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 pregnanediol 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-hydroxypregnenolone 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 into 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, Δ^4-pregnene-3,20-dione; pregnenolone, 3β-hydroxy-5-pregnen-20-one; 16α-hydroxypregnenolone, 3β,16α-dihydroxy-5-pregnen-20-one; 17α-hydroxypregnenolone, 3β,17α-dihydroxy-5-pregnen-20-one; 21-hydroxypregnenolone; 3β,21-dihydroxy-5-pregnen-20-one.

TABLE I

Weights and Sex of the Babies Studied

	Weight upon delivery at 35–37 wk			Weight upon delivery at 38–41 wk	
	Mean	Range		Mean	Range
	g			g	
Maternal cholestasis					
8 males and 8 females	2,800	2,750–3,230	5 males and 6 females	3,180	2,500–3,970
Control group					
5 males and 7 females	2,710	2,240–3,150	15 males and 15 females	3,440	2,870–4,540

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 oxaloacetic 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 administered for relief of pruritus. The other patients took no medicines.

These 27 patients delivered 13 male and 14 female infants. 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 cardiotocography 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 distress. 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 container. The plasma was immediately separated by centrifugation and stored at −20°C until analyzed.

The analytical procedure was described in detail previously (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 chromatographed 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 fractionated on 200-mg columns of silicic acid. After formation of trimethylsilyl or *O*-methyl oxime trimethylsilyl derivatives, steroids were quantified by gas-liquid chromatography with 2.2% SE-30 and 3% QF-1 liquid phases. The specificity of the quantifications was tested by gas chromatography-mass spectrometry with an LKB 9000 gas chromatograph-mass spectrometer (LKB Produkter AB, Stockholm, Sweden).

RESULTS

The specificity of the steroid determinations in cord plasma samples collected after pregnancies complicated by intrahepatic cholestasis was tested by gas chromatography-mass spectrometry. No impurities were found in the peaks of the steroids determined previously in cord plasma after uncomplicated pregnancies (7), except in that of 21-hydroxypregnenolone. 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 complicated by cholestasis and after uncomplicated pregnancies. 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 dehydroepiandrosterone and 16α-hydroxydehydroepiandrosterone sulfates in the group of 35–37 wk of gestation 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 obtained (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 fraction, the levels of 5β-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-hydroxypregnenolone sulfate were about half those found in the control group. Furthermore, concentrations of pregnenolone sulfate,

TABLE II

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

	Delivery at 35–37 wk of gestation			Delivery at 38–41 wk of gestation		
	Cholestasis group, $N = 16$	Control group, $N = 12$	P*	Cholestasis group, $N = 11$	Control group, $N = 30$	P*
Unconjugated steroids						
Progesterone	59±5.8	59±5.9	NS	65±6.4	59±4.6	NS
Estriol	18±1.8	15±1.5	NS	18±1.8	16±1.3	NS
Monosulfates						
Estriol	171±15.6	121±10.8	<0.05	169±20.9	135±8.2	NS
Dehydroepiandrosterone	50±5.5	58±5.7	NS	43±6.7	76±4.2	<0.001
16α-Hydroxydehydroepiandrosterone	153±23.7	235±26.2	<0.05	140±29.2	305±17.7	<0.001
3β,17β-Dihydroxy-5-androsten-16-one	69±20.5	66±20.0	NS	39±11.2	37±2.8	NS
5-Androstene-3β,16α,17β-triol	19±2.6	23±2.9	NS	18±2.7	25±1.6	<0.05
Pregnenolone	53±6.4	59±9.4	NS	44±7.1	74±6.0	<0.01
16α-Hydroxypregnenolone	73±10.0	101±24.5	NS	53±8.3	101±7.6	<0.001
17α-Hydroxypregnenolone	46±6.8	55±13.1	NS	34±6.3	56±5.6	<0.05
5-Pregnene-3β,20α-diol	38±5.6	40±5.9	NS	32±6.6	41±2.6	NS
5-Pregnene-3β,20α,21-triol	32±6.8	39±6.3	NS	22±5.3	37±3.2	<0.05
5α-Pregnane-3α,20α-diol	28±5.3	17±3.7	NS	31±9.2	21±1.8	NS
5α-Pregnane-3β,20α-diol	14±1.8	17±2.4	NS	14±1.7	18±1.5	NS
5β-Pregnane-3α,20α-diol	17±2.0	23±5.8	NS	20±3.7	17±1.3	NS
5α-Pregnane-3α,20α,21-triol	163±20.8	138±21.8	NS	136±33.1	110±10.7	NS
Disulfates						
5-Androstene-3β,17α-diol	224±18.5	251±32.5	NS	245±22.4	279±13.5	NS
5-Androstene-3β,17β-diol	126±18.6	146±25.1	NS	169±27.8	211±19.8	NS
16β-Hydroxydehydroepiandrosterone	27±4.2	49±11.4	NS	48±5.7	48±4.5	NS
3β,16β-Dihydroxy-5-androsten-16-one	20±2.9	30±5.5	NS	33±3.4	29±2.7	NS
5-Androstene-3β,16β,17α-triol	16±2.1	23±4.1	NS	20±2.0	31±2.4	<0.01
5-Pregnene-3β,20α-diol	41±5.2	69±15.3	<0.05	58±9.5	68±7.8	NS
5α-Pregnane-3α,20α-diol	252±27.9	53±8.3	<0.001	218±28.1	54±3.8	<0.001
5α-Pregnane-3β,20α-diol	103±12.5	95±18.7	NS	118±14.9	75±7.2	<0.05
5β-Pregnane-3α,20α-diol	77±9.8	<15		56±7.6	<15	
5α-Pregnane-3α,20α,21-triol	25±4.5	27±9.7	NS	32±4.6	22±3.6	NS

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 uncorrected for methodological losses.

* Groups were compared by means of Student's t test. NS = not significant.

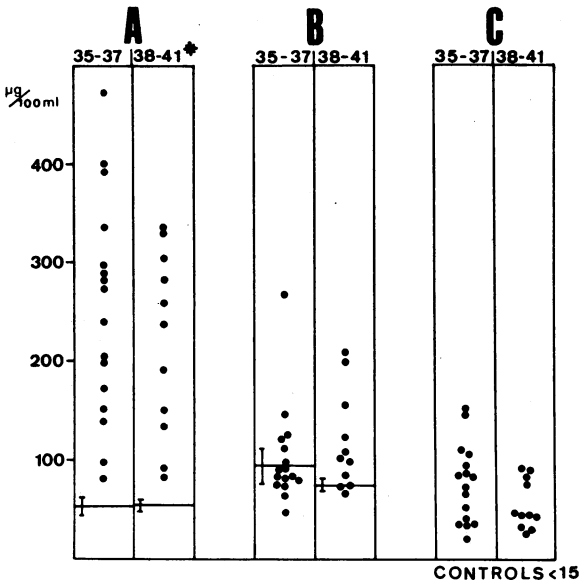

FIGURE 1 Concentrations of three pregnanediol isomers, 5α-pregnane-3α-20α-diol (A), 5α-pregnane-3β,20α-diol (B), and 5β-pregnane-3α,20α-diol (C), in the disulfate fraction of cord plasma samples collected after pregnancies complicated by maternal intrahepatic cholestasis. Transverse lines represent mean values and bars standard errors of results obtained in the control groups. (*) Gestational age (wk).

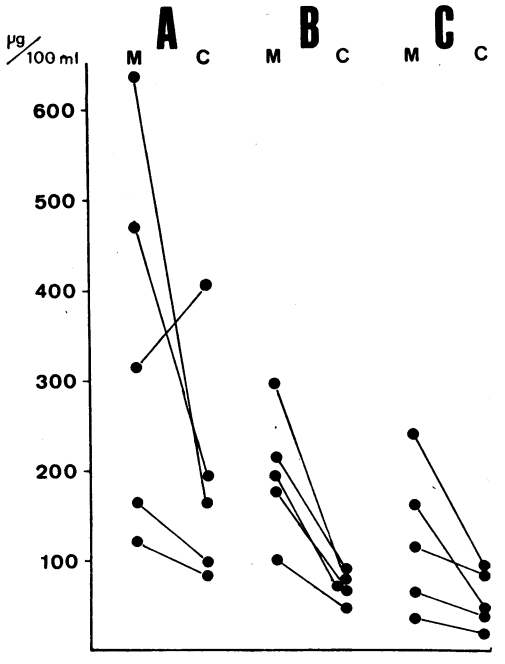

FIGURE 2 Comparison of the concentrations of 5α-pregnane-3α,20α-diol (A), 5α-pregnane-3β,20α-diol (B), and 5β-pregnane-3α,20α-diol (C) disulfates in plasma samples collected before delivery from five mothers with cholestasis (M) with those found in cord plasma of their babies (C).

dehydroepiandrosterone sulfate, 5-androstene-3β,16α,17β-triol monosulfate, and 5-androstene-3β,16β,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 estriol in cord plasma (Table II). The mean levels of estriol 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

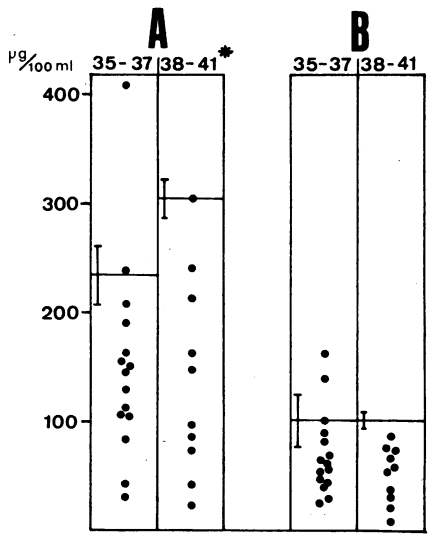

FIGURE 3 Levels of 16α-hydroxydehydroepiandrosterone sulfate (A) and 16α-hydroxypregnenolone sulfate (B) in cord plasma samples after pregnancies complicated by maternal cholestasis. Transverse lines with standard errors represent mean values obtained in the corresponding control groups. (*) Gestational age (wk).

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 C_{21}-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-pregnan-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 3β-hydroxy-Δ^5 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

part in the metabolism of the 16α-hydroxylated 3β-hydroxy-Δ^5 steroid sulfates circulating in the feto-placental unit.

We found no differences in the concentrations of unconjugated estriol in cord plasma between the cholestasis and control groups. In view of the low levels of the main estriol precursor, 16α-hydroxydehydroepiandrosterone 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 sulfate 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 metabolism in intrahepatic cholestasis observed by Sjövall and Sjövall (4). But other changes, which can not 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 complicated by intrahepatic cholestasis (5, 6).

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