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CORTISOL, CORTISONE AND DEHYDROEPIANDROSTERONE SULFATE LEVELS IN UMBILICAL CORD AND MATERNAL PLASMA BETWEEN 21 AND 30 WEEKS OF PREGNANCY

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Summary—Cortisol (F), Cortisone (E) and dehydroepiandrosterone sulfate (DHAS) were determined in maternal peripheral plasma and umbilical cord plasma between 21 and 30 weeks of amenorrhea. In fetal plasma, DHAS levels were the highest and those of F the lowest. E always exceeded F. The pattern of all these steroids was characterized by a plateau throughout the period considered. In maternal plasma, F levels were more elevated than those of E but lower than DHAS concentrations. All the steroids plateaued as in the fetus. The study of the correlation between the steroids in either the same milieu or in maternal and umbilical cord plasma demonstrated that fetal E was correlated with maternal F and with fetal F while fetal DHAS was inversely correlated with maternal E.

INTRODUCTION

In animals as well as in man, fetal lung maturation *in utero* is influenced by glucorticoids [1] and it was demonstrated that, at birth, cord plasma cortisol (F) and, to a lesser extent cortisone (E) levels were lower in infants with respiratory distress syndrome than in healthy infants [2, 3]. Fetal F and E patterns throughout pregnancy are however not entirely known as the data available in the literature concern only the levels observed either before the 20th or beyond the 30th week [1]; it appeared useful to fill the gap between 20 and 30 weeks. This study could be carried out thanks to a new technique which allowed us to collect umbilical cord blood *in utero* [4]. The fetal levels of F and E were compared with those obtained in the peripheral maternal blood collected simultaneously. In addition dehydroepiandrosterone sulfate (DHAS) was determined in the fetus as well as in the mother and the levels were correlated with those of the glucocorticoids. A part of DHAS levels was previously reported [5].

EXPERIMENTAL

Subjects

Forty-one pregnant women were referred to us for prenatal diagnosis of congenital toxoplasmosis. The gestational age assessed from the first day of the last menstrual period ranged from 21 to 30 weeks of amenorrhea.

Fetal blood was sampled by direct puncture of the umbilical cord vessels near the placental insertion of the cord under ultrasound guidance as previously described [4, 5] and any contamination by either maternal blood or amniotic fluid was checked. Though the umbilical vein was generally punctured, the origin of the blood samples obtained could not always be considered as uniquely venous. Peripheral blood was sampled simultaneously from an antecubital vein in 29 of the 41 corresponding mothers.

All samples collected on EDTA were immediately centrifuged and the plasma stored at −20°C until assays were carried out.

As congenital toxoplasmosis could be ruled out, the pregnancies were allowed to proceed until term when healthy infants were delivered.

METHODS

Determination of plasma E and F in the fetus and the mother

Plasma E and F levels were determined according to a modification of the previously reported radioimmunoassay for plasma and urine 6β-hydroxycortisol [6].

In addition to the reagents already described [6] tritiated E with a sp. act. of 53.4 Ci/mmol (New England Nuclear) was used. The antiserum was raised in rabbits injected with cortisol-3 (*O*-carboxymethyl)oxime BSA. It was used for F and E determination since the cross-reaction with the latter was 100%.

Approximately 1000 cpm of tritiated E and F were added to 0.02 ml of umbilical cord plasma or 0.01 ml

The following trivial names and abbreviations have been used: Cortisol: 11β-17,21-trihydroxy-4-pregnene-3,20-dione (F); Cortisone: 17,21-dihydroxy-4-pregnene-3,11,20-trione (E); Dehydroepiandrosterone: 3β-hydroxy-5-androsten-17-one (DHA); Dehydroepiandrosterone sulfate (DHAS); 11-Deoxycortisol: 17,21-dihydroxy-4-pregnene-3,20-dione; 21-Deoxycortisol: 11β, 17-dihydroxy-4-pregnene-3,20-dione; Estriol: 1,3,5 (10)-estratriene 3,16α, 17β-triol (E_3); Prednisolone: 11β, 17,21-trihydroxy-1,4-pregnadiene-3,20-dione.

Table 1. Evaluation of the precision

	Intra-assay variability				Inter-assay variability			
	n	mean	SD	CV (%)	*n*	mean	SD	CV (%)
Cortisone (ng/ml)	20	74	5.4	7.3	8	59	8.6	14.6
	20	150	12.3	8.2	8	112	11.4	10.2
Cortisol (ng/ml)	8	5.50	0.53	9.70				
	20	30	2.3	7.7	8	29	4.4	15.0
	20	99	8.1	8.2	8	83	12.1	14.6
	8	341	24.4	7.1				
DHAS (ng/ml)	8	824	35.4	4.0	12	1292	64.3	5.0

of peripheral maternal plasma and extraction performed with 5 ml of methylene chloride. After evaporation of the solvent the extracts were dissolved with 2×0.1 ml of methylene chloride and applied on Sephadex LH 20 columns (height: 168 mm; internal dia: 5 mm). The columns were washed with 7 ml and E eluted with 5 ml. The next 1 ml was discarded then F was eluted with 6 ml. The eluates were evaporated to dryness and the extracts redissolved with 2 ml of methylene chloride. Duplicate 0.2 ml aliquots were pipetted for radioimmunoassay and 1 ml for recovery estimation.

The radioimmunoassay was performed by adding to the dried eluate aliquots approx 3000 cpm of the corresponding steroid and 0.2 ml of the appropriate dilution of the antiserum (1:1,000 for E and 1:1,500 for F). All tubes were mixed, incubated for 30 min in a water-bath at 37°C and then kept at 4°C for 30 min. Free and bound steroids were separated by extracting the free fraction with 2 ml of chloroform. The aqueous phase was mixed with the scintillating mixture and counted. Water blanks were similarly prepared and a standard curve consisting of increasing amounts (10–800 pg) of either F or E was established with each series.

The sensitivity of the assay was estimated to be 100 pg/ml for either steroid and the blank value was generally similar to B_0. Accuracy was assessed by the addition of increasing amounts of either F or E to aliquots of a charcoal-treated plasma pool. The calculated regression lines were represented by:

$y = (0.999 \pm 0.032)\,x + (-0.037 \pm 0.599)$ and
$y = (1.013 \pm 0.019)\,x + (-0.541 \pm 0.352)$ for F and E respectively.

The study of joint confidence region for slopes and intercepts showed they were not significantly different from 1 and 0 respectively. The mean recovery of tritiated steroids added to aliquots of pooled plasma was $84.6 \pm 6.9\%$ (range: 73–95; $n = 11$) and $79.6 \pm 5.3\%$ (range: 69–85; $n = 11$) for E and F respectively. According to the intra- and interassay variabilities reported on Table 1, the method proved to be highly reproducible for both steroids. Concerning the specificity, the only significant cross-reactions of the antiserum were demonstrated with prednisolone (100%), 11-deoxycortisol (23.8%) and 21-deoxycortisol (2.7%). However, none of these steroids interfered in any assay since they were well separated from E and F by the chromatography on Sephadex LH 20 columns in methylene chloride.

Determination of plasma DHAS

Fetal and maternal plasma DHAS was determined after a preliminary extraction of unconjugated steroids with diethyl-ether and hydrolysis at pH 4.7 according to Bitman and Cohen[7]. The liberated DHA was extracted with diethyl-ether, then isolated by celite micro-column chromatography and radioimmunoassayed as described previously [8, 9]. The intra- and interassay variabilities of the technique are depicted on Table 1.

Statistical analysis

Results were expressed as the arithmetic mean ± SD. Statistical differences between steroid levels in the fetus and in the mother were evaluated using paired Student's *t*-test.

RESULTS

Mean levels of E, F and DHAS in umbilical cord plasma and in maternal peripheral plasma are reported on Tables 2 and 3 respectively.

In the fetus DHAS concentrations were the highest while those of F were the lowest whatever was the

Table 2. Concentrations (ng/ml) of cortisone (E) cortisol (F) and dehydroepiandrosterone sulfate (DHAS) in umbilical cord plasma

	Gestational age (weeks)				
	21–22 (10)*	23–24 (12)	25–26 (7)	27–28 (7)	29–30 (5)
E	23.1 (11.0–38.5)§	28.5 (7.0–52.0)	34.4 (21.0–69.0)	35.1 (23.0–60.0)	21.0 (12.0–29.0)
F	7.9 (4.0–13.4)	10.1 (4.3–15.8)	7.8 (4.0–12.0)	7.9 (5.0–10.0)	5.5 (4.0–8.0)
DHAS	845 (540–1234)	777 (392–1370)	739 (430–1135)	800 (310–1170)	552 (419–980)

*Numbers of cases. § Range.

Table 3. Concentrations (ng/ml) of cortisone (E) cortisol (F) and dehydroepiandrosterone sulfate (DHAS) in maternal peripheral plasma

	Gestational age (weeks)				
	21–22 (10)*	23–24 (12)	25–26 (4)	27–28 (2)	29–30 (1)
E	30.6 (20–54)§	30.1 (16–46)	27.8 (18–33)	40.5 (40–41)	23
F	261 (169–358)	315 (123–477)	308 (187–498)	413 (332–493)	192
DHAS	593 (241–988)	657 (193–1340)	573 (222–1400)	758 (706–809)	480

*Numbers of cases. §Range.

Table 4. Correlation coefficients (r) between the levels of steroids in umbilical cord plasma and in maternal plasma

	Steroids	n	Correlation coefficient (r)	P
Umbilical cord plasma	F versus E	40	0.38	$0.01 < P < 0.05$
	F versus DHAS	38	0.12	NS*
	E versus DHAS	39	−0.26	NS
Maternal plasma	F versus E	29	0.32	NS
	F versus DHAS	29	0.12	NS
	E versus DHAS	29	−0.01	NS

*Not significant.

gestational age. Mean E/F ratio was 3.83 ± 1.54 (range: 1.49–7.20). Throughout the period studied, namely between 21 and 30 weeks after the last menstrual period, no significant variations could be demonstrated for any of the three steroids. F and E levels were correlated (Table 4) [$0.01 < P < 0.05$] but no significant correlation could be shown between any of them and DHAS.

In maternal peripheral plasma, mean DHAS concentrations were the most important but, in contrast to what was observed in umbilical cord plasma, E was considerably lower than F, the mean E/F ratio being: 0.11 ± 0.05 (range: 0.06–0.32). As in the fetus, either steroid concentrations plateaued throughout the period considered. No correlation could be found among maternal steroids.

In comparison with the fetus, maternal F was found to be markedly elevated (mean maternal F/fetal F ratio: 38.3 ± 15.2; range: 12.8–61.8) and E only slightly but not significantly increased (mean maternal E/fetal E ratio: 1.26 ± 0.51). Conversely DHAS levels were higher in the fetus than in the mother (mean fetal DHAS/maternal DHAS ratio: 1.38 ± 0.92; range: 0.31–3.73) but the difference was not statistically significant. Among the correlations studied between fetal and maternal steroids (Table 5), the most significant one was observed between fetal E and maternal F ($P < 0.01$). Moreover, fetal DHAS was inversely correlated with maternal E ($0.01 < P < 0.05$). A similar relation, though not significant, was also demonstrated between fetal DHAS and maternal F.

DISCUSSION

The new procedure for fetal blood sampling *in utero* has proved to be a valuable tool in prenatal diagnosis [4, 5, 10–12] and besides it offers the best means to study fetal circulating steroid levels. Moreover, since all these pregnancies were terminated with the delivery of healthy children, the levels observed may be considered as normal. Indeed some interesting data concerning the levels of the sulfates of pregnenolone, 17-hydroxypregnenolone and dehydroepiandrosterone could recently be obtained [5].

In umbilical cord plasma, the determination of F cannot be performed by the techniques usually used for human adult plasma. In fact in fetal plasma F is at low levels in comparison with other steroids so direct techniques may give spurious results [13, 14]. However, a chromatographic step does not seem to be mandatory provided that plasma is prewashed with hexane then extracted with methylene chloride [13, 15] and that the antiserum is highly specific [15]. In the case of the antiserum used here a prewash purification was not sufficient so a chromatographic step had to be included in the procedure. Isolation of F and its separation from E can be achieved by different chromatographic techniques yet chromatography on Sephadex LH20 column appears to be the most convenient as already

Table 5. Correlation coefficients (r) between umbilical cord plasma and maternal plasma steroid levels

Mother \ Fetus	F	E	DHAS
F	0.11	0.54	−0.31
	(29)*	(29)	(28)
	NS†	$P < 0.01$	NS
E	0.19	0.35	−0.40
	(29)	(29)	(28)
	NS	NS	$0.01 < P < 0.05$
DHAS	0.01	−0.08	0.28
	(29)	(29)	(28)
	NS	NS	NS

*Number of cases. †Not significant.

outlined [16–18]. However, the reported techniques are rather cumbersome and time-consuming since big columns have to be used. In the present method, the use of methylene chloride without any addition of methanol has afforded the separation of F and E on rather small columns. This proved to be quick, simple and low-costing and besides the technique was highly reproducible.

In umbilical cord, the data concerning either total corticosteroids [19] or F and E [1, 14] concentrations between 20 and 30 weeks of gestation are rather limited. Thus comparison of the present results with literature data cannot be made. However, it is noteworthy that mean F and E levels were markedly similar to those observed in early gestation between 10–20 weeks [14, 20–22] and obtained at elective Cesarean section without labor. As in patients delivered vaginally after spontaneous onset of labor umbilical F was significantly higher [14] it may be concluded that the procedure used here to collect umbilical cord blood may be considered unstressful.

Whatever was the gestational age, E levels were found to be higher than F values and this is in agreement with all the data reported either in the previable fetus or at term [3, 20–22] and is related to the extensive conversion of F to E in the placenta and the fetal tissues [22–26]. This reaction has been proposed as a mechanism protecting the fetus from exceedingly high levels of F [18].

The plateau displayed by fetal F and E levels throughout the period studied is in keeping with the pattern described until 33–34 weeks by Smith and Shearman [19] for total corticosteroids. Besides such a pattern was also demonstrated in amniotic fluid between 20 and 30 weeks for either unconjugated F [27] or total F [28] and this fact is in agreement with our results since amniotic fluid F can be considered as a good reflection of fetal F, a significant correlation having been established between F levels in these two milieu [27]. In addition, in comparison with normal fetuses, there is a simultaneous decrease of F levels in amniotic fluid and in umbilical artery plasma in anencephalic fetuses [29].

The existence of a significant correlation between fetal F and E might be surprising since most of E originates mainly from the mother while the fetus secretes about three-quarters of its F at term [30]. Moreover no such a correlation could be found at term [21]. However, it should be noted that fetal E may come also from fetal F since, as already mentioned, the latter is extensively oxidized in most fetal tissues. On the other hand, the chorionic membrane converts E to F [31] and this may contribute to the overall production of F in the fetus since amniotic fluid is swallowed frequently throughout gestation [1]. Thus these interrelationships between E and F would account for the correlation observed in our series.

Concerning DHAS, our present data, in regard to the concentrations and the profile, are comparable to those observed by Parker *et al.*[32]. In our previous study, DHAS was found at higher levels and a decreasing trend with advancing gestational age was disclosed. The difference with the present results may be accounted for by the limited number of cases in the preceding series [5]. The absence of any significant correlation between fetal F and fetal DHAS might be relevant to the fact that the definitive and the fetal zones of the adrenals are functionally specialized, the former synthesizing primarily corticoids while the latter secreting mainly DHAS [33–35].

In maternal plasma, mean F levels and the plateau they display throughout the period studied are in agreement with already reported data [36–38]. The E concentrations observed in maternal plasma seem to be reported for the first time in such a large series. To our knowledge, the only available data concern the levels obtained in 9 patients in the third trimester of pregnancy with a mean rather higher than ours [39]. The similarity between the patterns of E and F mean levels is in agreement with the fact that blood production of E was demonstrated to be entirely contributed by that of F [30]. However this is in contradiction with the non significant correlation between F and E levels. This might be accounted for by the difference of binding of these steroids with CBG since the radioimmunoassay evaluates as a bulk the bound and the unbound fractions of each steroid. Indeed it was shown that in normal women in the third trimester of pregnancy 59.1% of total E while 95.2% of total F was bound to CBG [40].

Concerning maternal DHAS, no data is available before the 26th week. After this date the results observed here are lower than what was reported by Buster *et al.*[41]. This variance might be due to the technique used by these authors since it did not include a chromatographic step [42]. Nevertheless DHAS levels did not display any decreasing trend with gestational age [41] as can be expected in view of the increasing metabolic clearance rate [43] and the subsequent decreasing of DHAS half life in pregnancy [44]. This discrepancy might be related to the limited number of the cases studied here after 26 weeks.

The comparison of maternal and fetal steroid levels demonstrating higher F levels in the mother than in the fetus and the contrary for E concentrations is in agreement with what has been observed at 16–20 weeks [25] and at term [15, 18, 45–47].

The study of the interrelationships between steroid concentrations in maternal plasma and those in cord plasma has shown a significant correlation between fetal E and maternal F while maternal E and fetal DHAS were inversely correlated. The fact that maternal F is significantly correlated with fetal E is in keeping with the demonstration that most of the fetal circulating E was derived from the mother [30]. The inverse correlation found between fetal DHAS and maternal E and that, though not significant, between fetal DHAS and maternal F are in agreement with the

observations suggesting that maternal adrenal function might influence the fetal pituitary adrenal axis. In fact F administration to the mother resulted in a decrease of cord plasma DHAS [48] and besides maternal plasma F was shown to be inversely correlated with estriol [49–51] a steroid known to be derived from fetal adrenal precursors aromatized in the placenta [52]. That maternal plasma E was better correlated with fetal DHAS than maternal plasma F might be related to the fact that the importance of the fraction bound to CBG is different for these two steroids as already outlined [40]. In view of the aforementioned facts it would be expected to find also an inverse correlation between fetal F and maternal F. The absence of such a correlation might be related to the extensive conversion of F to E in the fetal tissues [25] so that the circulating F represents only a fraction of that secreted by the adrenals.

In conclusion, most of the data obtained here are reported for the first time in such a large series and in healthy fetuses. This could be carried out thanks to the use of a new technique for fetal blood sampling *in utero*. The correlations observed between the different steroid levels either in cord plasma or in maternal plasma or between both were found to agree with preceding metabolic and *in vitro* studies.

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