

## DETERMINATION OF CORTISOL, TETRAHYDROCORTISOL, TETRAHYDROCORTISONE, CORTICOSTERONE, AND ALDOSTERONE IN HUMAN AMNIOTIC FLUID

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### SUMMARY

Little is known about corticosteroid concentration in human amniotic fluid (AF) during the last trimester of pregnancy. 170 samples of AF were analyzed for cortisol, tetrahydrocortisol (THF), tetrahydrocortisone (THE), corticosterone and aldosterone by means of radioimmunoassay using highly specific antibodies raised in rabbits immunized by corticosteroid-21-hemisuccinate or -20-oxime bovine serum albumin (BSA) complexes. Samples were prepared in four different ways: 1:50 dilution, heating at 70°C, ethanolic and dichlormethanic extraction.

Concentration of cortisol in AF rises impressively throughout 32-40 weeks of gestation and shows a significant correlation to gestational age as well as to AF-lecithin-sphingomyelin ratio. THF, THE, showed weaker correlations to gestational age, whereas corticosterone and aldosterone did not. Some methodological remarks will be made. The problem of 6 $\beta$ -hydroxycortisol as a cross-reacting cortisol metabolite is reported. Clinical value of the results will be discussed.

Few reports of corticosteroids in amniotic fluid (AF) have appeared until today. Studies by Fencel *et al.* [1] and Murphy *et al.* [2] showed a sharp rise in cortisol concentration in amniotic fluid occurring after the 34th week of gestation. A good rank correlation has been demonstrated between cortisol and lecithin/sphingomyelin ratio. The rise in amniotic cortisol has been suggested reflects the initiation of fetal lung maturation. But up till now there is no direct evidence of cortisol being physiologically responsible for fetal lung maturation. Other corticoids have not been studied to such an extent as cortisol. In this study a survey of amniotic fluid levels of cortisol, tetrahydrocortisol (THF), tetrahydrocortisone (THE), corticosterone, and aldosterone has been made.

Amniotic fluid cortisol of 170 samples was determined by modifications of the radioimmunoassay for plasma cortisol [3]. Antibodies were obtained by immunizing rabbits with cortisol-21-hemisuccinate coupled to BSA. Cross reactions with some C-21 steroids are shown in Table 1. Other steroids like cortisol metabolites or C-19 steroids did not cross react. 25 pg could be clearly distinguished from zero level. The specificity of the antibodies allowed an assay without special purification steps.

Samples were prepared in four different ways in order to find the most simple and reliable method.

In the first procedure untreated amniotic fluid was diluted 1:50 and 100  $\mu$ l were taken for the assay. In this way the best correlation of cortisol values with gestational age was obtained (Fig. 1). Similar values

and correlation were found by the 2nd procedure in which ethanol was added to AF in order to precipitate proteins. After centrifugation the supernatant was 1:20 diluted with assay buffer and aliquots were assayed for cortisol.

There is an impressive rise of amniotic cortisol around the 34th week of gestation. Open circles lying in the lower area represent values of respiratory distress syndrome (RDS).

In the 3rd procedure samples were extracted by methylene chloride. After drying down the extracts were taken into assay buffer and aliquots were assayed. Cortisol values determined by this method were around 40-60% lower as only free unconjugated steroids were extracted from AF. We did not receive a good correlation to gestational age indicating varying portions of conjugated cortisol. Enzymatic splitting by  $\beta$ -glucuronidase followed by methylene chloride extraction of 10 samples produced slightly higher

Table 1. Cross reactions (in %) of cortisol antiserum with several steroids

Aldosterone	0.36
Cortisol	100
Desoxycorticosterone	0
Corticosterone	1.4
Progesterone	5.5
21-Deoxycortisol	10
11-OH-progesterone	0.4
18-OH-corticosterone	0
18-OH-desoxycorticosterone	0.42
6-OH-cortisol	4

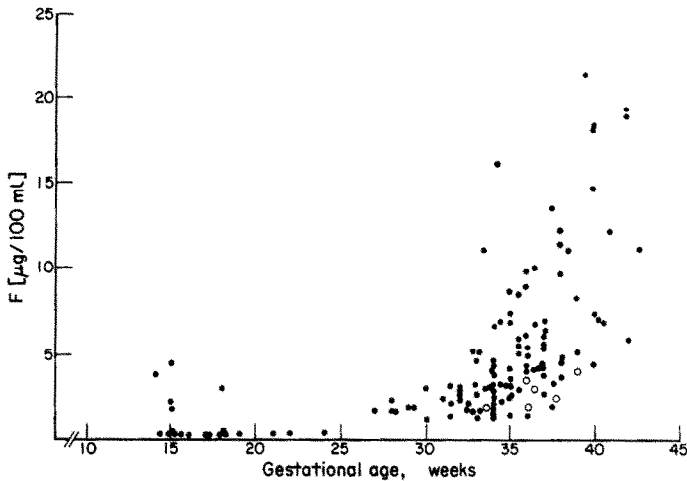


Fig. 1. Cortisol concentrations in amniotic fluid during pregnancy.  $\circ$ : cases with RDS.

values than extraction only. By acid catalyzed hydrolysis of the same samples we received values comparable with those of the 1:50 dilution procedure. Thus sulfo-conjugation seems to be more important for cortisol than glucuronic acid conjugation. Furthermore evidence is given for our antibodies cross reacting with C-21-glucuronides and C-21-sulfates.

In a 4th procedure samples were diluted 1:50 and heated to 70°C in order to denature proteins. Results of these measurements were not clearly understood as there is a correlation to gestational age but a considerable part of values were lower than the corresponding ones obtained by dilution only. There is no convincing explanation for this observation, which might be due to an oxidation of cortisol like material or to thermal solvolysis. Our assumption that 6- $\beta$ -hydroxycortisol interferes with cortisol radioimmunoassay was proved wrong. This steroid is known to occur in urine of newborns but it makes only 4% cross reaction. The development of a RIA for 6 $\beta$  hydroxycortisol is in progress [6].

In Fig. 2 the concentration of AF-cortisol is plotted against L/S ratio. The arrows point our cases with

RDS. These cortisol values did not exceed 4  $\mu\text{g}/100\text{ ml}$ . In samples with high cortisol content we normally found a high L/S ratio. However, high L/S ratios were not necessarily connected with high cortisol values. Cortisol does not appear to affect the L/S ratio directly.

THE and THF in AF were determined by specific radioimmunoassays recently developed in our laboratory [4]. Table 2 shows cross reactions of THE and THF antibodies raised in rabbits immunized by THE and THF-20-oximes coupled to BSA. Cross reactions with other tetrahydro, dihydro and allo corticosteroids as well as with cortisol, corticosterone, and cortisone were extremely low. But C-21-glucuronide conjugates of THE and THF cross reacted by 100%. The clinical value of THE and THF determination has been established by studies in various states of adrenal hypo- and hyperfunction. The assay was performed in unprocessed amniotic fluid, in which both steroids are present mainly as glucuronide conjugates [5]. THE and THF show a correlation with the duration of pregnancy similar to cortisol (Fig. 3). However, the values scatter more widely and the rise

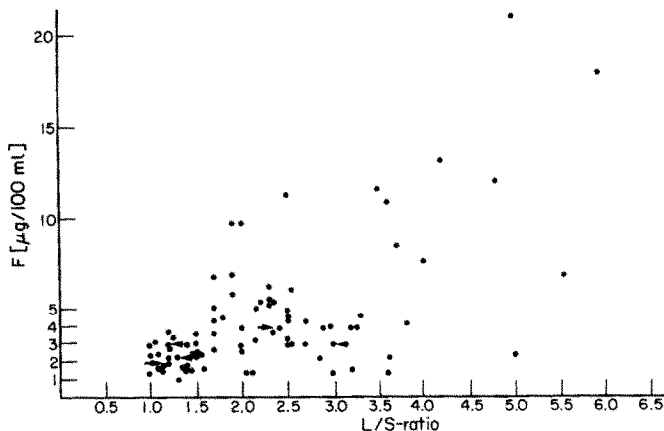


Fig. 2. Concentration of amniotic fluid cortisol plotted against L/S ratio.  $\rightarrow$  cases with RDS.

Table 2. Percent cross reaction at 50% displacement of [1,2,-<sup>3</sup>H] THE or THF

Antisera	THE	THF
Tetrahydro-cortisol (THF)	0.76	100
Tetrahydrocortisone (THE)	100	0.32
Tetrahydro-corticosterone (THB)	<0.01	1.5
Tetrahydro-deoxycortisol (THS)	0.31	3.97
5 $\alpha$ -Dihydrocortisol (DHF)	<0.01	0.05
5 $\alpha$ -Dihydrocortisone (DHE)	<0.01	<0.01
5 $\beta$ -Dihydrocortisol (DHF)	<0.01	8.18
5 $\beta$ -Dihydrocortisone (DHE)	1.1	0.02
Allo-Dihydrocortisol (ADHF)	<0.1	0.36
Allo-Tetrahydrocortisone (ATHE)	0.3	<0.01
Allo-Tetrahydrocorticosterone (ATHB)	<0.1	1.5
Allo-Tetrahydrodeoxycortisol (ATHS)	<0.1	3.97
Cortisol (F)	<0.01	0.13
Cortisone (E)	<0.01	<0.01
Corticosterone (B)	<0.01	<0.01

seems to appear somewhat earlier than the increase in amniotic fluid cortisol concentration.

There is a good correlation between THE and THF values (Fig. 4). The ratio is 4.6:1, reflecting the increased activity of 11- $\beta$ -hydroxy steroid dehydrogenase and that fetal urine is the main source of amniotic fluid. In premature neonates the THE/THF ratio ranges from 4:1 to 2:1 as shown in Fig. 5.

It shows the urinary excretion of THE and THF of 10 healthy premature neonates with gestational age of 32-36 weeks and of 10 infants with severe RDS. Both THE and THF are significantly higher in the group with RDS indicating the stress situation and

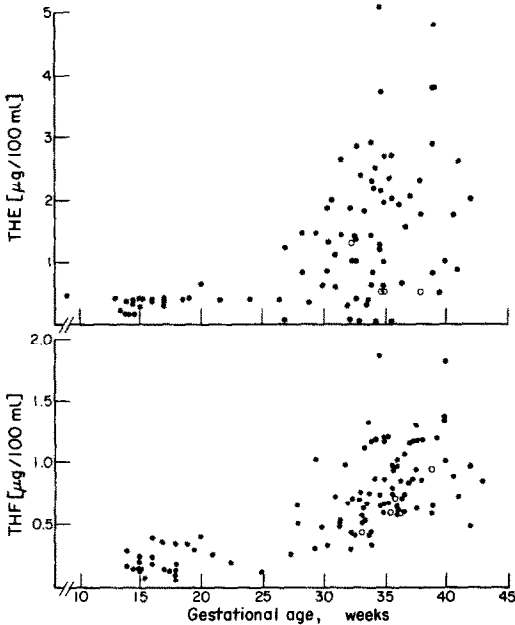


Fig. 3. THF and THE concentrations in amniotic fluid during pregnancy. O: cases with RDS.

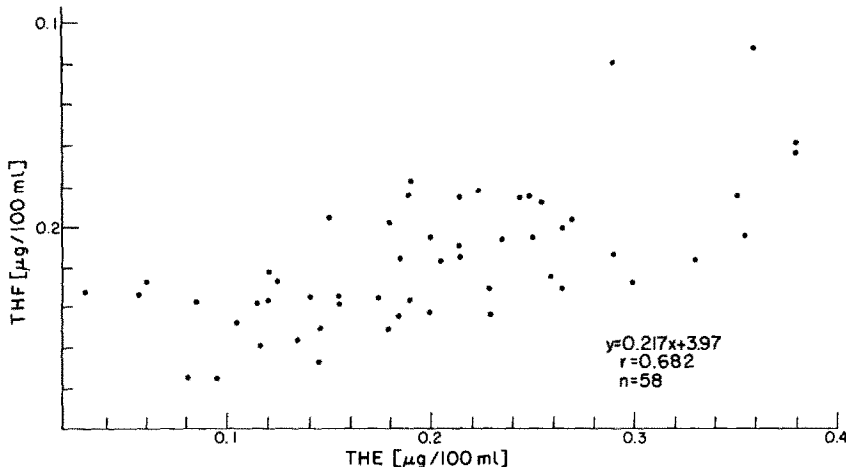


Fig. 4. Correlation between THF and THE values in amniotic fluid.

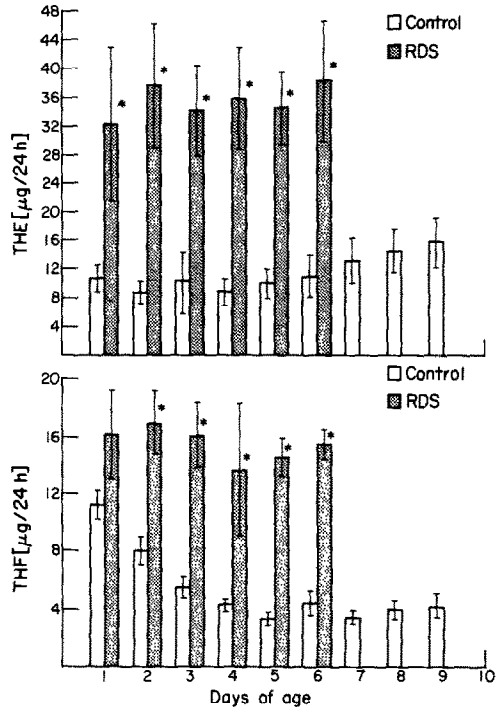


Fig. 5. Urinary excretion of THE and THF of 10 healthy premature neonates with gestational age of 32-36 weeks and 10 infants with severe RDS.

normal adrenal function of these infants. Compared with adults whose excretion rates of THE and THF range from 0.5–5.0 mg/day those of premature neonates are extremely low. THE/THF ratio in adults is approximately 1. These findings confirm the considerable differences in metabolism of steroid hormones between adults and neonates.

Corticosterone in AF was determined by our routine radioimmunoassay without chromatography [7]. Aldosterone was determined by measuring the 18-oxo-glucuronide by chromatography and radioimmunoassay as described by Vecsei in 1972 [8]. Both steroids did not show a correlation to gestational age. The range for corticosterone was 0.2–2  $\mu\text{g}/100\text{ ml}$ . Aldosterone was found between 0.1 and 1  $\mu\text{g}/100\text{ ml}$ .

Steroid measurements in AF need a careful evaluation as the content for a given steroid depends on the total vol., which is regulated by several mechanisms and which varies throughout pregnancy. The velocity of elimination of steroids from AF *via* fetal membranes depends on the kind of conjugates as shown by Katz in 1965 [9].

Our results can be summarized as follows. The rise in amniotic fluid cortisol, THE and THF during pregnancy and the finding of low values in cases of RDS are compatible with the suggestion that glucocorticoids play a role in fetal lung maturation. Best results are obtained by simple dilution of samples for the

radioimmunoassays. Cortisol in AF is mostly sulfate conjugated. There is a correlation between cortisol values and L/S ratio but the information provided by amniotic fluid cortisol determination may not be a substitute for that based on L/S ratio.

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