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PLASMA ANDROGENS (TESTOSTERONE AND 4-ANDROSTENEDIONE) AND 17-HYDROXYPROGESTERONE IN THE NEONATAL, PREPUBERTAL AND PERIPUBERTAL PERIODS IN THE HUMAN AND THE RAT: DIFFERENCES BETWEEN SPECIES

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SUMMARY

The main features of the developmental pattern of three Δ^4-steroid hormones in human and rat are as follows: In both species testosterone (T) is more elevated in the male than in female at birth. In early infancy values in the human are only half those of adults while they are 1/10 in the rat. Although decreasing after infancy, T levels remain higher in the male than in the female rat during the prepubertal period while they are identical in both sexes in the human. In the human, 4-androstenedione (Δ^4) and 17OH-progesterone (OHP) levels in both sexes are at their lowest during early childhood and their prepubertal rise (adrenarche) is only 2-fold. In the rat both Δ^4 and OHP increase to reach peak levels, by 25 days of life. At the onset of puberty Δ^4 and OHP further increase in the human while they decrease in the rat. These data suggest that; (1) the perinatal testicular activity is greater in man than in the rat; (2) The high levels of Δ^4 and 17OHP observed from 3 to 5 weeks of age in the rat might represent a prepubertal activation of the adrenal, "equivalent" to the human adrenarche. (3) The testicular contributions to the peripheral pool of OHP also differs between the two species but is probably minimal, if significant, in the rat.

INTRODUCTION

It is now well established that the hypothalamus of the rat is not yet sex-differentiated at birth and can be modified by a neonatal administration of testosterone [1–4] which produces an irreversible sterilization in the female. On the other hand, male castrated at birth exhibit a female-like neuro-endocrine pattern when given ovarian implants in adulthood. This suggests that a testicular endocrine secretion occurs at birth and is necessary to complete the male differentiation of the hypothalamus, while a passive role for the ovary at birth could be inferred.

Departing from this concept, although it has since been demonstrated that the situation was not the same in mammals [5] we have conducted studies which have shown that in the human there is a marked perinatal testicular activity under hypothalamo–hypophyseal control which stops at 7 months of age [6–9]. In previous works we also presented evidence indicating that the human ovaries might have a certain endocrine function during the second trimester of life [9, 10]. In human long before the onset of puberty there is also a selective increase in the production of adrenal androgens of the Δ^5 pathway (DHA and DHA-sulfate) [11–13].

In the rat it has been reported that a compensatory hypertrophy of the ovary follows hemicastration [14, 15], that early administration of antibodies to estradiol affects ovarian morphology [16], that *in vitro* a peak of ovarian endocrine activity occurs between 8 and 12 days post partum [17] and that in the male, neonatal [18] or prepubertal [19] surges of testosterone occur. On the other hand, other steroids such as progesterone [20, 21] or of unknown nature [21, 22] also exhibit surges in prepubertal rats in both sexes.

The role of the adrenal in sexual maturation has been investigated by various authors for both human [12] and rat [23]. We, therefore, reinvestigate the rat as a possible model to study the mechanism of the neuroendocrine changes occurring during sexual differentiation and maturation.

The patterns of testosterone* and those of its two immediate precursors, namely 4-androstenedione and 17α-hydroxyprogesterone have been established from birth to sexual maturation in Sprague–Dawley rats and are compared to those observed in the human.

MATERIAL AND METHODS

Blood was obtained from a peripheral vein of 550 normal human subjects of various ages between 9.00 and 11.00 [6–10, 24].

Sprague–Dawley rats, born in the laboratory, were maintained under conditions of controlled tempera-

*The following trivial names and abbreviations are used: T, testosterone = 17β-hydroxy-4-androsten-3-one; Δ^4, androstenedione = 4-androstene-3,17-dione; OHP, 17-hydroxyprogesterone = 17α-hydroxy-4-pregnen-3-one.

ture and lighting (12 h–12 h) with free access to food and water. Blood was collected by decapitation from 3000 rats between 12.00 and 13.00. Plasma was immediately decanted after centrifugation and stored at −25°C until analyzed. So as to obtain sufficient amouts of plasma, specimens were pooled, 4–35 according to age. For each day of life 6–8 pools were made.

Testosterone (T), 4-androstenedione (Δ^4) and 17α-hydroxyprogesterone (OHP) were separated by celite column chromatography as previously described [25]. Δ^4 was reduced to T [7], repurified on a second celite column chromatography and measured as T. Specific radioimmunoassays, described in detail elsewhere [7, 24, 25], were used to determine the plasma concentrations of T, Δ^4 and OHP in both species. Determinations were in all cases made in triplicate on progressive aliquots. In this study inter assay variations were 6.5% for T, 6.9% for Δ^4 and 8.4% for OHP.

RESULTS AND DISCUSSION

In the human

From the large gradient between cord (35.7 ± 10.5 ng/dl) and peripheral plasma concentrations (228 ± 128) it was concluded that the testes are still very active at birth [8]. The patterns thereafter observed for T, Δ^4 and OHP throughout infancy are summarized in Fig. 1. In male infants T, Δ^4 and OHP levels, after a rapid decrease during the first week of life, increase to peak levels of respectively 248 ± 49, 35 ± 10.6 and 201 ± 79 ng/dl at 1–3 months of age, decrease thereafter and reach at 6 months of life the lowest life values (respectively 6.8 ± 2.5, 11 ± 4 and 29 ± 21 ng/dl for T, Δ^4 and OHP). In female infants while T and Δ^4 are by 2 to 3 weeks of age in the same range as for males, OHP levels remained significantly higher at 7–9 months of age (60 ± 42 ng/dl).

The marked differences observed between sexes in these hormonal patterns indicate that in both sexes the gonads are active during infancy but at different ages: the testis exhibiting a large secretory activity

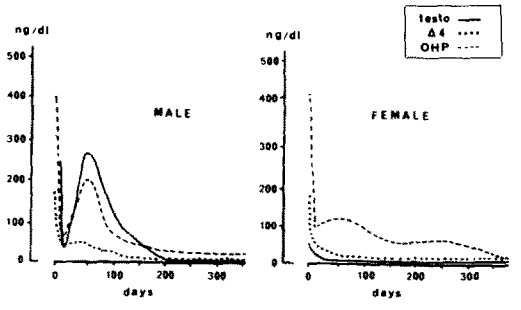

Fig. 1. Mean plasma levels of testosterone (testo), 4-androstenedione (Δ^4) and 17-hydroxyprogesterone (OHP) in normal male and female infants (compiled data from previous works [6–10]). The curves join the mean values observed in detailed age groups (days × 5, week × 2, then every month).

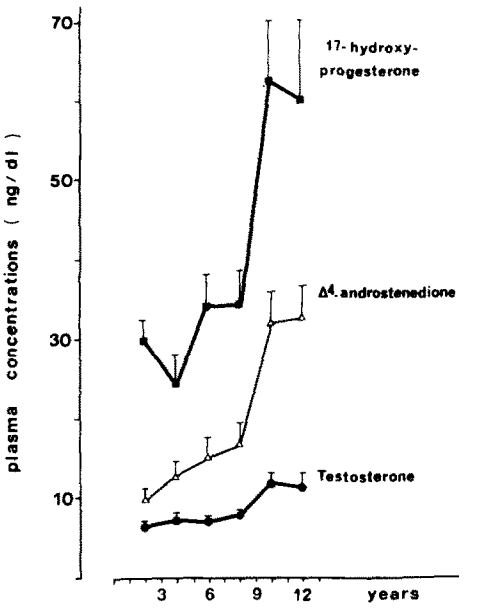

Fig. 2. Detailed changes (adrenarche) of the plasma concentrations (mean ± SD) of testosterone, 4-androstenedione and 17-hydroxyprogesterone in 98 normal prepubertal boys.

at 1–3 months, the ovary a more discrete one at 6–12 months of age.

The prepubertal period, from the second year of life to the onset of puberty, is characterized by 2 stages: during the first, which we call stage P_0 of pubertal development, T, Δ^4 and OHP remain at the lowest levels observed in life with no difference between sexes. The second stage (P_1 of pubertal development) is characterized by a rather abrupt and continued rise in the plasma levels of these hormones. The selective increase in adrenal Δ^5-androgens (DHA and DHA sulfate) is well know (adrenarche) and its exact chronology (abrupt rise of DHA at the 7th year of life) has been recently documented [11–13]. In a previous work [12] it has been shown that the levels of Δ^4 also increase during stage P_1, but about 2 years later. It appeared to us that the low peripheral conversion of DHA into Δ^4 [26] was not the only explanation for these chronologic differences, but rather that the "adrenarcheal changes" also involve the 4-ene pathway. We therefore investigate another group of normal children. The results of this study in males are presented in Fig. 2. The changes were similar in females. From these results it appears that Δ^4 and OHP increase at the same time (between 8 and 9 years of age). T also increases slowly probably at the same period but only becomes significant when a large series of children are studied.

The pubertal changes in androgens are well documented in the literature. We report our personal experience (Fig. 3) for comparison of absolute values. In male, T increases first rather slowly from stage P_2 to P_3, then very steeply from stage P_3 to P_5. Due to the large contribution of the adrenal production of the adrenal production [26] Δ^4 and OHP levels increase slowly throughout puberty in both sexes.

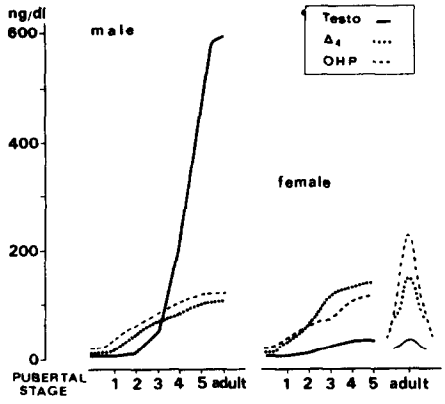

Fig. 3. Hormonal changes throughout puberty in human. The curves join the mean values observed at each stage of pubertal development.

In the rat

At birth, T levels were significantly higher in males (41.2 ± 6.7 ng/dl) than in females (7.3 ± 1.1 ng/dl). The sex difference was not significant for Δ^4 and OHP (respectively 28 ± 9 and 55 ± 30 in males and 17 ± 11 and 82 ± 29 ng/dl in females).

The daily changes in the levels of the 3 hormones from day 1 to puberty were analyzed from 5 to 7 days intervals and results are presented in Figs 4 and 5. In the male plasma T levels declined somewhat during the first days of life but remained similar until the onset of puberty. In the female T levels remained at the same low level (8–9 ng/dl) until day 18 when they rose slightly but significantly. Δ^4 levels were slightly but significantly higher in the male (19 ± 6 ng/dl) than in the female (12 ± 5 ng/dl) the first two weeks of life, after which they rose in both. Peak levels observed between days 20 and 30 were the same in both sexes (25.4 ± 16 and 28 ± 12 ng/dl). Comparable changes were observed for OHP which

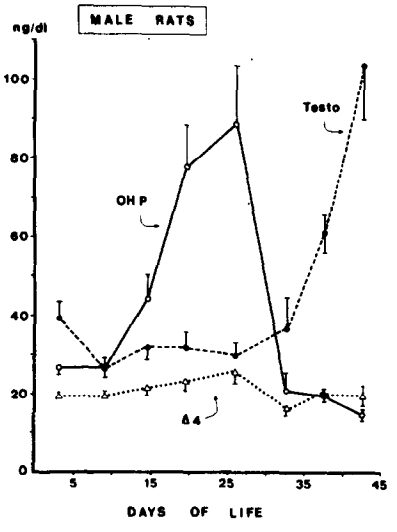

Fig. 4. Mean (±SE) levels of testosterone (testo), 4-androstenedione (Δ^4) and 17-hydroxyprogesterone (OHP) from birth to puberty in the male rat.

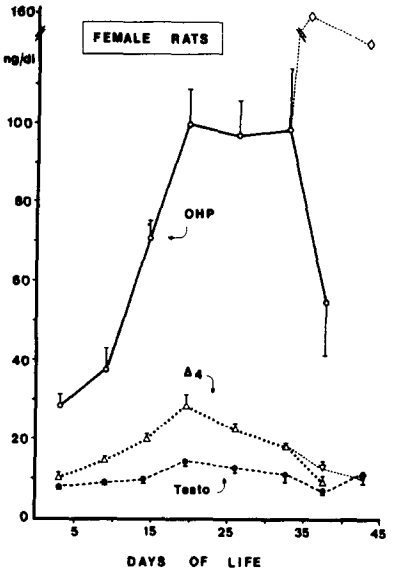

Fig. 5. Hormonal prepubertal changes in the female rat. The smallest dotted lines represent female with open vagina. See Fig. 4 for other legends.

showed peak levels of respectively 89 ± 67 and 100 ± 36 ng/dl in males and females between 20 and 30 days of age. Thereafter the plasma concentrations of OHP dropped abruptly in the male to 15.4 ± 2.2 ng/dl and remained at these low levels in adults. A similar drop in OHP after 32 days of age was observed in females but only in those with a closed vagina.

In the male rat, T levels rose abruptly at the onset of puberty (day 34–35) reaching adult values (248 ± 14 ng/dl) by 45 days of age. A slight rise in Δ^4 was also observed (adult values = 26 ± 2 ng/dl).

We were unable to find any significant late neonatal or prepubertal surge of T as described in Holtzman rats [19] for male Sprague–Dawley rats.

To our knowledge, OHP levels have not been reported in the rat, and no detailed simultaneous patterns of T, Δ^4 and OHP appeared in the literature. The most interesting findings were the large increase of OHP and to a minor extent of Δ^4 during the prepubertal period. Its occurrence in both sexes as the decline of OHP after day 32 in immature female and in males suggests its adrenal origin. A similar prepubertal rise in progesterone levels (maximal at 25 days of age) has been described in the same strain of rats raised in the same conditions and same housing and was proven to be of adrenal origin [21]. Thus in the rat there is a prepubertal increase of production of all steroids of the 4-ene pathway. We consider this phenomenon as the phyllogenic variant of the human adrenarche. The possible physiological role of these steroids in sexual maturation still remains to be established.

Comparison between the two species

A tentative comparison of the evolution of plasma concentrations of T, Δ^4 and OHP throughout life in

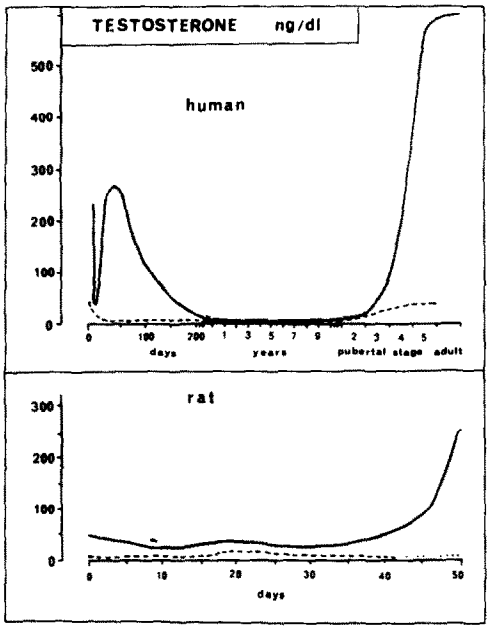

Fig. 6. Patterns of the plasma levels of testosterone from birth to adulthood in male (solid line) and female (dotted line) humans and rats.

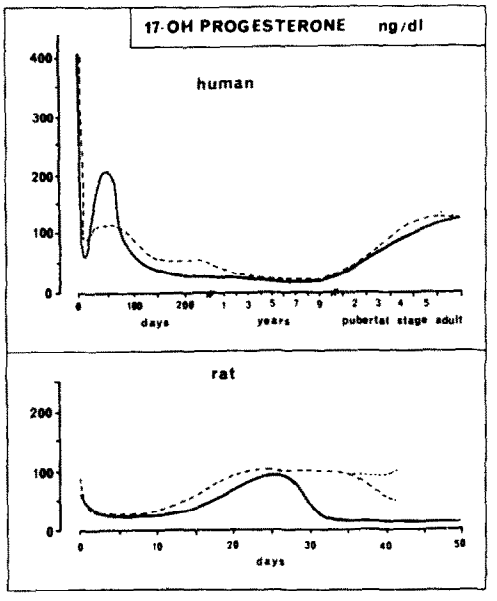

Fig. 8. Developmental changes in 17-hydroxyprogesterone levels in the human and the rat. Mean values are joined in solid lines for males and in dotted lines for females.

man and rats is presented in Fig. 6–8. Such a comparison may be artefactual since life span is so different in the 2 species; in particular steps in sexual maturation are more difficult to delineate in the rat than in human. However, when comparing absolute hormonal levels at different periods of life within the same species, two points can be discussed.

(1) At birth, the rat is very immature as compared to the human. In this species testicular testosterone directly or *via* its conversion into other steroids in target cells, irreversibly programs the hypothalamic centers involved in the hypothalamic–hypophyseal control of the gonads [1–4] and of hepatic sex-dependant steroid and drug metabolism [27]. The period during which this effect is exerted is "critically" limited to the first 5 days of life. Hypothetized sequence of neonatal "critical periods" of CNS imprinting or organization which affect growth rate of developing rat [28] and sexual behavior [29] have been demonstrated to be under dose-dependent androgen control.

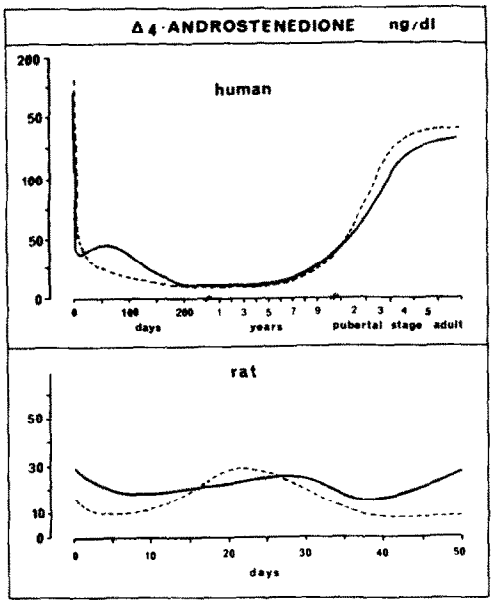

Fig. 7. Developmental changes in 4-androstenedione levels in the human and the rat (note the different scales in the ordinates). Solid lines represent mean values for males while dotted lines represent those for females.

When considering the rather low levels of testosterone present in the blood stream of the male rat at birth (41 ng/dl) and during the first week of life (39 ± 19 ng/dl) which are not considerably higher than those (8 ± 4 ng/dl) observed in the female counterpart and 8–10 times lower than those observed in adult males (248 ± 14 ng/dl), it is surprising that such small quantities of hormone could exert such irreversible effects.

In human, sexual differentiation of the hypothalamus appears complete at birth. However, in the male infant during the first 3 months of life T levels are five times higher than in the male newborn rat.

We wondered if in the rat the unbound fraction of T (biologically active fraction) was not considerably higher than in man. Using equilibrium dialysis we measured the percentages of T and Δ^4 bound by plasma proteins at two different ages in both species. The unbound fractions were calculated and the results are presented in Table 1. As can be seen, the unbound fraction of T is somewhat higher in the rat than in the human, but is in both species much lower than that of Δ^4. These data would suggest that in the rat

Table 1. Percentages of unbound levels of testosterone (T) and 4-androstenedione (Δ^4) in the plasma of male men and rats according to the stage of pubertal development

	Human		Rats	
	T	Δ^4	T	Δ^4
I. Prepubertal	0.62 ± 0.18	4.64 ± 0.94	3.15 ± 0.3	7.19 ± 0.68
(*n*)	(40)	(40)	(6)*	(6)*
II. Adult	1.45 ± 0.31	4.84 ± 0.5S	4.0 ± 0.08	7.14 ± 1.33
(*n*)	(25)	(25)	(6)*	(6)*
I vs II *P*	<0.01	NS	=0.05	NS

* Pools.

Table 2. Changes in the mean ratios of the plasma concentrations of testosterone over those of 4-androstenedione or 17α-hydroxyprogesterone during development

	Human		Rat	
	T/Δ	T/OHP	T/Δ	T/OHP
Prepubertal	0.55	0.25	1.3	0.33
Pubertal	5.2	4.9	9.9	16.7

there is also a specific binding for T, the nature of which remains to be elucidated.

By calculating the unbound concentrations of T in young and adult rats it would appear that the neonatal sexual differentiation of the brain requires a much lower level of androgens than do the establishment and the maintenance of adult sexual characteristics.

In human what are the actions of such high levels of T during early infancy? This question is still unanswered. However, in the Rhesus monkey, a model often used for man [30], it is possible that the postnatal period might be a critical one during which the CNS may be organized by androgens for control of pubertal timing and sexual behavior.

(2) In adulthood, the human testis secrete a number of sex steroids among which the predominant one is clearly testosterone [31]. OHP is also secreted *in vivo* in large quantity by the human testes [32]. This is reflected by the high concentrations of both T and OHP at 2 periods of active testicular activity, infancy and adulthood.

In the rat, the pubertal period and adulthood are characterized by testosterone dominance in peripheral plasma concentrations, whereas the prepubertal period appears to be characterized by the dominance of OHP (Table 2). In the rat levels of T in adults are half of those observed in the human while those of OHP are remarkably low. Differences in the concentration of these hormones in peripheral blood are not as conclusive as concentrations in spermatic venous blood used by others [33]. However, these data, together with *in vitro* studies [34], suggest that, contrary to the human, the testicular secretion of OHP is minimal if present in the rat.

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REFERENCES

1. Barraclough C. A.: Modifications in reproductive function after exposure to hormones during the prenatal and early postnatal period. In *Neuroendocrinology* (Edited by L. Martini and W. F. Ganong). Academic Press, New York, Vol. II (1967) pp. 61–69.
2. Harris G. W. and Naftolin F.: The hypothalamus and control of ovulation. *Brit. Med. Bull.* **26** (1970) 3–9.
3. Flerko B.: Steroid hormones and the differentiation of the central nervous system. In *Current Topics in Experimental Endocrinology* (Edited by L. Martini and V. H. T. James). Academic Press, New York (1971) pp. 41–80.
4. Gorki R. A.: Gonadal hormones and the perinatal development of neuroendocrine function. In *Frontiers in Neuroendocrinology* (Edited by L. Martini and W. F. Ganong). Oxford University Press, New York (1971) pp. 237–290.
5. Karsh F. J., Dierscкike D. J. and Knobil E.: Sexual differentiation of pituitary function: apparent difference between primates and rodents. *Science* **179** (1973) 484–489.
6. Forest M. G., Cathiard A. M. and Bertrand J.: Evidence of testicular activity in early infancy. *J. clin. Endocr. Metab.* **37** (1973) 148–151.
7. Forest M. G., Sizonenko P. C., Cathiard A. M. and Bertrand J.: Hypophyso-gonadal function in humans during the first year of life—I. Evidence for testicular activity in early infancy. *J. clin. Invest.* **53** (1974) 819–828.
8. Forest M. G. and Cathiard A. M.: Pattern of plasma testosterone and Δ^4-androstenedione in normal newborns: evidence for testicular activity at birth. *J. clin. Endocr. Metab.* **41** (1975) 977–980.
9. Forest M. G. and Bertrand J.: Ontogeny on gonadal steroids in human: perinatal period. In *Research on Steroids* (Edited by A. Vermeulen *et al.*). Elsevier/North Holland, Vol. VII (1977) pp. 559–570.
10. Forest M. G. and Cathiard A. M.: Ontogenic study of plasma 17α-hydroxyprogesterone in the human—I. Post-natal period: evidence for a transient ovarian activity in infancy. *Ped. Res.* **12** (1978) 6–11.
11. de Peretti E. and Forest M. G.: Unconjugated dehydroepiandrosterone plasma levels in normal subjects from birth to adolescence in human: the use of a sensitive radioimmunoassay. *J. clin. Endocr. Metab.* **43** (1976) 982–991.
12. Ducharme J. R., Forest M. G., de Peretti E., Sempé M., Collu R. and Bertrand J.: Plasma adrenal and gonadal sex steroids in human pubertal development. *J. clin. Endocr. Metab.* **42** (1976) 468–476.

13. de Peretti E. and Forest M. G.: Pattern of plasma dehydroepiandrosterone sulfate levels in humans from birth to adulthood. Evidence for testicular production. *J. clin. Endocr. Metab.* **47** (1978) 572–577.
14. Gerall A. A. and Dunlap J. L.: Evindence that the ovaries of the neonatal rat secrete active substances. *J. Endocr.* **50** (1971) 529–530.
15. Dunlap J. L., Preis L. K. Jr. and Gerall A. A.: Compensatory ovarian hypertrophy as a function of age and neonatal androgenization. *Endocrinology* **90** (1972) 1309–1314.
16. Reiter E. O., Goldenberg R. L., Vaitukaitis J. L. and Ross G. T.: A role for endogenous estrogen in normal ovarian development in the neonatal rat. *Endocrinology* **91** (1972) 1537–1539.
17. Smeaton T. C., Ariondoulis D. E. and Steele P. A.: The synthesis of testosterone and estradiol-17β by the gonads of neonatal rats *in vitro*. *Steroids* **26** (1975) 181–192.
18. Resko J. A., Feder H. H. and Goy R. W.: Androgen concentration in plasma and testis of developing rats. *J. Endocr.* **40** (1968) 485–491.
19. Miyachi Y., Nieschlag E. and and Lipsett M.: The secretion of gonadotropins and testosterone by the neonatal male rat. *Endocrinology* **92** (1973) 1–5.
20. Döhler K. D. and Wuttke W.: Change with age in levels of serum gonadotropins, prolactin and gonadal steroids in prepubertal male and female rats. *Endocrinology* **97** (1975) 898–907.
21. Morera A. M., Audi L., Bertrand J. and Saez J. M.: Estrogen-like compounds and progesterone in male and female rats before puberty—I. Pattern and origin. *J. steroid Biochem.* **9** (1978) 623–629.
22. Weisz J. and Gunsalus P.: Estrogen levels in immature female rats: true or spurious—ovarian or adrenal? *Endocrinology* **93** (1973) 1057–1065.
23. Gorki M. E. and Lawton I. E.: Adrenal involvement in determining the time of onset of puberty in the rat. *Endocrinology* **93** (1973) 1232–1234.
24. Forest M. G.: Use of highly specific antibodies against 17α-OH progesterone in a simplified non chromatographic RIA and in the simultaneous determination of sex hormones in human plasma. *Hormone Res.* 7 (1976) 260–273.
25. Forest M. G., Cathiard A. M. and Bertrand J.: Total and unbound testosterone levels in the newborn and in normal and hypogonadal children: use of a sensitive radioimmunoassay for testosterone. *J. clin. Endocr. Metab.* **36** (1973) 1132–1142.
26. Horton R.: Sex steroid production and secretion in the male *Andrologia* **10** (1978) 183–194.
27. Gustafsson J. A., Eneroth P., Pousette A., Skett P., Sonnenschein C., Stenberg A. and Ahlen A.: Programing and differentiation of rat liver enzymes. *J. steroid Biochem.* **8** (1977) 429–443.
28. Tarttelin M. F., Shryne J. E. and Gorski R. A.: Patterns of body-weight change in rats following neonatal hormone manipulation: a "critical period" for androgen induced growth increases. *Acta Endocr., Copenh.* **79** (1975) 177–191.
29. Feder H. H., Naftolin F. and Ryan K. J.: Male and female sexual responses in male rats given estradiol benzoate and 5-α-androstan-17β-ol-3-one propionate. *Endocrinology* **94** (1974) 136–150.
30. Wilen R., Goy R. W., Resko J. A. and Naftolin F.: Pubertal body-weight and growth in the female Rhesus pseudohermaphrodite. *Biol. Reprod.* **16** (1977) 470–473.
31. Lipsett M.: Steroid secretion by the testis in man. In *The Endocrine Function of the Human Testis* (Edited by V. H. T. James, M. Serio and L. Martini). Academic Press, New York (1974) pp. 1–11.
32. Jönsson G., Ólsson A. M., Luttrop W., Cekan Z., Purvis K. and Diczfalusy E.: Treatment of prostatic carcinoma with various types of estrogens derivatives. *Vitamins and Hormones* **33** (1975) 351–376.
33. de la Torre B., Hedman M. and Diczfalusy E.: Species differences in steroidogenesis following the *in vitro* incubation of decapsulated testes. *Acta Endocr., Copenh.* **86** (1977) 851–864.
34. Hashimoto T. and Suzuki Y.: Androgens in testicular venous blood in the rat, with special reference to puberal change in the secretory pattern. *Endocr. Jap.* **13** (1966) 326–337.