The sources of rat biliary cholesterol and bile acid

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Abstract The precursor sources of bile acid and bile neutral sterol were evaluated in the rat using Triparanol to inhibit the terminal reduction in the synthesis of cholesterol. During the initial period of Triparanol administration, the accumulation of hepatic desmosterol acts to segregate relatively newly synthetic hepatic sterol from the bulk of the equilibrated sterol mass. Biliary excretion of newly synthetic sterol can then be determined in acute studies, assuming no great differences between desmosterol and cholesterol as precursors of biliary neutral sterol or bile acid. It has been determined in this model that newly synthetic sterol comprises a mean of about 28% of the total biliary neutral sterol output. This fraction fell when hepatic cholesterogenesis was suppressed by prior cholesterol feeding. By using this approach in conjunction with the administration of labeled mevalonate to a renal pedicle-ligated rat, it was possible to calculate the amount of bile acid produced from either newly synthesized sterol or the equilibrated sterol pool. It has been estimated that the bulk of bile acid synthesis arises from this equilibrated source when these determinations were made within two hours of creating the fistula. With more prolonged fistula times, more of the bile acid originated from the newly synthesized sterol.

Supplementary key words desmosterol · Triparanol

The kinetic analysis of human biliary bile acid and cholesterol specific activities after injecting radioactive cholesterol suggests that the primary bile acids and biliary cholesterol are solely derived from the rapidly miscible sterol pool (1). However, this pool is apparently made up of several anatomically and kinetically distinct subpools, some of which may be the preferred source of biliary lipid. A number of studies (2–4) indicate that bile acid does not come from the entire rapidly miscible pool but from one or more of these subpools. Some data (2) suggest that the preferred substrate[1] for biliary bile acid is a newly synthesized cholesterol molecule which is converted to bile acid prior to equilibration with the entire rapidly miscible cholesterol pool. Since it is difficult to ascertain the mass of newly synthesized cholesterol and its corresponding specific activity, semiquantitative estimations of the amount of synthesized cholesterol that is converted to bile acid prior to equilibration with body pools are not available.

The origin of bile neutral sterol is also not established. When labeled cholesterol is injected in the plasma of humans (1), the biliary cholesterol has a specific activity similar to that of plasma after 24 hr, suggesting ready equilibration within the rapidly miscible pool. However, little activity data are available at early times, which would be necessary to determine the subpool source of biliary cholesterol. The precursor sources of bile neutral sterol and bile acid probably significantly influence the net biliary secretion of both these lipids. If synthesized cholesterol were a preferred substrate for bile acid production and not for biliary neutral sterol delivery, enhanced hepatic cholesterol synthesis might be anticipated to provoke higher ratios of bile acid to neutral sterol in bile. If the reverse were the case, the opposite observation should follow for bile.

When Triparanol is acutely administered to an animal to block reduction of desmosterol to cholesterol, an estimate of the mass of newly synthesized sterol can be made. This newly synthesized sterol by definition has not equilibrated with the bulk of the body cholesterol (i.e., equilibrated cholesterol) and its appearance in bile in either neutral or acidic sterol form in effect short circuits its metabolism. The relative amount of desmosterol that has equilibrated with plasma in comparison to that in bile permits a semiquantitative estimate of the newly synthesized contribution to bile neutral sterol. When the synthesized and equilibrated cholesterol pools are separately labeled, the precursor role of these two pools for the bile acids of bile can be estimated.

Three major assumptions underly this approach. It is first assumed that brief exposure to Triparanol has no toxic effect on the animal. Second, it is assumed the desmosterol that accumulates in response to this treatment is metabolized by the liver similarly to cholesterol, at least as far as the biliary system is concerned. Third, the calculation of bile acid synthesis assumes that the labeled precursor of synthesized sterol is in large measure diverted to desmosterol after brief

[1] Preferred substrate, in effect, means that the sterol is converted to biliary lipid prior to equilibrating with the entire rapidly miscible sterol pool or is enriched in bile in comparison to this pool.

(<48 hr) exposures to Triparanol. This experimental approach was used in the rat to define the origin of these biliary lipids shortly after bile duct interruption when the enterohepatic cycle was relatively intact, and after a period of fistula drainage when the enterohepatic bile acid pools were depleted.

MATERIALS AND METHODS

Male Sprague-Dawley rats (150–250 g) were housed under controlled lighting conditions. The basic diet of rat chow (Ralston Purina, St. Louis, MO) was supplemented with 2% (w/w) cholesterol, or 0.1% Triparanol, or both for varying periods in the experimental groups. Triparanol was obtained from Richardson Merrill (Cincinnati, OH) and dietary cholesterol from Fisher (Pittsburgh, PA). Bile duct cannulations were performed under light ether anesthesia with a PE-50 tube (Clay Adams, Parsippany, NJ) inserted into the common duct at 1 cm distal to the bifurcation of the main hepatic ducts. Some of the animals had cannulas implanted in the femoral veins for intravenous injections and blood collection. Most of the animals had ligation of both renal pedicles to obstruct arterial inflow and delete the renal influence on systemic sterol metabolism. Only those animals having renal pedicle ligations were used to calculate the fraction of bile acid synthesis arising from different precursor sources. The animals were placed in restraining cages and allowed access to water and appropriate chow.

Immediately after cannulating the bile duct, chromatographically pure radioactive cholesterol or desmosterol dispersed on rat lipoproteins (5) and either [5-^{3}H] or [2-^{14}C]DL-mevalonic acid was injected intravenously in animals in many of the studies. The [4-^{14}C]- and [1,2-^{3}H]cholesterol, [5-^{3}H]- and [2-^{14}C]DL-mevalonate, and [26-^{14}C]desmosterol used were obtained from New England Nuclear, Boston, MA. The mevalonate was recovered from its DBED salt by an established method (6) prior to use. Bile was collected at regular intervals (hourly for the first 4 hr and every 2 hr thereafter) by use of a fraction collector (LKB, Sweden). The bile was collected in methanol (4 volumes of methanol per volume of bile) at room temperature and, after recording volume, was stored at 4°C prior to further analyses. All solvents used were reagent grade and were redistilled before use.

At the end of the experiment, the animals were killed and the livers were removed. The hepatic venous system was perfused with iced 0.1 M phosphate buffer at pH 7.4 to remove blood and aliquots of whole liver and various homogenate fractions were taken for neutral sterol analysis. Hepatic sediment and mitochondrial, microsomal, and supernatant fractions were prepared as previously described (7). The plasma was separated from the red cells by centrifugation at 3,000 rpm in an L 2-B centrifuge (Sorval, Norwalk, CT) at 7°C; it was frozen before analysis.

The neutral sterols were extracted from 4 ml of the bile-methanol mixture by adding 20 ml of petroleum ether (bp 40–60°C), agitating for 4 min, and centrifuging for 10 min at 2,000 rpm in an International centrifuge (Model K, International Equipment Co., Boston, MA) at room temperature. The petroleum ether fraction was removed and the infranatant was re-extracted with an additional 20 ml of petroleum ether. The combined petroleum ether fractions were evaporated and backwashed with methanol–water 1:1. Experiments using labeled taurocholate showed that this procedure resulted in recoveries of more than 95% of neutral sterols with less than 1% bile acid contamination, and more than 92% of the bile acid was recovered in the infranate with no neutral sterol contamination. Thirty minutes after the labeled mevalonate injection, all of the radioactivity in the acidic sterol fraction chromatographed as bile acid. Plasma and liver homogenates were extracted with chloroform methanol 2:1 (20 volumes per volume) by the method of Folch, Lees, and Sloane Stanley (8).

The neutral sterols were quantitated by colorimetry (9, 10) or by gas–liquid chromatography (11) on 3% SP-2250 on 100/120 mesh Supelcoport (Supelco Inc., Bellefonte, PA) using a Packard 7400 series gas chromatograph (Downers Grove, IL). Bile acids were methylated and acetylated and analyzed by gas–liquid chromatography (12) using 3α,7α-dihydroxy-12-keto-5β-cholanoic acid as the reference standard. For subsequent specific activity determinations, desmosterol was readily separated from cholesterol by thin-layer chromatography (13). No cross contamination of the two neutral sterols was observed in this system. Radioactivity was assayed in a Packard model 3375 liquid scintillation spectrometer using Aquasol (New England Nuclear) as the fluor. Experiments with ^{14}C and ^{3}H in the same sample were counted under double-label conditions (14) and appropriate corrections were used to calculate disintegrations per minute.

The biliary secretion of unequilibrated newly synthesized sterol was estimated by correcting the desmosterol output in bile by the amount of desmosterol that had equilibrated with the plasma using Equation A.

$$\text{Synthetic sterol } (\mu\text{mol/hr}) = \text{bile desmosterol } (\mu\text{mol/hr}) - \frac{\text{Bile cholesterol } (\mu\text{mol/hr})}{\text{Plasma cholesterol/Desmosterol ratio}}. \quad \text{Eq. A}$$

TABLE 1. Rat bile neutral and acidic sterol outputs with Triparanol feeding[a]

	Control	Triparanol-fed		
		1 day	5–6 days	10–14 days
			μmol/hr	
Neutral sterols	0.30 (0.06)	0.31 (0.04)	0.30 (0.05)	0.28 (0.04)
Acidic sterols	14.18 (6.1)	13.24 (6.0)	12.3 (4.0)	17.6 (4.8)

[a] The outputs of both acidic and neutral sterols were determined between hours 1 and 2 after the fistula was established (9 animals in each group). The numbers within the parentheses represent the mean ± SEM of nine determinations.

The amount of bile acid derived from newly synthetic sterol was calculated using Equation B after administering [^{3}H]mevalonate.

$$\text{Synthetic sterol bile acid } (\mu\text{mol/hr}) = \frac{^3\text{H-labeled bile acid (dpm)/hr}}{\text{bile desmosterol specific activity}\left(\frac{\text{dpm}}{\mu\text{mol}}\right)} \quad \text{Eq. B}$$

The amount of bile acid derived from equilibrated cholesterol was calculated using Equation C after administering [^{14}C]cholesterol.

$$\text{Equilibrated sterol bile acid } (\mu\text{mol/hr}) = \frac{^{14}\text{C-labeled bile acid (dpm)/hr}}{\text{bile cholesterol specific activity}\left(\frac{\text{dpm}}{\mu\text{mol}}\right)} \quad \text{Eq. C}$$

RESULTS

Three major assumptions are implicit in the use of Triparanol to segregate newly synthesized cholesterol in these studies. It is important to document that Triparanol is not toxic to the animal and, in particular, that it does not influence the process of biliary secretion. Second, to make analogies to the physiologic situation, desmosterol must not be favorably or unfavorably converted to bile acid or secreted as neutral sterol, since desmosterol is the synthetic product in this model. The third major assumption is that the Triparanol dose used during these brief times does, in fact, stop the terminal conversion of mevalonic acid to cholesterol.

Toxic effects from the brief exposures to Triparanol were not observed either by gross or histologic assessment of the livers of treated rats. The bile flows and the total biliary outputs of neutral and acidic sterol were not significantly different for controls and animals fed Triparanol for different periods of time (**Table 1**). To assess the relative degree of biliary secretion of desmosterol and cholesterol, [26-^{14}C]desmosterol was mixed with [1-2^{3}H] cholesterol, dispersed on rat lipoproteins and injected into bile-fistula Triparanol-treated animals. The ratio of [^{3}H]cholesterol/[^{14}C]desmosterol in bile was similar to that of plasma for up to 8 hr (**Table 2**). This observation, and the neutral sterol outputs of animals treated with Triparanol (Table 1) and controls suggest that the transports of both neutral sterols into bile are not appreciably different. The similarity of biliary bile acid hourly outputs from 1 to 3 hr after surgery for Triparanol and control animals (Table 1) also suggests that desmosterol is handled similarly to cholesterol in terms of net conversion to bile acid and biliary excretion. The similarity of desmosterol and cholesterol as precursors for bile acid transport is also documented by the similarity of mevalonate radioactivity recovered in bile acid in Triparanol-treated and control animals during the initial 12 hr of bile collection (**Table 3**). The bile acid compositions of these Triparanol-treated animals were similar to controls containing predominantly cholic acid.

The final assumption of the model, that Triparanol blocks the completion of cholesterol synthesis, was tested in a number of animals that had been fed Triparanol for only 24 hr prior to receiving [^{14}C]mevalonic acid. It was recognized that the relative incorporations of labeled mevalonate into desmosterol and cholesterol in the Triparanol-treated animals were dramatically affected by whether or not the kidneys were able to metabolize the mevalonate. If the kidneys were intact when mevalonate was injected, an appreciable amount of the mevalonate activity was recovered in biliary cholesterol despite 2 days of Triparanol treatment (**Table 4**). However, if the renal pedicles of both kidneys were ligated just prior to injecting the labeled mevalonate, the recovery of label was predominantly in desmosterol. Over 93% of the label recovered in biliary neutral sterol was in desmosterol in 1–3 hr after the bolus. This would suggest that desmosterol was in fact the predominant synthesized

TABLE 2. Plasma and bile neutral sterol radioactivity ratios after [^{3}H]cholesterol and [^{14}C]desmosterol injection[a]

Time (hr)	$\frac{\text{Bile } ^3\text{H}/^{14}\text{C}}{\text{Plasma } ^3\text{H}/^{14}\text{C}}$
1	0.94 (12)
3	1.12 (13)
5	1.15 (13)
8	1.20 (14)

[a] The data represent the means of the ^{3}H and ^{14}C ratios of bile divided by those of plasma in eight animals. The number within parentheses is the fractional standard deviation of this value expressed as a percent.

TABLE 3. [^{3}H]Mevalonate incorporation into acidic sterols of control and Triparanol-treated rats[a]

	1–3 hr	9–11 hr after fistula
	fraction injected $^3H/2$ hr × 10^3	
Control (6)[b]	0.84 (0.35)[c]	0.09 (0.02)
48 hr Triparanol (6)[b]	1.1 (0.21)	0.13 (0.04)

[a] All 12 animals were injected with 20 μCi of [^{3}H]DL-mevalonate.
[b] Number of animals.
[c] Standard deviation.

neutral sterol of bile after 24 hr of Triparanol exposure in a renal pedicle-ligated rat. The specific activity of desmosterol after labeled mevalonate in these acute Triparanol feeding studies should provide a fair reflection of the synthetic pool. Although it is impossible to define the newly synthesized sterol pool that is the substrate for the 7α-hydroxylation reaction and thus impossible to determine the precise amount of bile acid arising from this source, the pool defined by this intervention provides an approximation of newly synthesized sterol when acute studies are performed. The data arising from the calculations can be regarded only as an approximation.

As would be anticipated, the relative amounts of plasma desmosterol would increase the longer the exposure to Triparanol. This is reflected by a decreasing cholesterol/desmosterol ratio in all tissues at longer times (**Table 5**). As was previously observed (11), the plasma appeared to contain a greater concentration of desmosterol relative to cholesterol than the liver after Triparanol administration. In each of the present studies, the bile was always found to contain significantly more desmosterol than the plasma. The liver was always noted to contain less desmosterol relative to cholesterol than plasma and this was true for specific organelles as well. As was previously documented (11), cholesterol feeding markedly decreased the amount of desmosterol in plasma (Table 5), but the bile still had relatively more desmosterol than did plasma.

TABLE 4. [^{14}C]Mevalonate recovery in neutral sterols of Triparanol-treated rats[a]

	Mean % Total Recovered Biliary Neutral Sterol (Range)	
	Desmosterol	Cholesterol
	% recovery[b]	
Intact kidneys	49 (38–64)[c]	51 (36–62)
Renal pedicle ligated	93 (88–96)	7 (4–12)

[a] The values presented are the mean percents of a 1–3-hr bile collection after a 48-hr Triparanol exposure for eight rats in each group.
[b] Percentage recovery (mean) of ^{14}C in biliary neutral sterols.
[c] Values in parentheses represent ranges.

TABLE 5. Plasma and bile neutral sterol ratios in Triparanol-fed rats[a]

Time of Triparanol Feeding	Cholesterol/Desmosterol Molar Ratio		
	Liver	Plasma	Bile
days			
1		3.22	2.24
1		4.31	2.17
5	2.56	1.69	0.84
5		2.36	1.69
6		1.68	1.28
10		1.10	0.82
11	1.10	1.02	0.52
14	2.11	0.72	0.36
7 (2% cholesterol)		69.2	21.06
10 (2% cholesterol)		39.5	14.65

[a] Concentrations were determined by gas–liquid chromatography as described in the text. Each indicated time represents the studies on a single rat.

The previous data (Table 1 and 2) suggest that desmosterol is not a preferred substrate for bile neutral sterol transport. The marked enrichment of bile with desmosterol would then suggest that some newly synthesized neutral sterol is secreted prior to becoming equilibrated with body sterol. When the bile desmosterol content is corrected for that equilibrated fraction richest in desmosterol (i.e., plasma), the contribution of synthesized unequilibrated desmosterol to total neutral sterol secretion can be estimated. These calculations (**Table 6**) indicate that from 10 to 40% of the biliary neutral sterol appears to arise from a newly synthesized source when the animal is fed the usual chow diet. With cholesterol feeding, the fraction from this source was less. Biliary neutral sterol outputs 24 hr after creating the fistula were substantially less than the acute values, but the fractions com-

TABLE 6. Newly synthesized neutral sterol transport in bile

Triparanol Feeding	Fistula Time	Total Neutral Sterol	Synthesized[a] Neutral Sterol μM/hr	Percent Synthetic
days	*hr*	*μmol/hr*		
1	2	0.343	0.073	21
	24	0.125	0.020	16
5	2	0.286	0.030	10
	24	0.121	0.036	30
11	2	0.201	0.086	43
	24	0.075	0.024	32
14	2	0.197	0.065	33
	24	0.052	0.019	36
7 (2% chol.)	2	0.331	0.011	3
	24	0.281	0.009	3

[a] The synthesized neutral sterol was determined by the formula described in the text. Data for days 1 and 5 are the means of two animals; data for days 11, 14, and 7 were from single animals.

TABLE 7. Origins of bile acid in rats fed Triparanol for 1 day[a]

Experiment	Acute Fistula (1–3 hr)			Chronic Fistula (9–11 hr)		
	Synthetic Source	Equilibrated Source	Total Bile Acid Output	Synthetic Source	Equilibrated Source	Total Bile Acid Output
		μmol/hr	*μmol*		*μmol/hr*	*μmol*
28	0.47	0.85	7.4	0.72	0.48	1.5
29	0.19	0.34	4.9	0.78	0.38	1.4
30	0.33	0.59	6.7	0.59	0.40	1.8
32	0.43	0.32	9.4	1.00	0.49	2.3
33	0.19	1.21	8.56	0.32	0.59	2.2
34	0.17	1.08	8.44	0.52	1.10	1.45
35	0.21	1.25	9.25	0.32	0.84	1.65
36	0.16	0.77	9.11	0.47	0.90	1.6

[a] Calculated from the total acidic sterol counts between hours 1–3 or 9–11 and the mean [^{3}H]desmosterol and [^{14}C]cholesterol at 2 and 10 hr, respectively, as described in the text. Each experiment represents the results of a single animal.

ing from the synthesized source were similar (Table 6). Since the hepatic synthesis of cholesterol (15) is significantly enhanced in control rats after 18 hr of fistula drainage, it might be anticipated that the fraction of newly synthesized biliary neutral sterol would increase. This appeared to be the case for both of the rats fed Triparanol for 5 days (Table 6) but for no other set of animals. However, the time for derepression of cholesterol synthesis in a Triparanol-treated animal is unknown. Although enhanced hepatic cholesterol synthesis was not reflected in the bile, the suppression of synthesis with cholesterol feeding was seen in the bile neutral sterol partition.

The ability to identify a presumed synthesized pool of cholesterol makes possible the calculation of the conversion of this pool to bile acid. The specific activity of this pool, after a labeled cholesterol precursor such as mevalonate, permits the sequential non-steady state estimate of synthetic sterol conversion to bile acid. Although these calculations imply that bile desmosterol specific activity reflects newly synthesized sterol, obviously an approximation, it is a better estimate of synthesized sterol specific activity than that using total biliary sterol mass. The conversions of unequilibrated synthesized sterol and equilibrated cholesterol to bile acid were estimated after brief Triparanol exposures (24 hr). These estimates were made between 1 and 3 hr after the acute fistula, at a time when the animal and bile flows recovered from the effects of surgery. Although substantial variability was noted in the hourly bile acid outputs (**Table 7**), and substantial differences were seen for the acute bile acid turnover, these acute fistula estimates revealed a greater contribution from the equilibrated sterol source than from newly synthesized sterol. Approximately 75% of newly synthesized bile acid appears to arise from this equilibrated source. The bile acid turnover in these animals varied from 10 to 20% in the acute studies. At later fistula times (Table 7, 9–11 hr), a reversal of the contribution from newly synthesized or equilibrated sterol sources for bile acid synthesis was observed for many of the animals. After depletion of much of the bile acid pool, there was an increment in the newly synthesized sterol contribution to bile acid synthesis in each animal when contrasted to the 2-hr fistula data. On the other hand, the relative amount of synthesized bile acid coming from equilibrated sterol appeared to decrease or remain stable with fistula drainage in most animals.

DISCUSSION

The precursor source of biliary neutral and acidic sterols may be important in determining the relative amounts of these materials in bile. If either biliary bile acid or neutral sterol was preferentially derived from newly synthesized cholesterol, a tight coupling of hepatic synthesizing activity and respective biliary lipid output would be anticipated. It has been observed that humans with gallstone disease who have high biliary ratios of cholesterol to bile acids (16) have enhanced hepatic HMG CoA reductase or cholesterol synthesis (17). One might infer from such data that newly synthesized sterol would be a tightly coupled secretory source for biliary cholesterol. Recent studies using labels for the synthesized and equilibrated cholesterol pools, and compartmental analysis suggested that the bile acids and neutral sterols of humans with chronic bile fistulae had distinct precursor pools, both of which received equal inputs from newly synthesized cholesterol (4). Data obtained from rats indicate that bile acids preferentially arise from synthesized cholesterol (2).

The use of Triparanol-treated animals in relatively acute studies enables the segregation of a synthesized pool of cholesterol from the bulk of equilibrated sterol. This assists in estimating the relative importance of these two sources of bile neutral sterol and acidic sterol but introduces some hazards in interpreting the data. Although the present studies would indicate that Triparanol does not significantly affect the process of biliary lipid secretion and that desmosterol is handled in a manner similar to cholesterol in this system, the identity of these preparations with normal physiology cannot be definitely established. The calculation of bile neutral and acidic sterols arising from the "synthesized" and "equilibrated" sterols source can only be regarded as approximations.

The mean enrichment of bile neutral sterol with 28% of newly synthesized sterol is equivalent to a mean daily biliary output of unequilibrated synthesized neutral sterol of about 0.6 mg. In these 200-g rats on sterol-free diets, it would be anticipated that about 4 mg of bile acid and 4 mg of neutral sterol would be lost in the feces daily (18). In the rat, half of the fecal neutral sterol is not from the synthesized pool (19), leaving a systemic turnover of about 6 mg/day in this size animal. This would mean that about 10% of the total daily synthesis enters the bile of the rat prior to equilibration with the systemic pool.

These results indicate that inhibiting cholesterol synthesis significantly decreased the synthesized neutral sterol fraction of bile but accelerated synthesis is not accompanied by a larger synthesized fraction. Data of Wilson (20) indicate that cholesterol feeding, which increases the hepatic content of cholesteryl esters, enhances the biliary output of both neutral sterols and bile acids. When hepatic cholesterol synthesis is accelerated by either a bile fistula or lecithin perfusions (7), a lower net biliary neutral sterol output is observed. In most of the animals the newly synthesized fractions of biliary neutral sterol do not increase with increasing hepatic synthesis. This suggests that hepatic cholesterol synthesis is a poor stimulus for increasing biliary neutral sterol outputs. In fact, greater biliary cholesterol outputs appear to be correlated with larger hepatic cholesterol contents (i.e., cholesterol feeding), a condition associated with suppressed cholesterogenesis.

The finding of approximately a 3:1 preference for equilibrated cholesterol in the formation of bile acids in rats with relatively intact enterohepatic circuits contrasts with data that suggest a preference for newly synthesized sterols (2). It is similar to estimates in the chronically bile-fistulized human who, by simulation and analysis (4), appears to have a 2:1 preference for the equilibrated source. However, our data would indicate that the imposition of a fistula in the system shifts the preference for bile acid formation to newly synthesized sterol. Such a state might also occur in a stagnant enterohepatic circuit as occurs postprandially. In light of the accelerated cholesterol synthesis preceding the recovery of bile acid synthesis of chronic bile fistula rats described by Myant and Eder (15), it was anticipated that newly synthesized cholesterol would be a major source for bile acid production in this state.

The bile acid fractional turnover obtained after these acute studies (i.e., 0.08–0.18) are somewhat lower than those calculated by Lindstedt (21) (i.e., 0.24–0.36) using fecal data and following the exponential decay. The acute turnovers determined here are similar to the extrapolations of Myant and Eder's data (15), assuming that the bile acid output occurring immediately after drainage of the pool represents synthesis.

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