Journal of Steroid Biochemistry, 1977, Vol. 8, pp. 537–544. Pergamon Press. Printed in Great Britain

THE GONADOTROPHINS AND THEIR SUBUNITS IN FOETAL PITUITARY GLANDS AND CIRCULATION.

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SUMMARY

Specific radioimmunoassays and column chromatography on Sephadex G-100 have been used to assess the changes in foetal pituitary content and serum concentration of LH, FSH and LH β-subunit and the common α-subunit during gestation. All pituitary glands contained free α-subunit and intact LH. The free α-subunit: LH ratio reached a peak between 10 and 14 weeks of gestation and decreased to adult ratios by term. No FSH was detected in pituitary ($n = 7$) extracts from 9.5–12.5 weeks while FSH was detectable in all foetal pituitaries at term. No significant sex difference in intact hormone or subunit concentrations in the pituitary was seen. In contrast to these results the pituitary of an anencephalic foetus (36 week of gestation) contained mainly free α-subunit.

During early gestation foetal blood levels of LH-HCG, FSH and free α-subunit were significantly higher than at term. The immunoreactive LH-HCG consisted of both LH presumably from foetal pituitary origin and HCG.

These results suggest that during foetal development the first gonadotrophin substance synthetized in the foetal pituitary gland is the common α-subunit. Under hypothalamic influence the synthesis of the β-subunit then takes place leading to the production of the intact gonadotrophins.

INTRODUCTION

The pituitary glycoprotein hormones, luteinizing hormone (LH), follicle stimulating hormone (FSH), thyrotrophin (TSH) and human chorionic gonadotrophin (HCG) produced by the placenta during pregnancy all consist of two non-identical smaller components, designated α- and β-subunit [1,2]. The α-subunit has almost identical protein structure in all the hormones within any species; in contrast, the β-subunit is characteristic of a particular hormone and varies in structure from hormone to hormone [3–5].

The adenohypophysis develops early in embryonic life from an evagination of the ectoderm, Rathske's pouch, which extends towards the base of the brain where it undergoes proliferation. The presence of basophil cells with the capacity to synthetize and store protein hormones soon after the anatomical formation of the human anterior pituitary gland at 8 weeks of gestation has been demonstrated by various methods [6–8].

Immunoreactive LH and FSH have been detected in foetal pituitary extracts as early as 9.5–10 weeks of gestation—the youngest foetuses studied [9, 10]. Furthermore, from the 16th to 40th week of gestation foetal pituitary tissue cultured *in vitro*, secretes, in molar terms, about 200 times as much of α-subunit as intact LH and FSH, and only small amounts of the LHβ subunit [11]. That a considerable amount of gonadotrophic substance in the foetal pituitary gland is the common α-subunit has been confirmed by Hagen and McNeilly[10].

In this presentation we will review our findings and the evidence for the existence of free subunits in the human foetal pituitary gland and circulation. In addition, the physiological implications of these findings on the development of the hypothalamic-pituitary control mechanism will be discussed.

Radioimmunological determination of various proteins implies the use of different heterogenous standards, therefore, comparison of concentrations of different hormones will always be arbitrary. In addition, the limitation in measuring the hormone content of an endocrine gland and not the synthesis and secretion and the accuracy of the assessment of foetal age will affect the results.

MATERIALS AND METHODS

Pituitary glands: Twenty foetal pituitary glands were obtained from patients undergoing hysterotomy during pregnancy at the age of gestation of 9.5–32 weeks, and pituitaries were removed within 30 min of incision of the uterus. Extraction of pituitary glands were performed as described [10].

Serum measurements: Blood samples were collected at the time of delivery from 26 foetal cord arteries and veins (period of gestation 37–41 weeks; 16

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Table 1. The specificity of the radioimmunoassays for LH, FSH, LHα and LHβ.

Hormone or subunit preparation	Assay*			
	LH	FSH	LHα	LHβ
LH IRC2/69 (Dr. A. S. Hartree)	100	<1	8	25
HCG CR115 (Dr. R. E. Canfield)	100	<0.1	4	0.2
FSH CPDS6 (Dr. W. R. Butt)	2	100	1	<1
LHα 4.1.72 (Dr. A. S. Hartree)	25	<1	100	1
HCGα CR115 (Dr. R. E. Canfield)	12	<1	100	<1
LHβ 18.10.72 (Dr. A. S. Hartree)	60	<1	<1	100

* Percentage inhibition.

females and 10 male foetuses) and from 17 early foetuses (period of gestation 10–16 weeks; 5 female and 11 male).

Chromatography. Pituitary extracts and serum samples were chromatographed on a Sephadex G-100 column (1.5 × 90 cm). The column was standardized with LH IRC2/69 (Dr. A. Stockell-Hartree), LHα (Dr. A. Stockell-Hartree) and HCG CR 115 (Dr. R. E. Canfield) as described [10, 12].

Radioimmunoassays. Serum LH, FSH, LHβ and the common α-subunit were measured by double antibody radioimmunoassays [10, 12, 13]. The specificity of the radioimmunoassays are shown in Table 1. In the LH assay equipotency on a weight basis of LH IRC2/69 (Dr. A. Stockell-Hartree) and HCG CR 115 (Dr. R. E. Canfield) was found. All results are expressed in ng of LH IRC2/69 (1 ng LH IRC2/69 = 2 mU MRC 68/40). In the FSH assay, FSH MRC 69/104 (assuming 10 I.U./ampoule) was used as standard but all results are expressed in ng FSH CPDS6/ml (1 ng FSH CPDS6 (immunopotency 5.000 I.U./mg) = 5 mU FSH 69/104). In the α-subunit assay LHα 1.4.72 (Dr. A. Stockell-Hartree), FSHα N611C (Dr. A. F. Parlow), TSHα N785B (Dr. A. F. Parlow) and HCGα CR 115 (Dr. R. E. Canfield) were equipotent on a weight basis. In the LHβ assay the LH preparation IRC2/69 caused significant displacement of I^{125} labelled LHβ subunit when it was present at concentrations > 4 ng/ml serum.

RESULTS

Pituitary extracts. Immunoreactive LH and α-subunit were detected in pituitary extracts as early as 9.5 weeks of gestation—the earliest extracts studied. However, the amount of LHβ subunit measured in the 7 extracts from 9.5–12.5 week old foetuses could, in 5 of the extracts, be due to crossreaction of LH in the LHβ assay. No FSH was detected in the 7 extracts from 9.5–12.5 week old foetuses.

In Fig. 1 the ratio between LH and the subunits in 19 foetal pituitary extracts and the values of 4 normal adult extracts are shown.

The α-subunit to LH ratio seems to reach peak values between the 10th to 14th week of gestation, and then to decrease towards term. In contrast to the adult pituitary extracts in which the amount of LH on a weight basis was double that of the α-subunit, the foetal extracts contained significantly ($p < 0.01$) more α-subunit than LH. No significant change in the ratio of LH:LHβ subunit could be detected from the 9.5–32 weeks of gestation. The adult pituitaries contained 100 to 1000 times more LH and α-subunit than pituitaries obtained from foetuses of 9.5–11.5 weeks. Pituitary extracts from three older foetuses (19.5–32 weeks) showed hormone and subunit ratios similar to those of the adult extracts.

Unlike normal pituitary extracts, the extract from an anencephalic foetus (age of gestation 36 weeks) contained predominantly α-subunit, but also significant amount of LH with an α-subunit:LH ratio of

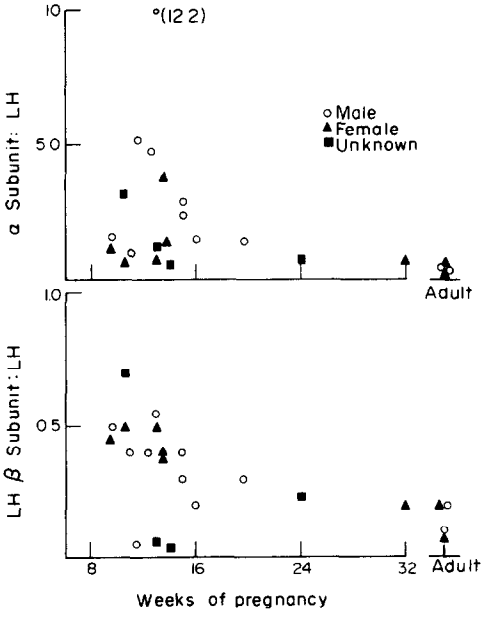

Fig. 1. The ratios of α-subunit: LH and LHβ subunit:LH in foetal and adult pituitary extracts.

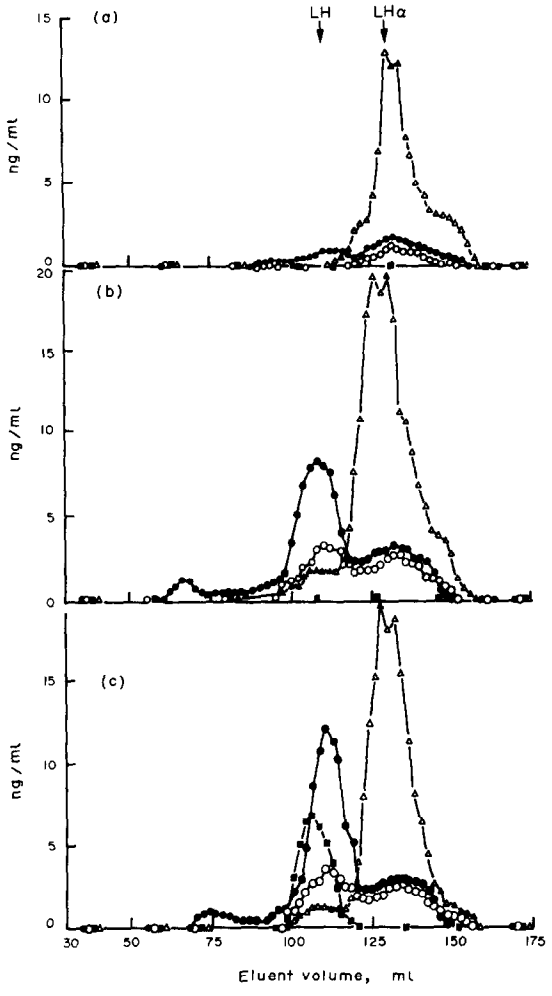

Fig. 2. Elution pattern after column chromatography on Sephadex G-100 of pituitary extracts from a 9.5-week old male (a), a 13.5-week old female (b) and a 32-week old female (c) foetus. All fractions were assayed by specific radioimmunoassays for LH (●), FSH (■), LHβ- (○) and α-subunits (△). The peaks for standard hormone preparations LH (68/40) and LHα subunit (A. Stockell-Hartree) are indicated (↓) at the top. (From *J. Endocr.* page 54, 1975).

7.8. No FSH could be detected. The ratio of LHβ:LH was 0.20, which is within the normal foetal range (0.02–0.7).

It has been confirmed by gel filtration that a considerable amount of gonadotrophic substance in the foetal pituitary gland is a molecular species recognized both immunologically and physically as the common α-subunit (Fig. 2). After column chromatography the ratio between LH and the α-subunit in the pituitaries in molar terms seems to decrease from about 1:30 at 9.5 weeks of gestation to 1:2 at 32 weeks.

All foetal pituitary extracts of more than 12 weeks of gestation contained not only a form of LH which behaved immunologically and physically like standard LH, but also a molecular species which eluted before LH, but reacted in the LH assay [10]. Whether

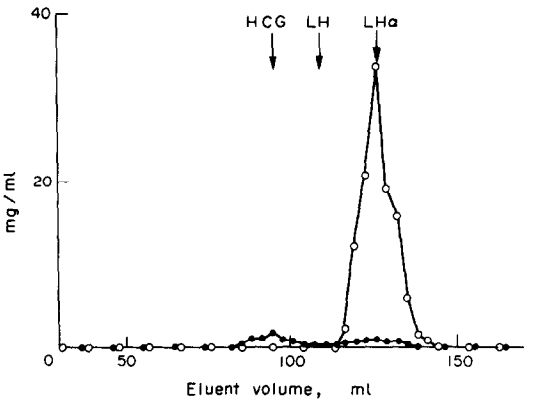

Fig. 3. Elution pattern after column chromatography on Sephadex G-100 of foetal cord serum at term. All fractions were assayed by specific radioimmunoassays for LH-HCG (●) and α-subunit (○). The peaks for standard HCG (CR 115), LH (68/40) and LHα subunit (A. Stockell-Hartree) are indicated (↓) at the top.

this 'big LH' is aggregated LH, LH bound to proteins or quite another form of LH remains to be seen. Only one form of α- and LHβ-subunit could be recognized in these extracts.

Foetal serum levels. As in the pituitary gland a molecular species, which behaves both immunologically and physically as the common α-subunit, is found in the foetal circulation (Fig. 3). Although a wide range of values is found, higher blood levels of α-subunit occur at the age of 10–16 weeks than at term (Fig. 4). The α-subunit concentrations in the foetal circulation is 50–500 times higher than those seen in adult premenopausal women and men. Low to undetectable levels of the LHβ subunit was found (Fig. 5). This might be due to crossreaction of LH-HCG and α-subunit in the LHβ-assay.

The concentrations of LH-HCG and FSH in the foetal circulation varies, but higher levels of these hormones were measured between the 11.5 to 16 weeks of gestation than at term (Figs. 6 and 7). The levels of FSH at term are within the range of FSH measured in premenopausal women.

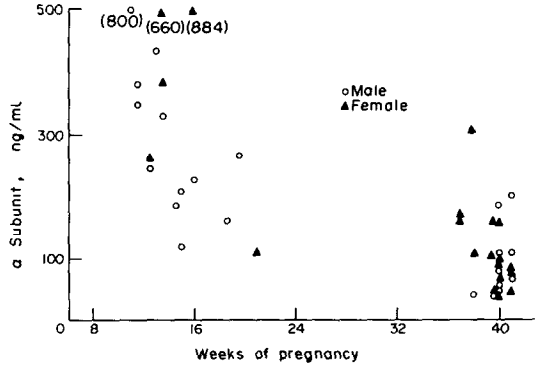

Fig. 4. The levels of α-subunit in the circulation of 17 early foetuses (age of gestation 11 to 21 weeks) and in cord veins of 26 foetuses at term are shown. The concentrations of the subunit were measured by radioimmunoassay.

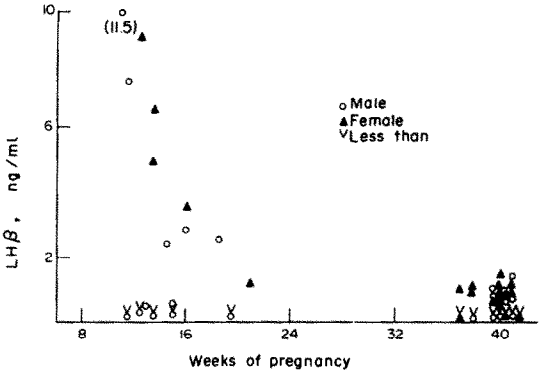

Fig. 5. The levels of LHβ subunit in the circulation of 17 early foetuses (age of gestation 11 to 21 weeks) and in cord veins of 26 foetuses at term are shown. The concentrations of the subunit were measured by radioiommunoassay.

Immunoreactive LH-HCG in foetal serum seems to consist of both LH of foetal origin and HCG from the placenta. Firstly, the levels of LHβ subunits obtained in the foetal circulation represented only 2% of the LH levels recorded, and with the known 25% crossreaction of LH in the LHβ assay, the foetal LH must consist of molecular species other than normal LH. Secondly, foetal serum at term contains a molecular species similar to HCG when examined by gel filtration and immunoreactivity, while only a small fraction is similar to standard LH (Fig. 3).

DISCUSSION

Pituitary content. The proposition that free α-and β-subunits in pituitary extracts are not due to dissociation of the intact hormones has been discussed in detail [10, 14, 15].

Immunoreactive LH and FSH have been detected in foetal pituitary extracts as early as 9.5 to 10 weeks of gestation—the youngest foetuses studied [10].

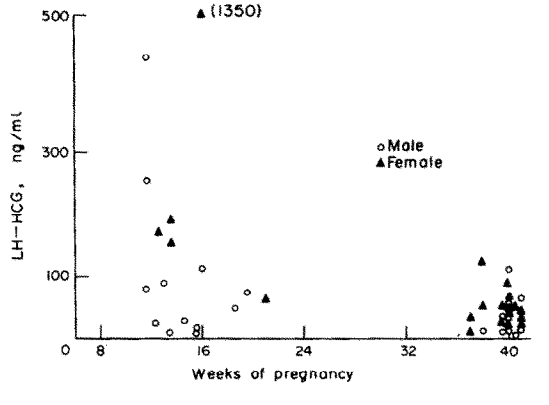

Fig. 6. The levels of LH-HCG in the circulation of 17 early foetuses (age of gestation 11 to 21 weeks) and in cord veins of 26 foetuses at term are shown. The concentrations of the hormone were measured by radioimmunoassay.

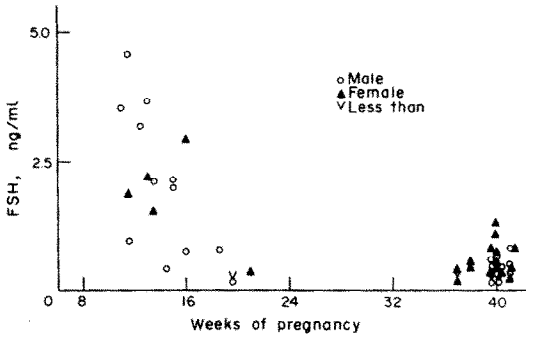

Fig. 7. The levels of FSH in the circulation of 17 early foetuses (age of gestation 11 to 21 weeks) and in cord veins of 26 foetuses at term are shown. The concentrations of the hormone were measured by radioimmunoassay.

Siler-Khodr *et al.* [16] demonstrated LH and FSH activity in foetal pituitary tissue of 5–7 weeks of gestation cultured *in vitro*; therefore, the onset of gonadotrophin synthesis seems to precede the anatomical development of the pituitary gland. Immunoreactive α-subunit and LH were present in all pituitary extracts studied (period of gestation 9.5–32 weeks), however, the α-subunit: LH ratio after gelfiltration seem to decrease with advancing age (Fig. 2). This indicates that the synthesis of the common α-subunit precedes that of the intact hormones.

Both the content and the amount of LH released in culture from the foetal pituitary gland increase with gestational age to a maximum between the 20th and 30th weeks of gestation, thereafter decreasing towards term [9, 16]. Before the 20th week of gestation no sex difference could be detected in the amount of the gonadotrophins released from the pituitary gland or in their content. However, from the 20th to 30th week of gestation female pituitaries contained and released more of the gonadotrophins than male [9, 16]. No sex difference in the ratio of α-subunit to LH was recorded, indicating that female pituitaries also contained more of the α-subunit than male. Unlike normal pituitary extracts the extract from an anencephalic foetus at 36 weeks of gestation contained predominantly α-subunit and hardly any of the intact hormones [10]. This agrees with the approximately 2% of LH and FSH found in the pituitary gland of an anencephalic foetus compared with the glands of normal newborn infants [9].

Serum levels. Circulating subunit levels are not due to the dissociation of intact hormones. This has been verified in adults by the infusion of purified LH in normal subjects and the observation that this was not followed by a rise in α- and LHβ-subunit [17]. Furthermore, it has been shown that the handling of the sample has no influence on the result. This has been demonstrated on serum samples obtained from postmenopausal women in which the levels of LH, FSH and α-subunits were compared both immediately and after having been left at 4°C or 37°C for one week (Hagen, unpublished observation). It has also been

demonstrated in tests carried out for the purpose of comparing the levels of TSH, TSHβ- and α-subunits after multiple freezing and thawing of a single serum sample [18].

The highest levels of LH-HCG and FSH in the circulation during foetal life occur when the content of gonadotrophins in the pituitary gland is increasing i.e. between 12 and 20 weeks of gestation [9, 19]. The present results confirm these reports since higher blood levels of both intact hormones (LH and FSH) and α-subunit occurred in early foetal life (age of gestation 10–16 weeks) than at term (Figs. 4, 6 and 7). However, due to the relatively small number of samples, it was not possible to confirm [9, 19, 20] the higher levels of FSH in the circulation of female than male foetuses. Foetal LH-HCG is not influenced by the sex of the foetus.

The blood concentration of the glycoproteins is dependent on the rate of secretion, their distribution within the body and their metabolism. There is no information on these parameters in the foetus. In adult males the initial half life of LH is from 21–60 min [21, 22] and that of the LHα and LHβ subunit from 14–17 min [22]. If the values for the half lives of subunits and intact hormones measured in adults apply to the foetus as well, the high circulating levels of α-subunit compared to the intact hormones found in the foetus indicate that the secretion of α-subunit in molar terms is 5–10 times that of LH.

Transfer of glycoproteins across the placental barrier. The observation that the respective concentrations of LH-HCG and α-subunit in maternal blood at term are 200 and 20 times higher than those of foetal blood [12, 23] indicate that the intact hormones and their subunits are transferred between maternal and foetal circulation in only small amounts if any. Secondly, the α-subunit levels in the mother increase steadily during pregnancy [23], whereas the levels in the foetus are lower at term than at the 10th to 16th week of gestation. Thirdly, no difference in the levels of the intact glycoprotein hormones or their subunits are seen between foetal cord artery and vein (Table 2) and no correlation between hormone or subunit levels in the maternal and the foetal circulation have been demonstrated [12]. On the other hand, most LH-HCG found in the foetal circulation at term is of a molecular species similar to HCG when examined by gel filtration and immunoreactivity while a small fraction is similar to LH [12, 24]. However, whether this is true HCG or LH of a bigger molecular species than normal LH remains to be seen. In addition, no physical or immunological difference between the α-subunit found in the foetus and the mother at term has been demonstrated [12]. From these studies it seems likely that the foetal pituitary gland contributes, at least in part, to the intact hormones and α-subunit found in the foetal circulation.

Table 2. The concentration of LH-HCG, α-subunit and LHβ-subunit in foetal cord artery and vein at term

Hormone or subunit	Artery* (ng/ml)	Vein* (ng/ml)
'LH-HCG'	39.6 (20.7–97)	38.5 (18.8–124)
α-subunit	99 (40–204)	97 (43–316)
LHβ subunit	1.0 (0.5–1.4)	0.9 (0.8–1.6)

* mean and range are shown; $n = 22$.

Ontogeny of gonadotrophin control. In adults the production of hormones from the anterior pituitary gland is to a large extent regulated by signals from the midbrain and the cerebral cortex; this control is mediated by the monoaminergic neurons and their neurohumeral chemotransmitters, which terminate in the median eminence and the pituitary gland [25], and by neurohormones secreted by the hypothalamus carried through the portal vessels to the anterior pituitary gland [26]. The secretion of both LH and FSH is apparently controlled by only one releasing hormone, the decapeptide gonadotrophin-releasing hormone (LHRH) [27]. The sex hormones oestrogen, progesteron and testosteron secreted by the gonads exert a predominantly negative feedback on the hypothalamus and pituitary [28]; however, oestrogens have a stimulatory effect as well [29, 30].

Hypothalamic control of the foetal adenohypophysis depends at any rate on the presence of an intact portal circulation, the development of nerve endings of tuberal neurons containing monoamines in the median eminence and pituitary gland, and the hypothalamic content of the hypophysiotrophic hormones [25, 31]. In humans, portal vessels appear about the 4th month of gestation, but the portal circulation is not completed before the second half of intrauterine life [32, 33]. Neurosecretory nerve fibres and structures containing monoamines are seen in the median eminence at the 15th to 18th week of gestation [33, 34]; however, Hyyppä[35] found an accumulation of nerve fibres containing monoamines in the median eminence at the 11th week of gestation. At the same time he found that the portal vessels penetrated the median eminence. Furthermore, the age of gestation at which LHRH is present in the human hypothalamus is controversial. Winters *et al.* [36] demonstrated immunoreactive LHRH in foetal hypothalami as early as 4.5 weeks of gestation. Gilmore and Dobbie[37] on the other hand were unable to demonstrate biologically active LHRH in hypothalamic extracts before the 16th week of gestation.

These studies indicate that releasing hormones and portal vessels exist in foetal life, but that the hypothalamic-pituitary control system is not developed until mid- to late-pregnancy. However, it is still possible that the pituitary gland is under influence of the hypothalamic hormones at a very early stage, because the small hypothalamic peptides could reach the pituitary gland by diffusion, or *via* the cerebro-spinal fluid circulation during this period [38].

The stage of foetal development at which the inhibitory feedback mechanisms of the sex hormones mature is not known. Steriodogenic activity in the foetal testis can be demonstrated from the 6th week of gestation, leading to testosterone production at the age of 12 weeks [19, 39, 40]. The steroid synthesis of the human foetal ovary is practically negligible [41]; however, in the foetal circulation oestrone, oestradiol-17β and oestriol are present around the 12th to 15th week of gestation, with increasing levels at the age of 20 weeks [19, 42]. The increase in foetal sex hormones correlates with the fall in pituitary content and the serum concentrations of LH and FSH; this might indicate the development of a sex hormone-hypothalamic-pituitary feedback mechanism at this time [9].

The pattern of change in the ratio of pituitary LH, FSH, LHβ subunits and α-subunits during foetal life suggests that the first gonadotrophin substance synthetized in the foetal pituitary gland is the common α-subunit, after which, under hypothalamic influence, the β-subunits synthetize, and this leads to the production of the intact hormones. This is supported by the fact that, in normal pituitaries, the hypothalamic gonadotrophin-releasing hormone not only releases LH and FSH but also stimulates their synthesis [27]. Furthermore, the pituitary extract from an anencephalic foetus (period of gestation 36 weeks) contained large amounts of α-subunit but practically no intact hormones [10].

Acknowledgements—We thank Drs. D. Gilmore and D. G. Evans for supplying some of the specimens. We also thank Drs. W. R. Butt, C. R. Canfield, A. S. Hartree, H. S. Jacobs, R. M. Lequin, S. S. Lynch and A. F. Parlow and the MRC for reagents used in the radioimmunoassays. We also thank the Wellcome Trust and the Danish Medical Research Council for financial support.

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DISCUSSION

Posner. Do you know of measurements of the kind made in amniotic fluid?

Hagen. I suppose you mean of the subunits. Yes, we have measured the α-and HCGβ-subunit concentration in a few samples of the amniotic fluid around term. The α-subunit concentration was very high (more than 500 ng/ml) and no HCGβ-subunit was found.

Thorburn. I just wondered what evidence you have that the fetal pituitary actually secretes the α-subunit. You measure high levels in the blood but this doesn't say it's secreted, have you any studies on perfusion of pituitaries or other experimental evidence to support your contention.

Hagen. No, we have not performed such studies, but Franchimont and coworkers have shown that the foetal pituitary *in vitro* is able to secrete free α-subunit. What we have shown is, that both the α-subunit and the intact gonadotrophins are present in the pituitary gland, and that we can pick up FSH, presumably LH and free α-subunit in the circulation. We can not distinguish between α-subunit of foetal and maternal origin, but I think it is like the LH-HCG story, that the α-subunit that we find in the foetal circulation is mainly of placental origin.

Jaffe. I can corroborate one of your latter comments. Some years ago Dr. Midgley and I performed gel filtration and radioimmunoassay of umbilical cord blood at term and did indeed find that the bulk of the gonadotrophin was HCG rather LH. I think also it might be helpful for people not working in this field if we labeled α-subunit just as α-subunit rather than as LH α-subunit. The term LH α-subunit might be misleading.

Hagen. I agree, when we are talking about circulating levels of α-subunit. But I still think it is nice to know, whether standards used in the assays or for standardizing columns, are HCGα, TSHα or FSHα.

Friesen. Do you know anything about the recovery in your extractions of pituitary α and β subunits? Is there a differential extraction of the two?

Hagen. I agree this is an important question. Because of the mild extraction procedures used, it is not likely that a dissociation of intact hormones into subunits has occurred. No, we have not performed any recovery studies. However, we have extracted pieces of the same pituitary gland on different days, and shown that this had no influence on the elution pattern of the hormones and their subunits. This finding does not exclude the possibility, that the extraction procedure and gel filtration influence our results, but I do not think it is very likely.

Friesen. In view of the possibility that α-subunit is secreted independently do you know of any suggestion that the α-subunit might itself have an independent action apart from something to do with the gonadal function?

Hagen. In adults, you can see a rise in circulating levels of α-subunit after the administration of LHRH and after large doses of TRH. We have shown that the rise in α-subunit precedes that of the intact hormones. Therefore, I think that the α-subunit secreted is of pituitary origin. In collaboration with Dr. Ken McNatty from Edinbourgh we have looked at the subunit concentration during the normal menstrual cycle. We found that in the circulation the α- and perhaps the LHβ-subunit showed a midcycle peak, similar to those seen for LH and FSH, but no changes during the follicular- and luteal phase. Secondly, in the follicular fluid we found the same concentration of α-subunit as in the corresponding blood samples, and in a few follicles even higher. Because of the non-specificity of our LHβ-assay, we have not been able to demonstrate free LHβ-subunit neither in the circulation nor in the follicular fluid. *In vitro* studies performed by Ken McNatty and others have not been able to show any biological function of the subunits themselves. However, we have speculated that in the normal menstrual cycle the subunits could penetrate the follicle and inside the follicle recombine and act as the intact hormones. I do not think it is an important function, but only a minor thing. Whether the α-subunit in the foetus and the mother has a biological function I do not know.