Estimation of bile acid excretion in man: comparison of isotopic turnover and fecal excretion methods

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Abstract Bile acid excretion was studied in 9 human subjects simultaneously by the Lindstedt (Lindstedt, S. 1957. *Acta Physiol. Scand.* **40**:1–9) isotopic turnover method and by fecal chemical analysis during a balance study. The identities of the fecal bile acids were confirmed by combined gas–liquid chromatography/mass spectrometry. Under the steady state conditions of the patient studies, bile acid excretion values obtained by fecal analysis were lower (by 18.1 to 44.2%) than the values obtained by the isotopic turnover method. This difference persisted even in those patients given [^{14}C]chenodeoxycholic acid instead of [^{3}H]chenodeoxycholic acid. The fecal excretion values were similar when calculated using either β-sitosterol or chromium sesquioxide as fecal flow markers. The fecal excretion values during the earlier part of the isotopic study were higher than those during the latter part of the study. The lower values of bile acid excretion obtained from fecal analysis could not be explained by the loss of bile acids as sulfate conjugates or by losses due to bile acid degradation in the intestine. This study suggests that bile acid turnover is consistently higher than bile acid excretion under experimental conditions. It is recommended that the data obtained from the isotopic turnover method should not be compared with fecal excretion data.

Supplementary key words Bile acid turnover•nonabsorbable markers•gas–liquid chromatography•mass spectrometry

Measurement of bile acid excretion or turnover is of importance during studies of sterol balance in patients (1) with various types of hyperlipoproteinemia (2, 3) and patients with cholelithiasis (4, 5). Currently, there are two ways of estimating bile acid excretion in man. The first is the isotopic turnover method described by Lindstedt (6) in which labeled cholic and chenodeoxycholic acids are administered to the patient and, on the basis of specific activity decay curves of these bile acids in the bile, the pool size and the turnover of the primary bile acids are calculated. The other technique involves the chemical measurement of bile acids excreted in the feces by a variety of methods (7–10). The values are corrected for fecal flow using nonabsorbable markers. Whether or not the values of bile acid excretion obtained by these two methods are similar has not been documented. For the purpose of interlaboratory comparison of data under various conditions, it is essential that the variability, if any, between the two methods be established. In the study reported herein, bile acid excretion was estimated by both methods simultaneously in the same human subjects.

METHODS

The studies were carried out in a total of nine human subjects. Among these were two type IIa, one type IIb, two type III, two type IV, one type V patient with hyperlipoproteinemia and one normal control. The clinical details, serum lipids and body weights of the subjects are shown in **Table 1.** All were eating solid food diets which were similar in composition to diets they had followed for at least one month prior to the study. Dietary cholesterol content ranged from 150 to 275 mg per day and the fat content from 50 to 60 g per day. The phytosterol content of the diet was 187 mg per 2,200 calories. Patients were started on fecal flow markers at least one month prior to admission for the study.

All patients were admitted to the Metabolic Unit and further equilibrated on the diet and fecal flow markers for at least five days prior to the radioisotope study.

Serum cholesterol and triglyceride levels were determined thrice during each study using standard methods (11, 12).

Bile acid kinetics of each of the two primary bile acids, cholic and chenodeoxycholic acids, were independently determined by the isotope dilution technique described by Lindstedt (6) and Danielsson and associates (13). A cocktail containing 30–47 μCi of randomly tritiated chenodeoxycholic acid (sp act 0.096–1.6 Ci/mmole) and 15–41 μCi of [^{14}C]cholic acid (sp act 2.5–56.3 mCi/mmole) in ethanol was given to the patient prior to breakfast (isotopes were obtained from New England Nuclear, Boston, Mass.). In three patients [24-^{14}C]-chenodeoxycholic acid was also used. Each lot of isotope was checked for purity by thin-layer chromatography. The solvent system isooctane–ethyl acetate–glacial acetic acid 5:5:1

Abbreviations: TFA, trifluoroacetate

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TABLE 1. Characteristics of the patients

Patient	Age (yr.) Sex	Weight (kg)	Type	Serum Cholesterol	Serum Triglyceride	Comments[a]
				mg/100 ml		
C. E.	62/F	54	IIa	466	106	Tendon xanthomas
T. W.	61/F	57	IIa	451	91	Chronic porphyria (cutanea tarda)
H. M.	50/M	80	IIb	368	159	Tendon xanthomas
M. P.	50/M	73	III	374	392	Arteriosclerosis obliterans and carotid atherosclerosis
M. P.	55/M	68	III	165	115	No vascular complication
I. S.	54/F	60	IV	214	670	Borderline diabetes mellitus, ASO, CAD with coronary bypass surgery
E. J.	48/M	104	IV	277	119	Diabetes mellitus
J. L.	60/M	67	V	483	2780	Diabetes mellitus
K. K.	30/M	61	N	167	84	Partial post-trauma hemiparesis

[a] CAD = coronary artery disease, ASO = atherosclerosis.

(v/v/v) was used in all cases and in recent studies additional purity checks were made using the solvent system: isooctane–isopropyl ether–acetic acid 10:5:8 (v/v/v), and benzene–dioxane-acetic acid 75:20:2 (v/v/v).

Over the subsequent eight to ten days, five to ten fasting bile samples, 5 to 15 ml each, were aspirated through a naso-duodenal tube. After extraction, bile acids of each sample were hydrolyzed enzymatically as described by Nair, Gordon, and Reback (14); thus, all bile acids were analyzed in the unconjugated form. The mass of the two primary bile acids in each bile sample was determined using thin-layer chromatography followed by spectrophotometric measurement of their sulfuric acid chromogens (15, 16) or by gas–liquid chromatography as trifluoroacetates (10). Radioactivity was determined by liquid scintillation counting. As described by Lindstedt (6), the excretion of the primary bile acids follows first order kinetics. Logarithms of the specific activities of each bile acid, plotted against time, were applied to the regression equation, $I_t = I_o + bt$ (I = specific activity; t = time; o = zero time; and b = slope), to determine the specific activity of each bile acid at time zero as well as the slope of the decay curve. The pool size (P) in mg, half-life ($t_{1/2}$) in days and synthesis (S) in mg/day of each primary bile acid were calculated using the following equations: P = initial dose/I_o; $t_{1/2} = log\, 2/b$; and $S = 2.30\,(b)P$ (2, 6).

In all of the subjects, fecal bile acid excretion rates were determined simultaneously with the bile acid turnover studies using chemical sterol balance methods (17). Patients were started on the fecal flow markers, β-sitosterol given as "Cytellin" (1 g/5 ml daily) (Cytellin was generously furnished by Eli Lilly Company, Indianapolis, Ind., and had 62% β-sitosterol, 31% compesterol and 7% stigmasterol), and chromium sesquioxide, two weeks prior to entry into the Study Unit. Individual daily 24-hr stool collections were analyzed for bile acids by combined thin-layer and gas–liquid chromatographic techniques as described previously (10). In this method the internal standard (hyocholic acid) was added to the fecal homogenates and followed through the entire extraction and purification procedure of bile acids. The individual bile acids were tentatively identified and quantitated by gas–liquid chromatography as methylester trifluoroacetates on 3% QF-1 columns (10, 18). The known bile acid peaks were identified and their concentration was calculated against the area of hyocholic acid with appropriate detector response corrections for each of the different bile acids. In all instances, the recovery of β-sitosterol was equal to or slightly greater than that of chromium sesquioxide indicating that no sterol ring breakdown occurred. Bile acid excretion rates were calculated for each 24-hr stool collection on the basis of β-sitosterol values in all patients and also on the basis of chromium sesquioxide in some patients. These values were averaged for each patient and the coefficient of variation (day to day variability) was calculated.

Gas–liquid chromatography–mass spectrometry of bile acids

Combined gas–liquid chromatographic–mass spectrometric analyses were done on a Varian MAT CH-5 single focusing mass spectrometer coupled with Varian Data 620i Computer (19) (Varian Associates, Palo Alto, Cal.).

The gas–liquid chromatographic separations were obtained on a Varian model 2700 Moduline gas chromatograph equipped with a 3 ft × 2 mm ID glass column packed with pretested QF-1 packing/3% on Gas-Chrom Q, mesh 80–100, Applied Science Laboratories Inc., State College, Pa., using helium as carrier gas (10 ml/min).

The gas chromatograph, which did not have a separate detector, was run at 225°C isothermally with the injector (on column) at 210°C. The transfer line and the Wattson–Biemann dual stage helium separator was kept at 275°C.

RESULTS

Identification of bile acids

One of the inherent difficulties involved in the accurate estimation of fecal bile acids is that the peaks on the gas chromatogram must be carefully identified, since it is possible to have peaks which do not correspond to the retention time of any known bile acids (10, 20). In this study, fecal bile acids were identified on the basis of migration on thin-layer chromatography (as methyl esters) using the following solvent

TABLE 2. Gas–liquid chromatography of human fecal bile acids

Bile Acid	Retention Times (Relative to Deoxycholate ME-TFA[a])	
	standard	*fecal component*
Lithocholic	0.64	0.64
3β,12α-Dihydroxycholanic	0.85	0.84
Deoxycholic	1.00	1.00
3α,12β-Dihydroxycholanic	1.12	1.10
Chenodeoxycholic	1.26	1.22
Ursodeoxycholic	1.48	1.43
Cholic	2.10	2.09
7-Keto-lithocholic	2.44	2.43
3-Keto,7α-hydroxycholanic	2.66	2.69
3β,12-Keto-cholanic	1.97	1.98
3α,12-Keto-cholanic	2.23	2.20

[a] Trifluoroacetates of bile acids were chromatographed on 3% QF-1 (on 100–120 mesh Chromosorb W). Column, 225° C; flash heater, 245° C; detector, 295° C; carrier gas, helium, 50 ml/min.

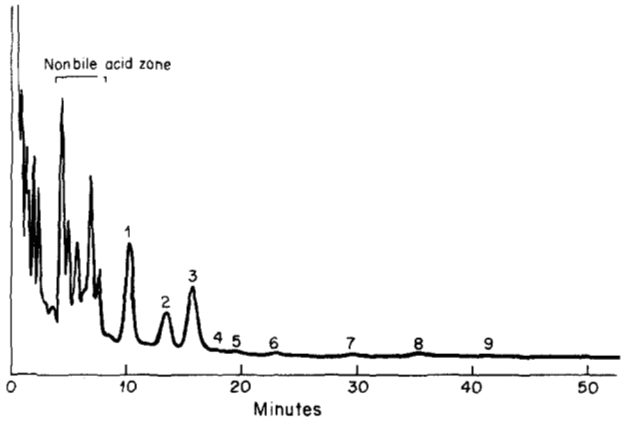

Fig. 1. Gas–liquid chromatographic separation of human fecal bile acids as their methyl ester trifluoroacetates on QF-1 column. Peak identification: 1. lithocholate; 2. 3β,12α-dihydroxycholanate; 3. deoxycholate; 4. 3α,12β-dihydroxycholanate; 5. chenodeoxycholate; 6. ursodeoxycholate; 7. 3β-hydroxy-12 ketocholanate; 8. 3α-hydroxy-12-keto-cholanate; and 9. 3 keto-7α-hydroxycholanate. Column conditions as described in the text.

systems: isooctane–isopropyl ether–acetic acid 50:25:40 (v/v/v) and isooctane–ethyl acetate–acetic acid 50:50:10 (v/v/v) followed by gas–liquid chromatography as trifluoroacetates on QF-1 columns **(Table 2).**

The bile acid fractions were further purified into groups by thin-layer chromatography, eluted and analyzed by gas–liquid chromatography. Their retention times were then compared to those of authentic standards. In this study, the following bile acids were noted most frequently: deoxycholic acid, lithocholic acid, 3β,12α-dihydroxycholanic, 3β,12-keto-cholanic, 3α,12-keto-cholanic and 7-keto-lithocholic acids. Occasionally, small amounts of chenodeoxycholic acid, cholic acid, 3α,12β-dihydroxycholanic acid and 3-keto-7α-hydroxy-cholanic acid were noted. The identities of the major bile acids were established by gas–liquid chromatography–mass spectrometry.

Gas–liquid chromatography–mass spectrometry

The bile acid methyl ester trifluoroacetates were chromatographed on 3% QF-1 columns **(Fig. 1)** and each component was subjected to mass spectrometry. The fragmentation patterns were then compared to those of the authentic standards. The peak corresponding to lithocholate (retention time 0.64 relative to deoxycholate) upon mass spectrometry gave a base peak at *m/e* 372 and a molecular ion *m/e* 486 characteristic of 3-hydroxycholanates. The ion peaks at *m/e* 257 were much less intense than what has been noticed for 7α and 12α monosubstituted cholanic acids (21). The mass spectrum corresponded well to that of authentic lithocholic acid. The absence of 7α,12α or 3β-monohydroxy bile acids was also indicated by the lack of corresponding peaks on gas chromatograms. The second major peak was identified as deoxycholic acid (retention time similar to that of authentic deoxycholate) by mass spectrometry. It gave a base peak at *m/e* 369 (loss of TFA and side chain). It also gave major peaks at *m/e* 342, 255, and 154. The mass spectrum corresponded to that of authentic deoxycholic acid. The third major peak corresponded to the retention time of 3β,12α-hydroxycholanic acid (retention time 0.85 relative to deoxycholate, different from that of 3α,12α- and 3α,12β-dihydroxycholanic acids). Upon mass spectrometry it gave a base peak at *m/e* 369 characteristic of 3,12-hydroxycholanates. It also gave ion peaks of *m/e* 342, 255, 241, and 154. Its ion spectrum was very similar to that of deoxycholic acid. Similarity in the mass spectrum of 3,12-dihydroxycholanic acids has been noted by Sjövall (21). Hence, on the basis of its retention time, it was identified as 3β,12α-dihydroxycholanic acid. These three bile acids represented more than 90% of the total fecal bile acids.

Two other bile acids were identified as 3α-hydroxy-12-keto and 3β-hydroxy-12-keto-cholanic acids. These two compounds had retention times (relative to deoxycholate) of 2.20 and 1.97, respectively, which corresponded to the known retention times of the standard. Both the compounds gave similar mass spectra with molecular ion at *m/e* 500 characteristic of TFA of methylated monohydroxymonoketo bile acids **(Fig. 2).** Also both these compounds gave a major peak at *m/e* 345 (M − 155) characteristic of 12 keto compounds due to the loss of side chain and cleavage through D-ring (21). It also gave major peaks at *m/e* 427, 386, and 231. The mass spectrum was similar to that of 3α-hydroxy-12-keto-cholanic acid. The identity of the 3β-hydroxy-12-keto compound was based on its retention time by gas–liquid chromatography and similarity of its mass spectrum to that of the 3-hydroxy-12-keto compound.

Occasionally, peaks corresponding to other bile acids (chenodeoxycholic, ursodeoxycholic, 3-keto-7α-hydroxycholanic and cholic acids) were noticed. These usually represented less than 3% of the total bile acids. The identity of the major bile acids corresponded well with the reports of Eneroth et al. (22, 23) except that peaks corresponding to dihydroxy-monoketo bile acids were not encountered.

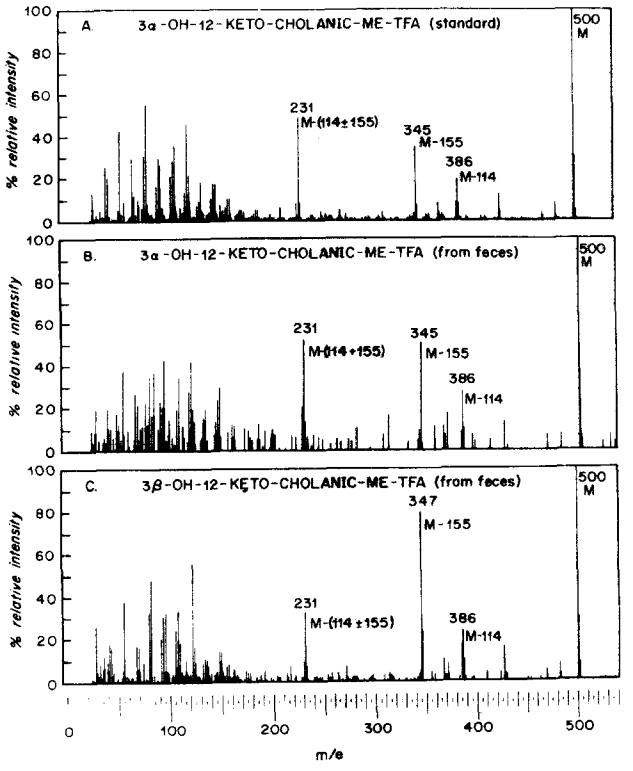

Fig. 2. Mass spectra of (*a*) authentic 3α-hydroxy-12-keto-cholanate; (*b*) 3α-hydroxy-12-keto-cholanate isolated from feces; and (*c*) 3β-hydroxy-12-keto-cholanate isolated from feces as methyl ester trifluoroacetates. Conditions used for gas–liquid chromatography–mass spectrometry are as described in the text.

Bile acid excretion

Bile acid excretion obtained by fecal excretion studies was compared with the turnover values in the same patients **(Table 3).** As can be seen, the values obtained by the turnover method were consistently higher (18.1–44.2%) in all the nine patients studied. These differences were calculated by relating differences to the Lindstedt method (6). The differences would be greater if the difference in excretion values is calculated against fecal excretion data. This difference was noticed over a wide range of pool sizes (600–3,591 mg) encountered in the patients. The correlation coefficients of the specific activity decay curves were >0.98 for [^{14}C]cholic and chenodeoxycholic acids and >0.93 for [^{3}H]chenodeoxycholic acid. It should be noted that in three patients [^{14}C]-chenodeoxycholic acid was used instead of [^{3}H]chenodeoxycholic acid. Even in these patients the fecal bile acid values were lower. These data are in agreement with the reports of Miller and Nestel (24) who noticed similar results in four subjects. Fecal excretion data of bile acids in Table 3 were obtained using β-sitosterol as a nonabsorbable marker to correct for variations in fecal flow. Attempts were then made to compare the fecal excretion data obtained on the basis of β-sitosterol to those calculated on the basis of another fecal marker, chromium sesquioxide, in six of the patients **(Table 4).** As can be seen, the fecal excretion data obtained on the basis of two separate markers were similar.

TABLE 3. Bile acid excretion in patients

Patient	Type	Bile Acid Excretion mg/day: Isotopic Turnover[a]	Bile Acid Excretion mg/day: Fecal Excretion ± SD (β-sitosterol corrected)
C. E.[c]	II_a	132.7	108.9 ± 25.3 (4/8)[b]
T. W.[c]	II_a	218.9	160.4 ± 43.9 (5/9)
H. M.[c]	II_b	442.0	353.5 ± 199.9 (9/9)
M. P.[d]	III	287.4	160.5 ± 43.4 (3/9)
Mi. P.[d]	III	305.4	227.6 ± 74.7 (3/9)
I. S.[c]	IV	234.0	175.6 ± 42.3 (6/8)
E. J.[c]	IV	623.0	526.4 ± 157.3 (9/10)
J. L.[d]	V	1720.9	1274.2 ± 267.9 (8/9)
K. K.[c]	N	559.0	312.9 ± 87.8 (4/6)

[a] Sum of the turnover values of cholic and chenodeoxycholic acids.
[b] Numbers in parentheses indicate the number of stool samples analyzed over the days of collection period.
[c] Patients given [^{14}C]cholic and [^{3}H]chenodeoxycholic acids.
[d] Patients given [^{14}C]cholic and [^{14}C]chenodeoxycholic acids.

TABLE 4. Comparison of fecal bile acid excretion using two "nonabsorbable" markers[a]

Patient	Type	Bile Acid Excretion mg/day ± SD: *β-sitosterol basis*	Bile Acid Excretion mg/day ± SD: *Cr_2O_3 basis*
C. E.	II_a	108.9 ± 25.3	92.9 ± 20.5
T. W.	II_a	160.4 ± 43.9	174.0 ± 38.7
H. M.	II_b	353.5 ± 199.9	366.2 ± 176.1
I. S.	IV	175.6 ± 42.3	186.7 ± 48.4
E. J.	IV	526.4 ± 157.3	480.9 ± 125.7
K. K.	N	312.9 ± 87.8	319.9 ± 85.2

[a] Daily doses of markers given were 1.5 g and 1 g for chromium sesquioxide and β-sitosterol, respectively.

TABLE 5. Calculation of fecal bile acid excretion in early and late periods of isotopic turnover study[a]

Patient	Isotopic Turnover Value of Bile Acids (mg/day)	Fecal Bile Acid Excretion mg/day[b]: *early period*[c]	Fecal Bile Acid Excretion mg/day[b]: *late period*[c]
C. E.	132.7	104.7 ± 18.5	78.2 ± 13.1
T. W.	218.9	185.7 ± 7.5	122.5 ± 53.0
H. M.	442.0	366.5 ± 253.0	335.7 ± 141.9
M. P.	287.4	152.6 ± 48.3	168.5 ± 35.5
Mi. P.	305.4	267.8 ± 80.9	187.5 ± 71.3
I. S.	234.0	186.5 ± 24.9	164.8 ± 48.3
E. J.	623.0	582.8 ± 88.0	470.1 ± 157.4
J. L.	1720.9	1331.2 ± 397.3	1217.3 ± 109.1
K. K.	559.0	237.1 ± 9.0	376.2 ± 52.2

[a] Fecal collection was started on the day labeled bile acids were administered for isotopic turnover study.
[b] Calculated by the analysis of daily fecal samples on the basis of β-sitosterol recovery.
[c] First and second half of the study period as indicated in parenthesis in Table 2.

TABLE 6. Comparison of excretion values of cholic and chenodeoxycholic acids obtained by isotopic turnover to the values calculated on the basis of their metabolites excreted in the feces[a]

Patient	Cholic Acid Excretion mg/day		Chenodeoxycholic Acid Excretion mg/day	
	Isotopic Turnover	Fecal Excretion Value	Isotopic Turnover	Fecal Excretion Value
M. P.	130.9	77.4	156.5	89.0
Mi. P.	159.7	112.3	145.7	114.9
C. E.	63.7	50.4	69.0	41.3

[a] Values of fecal excretion of cholic and chenodeoxycholic acids were calculated on the basis of the concentration of these primary bile acids and their respective known metabolites in the feces.

Fecal bile acid data were obtained by analysis of individual 24-hr collections of fecal samples over a mean of 9–10 days. An attempt was made to see whether the fecal bile acid data from an earlier part of the study would be more comparable to the isotopic turnover data **(Table 5).** This is based on the concept that on a logarithmic scale the specific activity curve would be more sensitive to changes during the first few days after isotope administration. It was found that the fecal bile acid data during the early part of the study were higher than that obtained during the later part of the study (except in two patients). However, the values from the earlier part of the study were still lower than the isotopic turnover values.

The total bile acid turnover recorded in Tables 2 and 4 is the sum of the turnovers of cholic and chenodeoxycholic acids. In three of the patients, the isotopic turnover of cholic and chenodeoxycholic acids was compared to the values of excretion obtained by the sum of the concentrations of their respective metabolites in the feces **(Table 6.)** Lithocholic, chenodeoxycholic, ursodeoxycholic, 7-keto-lithocholic and 3-keto,7α-hydroxycholanic acids were considered to be derived originally from chenodeoxycholic acid, while 3β,12α-dihydroxycholanic, deoxycholic, 3α,12β-dihydroxycholanic, cholic, 7-keto,3α,12α-dihydroxycholanic, 3α,12-keto-, 3β,12-keto-3-keto,12α- and 12-keto,3α,7α-dihydroxycholanic acids were considered derived from cholic acid. This rationale is in agreement with the known alterations of bile acids in the intestine (13, 25). As shown in the table, differences between the isotopic turnover and fecal excretion data persisted for both cholic and chenodeoxycholic acids indicating that it is not specific for any bile acid. The lower excretion value for fecal bile acids could not be explained by the loss of sulfated bile acids during extraction without solvolysis. This was indicated by the trace amounts of bile acids obtained in the feces of three patients following solvolysis and extraction of the aqueous phase of fecal homogenate left following the routine extraction of fecal bile acids.

In all the patients the turnover of chenodeoxycholic acid was calculated by using [^{3}H]chenodeoxycholic acid. Einarsson, Hellström, and Kallner (26) noted that the turnover values obtained using the tritiated compound were much higher (by 14.2%) than those obtained using the ^{14}C compound. Hence, in three patients [^{14}C]chenodeoxycholic acid was used. As can be seen from Table 3, in all three patients the fecal bile acid values were still lower. Also, in one patient (J.L.) bile acid turnover was determined by using both [^{14}C] and [^{3}H] chenodeoxycholic acid. It was found that the values obtained by using [^{14}C]chenodeoxycholic acid were lower (1720.9 vs. 2201.3 mg). But still the values obtained using the [^{14}C] chenodeoxycholic acid were higher than the fecal excretion values (1274.2 mg).

DISCUSSION

The results of the study demonstrate that the values of bile acid excretion in man obtained by the chemical analysis of feces are lower than the values obtained by the isotope turnover method. Similar discrepancies in bile acid excretion values using isotopic turnover and fecal bile acid values have been noticed in the rabbit by Hellström and Sjövall (27).

Studies by Einarsson et al. (26) have shown that isotopic turnover values of bile acids obtained using tritiated bile acids have higher values (by 14.2%) than those obtained by ^{14}C-labeled bile acids because of a loss of tritium due to exchange. Panveliwalla, Pertsemlidis, and Ahrens (28) noted a discrepancy in the turnover of [^{14}C]- and [^{3}H]chenodeoxycholic acids simultaneously. The value of the bile acid excretion rate with [^{14}C]chenodeoxycholic acid was lower than that obtained using the tritiated bile acid but still was higher than the fecal bile acid excretion rate. Also, in the three patients given [^{14}C]chenodeoxycholic acid the fecal bile acid values were still lower. Hence, this difference cannot be explained on the basis of the isotopes used. Our experience, however, supports the findings of Panveliwalla et al. (28) that tritiated bile acids give higher values for pool size and turnover than those obtained with [^{14}C]-labeled bile acids.

Before drawing conclusions regarding the superiority of one method over another, it is essential that one examine critically the limitations of both the methods. In the fecal excretion method lower values of bile acid could be obtained for the following reasons: (*a*) incomplete stool collection; (*b*) the markers used (β-sitosterol and chromium sesquioxide) may not correct for the degradative loss of acidic steroids during the intestinal transit; (*c*) excretion of bile acids via routes other than intestinal tract, i.e., urine, skin, etc; (*d*) failure of the procedure used to extract bile acids present as bile acid sulfates, etc.; and (*e*) lack of steady state conditions of patient study. The patients were all studied in the Clinical Study Unit with careful supervision and as far as we could tell there was no problem with incomplete stool collection. The lower values of bile acids could not be due to sterol ring breakdown as has been shown to occur in formula diets (29) because the recoveries of β-sitosterol and chromium sesquioxide were comparable. It is, however, still possible that chromium sesquioxide and β-sitosterol may not be exactly accountable for acidic steroid losses. Ideally, one needs to develop a non-absorbable bile acid marker to accurately reflect acidic steroid losses. Further work in this direction is needed to rule out the possible marker effects on fecal excretion calculations. The lower fecal values obtained could not be explained due

to the failure of our procedure to extract bile acid sulfates since solvolysis of the aqueous extract did not yield higher bile acid recovery. Also, we could not detect any significant losses of bile acids in our fecal analytical procedure using hyocholic acid as an internal standard. One would not anticipate losses of bile acid via urine and skin in the patients studied since none of our patients had biliary obstruction. The patients used in our study were in a reasonably good steady state. They followed the dietary regimen (used during the study period) for at least one month prior to their entry into the Study Unit (pre-equilibration) and their body weights remained constant. Under these conditions a fairly steady excretion of neutral sterols has been noted (17).

The isotopic turnover method could give an overestimation of bile acid excretion due to: (*a*) incomplete mixing of isotope with the pre-existing pool of bile acids and difficulties in determining the time of sample collection; (*b*) any deviation from the one pool model; and (*c*) inability to account for losses of labeled bile acid into serum. Incomplete mixing of the label with the pre-existing pool would undoubtedly give higher values. We, however, administered our isotope in a fasting state and the amino acid mixture is given to induce gallbladder contraction. The first sample of bile is obtained after 24 hr (in a fasting state). Studies by Duane et al. (30) have shown that after 12 hr complete mixing of the labeled bile acids occurs. Avoiding an earlier sample of bile might also decrease problems due to inadequate mixing. The Lindstedt technique assumes a one-pool model with a constant pool size, synthesis, and composition. If there are differences in any of the above mentioned features, it could affect the turnover rate. In this regard one possibility has to be considered in the loss of bile acids into the serum. Even though the serum bile acid concentration is low (2.5–5.0 μg/ml), in view of the large plasma volume (2.4 l in a 70 kg man) this could represent a considerable amount (6–12 mg). Whether or not the loss of this amount of bile acids into serum is significant enough to account for the greater decay of biliary radioactivity (hence a higher turnover), is not apparent at the moment. Preliminary studies by Mok et al. (31) have shown that the Lindstedt technique labels nonavailable bile acids outside the enterohepatic circulation, thus giving a higher turnover value.

In conclusion, both the methods used in this study have their limitations. Only further work will clearly establish the superiority of one method over the other. It is, however, recommended that the same methods be used for the comparison of bile acid excretion data in patient studies dealing with the effect of diet, drugs, etc. Also, interlaboratory comparison of data derived from different methods on a given kind of patient should only be made with caution.

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