J. steroid Biochem. Vol. 35, No. 2, pp. 301–305, 1990
Printed in Great Britain.

0022-4731/90 $3.00 + 0.00

STEROIDOGENESIS IN THE TESTES AND THE ADRENALS OF ADULT MALE RATS AFTER γ-IRRADIATION *IN UTERO* AT LATE PREGNANCY

KEIKO SUZUKI,* MASAKAZU TAKAHASHI,† HIROKO ISHII-OHBA, KIYOMI IKEDA and HIROSHI INANO

National Institute of Radiological Sciences, 9–1, Anagawa-4-chome, Chiba-shi 260, Japan

(*Received* 17 *January* 1989; *received for publication* 18 *September* 1989)

Summary—Pregnant rats were irradiated with 2.1 Gy γ-ray of ^{60}Co at day 20 of gestation. Seventy days after birth, the body weight of the fetally irradiated male pups was significantly lower than the control. The testes, ventral prostates and seminal vesicles were atrophied by irradiation, whereas no decreased weight of the adrenals was observed. Histological examination of the testes of the irradiated rats revealed a complete disappearance of germinal cells. Sertoli cells and Leydig cells appeared normal, and no apparent histological difference was observed in the adrenals between the control and the irradiated rats.

Activities of microsomal Δ^5-3β-hydroxysteroid dehydrogenase (HSD) + isomerase, 17α-hydroxylase/$C_{17,20}$-lyase, 17β-HSD and 7α-hydroxylase per pair of testes were decreased in the irradiated rats (36–86% of the control). In contrast, no decreased activity of 20α-HSD in the cytosol fraction was observed by irradiation. No decreased activity of adrenocortical enzymes, such as Δ^5-3β-HSD + isomerase, 21-hydroxylase, 11β-/18-hydroxylase and 5α-reductase, was also observed in the irradiated group.

Concentrations of LH, FSH, TSH, prolactin, testosterone, progesterone and aldosterone in serum were measured by radioimmunoassay. Only the FSH concentration was significantly increased by the irradiation, while no difference was found in the concentration of other hormones.

It was concluded that irreversible damage was induced in spermatogenesis and androgen production by the fetal irradiation, whereas corticoidogenesis was not affected.

INTRODUCTION

It is known that spermatogenic cells are radiosensitive [1]. On the other hand, it was supposed that androgen secretion was radioresistant [2–4], because the accessory sex organs were not atrophied by a local testicular irradiation of X-ray (5–15 Gy) or γ-ray (5.27 Gy) to adult rats. However, local testicular X-irradiation of 10 Gy to prepubertal rats caused a marked reduction of androgen production after maturation, as assessed by decreased weight of ventral prostates and seminal vesicles [5]. Although there are abundant reports concerning radiation effects upon gonads, not many studies have been carried out on the effect of irradiation upon corticoidogenesis. Recently, we have examined the effect of radiation during the fetal period upon steroidogenic enzymes after birth, and have reported already that γ-irradiation of a rat fetus *in utero* markedly decreased steroidogenic enzyme activities in testes, ovaries and adrenals as measured in the prepubertal period [6].

In this paper we examined steroidogenesis in the testes and adrenals of mature rats which were irradiated previously by 2.1 Gy γ-ray *in utero* on day 20 of pregnancy. The present results were compared with our previous findings obtained from irradiation in the fetal [6], prepubertal [5] and adult [2] periods.

EXPERIMENTAL

Radiochemicals

[4-^{14}C]-Labeled pregnenolone (57.2 mCi/mmol), 17α-hydroxyprogesterone (50 mCi/mmol), androstenedione (52 mCi/mmol) and DOC (60 mCi/mmol) were purchased from New England Nuclear (Boston, Mass). [4-^{14}C]Progesterone (56 mCi/mmol) and [4-^{14}C]testosterone (56.9 mCi/mmol) were obtained from Amersham International (Amersham, U.K.). The radiochemical purity was confirmed with TLC immediately before use.

Animals and enzyme preparation

Fetal rats of the Wistar strain were given *in utero* a dose of 2.1 Gy whole body γ-irradiation by ^{60}Co on day of gestation. When male pups were 70-days old, they were bled by cardiac puncture under pento-

*Author to whom all correspondence should be addressed.
†Visiting scientist from Sasaki Institute, Kanda-Surugadai, Chiyoda-ku, Tokyo 101, Japan.

barbital anesthesia. The sera were stored at −20°C until assay of hormones. Each pair of testes was individually homogenized in 5 ml of 0.25 M sucrose–10 mM Tris–HCl buffer (pH 7.4). The microsomal fraction was obtained from the homogenate as the precipitate at 10,000–105,000 *g*, and was suspended in 1 ml of the sucrose–Tris buffer. The cytosol fraction was obtained as the supernatant of the centrifugation at 105,000 *g*. A pair of adrenals was homogenized in 2 ml of 0.33 M sucrose–10 mM Tris–HCl buffer (pH 7.4). The mitochondrial fraction (a precipitate at 800–6000 *g*) obtained from the homogenate was suspended in 1 ml of the 0.33 M sucrose–Tris buffer. The adrenal microsomal fraction was also obtained in the same manner as in the case of the testis.

Incubation

Enzyme reaction was performed at 37°C for 30 min in 2 ml of 0.25 M sucrose–10 mM Tris–HCl buffer (pH 7.4), if not specified. The testicular microsomal fraction (0.1–0.7 mg protein) was incubated with [4-^{14}C]pregnenolone (50 nmol, 5.0×10^4 cpm) in the presence of 1.5 μmol of NAD^+. The microsomal fraction was also incubated with [4-^{14}C]-labeled progesterone (50 nmol, 5.0×10^4 cpm), 17α-hydroxyprogesterone (50 nmol, 5.0×10^4 cpm), or androstenedione (50 nmol, 5.0×10^4 cpm) individually in the presence of 1.2 μmol NADPH, or with [4-^{14}C]-testosterone (50 nmol, 5.0×10^4 cpm) in the presence of 1.3 μmol $NADP^+$. The testicular cytosol fraction (1.4–9.4 mg protein) was incubated with [4-^{14}C]17α-hydroxyprogesterone in the presence of 1.2 μmol NADPH.

The adrenal microsomal fraction (0.03–0.08 mg protein) was incubated with [4-^{14}C]pregnenolone (25 nmol, 6.4×10^4 cpm) in the presence of 1.5 μmol NAD^+, or with [4-^{14}C]progesterone (50 nmol, 7.8×10^4 cpm) in the presence of 1.2 μmol NADPH. The mitochondrial fraction (1.1–1.4 mg protein) was incubated with [4-^{14}C]DOC (50 nmol, 9.0×10^4 cpm) in the presence of 1.2 μmol NADPH. Only when the mitochondrial fraction was employed, the concentration of sucrose in the buffer was 0.33 M. Enzyme reactions were terminated by mixing with 5 ml of CH_2Cl_2.

Extraction, separation and quantitation of radioactive metabolites

Steroids were extracted twice from the incubation mixture with 5 ml of CH_2Cl_2. Nonradioactive progesterone, 17α-hydroxyprogesterone, androstenedione, testosterone and 11-deoxycortisol were added to the extract as markers (70 nmol each) if not specified. Corticosterone was also added, when DOC was used as a substrate. The extract was subjected to TLC on silica gel plates (0.25 mm in thickness, 5 × 20 cm, E. Merck, Darmstadt, Germany). The solvent system was benzene–acetone (4:1, v/v) if not specified. When progesterone was incubated with adrenal microsomes, 70 nmol of DOC were added to the extract as markers for TLC, and the plate was developed in a cyclohexane–ethylacetate (1:1, v/v) system. Marker steroids were detected under an u.v. lamp (wavelength 254 nm). Radioactive spots on the chromatogram were autoradiographically detected. Each radioactive spot was scraped off the plate, and the radioactivity was measured with a liquid scintillation spectrometer. Enzyme activity was calculated by summing up amounts of all metabolites which were produced by the enzyme, and expressed on the basis of a pair of organs. The protein concentration of a tissue preparation was measured by the Bradford method [7] using bovine plasma γ-globulin as a standard.

Measurement of hormones in serum

LH, FSH, TSH and prolactin were assayed with NIADDK radioimmunoassay kits kindly supplied by Dr A. F. Parlow, Pituitary and Antisera Center, Harbor-UCLA Medical Center, Torrance, Calif. and National Hormone and Pituitary Program, Baltimore, Md, U.S.A.

For measurement of steroid hormones in serum, "Progesterone-I-125-kit" (Sorin Biomedica, France), "Testosterone Eiken kit" (Eiken Chemical Co., Tokyo) and "Aldosterone·RIAKIT II" (Dainabot, Tokyo) were used.

Histology

Testes and adrenals were fixed in 10% formalin and embedded in paraffin. 4-μm sections in thickness prepared from the fixed organs were stained with hematoxylin and eosin.

Statistical analysis

The level of significance of the difference was assessed by Student's *t*-test after homogeneity of the variances between the two groups had been confirmed.

RESULTS

Effect of γ-irradiation upon body and organ weights

As shown in Table 1, the body weight of the irradiated male rats was significantly lower compared with the control when they were 70-days old. Not only testes, but also the weight of seminal vesicles and ventral prostates was significantly decreased in the irradiated group. But no effect upon the adrenal weight was observed by the fetal irradiation.

Histological observations

While active spermatogenesis was observed in the control testis (Fig. 1A), germinal epithelium had disappeared completely in seminiferous tubules of the irradiated testis (Fig. 1B). The diameter (170 μm) of the seminiferous tubules in the irradiated testis was smaller than the non-irradiated one (300 μm). Leydig cells and Sertoli cells appeared histologically intact in the irradiated testis.

Table 1. Biological effect of fetal irradiation on rats

Group	No. of rats	Body wt (g)	Testes wt (g)	Prostates wt (mg)	Seminal vesicles wt (mg)	Adrenals wt (mg)
Control	5	282 ± 7[a]	2.25 ± 0.03	158 ± 17	451 ± 40	37.6 ± 1.7
Irradiation	5	227 ± 7	0.48 ± 0.02	44 ± 7	179 ± 38	37.3 ± 0.6
Difference		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.01$	NS[b]

[a]Mean ± SE; [b]Non-significant.

No apparent morphological change of the adrenals was observed after irradiation. Adrenocortical cells of the irradiated rat appeared normal, and zonal structure of the cortex was also intact.

Steroid metabolism in testes of the irradiated rats

The amounts of the metabolites produced by a pair of testes of the control and the irradiated rats are listed in Table 2. A similar metabolic pattern was qualitatively observed in both groups. Incubation of pregnenolone with the microsomal fraction yielded only progesterone. After irradiation, the production of progesterone was decreased to 60% of the control. Progesterone was further metabolized to 17α-hydroxyprogesterone, androstenedione and testosterone. The production of each metabolite was reduced in the irradiated rats. The amount of testosterone was especially reduced to one-third of the control. From 17α-hydroxyprogesterone, androstenedione production in the irradiated rats was 71% of the control, whereas testosterone was 37%. Using androstenedione as a substrate, testosterone and 7α-hydroxyandrostenedione were produced, and their production rates were also decreased to 36 and 41% respectively by irradiation. When testosterone was incubated with the microsomal fraction to examine the reverse reaction, androstenedione production was reduced to 36% by irradiation. No 5α-reduced metabolites were obtained throughout the incubations using the microsomal fraction. When the testicular cytosol was incubated with 17α-hydroxyprogesterone, only one product, 17α,20α-dihydroxy-4-pregnen-3-one, was obtained. The production rate of the metabolite was not affected by irradiation.

Steroidogenic enzyme activities in the testis of the irradiated rats

Testicular enzyme activities of the irradiated and the control rats were shown in Fig. 2. Fetal γ-irradiation in the late pregnancy caused significant damage to the enzymes related to testosterone production. The activity of Δ^5-3β-HSD coupled with Δ^5–Δ^4 isomerase was depressed to 60% of the control level. Both of the activities of 17α-hydroxylase and $C_{17,20}$-lyase, which are derived from a single enzyme, were also significantly decreased by 49 and 44% respectively. 17β-HSD, which catalyzes both the forward and reverse reactions between androstenedione and testosterone, was damaged to 36% of the unirradiated value as assessed with both substrates. The irradiation also reduced the activity of 7α-hydroxylase as low as 40%. Among the enzymes assayed, 17β-HSD was the most sensitive to radiation, while 20α-HSD was radioresistant.

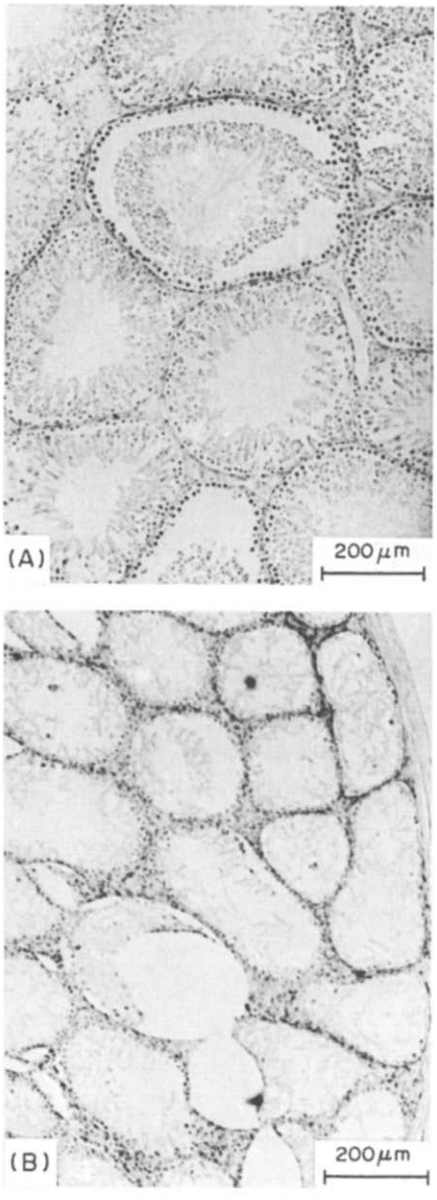

Fig. 1. Micrographs of testes stained with hematoxylin and eosin. (A) Intact testis and (B) fetally irradiated testis.

Table 2. Steroid metabolism in testes and adrenals obtained from irradiated rats

Substrate	Metabolite	Control (n = 5)	Irradiation (n = 5)	Difference
		nmol/pair of testes		
Pregnenolone	Progesterone	45.8 ± 4.5[a]	27.7 ± 2.9	$P < 0.01$
Progesterone	17α-Hydroxyprogesterone	57.4 ± 1.7	29.6 ± 4.8	$P < 0.001$
	Androstenedione	3.6 ± 0.2	3.1 ± 0.4	NS[b]
	Testosterone	9.7 ± 1.0	3.5 ± 0.5	$P < 0.001$
17α-Hydroxyprogesterone	Androstenedione	34.0 ± 3.6	24.0 ± 2.6	NS
	Testosterone	25.3 ± 0.5	9.4 ± 0.8	$P < 0.001$
Androstenedione	Testosterone	29.0 ± 2.0	10.4 ± 1.7	$P < 0.001$
	7α-Hydroxyandrostenedione	4.6 ± 0.3	1.9 ± 0.1	$P < 0.001$
Testosterone	Androstenedione	36.3 ± 2.1	13.2 ± 2.3	$P < 0.001$
17α-Hydroxyprogesterone	17α,20α-Dihydroxy-4-pregnen-3-one	64.8 ± 5.7	61.9 ± 8.1	NS
		nmol/pair of adrenals		
		(n = 4)	(n = 4)	
Pregnenolone	Progesterone	278 ± 44	288 ± 26	NS
Progesterone	DOC	25.9 ± 4.8	21.9 ± 3.6	NS
	5α-Pregnane-3,20-dione	5.9 ± 1.5	8.2 ± 2.5	NS
DOC	Corticosterone	1.4 ± 0.2	1.1 ± 0.1	NS
	18-Hydroxy-DOC	1.1 ± 0.2	0.8 ± 0.1	NS

[a]Mean ± SE; [b]non-significant.

Steroid metabolism in the adrenal of the irradiated rats

Steroid metabolism by the adrenal of the irradiated rats (Table 2) showed the same pattern as the control. Pregnenolone was transformed to progesterone by Δ^5-3β-HSD coupled with Δ^5–Δ^4 isomerase in the microsomal fraction, and progesterone was further metabolized to DOC by 21-hydroxylase and to 5α-pregnane-3,20-dione by 5α-reductase in the same fraction. The mitochondrial fraction converted DOC to corticosterone (11β-hydroxylase) and 18-hydroxy-DOC (18-hydroxylase). The enzyme activity in the adrenal of the irradiated rats was not different from the control.

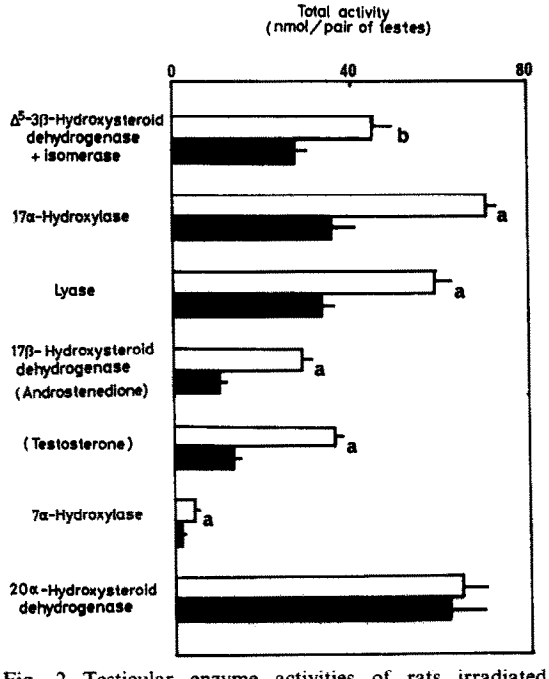

Fig. 2. Testicular enzyme activities of rats irradiated with ^{60}Co at the late pregnancy. □ Control (n = 5); ■ γ-irradiation (n = 5). (a) $P < 0.001$; (b) $P < 0.05$.

Effect of fetal irradiation upon peptide hormone and steroid hormones in serum

Among the measured adenohypophyseal peptide hormones, the concentration of FSH in serum was significantly increased by the irradiation (Table 3). However, no statistical difference was assessed in the concentration of LH, prolactin and TSH between both groups. The concentration of testosterone, progesterone and aldosterone in serum of the irradiated rat was not affected by the fetal irradiation.

DISCUSSION

In adult rats, it has been discussed whether irradiation to testes causes steroidogenic dysfunction in Leydig cells. Wang *et al.* [8] observed a maximum decrease in the weight of seminal vesicles at 21 days after 5.27 Gy γ-irradiation, and then a restoration to the control level until 52 days. On the other hand, 5 Gy of X-ray induced a weight loss of prostates of adult rats between 8 and 24 weeks after irradiation, but no significant decrease at 2 and 36 weeks [9]. Therefore, the time when androgen production is decreased by the irradiation cannot be determined from the two experiments. In contrast, many papers reported that the testicular androgen production was radioresistant [2–4]. This discrepancy might partly arise from different doses of radiation used during the

Table 3. Concentration of pituitary peptide hormones and steroid hormones in serum

Hormone	Control (n = 5)	Irradiation (n = 5)
LH (ng RP-2/ml)	0.57 ± 0.06[a]	0.70 ± 0.04
FSH (ng RP-2/ml)	10.9 ± 0.5	15.7 ± 0.4**
Prolactin (ng RP-3/ml)	19.1 ± 7.4	16.2 ± 1.7
TSH (ng RP-2/ml)	2.16 ± 0.59	2.03 ± 0.25
Progesterone (ng/ml)	8.14 ± 0.87	9.65 ± 1.25
Testosterone (ng/ml)	4.48 ± 0.89	3.81 ± 0.79
Aldosterone (ng/ml)	0.27 ± 0.04	0.33 ± 0.02

[a]Mean ± SE; **$P < 0.001$.

experiments. Concerning the case in which a reduction of the activity of steroidogenic enzymes was not detected, it is possible that the investigator used the animals before an appearance of dysfunction or after the functional restoration of Leydig cells. On the other hand, the irradiation to the prepubertal animals markedly damaged androgen production in their testes as measured after puberty [5]. Furthermore, it was recognized in the present paper and our previous study [6] that a fetal irradiation decreased the activities of Δ^5-3β-HSD (+isomerase), 17α-hydroxylase/lyase, 17β-HSD and 20α-HSD as assessed before puberty, and that the activities were not restored in the postpubertal period, except for that of 20α-HSD. These results confirmed a higher radiosensitivity of androgen production in the fetal and the prepubertal rat testes.

The adrenal weight and corticoidogenesis of the immature rats irradiated *in utero* were significantly lower than the control [5], and then subsequently recovered during the maturation as shown in this study despite the observed lower body weight of the treated animals. Studies have demonstrated that even the fetal γ-irradiation of a higher dose (2.6 Gy) revealed a recovery of the weight and corticoidogenesis of adult rat adrenals (unpublished data).

Among the testicular enzymes measured, 20α-HSD was the only one which was not affected by the irradiation. This may be due to a different intratesticular distribution of 20α-HSD from the other affected enzymes, as 20α-HSD is located in Sertoli cells, and 17α-hydroxylase/lyase in Leydig cells [10].

An increased FSH secretion after the local irradiation to testes had been reported previously [11, 12]. Secretion of FSH is regulated by inhibin produced in Sertoli cells [13]. The decreased concentration of androgen binding proteins (ABP), which are secreted from Sertoli cells, has also been demonstrated following fetal γ-irradiation [11], suggesting a radiation-induced dysfunction of Sertoli cells. Therefore, it is possible that the decrement of the inhibin secretion from Sertoli cells after irradiation resulted in an elevated FSH secretion in this experiment. In contrast, the activity of 20α-HSD in Sertoli cells was restored after puberty, while it was reduced in the prepubertal period [6]. It may be due to a different regulation of 20α-HSD from inhibin and ABP in Sertoli cells.

The testosterone concentrations in serum of the control and the irradiated rats were not significantly different in the present study, while the enzyme activities related to the synthesis of testosterone were severely damaged by irradiation. One of the reasons for the discrepancy might be the lesser blood volume due to the body weight reduced by irradiation. Weights of prostates and seminal vesicles were markedly reduced in the irradiated rats, probably because of the decreased androgen production and/or the direct effect of γ-ray upon the accessory sex organs.

Delic *et al.* reported that spermatogenesis partially continued in some seminiferous tubules 24 weeks after a local X-irradiation (5 Gy) to adult rat testes [9]. However, our results showed that fetal germinal cells were more sensitive to radiation than those of adult rats, as no spermatogenesis was observed.

Acknowledgements—We are indebted to Misses Yoshiko Imada, Reiko Kumagai, Shuko Kurihara and Atsuko Sato, Kyoritsu College of Pharmacy, for their technical assistance. We thank Dr Richard Roy, Laval University, Canada, for his advice in preparation of this manuscript.

REFERENCES

1. Bianchi M.: Cytotoxic insult to germinal tissue. In *The Testis; Part I, Cytotoxic Insult to Tissue: Effect of Cell Lineage* (Edited by C. S. Potten and J. H. Hendry). Churchill, Edinburgh (1983) pp. 258–328.
2. Inano H. and Tamaoki B.: *In vitro* biosynthesis of androgens by X-irradiated testes. *Endocr. Jap.* **15** (1968) 197–207.
3. Main S. J., Davies R. V. and Setchell B. P.: Feedback control by the testis of gonadotrophin secretion: an examination of the inhibin hypothesis. *J. Endocr.* **79** (1978) 255–270.
4. Verjans H. L. and Eik-Nes K. B.: Hypothalamic-pituitary-testicular system following testicular X-irradiation. *Acta Endocr. (Copenh.)* **83** (1976) 190–200.
5. Suzuki K., Inano H. and Tamaoki B.: Testicular function at puberty following prepubertal local X-irradiation in the rat. *Biol. Reprod.* **9** (1973) 1–8.
6. Inano H., Suzuki K., Ishii-Ohba H., Imada Y., Kumagai R., Kurihara S. and Sato A.: Steroid hormone production in testis, ovary, and adrenal gland of immature rats irradiated *in utero* with ^{60}Co. *Radiat. Res.* **117** (1989) 293–303.
7. Bradford M. M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analyt. Biochem.* **72** (1976) 248–254.
8. Wang J., Galil K. A. A. and Setchell B. P.: Changes in testicular blood flow and testosterone production during aspermatogenesis after irradiation. *J. Endocr.* **98** (1983) 35–46.
9. Delic J. I., Hendry J. H., Morris I. D. and Shalet S. M.: Dose and time relationships in the endocrine response of the irradiated adult rat testis. *J. Androl.* **7** (1986) 32–41.
10. Inano H.: Studies on enzyme reactions related to steroid biosynthesis-III. Distribution of the testicular enzymes related to androgen production between the seminiferous tubules and interstitial tissue. *J. Steroid Biochem.* **5** (1974) 145–149.
11. Rich K. A. and de Kretser D. M.: Effect of differing degrees of destruction of the rat seminiferous epithelium on levels of serum follicle stimulating hormone and androgen binding protein. *Endocrinology* **101** (1977) 959–968.
12. Pinon-Lataillade G., Viguier-Martinez M. C. and Maas J.: Endocrinological and histological changes induced by continuous low dose γ-irradiation of the rat testis. *Acta Endocr. (Copenh.)* **109** (1985) 558–562.
13. Steinberger A. and Steinberger E.: Secretion of an FSH-inhibiting factor by cultured Sertoli cells. *Endocrinology* **99** (1976) 918–921.