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The effect of biliary drainage upon the synthesis of cholesterol in the liver*

N. B. Myant and Howard A. Eder

Medical Research Council, Experimental Radiopathology Research Unit, Hammersmith Hospital, London, W. 12, England; and Departments of Medicine and Radiology, Albert Einstein College of Medicine, Yeshiva University, New York 61, N.Y.

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SUMMARY

Synthesis of cholesterol and fatty acids was measured *in vitro* in the livers of rats from which the bile had been drained for various periods of time, and in control rats with normal enterohepatic circulation. Cholic and chenodeoxycholic acids were excreted at an initial rate of more than 5 mg per hour. The rate fell rapidly to a minimum, followed by a secondary rise beginning between the twentieth and thirtieth hour after cannulation. After draining the bile for 12 hours or longer, there was an increase in the synthesis of cholesterol from acetate, but not from mevalonate, and a slight depression of fatty acid synthesis. Liver cholesterol content was unchanged. Although these data suggest that the increased production of bile acids may be secondary to an increase in cholesterol synthesis, other explanations cannot be excluded.

When the common bile duct of a rat is drained continuously, the rate of excretion of bile acids decreases during the first day (1) and then increases (2), reaching a plateau by about the third day after cannulation. Thompson and Vars (2) assumed that the early fall in the rate of excretion was due to temporary damage to the liver caused by the operation, and that the plateau value indicated the normal rate of bile acid formation in the intact animal. Eriksson (3), however, has put forward evidence that the normal rate is represented by the minimum value observed toward the end of the first day, and that the plateau value represents an abnormally high rate of formation, brought about by the continual removal of bile acids through the cannula.

All the bile acids are derived from cholesterol. Moreover, oxidation to bile acids is the most important pathway for the elimination of cholesterol in rats (4). A marked increase in bile-acid formation, such as that supposed by Eriksson to occur after cannulation of the bile duct, should therefore be accompanied by an increase in the synthesis of cholesterol, provided that there is no corresponding decrease in the cholesterol content of the liver.

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In this paper we describe experiments designed to test whether there is an increase in the synthesis of cholesterol in rat liver when the bile duct is cannulated. Another object of this work was to study the time relationship between changes in bile acid excretion and cholesterol synthesis under these conditions.

METHODS

Two male albino rats of the Wistar strain (Carworth Farms), paired by weight (about 300 g), were used for each experiment. In one of each pair, the bile duct was exposed under ether anesthesia and cannulated with a fine polyethylene tube. To prevent the cannula from being pulled out, the outer end of the tube was inserted between the muscle and skin of the abdominal wall and brought to the outside through an incision in the skin of the back. After closure of the muscle and skin in separate layers, the rat was placed in a restraining cage of wire netting. Bile was collected continuously over $2^1/_2$ hour intervals in centrifuge tubes arranged in an automatic fraction collector, each tube containing 2 ml of ethanol to sterilize the bile. The other rat, used as a control, was anesthetized and the bile duct was exposed but not cannulated. After closure of the abdominal wound, it was placed in a restraining cage for the same length of time as the rat whose bile duct was

cannulated. At the end of the period of bile collection, both rats were bled from the heart and then killed by a blow on the head. The livers were removed immediately and put into ice-cold buffer solution. All the animals were given food (Rockland Rat Diet Pellets) and water ad libitum throughout the experiment. Both groups of animals lost about 5% of their total body weight per day during the experimental period. The weight losses in the control and treated animals did not differ significantly.

Preparation and Subsequent Treatment of Slices. Slices were cut by hand with a Stadie-Riggs tissue slicer (5) and were incubated in duplicate for 3 hours in 10 ml of buffer solution (6) at 37°. The incubation flask contained either sodium acetate-1-C^{14} (40 μmoles + 3 to 5 μc/g tissue) or mevalonic acid-2-C^{14} (1.5 to 4.0 μmoles + 0.10 to 0.15 μc/g tissue). At the end of the incubation, 10 ml of ethanol and 1 ml of 33% KOH were added to each flask. The flasks were then left overnight on a water bath. Cholesterol was extracted from the hydrolyzate and precipitated as the digitonide, essentially according to the method of Gould *et al.* (7). After extraction of cholesterol, the hydrolyzate was acidified with 40% H_2SO_4 and the fatty acids were extracted into redistilled light petroleum ether. The petroleum ether was washed once with 0.1% acetic acid and twice with water, and then evaporated to dryness.

Measurement of Radioactivity. In some experiments radioactivity was measured with a mica-window Geiger counter (EHM 2/S, G.E.C. Research Laboratories). The precipitates of digitonide were prepared for assay by filtering them onto perforated polyethylene planchets. The fatty acids were dissolved in 3 ml of redistilled chloroform and neutralized with 0.2 N ethanolic NaOH, using phenolphthalein as indicator. The soaps were then evaporated to dryness, dissolved in 0.5 ml of water, and transferred to planchets. The water was evaporated before measurement of the radioactivity. The yield of cholesterol digitonide or total soap was found by weighing the planchet before and after plating out the sample. The results were expressed in microcurie per gram by correcting the observed counting rate to that of an "infinitely thick" sample by means of a standard self-absorption curve, and comparing this with the counting rate given by an "infinitely thick" disk of poly(methyl-C^{14})methacrylate of known specific activity. In other experiments the precipitates of digitonide were washed and dried and then dissolved in the dioxane: phosphoric acid: absolute ethanol reagent of Kabara (8). Aliquots of these were added to a counting vial and 13 ml of the phosphor solution [0.4% phenylbiphenyloxadiazole-1,3,4(PBD) and 0.01% 1,4 bis-2(phenyloxazole)-benzene (POPOP) in toluene] was added and the sample counted in a Packard Tri-Carb liquid scintillation spectrometer. Cholesterol was determined in another aliquot using a modified Liebermann-Burchard reagent. The results were expressed in microcurie per gram by referring the observed counting rates to that given by a standard sample of known activity. The fatty acids were dissolved in 100 ml of heptane and 1-ml portions were taken for measurement of titratable acidity by the procedure recommended by Gordon (9). Five-ml samples of the heptane solution were mixed with 10 ml of the liquid phosphor in counting vials and their radioactivity was measured. The same volumes of heptane and liquid phosphor were used for measuring the background counting rate. In order to convert the results from microcurie per milliliter titratable acidity to microcurie per gram fatty acids, it was assumed that 1 equivalent of fatty acids $\cong$ 280 g.

Estimation of Bile Acids. The tubes containing the bile samples were centrifuged to remove sediment and the supernatant was decanted into 25-ml Erlenmeyer flasks. The alcohol was evaporated by heating the flasks on a steam bath for 2 hours. The procedure of Mosbach *et al.* (10) was followed for hydrolysis, extraction, and spectrophotometric estimation of cholic and chenodeoxycholic acids. When known amounts of cholic and chenodeoxycholic acids were taken through the whole procedure, including hydrolysis and extraction into ether, about 80% of each acid was recovered.

The total cholesterol in liver and plasma was determined in duplicate samples by the method of Abell *et al.* (11). In a few cases total cholesterol in the liver was determined from the cholesterol digitonide prepared during the assay for radioactivity (8).

RESULTS

Excretion of Bile and Bile Acids. The initial rate of flow of bile, estimated from the first sample obtained after insertion of the cannula, was about 1.0 ml/hour. During the first few hours the rate fell to about 0.5 ml/hour and then remained more or less constant as long as the cannula remained in position (Fig. 1).

The initial rate of excretion of cholic acid averaged 5.1 ± 0.44 mg/hour, decreasing rapidly during the first few hours to a minimum of 0.34 ± 0.08 mg/hour. The fall was usually followed by a secondary rise beginning some time between the twentieth and thirtieth hour after cannulation. During this period the rate rose to four or five times the minimum rate, but never reached values as high as those observed immediately after insertion of the cannula (Fig. 1).

The initial rate of excretion of chenodeoxycholic acid was 1.1 ± 0.08 mg/hour. Thereafter the rate followed a curve similar to that of cholic acid, but at a correspondingly lower level. The rate at the lowest part of the curve averaged 0.12 ± 0.03 mg/hour.

In two out of nine experiments continued for more than 20 hours there was no secondary rise in the rate of excretion of bile acids, although the rate of flow of bile continued at the normal rate.

Synthesis of Cholesterol. At intervals varying from 3 to 50 hours after cannulation of the bile duct, the treated rat and its control were killed, and the rate of synthesis of cholesterol from acetate and mevalonate compared in liver slices from the two animals (Table 1). The result of each experiment was expressed as the ratio of the specific activity in the cholesterol from the treated rat to that from the control rat (Fig. 2).

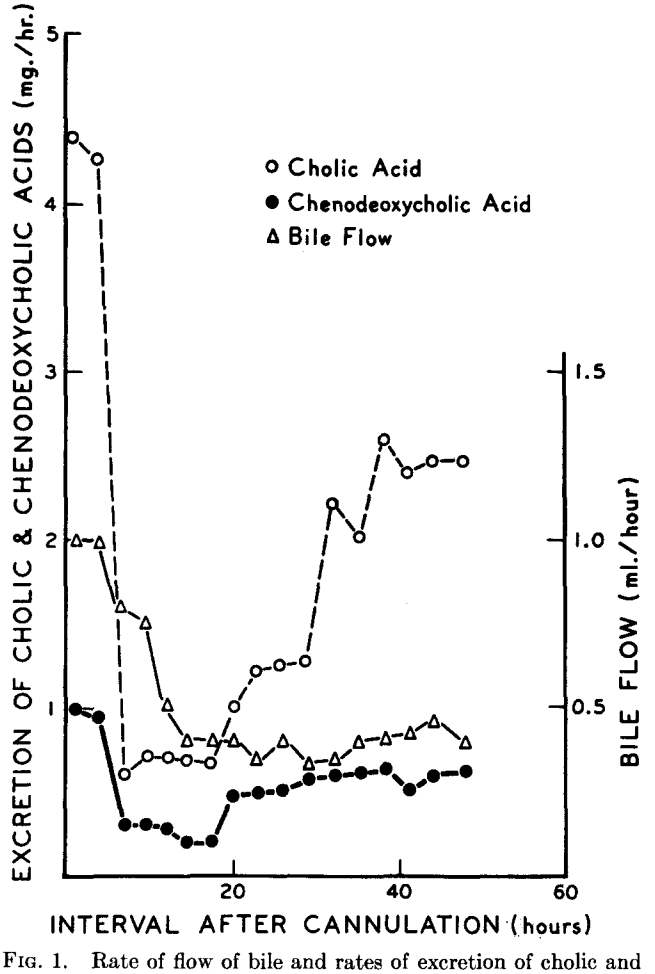

FIG. 1. Rate of flow of bile and rates of excretion of cholic and chenodeoxycholic acids from bile fistula of rat. Size of cholic acid pool in this rat estimated as follows: 25.8 mg cholic acid was excreted during 8-hour fall to minimum rate (0.7 mg/hour). If minimum rate assumed equal to rate of new formation during first 8 hours, 5.6 mg of the 25.8 mg came from new formation, therefore 20.2 mg came from preformed pool.

TABLE 1. *In Vitro* SYNTHESIS OF CHOLESTEROL AND FATTY ACIDS BY LIVERS OF TREATED (T) AND CONTROL (C) ANIMALS AT VARIOUS INTERVALS AFTER CANNULATION OF THE BILE DUCTS

	Interval	Cholesterol		Fatty Acids
		From Acetate	From Mevalonic Acid	From Acetate
	hours	*μc/g*	*μc/g*	*μc/g*
T	3	0.51	2.37	0.186
C		1.08	3.31	0.407
T	4	1.09	5.65	0.096
C		1.76	7.24	0.164
T	4	0.87	4.99	0.057
C		1.30	6.66	0.225
T	6	0.60	1.84	
C		0.98	2.79	
T	6	0.34	5.30	0.129
C		0.46	4.20	0.072
T	8	2.52	3.47	
C		0.98	2.62	
T	12	0.65	5.64	0.169
C		0.91	6.55	0.176
T	12	1.91	3.50	0.610
C		0.82	3.36	0.741
T	12	1.18	3.26	0.077
C		0.87	3.09	0.123
T	12	1.85	1.72	0.085
C		1.11	1.35	0.073
T	18	3.16	5.10	0.286
C		1.08	5.64	0.747
T	18	1.02	1.67	0.152
C		0.58	2.58	0.376
T	18	2.00	2.38	1.554
C		0.62	1.86	2.851
T	18	1.13	5.88	1.380
C		1.10	7.15	1.459
T	24	2.64	9.00	0.258
C		0.96	6.93	0.525
T	26	0.22		
C		0.16		
T	28	3.71	8.49	0.874
C		0.96	7.06	0.551
T	28	1.09	1.55	0.134
C		0.53	1.61	0.134
T	30	1.84	0.92	0.288
C		1.59	0.90	1.297
T	47	1.87	0.83	0.205
C		0.55	0.74	0.405
T	47	1.27	2.63	0.169
C		0.37	1.98	0.119
T	50	1.03	1.03	0.486
C		1.20	3.38	0.648
T	50	1.15	1.79	
C		0.31	2.77	

Results expressed as specific activity of lipid isolated at the end of incubation. Each value is the average from paired incubations.

When the period of cannulation was longer than 12 hours, the specific activity of the cholesterol derived from acetate in the treated animals was over twice that in the control animals in eight of the thirteen experiments (mean treated/control ratio 2.43 ± 0.29). After cannulation for 12 hours there was a slight increase (the mean treated/control ratio being 1.51 ± 0.39). After shorter intervals, the results were rather variable. In one experiment at 8 hours there was an increase, but in five experiments at earlier intervals there was a slight fall.

There are not enough observations to show exactly when the rise in cholesterol synthesis occurs. However, Figure 2 suggests that it probably begins by the eighteenth hour and possibly by the twelfth, that is, before the secondary rise in bile acid excretion. This point is illustrated by an experiment shown in Figure 3 and Table 2. Two rats were killed at the eighteenth hour after cannulation and before there was any rise in bile acid excretion, yet the specific activity of the cholesterol from the treated rat was more than three times that in the control slices.

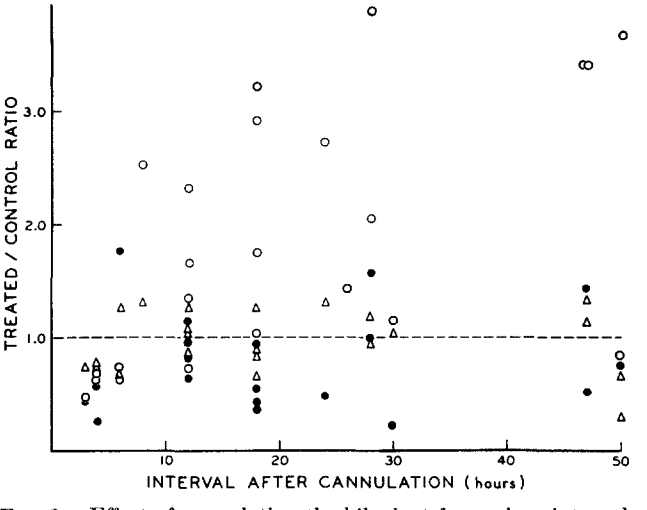

FIG. 2. Effect of cannulating the bile duct for various intervals upon the synthesis of cholesterol (○) and fatty acids (●) from acetate and of cholesterol (△) from mevalonic acid. Each value shows ratio of specific activity of cholesterol or fatty acids in treated liver to that in control liver.

When mevalonate was used as the precursor of cholesterol, the specific activity in the treated rats was, on the average, less than that in the controls, when all the results were considered together (treated/control ratio: 0.97), and when the results of experiments continued for more than 12 hours were considered separately (treated/control ratio: 0.96). In neither case was the difference between the values from treated and control rats statistically significant.

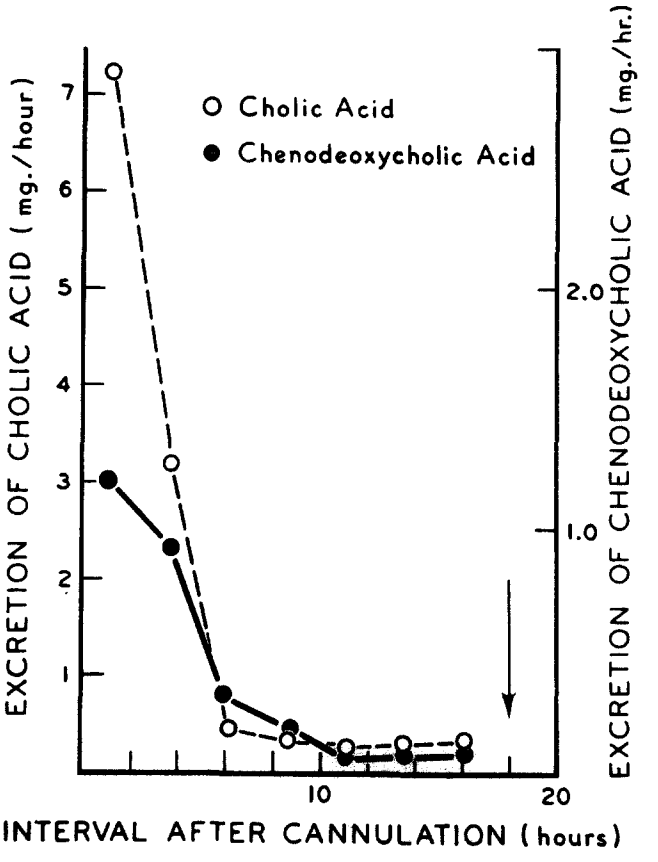

FIG. 3. Rates of excretion of cholic and chenodeoxycholic acids from rat with bile duct cannulated for 18 hours. Arrow shows time at which livers of treated and control rat removed for measurement of synthesis (see Table 2).

Synthesis of Fatty Acids. When the results of all experiments were considered together, there was a slight depression of fatty acid synthesis in the treated livers (treated/control ratio: 0.79 ± 0.10; Table 1 and Fig. 2). The difference between the values from treated and control livers was statistically significant ($0.02 < p < 0.05$).

TABLE 2. *In Vitro* SYNTHESIS OF CHOLESTEROL AND FATTY ACIDS BY LIVERS REMOVED 18 HOURS AFTER CANNULATION OF THE BILE DUCTS

Rat	Cholesterol		Fatty Acids
	From Acetate	From Mevalonic Acid	From Acetate
	μc/g	μc/g	μc/g
Treated (T)	2 17	2.26	1.55
	1.83	2.49	
Control (C)	0.74	1.71	3.00
	0.50	2.00	2.70
T/C	3.22	1.28	0.55

Results expressed as specific activity of lipid isolated at the end of incubation.

Liver and Plasma Cholesterol. The average concentration of total cholesterol in the livers of the treated animals (186.7 ± 16.7 mg/100 g wet weight) was a little lower than that in the control livers (189.2 ± 10.4 mg/100 g wet weight), but the difference was not statistically significant. The average concentration of total cholesterol in the plasma from the treated animals (96.8 ± 8.3 mg/100 ml) was higher than that from the controls (88.5 ± 6.1 mg/100 ml). The difference was not statistically significant.

DISCUSSION

In these experiments the initial rate of excretion of cholic and chenodeoxycholic acids averaged 6.2 mg/hour. This is more than twice as high as the initial rate of excretion of bile salts observed by Eriksson (3). The difference may be due, at least in part, to the fact that in our experiments the samples were collected over shorter intervals of time. Eriksson (3) has pointed out that the first phase of excretion can hardly represent the normal rate of formation of bile acids in the intact animal, since the amount collected during the first few hours after cannulation is much greater than the amount formed during the same period, as estimated from the total exchangeable pool and the turnover time of the pool. In our rats, for instance, the initial rate of excretion of cholic and chenodeoxycholic acids is equivalent to about 150 mg/day, whereas Eriksson (12) has estimated that the amount of bile acid formed per day in the intact rat is only 2 to 3 mg/100 g rat.

It is more probable that the first samples of bile collected from the cannula come mainly from a store already present in the bile ducts, intestine, liver, and portal circulation, and that it is only when this store has been drained away that the rate of excretion truly represents the rate of formation. On this view it is possible to estimate the amount of bile acid in the preformed store, from the total collected during the early phase of excretion, less the amount newly formed during the same period, the latter being calculated on the assumption that formation occurs at a constant rate equal to the minimum observed (Fig. 1). Eriksson (3) has shown that the amount so estimated agrees roughly with the amount estimated from the extent of dilution of a labeled sample of bile acid given to a rat. This may be taken as further evidence in favor of the above interpretation of the early phase of the excretion curve. When this method of calculation was applied to our results, the amounts of cholic and chenodeoxycholic acids in the preformed store were found to average 23 ± 2.3 and 5.2 ± 1.3 mg, respectively. This agrees with the more recent results of Eriksson (12), who obtained a value of 8 mg conjugated bile acids/100 g rat.

After the initial fall in bile acid excretion, there was a second phase, lasting a few hours, during which excretion continued at a more or less constant rate, as shown in Figures 1 and 3. At this time the average rate of excretion of cholic and chenodeoxycholic acids was equivalent to 3.7 mg/day/100 g rat. This is so close to Eriksson's estimate (12) that it seems reasonable to assume that the rate of excretion observed during the second phase represents the normal rate of formation of bile acids. If this is the case, the high rates observed during the second day must reflect an increase in bile acid formation above the normal level. On this interpretation of the excretion curve, it also follows that the rise in bile acid formation does not begin until the bile duct has been drained for 20 hours or more.

The rise in cholesterol synthesis from acetate, with little or no change in synthesis from mevalonate, suggests that the increase in the treated rats is due to stimulation of a rate-limiting step between acetate and mevalonate. The existence of such a rate-limiting step in this part of the pathway from acetate to cholesterol has also been inferred by other workers who observed changes in cholesterol synthesis in the rat's liver by X-irradiation (13), injections of Triton WR-1339 (14), treatment with thyroxine (6), and cholesterol feeding (15). The observations of Bucher *et al.* (16) suggest that the rate-limiting step is the conversion of hydroxy methyl glutaryl coenzyme A to mevalonic acid. Our finding that there is a rise in cholesterol synthesis in the liver after cannulation of the bile duct is in agreement with the observations of Bergström and Gloor.[1] These workers estimated the rate of synthesis of cholesterol *in vivo* by measuring the incorporation of an intraperitoneal injection of C^{14}-acetate into the plasma cholesterol. They found an increase in cholesterol synthesis 3 to 4 days after cannulation of the bile duct.

Bergström and Danielsson (17) have shown that infusion of bile salts into the distal portion of the severed bile duct prevents the secondary rise in the bile acid excretion that occurs in a rat whose bile duct has been cannulated. They concluded that the concentration of bile acids supplied to the liver via the portal blood influences the rate of formation of bile acids in the liver and that the increase in bile acid excretion in the cannulated rat is due to release from this control. Control of the rate of formation of bile acids might be exerted upon the conversion of cholesterol to bile acids, or it might act primarily upon the synthesis of choles-

[1] Personal communication.

terol leading secondarily to an increase in the production of bile acids. Our results, suggesting that the rise in cholesterol synthesis begins before the rise in bile acid excretion, are consistent with the latter alternative. Against it, however, Gilon and Eder[2] have shown that in a cholesterol-fed rat whose bile duct was cannulated, a secondary rise in bile acid excretion occurred at about 20 hours, although cholesterol synthesis from acetate was negligible. This observation might suggest that primary control of bile acid formation is exerted upon the conversion of cholesterol to bile acids. Control at this level, however, does not explain the early rise in cholesterol synthesis in cannulated rats. Possibly the rise is due to loss of cholesterol in the bile, though this is not supported by our finding that the concentration of cholesterol in the livers of the cannulated rats is as high as that in the controls. In conclusion, our results do not exclude the possibility that control is exerted at more than one step in the formation of bile acids.

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[2] Unpublished experiments.

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