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CORTICOSTEROID BIOSYNTHESIS BY THE HUMAN FETAL ADRENAL: EVIDENCE FROM MEASUREMENTS *IN VIVO* AND *IN VITRO*

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SUMMARY

In late pregnancy the mean excretions of tetrahydrocorticosterone, tetrahydro-11-deoxycortisol, pregnanetriol and 17-hydroxy-pregnanolone by women pregnant with an anencephalic fetus were significantly lower than the excretion of these steroids by women pregnant with a normal fetus. In contrast, there was no significant difference between these groups of subjects in the excretion of tetrahydrocortisol, tetrahydrocortisone, cortolone (20α + 20β), cortol (20α + 20β) and tetrahydrodeoxycorticosterone. From this evidence it is concluded that the fetal zone of the human fetal adrenal near term contributes to the secretion of corticosterone and 17-hydroxyprogesterone or their precursors, but not to the secretion of cortisol (or its precursors). Radioactive cortisol was formed during incubation of radioactive progesterone and pregnenolone with adrenal tissue (largely definitive zone) from newborn anencephalic infants. In contrast, the incorporation of [^{14}C]-acetate into 4-en-3-oxo steroids by adrenal tissue from fetuses of eighteen weeks gestation could not be demonstrated. The evidence presented suggests that any synthesis of cortisol by the human fetal adrenal occurs largely in the definitive zone.

INTRODUCTION

There is now good evidence, from analysis of samples obtained through catheters implanted in the fetal circulation, that parturition in the sheep, cow and goat is preceded by increased concentrations of cortisol in fetal blood [1–5]. During the same period there is little change in the cortisol concentration in maternal blood. In these species the increase in fetal cortisol is generated by the fetal adrenal.

In human pregnancy, where direct sampling of the fetus is inadmissible, the evidence on these points is less clear. For example, neither Dormer and France[6] nor Cawson *et al.*[7] were able to show a significant concentration gradient for cortisol in the umbilical circulation. Murphy[8] reported that at 10–20 weeks gestation and at vaginal delivery at term [9] the concentration of cortisol in umbilical arterial blood was greater than that in umbilical venous blood. In contrast, Smith and Shearman[10, 11] found higher concentrations of corticosteroids in umbilical venous samples at term when compared with umbilical arterial blood.

Murphy[12] interpreted the greater concentrations of cortisol in cord serum from infants delivered after spontaneous labour when compared with delivery without spontaneous labour, as demonstrating a surge of cortisol in the fetus before normal parturition. These findings were confirmed by other workers [7].

However, there are difficulties in interpreting these results either as evidence of cortisol secretion by the fetus or as indicative of increased cortisol production by the fetus before delivery. An important reservation is that cord samples are necessarily obtained after delivery of the infant and usually after separation of the placenta. Analysis of these samples is therefore unlikely to reflect accurately steroid production by the *fetus in utero*. Labour and delivery are stressful experiences for both mother and infant and marked changes in cortisol concentrations in maternal blood occur [13–15]. Cortisol passes to the fetus at this time [16] and transfer of this steroid may be reflected in the concentration of cortisol in cord blood as emphasised by Pokoly[17] and Talbert, Easterling and Potter[18]. In addition, the concentration of cortisol in the plasma of the newborn calf was found to be much greater than that of fetal samples [5]. This increase is associated with delivery and emphasises the difficulty of interpreting cortisol concentrations in umbilical cord samples as reflecting values before delivery.

In view of these uncertainties other approaches which could distinguish cortisol secretion by the fetal adrenal would be helpful.

This paper reviews an investigation carried out for this purpose [19]. A comparison was made of the quantities of individual corticosteroid metabolites excreted, near term, by women who later delivered

Nomenclature

The systematic nomenclature is provided for steroids referred to by the following trivial names: 17-hydroxypregnanolone = 3α,17-dihydroxy-5β-pregnan-20-one; 11-deoxycortisol = 17α,21-dihydroxy-4-pregnene-3,20-dione; tetrahydro-11-deoxycortisol = 3α,17,21-trihydroxy-5β-pregnan-20-one; pregnenolone sulphate = pregnenolone-3β-yl sulphate; 17-hydroxypregnenolone = 3β,17-dihydroxy-5-pregnen-20-one; 17-hydroxypregnenolonesulphate = 3β,17-dihydroxy-5-pregnen-20-one 3β-yl sulphate.

an anencephalic infant with those excreted by women who subsequently delivered a normal infant. This experimental design follows that described by Frandsen and Stakemann[20] who demonstrated a reduced excretion of oestrogens in this condition, relative to normal pregnancy. Their observation led to the recognition of the importance of precursors from the fetal adrenal for oestrogen biosynthesis. The information obtained on the nature of the C_{21} steroids contributed to maternal urine by the fetus near term is assessed in relation to steroid biosynthesis *in vitro* by adrenal tissue from newborn anencephalic infants [21] and from normal fetuses at eighteen weeks of gestation [22].

EXPERIMENTAL

A. Corticosteroid excretion by women with a normal fetus and by women with an anencephalic fetus

A 24-hour urine specimen was obtained at about thirty eight weeks gestation from nine women with an uncomplicated pregnancy who gave birth to healthy surviving infants (mean birth weight 3.20 kg) and from nine women who subsequently delivered an anencephalic infant (mean birth weight 1.80 kg). A portion of each 24-hour urine sample was acidified to pH 2, adjusted to 50% w/v with $(NH_4)_2SO_4$ and shaken with ether/ethanol (3:1 v/v). The extract, which contains free and conjugated steroids, was incubated with β-glucuronidase (devoid of sulphatase activity) for 48-hour in sodium acetate buffer. Tritium labelled corticosteroid metabolites were added and unconjugated steroids, released during hydrolysis were extracted into ethyl acetate. Nine corticosteroid metabolites were isolated by multiple chromatography, and derivative formation. Final estimation was by the blue tetrazolium reaction (for tetrahydrocorticosterone, tetrahydrodeoxycorticosterone, tetrahydrocortisone and tetrahydrocortisol) or the Zimmerman reaction (for 17-oxosteroids derived from pregnanetriol, 17α-hydroxypregnanolone, tetrahydro-11-deoxycortisol, cortol (20α + 20β) and cortolones (20α + 20β). Manipulative losses were corrected by the measured loss of [^{3}H]-labelled tetrahydrocortisol, tetrahydrocortisone, tetrahydrocorticosterone, cortol-20α and cortolone-20α added after hydrolysis or by adjusting for material used in detection (10% for each chromatogram).

The recovery of radioactive steroids was similar from samples provided by both groups of patients, demonstrating a lack of bias in the analysis.

B. Corticosteroid biosynthesis from pregnenolone and progesterone by adrenal tissue from newborn anencephalic infants

Adrenal tissue from five newborn anencephalic infants was obtained within 0.5 h of death. Histological examination showed definitive zone tissue with only remnants of fetal zone present.

Portions of tissue (145–200 mg) were sliced and incubated in Krebs–Ringer phosphate buffer (5 ml, pH 7.3) for 5 h with either [^{14}C]-pregnenolone (1.9 μCi, 80 nmol), [^{3}H]-pregnenolone (19 μCi, 68 nmol) or [^{3}H]-progesterone (9 μCi, 68 nmol) which had been purified by paper chromatography before use. The incubations were stopped by addition of acetone (40 ml) containing pregnenolone, progesterone, 17-hydroxyprogesterone, corticosterone, deoxycorticosterone, cortisol and 11-deoxycortisol (300 nmol of each). The carrier steroids were re-isolated by sequential chromatography. Formation of derivatives was also included in the scheme of isolation. The identity of each radioactive metabolite associated with carrier steroid after the isolation process was established by crystallization to constant S.A. (after addition of 300 μmol of the appropriate carrier steroid) (Table 1).

C. Steroid biosynthesis in vitro *from [^{14}C]-acetate by adrenal tissue from fetuses of eighteen weeks gestation*

Portions of adrenal tissue (125–210 mg) from two fetuses of eighteen weeks gestation were incubated with [^{14}C]-acetate (27 or 50 μCi, 580–1010 nmol from The Radiochemical Centre, Amersham) in Krebs–Ringer phosphate buffer (pH 7.4, 10 ml) containing glucose (0.1 mol/l). Porcine ACTH (25 or 75 i.u.) was added to some incubation flasks. Boiled tissue was also incubated with [^{14}C]-acetate to serve as a control for the assessment of the purification procedures. After 3 h the tissue enzymes were denatured by addition of acetone and the following [^{3}H]-labelled carrier steroids were added to each flask: pregnenolone,

Table 1. Identification of metabolites of [^{3}H]-progesterone and [^{3}H]- or [^{14}C]-pregnenolone incubated with adrenal tissue from newborn anencephalic infants. The values shown are specific activities (d.p.m./μmol) or ^{3}H/^{14}C ratios

	Precursor							
	[^{3}H]-progesterone				[^{3}H]- or [^{14}C]-pregnenolone			
Crystallization	1	2	3	4	1	2	3	4
Metabolite								
17-Hydroxyprogesterone	1.64	1.65	1.59	1.57	146	155	150	146
Deoxycorticosterone	27.5	27.4	25.9	25.5	85	84	89	87
11-Deoxycortisol	1.18	1.15	1.18	1.18	0.040	0.043	0.044	0.045
Cortisol	12.7	12.2	12.1	11.8	11.7	10.5	10.8	11.1
Corticosterone	405	408	405	421	12.0 •	11.4	11.8	11.7

cortisol, deoxycorticosterone, 11-deoxycortisol and corticosterone. After evaporation of the acetone the contents of each flask were shaken with ether. This extract was examined for [^{14}C]-labelled non-conjugated steroids. The aqueous residue remaining after removal of unconjugated steroids was saturated with NaCl and shaken with ethyl acetate-*n*-butanol (3:1, v/v). Steroid sulphates were isolated from this extract by chromatography on Florisil [23]. Free steroids were obtained from the sulphates by solvolysis, after which [^{3}H]-pregnenolone was added to aid the characterization of any [^{14}C]-pregnenolone released from the sulphate fraction.

RESULTS

A. Corticosteroid excretion by women with a normal fetus and by women with an anencephalic fetus

The mean 24-hour excretions of each of the nine compounds assayed in the two groups of women are shown in Fig. 1 (compounds with a significant difference) and Fig. 2 (compounds without a significant difference). These data demonstrate significant reductions in the mean excretion of pregnanetriol, 17-hydroxypregnanolone, tetrahydrocorticosterone and tetrahydro-11-deoxycortisol by women with an anencephalic fetus. In contrast there was no significant difference between the two groups in the mean excretion of tetrahydrocortisol, tetrahydrocortisone, tetrahydrodeoxycorticosterone and cortol (20α + 20β). The decrease in the excretion of cortolone (20α + 20β) was at the limit of significance.

B. Corticosteroid biosynthesis from pregnolone and progesterone by adrenal tissue from newborn anencephalic infants

During the incubation [^{3}H]-progesterone was converted to 17-hydroxyprogesterone, deoxycorticosterone, corticosterone, 11-deoxy-cortisol and cortisol. Carrier steroids were shown to be free of radioactivity after incubation of [^{3}H]-progesterone with boiled adrenal tissue. This evidence demonstrates that definitive zone tissue contains 11β-, 17α- and 21-hydroxylase enzymes and has the potential for biosynthesis of cortisol, corticosterone and intermediate steroids from progesterone. The relative yields of each metabolite are shown in Table 2 and are corrected for manipulative loss by the isotope dilution principle. There was no evidence for the formation of 18-hydroxy or 16-hydroxy steroids.

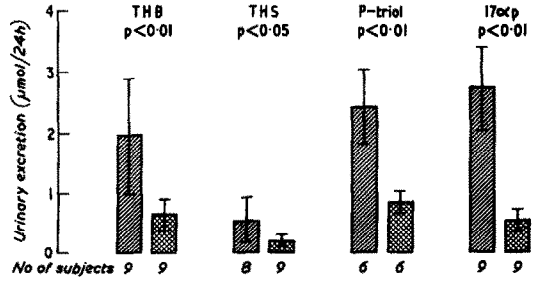

Fig. 1. Excretion of tetrahydrocorticosterone (THB), tetrahydro-11-deoxycortisol (THS), pregnanetriol (P-triol) and 3α,17-dihydroxy-5β-pregnan-20-one (17α-p) as glucuronides by women with a normal (▨) or an anencephalic (▩) fetus. Values are means in μmol/24h. Bars show ± S.D. Number of subjects and significance of differences in excretion are shown.

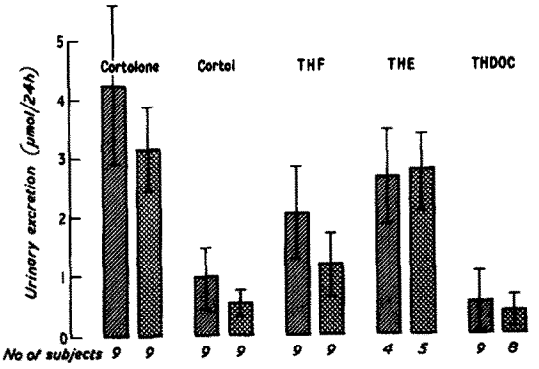

Fig. 2. Excretion of cortolone, cortol, tetrahydrocortisol (THF) tetrahydrocortisone (THE) and tetrahydrodeoxycorticosterone (THDOC) as glucuronides by women with a normal (▨) or an anencephalic (▩) fetus. Values are means in μmol/24 h. Bars show ± S.D. Number of subjects in each group is shown. There was no significant difference ($P > 0.05$) in excretion between the groups for any of these steroids.

[^{3}H]- or [^{14}C]-pregnenolone was converted to progesterone, 17-hydroxyprogesterone, deoxycorticosterone, corticosterone, 11-deoxycortisol and cortisol, during these incubations. These results demonstrate that the tissue contains a 3β-hydroxysteroid dehydrogenase-isomerase enzyme in addition to the hydroxylases noted previously. The yields of each metabolite, corrected for manipulative loss are shown in Table 2.

C. Steroid biosynthesis in vitro *from [^{14}C]-acetate by adrenal tissue from fetuses of eighteen weeks gestation*

Progesterone, deoxycorticosterone, cortisol, 11-deoxycortisol and corticosterone were re-isolated from all incubations by sequential paper chromatography. The purification process included formation of acetate derivatives or reduction with sodium borohydride where appropriate. Initially, each compound

Table 2. Yields of radioactive metabolites (% of substrate) isolated after incubation of adrenal tissue from newborn anencephalic infants with [^{3}H]-progesterone, [^{3}H]-pregnenolone or [^{14}C]-pregnenolone

	Substrate	
Metabolite	Progesterone	Pregnenolone
Progesterone	—	1.5
17-Hydroxyprogesterone	6.1	4.1
Deoxycorticosterone	21.0	8.0
Corticosterone	17.0	8.1
11-Deoxycortisol	10.9	3.0
Cortisol	7.3	1.4

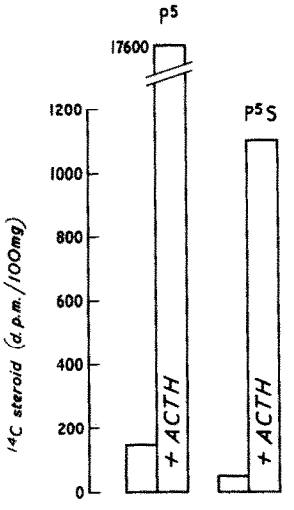

Fig. 3. Yields of [^{14}C]-pregnenolone (P^5) and [^{14}C]-pregnenolone sulphate (P^5S) during incubation of fetal adrenal tissue (eighteen weeks of gestation) with [^{14}C]-acetate, showing the effect of added porcine ACTH (25 i.u.). Yields (d.p.m./100 mg tissue) are corrected for manipulative loss and are calculated from the $^{14}C/^3H$ ratio of the last crystallization.

was associated with ^{14}C suggesting incorporation of [^{14}C]-acetate into these 4-en-3-oxo steroids. However, subsequent manipulations failed to confirm this suggestion. For example, after paper chromatography in a sequence of 4 solvent systems and treatment with acetylating reagent, ^{14}C was still associated with carrier progesterone. However, recrystallization showed a dissociation between ^{14}C and carrier (Table 3). Cortisol was purified by chromatography using four different solvent systems. The carrier steroids isolated contained only 490 c.p.m. (incubation of tissue without additions) and 350 c.p.m. (incubation of tissue with added ACTH). In view of the limited quantities of radioactivity associated with carrier at this stage recrystallization was not undertaken. Similar results were obtained for corticosterone. [^{14}C] labelled material remained with this carrier after sequential paper chromatography in four solvent systems. Recovery of carrier corticosterone at this stage varied from 22–42%. The quantities of ^{14}C associated with corticosterone were 37 c.p.m. (incubation of tissue) and 310 c.p.m. (incubation of tissue with added ACTH). In view of the minimal amount of radioactivity present recrystallizations were not carried out.

In contrast to these unsuccessful attempts to detect incorporation of [^{14}C]-acetate into 4-en-3-oxo steroids, formation of [^{14}C]-pregnenolone and its sulphate was readily demonstrated. For example, after an initial sequence of chromatographic purification, including acetylation, the quantities of ^{14}C associated with [^{3}H]-pregnenolone acetate carrier were 530 c.p.m. (incubation of tissue) and 7950 c.p.m. (incubation of tissue with added ACTH). This material, more abundant than that associated with the 4-en-3-oxo steroids at a comparable stage of purification, was recrystallised. Constant $^{14}C/^3H$ ratios were achieved (Table 3). Similarly, sufficient ^{14}C remained associated with carrier 5-pregnene-3β,20β-diol (formed by reduction of pregnenolone added after solvolysis of the sulphate fraction) for recrystallization to constant isotope ratio (Table 3). The extent of incorporation of [^{14}C]-acetate into pregnenolone and pregnenolone sulphate is shown in Fig. 3. Addition of porcine ACTH stimulated the incorporation of substrate into pregnenolone and its sulphate 100- and 20-fold, respectively. Despite this marked increase in the presence of ACTH, no incorporation of [^{14}C]-acetate into 4-en-3-oxo steroids could be demonstrated in these incubations.

DISCUSSION

Information on the nature of the corticosteroids secreted by the undisturbed human fetus is relevant to the understanding of the initiation of parturition and the cause of the respiratory distress syndrome. In view of the difficulties in obtaining blood samples from the human fetus, indirect methods must be used. One approach to this problem is to compare the quantities of corticosteroids in the urine of women with a normal fetus with those excreted by women with an anencephalic fetus, where the fetal adrenal is usually small at term due to failure of the fetal zone to develop properly. Any differences may be due to a failure of synthesis by the abnormal adrenal glands of the anencephalic fetus and, by implication, provide evidence of secretion by the adrenal glands of the normal fetus. This approach has the advantage of avoiding any stress or disturbance to the fetus. In addition, the umbilical circulation is maintained intact during sampling.

A similar experimental design has proved invaluable in understanding oestrogen biosynthesis in preg-

Table 3. $^{14}C/^3H$ ratios of crystals during recrystallization of [^{3}H]-labelled carrier steroids and associated ^{14}C after incubation of fetal tissue (eighteen weeks of gestation) with [^{14}C]-acetate.

	Crystallization				
Carrier steroid	1	2	3	4	5
Progesterone	0.122	0.098	0.086	0.061	0.029
5-Pregnene-3β,20β-diol	0.58	0.46	0.46	0.43	0.41
Pregnenolone acetate (from unconjugated fraction)	0.041	0.028	0.018	0.018	0.016

nancy [20]. Its application to the study of corticosteroid production by the fetal adrenal is more complicated since oestrogen ingestion alters corticosteroid metabolism. The alterations of corticosteroid metabolism which follow oestrogen treatment are due to synthesis of hepatic enzymes and an increased production of corticosteroid binding globulin, which in turn leads to an increased binding capacity of plasma. Women pregnant with a normal fetus excrete, near term, 1000 times the quantity of oestrogens excreted by a non-pregnant woman. Women with an anencephalic fetus excrete only 100 times that of a non-pregnant woman. The ratio of the concentrations of unconjugated oestrogen in blood are—normal pregnancy (term) 25: pregnancy with an anencephalic fetus (term) 7: non-pregnant woman (mid-cycle) 1 [24]. From this evidence both the quantity of oestrogen produced and the level of exposure of the mother to oestrogen are clearly greater in normal pregnancy than in pregnancy with an anencephalic fetus. Despite this, by term there is no significant difference in the cortisol binding capacity of maternal plasma between the two groups [24]. Differences in corticosteroid excretion in the two groups cannot therefore be explained as due to differences in plasma cortisol binding capacity, reflecting exposure to different concentrations of oestrogens. Information on the activities of hepatic enzymes in the two groups is not available.

Our earlier investigations were concerned with measurement of groups of steroids. It had already been established that the excretion of 17-oxogenic steroids, 17-deoxycorticosteroids and steroid 21-deoxyketols increase toward the end of pregnancy [25,26]. In pregnancies with an anencephalic fetus the excretion of these groups of steroids, near term, is markedly reduced when compared with that of women with a normal pregnancy [27–29]. For example, the excretion of these compounds in normal pregnancy and pregnancy with an anencephalic fetus are (mean $\pm$ S.D., μmol/24h).

17-oxogenic steroids:	46.0 ± 11.2, 25.0 ± 3.7;
17-deoxycorticosteroids:	45.0 ± 12.0, 10.9 ± 3.7;
21-deoxyketols:	4.10 ± 1.70, 1.40 ± 0.63.

The excretion of these groups of steroids by women with an anencephalic fetus and by women who had been taking oestrogen based oral contraceptives was not significantly different. It was considered reasonable to attribute the diminished corticosteroid excretion in pregnancy with an anencephalic fetus to lack of precursors rather than to an altered metabolism in that condition.

Separation of individual corticosteroids revealed reduced excretion of 17-hydroxypregnanolone and pregnanetriol (which contribute to the 17-oxogenic and the 21-deoxyketol groups), of tetrahydrocorticosterone (which contributes to the 17-deoxycorticosteroid group) and of tetrahydro-11-deoxycortisol (which contributes to the 17-oxogenic steroid group). These steroids were all found in the glucuronoside fraction, since the enzyme preparation used to hydrolyse the urinary conjugates was free from sulphatase. Consequently, the steroids measured do not include the C(21)-sulphates of corticosterone and cortisol [30] and the sulphates of 3β-hydroxy-5-en steroids which account for about 12% of steroids in late pregnancy urine [31].

It may be concluded, therefore, that the fetal zone of the fetal adrenal, near term, contributes to the excretion of 17-hydroxypregnanolone, pregnanetriol, tetrahydrocorticosterone and tetrahydro-11-deoxycortisol found in maternal urine. These compounds are unlikely to be secreted directly by the fetal adrenal and presumably arise by peripheral metabolism (reduction and conjugation) in the maternal and fetal compartments of the appropriate precursors 17-hydroxyprogesterone, corticosterone and 11-deoxycortisol. These precursors, in turn, may be secreted directly by the fetal zone of the fetal adrenal or may be secreted as the 3β-sulphates of the corresponding 3β-hydroxy-5-en steroids which are converted into 4-en-3-oxo steroids in the placenta. Our experimental design does not enable us to distinguish between these possibilities. Two pieces of evidence which bear on this point are contradictory. There is a pathway from fetal 3β-hydroxy-5-en steroids to urinary tetrahydrocorticosteroids. This was demonstrated by Reynolds *et al.*[32], who isolated, after hydrolysis with β-glucuronidase, tritium labelled pregnanetriol and 17-hydroxypregnanolone from the urine of a pregnant woman following injection of [^{3}H]-17-hydroxypregnenolone into the amniotic cavity. The corresponding 3β-sulphate would also be expected to serve as a precursor of urinary pregnanetriol following hydrolysis of the sulphate group by placental sulphatase enzymes. However, in a patient with deficiency of sulphatase enzymes for dehydroepiandrosterone sulphate, oestrone sulphate and pregnenolone sulphate, excretion of 17-oxogenic steroids and steroid 21-deoxyketols were within the ranges for normal pregnancy [29,33]. This might imply that hydrolysis of 3β-steroid sulphates is not obligatory for a continued fetal contribution to urinary corticosteroid excretion.

The secretion of corticosterone, or its 3β-hydroxy-5-en sulphate precursor, by the fetal zone of the fetal adrenal, near term, is of interest. Although measurement of corticosterone in cord blood has not yet been reported this compound has been recognised as a major secretory product in the newborn infant [34,35].

17-hydroxyprogesterone has already been recognized as a secretion product of the fetoplacental unit near term [36]. These authors concluded that a major source for production of this steroid in the placenta was 17-hydroxypregnenolone sulphate secreted by

the fetal adrenal. It was also noted that in two pregnant women with an anencephalic fetus the concentration of 17-hydroxyprogesterone in maternal plasma was only 20% that of normal pregnant women. Our findings of a reduced excretion of pregnanetriol and of 17-hydroxypregnanolone in pregnancies with an anencephalic fetus corroborates these conclusions and emphasises the importance of the fetal adrenal, and in particular, of the fetal zone in the production of 17-hydroxyprogesterone.

The secretion of 11-deoxycortisol or the corresponding 3β-hydroxy-5-en steroid sulphate, implied by our results, does not appear to have been previously reported. Eberlein[37] detected relatively low concentrations (3 μg/100 ml) of a compound with properties similar to the sulphate in cord blood. The significance of this material remains to be determined. In adults 11-deoxycortisol is considered to be an intermediate in cortisol production, rather than an end product of secretion.

There was no evidence for a diminished excretion of tetrahydrocortisol, tetrahydrocorticosterone, cortol (20α + 20β), cortolone (20α + 20β) or tetrahydrodeoxycorticosterone in pregnancy with an anencephalic fetus. It is concluded therefore, that the fetal zone of the fetal adrenal plays little part, if any, in the secretion of these urinary steroids or their precursors. Consequently, any cortisol or deoxycorticosterone secreted by the fetus must be presumed to be derived from the definitive zone.

Study of definitive zone tissue is difficult. Steroid biosynthesis by fetal zone tissue and fetal plus definitive zone tissues obtained from fetuses at mid-gestation, has been measured [38,39]. Evidence more relevant to our purpose is provided by the measurement of steroid biosynthesis in adrenal tissue from newborn anencephalic fetuses. This tissue, confirmed histologically as predominantly definitive zone tissue, had the capacity to synthesize cortisol from both pregnenolone and progesterone. Whole adrenal glands from a newborn infant delivered at term were also capable of converting pregnenolone and progesterone to cortisol *in vitro* [40].

Our results indicate that earlier in pregnancy, at eighteen weeks of gestation, whole fetal adrenals had little or no ability for cortisol synthesis from acetate, even in incubations where the incorporation of this precursor into pregnenolone and its sulphate were readily detectable. Other workers have shown minimal conversions of pregnenolone [41] or acetate [42] to cortisol at this stage of gestation. The effect of the length of gestation on the activity of the necessary enzymes is illustrated by the finding of Milner and Mills[43,44]. These authors reported increasing conversions of pregnenolone and progesterone to cortisol by whole fetal adrenal glands over the period 12–27 weeks of gestation. Histochemical evidence generally supports the findings of Goldman *et al.*[45] who demonstrated that a 3β-hydroxysteroid dehydrogenase, for dehydroepiandrosterone, appeared in the definitive zone at about sixteen weeks of gestation. This enzyme was absent from the fetal zone throughout pregnancy. The absence of 3β-hydroxysteroid dehydrogenase for pregnenolone and hydroxylated derivatives in this zone remains to be established histochemically. If this is correct then any contribution to cortisol biosynthesis from pregnenolone in the fetal zone could be ruled out. The conversions of pregnenolone [41], 17-hydroxypregnenolone [46] and 3β,17,21-trihydroxy-5-pregnen-20-one [47] to cortisol detected with whole adrenal tissue at mid-gestation would therefore be presumed to occur in the definitive zone only.

Similarly, there is an absence of reports of the isolation of cortisol after incubation of progesterone with *fetal zone* tissue isolated from mid-gestation fetal adrenals or from fetuses at term. The importance of this precursor for cortisol synthesis in this zone cannot be assessed. It is generally considered that the fetal zone has the complement of 11β-, 17α- and 21-hydroxylase enzymes necessary for cortisol synthesis although most of the evidence for this is derived from incubations containing both fetal and definitive zone tissues [48,49]. However, from our results of analysis of urine, which failed to demonstrate any contribution to cortisol secretion by the fetal zone irrespective of precursor, the role of progesterone in cortisol production by the fetal zone appears to be minimal.

It is concluded from the evidence presented in this paper that any synthesis of cortisol by the fetal adrenal occurs predominantly in the definitive zone. The potential for cortisol synthesis is low at eighteen weeks of gestation, but is readily detectable after birth. The fetal zone appears to make a substantial contribution to the secretion of corticosterone, 17-hydroxyprogesterone and 11-deoxycortisol (or their precursors).

Further work is required to substantiate these conclusions and this should include investigation of C_{21} steroid biosynthesis *in vitro* by fetal zone tissue from mid-term and mature fetuses.

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DISCUSSION

Jaffe. In the adrenal of the anencephalic after delivery, you didn't discuss with us the possibility of ACTH further stimulating cortisol production and I wondered if you had done those studies. In light of Dr. Huhtaniemi's studies and the 5-ene-steroid sulfate concentrations, would you tend to favour the 5-ene-3β hydroxysteroid precursors as the major factors in the fetal zone rather than the 4-ene-3-keto steroids?

Oakey. We haven't done similar experiments with the addition of ACTH to adrenal tissue from newborn anencephalic infants. At the time we did the experiments with this tissue we were more concerned with biosynthetic potential and used C_{21} precursors. To see an effect of ACTH one probably needs incubations with cholesterol or acetate. The fetal zone, like Dr. Huhtaniemi has said, in the human near term is biased towards the production of 5-ene-3β-hydroxysteroids. I think the possibility of a corticosterone producing pathway from progesterone in this tissue must also be considered, because tetrahydrocorticosterone was one of the compounds missing in the urine of women bearing anencephalic fetuses. It is difficult to see just how that's going to come down the 5-ene-pathway when other corticosteroids won't.

Solomon. Some years ago we perfused pregnenolone and pregnenolone sulfate. Actually we didn't perfuse we injected it into the umbilical vein and clamped the circulation at various times. When pregnenolone sulfate was injected into the fetal circulation with the fetus *in situ* it was not very metabolized to any extent as a free compound but stayed as a sulfate. When pregnenolone was injected by itself and the circulation was clamped, 20 seconds after injection 40% of radioactivity was already in the maternal circulation. Of some 17 products that were isolated, pregnenolone went to pregnenolone sulfate in a large part and then to a large number of sulfates, including dehydroepiandiosterone sulfate, 16 hydroxy dehydroepiandrosterone sulfate. But not a single one of these had a 4-ene-3 keto function. I wonder if you could comment on these studies.

Oakey. I think clearly there's a difference in approach here, in that you were perfusing the whole fetus and other tissues were liable to metabolise the substrates as well as the adrenal, whereas our studies were concerned purely with adrenal. The failure to produce the 4-ene-3-oxo steroids from pregnenolone and metabolism of that towards the sulfate pathway was what I would expect.

Solomon. I've never seen 11-oxygenated steroids in any tissue other than the adrenal. So when I get the 11-oxygenated steroids they are adrenal in origin.

Oakey. I am just a bit lost on the point that you are making for comment.

Solomon. The point is Dr. Jaffe put his finger on it earlier when he was talking. We have described the possible pathways in the fetus but we haven't done anything about saying which is more important or which is operative at this stage for this type of measurement. I agree with Dr. Jaffe, you need to do a different type of thing than has been done in the past. One needs to do quantitative studies if one has to go into the Fisher model and put it on the computer and get some production rates. But one has to do quantitative studies to find out which pathways operates at which stage. Our work largely described the pathways as we saw them in the intact fetus. Of course there are variance with other techniques, that's why I want you to comment on them.

Oakey. I think if one wants to look at pregnenolone in the fetal circulation as a possible precursor, one immediately has to ask where it's coming from. It appears to be produced by the fetal adrenal; the pregnenolone is not being sent from the placenta to the fetal adrenal for uptake and metabolism. The measurements that have been made on umbilical vein and artery steroids have shown virtually no difference in pregnenolone concentrations as distinct from pregnenolone sulfate concentrations. This steroid appears to be secreted by the fetus to the placenta. Pregnenolone appears to be present in similar concentrations in the umbilical artery and umbilical vein. This is why we have looked for intraadrenal pathways either from acetate or from cholesterol for production of steroids. In the fetal zone these pathways appear to pass through the 5-ene steroids. There is a possibility in the definitive zone for production of 4-ene-steroids and we feel that is where any cortisol is being produced.

Jaffe. Is there anything inconsistent with the following scheme in terms of your data: the 5-ene-steroids are utilized in the adrenal for further metabolism both to other unconjugated 5-ene steroids and to sulfurylated 5-ene steroids. The 4-ene-3 keto steroids that you find are products of placental progesterone and the excretory products that you find in the urine derive from 4-ene-3 keto steroids precursors which are all formed in the placenta from 5-ene 3β hydroxysteroids from the fetus, including your unique metabolite.

Oakey. There is nothing in our data I think that wouldn't go along with it. We've always said that our measurements, made on maternal urine, cannot tell us whether the cortisol is being produced by the fetal adrenal. What we have been looking at is essentially a combination of the fetus and the placenta and the opportunity both of them have for metabolism. The tetrahydro steroids that we find missing in the women with an anencephalic fetus may be derived, in normal pregnancy, from fetal 5-ene steroids which pass to the placenta for conversion to 4-ene-3-oxo steroids and are then reduced.

Jaffe. Our superfusion studies, in which cortisol was produced and subsequently augmented by ACTH in the absence of exogenous precursors, would suggest either cortisol was being formed in the adrenal from endogenous precursors such as placental precursors or *de novo* in the adrenal.