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FORMATION OF 5α-REDUCED C_{19}-STEROIDS FROM PROGESTERONE *IN VIVO* BY 5α-REDUCED PATHWAY IN IMMATURE RAT OVARIES

T. INABA, T. IMORI and K. MATSUMOTO*

Department of Surgery and Obstetrics, School of Veterinary Science, College of Agriculture, University of Osaka prefecture, Osaka, Japan; and
*Institute for Cancer Research, Osaka University Medical School, Osaka, Japan

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SUMMARY

Either [^{3}H]-progesterone (0.6 nmol/30 μCi), or [^{14}C]-progesterone (6.6 nmol/0.2 μCi) plus [^{3}H]-5α-pregnane-3,20-dione (0.5 nmol/0.5 μCi), suspended in 5 μl of physiological saline solution, was directly injected into the ovary at the central portion in 28- and 140-day-old rats. Following injection, radioactive metabolites in ovary and ovarian vein blood were extracted, isolated, measured and identified in the processes of column and paper chromatography, derivative formation, and finally recrystallization to constant specific activity.

In the ovarian vein blood and ovaries of immature rats, major 17-OH-C_{21}- and C_{19}-metabolites of progesterone were 5α-reduced steroids such as 3α,17-dihydroxy-5α-pregnan-20-one, 3α-hydroxy-5α-androstan-17-one and 5α-androstane-3α,17β-diol. Following injection of [^{14}C]-progesterone plus [^{3}H]-5α-pregnane-3,20-dione into 28-day-old rat ovary, no significant augmentation of the isotope from progesterone was observed in 5α-reduced C_{19}-steroids in the ovary as compared with 5α-reduced 17-OH-C_{21}-steroids, as one compared the ratios of ^{3}H/^{14}C in 5α-reduced C_{19}-steroids with that of 5α-reduced 17-OH-C_{21}-steroids, which were about the same. In the ovarian vein blood and ovaries of adult rats, only small amounts of 5α-reduced 17-OH-C_{21}- and C_{19}-metabolites derived from progesterone were found, while 20α-hydroxy-4-pregnen-3-one was the major metabolite.

These findings suggest that the formation of 5α-reduced C_{19}-steroids from progesterone by the 5α-reduced pathway is dominant in androgen biosynthesis of the immature rat ovary *in vivo*.

INTRODUCTION

Previous studies of androgen biosynthesis *in vitro* have demonstrated that rat testes are capable of a high rate of progesterone conversion to testosterone during fetal development [1], during the period up to 10 days after birth [1–3] and in the adult [2–5]. On the other hand, testicular homogenates from 20- to 40-day-old immature rats yield 5α-reduced C_{19}-steroids such as 3α-hydroxy-5α-androstan-17-one and 5α-androstane-3α,17β-diol as major products from progesterone [2, 3, 5]. Our *in vitro* studies on mouse testes indicated a similar age-dependent pattern of progesterone metabolism [6]. We also found that progesterone was converted to these 5α-reduced C_{19}-steroids primarily by a pathway through 5α-reduced C_{21}-steroids in testes of immature rats and immature mice *in vitro* [7, 8]. The 5α-reduced pathway was not present in testes of adult rats and mice [7, 8].

Previous studies of the ovary *in vitro* have demonstrated that immature rat ovary contains high 5α-reductase activity [9] and that ovarian homogenates from immature rats form large amounts of 5α-reduced C_{19}-steroids from 3β-hydroxy-pregn-5-en-20-one and progesterone while adult rat ovaries are unable to form a significant amount of 5α-reduced C_{19}-steroids [10]. Furthermore, 5α-androstane-3α,17β-diol and its 3β epimer were found at high levels (100–200 ng/ml) in the peripheral circulation of immature female rats but they disappeared after the onset of puberty [11, 12]. Since these 5α-reduced C_{19}-steroids, which are of no use for oestrogen biosynthesis, have been shown to exert a negative feedback on gonadotrophin release [13, 14] and to induce precocious ovulation in rats [11, 12, 14, 15], the formation of 5α-reduced C_{19}-steroids in immature rat ovary seem to have a biological significance.

Recently, we [16] and Lerner and Eckstein [17] found that progesterone was converted to these 5α-reduced C_{19}-steroids primarily by a pathway through 3α,17-dihydroxy-5α-pregnan-20-one in homogenates of immature rat ovaries. A study *in vitro*, however, is not physiological. Information obtained in such a system only suggests that a given biosynthetic pathway can occur in the living cell, but whether it does so remains to be determined. This paper deals with the formation of 5α-reduced C_{19}-steroids by the 5α-reduced pathway *in vivo* in the ovary of 28-day-old rats, following a single injection of radioactive progesterone and/or 5α-pregnane-3,20-dione into the ovary.

Correspondence should be sent to: Dr. K. Matsumoto, Institute for Cancer Research, Osaka University Medical School, Kita-ku, Osaka 530, Japan.

MATERIALS AND METHODS

Animals. Female rats of the Sprague–Dawley strain (28 days of age and 140 days of age at estrus) were used. The onset of puberty in the strain of rats used was at 35–38 days of age.

Chemicals. [1,2-^{3}H]-progesterone (45 Ci/mmol) and [4-^{14}C]-progesterone (30 mCi/mmol), obtained from Daiichi Pure Chemical Co. Ltd, Japan, were purified by paper chromatography with the hexane–formamide system [18] just before use. Using testicular homogenates collected from immature rats, [1,2-^{3}H]-5α-pregnane-3,20-dione was biosynthesized from [1,2-^{3}H]-progesterone and purified as previously reported [7]. The radiochemical purity of the purified [^{3}H]-5α-pregnane-3,20-dione was 99% by recrystallization. The [^{3}H]-5α-pregnane-3,20-dione was tested further by paper [18] and column [19] chromatography with derivative formation and recrystallization [7, 8] for the presence of compounds which might have been a cause of spurious findings. The substrate behaved as a single compound and there were very little or no radioactive steroids (less than 0.2% of each) in 16 fractions of neutral steroids examined in the present study. The radiochemical purity of the purified [^{3}H]-progesterone and [^{14}C]-progesterone was also examined by the same methods. The substrate behaved as a single compound and there were very little or no radioactive steroids in 16 steroid fractions examined. Non-radioactive steroids were obtained from Steraloids, Inc. and Ikapharm. Other reagents were of analytical grade.

Injection of radioactive steroids, removal of ovary, collection of ovarian vein blood and extraction of steroids. [^{3}H]-progesterone (12 nmol/600 μCi), or [^{14}C]-progesterone (132 nmol/4 μCi) plus [^{3}H]-5α-pregnane-3,20-dione (10 nmol/10 μCi), each dissolved in 0.01 ml ethanol, was suspended in 0.09 ml of physiological saline solution, and 5 μl of this suspension was directly injected into the left ovary at the central portion. The left ovary was removed 2 or 5 min after injection. A blood sample from the left ovarian vein of other rats was collected 1–3 or 3–5 min following injection from incisions of the ovarian vein using heparinized capillary tubes. These procedures were performed under ether anesthesia of the animals. The ovaries and ovarian vein blood were immediately homogenized or mixed with 0.5 ml of water and 0.2 ml of 1 N HCl. When [^{3}H]-progesterone was used as substrate, 0.01 μCi of [^{14}C]-progesterone was added as internal [^{14}C]-labeled tracer for recovery of extraction. The extraction of radioactive steroids in the ovaries and ovarian vein blood was the same as previously described [20]. The recoveries for extraction were 84–95%. Since the radioactivity in the water fraction was proved to be less than 1% of that in the corresponding extract, no further analysis of it was attempted.

Analysis and identification of steroids. To the extract, 5–50 μg quantities of 17 non-radioactive neutral steroids shown in Table 1 were added as carriers. The analysis of these 17 neutral steroids by paper [18] and elution [19] chromatography with acetylation of steroids were the same as previously de-

Table 1. Radioactivity of steroids in ovarian vein blood following injection of [^{3}H]-progesterone into rat ovary (mean of two experiments)

Age (days):	28		140	
Time after injection (min):	1–3	3–5	1–3	3–5
Total [^{3}H]-steroids extracted from each collection of blood (pmol):	6.2	4.4	5.8	4.2
		(pmol)		
Progesterone (unchanged)	3.95	1.80	4.39	1.56
20α-hydroxy-4-pregnen-3-one	0.06	0.06	0.33	0.94
5α-pregnane-3,20-dione	0.12	0.11	0.08	0.11
3(α or β)-hydroxy-5α-pregnan-20-one	0.21	0.62	0.06	0.32
5α-pregnane-3α,20α-diol	<0.01	<0.01	0.02	0.19
17-hydroxy-4-pregnene-3,20-dione	0.05	0.01	<0.01	<0.01
17-hydroxy-5α-pregnane-3,20-dione	0.02	0.03	<0.01	0.02
3α,17-dihydroxy-5α-pregnan-20-one	0.07	0.28	0.00	0.03
3β,17-dihydroxy-5α-pregnan-20-one	0.00	0.00	0.00	0.00
4-androstene-3,17-dione	0.07	0.02	<0.01	0.01
Testosterone	<0.01	<0.01	0.00	0.01
5α-androstane-3,17-dione	0.01	<0.01	<0.01	<0.01
3α-hydroxy-5α-androstan-17-one	0.13	0.44	0.00	<0.03
3β-hydroxy-5α-androstan-17-one	0.01	<0.01	0.00	0.01
17β-hydroxy-5α-androstan-3-one	0.02	<0.01	<0.01	<0.01
5α-androstane-3α,17β-diol	0.01	0.04	<0.01	0.00
5α-androstane-3β,17β-diol	0.00	<0.01	0.00	0.00
Oestrone	0.00	0.00	0.00	0.00
Oestradiol-17β	0.00	0.00	0.00	0.00

[^{3}H]-progesterone (0.6 nmol/30 μCi per 5 μl) was directly injected into the ovary. A portion of ovarian vein blood was collected 1–3 or 3–5 min after injection. Values were obtained after 4 repeated crystallizations to constant specific activity.

scribed [7, 21]. In addition, when oestrogen formation was examined, the extracts were separated into neutral and phenolic fractions. The phenolic fractions were separated into oestradiol-17β and oestrone by elution chromatography [19] following the addition of non-radioactive carriers. The percentage of the total radioactivity present in each zone on the first chromatogram was calculated. This percentage was distributed among those compounds which showed significant radioactivity, according to the proportion of each compound present, as indicated by succeeding chromatograms. Finally, all the purified radioactive steroids or their acetates except for 3(α or β)-hydroxy-5α-pregnan-20-one and 17-hydroxy-5α-pregnan-3,20-dione were recrystallized with 10–15 mg non-radioactive standard steroids to constant specific activity in order to identify the steroids formed and to obtain accurate $^3H/^{14}C$ ratios in the isolated compounds. Each of 3(α or β)-hydroxy-5α-pregnan-20-one and 17-hydroxy-5α-pregnane-3,20-dione was tentatively identified by derivative formation (acetylation and oxidation with chromium trioxide) and recrystallization of the derivatives formed with chromium trioxide to constant specific activity, as previously described [21]. Final evidence of identification or tentative identification required that the specific activities of three consecutive crystallizations were within ±5% of the mean. The amount of each steroid on the final chromatogram was corrected by the decrease of specific radioactivity on repeated crystallizations to constant specific activity. Although the present procedure for calculating the rate of formation of metabolites could permit an approximate estimation of the percent formation, this seems to be of use for the present purpose.

RESULTS

1. *Radioactivity of steroids in ovarian vein blood and ovary following injection of [^3H]-progesterone into rat ovary*

The radioactivities of unconjugated steroids in ovarian vein blood (Table 1) and in ovaries (Table 2) after injection of [^{3}H]-progesterone (0.6 nmol/30 μCi per 5 μl) into rat ovary were measured in 28- and 140-day-old rats. Mean values of the two experiments under the same condition are presented in Tables 1 and 2, since each experiment showed fundamentally similar results. Amounts of each steroid or steroids is shown in Tables 1 and 2 as pmol steroid isolated from each collection of ovarian vein blood or each ovary. The total radioactivities in ovarian vein blood collected 1–3 and 3–5 min after injection were approx. 1.0% and 0.7% of the injected dose (the total initial radioactive substrate directly injected into the ovary: 0.6 nmol/30 μCi), respectively (Table 1), and those in ovaries removed 2 and 5 min after injection were 30% and 4% of the injected dose, respectively. Major C_{21}- and C_{19}-metabolites of progesterone were 5α-reduced steroids such as 3(α or β)-hydroxy-5α-pregnan-20-one, 3α,17-dihydroxy-5α-pregnan-20-one and 3α-hydroxy-5α-androstan-17-one in ovarian vein blood and ovaries collected from 28-day-old rats, while 20α-hydroxy-4-pregnen-3-one and 3(α or β)-hydroxy-5α-

Table 2. Radioactivity of steroids in ovary 2 or 5 min after injection of [^{3}H]-progesterone into rat ovary (mean of two experiments)

Age (days):	28		140
Time after injection (min):	2	5	5
Total [^{3}H]-steroids extracted from each ovary (pmol):	177.2	25.4	24.4
	(pmol per ovary)		
Progesterone (unchanged)	93.74	9.88	10.83
20α-hydroxy-4-pregnen-3-one	0.53	0.18	4.10
5α-pregnane-3,20-dione	14.71	1.02	1.51
3(α or β)-hydroxy-5α-pregnan-20-one	30.48	4.09	3.15
5α-pregnane-3α,20α-diol	0.18	0.05	1.10
17-hydroxy-4-pregnene-3,20-dione	0.89	0.03	0.02
17-hydroxy-5α-pregnane-3,20-dione	1.06	0.15	0.07
3α,17-dihydroxy-5α-pregnan-20-one	4.61	0.71	0.10
3β,17-dihydroxy-5α-pregnan-20-one	0.00	0.00	0.00
4-androstene-3,17-dione	0.89	0.28	0.07
Testosterone	0.18	0.10	0.02
5α-androstane-3,17-dione	0.53	0.08	<0.02
3α-hydroxy-5α-androstan-17-one	2.13	1.24	0.10
3β-hydroxy-5α-androstan-17-one	0.53	0.08	<0.02
17β-hydroxy-5α-androstan-3-one	0.18	<0.03	0.05
5α-androstane-3α,17β-diol	0.18	0.10	0.00
5α-Androstane-3β,17β-diol	0.00	0.00	0.00
Oestrone	0.00	0.00	0.00
Oestradiol-17β	0.00	0.00	0.02

[^{3}H]-progesterone (0.6 nmol/30 μCi per 5 μl) was directly injected into ovary. Values were obtained after 4 repeated crystallizations to constant specific activity.

pregnan-20-one were major products in ovarian vein blood and ovaries obtained from 140-day-old rats (Tables 1 and 2). The amounts of 5α-reduced 17-OH-C_{21} and C_{19}-steroids were much higher in ovarian vein blood and ovaries from immature rats than in those from adult rats (Tables 1 and 2). The conversion of progesterone to oestrogen took place a little in ovaries of 140-day-old rats but it was not detectable in ovaries of 28-day-old rats (Table 2).

2. Ratios of 5α-pregnane-3,20-dione to progesterone as precursors of metabolites in 28-day-old rat ovary

Experiments were attempted to determine $^3H/^{14}C$ ratios of products in ovary following injection of [^{14}C]-progesterone plus [^{3}H]-5α-pregnane-3,20-dione into 28-day-old rat ovary. The values shown in Table 3 were obtained by analyses of radioactive metabolites in pooled ovaries collected from 12 rats 2 min after injection. In Table 3 the isotope ratio is expressed as the amount of radioactivity from [^{3}H]-5α-pregnane-3,20-dione divided by the amount of radioactivity from [^{14}C]-progesterone. The molar ratio expressed as pmol steroid formed from [^{3}H]-5α-pregnane-3,20-dione divided by pmol steroid from [^{14}C]-progesterone is also given in Table 3. Since isotope and molar ratios in original mixture are 0.076 and 2.50, respectively, a ratio below 0.076 or 2.50 in a compound indicates that a higher percentage of radioactive progesterone than 5α-pregnane-3,20-dione has been incorporated into the steroid. In 28-day-old rat ovaries, significant augmentation of the isotope from progesterone was observed in 3α,17-dihydroxy-5α-pregnan-20-one as compared with 3(α or β)-hydroxy-5α-pregnan-20-one, indicating that the conversion of 3(α or β)-hydroxy-5α-pregnan-20-one and 17-hydroxy-4-pregnene-3,20-dione to 5α-reduced 17-OHC_{21}-steroids occurred. The ratios in 5α-reduced 17-OH-C_{21}-steroids and 5α-reduced C_{19}-steroids showed slight differences. This finding seems to indicate that 5α-reduced C_{19}-steroids were formed mainly from 5α-reduced 17-OH-C_{21}-steroids such as 3α,17-dihydroxy-5α-pregnane-20-one and 17-hydroxy-5α-pregnane-3,20-dione. No radioactivity from [^{3}H]-5α-pregnane-3,20-dione was associated with 4-ene-3-keto-steroids in ovaries from immature rats. Reversal of the 5α-reduced system was ruled out.

DISCUSSION

In our previous paper [16], we showed that in ovarian homogenates of immature rats, 3α,17-dihydroxy-5α-pregnan-20-one, which was formed from progesterone via 3α-hydroxy-5α-pregnan-20-one and 17-hydroxy-4-pregnene-3,20-dione, was the major intermediate in the formation of 5α-reduced C_{19}-steroids from progesterone. The present results shown in Tables 1–3 seem to provide the evidence that the same pathway operates in the living ovarian cells of immature rats. To the best of our knowledge, the present results demonstrate for the first time the formation of 5α-reduced C_{19}-steroids by the 5α-reduced pathway in the ovary *in vivo*. However, the injection of progesterone directly into the ovary does not represent the true *in vivo* state and there is no evidence to show that the exogenously administered steroids would behave in the same manner as the endogenously produced precursors. But studies of steroid metabolism *in vivo*, as in the present experiment, would eliminate some of the problems encountered with tissue homogenates and slices. The present study might provide more direct evidence that active formation of 5α-reduced C_{19}-steroids by the 5α-reduced pathway can occur in the living ovarian cells of immature rats. These experiments were carried out in

Table 3. Ratios of 5α-pregnane-3,20-dione (5α-P) to progesterone (P) as precursors of metabolites in pooled ovaries collected from 12 immature rats

	[^{3}H]-5α-P (pmol per ovary)	[^{14}C]-P (pmol per ovary)	pmol from 5α-P /pmol from P	$^3H/^{14}C$*
Original mixture			0.076	2.50
Total radioactive steroids extracted from one ovary†	73.5	976.8	0.075	2.48
5α-pregnane-3,20-dione	48.05	41.61	1.155	38.38
3(α or β)-hydroxy-5α-pregnan-20-one	13.77	30.86	0.446	14.83
17-hydroxy-5α-pregnane-3,20-dione	0.15	1.95	0.077	2.50
3α,17-dihydroxy-5α-pregnan-20-one	0.35	4.88	0.072	2.38
5α-androstane-3,17-dione	0.17	2.15	0.079	2.63
3α-hydroxy-5α-androstan-17-one	0.49	6.25	0.078	2.60
3β-hydroxy-5α-androstan-17-one	0.06	0.98	0.061	2.00
Progesterone	0.00	697.14	0.000	0.00
20α-hydroxy-4-pregnen-3-one	0.00	3.22	0.000	0.00
17-hydroxy-4-pregnene-3,20-dione	0.00	4.30	0.000	0.00
4-androstene-3,17-dione	0.00	7.52	0.000	0.00

[^{14}C]-progesterone (6.6 nmol/0.2 μCi) plus [^{3}H]-5α-pregnane-3,20-dione (0.5 nmol/0.5 μCi) suspended in 5 μl of physiological saline solution was directly injected into the ovary. The ovary was removed 2 min after injection. Ratios were obtained after 4 repeated crystallizations to constant specific activity.

* Ratio of 5α-pregnane-3,20-dione isotope: progesterone isotope in isolated compound from 12 ovaries.

† Total radioactive steroids extracted from pooled 12 ovaries are divided by 12.

anesthetized rats. It may still be possible that the difference in biosynthetic pathways leading to C_{19}-steroids from progesterone between the two groups, 28- and 140-day-old rats, might be explained by a difference in response to anesthesia. But we think this is unlikely, since our previous studies using ovarian homogenates obtained from 30- and 150-day-old rats under non-stressed conditions clearly demonstrated the same difference in the biosynthetic pathway [16] as that found in the present *in vivo* study. Following injection of [^{3}H]-progesterone into the ovary, the major radioactive metabolites in ovarian vein blood were 20α-hydroxy-4-pregnen-3-one in adult rats and 3(α or β)-hydroxy-5α-pregnan-20-one, 3α,17-dihydroxy-5α-pregnan-20-one and 3α-hydroxy-5α-androstan-17-one in immature rats. This finding seems to suggest that 5α-reduced C_{19}-steroids are the major C_{19}-steroids secreted from immature rat ovaries. In connection with this finding, high levels of 5α-androstanediols have been found in peripheral circulation of normal immature female rats [11, 12].

It was reported that the degree of formation *in vitro* of labeled oestrogens from labeled progesterone by rat ovarian tissue was high at 5–10 and 100–200 days of age, but low at 20–30 days [22, 23]. Similarly, plasma oestradiol-17β levels in female rats were always the lowest (almost undetectable) from day 20 to day 35 [24]. However, ovariectomy on day 15 results in a marked increase in gonadotropin concentration at 27 days of age [24]. Since 5α-androstanediols, which are of no use for oestrogen biosynthesis, have been shown to have an inhibitory effect on gonadotropin secretion in rats [13, 25], these observations can be explained by the present results, in which very active 5α-reduction of C_{21}- and C_{19}-4-ene-3-ketosteroids in the ovaries of immature rats are demonstrated (Tables 1–3). The prepubertal state in female rats is thought to be maintained by an increase in sensitivity to the inhibitory effects of oestradiol-17β on gonadotropin secretion [26, 27]. However, the present and previous [9–12, 14–17] results suggest that the active 5α-reduction of C_{19}-4-ene-3-ketosteroids and the formation of 5α-reduced C_{19}-steroids by the pathway not involving 4-androstene-3,17-dione and testosterone in ovaries seem to have an additional biological significance in immature female rats.

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REFERENCES

1. Noumura T., Weisz J. and Lloyd C. W.: *In vitro* conversion of [7-^{3}H]-progesterone to androgens by the rat testis during the second half of fetal life. *Endocrinology* **78** (1966) 245–253.
2. Ficher M. and Steinberger E.: *In vitro* progesterone metabolism by rat testicular tissue at different stages of development. *Acta endocr., Copenh.* **68** (1971) 285–292.
3. Coffey J. C., French F. S. and Nayfeh S. N.: Metabolism of progesterone by rat testicular homogenates. IV. Further studies of testosterone formation in immature testis *in vitro*. *Endocrinology* **89** (1971) 865–872.
4. Slaunwhite W. R. Jr and Samuels L. T.: Progesterone as a precursor of testicular androgens. *J. biol. Chem.* **220** (1956) 341–352.
5. Inano H., Hori Y. and Tamaoki B.: Effect of age on testicular enzymes related to steroid bioconversion. *Ciba Fedn Colloq. Endocr.* **16** (1967) 105–119.
6. Tsujimura T. and Matsumoto K.: Progesterone metabolism *in vitro* by mouse testes at different stages of development. *Endocrinology* **94** (1974) 288–290.
7. Yamada M. and Matsumoto K.: Pathway from progesterone to 5α-reduced C_{19}-steroids not involving androstenedione and testosterone in immature rat testes *in vitro*. *Endocrinology* **94** (1974) 777–784.
8. Tsujimura T., Mizutani S. and Matsumoto K.: Pathway from progesterone to 5α-reduced C_{19}-steroids not involving androstenedione and testosterone in immature mouse testes *in vitro*. *Endocrinology* **96** (1975) 515–518.
9. Mason N. R.: Steroid A-ring reduction by rat ovaries. *Endocrinology* **87** (1970) 350–355.
10. Springer C. and Eckstein B.: Regulation of production *in vitro* of 5α-androstane-3α,17β-diol in the immature rat ovary. *J. Endocr.* **50** (1971) 431–439.
11. Eckstein B. and Ravid R.: On the mechanism of the onset of puberty: Identification and pattern of 5α-androstane-3β,17β-diol and its 3α epimer in peripheral blood of immature female rats. *Endocrinology* **94** (1974) 224-229.
12. Ravid R. and Eckstein B.: Androstanediol sulphates in peripheral blood of immature rats and some of their biological effects. *J. Endocr.* **71** (1976) 299–304.
13. Swerdloff R. S., Grover P. K., Jacobs H. S. and Bain J.: Search for a substance which selectively inhibits FSH- effects of steroids and prostaglandins on serum FSH and LH levels. *Steroids* **21** (1973) 703–722.
14. Eckstein B.: Studies on the mechanism of the onset of puberty in the female rat. *J. steroid Biochem.* **6** (1975) 873–878.
15. Eckstein B., Golan R. and Shani (Mishkinsky) J.: Onset of puberty in the immature female rat induced by 5α-androstane-3β,17β-diol. *Endocrinology* **92** (1973) 941–945.
16. Karakawa T., Kurachi K., Aono T. and Matsumoto K.: Formation of 5α-reduced C_{19}-steroids from progesterone *in vitro* by a pathway through 5α-reduced C_{21}-steroids in ovaries of late prepubertal rats. *Endocrinology* **98** (1976) 571–579.
17. Lerner N. and Eckstein B.: Identification of two 5α-reduced pregnanes as major metabolites of progesterone in immature rat ovaries (1000 × *g* supernatant) *in vitro*. *Endocrinology* **98** (1976) 179–188.
18. Zaffaroni A. and Burton R. B.: Identification of corticosteroids of beef adrenal extract by paper chromatography. *J. biol. Chem.* **193** (1951) 749–767.
19. Seki T. and Matsumoto K.: Chromatographic separation of 17-hydroxycorticosteroids and 17-ketosteroids. *J. Chromat.* **27** (1967) 423–430.
20. Yamada M., Miyaji H., Kasai H. and Matsumoto K.: Formation of 5α-reduced C_{19}-steroids from progesterone *in vivo* by 5α-reduced pathway in older prepubertal rat testis. *Biochim. biophys. Acta* **424** (1976) 82–89.
21. Yamada M., Yasue S. and Matsumoto K.: Formation of C_{21}-17-hydroxysteroids and C_{19}-steroids from 3β-hydroxypregn-5-en-20-one and progesterone *in vitro* by germ cells from immature rat testes. *Endocrinology* **93** (1973) 81–87.
22. Quattropani S. L. and Weisz J.: Conversion of progesterone to estrone and estradiol *in vitro* by the ovary of the infantile rat in relation to the development of

its interstitial tissue. *Endocrinology* **93** (1973) 1269–1276.
23. Weisz J. and Lloyd C. W.: Estrogen and androgen production *in vitro* from [7-^{3}H]-progesterone by normal and polycystic rat ovaries. *Endocrinology* **77** (1965) 735–744.
24. Meijs-Roelofs H. M. A., Uilenbroek J. T. J., De Jong F. H. and Welschen R.: Plasma oestradiol-17β and its relationship to serum follicle stimulating hormone in immature female rats. *J. Endocr.* **59** (1973) 295–304.
25. Zanisi M., Motta M. and Martini L.: Inhibitory effect of 5α-reduced metabolites of testosterone on gonadotrophin secretion. *J. Endocr.* **56** (1973) 315–316.
26. Ramirez V. D. and McCann S. M.: Comparison of the regulation of luteinizing hormone (LH) secretion in immature and adult rats. *Endocrinology* **72** (1963) 452–464.
27. Steele R. E. and Weisz J.: Changes in sensitivity of the estradiol-LH feedback system with puberty in the female rat. *Endocrinology* **95** (1974) 513–520.