Regulation of biliary cholesterol output in the rat: dissociation from the rate of hepatic cholesterol synthesis, the size of the hepatic cholesteryl ester pool, and the hepatic uptake of chylomicron cholesterol

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Abstract These studies were designed to determine the importance of the rate of hepatic cholesterol synthesis, the size of the hepatic cholesteryl ester pool, the amount of chylomicron cholesterol reaching the liver, and the rate of bile acid transport into bile as determinants of the rate of biliary cholesterol output. Female rats that had been subjected to diurnal light cycling, fasting for 48 hr, intravenous administration of chylomicrons, and diets containing either cholestyramine, cholesterol, or bile acid underwent total biliary diversion for 2 hr. The animals were then killed and the rates of hepatic cholesterol synthesis and levels of hepatic esterified cholesterol were measured along with biliary lipid concentrations. Despite a 1000-fold variation in the rate of hepatic cholesterogenesis and a 100-fold variation in the levels of cholesteryl esters, the output and molar percentage of cholesterol in bile remained essentially constant with the exception of an approximate doubling in the output of cholesterol, as well as of bile acid and phospholipid in those animals fed bile acid. However, in this latter group the molar percentage of each component was unchanged. The administration of a bolus of chylomicrons did not alter output or molar percentage of cholesterol. Total biliary diversion for 36 hr and bile acid infusion were used to markedly vary the rate of biliary bile acid output. Cholesterol and phospholipid output remained tightly coupled to bile acid output over almost a 40-fold range. In other experiments it was shown that biliary cholesterol output could be driven by bile acid infusion to a similar extent in rats in which the rate of hepatic cholesterogenesis had been varied over a 26-fold range. It was concluded that the rate of hepatic cholesterol synthesis, the level of hepatic cholesteryl esters, and the amount of cholesterol absorbed from the diet play no role in determining the rate of biliary cholesterol secretion, at least in this species.—**Turley, S. D., and J. M. Dietschy.** Regulation of biliary cholesterol output in the rat: dissociation from the rate of hepatic cholesterol synthesis, the size of the hepatic cholesteryl ester pool, and the hepatic uptake of chylomicron cholesterol. *J. Lipid Res.* 1979. **20:** 923–934.

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Under normal physiological circumstances, cholesterol is present in bile in the form of mixed micelles, the other components of which are bile acid and phospholipid. Under pathological conditions, where the proportion of cholesterol increases so that it exceeds the solubilizing capacity of the bile acid and phospholipid, the bile becomes saturated with cholesterol, predisposing to the formation of gallstones (1–4). Although the physicochemical interactions between cholesterol, bile acid, and phospholipid have been examined in detail (1, 5), the transport mechanisms whereby these lipids enter bile and, more particularly, the factors that ultimately dictate the rate of biliary cholesterol output are poorly understood (6). It is, nevertheless, clear that there is tight coupling of the biliary secretion rates of all three lipids (7–9).

The production of saturated or lithogenic bile has been attributed to an elevated rate of hepatic cholesterol synthesis. This view is based on the finding that during the administration of either chenodeoxycholic acid or ursodeoxycholic acid to patients with gallstones there is a dramatic reduction in the biliary cholesterol saturation index and a fall in the level of activity of 3-hydroxy-3-methylglutaryl CoA reductase (HMG CoA reductase) in the liver as measured in biopsy specimens (10–12). Chenodeoxycholic acid has similar effects in hamsters that have been fed a lithogenic diet (13). However, during bile acid feeding

Abbreviation: HMG, 3-hydroxy-3-methylglutaryl.

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there is actually a diminished requirement for the synthesis of cholesterol in the liver, since less sterol is converted to bile acid, and there may also be increased cholesterol absorption from the gastrointestinal tract (14). Thus, a decrease in the rate of hepatic cholesterol synthesis would be expected, but this need not necessarily be the cause of decreased biliary cholesterol secretion.

Studies in the rat and man in which the specific activity of sterol in the blood, bile, and liver was measured following the intravenous administration of isotopically labeled mevalonate and cholesterol suggested that at least a portion of newly synthesized sterol is secreted directly into the bile (15, 16). This finding was supported by the experiments of Long et al. (17) with triparanol-fed rats in which it was demonstrated that, under basal conditions, about 28% of biliary sterol is derived directly from newly synthesized sterols. Furthermore, in man both the rate of biliary cholesterol secretion and total body cholesterol synthesis correlate directly with body weight (18). However, in this latter study, it is not known how the rate of cholesterol synthesis in the liver varies with body weight and, in other species, there is no definitive evidence that the rate of biliary cholesterol output is dictated primarily by the rate of hepatic cholesterol synthesis. Although this problem could be readily evaluated by measuring the rate of biliary cholesterol secretion under conditions where the rate of hepatic cholesterogenesis is varied over a wide range, this has not yet been done.

In addition to newly synthesized cholesterol, the liver also derives cholesterol from the uptake of chylomicrons and serum lipoproteins (19–21). Although part of the cholesterol secreted in bile probably originates from these lipoproteins, the importance of the rate of hepatic lipoprotein uptake in determining the rate of biliary cholesterol output also is poorly understood. Similarly no studies have been undertaken to determine whether the size of the cholesteryl ester pool, which almost invariably reflects changes in the total cholesterol content of the hepatocyte, has any direct influence on the size of the pool of free cholesterol from which biliary cholesterol is derived.

In summary, biliary cholesterol secretion ultimately must be regulated either by intrahepatic factors such as the rate of cholesterol synthesis or the size of the cholesteryl ester pool, by the amount of lipoprotein cholesterol entering the hepatocyte, or by the degree of coupling to bile acid or phospholipid transport into bile. The purpose of the present studies was to systematically evaluate the role of each of these variables in dictating the rate of biliary cholesterol output in the rat.

MATERIALS AND METHODS

Animal preparations

Female Sprague-Dawley-derived rats (Charles River Breeding Laboratories, Inc., Wilmington, MA), originally purchased in the weight range of 125–150 g, were housed in a room with alternating 12-hr periods of light (1500–0300 hr) and darkness (0300–1500 hr) and were allowed free access to water and Formulab rat chow (Ralston Purina Co., St. Louis, MO) for at least 3 weeks before being used in specific experiments. Groups of rats were then subjected to various experimental manipulations which included the feeding, ad libitum, of diets containing one of the following: 2% (w/w) cholestyramine (Mead Johnson Research Center, Evansville, IN) for 14 days; 2% cholesterol (Nutritional Biochemicals Corp., Cleveland, OH) and 10% corn oil for 12 days; and 1% sodium taurocholate (Calbiochem, San Diego, CA) for 3 days. Other groups of rats were either fasted for 48 hr or, 12 hr before use, were administered intravenously a bolus of chylomicrons which had been prepared from rat intestinal lymph as previously described (22, 23). In another series of experiments rats were subjected to either total biliary diversion for 36 hr or were infused with bile acid.

Rats were anesthetized with ether and the bile duct was cannulated with PE-50 polyethylene tubing. In the experiments involving total biliary diversion for 36 hr and bile acid infusion, a catheter was also placed in the tail vein using PE-10 tubing. The rats were placed in individual restraining cages and allowed to awaken. Unless otherwise indicated, bile was collected during the following 2 hr and flow rates were measured. All operative procedures were carried out at about the mid-dark phase of the light cycle and, except for the group fasted for 48 hr, all rats had access to food up until the time of the experiment.

In the experiment on bile acid depletion the rats were continuously infused with a solution containing NaCl (145 mM) and KCl (5 mM) at a rate of 1 ml/hr and the bile was collected hourly for 36 hr. Two experiments involving bile acid infusion were carried out. In one, a group of rats fitted with bile duct cannulas was infused with 0.9% NaCl solution for 1 hr and then with solutions containing different concentrations of sodium taurocholate (pH 7.4) for 5 hr. The amount of bile acid infused was in the range of 22.5–90 μmol/rat per hr. Bile was collected hourly and the flow rate was measured. In the second experiment, rats that had been either fasted for 48 hr or fed a diet containing 2% cholestyramine for 1 week, as well as a group of control rats, were infused first with 0.9% NaCl solution for 1 hr and then with a

solution of sodium taurocholate at a rate of 40 μmol/rat per hr for an additional 2 hr. Bile was collected at hourly intervals and the flow rate was measured.

Incubation techniques and analytic procedures

After collection of bile the rats were killed, blood samples were obtained from the abdominal aorta, and the liver was quickly removed and chilled in 0.9% NaCl solution. Liver slices were prepared and incubated with 1 mM [1-^{14}C]octanoate as described in detail elsewhere (24, 25). The incorporation of this substrate into digitonin-precipitable sterols, CO_2, and ketones was measured and, after correction for the specific activity of the intracellular acetyl CoA pool, these data were used to calculate the incorporation rate of acetyl CoA units, i.e., C_2 units, into CO_2 and cholesterol (24, 25). The free and esterified cholesterol[2] content of the liver and plasma cholesterol levels were assayed as previously described (26). To measure the bile acid pool size in each animal, the entire small intestine and its contents were cut into small pieces into ethanol containing a known amount of [G-^{3}H]-taurocholic acid (New England Nuclear Corp., Boston, MA). The mixture was boiled vigorously for 4 hr, filtered, and taken to a known volume. An aliquot of the extract was washed with diethyl ether and this layer was discarded. The lower phase was then evaporated under air, the residue was dissolved in methanol, and aliquots were taken for measurement of radioactivity and bile acid content. The concentration of total bile acids in the intestinal extracts and in bile was determined using the hydroxysteroid dehydrogenase assay as described in detail elsewhere (27). After saponification and extraction with petroleum ether, biliary cholesterol was precipitated with digitonin and quantitated using the $FeCl_3$–H_2SO_4 method (28). Biliary phospholipid concentration was assayed by colorimetric determination of inorganic phosphate with malachite green (29).

Mathematical treatment of data

Where appropriate, mean values ±1 SEM for groups of data are given. For correlating two variables, linear regression lines were fitted by the method of least squares to the data obtained from individual animals and have the usual form $y = a + bx$. As there was wide variation in the individual rates of hepatic cholesterogenesis and hepatic cholesteryl ester levels, the data relating these two variables to biliary cholesterol output and molar percentage of cholesterol were plotted on a semilogarithmic scale, e.g., Fig. 1 and 2.

[2] Cholesteryl ester concentrations refer to concentrations of esterified cholesterol and not to the mass of cholesteryl esters.

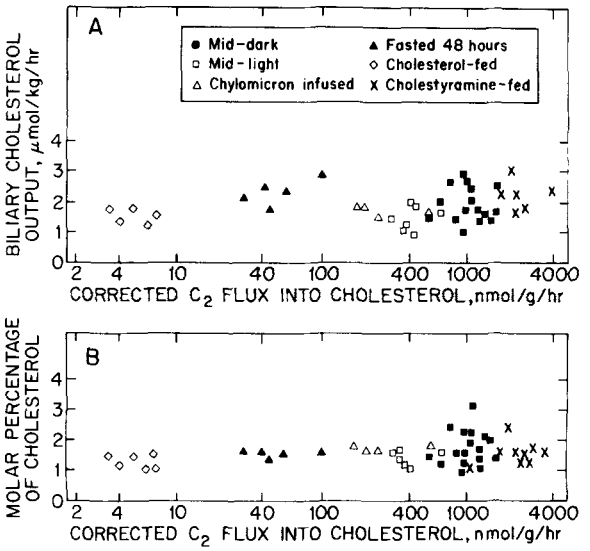

Fig. 1. Biliary cholesterol output and molar percentage of cholesterol in the bile of rats with widely different rates of hepatic cholesterogenesis. Female rats weighing 190–230 g, which had been subjected to various experimental manipulations including diurnal light cycling, fasting for 48 hr, feeding of diets containing either 2% cholestyramine or 2% cholesterol and 10% corn oil, and the intravenous administration of a bolus of chylomicrons, were anesthetized with ether. The bile duct was cannulated and bile was collected for the following 2 hr. The rats were then killed and rates of hepatic cholesterogenesis were measured as described in Materials and Methods. Panel *A* shows the rate of biliary cholesterol output as a function of the rate of hepatic cholesterol synthesis in 45 individual animals. The regression equation fitted to these data is $y = 1.72 + 0.0002x$ ($r = 0.27$, $P > 0.05$). Panel *B* shows the molar percentage of cholesterol in the bile as a function of the rate of hepatic cholesterol synthesis in 50 individual animals. The regression equation fitted to these data is $y = 1.57 + 0.00004x$ ($r = 0.09$, $P > 0.05$). In both panels the data are plotted on a semilogarithmic scale. Thus the regression lines, which were derived using the actual values rather than the logarithms, are not shown.

However in the derivation of the regression equations, the actual values for rates of synthesis and levels of esters, and not the natural logarithms of these values, were used.

RESULTS

The first series of studies was concerned with establishing whether biliary cholesterol output could be altered by varying either the rate of hepatic cholesterol synthesis, the size of the hepatic cholesteryl ester pool, or the amount of chylomicron cholesterol reaching the liver. **Fig. 1** shows the rate of biliary cholesterol output (panel *A*) and the molar percentage of cholesterol (panel *B*) in bile as a function of the corresponding rate of hepatic cholesterogenesis in individual rats subjected to various experimental manipulations. The mean values for each of these experimental groups are given in **Table 1.**

TABLE 1. Effect of various experimental manipulations on biliary lipid output and composition, bile acid pool size, plasma and liver cholesterol concentrations and hepatic cholesterogenesis in rats[a]

Experimental Group	Bile Secretion	Bile Acid in Bile			Phospholipid in Bile		
		Conc.	Output	Molar Percentage	Conc.	Output	Molar Percentage
	ml/kg/hr	*μmol/ml*	*μmol/kg/hr*	%	*μmol/ml*	*μmol/kg/hr*	%
A. Control (mid-dark)	4.3 ± 0.1[b] (22)	24.4 ± 1.3 (23)	104 ± 6 (22)	86.7 ± 0.6 (23)	3.2 ± 0.2 (23)	14.0 ± 1.2 (22)	11.5 ± 0.5 (23)
B. Control (mid-light)	4.0 ± 0.2 (12)	23.8 ± 1.0 (12)	95 ± 7 (12)	87.4 ± 0.5 (12)	3.0 ± 0.2 (12)	12.4 ± 1.1 (12)	11.0 ± 0.5 (12)
C. Fasted (48 hr)	3.7 ± 0.1*[c] (5)	32.3 ± 2.3* (5)	120 ± 8 (5)	82.4 ± 0.7* (5)	6.4 ± 0.6* (5)	23.4 ± 2.2* (5)	16.0 ± 0.6* (5)
D. Cholesterol-fed	3.8 ± 0.2 (6)	25.2 ± 1.0 (6)	96 ± 5 (6)	85.2 ± 1.1 (6)	4.0 ± 0.2 (6)	15.2 ± 1.0 (6)	13.5 ± 1.0 (6)
E. Chylomicron infusion	4.5 ± 0.1 (4)	20.0 ± 1.1 (4)	89 ± 3 (4)	84.9 ± 0.6 (4)	3.1 ± 0.1 (4)	13.8 ± 0.5 (4)	13.3 ± 0.6 (4)
F. Cholestyramine-fed	4.6 ± 0.1 (8)	26.9 ± 1.4 (12)	115 ± 6 (8)	88.4 ± 0.5 (12)	3.0 ± 0.1 (12)	15.0 ± 1.3 (8)	10.0 ± 0.4 (12)
G. Bile acid-fed	5.9 ± 0.3* (6)	36.2 ± 2.7* (6)	209 ± 15* (6)	86.5 ± 1.1 (6)	4.9 ± 0.3* (6)	28.8 ± 2.8* (6)	11.9 ± 1.1 (6)

[a] Female rats of 190–230 g were subjected to various experimental manipulations which included diurnal light cycling, fasting for 48 hr, intravenous administration of a bolus of chylomicrons, and feeding ad libitum of diets containing one of the following; 2% cholestyramine for 14 days, 2% cholesterol and 10% corn oil for 12 days, and 1% sodium taurocholate for 3 days. All rats were anesthetized with ether, the bile duct was cannulated, and bile was collected during the following 2 hr. They were then killed and rates of hepatic cholesterogenesis and various

Although rats at the mid-dark phase showed a 2.5-fold higher rate of hepatic cholesterogenesis than those at the mid-light phase, there was no difference in either biliary cholesterol output or the molar percentage of cholesterol in bile between the two groups (Groups A and B). Similarly, fasting for 48 hr, which decreased the rate of synthesis to about 5% of control values, had no effect on the output or molar percentage of cholesterol in bile (Group C). However, the fasted rats did show, in addition to decreased plasma cholesterol levels, a marked increase in the biliary concentration, output, and molar percentage of phospholipid with a concomitant decrease in the molar percentage of bile acid (Table 1).

The feeding of a diet containing added cholesterol and corn oil resulted in almost complete inhibition of hepatic cholesterol synthesis (Group D). Although the biliary concentration, output and molar percentage of cholesterol were somewhat lower compared to control values, the degree of change in these variables was small compared to that in cholesterol synthesis which decreased from a rate of 1091 nmol/g per hr to only 6 nmol/g per hr.

The experiment on the intravenous administration of chylomicrons was designed to determine whether an acute change in the amount of cholesterol reaching the liver had any effect on biliary cholesterol output. Approximately 12 hr after the rats had been given a bolus of chylomicrons containing 20 mg of cholesterol, the rate of cholesterol synthesis in the liver had decreased about 75% (Group E). There was a slight decrease in biliary cholesterol concentration but total cholesterol output was not significantly different from that in control animals.

In the next experiment the enterohepatic circulation of bile acid was varied. Cholestyramine feeding doubled the rate of hepatic cholesterogenesis but biliary lipid output and composition remained unchanged (Group F). The results of the experiment on bile acid feeding provided further evidence of a dissociation of the rate of biliary cholesterol secretion from the rate of hepatic cholesterogenesis. After 3 days of bile acid feeding, cholesterol synthesis in the liver decreased to only 17% of the control value, yet biliary cholesterol output approximately doubled. The increased output resulted from the combined effects of an increase in bile secretion rate as well as an increase in the concentration of cholesterol in bile (Group G). The biliary concentration and output of both bile acid and phospholipid were similarly increased. Thus the molar percentage of all three components remained normal.

Although it was possible to markedly alter hepatic cholesterol synthesis without significantly changing the level of cholesteryl ester by manipulations such as diurnal light cycling, fasting, and cholestyramine

Cholesterol in Bile			Bile Acid Pool Size	Plasma Cholesterol Concentration	Liver Cholesterol Concentration		C_2 Flux into	
Conc.	Output	Molar Percentage			Free	Esterified	CO_2	Cholesterol
μmol/ml	*μmol/kg/hr*	*%*	*μmol/kg*	*mg/dl*	*mg/g*	*mg/g*	*μmol/g/hr*	*nmol/g/hr*
0.48 ± 0.02 (23)	2.0 ± 0.1 (22)	1.8 ± 0.11 (18)	679 ± 37 (14)	68.8 ± 3.1 (15)	2.20 ± 0.06 (18)	0.15 ± 0.01 (18)	11.9 ± 0.4 (18)	1,091 ± 70 (18)
0.45 ± 0.03 (12)	1.8 ± 0.2 (12)	1.6 ± 0.08 (12)	639 ± 44 (7)	77.7 ± 2.5 (6)	2.04 ± 0.08 (7)	0.13 ± 0.01 (7)	10.6 ± 0.4 (7)	420 ± 46* (7)
0.61 ± 0.05 (5)	2.3 ± 0.2 (5)	1.6 ± 0.04 (5)	759 ± 40 (5)	47.0 ± 7.9 (3)	2.44 ± 0.09 (5)	0.29 ± 0.06 (5)	8.2 ± 0.5* (5)	54 ± 13* (5)
0.39 ± 0.02* (6)	1.5 ± 0.1* (6)	1.3 ± 0.08* (6)	770 ± 74 (6)	111.5 ± 8.4* (6)	3.14 ± 0.04* (6)	11.9 ± 0.78* (6)	10.7 ± 0.8 (5)	6 ± 0.6* (6)
0.40 ± 0.02* (4)	1.7 ± 0.1 (4)	1.8 ± 0.03 (4)	879 ± 73 (3)	69.2 ± 8.1 (4)	2.44 ± 0.06 (4)	1.19 ± 0.09* (4)	10.4 ± 0.6 (4)	298 ± 94* (4)
0.47 ± 0.02 (12)	2.1 ± 0.2 (8)	1.6 ± 0.10 (12)	776 ± 46 (7)	65.4 ± 3.0 (8)	2.36 ± 0.11 (10)	0.19 ± 0.02 (10)	13.1 ± 0.7 (10)	2,304 ± 251* (10)
0.65 ± 0.05* (6)	3.8 ± 0.3* (6)	1.6 ± 0.10 (6)	1,269 ± 78* (6)	63.1 ± 5.7 (6)	2.23 ± 0.08 (5)	0.61 ± 0.14* (5)	10.2 ± 0.8 (5)	189 ± 6* (5)

other parameters were measured as described in Materials and Methods.

[b] Values are the mean ± 1 SEM for the number of animals indicated in parentheses.

[c] The asterisk indicates that the value is significantly different from the corresponding value for animals in the control (mid-dark) group at the $P < 0.05$ level.

feeding, it was not possible to independently alter the cholesteryl ester concentration. Thus, the administration of chylomicrons, cholesterol feeding, and bile acid feeding, while increasing hepatic esterified cholesterol levels, also resulted in a decreased rate of cholesterogenesis. However, if the size of the cholesteryl ester pool was an important determinant of the rate of biliary cholesterol secretion then, regardless of the concomitant decrease in the synthesis rate, expansion of the cholesteryl ester pool should have altered the amount of cholesterol secreted in the bile. As shown in **Fig. 2,** variation of the hepatic cholesteryl ester concentration over a 100-fold range was not associated with any consistent change in either the rate of biliary cholesterol output or the molar percentage of cholesterol in bile. Comparison of the data for the cholesterol-fed and bile acid-fed groups (Table 1) clearly suggests a dissociation of these variables. For example, rats fed the cholesterol diet showed an 80-fold increase in esterified cholsterol concentration and a decreased biliary cholesterol output. Bile acid-fed rats also showed significantly higher hepatic esterified cholesterol levels but in these animals biliary cholesterol output was significantly increased.

Regression equations were derived for the data shown in Fig. 1 and Fig. 2 and these are given in the respective figure legends. The slopes of these regression lines do not differ significantly from zero.

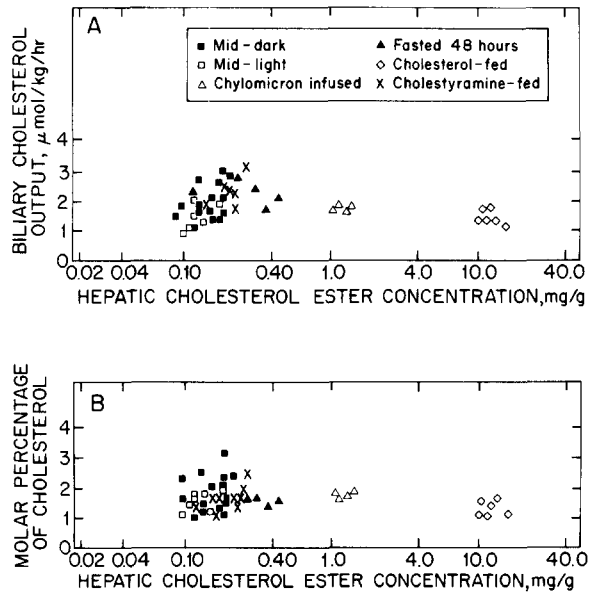

Fig. 2. Biliary cholesterol output and molar percentage of cholesterol in the bile of rats with widely different hepatic cholesteryl ester concentrations. The animal preparations in these studies were identical to those described in the legend to Fig. 1. Panel *A* shows the rate of biliary cholesterol output as a function of the hepatic cholesteryl ester level in 45 individual animals. The regression equation fitted to these data is $y = 1.94 - 0.040x$ ($r = -0.31, P > 0.05$). Panel *B* shows the molar percentage of cholesterol in the bile as a function of the hepatic cholesteryl ester level in 50 individual animals. The regression equation fitted to these data is $y = 1.64 - 0.028x$ ($r = -0.26, P > 0.05$). The regression lines are not shown for the same reason as given in the legend to Fig. 1.

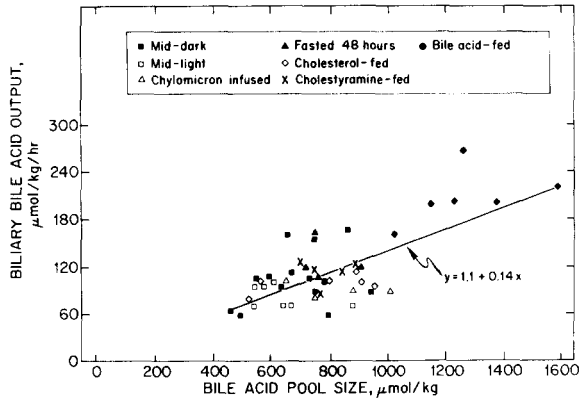

Fig. 3. Relationship between biliary bile acid output and bile acid pool size in rats subjected to various experimental manipulations. The animal preparations in these studies were identical to those described in the legend to Fig. 1 except that an additional group of rats that had been fed a diet containing 1% sodium taurocholate for 3 days was included. Biliary bile acid concentrations and the size of the bile acid pool in the small intestine were measured as described in Materials and Methods. Each point represents the value for a single animal. The regression line was fitted to the data obtained in 47 individual animals ($r = 0.73$, $P < 0.001$).

Thus, it is clearly evident that neither the rate of hepatic cholesterol synthesis nor the size of the hepatic cholesteryl ester pool is a major determinant of the rate of biliary cholesterol output in this animal model.

The relationship between the rate of biliary bile acid output and the size of the bile acid pool in animals subjected to the various experimental manipulations is shown in **Fig. 3.** As anticipated, bile acid output increased in direct proportion to bile acid pool size. From the slope of the regression line it was calculated that the bile acid pool cycled approximately 3.5 times every 24 hr.

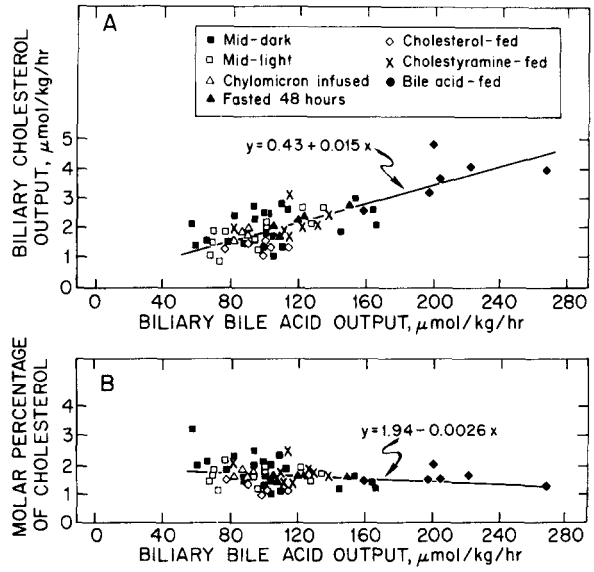

Fig. 4. Biliary cholesterol output and molar percentage of cholesterol in the bile of rats with widely different rates of bile acid output. The animal preparations in these studies were identical to those described in the legend to Fig. 3. Panel *A* shows the rate of biliary cholesterol output and Panel *B* the molar percentage of cholesterol in the bile, each as a function of the corresponding rate of biliary bile acid output. Each point represents the value for a single animal. Both regression lines were fitted to the data obtained in 47 individual animals. Panel *A* ($r = 0.79, P < 0.001$). Panel *B* ($r = -0.26$, $P > 0.05$).

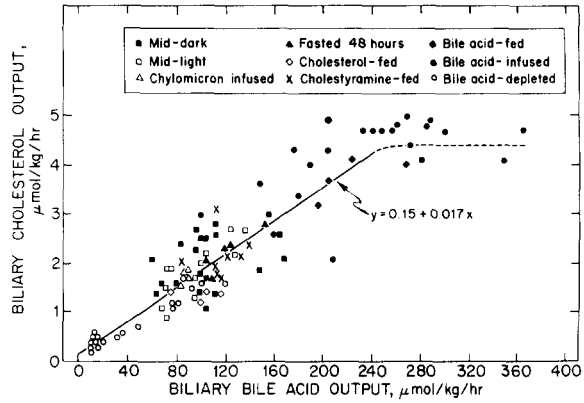

Fig. 5. Biliary cholesterol output in rats with widely different rates of bile acid output. In addition to the animal preparations described in Fig. 3, two further manipulations were included to produce extreme variations in the rate of biliary bile acid output. One group of rats was subjected to total biliary diversion for 36 hr during which time the animals were continuously infused with a solution containing NaCl (145 mM) and KCl (5 mM). Bile was collected at hourly intervals. Another group was infused continuously for 5 hr with solutions containing different concentrations of sodium taurocholate so that the total amount of bile acid administered to different rats was in the range of 22.5–90 μmol/rat per hr. Bile was collected hourly. Biliary lipid concentrations were assayed as described in Materials and Methods. In this figure, as well as in Figs. 6–8, all points except those for the bile acid-depleted rats represent a single value for an individual animal. The points for the bile acid-depleted animals represent the mean hourly values for determinations in five rats. The regression line was fitted to a total of 232 individual values ($r = 0.78$, $P < 0.001$). The data corresponding to bile acid outputs above 240 μmol/kg per hr were not included in the derivation of this regression equation because of the apparent deviation of these points from the overall linear relationship.

Although biliary cholesterol output did not vary consistently with either the rate of hepatic cholesterol synthesis or the level of cholesteryl esters, it was clearly coupled to the amount of bile acid secreted in the bile. This is shown in panel *A* of **Fig. 4** where biliary cholesterol output in individual animals subjected to various manipulations is plotted as a function of the corresponding rate of biliary bile acid output. Regression analysis of these data showed that, within the limits of this range of bile acid secretion rates, cholesterol output increased in direct proportion to bile acid output. However, as shown in panel *B*, the molar percentage of cholesterol remained constant over this range of bile acid secretion rates, indicating a close coupling between bile acid, phospholipid, and cholesterol output.

The quantitative aspects of this coupling were further studied by subjecting rats to bile acid depletion and bile acid infusion to produce a greater variation in biliary bile acid output. The data from these studies were pooled with those from the preceding experiments. As shown in **Fig. 5,** cholesterol output remained tightly coupled to bile acid output which varied over almost a 40-fold range. At bile acid outputs above 240 μmol/kg per hr the output of cholesterol appeared to deviate from the overall linear relationship. Thus, these data were not included in the regression analysis which showed that 1.7 μmol of cholesterol was secreted for every 100 μmol of bile acid.

The relationship between phospholipid and bile acid output over the same range of bile acid secretion rates is shown in **Fig. 6.** Again, because of apparent deviation from the overall linear relationship, phospholipid secretion rates corresponding to rates of bile acid output above 240 μmol/kg per hr were not included in the regression analysis. Phospholipid output, like cholesterol output, also was tightly coupled to bile acid secretion rates over a wide range so that 12.3 μmol of phospholipid were secreted for every 100 μmol of bile acid.

The linear relationship between biliary cholesterol and phospholipid output, shown in **Fig. 7,** did not hold when bile acid output rates fell below 20 μmol/kg per hr. This is clearly demonstrated in **Fig. 8** which shows the molar ratio of the concentration of cholesterol to that of phospholipid in bile as a function of the corresponding rate of bile acid output. At critically low rates of bile acid secretion the amount of cholesterol in the bile relative to that of phospholipid increased

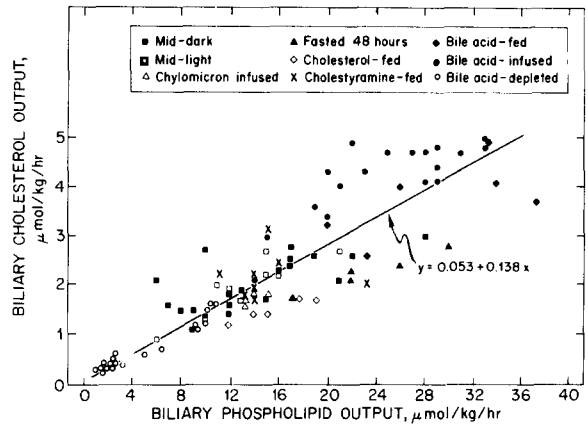

Fig. 7. Relationship between biliary cholesterol and phospholipid output in rats with widely different rates of bile acid output. The regression line was fitted to a total of 135 individual values ($r = 0.90$, $P < 0.001$). Those rates of biliary cholesterol and phospholipid output corresponding to bile acid outputs less than 20 μmol/kg per hr are not shown here and thus were not included in the derivation of this regression equation because, as demonstrated in Fig. 8, biliary secretion of cholesterol and phospholipid become uncoupled at low rates of bile acid output.

significantly. Thus the data presented in Fig. 7 do not include cholesterol and phospholipid secretion rates corresponding to bile acid outputs of less than 20 μmol/kg per hr. However, above this limit the cholesterol:phospholipid ratio remained fixed at a value of approximately 0.14 over a wide range of rates of bile acid output.

As the amount of cholesterol secreted in the bile was found to depend critically upon the rate of biliary bile acid output, it was important to determine the extent to which cholesterol output in bile could be

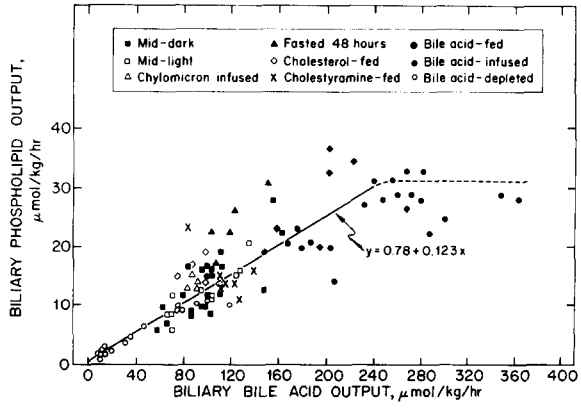

Fig. 6. Biliary phospholipid output in rats with widely different rates of bile acid output. The animal preparations used in these studies were identical to those described in Fig. 5. The regression line was fitted to a total of 247 individual values ($r = 0.91, P < 0.001$). The data corresponding to bile acid outputs above 240 μmol/kg per hr were not included in the derivation of this regression equation because of the apparent deviation of these points from the overall linear relationship.

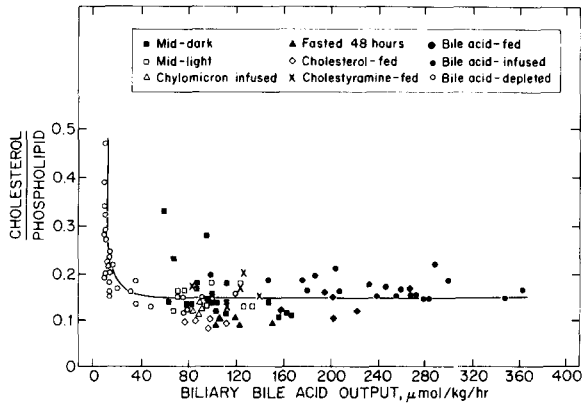

Fig. 8. Relationship between biliary cholesterol and phospholipid output in rats with wide differences in bile acid output. The animal preparations used in these studies were identical to those described in Fig. 5. The molar ratio of the cholesterol concentration to the phospholipid concentration in the bile was calculated and is plotted as a function of the corresponding rate of bile acid output. The line was fitted by eye. Only about half of the 243 values obtained have been plotted because many of the values overlapped.

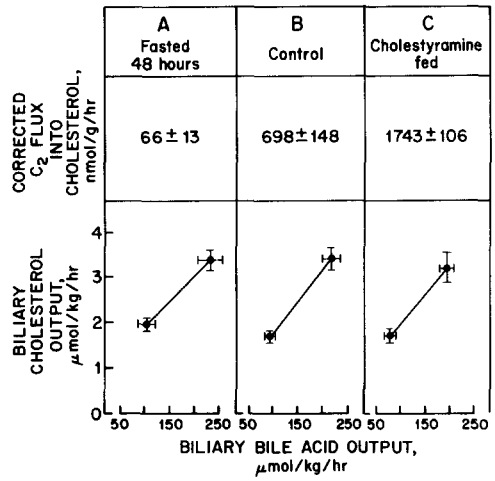

Fig. 9. Effect of bile acid infusion on rates of biliary cholesterol output in rats with widely different rates of hepatic cholesterogenesis. Female rats that had been either fasted for 48 hr or fed a diet containing 2% cholestyramine for 1 week, as well as a group of control rats, were infused first with 0.9% NaCl solution for 1 hr and then with taurocholate at a rate of 40 μmol/rat per hr for an additional 2 hr. Bile was collected at hourly intervals and the flow rate was measured. The rats were then killed and rates of hepatic cholesterogenesis were measured as described in Materials and Methods. The lower point in each panel represents the rate of biliary cholesterol secretion, expressed as a function of the corresponding rate of bile acid secretion, during the first hour while the upper point corresponds to the rates obtained with bile acid infusion. The rates of biliary cholesterol output during the 2nd and 3rd hourly intervals were averaged as were the rates of bile acid output. The values for the rates of hepatic cholesterol synthesis represent the mean ± SEM for determinations in 6 animals in each group whereas the values for the rates of biliary cholesterol and bile acid output represent the mean ± SEM for determinations in 10 animals in the control group and 12 animals in both the fasted and cholestyramine-fed groups. The vertical bars represent the SEM for the rates of cholesterol output and the horizontal bars, the SEM for the rates of bile acid output.

driven by bile acid infusion under circumstances where the rate of hepatic cholesterol synthesis was varied markedly. This was studied by measuring biliary lipid output before and during the intravenous administration of bile acid to fasted, cholestyramine-fed, and control rats. The results of this study are shown in **Fig. 9.** The lower point in each panel represents the rate of biliary cholesterol output, expressed as a function of the corresponding rate of bile acid output, during the first hour of diversion when the rats were infused with only 0.9% NaCl. The upper points represent the average rates of cholesterol secretion, also expressed as a function of the corresponding rates of bile acid secretion, during the 2nd and 3rd hours of diversion when taurocholate was infused at a rate of 40 μmol/rat per hr. Despite a 26-fold difference in the rate of hepatic cholesterogenesis, the net increase in biliary cholesterol secretion during the period of bile acid infusion did not differ significantly between the three groups (controls, 1.8 ± 0.2 μmol/kg per hr; fasted 1.4 ± 0.22 μmol/kg per hr; and cholestyramine-fed, 1.5 ± 0.2 μmol/kg per hr). This finding further showed that biliary cholesterol output was not dependent on the rate at which the liver synthesized cholesterol but was dictated by the rate of transport of bile acid into bile.

DISCUSSION

In the present studies, acute interruption of the enterohepatic circulation was used to examine the relationships between biliary secretion of bile acid, phospholipid, and cholesterol, and to determine the extent to which intracellular metabolic events related to cholesterol metabolism influence the rate of biliary cholesterol secretion. The validity of the conclusions drawn from these experiments is based upon the assumption that the rates of biliary cholesterol output, as well as those of bile acid and phospholipid, observed during acute total interruption of the enterohepatic circulation truly reflect the rates occurring in the intact animal. Although experiments were not carried out to determine the rates of biliary lipid secretion in rats with chronic partial interruption of the enterohepatic circulation, studies by Dowling, Mack, and Small (9) with rhesus monkeys have shown that during the first 2–3 hr after acute total biliary diversion the absolute rates of secretion of bile acid, phospholipid, and cholesterol are essentially the same as those measured in animals with an "intact" enterohepatic circulation (5% interruption).

The relationships between the biliary secretion of bile acid, phospholipid, and cholesterol found in the present experiments can be summarized as follows. First, there is close coupling between the outputs of cholesterol and bile acid, and between phospholipid and bile acid over a wide range of bile acid secretion rates. Second, there is a fixed proportion of cholesterol to phospholipid in bile except at very low bile acid outputs when cholesterol secretion continues in the virtual absence of phospholipid secretion so that the ratio of cholesterol to phospholipid increases greatly. Third, at very high rates of bile acid secretion the ratio of cholesterol to phospholipid remains constant but the absolute rates of output of each become proportionately less. These general relationships are similar to those reported for the chronic partial biliary fistula preparations in the rhesus monkey (9). They also agree well with previous reports in the rat as well as in other species, including the dog and man, where total acute interruption of the enterohepatic circulation also was used (7, 8, 30–34). Thus, the major conclusion to be drawn from these initial observations is

that the qualitative and quantitative characteristics of biliary lipid secretion found in these acute studies in the rat reflect closely similar findings reported in a number of other species, including man, utilizing a variety of experimental procedures.

In theory, cholesterol secreted across the canalicular membrane of the liver cell can originate from only a relatively few sources. These are outlined in the model shown in **Fig. 10.** For simplicity, in this model we have assumed that there is only one pool of metabolically active cholesterol that is the source of the sterol secreted into bile (pathway 6), incorporated into lipoproteins (pathway 7), and used as a substrate for bile acid synthesis (pathway 5). Within the hepatocyte, cholesterol could be derived from the hydrolysis of cholesterol esters (pathway 2) or from the synthesis of cholesterol from acetyl CoA in the cytosolic compartment (pathway 1). Both of these sources of cholesterol undergo marked variation in activity depending upon the animal's need for sterol. For example, increased delivery of cholesterol to the liver or decreased utilization of sterols for bile acid or lipoprotein synthesis is associated with suppression of cholesterol synthesis and expansion of the cholesterol ester pool (14, 21, 35–41). Contrariwise, decreased delivery of cholesterol to the liver or enhanced utilization of sterol for synthetic purposes causes increased de novo cholesterol synthesis and a reduction in the amount of cholesteryl esters stored in the cell. There may also be net cholesterol entry into the metabolically active pool from three major sources outside the liver cell. Dietary cholesterol or cholesterol synthesized from acetyl CoA in the intestinal mucosa enters the body carried principally in chylomicrons (42). This lipoprotein is metabolized in the peripheral capillary beds by lipoprotein lipase to the chylomicron remnant which, in turn, is rapidly taken up into the liver by a high-velocity, saturable transport system (pathway 3) (19, 20). Cholesterol is also synthesized at low, although easily detectable, rates in various other tissues in the carcass besides the liver and intestine (35, 36, 43). Except for the endocrine glands and skin, this newly synthesized cholesterol ultimately must also be transported to the liver for excretion from the body (pathway 4). Whether the vehicle for the transport is high or low density lipoproteins or even the remnants of chylomicrons or very low density lipoproteins is not clear. Cholesterol carried in all of these particles can be transported into the liver, although that carried in the high and low density lipoproteins is taken up at very low rates relative to those manifested by the remnant particles (20, 21).

The present studies were designed specifically to test the possibility that changes in the rate of entry of

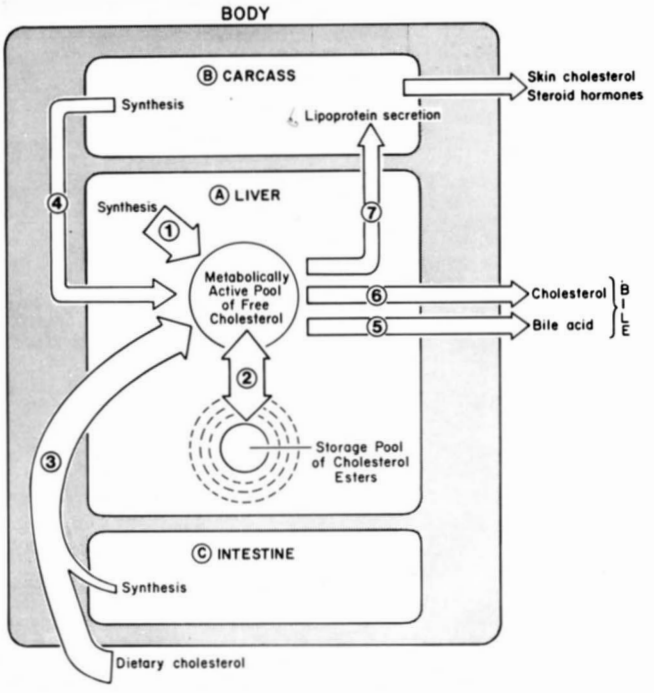

Fig. 10. Model describing the processes involved in the maintenance of cholesterol balance across the liver and how these relate to the regulation of biliary cholesterol output in the rat. The body is represented as three compartments; *A*, the liver; *B*, the carcass which includes the blood; and *C*, the intestine. The liver contains a pool of metabolically active free cholesterol which is in equilibrium with the cholesteryl ester pool, the size of which can fluctuate greatly depending on the metabolic state of the liver. Cholesterol entering the pool of metabolically active free cholesterol is derived from synthesis within the liver (pathway 1), synthesis in the carcass (pathway 4), and synthesis in the intestine together with the cholesterol absorbed across the intestinal wall (pathway 3). The entry of cholesterol into this pool is balanced by output in three pathways: lipoprotein secretion (pathway 7), the secretion of free cholesterol in bile (pathway 6), and the conversion of cholesterol to bile acids and their subsequent secretion in bile (pathway 5). A portion of the cholesterol and bile acid in bile is lost from the body by fecal excretion. Sterol is also lost through urinary and biliary excretion of steroid hormones and through the sloughing of skin.

cholesterol into the metabolically active pool from any of these intra- or extracellular sources plays a major role in determining the rate of hepatic cholesterol secretion in the bile. Inherent in this thesis is the assumption that the size of the intrahepatic cholesterol pool somehow "drives" the transport process responsible for sterol movement into the bile. It is evident from the results of these studies, however, that in this animal model this was not the case. The rate of de novo cholesterol synthesis was changed over a nearly 1000-fold range by such diverse maneuvers as light cycling, fasting, chylomicron administration, and cholestyramine feeding without any significant change in either the absolute secretory rate or molar percentage of biliary cholesterol. That the rate of biliary cholesterol secretion is independent of the rate of hepatic

cholesterol synthesis was further supported by the studies shown in Fig. 9. Cholesterol secretion in bile could be increased to a similar extent by increasing biliary bile acid output regardless of whether the C_2 flux into cholesterol by the liver was 66, 698, or 1743 nmol/g per hr. Similar negative findings were seen when the cholesteryl ester pool in the liver cell was expanded over a 100-fold range and when relatively massive amounts of cholesterol were delivered acutely to the liver in the form of chylomicron remnants. It should be noted, however, that while these latter manipulations failed to increase the delivery of cholesterol into the bile, they did result in increased movement of cholesterol out of the liver incorporated in lipoproteins (resulting in an elevation of the plasma lipoproteins with a density >1.020 g/ml) and as bile acids (with increased bile acid synthesis rates) (44). Clearly, these findings demonstrate that increasing availability of cholesterol in the metabolically active pool in the hepatocyte by whatever means does not "drive" cholesterol into the bile. Rather, the rate of hepatic cholesterol synthesis changes over a broad range to compensate for changes in the rate of lipoprotein-cholesterol delivery to the liver (as occurs with alterations of dietary cholesterol intake) or for changes in the rate of cholesterol conversion to bile acids (as occurs with bile acid or cholestyramine feeding).

While biliary sterol output is independent of these major metabolic events within the hepatocyte, it is equally apparent from these studies that there is very tight coupling of cholesterol secretion in bile to the rate of bile acid output. Furthermore, this coupling remains intact under essentially all physiological conditions that were examined in these studies, even though these manipulations markedly altered various aspects of lipid metabolism in the experimental animals. For example, insofar as could be detected, the rate of biliary cholesterol output was principally determined by the rate of bile acid output under circumstances where the rate of hepatic cholesterol synthesis was altered over a wide range (Figs. 5 and 9); where the level of cholesteryl esters varied markedly (Fig. 2); where the serum cholesterol levels ranged between 47 and 112 mg/dl (Table 1); where the animals were fed large amounts of dietary cholesterol or were administered cholesterol in chylomicrons; and where rates of fatty acid oxidation and ketone body production had been markedly changed (Table 1). As noted above, the relationship between cholesterol and bile acid secretion into bile was disrupted only at very high or very low rates of bile acid output.

These data strongly support the contention articulated by such workers as Hardison and Francis (45) and Wheeler and King (7) that the coupling of cholesterol and bile acid output must be primarily explained by events occurring at or near the canalicular membrane during the secretion of biliary lipids. Whether this coupling can be attributed to events within the membrane during the translocation step or to events occurring within the newly secreted bile just adjacent to the canalicular membrane remains to be elucidated. However, if this contention is correct, then differences in the molar percentage of cholesterol in bile in patients or animals fed different bile acids probably also must be explained in terms of differences in this coupling reaction.

Finally, it should be emphasized that while many of the qualitative and quantitative aspects of biliary cholesterol secretion are similar in man, the rat, and other animals, there are also important differences thay may limit the extent to which these data can be generalized to other species. For example, on a low dietary cholesterol intake, the degree of biliary cholesterol saturation varies in different species; animals like the rat and dog have bile that is less saturated with cholesterol than that of several primates, including man (2, 7, 9). When the load of dietary cholesterol is increased, man and other species such as the hamster and squirrel monkey increase biliary cholesterol output while the rate of biliary cholesterol secretion in the rat may actually decrease (13, 46–48). Similar variability is found in the metabolic response of animals given various bile acids. The administration of a specific bile acid to different animal species has variable effects both on the rate of hepatic cholesterol synthesis and on biliary cholesterol secretory rates (11–13, 30–32, 41, 49). Whether these variations in the handling of biliary cholesterol reflect important species differences in the fundamental processes responsible for biliary cholesterol secretion or result from differences in some other aspect of cholesterol metabolism such as the rate of intestinal cholesterol absorption or the rate of degradation of lipoprotein cholesterol remains to be elucidated.

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REFERENCES

1. Hofmann, A. F., and D. M. Small. 1967. Detergent properties of bile salts: correlation with physiological function. *Ann. Rev. Med.* **18:** 333–376.
2. Admirand, W. H., and D. M. Small. 1968. The physicochemical basis of cholesterol gallstone formation in man. *J. Clin. Invest.* **47:** 1043–1052.
3. Shaffer, E. L., and D. M. Small. 1977. Biliary lipid secretion in cholesterol gallstone disease. The effect of cholecystectomy and obesity. *J. Clin. Invest.* **59:** 828–840.
4. Schoenfield, L. J. 1977. Diseases of the Gallbladder and Biliary System. Wiley and Sons, Inc., New York, N.Y. 81–107.
5. Small, D. M., M. Bourgès, and D. G. Dervichian. 1966. Ternary and quaternary aqueous systems containing bile salt, lecithin, and cholesterol. *Nature.* **211:** 816–818.
6. Forker, E. L. 1977. Mechanisms of hepatic bile formation. *Ann. Rev. Physiol.* **39:** 323–347.
7. Wheeler, H. O., and K. K. King. 1972. Biliary excretion of lecithin and cholesterol in the dog. *J. Clin. Invest.* **51:** 1337–1350.
8. Hardison, W. G. M., and J. T. Apter. 1972. Micellar theory of biliary cholesterol excretion. *Am. J. Physiol.* **222:** 61–67.
9. Dowling, R. H., E. Mack, and D. M. Small. 1971. Biliary lipid secretion and bile composition after acute and chronic interruption of the enterohepatic circulation in the rhesus monkey. IV. Primate biliary physiology. *J. Clin. Invest.* **50:** 1917–1926.
10. Coyne, M. J., G. G. Bonorris, L. I. Goldstein, and L. J. Schoenfield. 1976. Effect of chenodeoxycholic acid and phenobarbital on the rate-limiting enzymes of hepatic cholesterol and bile acid synthesis in patients with gallstones. *J. Lab. Clin. Med.* **87:** 281–291.
11. Makino, I., and S. Nakagawa. 1978. Changes in biliary lipid and biliary bile acid composition in patients after administration of ursodeoxycholic acid. *J. Lipid Res.* **19:** 723–728.
12. Maton, P. N., G. M. Murphy, and R. H. Dowling. 1977. Ursodeoxycholic acid treatment of gallstones. Dose-response study and possible mechanism of action. *Lancet* **2:** 1297–1301.
13. Coyne, M. J., G. G. Bonorris, A. Chung, H. Cove, and L. J. Schoenfield. 1977. Dietary cholesterol affects chenodeoxycholic acid action on biliary lipids. *Gastroenterology* **72:** 927–931.
14. Nervi, F. O., and J. M. Dietschy. 1978. The mechanisms of and the interrelationship between bile acid and chylomicron-mediated regulation of hepatic cholesterol synthesis in the liver of the rat. *J. Clin. Invest.* **61:** 895–909.
15. Normann, P. T., and K. R. Norum. 1976. Newly synthesized hepatic cholesterol as precursor for cholesterol and bile acids in rat bile. *Scand. J. Gastroenterol.* **11:** 427–432.
16. Schwartz, C. C., M. Berman, Z. R. Vlahcevic, L. G. Halloran, D. H. Gregory, and L. Swell. 1978. Multicompartmental analysis of cholesterol metabolism in man. Characterization of the hepatic bile acid and biliary cholesterol precursor sites. *J. Clin. Invest.* **61:** 408–423.
17. Long, T. T., L. Jakoi, R. Stevens, and S. Quarfordt. 1978. The sources of rat biliary cholesterol and bile acid. *J. Lipid Res.* **19:** 872–878.
18. Grundy, S. M., H. Y. I. Mok, and K. von Bergmann. 1976. Regulation of biliary cholesterol secretion in man. *In* The Liver. Quantitative Aspects of Structure and Function. R. Preisig, J. Bircher, and G. Paumgartner, editors. Editio Cantor, Aulendorf, Germany. 393–403.
19. Cooper, A. D. 1977. The metabolism of chylomicron remnants by isolated perfused rat liver. *Biochim. Biophys. Acta.* **488:** 464–474.
20. Sherrill, B. C., and J. M. Dietschy. 1978. Characterization of the sinusoidal transport process responsible for uptake of chylomicrons by the liver. *J. Biol. Chem.* **253:** 1859–1867.
21. Andersen, J. M., S. D. Turley, and J. M. Dietschy. 1979. Low and high density lipoproteins and chylomicrons as regulators of rate of cholesterol synthesis in rat liver in vivo. *Proc. Natl. Acad. Sci. USA* **76:** 165–169.
22. Andersen, J. M., and J. M. Dietschy. 1977. Regulation of sterol synthesis in 16 tissues of rat. I. Effect of diurnal light cycling, fasting, stress, manipulation of enterohepatic circulation, and administration of chylomicrons and triton. *J. Biol. Chem.* **252:** 3646–3651.
23. Andersen, J. M., and J. M. Dietschy. 1977. Regulation of sterol synthesis in 15 tissues of rat. II. Role of rat and human high and low density lipoproteins and of rat chylomicron remnants. 1977. *J. Biol. Chem.* **252:** 3652–3659.
24. Dietschy, J. M., and J. D. McGarry. 1974. Limitations of acetate as a substrate for measuring cholesterol synthesis in liver. *J. Biol. Chem.* **249:** 52–58.
25. Dietschy, J. M., and M. S. Brown. 1974. Effects of alterations of the specific activity of the intracellular acetyl CoA pool on apparent rates of hepatic cholesterogenesis. *J. Lipid Res.* **15:** 508–516.
26. Andersen, J. M., and J. M. Dietschy. 1978. Relative importance of high and low density lipoproteins in the regulation of cholesterol synthesis in the adrenal gland, ovary, and testis of the rat. *J. Biol. Chem.* **253:** 9024–9032.
27. Turley, S. D., and J. M. Dietschy. 1978. Re-evaluation of the 3α-hydroxysteroid dehydrogenase assay for total bile acids in bile. *J. Lipid Res.* **19:** 924–928.
28. Tonks, D. B. 1967. The estimation of cholesterol in serum: a classification and critical review of methods. *Clin. Biochem.* **1:** 12–29.
29. Chalvardjian, A., and E. Rudnicki. 1970. Determination of lipid phosphorus in the nanomolar range. *Anal. Biochem.* **36:** 225–226.
30. Scherstén, T., L. Lindblad, and K. Lundholm. 1976. The influence of cholic and chenodeoxycholic acids on the bile flow and the biliary secretion of lecithin and cholesterol in man. *In* The Liver. Quantitative Aspects of Structure and Function. R. Preisig, J. Bircher, and G. Paumgartner, editors. Editio Cantor, Aulendorf, Germany. 275–286.
31. Hoffman, N. E., D. E. Donald, and A. F. Hofmann. 1975. Effect of primary bile acids on bile lipid secretion from perfused dog liver. *Am. J. Physiol.* **229:** 714–720.
32. Poupon, R., R. Poupon, M. L. Grosdemouge, M. Dumont, and S. Erlinger. 1976. Influence of bile acids

upon biliary cholesterol and phospholipid secretion in the dog. *Eur. J. Clin. Invest.* **6:** 279–284.
33. Wagner, C. I., B. W. Trotman, and R. D. Soloway. 1976. Kinetic analysis of biliary lipid excretion in man and dog. *J. Clin. Invest.* **57:** 473–477.
34. LaRusso, N. F., P. A. Szczepanik, and A. F. Hofmann. 1977. Effect of deoxycholic acid ingestion on bile acid metabolism and biliary lipid secretion in normal subjects. *Gastroenterology.* **72:** 132–140.
35. Dietschy, J. M., and M. D. Siperstein. 1967. Effect of cholesterol feeding and fasting on sterol synthesis in seventeen tissues of the rat. *J. Lipid Res.* **8:** 97–104.
36. Dietschy, J. M., and J. D. Wilson. 1968. Cholesterol synthesis in the squirrel monkey: relative rates of synthesis in various tissues and mechanisms of control. *J. Clin. Invest.* **47:** 166–174.
37. Weis, H. J., and J. M. Dietschy. 1969. Failure of bile acids to control hepatic cholesterogenesis: evidence for endogenous cholesterol feedback. *J. Clin. Invest.* **48:** 2398–2408.
38. Weis, H. J., and J. M. Dietschy. 1975. The interaction of various control mechanisms in determining the rate of hepatic cholesterogenesis in the rat. *Biochim. Biophys. Acta.* **398:** 315–324.
39. Nervi, F. O., H. J. Weis, and J. M. Dietschy. 1975. The kinetic characteristics of inhibition of hepatic cholesterogenesis by lipoproteins of intestinal origin. *J. Biol. Chem.* **250:** 4145–4151.
40. Nervi, F. O., and J. M Dietschy. 1975. Ability of six different lipoprotein fractions to regulate the rate of hepatic cholesterogenesis in vivo. *J. Biol. Chem.* **250:** 8704–8711.
41. Carrella, M., and J. M. Dietschy. 1977. Comparison of the effect of cholic acid and chenic acid feeding on rates of cholesterol synthesis in the liver of the rat. *Am. J. Dig. Dis.* **22:** 318–326.
42. Fielding, C. J. 1978. Origin and properties of remnant lipoproteins. *In* Disturbances in Lipid and Lipoprotein Metabolism. J. M. Dietschy, A. M. Gotto, and J. A. Ontko, editors. American Physiological Society, Bethesda, Md. 83–98.
43. Andersen, J. M., and J. M. Dietschy. 1979. Absolute rates of cholesterol synthesis in extrahepatic tissues measured with [^{3}H]water and [^{14}C]substrates. *J. Lipid Res.* **20:** 740–752.
44. Wilson, J. D. 1964. The quantification of cholesterol excretion and degradation in the isotopic steady state in the rat: the influence of dietary cholesterol. *J. Lipid Res.* **5:** 409–417.
45. Hardison, W. G. M., and T. I. Francis. 1969. The mechanisms of cholesterol and phospholipid excretion in bile. *Gastroenterology.* **56:** 1164 (abst.).
46. DenBesten, L., W. E. Connor, and S. Bell. 1973. The effect of dietary cholesterol on the composition of human bile. *Surgery.* **73:** 266–273.
47. Osuga, T., O. W. Portman, N. Tanaka, M. Alexander, and A. J. Ochsner. 1976. The effect of diet on hepatic bile formation and bile acid metabolism in squirrel monkeys with and without cholesterol gallstones. *J. Lab. Clin. Med.* **88:** 649–661.
48. Cayen, M. N., and D. Dvornik. 1979. Effect of diosgenin on lipid metabolism in rats. *J. Lipid Res.* **20:** 162–174.
49. Smallwood, R. A., and N. E. Hoffman. 1976. Bile acid structure and biliary secretion of cholesterol and phospholipid in the cat. *Gastroenterology.* **71:** 1064–1066.