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Biliary lipid secretion and bile composition after acute and chronic interruption of the enterohepatic circulation in the rhesus monkey: *IV. Primate biliary physiology*

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IV. PRIMATE BILIARY PHYSIOLOGY

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ABSTRACT Bile salts and phospholipids are both required to solubilize biliary cholesterol. Since interruption of the enterohepatic circulation (EHC) depletes bile of bile salts, we have examined in the rhesus monkey the effects of controlled interruption of the EHC on biliary secretion of bile salt, phospholipid, and cholesterol and on the relative proportions of these components in bile.

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When the EHC was only partially interrupted, secretion rates and the relative concentration of bile salt, phospholipid, and cholesterol did not change significantly from control values until more than 20% of the bile was diverted. Modest changes in the relative composition of bile occurred when 33 and 66% of the bile was diverted, and these changes were very similar to those produced by resection of the distal small bowel.

INTRODUCTION

Cholesterol is virtually insoluble in water but can be held in a mixed micellar solution by a mixture of phospholipids and bile salts (1, 2). With respect to cholesterol solubility, human gallbladder bile behaves like a mixture of bile salts, lecithin, and cholesterol in water (3). In normal bile the relative concentrations of these three substances is such that cholesterol is completely soluble, and bile normally exists as one phase, a clear liquid. In bile from patients with cholesterol gallstones, however, there occurs either a relative excess of cholesterol, a relative depletion of bile salts or phospholipids, or a combination of these changes. Such changes in relative composition result in decreased cholesterol solubility. Thus, in addition to the liquid phase, bile from these patients also contains a solid phase of precipitated cholesterol (3).

In situations where bile is depleted of bile salts, one might expect that cholesterol solubility would be jeopardized, thereby favoring cholesterol gallstone formation. When the ileum is diseased or resected, for example, bile salts are poorly absorbed and the bile salt pool may be depleted. (The bibliography of this subject has been reviewed in a previous paper [4]). Since interruption of

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the enterohepatic circulation (EHC)[1] is poorly compensated by increased hepatic bile salt synthesis (4), the effects of major interruptions of EHC are decreased secretion of biliary-bile salts and decreased bile salt pool size (4). In fact it has been recently implied that a decreased bile salt pool may be responsible for cholesterol gallstones (5).

It seemed important, therefore, to study the effects of an interrupted EHC on the bile salt, phospholipid, and cholesterol content of bile and to see if the reduced bile salt secretion and pool size produced by interruption of the EHC would jeopardize cholesterol solubility in bile. We, therefore, examined the effects of both *acute* and *chronic* interruption of the EHC on the composition of bile in the rhesus monkey.

The EHC was mechanically interrupted to varying degrees under controlled conditions (4, 6) and the resultant changes in bile composition were compared with those produced by resection of the distal one-third or two-thirds of the small intestines. When chronic interruption of the EHC was produced, either mechanically or by ileal resection, there was a relative bile salt deficiency and concomitant relative increase in phospholipid and cholesterol. However, even though the bile contained relatively more cholesterol, the cholesterol remained soluble because of the relative increase of phospholipid. However, acutely after complete interruption of the EHC the bile transiently became supersaturated with cholesterol thus creating conditions temporarily conducive to cholesterol precipitation in bile.

METHODS

Experimental model. Mechanical interruption of the EHC of bile was produced in 17 bile fistula rhesus monkeys using an electronic stream splitter as previously described (4, 6).

The secretion rates in mmoles/24 hr of bile salts, phospholipids, and cholesterol in bile were measured with interruptions of 5, 10, 20, 33, 66, and 100% of the EHC.

The animals were considered to be in the "steady state" with respect to secretion of biliary lipids when constant bile flow and secretion rates of bile salt, phospholipids, and cholesterol were obtained for a period of no less than 5 consecutive days. After steady-state secretory-rates were obtained, five or more animals were studied at each level of interruption of the EHC for periods of 5 to 14 days. These animals are defined as having "chronic" interruptions of the EHC. From the secretion rates the relative molar proportions of bile salt, phospholipid, and cholesterol were calculated for each of the different levels of biliary diversion and compared with the normal molar ratios of bile salts, phospholipids, and cholesterol as measured in gallbladder bile obtained by needle aspiration at the time of the original operation.

The effects of chronic interruption of the EHC with the electronic stream splitter were then compared with those produced by one-third or two-thirds distal small bowel resection when all but 5% of the bile was returned to the shortened intestine (4).

The secretion rates and molar ratios of bile salts, phospholipids, and cholesterol were also followed at 30 to 120-min intervals after the EHC was acutely interrupted by changing abruptly from an "intact" EHC (1 or 5% biliary diversion) to a complete bile fistula (100% bile diversion) (4).

With both acute and chronic interruption of the EHC, the volume of bile was recorded and after mixing, samples of bile were frozen at $-20°C$ until analyzed for bile salt, phospholipid, and cholesterol content.

Laboratory procedures. Bile salts in bile were measured enzymatically using the hydroxysteroid method of Talalay (7) as modified by Admirand and Small (3). Phospholipids were measured as inorganic phosphorus by the method of Bartlett (8). Cholesterol was measured using Carr and Drekter's (9) modification of the original method of Abell, Levy, Brody, and Kendall. (10).

Expression of results using triangular coordinates. When only two variables are present, any mixture of the two may be represented by a single point on the conventional graph with ordinate and abscissa. When, as in these studies, three variables are present, bile salts, phospholipids, and cholesterol, triangular coordinates may be used to represent any mixture of the three as a single point *provided* that each individual substance is expressed as a percentage of the total.

Small, Bourges, and Dervichian (1, 2) and Admirand and Small (3) used this method to express cholesterol solubility in vitro. These authors (3) examined a large range of synthetic mixtures of bile salt, lecithin, and cholesterol made up to simulate bile and found that for mixtures of these components present at a total concentration similar to that found in gallbladder bile (5–20 g per 100 ml), the triangle could be divided into two zones on the basis of the physical properties of the mixtures (Fig. 1).

In mixtures containing large proportions of bile salt and lecithin, and small proportions of cholesterol, cholesterol was completely soluble in a one-phase micellar solution. In all other mixtures there was insufficient bile salt and phospholipid present to hold the cholesterol in solution so that, in addition to the micellar phase, other phases containing insoluble cholesterol also were present. These authors went on to show that the in vitro findings also generally held true for human gallbladder bile. The bile salt, phospholipid, and cholesterol content of bile from normal subjects was represented by points falling in the one-phase micellar zone while bile from patients with cholesterol gallstones was, in general, either nearly saturated with cholesterol or contained frank cholesterol microcrystals.

The same technique of expressing the relative molar concentrations of bile salts, phospholipids, and cholesterol on triangular coordinates has been used in the present study. However, hepatic bile from monkeys is more dilute than human gallbladder bile. The line of maximum cholesterol solubility described by Admirand and Small (3) refers to in vitro systems whose total bile salts, phospholipid, and cholesterol was between 5 and 20 g per 100 ml. In their study of human gallbladder bile they specifically excluded dilute biles (3). However, the mean total concentration of these three components in our monkeys was 4 g per 100 ml for bile from animals with "intact" EHC's and 2 g per 100 ml for animals with total bile fistula. While cholesterol solubility in systems containing 4 g per 100 ml is virtually the same as that for systems containing 5–20 g per 100 ml, cholesterol solubility in the more dilute systems is signifi-

[1] *Abbreviation used in this paper:* EHC, enterohepatic circulation.

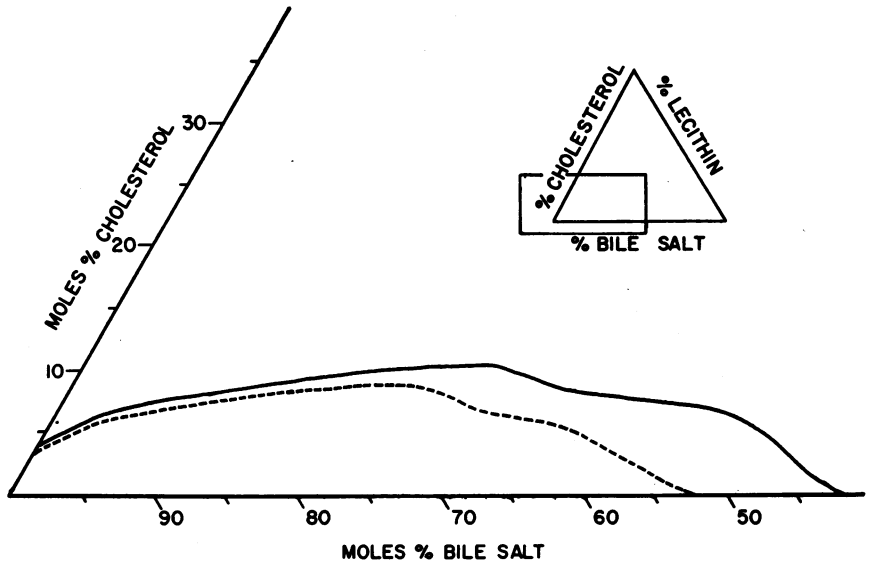

FIGURE 1 Maximum solubility of cholesterol in bile salt–lecithin systems containing 10% (solid line) and 2% (broken line) total bile salt + lecithin + cholesterol (12). Graph shows the details of the lower left hand corner of the entire plot (insert).

cantly less. The reason for this fact is that as the solution becomes more dilute, proportionately less bile salts form micelles and proportionately more are present as individual ions. These individual bile salt ions are unable to solubilize phospholipid and cholesterol. In concentrated solution (i.e., 4–20 g per 100 ml) virtually all the bile salt molecules form mixed micelles with phospholipids and cholesterol. In dilute solutions (3 g per 100 ml or less), the proportion of non-micelle associated-bile salts becomes important. Since this proportion of the bile salt molecules is not available for the formation of mixed phospholipid–cholesterol–bile salt micelles, cholesterol (and phospholipid) solubility decreases. Below the critical micellar concentrations of the mixed micelle there would be no cholesterol solubilization. Using the critical micellar concentrations of a mixture of conjugated bile salts at appropriate temperature and counterion concentration (11) the maximum solubility of cholesterol in a system containing 2 g (total bile salt, phospholipid, and cholesterol) per 100 ml has been calculated (12). This line, shown as a broken line, is given in Fig. 1. Both the line

TABLE I

*Effects of Chronic Interruption of the Enterohepatic Circulation by the Stream Splitter on Bile Salt, Phospholipid, and Cholesterol Secretion Rates and Molar Ratios**

Per cent of bile diverted	Number of observation periods‡	Number of animals	Secretion rate			Relative molar ratio		
			Bile salts	Phospholipids	Cholesterol	Bile salts	Phospholipids	Cholesterol
			mmoles/24 hr			*moles per cent*		
GB bile§		12				83 ±2.0	11 ±1.5	6 ±0.6
1 or 5, base line	22	13	9.98 ±0.62	1.11 ±0.06	0.73 ±0.06	84 ±0.7	10 ±0.5	6 ±0.3
10	6	5	9.45 ±0.88	1.16 ±0.12	0.67 ±0.09	82 ±2.0	11 ±1.4	6 ±0.7
20	5	5	8.74 ±1.51	1.07 ±0.14	0.67 ±0.07	83 ±1.9	10 ±1.2	7 ±0.8
33	8	8	5.71 ±0.53	0.81 ±0.08	0.50 ±0.05	81 ±1.3	11 ±0.7	7 ±0.5
66	7	7	3.21 ±0.33	0.59 ±0.06	0.35 ±0.05	78 ±1.0	15 ±0.8	8 ±0.5
100	11	9	1.91 ±0.13	0.52 ±0.04	0.20 ±0.02	72 ±2.2	20 ±2.0	8 ±0.6

* All values listed represent mean ±SEM.

‡ Each observation period consists of 5 to 14 consecutive 24-hr periods. During the 5 to 14 days the animal was in a steady state relative to bile salt, phospholipid, and cholesterol secretion rates and bile salt pool size.

§ Obtained at original surgery.

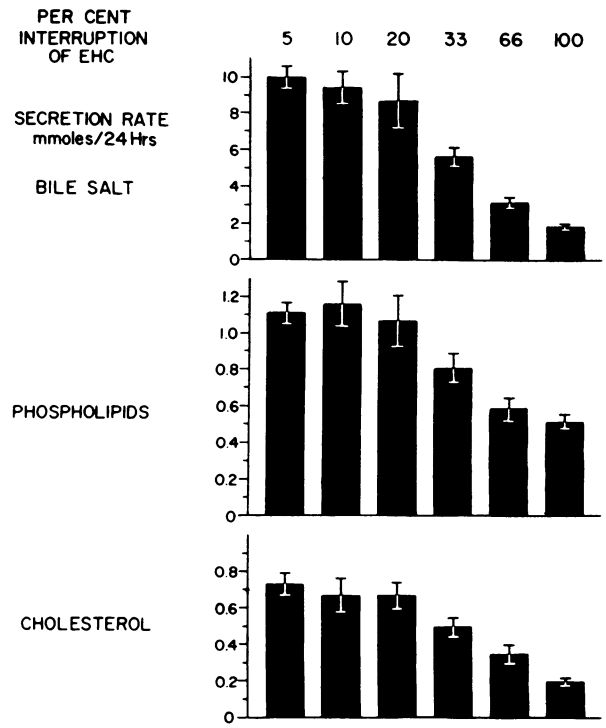

FIGURE 2 Mean secretion rates (mmoles/24 hr ±1 SEM) of total bile salts, phospholipids, and cholesterol with varying degrees of chronic interruption of the EHC.

referring to the maximum solubility of cholesterol in more concentrated solution (4–20 g per 100 ml) and the broken line referring to dilute solutions will be shown on the subsequent diagrams for the purpose of comparing bile composition to cholesterol solubility.

RESULTS

Bile salt, phospholipid, and cholesterol secretion in response to controlled interruption of the EHC. Table I and Fig. 2 summarize the effcts of different levels of chronic interruption of the EHC on bile salt, phospholipid, and cholesterol secretion when steady state conditions were reached.

As was shown previously (4), bile salt secretion did not change when 10 and 20% of the bile was diverted. Similarly, the secretion rates of phospholipids and cholesterol at these two levels of interruption of the EHC were not significantly different from the base line values of 1.11 mmoles/24 hr for phospholipids, and 0.73 mmoles/24 hr for cholesterol.

However, just as diversion of 33% or more of the biliary output caused a progressive fall in both bile volume and bile salt secretion, so with 33% or greater interruption of the EHC, there was also a progressive fall in the secretion rates of both phospholipids and cholesterol at the corresponding levels of interruption.

Relative proportions of bile salts, phospholipids, and cholesterol in response to controlled interruption of the EHC. The ratios of the three substances at the different levels of biliary diversion are illustrated in Fig. 3. The number of moles of each individual substance is expressed as a percentage of the total moles of bile salts, phospholipids, and cholesterol.

Normal gallbladder bile contained 83 ±2.0 (SEM)% bile salts; 11 ±1.5% phospholipids, and 6 ±0.6% cholesterol. When these results were plotted on triangular coordinates, the composition of the bile was represented by a single point in the one-phase micellar zone which was well within the zone of cholesterol solubility.

The relative proportions of bile salts, phospholipids, and cholesterol in bile were not significantly different at 5, 10, and 20% biliary diversion from the proportions observed in normal gallbladder bile (Table I, Fig. 3).

As the per cent of bile diverted increased from 33 to 66 to 100% there was a progressive fall, not only in the absolute amount of bile salts secreted (refer to Fig. 2) but also in the relative proportion of bile salts present in bile (Fig. 3). During these larger bile diversions there was a modest increase in the relative percentage of cholesterol as well as a relative increase in phospholipids. As a result, the bile although more saturated with cholesterol, did not fall outside the one-phase micellar zone even at the 2 g/100 ml line (Fig. 3).

Thus, although major interruptions of the EHC deplete the bile salt content of bile, they probably do not jeopardize cholesterol solubility *in these monkeys* since the relative proportion of phospholipids in bile is increased.

Bile composition after chronic interruption of the EHC by ileal resection. Table II indicates the secretion rates in mmoles/24 hr of bile salt, phospholipids, and cholesterol, and the relative bile composition in monkeys subjected to distal small bowel resection. In these experiments all but 5% of the bile was returned to the

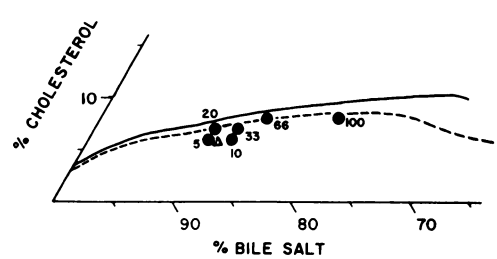

FIGURE 3 Relative mean composition of bile plotted on triangular coordinates as a function of the degree of chronic interruption of the EHC. Solid and broken lines have same meaning as in Fig. 1. Open triangle represents mean composition of gallbladder bile taken at surgery. Closed circles represent mean composition of biles during chronic partial (5, 10, 20, 33, and 66%) and complete (100%) interruption of the EHC.

TABLE II

Secretion of Biliary Lipids and Bile Composition after One-Third and Two-Thirds Distal Small Bowel Resection

Monkey Number	Extent of resection	Number of 24-hr observation periods	Mean secretion rate			Mean molar ratio		
			Bile salts	Phospho-lipids	Choles-terol	Bile salts	Phospho-lipids	Choles-terol
			mmoles/24 hrs			*moles per cent*		
9	Distal one-third	46	3.80	0.79	0.28	78	16	6
18	Distal one-third	38	4.60	0.83	0.42	79	14	7
19	Distal two-thirds	9	2.60	0.76	0.25	72	21	7
20	Distal two-thirds	10	2.59	0.73	0.28	72	20	8

shortened intestine. Small bowel resection produced significant reductions in secretion rates of the biliary components. Removal of the distal two-thirds of the small bowel produced, as might be expected, somewhat greater changes in bile salt, cholesterol, and phospholipid secretion rates than did one-third resection.

These results are plotted on triangular coordinates in Fig. 4 to show the relative composition of the bile after resection. While the relative proportion of bile salts decreased, there was only a slight rise in the relative proportion of the cholesterol present, the difference again being made up by a relative increase of phospholipid. Thus, the bile is more saturated with cholesterol but still falls within the one-phase zone of cholesterol solubility.

Although the number of animals studied is small, it appears that the bile composition after more major resections is comparable to that found with a complete bile fistula (100% diversion of bile). On the other hand, removal of the distal one-third of small intestine produced less marked changes, the relative composition being similar to that found between 33 and 66% bile diversion with the stream splitter.

Changes in bile salt, phospholipid, and cholesterol secretion after acute interruption of the EHC. The absolute secretion rates of bile salts, phospholipids, and cholesterol were followed at 0.5 to 2-hr intervals after the production of total biliary fistula. The bile flow rate (milliliter per hour) and secretion rates (micromoles per hour) of lipids for each collection period is given for four different animals in Table III. As an example of the change in biliary flow and absolute secretion rates after 100% interruption of the EHC, data from a fifth animal (monkey 19) are plotted in Fig. 5. During the 1st 2 hr after biliary diversion, volume and secretory rates remain high. In the animals studied at 0.5-hr intervals a rather sharp increase in all values was noted at 1 or 1.5 hr. After 2 hr, the volume falls moderately but secretion rates decrease markedly. At about 4 hr the secretion rates of bile salt and phospholipid reach a low point. Secretion remains at this low level for about 2 hr and thereafter increases steadily over the next several hours. This change is most marked for bile salts but also occurs with phospholipid secretion in all animals. Cholesterol secretion, on the other hand, shows no consistent low point or subsequent rise. Secretion rates become constant 24–48 hr after interruption of the EHC and are the same as those for the animal with a chronic bile fistula (refer to Table I, 100% interruption of the EHC).

Changes in relative proportions of bile salts, phospholipids, and cholesterol after acute interruption of the EHC. The changes in relative composition of bile after complete biliary diversion in all five animals are illustrated in Fig. 6 by plotting relative composition on triangular coordinates as a function of time after onset of biliary diversion. For the 1st 2–3 hr after acute interruption of the EHC, the molar ratios of bile salt, phospholipid, and cholesterol remained essentially the same as those measured in the steady state with an "intact" enterohepatic circulation (5% interruption of the EHC). Then, as the circulating bile salt pool was drained out, the proportion of bile salts fell and while this was par-

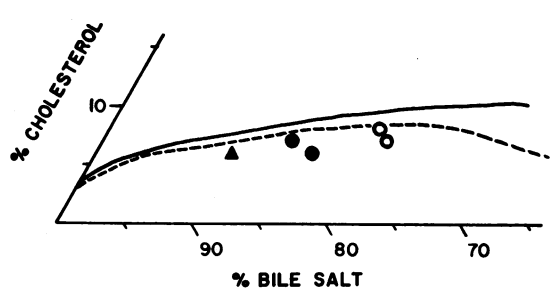

FIGURE 4. Relative composition of bile in animals having one-third distal small bowel resection (solid circles) or two-third distal small bowel resection (open circles). Triangle represents the mean composition of bile in these animals with an "intact" (5% interruption of EHC) before small bowel resection. Solid and broken lines have same meaning as in Fig. 1.

TABLE III

Effects of Acute Total Interruption of the EHC on Bile Volume and on Secretion Rates and Molar Ratios of Bile Salt, Phospholipid, and Cholesterol

Monkey number, weight	Time after interruption of EHC	Vol.	Secretion rate		
			BS*	PL‡	C§
	hr	*ml/hr*	*μmoles/hr*		
15 (5.0 kg)	2.0	10.5	608.0	63.6	41.8
	4.0	7.5	183.6	54.3	26.4
	6.0	3.5	12.5	14.2	9.5
	24.0	3.1	16.7	21.8	13.2
	48.0	3.8	54.0	24.2	7.9
18 (5.8 kg)	1.5	9.5	601.0	42.1	40.1
	3.0	4.3	56.3	18.5	6.6
	4.5	5.3	24.2	14.8	5.9
	6.0	5.3	24.2	15.1	5.8
	7.75	5.0	46.7	17.2	5.6
	9.25	5.3	49.6	17.2	3.7
	10.75	6.0	34.3	16.5	3.0
	12.25	6.3	52.1	19.4	4.7
	13.75	5.7	58.7	20.8	5.4
21 (5.2 kg)	0.5	3.6	242.0	48.5	20.8
	1.0	3.2	254.1	48.3	18.7
	1.5	4.9	387.8	77.3	37.2
	2.0	3.8	271.0	56.9	24.2
	2.5	2.4	150.0	31.3	14.7
	3.0	2.1	166.7	27.1	10.5
	3.5	2.0	79.2	26.2	9.5
	4.0	2.0	54.2	21.0	7.3
	4.5	2.3	41.7	19.4	6.7
	5.5	2.1	33.3	14.2	7.5
	6.0	1.8	25.0	12.4	6.0
	7.0	1.8	29.2	13.8	6.7
	8.0	2.2	54.2	23.8	11.5
	10.0	2.8	45.8	24.3	9.8
	24.0	3.8	80.0	21.7	7.9
40 (4.0 kg)	2.0	4.7	263.5	23.8	14.5
	2.5	6.0	183.2	21.8	13.1
	3.0	3.6	133.2	17.9	7.7
	3.5	4.4	84.2	14.8	7.5
	4.0	4.0	71.8	14.8	7.5
	4.5	4.6	68.0	14.0	9.5
	5.0	4.8	37.1	9.5	5.9
	5.5	5.0	27.5	9.5	6.0
	6.0	5.2	29.6	10.0	4.4
	6.5	3.8	28.9	10.0	6.8
	7.0	4.6	31.7	10.1	3.3
	8.0	2.4	34.2	9.5	4.9

* BS, total bile salts.
‡ PL, phospholipids.
§ C, cholesterol.

tially offset by a relative increase in phospholipids, for a period beginning 3–4 hr after the acute interruption, there was insufficient bile salt and phospholipid to solubilize the cholesterol. As collected from the stream splitter, these biles appeared as clear solutions. However, cholesterol could be precipitated from these solutions by freezing and thawing suggesting that they were supersaturated with cholesterol when secreted. In most animals (monkeys 18, 19, 21, and 40) the period during which supersaturated bile was secreted lasted only a few hours, but monkey 15 took much longer to return toward normal. Even 48 hr after acute interruption, her bile was supersaturated with respect to the 2 g per 100 ml line (Fig. 6). The return of relative bile composition toward normal values was coincidental with the adaptive increase in hepatic bile salt–synthesis (Table III, Fig. 5). As bile salt synthesis increased, the proportion of bile salt increased until a new steady state was reached. The relative composition in this new state was the same as that found for 100% diversion in the steady state (Table I and Fig. 3).

DISCUSSION

The present studies were designed to explore the physiology of bile secretion and to examine the changes in

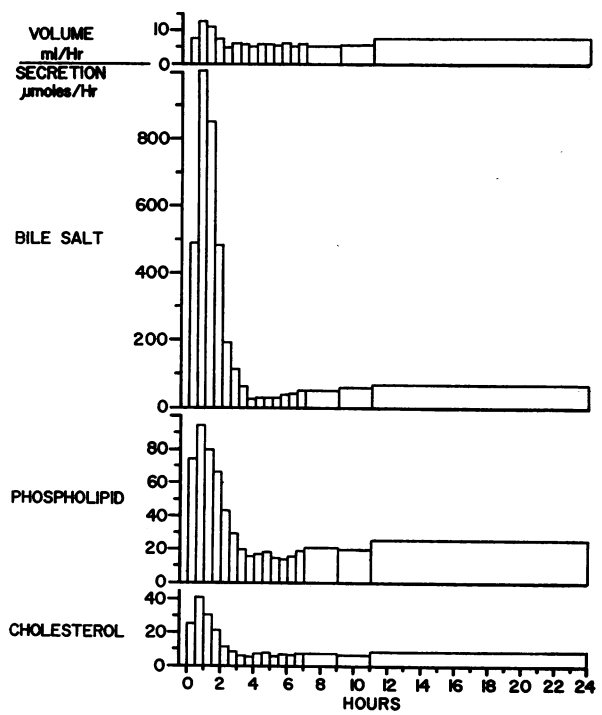

FIGURE 5 Changes in the bile flow (volume in milliliter per hour) and secretion (micromoles per hour) of total bile salt, phospholipid, and cholesterol in monkey 19, immediately after complete interruption of the EHC. Monkey 19 weighed 5.1 kg.

bile lipid composition (with particular respect to cholesterol solubility) produced by interruption of the EHC both by biliary diversion and by ileal resection. We have studied bile salt, phospholipid, and cholesterol secretion in acute and chronic controlled interruptions of the EHC and related the relative proportions of lipids secreted to cholesterol solubility in bile.

Our results show that after chronic interruption of the EHC in the Rhesus monkey, the over-all pattern of cholesterol and phospholipid secretion is similar to that of bile salts (4). There were no significant differences in cholesterol and phospholipid secretion at 5, 10, and 20% interruption of the EHC, but with diversion of 33% or more of the biliary output, there was not only a fall in bile salt secretion, but also a progressive fall in the amounts of cholesterol and phospholipids secreted.

The similarity in the over-all pattern of bile salt, cholesterol, and phospholipid secretion rates and their intimate relationship has been emphasied in the past. For example, Kay and Entenman (13) studied the isolated perfused rat liver and found that the addition of cholic acid to the perfusate not only increased bile acid excretion but also increased the excretion of both phospholipid and free cholesterol in bile. Using the conjugated bile salt, sodium taurocholate, Hardison and Francis (14) confirmed these findings in bile fistula rats. They also reported that sodium dehydrocholate (a bile salt which does not form micelles (15) or solubilize lecithin) does not increase phospholipid or cholesterol excretion, a fact that suggests that phospholipid and cholesterol are secreted as mixed micelles. Swell, Bell, and Entenman (16) used similar techniques to study the dog liver and found that secretion of hepatic phospholipids in bile was dependent on the presence of the bile salt sodium taurocholate in the infusion mixture. These authors, therefore, suggested that the secretion of biliary lipids by the liver was regulated by bile acid availability thus accounting for the "relatively fixed proportions of free cholesterol, phospholipids, and bile acids in bile (16)." Observing the changes in secretions produced by bile fistula in the rat, Thompson and Vars (17) and Eriksson (18) found that biliary cholesterol secretions closely followed the fluctuations in bile acid secretion. Light, Witner, and Vars (19) emphasized that in the rat, for several hours after acute interruption of the EHC, the bile salt: cholesterol ratio decreased. Unfortunately, phospholipids were not measured in these studies (17, 18, 19).

In the current studies, if the secretion rates of all three substances had fallen to an equal extent with major interruptions of the EHC (greater than 33% diversion) then the ratio of bile salts: phospholipids: cholesterol would have remained the same and no change would have been noted in the relative composition when expressed on triangular coordinates.

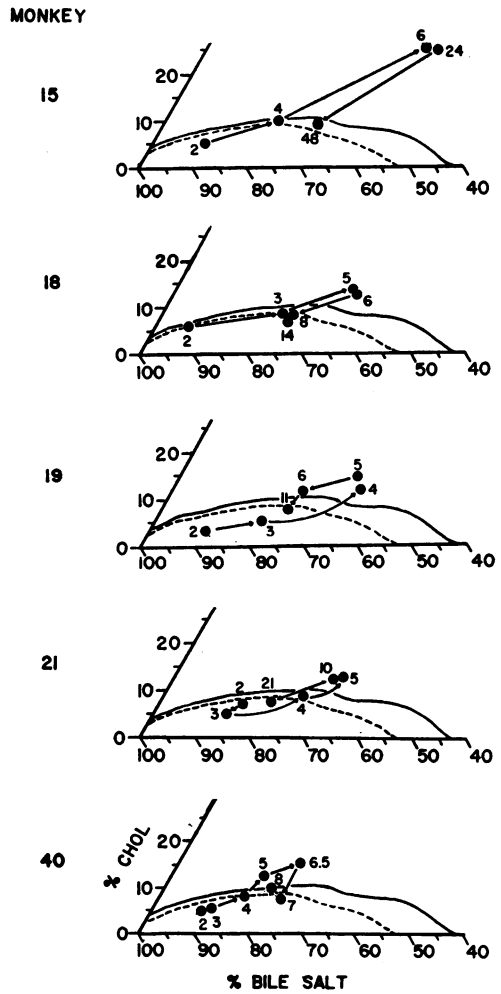

FIGURE 6 Changes in relative composition of bile in five monkeys after acute interruption of the EHC. Black dots represent the relative composition of bile at times (2, 3, 4, . . . 48) hr after initiation of total bile fistula. Note that except for animal 15, bile composition returns toward normal within 24 hr of acute interruption of the EHC.

However, when large interruptions of the EHC were produced, a new steady state of secretion (Fig. 2) and a new relative bile composition (Figs. 3 and 4) were established. While secretion of all components was decreased, bile salt secretion was relatively more depressed than phospholipid and cholesterol secretion. Note that this "uncoupling" of fixed secretory ratios occurred in spite of maximum bile salt synthesis.

In the nonsteady state, that is for 24 hr immediately after conversion to total bile fistula (Fig. 5, Table III), the discrepancy between secretion of biliary lipids becomes so marked that the large decrease in bile salt and relative increase in phospholipid and cholesterol lead to a bile supersaturated with cholesterol capable of pre-

cipitating as crystals. These transient changes in bile composition raise many questions concerning the nature and control of secretion of biliary lipids. It is clear from bile salt infusion–studies that increased bile salt secretion leads to increased phospholipid and cholesterol secretion (13, 14, 16). This was also true in our animals for, when the EHC of bile salt was reestablished, phospholipid and cholesterol increased simultaneously with bile salts. Thus, bile salts returning through the portal vein to the liver stimulated phospholipid and cholesterol secretion. However, the transient changes seen after bile fistula are more complex. At first, as the bile salt pool which was distributed in the animal's EHC returned to the liver and was secreted, phospholipid and cholesterol secretion were unchanged from the preinterruption state. This effect appears to be due to the circulating pool of bile salts passing through the liver. When the pool had been drained out and no bile salt was returning to the liver, at first bile salt synthesis and, therefore, secretion were depressed (4). During this period phospholipid and cholesterol secretion was also depressed in terms of absolute amounts but proportionately much less than bile salt. However, as bile salt synthesis began to increase toward a new steady state, the relative proportions of phospholipid and cholesterol decreased toward, but did not attain the prefistula state. What these intricate changes in biliary lipids mean in terms of hepatic phospholipid and cholesterol metabolism cannot be answered from the present data.

While a study of controlled chronic interruption of the EHC has not been carried out in normal humans, differences in bile composition have been studied in gallstone patients recovering from cholecystectomy. Thureborn (20) and Isaksson and Thureborn (21), whose bile sampling techniques inspired the present study, and more recently Nilsson and Schersten (22) examined the changes in bile composition after acutely interrupting the EHC in bile fistula patients. After acute interruption the EHC in bile fistula pataients. After acute interruption of the EHC was produced by inflating a balloon in the common duct, the secretion of all three substance decreased. But, as in the present monkey studies, bile salt secretion was depressed the most and a relative increase in phospholipid and cholesterol secretion occurred. In the cholesterol gallstone patient acute diversion led to very abnormal bile, but unfortunately, no studies concerning the physical state of this bile were published.

The biles of our monkeys and that of gallstone patients differ markedly. Unlike the monkeys many of these gallstone patients (20, 22) had biles, collected with the EHC intact, which were abnormal, since, when plotted on triangular coordinates, their composition fell at the limits of or outside the micellar zone (23). This fact supports the recent finding that hepatic bile of gallstone patients collected at surgery is supersaturated with cholesterol. Abnormal bile secretion persists even after cholecystectomy and the removal of gallstones at a time when the patient has largely recovered from surgery (23). It has, therefore, been suggested that patients with cholesterol gallstone disease have a subtle metabolic defect of the liver which results in the secretion of abnormal bile with respect to cholesterol solubility (12, 23, 24). While we have thus far found no intact monkeys with abnormal bile, the changes in bile composition after chronic interruptions of the EHC result in decreased capacity of the bile produced to solubilize cholesterol and to frank production of supersaturated bile in acute interruptions. Could the abnormal hepatic bile seen in gallstone patients be due to a depressed bile salt–secretion rate? It has been suggested that a decreased bile salt pool could lead to depressed bile salt secretion and thus to abnormal bile formation (5), but it has not been established that the pool or bile salt secretion is depressed in potential gallstone patients *before* the development of stones. Thus, the work of Vlahcevic, Bell, Buhac, Farrar, and Swell (5) could reasonably be interpreted as showing that gallstones cause a depressed bile salt pool rather than result from it. Nevertheless, the possibility exists that in a patient whose bile was nearly saturated with cholesterol, interruption of the EHC, for instance by ileal resection or ileal disease, could make the bile supersaturated and thus abnormal. In patients whose bile is already abnormal, interruption of the EHC would be expected to augment supersaturation and thus accentuate the defect which ultimately leads to cholesterol gallstones.

The concept that interruption of the enterohepatic circulation might, by reducing bile salt concentration in bile, lead to precipitation of cholesterol and thus to gallstone formation was postulated by Hofmann (25) who suggested that ileal dysfunction might promote gallstone formation. This hypothesis has equivocal support from clinical observations and from the results of studies in experimental animals. In animals, cholestyramine produced cholesterol gallstones in guinea pigs (26), promoted gallstone dissolution in hamsters (27, 28) but had no effect on stone dissolution in mice (29). Recently, Heaton and Read (30) and Cohen, Kaplan, Gottleib, and Patterson (31) reported that about 30% of patients with distal small bowel disease had clinical or cholecystographic evidence of cholelithiasis. However, since the *true* prevalence of gallstone disease in the general population is unknown (32), it cannot be stated categorically that patients with disorders of the ileum have a greater prevalence of gallstone disease than the general population. In fact, the high prevalence of silent stones in the Pima Indians of the Southwest (33) suggests that the *true* prevalence of gallstones may approach 25–30% in

the general adult population. Further, little is known about the chemical nature of the gallstones nor about the concentrations of bile salt, lecithin, and cholesterol in bile of patients with ileal dysfunction (31). Finally, a large number of patients with ileal disease also have chemical or biopsy evidence of liver disease (31) which by itself may influence the prevalence of gallstones.

Acute interruption of the EHC in humans could conceivably be related to gallstone formation (20). During prolonged fasting, the bile salt pool rests in the gallbladder and does not circulate. This "physiologic" interruption of the EHC could lead in humans, as in our animals, to a transient production of supersaturated hepatic bile. The amount of supersaturated bile formed would be small compared to that quantity of normal bile resting in the gallbladder and thus, if mixing of gallbladder bile with the newly formed hepatic bile occurred, the overall composition would be normal. However, it is possible that the newly formed bile could layer on top of the normal gallbladder bile (34) and if this supersaturated bile were nucleated and cholesterol crystals precipitated, then stone formation could be initiated in an individual with usually normal bile. Of course, if no nucleation occurred then hepatic bile composition would return to normal shortly after the EHC was reestablished by contraction of gallbladder and the subsequent return of bile salts to the liver.

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