Increased urinary excretion of bile alcohol glucuronides in patients with primary biliary cirrhosis[1]

S. Weydert-Huijghebaert, G. Karlaganis,[2] E. L. Renner, and R. Preisig

Department of Clinical Pharmacology, University of Berne, CH-3010 Berne, Switzerland

Abstract The bile alcohol glucuronides in urine of 12 patients with primary biliary cirrhosis (PBC), 10 patients with chronic active hepatitis (CAH), and 6 healthy volunteers were analyzed by capillary gas-liquid chromatography-mass spectrometry. In all subjects studied, the major urinary bile alcohol was found to be 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol (C_{26} pentol). In PBC patients, the excretion of C_{26} pentol (main isomer) was significantly increased above values observed in healthy volunteers (mean ± SD = 5.2 ± 3.5 μmol/24 h, range 1.0–13.4; versus 0.6 ± 0.3, range 0.4–1.0). In addition, PBC patients excreted increased amounts of other bile alcohols such as isomers of C_{26} pentol, pentahydroxylated C_{27} bile alcohols (5β-cholestane-3α,7α,12α,24,25-pentol) and 5β-cholestane-3α,7α,12α, 25, 26-pentol) and a hexahydroxylated C_{26} bile alcohol (27-nor-5β-cholestane-3α,7α,12α,24,25,26-hexol). In CAH patients, the excretion of the C_{26} pentol main isomer ranged from 0.3 to 2.0 μmol/ 24 h (mean ± SD = 0.7 ± 0.5) and did not significantly differ from that in healthy volunteers. Moreover, the bile alcohol profile was comparable to those found in healthy volunteers and PBC patients. These findings show that total urinary bile alcohol glucuronide excretion is significantly increased in primary biliary cirrhosis. A PBC-specific urinary bile alcohol profile, however, does not exist. —**Weydert-Huijghebaert, S., G. Karlaganis, E. L. Renner, and R. Preisig.** Increased urinary excretion of bile alcohol glucuronides in patients with primary biliary cirrhosis. *J. Lipid Res.* 1989. **30:** 1673–1679.

Supplementary key words sterols • primary biliary cirrhosis • capillary gas-liquid chromatography-mass spectrometry

Bile alcohols are known to be produced as intermediary products in the metabolism of cholesterol to bile acids (1, 2). It was demonstrated in children that bile alcohols may also be excreted as glucuronide conjugates in urine; a glucuronide of the major bile alcohol 27-nor-5β-cholestane-3α,7α,12α, 24,25-pentol was excreted by healthy children and in increased amounts by children with α_1-antitrypsin deficiency and subsequent cirrhosis (3, 4). This bile alcohol glucuronide was also found in urine of a child with neonatal cholestasis and cirrhosis (5) and an adult patient with cirrhosis and Wilson's disease (6). In addition, Kuwabara and coworkers identified it in urine from healthy humans (7) and Kuroki et al. identified it in bile from patients with cholelithiasis (8). Only short chain bile alcohols (26,27-nor-cholestanols) have been described by Kibe et al. (9) in the bile of a patient with extrahepatic cholestasis due to gallstone obstruction in the common bile duct.

Patients with cerebrotendinous xanthomatosis, a rare inherited lipid storage disease with impaired bile acid metabolism, excrete large amounts of bile alcohols in bile and feces due to inborn defects in the activities of 26-hydroxylase (10–15) and 24S-hydroxylase (16). The side chain hydroxylations of cholestanols are carried out by microsomal or mitochondrial liver enzymes (12, 17–19). Bile alcohols are polyhydroxylated compounds with hydroxyl groups at different positions in the side chain. Urinary bile alcohol profiles might, therefore, reflect not only bile alcohol excretion but rather hepatic hydroxylase activities.

PBC is a liver disease predominantly of middle-aged females, characterized by severe intrahepatic cholestasis and elevated plasma and urinary levels of bile acids (20–22). To examine whether the urinary excretion of bile alcohol glucuronides is altered also in adult cholestasis, we performed both qualitative and quantitative studies in patients with PBC. To investigate to what extent the bile alcohol profile is specific for PBC, urinary bile alcohol glucuronide excretion was also determined in 10 patients with chronic active hepatitis (CAH) and in 6 healthy volunteers.

Abbreviations: GLC-MS, gas-liquid chromatography-mass spectrometry; CAH, chronic active hepatitis; PBC, primary biliary cirrhosis; n.s., not significant.

[1]Presented in part at the VIII International Bile Acid Meeting held in Berne, Switzerland, 1984.

[2]To whom correspondence should be addressed.

MATERIALS AND METHODS

Subjects

Twelve female patients with PBC were studied (**Table 1**). The diagnosis was based on typical clinical and laboratory findings, including positive antimitochondrial antibodies with titers over 1:100, elevated IgM levels in plasma over 3.8 g/l, and compatible histology. Seven of the PBC patients were in stage IV of their disease exhibiting cirrhosis: five were in stages II or III (without cirrhosis). Ten female patients with biopsy-proven CAH were also included in the study, five with and five without cirrhosis (Table 1). To assess functional impairment of the liver, galactose elimination capacity was measured (23) and/or aminopyrine breath test was performed (24, 25). Six healthy age-matched female volunteers served as controls (Table 1).

Collection of samples

Urine samples (24 h) were collected and kept refrigerated at −20°C until analysis. After an overnight fast, 10 ml of blood was taken (in the morning of the same day of urine collection) for determination of conjugated serum bile acids (radioimmunoassay) and creatinine.

Analysis of bile alcohol glucuronides

Fifty ml of filtered urine was submitted to analysis of bile alcohol glucuronides, as previously described by Karlaganis et al. (4). Briefly, [^{14}C]testosterone glucuronide (kind gift from Prof. S. Matern, Aachen, FRG) was added for estimation of recoveries. Bile alcohol conjugates were extracted using Sep-Pak C_{18} cartridges. The extract was filtered over a column of SP-Sephadex C25 in the H^+ form. The glucuronide fraction was isolated by ion-exchange chromatography on Lipidex-DEAP. Bile alcohol glucuronides were hydrolyzed at 37°C for 20 h with β-glucuronidase from *Helix pomatia* intestinal juice (purified over Amberlist XAD-2 in 0.5 M sodium acetate buffer, pH 4.5). Hydrolyzed steroids were extracted with Sep-Pak C_{18} cartridges and further purified by filtration through Lipidex-DEAP. Twenty-five nmol of coprostanol was added as internal standard for analysis by computerized combined capillary gas–liquid chromatography–mass spectrometry (GLC-MS). Bile alcohols were converted to trimethylsilyl ethers and dissolved in toluene–hexane 1:1 (v/v).

GLC–MS was carried our using a Finnigan 1020 instrument equipped with a (self-made) 15 m × 0.3 mm i.d. persilanized glass capillary column (26) coated with OV-73 (0.2% static coating). The temperature of injector and GLC–MS interface was 250°C; splitless injection was performed. Column temperature was increased from 110 to 260°C by 20°C/min. Helium was used as carrier gas. The energy of bombarding electrons was 22 eV. Repetitive magnetic scanning was carried out over the mass range of 34–800 atomic mass units within 3 sec. The structures of the measured bile alcohols are shown in **Fig. 1**. Peak areas of mass chromatograms were quantified by the data system of the GLC–MS system for *m/z* 321 (C_{26} pentol 2nd isomer, C_{26} pentol 3rd isomer, C_{26} hexol), *m/z* 131 (C_{27}-24,25-pentol, *m/z* 349 (C_{27}-25,26-pentol), and *m/z* 370 (internal standard coprostanol), and ratios were calculated. Cholic acid methyl ester trimethylsilyl ether (*m/z* 253) was used as external standard for calibration. Percentage ionization of the mass fragments given above compared to total ionization was taken from the corresponding mass spectra, and the results were corrected for this difference of percentage ionization between the measured bile acids and the external standard (27), as well as for losses during sample preparation from the recovery of liberated [^{14}C]testosterone. Recoveries and quantitations are tentative, since there were no bile alcohols as reference compounds.

Statistics

One-way analysis of variance was performed for comparison of groups; differences between group means were shown with the Student's *t*-test. Correlations were investigated using Pearson correlation coefficients. $P < 0.05$ was considered as statistically significant.

RESULTS

Bile alcohol profiles

Fig. 2 shows a representative example of a bile alcohol profile from the glucuronide fraction of urine of a patient with PBC as determined by capillary GLC–MS. Peaks of interest were identified by comparison with mass spectra of reference bile alcohols (both C_{27} pentols; kind gift from Prof. J. Sjövall, Stockholm) and/or by comparison with published spectra (5, 28). The profiles contained 15 to 20 peaks between 3430 and 3800 retention index. (Kovats index; methylene units × 100).

The compounds that were quantified (Table 1) had the same ring structure with hydroxyl groups in positions 3α,7α,12α, as in cholic acid. The side chain contained seven or eight carbon atoms, resulting in C_{26} (= 27-nor) compounds or C_{27} compounds, respectively. In all subjects studied, the major bile alcohol was shown to be 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol (C_{26} pentol, 3rd isomer), which appeared approximately at retention index 3485. Two other isomers of this C_{26} pentol were identified, appearing at retention indices 3436 and 3460, respectively. The 4th isomer of C_{26} pentol was mostly present only as a small shoulder of the major C_{26} pentol peak. Other compounds in the profile of patients with PBC were pentahydroxylated C_{27} bile alcohols with two hydroxyl groups in the side chain at C-24,25 (C_{27}-24,25-pentol) or C-25,26 (C_{27}-25,26-pentol) and a hexahydroxylated C_{26} bile alcohol, named C_{26} hexol,

TABLE 1. Urinary bile alcohol excretion in patients with primary biliary cirrhosis, patients with chronic active hepatitis, and healthy volunteers[a]

Subject	Age	Body Weight	Creatinin Clearance	Conjugated Bile Acids	Urinary Excretion in 24 h of: Unconjugated C_{26} Pentol 3rd Isomer	Glucuronide C_{26} Pentol 2nd Isomer	Glucuronide C_{26}Pentol 3rd Isomer	Glucuronide C_{27} 24,25-Pentol	Glucuronide C_{27} 25,26-Pentol	Glucuronide C_{26} Hexol	Sum of Five Major Glucuronidated Bile Alcohols	Galactose Elimination Capacity	Aminopyrine Breath Test
	yr	*kg*	*ml/min/1.73 m²*	*μmol/l*	*μmol*	*μmol*	*μmol*	*μmol*	*μmol*	*μmol*	*μmol*	*mg/min/kg*	*% dose × kg/mmol CO_2*
Normal values			75–127	6								6.0–9.1	0.60–1.00
Primary biliary cirrhosis (with cirrhosis; n = 7)													
A	43	65	72	172.0	0.03	1.60	13.43	1.91	7.42	2.55	26.91	5.1	0.29
B	45	50	91	53.3	0.15	1.16	8.12	1.46	4.40	1.71	16.85	5.8	0.82
C	59	70	59	41.0	0.11	0.71	6.60	0.85	1.16	1.68	11.00	7.3	0.83
D	59	48	66	26.0	0.12	1.16	6.05	0.65	1.61	1.33	10.80	5.7	0.30
E	57	49	61	18.0	n.d.	0.51	4.88	0.76	1.30	0.92	8.37	4.0	0.36
F	54	76	45	2.0	0.08	0.18	2.18	0.31	0.53	0.99	4.19	5.9	0.53
G	57	83	51	45.0	0.07	0.20	1.44	0.17	0.44	0.27	2.52	3.8	0.08
Mean	53	63	64	51.0[b]	0.09	0.79[c,d]	6.10[c,e]	0.87[b,d]	2.41[b,d]	1.35[c,e]	11.52[c,d]	5.4	0.46
SD	7	14	15	56.1	0.04	0.54	4.01	0.62	2.58	0.73	8.27	1.2	0.28
Primary biliary cirrhosis (without cirrhosis; n = 5)													
H	59	74	108	40.0	0.27	0.73	6.44	1.46	2.03	1.46	12.12	5.0	0.63
I	74	60	46	5.0	0.04	0.57	6.23	0.97	1.28	0.97	10.02	7.5	0.85
J	76	54	44	9.2	0.09	0.52	4.24	0.78	1.09	0.79	7.42	5.3	0.54
K	51	66	65	6.2	0.08	0.25	2.22	0.50	0.56	0.59	4.12	4.1	0.49
L	63	58	42	6.4	0.07	0.13	0.99	0.32	0.20	0.32	1.96	5.7	0.46
Mean	65	62	61	13.4[b]	0.11	0.44[c,d]	4.02[c,e]	0.81[c,e]	1.03[b,d]	0.83[c,e]	7.13[c,e]	5.5	0.59
SD	10	8	28	15.0	0.09	0.24	2.41	0.44	0.70	0.43	4.16	1.3	0.16
Chronic active hepatitis (with cirrhosis; n = 5)													
M	72	68	51	21.0	0.12	0.32	1.98	0.20	0.53	0.30	3.33	3.9	0.32
N	59	55	102	16.0	0.20	0.24	0.87	0.14	0.32	0.19	1.76	4.0	0.18
O	54	79	78	4.1	n.d.	0.23	0.55	0.12	0.24	0.19	1.33	3.0	0.32
P	63	64		36.0	0.10	0.20	0.53	0.13	0.22	0.19	1.27	3.3	0.34
Q	53	46	69	4.0	n.d.	0.12	0.36	0.07	0.12	0.10	0.77	5.6	0.45
Mean	60	62	75	16.2		0.22[d]	0.86[e]	0.13[d]	0.29[d]	0.19[e]	1.69[d]	4.0	0.32
SD	8	13	21	13.3		0.07	0.65	0.05	0.15	0.07	0.98	1.0	0.10
Chronic active hepatitis (without cirrhosis; n = 5)													
R	33	53	109	8.0	0.09	0.30	0.91	0.20	0.24	0.10	1.75	5.4	
S	64	66		2.2	n.d.	0.18	0.73	0.11	0.20	0.17	1.39	4.1	
T	38	58	81	4.4	n.d.	0.23	0.49	0.18	0.28	0.22	1.40	4.6	0.14
U	29	46	84	1.9	0.13	0.12	0.36	0.21	0.10	0.06	0.85		
V	33	63	101	0.9	0.06	0.14	0.35	0.12	0.15	0.11	0.87	7.5	
Mean	39	57	94	3.5		0.19[d]	0.57[e]	0.16[e]	0.19[d]	0.13[e]	1.25[e]	5.4	
SD	14	8	13	2.8		0.07	0.24	0.05	0.07	0.06	0.39	1.5	
Healthy volunteers (n = 6)													
1	49	57	91	0.6	n.d.	0.10	1.02	0.32	0.31	0.42	2.17		
2	60	61	63	1.6	n.d.	0.06	0.90	0.17	0.10	0.10	1.33		
3	50	54	74	2.1	n.d.	0.09	0.53	0.28	0.15	0.13	1.18		
4	60	60	79	1.8	n.d.	0.07	0.44	0.08	0.08	0.17	0.84		
5	55	70	67	0.5	n.d.	0.09	0.40	0.06	0.10	0.12	0.77		
6	45	57	57	1.5	n.d.	0.08	0.38	0.15	0.10	0.10	0.81		
Mean	53	60	72	1.4		0.08	0.61	0.18	0.14	0.17	1.18		
SD	6	6	12	0.6		0.01	0.28	0.10	0.09	0.12	0.53		

[a]The patients are listed according to decreasing excretion of glucuronidated C_{26} pentol 3rd isomer: n.d., not detectable. Group comparisons by *Student's t-test*: [b]Patients versus healthy volunteers, $P < 0.05$. [c]Patients versus healthy volunteers, $P < 0.01$. [d]PBC versus CAH (with/without cirrhosis), $P < 0.05$. [e]PBC versus CAH (with/without cirrhosis), $P < 0.01$.

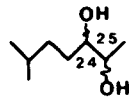
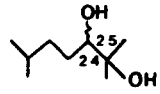
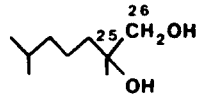
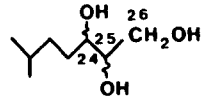
NAME	ABBREVIATION	SIDE CHAIN STRUCTURE
27-Nor-5β- cholestane-3α,7α,12α,24,25-pentol	C_{26} pentol	
5β-Cholestane-3α,7α,12α,24,25-pentol	C_{27}-24,25-pentol	
5β-Cholestane-3α,7α,12α,25,26-pentol	C_{27}-25,26-pentol	
27-Nor-5β-cholestane-3α,7α,12α,24,25,26-hexol	C_{26} hexol	

Fig. 1. Bile alcohols found in the glucuronide fraction of urine from adults, as described in Table 1. The ring structure is the same as in cholic acid.

having three hydroxyl groups in the side chain in positions 24, 25, and 26. The positions of the hydroxyl groups in the C_{26} hexol have been identified previously (5).

All compounds detected in the urine of PBC patients were also present as small peaks in the mass chromatograms of the healthy volunteers. The urinary bile alcohol profile of patients with CAH again showed a similar pattern, and the structures of the identified bile alcohols were the same as in PBC and healthy volunteers.

Quantitative analysis

The urinary excretion of the identified bile alcohols in patients and healthy volunteers is given in Table 1. The excretion of all five bile alcohol glucuronides measured was significantly increased in patients with primary biliary cirrhosis (with and without cirrhosis) compared to healthy volunteers despite comparable creatine clearances in both groups.

The mean urinary excretion of total bile alcohols was increased 10-fold in PBC with cirrhosis ($P < 0.01$) and 6-fold in PBC without cirrhosis ($P < 0.005$) compared to controls (Table 1). It was increased 7-fold in PBC with cirrhosis compared to CAH with cirrhosis ($P < 0.05$) and increased 6-fold in PBC without cirrhosis compared to CAH without cirrhosis ($P < 0.01$), respectively. The excretion of bile alcohols by CAH patients with, as well as without, cirrhosis was as low as in healthy volunteers.

The excretion of the major C_{26} pentol isomer in PBC was between 48 and 62% of the total bile alcohol profile. The second major bile alcohol was C_{27}-25,26-pentol (10–28%), but its excretion varied considerably. In healthy volunteers and patients with lower bile alcohol excretion rates, its excretion was comparable to that of C_{26} hexol. The C_{26} pentol 1st isomer was present only in trace amounts and is therefore not given in Table 1. The C_{26} pentol 2nd isomer, however, constituted approximately one-tenth of the major C_{26} pentol isomer.

In PBC, 98% of the C_{26} pentol was excreted as glucuronide, and only 2% in its unconjugated form (Table 1). In CAH, six of ten patients excreted trace amounts of unconjugated C_{26} pentol, and in healthy volunteers none could be detected in the unconjugated fraction.

Regression analysis

Pearson's correlation coefficients were calculated in patients with PBC between the 24-h urinary excretion of total bile alcohols and several laboratory parameters. Significant correlations were found with serum bile acids ($P < 0.001$), serum bilirubin ($P < 0.01$), serum aspartate aminotransferase ($P < 0.001$), and alkaline phosphatase ($P < 0.05$). Moreover, significant correlations ($P < 0.05$) were obtained when the 24-h urinary excretion of each individual bile alcohol (shown in Table 1) was plotted versus the above parameters. No significant correlation was found between 24-h urinary excretion of either total or individual bile alcohols and duration of the disease, creatinine clearance, aminopyrine breath test, and galactose elimination capacity.

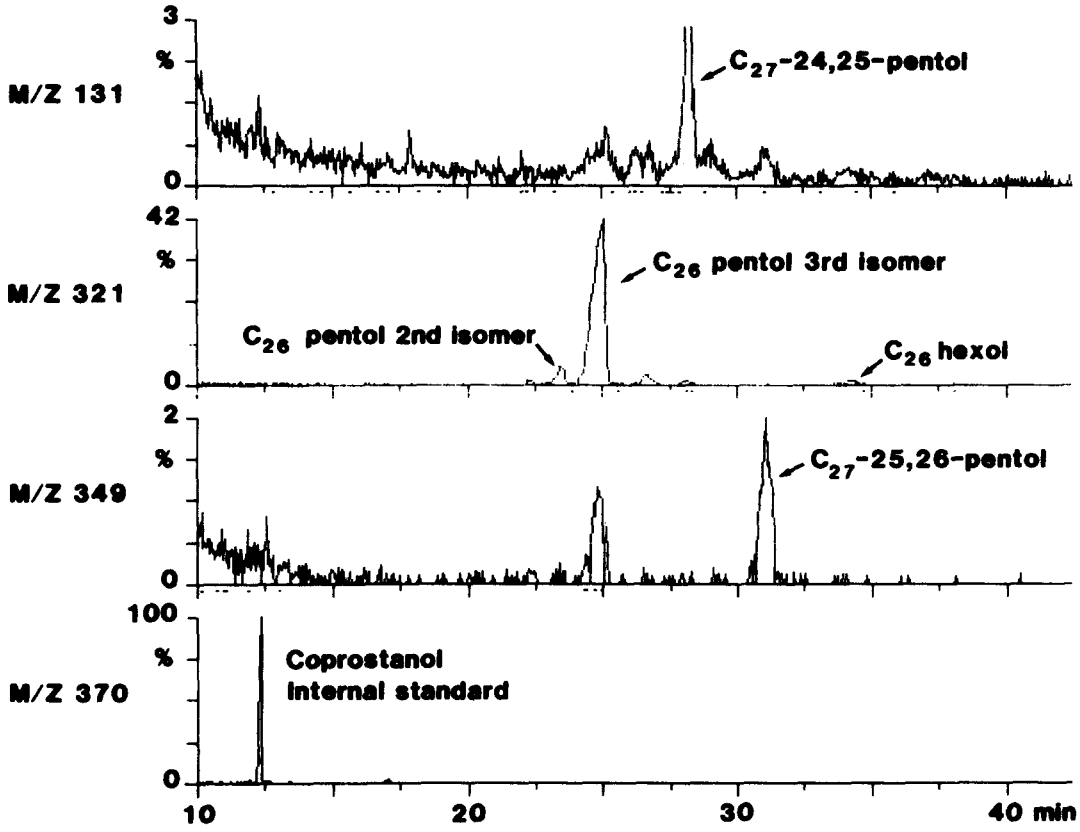


Fig. 2. Mass chromatograms of the trimethylsilyl ethers of bile alcohols from the glucuronide fraction of urine from a patient with primary biliary cirrhosis.

DISCUSSION

Children with neonatal cholestasis excrete larger amounts of bile alcohol glucuronides in urine than healthy infants (4). Our study clearly demonstrates that the urinary bile alcohol glucuronide excretion may also be increased in adult cholestatic liver disease. Thus, patients with primary biliary cirrhosis excreted significantly larger amounts of bile alcohol glucuronides in urine than patients with CAH or healthy, sex- and age-matched controls. The urinary bile alcohol profile common to PBC patients, however, was not specific for the disease, since in patients with CAH and in healthy volunteers a similar profile was observed.

The C_{27} bile alcohols in urine of patients with PBC were 5β-cholestane-3α,7α,12α,24,25-pentol and 5β-cholestane-3α,7α,12α,25,26-pentol. The mass spectra of the trimethylsilyl ethers of the 5β-cholestane-pentols were previously published by Tint et al. (28). The spectra of the C_{27} bile alcohols in PBC were in agreement with these data. The C_{27}-25,26-pentol has been described previously in urine from healthy volunteers (7) and in a cirrhotic patient (6), as well as in a cirrhotic child (5). C_{27}-24,25-pentol has been found in urine from healthy volunteers and from patients with cerebrotendinous xanthomatosis, a rare inherited lipid storage disease (15).

The five to eight peaks in the area of the ketonic bile alcohols (unidentified peaks between retention indices 3660 and 3800) contributed only 5–22% to the total urinary bile alcohol excretion in PBC and CAH patients. This contrasts with the observation of more than 50% of total urinary bile alcohols being ketonic in a child with severe cholestasis (5). Further studies are needed to decide whether this difference in urinary excretion of ketonic bile alcohols between infant and adult cholestatic liver disease reflects age-related or disease-related differences in bile alcohol metabolism.

Kibe et al. (9) identified by GLC–MS three 26,27-dinor-polyhydroxy-cholestanes in the bile of a patient with cholestasis due to gallstone obstruction. These compounds were not detected in the bile alcohol profile of our patients with PBC, nor were they detected when the early part of the profiles from retention index 3220 to 3400 was examined. These short chain bile alcohols, however, are presumably sulfated (9) and may have, therefore, been lost during the extraction procedure used in isolating the glucuronide fraction analyzed in the present study.

In patients with cerebrotendinous xanthomatosis, the large amounts of unusual bile alcohols found in feces, bile (10, 11, 14, 29) and urine (15), are due to impaired bile acid synthesis because of the lack of 26-hydroxylase (13) and a defective microsomal C-24S hydroxylase (16). The existence of similar metabolic defects in PBC in unlikely, since *1*) bile alcohol profiles were similar in PBC patients, CAH patients, and in healthy subjects, and *2*) patients with PBC and CAH, as in healthy volunteers, excreted bile alcohols hydroxylated at C-24, C-25, and C-26, in contrast to the patients with cerebrotendinous xanthomatosis who excreted large amounts of polyhydroxylated bile alcohols with hydroxyl groups at C-23, C-24 and/or C-25, but not at C-26 (15).

In PBC patients, urinary excretion of the major C_{26} pentol isomer did not correlate with the aminopyrine breath test or the galactose elimination capacity, which estimate microsomal liver function and functional liver cell mass, respectively (23–25). Urinary bile alcohol excretion correlated best with conjugated serum bile acid levels, suggesting that cholestasis itself may be the main reason for increased urinary bile alcohol excretion (30).

In urine from healthy volunteers, Kuwabara et al. (7) found 95% of C_{26} pentol in the glucuronide and 5% in the unconjugated fraction. In the present study of PBC patients, similarly, 98% of the C_{26} pentol was excreted as glucuronide, and only 2% in its unconjugated form. The absolute amounts of C_{26} pentol excreted in urine of PBC subjects were several fold increased compared to healthy controls, indicating that bile alcohol glucuronidation remains highly effective even in advanced stages of this disease. Our data, however, do not allow us to draw any conclusion about the site, i.e., hepatic versus extrahepatic (e.g., kidney), of bile alcohol glucuronidation in PBC.

C_{27}-bile alcohols in man are known to be formed from cholesterol and it was shown that cholesterol is also the precursor of the C_{26} pentol (31). The sequence of the reactions from cholesterol to these bile alcohols is not yet clear and different pathways over 5β-cholestane-3α,7α,12α,25- or -26-tetrol have been proposed (5–8). It remains to be determined, however, whether these bile alcohols and their glucuronide metabolites are end products in cholesterol metabolism that are synthesized in increased amounts in cholestatic liver disease, or whether they are intermediary products of bile acid synthesis and simply accumulate in the body in cholestasis.

This investigation was supported by the Swiss National Science Foundation and the Sandoz Stiftung. S. W-H. was the recipient of a grant from the Belgium National Fund for Scientific Research. The skillful technical assistance of Mr. T. Weber and the secretarial help of Ms. M. Dick are gratefully acknowledged. The authors would like to thank Dr. C. Minder for support during the statistical analysis of the data.

Manuscript received 24 June 1987 and in revised form 9 May 1989.

REFERENCES

1. Danielsson, H., and J. Gustafsson. 1981. Biochemistry of bile acids in health and disease. *Pathobiol. Annu.* **11:** 259–298.
2. Karlaganis, G., and J. Sjövall. 1984. Formation and metabolism of bile alcohols in man. *Hepatology.* **4:** 966–973.
3. Karlaganis, G., B. Almé, V. Karlaganis, and G. Sjövall. 1981. Bile alcohol glucuronides in urine. Identification of 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol in man. *J. Steroid Biochem.* **14:** 341–345.
4. Karlaganis, G., A. Nemeth, B. Hammarskjöld, B. Strandvik, and J. Sjövall. 1982. Urinary excretion of bile alcohols in normal children and patients with α_1-antitrypsin deficiency during development of liver disease. *Eur. J. Clin. Invest.* **12:** 399–405.
5. Karlaganis, G., V. Karlaganis, and J. Sjövall. 1984. Identification of 27-nor-5β-cholestane-3α,7α,12α,24ξ,25ξ,26-hexol and partial characterization of the bile alcohol profile in urine. *J. Lipid Res.* **25:** 693–702.
6. Ludwig-Köhn, H., H. V. Henning, A. Sciedat, D. Matthaei, G. Spiteller, J. Reiner, and H. J. Egger. 1983. The identification of urinary bile alcohols by gas chromatography–mass spectrometry in patients with liver disease and in healthy individuals. *Eur. J. Clin. Invest.* **13:** 91–98.
7. Kuwabara, M., T. Ushiroguchi, K. Kihira, T. Kuramoto, and T. Hoshita. 1984. Identification of bile alcohols in urine from healthy humans. *J. Lipid Res.* **25:** 361–368.
8. Kuroki, S., K. Shimazu, M. Kuwabara, M. Une, K. Kihira, T. Kuramoto, and T. Hoshita. 1985. Identification of bile alcohols in human bile. *J. Lipid Res.* **26:** 230–240.
9. Kibe, A., S. Nakai, T. Kuramoto, and T. Hoshita. 1980. Occurrence of bile alcohols in the bile of a patient with cholestasis. *J. Lipid Res.* **21:** 594–599.
10. Setoguchi, T., G. Salen, G. S. Tint, and E. H. Mosbach. 1974. A biochemical abnormality in cerebrotendinous xanthomatosis. Impairment of bile acid biosynthesis associated with incomplete degradation of the cholesterol side chain. *J. Clin. Invest.* **53:** 1393–1401.
11. Shefer, S., B. Dayal, G. S. Tint, G. Salen, and E. H. Mosbach. 1975. Identification of pentahydroxy bile alcohols in cerebrotendinous xanthomatosis: characterization of 5β-cholestane-3α,7α,12α,24ξ, 25-pentol and 5β-cholestane-3α,7α,12α,23ξ,25-pentol. *J. Lipid Res.* **16:** 280–286.
12. Shefer, S. F., W. Cheng, B. Dayal, S. Hauser, G. S. Tint, G. Salen, and E. H. Mosbach. 1976. A 25-hydroxylation pathway of cholic acid biosynthesis in man and rat. *J. Clin. Invest.* **57:** 897–903.
13. Oftebro, H., I. Björkhem, S. Skrede, A. Schreiner, and J. I. Pedersen. 1980. Cerebrotendinous xanthomatosis. A defect in mitochondrial 26-hydroxylation required for normal biosynthesis of cholic acid. *J. Clin. Invest.* **65:** 1418–1430.
14. Hoshita, T., M. Yasuhara, M. Une, A. Kibe, E. Itoga, S. Kito, and T. Kuramoto. 1980. Occurrence of bile alcohol glucuronides in bile of patients with cerebrotendinous xanthomatosis. *J. Lipid Res.* **21:** 1015–1021.
15. Wolthers, B. G., M. Volmer, J. Van der Molen, B. J. Koopan, A. E. J. de Jager, and R. J. Waterreus. 1983. Diagnosis of cerebrotendinous xanthomatosis (CTX) and effect of chenodeoxycholic acid therapy by analysis of urine using capillary gas chromatography. *Clin. Chim. Acta.* **131:** 53–65.
16. Dayal, B., G.S. Tint, V. Toome, A. K. Batta, S. Shefer, and G. Salen. 1985. Synthesis and structure of 26 (or 27)-nor-5β-cholestane-3α,7α,12α,24S,25ξ-pentol isolated from the urine and feces of a patient with sitosterolemia and xanthomatosis. *J. Lipid Res.* **26:** 298–305.

17. Björkhem, I., and J. Gustafsson. 1974. Mitochondrial omega-hydroxylation of cholesterol side chain. *J. Biol. Chem.* **249:** 2528–2535.
18. Gustafsson, J. 1975. Biosynthesis of cholic acid in rat liver: 24-hydroxylation of 3α,7α,12α-trihydroxy-5β-cholestanoic acid. *J. Biol. Chem.* **250:** 8243–8247.
19. Gustafsson, J., and S. Sjöstedt. 1978. On the stereospecificity of microsomal "26"-hydroxylation in bile acid biosynthesis. *J. Biol. Chem.* **253:** 199–201.
20. Almé, B., A. Bremmelgaard, J. Sjövall, and P. Thomassen. 1977. Analysis of metabolic profiles of bile acids in urine using a lipophilic anion exchanger and computerized gas–liquid chromatography–mass spectrometry. *J. Lipid Res.* **18:** 339–362.
21. Bremmelgaard, A., and J. Sjövall. 1979. Bile acid profiles in urine of patients with liver diseases. *Eur. J. Clin. Invest.* **9:** 341–348.
22. Van Berge Hengouwen, G. P. 1974. Bile acids and cholestasis. Thesis, Drukkery Eleric, Bennekom, The Netherlands.
23. Bircher, J., R. Blankart, A. Halpern, W. Häcki, J. Laissue, II and R. Preisig. 1973. Criteria for assessment of functional impairment in patients with cirrhosis of the liver. *Eur. J. Clin. Invest.* **5:** 72–85.
24. Hepner, G. W., and E. S. Vessell. 1975. Quantitative assessment of hepatic function by breath analysis after oral administration of ^{14}C-aminopyrine. *Ann. Int. Med.* **83:** 632–638.
25. Bircher, J., A. Küpfer, I. Gikalov, and R. Preisig. 1976. Aminopyrine demethylation measured by breath analysis in health and in cirrhosis of the liver. *Clin. Pharmacol. Ther.* **20:** 484–492.
26. Grob, K., G. Grob, and K. Grob, Jr. 1979. Deactivation of glass capillaries by persilylation. Part 2: Practical recommendations. *J. High Resolut. Chromatogr. Chromatogr. Commun.* **2:** 677–678.
27. Axelson, M., T. Cronholm, T. Curstedt, R. Reimendal, and J. Sjövall. 1974. Quantitative analysis of unlabelled and polydeuterated compounds by gas chromatography–mass spectrometry. *Chromatographia.* **7:** 502–509.
28. Tint, G. S., B. Dayal, A. K. Batta, S. Shefer, F. W. Cheng, G. Salen, and E. H. Mosbach. 1978. Gas–liquid chromatography–mass spectrometry of trimethylsilyl ethers of bile alcohols. *J. Lipid Res.* **19:** 956–966.
29. Yasuhara, M., T. Kuramoto, and T. Hoshita. 1978. Identification of 5β-cholestane-3α,7α,12α,23-tetrol, 5β-cholestane-3α,7α,12α,24α-tetrol, and 5β-cholestane-3α,7α,12α,24β-tetrol in cerebrotendinous xanthomatosis. *Steroids.* **31:** 333–345.
30. van Berge Henegouwen, G. P., K-H. Brandt, H. Eyssen, and G. Parmentier. 1976. Sulphated and unsulphated bile acids in serum, bile and urine of patients with cholestasis. *Gut.* **17:** 861–869.
31. Karlaganis, G., A. Bremmelgaard, V. Karlaganis, and J. Sjövall. 1983. Precursor of 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol in man. *J. Steroid Biochem.* **18:** 725–729.