Effect of chenodeoxycholic acid on biliary and urinary bile acids and bile alcohols in cerebrotendinous xanthomatosis; monitoring by high performance liquid chromatography

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Abstract Biliary and urinary bile alcohol and bile acid composition has been determined by high performance liquid chromatography in patients with cerebrotendinous xanthomatosis before and after treatment with chenodeoxycholic acid. Most of the bile acids and bile alcohols in the bile and urine were separated in less than 30 min using a radial pack C_{18} μBondapak 5 μm particle size column with a mobile phase of acetonitrile–water–methanol–acetic acid 70:70:20:1 (v/v/v/v) at a flow rate of 2 ml/min, and a refractive index detector. Before treatment, cholic acid (49%) and 5β-cholestane-3α,7α,12α,25-tetrol (27%) were the major biliary bile acid and bile alcohol, respectively, but were not detected in the urine of five patients. 5β-Cholestane-pentols were, instead, the major urinary bile alcohols with 5β-cholestane-3α,7α,12α,23ξ,25-pentol (56%) predominating. Whereas 5β-cholestane-3α,7α,12α,24S,25-pentol was not detected in the bile, it was isolated in the urine of all patients (27%). The only urinary bile acid isolated by high performance liquid chromatography was nor-cholic acid. After 1 month of treatment with chenodeoxycholic acid, 0.75 g/day, chenodeoxycholic acid became the major bile acid in the bile of all patients (71%) along with its metabolite, ursodeoxycholic acid (21%). Cholic acid and 5β-cholestane-3α,7α,12α,25-tetrol were drastically reduced and were only 3% each. The excretion of 5β-cholestane-pentols in the urine was also drastically reduced from 130 mg/day to 15 mg/day. — **Batta, A. K., S. Shefer, M. Batta, and G. Salen.** Effect of chenodeoxycholic acid on biliary and urinary bile acids and bile alcohols in cerebrotendinous xanthomatosis; monitoring by high performance liquid chromatography. *J. Lipid Res.* 1985. **26:** 690–698.

Supplementary key words 5β-cholestane-pentols • 5β-cholestane-tetrols • cholic acid • ursodeoxycholic acid

Owing to a block in bile acid synthesis, patients with cerebrotendinous xanthomatosis (CTX) excrete subnormal quantities of bile acids in their bile and accumulate large amounts of bile alcohol intermediates hydroxylated at C-25 (1–4). 5β-Cholestane-3α,7α,12α,25-tetrol is the major biliary bile alcohol and substantial quantities of 5β-cholestane-3α,7α,12α,23R,25-pentol and 5β-cholestane-3α,7α,12α,24R,25-pentol have also been isolated from the bile of CTX patients (5, 6). Recently, the presence of several 5β-cholestane-pentols hydroxylated at C-22, C-23, C-24, C-25, and C-26 has been reported in the urine of these patients (7, 8) and it has been suggested that the urinary bile alcohol pattern could be used for diagnosis of CTX (8). Since at least some of these bile alcohols are produced by the liver, it seems important to compare the two excretory pathways in the same patient with respect to composition and quantities. In this study, the major bile alcohols and bile acids in the bile and urine of five untreated CTX patients have been isolated by high performance liquid chromatography and the effect of chenodeoxycholic acid therapy on the excretion of these compounds in the bile and urine of these patients has been assessed.

MATERIALS AND METHODS

Materials

Cholic acid, nor-cholic acid, chenodeoxycholic acid, deoxycholic acid, and ursodeoxycholic acid were purchased from Steraloids Inc. (Wilton, NH). The acetone powder of cholylglycine hydrolase [from *Clostridium perfringens* (welchii)] and β-glucuronidase (from *Helix pomatia*) were purchased from Sigma Chemical Co. (St. Louis, MO). Sep-pak C_{18} cartridges were from Waters Associates, Inc. (Milford, MA). The solvents used for HPLC

Abbreviations: The following abbreviations or trivial names were used: cholic acid (CA), 3α,7α,12α-trihydroxy-5β-cholanoic acid; chenodeoxycholic acid (CDCA), 3α,7α-dihydroxy-5β-cholanoic acid; ursodeoxycholic acid (UDCA), 3α,7α-dihydroxy-5β-cholanoic acid; TLC, thin-layer chromatography; HPLC, high performance liquid chromatography; GLC, gas–liquid chromatography; TMSi, trimethylsilyl; CTX, cerebrotendinous xanthomatosis; CD, circular dichroism.

analysis were all HPLC grade and were purchased from Waters Associates, Inc. [24-^{14}C]Cholic and [24-^{14}C]chenodeoxycholic acid were purchased from Amersham (Arlington Heights, IL) and were >99% pure by thin-layer chromatography (TLC).

The bile alcohols, 5β-cholestane-3α,7α,12α,25-tetrol and 5β-cholestane-3α,7α,12α,24(R and S), 25-pentols were synthesized from cholic acid as described by Dayal et al. (9). 5β-Cholestane-3α,7α,12α,23R,25-pentol was isolated from the bile of a CTX patient as follows. One ml of the bile was diluted with 9 ml of 0.1 M sodium acetate buffer, pH 5.0, and incubated with 0.1 mg of β-glucuronidase in 0.1 ml water at 37°C for 36 hr. The contents were then applied on a Sep-pak C_{18} cartridge, which was previously washed with 5 ml of methanol followed by 5 ml of distilled water. The flow rate through the Sep-pak cartridge was kept at 20 drops per min. After washing with 5 ml of distilled water, the cartridge was eluted with 5 ml of methanol. The eluate was evaporated to dryness at 50°C under nitrogen and redissolved in 100 μl of methanol. The methanol solution was then applied as a streak on a 20 × 20 cm silica gel G thin-layer chromatography plate (0.25 mm thickness, Analabs, North Haven, CT) and the plate was developed in a solvent system of chloroform–acetone–methanol–acetic acid 70:50:18:1 (v/v/v/v). The solvent system was allowed to run up to 17 cm on the plate and then the plate was removed, dried with hot air, and the bands were visualized by spraying evenly with water. The band with R_f 0.50 was scraped and the compound was eluted with methanol (20 ml). After evaporation of methanol at 50°C under nitrogen, the residue was found to be pure on TLC and showed GLC and mass spectral fragmentation patterns identical with those described for 5 β-cholestane-3α,7α,12α,23R,25-pentol (6).

27-Nor-5β-cholestane-3α,7α,12α,24ξ,25-pentol was isolated from the urine of a normal subject (10). The urine (100 ml) was applied onto a pre-washed Sep-pak C_{18} cartridge and eluted at a rate of 20 drops per min. The cartridge was washed with 10 ml of distilled water and then eluted with 10 ml of methanol. Methanol was evaporated at 50°C under nitrogen and the residue was dissolved in 10 ml of 0.1 M sodium acetate buffer, pH 5.0, and incubated with 0.1 mg of β-glucuronidase for 36 hr at 37°C. The contents were then passed through a pre-washed Sep-pak C_{18} cartridge and the products were eluted with methanol as described above for the isolation of 5β-cholestane-3α,7α,12α,23R,25-pentol. Methanol was evaporated at 50°C under nitrogen, the residue was dissolved in 200 μl of methanol and subjected to preparative TLC on two 20 × 20 cm silica gel G plates. The plates were developed in the solvent system described above and the band corresponding to R_f 0.37 was scraped and eluted with 50 ml of methanol. The residue after evaporation of solvent yielded a pure compound which showed GLC and mass spectral characteristics identical with those described for 27-nor-5β-cholestane-3α,7α,12α,24ξ,25-pentol (10, 11).

Labeled compounds

[24-^{14}C]Taurocholate and [24-^{14}C]taurochenodeoxycholate were prepared from [24-^{14}C]cholic acid and [24-^{14}C]chenodeoxycholic acid, respectively, according to Tserng, Hachey, and Klein (12) and purified by preparative TLC (13). 5β-[26,27-^{14}C]Cholestane-3α,7α,12α,25-tetrol was prepared from cholic acid as described by Cheng et al. (14) (sp act 1.49 × 10^6 dpm/mg; radioactive purity, 98%). The glucuronide of the labeled 5β-cholestanetetrol at C-3 was prepared according to Hoshita et al. (15) and the final product was purified by preparative TLC (15). The pure glucuronide thus obtained had a specific activity of 1.4 × 10^6 dpm/mg and was 98.7% pure.

Samples

Bile was collected from the control and CTX subjects before and after bile acid therapy and stored at −20°C until analysis. Twenty-four-hour urine samples from all patients were pooled and stored at −20°C until analysis.

Isolation of urinary bile alcohols and bile acids

Forty ml of centrifuged urine was passed through a pre-washed Sep-pak C_{18} cartridge at a rate of 20 drops per min. The cartridge was then washed with 10 ml of distilled water and compounds were eluted with 10 ml of methanol. Methanol was evaporated and the residue was dissolved in 10 ml of 0.1 M sodium acetate buffer, pH 5.0, and 0.1 mg of β-glucuronidase and 0.1 mg of cholylglycine hydrolase were then added and the mixture was incubated at 37°C for 36 hr. The mixture was then passed through a Sep-pak cartridge as described above and the compounds were eluted with 10 ml of methanol. The methanol was evaporated and the residue was dissolved in 1 ml of methanol and used for chromatographic analysis. In order to separate the bile acids from bile alcohols, the residue obtained after passing the products of enzymatic hydrolysis through Sep-pak followed by elution with methanol was dissolved in ethyl acetate (5 ml) (or in another experiment, first solvolyzed and then dissolved in ethyl acetate). The ethyl acetate solution was extracted with 2% sodium hydroxide (3 × 2 ml) and evaporated to dryness. The residue was dissolved in 1 ml of methanol and used for chromatographic analysis of bile alcohols. The sodium hydroxide layer was acidified with 1 N hydrochloric acid to pH 1 and then extracted with ethyl acetate (2 × 5 ml). The ethyl acetate extract was washed with water (3 × 3 ml) and then evaporated to dryness. The residue was dissolved in 0.5 ml of methanol and used for chromatographic analysis of bile acids.

Solvolysis

The residue obtained after hydrolysis of 40 ml of urine with β-glucuronidase and cholylglycine hydrolase as described above for the isolation of urinary bile alcohols and bile acids was dissolved in 9 ml of acetone containing 1 ml of methanol and solvolyzed as described by Batta et al. (16). The product was dissolved in ethyl acetate (5 ml) and the acidic and non-acidic components were separated as described above.

Isolation of biliary bile alcohols and bile acids

Bile (1 ml) was diluted with 9 ml of 0.1 M sodium acetate buffer, pH 5.0, and incubated with 0.1 mg of β-glucuronidase and 0.1 mg of cholylglycine hydrolase at 37°C for 36 hr. The products were isolated exactly as described above for the isolation of products in urine. The final residue was dissolved in 1 ml of methanol and used for chromatographic analysis.

Thin-layer chromatography

The TLC of the urinary and biliary bile alcohols and bile acids was performed on silica gel G plates in solvent system A consisting of chloroform–acetone–methanol–acetic acid 70:50:18:1 (v/v/v/v). The spots were visualized by spraying the plate with 10% H_2SO_4 followed by a solution of 3.5% phosphomolybdic acid in isopropanol and then heating the plate for 2 min at 110°C. The following R_f values were obtained for the reference compounds: cholic acid, 0.61; nor-cholic acid, 0.59; chenodeoxycholic acid, 0.84; ursodeoxycholic acid, 0.85; 5β-cholestane-3α,7α,12α,25-tetrol, 0.71; 5β-cholestane-3α,7α,12α,24R,25-pentol, 0.46; 5β-cholestane-3α,7α,12α,24S,25-pentol, 0.50; 5β-cholestane-3α,7α,12α,23R,25-pentol, 0.50; and 27-nor-5β-cholestane-3α,7α,12α,24ξ,25-pentol, 0.37. In order to separate cholic acid from nor-cholic acid, a solvent system of chloroform–methanol–acetic acid 40:3:2 (v/v/v) (solvent system B) was used. Cholic acid showed an R_f value of 0.53 and nor-cholic acid, 0.45 in this solvent system.

High performance liquid chromatography

HPLC of the underivatized bile acids and bile alcohols was performed on a Waters Associates ALC 201 system employing a Waters Associates model 401 refractive index detector and a Hewlett-Packard (Lexington, MA) Model 3380 integrator. A Waters Associates radial pack μBondapack C_{18} reversed-phase column (5 μm particle size) was used for all separations.

Ten to one hundred μg of the bile acid or bile alcohol dissolved in 10–20 μl of methanol (or 50 μl of the methanol solution of the urinary or biliary extract as obtained above) was injected into the column for HPLC analysis. The mobile phase consisted of acetonitrile–water–methanol–acetic acid 70:70:20:1 (v/v/v/v) and the flow rate was maintained at 2 ml/min (operating pressure, ca. 2000 psi).

When mixtures containing nor-cholic acid, cholic acid, and chenodeoxycholic acid were injected into the HPLC column, the detection limit of nor-cholic acid was found to be of the order of 1 μg, of cholic acid, 2 μg, and of chenodeoxycholic acid, 4 μg. The detection limits for the 5β-cholestane-pentols were 1–1.4 μg, whereas that of 5β-cholestane-3α,7α,12α,25-tetrol was 6 μg. The detection limits refer to a signal/noise ratio of 3 or above.

Gas–liquid chromatography (GLC), mass spectrometry, and circular dichroism (CD)

The various bile acid and bile alcohol fractions were collected during HPLC and the solvents were evaporated under reduced pressure. The residue in each case was analyzed by GLC as the TMSi ether (for bile alcohols) or the methyl ester–TMSi ether (for bile acids). GLC was performed on a Hewlett-Packard Model 7610A gas chromatograph as described previously (17) with columns (180 cm × 4 mm) packed with either 3% QF-1 or 1% HiEFF8BP on 80/100 mesh Gas Chrom Q. Instrumentation and conditions for mass spectrometry (17, 18) and circular dichroism (6) were as previously described.

RESULTS

To minimize losses caused during extractions, attempts were made to analyze the biliary and urinary bile acids and bile alcohols together, without separation into the acidic and neutral fractions. On TLC of the bile acids and alcohols obtained after treatment of the bile and urine of CTX patients with cholylglycine hydrolase and β-glucuronidase, it was found that 5β-cholestane-3α,7α,12α,24S,25-pentol and 5β-cholestane-3α,7α,12α,23R,25-pentol had the same relative mobilities in solvent systems consisting of chloroform–acetone–methanol–acetic acid 70:50:18:1 and n-hexane–ethyl acetate–acetic acid 50:50:10 (v/v/v) (19). Nor-cholic acid and cholic acid could be separated when the TLC plate was developed in chloroform–methanol–acetic acid 40:3:2 and only nor-cholic acid was detected in the urine.

When examined on HPLC, it was found that most of the bile acids and bile alcohols reported in the bile and urine of patients with CTX could be separated in a single chromatogram when a 5 μm C_{18} radial pack reversed-phase column was used (**Fig. 1**). The retention volumes were reproducible and for amounts of bile acids and alcohols ranging from 5 to 100 μg, the detector response measured by peak area was linear. When ^{14}C-labeled taurocholate, taurochenodeoxycholate, or 5β-[26,27-^{14}C]cholestane-3α,7α,12α,25-tetrol-3-glucuronide was added to the untreated bile or urine before passing through Sep-pak, 90–98% radioactivity was recovered. Similarly, when ^{14}C-labeled cholic acid or 5β-cholestane-3α,7α,12α,25-tetrol was added to the products of enzymatic hydrolysis of the bile or urine and then passed

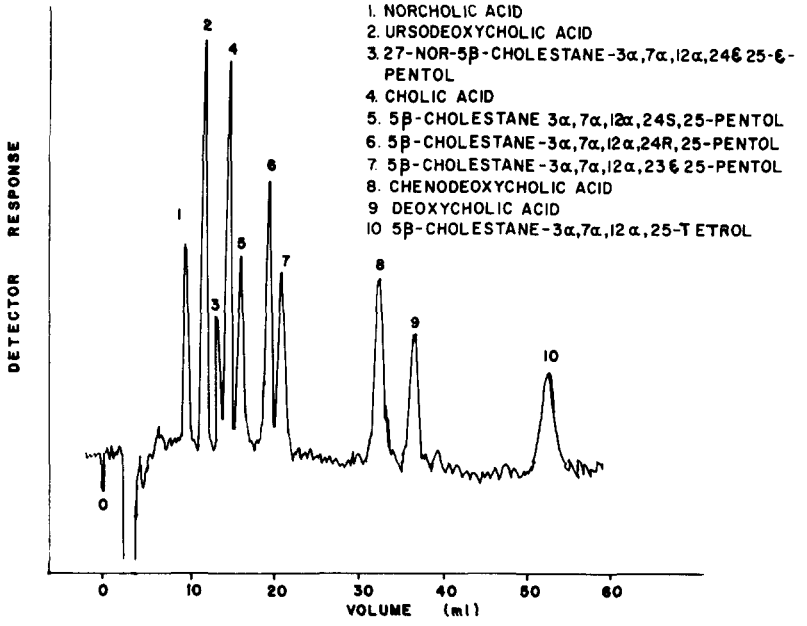

Fig. 1. HPLC of standard bile acids and bile alcohols. Column: 100 × 8 mm I.D. Radial pack C_{18} cartridge (5 μm) attached to a guard column (49 × 4.6 mm I.D.) dry packed with 37–50 μm C_{18} Corasil reversed-phase material. Eluent: acetonitrile–water–methanol–acetic acid 70:70:20:1. Flow rate: 2 ml/min.

through Sep-pak, 93–98% radioactivity was recovered and on further passage through the HPLC column, 94–97% radioactivity was recovered. The HPLC method was further validated by quantitating known amounts of standard bile acids and bile alcohols by HPLC as well as by GLC (17, 18); an excellent correlation between the two methods was found. Since all components did not show the same detector response (peak height was maximum for nor-cholic acid and minimum for 5β-cholestane-3α,7α,12α,25-tetrol when equal amounts of the standard bile acids and bile alcohols were injected), each biliary and urinary component was quantitated by taking into consideration the slope of the detector response versus peak height curve. The various bile acids and bile alcohols as eluted from the HPLC column were collected and further characterized by gas–liquid chromatography–mass spectrometry (5, 17, 18).

The HPLC patterns of the biliary bile acids and bile alcohols in a CTX patient before and after chenodeoxycholic acid therapy are shown in **Fig. 2A and B** and the data on five patients are listed in **Table 1.** Cholic acid was the major bile acid (49%) and 5β-cholestane-3α,7α,12α,25-tetrol was the major bile alcohol (27%) in the bile of untreated patients. 5β-Cholestane-3α,7α,12α,23R,25-pentol was found to be the major 5β-cholestane-pentol (10%) and, of the two diastereoisomers of 5β-cholestane-3α,7α,12α,24,25-pentol at C-24, only the 24R-diastereoisomer could be isolated (4%). After feeding chenodeoxycholic acid 0.75 g/day for 1 month, this bile acid and its bacterial metabolite, ursodeoxycholic acid, became the predominant components of the bile (chenodeoxycholic acid, 71% and ursodeoxycholic acid, 21% (20)), and the relative proportions of cholic acid and 5β-cholestane-3α,7α,12α,25-tetrol were reduced to 3% each. Bile alcohols could not be detected in five control bile specimens, and cholic acid and chenodeoxycholic acid were the major bile acids before chenodeoxycholic acid feeding (Table 1). After feeding 0.75 g/day chenodeoxycholic acid, as expected, this bile acid became the predominant bile acid in the bile, and substantial amounts of ursodeoxycholic acid were also formed. Cholic acid became a minor bile acid.

Fig. 3A and B shows the HPLC pattern of the urinary bile acids and bile alcohols in a CTX patient before and after chenodeoxycholic acid therapy. The major urinary product in untreated patients was found to be 5β-cholestane-3α,7α,12α,23ξ,25-pentol (74 mg/day). Both 24R- and 24S-diastereoisomers of 5β-cholestane-3α,7α,12α,24,25-pentol were identified; the 24S-diastereoisomer was excreted in larger amounts than the 24R-diastereoisomer (40 mg/day of 24S-diastereoisomer versus 16 mg/day of the 24R-diastereoisomer). 5β-Cholestane-3α,7α,12α,25-tetrol was excreted in very small amounts and could be detected only by TLC. The only urinary bile acid that could be detected by HPLC was nor-cholic acid and it was isolated by treatment of the urine with cholylglycine hydrolase and β-glucuronidase followed by separation of the acidic and non-acidic components with dilute NaOH. The crude acidic fraction was subjected to preparative TLC in solvent system A and the band corresponding to

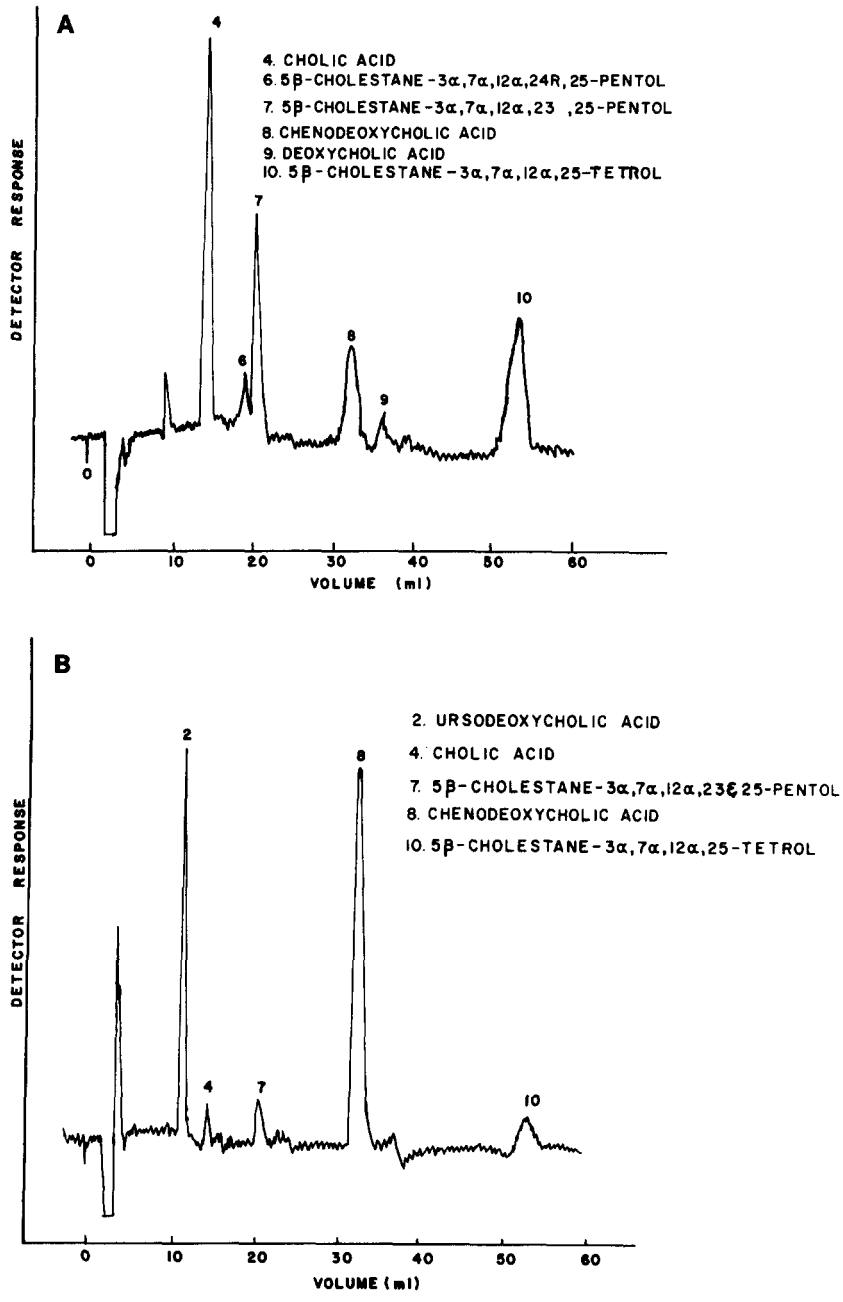

Fig. 2. HPLC of the bile acids and bile alcohols in the bile of a CTX patient. A, Before treatment; B, after treatment with chenodeoxycholic acid; conditions same as in Fig. 1.

the mobility of nor-cholic acid was eluted with methanol. TLC (solvent system B) and HPLC analysis showed the presence of only nor-cholic acid and no detectable amount of cholic acid was found to be present. The average daily excretion of nor-cholic acid in the urine of three untreated patients was found to be 14 mg (8). Feeding of chenodeoxycholic acid resulted in approximately a 9-fold reduction in the daily excretion of bile alcohols in the urine of CTX patients and 5β-cholestane-3α,7α,12α,25-tetrol could not be detected by TLC or HPLC. It should be pointed out that, due to lower sensitivity of HPLC compared to GLC, small changes in bile acid and bile alcohol concentrations may not be accurately determined.

Owing to the lack of a reference standard, the 23S-diastereoisomer of 5β-cholestane-3α,7α,12α,23,25-pentol could not be characterized in the bile and urine of CTX patients. But, in the bile as well as urine of CTX patients, the fraction in the HPLC chromatogram with retention volume corresponding to that of 5β-cholestane-3α,7α,12α,23R,25-pentol was found to be completely

TABLE 1. Bile acids and bile alcohols in the bile of CTX patients before and after CDCA treatment[a]

Patient	Treatment	Bile Acids				Bile Alcohols 5β-Cholestane-3α,7α,12α-			
		CA	CDCA	DCA	UDCA	23R,25-Pentol	24R,25-Pentol	24S,25-Pentol	25-Tetrol
						percent			
A	Pre[b]	31	5			13	9		42
	After[c]	2	72		19	2	1		4
B	Pre[b]	60	10	3		6	2		19
	After[c]	5	68		22	2	1		2
C	Pre[b]	70	5			9	2		14
	After[c]	2	75		18	2			3
D	Pre[b]	35	12	3		12	4		34
	After[c]	3	76		14	2	1		4
E	After[d]	1	62		34	1	1		1
Mean	Pre	49	8	2		10	4		27
	After	3	71		21	2	1		3
Control[e]	Pre[b]	46	40	11	3				
	After[d]	4	88	2	6				

[a]Bile was analyzed by HPLC after hydrolysis with cholylglycine hydrolase and β-glucuronidase (see Materials and Methods). Values are calculated as percent of total bile acids and bile alcohols.
[b]Bile was obtained before starting bile acid treatment.
[c]Bile was obtained after 1 month of 0.75 g/day CDCA feeding.
[d]Bile was obtained after 12 months of 0.75 g/day CDCA feeding.
[e]Average of five gallstone patients.

identical by TLC, HPLC, GLC, and circular dichroism with a sample of 5β-cholestane-3α,7α,12α,23R,25-pentol whose configuration at C-23 had already been established by circular dichroism (6). The HPLC fractions as obtained from the bile or urine with retention volumes corresponding to those of the 24R- and 24S-diastereoisomers of 5β-cholestane-3α,7α,12α,24,25-pentol were compared by TLC and gas-liquid chromatography-mass spectrometry with standard samples of 5β-cholestane-3α,7α,12α,24(R and S), 25-pentols whose configuration at C-24 had been established by circular dichroism (6). Only the 24R-diastereoisomer of this bile alcohol was found in the bile, whereas both 24R- and 24S-diastereoisomers were characterized in the urine. The only bile alcohol present in appreciable quantities in the urine of five control subjects was 27-nor-5β-cholestane-3α,7α,

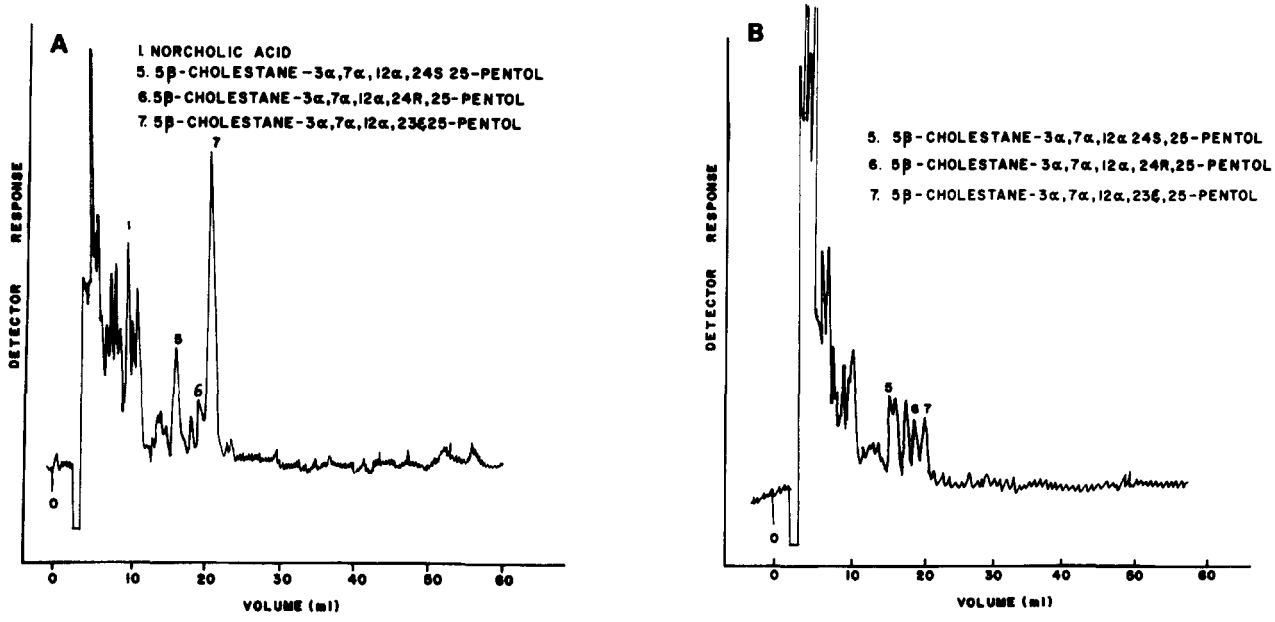

Fig. 3. HPLC of the bile acids and bile alcohols in the urine of a CTX patient. A, Before treatment; B, after treatment with chenodeoxycholic acid; conditions same as in Fig. 1.

12α,24ξ,25-pentol and it was estimated that approximately 1.5 mg/day was excreted in the urine.

It may be pointed out that the HPLC chromatograms obtained may not exhibit all the biliary and urinary bile alcohols and bile acids present, since relatively larger amounts of compounds with larger retention volumes must be present in the injection mixtures in order to be detected by HPLC. Therefore, some of the minor components may not have been detected. Furthermore, although several of the isomeric 5β-cholestane-pentols are separable by GLC (17, 18), the HPLC peaks due to the 5β-cholestane-pentols may contain other isomers which might be separable by capillary GLC (8, 11). However, these components are expected to be in very small amounts (8) and are therefore physiologically less important.

DISCUSSION

Wolthers et al. (8) have shown that significant amounts of 5β-cholestane-pentols are excreted in the urine of patients with CTX and that feeding chenodeoxycholic acid suppresses their excretion. Our results confirm these findings and we have compared the biliary and urinary excretion products in the same patient. For isolation of the bile alcohols and bile acids, HPLC was used and the results of the present investigation demonstrate that HPLC can be used for the analysis of bile acids and bile alcohols together in the bile and urine of CTX patients. The bile or the urine was enzymatically hydrolyzed and the bile acids and bile alcohols were obtained by passage through Sep-pak (15) and subjected to HPLC on a reversed-phase (5 μm) column. With the use of a solvent system of acetonitrile–water–methanol–acetic acid 70:70:20:1 (v/v/v/v), it was found that the biliary and urinary bile acids and bile alcohols could be separated and quantitated by one passage through the column.

Significant differences were noted in the biliary and urinary products (Table 1 and **Table 2**). Thus, cholic acid, the major biliary bile acid, was virtually absent in the urine. Even after solvolysis, cholic acid and chenodeoxycholic acid could not be detected by HPLC. Since the β-glucuronidase used also contained sulfatase activity (15,000–40,000 units/g), the glycine and taurine conjugates of sulfated bile acids, if present, would also be hydrolyzed. Similarly, the major biliary bile alcohol, 5β-cholestane-3α,7α,12α,25-tetrol, was present only in very small amounts in the urine. Although the 24R diastereoisomer of 5β-cholestane-3α,7α,12α,24,25-pentol was present in both bile and urine, the 24S isomer was present only in the urine of the CTX patients. Between 108 and 153 mg/day of 5β-cholestane-pentols was excreted each day. Since these compounds are conjugated with glucuronic acid and excreted in the urine in large amounts, a considerable plasma transport must exist. Thus, in addition to cholestanol (21), bile alcohol glucuronides may also be present in plasma. Feeding chenodeoxycholic acid to the CTX patients for just 1 month drastically reduced the excretion of 5β-cholestane-pentols; only 15 mg/day was excreted. On the other hand, the bile became rich in chenodeoxycholic acid and its bacterial metabolite ursodeoxycholic acid. The proportions of cholic acid and 5β-cholestane-3α,7α,12α,25-tetrol were very low after chenodeoxycholic acid feeding.

TABLE 2. Effect of CDCA feeding on urinary bile alcohol glucuronides in CTX patients[a]

Patient	Treatment	Nor-CA[b]	23ξ,25-Pentol	Bile Alcohols 5β-Cholestane-3α,7α,12α- 24R,25-Pentol	24S,25-Pentol	25-Tetrol	Total 5β-Cholestane Pentols
				mg/day			
A	Pre[c]	9	76	5	27	1	108
	After[d]	n.d.[e]	7	3	4		14
B	Pre[c]	16	60	19	41	2	120
	After[d]	n.d.	8	3	4		15
C	Pre[c]	n.d.	75	17	48	2	140
	After[d]	n.d.	9	5	7		21
D	Pre[c]	17	86	21	46	2	153
	After[d]	n.d.	8	4	5		17
E	After[f]	n.d.	5	2	4		11
Mean	Pre	14	74	16	40	2	130
	After	n.d.	7	3	5		15

[a]Urine was analyzed by HPLC after hydrolysis with cholylglycine hydrolase and β-glucuronidase.
[b]Nor-CA was quantitated by HPLC of the NaOH-soluble fraction of the enzymatic hydrolysis products of urine.
[c]Twenty-four-hr urine collection before starting bile acid treatment.
[d]Twenty-four-hr urine collection after 1 month of 0.75 g/day CDCA feeding.
[e]Not determined.
[f]Twenty-four-hr urine collection after 12 months of 0.75 g/day CDCA feeding.

Treatment with chenodeoxycholic acid has been shown to reduce the clinical symptoms in CTX and markedly reduce plasma cholestanol levels (21). The results of this investigation indicate that the abnormal bile acid synthesis is suppressed, as evidenced by the marked reduction in both biliary and urinary bile alcohol excretion and in biliary cholic acid. The mechanism of bile acid suppression is not clear. However, both the inhibition of HMG-CoA reductase activity and cholesterol 7α-hydroxylase activity, actions which have been attributed to chenodeoxycholic acid (21, 22), may be responsible. The net result is that less bile acid precursor, either cholesterol or 7α-hydroxycholest-4-en-3-one is produced and, as a consequence, the formation of bile alcohols is substantially reduced. Obviously, treatment with cholestyramine, which promotes bile acid excretion and activates bile acid synthesis (23, 24), will be deleterious in CTX and aggravate bile acid synthetic pathway. The demonstration that cholestanol levels rise during cholestyramine therapy supports this hypothesis (23).

In summary, increased amounts of bile alcohol glucuronides are excreted in the bile and urine of patients with CTX. In contrast to bile, where the predominant bile alcohol is 5β-cholestane-3α,7α,12α,25-tetrol, the urinary pattern shows a predominance of 5β-cholestane-pentols and probably hexols and heptols (7, 8). The large quantities probably reflect a defect in bile acid synthesis involving incomplete oxidation of side chain. Treatment with chenodeoxycholic acid inhibits bile acid synthesis and thereby virtually eliminates bile alcohols from both bile and urine. These findings are in complete agreement with the findings of Wolthers et al. (8) who have shown that chenodeoxycholic acid treatment markedly reduces bile alcohol excretion in the urine of CTX patients. The virtual absence of these bile alcohols in the bile supports the conclusion that chenodeoxycholic acid suppresses the hepatic synthesis of bile alcohols. It is not clear why 5β-cholestane-3α,7α,12α,25-tetrol is preferentially excreted in the bile and feces (2, 5) with only trace amounts appearing in the urine while large amounts of 5β-cholestane-pentols are excreted in the urine. We have shown that only 24S-diastereoisomer of 5β-cholestane-3α,7α,12α,24,25-pentol is effectively converted into cholic acid (25), which may explain why this isomer is not detected in the bile. However, we do not know why both isomers of this bile alcohol are excreted in the urine.

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