Effect of bile duct ligation on bile acid metabolism in rats

Tatsuya Kinugasa, Kiyohisa Uchida,[1] Masumi Kadowaki, Haruto Takase, Yasuharu Nomura, and Yoichi Saito

First Department of Surgery, Kobe University School of Medicine, Ikuta-ku, Kobe, 650[2] and Shionogi Research Laboratories, Fukushima-ku, Osaka, 553[3] Japan

Abstract The effect of bile duct ligation on the quantitative and qualitative changes of bile acids in serum, liver, urine, and feces, and the concentration of cholesterol and phospholipids in serum and liver were examined in male rats. The concentration of bile acids in serum increased over 100-fold on day 5 but was lower than the 5-day level on days 10 and 15. The concentration in the liver also increased about 10-fold. β-Muricholic acid predominantly increased but the secondary bile acids, deoxycholic acid and hyodeoxycholic acid, decreased. The urinary excretion of bile acids increased to about 40 mg/day per rat on the first day of bile duct ligation but this increase was reduced on day 2 to about half and remained at that level until day 24. These values exceeded that of fecal bile acids, 12 mg/day per rat, before bile duct ligation. The amount of bile acid sulfates in the urine was as low as 1% of the total. The urinary nonsulfated bile acids consisted mainly of β-muricholic acid (60%) and cholic acid (20%), while the sulfates contained a considerable amount of unidentified acidic substance (40%) in addition to cholic acid and β-muricholic acid. The concentration of cholesterol and phospholipids in serum markedly increased on day 5 but declined gradually thereafter. The liver cholesterol concentration did not change but the phospholipid concentration decreased. Fecal sterols did not change in both the total amount and composition. These data indicated that daily synthesis of bile acids, especially β-muricholic acid, was accelerated in bile duct-ligated rats.—**Kinugasa, T., K. Uchida, M. Kadowaki, H. Takase, Y. Nomura, and Y. Saito.** Effect of bile duct ligation on bile acid metabolism in rats. *J. Lipid Res.* 1981. **22:** 201–207.

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Bile acids, which are synthesized from cholesterol in the liver, are mainly located in a closed system, the enterohepatic circulation. When the bile duct is obstructed, bile acids enter the systemic blood and are excreted into urine and cholesterol and bile acid metabolism is greatly disturbed. The concentration of cholesterol and bile acids in serum increases (1–5) and the rate of cholesterol synthesis rises several-fold (2, 3, 6). The concentration of cholesterol in the liver does not change, but that of bile acids increases (7). In rats, the synthesis of β-muricholic acid is enhanced (8) and this bile acid becomes the major component in the liver (7), being consistent with the increase in 6β-hydroxylation activity (9). Gustafsson (10) has demonstrated an increase in the mitochondrial 26-hydroxylation to support the enhancement of the synthesis of chenodeoxycholic acid, which is further metabolized to β-muricholic acid in rat. Lutton, Mathé, and Chevallier (11) have observed that bile acid formation is stimulated in bile duct ligated rats.

Boyd, Eastwood, and MacLean (1) have determined the time course changes in urinary excretion of bile acids after bile duct ligation in rats that showed a rapid loss of bile acids in the enterohepatic circulation into the urine, though β-muricholic acid was not studied and the experiments ended by 96 hr. Therefore, we examined the changes in urinary and fecal excretion of bile acids, in addition to the changes in serum and liver concentrations of bile acids, for a longer period up to the death of the animals.

MATERIALS AND METHODS

Animals and maintenance

Wistar strain male rats weighing approximately 300 g were kept in an air-conditioned room (25 ± 1°C, 50–60% humidity) lighted 12 hr a day (8:00 AM to 8:00 PM) and maintained on a commercial balanced stock diet (Japan CLEA CA-1, Tokyo, Japan). The composition of the diet was 25.5% protein, 4.0% lipid, 53.5% carbohydrate, 4.0% fiber, 7.0% ash, and 6.0% water. The cholesterol content was 0.04–0.05%.

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography; GLC–MS, gas–liquid chromatography–mass spectrometry.

[1] To whom reprint requests should be addressed.

[2] T. Kinugasa and Y. Saito.

[3] K. Uchida, M. Kadowaki, H. Takase, and Y. Nomura.

The animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and the common bile duct was ligated three times. The animals were kept individually in metabolic cages to collect the urine and feces daily. In one series of experiments, the rats were kept until their death. In another series, five rats each were killed on days 5, 10, and 15. The control rats were killed on the day corresponding to day 15. The autopsies were performed between 9:00 AM and 11:00 AM to avoid variation due to the circadian rhythm.

Cholesterol and phospholipid determinations

Serum was separated by centrifugation at 3000 rpm for 15 min after the blood had been left at least 30 min at room temperature. After the bile in the common bile duct had been removed, a portion of the largest lobe of the liver (*lobus sinistra externa*), about 1 g, was excised and homogenized with nine volumes of ice-chilled physiological saline using an ULTRA-TURRAX TP 18-10 (IKE-WERK, Janke & Kunkel KG, West Germany). The serum and liver homogenate were extracted with 10 volumes of ethanol by refluxing for 20 min at 90–95°C.

Total cholesterol was determined with portions of the lipid extracts as previously reported (12, 13). Phospholipids were determined by the method of Gomori (14).

Sterol and bile acid determination

Fecal sterols and bile acids were determined as reported previously (15, 16). Briefly, dried and powdered feces were extracted with absolute ethanol and hydrolyzed in 1.25 N sodium hydroxide solution at 120°C for 6 hr under pressure. After the extraction of sterols with 10 volumes of ethyl ether three times, the hydrolyzate was acidified to pH 2 or below with 2 N hydrochloric acid, and bile acids were extracted with 10 volumes of ethyl ether three times.

The sterols were quantified by GLC using a 1% SE-30 column. The bile acids were methylated with freshly prepared diazomethane, then trifluoroacetylated with trifluoroacetic anhydride and quantified by GLC using a 3% QF-1 column (16). Since cholic acid and β-muricholic acid showed almost the same retention time in the present GLC, both bile acids were initially separated by TLC. Precoated silica gel GF plates (0.25 mm, Uniplate, Analtech, Newark, NJ) were used and developed in benzene–acetone 7:3.

Serum bile acids were isolated by the use of Amberlyst A-26 column (17) and quantified by GLC after enzymic hydrolysis.

The urine was applied to an Amberlite XAD-2 column and bile acids were eluted with methanol according to the method of Makino et al. (18). The bile acids thus obtained were applied to an LH-20 column and separated into nonsulfate and sulfate fractions (19). The sulfate fraction was evaporated to dryness and the residue was dissolved in 30 ml of acetone–ethanol 9:1 containing 0.01 volume of 2 N hydrochloric acid. The solution was left at room temperature for 2 to 3 days to complete solvolysis. The reaction mixture was then evaporated to dryness and the residue was subjected to alkaline hydrolysis. Bile acids in both nonsulfate and sulfate fractions were analyzed by GLC.

The remaining liver, 10 g or so, was homogenized with two volumes of distilled water, lyophilized, and then extracted with ethanol containing 0.1% ammonia. The extract was dissolved in 70% methanol and the neutral lipids were removed with n-hexane twice. Next, the 70% methanol layer was evaporated to dryness and the bile acids were determined by GLC after hydrolysis.

Extraction efficiency of sterols and bile acids from feces

In order to determine the extraction efficiency of sterols and bile acids from feces, a dose of 10 μCi/rat of [4-^{14}C]cholesterol (Amersham, England, 57.2 μCi/mmol) was administered once orally to intact male rats and the feces were collected daily for 2 weeks. The feces on days 1 and 11, which contained labeled sterols and bile acids, were extracted by procedures described previously (15, 16). Portions of the feces before extraction and the residues after extraction were combusted utilizing a Sample Oxidizer Model 400 (AMCO Co. Ltd., Tokyo, Japan) and the radioactivities were determined. As shown in **Table 1,** the extraction efficiency was 98% on day 1 and 93% on day 11. The efficiencies differed probably because the day-1 feces contained a larger amount of sterols while the day-11 feces contained a larger amount of bile acids.

RESULTS

As shown in **Table 2,** the body weight decreased after bile duct ligation but the liver weight increased progressively. In the present experiment, all the rats were killed by day 15, but the mean life span after bile duct ligation was 23.2 days in our rats. The concentration of serum cholesterol and phospholipids markedly increased by day 5, but the increases were reduced on day 10 and further on day 15. The liver cholesterol level remained unchanged but the phospholipid level decreased gradually.

TABLE 1. Extraction efficiency of sterols and bile acids from feces

Feces[a]	No. of Experiment	Before Extraction	After Extraction	Extraction Efficiency	BA/S[b]
		10^4 dpm/g dry feces			
Day 1	5	92.4 ± 2.91[c]	1.55 ± 0.428[c]	98.4%	1.61
Day 11	5	6.73 ± 0.149	0.51 ± 0.016	92.5%	6.84

[a] A 24-hour feces was collected after oral administration of 10 μCi/rat (ca. 300 g body weight) of [4-^{14}C]cholesterol.
[b] Bile acid/sterol ratio.
[c] Mean ± S.E.

The concentration of bile acids in serum and liver and the urinary excretion of bile acids markedly increased after the operation, while the fecal excretion of bile acids decreased. The bile acids in the urine were mostly found in the nonsulfate fraction. The amount of urine almost doubled by day 5 and remained high thereafter. The feces dry weight gradually decreased after bile duct ligation but the fecal sterol levels of both cholesterol and coprostanol did not decrease but rather increased on day 10. The diet intake was not recorded in the present experiment but, since the feces dry weight usually changed in parallel with the amount of diet, it was presumed that the diet intake decreased after bile duct ligation to a half of the control by day 15.

Table 3 shows the changes in the composition of bile acids in serum and liver. Before bile duct ligation, the serum bile acids consisted of cholic acid plus β-muricholic acid (about 50%), hyodeoxycholic acid (40%), and appreciable amounts of deoxycholic acid and α-muricholic acid. Chenodeoxycholic acid was not detectable. After bile duct ligation, cholic acid plus β-muricholic acid and chenodeoxycholic acid increased, but the other bile acids decreased. In the serum samples, cholic acid and β-muricholic acid were not determined separately, but the results for the liver (Table 3) and the urine (Table 4) indicated that most of the increase was in the β-muricholic acid level.

The liver bile acids in the control rats consisted of cholic acid (about 40%), β-muricholic acid (35%), hyodeoxycholic acid (10%), and minor amounts of deoxycholic acid, α- and ω-muricholic acids and chenodeoxycholic acid. After bile duct ligation, β-muricholic acid markedly increased but the other bile

TABLE 2. Changes in body weight, liver weight, serum and liver lipid levels, and urinary and fecal bile acid excretion after bile duct ligation in rats

	Control	Day-5	Day-10	Day-15
Body weight (g)	338 ± 5.0[a]	278 ± 4.7[a]	299 ± 10.5[a]	287 ± 6.8[a]
Liver weight (g)	9.1 ± 0.28	9.9 ± 0.34	12.1 ± 0.71	14.0 ± 0.42
Serum				
cholesterol (mg/100 ml)	69 ± 3.0	200 ± 3.1[b]	183 ± 6.4[b]	125 ± 19.1[b]
phospholipids (mg/100 ml)	137 ± 0.5	287 ± 8.4[b]	274 ± 15.5[b]	182 ± 24.9[b]
bile acids (μg/ml)	0.66[c]	80.6 ± 21.9	51.8 ± 3.34	49.0 ± 8.01
Liver				
cholesterol (mg/g)	4.1 ± 0.19	3.9 ± 0.06[a]	3.6 ± 0.19[a]	4.0 ± 0.11[a]
phospholipids (mg/g)	47.3 ± 0.77	43.1 ± 1.52	39.4 ± 0.85	39.0 ± 1.30
bile acids (mg/rat)	0.34 ± 0.04	3.04 ± 0.22	4.17 ± 0.50	3.56 ± 0.34
Urine				
amount (ml/day/rat)	7.2[c]	13.9 ± 2.7	15.6 ± 2.9	17.3 ± 1.5
nonsulfated bile acids (mg/day/rat)	0.005	10.5 ± 1.57	18.8 ± 2.27	20.3 ± 1.94
sulfated bile acids (mg/day/rat)	<0.001	0.1 ± 0.02	0.2 ± 0.03	0.2 ± 0.01
Feces				
dry weight (g/day/rat)	5.2 ± 0.13	4.2 ± 0.15[d]	3.6 ± 0.12[d]	2.6 ± 0.98
cholesterol (mg/day/rat)	5.4 ± 0.21	6.7 ± 1.34	7.0 ± 0.87	7.0 ± 1.02
coprostanol (mg/day/rat)	6.1 ± 0.42	7.1 ± 1.78	8.9 ± 1.13	4.7 ± 1.54
bile acids (mg/day/rat)	12.6 ± 0.52	1.0 ± 0.11[d]	1.3 ± 0.08[d]	0.5 ± 0.12[d]

[a] Mean ± S.E. in five rats.
[b] Mean ± S.E. in four rats.
[c] Samples from five rats were combined.
[d] Statistically significant ($P < 0.05$).

TABLE 3. Changes in serum and liver bile acid composition after bile duct ligation in rats

	Serum				Liver			
	Control	Day-5	Day-10	Day-15	Control	Day-5	Day-10	Day-15
	%				%			
Lithocholic acid	1.9[a]	0.4 ± 0.07[b]	0.3 ± 0.08	0.5 ± 0.15	0.9 ± 0.22	0.4 ± 0.09	0.3 ± 0.02	0.4 ± 0.07
Deoxycholic acid	3.7	nd	nd	nd	4.9 ± 0.68	0.1 ± 0.04	0.1 ± 0.03	0.4 ± 0.09
α-Muricholic acid	4.7	1.8 ± 0.16	2.3 ± 0.10	2.1 ± 0.09	2.6 ± 0.30	1.5 ± 0.10	2.0 ± 0.18	1.8 ± 0.21
Chenodeoxycholic acid	nd	3.6 ± 0.38	6.5 ± 0.80	5.8 ± 0.73	4.6 ± 0.63	2.8 ± 0.18	3.5 ± 0.56	3.5 ± 0.45
Hyodeoxycholic acid	38.8	0.5 ± 0.08	0.5 ± 0.07	0.4 ± 0.04	11.2 ± 1.16	1.9 ± 0.73	1.2 ± 0.09	1.2 ± 0.10
Ursodeoxycholic acid	nd	nd	nd	nd	nd	nd	nd	nd
Peak 8[c]	nd	nd	nd	nd	nd	nd	nd	nd
Cholic acid	50.9	93.7 ± 0.39	90.4 ± 1.30	91.1 ± 1.49	37.7 ± 1.03	11.8 ± 0.16	13.7 ± 0.11	32.7 ± 0.17
β-Muricholic acid					34.8 ± 0.94	79.2 ± 1.12	77.5 ± 0.66	58.2 ± 0.29
ω-Muricholic acid	nd	nd	nd	nd	3.2 ± 0.14	2.2 ± 0.30	1.6 ± 0.03	1.7 ± 0.10

[a] Samples from five rats were combined before analysis.
[b] Mean ± S.E. in five rats.
[c] Not identified.

acids decreased. Among them, the decrease of hyodeoxycholic acid was most predominant in both serum and liver.

Table 4 shows the changes in the urinary bile acid composition. Although the amounts of urinary bile acids in the control rats were very low as shown in Table 2, a striking difference was found in the composition of bile acids between the nonsulfates and the sulfates. Cholic acid, β-muricholic acid, and even chenodeoxycholic acid were excreted without sulfation but lithocholic acid, deoxycholic acid, and ursodeoxycholic acid were sulfated. After bile duct ligation, the percentage of lithocholic acid decreased and those of peak 8 and cholic acid plus β-muricholic acid increased in both nonsulfate and sulfate fractions. These changes seemed to be much influenced by the large increase of β-muricholic acid. Peak 8 was as not yet identified, but it seemed to be trihydroxycholanic acid having mono-unsaturated side chain from the preliminary GLC–MS analysis.

Fig. 1 shows the time course changes in urinary excretion of bile acids up to day 24, in comparison with the change in the total bile acids in the feces. In this experiment, the urinary bile acids were determined without solvolysis since most of the urinary bile acids were not sulfated (Table 2). As is obvious in Fig. 1, an enormous amount of bile acids of over 40 mg/day per rat was excreted into urine on day 1. It decreased to around 20 mg/day per rat on day 2 and remained at this level thereafter. These values were almost twice the amount of fecal bile acids before bile duct ligation (day 0). Much cholic acid was excreted on day 1 but the amount decreased on day 2 and reached the lowest value of 3.8 mg/day per rat on day 4. It remained at this level thereafter but showed a tendency to increase. On the other hand, the excretion of β-muricholic acid was 13.1 mg/day per rat on day 1 and, though it slightly decreased on day 2, remained at the level of 12 to 14 mg/day per rat thereafter for 2 weeks, then seemed to decrease but with much fluctuation.

DISCUSSION

In a steady state, the amount of fecal bile acids is assumed to represent the amount of bile acids synthesized daily. From this definition, our rats synthesized about 12 mg/rat of bile acids in a day. Similarly, the amount of urinary bile acids in bile duct-obstructed rats is considered to correspond to that of daily synthesis.

We determined urinary and fecal bile acids before and after bile duct ligation in rats and found that the

TABLE 4. Changes in urinary non-sulfated and sulfated bile acid composition after bile duct ligation in rats

	Non-sulfate				Sulfate			
	Control	Day-5	Day-10	Day-15	Control	Day-5	Day-10	Day-15
	%				%			
Lithocholic acid	14.9[a]	1.2 ± 0.16[b]	1.2 ± 0.10	0.4 ± 0.16	37.5	1.9 ± 0.25	2.7 ± 0.35	2.6 ± 0.37
Deoxycholic acid	4.3	0.8 ± 0.03	0.9 ± 0.09	0.5 ± 0.09	25.0	0.8 ± 0.21	0.9 ± 0.16	1.4 ± 0.13
α-Muricholic acid	nd	1.9 ± 0.07	1.7 ± 0.07	1.6 ± 0.18	nd	1.9 ± 0.32	0.9 ± 0.16	0.6 ± 0.13
Chenodeoxycholic acid	20.2	0.5 ± 0.04	0.5 ± 0.04	0.5 ± 0.04	nd	6.8 ± 1.96	2.1 ± 0.21	1.3 ± 0.15
Hyodeoxycholic acid	nd	1.4 ± 0.19	1.3 ± 0.07	0.9 ± 0.39	nd	2.5 ± 0.62	4.2 ± 0.52	6.7 ± 0.49
Ursodeoxycholic acid	nd	4.8 ± 0.12	4.4 ± 0.24	3.2 ± 1.44	37.5	2.3 ± 0.74	1.4 ± 0.32	1.7 ± 0.09
Peak 8[c]	nd	4.5 ± 0.51	3.4 ± 0.51	5.2 ± 1.43	nd	42.1 ± 2.44	37.8 ± 1.61	30.8 ± 2.24
Cholic acid	60.5	22.0 ± 1.25	22.3 ± 0.94	37.2 ± 4.40	nd	40.8 ± 3.86	50.1 ± 1.26	54.9 ± 1.84
β-Muricholic acid		62.9 ± 1.70	64.3 ± 1.48	50.3 ± 4.40				

[a] Samples from five rats were combined before analysis.
[b] Mean ± S.E. in five rats.
[c] Not identified.

level of urinary bile acids, which greatly exceeded the level of fecal bile acids before bile duct ligation, remained high for over 3 weeks until the death of the animals.

The enormous increase of urinary bile acids on day 1 (Fig. 1) is considered to show the loss of bile acids in the enterohepatic circulation as initially shown by Boyd et al. (1). The pool size of bile acids in rats is about 40 to 45 mg/rat and is mainly located in the intestine (16, 20). These bile acids are absorbed, enter the systemic blood flow, and are excreted into urine. Part of the bile acids may accumulate in blood and tissues after bile duct ligation, but the amounts are not very large. In this study, the concentration in the blood serum was about 100 μg/ml and therefore the total amount in blood was presumed to be about 1 to 2 mg. The maximum amount in the liver, which might include bile acids in the intrahepatic bile duct, was 4 mg/rat (Table 2).

The bile acid composition in the urine was different between day 1 and the following days. Cholic acid was the largest component, being about 50% in the day 1 urine, but decreased to below 5 mg/day per rat in the following days; while the amount of β-muricholic acid was 13.1 mg/day per rat on Day 1 and remained at this level thereafter. Boyd et al. (1), although they did not determine β-muricholic acid, reported the increase of cholic acid excretion into urine on the first day of bile duct ligation and a subsequent decline of its excretion. In the bile acid pool of intact male rats, cholic acid and β-muricholic acid are major components of about 45% and 28%, respectively. These values resembled those in the day-1 urine, suggesting that the enormous amount of bile acid in that urine came from the pool of bile acids.

After the excretion of pool bile acids, the urinary excretion of bile acids reflects the synthesis. As mentioned above, the urinary excretion of bile acids after bile duct ligation (20 mg/day per rat) exceeded the fecal excretion before bile duct ligation (12 mg/day per rat) by about 70%, suggesting that bile acid synthesis was elevated in the bile duct-ligated rats. This was consistent with the observation of Lutton et al. (11) and the fact that cholesterol 7α-hydroxylase activity in rats is elevated after bile duct ligation (9). The increase of the activity was confirmed in our rats (0.38 versus 1.39 nmol/hr per mg protein) (21).

Biliary obstruction produces an acceleration of hepatic cholesterol synthesis (2, 3, 6) and an increase in serum cholesterol level (1–4). Cooper and Ockner (3) showed that the elevation of plasma cholesterol level during obstruction was due to newly synthesized cholesterol. In our rats, the serum cholesterol level,

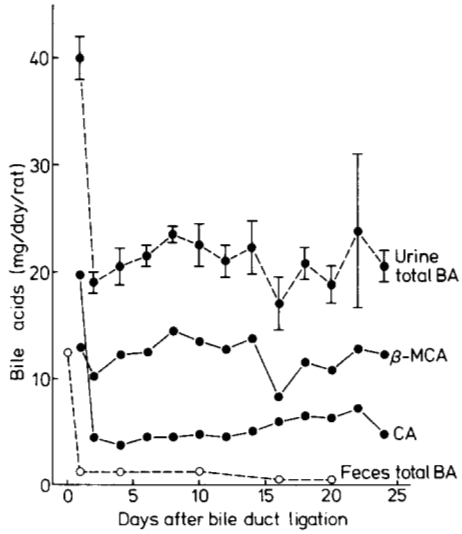

Fig. 1. Changes in urinary and fecal excretion of bile acids in rats after bile duct ligation. Each point and bar indicates mean ± SE in five rats.

though it declined gradually with time (Table 2), markedly increased and the acetate incorporation into cholesterol by liver slices increased about 7-fold (21). On the other hand, newly synthesized cholesterol is postulated to become a preferable substrate for cholesterol 7α-hydroxylase (22–25). Therefore, the acceleration of cholesterol synthesis is consistent with the increase in bile acid synthesis.

Some bile acids are known to inhibit bile acid synthesis in vivo (26–28). Since bile acid concentration in the liver increased during cholestasis (7), the bile acid synthesis was presumed to decrease as Redinger, Strasberg, and Small (29) demonstrated in rhesus monkeys, but actually increased according to the comparison of urinary and fecal excretion of bile acids. This conflict can be explained if we assume that *a*) β-muricholic acid which becomes the major bile acid after bile duct ligation (7) is ineffective in inhibiting bile acid synthesis, or *b*) the increase in the liver bile acid levels is produced by the increase of bile remaining in the intrahepatic bile duct but not in hepatocytes. Only 0.2 ml bile would be responsible for the rise of bile acid levels by 2 to 3 mg. However, no significant experimental evidence is available to support these speculations. In addition, Hayakawa (30) has reported that bile acids are degraded to acidic substances of smaller molecules by soil micro-organisms. This has not yet been studied with intestinal bacteria, but if such a degradation reaction takes place in the intestine, the amount of bile acids in the feces should be lower than that of those synthesized. Although the extraction efficiency from feces was over 90% (Table 1), we could not quantify such small molecule substances by our GLC analysis.

Redinger et al. (29) reported entirely opposite results; they showed that bile acid synthesis was suppressed during biliary obstruction in rhesus monkeys. It is not known whether the difference is species-related or not, but the bile acid profiles of these animals are much different. Since β-muricholic acid, which markedly increased during obstruction, is a bile acid found only in rats and mice and not in the other species, the formation of this species-specific bile acid may be related to the difference between rats and monkeys. The duration of obstruction would be another factor to bring about such a difference. Redinger et al. (29) examined the changes during acute obstruction, while the present experiment demonstrated those during chronic obstruction. If the duration of ligation has a role in this problem, it is presumed that bile acid synthesis is suppressed shortly after the obstruction but then increases, probably with the onset of an alternative pathway of bile acid synthesis. It is likely that bile acids are synthesized through an alternative pathway in cholestasis (31). The fact that the bile acid composition in the bile duct-ligated rats is different from that in intact animals may support the presence of the alternative pathway.

Bile acid synthesis increased after bile duct ligation but the increase was far less than that observed in bile fistula animals (32), suggesting that some inhibition of bile acid synthesis remained in biliary-obstructed animals. The increase of bile acid synthesis in bile fistula animals is due to the depression of feedback inhibition (33), but the mechanism for the increase during biliary obstruction is not known, though we assume that the increase is brought by the onset of the alternative pathway of bile acid synthesis, as mentioned above.

From the analysis of urinary bile acid composition, we concluded that the formation of β-muricholic acid was predominant, and the ratio of synthesized cholic acid and β-muricholic acid was about 1:3. Since β-muricholic acid is formed from chenodeoxycholic acid (8), the above ratio may indicate the ratio of synthesized cholic acid and chenodeoxycholic acid. The increase in the synthesis of β-muricholic acid has already been reported (6–8). The increase is consistent with the increase of hepatic 6β-hydroxylation activity (9, 10). Gustafsson (10) has demonstrated that microsomal 26-hydroxylation decreases but mitochondrial 26-hydroxylation and microsomal 7α- and 6β-hydroxylations increase after bile duct ligation, and he concluded that chenodeoxycholic acid synthesis increases but cholic acid synthesis decreases in cholestatic rats, based on the assumption that chenodeoxycholic acid is formed by the pathway starting from mitochondrial 26-hydroxylation of cholesterol.

In our rats, 4 to 5 mg of cholic acid was excreted daily into urine from day 2 through day 24. The value was very comparable to the amount of fecal deoxycholic acid in intact rats, which amounted to about 4 mg/day/rat (16). Therefore we concluded that if bile acids were not decomposed by intestinal bacteria, the synthesis of cholic acid was not depressed and that of chenodeoxycholic acid, which was further metabolized to β-muricholic acid, was much enhanced in bile duct-ligated rats.

Urinary bile acids are mostly sulfated in man (19) but not in rats (Tables 2 and 3). β-Muricholic acid which actually increased after bile duct ligation in rats is postulated to be nontoxic and therefore safely excreted into urine without sulfation (7), but no data on the biological effect of this bile acid have been reported.

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REFERENCES

1. Boyd, G. S., M. A. Eastwood, and N. MacLean. 1966. Bile acids in the rat: studies in experimental occlusion of the bile duct. *J. Lipid Res.* **7:** 83–94.
2. Harry, D. S., M. Dini, and N. McIntyre. 1973. Effect of cholesterol feeding and biliary obstruction on hepatic cholesterol biosynthesis in the rat. *Biochim. Biophys. Acta.* **296:** 209–220.
3. Cooper, A. D., and R. K. Ockner. 1974. Studies of hepatic cholesterol synthesis in experimental acute biliary obstruction. *Gastroenterology.* **66:** 586–595.
4. Adler, R. D., F -J. Wannagat, and R. K. Ockner. 1977. Bile secretion in selective biliary obstruction. Adaptation of taurocholate transport maximum to increased secretory load in the rat. *Gastroenterology.* **73:** 129–136.
5. Parl, F., W. H. Gutstein, A. F. D'Aguillo, and A. Baez. 1975. Endothelial injury. Association with elevations of serum bile acid and cholesterol concentration in biliary-obstructed rats. *Atherosclerosis.* **21:** 135–146.
6. Weis, H. J., and J. M. Dietschy. 1971. Presence of an intact cholesterol feedback mechanism in the liver in biliary stasis. *Gastroenterology.* **61:** 77–84.
7. Greim, H., D. Trulzsch, J. Roboz, K. Dressler, P. Czygan, F. Hutterer, F. Schaffner, and H. Popper. 1972. Mechanism of cholestasis. 5. Bile acids in normal rat livers and in those after bile duct ligation. *Gastroenterology.* **63:** 837–845.
8. Mahowald, T. A., J. T. Matschiner, S. L. Hsia, E. A. Doisy, Jr., W. H. Elliot, and E. A. Doisy. 1957. Bile acids. III. Acid I; The principal bile acid in urine of surgically jaundiced rats. *J. Biol. Chem.* **225:** 795–802.
9. Danielsson, H. 1973. Effect of biliary obstruction of formation and metabolism of bile acids in rat. *Steroids.* **22:** 567–579.
10. Gustafsson, J. 1978. Effect of biliary obstruction on 26-hydroxylation of C_{27}-steroids in bile acid synthesis. *J. Lipid Res.* **19:** 237–243.
11. Lutton, C. L., D. Mathé, and F. Chevallier. 1973. Vittesses des processus de renouvellement du cholestérol contenu dans son espace de transfert, chez le rat. VI. Influence de la ligature du cholédoque et de l'ingestion d'acides biliares ou de cholestyramine. *Biochim. Biophys. Acta.* **306:** 483–496.
12. Uchida, K., and T. Miyake. 1961. Effect of various steroids on the plasma lipid levels of castrated male rats. *Annu. Rep. Shionogi Res. Lab.* **11:** 119–132.
13. Uchida, K., M. Kadowaki, and T. Miyake. 1965. Failure of estrogen to produce hypocholesterolemic effect in immature rats. *Endocrinology.* **76:** 766–770.
14. Gomori, G. 1942. A modification of the colorimetric phosphorus determination for use with the photoelectric colorimeter. *J. Lab. Clin. Med.* **27:** 955–960.
15. Uchida, K., Y. Nomura, M. Kadowaki, N. Takeuchi, and Y. Yamamura. 1977. Effect of dietary cholesterol on cholesterol and bile acid metabolism in rats. *Jpn. J. Pharmacol.* **27:** 193–204.
16. Uchida, K., Y. Nomura, M. Kadowaki, H. Takase, K. Takano, and N. Takeuchi. 1978. Age-related changes in cholesterol and bile acid metabolism in rats. *J. Lipid Res.* **19:** 544–552.
17. Sandberg, D. H., J. Sjövall, K. Sjövall, and D. A. Turner. 1965. Measurement of human serum bile acids by gas–liquid chromatography. *J. Lipid Res.* **6:** 182–192.
18. Makino, I., K. Shinozaki, S. Nakagawa, and K. Mashimo. 1974. Measurement of sulfated and nonsulfated bile acids in human serum and urine. *J. Lipid Res.* **15:** 132–138.
19. Makino, I., H. Hashimoto, K. Shinozaki, K. Yoshino, and S. Nakagawa. 1975. Sulfated and nonsulfated bile acids in urine, serum and bile of patients with hepatobiliary disease. *Gastroenterology.* **68:** 545–553.
20. Uchida, K., I. Okuno, H. Takase, Y. Nomura, M. Kadowaki, and N. Takeuchi. 1978. Distribution of bile acids in rats. *Lipids.* **13:** 42–48.
21. Kadowaki, M., K. Uchida, N. Takeuchi, and T. Kinugasa. 1979. Changes in bile acid metabolism in bile duct ligated rats. *Proc. Jpn. Cont. Biochem. Lipids.* **21:** 36–39 (in Japanese)
22. Mackinnon, M., and F. Simon. 1975. Pharmacological reversal of cholestasis-associated decrease in hepatic cytochrome P-450. *Biochem. Pharmacol.* **24:** 748–749.
23. Balasubramaniam, S., K. A. Mitropoulos, and N. B. Myant. 1973. Evidence for the compartmentation of cholesterol in rat-liver microsomes. *Eur. J. Biochem.* **34:** 77–83.
24. Mitropoulos, K. A., N. B. Myant, G. F. Gibbons, S. Balasubramaniam, and B. E. A. Reeves. 1974. Cholesterol precursor pools for the synthesis of cholic and chenodeoxycholic acids in rats. *J. Biol. Chem.* **249:** 6052–6056.
25. Björkhem, I., and H. Danielsson. 1975. 7α-Hydroxylation of exogenous and endogenous cholesterol in rat-liver microsomes. *Eur. J. Biochem.* **53:** 63–70.
26. Danielsson, H. 1973. Influence of dietary bile acids on formation of bile acids in rat. *Steroids.* **22:** 667–676.
27. Shefer, S., S. Hauser, V. Lapar, and E. H. Mosbach. 1973. Regulatory effects of sterols and bile acids on hepatic 3-hydroxy-3-methylglutaryl CoA reductase and cholesterol 7α-hydroxylase in the rat. *J. Lipid Res.* **14:** 573–580.
28. Danielsson, H., and G. Johansson. 1974. Effects of long term feeding of chenodeoxycholic acid on biosynthesis and metabolism of bile acids in the rat. *Gastroenterology.* **67:** 126–134.
29. Redinger, R. N., S. M. Strasberg, and D. M. Small. 1974. Primate biliary physiology. IX. Effects of acute biliary obstruction on biliary lipid metabolism in the monkey. *Am. J. Physiol.* **226:** 776–783.
30. Hayakawa, S. 1973. Microbiological transformation of bile acids. *Adv. Lipid Res.* **11:** 143–192.
31. Makino, I., J. Sjövall, A. Norman, and B. Strandvik. 1971. Excretion of 3β-hydroxy-5-cholenoic acid and 3α-hydroxy-5α-cholanoic acids in urine of infants with biliary atresia. *Fed. Eur. Biochem. Soc. Lett.* **15:** 161–164.
32. Eriksson, S. 1957. Biliary excretion of bile acids and cholesterol in bile fistula rats. *Proc. Soc. Exp. Biol. Med.* **94:** 578–582.
33. Shefer, S., S. Hauser, I. Bekersky, and E. H. Mosbach. 1969. Feedback regulation of bile acid biosynthesis in the rat. *J. Lipid Res.* **10:** 646–655.