EXCRETION OF 3β-HYDROXY-5-CHOLENOIC AND 3α-HYDROXY-5α-CHOLANOIC ACIDS IN URINE OF INFANTS WITH BILIARY ATRESIA

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1. Introduction

Infants with extrahepatic bile duct atresia have no enterohepatic circulation of bile acids. Conjugates of cholic (3α, 7α, 12α-trihydroxy-5β-cholanoic) and chenodeoxycholic (3α, 7α-dihydroxy-5β-cholanoic) acids are the main bile acids excreted in urine [1]. In addition, several compounds with chromatographic properties of mono- and dihydroxycholanoic acids have been detected [1]. This paper reports the identification of 3β-hydroxy-5-cholenoic and 3α-hydroxy-5α-cholanoic (allolithocholic) acids and 5-cholestene-3β, 24ξ-diol as the major components among the less polar compounds isolated by aluminum oxide chromatography. These compounds contained little or no radioactivity in samples from a patient given 4-^{14}C-cholesterol. The results indicate that 3β-hydroxy-5-cholenoic acid is formed in considerable amounts in infants with biliary atresia, and that the major part is not formed from the cholesterol pool used in the synthesis of cholic and chenodeoxycholic acids.

2. Materials and methods

Four children with extrahepatic biliary atresia were studied: A.L. (63 weeks old), A.M. (13 weeks), K.J. (8 weeks) and C.E. (15 weeks) [see 1].

Urine, 30 ml, was passed through an Amberlite XAD-2 column, which retains steroids and bile acids [1–3]. These compounds were eluted with methanol and were subjected to solvolysis, alkaline hydrolysis and methylation [1]. The resulting material was fractionated on aluminum oxide. All fractions were analysed by thin-layer chromatography and those which contained compounds with mobilities similar to that of methyl monohydroxycholanoates were analysed by gas chromatography–mass spectrometry after conversion into trimethylsilyl ethers [see 4]. A compound was considered to be identified when the thin-layer chromatographic mobilities, the retention time on SE-30, and the mass spectrum of the silyl ether were identical with those of the reference compound. When the identity and purity of the gas chromatographic peaks had been established, peak areas were measured and were compared with that given by a standard amount of the silyl ether of methyl chenodeoxycholate.

3. Results

Table 1 summarizes results of the aluminum oxide chromatography of material from patient A.L. 2. Five compounds were identified: methyl 3β-hydroxy-5-cholenoate, methyl 3α-hydroxy-5α- and 3α-hydroxy-5β-cholanoates, and 24ξ- and 26-hydroxycholesterol. Two unidentified compounds gave mass spectra indi-

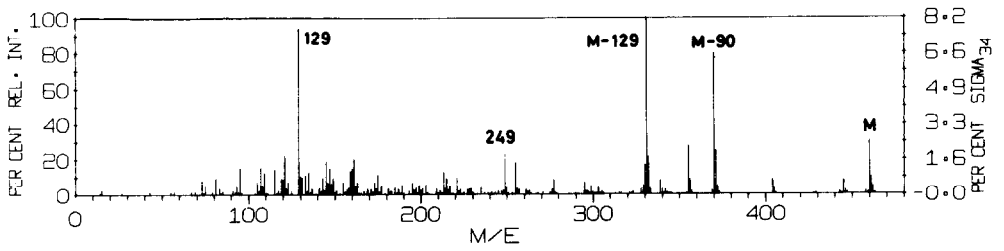

Fig. 1. Mass spectrum of the silyl ether of methyl 3β-hydroxy-5-cholenoate isolated from urine of patient A.L.

Table 1
Chromatography of solvolysed, hydrolysed and methylated bile acids and steroids in urine from patient A.L. (sample 2). Column: 20 g of aluminum oxide, grade V; 100 ml fractions were collected.

Fractions	Solvent[a]	Main compounds[b] found by gas chromatography–mass spectrometry
1–5	H/B 9:1	–
6–7	H/B 8:2	–
8–9	H/B 7:3	–
10	H/B 6:4	–
11	H/B 6:4	(B^5-3β-ol)
12	H/B 5:5	B^5-3β-ol
13	H/B 5:5	B^5-3β-ol
14	H/B 4:6	5αB-3α-ol Unknown bile acid
15	H/B 4:6	5βB-3α-ol Unknown bile acid C^5-3β, 24ξ-ol
16	H/B 3:7	C^5-3β, 24ξ-ol
17	H/B 3:7	C^5-3β, 24ξ-ol C^5-3β, 26ξ-ol

[a] H = hexane; B = benzene
[b] C = cholestane; B = methyl cholanoate; superscript indicates position of double bond, greek letters denote configuration of hydrogen or hydroxyl groups.

cative of bile acid structures. One was a trace component and none of these compounds was found in the other patients. The spectrum of the Δ^5 bile acid derivative (fig. 1) showed peaks at *m/e* 129 and M-129 typical of this structure [see 4]. The relative intensity of the molecular ion was higher and that of the ABCD-ring ion (*m/e* 257) lower in the spectrum of methyl 3α-trimethylsiloxy-5α-cholanoate than in that of the 5β epimer [see 4]. After oxidation, the mass spectra of the 3-keto-5α- and 3-keto-5β-cholanoates formed were more easily distinguished than those of the parent epimers (fig. 2). The spectra of the silyl ethers of 24- and 26-hydroxycholesterol were identical to those published previously [5].

The quantitative estimations are given in table 2. It should be emphasized that the values are minimum figures, since loss of Δ^5 steroids probably occurred during the isolation procedure. All patients excreted large amounts of 3β-hydroxy-5-cholenoic acid; in K.J. and C.E. the amounts were the same as that of chenodeoxycholic acid. Allolithocholic acid was the major saturated monohydroxycholanoic acid and lithocholic (3α-hydroxy-5β-cholanoic) acid could be identified only in one patient. 24ξ-Hydroxycholesterol was the predominant dihydroxysterol in all samples. Minor amounts of 22ξ- and 26-hydroxycholesterol were found in patients C.E. and A.L., respectively.

4-^{14}C-Cholesterol was given to patient A.L. four days before the A.L.2 sample was collected [1]. The combined fractions containing monohydroxycholanoates and dihydroxysterols contained less than 5 percent of the amount of radioactivity present in the di- and trihydroxycholanoate fractions. The same was true for a sample collected 13 days later.

4. Discussion

The occurrence of side chain hydroxylated cholesterol derivatives in urine of infants with biliary atresia is probably explained by the absence of biliary excretion. Normally, sulphates of these compounds are excreted in faeces [5]. The atresia also leads to excretion in urine of conjugated cholic and chenodeoxycholic acids which are the major bile acids in infant bile [6].

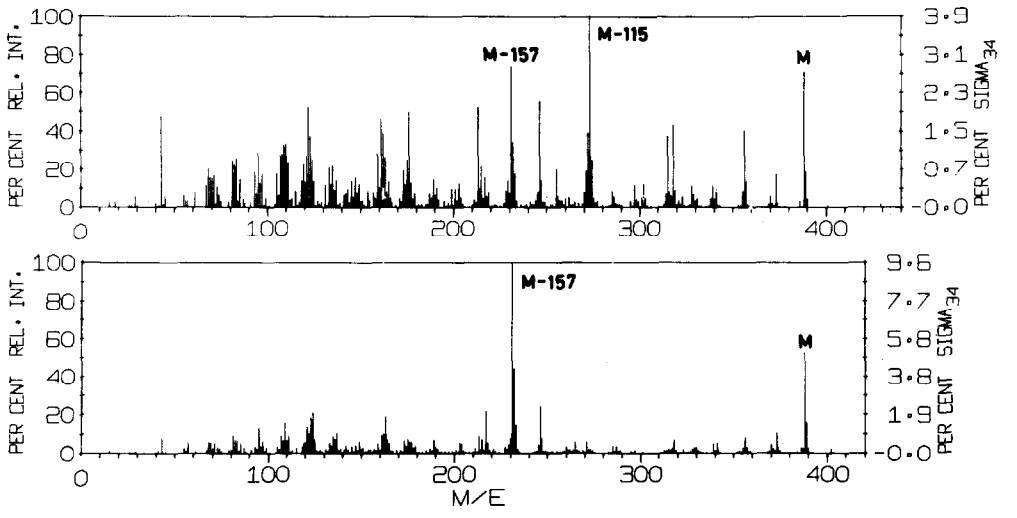

Fig. 2. Mass spectra of the methyl 3-ketocholanoates obtained after oxidation of 3α-hydroxy-5β- (upper spectrum) and 3α-hydroxy-5α- (lower spectrum) cholanoates isolated from urine of patient A.L.

Table 2

Approximate daily excretion of monohydroxylated cholesterol derivatives and monohydroxy bile acids in urine of infants with extrahepatic biliary atresia. Values for cholic and chenodeoxycholic acids are given for comparison.

Bile acid or sterol[a]	Excretion of bile acids and sterols (mg/24 hr)				
	Patient				
	A.L. 1[b]	A.L. 2[b]	A.M.	K.J.	C.E.
C^5-3β, 22ξ-ol	–	–	–	–	0.03
C^5-3β, 24ξ-ol	0.37	4.49	0.02	0.17	0.43
C^5-3β, 26ξ-ol	0.04	0.27	–	–	–
Total hydroxy-cholesterols	0.41	4.76	0.02	0.17	0.46
5βB-3α-ol	0.25	–[d]	–	–	–
5αB-3α-ol	0.23	0.63	0.11	0.14	–
B^5-3β-ol	0.63	1.95	1.00	4.39	1.55
Total monohydroxy acids	1.11	2.58	1.11	4.53	1.55
5βB-3α, 7α-ol	2.05	7.20	2.45	4.00	1.57
5βB-3α, 7α, 12α-ol	0.88	0.96	7.20	5.00	2.70

[a] For abbreviations see table 1

[b] Two separate 24 hr specimens were analysed

[c] Indicates that the compound was not found by gas chromatography–mass spectrometry

[d] Small amounts included in 5αB-3α-ol

The occurrence of 3β-hydroxy-5-cholenoic acid in the human has not previously been reported. It is therefore surprising to find large amounts of this bile acid in the urine of infants with biliary atresia. Its formation is not restricted to this disease since preliminary studies have indicated that it is also excreted in the urine of infants with other liver diseases. The formation of 3β-hydroxy-5-cholenoic acid from cholesterol in rat liver mitochondria has been demonstrated [7], and studies with bile

fistula rats have indicated that 26-hydroxycholesterol is a better precursor of this bile acid than cholesterol [8]. The low incorporation of radioactivity from 4-^{14}C-cholesterol into 3β-hydroxy-5-cholenoic acid and 24ξ-hydroxycholesterol excreted by patient A.L. suggests that cholesterol may not be the direct precursor of these two compounds. They may be formed from a cholesterol precursor or from some other sterol.

Allolithocholic acid was the major saturated monohydroxycholanoic acid. Lithocholic acid, claimed to be present in meconium [9], was found only in one patient. The precursors of these acids are unknown, but it is possible that they are metabolites of the Δ^5 bile acid. Thus, lithocholic acid can be formed from this precursor in rat liver mitochondria [7]. Preliminary studies indicate that the three monohydroxylated bile acids are excreted as solvolysable conjugates, possibly sulphates.

Acknowledgements

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