

# Increased plasma bile alcohol glucuronides in patients with cerebrotendinous xanthomatosis: effect of chenodeoxycholic acid

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**Abstract** Large quantities of C<sub>27</sub> bile alcohols hydroxylated at C-25 are excreted in the bile and urine of patients with cerebrotendinous xanthomatosis, a lipid storage disease that results from defective bile acid synthesis. The presence of both biliary and urinary bile alcohols reflects impaired bile acid synthesis. After treatment of samples with  $\beta$ -glucuronidase, plasma bile alcohols were quantitated by gas-liquid chromatography-mass spectrometry. 5 $\beta$ -Cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,25-tetrol (334  $\mu$ g/dl) was found to be the major bile alcohol, followed by 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,23R,25-pentol (65  $\mu$ g/dl), and 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,24(R and S),25-pentols (62.5  $\mu$ g/dl and 64.5  $\mu$ g/dl, respectively) in the plasma of these patients. When compared to biliary and urinary bile alcohol excretions, the plasma pattern resembled bile where 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,25-tetrol glucuronide predominated. In contrast, urinary bile alcohols were composed chiefly of 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,25-tetrol glucuronides with only small amounts of 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,25-tetrol glucuronide. Treatment with chenodeoxycholic acid, which suppresses abnormal bile acid synthesis in these patients, reduced plasma bile alcohol concentrations dramatically. ■ These results show that large quantities of bile alcohol glucuronides, particularly 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,25-tetrolglucuronide, circulate in plasma of patients with cerebrotendinous xanthomatosis. The plasma bile alcohols closely resemble biliary bile alcohols which indicates their hepatic origin. The large quantities of polyhydroxylated bile alcohols in the urine may suggest their formation, at least in part, from 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,25-tetrol by renal hydroxylating mechanisms. Treatment with chenodeoxycholic acid inhibits hepatic bile alcohol synthesis and reduces plasma concentration of bile alcohol glucuronides.—Batta, A. K., G. Salen, S. Shefer, G. S. Tint, and M. Batta. Increased plasma bile alcohol glucuronides in patients with cerebrotendinous xanthomatosis: effect of chenodeoxycholic acid. *J. Lipid Res.* 1987. 28: 1006–1012.

**Supplementary key words** urinary bile alcohols • plasma bile alcohols

Cerebrotendinous xanthomatosis (CTX), a familial sterol storage disease, results from a block in bile acid synthesis (1) and is characterized by the accumulation of cholesterol and cholestanol in most tissues (2). Large amounts of bile alcohols hydroxylated at C-25 have been detected as glucuronides in bile and urine of CTX patients (3, 4) while,

in feces, free bile alcohols are excreted presumably due to bacterial hydrolysis (1). The major biliary and fecal bile alcohols excreted in CTX are 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,25-tetrol and 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,23 $\xi$ ,25-pentols along with substantial amounts of 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,24R,25-pentol (1, 3). In contrast, Wolthers et al. (4) and Batta et al. (5) have found that the urine of these patients contains 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,25-tetrol as the major bile alcohols with only small amounts of 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,25-tetrol. The isolation of large amounts of bile alcohol glucuronides from the urine suggests a plasma pool of these compounds in CTX. The purpose of this investigation was to measure plasma bile alcohol composition and compare the plasma pattern with that in urine and bile. Also, the effect of chenodeoxycholic acid (CDCA), which suppresses abnormal hepatic bile acid synthesis in CTX, was determined.

## EXPERIMENTAL PROCEDURES

### Materials

Sep-Pak C<sub>18</sub> cartridges were purchased from Waters Associates (Milford, MA). The acetone powder of cholesteryl-glycine hydrolase (from *C. perfringens* (welchii)) and  $\beta$ -glucuronidase (from *Helix pomatia*) were from Sigma Chemical Co. (St. Louis, MO). Chenodeoxycholic acid was obtained from G. Pharmix, Milan, Italy.

The bile alcohols, 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,25-tetrol and 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,24(R and S)25-pentols were syn-

Abbreviations: CTX, cerebrotendinous xanthomatosis; CDCA, chenodeoxycholic acid; TLC, thin-layer chromatography; GLC, gas-liquid chromatography; TMSi, trimethylsilyl. The following trivial names were used: chenodeoxycholic acid, 3 $\alpha$ ,7 $\alpha$ -dihydroxy-5 $\beta$ -cholanoic acid; cholesterol, 5-cholesten-3 $\beta$ -ol; cholestanol, 5 $\alpha$ -cholestan-3 $\beta$ -ol.

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thesized from cholic acid according to Dayal et al. (6). 5 $\beta$ -Cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,23R,25 pentol was isolated from the bile of a CTX patient as described by Batta et al. (5). The glucuronide of 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,25-tetrol was prepared according to Hoshita et al. (7).

## Patients

Nine untreated CTX patients (five male and four female, aged between 28 and 60 years) were examined. All exhibited tendon xanthomas, dementia, and cataracts. Elevated plasma cholestanol levels were present in all cases.

## Samples

Bile and plasma were collected from fasting CTX subjects before and after feeding 1 g/day of chenodeoxycholic acid for 1 month and stored at  $-20^{\circ}\text{C}$  until used. Twenty four-hour urine collections from these patients were obtained before and after bile acid therapy and stored at  $-20^{\circ}\text{C}$  until analyzed.

## Thin-layer chromatography

The TLC of the urinary, biliary, and plasma bile alcohol glucuronides was performed on silica gel G plates in a solvent system of chloroform-methanol-acetic acid-water 26:10:4:2 (v/v/v/v) (7). The spots were visualized by spraying the plate with 10%  $\text{H}_2\text{SO}_4$  followed by a solution of 3.5% phosphomolybdic acid in isopropanol and then heating the plate at  $110^{\circ}\text{C}$  for 2 min. Alternatively, the plate was sprayed with a 0.2% solution of naphthoresorcinol in ethanol-phosphoric acid 9:1 (v/v) followed by heating at  $110^{\circ}\text{C}$  for 5 min. The glucuronides gave blue spots. Synthetic 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,25-tetrol-3-glucuronide was used for TLC comparison. The identification of the various bile alcohol glucuronides was made after their extraction from the respective bands on the TLC plate with methanol, hydrolysis with  $\beta$ -glucuronidase, and gas-liquid chromatography of the liberated unconjugated bile alcohols as their trimethylsilyl ether derivatives. These methodologies are described below.

## Gas-liquid chromatography

The various bile alcohols in the plasma, bile, and urine were silylated with 100  $\mu\text{l}$  of Sil-Prep (Applied Science Labs), for 30 min at  $55^{\circ}\text{C}$ . After evaporation of solvents under  $\text{N}_2$ , the silyl ethers were dissolved in 100  $\mu\text{l}$  of hexane and 1–5  $\mu\text{l}$  was injected into a Hewlett Packard 5880A gas chromatograph equipped with a split/splitless device for capillary columns. A fused silica CP-Sil-5 capillary column (25 m), i.d. 0.20–0.22 mm was employed and helium was used as the carrier gas with a flow rate of 0.9 ml/min. The GLC operating conditions were as follows: injector and detector temperatures were set at  $260^{\circ}\text{C}$  and  $290^{\circ}\text{C}$ , respectively. Following injection, the oven temperature was kept at  $100^{\circ}\text{C}$  for 2 min, then programmed at a rate of  $25^{\circ}\text{C}/\text{min}$  to a temperature of  $265^{\circ}\text{C}$ , and subse-

quently at a rate of  $1.5^{\circ}\text{C}/\text{min}$  to a final temperature of  $280^{\circ}\text{C}$  (4). The retention times of some of the standard bile alcohols relative to that of 5 $\alpha$ -cholestane (internal standard) are given in Table 1. The retention times of the bile alcohols were reproducible and, for amounts of bile alcohols varying from 10 ng to 140 ng injected onto the column, the detector response as shown by the integrator was linear (the retention time of the bile alcohol slightly increased when a relatively large amount of the TMSi ether of the bile alcohol was injected). The response factors of the various bile alcohols (5 $\beta$ -cholestanetetrol and 5 $\beta$ -cholestanepentols) were found to be similar and the correction factor corresponding to the peak area of 5 $\alpha$ -cholestane was found to be of the order of 1.05 for 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,25-tetrol as well as the 5 $\beta$ -cholestanepentols. This correction factor was used for the quantitation of the bile alcohols in the plasma, bile, and urine of the patients.

## Mass spectra

The mass spectra were obtained from a Varian MAT-III, gas chromatograph-mass spectrometer (Varian Associates, Palo Alto, CA) as described previously (8).

## Isolation of plasma bile alcohols

Plasma (1 ml) was slowly passed through a prewashed Sep-Pak C<sub>18</sub> cartridge and the bile acids and bile alcohols were eluted from the Sep-Pak with methanol as described before (5). Methanol was evaporated, the residue was dissolved in 2 ml of 0.1 M sodium acetate buffer, pH 5.0, and 0.05 mg of  $\beta$ -glucuronidase was added and the products were incubated at  $37^{\circ}\text{C}$  for 18 hr. After another filtration through Sep-Pak, the bile acids and bile alcohols were eluted with methanol. Methanol was evaporated and the residue was dissolved in ethyl acetate (5 ml). The ethyl acetate solution was washed with 2% sodium hydroxide solution (1 ml  $\times$  3) followed by water washing (1 ml  $\times$  3). The

TABLE 1. GLC retention times of the trimethylsilyl ethers of bile alcohols<sup>a</sup>

5 $\beta$ -Cholestane-	GLC (RRT) <sup>b</sup>
3 $\alpha$ ,7 $\alpha$ ,25-triol	1.54
3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,25-tetrol	1.56
3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,26-tetrol (25R + 25S)	1.62
3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,23R,25-pentol	1.78
3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,24S,25-pentol	1.83
3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,24R,25-pentol	1.84

<sup>a</sup>Operating conditions for GLC were as follows: column, fused silica CP-Sil-5 capillary column (25 m), i.d., 0.20–0.22 mm; carrier gas,  $\text{H}_2$ , at flow rate of 0.9 ml/min; injector temperature,  $260^{\circ}\text{C}$ ; detector temperature,  $290^{\circ}\text{C}$ ; oven temperature,  $100^{\circ}\text{C}$  for 2 min, then programmed at a rate of  $25^{\circ}\text{C}/\text{min}$  to a temperature of  $265^{\circ}\text{C}$ , and subsequently at a rate of  $1.5^{\circ}\text{C}/\text{min}$  to a final temperature of  $280^{\circ}\text{C}$ .

<sup>b</sup>Retention time relative to that of 5 $\alpha$ -cholestane (retention time, 11.22 min). RRT of cholesterol under these conditions is 1.23.

residue obtained after evaporation of solvent was silylated, taken up in 100  $\mu$ l of hexane, and 1–5  $\mu$ l was injected onto a GLC column.

#### Isolation of biliary bile alcohols

Bile (0.2 ml) was diluted with 1 ml of 0.1 M sodium acetate buffer, pH 5.0, and incubated with 0.05 mg of  $\beta$ -glucuronidase at 37°C for 18 hr. The bile alcohols were then isolated as described above for the isolation of plasma bile alcohols. The final residue was similarly silylated and used for GLC analysis.

#### Isolation of urinary bile alcohols

Two milliliters of centrifuged urine was slowly filtered through Sep-Pak and the methanol solution of the bile acids and bile alcohols obtained was subjected to hydrolysis with  $\beta$ -glucuronidase as described above. The bile alcohols were isolated and used for GLC analysis as described for the plasma bile alcohols.

In order to determine the recovery of the bile alcohols and their glucuronides during the Sep-Pak and  $\beta$ -glucuronidase treatment, 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,25-tetrol-3-glucuronide (0.5 mg) was added to 1 ml of plasma from a healthy control before passing through Sep-Pak.  $\beta$ -Glucuronidase treatment of the products followed by another Sep-Pak filtration resulted in the recovery of 86% of the glucuronide as free 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,25-tetrol.

#### Isolation of bile alcohol glucuronides

In order to isolate the bile alcohol glucuronides, the plasma (1 ml), bile (0.4 ml), or urine (2 ml) was filtered through Sep-Pak and the products were eluted with methanol. The residue after evaporation of methanol was either used as such for TLC (bile alcohol glucuronides plus bile acid conjugates) or was first subjected to cholyglycine hydrolase as follows. The residue was dissolved in 1 ml of 0.1 M sodium acetate buffer, pH 5.6, and incubated with 0.05 mg of cholyglycine hydrolase for 18 hr at 37°C. The products were again passed through Sep-Pak and the bile acid plus bile alcohol fraction was eluted with methanol. The residue after evaporation of methanol contained bile alcohol glucuronides plus unconjugated bile acids and was used for TLC examination.

In order to determine whether unconjugated bile alcohols were present in the plasma, 2 ml of the plasma was passed through Sep-Pak and the products were applied as a band on a TLC plate and the plate was developed in chloroform–acetone–methanol 70:50:7 (9). Standards of 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,25-tetrol and 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,24(R and S) 25-pentols were applied on the sides of the plate. Silica gel was scraped from the plate corresponding to the areas where the unconjugated bile alcohols appeared and the products were eluted with methanol. The residue, after evaporation of methanol, was dissolved in ethyl acetate (5 ml) and washed with 2% NaOH (2 ml) followed

by water washing (2  $\times$  2 ml). The organic layer was evaporated and the products were silylated and used for GLC.

## RESULTS

The plasma bile alcohols in the CTX patients were isolated by the same method used for the isolation of the urinary bile alcohols in these patients (5). Thus, the plasma from the CTX patients was passed through Sep-Pak. After washing the Sep-Pak with water, bile acids and bile alcohols were eluted with methanol. In this way, proteins and over 90% of the cholesterol and cholestanol were removed. The bile alcohol glucuronides in the methanol extract were hydrolyzed with  $\beta$ -glucuronidase, and after a second Sep-Pak treatment, the free bile alcohols were separated from the bile acid conjugates by extraction of the latter with sodium hydroxide. The bile alcohol fraction was then silylated and subjected to gas chromatography.

Fig. 1A, shows the chromatogram of the TMSi ethers of the bile alcohols in the plasma of an untreated CTX patient (A) and in Table 2 are shown the amounts ( $\mu$ g/dl) of the bile alcohols present in the plasma of nine untreated patients. As seen from the figure, several peaks were observed in the region of tetra- and pentahydroxy bile alcohols. However, the major peak corresponded with the retention time due to the TMSi ether of 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,25-tetrol (peak a). In addition, small amounts of 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,23R,25-pentol (peak b), 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,24S,25-pentol (peak c), and 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,24R,25-pentol (peak d) were also present. The major fragments in the mass spectra of the TMSi ether derivatives of these compounds are given in Table 3. The fragmentation pattern was completely consistent with the structures of these compounds. The detailed interpretation of the mass spectra of these compounds has been previously described by us (8).

As seen from Table 2, the plasma concentration of 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,25-tetrol was approximately twice that of the total of the three 5 $\beta$ -cholestanepentols ( $334 \pm 174$   $\mu$ g/dl 5 $\beta$ -cholestanetetrol vs.  $191.8 \pm 148$   $\mu$ g/dl 5 $\beta$ -cholestanepentols). 5 $\beta$ -Cholestan-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,25-tetrol was always the predominant plasma bile alcohol and although the ratio of 5 $\beta$ -cholestanetetrol to the 5 $\beta$ -cholestanepentols varied from patient to patient, it was always found to be greater than 1. Bile alcohols were not detected in the plasma from three control subjects when 2 ml of the plasma was analyzed in an identical way.

Figs. 1B and 1C show the GLC pattern of the TMSi ethers of bile alcohols present in the bile and urine, respectively, of patient A. 5 $\beta$ -Cholestan-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,25-tetrol was again the major biliary bile alcohol accompanied with smaller amounts of 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,23R,25-pentol and 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,24R,25-pentol. The proportions of the various bile alcohols were similar in the chro-

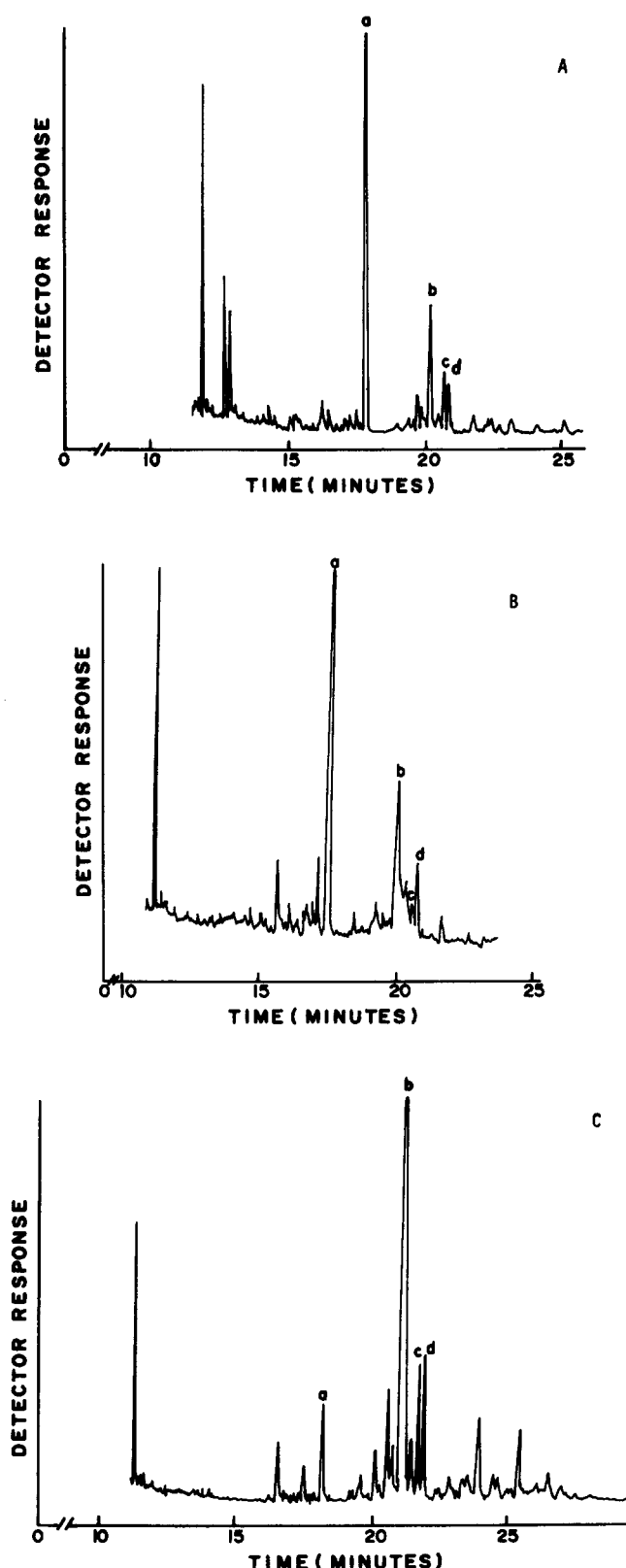


Fig. 1. GLC of the bile alcohols in the plasma, bile and urine of CTX patient A before treatment. A, Plasma; B, bile; and C, urine. For GLC operating conditions, see Table 1. Peaks a,  $5\beta$ -cholestane- $3\alpha,7\alpha,12\alpha,25$ -tetrol; b,  $5\beta$ -cholestane- $3\alpha,7\alpha,12\alpha,23\beta,25$ -pentol; c,  $5\beta$ -cholestane- $3\alpha,7\alpha,12\alpha,24S,25$ -pentol; and d,  $5\beta$ -cholestane- $3\alpha,7\alpha,12\alpha,24R,25$ -pentol.

matograms of the biliary and the plasma bile alcohols. In contrast,  $5\beta$ -cholestane- $3\alpha,7\alpha,12\alpha,25$ -tetrol was only a minor constituent. The urine also contained several more polar bile alcohols (probably  $5\beta$ -cholestane- $3\alpha,7\alpha,12\alpha,23\beta,25$ -pentol and  $5\beta$ -cholestane- $3\alpha,7\alpha,12\alpha,24S,25$ -pentol) (4). Thus, the plasma mirrored the biliary bile alcohol pattern rather than the urinary bile alcohol pattern in that  $5\beta$ -cholestane- $3\alpha,7\alpha,12\alpha,25$ -tetrol was the major bile alcohol in both plasma and bile while this bile alcohol was present only in small quantities in the urine. Also, the ratios of  $5\beta$ -cholestane- $3\alpha,7\alpha,12\alpha,25$ -tetrol in the plasma and bile were similar while the urine contains a much larger proportion of the pentahydroxy bile alcohols.

The bile alcohols isolated from the plasma were present as glucuronides with no appreciable amounts of unconjugated bile alcohols, as was demonstrated by direct TLC examination of a plasma sample after concentration of the bile alcohols and bile acids by Sep-Pak and hydrolysis of bile acid conjugates with cholesteryl glycidyl hydrolase. A comparison with the biliary bile alcohol glucuronide fraction similarly obtained showed an identical pattern between the plasma and the bile in that the  $5\beta$ -cholestane- $3\alpha,7\alpha,12\alpha,25$ -tetrol glucuronide was the major compound and the  $5\beta$ -cholestane- $3\alpha,7\alpha,12\alpha,23\beta,25$ -pentol glucuronides were comparatively less. On the other hand, the TLC of the urinary bile alcohol glucuronides showed a preponderance of  $5\beta$ -cholestane- $3\alpha,7\alpha,12\alpha,25$ -tetrol glucuronides with only a small proportion of the glucuronide of  $5\beta$ -cholestane- $3\alpha,7\alpha,12\alpha,23\beta,25$ -pentol.

The effect of chenodeoxycholic acid feeding on the plasma bile alcohol levels in six CTX subjects is also shown in Table 2. A comparative gas chromatogram of the bile alcohols in the plasma, bile and urine of CTX patient A after 1 month of 1 g/day of chenodeoxycholic acid feeding is shown in Figs. 2A, 2B, and 2C. After 1 month, plasma bile alcohol glucuronide concentrations declined markedly (Table 2). Similarly, urinary and biliary excretion of bile alcohols also diminished (Figs. 2A-2C).

## DISCUSSION

The results of the present investigation show that substantial concentrations of bile alcohol glucuronides circulate in the plasma of patients with CTX. The large quantities of bile alcohol glucuronides that result from a defect in the bile acid synthesis are not only excreted in the bile but also overflow into the plasma and are then excreted into the urine. Of importance was the demonstration that the urinary bile alcohol pattern differed markedly from the plasma and biliary bile alcohol pattern. Although the precise bile acid synthetic defect in CTX remains controversial, it is agreed that cholic acid biosynthesis in patients with this disease occurs via 25-hydroxylated intermediates (10). As a result, large amounts of  $5\beta$ -cholestane- $3\alpha,7\alpha,12\alpha,25$ -tetrol



TABLE 2. Bile alcohols in the plasma of CTX patients before and during CDCA feeding<sup>a</sup>

Patient	Bile Alcohol: 5 $\beta$ -Cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ - <sup>b</sup>			
	25-Tetrol	23 $\xi$ ,25-Pentol	24R,25-Pentol	24S,25-Pentol
A	350 (60) <sup>c</sup>	107 (2)	117 (29)	116 (16)
B	280 (28)	100 (-) <sup>d</sup>	28 (-)	50 (-)
C	160 (89)	65 (10)	16 (-)	56 (-)
D	350 (10)	40 (10)	47 (-)	47 (-)
E	210 (20)	52 (25)	28 (-)	44 (-)
F	300 (10)	25 (5)	10 (-)	10 (-)
G	250	40	35	30
H	340	80	65	60
I	770	76	217	168
Mean	334 (36) $\pm$ 176 ( $\pm$ 32)	65 (9) $\pm$ 28 ( $\pm$ 9)	63 (5) $\pm$ 66 ( $\pm$ 12)	65 (3) $\pm$ 48 ( $\pm$ 7)
	$P < 0.002^e$	$P < 0.02$	$P < 0.05$	$P < 0.01$

<sup>a</sup>Fasting plasma samples were obtained before and after 1 month of 1 g/day CDCA treatment. Plasma was analyzed by capillary GLC after hydrolysis with  $\beta$ -glucuronidase. Values are calculated as  $\mu$ g/dl plasma. For GLC operating conditions see Table 1, footnote (a).

<sup>b</sup>Small amounts of 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,23-tetrol (15 + 10  $\mu$ g/dl) were also found in the plasma of all patients.

<sup>c</sup>Values shown in parentheses are those obtained after feeding CDCA.

<sup>d</sup>Not detected.

<sup>e</sup>Significance levels calculated for first six subjects only (paired Student's *t*-test).

are produced from 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ -triol. According to Salen et al. (11), a defect in hepatic microsomal 24S-hydroxylation blocks the transformation of 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,25-tetrol into 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,24S,25-pentol and cholic acid. As a result, the 5 $\beta$ -cholestanetetrol accumulates in the liver where it undergoes glucuronidation. A portion of the newly formed 5 $\beta$ -cholestanetetrol glucuronide is secreted into the bile with the remainder

overflowing into the plasma where it reaches the kidney. In previous studies, it was demonstrated that between 300 and 1000 mg/day of bile alcohol glucuronides is excreted in the urine and only about 100 mg/day of the bile alcohols is excreted in the feces (1, 4, 5). Thus, the urine represents the major pathway for the elimination of the bile alcohol glucuronides. However, the major bile alcohol glucuronides excreted in the urine are 5 $\beta$ -cholestanepentols and probably

TABLE 3. Percent relative intensity for major fragments in the mass spectra of the trimethylsilyl ethers of bile alcohols

m		5 $\beta$ -Cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ -			
e	Fragmentation	25-Tetrol <sup>a</sup>	23 $\xi$ ,25-Pentol <sup>b</sup>	24S,25-Pentol <sup>c</sup>	24R,25-Pentol <sup>d</sup>
		% relative intensity			
724	M <sup>+</sup>	0.05			
681	M <sup>+</sup> - 131			2	2
667	M <sup>+</sup> - 143		3.6		
634	M <sup>+</sup> - 90	0.5			
591	M <sup>+</sup> - (90 + 131)			8	7.5
577	M <sup>+</sup> - (90 + 145)		0.27		
544	M <sup>+</sup> - (2 $\times$ 90)	2			
501	M <sup>+</sup> - (2 $\times$ 90 + 131)			1	1
487	M <sup>+</sup> - (2 $\times$ 90 + 145)		0.53		
454	M <sup>+</sup> - (3 $\times$ 90)	14			
411	M <sup>+</sup> - (3 $\times$ 90 + 131)			1	1
397	M <sup>+</sup> - (3 $\times$ 90 + 145)		1		
364	M <sup>+</sup> - (4 $\times$ 90)	14			
343	M <sup>+</sup> - (2 $\times$ 90 + side chain)	10			
321	M <sup>+</sup> - (4 $\times$ 90 + 131)			4.8	4.8
307	M <sup>+</sup> - (4 $\times$ 90 + 145)		3.3		
253	M <sup>+</sup> - (3 $\times$ 90 + side chain)	48			
131	(CH <sub>3</sub> ) <sub>3</sub> Si-O-C <sup>+</sup> (CH <sub>3</sub> ) <sub>2</sub>	100	100	100	100

<sup>a</sup>GLC peak 'a'.

<sup>b</sup>GLC peak 'b'.

<sup>c</sup>GLC peak 'c'.

<sup>d</sup>GLC peak 'd'.

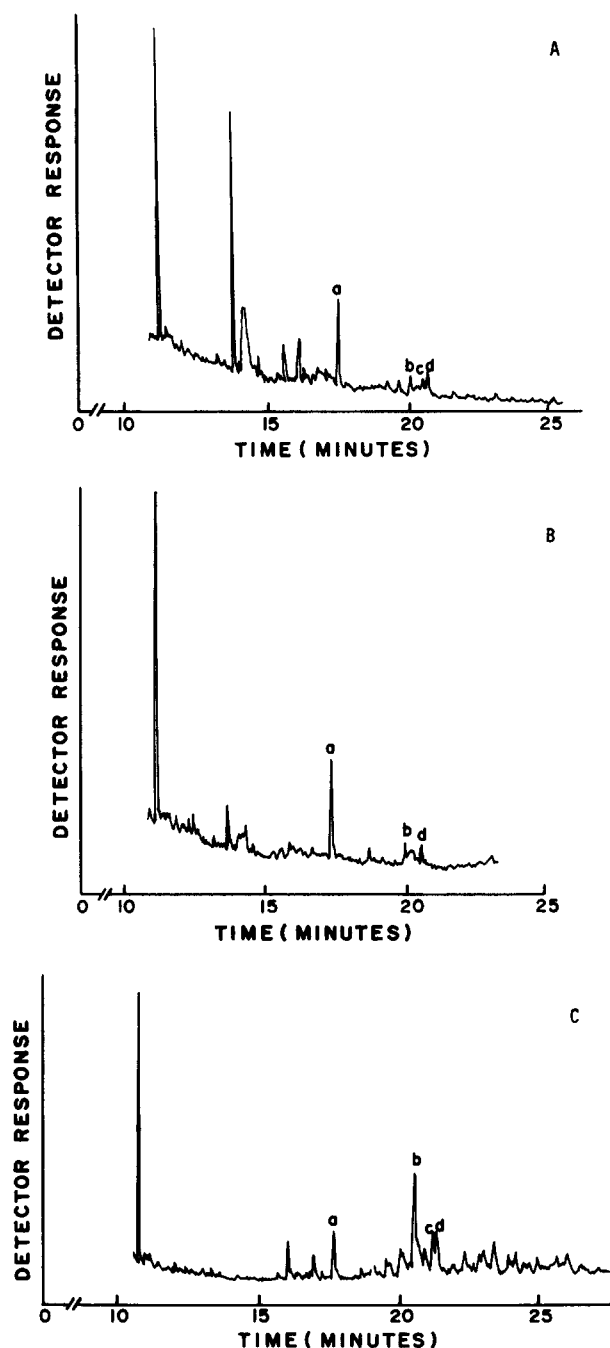


Fig. 2. GLC of the bile alcohols in the plasma, bile and urine of CTX patient A after feeding 1 g/day of chenodeoxycholic acid for 1 month. A, Plasma; B, bile; and C, urine. For GLC operating conditions, see Table 1. For identification of the GLC peaks, see legend to Fig. 1.

$5\beta$ -cholestanhexols and -heptols (4, 5). Thus, the urinary bile alcohol pattern differs qualitatively from that of the plasma and bile where  $5\beta$ -cholestane- $3\alpha,7\alpha,12\alpha,25$ -tetrol glucuronide is the major component. Two possible explanations exist. Since small amounts of  $5\beta$ -cholestanepentols are present in the bile and plasma, the urine may selectively secrete these pentahydroxy (and more polar) bile alcohol

glucuronides. Alternatively,  $5\beta$ -cholestanetetrol glucuronide, which is produced in the liver and circulates in the plasma, may be hydroxylated by renal hydroxylases and excreted in part in the urine, while a portion may be reabsorbed from the kidney and circulate in the plasma to be excreted by the liver. The important point is that a substantial amount of  $5\beta$ -cholestane- $3\alpha,7\alpha,12\alpha,25$ -tetrol glucuronide that is produced in the liver is transported in the plasma but is not found in the urine.

Existence of bile alcohol glucuronides in the plasma has important diagnostic and pathogenic implications. In some patients with CTX, plasma cholestanol levels are only modestly elevated. The demonstration of bile alcohol glucuronides in the plasma will lead to more definite diagnosis of CTX. Furthermore, in certain cases of liver disease, plasma cholestanol concentrations tend to be relatively high (12). The simultaneous determination of plasma bile alcohols helps establish the correct diagnosis. The same plasma sample can be used for the determination of the bile alcohol glucuronides that was used for sterol determination, after extraction of the sterol fraction with hexane.

Treatment with chenodeoxycholic acid reduces plasma cholestanol levels to near normal in the CTX patients (13) and virtually eliminates both biliary and urinary bile alcohols (4, 5). A similar effect on plasma bile alcohol glucuronide levels was seen after feeding chenodeoxycholic acid. As shown in Table 2, plasma bile alcohol glucuronide concentrations were markedly suppressed in just 1 month of chenodeoxycholic acid therapy (526  $\mu\text{g}/\text{dl}$  plasma to 52  $\mu\text{g}/\text{dl}$  plasma, determined as the free bile alcohols). Figs. 2A–2C show gas chromatograms of the plasma, biliary, and urinary bile alcohols in CTX patient A after 1 month of chenodeoxycholic acid therapy. A comparison of Figs. 1 and 2 clearly shows the marked reduction in the plasma and biliary as well as urinary bile alcohol glucuronides in the patient after CDCA treatment. Thus, determination of plasma bile alcohol glucuronides gives an additional parameter for the evaluation of the effect of chenodeoxycholic acid therapy.

The presence of bile alcohols in the plasma may also have a pathological significance. Normally the blood-brain barrier is highly impermeable to plasma lipoproteins (14, 15). It is possible that the large plasma bile alcohol glucuronides pool plays a role in the abnormal blood-brain barrier permeability in these patients, since we have recently found increased amounts of albumin and apolipoprotein B in the cerebrospinal fluid of eight untreated CTX patients (Batta, A. K., et al., unpublished observations). This may lead to the increased transport of cholestanol and cholesterol in the brain, a situation that may be corrected by feeding chenodeoxycholic acid since the plasma levels of bile alcohol glucuronides are greatly reduced with this treatment.

In summary, we have demonstrated the presence of bile alcohol glucuronides in the plasma of CTX patients. The major plasma bile alcohol glucuronide is  $5\beta$ -cholestan-

3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,25-tetrol and the pattern is very different from that in the urine where 5 $\beta$ -cholestane-pentols predominate, but closely resembles the biliary pattern. We hypothesize that renal hydroxylation of 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,25-tetrol to the 5 $\beta$ -cholestane-pentols, -hexols and -heptols is responsible for this difference. The presence of plasma bile alcohol glucuronides may be useful not only in establishing the diagnosis of CTX but may also play a role in affecting blood-brain barrier permeability. Treatment with chenodeoxycholic acid, which suppresses abnormal bile acid formation in CTX, reduces plasma bile alcohol glucuronide concentrations. ■

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