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STUDIES ON STEROIDOGENESIS AND ITS REGULATION IN HUMAN FETAL ADRENAL AND TESTIS

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SUMMARY

Endogenous neutral steroids and tropic regulation of their formation were studied in the adrenals and testis of human fetuses of early and mid-gestation. The steroids were analyzed using gas–liquid chromatography and gas chromatography–mass spectrometry. In the adrenals, pregnenolone, pregnenolone sulfate and dehydroepiandrosterone sulfate were present in highest concentrations. No 3-keto-4-ene steroids were found. Adrenal incubations demonstrated active endogenous formation of dehydroepiandrosterone sulfate. Thus, production of sulfated 3β-hydroxy-5-ene steroids was found to be quantitatively the dominating steroidogenic function of the fetal adrenal *in vivo* and in incubations. In adrenal tissue cultures, sulfated 3β-hydroxy-5-ene steroids were also the main metabolites synthesized on days 0–5. During days 6–11 steroid formation decreased. However, when ACTH was added to the media, differentiation of adrenal definitive zone cells and increase in steroid formation were observed. Both 3-keto-4-ene and 3β-hydroxy-5-ene steroids were produced during ACTH stimulation, and cortisol was the major steroid formed. Fetal testes contained several unconjugated and sulfated neutral steroids, with highest concentrations of testosterone and pregnenolone. In testicular incubations, hCG stimulated endogenous biosynthesis of testosterone. Maximal response was achieved with concentrations of 50 mIU/ml and above. These observations provide further evidence of active androgen formation by the testis of early and mid-term fetuses and for the possible role of hCG in its regulation.

INTRODUCTION

The active role of the fetus in steroid production of the human fetoplacental unit is well established (for a recent review see [1]). Extensive information on the fetoplacental steroid metabolism has been obtained through a variety of *in vitro* incubation and perfusion experiments, using labeled steroids and other precursors. The relative contribution of the metabolic pathways observed in these *in vitro* studies to the steroid metabolism *in vivo* has been difficult to determine.

The human placenta produces a number of tropic hormones throughout gestation. In addition, the maturation of hormone synthesis and secretion of the fetal pituitary occurs during the first and second trimesters of pregnancy [2, 3]. Significant concentrations of these placental and pituitary tropic hormones have been demonstrated in the fetal circulation [2–5]. However, their role in the regulation of fetal endocrine gland development and function is poorly understood.

We expected that determination of endogenous steroid levels in fetal tissues, coupled with observations about the metabolism of these steroids, would increase our understanding of *in vivo* steroid metabolism of the fetus. For this purpose, we developed a method for the identification and measurement of endogenous neutral steroids in fetal tissues, using gas–liquid chromatography and gas chromatography–mass spectrometry [6]. This paper summarizes the results of endogenous steroid determination in fetal adrenals and testes, as well as of observations about the *in vitro* metabolism of these steroids. Furthermore, in order to better understand the regulation of fetal endocrine glands, we studied the effect of ACTH on the differentiation and steroidogenesis of fetal adrenal cortex in tissue culture and the effect of hCG on fetal testicular testosterone production in incubations.

EXPERIMENTAL

Tissue. Fetuses (10–20 weeks gestational age) were obtained from interruptions of pregnancies for sociomedical reasons. Adrenal and testicular endogenous steroid determinations [6–8] and adrenal *in vitro* studies [9, 10] were performed with tissue from fetuses delivered by abdominal hysterotomy. Testicular incubations [11] were performed with tissue obtained following prostaglandin-induced pregnancy terminations.

Analytical procedures. A detailed description of the gas–liquid chromatographic (g.l.c.) and gas chromatographic–mass spectrometric (g.c.–m.s.) methods used for the analysis of endogenous steroids in adrenals and testes and for the steroids formed by the adrenal *in vitro* studies has been presented previously [6, 12]. In brief, the procedure was as follows. Tissue homogenate, incubation or culture medium was extracted with acetone/ethanol (1:1, v/v). The dried residue was chromatographed on Sephadex LH-20 and fractions of unconjugated, mono- and disulfated steroids were obtained. The unconjugated steroid fraction was purified in solvent partition between 0.1 M sodium hyd-

roxide and ethyl acetate and the sulfate conjugates were solvolyzed. Final purification of the steroid fractions was carried out by silicic acid chromatography. The compounds were then converted to either trimethylsilyl or methoxime-trimethylsilyl ethers and analyzed by g.l.c. and g.c.–m.s.

Testosterone was measured in fetal testicular incubations with radioimmunoassay, described in more detail elsewhere [13]. In short, duplicate samples of incubation medium were extracted with diethyl ether, as were the testosterone standards used for the standard curve. No further purification of the samples was done. An antiserum against testosterone-3-oxime-bovine serum albumin was used. Of the structurally related steroids studied, the antiserum has a 70% cross reactivity with 5α-dihydrotestosterone. Since no endogenous 5α-dihydrotestosterone was detected in fetal testes [8], the possible contamination of testosterone values by this steroid is negligible.

In vitro *experiments.* The tissue culture procedure has been described elsewhere [10, 14]. Both explant and suspension cultures were made. The medium was changed every fifth day during cultivation. ACTH was added to the culture dishes at a rate of 0.1 IU/ml/day on days 6–11. Pooled culture media were analyzed for neutral steroids by g.l.c. and g.c.–m.s. (see above) and the cells grown were prepared for electron microscopic study (see [10]).

In vitro incubations of the adrenal and testis were carried out as follows: Minced tissue was incubated in Krebs–Ringer phosphate (adrenal) or bicarbonate (testis) buffer, both containing 0.1% glucose, for 3 or 4 h in +37°C in an atmosphere of 95% O_2/5% CO_2. A single piece of the adrenal tissue was taken for endogenous steroid determination prior to the incubation and after the incubation the medium and tissue were analyzed for neutral steroids. The testis tissue was preincubated for 30 min in an excess of buffer. Thereafter, the medium was changed to one containing 0, 0.5, 5 or 50 ng/ml of NIH–hCG (10,000 IU/mg). Aliquots of the medium were taken for testosterone radioimmunoassay at 1, 2 and 3 h of the incubation. No exogenous substrate was added to the media in the *in vitro* studies.

Table 1. Endogenous neutral steroids in a pool of human fetal adrenals (gestational age 12–20 weeks). The concentrations are expressed as μg/100 g wet tissue [6, 7]

Unconjugated:		
pregnenolone		180
17-hydroxypregnenolone		130
	Total	310
Monosulfates:		
dehydroepiandrosterone		130
16α-hydroxydehydroepiandrosterone		63
pregnenolone		560
17-hydroxypregnenolone		130
	Total	883

RESULTS

Adrenal and testicular endogenous steroids [6–8]. Four 3β-hydroxy-5-ene steroids were found in fetal adrenal tissue (Table 1). The C_{21} steroids, pregnenolone and 17-hydroxypregnenolone, were present both in the unconjugated and monosulfated forms. The C_{19} steroids, dehydroepiandrosterone and 16α-hydroxydehydroepiandrosterone, were present as monosulfates only. Pregnenolone sulfate was present in highest concentration, 560 μg/100 g wet tissue. Neither cortisol nor other 3-keto-4-ene steroids were detected.

In the pool of testes analyzed (Table 2) the unconjugated steroid fraction was quantitatively the most significant, containing pregnenolone and testosterone in high concentrations. The major monosulfates in this tissue were dehydroepiandrosterone and its 16α-hydroxylated derivative. Disulfates were found in trace amounts only.

Adrenal incubations [9]. Active steroid production of adrenal minces was observed without any exogenous substrates. The changes in fetal adrenal endogenous steroid concentrations during the *in vitro* incubations are depicted in Fig. 1. The concentrations of the two unconjugated steroids, pregnenolone and 17-hydroxypregnenolone, decreased while those of their sulfate conjugates and especially of the third sulfate conjugate, dehydroepiandrosterone sulfate, increased. The concentration of the latter steroid increased 10–60-fold during the 4 h incubation. No 16α-hydroxydehydroepiandrosterone sulfate, detected in the pool of adrenal tissue (Table 1), was observed in the individual tissue samples of these experiments. Only traces of unconjugated dehydroepiandrosterone and progesterone, and no cortisol, were found in the incubations.

Adrenal tissue culture [10]. The steroid patterns detected in the media of two fetal adrenal cultures

Table 2. Endogenous neutral steroids in a pool of human fetal testes (gestational age 12–24 weeks). The concentrations are expressed as μg/100 g wet tissue [8]

Unconjugated:		
testosterone		170
androstenedione		18
pregnenolone		180
	Total	368
Monosulfates:		
dehydroepiandrosterone		45
16α-hydroxydehydroepiandrosterone		66
pregnenolone		24
5-pregnene-3β,20α-diol		22
	Total	157
Disulfates:		
5-androstene-3β,17α-diol		2.6
5-androstene-3β,17β-diol		1.6
	Total	4.2

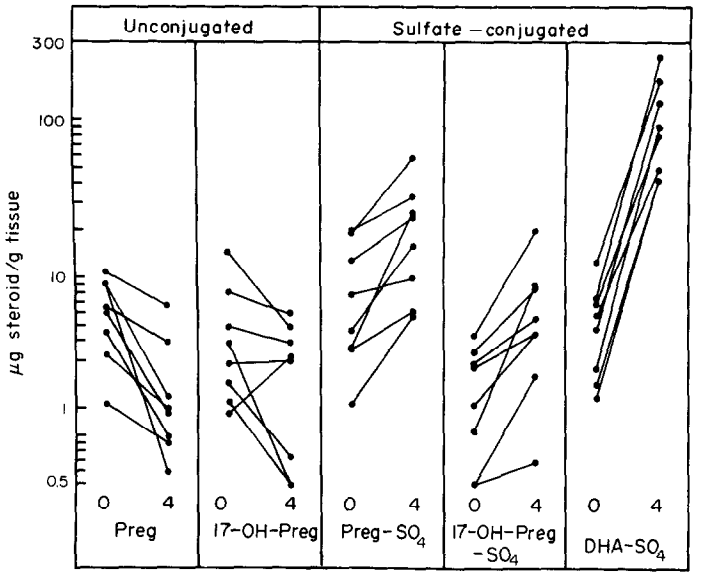

Fig. 1. Formation of neutral steroids from endogenous precursors in human fetal adrenals *in vitro* (fetal gestational ages 10–18 weeks). 0 = endogenous steroid concentration per g tissue prior to the incubation, 4 = steroid concentration in the tissue and medium per g tissue after 4 h incubation, Preg = pregnenolone, 17-OH-Preg = 17-hydroxypregnenolone, DHA = dehydroepiandrosterone, SO_4 = sulfate-conjugate [9].

are depicted in Table 3. Unlike the endogenous steroids in the adrenal tissue or those detected after incubation of minced tissue, the steroids formed in tissue culture included both 3β-hydroxy-5-ene and 3-keto-4-ene steroids. The two steroids found in highest concentration were dehydroepiandrosterone sulfate and cortisol.

Distinct changes were seen in the steroid patterns of the culture media with time. During the first 5 days of culture, a dominant part of the steroids were monosulfated 3β-hydroxy-5-ene steroids (Table 3), dehydroepiandrosterone sulfate being the major metabolite. During subsequent 5 days, there was a clear decrease in the total concentration of all steroids. However, when ACTH was administered to the 6–11 day cultures, a dramatic increase was seen in the concentration of each steroid. This increase was particularly pronounced with cortisol and other 3-keto-4-ene steroids. Compared with values on days 0–5, the concentration of cortisol increased 30–200-fold.

Also, morphologically distinct changes were seen in the tissue during culture (for a detailed description, see [10]). In the beginning, two different cell types were seen: fetal zone cells and definitive zone cells. No significant growth was seen in the fetal zone cells.

Table 3. Neutral steroids secreted into the culture medium by human fetal adrenal cortical cells grown in tissue culture. The amounts are as ng/ml medium [10]

Steroids identified	Experiment I*			Experiment II*		
	0–5 days culture	6–11 days culture	6–11 days culture + ACTH†	0–5 days culture	6–11 days culture	6–11 days culture + ACTH†
Unconjugated:						
Androstenedione	100	29	40	31	21	230
11β-hydroxyandrostenedione	37	—	1700	—	—	400
Dehydroepiandrosterone	180	38	490	100	61	120
17-hydroxyprogesterone	—	—	1600	—	—	180
17-hydroxypregnenolone	29	32	350	28	37	540
Cortisol	110	200	740	48	49	1900
Total	459	299	12,380	207	168	3370
Monosulfates:						
Dehydroepiandrosterone	1500	15	2400	1000	83	1300
5-androstene-3β,17α-diol	25	—	100	6.0	10	25
Pregnenolone	380	11	1100	200	71	920
17-hydroxypregnenolone	160	4.2	500	69	9.0	500
Total	2065	30.2	4100	1275	173	2745

* The fetal gestational age was in Experiment I 14 weeks and Experiment II 16 weeks.
† ACTH was added 0.1 IU/ml medium/day days 6–11.

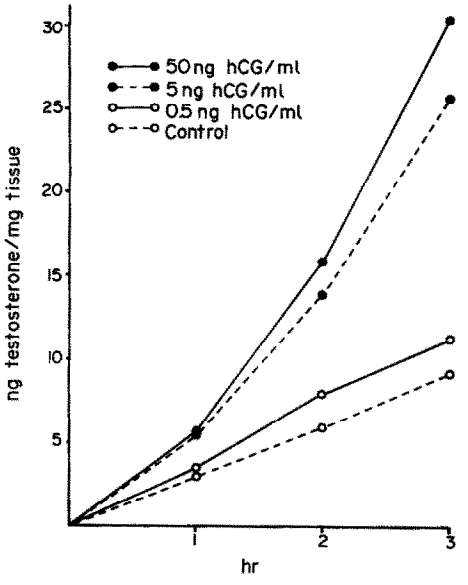

Fig. 2. Effect of different concentrations of hCG on endogenous testosterone production of minced human fetal testis (fetal gestational age 16 weeks). Each point represents the mean of duplicate incubations [11].

Definitive zone cells, similar to adult adrenal zona glomerulosa cells, seemed to be the only cortical cells growing in the culture. ACTH induced a differentiation of these cells into the zona fasciculata type.

Testicular incubations [11]. Aliquots of fetal testicular tissue were incubated in medium containing 0, 0.5, 5 and 50 ng of hCG/ml. Preincubation of the tissue for 30 min was found to be necessary to see differences between the control and hCG stimulated samples. As Fig. 2 shows, a clear dose-related response was seen in testicular testosterone production. The steepest increase in testosterone output was usually seen between hCG levels of 0.5 and 5 ng/ml. Between the two highest doses (5 and 50 ng hCG/ml) the increase in testosterone production was minimal. Maximal output of testosterone in hCG stimulated samples varied from 10–30 ng/mg tissue/3 h. In controls, testosterone was formed at a rate of 2–9 ng/mg tissue/3 h.

DISCUSSION

All endogenous steroids detected in the human fetal adrenal had a 3β-OH-5-ene structure (Table 1). Furthermore, the bulk of these compounds were sulfate-conjugated. These findings agree with previous observations about a relative lack of 3β-hydroxysteroid dehydrogenase [15, 16] and about the active sulfate conjugation [17] of fetal tissues. No cortisol was found in our study. Murphy and Diez d'Aux [18] have shown recently that the concentration of cortisol in fetal adrenal tissue is of the order of 20 μg/100 g tissue, which is for this steroid at the limit of detection of the g.l.c. methods used.

Both circulating steroids coming from the mother, placenta and other fetal tissues and steroids formed *de novo* by the adrenal [19–21] can contribute to the endogenous steroid formation. The purpose of the adrenal *in vitro* incubations was to examine the origin and routes of formation of the endogenous steroids detected. The concentrations of the two unconjugated steroids, pregnenolone and 17-hydroxypregnenolone, decreased during the incubation while those of the sulfate conjugates and especially of dehydroepiandrosterone sulfate increased (Fig. 1). No unconjugated dehydroepiandrosterone was found. One explanation for this is that the synthesis of dehydroepiandrosterone sulfate from adrenal endogenous precursors proceeds via sulfated intermediates, *i.e. via* pregnenolone sulfate and 17-hydroxypregnenolone sulfate. *De novo* steroid sulfate biosynthesis [20] and direct conversion of steroid sulfates to other steroid sulfates [22–24] have been demonstrated in fetal tissues *in vitro*. This would also agree with the theory of Telegdy *et al.* [21] suggesting that steroids formed *de novo* in the fetus are mainly metabolized via sulfated intermediates while exogenous steroids show a preponderance of the unconjugated pathway.

No endogenous 3-keto-4-ene steroids could be detected in adrenal tissue nor could their formation be demonstrated in the incubations. This would suggest that formation of sulfated 3β-hydroxy-5-ene steroids quantitatively plays a dominant role in human fetal adrenal steroidogenesis. The possibility of biosynthesis of corticosteroids cannot, however, be excluded, but their synthesis evidently needs either exogenous steroidal precursors having a 3-keto-4-ene structure, most likely placental progesterone, and/or the presence of a tropic stimulus.

Distinct differences were seen between the adrenal endogenous steroid pattern and that found in the tissue culture media (Tables 1 and 3). The nature of steroid conjugation was the same in both cases; both unconjugated and monosulfated steroids were found. The clearest difference was that all the endogenous steroids detected had a 3β-hydroxy-5-ene structure whereas a number of 3-keto-4-ene steroids also were present in the culture media. The distribution of 3β-hydroxysteroid dehydrogenase in fetal adrenal and definitive zone cells could be an explanation for this finding. The fetal zone, which lacks this enzyme [15, 16] and is thus unable to synthesize 3-keto-4-ene steroids, forms the main part of the fetal adrenal cortex *in vivo* [25]. In tissue culture, the new generation of cells has characteristics of the adult zone cells which are known to have an active 3β-hydroxysteroid dehydrogenase system [15].

Also in adrenal cultures, the 3β-hydroxy-5-ene steroids were dominant in the beginning (days 0–5 of cultivation). These steroids were most likely formed by the fetal zone cells which were still present in the flasks during the first days of culture. During days 6–11 the steroidogenic capacity of the cells decreased. The decreased steroidogenic capacity was reflected

also in the morphology of the tissue as the cells grown were ultrastructurally fairly undifferentiated, of the adult zona glomerulosa type. ACTH induced the differentiation of these cells to a type reminiscent of the adult zona fasciculata. High steroidogenic capacity of these cells was evident both from their ultrastructure (see [10]) and from the number and amount of steroids detected in the culture media. Particularly striking was the active production of cortisol and other 3-keto-4-ene steroids. However, the cells also produced high concentrations of sulfated 3β-hydroxy-5-ene steroids.

Our observations suggest that the adrenal cortex of early and mid-term human fetuses, which consists mainly of the fetal zone, actively synthesizes sulfated 3β-hydroxy-5-ene steroids. The major end-product of this metabolism is dehydroepiandrosterone sulfate, an important fetal precursor of placental estrogen biosynthesis. The definitive zone cells were the only adrenal cortical cells growing in tissue culture. These cells were able to produce cortisol and other 3-keto-4-ene steroids as well as 3β-hydroxy-5-ene steroids. Their differentiation from a steroidogenically inactive form to actively steroid producing cells was observed to be dependent upon the effect of ACTH.

The endogenous steroid pattern of fetal testes included both 3β-hydroxy-5-ene steroids and 3-keto-4-ene steroids (Table 2). Testosterone was one of the main compounds detected. The testis also was the only fetal tissue in which we could find endogenous 3-keto-4-ene steroids [6–8, 26, 27]. These findings agree with previous observations that there is a peak of 3β-hydroxysteroid dehydrogenase activity in fetal testes during early and mid-pregnancy, and that this enzyme is lacking nearly completely in other fetal tissues during the same period [15, 16, 28]. Analysis of testosterone concentration in individual fetal testes has shown recently that it reaches its peak levels in 11–13 week old fetuses [29]. At the same gestational age, fetal testes are able to form testosterone *de novo* from acetate [30] and their capacity to convert radioactive pregnenolone and progesterone to testosterone has its maximum [31]. This period of testicular activity is coincident with the differentiation of male genitalia for which testicular androgens are necessary [32].

Since pregnenolone and dehydroepiandrosterone, but no progesterone, were present in the testes, the 5-ene-pathway, *via* pregnenolone and dehydroepiandrosterone, and not the 4-ene-pathway, via progesterone, may be the route of fetal testicular testosterone formation *in vivo*. Based on fetal perfusion studies, Mathur *et al.* [33] reached the same conclusion. The function of such a pathway in human fetal testis has been demonstrated *in vitro* [34, 35].

The coincidence of peak levels of hCG in the maternal and fetal circulation and the active steroidogenic phase of fetal testes suggest that hCG may be the main tropic stimulus of fetal testoterone synthesis. In addition, levels of LH in the fetal circulation at this time are low [3, 5]. Qualitatively, the response of fetal testicular testosterone synthesis to high concentrations of hCG has been demonstrated in incubations with radioactive tracers [36] and in tissue culture [37]. Using physiological concentrations of hCG, we observed that a clear increase in the output of testosterone was achieved *in vitro* with hCG concentrations of 5 ng/ml or more (Figure 2). Since mean concentration of hCG in the circulation of 12–20 week old fetuses is about 35 ng/ml (1 ng = 10 mIU) [5], testicular steroidogenesis seems to be under very active tropic stimulation *in vivo*. This is further supported by our inability to observe a clear difference in testosterone formation between controls and hCG stimulated incubations unless the tissue samples were preincubated in an excess of buffer. The data suggest that this procedure may remove a part of the hCG bound to the testis *in vivo*.

No exogenous substrate was used in the incubations. Since the testis contains only limited amounts of possible C_{19} and C_{21} steroid precursors for testosterone formation (Table 2), it seems that the tissue is able to form testosterone either *de novo* or from sterol precursors. *De novo* synthesis of testosterone from acetate has been previously demonstrated in fetal testes *in vitro* [30].

Thus, in the testicular experiments we demonstrated the ability of early and mid-term fetal testis to form testosterone. Testes form this androgen possibly through a 5-ene-pathway, thus excluding placental progesterone as the intermediate. Furthermore, testicular testosterone biosynthesis was clearly stimulated by physiological levels of hCG *in vitro*.

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DISCUSSION

De Hertogh. What was the age of the fetus from which you studied the adrenals? Secondly, have you studied the effect of ACTH during the first five days of culture, instead of the 6th to 11th day period, when apparently the formation of the delta five compounds has diminished quite a lot?

Huhtaniemi. The ages of the fetuses used in this study varied from 15–20 weeks of gestational age. Within this narrow age range and with the small number of individual adrenals cultured we could not find any changes of steroidigenesis correlated to fetal age. We have not yet studied the effect of ACTH during the first days of culture but these studies are in progress.

De Hertogh. The reason for this question was to know whether immediately after the culture is begun there may be a more important stimulation of dehydroepiandroster-

one sulfate than 5 days afterwards, when, as you showed, cortisol is secreted in very large amounts. It is a question of survival of the cells involved in DHEAS secretion in comparison with those which are involved in cortisol secretion.

Huhtaniemi. During the first 5 days of culture DHAS secretion is high without added exogenous tropic hormones. A possible explanation for this is that the endogenous stimulation of these cells is sufficient to maintain steroidogenesis. Later during cultivation the stimulation seems to be lost and dedifferentiation of the cells and disappearance of their active steroidogenesis are observed. It is possible that administration of ACTH to the culture media from the beginning of cultivation could counteract this dedifferentiation and maintain the high DHAS production longer. This is an important question since we do not know yet whether ACTH has effect on fetal zone cells present during the first days of culture. We could not find any growth of the fetal cells in our cultures and they had disappeared completely from the dishes until the fifth culture day. ACTH seemed to stimulate only the growth of adult zone cells, when administered during 6 to 11 culture days. A possible explanation for this is that both the fetal and adult zone cells are formed from the same undifferentiated stem cell. ACTH stimulates their differentiation to adult zone cells. The fetal zone cells may need in addition to ACTH an inhibitor of some of their enzyme systems (e.g. 3β-hydroxysteroid dehydrogenase). Such inhibited cells would look morphologically like fetal zone cells. The placental hormones (e.g. estriol or progesterone) could be such inhibitors. For this reason the fetal adrenal cortex would consist *in vivo* mainly of fetal zone cells but ACTH alone in culture could not stimulate their growth.

Grenier. Have you looked at the possibility of any synthesis of estradiol 17β by the fetal testis.

Huhtaniemi. No, we haven't.

Grenier. This might be an interesting point since at puberty estradiol-17β response to HCG seems a better index of complete endocrine maturation of the testis than testosterone response (Scholler *et al., J. steroid Biochem.* **6** (1975) 95–99). So I was wondering if the fetal testis had a capacity to synthesize estradiol 17β?

Huhtaniemi. As far as I know, no estrogen formation in testes of early and mid-term human fetuses has been demonstrated. In fetal sheep testes Attal (*Endocrinology* **86** (1969) 280) found endogenous estrone and estradiol much later during gestation than he was able to find testosterone.

Tamaoki. In your last slide, you mentioned formation of testosterone and *other androgens* after incubation. What are the *other androgens*?

Huhtaniemi. I did not mean results from the incubations but from the endogenous steroid determinations. The other androgens present endogenously in human fetal testes were androstenedione and dehydroepiandrosterone. In the incubation studies we measured only testosterone formation.

Tamaoki. Is there any possibility that you may have found the other 5α-reduced androgens among these C-19 steroids that you mentioned?

Huhtaniemi. Siiteri and Wilson (*J. clin. Endocr. Metab.* **38** (1974) 113) have performed fetal testicular incubations using pregnenolone and progesterone as substrates and they could not find conversion of these substrates to 5α-reduced androgens. So if you mean 5α-dihydrotestosterone, there evidently is not formation of this androgen in human fetal testes.

Tamaoki. As you may know, after the birth and in immature state, activity of 5α-reductase in the testes was remarkably high, being almost comparable with the activity in the prostate of adult animal. Therefore, I am wondering whether the activity of 5α-reductase in the fetal testes is quantitated in your experiment or not.

Huhtamiemi. In the data of Siiteri and Wilson they showed that there is no 5 alpha reduced androgens, during the first 3 months of gestation at least.

Osawa. I wonder if you detected 20α-hydroxy- and 20,22-dihydroxycholesterol and also the 22-hydroxy compound which is supposed to be the precursor for pregnenolone biosynthesis in your analysis.

Huhtaniemi. No, we could not have detected them since our chromatographic method separates C_{27} and C_{21} and C_{19} steroids into different fractions and we measured only compounds in the fraction of C_{21} and C_{19} steroids.

Pasqualini. You say that cortisol came only from the 4-ene-pathway. Did you check if 17,21-dihydroxypregnenolone can be converted to cortisol?

Huhtaniemi. This possible intermediate of cortisol formation was not detected but as your studies have demonstrated (Pasqualini *et al., Biochim. biophys. Acta* **162** (1968) 648; *Acta endocr.* **63** (1970) 11), 3β-hydroxy-5-ene-steroids having either 17α and/or 21-hydroxyl group can be converted to 3-keto-4-ene-steroids by human fetal adrenals.

Gustafsson. Would you care to speculate about any possible physiological role of 3β-hydroxy-5-ene steroids in sulfated form secreted by the fetal adrenal, in addition to acting as precursors?

Huhtaniemi. I do not think that other functions than being precursors for placental estrogen synthesis have been demonstrated for fetal adrenal steroid sulfates. They can also be substrates for steroid metabolism of other fetal tissues. As far as I know, steroid sulfates themselves do not have biological effects.