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Composition of Basal and Stimulated Hepatic Bile in Baboons, and the Formation of Cholesterol Gallstones

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ABSTRACT **The baboon, *Papio*, has been found to be a model for the study of the pathogenesis of cholesterol cholelithiasis in man. Studies of the physiologic variations in hepatic bile composition indicate a cyclic pattern to the proportions of cholesterol, lecithin, and bile salt in hepatic bile. During reabsorption of the bile salt pool from the intestines (stimulated flow), hepatic bile is characteristically undersaturated with cholesterol. After reabsorption of the bile salt pool (basal flow), hepatic bile is characteristically supersaturated with cholesterol. This typical pattern of basal and stimulated hepatic bile occurs irrespective of the presence of cholesterol stones in the baboon. Recognition of these two types of hepatic bile and their interrelationship during admixture in the gallbladder provides new insight into the pathogenesis of gallstone formation.**

It has been recognized for many years that the cholesterol content of human bile is greater than that found in most other species. Thus the early studies of stone formation focused on those factors, bile salts and lecithin, that maintained cholesterol in solution. The relationship of these compounds to cholesterol solubility has recently been stated most clearly and comprehensively by Small and his associates (1). Because a number of people with cholesterol cholelithiasis were found to have hepatic and gallbladder bile that was supersaturated with cholesterol it was suggested that the difference between the normal subjects and the stone-formers was in the type of bile secreted by the liver (2). However, the analysis of gallbladder bile in normal and stone-forming individuals by Dam and his coworkers (3) failed to reveal a significant difference in bile composition in these two groups. Elucidation of the physical properties of bile has therefore provided a background for stone formation, but has failed thus far to establish critical determinants that distinguish the normal population from those who form stones.

In 1970, Glenn and McSherry reported the occurrence of spontaneous cholesterol gallstones in baboons (4). Gallstones ranging in cholesterol content from 86 to 97% were found in three adult female baboons. With the development of an animal model that is relevant to cholesterol stone formation in man, it has become possible to monitor the physiologic variations in bile composition during a 24-hr period. The relevance of this animal model has been established by finding that the baboon spontaneously forms pure cholesterol stones both in the wild and in captivity and that the proportion of cholesterol, bile salt, and lecithin in gallbladder and hepatic bile is indistinguishable from that of man (5).

We now report on the enterohepatic regulation of bile composition, which provides new insights into the pathogenesis of gallstone formation.

MATERIALS AND METHODS

The baboons utilized in these investigations at The Laboratory for Experimental Medicine and Surgery in Primates, Sterling Forest, N.Y. and at the Southwest Foundation for Research and Education, San Antonio, Texas, were selected solely on the basis of their availability at the time these studies were undertaken.

Group A: Composition of gallbladder and hepatic bile in normal baboons

The 27 female and 3 male baboons studied were sedated with surnylan, and anesthesia was maintained by intravenous sodium pentobarbital. The body cavity was opened with sterile technique, and all of the bile present in the gallbladder was aspirated with needle and syringe. The common bile duct was then cannulated with a polyethylene catheter (P.E. no. 190), and 3 ml of hepatic bile was collected. The choledochotomy incision was repaired with 6-0 silk. All animals survived the procedure. Bile samples were analyzed for cholesterol, phospholipid, and bile salt content by methods previously described (5). In addition to the determination of total bile salt by the enzymatic procedure of Javitt and Emerman (6), individual bile salts were quantitatively analyzed in selected samples by gas–liquid chromatography with internal standardization (7). The relative amounts of cholesterol, bile salts, and phospholipid as percentages of the total quantity of all three components in each bile sample were expressed as a single point on triangular coordinates as described by Admirand and Small (1).

Group B: Composition of hepatic bile after instillation of gallbladder bile into the ileum

In the fasting state, most of the bile salt pool is present in the gallbladder bile. The most convenient technique for presenting the liver with a bile salt challenge is to instill the animal's gallbladder bile into the ileum. The absorption of bile salt from the ileum is both rapid and efficient. Five animals were selected for this study. At laparotomy, all of the bile present in the gallbladder was aspirated with a needle and syringe. The volumes of gallbladder bile ranged from 9 to 13 ml. Hepatic bile was completely diverted by cannulating the common hepatic duct. After baseline samples of hepatic bile were obtained, the animal's gallbladder bile was instilled into the distal small bowel through an enterotomy. A 2-ml aliquot of gallbladder bile was retained for analysis. After instillation of gallbladder bile into the ileum, hepatic bile samples were collected for 30-min periods over 1.5–4 hr. Bile samples were analyzed as in Group A.

Group C: Composition of hepatic bile after intraportal infusion of bile salt

These experiments were designed to define more precisely the role of bile salts in regulating cholesterol solubility in hepatic bile. In two animals, gallbladder bile was instilled into the ileum, and hepatic bile samples were collected for 2 hr as described for Group B. At this point, 1 g of sodium taurocholate, as a 1.3% solution in dextrose and water, was infused over a 30-min period into the portal circulation through a mesenteric vein. Hepatic bile samples were collected for 2 hr after the completion of the bile salt infusion.

Group D: Composition of hepatic bile after systemic infusion of bile salt

Two animals were selected for this study to determine if the hepatic response to a bile salt load was dependent upon the enterohepatic circulation, or whether changes in cholesterol solubility could also be induced by the systemic administration of bile salt. In these animals, control samples of hepatic bile were obtained after a cannula was inserted into the common hepatic duct. The sodium taurocholate solution, identical with that described above, was infused over a 30-min period into an arm vein. Hepatic bile samples were collected at 30-min intervals for 2 hr.

RESULTS

Group A

Samples of hepatic bile were available from 20 baboons, and gallbladder bile from 26 (Fig. 1). When the relative concentrations of cholesterol, phospholipid, and bile salts in each of these samples are plotted as a single point on triangular coordinates, several conclusions are possible. With respect to hepatic bile, cholesterol solubility in 10 baboons (50% of the group) was outside the micellar zone and therefore present in a supersaturated state. However, the cholesterol solubility in gallbladder bile was such that in 22 of 26 animals (84.6%), it fell within the micellar zone.

Group B

After instillation of gallbladder bile into the terminal ileum, the bile salt content of hepatic bile promptly increased (Fig. 2). In three of the five animals this increase was maximal by 30 min. In the remaining two baboons, the maximum bile salt content of hepatic bile was reached in 60 min. The effect of this increase in bile salt content on cholesterol solubility in hepatic bile is depicted in Fig. 2. In all the animals the cholesterol-solubilizing capacity of bile was increased, as evidenced by a shift further into the micellar zone. After 60 min, cholesterol solubility decreased as the bile salt content of hepatic bile decreased.

Group C

The effect of an infusion of bile salt, sodium taurocholate, into the portal circulation is depicted in Fig. 3. After cholesterol solubility in hepatic bile had reached a maximum after instillation of gallbladder bile into the ileum, the infusion of bile salt into the portal circulation produced a prompt further increase in the bile salt concentration of hepatic bile and an additional increase in its cholesterol-solubilizing capacity.

Group D

The infusion of sodium taurocholate into the systemic circulation produced a marked increase in the proportion of bile salt in hepatic bile, and a corresponding shift in its cholesterol-solubilizing capacity similar to that noted in the animals in Group C (Fig. 4).

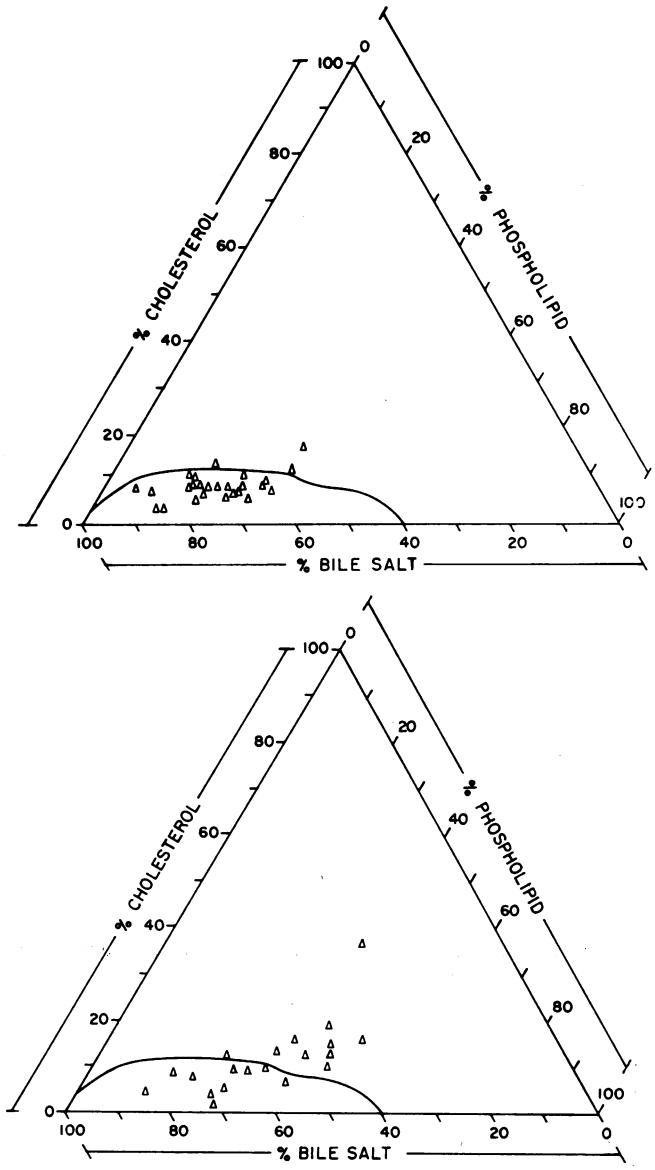

FIG. 1. The cholesterol, bile salt, and phospholipid (weight) percentages in gallbladder (*above*) and hepatic (*below*) bile samples from normal baboons, plotted as a percentage of the total quantity of all three components on triangular coordinates. The cholesterol in bile samples located below the solid line is within the micellar zone. Above this line, cholesterol is present in a supersaturated state.

DISCUSSION

There are very few data in man concerning the relative importance of the liver and gallbladder in gallstone formation. Burnett obtained bile samples simultaneously from the gallbladder and common duct in patients with cholesterol stones and in patients with a normal biliary tree (8). The hepatic bile of the patients with stones contained a low concentration of bile acids in about one-half of the patients and the concentration of phospholipids was occasionally lower than normal. Burnett suggested that the liver produced a potentially lithogenic hepatic bile in patients with gallstones.

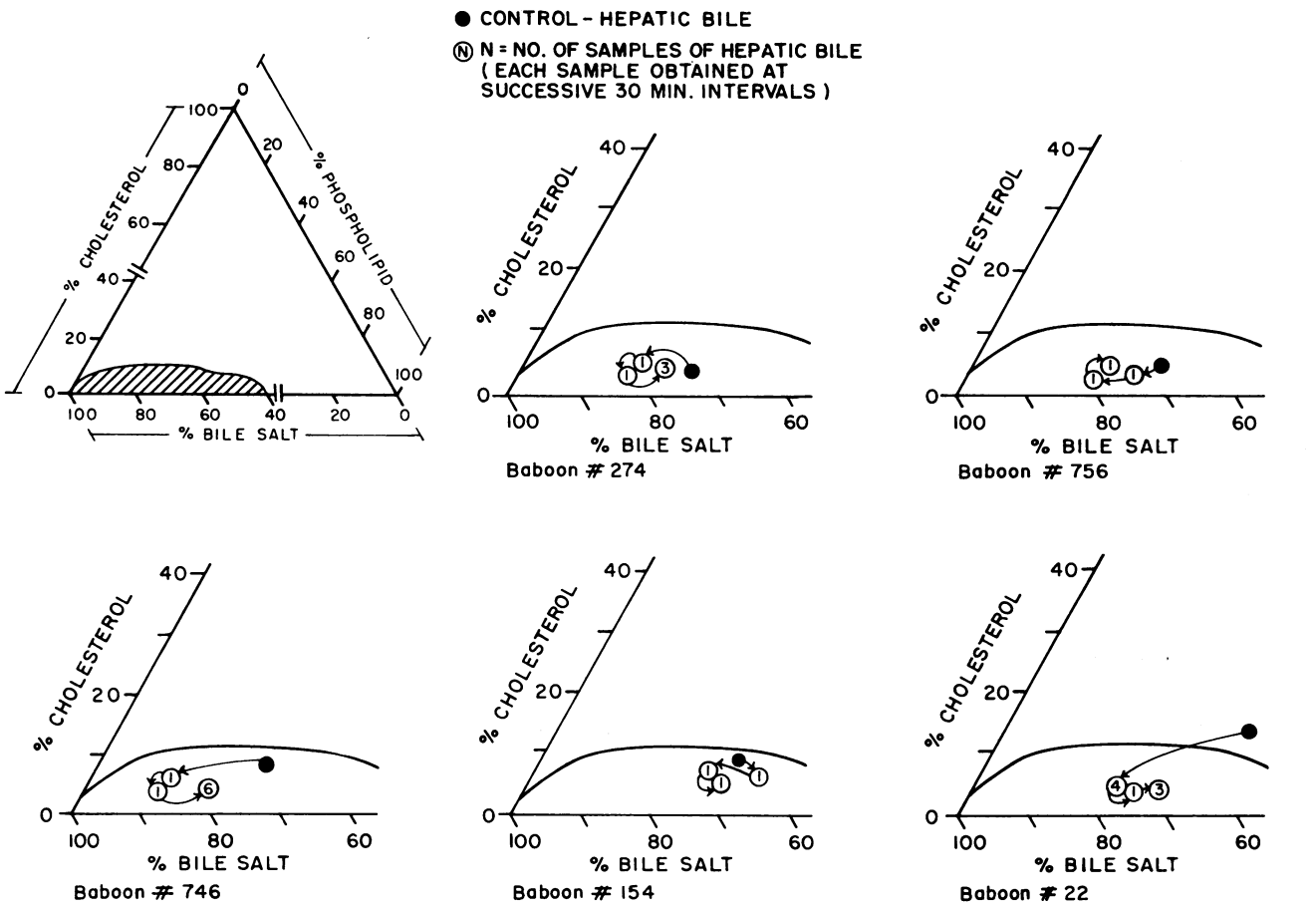

FIG. 2. Cholesterol solubility in hepatic bile after instillation of gallbladder bile into the ileum. The triangle at the upper left represents the three-component system and the shaded area, the micellar zone.

However, when Small plotted Burnett's data on triangular coordinates, both hepatic bile and gallbladder bile of patients with gallstones were in the micellar range, which indicates that the hepatic bile was not abnormal in these patients (9). Vlahcevic, Bell, and Swell (10) analyzed the composition and micellar state of gallbladder and hepatic bile in 14 patients with cholesterol gallstones, four patients with pigmented stones, and two patients without stones. In the patients with cholesterol gallstones, the gallbladder and hepatic bile samples were either out of the micellar zone or near the level of maximum solubility for cholesterol. The gallbladder and hepatic bile of the four patients with pigmented stones fell within the micellar zone. The cholesterol solubility of the gallbladder and hepatic bile obtained from the two patients without gallstones was similar to that of the patients with stones, in that it was also supersaturated. The authors concluded that these patients may form stones in the future. Recently, Small and Rapo (2) reported their observations on 30 American Indians of the Southwest with gallstones. Samples of gallbladder bile were available from 19 of these patients and hepatic bile from 23. Gallbladder bile was saturated and hepatic bile supersaturated with cholesterol. The authors concluded that in patients with cholesterol gallstones, the liver secreted a lithogenic bile. The validity of this conclusion is dependent, in part, on the constancy of composition of hepatic bile. Our findings in the baboon indicate that hepatic bile is continually varying in composition. Therefore one cannot assume, because cholesterol is present in a supersaturated state in hepatic bile obtained from fasting individuals with gallstones, that they produce a bile which is significantly different from the normal population.

Because of the episodic cycling of bile salts and their effect on bile composition, it is reasonable to distinguish hepatic bile secreted under basal conditions from that occurring under stimulated conditions. Basal bile flow is defined as the type of bile secreted by the liver after 90% of the bile salt pool has returned to the gallbladder. Stimulated bile flow occurs during the reabsorption from the intestines and secretion into bile of 90% of the bile salt pool. In our studies in the baboon and from limited data in man it appears that basal hepatic bile composition can be characterized by having more cholesterol in solution than can be accounted for by micellar solubility. Stimulated hepatic bile, in contrast, is less than saturated with cholesterol. These differences between basal bile and stimulated bile are present irrespective of the occurrence of stones in the baboon. In man, only the population with stones has been adequately studied, and in the majority of instances the hepatic bile obtained under basal conditions was different from gallbladder bile, which when expressed as the relative proportions of bile salt, cholesterol, and lecithin can be considered to be stimulated hepatic bile. Until similar studies are done in humans without stones, interpretation of the significance of these differences in basal and stimulated bile composition is difficult. Perhaps it is related to the pres-

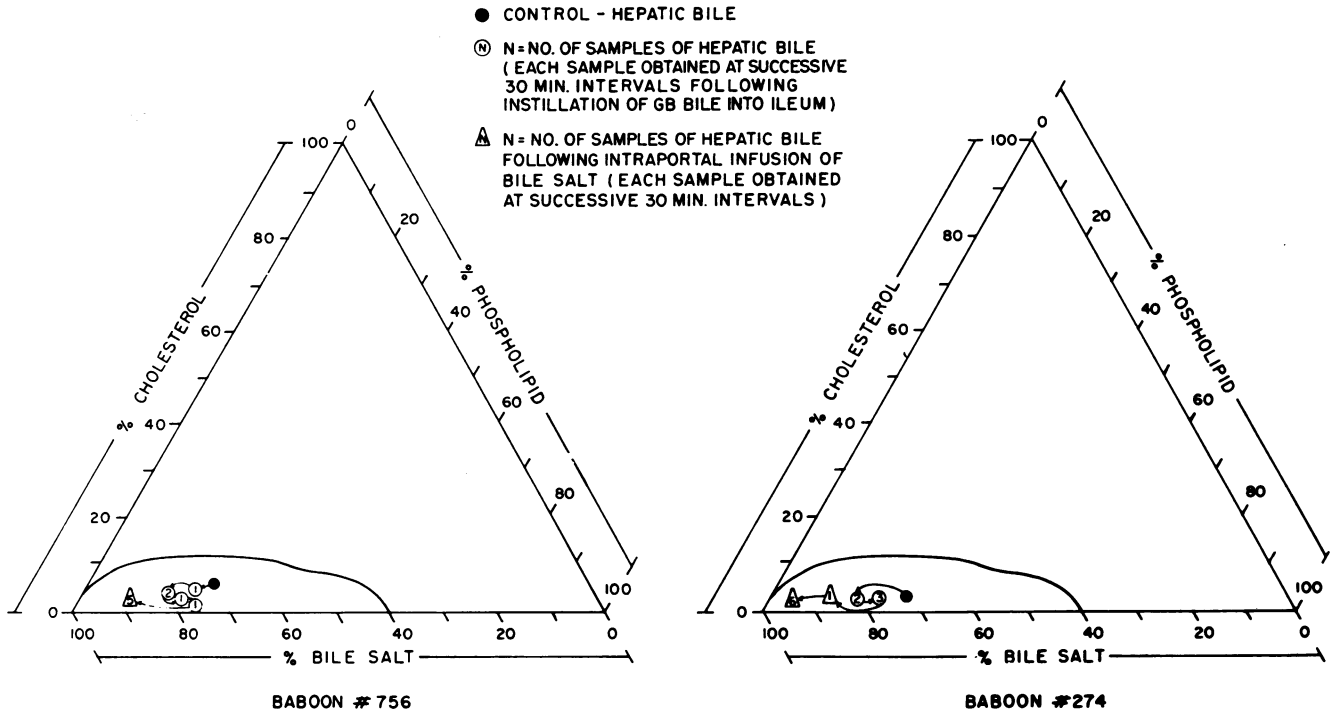

FIG. 3. Increase of cholesterol solubility in hepatic bile caused by intraportal infusion of bile salt.

ence of stones or alternatively, as in the baboon, represents the physiologic consequence of the enterohepatic cycling of bile salts.

With the recognition that hepatic bile is of continually varying composition, it becomes important to speculate on what role this may play in stone formation. Recently, it has been proposed (11) that there is nonmixing of bile in the gallbladder, and layering of bile relatively rich in cholesterol near the gallbladder epithelium. Because of this proposed phenomenon, the authors minimized the solubilizing capacity of lecithin and bile salts in gallbladder bile as an important determinant of stone formation. Our data support the view that gallbladder bile may be a heterogeneous mixture of basal and stimulated biles from which salt and water have been removed. In the absence of mechanical stirring in the gallbladder, it is possible that rates of diffusion of cholesterol from a nonmicellar to a micellar phase, especially when cholesterol concentrations in the two phases are similar, may

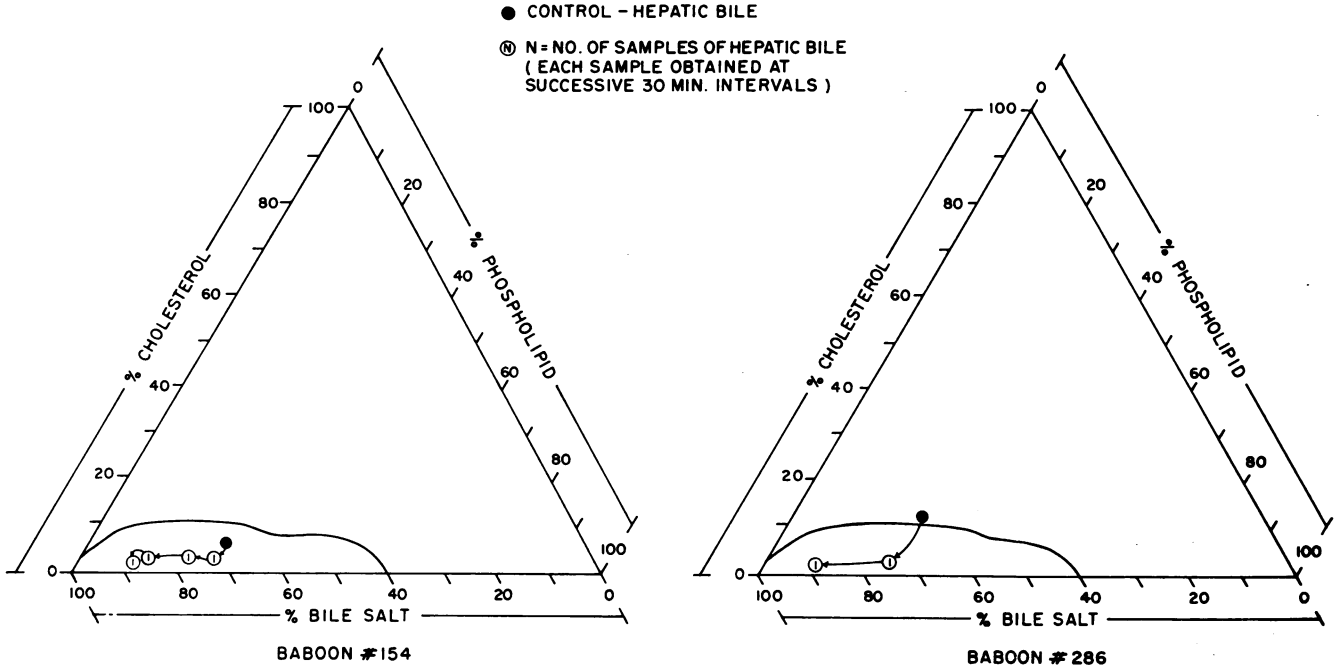

FIG. 4. Increase of cholesterol solubility in hepatic bile caused by systemic infusion of bile salt.

be very slow, and a single homogeneous micellar composition does not form. Clearly, the ratio of basal hepatic bile to stimulated hepatic bile will determine the final proportions of cholesterol, phospholipid, and bile salt. Because of a lack of knowledge concerning the rate at which equilibrium would occur under the described conditions, it is not possible at present to fully evaluate the concept of the heterogeneity of gallbladder bile composition. However, the relatively poor correlation between the cholesterol-solubilizing capacity of bile and the occurrence of stones indicates that more critical determinants may be the relationship of basal hepatic bile secretion to stimulated secretion and the events that follow their entry into the gallbladder.

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