

Sterol metabolism studies in the rat. Effects of primary bile acids (sodium taurochenodeoxycholate and sodium taurocholate) on sterol metabolism

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Abstract Sterol metabolism studies using a combination of isotopic and chromatographic procedures were carried out in rats fed either a low-cholesterol stock diet or a stock diet containing 0.1% cholesterol. The primary bile acids, sodium taurochenodeoxycholate and sodium taurocholate, were added to the stock diets at a level of 0.5%, as required. Feeding sodium taurochenodeoxycholate and sodium taurocholate led to a decrease in acidic steroid synthesis, cholesterol turnover, and cholesterol balance, compared to controls. Sodium taurochenodeoxycholate feeding did not influence cholesterol absorption, but rats fed sodium taurocholate showed a twofold increase in cholesterol absorption as well as an accumulation of cholesterol in the liver. Rats receiving diets containing sodium taurochenodeoxycholate or sodium taurocholate plus cholesterol (0.1%) showed decreased acidic steroid synthesis, cholesterol turnover, and cholesterol balance, compared to the corresponding controls (Group 2, high cholesterol diet). Significantly larger amounts of cholesterol were absorbed in the taurocholate group (34.1 mg/day); these animals increased their concentrations of cholesterol in liver and plasma. The rats fed taurocholate plus 0.1% cholesterol differed from those fed taurochenodeoxycholate plus 0.1% cholesterol in the following respects: *a*) increased cholesterol absorption (35%), and *b*) accumulation of cholesterol in liver and plasma.

Supplementary key words Sterol metabolism · cholesterol turnover · bile acid synthesis · cholesterol absorption · cholesterol balance.

The primary bile acids formed from cholesterol are chenodeoxycholic acid and cholic acid. The pathways for this conversion have been well studied (1–3). Effects of bile acids on their own synthesis and metabolism have been examined in a variety of species including man, hamsters, and rats. A useful technique to measure various aspects of cholesterol–bile acid metabolism, the sterol balance method (4), provides a tool for determining cholesterol balance, cholesterol absorption, and bile acid synthesis *in vivo*. The sterol

metabolism studies in this investigation were undertaken to explore possible differential effects of the two primary bile acids, sodium taurocholate and sodium taurochenodeoxycholate, on cholesterol and bile acid metabolism and cholesterol absorption in the rat. A low as well as a high cholesterol diet was employed in order to study changes in bile acid output in response to changes in available substrate for hepatic bile acid synthesis.

MATERIALS AND METHODS

Animals and diet

Male Sprague-Dawley derived rats weighing an average of 230–270 g were purchased from Charles River Breeding Laboratories, Wilmington, MA. The animals were placed in metabolic cages and were studied during the experimental period by procedures previously described (5, 6). They were fed the experimental diets over the entire 14-day experimental period. The basal diet consisted of Rockland rat chow supplemented with 5% corn oil, and it contained an average of 0.30 mg/g of cholesterol and 0.64 mg/g of β -sitosterol (7). This diet was further supplemented with either 0.5% sodium taurocholate or 0.5% sodium taurochenodeoxycholate by dissolving these bile acids in ethanol and adding them to the food. The food was thoroughly mixed and the alcohol was allowed to evaporate. In some studies the basal diet of Rockland rat chow and corn oil was further supplemented with a small amount of cholesterol (0.1%).

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

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On day 1 of the experiment, each rat was injected intraperitoneally with 10 μCi of DL-[2- ^{14}C]mevalonolactone (Amersham-Searle, Arlington Heights, IL) and feeding of the experimental diet was begun several hours later. Control rats were always studied concurrently with the bile acid-fed groups and were maintained under identical conditions. Feces were collected in three 2-day pools on days 10, 12, and 14 after isotopic labeling. Beginning on day 4 of the experimental period and every 2 days thereafter, plasma was obtained from the tail vein for determination of plasma cholesterol concentration and cholesterol specific activity. At the end of the experiment (day 14), samples of bile and liver were obtained for the determination of biliary cholesterol and liver cholesterol concentrations and specific activities using procedures described earlier (5–7).

Labeled compounds

DL-[2- ^{14}C]Mevalonolactone in benzene was found to be greater than 95% pure on silica gel thin-layer chromatography with acetone–benzene 1:1 (v/v). All solvents were evaporated from the mevalonolactone which was then redissolved in sterile isotonic saline to give a final concentration of 10 $\mu\text{Ci}/\text{ml}$.

Reference compounds

Cholesterol (U.S.P., Nutritional Biochemicals Corp., Cleveland, OH) was recrystallized twice from ethanol. The material was dried in vacuo and stored in a nitrogen atmosphere.

Taurocholic acid (sodium salt) was prepared from cholic acid (Weddell, London) using the procedure of Norman (8).

Taurochenodeoxycholic acid (sodium salt) was synthesized from chenodeoxycholic acid (Intellectual Property Development Corp.) using the methods of Norman (8, 9) with modifications (9).

5 α -Cholestane (Applied Science Laboratories, State College, PA) was used as an internal standard for the gas–liquid chromatographic separations of the neutral sterols and acidic steroids after preparation of trimethylsilyl ether derivatives.

3 α ,7 α -Dihydroxy-12-keto-5 β -cholanoic acid was synthesized according to the procedure of Fieser (10).

Thin-layer chromatography

The thin-layer chromatographic separations were carried out exactly as previously described (7).

Gas–liquid chromatography

The methods and conditions of the gas–liquid chromatography analyses were those previously described by us (5–7, 11). All analyses were carried

out on a Hewlett-Packard (Palo Alto, CA) 7610A gas chromatograph.

Methods for isolation and quantitation of neutral and acidic steroids from feces

The methods used for the extraction of the neutral and acidic steroids from the feces have been described in detail (11–13). Quantitation of the material in the neutral steroid fraction was carried out by gas–liquid chromatography of the trimethylsilyl ether derivatives. 5 α -Cholestane was added to each sample as an internal standard. The recovery of dietary β -sitosterol was used as an index to correct for losses of neutral steroids during the procedure. Since recovery of the β -sitosterol was good (93% or better), no corrections for losses of neutral steroids due to degradation in the intestinal tract were required.

Quantitation of the material in the fecal bile acid fraction was carried out by isotopic techniques similar to those already described (4, 14). The specific activity of plasma cholesterol was determined 6 days before the collection of the fecal samples. The total radioactivity in each fecal bile acid fraction (dpm) divided by the plasma cholesterol specific activity (dpm/mg) was employed to determine the amount of bile acids synthesized during each period. 3 α ,7 α -Dihydroxy-12-keto-5 β -cholanoate served as recovery standard in each sample. Corrections for losses were made as required.

Radioactivity measurements

All radioisotope measurements were made using new scintillation glass vials on a Beckman LS-200 liquid scintillation system (Beckman Instruments, Fullerton, CA). The radioactivity in each sample was obtained after evaporation of the solvent by the addition of 10–12 ml of 2,5-bis[2-(5-*tert*-butylbenzoxazolyl)]thiophene (BBOT) solution (4 g/ml in toluene). Each sample was corrected for background and quenching effects using appropriate blanks and standards.

Calculations

The fecal acidic steroid synthesis was calculated using isotopic techniques described above. The neutral steroid output was determined by chromatographic techniques (combined thin-layer and gas–liquid chromatography). Endogenous neutral sterol production was estimated as previously described (4, 6, 7). Cholesterol absorption was determined as the difference between the dietary intake of cholesterol (determined chromatographically) and the unabsorbed neutral steroids in the feces. The unabsorbed dietary neutral steroids (mg), cholesterol turnover

(mg/day), and cholesterol balance (mg/day) were calculated as described below (4, 6, 7). Cholesterol turnover (mg/day) = daily fecal endogenous neutral steroid output (isotopic) (mg/day) + fecal acidic steroid synthesis (isotopic) (mg/day); cholesterol balance (mg/day) = cholesterol output - cholesterol input = fecal neutral sterols (mg/day) + endogenous fecal bile acids (mg/day) - cholesterol intake (mg/day). Our animals were allowed to gain weight, so the measurement of absolute cholesterol synthesis was not possible. The calculation of absolute cholesterol synthesis would require the addition of the amount of cholesterol retained in the animal (retention, mg/day) over the 14-day experimental period. Our rats were maintained under similar experimental conditions and gained similar amounts of weight throughout the 14-day study period.

RESULTS

Rats weighing an average of 230 g were injected with DL-[2-¹⁴C]mevalonolactone to label their cholesterol pool. The rats were then fed the experimental diets for 14 days. In certain cases, a small amount of cholesterol (0.1% by weight) was added to the chow in addition to the bile acids (either sodium taurochenodeoxycholate or sodium taurocholate). The weight gain for the animals in each group was similar (averaging 100 g over the 14-day period, **Table 1**). Added dietary cholesterol did not significantly alter the weight gain over the experimental period. In addition, the food intakes and fecal outputs

of the bile acid-fed and control rats with and without the supplemental cholesterol did not show any significant differences ($P > 0.01$).

Sodium taurochenodeoxycholate-fed rats (with and without supplemental cholesterol, Groups 3 and 4)

Four rats in each group were fed the experimental diet for 14 days. This experimental period permitted us to obtain sufficient fecal samples to compare the effects of the different regimens. The animals receiving taurochenodeoxycholate alone (Group 3) ingested 5.7 mg/day of exogenous cholesterol compared to 42.8 mg/day for the cholesterol plus bile acid-fed rats (Group 4). The difference in cholesterol intake did not affect tissue cholesterol levels in bile acid-fed vs. control animals (Group 1 vs. Group 3 and Group 2 vs. Group 4). These results are summarized in **Table 2**. The animals' liver cholesterol levels remained near normal (2.1 mg/g of liver) except in those fed bile acid plus cholesterol (Group 4) where liver cholesterol rose to 4.5 mg/g. Plasma cholesterol and biliary cholesterol concentrations were not significantly altered by taurochenodeoxycholate feeding with or without the added cholesterol in the diet. Isotopic measurements were made at the end of the experiment to determine the specific activities of liver cholesterol, plasma cholesterol, and biliary cholesterol. These specific activities were found to be similar, as observed previously (5, 6) (See also Table 5).

Data for the acidic steroid synthesis, neutral steroid output, cholesterol turnover, and cholesterol balance of the taurochenodeoxycholate-fed groups (3 and 4) are summarized in **Table 3**. The acidic steroid syn-

TABLE 1. Weights, food intake, and fecal outputs of rats

Group	No. of Rats	Animals + Diet	Initial Weight	Weights at Death	Food Intake	Fecal Output
			g ^a	g ^a	g/day ^b	
1	6	Controls ^c . No added cholesterol	238 ± 27 (201–271)	332 ± 9 (282–351)	25.9 ± 3.3 (19.4–32.7)	7.1 ± 0.9 (5.3–9.2)
2	6	Controls + 0.1% cholesterol	269 ± 32 (247–292)	368 ± 18 (355–381)	27.9 ± 2.1 (26.3–29.1)	7.6 ± 0.8 (7.4–8.9)
3	4	Sodium taurochenodeoxycholate (0.5%)	238 ± 27 (231–249)	325 ± 16 (320–348)	23.0 ± 2.1 (19.1–25.3)	5.9 ± 1.0 (4.6–7.8)
4	4	Sodium taurochenodeoxycholate (0.5%) + 0.1% cholesterol	233 ± 15 (224–256)	346 ± 19 (322–364)	26.3 ± 3.7 (23.7–28.6)	8.1 ± 1.7 (7.3–9.0)
5	4	Sodium taurocholate (0.5%)	237 ± 12 (220–244)	330 ± 20 (323–360)	24.4 ± 2.7 (20.8–28.8)	6.8 ± 0.8 (5.8–7.8)
6	4	Sodium taurocholate (0.5%) + 0.1% cholesterol	244 ± 10 (232–255)	345 ± 11 (315–369)	28.0 ± 11.0 (23.0–32.2)	8.1 ± 3.0 (5.5–9.5)

^a Values represent the average ± SD; numbers in parentheses represent the range.

^b Values represent the average ± SD over the 14-day experimental period. Numbers in parentheses represent the range.

^c Ground Rockland rat chow + 5% corn oil.

TABLE 2. Effect of diet on cholesterol concentration in body tissue^a

Group No.	Animals + Diet	Liver Cholesterol	Plasma Cholesterol	Bile Cholesterol
		mg/g ^b	mg/100 ml ^b	mg/ml ^b
1	Controls. No added cholesterol	2.2 ± 0.24	73 ± 13.0	0.19 ± 0.04
2	Controls + 0.1% cholesterol	2.5 ± 0.15	86 ± 30	0.15 ± 0.00
3	Sodium taurochenodeoxycholate (0.5%)	2.1 ± 0.24	67 ± 10	0.15 ± 0.02
4	Sodium taurochenodeoxycholate (0.5%) + 0.1% cholesterol	4.5 ± 0.27 ^c	90 ± 2.0	0.17 ± 0.01
5	Sodium taurocholate (0.5%)	3.0 ± 0.62 ^d	72 ± 7	0.15 ± 0.01
6	Sodium taurocholate (0.5%) + 0.1% cholesterol	8.0 ± 1.1 ^e	114 ± 5.0 ^e	0.18 ± 0.01

^a Determined by combined TLC/GLC techniques.

^b Values represent the average ± SD for the animals in each group.

^c Differs significantly from controls (Groups 1 and 2) and bile acid-fed (Group 3) rats ($P < 0.01$).

^d Differs significantly from Group 1 controls ($P < 0.025$), and bile acid-fed rats (Group 3) ($P < 0.025$).

^e Differs significantly from controls (Groups 1 and 2) and bile acid-fed groups 3, 4, and 5) ($P < 0.01$).

thesis dropped 75% (from 12.0 mg/day in controls (Group 1) to 3.2 mg/day in Group 3). The daily neutral steroid outputs for controls and the taurochenodeoxycholate-fed rats (Group 3) were not significantly different ($P > 0.01$). Endogenous neutral steroid output decreased from 7.7 mg/day to 5.1 mg/day ($P < 0.025$). The rats in Groups 1 and 3 ingested 6.8 and 5.7 mg/day respectively of exogenous cholesterol and absorbed 1.3–1.2 mg/day (14–16%) of this cholesterol. Large decreases in cholesterol turnover and cholesterol balance for taurochenodeoxycholate-fed rats were observed (from 19.5 mg/day to 8.2 mg/day, and 19.5 mg/day to 7.6 mg/day, respectively) (Group 1 vs. Group 3).

Adding cholesterol (0.1%) to the bile acid diet caused large changes in the sterol metabolism of the rats. The animals in Group 2 (control diet plus 0.1% cholesterol) and Group 4 (taurochenodeoxycholate plus cholesterol) ingested 45.0 or 42.8 mg/day of cholesterol and absorbed 26.3 mg/day and 25.2 mg/day, respectively. The rats compensated for this large increase in dietary cholesterol by *a*) increasing their acidic steroid synthesis (47%, Group 1 controls compared to Group 2 controls; or 3-fold, when comparing taurochenodeoxycholate without and with added cholesterol); *b*) increasing neutral steroid output (71%, Group 1 controls compared to Group 2 controls; or 2-fold, when comparing taurochenodeoxy-

TABLE 3. Comparative sterol metabolism data^a

Group No.	Animals + Diet	Daily Acidic Steroid Output	Daily Neutral Steroid Output	Daily Endogenous Neutral Steroid Output	Daily Cholesterol Absorption	Cholesterol Turnover	Cholesterol Intake	Cholesterol Balance
		mg/day	mg/day	mg/day	mg/day	mg/day	mg/day	mg/day
1	Controls. No added cholesterol	12.0 ± 3.6 (7.9–16.9)	14.3 ± 4.7 (6.9–25.9)	7.7 ± 2.8 (3.7–11.8)	1.3 ± 0.99 (0–2.4)	19.5 ± 5.0 (12.7–27.8)	6.8 ± 1.3 (5.3–8.9)	19.5 ± 5.2 (12.7–27.8)
2	Controls + 0.1% cholesterol	18.9 ± 4.3 (14.7–24.6)	24.3 ± 0.9 (22.1–26.5)	5.6 ± 0.9 (4.5–6.8)	26.3 ± 1.1 (21.3–28.9)	24.6 ± 4.3 (21.5–30.8)	45.0 ± 1.7 (42.3–46.9)	–1.8 ± 6.1 [+0.5–(–7.7)]
3	Sodium taurochenodeoxycholate (0.5%)	3.2 ± 0.7 ^b (2.2–3.8)	10.1 ± 1.1 (8.5–11.4)	5.1 ± 0.8 (3.6–6.8)	1.0 ± 0.82 (0–2.5)	8.2 ± 0.9 (7.2–9.5)	5.7 ± 0.5 (4.6–6.2)	7.6 ± 1.0 ^b (6.5–9.0)
4	Sodium taurochenodeoxycholate (0.5%) + 0.1% cholesterol	9.7 ± 2.1 ^c (6.2–13.8)	24.0 ± 1.2 (18.7–32.8)	6.4 ± 1.2 (5.4–9.1)	25.2 ± 0.6 (21.8–27.0)	16.1 ± 2.5 ^c (14.0–19.9)	42.8 ± 2.7 (38.2–46.0)	–9.1 ± 4.3 [–0.7–(–13.5)]
5	Sodium taurocholate (0.5%)	4.5 ± 1.6 ^b (3.6–7.3)	7.0 ± 1.2 ^b (5.5–8.5)	3.7 ± 0.7 ^b (2.9–5.1)	2.8 ± 0.5 ^b (2.1–3.7)	8.1 ± 1.4 ^b (7.6–10.2)	5.8 ± 0.6 (5.0–6.8)	5.7 ± 1.7 (2.9–8.6)
6	Sodium taurocholate (0.5%) + 0.1% cholesterol	11.0 ± 3.1 ^d (5.5–15.9)	14.0 ± 0.6 ^d (11.6–18.7)	5.7 ± 1.0 (5.0–7.6)	34.1 ± 2.4 ^d (28.4–44.8)	16.7 ± 3.4 ^d (13.6–22.3)	41.6 ± 8.8 (17.1–52.8)	–16.6 ± 8.8 ^d [4.0–(–26.1)]

^a Values reported are for the last 6 days of the experimental period; numbers in parentheses represent the range.

^b Differs significantly from controls (Group 1) ($P < 0.01$).

^c Differs significantly from controls (Group 2) and bile acid-fed group (Group 3) ($P < 0.01$).

^d Differs significantly from controls (Group 2) and bile acid-fed group (Group 5) ($P < 0.01$).

TABLE 4. Determination of bile acids by isotopic measurements

Animal No. and Fecal Pool ^a	Acidic Steroids in Feces ^b	Acidic Steroid Synthesis ^c	Fecal Bile Acid Specific Activity ^d	Plasma Cholesterol Specific Activity 6 Days Earlier ^e	Plasma Cholesterol Specific Activity 4 Days Earlier ^f
	dpm/day	mg/day	dpm/mg	dpm/mg	dpm/mg
1—Day 12	16,170	16.5	980	1,030 (5)	960 (2)
2—Day 12	11,150	10.2	1,090	960 (14)	790 (28)
3—Day 14	13,410	7.7	1,740	1,820 (4)	1,480 (15)
4—Day 12	25,000	13.2	1,890	2,010 (6)	1,500 (21)
5—Day 12	20,000	14.1	1,420	1,500 (5)	1,020 (28)
6—Day 14	45,700	12.8	3,570	3,710 (4)	2,330 (35)
7—Day 14	86,400	12.6	6,860	5,960 (15)	4,370 (36)
8—Day 12	64,300	8.9	7,230	6,900 (6)	5,370 (26)
9—Day 12	81,100	7.3	11,120	10,320 (8)	6,090 (45)

^a Represents the 2-day fecal pool in nine rats fed stock chow either 12 or 14 days after isotope labeling.

^b Determined on fecal extract by liquid scintillation techniques described in the text.

^c Determined by chromatographic measurements on fecal extract as described in the text.

^d Determined by dividing acidic steroids in feces (dpm/day)/acidic steroid in feces (mg/day).

^e Plasma cholesterol specific activity measured 6 days prior to fecal bile acid specific activity. Numbers in parentheses represent the percent difference between fecal bile acid specific activity and plasma cholesterol specific activity 6 days earlier.

^f Plasma cholesterol specific activity measured 4 days prior to fecal bile acid specific activity. Numbers in parentheses represent the percent difference between fecal bile acid specific activity and plasma cholesterol specific activity 4 days earlier.

cholate alone and with supplemental cholesterol); *c*) increasing cholesterol turnover (26%, Group 1 compared to Group 2; or 2-fold, comparing taurochenodeoxycholate alone and with cholesterol); and *d*) negative cholesterol balance (see Table 3). Cholesterol balance was more negative in the group given bile acid plus 0.1% cholesterol (from 7.6 mg/day to -9.1 mg/day). Rats given the additional cholesterol alone showed cholesterol balances lower by an average of 21.3 mg/day compared to controls (Group 1).

Sodium taurocholate-fed rats (with and without supplemental cholesterol, Groups 5 and 6)

The rats in Groups 5 and 6 were fed sodium taurocholate (0.5%) and sodium taurocholate with 0.1% cholesterol. The corresponding controls were studied concurrently (Groups 1 and 2). The rats on the low cholesterol diet (and low cholesterol plus bile acid) received 6.8 or 5.8 mg/day of cholesterol compared to 41.6 mg/day ingested by the cholesterol-supplemented group. The liver cholesterol concentrations of the sodium taurocholate-fed rats were significantly different from the corresponding controls (3.0 mg/g compared to 2.2 mg/g, respectively) ($P < 0.025$). When cholesterol was added to the diet, the liver cholesterol concentrations of the bile acid

group showed the expected increase (from 2.5 mg/g to 8.0 mg/g). Taurocholate administration did not alter biliary cholesterol levels, which ranged 0.15–0.19 mg/ml for the animals in each group. Taurocholate alone did not alter plasma cholesterol levels but when it was in combination with the supplemental 0.1% cholesterol, plasma cholesterol rose from 86 mg% (Group 2) to 114 mg% (Group 6).

Sterol metabolism data for the rats fed the taurocholate diets are summarized in Table 3. Acidic steroid output decreased significantly in both taurocholate-fed groups (63% in the taurocholate Group 5 and 30% in the taurocholate plus cholesterol Group 6). The supplemental cholesterol caused a significant increase in acidic steroid synthesis (59%) compared to rats receiving the bile acid alone. The fecal neutral steroid output was also decreased in both of the taurocholate-fed groups (51% in Group 5 and 58% in Group 6, Table 3).

Cholesterol absorption increased dramatically in the taurocholate-fed rats. Rats ingesting taurocholate alone increased cholesterol absorption by 1.5 mg/day (from 1.3 mg/day to 2.8 mg/day), while rats receiving the bile acid with 0.1% cholesterol increased their cholesterol absorption by 7.8 mg/day (Group 2 vs. Group 6). Cholesterol turnover and cholesterol bal-

TABLE 5. Representative specific activities of cholesterol in liver, plasma, and bile on day 14^a

Group No. and Diet	Animal ^b	Liver	Plasma	Bile
		Cholesterol Specific Activity	Cholesterol Specific Activity	Cholesterol Specific Activity
		dpm/mg	dpm/mg	dpm/mg
1 Controls. No added cholesterol	A	1680	1400	1740
	B	1990	2060	1900
	C	3810	3870	3440
	D	1990	2050	1900
2 Controls + 0.1% cholesterol	A	1300	1000	1270
	B	1560	1690	1700
	C	2390	2790	2450
	D	360	350	350
3 Sodium taurochenodeoxycholate (0.5%)	A	7260	7630	7300
	B	6270	7240	7150
	C	7190	7310	7580
	D	7260	7100	7200
4 Sodium taurochenodeoxycholate (0.5%) + 0.1% cholesterol	A	5600	5810	5570
	B	4730	5570	5230
	C	5630	5180	5430
	D	6070	6300	6670
5 Sodium taurocholate (0.5%)	A	2490	1900	2160
	B	1830	1700	1690
	C	1960	1670	1650
	D	2730	1970	2210
6 Sodium taurocholate (0.5%) + 0.1% cholesterol	A	2280	1740	1830
	B	1660	1370	1410
	C	2110	1600	1620
	D	1550	1290	1300

^a Determined by combined TLC/GLC techniques and liquid scintillation counting on day animals were killed.

^b Represents the animals in each group.

ance were depressed in both taurocholate-fed groups (see Table 3). Cholesterol balance was lowest in the group receiving the taurocholate with the supplemental cholesterol in the diet, indicating accumulation of cholesterol in the body.

To determine on which day the specific activity of the cholesterol in plasma reflected the specific activity of previously synthesized bile acids in feces, the following experiment was carried out. Total bile acids in the feces (mg/day) were determined by chromatographic methods. Plasma cholesterol specific activity was determined as described above. We subsequently matched the day on which the specific activity of synthesized fecal bile acids was closest to that of plasma cholesterol. It appeared that the specific activity of plasma cholesterol 6 days before fecal collection gave the most consistent correlation. These results are summarized in **Table 4**.

The isotopic method permits us to determine daily bile acid synthesis when exogenous bile acids are ad-

ministered. We have consequently used this method to determine bile acid synthesis in the bile acid-fed rats where we cannot apply the chromatographic method. We observed that the specific activity decay of plasma cholesterol for the animals in the bile acid groups was slower than for the control animals receiving no bile acid. This tended to support our method for measurement of bile acids by isotope techniques since less dpm/day were excreted in the feces of rats receiving bile acids compared to controls and the specific activity of plasma cholesterol on any particular day was higher for bile acid-fed animals compared to controls. We should indicate that the 6-day specific activity measurement should be applied with caution since it could vary with *a*) type of animal, *b*) type of diet (solid vs. liquid), and *c*) age and physiological condition of the animals.

The specific activities of cholesterol in plasma, liver, and bile for the animals in each group on day 14 are shown in **Table 5**. Each animal was injected on day 1 of the experiment with 10 μ Ci of DL-[2-¹⁴C]-mevalonolactone. The values for the specific activities on day 14 were similar and indicated that an isotopic equilibrium of cholesterol had been achieved in these tissues during this short-duration metabolism study.

DISCUSSION

The present study reports the differential effects of 0.5% sodium taurochenodeoxycholate and 0.5% sodium taurocholate administration on *a*) bile acid synthesis, *b*) cholesterol absorption, *c*) endogenous neutral sterol production, and *d*) cholesterol balance. In certain cases, additional amounts of cholesterol (0.1% of the diet) were fed along with the bile acid to enable us to obtain more precise determinations of cholesterol absorption. Previous studies on the effects of bile acid feeding on sterol metabolism have been made using isotopic measurements (14), while other studies have been based on the hypothesis that measurement of the activities of the rate-limiting enzymes of cholesterol and bile acid synthesis reflected rates of *in vivo* cholesterol/bile acid metabolism (5, 6). We studied cholesterol/bile acid balance in rats fed sodium taurocholate and sodium taurochenodeoxycholate using a combination of isotopic and chromatographic techniques. These measurements are thought to reflect more accurately the state of cholesterol metabolism *in vivo* (4-6).

Sterol balance measurements are usually made under the condition of a metabolic "steady state",

which has been defined as follows (4): *a*) constant plasma cholesterol concentrations, *b*) unvarying output of fecal neutral sterols, and *c*) constant body weight over a period of time. Our rats were in a "steady state" (constant plasma cholesterol concentrations for each rat over days 6–14, and constant fecal neutral sterol output for each rat over days 10–14), except that they gained weight during the experimental period.

Rats normally gain weight during most of their life span and it is probably preferable to allow them to do so rather than to keep them at constant weight by dietary deprivation. We have reported that valid comparisons of sterol balance data can be made between groups of rats treated under similar conditions and allowed to gain weight (5, 6). Consequently, the rats in the present studies were permitted to gain weight. We have not made corrections for this fact, thus accounting for the negative cholesterol balance in certain groups. Beher et al. determined that there were no significant differences in carcass cholesterol in rats fed *a*) a control diet, *b*) a control diet + cholic acid, *c*) a control diet + cholic acid + cholesterol, or *d*) a control diet + chenodeoxycholic acid (15). Thus, we report values for cholesterol balance rather than cholesterol synthesis since cholesterol retention was not measured and no increase in tissue cholesterol (except liver and plasma cholesterol) occurs in the rat (15). (It is apparent that, during balance studies in man, changes of cholesterol tissue pools could occur during a given experimental manipulation but would not necessarily be detected.)

Sterol metabolism studies in growing rats (16) and growing swine (17, 18) have been reported. The studies in swine measured parameters similar to those reported in the present study, with the additional calculation of sterol retention. Sterol retention values make it possible to obtain absolute measurement of cholesterol synthesis in the growing animal. In our studies, we did not make this measurement; consequently our values for cholesterol balance do not include cholesterol retention and can be used *only for comparison* between groups of animals maintained under similar experimental conditions.

Taurochenodeoxycholic acid feeding [with and without supplemental cholesterol (Groups 3 and 4)]

Under the conditions of the present study, the rats receiving taurochenodeoxycholate diets ingested an average of 125 mg/day of this bile acid along with either 5.7 mg/day of cholesterol (no supplemental cholesterol added, Group 3) or 42.8 mg/day (0.1% cholesterol added, Group 4). The animals on these diets showed no significant differences from the

corresponding controls, Groups 1 and 2, in their weight gain, food intake, or fecal outputs for the entire experimental period (Table 1, $P > 0.1$). Biliary and plasma cholesterol levels did not rise significantly during taurochenodeoxycholate feeding (Group 3 vs. Group 1 controls). Liver cholesterol levels increased in the rats ingesting taurochenodeoxycholate plus cholesterol. This increase is attributed to exogenous cholesterol that was absorbed by these animals and not converted to bile acids (see below).

Sterol metabolism measurements reflected certain important changes in the bile acid-fed groups compared to the controls. On the low cholesterol diets, significant decreases in bile acid synthesis and overall cholesterol balance were found in the bile acid-fed rats (Table 3, Group 1 vs. Group 3). The decrease in bile acid synthesis in the taurochenodeoxycholate-fed rats (Group 3) could be due to the decrease in available cholesterol substrate for the conversion to bile acids. Mitropoulos et al. reported that newly synthesized cholesterol might be the preferred substrate for bile acid synthesis (19). It was also previously reported that taurochenodeoxycholate feeding for a period of one week inhibited the rate-controlling enzyme of cholesterol synthesis, HMG-CoA reductase (20, 21). In previous studies, cholesterol 7 α -hydroxylase activity was only mildly inhibited after taurochenodeoxycholate administration for one week (1, 20, 21). Therefore, the activity of the 7 α -hydroxylase may not reflect the *in vivo* rates of bile acid synthesis when insufficient amounts of endogenous cholesterol are available to the enzyme. However, the presence of moderate amounts of exogenous cholesterol in the diet (Groups 2 and 4) is apparently not sufficient to maintain the increased level of bile acid synthesis in the presence of large amounts (0.5%) of exogenous bile acid (taurochenodeoxycholate). It should be noted that the feeding experiments reported here were carried out for two weeks whereas the enzyme studies were carried out after one week of bile acid feeding (20, 21).

Feeding sodium taurochenodeoxycholate with 0.1% cholesterol revealed differences in the sterol metabolism of the rat not observed with the bile acid alone. The animals in Group 4 showed a significant increase in liver and plasma cholesterol levels compared to corresponding controls ($P < 0.01$) (Group 2). We believe that this cholesterol is exogenous cholesterol that was absorbed, equilibrated with plasma cholesterol, and stored in the liver and plasma. This conclusion is supported by the finding that the specific activities of liver cholesterol and plasma cholesterol were similar on day 14 of the experi-

ment (Table 5). Control rats receiving only the added cholesterol (Group 2) appeared to compensate for the added influx of dietary cholesterol (26.3 mg/day) by increasing bile acid synthesis from 12.0 mg/day (Group 1) to 18.9 mg/day (Group 2). The presence of sodium taurochenodeoxycholate prevented an elevation in bile acid production to the level present in the Group 2 controls rats. In addition, the bile acid plus cholesterol depressed cholesterol balance. This was attributed to the presence of the cholesterol in the diet, resulting in an accumulation of cholesterol in the liver. Since exogenous bile acid tends to suppress bile acid synthesis while exogenous cholesterol stimulates bile acid production, the results obtained when bile acid and cholesterol were fed in combination would be expected to depend to a considerable extent on the relative proportions of bile acid and cholesterol added to the diet. The amount of cholesterol absorbed by these rats (Group 4) was not significantly different from that of rats receiving no supplemental bile acids (Group 2) ($P > 0.1$).

Sodium taurocholate feeding with and without supplemental cholesterol

Rats receiving sodium taurocholate-enriched diets (Groups 5 and 6) ingested an average of 125 mg/day of this bile acid. They behaved similarly to the chenodeoxycholate animals in that there was no significant increase in weight, food intake, or fecal output over the entire experimental period compared to corresponding controls. Plasma cholesterol levels in the taurocholate group (Group 5) were not significantly different from the taurochenodeoxycholate group (Group 3) or the corresponding controls (Group 1). Liver cholesterol levels were significantly different ($P < 0.025$) when cholesterol was administered in addition to the bile acid (taurocholate). Increases in both liver and plasma cholesterol levels had been observed previously (20, 21). We believe that these increases can be ascribed to a combination of increased cholesterol absorption and decreased conversion of cholesterol to bile acids (see below) (22).

The sterol metabolism studies using sodium taurocholate revealed an important difference between sodium taurocholate and sodium taurochenodeoxycholate. Cholesterol absorption was increased 30% in the taurocholate plus cholesterol group (Group 6) compared to the corresponding controls (Group 2) and to the rats fed taurochenodeoxycholate plus cholesterol (Group 4). Part of this increase in daily cholesterol absorption with taurocholate can be seen in the increase in both liver cholesterol and plasma cholesterol levels. The increased cholesterol

absorption was also reflected in a decreased output of fecal neutral sterols (14.0 mg/day vs. 24.3 mg/day for Group 2 controls). The effect of the exogenous cholesterol acting as a substrate for bile acid synthesis was also seen in the taurocholate-fed rats (Group 6). Taurocholate administered without supplemental cholesterol gave a calculated bile acid synthesis rate of 4.5 mg/day; with the added cholesterol (Group 6), bile acid synthesis increased to 11 mg/day.

The negative value for cholesterol balance was the result of the weight gain and body tissue accumulation of cholesterol of the rats during the experimental period. Apparently, rats can utilize exogenous cholesterol to produce bile acids. This is not entirely in accord with the conclusions of Mitropoulos et al. (19) who suggested that newly synthesized cholesterol was the preferred substrate for bile acid synthesis. However, this difference might be attributable to the *in vitro* methodology used by Mitropoulos compared to the *in vivo* balance technique (19). The activities of the rate-limiting enzyme of cholesterol synthesis reported previously showed that cholesterol synthesis was decreased over a 1-week feeding period (20, 21). However, for reasons remaining to be determined, the activity of cholesterol 7 α -hydroxylase, the rate-limiting enzyme of bile acid synthesis, did not correspond to observed rates of bile acid production in certain groups of animals. Further studies are required in this area.

In summary, we have compared the effects of feeding sodium taurochenodeoxycholate and sodium taurocholate (with low and high cholesterol diets) on sterol metabolism in the rat. We observed that *a*) sodium taurocholate causes increased cholesterol absorption compared to sodium taurochenodeoxycholate, *b*) both sodium taurochenodeoxycholate and sodium taurocholate depress bile acid synthesis and lead to a lower cholesterol balance, and *c*) in the presence of exogenous (0.1%) cholesterol, bile acid synthesis remains depressed when sodium taurochenodeoxycholate (0.5%) and sodium taurocholate (0.5%) are present in the diet. ■■

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