. VIII. The effect of phenobarbital upon bile salt synthesis and pool size, biliary lipid secretion, and bile composition. *J. Clin. Invest.* **52:** 161–172.
13. Davignon, J., W. J. Simmonds, and E. H. Ahrens, Jr. 1968. Usefulness of chromium oxide as an internal standard for balance studies in formula-fed patients and for assessment of colonic function. *J. Clin. Invest.* **47:** 127–138.
14. Talalay, P. 1960. Enzymatic analysis of steroid hormones. *Methods Biochem. Anal.* **8:** 119–143.
15. Shefer, S., G. Nicolau, and E. H. Mosbach. 1975. Isotope derivative assay of microsomal cholesterol 7α-hydroxylase. *J. Lipid Res.* **16:** 92–96.
16. Bolin, D. W., R. P. King, and R. W. Klosierman. 1952. A simplified method for the determination of chromium oxide when used as an index. *Substance Sci.* **116:** 34.
17. Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. 6th edition. Iowa State University Press, Ames, IA.
18. Einarsson, K. A., S. M. Grundy, and W. G. M. Hardison. 1979. Enterohepatic circulation rates of cholic acid and chenodeoxycholic acid in man. *Gut.* **20:** 1078–1082.
19. Mok, H. Y., K. Von Bergman, and S. M. Grundy. 1980. Kinetics of the enterohepatic circulation during fasting: biliary lipid secretion and gallbladder storage. *Gastroenterology.* **78:** 1023–1033.
20. Redinger, R. N., A. H. Hermann, and D. M. Small. 1973. Primate biliary physiology. X. Effects of diet and fasting on biliary lipid secretion and relative composition and bile salt metabolism in the rhesus monkey. *Gastroenterology.* **64:** 610–621.
21. Stanley, M. M. 1970. Quantitation of intestinal functions during fasting: estimations of bile salt turnover, fecal calcium and nitrogen excretions. *Metabolism.* **19:** 865–875.
22. Mok, H. Y., K. Von Bergmann, and S. M. Grundy. 1977. Regulation of pool size of bile acids in man. *Gastroenterology.* **73:** 684–690.
23. Hislop, I. G. 1970. The absorption and enterohepatic circulation of bile salts. An historical review. *Med. J. Aust.* **1:** 223–226.
24. Bergstrom, S., and H. Danielsson. 1958. On the regulation of bile acid formation in the rat liver. *Acta Physiol. Scand.* **43:** 1–7.
25. Duane, W. C., M. L. Gilberstadt, and D. M. Wiegand. 1979. Diurnal rhythms of bile acid production in the rat. *Am. J. Physiol.* **236:** R175–179.
26. Duane, W. C., R. L. Ginsberg, and L. J. Bennion. 1979. Effects of fasting on bile acid metabolism and biliary lipid composition in man. *J. Lipid Res.* **17:** 211–219.
27. Aldini, R., A. Roda, A. M. Labate, G. Cappelleri, E. Roda, and L. Barbara. 1982. Hepatic bile acid uptake: effect of conjugation, hydroxyl and keto groups, and albumin binding. *J. Lipid Res.* **23:** 1167–1173.