

## Effect of Bile Acids on Biliary Excretion of Cholesterol in Rabbits

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### ABSTRACT

The effects of cholic acid and eight related cholanic acid analogs on bile flow and biliary excretion of bile salts and cholesterol were studied in rabbits. Bile acids were infused intravenously in anesthetized rabbits. In all except hyodeoxycholic or lithocholic acid treated animals increases in bile flow were recorded within 10 minutes during infusion of bile acid. The increase in bile flow associated with an increase in bile salt level in bile after cholic acid infusion was observed, however, there were little changes in biliary cholesterol levels. Bile salt level in bile was not associated with bile flow after chenodeoxycholic acid infusion but the cholesterol level in bile was significantly increased. Ursodeoxycholic acid similarly increased cholesterol but to a lesser extent. Keto-forms of chenodeoxycholic acid were without action. These results indicate that both cholic and chenodeoxycholic acids have the capacity to alter specific biliary excretion of bile components, the former on bile salts and the

latter on cholesterol—a precursor of bile acids in bile.

### INTRODUCTION

In a study of choleretic potencies of several bile acids in rabbits, two of these substances—cholic acid and chenodeoxycholic acid had definite but differing effects. Cholic acid caused a substantial increase in the bile salt level in bile. On the other hand, chenodeoxycholic acid increased biliary cholesterol without increasing bile salts. This dihydroxycholanic acid has been claimed to form larger micelles with less surface charge and consequently less stability, and a shift toward this acid may therefore favor gallstone formation (Bouchier and Freston, 1968; Van der Linden, 1971). Recently Danzinger *et al.* (1972) found a contradictory result in that when chenodeoxycholic acid was given at high doses (0.75 to 4.5g per day) and for a period of 6 to 22 months to seven women with gallstones, radiologic evidence of stone dissolution was obtained in four of the patients.

In the present study the choleretic potencies of cholic acid and its analogs following intravenous infusion have been investigated in rabbits. In addition the effect of choles-

\* This study was supported in part by the China Medical Board of New York, Inc. (Grant 70-149-4), U.S.A., and in part by the College of Medicine, Yonsei University, Seoul, Korea.

Table 1. Effects of bile acid infusions on bile flow and bile components in rabbits

Group	No. Exp.	Bile component							
		Volume, ml/10min		Bilirubin, mg/dl		Cholate, mg/dl		Cholesterol, mg/dl	
		before	after	before	after	before	after	before	after
Cholic acid	8	1.5 ±0.13	1.8 ±0.21	2.8 ±0.47	3.1 ±0.54	31.6 ±4.51	108.1*** ±14.53	30.3 ±2.54	33.4 ±3.18
Deoxycholic acid	6	1.2 ±0.12	2.1** ±0.22	3.1 ±0.86	2.2 ±0.48	22.9 ±3.11	21.8 ±2.54	24.3 ±6.57	22.2 ±5.43
Chenodeoxycholic acid	10	1.7 ±0.13	2.1 ±0.16	1.6 ±0.34	1.7 ±0.44	25.7 ±2.32	24.5 ±2.15	25.6 ±2.85	55.3** ±6.86
Ursodeoxycholic acid	7	2.0 ±0.22	2.4 ±0.30	1.2 ±0.21	1.2 ±0.31	26.3 ±4.03	23.3 ±3.17	24.6 ±2.65	29.9 ±3.54
7-Ketochenodeoxycholic acid	6	1.8 ±0.16	2.5* ±0.25	1.4 ±0.15	1.1 ±0.18	18.9 ±2.10	16.2 ±2.57	22.9 ±3.78	18.6 ±3.54
3,7-Diketochenodeoxycholic acid	6	1.4 ±0.22	1.6 ±0.26	2.4 ±0.63	2.1 ±0.65	31.2 ±5.70	25.5 ±4.64	34.9 ±6.33	26.1 ±4.60
Dehydrocholic acid	7	1.5 ±0.01	2.1** ±0.15	2.5 ±0.52	1.8 ±0.33	26.5 ±2.08	21.4 ±2.47	27.9 ±3.42	24.3 ±2.99
Lithocholic acid	6	1.9 ±0.63	2.0 ±0.38	1.1 ±0.16	1.3 ±0.24	22.3 ±3.29	19.9 ±3.64	24.6 ±1.29	22.2 ±2.39
Hyodeoxycholic acid	4	1.5 ±0.05	1.6 ±0.09	1.3 ±0.11	1.2 ±0.13	23.0 ±0.45	22.5 ±0.01	23.4 ±1.16	24.0 ±2.36

Values are means±S.E.

\* P&lt;0.05    \*\* P&lt;0.01    \*\*\* P&lt;0.001

terol excretion in bile has been studied.

## METHODS

Albino rabbits of both sexes, weighing approximately 2 kg were used. In the rabbit, in spite of its smaller size than dog or cat, the bile flow is much larger than either. It is usually in excess of 1.0 ml per 10 minutes. The abdomen was opened through a 4.0 to 6.0 cm right rectus incision under secobarbital anesthesia. The common bile duct was cannulated with a small polyethylene cannula and the cystic duct was ligated. Physiological saline solution was infused intravenously through the experiment. Hepatic bile was collected every 10 minutes for 30 minutes and then between 10 and 14 consecutive 10-minute samples were obtained following infusion of bile acid in the doses of 5 mg/kg per 10 minutes in each animal.

The specimen was selected when maximum response in flow occurred during infusion. The bile acids used are as follows: Cholic acid(Nutritional Biochem. Corp., Cleveland, Ohio); Chenodeoxycholic acid(Mann Res. Lab., New York, N.Y.); Lithocholic acid(K & K Lab., Plainview, N.Y.); Ursodeoxycholic acid(K & K Lab.); Hyodeoxycholic acid(K & K Lab.); Sodium deoxycholate(Difco Lab., Detroit, Michigan); Dehydrocholic acid<sup>1)</sup>; 7-Keto and 3,7-Diketo chenodeoxycholic acids<sup>2)</sup>. The bile acids were prepared as solution of sodium salt with a pH 7.4 for infusion. The levels of cholate and bilirubin in bile were determined by the methods of Irvin *et al.*(1944) and Magee *et al.*(1952), respectively. In order to determine

1) 2) Courtesy of Professor S. U. Hong, Sung Kyun Kwan University, College of Pharmacy, Seoul, Korea.

Table 2. Percentage changes of bile volume, cholate and cholesterol levels with bile acid infusions

Cholanic acid deriv.	Radicals in position			% difference from control		
	3	7	12	Volume	Cholate Conc.	Cholesterol Conc.
Cholic acid	OH	OH	OH	20.0	242.0	10.2
Deoxycholic acid	OH	H	OH	75.0	-4.8	-8.6
Chenodeoxycholic acid	OH	OH	H	23.5	-4.6	116.0
Ursodeoxycholic acid	OH	$\beta$ OH	H	20.0	-11.3	21.5
7-Ketochenodeoxycholic acid	OH	=O	H	38.8	-14.2	-18.7
3,7-Diketochenodeoxycholic acid	=O	=O	H	14.2	-18.2	-25.2
Dehydrocholic acid	=O	=O	=O	40.0	-19.2	-12.9
Lithocholic acid	OH	H	H	5.2	-10.7	-9.7
Hyodeoxycholic acid	OH	6-OH	H	6.6	-2.1	2.5

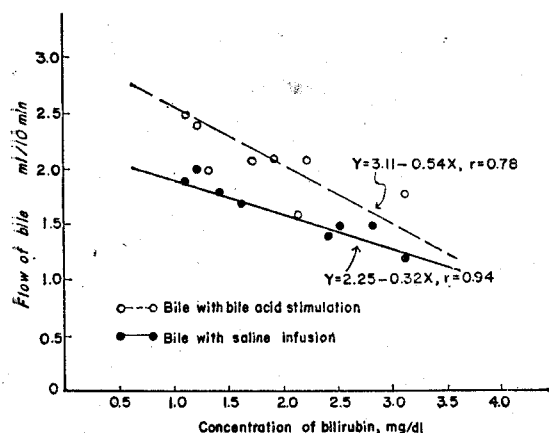


Fig. 1. To show the relation between flow and bilirubin level in bile of rabbits with and without bile acid infusions.

the level of cholesterol in bile, an aliquot of the hepatic bile was extracted with 2:1 chloroform-methanol mixture according to the method of Folch *et al.* (1957) and the cholesterol content was measured by the method of Chiamori and Henry (1959). In four animals blood pressure was recorded after intravenous injection of bile acids in the doses of 5 mg/kg. The change of blood

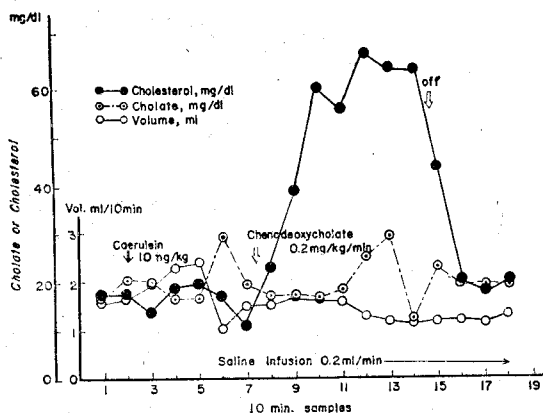


Fig. 2. Biliary cholesterol excretion during intravenous infusion of chenodeoxycholate in rabbit. A slight increase of bile flow was seen following intravenous injection of caerulein, but no changes of bile components were elicited. Chenodeoxycholic acid infusion induced a marked increase of cholesterol level. This response ended quickly after stopping the infusion. The bile flow and cholate level in bile were not much changed.

pressure was slight.

## RESULTS

### 1. Bile flow and bilirubin concentration;

In all the rabbits anesthetized with secobarbital, increases in bile flow were recorded during intravenous infusion of bile acids. The bile flow in response to deoxycholic ( $p < 0.01$ ), dehydrocholic ( $p < 0.01$ ) or 7-ketochenodeoxycholic ( $p < 0.05$ ) acids was increased significantly (Table 1.). The hydrocholeretic action of investigated bile acids occurs in a decreasing order; deoxycholic acid, dehydrocholic acid, 7-ketochenodeoxycholic acid, chenodeoxycholic acid, cholic acid and others. The output of bilirubin in bile did not change significantly during the period of choleresis. There was an inverse relation between bilirubin concentration and flow rate (Fig. 1).

2. Biliary excretion of bile salts and cholesterol: Although the bile flow in response to various bile acid infusions increased, the concentration of bile salts during the period of choleresis with cholic acid infusion was increased by 242.0% (Table 2). The increment in bile salt excreted did not exceed the amount administered. The control biliary excretion of cholesterol was not significantly different from that seen after cholic acid. The cholesterol level in bile was raised during bile acid infusions particularly with chenodeoxycholic acid (Fig. 2). Chenodeoxycholic acid caused a great increment (116.0%) in biliary cholesterol (Table 1 & 2). Ursodeoxycholic acid which differs only in  $7\beta$ -OH from chenodeoxycholic acid increased cholesterol levels but to a much lesser extent. Keto forms of chenodeoxycholic acid had reduced or absent capacities to increase biliary cholesterol. Little changes in bile flow or composition were seen with lithocholic or hyodeoxycholic acid infusion in rabbits.

## DISCUSSION

Gregg and Poley (1966) clearly documented that in intact rabbit bile deoxycholic acid is the principal acid and that chenodeoxycholic acid is present in small amounts; however, in animals with prolonged fistula drainage deoxycholic acid and other bacterial metabolites were no longer present and the cholic acid becomes the main bile acid.

The results in this study indicate that intravenous infusion of bile salts increases the rate of bile flow but to different degrees, e.g., cholic acid and chenodeoxycholic acids were much less potent than deoxycholic acid at the same dose. However, in contrast to the flow both cholic and chenodeoxycholic acids profoundly enhanced the secretion of bile salts or the precursor, cholesterol, in bile. The choleresis from these acids was not unexpected, however, it was surprising to note that only chenodeoxycholic acid caused a marked elevation in biliary cholesterol.

Bergström and Danielsson (1958) found that a continuous intraduodenal infusion of taurochenodeoxycholate into bile-fistula rats which synthesize bile acids at greatly accelerated rates in compensation for the loss via the fistula reduced the biliary excretion of bile salts to about the same level as in the intact rat. Lee *et al.* (1965) also confirmed that chenodeoxycholic acid inhibits the conversion of cholesterol into bile acids in rats much more powerfully than does cholic acid. From this it was claimed that chenodeoxycholic acid has a specific effect on the excretion of cholesterol, the precursor of bile salts themselves.

The principal primary bile acids of human bile are cholic acid—a trihydroxycholanolic acid and chenodeoxycholic acid—a dihydroxycholanolic acid. It is claimed that the micelles formed with trihydroxy bile acids differ from those with dihydroxy bile acids, e.g., the more polar trihydroxy complexes are smaller, more stable and therefore resist any tendency to coalesce. The dihydroxy bile salts are more non-polar, and the critical micellar concentration is lower. The larger micelles which may be presumed to form will have less surface charge, be less stable, and will coalesce more readily to come out of solution (Bouchier and Freston, 1968; Van der Linden, 1971). This fact, together with our finding that chenodeoxycholic acid raises biliary cholesterol saturation implies that this acid should enhance gallstone formation. However, Danzinger *et al.* (1972) are convinced that cholelithiasis is a disease of decreased bile acid secretion rather than a disease of increased cholesterol secretion in view of the fact that reduced secretion of bile acid and lecithin lowers the capacity of bile to dissolve cholesterol. They found in four patients out of seven who ingested from 0.75 to 4.5 g of chenodeoxycholic acid daily for a period of 6 to 22 months, progressive dissolution of their gallstones. Our observation does not correlate directly with their finding, but depletion of liver cholesterol, increase of biliary bile salts and therefore expansion of the bile acid pool by the acid supplement would increase the secretion of both bile acid and lecithin and lower biliary cholesterol saturation. Consequently further development of gallstone would be prevented. Nevertheless, the question of how the dis-

solution of persisting gallstones could occur is still obscure.

The conflicting views on gallstone formation with chenodeoxycholic acid, together with our result which showed an increment of biliary cholesterol excretion with the bile acid, may indicate that the chenodeoxycholic acid has dual effects on gallstone formation, namely, the acid, when administered intermittently, favors gallstone formation owing to increased cholesterol levels in bile while with long-term administration of the acid in high doses further formation of the stones is prevented due to depletion of liver cholesterol, increased bile acid secretion, and therefore an increased capacity to dissolve cholesterol.

The keto forms in 7-OH or 3- and 7-dihydroxy radicals of chenodeoxycholic acid had no specific action on biliary cholesterol secretion in our experiment. Ursodeoxycholic acid, a 7 $\beta$ -OH steric isomer of chenodeoxycholic acid showed effects similar to chenodeoxycholic acid but less in extent. Hyodeoxycholic acid which is also a dihydroxycholic acid with hydroxy radicals in 3 and 6 positions had no effect on either bile flow or biliary composition in our rabbits.

Entemnan *et al.* (1968) showed that infusion of cholate, deoxy, chenodeoxy, hyodeoxy or litho cholates into the circulation of isolated perfused rat livers increased the excretion per hour of free biliary cholesterol and phospholipid. These authors do not record the volume of bile secreted so it is difficult to compare their data with ours but, in two of their three series, (the differences between which are undescribed) chenodeoxycholic

acid produced a much higher cholesterol output than any of the other acids.

Recently Wheeler(1973) Observed in hamsters that the incidence of cholesterol gallstones was 100% when 0.1% chenodeoxycholic acid was added in an essential fatty acid-deficient diet (68% incidence with deficient diet alone), and zero with 0.1% hyodeoxycholic acid. Moreover, cholesterol output was markedly increased after intravenous hyodeoxycholic acid in his experiment. Formerly this bile acid was reported by Behr *et al*, (1960) to promote liver cholesterol mobilization in mice and by Dam and Christensen (1962) to prevent gallstone formation in hamsters. These findings are different from our results observed in rabbits.

Both dehydrocholic and 7-ketochenodeoxycholic acids showed moderately potent hydrocholeretic effects but neither lithocholic nor hyodeoxycholic acid had any effect on bile secretion in our experiments.

### ACKNOWLEDGEMENTS

We wish to express our thanks to Professor D.F. Magee, Department of Physiology-Pharmacology, Creighton University, School of Medicine, Omaha, Nebraska, U.S.A., for his helps in preparing the manuscript and also our gratitude to Dr. I.P. Lee of National Institute of Environmental Health Sciences, Research Triangle Park, N.C., U.S.A. for the generous supply of bile acids used in this study.

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