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Taurine conjugate of 3α,6β,7β-trihydroxy-5β,22-cholen-24-oic acid (tauro-Δ^{22}-β-muricholate): the major bile acid in the serum of female rats treated with α-naphthylisothiocyanate and its secretion by liver slices

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Abstract The taurine conjugate of 3α,6β,7β-trihydroxy-5β,22-cholen-24-oic acid (tauro-Δ^{22}-β-muricholate) has been identified in the serum of female rats treated with α-naphthylisothiocyanate. Using a high performance liquid chromatographic/enzymatic method for measurement of bile acids, tauro-Δ^{22}-β-muricholate was the predominant bile acid in the serum of female Fischer 344 rats treated for 3 days with α-naphthylisothiocyanate. Other significant changes in concentrations of serum bile acids included increases in tauro-α-muricholate, tauro-β-muricholate, taurocholate, taurochenodeoxycholate, and several unknown bile acids. The formation of tauro-Δ^{22}-β-muricholate was examined in vitro using liver slices from control rats and rats treated with α-naphthylisothiocyanate. Slices were incubated for 7 h in William's E medium containing no bile acids or 25 μmol/l of one of the following: β-muricholate, tauro-β-muricholate, or cholate. Tauro-Δ^{22}-β-muricholate was secreted by slices from control and treated rats and the rate was increased significantly by the addition of β-muricholate (2.9–5.6-fold) but not tauro-β-muricholate or cholate to the medium. Tauro-Δ-β-muricholate was formed by liver slices from endogenous precursors and from exogenous β-muricholate. Pretreatment of rats with α-naphthylisothiocyanate did not alter total secretion rates but those of some important individual bile acids were affected. Because of the increased secretion of tauro-Δ-β-muricholate by liver slices with the addition of β-muricholate to the medium, the liver may be the primary site of formation for this unsaturated bile acid.—**Thompson, M. B., D. G. Davis, and R. W. Morris.** Taurine conjugate of 3α,6β,7β-trihydroxy-5β,22-cholen-24-oic acid (tauro-Δ^{22}-β-muricholate): the major bile acid in the serum of female rats treated with α-naphthylisothiocyanate and its secretion by liver slices. *J. Lipid Res.* 1993. **34:** 553–561.

Supplementary key words bile acids and salts • HPLC • cholestasis • β-muricholate • Δ^{22}-β-muricholate

In a previous study (1), we detected low concentrations of an unidentified bile acid (unknown 3) in serum of control female rats. The bile acid eluted early during the analytical procedure (HPLC), soon after tauro-β-MC (Tβ-MC). It became one of the major forms in serum in female rats treated with α-naphthylisothiocyanate (ANIT), a chemical that produces bile duct necrosis and cholestasis (2–6). This bile acid has been isolated and identified by NMR spectroscopy as the taurine conjugate of 3α,6β,7β-trihydroxy-5β,22-cholen-24-oic acid (tauro-Δ^{22}-β-muricholate; T-Δ^{22}-β-MC) (7).

In recent pilot experiments in our laboratory, T-Δ^{22}-β-MC was secreted by cultured hepatocytes and slices of rat liver. Because addition of β-MC to the medium increased its formation, β-MC was considered to be a probably precursor for T-Δ^{22}-β-MC. We have conducted in vivo and in vitro studies to investigate the occurrence and formation of T-Δ^{22}-β-MC. Concentrations of individual bile acids in serum and rates of secretion by cultured slices of liver from control and ANIT-treated female rats were determined. Slices of rat liver were cultured in medium lacking exogenous bile acids and in medium to which β-MC, cholate (C), or Tβ-MC had been added. We report that T-Δ^{22}-β-MC was the predominant bile acid in the serum of female rats treated with ANIT, that it was secreted by liver slices from normal and cholestatic female rats, and

Abbreviations: T, taurine (tauro-); G, glycine (glyco-); Δ^{22}-β-MC, 3α,6β,7β-trihydroxy-5β,22-cholen-24-oic acid (Δ^{22}-β-muricholate); ω-MC, ω-muricholate; α-MC, α-muricholate; β-MC, β-muricholate; 3α,6α,7K-, 3α,6α-dihydroxy-7-oxo-5β-cholanoate; DMC, deoxymuricholate; UDC, ursodeoxycholate; HDC, hyodeoxycholate; C, cholate; THC, taurohyocholate; IS, internal standard; alloCDC, allochenodeoxycholate; CDC, chenodeoxycholate; DC, deoxycholate; LC, lithocholate; HPLC, high performance liquid chromatography; ANIT, α-naphthylisothiocyanate; CGH, cholylglycine hydrolase; 3α-HSD, 3α-hydroxysteroid dehydrogenase.

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that its secretion was increased markedly by the addition of β-MC to the medium. These studies reveal that T-Δ^{22}-β-MC can be formed in the liver from β-MC and that it need not be a product of intestinal microorganisms as previously reported (8, 9).

MATERIALS AND METHODS

Animals and treatments

In vivo studies. Five female Fischer 344 rats (Charles River Laboratories, Raleigh, NC), weighing between 181 and 201 g, were administered 20 mg/kg ANIT (Sigma Chemical Co., St. Louis, MO) in corn oil (5 mg/ml) two times a day for 3 days by gavage. Animals were provided water and feed (NIH-31, Zeigler Bros., Gardners, PA) ad libitum. On the morning of the first treatment, the rats were anesthetized with CO_2 and blood samples (~1.0 ml) were collected from the retroorbital sinus for determination of pretreatment concentrations of individual bile acids in serum. Six hours after the last treatment on the third day, rats were anesthetized with CO_2 and blood samples were collected from the right cardiac ventricle. Serum was stored at −70°C for later analysis of bile acid concentrations.

In vitro studies. Four female Fischer 344 rats weighing between 220 and 246 g were used. Two were treated with ANIT or corn oil as described for the previous experiment. Four hours after the last treatment, rats were killed with CO_2 and the livers were removed immediately and placed in cold (4°C) William's E medium (Sigma Chemical Co.). Two cores, 11 mm in diameter, were removed from the middle lobe of each liver and slices (~300 mm thick) were prepared in cold William's E medium using a Krumdieck tissue slicer (K and F Research Corp., Birmingham, AL). Three or four slices from each liver were incubated in each medium. Each slice was placed individually in a culture well (Transwell cell culture chambers, Costar, Cambridge, MA) containing 1.0 ml of medium supplemented with 20 g/l BSA, 50 μmol/l taurine, and 25 μmol/l of one the following: β-MC; Tβ-MC; C; or no bile acid. All media were preconditioned for 1 h at 37°C in an atmosphere of 5% CO_2/95% air. Slices were incubated for 1 h in the appropriate medium to remove recirculating bile acids from the tissue. The medium was removed and discarded and each slice and well was rinsed with 1.0 ml of fresh medium. Then, 1.0 ml of fresh, preconditioned medium was added and a 7-h incubation was begun. At the end of this period, medium was stored at −70°C until extraction and analysis of bile acids. Slices were sonicated for 5 sec with a Virsonic 300 cell disrupter (Virtis Co., Inc., Gardiner, NY) in 1.0 ml RPMI buffer and frozen at −70°C for later analysis of total protein content by the Coomassie blue method (Bio-Rad, Richmond, CA).

Sample preparation and HPLC analysis

Bile acids in sera and media were extracted using C_{18} cartridges (Sep-Pak, Waters Associates, Milford, MA) and concentrations of individual bile acids were determined by HPLC/enzymatic analysis with fluorescence detection as previously described (1) with several modifications (see below). Portions of pre- and post-treatment serum samples from one rat were incubated with cholylglycine hydrolase (EC 3.5.1.24, Sigma Chemical Co.) and profiles of unconjugated bile acids were determined using the same HPLC method.

The HPLC system consisted of three pumps (Model 510), an automatic sample injector (WISP 712), and three radial compression modules with C_{18} Radial-Pak cartridges (all from Waters Associates). 3α-Hydroxysteroid dehydrogenase (Sigma Chemical Co.) was immobilized to aminopropyl beads and packed in a 4 mm by 10 cm column. The mobile phase consisted of ammonium phosphate buffer (30.0 mmol/l, pH 8.35) and acetonitrile. During the run (480 min), percentages of ammonium phosphate to acetonitrile increased from 81:19 to 50:50, respectively, and the flow rate increased from 1.8 to 2.7 ml/min. The enzyme column was placed in-line after the analytical columns and after the introduction of Tris buffer (100.0 mmol/l, pH 8.5) at 2 ml/min containing dithiothreitol (6.5 mmol/l) and β-NAD^+ (1.0 mmol/l). NADH was detected fluorometrically (Model FL-750 Detector, McPherson Instrument, Acton, MA) at 365 nm for excitation and at zero order with a 420 nm cut-off filter for emission.

Bile acids in the standard and those used in the liver-slice study were from Sigma Chemical Co. (St. Louis, MO) and Steraloids (Wilton, NH). Taurine and glycine conjugates for α-MC, β-MC, deoxymuricholate (DMC, taurine only), and hyocholate (HC, taurine only) were prepared by the method of Lack et al. (10). Allochenodeoxycholate (alloCDC) was a gift from Dr. W. H. Elliott.

Statistical analysis

Analysis of variance methods were used to analyze the square root of individual and total bile acid concentrations in serum and secretion rates in media. For the analysis of secretion rates, a fixed-effects model with tissue slices nested within animals was assumed (11). Tests for three effects were computed for each bile acid: ANIT, medium, and animal:medium interaction. Dunnett's test was used to compare each medium with the control (12). Differences in serum bile acid concentrations resulting from ANIT treatment were tested with a paired *t*-test. All tests, including Dunnett's, were performed at the 0.05 level of significance using SAS® (13).

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RESULTS

In vivo: bile acids in serum

Bile acids in serum samples collected from animals prior to treatment were predominantly unconjugated. These included α-MC (2.05 μmol/l), hyodeoxycholate (HDC, 3.72 μmol/l), C (8.41 μmol/l), and CDC (4.24 μmol/l) (**Table 1**). Conjugated forms of C (glycocholate, GC, 1.46 μmol/l; TC, 1.52 μmol/l), were present in moderate amounts. Treatment of animals with ANIT increased concentrations of total bile acids greater than 7-fold (228.21 μmol/l). Of the 28 bile acids reported, 13 were significantly increased in rats treated with ANIT versus controls and 7 were significantly decreased. T-Δ^{22}-β-MC, which eluted soon after Tβ-MC, increased from 0.93 to 79.80 μmol/l and became the major bile acid in serum (35% of total). After deconjugation with CGH, Δ^{22}-β-MC was identified as the largest peak in the sample which eluted soon after β-MC (**Fig. 1**).

In treated animals, Tβ-MC and TC also were quantitatively important bile acids. Concentrations of Tβ-MC increased from 0.10 to 24.28 μmol/l (11% of total) and those of TC, from 1.52 to 49.94 μmol/l (22% of total). After deconjugation with CGH, a peak from an unknown bile acid (UK 4), which had eluted soon before TCDC in the samples from treated rats, moved to a similar position (UK 3) before CDC and corresponded to alloCDC in the standard (Fig. 1). Mean concentration of this unknown was 10.80 μmol/l (4.7% of total) in treated rats. Unknown forms appeared or increased in concentration after treatment and represented approximately 12% of the total concentration.

In vitro: bile acids secreted by liver slices

Slices of liver from rats treated with corn oil and placed in control medium (control:control) secreted bile acids at rates of 207.8 and 160.3 pmol/mg protein per h (**Table 2**). Unconjugated bile acids were either absent or present only in trace amounts in all samples whether from control or treated rats. Major bile acids were TC, Tβ-MC, taurohyodeoxycholate (THDC), and T-Δ^{22}-β-MC (**Fig. 2**). Slices from rats treated with ANIT that were placed in control medium (ANIT:control) secreted bile acids at total rates (160.7 and 214.4 pmol/mg protein per h) similar to those from control:controls. Regardless of the medium used, rates of secretion for Tα-MC, TCDC, and several unknowns were significantly increased by slices from ANIT-treated rats. The increased rate of secretion for Tβ-MC in treated rats versus controls approached significance (P = 0.056). For TC, there was significant interaction between animal treatment and medium and a trend for secretion rates to decrease in slices from ANIT-treated rats. Slices from rats treated with ANIT versus controls had lower secretion rates for TC in control, β-MC-, and Tβ-MC-containing media and higher rates in cholate-containing medium.

Addition of β-MC to the medium resulted in significant increases in secretion of Gβ-MC, G-Δ^{22}-β-MC, Tβ-MC, Tα-MC, and T-Δ^{22}-β-MC regardless of pretreatment (corn oil or ANIT). For slices from control rats, addition

TABLE 1. Concentrations of selected bile acids in serum of control and ANIT-treated rats

Group	Bile Acid					
			μmol/l			
	α-MC	β-MC	Δ^{22}-β-MC	Gα-MC	Gβ-MC	G-Δ^{22}-β-MC
Control	2.05(0.99)	0.85(0.43)	0.51(0.22)	0.56(0.28)	0.67(0.73)	0.87(0.48)
ANIT	0.88(0.28)[a]	4.98(1.90)[a]	2.43(1.91)	0.06(0.13)[a]	0.62(0.86)	3.98(4.26)
	Tα-MC	Tβ-MC	T-Δ^{22}-β-MC	UK-5	UK-6	UDC
Control	0.57(0.36)	0.10(0.19)	0.93(0.55)	0(0)	0(0)	0.33(0.17)
ANIT	7.10(1.76)[a]	24.28(10.29)[a]	79.80(51.90)[a]	3.59(1.74)[a]	3.44(2.86)[a]	0.21(0.24)
	UK-7	HDC	C	GHDC	GC	THDC
Control	0(0)	3.72(1.99)	8.41(2.81)	0.62(0.37)	1.46(0.69)	0.34(0.16)
ANIT	6.48(2.91)[a]	0.73(0.50)[a]	8.14(7.63)	0(0)[a]	1.84(0.93)	2.53(1.73)[a]
	UK-2	TC	UK-3	CDC	DC	GCDC
Control	0(0)	1.52(1.40)	0.67(0.34)	4.24(1.83)	0.29(0.13)	0.23(0.09)
ANIT	3.39(1.74)[a]	49.94(38.84)[a]	0.71(0.54)	0.61(0.44)[a]	0.02(0.05)[a]	0.10(0.14)
	UK-4	TCDC	TDC	LC		Total
Control	0.14(0.04)	0.28(0.17)	0.03(0.07)	0.09(0.01)		31.64(12.20)
ANIT	10.80(2.40)[a]	5.18(1.51)[a]	0.41(0.20)[a]	0(0)[a]		228.21(128.93)[a]

Data are mean and (SD) of concentrations of individual bile acids in serum samples from five rats bled before and on the third day of treatment with 20 mg/kg ANIT as described in Materials and Methods. Identification of bile acids was based on comparison of elution times with authentic standards.
[a]Significant effects were identified by a paired t-test at $P < 0.05$.

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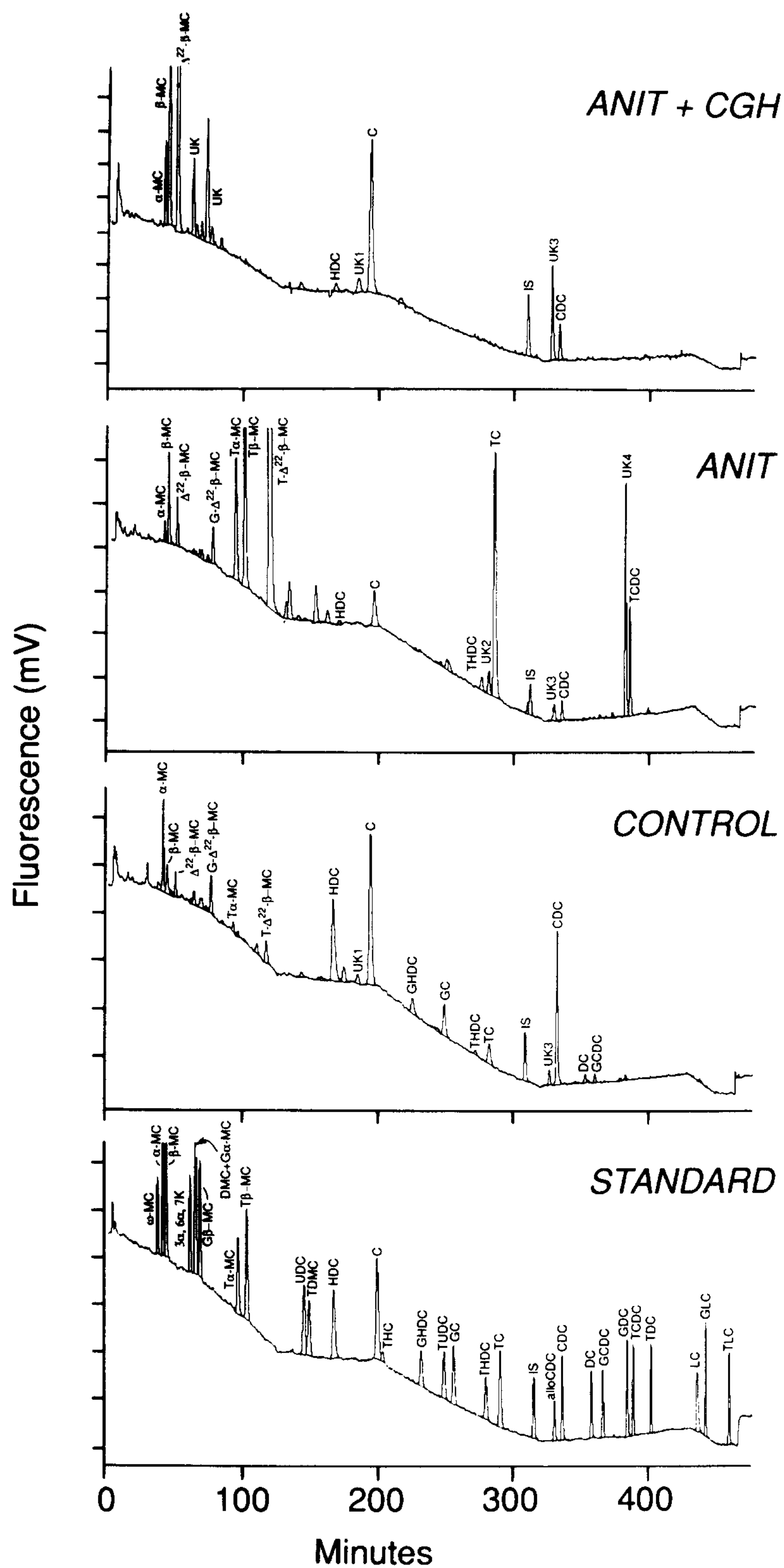

Fig. 1. High performance liquid chromatograms of individual bile acids in serum of control and ANIT-treated rats. HPLC method has reversed-phase separation, enzymatic reaction with 3α-HSD, and fluorescence detection of NADH. Amounts of bile acids in standard are 500 pmol with exceptions of all muricholates, THC, and alloCDC. Control and ANIT samples were collected from rats before and after treatment, respectively. ANIT + CGH sample was serum from an ANIT-treated rat that was incubated with CGH to deconjugate bile acids. Fluorescence is in mv with scale of 50 mv/increment. Negative baseline drift is produced by flow gradient of mobile phase.

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TABLE 2. Secretion rates of selected bile acids by liver slices from control and ANIT-treated rats

Treatment:Medium	Bile Acid											
	G-β-MC	Δ^{22}-Gβ-MC	Tα-MC	Tβ-MC	Δ^{22}-Tβ-MC	GC	THDC	UK-2	TC	UK-4	TCDC	Total
	pmol/mg protein/hr											
Control:control												
Rat #1	3.7(0.7)	13.4(1.8)	7.3(2.2)	18.7(3.9)	43.5(7.3)	24.0(5.1)	15.7(4.1)	0.0(0.0)	76.2(14.6)	0.5(0.5)	2.7(0.4)	207.8(39.5)
Rat #2	0.0(0.0)	2.6(0.7)	6.8(2.2)	27.0(4.5)	27.9(6.2)	2.7(2.5)	16.8(3.5)	0.4(0.7)	70.2(25.1)	0.4(0.6)	0.9(1.2)	160.3(50.1)
ANIT:control												
Rat #3	1.3(1.1)	1.1(1.5)	9.8(4.6) [a]	39.5(16.4)	41.3(21.3)	2.9(2.7)	2.1(1.5) [a]	3.4(1.8) [a]	39.2(4.1)	6.8(3.0) [a]	3.4(1.6) [a]	160.7(59.0)
Rat #4	1.7(0.8)	2.2(1.1)	13.5(0.8)	55.7(16.1)	46.6(14.6)	3.6(2.7)	2.7(3.8)	6.3(2.3)	52.6(6.7)	12.3(1.3)	4.1(1.3)	214.4(46.1)
Control:β-MC												
Rat #1	97.4(12.8) [b]	62.3(10.4) [b]	8.7(3.5) [b]	235.9(33.7) [b]	156.4(37.0) [b]	13.4(7.2)	12.0(3.3)	0.0(0.0)	47.0(20.2)	0.2(0.4)	1.2(1.6)	635.9(106.4)
Rat #2	86.6(22.8)	39.7(8.8)	9.0(2.9)	249.3(57.2)	132.4(27.3)	1.9(2.1)	15.3(3.3)	0.0(0.0)	57.2(13.0)	0.2(0.4)	1.1(1.3)	597.0(121.8)
ANIT:β-MC												
Rat #3	106.0(28.8) [b]	26.8(12.5) [b]	17.2(1.8) [a,b]	424.5(41.4) [b]	157.7(21.2) [b]	2.5(2.9)	3.9(0.4) [a]	3.7(1.4) [a]	42.9(5.0)	9.0(0.3) [a]	4.6(0.7) [a]	806.7(104.9)
Rat #4	112.6(12.3)	26.6(4.6)	17.2(2.6)	340.2(13.7)	117.3(6.5)	3.0(2.5)	0.3(0.7)	4.5(0.3)	33.9(5.5)	10.2(1.8)	5.2(0.8)	680.3(21.7)
Control:Tβ-MC												
Rat #1	3.8(3.9)	10.0(3.7)	5.9(1.4)	added	33.0(10.0)	16.0(7.9)	11.7(2.7)	0.0(0.0)	53.8(21.3)	0.1(0.3)	1.6(1.9)	142.7(44.8)
Rat #2	2.1(3.7)	1.3(1.5)	5.9(1.9)	added	24.2(7.0)	1.5(1.8)	14.5(4.1)	0.9(1.0)	46.9(13.2)	2.0(2.9)	1.9(2.2)	107.8(24.3)
ANIT:Tβ-MC												
Rat #3	3.0(0.6)	1.0(0.9)	12.0(2.9) [a]	added	37.2(6.3)	2.8(2.6)	2.0(0.7) [a]	3.5(0.9) [a]	36.2(8.7)	7.5(0.7) [a]	5.7(1.6) [a]	130.3(30.5)
Rat #4	3.1(1.4)	1.2(1.4)	14.0(3.1)	added	26.6(11.0)	2.1(2.6)	3.1(6.1)	7.8(3.6)	39.9(9.6)	8.4(4.3)	5.9(0.7)	136.9(48.3)
Control:C												
Rat #1	4.4(6.2)	3.2(0.4)	2.8(0.1)	12.7(5.6)	24.5(15.9)	164.3(0.1) [b]	10.1(6.8)	0.0(0.0)	349.2(19.3) [b,c]	1.5(1.3)	1.5(0.1)	598.2(62.4)
Rat #2	1.6(1.9)	1.9(1.5)	6.5(1.7)	25.7(4.0)	27.2(3.7)	119.1(17.5)	14.2(4.6)	0.0(0.0)	377.9(58.6)	0.0(0.0)	0.9(1.1)	586.9(81.1)
ANIT:C												
Rat #3	3.1(1.1)	2.3(2.0)	12.6(3.7) [a]	52.0(12.5)	62.6(19.4)	94.9(20.1) [b]	4.9(1.7) [a]	3.2(0.6) [a]	399.1(41.4) [b,c]	8.4(1.2) [a]	3.6(0.8) [a]	729.1(148.9)
Rat #4	1.0(1.3)	0.0(0.0)	9.2(2.5)	30.1(8.6)	20.3(7.1)	184.7(46.7)	2.7(3.1)	4.7(1.9)	470.4(76.4)	8.4(1.4)	4.2(0.9)	785.7(150.5)

Data are mean (SD) of rates from three to four slices/animal per medium. Rats were treated for 3 days with corn oil (Control:) or ANIT (ANIT:) as described in Materials and Methods. Medium was William's E supplemented with 20 g/l BSA and 50 μmol/l taurine (:Control); or control medium with one of the following: 25 μmol/l β-MC (:β-MC); 25 μmol/l Tβ-MC (:Tβ-MC); or 25 μmol/l C (:C).

[a] Indicates rates of bile acid secretion by slices from ANIT-treated rats that were significantly different ($P < 0.05$) from those of slices from control rats regardless of the medium.

[b] Indicates rates of bile acid secretion by slices in medium containing exogenous bile acids that were significantly different ($P < 0.05$) from rates of secretion by corresponding slices in control medium.

[c] Indicates rates of secretion for TC with significant interaction between animal treatment and medium.

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Fig. 2. High performance liquid chromatograms of individual bile acids secreted by liver slices into medium during 7-h incubation. Control:Control, Control:β-MC, ANIT:Control, and ANIT:Tβ-MC samples were bile acids secreted into medium by liver slices from control (Control:) or ANIT-treated (ANIT:) rats incubated in William's E medium containing no bile acid (:Control) or medium containing 25 μmol/l of β-MC or Tβ-MC. Fluorescence is in mv with scale of 50 mv/increment.

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of β-MC to the medium increased the formation of conjugated β-MC (glycine and taurine) and those of conjugated Δ^{22}-β-MC by 3.8-(rat #1) and 5.6-(rat #2) fold, respectively, and, for slices from ANIT-treated rats, by 4.4-(rat #3) and 2.9-(rat #4) fold, respectively. Addition of Tβ-MC to the medium, regardless of pretreatment, had no effect on basal secretion of T-Δ^{22}-β-MC. Similarly, addition of C to the medium had no effect on the secretion of T-Δ^{22}-β-MC by slices from control or ANIT-treated rats, but secretion rates of GC and TC were significantly increased.

DISCUSSION

Treatment of rats for 3 days with ANIT dramatically increased total bile acid concentrations in serum and changed the profiles from one consisting primarily of unconjugated forms (for example, α-MC, HDC, C, and CDC) to one predominated by T-Δ^{22}-β-MC, Tβ-MC, TC, and several unknown bile acids. T-Δ^{22}-β-MC became the major bile acid in serum. Unsaturated derivatives of β-MC in rats have been described in previous studies (14–20). Kern et al. (15) detected six metabolites of β-MC in the bile of rats treated with ethynylestradiol. Because an intact enterohepatic circulation was reported to be needed for the formation of these compounds (16), it has been assumed that these bile acids resulted from bacterial modification of β-MC in the intestinal tract (17).

In several studies, Eyssen and other investigators from the same laboratory (21, 22) reported finding either a derivative of β-MC with an unsaturation in the side chain or, in later studies, Δ^{22}-β-MC in the feces of normal and gnotobiotic rats inoculated with specific strains of *Eubacterium* and *Clostridium* (8, 9). An initial description of the mass spectrum of the unsaturated compound was presented (22) but we were unable to locate a reference that described the definitive identification of Δ^{22}-β-MC. Regardless, the investigators reported that Δ^{22}-β-MC was formed by the action of intestinal bacteria on β-MC. In rats that had high concentrations of Δ^{22}-β-MC (or the unknown) in the feces, a large portion of the bile acids had been deconjugated by the intestinal bacteria.

In the current in vitro study, slices from control rats in control medium secreted moderate amounts of T-Δ^{22}-β-MC and the addition of β-MC, but not Tβ-MC or C, dramatically increased the formation of glycine and taurine conjugates of this bile acid. The process of unsaturation occurred only with unconjugated and not conjugated β-MC. Consequently, conditions in vivo that favor the formation and return of β-MC to the liver (for example, deconjugation of Tβ-MC in the intestines) should enhance the hepatic synthesis of T-Δ^{22}-β-MC. Additionally, because T-Δ^{22}-β-MC was formed by slices in medium that did not contain exogenous bile acids, endogenous forms such as β-MC and possibly α-MC, CDC, and LC may be immediate (β-MC) or intermediate precursors. Although these findings do not preclude the possibility that the compound can be formed by intestinal bacteria, intestinal contribution to the pool may be small based on the large amount secreted by slices.

In addition to T-Δ^{22}-β-MC, slices of liver from control rats in control medium secreted moderate amounts of Tβ-MC, THDC, TC, and smaller amounts of other bile acids (Table 2). The mean secretion rates of total bile acids by slices from each control rat were 207.8 and 160.3 pmol/mg protein per h. A rate of 8 nmol/g of liver per h (for C + β-MC) has been reported for cultured rat hepatocytes (23). Based on a factor of 15% protein per wet weight of liver (value determined in our laboratory), this rate, which the investigators admit may be low, converts to 53.3 pmol/mg protein per h. Other published rates for bile acid synthesis are 12 pmol/mg protein per h and 170 pmol/mg protein per h for cultured hepatocytes from rats and rabbits, respectively (24). Unlike values from cultured hepatocytes, our higher values for liver slices compare favorably with measurements using cell suspensions (250 pmol/mg protein per h for cholate) (25), perfused liver (278 pmol/mg protein per h, our conversion from ref. 26), and intact liver (865 pmol/mg protein per h, our conversion from ref. 27 assuming liver weight is 3.6% of body weight in male rat). Although it is difficult to directly compare rates and results between studies, in part because of differences in analytical techniques and use of in vivo and in vitro systems, these results indicate that liver slices can provide a valuable mechanism for studying bile acid secretion and metabolism.

The status of bile acid synthesis in the cholestatic rat is unclear. Danielsson (14) noted that bile duct ligation resulted in an increase in activity of cholesterol 7α-hydroxylase that appeared to be accompanied by a decrease in bile acid synthesis. Similarly, data from a study by Boyd, Eastwood, and MacLean (28) in which excretion rates of bile acids were measured in rats with bile duct occlusion were consistent with a decrease in bile acid synthesis. Hepatocytes cultured from cholestatic rats (treated with ethynylestradiol) have impaired secretion of bile acids (29). Alternatively, other investigators have measured increases in bile acid synthesis in rats with bile duct ligation (30, 31). In the current study, slices of liver from rats treated with ANIT had rates of total bile acid secretion similar to those from rats treated with corn oil; however, those of some individual bile acids were different. Secretion rates of THDC and TC were lower in the ANIT-treated rats and those of Tα-MC, Tβ-MC, TCDC, and several unknowns were higher. Total secretion rates in ANIT-treated rats were maintained by increased secretion of specific bile acids. One possibility is that these higher rates were produced by increases in synthesis of certain bile acids via alternate pathways (16, 32–37). Additional studies are needed to explore this issue.

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The unsaturation in T-Δ^{22}-β-MC has little effect on the hydrophilic characteristics of Tβ-MC. Based on elution times using reversed phase HPLC, T-Δ^{22}-β-MC is slightly less hydrophilic than Tβ-MC and more than ursodeoxycholate. Concerning the unsaturation, we propose that it is formed through an unsuccessful attempt to cycle unconjugated β-MC through a second round of side chain oxidation in peroxisomes. As demonstrated by Tβ-MC in the slice experiments, conjugation of the side chain blocks this process. The synthesis of T-Δ^{22}-β-MC may depend directly on the availability of free β-MC that is either newly synthesized or recirculating. The possibility that other bile acids are metabolized to Δ^{22}-β-MC through or independent of β-MC has not been explored. Additionally, we do not know if the process of unsaturation itself can be increased or decreased by various treatments. Currently, increases in T-Δ^{22}-β-MC appear to have similar implications as those for increases in Tβ-MC (for example, cholestasis).

In conclusion, T-Δ^{22}-β-MC became the predominant bile acid during cholestasis produced by ANIT in female rats. It was secreted by cultured slices of rat liver and its formation was increased dramatically by the addition of β-MC to the medium. The cultured slices also were valuable in determining rates of bile acid secretion and in evaluating changes in profiles produced by previous treatment of the animals with a hepatobiliary toxin.

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