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Metabolism of steroid and amino acid moieties of conjugated bile acids in man: *II. Glycine-conjugated dihydroxy bile acids*

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Metabolism of Steroid and Amino Acid Moieties of Conjugated Bile Acids in Man

II. GLYCINE-CONJUGATED DIHYDROXY BILE ACIDS

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ABSTRACT Chenodeoxycholyl-2,4-^{3}H-glycine-1-^{14}C and deoxycholyl-2,4-^{3}H-glycine-1-^{14}C were synthesized and administered orally to 10 healthy subjects. Distribution of radioactivity among bile acids and specific activity of steroid and amino acid moieties were determined in bile samples. ^{3}H and ^{14}C were measured in feces. ^{14}C in breath was calculated from interval $^{14}CO_2$ specific activity determinations.

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INTRODUCTION

In the preceding paper (1) we characterized the metabolism of the steroid and glycine moieties of cholylglycine using the doubly labeled compound. In this paper, we describe the metabolism of the steroid and glycine moiety of two other major components of the human bile acid pool: chenodeoxycholylglycine and deoxycholylglycine. Although a few observations on the metabolism of the steroid moiety of these bile acids in man have been reported (2, 3), there have been no reports of studies on more than two subjects until the recent one by Vlahcevic, Miller, Farrar, and Swell (4), which showed that the chenodeoxycholic acid pool was smaller than the cholic acid pool and had a slower fractional turnover rate. Our studies, which were in progress at the time of their report, confirm and extend their findings, allowing the first description of the enterohepatic circulation of the three glycine-conjugated bile acids in man.

METHODS

Bile acids. Chenodeoxycholic acid-2,4-^{3}H and deoxycholic acid-2,4-^{3}H were prepared from methyl-7α-hydroxy-3-keto-5β-cholanoate and methyl-12α-hydroxy-3-keto-5β-cholanoate, respectively (5), and conjugated with glycine methyl ester (6) to give the ring-labeled conjugated bile acid methyl esters. These compounds were purified by preparative thin-layer chromatography (TLC), using benzene: acetone (70: 30) as solvent. They were gently saponified to remove the methyl moiety by dissolving in 95% ethanol, adding an equal volume of 2 N sodium hydroxide, and leaving overnight at 37°C. The solution was then adjusted to neutrality with HCl and evaporated to dryness. The cholylglycine was extracted from the residue with chloroform: methanol 2: 1. The final specific activity of the two compounds was 2.0 mCi/mmole. Glycine-1-^{14}C methyl ester was conjugated with chenodeoxycholic acid or deoxycholic acid to yield the glycine-labeled conjugates (SA, 0.5 mCi/mmole) which were purified similarly.

Experimental subjects and procedures. Subjects were carefully informed before giving consent to enter the study. Ten healthy volunteers participated in the study (Table I). Six of these had taken part in a previous study on the metabolism of cholylglycine. On the morning on which the study commenced, a nasoduodenal tube was passed until the tip reached the duodenojejunal flexure; the tube remained in that position for 7 days. Fasting subjects received 10 μCi ^{14}C and 15–20 μCi ^{3}H in either chenodeoxycholylglycine or deoxycholylglycine, dissolved in a milk shake and administered orally. A second study was performed 6 wk after the first, in some subjects, at which time radioactivity from the preceding study was no longer present in the bile. During the study the subjects carried out their usual activities and were instructed to eat three meals per day.

Analysis of bile samples. Samples of bile, obtained by duodenal drainage, were taken on the 7 days after the administration of the isotope: 2 ml of duodenal content was removed after the intravenous administration of 37.5 U of cholecystokinin (Dr. E. Jorpes, Stockholm, Sweden). Samples were collected in 20 ml ethanol, heated to precipitate protein, filtered, and dried. The residue was dissolved in 0.5 ml methanol and stored at 4°C.

A portion of bile was examined by TLC with propionic acid: *n*-propanol: water: iso-amyl acetate (30:20:10:40, v:v) as solvent using an automatic zonal scanner (7) in order to determine the distribution of radioactivity (^{3}H and ^{14}C) among the individual bile-acid fractions. The disintegrations per minute (dpm) in each 4 mm section were plotted and a histogram was obtained indicating the percentage of radioactivity in each component. Reference compounds were chromatographed simultaneously and radioactivity was assigned to chenodeoxycholylglycine and chenodeoxycholyltaurine in the subjects given labeled chenodeoxycholylglycine, and to deoxycholylglycine and deoxycholyltaurine in the subjects given labeled deoxycholylglycine. In some subjects, about 5–10% of ^{3}H activity exhibited the same chromatographic mobility as cholylglycine.

With the same solvent as the first, a second portion of bile was chromatographed on a 20 × 20 cm silicic-acid plate: standards of chenodeoxycholylglycine or deoxycholylglycine were run on either side of the bile portion. The region of the plate containing chenodeoxycholyl and deoxycholylglycine was scraped off and eluted with 0.5 ml phosphate buffer (pH 9.5.). 100 μl of the sample were analyzed for bile acid mass (chenodeoxycholic and deoxycholic acid) using the steroid dehydrogenase method (8), and actual mass was determined from the ratio of deoxycholic: chenodeoxycholic acid obtained by analyzing the bile sample with gas-liquid chromatography (9). The remaining half was counted for ^{3}H and ^{14}C to yield the specific activity of each moiety of each portion of the bile acid conjugate. Samples were run in duplicate and the mean of the duplicate was used. The coefficient of variation was 6%. Because of the rapid loss of ^{14}C label, valid specific activity for the glycine moiety could not be determined after 120 hr in most studies.

Bile acid turnover was calculated according to a first order kinetic model as previously described (10).

Recovery of radioactivity in breath and stool. $^{14}CO_2$ specific activity of breath was measured at 2, 4, 6, 8, 12, 18, and 24 hr as described (11, 12), and a semiquantitative estimate of excretion of $^{14}CO_2$ was obtained by multiplying the mean CO_2 specific activity for each time interval by the endogenous production of CO_2 (9 mmoles/kg per hr) (13). Two 4-day stool samples were collected from all subjects in the course of the study. After homogenization, a portion was used for measurement of ^{14}C and ^{3}H radioactivity by means of a Packard oxidizer (Packard Instrument Co., Downers Grove, Ill.).

TABLE I

Subjects Studied in This and Previous Study (1)

Subject	Age and sex	Weight	Studies		
			Chenodeoxycholylglycine	Deoxycholylglycine	Cholylglycine
	yr	*kg*			
C_1	37 M	89	−	+	+
C_2	50 F	75	+	−	+
C_3	34 M	78	−	−	+
C_4	57 F	59	+	+	+
C_5	23 M	94	−	−	+
C_6	34 M	82	+	+	+
C_7	37 M	68	+	+	+
C_8	26 M	89	+	+	+
C_9	22 M	68	+	+	−
C_{10}	21 M	92	+	+	−
C_{11}	22 M	110	+	+	−
C_{12}	56 F	75	+	+	−

RESULTS

Glycine moiety. The daily fractional turnover of the glycine moiety of all three conjugated bile acids was significantly greater than the daily fractional turnover of the steroid moiety (Table II and III) ($P = 0.001$). There was no difference in the daily fractional turnover rate of the glycine moieties of the three conjugated bile acids: for all three bile acids the mean daily fractional turnover rate (and synthesis) of the glycine moiety was about three times the mean daily fractional turnover rate of the respective steroid moiety. Because of the larger size of the cholylglycine pool, the daily synthesis of glycine for cholylglycine (3.18 mmoles ±0.61 SE) was more than the daily synthesis of glycine for chenodeoxycholylglycine (1.88 mmole±0.30 SE) and deoxycholylglycine (1.00 mmole±0.15 SE).

Steroid moiety. The pool size, daily fractional turnover, and daily synthesis of the steroid moiety of chenodeoxycholyl and deoxycholylglycine is shown in Table III together with the data on cholylglycine previously obtained. The pool size of chenodeoxycholylglycine (1.61±0.17 mmoles SE) was significantly smaller than that of cholylglycine, and significantly larger than that of deoxycholylglycine (0.89 mole±0.10). The average daily fractional turnover of the chenodeoxycholyl moiety of chenodeoxycholylglycine (29.9±3.0 SE) was similar to the average daily fractional turnover of the deoxycholyl moiety of deoxycholylglycine (3.1±4% SE), and in five of the eight subjects who received

TABLE II

Pool Size, Daily Fractional Turnover Rate, and Synthesis of Glycine Moiety of Glycine-Conjugated Bile Acids

Subject	Chenodeoxycholylglycine-1-^{14}C			Deoxycholylglycine-1-^{14}C			Cholylglycine-1-^{14}C*		
	Pool	Turnover	Synthesis	Pool	Turnover	Synthesis	Pool	Turnover	Synthesis
	mmoles	%	*mmoles/day*	*mmoles*	%	*mmoles/day*	*mmoles*	%	*mmoles/day*
C_1	—	—	—	0.28	110	0.31	4.95	128	6.34
C_2	2.34	114	2.67	—	—	—	4.07	64	2.61
C_3	—	—	—	—	—	—	3.02	61	1.34
C_4	1.70	63	1.07	1.03	125	1.29	2.20	46	1.01
C_5	—	—	—	—	—	—	2.18	164	3.58
C_6	2.20	135	2.97	1.20	140	1.68	2.75	164	4.51
C_7	1.40	130	1.82	0.97	74	0.72	3.02	128	3.87
C_8	1.00	216	2.16	0.86	168	1.44	1.83	92	1.68
C_9	1.33	115	1.53	0.43	150	0.65	—	—	—
C_{10}	1.25	70	0.88	0.74	130	0.96	—	—	—
C_{11}	2.17	160	3.47	0.60	104	0.62	—	—	—
C_{12}	1.14	122	1.37	1.26	118	1.35	—	—	—
Mean	1.61	125	1.98	0.82	124	1.00	3.00	106	3.18
SE	0.17	15	0.30	0.10	9	0.15	0.37	17	0.61

* Data from reference 1.

both chenodeoxycholylglycine and deoxycholylglycine the daily fractional turnover rate for the two steroid moieties differed by less than 10%. Because the pool size of chenodeoxycholylglycine was less than the pool size of cholylglycine, the daily synthesis of its steroid moiety (0.47 mmole±0.06) was significantly less ($P = 0.01$) than the daily synthesis of the cholylglycine (1.05 mmoles±0.18). For similar reasons, the daily "synthesis rate," that is intestinal input, of the deoxycholyl moiety of deoxycholylglycine (0.29 mmole±0.05) was significantly less than the daily synthesis rate of either of the other steroid moieties ($P = 0.01$).

TABLE III

Pool Size, Daily Fractional Turnover Rate, and Synthesis of Steroid Moiety of Glycine-Conjugated Bile Acids

Subject	Chenodeoxycholyl-^{3}H			Deoxycholyl-^{3}H			Cholyl-^{3}H*		
	Pool	Turnover	Synthesis	Pool	Turnover	Synthesis‡	Pool	Turnover	Synthesis
	mmoles	%	*mmoles/day*	*mmoles*	%	*mmoles/day*	*mmoles*	%	*mmoles/day*
C_1	—	—	—	0.28	10	0.03	4.95	12	0.59
C_2	2.34	19	0.44	—	—	—	4.07	50	2.03
C_3	—	—	—	—	—	—	3.02	39	1.18
C_4	1.70	16	0.27	1.03	17	0.18	2.20	19	0.42
C_5	—	—	—	—	—	—	2.18	66	1.44
C_6	2.20	30	0.66	1.20	31	0.37	2.75	31	0.85
C_7	1.40	26	0.36	0.97	25	0.24	3.02	28	0.85
C_8	1.00	28	0.28	0.86	32	0.28	1.83	55	1.01
C_9	1.33	32	0.43	0.43	32	0.34	—	—	—
C_{10}	1.25	33	0.41	0.74	40	0.30	—	—	—
C_{11}	2.17	40	0.87	0.60	41	0.24	—	—	—
C_{12}	1.14	45	0.51	1.26	48	0.60	—	—	—
Mean	1.61	29.9	0.47	0.89	30.7	0.29	3.00	37.5	1.05
SE	0.17	3.0	0.07	0.10	4.0	0.05	0.37	6.5	0.18

* Data from reference 1.

‡ Formed by bacterial 7-dehydroxylation of cholyl moiety.

Biotransformations. After administration of chenodeoxycholyl-2,4-^{3}H-glycine-1-^{14}C and deoxycholyl-2,4-^{3}H-glycine-1-^{14}C, ^{14}C appeared only in chenodeoxycholyl and deoxycholylglycine. ^{3}H, on the other hand, appeared in conjugates of taurine (Fig. 1) indicating that chenodeoxycholyl and deoxycholylglycines were being deconjugated and that some of the liberated steroid moiety was conjugated in the liver to taurine as well as to glycine. The percentage of ^{3}H appearing in the conjugates of taurine fraction of the bile acid reached a peak of 13.8% after 120 hr in the case of chenodeoxycholylglycine while it reached a peak of 12.5%, 168 hr after the administration of deoxycholyl-2,4-^{3}H-glycine-1-^{14}C.

^{3}H radioactivity, with chromatographic mobility corresponding to that of cholylglycine, appeared in the majority of subjects studied with either dihydroxy conjugate. Quantitatively, this fraction was always less than 10%.

Excretion of label. The percentage of $^{14}CO_2$ excreted in the breath in the first 24 hr after its administration was found to correlate with the daily fractional turnover rate of the glycine moiety of the bile acid (Fig. 2). The correlation between daily fractional turnover rate of the glycine moiety of chenodeoxycholylglycine and the recovery of $^{14}CO_2$ in the breath in the first 24 hr was 0.89, and the correlation between the fractional turnover rate of the glycine moiety of deoxycholyl glycine and the recovery of $^{14}CO_2$ in the breath was 0.82. In the first 24 hr, 23.9±2.4% of the administered dose of chenodeoxycholylglycine-1-^{14}C was recovered as $^{14}CO_2$ and 24.0±1.9% of the administered dose of deoxycholylglycine-1-^{14}C. By contrast, only 7.7±0.8% of the ^{14}C from chenodeoxycholylglycine-1-^{14}C and 6.8±0.6% of the ^{14}C from deoxycholylglycine-1-^{14}C was recovered in the stool in the entire 168 hr of the study (Table IV).

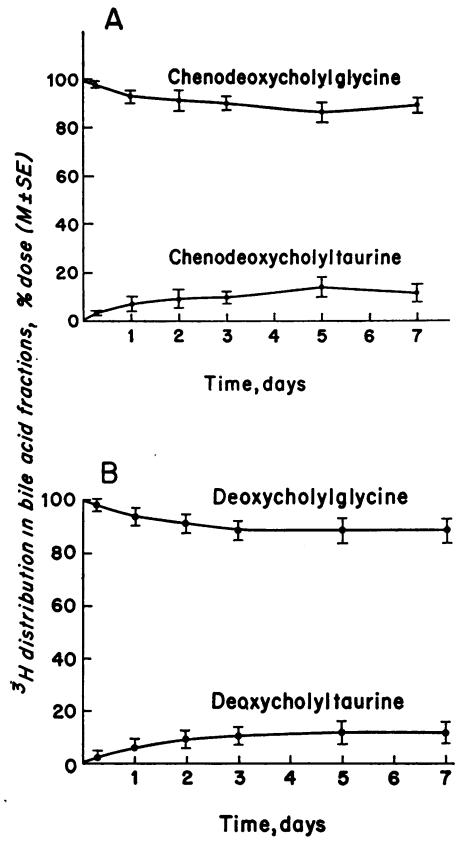

FIGURE 1 (*A*) Time course of distribution of ^{3}H among conjugated chenodeoxycholic acid classes as mean ±SE for subjects studied. (*B*) Time course of distribution of ^{3}H among conjugated deoxycholic acid classes as mean ±SE for subjects studied.

TABLE IV

Recovery of ^{3}H and ^{14}C in Stool after 168 Hr (% Dose)

Subject	Chenodeoxycholylglycine		Deoxycholylglycine		Cholylglycine*	
	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C
C_1	—	—	26	4	33	2
C_2	40	9	—	—	53	17
C_3	—	—	—	—	50	6
C_4	28	10	34	8	38	7
C_5	—	—	—	—	67	6
C_6	45	6	56	5	67	8
C_7	38	9	44	9	65	5
C_8	43	11	46	7	68	4
C_9	37	5	37	9	—	—
C_{10}	45	5	50	6	—	—
C_{11}	50	9	45	7	—	—
C_{12}	56	5	59	6	—	—
Mean	42.4	7.7	44.1	6.8	55.1	6.9
SE	2.7	0.8	3.5	0.6	4.9	1.6

* Data from reference 1.

The fecal recovery of ^{3}H derived from chenodeoxycholyl-2,4-^{3}H or deoxycholyl-2,4-^{3}H (42.4±2.7% and 44.1±3.5%, respectively) was significantly less than the recovery of ^{3}H from the cholyl-2,4-^{3}H moiety of cholylglycine in the previous study (55.1±4.9%) ($P = < 0.05$) (Table IV), and this was especially apparent in subjects who had undergone a previous study with cholylglycine as well as the present study with one or two dihydroxy bile acids. The fecal recovery of ^{3}H was not different in the subjects who received chenodeoxycholylglycine and deoxycholylglycine. The fecal recovery of ^{3}H from the chenodeoxycholyl and deoxycholyl moieties in 168 hr correlated significantly with the daily fractional turnover rate (Fig. 3).

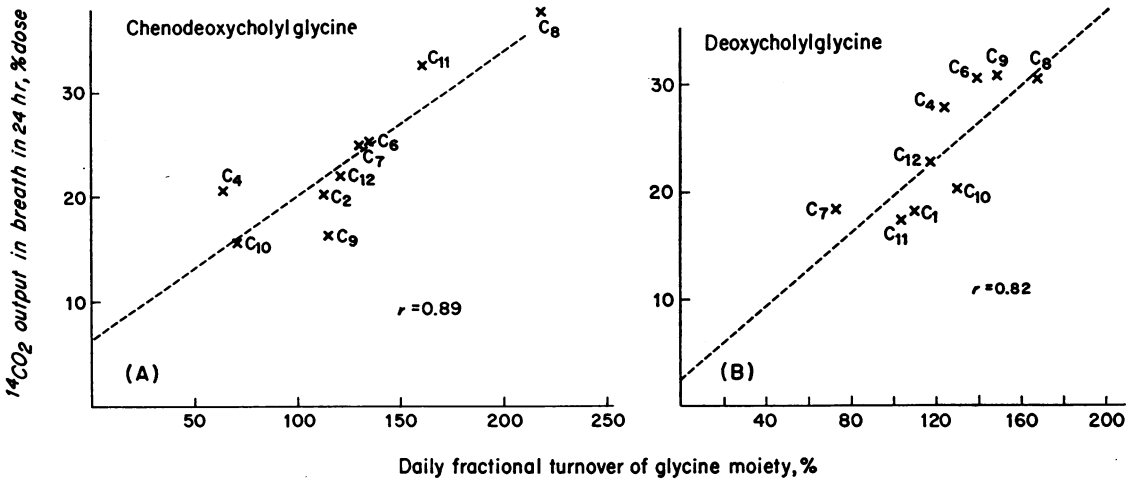

FIGURE 2 (*A*) Daily fractional turnover of glycine moiety of chenodeoxycholylglycine plotted against $^{14}CO_2$ excretion in breath in 24 hr as percentage of administered dose. The cumulative excretion of $^{14}CO_2$ in this period was calculated by multiplying the mean CO_2 specific activity between intervals at which this was measured by the endogenous production of CO_2 (9 mmoles/kg per hr). The regression line is: $y = 6.20 + 0.14x$. (*B*) Daily fractional turnover of glycine moiety of deoxycholylglycine plotted against $^{14}CO_2$ excretion in breath in 24 hr as percentage of administered dose. The regression line is: $y = 2.77 + 0.17x$.

Bile acid pool size. Four subjects (C_4, C_6, C_7, and C_8) had pool size determinations of cholylglycine, chenodeoxycholylglycine, and deoxycholylglycine in three separate studies. The ratio of cholic acid: chenodeoxycholic acid: deoxycholic acid also was determined by gas-liquid chromatographic analysis of a portion of their bile. In Table V, the pool sizes of the three bile acids in these four subjects are shown, together with the ratio of these pools both as determined from the measurement of the pool size and as determined by the ratio of cholic acid: chenodeoxycholic acid: deoxycholic acid in a portion of bile analyzed by gas-liquid chromatography. The ratio of the three bile acids in the bile reflects closely the ratio of the three pools as determined directly. Pool sizes of the bile acids that were not directly determined in other subjects were calculated by multiplying the size of the measured bile acid pool by the molar fraction in the bile of the bile acid whose pool size was being estimated (Table VI).

TABLE V
Composition of Glycine-Conjugated Bile Acid Pool

Subject	Cholylglycine*‡	Chenodeoxycholylglycine*	Deoxycholylglycine*	Molar ratio pools*	Molar ratio bile§
		mmoles			
C_4	2.20	1.70	1.03	45:35:20	48:35:17
C_6	2.75	2.20	1.20	45:36:19	48:34:18
C_7	3.02	1.40	0.97	56:26:18	55:25:20
C_8	1.83	1.00	0.86	50:27:21	53:26:21

* From isotope dilution procedure.
‡ Data from reference 1.
§ By gas-liquid chromatography of bile sample.

DISCUSSION

Glycine moiety. This study clearly shows that the steroid moiety of the dihydroxy bile acids is better conserved than the glycine moiety, since the daily fractional turnover of the chenodeoxycholyl and deoxycholyl moieties of the glycine-conjugated bile acid were 29.9±3.0% SE and 30.7±4.0% SE compared with a daily fractional turnover of the respective glycine moieties of 125±15% and 124±9% SE. This finding supplements our previous observation that the cholyl moiety of cholylglycine has a daily fractional turnover (37.5±6.5% SE) that is only one-third of the daily fractional turnover of the glycine moiety (106±17% SE).

Steroid moiety. Our data represent the first measurement of the turnover rates of the steroid moiety of the three glycine-conjugated bile acids in human bile. Heaton, Austad, Lack, and Tyor (14) and Garbutt, Wilkins, Lack, and Tyor (15) determined the fractional turnover rates of cholylglycine and cholyltaurine but did not distinguish turnover of the steroid and amino acid moieties. Vlahcevic et al. (4) performed a study in which turnover rates of cholic and chenodeoxycholic acids were determined after the bile acids had been deconjugated: our figures for bile acid synthesis refer only to the turnover rate of the individ-

ual glycine-conjugated bile acids. The mole fraction of taurine conjugates may be inferred from data in this and the previous paper to be about 0.10–0.15 so that total bile acid synthesis in our subjects is correspondingly larger. Our data, therefore, agree with those of Vlahcevic et al. (4), who calculated that total daily bile acid synthesis was 2.27±0.14 g/day (SE), while we calculated synthesis of glycine-conjugated bile acids to be 2.08±0.22 g/day (SE).

The absorbed fraction of the circulating bile acid pool may be calculated from the steady-state equation for the enterohepatic circulation, assuming 6 cycles/day, a figure which has recently been validated in man (16). The two dihydroxy bile acids are conserved equally by intestinal absorption (95.0±0.42% [SE] per enterohepatic cycle) and both are conserved as well as, or better than, cholic acid (93.8±1.1% [SE] per enterohepatic cycle). Since the fraction of the bile acid pool absorbed per cycle is equal to $(1 - k/C)$ where k is the daily fractional turnover rate and C is the daily number of enterohepatic cycles, the greater the number of cycles, the more efficient the intestinal absorption. For dihydroxy acids, the absorption efficiency for 3 cycles/day would be 90% and for 9 cycles/day, 97%. The greater conservation of chenodeoxycholic than cholic acid has been noted by others in healthy subjects (2,4) and in patients with hypercholesterolemia (3), although the data apply to the total bile acids in bile, both glycine and taurine conjugates. Deoxycholic acid kinetics do not appear to have been measured previously in man.

Differences were significant in cholic acid synthesis and deoxycholic acid input in the five subjects who were studied with both bile acids. In one subject (C_6) deoxycholic acid input was 44% of the loss (synthesis) of cholic acid, while in another subject (C_1) input of deoxycholic acid was only 5% of his cholic acid synthesis rate. As anticipated, these findings correlated with the distribution of radioactivity in the bile acid fractions after administration of cholyl-2,4-^{3}H-glycine: in subject C_6, 48% of the ^{3}H from the cholyl-2,4-^{3}H-glycine was in deoxycholylglycine after 7 days while in subject C_1, only 3% was in deoxycholylglycine at the same time.

From the data in this and the previous paper in this series it is possible to infer the role of the terminal portion of the ileum and the colon in the absorption of glycine-conjugated bile acids. Assuming six enterohepatic cycles daily and a bile acid pool of 5 mmoles, 30 mmoles of bile acids will be secreted daily into the small intestine. Fecal excretion, equivalent to daily synthesis, is about 1.8 mmoles or 0.3 mmole/cycle. Bile acid absorbed from the intestine, both free and conjugated, is 28.2 mmoles. 6 mmoles of glycine is used

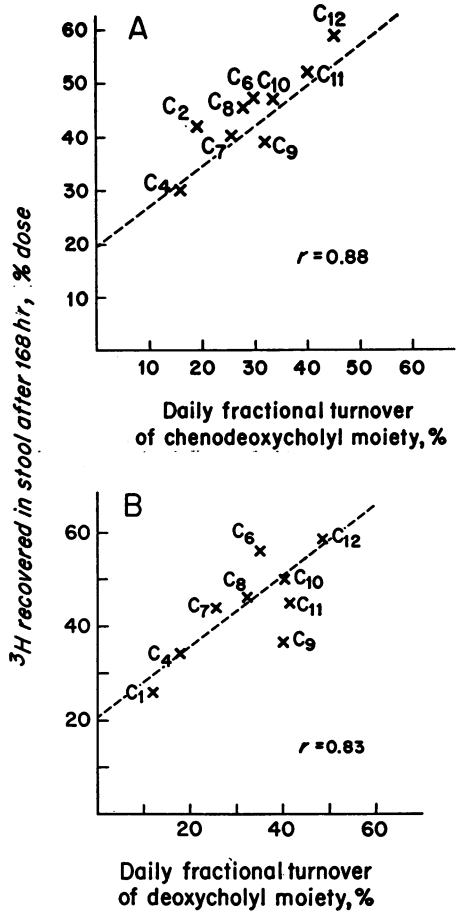

FIGURE 3 (*A*) Daily fractional turnover of chenodeoxycholyl moiety plotted against percentage administered ^{3}H recovered in stool after 168 hr. The regression line is: $y = 19.4 + 0.77x$. (*B*) Daily fractional turnover of deoxycholyl moiety plotted against percentage administered ^{3}H recovered in stool after 168 hr. The regression line is: $y = 21.8 + 0.73x$.

daily for bile acid conjugation, 1.8 for newly synthesized bile acid, and 4.2 mmoles for reconjugation of bile acid absorbed in unconjugated form from the intestine. Thus 4.2/28.2 or an average of about 15% of bile acids is absorbed in unconjugated form.

The site of absorption of unconjugated bile acids is not known, but presumably it is the ileum or the colon or both, whereas the site of absorption of deoxycholic acid, equal to 0.3 mmole/day, is most likely the colon, since it is formed only by strict anaerobes that are found predominantly in the colon (17). If all unconjugated bile acid and all deoxycholic acid are absorbed from the colon, they would amount to 4.5 mmoles daily or about 16% of the bile acids absorbed daily. If, however, unconjugated bile acid is absorbed from the ileum and deoxycholic acid from the colon, then colonic ab-

TABLE VI

Component of Glycine-Conjugated Bile Acid Pools

Subject	Cholylglycine*			Chenodeoxycholylglycine			Deoxycholylglycine			Total		
	mmoles	*g*	*mg/kg*	*mmoles*	*g*	*mg/kg*	*mmoles*	*g*	*mg/kg*	*mmoles*	*g*	*mg/kg*
C_1	4.95	2.02	22.7	2.85	1.12	12.6	0.28	0.11	1.2	8.08	3.25	36.5
C_2	4.07	1.66	22.2	2.34	0.95	12.3	0.86	0.34	4.5	5.27	2.94	39.0
C_4	2.20	0.90	15.2	1.70	0.67	11.3	1.03	0.40	6.8	4.93	1.97	33.3
C_6	2.75	1.12	13.7	2.20	0.86	10.5	1.20	0.47	5.7	6.15	2.45	29.9
C_7	3.02	1.23	18.1	1.40	0.55	8.1	0.97	0.38	5.6	5.39	2.16	31.8
C_8	1.83	0.75	8.4	1.00	0.39	4.4	0.86	0.34	3.8	3.69	1.48	16.6
C_9	1.52	0.62	9.1	1.33	0.52	7.7	1.11	0.44	6.4	4.96	1.58	23.2
C_{10}	1.61	0.66	7.2	1.25	0.49	5.3	0.74	0.29	3.2	3.60	1.44	15.7
C_{11}	2.28	0.93	8.5	2.27	0.85	7.7	0.60	0.24	2.1	5.05	2.02	18.3
C_{12}	1.46	0.60	8.0	1.14	0.45	6.0	1.26	0.50	6.6	3.86	1.55	20.6
Mean	2.57	1.05	13.3	1.75	0.68	8.6	0.89	0.35	4.6	5.10	2.08	26.5
SE	0.37	0.15	1.9	0.20	0.08	0.7	0.09	0.04	0.6	0.42	0.20	2.7

* Data from reference 1.

For conversion of mole values to mass, the molecular weight of the steroid moiety alone was used to permit comparison of these figures with published values.

sorption would be about 1% of the bile acids absorbed daily from the intestine. Thus colonic absorption of bile acids represents 1–15% of intestinal bile acid absorption in health, but the figure is likely to be between these two limits since unconjugated bile acids are present in the distant ileum in health (18) and, if so, should be absorbed there.

Biotransformation of steroid moiety. The significance of the ^3H-labeled compounds with the mobility of cholylglycine is unknown. According to available evidence, neither chenodeoxycholic nor deoxycholic acid is hydroxylated in the liver of man (19–21), and if rehydroxylation occurred in our studies its magnitude was small; 7-rehydroxylation of deoxycholic acid does occur in the liver of several other species (22–25).

Excretion of labels. As in the previous study with cholylglycine, it was found that most of the steroid moiety labeled with ^3H was excreted in the stool, the fecal recovery of ^3H correlating well with the daily fractional turnover rate of the steroid moiety.[1] In contrast, most of the ^{14}C-labeled glycine moiety was excreted in the breath, the recovery of $^{14}CO_2$ in the first 24 hr correlating well with the daily fractional turnover of the glycine moiety. Thus it appears feasible to estimate daily bacterial deconjugation from interval measurements of $^{14}CO_2$ specific activity in breath, an extremely simple procedure. In addition, the data presented here, together with that of our previous paper, indicate that the total amount of glycine used daily for bile acid conjugation is about 6 mmoles/day.

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[1] Subsequent studies (Hepner, G. W., J. A. Sturman, P. J. Thomas, and A. F. Hofmann, unpublished observations) have shown that up to 40% of ^3H may appear in body water after ingestion of 2,4-^3H-labeled bile acids. This finding probably indicates that intestinal bacteria in man can remove the labeled atoms at the 2 or the 4 position or both (27). It seems likely to us that this loss of ^3H does not occur until a bile acid has left the bile acid pool (defined operationally as that mass of bile acids with which an administered bile acid mixes) for the following reasons: (*a*) bile acid turnover rates in two healthy subjects when determined after simultaneous administration of ^{14}C and 2,4-^3H-labeled cholic and chenodeoxycholic acid were identical (5); (*b*) the turnover rates obtained in this and the preceding paper for cholic and chenodeoxycholic acid agree well with published figures based on ^{14}C-labeled bile acids (4); and (*c*) in other studies (26) we have simultaneously compared the turnover rates of cholic acid-^{14}C and chenodeoxycholic acid-^3H. The turnover rates of chenodeoxycholic acid was constantly below that of cholic acid, in agreement with the studies of Vlahcevic et al. (4) who carried out identical studies using bile acids labeled with ^{14}C only. Nonetheless, the fecal excretion rates of ^3H reported in this and the preceding paper were presumably accompanied by losses of ^3H in urine which were not measured.

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