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EXCRETION OF PROGESTERONE METABOLITES IN URINE AND BILE OF PREGNANT WOMEN WITH INTRAHEPATIC CHOLESTASIS

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SUMMARY

Progesterone metabolism was studied in five pregnant women with intrahepatic cholestasis by determining several free and conjugated epimeric pregnanolones and pregnanediols and their 16α-and 21-hydroxylated derivatives in urine, bile and plasma. Comparison of the steroid profiles of these patients with those of healthy pregnant women showed the following features of intrahepatic cholestasis:

(i) Biliary excretion of progesterone metabolites is impaired.
(ii) The conjugation of progesterone metabolites changes towards greater sulphate formation.
(iii) The metabolism of the steroid moiety of progesterone seems to differ from that occurring in normal pregnancy.

These changes are in part attributable to the diminished enterohepatic circulation of steroids and in part to the increased steroid content of the liver cells.

INTRODUCTION

SEVERAL clinical and biochemical findings in intrahepatic cholestasis of pregnancy resemble those seen in steroid induced jaundice [see 1, 2, 11]. The possibility of the similar pathogenesis in these diseases has stimulated studies on changes in maternal steroid metabolism in intrahepatic cholestasis of pregnancy. Biliary excretion of oestrogen conjugates has been found to be impaired [2, 3], and changes occur in the proportions of the various oestrogen conjugates in urine [2, 4]. In pregnant women with pruritus Sjövall and Sjövall [12] found high plasma levels of sulphated progesterone metabolites. Recently, Eriksson *et al.* [22] reported studies on neutral steroid pattern in urine and faeces of women with intrahepatic cholestasis of pregnancy. These two seem to be the only studies on progesterone metabolism in intrahepatic cholestasis of pregnancy.

In previous studies we have characterized and determined several neutral steroid conjugates in urine [6–8] and bile [10] of healthy pregnant women. Here we describe studies on progesterone metabolism in pregnant women with intrahepatic cholestasis. Several unconjugated and glucuronide, mono- and disulphate conjugates of progesterone metabolites were estimated in plasma, urine and bile of these patients and the values were compared with those occurring in normal pregnancy.

EXPERIMENTAL

Five pregnant patients with pruritus which appeared at 28–37 weeks of gestation were selected for this study. All these patients had mild jaundice. The diagnosis of obstetric hepatosis was based on the criteria described in Ikonen[11]. Some laboratory data on these patients are given in Table 1. The thymol turbidity test was negative in all cases. All these subjects were primigravidae and two of them had twins. Caesarean section was done in the case No. 5 (Table 1) because of placenta praevia and in the case of No. 4 because of breech presentation of foetus A. In these two patients bile samples were drawn from the gall-bladder with a thin needle during the operation.

The methods used for analysis of steroids in urine[6–9, 15] and bile[10] have been described in detail previously. After extraction, urine samples were desalted by chromatography on Sephadex G-25[8, 9]. Fractions containing unconjugated steroids, and glucuronide and mono- and disulphate conjugates of steroids were separated by chromatography on Sephadex LH-20[8, 10, 15]. The glucuronides were hydrolysed with Ketodase® and the sulphates were solvolysed. The steroids liberated were purified and fractionated by chromatography on silicic acid and quantified by gas-liquid chromatography[8, 10]. The specificity of these quantifications was tested by gas chromatography-mass spectrometry. Steroid sulphates were determined in pregnancy plasma as described in Sjövall and Sjövall[12].

RESULTS

Table 2 shows the concentrations of sulphate-conjugated pregnanolones and pregnanediols and their 16α- and 21-hydroxylated derivatives in the plasma of the subjects studied. The total amounts of these steroids in the monosulphate fraction varied from 0·5 to 0·9 mg and in the disulphate fraction from 0·2 to 1·6 mg per 100 ml of plasma.

In four subjects urinary excretion of progesterone metabolites was determined and the results are given in Table 3. For comparison, the amounts of the same steroids excreted in the urine of four healthy pregnant women at corresponding stages of gestation are given. The total amount of urinary steroids in these patients with cholestasis ranged from 54 to 83 mg/24 h, which was of about the same magnitude as the excretion in healthy pregnant women. The figure shows that the cholestasis patients excrete about the same amounts of unconjugated steroids in urine as healthy pregnant women do. In all patients the excretion of glucuronide conjugates was below the mean value of controls. The patients excreted far more sulphated progesterone metabolites than the healthy controls. The excretion of monosulphates by the former group was 0·7–4·5 mg/24 h, by the latter group it was 0·1–0·8 mg/24 h. The corresponding values for disulphates were 12–32 mg/24 h and 1–7 mg/24 h, respectively.

The two groups also differed in the urinary excretion of the individual progesterone metabolites (Table 3). The following steroids were excreted in the urine by all the patients in larger amounts than by any of the control subjects studied: 5α-pregnane-3α,20α-diol in the glucuronide and monosulphate fractions, 5β-pregnane-3α,20α-diol, 3α, 21-dihydroxy-5α (and 5β)-pregnan-20-one, and 5α-pregnane-3α,20α,21-triol in the disulphate fraction. In the disulphate fraction the main pregnanediol isomer in urine of healthy pregnant women was 5α-pregnane-3β,20α-diol, the mean to mean ratio of 5α-pregnane-3α,20α-diol to 5α-

Table 1. Data on patients studied

Subject No.	Age (years)	Onset of pruritus (weeks)	GOT[a] U/l	GPT[b] U/l	Alkaline phosphatase B–L units[c]	Bilirubin mg/ 100 ml	Delivery[d]	Child	
								sex	weight (g)
1	19	31	100–410	155–405	1·9–9·9	1·5	34	female	1450
2	22	32	105–190	200–255	3·3–5·7	2·1	37	female	3190
3	28	28	20–110	35–150	6·5–12·9	1·2	37	A: male B: male	2690 2460
4	21	37	120–150	100	7·9	5·3	38	A: female B: female	2850 2500
5	41	28	130–408	235–870	2·6–5·2	0·9	37	male	3450

[a]Serum glutamic oxaloacetic transaminase, International Units per liter, normal values < 20 U/l.
[b]Serum glutamic pyruvic transaminase, International Units per liter, normal values < 24 U/l.
[c]Bessey–Lowry units.
[d]Weeks from the first day of the last menstruation.

Table 2. Concentrations of mono- and disulphate conjugates of C_{21} steroids in plasma of pregnant women with intrahepatic cholestasis. The values are expressed as μg of free steroid/100 ml of plasma

	Subject No.						
	1	2	2	3	4	5	5
	34*	36 w	37 w	37 w	38 w	36 w	37 w
Monosulphates:							
3α-Hydroxy-5α-pregnan-20-one	68	96	70	117	122	93	91
3β-Hydroxy-5α-pregnan-20-one	2	8	9	< 5	25	34	26
3α-Hydroxy-5β-pregnan-20-one	1	18	15	23	32	12	12
5α-Pregnane-3α,20α-diol	150	156	88	309	208	160	175
5α-Pregnane-3β,20α-diol	1	6	6	1	25	59	51
5β-Pregnane-3α,20α-diol	196	117	73	149	179	63	56
3α,16α-Dihydroxy-5α-pregnan-20-one	46	75	52	132	73	23	31
5α-Pregnane-3α,20α,21-triol	59	339	272	121	151	102	100
Disulphates:							
5α-Pregnane-3α,20α-diol	404	164	124	582	820	165	62
5α-Pregnane-3β,20α-diol	78	115	100	245	344	200	126
5β-Pregnane-3α,20α-diol	231	68	64	128	412	30	21
5α-Pregnane-3α,20α,21-triol	14	45	28	21	68	17	12

*Weeks from the first day of the last menstruation.

pregnane-3β,20α-diol was 0·45. In the urine of the hepatosis patients the amount of 5α-pregnane-3α,20α-diol exceeded that of the corresponding 3β-hydroxy-epimer, and the corresponding ratio was 1·8.

Table 4 shows the concentrations of steroids in gall-bladder bile of two of the patients (Nos. 4 and 5) with intrahepatic cholestasis. For comparison, the mean values and range of the levels of the corresponding steroids in the bile of four healthy pregnant women [10] are given. The total amounts of bile steroids in the two patients were 18 and 34 mg/100 ml, compared with 57–65 mg/100 ml in the bile of the controls. The total bile steroid glucuronides amounted to 4·6 and 4·8 mg/100 ml in these two patients, compared with 10 to 36 mg/100 ml in the healthy pregnant women. The total amounts of bile steroid monosulphates was lower in both patients than in the controls. Patient No. 4 excreted considerable amounts of steroid disulphates in bile. The biliary levels of several individual sulphated steroid metabolites were of the same magnitude in both patients as in the control subjects (Table 4).

DISCUSSION

The steroids studied here have recently been shown to be the main neutral steroids in the plasma [5, 12, 13], urine [6, 7, 8, 14] and bile [10] of healthy pregnant women. The same isomers of pregnanolone and pregnanediol [15] and 16α- and 21-hydroxylated derivatives (Jänne *et al.* to be published) were found in plasma, urine and bile after intramuscular administration of progesterone to non-pregnant subjects. Hence, the steroid metabolites studied here are able to provide us with rather detailed information about the metabolism of the steroid moeity of progesterone and the conjugation of its metabolites. The values found in this study for urinary excretion of steroid conjugates agree with those reported

Table 3. Excretion of progesterone metabolites in urine of patients with intrahepatic cholestasis of pregnancy and of four healthy pregnant women. The values are expressed as mg of free steroid/24 h and are uncorrected for losses during the method

	Subject No.						Control group	
	1 36 w	2 36 w	3 37 w	5 36 w	5 37 w	mean*	mean	(range)
Unconjugated steroids								
3α-Hydroxy-5β-pregnan-20-one	0·2	0·3	0·1	0·1	0·2	0·2	0·05	(0·03–0·1)
5β-Pregnane-3α,20α-diol	1·7	1·4	0·2	1·1	1·0	1·1	0·6	(0·3 –1·0)
Total	1·9	1·7	0·3	1·2	1·2	1·3	0·6	(0·3 –1·0)
Glucuronides								
3α-Hydroxy-5α-pregnan-20-one	1·0	2·8	3·3	5·0	9·5	4·1	2·3	(1·4 –3·0)
3β-Hydroxy-5α-pregnan-20-one	2·7	1·4	0·7	2·5	2·6	1·8	2·3	(0·9 –3·2)
3α-Hydroxy-5β-pregnan-20-one	3·4	10·4	6·7	4·9	10·0	7·6	5·4	(4·5 –6·4)
5α-Pregnane-3α,20α-diol	1·4	4·0	4·1	4·9	4·0	3·4	0·2	(0·2 –0·3)
5α-Pregnane-3β,20α-diol	0·2	0·2	0·3	0·2	0·1	0·2	0·5	(0·3 –0·6)
5β-Pregnane-3α,20α-diol	28·2	32·4	36·5	14·0	8·0	26	49	(40–64)
Total	36·9	53·2	51·6	31·5	34·2	43·1	59·4	(53–73)
Monosulphates								
5α-Pregnane-3α,20α-diol	1·6	0·1	1·1	1·6	0·7	0·9	0·04	(0·02–0·08)
5α-Pregnane-3β,20α-diol	0·1	0·06	0·2	1·0	0·2	0·1	0·09	(0·05–0·15)
5β-Pregnane-3α,20α-diol	0·3	0·2	1·8	0·3	0·1	0·6	0·2	(0·02–0·5)
5α-Pregnane-3α,20α,21-triol	0·3	0·3	1·4	0·4	0·7	0·7	0·03	
Total	2·3	0·7	4·5	3·3	1·7	2·3	0·36	(0·1 –0·8)
Disulphates								
5α-Pregnane-3α,20α-diol	12·1	2·2	8·0	5·1	4·6	6·7	0·96	(0·2 –2·4)
5α-Pregnane-3β,20α-diol	5·8	1·9	6·5	1·1	0·6	3·7	2·15	(0·7 –3·9)
5β-Pregnane-3α,20α-diol	10·6	1·2	5·0	7·1	5·7	5·6	0·2	(0·04–0·8)
3α,21-Dihydroxy-5α (and β)-pregnan-20-one	0·6	0·6	2·3	1·2	0·7	1·0	0·1	(< 0·02–0·2)
3β,21-Dihydroxy-5α-pregnan-20-one	0·2	0·1	0·4	0·3	0·4	0·3	0·3	(0·02–0·4)
5α-Pregnane-3α,20α,21-triol	2·5	5·4	3·9	5·0	4·3	4·0	0·2	(< 0·02–0·4)
5α-Pregnane-3β,20α,21-triol	0·1	0·2	0·1	0·6	0·7	0·3	0·1	
Total	31·9	11·6	26·3	20·4	17·0	21·6	4·0	(1·0 –7·0)

*Values at 37 w of the case No. 5 were included.

by Eriksson *et al.*[14], whereas smaller amounts of unconjugated steroids were found in the urine of both groups in this study.

In bile samples obtained from two subjects with intrahepatic cholestasis the total amount of progesterone metabolites was considerably lower than in bile of healthy pregnant subjects. This indicates that biliary excretion of progesterone metabolites was impaired and at least partly explains the elevated plasma levels of several sulphated progesterone metabolites in such patients[12, Table 2]. After completion of this work, Eriksson *et al.*[22] reported studies on the neutral steroid excretion in urine and faeces of two pregnant subjects with intrahepatic cholestasis. Low faecal steroid excretion in these patients was found by these authors.

Table 4. Concentrations of progesterone metabolites in gall-bladder bile of two patients with intrahepatic cholestasis and healthy pregnant subjects. The values are given as mg of free steroid/100 ml of bile

	Subject No.		Control group*	
	4	5	mean	(range)
Glucuronides				
3α-Hydroxy-5β-pregnan-20-one	1·8	1·6	7·7	(4·6–9·8)
5α-Pregnane-3α,20α-diol	0·03	0·2	0·7	(0·2–1·3)
5β-Pregnane-3α,20α-diol	3·0	2·8	17·2	(5·0–25·8)
Total	4·8	4·6	23·0	(10–36)
Monosulphates				
3α-Hydroxy-5α-pregnan-20-one	1·1	0·9	3·6	(0·9–9·9)
3β-Hydroxy-5α-pregnan-20-one	0·1	0·2	0·7	(0·2–2·0)
3α-Hydroxy-5β-pregnan-20-one	0·4	0·2	1·0	(0·4–2·6)
5α-Pregnane-3α,20α-diol	3·9	2·7	7·0	(3·6–13·6)
5α-Pregnane-3β,20α-diol	0·1	0·1	0·3	(0·2–0·4)
5β-Pregnane-3α,20α-diol	1·6	1·6	2·2	(0·9–2·7)
3α,16α-Dihydroxy-5α-pregnan-20-one	0·2	0·3	0·5	(0·2–0·9)
5α-Pregnane-3α,16α,20α-triol	0·5	0·2	2·5	(1·0–5·8)
3α,21-Dihydroxy-5α(and 5β)-pregnan-20-one	0·07	0·04	0·2	(0·2–0·2)
5α-Pregnane-3α,20α,21-triol	0·6	0·2	1·0	(0·3–1·8)
Total	8·4	6·4	19	(10–39)
Disulphates				
5α-Pregnane-3α,20α-diol	10·1	3·4	9·1	(4·8–14·9)
5α-Pregnane-3β,20α-diol	1·1	1·0	3·0	(1·4–4·0)
5β-Pregnane-3α,20α-diol	6·4	0·8	4·1	(1·9–7·4)
3α,21-Dihydroxy-5α(and 5β)-pregnan-20-one	2·1	0·5	0·9	(0·2–1·5)
5α-Pregnane-3α,20α,21-triol	1·1	0·9	1·4	(0·9– 2·2)
Total	20·8	6·6	18·5	(13–27)

*From Laatikainen *et al.* [10].

They concluded that there is a specific defect in the biliary excretion of steroid sulphates in this condition. In our study, excretion of glucuronide conjugates in bile and urine of patients with obstetric hepatosis was found to be decreased, whereas urinary excretion of disulphate conjugates was considerably increased (Fig. 1). This indicates that conjugation of progesterone metabolites with sulphate is enhanced in obstetric hepatosis. It is possible that, owing to impaired biliary excretion of progesterone metabolites, the amount of progesterone in the liver cell increases. This increased load of progesterone might favour sulphate conjugation, because administration of increasing amounts of progesterone to non-pregnant subjects was found to lead to an increase in the proportion of sulphate conjugates [15, 17]. The high levels of sulphated progesterone metabolites in blood plasma of patients with cholestasis [12, Table 2] might in part be due to increased formation of sulphate conjugates in the liver and not merely to decreased biliary excretion of these conjugates.

There were differences in the excretion of individual steroid metabolites between the patients with obstetric hepatosis and the control subjects. The ratio of 5α-pregnane-3α,20α-diol to 5α-pregnane-3β,20α-diol was increased in urine and plasma [12, Table 2] of cholestasis patients as compared with their healthy controls. This difference is probably due to the diminished biliary excretion and enterohepatic circulation of steroids in patients with intrahepatic cholestasis,

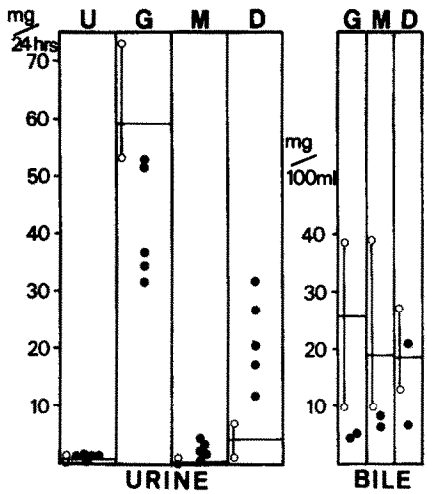

Fig. 1. Total amounts of progesterone metabolites in fractions of unconjugated (U) steroids, steroid glucuronides (G), and mono- (M) and disulphates (D) from urine (mg/24 h) and bile (mg/100 ml) of hepatosis patients. Transverse lines represent mean values and the bars the ranges obtained in four healthy controls.

because there is evidence that 5α-pregnane-3β,20α-diol is at least partly a metabolite formed from biliary 5α-pregnane-3α,20α-diol under the influence of the intestinal microflora [16, 18]. Large amounts of 5α-pregnane-3α,20α,21-triol were excreted in the urine of the patients, but the biliary levels of this compound in the two patients studied were of the same magnitude as in the controls (Table 4). This steroid is present in considerably elevated amounts in blood plasma of hepatosis patients [12, Table 2]. Therefore, it is probable that in patients with hepatosis the formation of this metabolite is increased.

It seems likely that these changes in the conjugation and metabolism of progesterone are not of any aetiological significance, but are secondary to the cholestatic condition. However, the altered profile of progesterone metabolites in maternal plasma in this condition may have secondary effects on progesterone metabolism in the uterine muscle and in the foeto-placental unit. This change in steroid metabolism may contribute to the increased rate of premature deliveries reported in obstetric hepatosis [19–21].

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