Effect of Maternal Intrahepatic Cholestasis on Fetal Steroid Metabolism

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Abstract

Estriol, estriol sulfate, progesterone, and 17 neutral steroid sulfates, including estriol precursors and progesterone metabolites, were determined in 27 cord plasma samples collected after pregnancies complicated by intrahepatic cholestasis of the mother. The levels of these steroids were compared with those in the cord plasma of 42 healthy controls.

In the cord plasma, the steroid profile after pregnancies complicated by maternal intrahepatic cholestasis differed greatly from that seen after uncomplicated pregnancy. Two main differences were found. In the disulfate fraction, the concentrations of two pregnane-diol isomers, 5α-pregnane-3α,20α-diol and 5β-pregnane-3α,20α-diol, were high after cholestasis. Other investigators have shown that, as a result of cholestasis, these pregnanediol sulfates circulate in greatly elevated amounts in the maternal plasma. Our results indicate that in cholestasis these steroids cross the placenta into the fetal compartment, where they circulate in elevated amounts as disulfates. Secondly, the concentrations of several steroid sulfates known to be synthesized by the fetus were significantly lower in the cholestasis group than in the healthy controls. This was especially true of 16α-hydroxydehydroepiandrosterone sulfate and 16α-hydroxyprogrenenolone sulfate. These results suggest that, in pregnancies complicated by maternal intrahepatic cholestasis, impairment of fetal steroid synthesis, and especially of 16α-hydroxylation, occurs in the fetal compartment.

Thus, the changes in maternal steroid metabolism caused by cholestasis are reflected in the steroid profile of the fetoplacental circulation. Furthermore, maternal intrahepatic cholestasis may result in the production of some substance which crosses the placenta and affects fetal steroid metabolism.

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Introduction

Intrahepatic cholestasis is a well-documented biochemical feature of the disease known as recurrent jaundice, or hepatosis, of pregnancy. In the maternal plasma, the direct fraction of bilirubin is elevated; (1), and the concentration of total bile acids rises from 10- to 100-fold above the levels found in normal pregnancy (2). The excretion of administered bromsulphalein in the bile is reduced and so is the capacity of the liver to take up this substance (3). Recently, Sjövall and Sjövall (4) have shown that, as a result of cholestasis, the levels of several neutral steroid sulfates, mostly metabolites of progesterone,1 are high in maternal plasma in this condition. It is not known whether these biochemical changes are reflected in the fetoplacental compartment. Information on this point would be of interest, because the rate of premature deliveries and risks to fetal well-being are reported to be increased in pregnancies complicated by maternal intrahepatic cholestasis (5, 6).

To obtain more detailed information on the metabolism and conjugation of steroids in the fetoplacental unit, we developed a method for determination of progesterone, estriol, estriol sulfate, and 13 neutral steroid monosulfates and 10 neutral steroid disulfates in the cord plasma (7). To study the fetoplacental steroid metabolism in pregnancies complicated by maternal intrahepatic cholestasis, we have analyzed 27 cord plasma samples collected after such pregnancies and compared the steroid levels with those obtained in the cord plasma after uncomplicated pregnancies.

1 Trivial and systematic names of steroids: dehydroepiandrosterone, 3β-hydroxy-5-androsten-17-one; 16α-hydroxydehydroepiandrosterone, 3β,16α-dihydroxy-5-androsten-17-one; progesterone, Δ4-pregnene-3,20-dione; pregnenolone, 3β-hydroxy-5-pregnen-20-one; 16α-hydroxyprogrenenolone, 3β-, 16α-dihydroxy-5-pregnen-20-one; 17α-hydroxyprogrenenolone, 3β,17α-dihydroxy-5-pregnen-20-one; 21-hydroxyprogrenenolone; 3β,21-dihydroxy-5-pregnen-20-one.
disulfates were
unconjugated
proportion
The unconjugated steroids
on extracted
The plasma
tainer.

METHODS
The series comprised 27 pregnant women admitted to the hospital because of pruritus, with onset at 24–37 wk of
gestation, and elevated serum levels of glutamic oxalo-acetic and glutamic pyruvic transaminase (GOT and GPT).
Six of the patients were slightly icteric. Serum levels of
GOT, GPT, and alkaline phosphatase were elevated in every
patient, and bilirubin was elevated in 20 (1). Hepatitis
was excluded by the thymol turbidity test, which was
negative in every case, and by serum electrophoresis, which
did not show an elevated γ-globulin peak in any of these
patients. In addition, serum Au-antigen was determined in
16 of these cases with consistent negative results. After
labor, pruritus disappeared quickly in every case, and the
levels of serum transaminases and bilirubin fell to normal
in 2 wk after delivery. As a result, intrahepatic cholestasis
of pregnancy was diagnosed in all 27 cases. To eight of
these patients, an antihistamine (feniramine) was adminis-
tered for relief of pruritus. The other patients took no
medicines.

These 27 patients delivered 13 male and 14 female in-
fants. 19 labors were spontaneous, and in the remaining
cases, labor was induced with oxytocin or by rupturing
the membranes. In two cases, fetal asphyxia was diagnosed
during labor by cardiocotography and by determining the
acid-base status in fetal blood. Cesarean section was done
in these cases. In addition, four babies had Apgar scores
of 3–6 at 1 or 6 min after delivery, indicating fetal dis-
tress. The other babies were in good condition. Because a
considerable proportion of these deliveries occurred at 35–
37 wk of gestation, the series was divided into two groups
according to time of delivery, as shown in Table I.

Control groups. Control groups consisted of 30 plasma
samples collected after normal pregnancies and deliveries
at 38–41 wk of gestation. The results of these analyses
were published previously (7). Further, 12 cord plasma
samples collected after deliveries at 35–37 wk of gestation
after uncomplicated pregnancies were analyzed in this study.

Method. Immediately after clamping of the umbilical
cord, mixed venous and arterial blood was allowed to drain
from the placental end of the cord into a heparinized con-
tainer. The plasma was immediately separated by centri-
fugation and stored at −20°C until analyzed.
The analytical procedure was described in detail previ-
sely (7). Briefly, the procedure was as follows: lipids
were extracted from a 5-ml sample of cord plasma with
acetone/ethanol 1:1 vol/vol. The extract was chromatog-
rapped on a 4-g column of Sephadex LH-20, and fractions
of unconjugated steroids, steroid monosulfates, and steroid
disulfates were obtained. Steroid sulfates were solvolyzed.
The unconjugated steroids and the steroids in the mono-
and disulfate fractions were separately purified and frac-
tionated on 200-mg columns of silicic acid. After forma-
tion of trimethylsilyl or O-methyl oxime trimethylsilyl der-
ivatives, steroids were quantified by gas-liquid chromatog-
raphy with 2.2% SE-30 and 3% QF-1 liquid phases. The
specificity of the quantifications was tested by gas chroma-
tography-mass spectrometry with an LKB 9000 gas chroma-
tography-mass spectrometer (LKB Produkter AB, Stock-
holm, Sweden).

RESULTS
The specificity of the steroid determinations in cord
plasma samples collected after pregnancies complicated by
intrahepatic cholestasis was tested by gas chroma-
tography-mass spectrometry. No impurities were found
in the peaks of the steroids determined previously in
cord plasma after uncomplicated pregnancies (7), ex-
cept in that of 21-hydroxyprogrenolone. Therefore,
this steroid was not determined in this study.

Table II lists the concentrations of steroids in cord
plasma samples collected in deliveries at 35–37 and
at 38–41 wk of gestation after pregnancies complica-
ted by cholestasis and after uncomplicated preg-
nancies. It is seen that in healthy pregnancies the
steroid pattern of the cord plasma depends on the
gestational age of the fetus. The mean levels of de-
hydroepiandrosterone and 16a-hydroxydehydroepi-
drosterone sulfates in the group of 35–37 wk of gesta-
tion were 58 and 235 μg/100 ml, respectively, whereas
at full term, higher mean values for these steroids, 76
and 305 μg/100 ml, respectively, were previously ob-
tained (7). These differences were significant (P <
0.05). In contrast, no rise in the levels of these steroid
conjugates with advancing gestation were found in the
pregnancies complicated with cholestasis (Table II).

Comparison of the steroid profiles in the cord plasma
of patients and controls (Table II) shows that the
concentrations of some pregnanediol isomers were
high in the cholestasis groups. In the disulfate frac-
tion, the levels of 5α-pregnane-3α,20α-diol were 77 and
56 μg/100 ml in the two cholestasis groups, whereas
only trace amounts of this steroid were found in the
control samples in which it never exceeded 15 μg/100
ml (7). The mean levels of 5α-pregnane-3α,20α-diol disulfate in the cholestasis groups were much higher than those in the control groups (Table II). Fig. 1 shows individual levels of pregnanediol isomers in the disulfate fraction of cord plasma samples in cholestasis. No differences in the levels of progesterone or pregnanediol monosulfates were found between the cholestasis and control groups (Table II).

15 maternal plasma samples of the cholestasis series were collected before delivery, and steroid sulfates were analyzed as described. Of these, five samples were obtained during the 2 days before delivery or at delivery itself. Maternal and cord plasma levels of pregnanediol disulfates in these samples are compared in Fig. 2. In all cases except one, the levels were higher in the maternal plasma.

The concentrations of some steroid sulfates in the cord plasma were found to be lower in the cholestasis groups than in their healthy controls (Table II). Especially large differences were found when the comparisons were made between results with samples obtained at full term. In the pregnancies complicated by cholestasis, the mean concentrations of 16α-hydroxydehydroepiandrosterone sulfate and of 16α-hydroxyprogrenolone sulfate were about half those found in the control group. Furthermore, concentrations of progrenolone sulfate,

| TABLE II |
| Concentrations of Progesterone, Estriol, Estriol Sulfate, and Neutral Steroid Mono- and Disulfates in Cord Plasma Samples |

<table>
<thead>
<tr>
<th>Delivery at 35-37 wk of gestation</th>
<th>Delivery at 38-41 wk of gestation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cholesterol group</td>
</tr>
<tr>
<td></td>
<td>N = 16</td>
</tr>
<tr>
<td>Unconjugated steroids</td>
<td></td>
</tr>
<tr>
<td>Progesterone</td>
<td>59±5.8</td>
</tr>
<tr>
<td>Estriol</td>
<td>18±1.8</td>
</tr>
<tr>
<td>Monosulfates</td>
<td></td>
</tr>
<tr>
<td>Estriol</td>
<td>171±15.6</td>
</tr>
<tr>
<td>Dehydroepiandrosterone</td>
<td>50±5.5</td>
</tr>
<tr>
<td>16α-Hydroxydehydroepiandrosterone</td>
<td>153±23.7</td>
</tr>
<tr>
<td>3β,17β-Dihydroxy-5-androsten-16-one</td>
<td>69±20.5</td>
</tr>
<tr>
<td>5-Androstone-3β,16α,17β-triol</td>
<td>19±2.6</td>
</tr>
<tr>
<td>Pregnenolone</td>
<td>53±6.4</td>
</tr>
<tr>
<td>16α-Hydroxyprogrenolone</td>
<td>73±10.0</td>
</tr>
<tr>
<td>17α-Hydroxyprogrenolone</td>
<td>46±6.8</td>
</tr>
<tr>
<td>5-Pregnen-3β,20α-diol</td>
<td>38±5.6</td>
</tr>
<tr>
<td>5-Pregnen-3β,20α,21-triol</td>
<td>32±6.8</td>
</tr>
<tr>
<td>5α-Pregnen-3α,20α-diol</td>
<td>28±5.3</td>
</tr>
<tr>
<td>5α-Pregnen-3β,20α-diol</td>
<td>14±1.8</td>
</tr>
<tr>
<td>5β-Pregnen-3α,20α-diol</td>
<td>17±2.0</td>
</tr>
<tr>
<td>5α-Pregnen-3α,20α,21-triol</td>
<td>163±20.8</td>
</tr>
<tr>
<td>Disulfates</td>
<td></td>
</tr>
<tr>
<td>5-Androstone-3β,17α-diol</td>
<td>224±18.5</td>
</tr>
<tr>
<td>5-Androstone-3β,17β-diol</td>
<td>126±18.6</td>
</tr>
<tr>
<td>16β-Hydroxydehydroepiandrosterone</td>
<td>27±4.2</td>
</tr>
<tr>
<td>3β,16β-Dihydroxy-5-androsten-16-one</td>
<td>20±2.9</td>
</tr>
<tr>
<td>5-Androstone-3β,16α,17α-triol</td>
<td>16±2.1</td>
</tr>
<tr>
<td>5-Pregnen-3β,20α-diol</td>
<td>41±5.2</td>
</tr>
<tr>
<td>5α-Pregnen-3α,20α-diol</td>
<td>252±27.9</td>
</tr>
<tr>
<td>5α-Pregnen-3α,20α,21-triol</td>
<td>103±12.5</td>
</tr>
<tr>
<td>5β-Pregnen-3α,20α-diol</td>
<td>77±9.8</td>
</tr>
<tr>
<td>5α-Pregnen-3α,20α,21-triol</td>
<td>25±4.5</td>
</tr>
</tbody>
</table>

Samples were collected after 27 pregnancies complicated by intrahepatic cholestasis. Previous results (7) obtained in 30 uncomplicated pregnancies at term are given for comparison. Values are expressed as μg of free steroid in 100 ml of plasma (mean±SE) and are not corrected for methodological losses.

* Groups were compared by means of Student's t test. NS = not significant.
dehydroepiandrosterone sulfate, 5-androstene-3β,16α,17β-triol monosulfate, and 5-androstene-3β,16α,17α-triol disulfate were lower in the cord plasma samples in the cholestasis group than in the controls (Table II). When corresponding comparisons were made between cord plasma samples of the cholestasis and control groups collected at 35–37 wk of gestation (Table II), the differences found were not so large. The mean concentration of 16α-hydroxydehydroepiandrosterone sulfate in cord plasma in the cholestasis group was also lower than in controls. Lower mean value for 16α-hydroxypregnenolone sulfate were also obtained here in the cholestasis group, but the variation between individuals was large, and the difference was not significant. The results of the individual analyses of these two steroids in cord plasma in the cholestasis groups are seen in Fig. 3. The cholestasis and control groups did not differ in the mean levels of unconjugated estradiol in cord plasma (Table II). The mean levels of estradiol sulfate were higher in the cholestasis than in their controls. Between the earlier delivery groups this difference was significant ($P < 0.05$).

**DISCUSSION**

In the 27 pathological pregnancies studied here, all the mothers had pruritus, a common symptom of intrahepatic cholestasis (1, 8). The serum levels of alkaline phosphatase, transaminases, and bilirubin were in accordance with those found earlier in this disease (1, 8). All the patients were in good condition, and after
delivery, the pruritus subsided rapidly, and pathological laboratory findings became normal in 2 wk. As a result, the diagnosis in all these cases was intrahepatic cholestasis of pregnancy. There is widespread agreement that patients chosen on the above mentioned criteria have a disease with a common etiology which is not known, but which may have a hormonal basis (9, 10).

In plasma of pregnant women with pruritus concentrations of 3α-steroid sulfates with a 3α-hydroxy-5α and 3α-hydroxy-5β structure were recently shown to be increased (4). The concentrations of 5α-pregnane-3α, 20α-diol, and 5β-pregnane-3α,20α-diol in the disulfate fraction were especially high. These progesterone metabolites are normally excreted in large amounts in the bile of pregnant women (11). In intrahepatic cholestasis of pregnancy, the biliary excretion (12) and the fecal elimination (13) of progesterone metabolites have decreased. The rise in the concentrations of sulfate conjugates of progesterone metabolites in maternal plasma has been ascribed to the impaired biliary excretion (12, 13) and possibly partly to the enhanced formation of steroid sulfates in the maternal liver (12). This change in maternal progesterone metabolism was confirmed in 15 patients with cholestasis of the present series from whom samples of maternal plasma were collected before delivery and steroid sulfates were analyzed as described. The cord plasma analyses showed that the levels of 5α-pregnane-3α,20α-diol and 5β-pregnane-3α,20α-diol disulfates in the fetal circulation were very much elevated after pregnancies complicated with cholestasis. With one exception, higher levels of these compounds were found in the maternal plasma when compared with the cord plasma in those subjects where maternal-fetal correlations were studied. This indicates that, as a result of maternal cholestasis, these progesterone metabolites are transferred from the maternal circulation to the fetal compartment. It is possible that, during transfer across the placenta, partial hydrolysis of these pregnanediol conjugates takes place as a result of the intensive sulfatase activities in this tissue (14). Unconjugated pregnanediols might have injurious effects on the fetus, because, in microsomal preparations of the liver, unconjugated 5β-pregnane-3α,20α-diol was shown to be an inhibitor of UDP-glucuronyl transferase (16), and its 20β-hydroxy epimer was reported to cause neonatal hyperbilirubinemia and to inhibit glucuronide formation in vitro (17). The fetal compartment sulfurylates steroids effectively (15) and so protects the fetus against any undesirable effects of steroids originating from the maternal compartment in cholestasis.

There is much evidence that dehydroepiandrosterone, 16α-hydroxydehydroepiandrosterone, pregnenolone, and 16α-hydroxypregnenolone sulfates in the fetoplacental circulation are of fetal origin. The fetus synthesizes steroids from acetate via "the conjugating pathway" and thus produces pregnenolone and dehydroepiandrosterone sulfates (18, 19). The fetal liver hydroxylates steroids in the 16α-position (20) and is regarded as the main site of production of 16α-hydroxypregnenolone and 16α-hydroxydehydroepiandrosterone (20, 21). The fetal origin of these steroid conjugates is confirmed by the higher levels of dehydroepiandrosterone (22), 16α-hydroxydehydroepiandrosterone (23, 24), and 16α-hydroxypregnenolone (24) sulfates in the umbilical artery, as compared with umbilical venous blood. When cord plasma steroid levels after pregnancies complicated with maternal cholestasis were compared with those obtained after uncomplicated pregnancy, it was found that in the cholestasis group the levels of 16α-hydroxydehydroepiandrosterone sulfate were significantly lower in the earlier delivery group, and at full term, the concentrations of all the above mentioned four steroid conjugates were depressed. These results suggest impairment of fetal steroid synthesis and especially of 16α-hydroxylation of steroids in the fetal liver in pregnancies complicated by maternal cholestasis. We suggest that maternal cholestasis leads to the production of some substance that crosses the placenta into the fetal compartment and affects fetal steroid metabolism, especially 16α-hydroxylation. A similar pathophysiological mechanism for the "poisoning" effect of cholestasis has been postulated for cortisol metabolism by Zumoff, Bradlow, Cassouto, Gallagher, and Hellman (25) and for estrogen metabolism in the adult liver by Hellman, Zumoff, Fishman, and Gallagher (26). In the latter study, depression of 16α-hydroxylation was the specific change in estrogen metabolism found to characterize cholestasis. Sjövall and Sjövall (4) found elevated levels of 3α,16α-dihydroxy-5α-pregn-20-one and 5α-pregnane-3α,16α,20α-triol monosulfates in the maternal plasma in cholestasis. These compounds were not found in the cord plasma in this study. With regard to the 16α-hydroxylated 3α-hydroxy-Δ4 steroids, it can be observed that, in maternal blood as compared with cord plasma, the amount of circulating 16α-hydroxydehydroepiandrosterone sulfate is small (27), and Sjövall and Sjövall (4) found no difference in maternal plasma concentrations of this steroid conjugate between pregnant women with cholestasis and healthy controls. 16α-Hydroxypregnenolone sulfate was not found in the plasma of pregnant women by Sjövall (28). Therefore, the maternal compartment is unlikely to play a great

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part in the metabolism of the 16α-hydroxylated 3β-
hydroxy-Δ4 steroid sulfates circulating in the foeto-
placental unit.

We found no differences in the concentrations of
unconjugated estriol in cord plasma between the cho-
lestasis and control groups. In view of the low levels
of the main estriol precursor, 16α-hydroxydehydroepi-
androsterone sulfate, in the fetal circulation in both
cholestasis groups, the estriol level would be expected
to be low. The bulk of the estriol synthesized in the
placenta is transferred to the maternal compartment,
and this may explain why the changes in the levels of
16α-hydroxydehydroepiandrosterone sulfates are not
closely followed by changes in the fetal estriol levels.

The present study shows that maternal intrahepatic
cholestasis leads to changes in fetoplacental steroid
metabolism. Some of these changes could be regarded
as secondary to the changes in maternal steroid metab-
olism in intrahepatic cholestasis observed by Sjövall
and Sjövall (4). But other changes, which cannot be
explained in this way, are suggested to be due to a
"toxic" effect of maternal cholestasis on fetal steroid
metabolism. Further studies on the pathogenesis of
these effects are indicated, because some authors state
that there is increased fetal risk in pregnancies com-
licated by intrahepatic cholestasis (5, 6).

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