Clinical Endocrinology (1976) **5**, 551–569.

REVIEW ARTICLE

HYPOTHALAMIC–PITUITARY–GONADAL RELATIONSHIPS IN MAN FROM BIRTH TO PUBERTY

MAGUELONE G. FOREST, EVELINE DE PERETTI AND JEAN BERTRAND

Unité de Recherches Endocriniennes et Métaboliques chez l'Enfant, Hôpital Debrousse, Lyon, France

We propose in this review to consider the four main phases of development of gonadal function: fetal, perinatal, prepubertal and pubertal.

1. THE OVERALL DEVELOPMENT OF GONADAL FUNCTION

Gonadal function depends upon activities at various levels. Thus there is: (a) a central level (extra-hypothalamic, hypothalamic, and anterior pituitary); (b) an intermediate level (gonadal and plasma), and (c) a peripheral level consisting of the target organs to which the hormonal message is directed. These determine both the expression of gonadal function (physical, physiological, or psychological) and the feedback regulation of the secretion of gonadotrophins.

(a) *Central level*

(i) *Extra-hypothalamic centres.* The production of LH releasing hormone (LHRH) is affected in the rat, and probably in primates as well, by extra-hypothalamic stimuli coming, in particular, from the amygdala and the hippocampus. Adrenergic regulation exists at this level, noradrenaline having a stimulatory and dopamine an inhibitory effect (Bliss *et al.*, 1972).

(ii) *Hypothalamic centres.* These are localized in particular in the pre-optic area. Immunochemical techniques have demonstrated that LHRH is secreted by neurones of the arcuate nucleus, and released into the capillary plexus proximal to the hypophyseal portal system at the level of the median eminence, thereby reaching the anterior pituitary (Barry & Dubois, 1974; Setalo *et al.*, 1975).

(iii) *Gonadotrophin secreting cells of the anterior pituitary.* These synthesize and secrete the gonadotrophins, LH and FSH, under the influence of LHRH (Schally *et al.*, 1972). The pituitary gonadotrophins stimulate the synthesis and the secretion of the gonadal

Correspondence: Dr Maguelone G. Forest, Unité de Recherches Endocriniennes et Métaboliques chez l'Enfant, INSERM-U.34, Hôpital Debrousse, 29 rue Soeur Bouvier, 69322 Lyon Cedex 1, France.

hormones by the Leydig cells of the testes, and by the interstitial and follicular tissue of the ovaries.

(b) *Intermediate level*

This involves the secretion of the sex steroid hormones, testosterone, oestradiol and progesterone by the gonads, and their pre-hormones of gonadal and adreno-cortical origin: Δ^4-androstenedione and dehydroepiandrosterone (DHA). The steroids are carried in plasma largely bound to plasma proteins. These include albumin (of high capacity and low affinity), and sex-hormone-binding-globulin (SHBG) for testosterone, oestradiol and other 17β-hydroxylated steroids.

(c) *Peripheral level*

This is composed of the different target organs which finally express the hormonal action. The target cells are characterized by the presence of a cytosol specific receptor which, after binding the steroid, is translocated into the nucleus and binds to chromatin. In order to become active, some of the gonadal steroids undergo prior metabolic transformation. Thus testosterone is converted into 5α-dihydrotestosterone in the prostate and the external genitalia, into 3α-5α-androstenediol in the pituitary (Loras *et al.*, 1974; Massa *et al.*, 1975), and into oestrogen in the pituitary and hypothalamus (Naftolin *et al.*, 1971). These hormones also regulate their own secretion by a dual mechanism of positive and negative feedback that is exerted centrally at extra-hypothalamic, hypothalamic and pituitary sites.

There is a process of self-regulation of the above apparatus which differs according to the sex of the individual. Cyclic activity as found in females, in contrast to the tonic activity of the male, seems to be under the control of central neuroamines and circulating gonadal steroids. For this reason the ensemble of the hypothalamic centres and the regulation of their activity by the concentration of circulating sex steroids has been called the 'gonadostat' (Grumbach *et al.*, 1974). Thus the pituitary response to LHRH is modified by plasma oestrogens and androgens. Androgens decrease while oestrogens increase the concentration of the plasma protein SHBG, and some of the steroid hormones also regulate the quantity of receptor or the activation of enzymes at the level of target tissue cells. This 'equilibrium' varies with age and is predominantly the subject of the present review. According to Grumbach *et al.* (1974), fetal gonadotrophic secretion is associated with relatively autonomous activity of the hypothalamus at the moment of sexual differentiation. Thereafter there is a progressive maturation of the central inhibitory factors which assume control of hypothalamic–pituitary activity. During childhood gonadal function operates at a very low level of activity, and this is followed by a progressive increase of hypothalamic–pituitary activity leading ultimately to puberty (Fig. 1).

Four successive phases may thus be described in the development of gonadal function in children: (a) the fetal phase of sexual differentiation and of autonomous central activity; (b) the perinatal phase of organization of this central system; (c) the pre-pubertal phase of maturation; (d) the pubertal phase with the change from immature to adult status and the acquisition of reproductive capacity.

The sex steroids play a major double role in development by: (a) A 'programming' effect in which the steroids apparently imprint a programme on a target cell that is not revealed until adulthood. Two particular examples of this have been studied in the rat. Firstly the programming at birth by testosterone of the male tonic activity of the hypothalamus, and

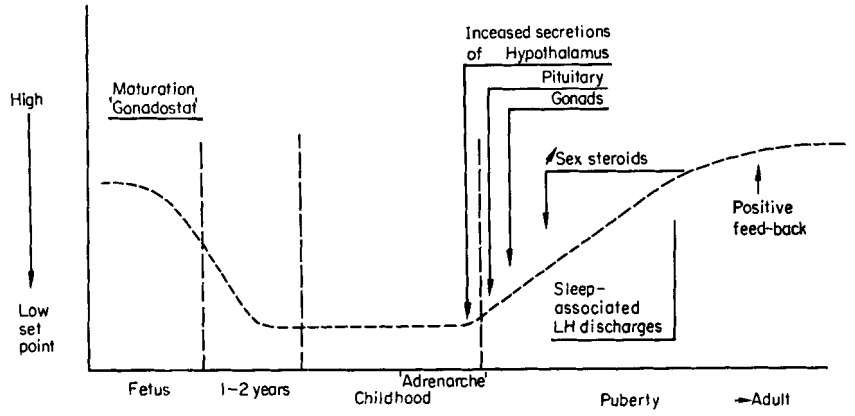

FIG. 1. Diagram illustrating the current concepts of the evolution of the regulatory factors of gonadal function during the course of the three principle stages of sexual development: fetal, perinatal and pubertal.

secondly the hypothalamic control of the secretion by the pituitary of a factor, named feminotropin (Gustafsson & Stenberg, 1974) which appears to be responsible for the sexual characteristics of the hepatic enzymes which are concerned with the metabolism of androgens in the adult (De Moor *et al.*, 1973; Gustafsson *et al.*, 1975a, b). (b) A better known regulatory activity in which the steroids de-repress part of the nuclear transcription of the specific target cell, thereby revealing its programme.

The use of radioimmunoassays has enormously facilitated investigations of the development of gonadal function. These are relatively simple and sensitive techniques which do not need large quantities of blood. They are, however, subject to certain criticisms. Thus they may not be entirely specific as seen in the cross reaction of antibodies to LH with placental HCG. Immunological activity may not always correspond to the biological activity of the polypeptide, and heterogeneous forms ('big' and 'little' molecules) may exist. The gonadotrophins are glycoproteins which are composed of two sub-units, α and β, of which only sub-unit β is specific, and the use of antibodies to this fraction allows a much more specific assay (Hagen & McNeilly, 1975).

Finally, other uncertainties must be emphasized. The concentration of a hormone in the blood is a function of its rate of production and of its metabolic clearance. The kinetic characteristics of the gonadotrophins in the fetus are not known, which makes it difficult to relate pituitary and plasma concentrations of the hormones. The placenta not only acts as a filter for the fetal steroids between mother and child, but also supplies steroids for the fetus. Little is known of the kinetics of disappearance of steroids in the fetus which, along with plasma steroid-binding–protein concentrations, may be different from those in children. The steroid concentration in umbilical cord blood is thus not identical with the peripheral concentration, as is seen with circulating testosterone in the new-born male (Forest & Cathiard, 1975).

2. GONADAL FUNCTION IN THE FETUS

(a) *In the male* (Fig. 2)

Jost's fundamental studies (1970) in the rabbit clearly demonstrated that normal sexual

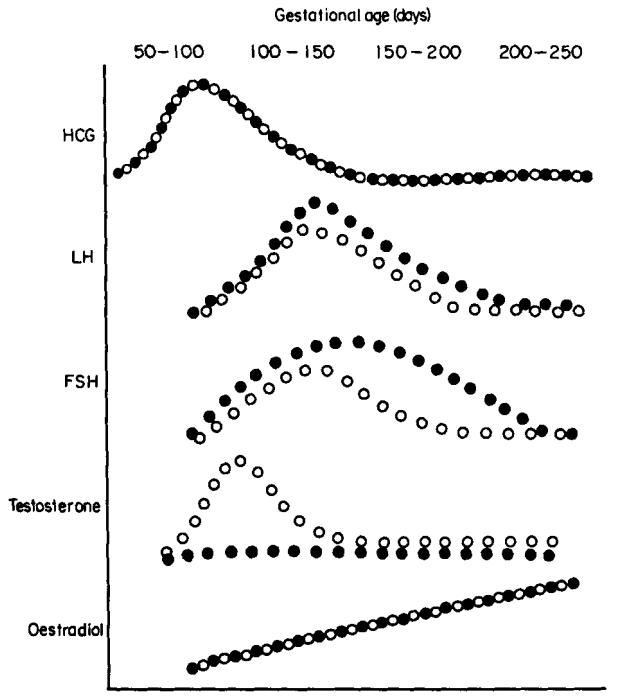

FIG. 2. Diagram showing the time course of changes of plasma concentrations of the gonadotrophins (HCG, LH and FSH), and the sex steroids (testosterone and oestradiol), in the human fetus. (Data from Abramovich & Rowe, 1973; Grumbach & Kaplan, 1973; Reyes *et al.*, 1974; Silhr-Khodr *et al.*, 1974.) ○, Male; ●, female.

differentiation in the male fetus depends upon testosterone secretion. His work has subsequently been confirmed in humans. Thus feminization of the external genitalia is found in the syndrome of testicular feminization caused in some cases by the absence of receptors for dihydrotestosterone (Amrheim *et al.*, 1975), and in enzyme deficiencies, in particular of 17-ketoreductase, with defective synthesis of testicular testosterone.

In the human fetus the differentiation of the male genitalia occurs between the ninth and tenth weeks. Masculinization of the external genitalia begins between the 65th and 66th day of gestation and continues until the 85th day, whilst differentiation of the internal genitalia terminates between the eleventh and twelfth weeks (Jirasek, 1970; Villee, 1969).

The testicle itself differentiates near the seventh week, and the Leydig cells appear about the 60th day. These cells increase rapidly both in size and number to a maximum between the tenth and eighteenth weeks. The steroidogenic activity of the gonads, as studied in incubates, begins towards the eighth week, and reaches its maximum between the eleventh and eighteenth weeks. A rapid diminution of the endocrine activity of the testes seems to follow thereafter, even though the volume of the gland continues to increase (Ahluwalia *et al.*, 1974; Serra *et al.*, 1970; Siiteri & Wilson, 1974). Similarly maximum amounts of intratesticular testosterone are found near the twelfth week (Abramovich & Rowe, 1973; Reyes *et al.*, 1973). As measured in the umbilical cord blood or peripheral blood, testosterone

values are at their maximum between the eleventh and eighteenth weeks (Abramovich & Rowe, 1973; Abramovich, 1974; Diez D'Aux & Murphy, 1974; Reyes *et al.*, 1974).

Thus the differentiation of the male genital tract coincides closely with the development of testicular endocrine function. During this period 5α-reductase activity, which is responsible for the transformation of testosterone into dihydrotestosterone, is especially important for the male differentiation of the urogenital tract, whilst testosterone itself appears to be the active androgen affecting the Wolffian ducts (Siiteri & Wilson, 1974). Additional evidence of the role of dihydrotestosterone in the male differentiation of the urogenital tubercle is provided by the observation of ambiguous external genitalia, with normal male internal structures, secondary to inherited enzyme defects of 5α-reductase (Imperato-McGinley *et al.*, 1974).

Testicular function in the fetus is partly dependent on the pituitary and the response of the Leydig cells to gonadotrophin stimulation (Abramovich *et al.*, 1974). Experimentally, Jost *et al.* (1973) have observed incomplete masculinization of the genital tract after decapitating male rabbit fetuses, which can be prevented by the injection of gonadotrophins. In man the situation is different (Siler-Khodr *et al.*, 1974). Thus an anencephalic fetus or a fetus which lacks the pituitary usually has basically normal external male genitalia, but these may be hypoplastic and the testes ectopic (Blizzard & Alberts, 1956; Reid, 1960; Zondek & Zondek, 1974). The probable explanation for these species differences is that chorionic gonadotrophins are not produced in the rabbit during gestation, whilst chorionic gonadotrophin in man initiates testicular activity, and stimulates sufficient testosterone production to permit the early masculinization of the external male genitalia. In fact, the placental as well as the maternal and the fetal circulating HCG concentrations reach a maximum just before and during the development of testicular secretion, and then decline after the fourteenth week. By contrast, the pituitary and plasma concentrations of LH are very low during this time, and do not attain their maximum values until the end of the second trimester of gestation (Reyes *et al.*, 1974).

The secretion of gonadotrophin by the fetal pituitary is thus delayed, so it is not until towards the 68th day that LH and FSH are detected in the human anterior pituitary. Their intrapituitary concentrations then increase until the twenty-fifth week. The levels of circulating LH and FSH begin to rise from the 84th day and attain their maximum values between the twelfth and twenty-fourth weeks, and twenty-eighth and thirty-second weeks, respectively, with levels similar to those of a castrated adult. During the second half of fetal life, the pituitary content of gonadotrophins remains high. The use of radioimmunoassays with specific antisera has demonstrated that the levels of plasma HCG decrease in the fetus before those of LH increase, and that the pituitary gonadotrophins predominate during the second half of the pregnancy (Gitlin & Biasucci, 1969; Grumbach & Kaplan, 1973; Hagen *et al.*, 1974; Reyes *et al.*, 1974; Rice *et al.*, 1968).

(b) *In the female* (Fig. 2)

Endocrine activity of the ovary is not necessary for the differentiation of the female genital tract. In the tenth week, the Wolffian ducts regress and the Müllerian ducts develop (Jost *et al.*, 1973). The differentiation of the ovary takes place somewhat later than that of the testis, and it is not until about the twelfth week that oocytes begin to appear. The formation of primordial follicles reaches a maximum between the twentieth and twenty-fifth weeks of gestation, and the first Graafian follicles have been noted by Pryse-Davies &

Dewhurst (1971) at the age of 26 weeks. During the second half of fetal life the ovaries increase in size to a much greater extent than the testes (Siiteri & Wilson, 1974).

Ovarian steroidogenesis in the fetus is minimal. *In vitro* studies have demonstrated only a slight production of androgens (Δ^4-androstenedione) (Payne & Jaffe, 1974), and there is a marked difference in the levels of plasma testosterone between male and female fetuses (Abramovich & Rowe, 1973; Abramovich, 1974; Ahluwalia *et al.*, 1974; Diez D'Aux & Murphy, 1974; Reyes *et al.*, 1974). The values of plasma oestrogens and especially those of oestradiol, however, are not significantly different between the two sexes (Reyes *et al.*, 1974). The levels increase steadily with age, and comparison of umbilical cord levels in the fetus and fullterm infant with venous and arterial values indicates that the placenta secretes about 3 mg/24 h of oestrogen, mainly oestriol, into the umbilical vein. This amount of oestrogen secretion is even more impressive since the physiologically active free fraction is considerably more elevated at this time than in an older infant or in an adult (Shutt *et al.*, 1974; Tulchinsky, 1973).

Although it has been shown in tissue culture that rat ovaries do not produce oestrogen after HCG stimulation (Levina *et al.*, 1975), fetal ovaries do possess the enzymes required for steroidogenesis, and can respond to stimulation by prostaglandin PGE_2 with increased cAMP formation (Lamprecht *et al.*, 1973). On the whole the role of pituitary secretion in ovarian endocrine function *in utero* seems limited. However, anencephalic fetuses habitually have hypoplastic ovaries with a markedly decreased number of primordial follicles. FSH and LH appear in the pituitary toward the 68th day, and the intrapituitary concentrations of FSH reported by Grumbach & Kaplan (1973) increase by about the twenty-ninth week and of LH by about the twenty-fourth week. The pituitary content of gonadotrophins is much greater in female fetuses than in the male, and this applies especially to FSH. Recent experiments with pituitary cultures confirm this finding (Siler-Khodr *et al.*, 1974), and have also demonstrated that secretion of gonadotrophins begins earlier in females than in males. This supports earlier biological data reported by Levina (1968, 1972) that the secretion of FSH begins 6 weeks earlier in the female than the male, and that plasma concentrations of FSH are higher in the female; these differences however are not so marked with LH.

(c) *Hypothalamic control of gonadal function in the fetus*

The fetal pituitary is capable of responding to hypothalamic activity, thus human fetal pituitaries transplanted into the sella turcica of hypophysectomized adult male rats are capable of increasing their gonadotrophin secretion (Levina, 1968). Furthermore, the application of hypothalamic extract, or of synthetic LHRH, to fetal pituitary cells in culture stimulates the production of gonadotrophins (Groom & Boyns, 1973), more so of FSH than of LH (Pasteels *et al.*, 1974).

According to Levina (1970) and Gilmore & Dobbie (1975), LHRH activity in the human fetal hypothalamus cannot be detected before the sixteenth week. Siler-Khodr *et al.* (1974) have shown that pituitaries obtained from anencephalic fetuses produce reduced amounts of gonadotrophins as compared with normal fetuses, which illustrates the role of the hypothalamus in fetal pituitary function. There is also some correlation between the morphological development of the hypothalamus and the hypophyseal portal system, and changes in the pituitary content and plasma levels of gonadotrophins (Grumbach & Kaplan, 1973). In this context it is of interest that the hypophyseal system in the rat is imperfectly developed

at time of birth (Goldman *et al.*, 1971). Finally, the hypothalamus can be considered as an androgen target organ, as it is capable of selective uptake of labelled testosterone (Abramovich, 1974), and of aromatization of Δ^4-androstenedione into oestrone (Naftolin *et al.*, 1971).

Two particular observations require explanation; namely the findings of elevated pituitary and plasma gonadotrophin concentrations in the female, and of the persistence of relatively high plasma gonadotrophin levels during the second half of fetal life, despite high levels of circulating oestrogens in both sexes (Shut *et al.*, 1974) and high levels of testosterone in the male (Reyes *et al.*, 1974). The difference of gonadotrophin secretion between the two sexes could be interpreted as indicating the establishment of the hypothalamic feedback, in the male fetus. Thus it is at the age of 6–8 weeks that the secretion of testosterone steadily rises, and it may be that this is effective in producing the more rapid maturation of the 'gonadostat', and the subsequent limitation of the production of gonadotrophins. In contrast, the female fetus does not have such high levels of testosterone in the plasma (Siler-Khodr *et al.*, 1974).

3. GONADAL FUNCTION IN THE NEWBORN INFANT

On the basis of histological studies (Pryse-Davies & Dewhurst, 1971; Vilar, 1970) the gonads have been considered to be inactive at birth. However, the use of radioimmunoassays has provided new data on this matter.

(a) *Circulating sex steroids and gonadotrophins in the infant* (Fig. 3)

(i) *Testosterone.* At birth there is a slight but significant ($P<0{\cdot}01$) difference in umbilical

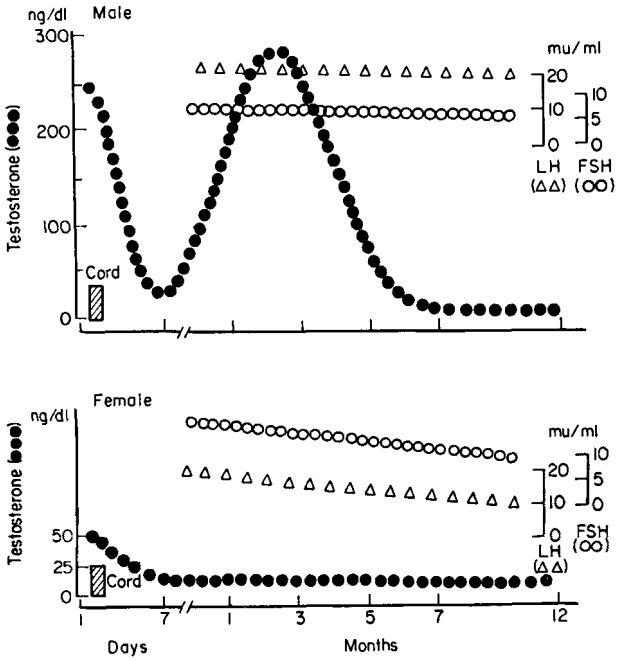

FIG. 3. Diagram illustrating changes of plasma testosterone, LH and FSH during the first year of life. (Data from Forest *et al.*, 1973b, 1974a, b; Forest & Cathiard, 1975.)

cord testosterone levels (mean $\pm$ 1 SD) between males and females ($33{\cdot}8 \pm 9{\cdot}45$ ng/dl in the male and $26{\cdot}4 \pm 7{\cdot}35$ ng/dl in the female) (Forest *et al.*, 1973b). This difference is even more striking when peripheral blood is examined (Forest & Cathiard, 1975); thus in males there is a marked difference in testosterone levels measured on the same day in peripheral (244 ng/dl) and in umbilical cord (34 ng/dl) blood. We have shown (Forest *et al.*, 1974a, 1975) that changes of plasma testosterone in the male newborn are very striking. For the first 5 or 6 days after birth there is a rapid decrease in levels. Subsequently, in the second week, an increase is found, and the levels continue to rise to a maximum value between the 30th and 60th day of $265 \pm 31{\cdot}3$ ng/dl, values which are comparable to those found in adolescence and about half the levels found in normal adult males (595 ± 141 ng/dl). From the 60th to the 120th day these values decrease again, and between the seventh and twelfth month the concentration of plasma testosterone is the same as is found in prepubertal children ($7 \pm 4{\cdot}1$ ng/dl; Forest *et al.*, 1973a).

In girls the changes are quite different. Levels of plasma testosterone at birth are similar to those of the normal adult female (Forest & Cathiard, 1975); however, as soon as the end of the first week, average testosterone levels are the same as in pre-pubertal children (Forest *et al.*, 1973a, 1974b). Thus there is not the transient rise in plasma testosterone levels in girls as is found in boys.

It is known that only the unbound testosterone fraction is physiologically active. It was therefore important to determine whether the pattern of total plasma testosterone levels found in the male infant was similar for free testosterone. Using an equilibrium dialysis method, we (Forest *et al.*, 1973a, 1974b) have measured changes of carrier protein binding capacity and have thereby calculated the levels of free testosterone. At birth a similar difference as with total testosterone is found in umbilical cord blood between male and female infants ($1{\cdot}1 \pm 0{\cdot}4$ ng/dl and $0{\cdot}89 \pm 0{\cdot}29$ ng/dl, respectively). Subsequently the same changes are found in the male infant as with total plasma testosterone. Thus, between the 35th and 50th days, the level of free testosterone rises to $1{\cdot}6 \pm 0{\cdot}2$ ng/dl, and after the sixth month the levels decline to those of prepubertal children. In girls free testosterone levels decrease during the first week to prepubertal levels (0·04 ng/dl). The gonadal origin of this secretion in boys has been clearly demonstrated as the characteristic rise is not observed in primary gonadal defects such as agonadism and some cases of male pseudo-hermaphroditism (Forest, unpublished data).

(ii) *Oestrogens.* Despite difficulties about the sensitivity of the radioimmunoassay of plasma oestrogens and the relatively large volumes of blood that are required (8–10 ml plasma), some results have been obtained (Bidlingmaier *et al.*, 1974; Kenny *et al.*, 1973). Umbilical cord blood oestrone and oestradiol levels are one thousand times higher than those of the adult. Conversely, peripheral blood concentrations are fifty times lower than those of umbilical cord blood. During the first week, blood levels fall rapidly, but plasma concentrations of oestradiol rise again during the second week. Although there are large fluctuations in blood levels, it appears that this rise continues for the whole of the first year of life, and is more marked in the female than the male. It is still not certain that this oestrogen rise (Bidlingmaier *et al.*, 1974) is ovarian in origin, although this is suggested by the finding of a large number of Graafian follicles at this time (Pryse-Davies & Dewhurst, 1971).

(iii) *Pituitary activity in the newborn.* Gonadal activity during the first 6 months of life is under pituitary control. There is an increase in the concentration of circulating gonado-

trophins during the first year with levels considerably higher than those of the prepubertal child; a difference between the sexes is found and the levels of circulating FSH are higher in the female than the male (Buckler & Clayton, 1970; Forest *et al.*, 1974b; Penny *et al.*, 1974). Pituitary activity is affected by several regulatory factors. Thus it can be stimulated by LHRH and suppressed by large doses of androgens (Forest *et al.*, 1975).

(b) *Control of gonadal function in the newborn*

Interpretation of the changes of circulating testosterone in the male and of oestradiol in the female, and the plasma levels of gonadotrophins during the first year presents three particular problems (Forest *et al.*, 1974b).

(i) The rapid fall of plasma testosterone is explained by the abrupt withdrawal of placental HCG, as reflected by its declining plasma concentration during the first 5–7 days (Winter *et al.*, 1975). It is, however, important to note that during this period a substantial amount of LH is already circulating (Hagen *et al.*, 1974). A high plasma prolactin concentration which is inhibitory to 5α-reductase activity is also found at this age (Guyda & Friesen, 1973), and could perhaps play a role in mediating the hypophyseal regulation of sexual differentiation of this enzyme (Gustafsson & Stenberg, 1975).

(ii) The secondary increase in the level of plasma testosterone during the second week is most readily explained by the high levels of plasma gonadotrophins at this time. It is interesting that plasma testosterone levels rise as high as 265 ng/dl and it would seem that the 'gonadostat' threshold to peripheral sex hormones is greatly elevated. This presumably indicates immaturity of the hypothalamic–pituitary–gonadal system at this age.

(iii) From the 60th day onwards, plasma testosterone concentrations decline, although blood LH and FSH levels remain higher than those of prepubertal children (Forest *et al.*, 1974b). This discrepancy could perhaps also be explained by an immaturity of the hypothalamic centres and pituitary secretion which control gonadal function at this age. Several arguments support this last hypothesis. Firstly, the absence of any correlation between plasma testosterone and gonadotrophin levels at this time (Forest *et al.*, 1974b). Secondly, the facts that, in the premature infant, there is a prolongation, even beyond the second month, of elevated plasma testosterone concentrations, and that the levels of plasma total and free testosterone is much higher than in the normal newborn infant (Forest *et al.*, 1974a). Thirdly, the similar response with age of the infantile testis to exogeneous stimulation by HCG (Forest *et al.*, 1975). Fourthly, the existence of a high percentage of biologically inactive LH-HCG-α sub-units in the pituitary presumably due to the immaturity of these cells (Hagen & McNeilly, 1975). Although this last observation supports our hypothesis of a progressive change in the biological potency of the gonadotrophin secretions throughout infancy, the apparent discrepancy between declining testicular activity and persistently high activity is still not fully understood.

The implications of the neonatal androgenic activity of the human newborn are still unknown. Their study is beyond the scope of this report but the work of Pfeiffer (1935), Barraclough (1961), Gorsky (1971) and McDonald & Doughty (1974) on sex differentiation of the neuro-endocrine axis in mammals is clearly relevant. There appear to be regulatory centres of gonadotrophic function in the hypothalamus of some rodents; a tonic centre which is inhibited by a negative feedback effect of the sex steroids, and a cyclic centre which is regulated by a positive feedback of oestradiol. The development in adulthood of this cycle centre is apparently repressed by the neonatal activity of the testicles, and the same

effect occurs with an injection of testosterone in the newborn female. This action of testosterone probably occurs after its aromatization since the administration of dihydrotestosterone (which cannot be aromatized), has no such effect (McDonald & Doughty, 1974). In contrast, however, the neonatal administration of a large dose of oestrogens produces masculinization of the hypothalamus and, in adult life, the development of the permanent oestrus syndrome (Gorski, 1971). A similar permanent 'programming' action of testosterone secreted at birth appears to occur in the hypothalamus, and will reveal itself in the skin and the liver enzyme activities in adulthood (De Moor *et al.*, 1973; Gustafsson *et al.*, 1975b).

In man it is difficult to draw definite conclusions from the physio-pathological model of the syndrome of congenital adrenal hyperplasia (CAH). In normal girls before puberty FSH levels are higher than in boys, and it now appears that the excessive prenatal androgen production which occurs in CAH does not change this pattern (Reiter *et al.*, 1975). These recent findings are contrary to those previously reported by some authors (Bongiovanni *et al.*, 1972; Kirkland *et al.*, 1974; Money & Ehrhardt, 1972; Penny *et al.*, 1973; Reiter *et al.*, 1974).

4. GONADAL FUNCTION IN THE CHILD AND ADOLESCENT

During the first 2 years of life, hypothalamic–pituitary–gonadal relations become established. The threshold of sensitivity of the gonadostat is very high, and the circulating concentrations of gonadotrophins and sex steroids are correspondingly low in both sexes. Puberty is the final act of maturation of gonadal function, and is defined by the appearance of secondary sexual characteristics and the capacity for reproduction. Important changes in the function of the gonadostat progressively appear. Initially there is a diminution of its sensitivity to the negative feedback action of sex steroids. Subsequently intermittent activity during sleep is found with episodic discharges of LH, and, finally, there is the development of the positive feedback effect of oestrogens. Parallel to this progressive maturation of the hypothalamic–pituitary–gonadal axis there is an increased secretion of the gonadotrophins and the sex steroids. This occurs simultaneously with the four stages of puberty described by Tanner, and correlates better with bone age than with chronological age (Canlorbe *et al.*, 1974; Forest *et al.*, 1974c; Grumbach *et al.*, 1974; Kantero *et al.*, 1975; Kulin & Reiter, 1973). The recently reported longitudinal studies are particularly useful and allow much more precise correlations between morphological phenomena and hormonal variations (Knorr *et al.*, 1974; Lee *et al.*, 1974).

The present section will be limited to the study of those phenomena which are directly responsible for changes of gonadal function during the development of puberty, namely the role of the adrenal cortex, the variations of response of the hypothalamic–pituitary–gonadal axis to different stimuli, the diminution of the sensitivity of the gonadostat, and the appearance of the positive feedback exerted by oestrogens.

(a) *The role of the adrenal cortex in the development of puberty*

It has been suggested that puberty is determined not only by hormonal but also by extra-hormonal factors, such as body weight (Frisch, 1974). As far as endocrine factors are concerned, it appears that the earliest changes of puberty begin from around the age of 6

years, with the output of the weak androgens dehydroepiandrosterone (DHA) and its sulphate (DHAS) and Δ^4-androstenedione (Δ^4) by the adrenal cortex.

This phenomenon was first recognized through certain clinical states. Thus in 'premature pubarche' which is the precocious development of pubic hair in young girls, an increase of urinary 17-oxosteroids, in particular of DHA, aetiocholanolone and of androsterone has been demonstrated (Visser & Degenhart, 1966). Similarly, blood levels of DHA and DHAS have been found to be elevated. The adrenocortical origin of these steroids has been demonstrated by ACTH stimulation and dexamethasone suppression (Rosenfield, 1971), and the finding of such changes in agonadal children (Korth-Schultz *et al.*, 1975). The adrenal phase of prepubertal development is termed the 'adrenarche'.

The prepubertal concentration of plasma testosterone is the same for the two sexes, i.e. $6{\cdot}62 \pm 2{\cdot}46$ ng/dl for the male and $6{\cdot}48 \pm 2{\cdot}48$ ng/dl for the female (mean $\pm$ SD). This secretion is also predominantly of adrenocortical origin as indicated by the observations that patients with Turner's syndrome or agonadal subjects have similar plasma testosterone levels (Forest *et al.*, 1973a, c). The prepubertal development of adrenal steroidogenesis is not under gonadotrophin control, neither does it seem to be under the direct control of ACTH. The secretion of prolactin also does not seem to be a factor since it remains fairly constant during puberty (Lee *et al.*, 1974).

During a recent study of the secretion of DHA, Δ^4, testosterone, oestrone and oestradiol in the child, we have been able to show that there is a sequence in the development of these different secretions. Thus plasma DHA increases first of all from the age of 6 years, followed by an increase of Δ^4 from 8 to 10 years. Plasma testosterone and oestradiol levels do not rise until later, at the time of onset of puberty (Forest *et al.*, 1973a; Ducharme *et al.*, 1976). This prepubertal adrenal activity is not specific to man and can also be found in animals, in particular the rat. Immunoreactive progesterone and oestrogens also seem to be of adrenal origin at the onset of puberty (Morera *et al.*, 1974; Ramaley, 1974), and adrenalectomy in female rats between the ages of 18 and 20 days significantly delays the opening of the vagina (Gorski & Lawton, 1973).

The role of the 'adrenarche' seems to be permissive, and perhaps this adrenal secretion is involved with the maturation of the gonadostat. DHA and, particularly, Δ^4-androstenedione could be effective either directly, or after their transformation into oestrogens at the level of the hypothalamus. The appearance of nyctohemeral rhythms of plasma LH, characteristic of normal puberty, in patients with gonadal dysgenesis (Boyar *et al.*, 1973b) and the frequent occurrence of delayed puberty in patients with chronic primary adrenal insufficiency strongly support this hypothesis.

(b) *Increase of hypothalamic–pituitary–gonadal sensitivity to stimuli*

This characterizes the second stage of pubertal development.

(i) *Increase of gonadal sensitivity to gonadotrophin stimulation.* In the rat an increase in the secretion of testosterone after identical doses of HCG can be demonstrated with the approach of puberty (Moger & Armstrong, 1974; Odell *et al.*, 1974). This could be explained in part by a progressive decline of testicular 5α-reductase activity (Strickland *et al.*, 1970), and in part by an increase of the number of receptor sites in the testis for gonadotrophins (Frowein & Engel, 1975). In the child the testis is also capable of responding to prolonged stimulation by chorionic gonadotrophin (Saez & Bertrand, 1968; Forest *et al.*, 1974c), and similarly when the response to a single dose of HCG is tested in children of

different ages, the rise of plasma testosterone increases with the early development of puberty (Anderson *et al.*, 1972; Attanasio *et al.*, 1974; Zachman, 1974; Canlorbe *et al.*, 1974; Scholler *et al.*, 1975). FSH may play an important part in this response as a positive correlation has been demonstrated in the hypopituitary child between the basal level of plasma FSH and the response to HCG (Sizonenko *et al.*, 1973). Furthermore, during the prepubertal period when the testes are increasing in size under the influence of FSH, there is an increased testicular response to gonadotrophin stimulation (Odell, 1974).

(ii) *Increase of pituitary sensitivity to LHRH stimulation.* Two groups have clearly shown that the pituitary response to LHRH is enhanced at the onset of puberty in man (Job *et al.*, 1972; Roth *et al.*, 1972), and this is also found in the rat (Odell, 1974). This increased sensitivity of the pituitary could be secondary to an increased endogenous secretion of LH-RH, but some authors believe the role of steroids to be more important (McCann, 1974; Shin & Kraicer, 1974). Thus the administration of sex steroids in low doses causes precocious puberty in rats (Ramirez, 1973). In humans oestrogens appear to play a special part in pituitary maturation and its response to hypothalamic stimulation (Guyda & Friesen, 1973; Odell, 1974). This effect upon the forebrain could also be due to the aromatic conversion of Δ^4-androstenedione into oestrogens (Naftolin *et al.*, 1971). These considerations could account for the findings in some patients with congenital adrenal hyperplasia, who have been untreated until the age of 6 years, of greatly advanced bone maturation. The institution of therapy in such patients leads to the rapid onset of true puberty, associated with the appearance of sensitivity of the pituitary to LHRH, and a nyctohemeral rhythm of plasma LH such as occurs during normal puberty (Boyar *et al.*, 1973b; Penny *et al.*, 1973; Reiter *et al.*, 1975). It also seems possible that long-standing exposure to high concentrations of sex hormones could lead to precocious maturation of the hypothalamic–pituitary axis and induce true precocious puberty (Boyar *et al.*, 1973a).

(c) *Maturation of the gonadostat*

(i) *Elevation of the threshold of sensitivity of the gonadostat to the negative feedback of steroids.* In the opinion of many authors, the basic phenomenon responsible for the onset of puberty is the progressive decrease of forebrain sensitivity to circulating steroids. This hypothesis, first suggested by Holweg & Dohrn (1932) and then by Donovan & van der Werff ten Bosch (1965), has been verified in animals (Bloch *et al.*, 1974; Eldridge *et al.*, 1974; Steele & Weisz, 1974) as well as in humans (Grumbach *et al.*, 1974). The threshold of sensitivity of the hypothalamic gonadostat progressively increases during puberty, with pituitary inhibition requiring increasing levels of oestrogen and testosterone as pubertal maturation develops. Simultaneous changes by hypothalamic neuroamines have been found experimentally (Coppola, 1969; De Moor *et al.*, 1973; Ramirez, 1973). Grumbach *et al.* (1974) have shown that the hypothalamic gonadostat in prepubertal children is six to fifteen times more sensitive to circulating oestrogens than after puberty. This can be illustrated by two clinical observations. Before puberty the administration of as small a dose of clomiphene as 0·1 mg daily for 1 week has been shown to be followed by a fall of gonadotrophin levels, due to the weak oestrogenic activity of the compound, whereas after puberty a dose of 100 mg daily of clomiphene produces the same rise of plasma testosterone and LH (Cathro *et al.*, 1971; Nankin *et al.*, 1971) that is found in adults.

Similarly, inhibition of gonadotrophin secretion can be produced in prepubertal children by the administration of 2 μg of ethinyloestradiol per day, whereas in the adult and adoles-

cent more than 10 μg daily per square metre of body surface are required to produce a similar effect (Kelch *et al.*, 1973). This phenomenon is a central one and cannot be explained by changes in the metabolic clearance of gonadotrophins and steroids with age.

(ii) *Nocturnal discharge of LH and testosterone.* The appearance of a nyctohemeral rhythm of the hypothalamic–pituitary–gonadal complex is also evidence for sexual maturation of the central nervous system. Boyar *et al.* (1972) first drew attention to the fact that, in pubertal children, nocturnal discharges of LH can be demonstrated, and that their amplitude is much greater than in adults. Furthermore, at stage II of puberty there is a characteristic nocturnal increase in plasma testosterone levels (Boyar *et al.*, 1974; Judd *et al.*, 1974).

(iii) *The appearance of the positive feedback control of the gonadostat by oestrogens.* The pubertal phase of gonadal function in the female ends with the development of the cyclic centre and its positive feedback control by oestrogens. In 1932, Holweg described the luteinizing effect of a single injection of oestrogens in the female rat. In rats (Caligaris *et al.*, 1972; Steele & Weisz, 1974), as well as sheep (Land *et al.*, 1970; Short, 1974) and monkeys (Knobil, 1974), the positive feedback mechanism which causes the preovulatory LH surge only becomes mature at the end of puberty. Likewise in girls this positive feedback effect of oestrogens on LH secretion does not exist before puberty (Reiter *et al.*, 1974). It first appears in a somewhat incomplete form, and its full development does not occur until later, in association with ovulatory cycles of the post pubertal girl (Land *et al.*, 1970). In lower animals such activity is limited to females and requires the absence of any androgenization during the perinatal period (Turgeon & Barraclough, 1974). In primates, on the other hand, the positive feedback effect of oestrogens is found in both males and females (Karsch *et al.*, 1973).

CONCLUSIONS

We feel that the study of gonadal function of the child should be regarded as a whole and should begin with the fetus and end with the adolescent. Its development is an uninterrupted phenomenon based upon the sensitivity of the gonadostat to the levels of circulating steroids. Four successive phases can be distinguished (Grumbach *et al.*, 1974) (Fig. 1).

(a) A fetal phase beginning with testosterone secretion around the eighth week *in utero* under the control of chorionic gonadotrophin; subsequently the secretion of gonadotrophins by the pituitary becomes established during the twelfth week. The hypothalamic–pituitary complex (gonadostat) acts autonomously until the twenty-second to twenty-fourth weeks and does not appear to be susceptible to inhibition. During the second half of fetal life the negative feedback becomes gradually established, appearing more rapidly in boys than in girls, presumably because of their higher concentrations of circulating testosterone.

(b) A perinatal phase during the course of which hypothalamic–pituitary activity accounts for the testicular and probably ovarian secretion that has been demonstrated to occur during the first 6 months of life.

The threshold of sensitivity of the gonadostat to steroid inhibition appears to be high during the first 3 months of life and then progressively decreases to the very low level characteristic of childhood. The discrepancy between the elevated circulating immunoreactive gonadotrophins and the low testosterone levels observed from 6 to 12 months of age is still not understood, but could perhaps indicate low biological potency of the gonadotrophins.

(c) A prepubertal phase when the gonadostat is least active. It subsequently becomes progressively more active, probably under the permissive influence of the adrenocortical steroids. This hypothalamic activity is responsible for the gradual increase of the pituitary and gonadal sensitivity to their respective stimuli.

(d) A pubertal phase which is characterized by a rapid elevation of the gonadostat threshold, and the development of secondary sexual characteristics and of reproductive capacity. This central pubertal maturation is manifested by the appearance of two specific phenomena, namely large nocturnal discharges of LH and of testosterone, and the establishment of positive feedback control by oestrogens which permits ovulation.

ACKNOWLEDGMENTS

The authors are indebted to J. Bois and M. Montagnon for their much appreciated secretarial assistance.

REFERENCES

ABRAMOVICH, D.R. (1974) Human sexual differentiation in utero influences. *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **81**, 448–453.

ABRAMOVICH, D.R., BAKER, T.G. & NEAL, P. (1974) Effect of HCG on testosterone secretion by the foetal human testis in organ culture. *Journal of Endocrinology*, **60**, 179–185.

ABRAMOVICH, D.R. & ROWE, P. (1973) Fetal plasma testosterone levels at mid-pregnancy and at term. Relationship to fetal sex. *Journal of Endocrinology*, **56**, 621–622.

AHLUWALIA, B., WILLIAMS, J. & VERMA, P. (1974) In vitro testosterone biosynthesis in the human fetal testis. II. Stimulation by cAMP and HCG. *Endocrinology*, **95**, 1411–1415.

AMRHEIN, J., KEENA, B., MEYER, W. & MIGEON, C.J. (1975) Androgen receptor deficiency in man. In: *Proceedings of the Symposium 'Adrenal Congenital Hyperplasia: a Quarter of Century Later', Baltimore*, 20–24 October.

ANDERSON, D.C., MARSHALL, J.C., YOUNG, J.L. & FRASER, T.R. (1972) Stimulation tests of pituitary–Leydig cell function in normal male subjects and hypogonadal men. *Clinical Endocrinology*, **1**, 127–140.

ATTANASIO, A., JENDRICKE, K., BIERICH, J.R. & GUPTA, D. (1974) Clinical and hormonal effects of HCG in prepubertal cryptorchid boys. (Abstract). *Journal of Endocrinology*, **63**, 50P.

BARRACLOUGH, C.A. (1961) Production of anovulatory sterile rats by single injection of testosterone propionate. *Endocrinology*, **88**, 62–67.

BARRY, J. & DUBOIS, M.P. (1974) Différenciation périnatale de l'hypothalamus gonadotrope chez les mammifères. *Colloque International sur l'Endocrinologie Sexuelle Périnatale* (ed. by M. G. Forest and J. Bertrand), Vol. 32, pp. 63–78. INSERM, Paris.

BIDLINGMAIER, F., VERSMOLD, H. & KNORR, D. (1974) Plasma estrogens in newborns and infants. *Colloque International sur l'Endocrinologie Sexuelle de la Période Périnatale* (ed. by M. G. Forest and J. Bertrand), Vol. 32, pp. 299–314. INSERM, Paris.

BLISS, E.L., FRISCHAT, A. & SAMUELS, L. (1972) Brain and testicular function. *Life Sciences*, Part I, **2**, 231–238.

BLIZZARD, R.M. & ALBERTS, M. (1956) Hypopituitarism, hypoadrenalism and hypogonadism in the newborn infant. *Journal of Pediatrics*, **48**, 782–789.

BLOCH, G.J., MASKEN, J., DRAGT, C.L. & GANONG, W.F. (1974) Effect of testosterone in plasma LH in male rats of various ages. *Endocrinology*, **94**, 947–951.

BONGIOVANNI, A.M., MOSHANG, T. & PARKS, J.S. (1972) Deferment of puberty in 'late' treated congenital adrenal hyperplasia. (Abstract). *Pediatric Research*, **6**, 352/92.

BOYAR, R., FINKELSTEIN, J., ROFFWARG, G., KAPEN, S., WEITZMAN, E. & HELLMAN, L. (1972) Synchronization of augmented luteinizing hormone secretion with sleep during puberty. *New England Journal of Medicine*, **287**, 582–586.

BOYAR, R.M., FINKELSTEIN, J.W., DAVID, R., ROFFWARD, H., KAPEN, S., WEITZMAN, E.D. & HELLMAN, L. (1973a) Twenty-four hour patterns of plasma luteinizing hormone and follicle stimulating hormone in sexual precocity. *New England Journal of Medicine*, **289**, 282–286.

BOYAR, R.M., FINKELSTEIN, J.W., ROFFWARG, H., KAPEN, S., WEITZMAN, E.D. & HELLMAN, L. (1973b) Twenty-four hour luteinizing hormone and follicle-stimulating hormone secretory patterns in gonadal dysgenesis. *Journal of Chemical Endocrinology and Metabolism*, **37**, 521–525.

BOYAR, R.M., ROSENFIELD, R.S., KAPEN, S., FINKELSTEIN, J.W., ROFFWARG, H.P., WEITZMAN, E.D. & HELLMAN, L. (1974) Human puberty. Simultaneous augmented secretion of luteinizing hormone and testosterone during sleep. *Journal of Clinical Investigation*, **54**, 609–618.

BUCKLER, J.M.H. & CLAYTON, B.E. (1970) Output of luteinizings hormone in the urine of normal children and those with advanced sexual development. *Archives of Disease in Childhood*, **45**, 478–484.

CALIGARIS, L., ASTRADA, J.J. & TALEISNIK, S. (1972) Influence of age on the release of luteinizing hormone induced by oestrogens and progesterone in immature rats. *Journal of Endocrinology*, **55**, 97–103.

CANLORBE, P., TOUBLANC, J.E., JOB, J.C., SCHOLLER, R., ROGER, M., CASTAGNIER, M. & LEYMARIE, P. (1974) La fonction endocrine du testicule chez l'enfant et l'adolescent. Taux de base et après gonadotrophins chorioniques de la testostérone et des oestrogènes plasmatiques. Variations spontanées de la testostérone. *Annales de Pédiatrie (Paris)*, **21**, 13–26.

CATHRO, D.M., SAEZ, J.M. & BERTRAND, J. (1971) The effect of clomiphene on the plasma androgens of prepubertal and pubertal boys. *Journal of Endocrinology*, **50**, 387–396.

COPPOLA, J.A. (1969) Turnover of hypothalamic catecholamines during various states of gonadotrophin secretion. *Neuroendocrinology*, **5**, 75–80.

DE MOOR, P., VERHOEVEN, G. & HEYNS, W. (1973) Permanent effects of foetal and neonatal testosterone secretion on steroid metabolism and binding. *Differentiation*, **1**, 241–253.

DIERSCHKE, D.J., KARSCH, F.J., WEICK, R.F., WEISS, G., HOTCHKISS, J. & KNOBIL, E. (1974) In: *The Control of the Onset of Puberty* (ed. by M. M. Grumbach, G. D. Grave and F. E. Mayer), pp. 104–114. John Wiley, New York.

DIEZ D'AUX, R.C. & MURPHY, B.E.P. (1974) Androgens in the human fetus. *Journal of Steroid Biochemistry*, **5**, 207–210.

DONOVAN, D.T. & VAN DER WERFF TEN BOSCH, J.J. (1965) *Physiology of Puberty*. Arnold, London.

DUCHARME, J.R., FOREST, M.G., DE PERETTI, E., SEMPE, M., COLLU, R. & BERTRAND, J. (1976) Plasma adrenal and gonadal sex steroid in human pubertal development. *Journal of Clinical Endocrinology and Metabolism*, **42**, 468–476.

ELDRIDGE, J.CH., MCPHERSON, J.C., III & MAHESH, V.B. (1974) Maturation of the negative feed-back control of gonadotropin secretion in the female rat. *Endocrinology*, **94**, 1536–1540.

FOREST, M.G., CATHIARD, A.M. & BERTRAND, J. (1973a) Total and unbound testosterone levels in the newborns and in normal and hypogonadal children: use of a sensitive radioimmunoassay for testosterone. *Journal of Clinical Endocrinology and Metabolism*, **36**, 1132–1142.

FOREST, M.G., CATHIARD, A.M. & BERTRAND, J. (1973b) Evidence of testicular activity in early infancy. *Journal of Clinical Endocrinology and Metabolism*, **37**, 148–151.

FOREST, M.G., CATHIARD, A.M., BOURGEOIS, J. & GENOUD, J. (1974a) Androgènes plasmatiques chez le nourrisson normal et prématuré. Relation avec la maturation de l'axe hypothalamo-hypophyso-gonadique. *Colloque International sur l'Endocrinologie Sexuelle de la Période Périnatale* (ed. by M. G. Forest and J. Bertrand), Vol. 32, pp. 315–336. INSERM, Paris.

FOREST, M.G., SIZONENKO, P.C., CATHIARD, A.M. & BERTRAND, J. (1974b) Hypophyso-gonadal function in human during the first year of life. *Journal of Clinical Investigation*, **53**, 819–828.

FOREST, M.G., SAEZ, J.M., SANN, L. & BERTRAND, J. (1974c) La fonction gonadique chez le nourrisson et l'enfant. Notions physiopathologiques et exploration. *Archives Françaises de Pédiatrie*, **31**, 587–617.

FOREST, M.G. & CATHIARD, A.M. (1975) Pattern of testosterone and androstenedione during the first month of life: evidence for testicular activity at birth. *Journal of Clinical Endocrinology and Metabolism*, **41**, 977–980.

FOREST, M.G., CATHIARD, A.M. & BERTRAND, J. (1975) Hypothalamo-hypophyso-testicular axis maturation during infancy in boys. *Xth Acta Endocrinologica Congress, Amsterdam* (26–29 August), p. 92

FRISCH, R.E. (1974) A method of prediction of age of menarche from height and weight at ages 9 through 13 years. *Pediatrics*, **53**, 384–390.

FROWEIN, J. & ENGEL, W. (1975) Binding of human chorionic gonadotrophin by rat testis. Effect of sexual maturation, cryptorchidism and hypophysectomy. *Journal of Endocrinology*, **64**, 59–66.

GILMORE, D.P. & DOBBIE, G.H. (1975) Absence of gonadotrophin releasing activity in the human foetal hypothalamus before 16 weeks. (Abstract). *Journal of Endocrinology*, **64**, 52P.

GITLIN, D. & BIASUCCI, A. (1969) Ontogenesis of immunoreactive growth hormone, follicle-stimulating-hormone, thyroid-stimulating hormone, luteinizing hormone, chorionic prolactin and chorionic gonadotropin in the human conceptus. *Journal of Clinical Endocrinology and Metabolism*, **29**, 926–935.

GOLDMAN, B.D., GRAZIA, Y.R., KAMBERI, I.A. & PORTER, J.C. (1971) Serum gonadotropin concentrations in intact and castrated neonatal rats. *Endocrinology*, **88**, 771–776.

GORSKI, R.A. (1971) Gonadal hormones and the perinatal development of neuroendocrine function. *Frontiers in Neuroendocrinology* (ed. by L. Martini and W. F. Ganong), pp. 237–290. Oxford University Press, London.

GORSKI, M.E. & LAWTON, I.E. (1973) Adrenal involvement in determining the time of onset of puberty in rat. *Endocrinology*, **93**, 1232–1234.

GROOM, G.C. & BOYNS, A.R. (1973) Effect of hypothalamic releasing factors and steroids on release of gonadotrophins by organ cultures of human fetal pituitaries. *Journal of Endocrinology*, **59**, 511–522.

GRUMBACH, M.M. & KAPLAN, S.L. (1973) Ontogenesis of growth hormone, insulin, prolactin and gonadotropin secretion in the human foetus. *Advances in Foetal and Neonatal Physiology* (ed. by K. W. Cross and P. Nathanielsz), pp. 462–487. Cambridge University Press.

GRUMBACH, M.M., ROTH, J.C., KAPLAN, S.L. & KELCH, R.P. (1974) Hypothalamic–pituitary regulation of puberty. Evidence and concepts derived from clinical research. *The Control of Onset of Puberty* (ed. by M. M. Grumbach, G. D. Grave and F. E. Mayer), pp. 115–116. Wiley, New York.

GUSTAFSSON, J.A., INGELMAN-SUNDBERG, M. & STENBERG, A. (1975a) Neonatal androgenic programming of hepatic steroid metabolism in rats. *Journal of Steroid Biochemistry*, **6**, 643–650.

GUSTAFSSON, J.A., LARSSON, A., SKETT, P. & STENBERG, A. (1975b) Influence of a novel hypophyseal factor on steroid metabolism in cultured hepatoma cells. *Proceedings of the National Academy of Sciences of the United States of America*, **72**, 4986–4990.

GUSTAFSSON, J.A. & STENBERG, A. (1974) Masculinization of rat liver enzyme activities following hypophysectomy. *Endocrinology*, **95**, 891–896.

GUSTAFSSON, J.A. & STENBERG, A. (1975) Influence of prolactin on the metabolism of steroid hormones in rat liver and adrenals. *Acta Endocrinologica*, **78**, 545–553.

GUYDA, H.J. & FRIESEN, H.G. (1973) Serum prolactin levels in human from birth to adult life. *Pediatric Research*, **7**, 534–540.

HAGEN, C. & MCNEILLY, A.S. (1975) The specificity and application of a radioimmunoassay for the α-subunit of luteinizing hormone in man. *Acta Endocrinologica*, **78**, 664–674.

HAGEN, C., MCNEILLY, A.S. & CHARD, T. (1974) Measurement and identification of gonadotrophins and their subunits in human maternal and fetal circulation around term. (Abstract). *Journal of Endocrinology*, **63**, 28P.

HOLWEG, W. & DOHRN, M. (1932) Uber die Beziehungen zwischen Hypophysenvorderlappen und Keimdrüsen. *Klinische Wochenschrift*, **11**, 233–235.

IMPERATO-MCGINLEY, J., GUERRERO, L., GAUTIER, T. & PETERSON, R.E. (1974) Steroid 5 α-reductase deficiency in man: an inherited form of male pseudohermaphroditism. *Science*, **186**, 1213–1215.

JIRASEK, J.E. (1970) The relationship between differentiation of the testicle, genital ducts and external genitalia in fetal and postnatal life. *The Human Testis* (ed. by E. Rosemberg and C. Paulsen), pp. 19–30. Plenum Press, New York.

JOB, J.C., GARNIER, P.E., CHAUSSAIN, J.L., BINET, E., RIVAILLE, P. & MILHAUD, G. (1972) Effect of synthetic luteinizing hormone-releasing hormone (LH-RH) on serum gonadotropins (LH and FSH) in normal children and in adults. *Revue Européenne d'Etudes Cliniques et Biologiques*, **17**, 411–414.

JOB, J.C., GUILHAUME, B., CHAUSSAIN, J.L. & GARNIER, P.E. (1975) Le développement isolé des seins chez les fillettes. Résultats de l'épreuve à la LH-RH. *Archives Françaises de Pédiatrie*, **32**, 39–48.

JOST, A. (1970) Hormonal factors in the development of the male genital system. *The Human Testis* (ed. by E. Rosemberg and C. Paulsen), pp. 11–18. Plenum Press, New York.

JOST, A., VIGIER, E., CREPIN, J. & PERCHELLET, J. (1973) Studies on sex differentiation in mammals. *Recent Progress in Hormone Research*, **29**, 1–41.

JUDD, H.L., PARKER, D.C., SILER, TH.M. & YEN, S.S.C. (1974) The nocturnal rise of plasma testosterone in pubertal boys. *Journal of Clinical Endocrinology and Metabolism*, **38**, 710–713.

KANTERO, R.L., WIDE, L. & WIDHOLM, O. (1975) Serum growth hormone and gonadotrophins and urinary steroids in adolescent girls. *Acta Endocrinologica*, **78**, 11–21.

KARSCH, F.J., DIERSCHKE, D.J. & KNOBIL, E. (1973) Sexual differentiation of pituitary function: apparent difference between primates and rodents. *Science*, **179**, 484–486.

KELCH, R.P., KAPLAN, S.L. & GRUMBACH, M.M. (1973) Suppression of urinary and plasma follicle-stimulating hormone by exogenous estrogens in prepubertal and pubertal children. *Journal of Clinical Investigation*, **52**, 1122–1128.

KENNY, F.M., ANGSUSINGHA, K., STINSON, D. & HOTCHKISS, H. (1973) Unconjugated estrogens in the perinatal period. *Pediatric Research*, **7**, 826–831.

KIRKLAND, J., KIRKLAND, R., LIBRICK, L. & CLAYTON, G. (1974) Serum gonadotropin levels in female adolescents with congenital adrenal hyperplasia. *Journal of Pediatrics*, **84**, 411–414.

KNOBIL, E. (1974) Maturation of the neuro-endocrine control of gonadotropin secretion in the Rhesus monkey. *Colloque International sur l'Endocrinologie Sexuelle de la Période Périnatale* (ed. by M. G. Forest and J. Bertrand), Vol. 32, pp. 205–218. INSERM, Paris.

KNORR, D., BIDLINGMAIER, F., BUTENANDT, O., FENDEL, H. & EHRT-WEHLE, R. (1974) Plasma testosterone in male puberty: I. Physiology of plasma testosterone. *Acta Endocrinologica*, **75**, 181–194.

KORTH-SCHULTZ, S., LEVINE, L.S. & NEW, M.I. (1975) Adrenal androgens in precocious adrenarche. *Acta Endrocrinologica*, **84**, Suppl. 193, Abstract No. 84.

KULIN, H.E. & REITER, E.O. (1973) Gonadotropins during childhood and adolescence: a review. *Pediatrics*, **51**, 260–271.

LAMPRECHT, S.A., ZOR, U., TSAFRIDI, A. & LINDNER, H.R. (1973) Action of prostaglandin E_2 and of luteinizing hormone on ovarian adenylate cyclase, protein kinase and ornithine decarboxylase activity during post-natal development and maturity in the rat. *Journal of Endocrinology*, **57**, 217–233.

LAND, R.B., THIMONIER, J. & PELLETIER, J. (1970) Possibilité d'induction d'une décharge de LH par une injection d'oestrogènes chez l'agneau femelle en fonction de l'âge. *Comptes Rendus de l'Académie des Sciences (Paris)*, Série D, **271**, 1549–1551.

LEE, P.A., JAFFE, R.B. & MIDGLEY, R.R. (1974) Serum gonadotropin, testosterone and prolactin concentration throughout puberty in boys: a longitudinal study. *Journal of Clinical Endocrinology and Metabolism*, **39**, 664–672.

LEVINA, S.E. (1968) Endocrine features in development of human hypothalamus, hypophysis and placenta. *General and Comparative Endocrinology*, **11**, 151–158.

LEVINA, S.E. (1970) Regulation of secretion of hypophyseal gonadotropins in human embryogenesis. *Problem Endokrinologia Moskowa*, **16**, 53–59.

LEVINA, S.E. (1972) Times of appearance of LH and FSH activities in human foetal circulation. *General and Comparative Endocrinology*, **19**, 242–251.

LEVINA, S.E., GYEVAI, A. & HORVATH, E. (1975) Responsiveness of the ovary to gonadotrophins in pre and perinatal life: oestrogen secretion in tissue and organ cultures. *Journal of Endocrinology*, **65**, 219–223.

LORAS, B., GENOT, A., MONBON, M., BUECHER, F., REBOUD, J.P. & BERTRAND, J. (1974) Binding and metabolism of testosterone in the rat brain during sexual maturation. II. Testosterone metabolism. *Journal of Steroid Biochemistry*, **5**, 425–431.

MCCANN, S.M. (1974) Sex steroids, pituitary and hypothalamic hormones during puberty in experimental animals. *The Control of the Onset of Puberty* (ed. by M. M. Grumbach, G. D. Grave and F. E. Mayer), pp. 1–20. Wiley, New York.

MCDONALD, P.G. & DOUGHTY, G. (1974) Effect of neonatal administration of different androgens in the female rat; correlation between aromatization and the induction of sterilization. *Journal of Endocrinology*, **61**, 95–103.

MASSA, R., JUSTO, S. & MARTINI, L. (1975) Conversion of testosterone into 5 α-reduced metabolites in the anterior pituitary and in the brain of maturing rats. *Journal of Steroid Biochemistry*, **6**, 567–571.

MOGER, W.H. & ARMSTRONG, D.T. (1974) Changes in serum testosterone levels following acute LH treatment in immature and mature rats. *Biology of Reproduction*, **11**, 1–6.

MONEY, J. & EHRHARDT, A.A. (1972) In: *Man and Woman, Boy and Girl*. Johns Hopkins University Press, Baltimore.

MORERA, A.M., AUDI, L. & SAEZ, J.M. (1974) Augmentation de la progestérone et pic de composés oestrogènes-immuno-réactifs dans le plasma des rats impubères. *Comptes Rendus de l'Académie des Sciences (Paris)*, Série D, **279**, 73–76.

NAFTOLIN, F., RYAN, K.J. & PETRO, Z. (1971) Aromatization of androstenedione by the diencephalon. *Journal of Clinical Endocrinology and Metabolism*, **33**, 368–370.

NANKIN, H.R., YANAIHARA, T. & TROEN, P. (1971) Response of gonadotropins and testosterone to clomiphene stimulation in a pubertal boy. *Journal of Clinical Endocrinology and Metabolism*, **33**, 360–363.

ODELL, W.D. (1974) The role of the gonads in sexual maturation. *The Control of the Onset of Puberty* (ed. by M. M. Grumbach, G. D. Grave and F. E. Mayer), pp. 313–332. Wiley, New York.

ODELL, W.D., SWERDLOFF, R.S., BAIN, J., WOLLESEN, F. & GROVER, P.K. (1974) The effect of sexual maturation on testicular response to LH stimulation of testosterone in the intact rat. *Endocrinology*, **95**, 1380–1384.

PASTEELS, J.L., GAUSSET, P., DANGUY, A. & ECTORS, F. (1974) Gonadotropin secretion by human feotal and infant pituitaries. *Colloque International sur l'Endocrinologie Sexuelle de la Période Périnatale* (ed. by M. G. Forest and J. Bertrand), Vol. 32, pp. 13–36. INSERM, Paris.

PAYNE, A.H. & JAFFE, R.B. (1974) Androgen formation from pregnenolone sulfate by the human fetal ovary. *Journal of Clinical Endocrinology and Metabolism*, **39**, 300–304.

PENNY, R., OLAMBIWONNU, N.O. & FRASIER, S.D. (1973) Precocious puberty following treatment in a six-year-old male with congenital adrenal hyperplasia. Studies of serum luteinizing hormone (LH), serum follicle-stimulating-hormone (FSH) and plasma testosterone. *Journal of Clinical Endocrinology and Metabolism*, **36**, 920–924.

PENNY, R., OLAMBIWONNU, N.O. & FRASIER, D. (1974) Serum gonadotropin concentration during the first year of life. *Journal of Clinical Endocrinology and Metabolism*, **38**, 320–321.

PFEIFFER, C.A. (1935) Origin of functional differences between male and female hypophyses. *Proceedings of the Society for Experimental Biology and Medicine*, **32**, 603–605.

PRYSE-DAVIES, J. & DEWHURST, C.J. (1971) The development of the ovary and uterus in the foetus, newborn and infant: a morphological and enzyme histochemical study. *Journal of Pathology*, **103**, 5–25.

RAMALEY, J.A. (1974) Adrenalgonadal interactions at puberty. *Life Sciences*, **14**, 1623–1633.

RAMIREZ, V.D. (1973) Endocrinology of puberty. *Handbook of Physiology* (ed. by R. O. Greep and E. B. Astwood), Vol. II, part 1, pp. 1–28. Williams & Wilkins, Baltimore.

REID, J.R. (1960) Congenital absence of the pituitary gland. *Journal of Pediatrics*, **56**, 658–664.

REITER, E.O., KULIN, H.E. & HAMWOOD, S.M. (1974) The absence of positive feed-back between estrogens and luteinizing hormone in sexually immature girls. *Pediatric Research*, **8**, 740–745.

REITER, E.O., SAVAGE, D.C.L., COMTE, F.A., DE GROPPA, M.I., KAPLAN, S.L. & GRUMBACH, M.M. (1974) The response to LRF in idiopathic precocious puberty (IPP) and congenital adrenal hyperplasia. (Abstract). *Pediatric Research*, **8**, 373/99.

REITER, E.O., GRUMBACH, M.M., KAPLAN, S.L. & CONTE, F.A. (1975) The response of pituitary gonadotropes to synthetic LRF in children with glucocorticoid treated congenital adrenal hyperplasia: lack of effect of intrauterine and neonatal androgen excess. *Journal of Clinical Endocrinology and Metabolism*, **40**, 318–325.

REYES, F.I., WINTER, J.S.D. & FAIMAN, C. (1973) Studies on human sexual development. I. Fetal gonadal and adrenal sex steroids. *Journal of Clinical Endocrinology and Metabolism*, **37**, 74–78.

REYES, F.I., BORODITSKY, R.S., WINTER, J.S.D. & FAIMAN, C. (1974) Studies on human sexual development. II. Fetal and maternal serum gonadotropin and sex steroid concentrations. *Journal of Clinical Endocrinology and Metabolism*, **38**, 612–617.

RICE, B.F., PONTHIER, R., JR & STERNBERG, W. (1968) Luteinizing hormone and growth hormone activity of human fetal pituitary. *Journal of Clinical Endocrinology and Metabolism*, **28**, 1071–1073.

ROSENFIELD, R.L. (1971) Plasma 17-ketosteroids and 17 β-hydroxysteroids in girls with premature development of sexual hair. *Journal of Pediatrics*, **79**, 260–266.

ROTH, J.C., KELCH, R.P., KAPLAN, S.L. & GRUMBACH, M.M. (1972) FSH and LH response to luteinizing hormone-releasing factor in prepubertal and pubertal children, adult males and patients with hypogonadotrophic and hypergonadotrophic hypogonadism. *Journal of Clinical Endocrinology and Metabolism*, **35**, 926–930.

SAEZ, J.M. & BERTRAND, J. (1968) Studies on testicular function in children: plasma concentrations of testosterone, dehydroepiandrosterone and its sulfate before and after stimulation with human chorionic gonadotrophin. *Steroids*, **12**, 749–761.

SCHALLY, A.V., KASTIN, A.J. & ARIMURA, A. (1972) The hypothalamus and reproduction. *American Journal of Obstetrics and Gynecology*, **114**, 423–442.

SCHOLLER, R., ROGER, M., LEYMARIE, P. & CASTANIER, M. (1975) Evaluation of Leydig-cell function in normal prepubertal and pubertal boys. *Journal of Steroid Biochemistry*, **6**, 95–99.

SERRA, G.B., PEREZ-PALACIOS, G. & JAFFE, R.B. (1970) De novo testosterone biosynthesis in the human fetal testis. *Journal of Clinical Endocrinology and Metabolism*, **30**, 128–130.

SETALO, G., VIGH, S., SCHALLY, A.V., ARIMURA, A. & FLERKO, B. (1975) LH-RH containing neural elements in the rat hypothalamus. *Endocrinology*, **96**, 135–142.

SHIN, S.H. & KRAICER, J. (1974) LH-RH radioimmunoassay and its applications. Evidence of antigenically distinct FSH-RH and diurnal study of LH-RH and gonadotropins. *Life Sciences*, **14**, 281–282.

SHORT, R.V. (1974) Sexual differentiation of the brain of the sheep. *Colloque International sur l'Endocrinologie Sexuelle de la Période Périnatale* (ed. by M. G. Forest and J. Bertrand), Vol. 32, pp. 121–142. INSERM, Paris.

SHUTT, D.A., SMYTH, L.D. & SHEARMAN, R.P. (1974) Oestrone, oestradiol-17β and oestriol levels in human foetal plasma during gestation and at term. *Journal of Endocrinology*, **60**, 333–341.

SIITERI, P.K. & WILSON, J.D. (1974) Testosterone formation and metabolism during male sexual differentiation in the human embryo. *Journal of Clinical Endocrinology and Metabolism*, **38**, 113–125.

SILER-KHODR, T.M., MORGENSTERM, L.L. & GREENWOOD, F.C. (1974) Hormone synthesis and release from human fetal adenohypophysis in vitro. *Journal of Clinical Endocrinology and Metabolism*, **39**, 891–905.

SIZONENKO, P.C., CUENDET, A. & PAUNIER, P. (1973) FSH. I. Evidence for its mediating role on testosterone secretion in cryptorchidism. *Journal of Clinical Endocrinology and Metabolism*, **37**, 68–73.

STEELE, R.E. & WEISZ, J. (1974) Changes in sensitivity of the estradiol LH feed-back system with puberty in the female rat. *Endocrinology*, **95**, 513–520.

STRICKLAND, A.L., NAYFEH, S.N. & FRENCH, F.S. (1970) Conversion of cholesterol to testosterone and androstanediol in testicular homogenates of immature and mature rats. *Steroids*, **15**, 373–387.

TULCHINSKY, D. (1973) Placental secretion of unconjugated estrone, estradiol and estriol into the maternal and the fetal circulation. *Journal of Clinical Endocrinology and Metabolism*, **36**, 1079–1087.

TURGEON, J.L. & BARRACLOUGH, C.A. (1974) Pulsatile plasma LH rhythms in normal and androgen sterilized ovariectomized rats. Effects of estrogen treatment. *Proceedings of the Society for Experimental Biology and Medicine*, **145**, 821–825.

VILAR, O. (1970) Histology of the human testis from neonatal period to adolescence. *The Human Testis* (ed. by E. Rosemberg and C. Paulsen), pp. 95–111. Plenum Press, New York.

VILLEE, D.B. (1969) Development of endocrine function in the human placenta and fetus (Part II). *New England Journal of Medicine*, **281**, 533–541.

VISSER, H.K.A. & DEGENHART, H.J. (1966) Excretion of six individual 17-ketosteroids and testosterone in four girls with precocious sexual hair (premature pubarche). *Helvetica Paediatrica Acta*, **21**, 409–422.

WINTER, J.S.D., FAIMAN, C., HOBSON, W.C., PRASAD, A.V. & REYES, F.I. (1975) Pituitary-gonadal relations in infancy I. Patterns of serum gonadotropin concentrations from birth to four years of age in man and chimpanzee. *Journal of Clinical Endocrinology and Metabolism*, **40**, 545–551.

ZACHMAN, M. (1974) Evaluation of gonadal function in childhood and adolescence. *Helvetica Paediatrica Acta*, Suppl. 34, 53–62.

ZONDEK, L.H. & ZONDEK, T. (1974) The influence of complications of pregnancy and of some congenital malformations on the reproductive organs of the male foetus and neonate. *Colloque International sur l'Endocrinologie Sexuelle de la Période Périnatale* (ed. by M. G. Forest and J. Bertrand), Vol. 32, pp. 79–96. INSERM, Paris.