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CORTICOSTEROIDS IN AMNIOTIC FLUID AND THEIR RELATIONSHIP TO FETAL LUNG MATURATION

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SUMMARY

Cortisol, progesterone, 11-deoxycortisol and deoxycorticosterone were measured in amniotic fluid from sixty-four subjects. Palmitic acid was measured as an index of fetal lung maturation. The correlation coefficient between cortisol and palmitate levels was 0.46. The ratio of progesterone to deoxycorticosterone decreased significantly ($P < 0.05$) between the 34–35th and 36–37th weeks of gestation, suggesting the induction of the steroid 21-hydroxylase enzyme system. This preceded the significant rise ($P < 0.05$) in cortisol levels between the 36–37th weeks and the 38–39th weeks. The greatest rise of palmitic acid did not occur until a week later, and after the period when lung maturation had presumably occurred.

INTRODUCTION

Recent investigations have suggested that corticosteroids may be implicated in the maturation of the fetal lung. However, evidence of the involvement of steroid hormones in this process has been difficult to assess, as much of the early work was with animals [1–3]. More recently the use of dexamethasone in human pregnancies has been shown to cause a rapid increase in lecithin/sphingomyelin (L/S) ratios [4]. Low levels of cortisol have also been found in infants developing respiratory distress syndrome at birth [5, 6]. The relationship between cortisol concentration in amniotic fluid and L/S ratio or palmitate levels has been investigated with different results [7–10]. Fencl *et al.*[8] found good correlation between total cortisol and L/S ratio, but Sharpe-Cageorge *et al.*[9] and Peltonen *et al.*[10] concluded that amniotic fluid cortisol levels could not be used with confidence to predict lung maturation as indicated by lecithin production.

In order to investigate fetal corticosteroid metabolism and its relation to lung maturation we have measured cortisol, progesterone, 11-deoxycortisol and deoxycorticosterone (DOC) in amniotic fluid. Amniotic fluid palmitate levels were used as an index of fetal lung surfactant [11].

EXPERIMENTAL

Materials

Fifty-one amniotic fluids were obtained by amniocentesis for antenatal diagnosis, ten specimens by artificial rupture of the membranes and three during spontaneous labour. Samples contaminated with meconium or blood were discarded. No infants born developed respiratory distress syndrome. The amniotic fluids were centrifuged at 1,000 *g* for 5 min to remove cells and debris. The supernatant was decanted and stored at −20°C until it was analysed. No significant changes were detected over the storage period.

Reagents

[1,2,6,7(*n*)−^{3}H]-cortisol, 11 deoxy[1,2(*n*) − ^{3}H]-corticosterone and [1,2(*n*) − ^{3}H]-progesterone were obtained from the Radiochemical Centre, (Amersham, England). Reference compounds were obtained in pure crystalline form from either Sigma London Chemical Co. Ltd U.K., or Steraloids Ltd U.K. Benzene and petroleum ether were redistilled. Other reagents were of Analar quality. Assay buffer—0.05 M sodium phosphate buffer, pH 7.4 containing 1 g/l gelatine and 0.02 M sodium azide.

Antisera

Antisera were raised in sheep at the University of Surrey using antigen prepared in this laboratory. Cortisol and 11-deoxycortisol were measured using an antiserum raised against cortisol-21-succinyl-ovalbumin. Deoxycorticosterone was measured using an antiserum raised against deoxycorticosterone-21-succinyl-ovalbumin, and progesterone from an antiserum raised against progesterone-11-succinyl-ovalbumin. Details of the cross-reaction of these antisera are given in Table 1.

METHODS

Methods for steroids

Progesterone was measured on the dichloromethane extract of amniotic fluid without chromatography. Cortisol, 11-deoxycortisol and DOC were

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extracted from 0.7 mls of amniotic fluid with 6 mls dichloromethane after addition of approximately 2000 c.p.m. of [^{3}H]-DOC and [^{3}H]-cortisol. The organic phase was removed, dried at 40°C, and redissolved in 0.2 ml of cyclohexane–benzene–ethanol (9:4:1, v/v). The extract was applied to a column of Sephadex LH20, 1 g (8 × 62 mm), equilibrated and eluted with the same solvent. The elution patterns of the steroids of interest were established using pure compounds. The following fractions were collected and vacuum dried:—

deoxycorticosterone (5.0–8.5 ml), 11-deoxycortisol (9.5–14.5 ml), cortisol (25–45 ml).

The dried fraction containing DOC was reconstituted in 1.0 ml of assay buffer and 0.2 ml aliquots taken in duplicate for assay. 0.5 ml was transferred directly into a counting vial to measure the procedural recovery. A blank and pooled samples were run with each batch. A binding solution was made containing anti-DOC antiserum (final concentration in tube 1:120,000) and [^{3}H]-DOC (1,250 c.p.m.) and 0.2 ml added to each tube. The contents of the tubes were mixed and left at 37°C for an hour, and subsequently for 30 minutes in an ice bath. Dextran coated charcoal (0.1 ml) was added to the tubes, which were mixed, stood in ice for 30 min and then centrifuged at 1200 *g* for 10 min at 4°C. The supernatants were decanted and counted in scintillation fluid for 10,000 counts. The results were calculated using a polynomial equation and incorporated a correction for dilution and recovery.

11-Deoxycortisol and cortisol were assayed in a similar manner, except a binding solution was made containing anticortisol antiserum (final concentration in tube 1:2000) and [^{3}H]-cortisol (4500 c.p.m.). The cross-reactivity of the anti-cortisol antiserum was adequate for measurement of 11-deoxycortisol.

Method for palmitic acid

Palmitic acid was estimated on 1 ml of amniotic fluid by a method similar to that of Warren *et al.*[12] using the centrifuged fluid. Greater precision was achieved by adding the internal standard, heptadecanoic acid, in the extraction solvent. DL-α-dipalmityl-lecithin was used as a calibration standard that could be treated as the samples. Palmitic acid was measured as the methyl ester using a 6 foot glass column containing 10% diethylene glycol succinate on Gaschrome Q 80–100 mesh on a Pye 104 Gas Chromatograph fitted with a flame ionisation detector.

The significance of differences between means was calculated by the *t*-test.

RESULTS

Validification of the analysis

The extent to which potentially interfering steroids cross-react with the antisera was investigated and the results are shown in Table 1. A relatively non-specific anti-cortisol antiserum was selected so that 11-deoxycortisol could be measured using the same assay system. The necessary specificity was obtained by including LH20 chromatography stage. The elution pattern of the pooled amniotic fluid sample analysed using the anti-cortisol antiserum is shown in Fig. 1. All peaks were confirmed by the use of reference steroids. It was established that less than 1% of the progesterone was eluted in the DOC fraction. The interference by progesterone in the DOC assay was therefore lower than 0.1%. A pooled amniotic fluid was analysed with each batch of samples ($n = 8$). The mean concentration and coefficients of variation are:— cortisol 72.6 nmol ± 11%, DOC 1.02 nmol ± 15%, 11-deoxycortisol 38 nmol ± 20%, progresterone 86.5 nmol ± 14%, and palmitic acid 161 μmol ± 3%.

Analysis of amniotic fluid

Cortisol, 11-deoxycortisol, DOC, progesterone and palmitic acid results are shown in Table 2.

There was a significant ($P < 0.05$) rise in cortisol levels between the 36–37th weeks and 38–39th weeks. Whereas palmitic acid levels increased throughout the study period, the greatest and only significant ($P < 0.05$) rise occurred after the 38–39th weeks. Levels of the other steroids measured tended to decrease with increasing time of gestation, except for progesterone, which increased again just before delivery.

The ratios of precursor to substrate for a number of enzymes concerned in the corticosteroid synthesis were calculated and given in Table 3. The progesterone to DOC ratio representing the 21-hydroxylase enzyme decreased significantly ($P < 0.05$) after the 34–35th week followed by a gradual rise, whereas the 11-deoxycortisol to cortisol ratio representing the

Table 1. Percentage cross-reactions of three different antisera used in assays

	Progesterone-11α-succinate-ovalbumin	DOC-21-succinate-ovalbumin	Cortisol-21-succinate-ovalbumin
Progesterone	100.0	10.0	16.0
Deoxycorticosterone	0.90	100.0	200.0
Cortisol	≪0.01	0.7	100.0
11-deoxycortisol	0.01	0.8	44.0
17-hydroxyprogesterone	0.30	0.9	41.0
Corticosterone	0.78	2.8	190.0
Cortisone	≪0.01	<0.1	—

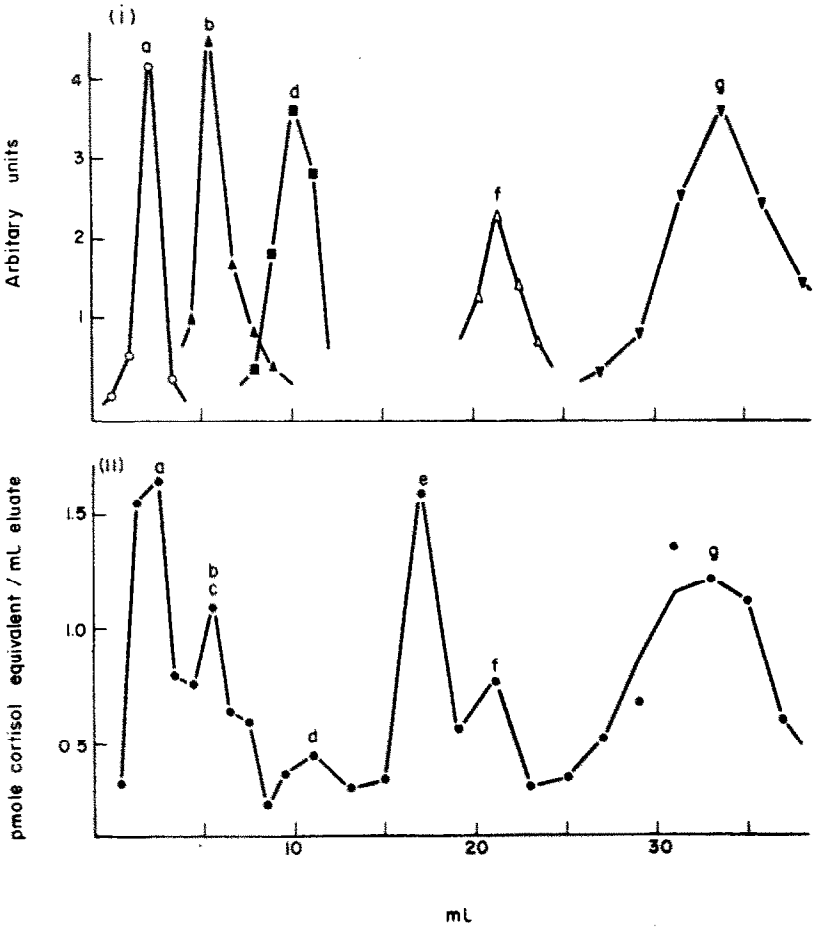

Fig. 1. The elution profiles from Sephadex LH20 of (i) pure steroids and (ii) extract of amniotic fluid. (a) progesterone, (b) deoxycorticosterone, (c) 17-OH progesterone, (d) 11-deoxycortisol, (e) corticosterone, (f) cortisone, (g) cortisol.

11-hydroxylase enzyme and the DOC to 11-deoxycortisol ratio representing indirectly the 17-hydroxylase enzyme decrease gradually during gestation.

The scatter diagram of palmitic acid against cortisol is shown in Fig. 2. The mean concentrations of cortisol and palmitic acid prior to their significant increases and the concentration of palmitic acid above which the lung can be assumed to be mature [12], are shown in the figure. There was a significant linear regression between cortisol and palmitate levels ($r = 0.46$ $P < 0.001$) indicating that the two parameters do not vary independently.

DISCUSSION

Cortisol levels, both free [9, 13], and total [8], have been shown to rise towards term. The amniotic fluid at this time will contain fetal urine and it is possible

Table 2. Concentration of corticosteroids and palmitic acid in amniotic fluid, grouped according to time of gestation (mean ± S.E.)

	Weeks of gestation				
	<33	34–35	36–37	38–39	>40
Cortisol	nM 32.5 ± 14.9	36.4 ± 7.9	37.4 ± 5.6*	50.3 ± 4.3	60.5 ± 8.9
	(n = 3)	(4)	(8)	(19)	(15)
DOC	nM 1.73 ± 0.45	0.93 ± 0.29	0.99 ± 0.11	0.92 ± 0.10	0.99 ± 0.14
	(n = 7)	(5)	(9)	(22)	(17)
11-Deoxycortisol	nM 27.6 ± 5.1	19.4 ± 5.5	19.8 ± 3.8	22.4 ± 3.5	21.9 ± 3.4
	(n = 7)	(5)	(10)	(21)	(17)
Progesterone	nM 224 ± 55	145 ± 35	101 ± 14	98.4 ± 6.5	127 ± 14
	(n = 8)	(5)	(10)	(22)	(16)
Palmitic acid	μM 54.0 ± 9.6	93.3 ± 32.5	116.0 ± 33.0	133.0 ± 23.0**	255.0 ± 65.0
	(n = 8)	(5)	(10)	(22)	(16)

* Compared with 38–40 weeks $P < 0.05$, ** Compared with >40 weeks $P < 0.05$.

Table 3. Ratios of concentrations of corticosteroids in amniotic fluid, grouped according to time of gestation (mean ± S.E)

	Weeks of gestation				
	<33	34–35	36–37	38–39	>40
Progesterone / DOC	182 ± 38 (n = 7)	199 ± 46* (5)	111 ± 10 (9)	131 ± 13 (22)	162 ± 23 (16)
DOC / 11-deoxycortisol	0.102 ± 0.057 (n = 6)	0.096 ± 0.046 (5)	0.074 ± 0.019 (9)	0.058 ± 0.011 (21)	0.053 ± 0.008 (17)
11-deoxycortisol / Cortisol	0.61 ± 0.09 (n = 3)	0.58 ± 0.29 (4)	0.53 ± 0.09 (8)	0.46 ± 0.06 (19)	0.41 ± 0.05 (15)

* Compared with 36–37 weeks $P < 0.05$.

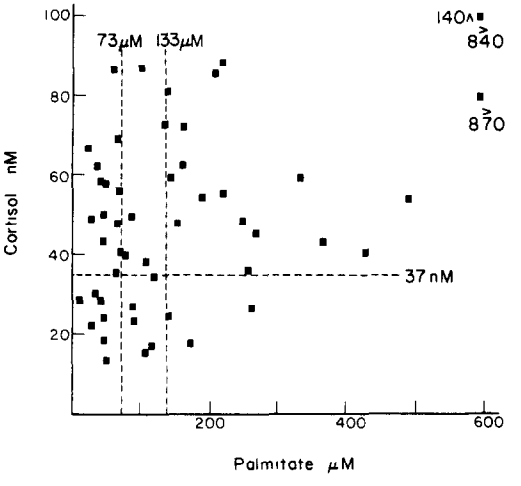

Fig. 2. Scatter diagram of amniotic fluid palmitate against cortisol concentrations. The following are indicated by broken lines:—Mean concentration of palmitic acid (38–39 weeks). Mean concentration of cortisol (36–37 weeks). Critical concentration of palmitic acid (73 μM) for lung maturity [12].

that this is a major source of amniotic fluid cortisol. As urinary unconjugated cortisol is related to the biologically active free fraction of plasma cortisol it was decided to investigate the levels of unconjugated steroids rather than total conjugated steroids.

The ratio of precursor to substrate for a number of enzymes involved in corticosteroid synthesis were calculated to obtain evidence for alteration in enzyme activity with time of gestation. The significant ($P < 0.05$) decrease in progesterone to DOC ratio after the 35th week is strongly indicative of the induction or activation of the steroid 21-hydroxylase enzyme system. It has been suggested that the same 21-hydroxylase system converts 17OH-progesterone to 11-deoxycortisol [14] which is the main pathway to cortisol synthesis. It is possible therefore, that the increase in the activity of the 21-hydroxylase after the 35th week may lead to greater cortisol production. This would account for the increased concentration found in amniotic fluid after the 37th week. The lack of any statistically significant changes with the other steroid ratios suggests that the activity of these enzymes rises gradually during gestation.

The correlation between palmitic acid and cortisol levels is similar to that reported by Sharpe-Cageorge *et al.*[8]. The mean value of palmitic acid at 36–37 weeks is double that at 33 weeks and is consistent with the occurrence of lung maturation during this period. However cortisol only rose from 32.5 to 37.4 nM during the same period. On the other hand, a significant increase in cortisol occurred at 38–39 weeks and preceded the greatest increase in palmitic acid. It is very probable that these increases occurred after lung maturation had taken place. It will also be seen from Fig. 2 that cortisol levels below the mean at 36 weeks were often associated with palmitic acid levels greater than 73 μM, indicating lung maturation [12]. But terminally mature palmitate levels were associated with low cortisol levels in only three cases. It is possible that this increase in endogenous cortisol produces physiologically the same proliferation of lung surfactant that dexamethasone has been shown to do pharmacologically. The induction of the steroid 21-hydroxylase enzyme system followed by the rise in fetal cortisol production prior to term may be important in initiating the maturation of other enzyme systems necessary for the independent existence of the fetus [10].

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