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ANDROGENS IN THE HUMAN FETUS

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SUMMARY

Umbilical cord blood and tissue were obtained at hysterotomy from human fetuses of 8–20 weeks gestation along with maternal peripheral blood. Umbilical cord and maternal blood were also obtained from healthy newborn babies and their mothers after spontaneous delivery. The samples were analyzed by competitive binding to human sex steroid-binding globulin to determine the total levels of unconjugated 17β-hydroxysteroids and, after column chromatography, testosterone (T). Androgen levels of maternal serum (20 ng/ml) both in mid-pregnancy and at parturition were much higher than those of non-pregnant women (1·8 ng/ml) but T was only slightly elevated (0·98 vs 0·37 ng/ml). Cord serum levels of total androgens in both male and female fetuses did not differ from maternal levels at the time of parturition but were lower in early gestation. Male cord serum at 8–18 weeks showed a higher level of T (3·7 ng/ml) than serum from females, newborn males or mothers. Testicular androgens were greatly elevated (2900 ng/g) compared with adrenal (214 ng/g or ovarian (272 ng/g) levels. The bulk of this material in the testis was testosterone. Thus the mean testicular level (1730 ng/g) was about 500 × the serum T level (3·7 ng/ml). There was relatively little material identifiable as T in the ovaries of any of the female fetuses studied (< 40 ng/g) or in the adrenal glands of fetuses of either sex (148 ng/g). It is concluded that the human fetal testis undergoes a period of intense secretory activity during early gestation but is relatively quiescent at birth.

INTRODUCTION

Very few studies to date have documented endogenous androgen blood or tissue levels in the human fetus. The first report of testosterone (T) being identified in the fetus came from Finland in 1970, when Huhtaniemi *et al.*, isolated several steroids (including T) from a homogenized pool of 33 fetal testes[1]. At the 1972 International Congress of Endocrinology. Reyes *et al.* [2, 3] and our own laboratory[4] reported finding T in individual fetal testes. We also noted high levels of testosterone-like material in male fetal blood[4].

MATERIALS AND METHODS

Subjects

Blood and tissues from normal human fetuses were obtained at elective hysterotomy performed at 5·5 to 19 weeks' gestation to achieve therapeutic abortion in subjects requiring sterilization. Maternal blood was taken just before or during operation. The gestational age expressed in weeks from the date of conception was derived from the weight and crown-rump length of the fetuses. One gonad from each fetus was fixed and stained for a histological determination of sex, while the remaining gonad and adrenals were weighed and put into ethanol. Maternal and umbilical cord samples were also obtained after normal delivery at term.

Materials

Tritiated steroids (S.A. 30–60 μCi/mmol) were obtained from the New England Nuclear Corp. and were used without purification. On arrival they were diluted to 10 μCi/ml with redistilled ethanol, and were stored at −10°C. Purity was checked by Sephadex LH-20 column chromatography[5]. Unlabeled steroids were obtained from the Sigma Chemical Co., diluted with redistilled ethanol, and stored at −10°C. Solvents were Fisher reagent grade.

Methods

The steroids were extracted from serum samples with 2 vol. diethyl ether and tracers of T and dihydrotestosterone (DHT) (about 10 pg each) were added. Tissues were weighed, homogenized in ethanol and the supernatant was evaporated to dryness under air and assayed directly.

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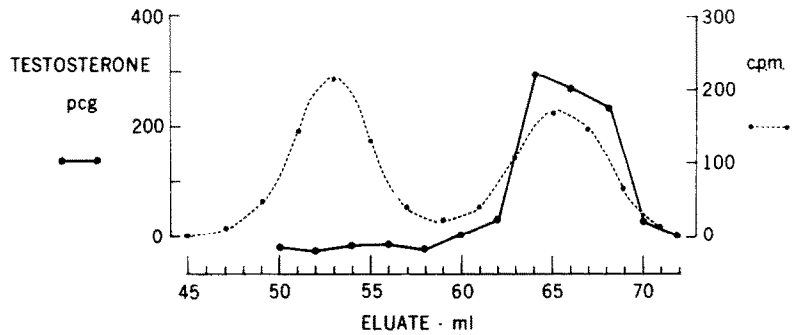

Fig. 1. Elution pattern of testosterone (63–69 ml) and dihydrotestosterone (49–57 ml) tracers added to 0·5 ml fetal serum. Alternate 1 ml samples were counted for radioactivity or assayed for binding to SSBG. The peak of binding activity corresponded exactly with the peak of the testosterone tracer.

For determination of total 17β-hydroxysteroids in early studies, the extracts were washed with NaOH to remove the phenolic steroids; however, since in most cases the estrogenic substances did not contribute significantly to the assayed level of androgens, the alkali wash was abandoned. Total 17β-hydroxysteroids were determined using competitive protein-binding (CPB) to the sex steroid-binding globulin (SSBG) of late pregnancy human plasma as described previously[6] but with the modification that the assay was performed in the presence of 0·5 g/l. gelatin. This technique measures unconjugated testosterone, dihydrotestosterone, androstanediols and androstenediols[6].

Fractionation was carried out on columns of 60 cm length, 0·8 cm dia., using Sephadex LH-20 and an organic solvent Methylene chloride–Heputane–Ethanol–Water. 50:50:1:6) as described previously[5]. Alternate 1 ml fractions were counted to obtain the pattern of radioactive tracers and to estimate recovery. The remaining aliquots corresponding to the T peak were assayed individually. Testosterone was determined by the same CPB method made more sensitive by decreasing the incubation vol to 0·1 ml and using 0·125 g % charcoal suspension as the adsorbent. Extensive studies in our laboratory[6–8] have indicated that the only known steroid which is eluted in the region of T in our chromatographic system and which can bind (about 2% compared to testosterone) to SSBG is dehydroepiandrosterone (DHEA). To eliminate the possibility of overlap with this steroid the pattern of the T peak was compared with that of the radioactive steroid in each sample as shown in Fig. 1. Although DHEA could be demonstrated to be present when large amounts of newborn serum (3·0 ml) were chromatographed, there was no detectable interference in the small amounts of fetal serum used (0·5 ml). DHT was clearly separated.

RESULTS

1. *Total androgens in serum*

Levels of total androgens for male and female fetuses and newborns and for their mothers are compared with those for adult men and non-pregnant women in Table 1. Maternal levels were extremely high both early in gestation and at delivery. Cord blood levels did not differ from maternal levels at the time of parturition ($P > 0{\cdot}05$) but maternal levels were higher than those for both male ($P < 0{\cdot}01$) and female ($P < 0{\cdot}001$) fetuses in early gestation. Levels in cord blood and maternal blood were considerably higher than those found in men ($P < 0{\cdot}001$) or non-pregnant

Table 1. Total androgens in serum

Source of serum		(n)	mean ± S.D. (ng/ml)
Early gestation	Maternal	14	20·3 ± 10·3
	Male fetus	13	11·1 ± 4·9
	Female fetus	6	8·0 ± 1·3
Newborn	Maternal	43	22·0 ± 15·0
	Male newborn	23	16·8 ± 7·7
	Female newborn	21	19·0 ± 7·0
Young adult	Male	50	6·5 ± 1·7
	Female (non-pregnant)	15	1·8 ± 0·6

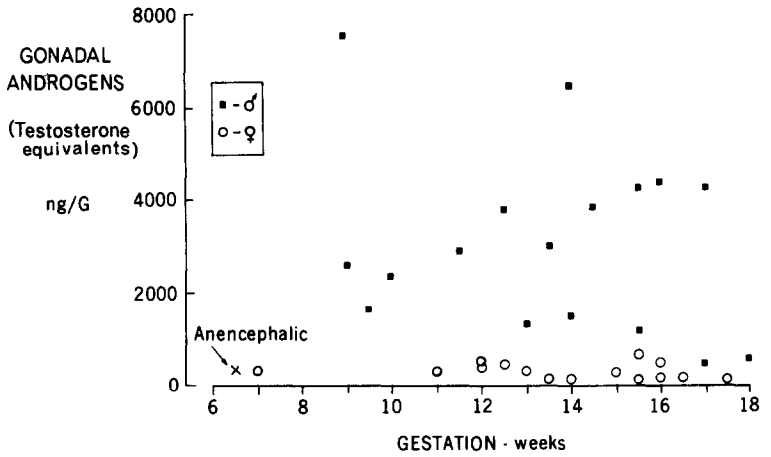

Fig. 2. Gonadal androgens in relation to gestational age.

Table 2. Testosterone levels in serum

Source of serum		(*n*)	mean ± S.D. (ng/ml)
Early gestation	Maternal	7	0·98 ± 0·37
	Male fetus	9	3·70 ± 0·92
	Female fetus	6	<0·96 ± 0·27
Newborn	Male	6	<0·8
	Female	7	<0·5
Young adult	Male	15	5·25 ± 2·21
	Female	8	0·37 ± 0·26

women ($P < 0·001$). Levels in newborn males ($P < 0·01$) and females ($P < 0·001$) were higher than in early gestation presumably due to increased androgens of adrenal origin.

2. *Testosterone levels in serum*

As shown in Table 2, testosterone levels in the serum of male fetuses (3·70 ng/ml) were comparable to, though somewhat lower ($P < 0·05$) than, those of normal men (5·75 ng/ml) and considerably higher than those of the mothers or female fetuses ($P < 0·001$). Testosterone levels of mothers and both male and female newborns were uniformly low by comparison ($P < 0·001$). The discrepancy between total androgens and testosterone levels in umbilical cord blood was much greater at term than in early gestation.

3. *Total androgen and testosterone levels in fetal tissues*

From Table 3, it may be seen that total androgen levels were considerably higher ($P < 0·001$) in the testis than in the ovary or adrenal. Testosterone made up the bulk of the material measured as total androgens in the testis but was not detected with confidence in the ovary. Adrenal levels were relatively low. The relationship of testicular androgen levels with gestational age is shown in Fig. 2. High levels were observed as early as 9 weeks and as late as 17 weeks. The lowest levels in males were seen at 17 and 18 weeks.

DISCUSSION

There is a large amount of material which binds to SSBG in the maternal and fetal circulation throughout pregnancy. Only a small amount of this is accounted for as testosterone in the blood although testosterone accounts for most of that found in the fetal testis and adrenal. The testicular ovarian and adrenal testosterone levels found in this study are in general agreement with those of Reyes *et al.*[3]. The serum testosterone levels in newborns are compatible with those found by Forest *et al.*[9].

The androgen material in serum which is not testosterone is probably mainly of adrenal origin. Only a small amount at term can be accounted for as DHEA. On

Table 3. Androgen levels of fetal tissues in early gestation (ng/gm)

	Total androgens		Testosterone	
	(*n*)	mean ± S.D.	(*n*)	mean ± S.D.
Testis	18	2900 ± 2040	5	1730 ± 1250
Ovary	16	272 ± 168	4	< 40
Adrenal	9	214 ± 136	4	148 ± 107

Sephadex LH-20 column chromatography several large peaks in the region of adrostenedione and the androstanediols were assayed and these remain to be identified. Increased adrenal function at the time of birth[12] probably accounts for the higher levels in the newborn compared with early gestation.

The high serum testosterone levels found in male fetuses indicate that the testosterone formed in the testes is secreted into the fetal circulation. This observation coupled with the *in vitro* demonstration by Kelch *et al.*[10] of rapid conversion of $T \rightarrow$ dihydrotestosterone in fetal perineal skin and other tissues and the morphological observations of Jost *et al.*[11] strongly suggest that fetal testosterone production plays an important role in human sexual differentiation.

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