IN VITRO BIOSYNTHESIS OF ANDROGENS BY X-IRRADIATED TESTES

HIROSHI INANO AND BUN-ICHI TAMAOKI

National Institute of Radiological Sciences, Chiba-shi 280.

SYNOPSIS

After X-irradiation, the testicular weight of mice (6 weeks in age) decreased gradually, being significantly reduced on the 14th day post-irradiation and thereafter, in comparison to the control group. The specific activity of the 17α -hydroxylase in the testicular homogenate of the X-irradiated mice became higher than in the non-irradiated animals. When testes of the rats were locally X-irradiated, the weight of testes decreased almost half, but the weight of accessory organs remained almost unchanged. However, further detailed analysis of the individual enzymes of rat related to biosynthesis of androgens revealed that the 17α -hydroxylase and the 17α -hydroxypregnene $C_{17}-C_{20}$ lyase activities per testis were significantly reduced by local X-irradiation, while the $\Delta^5-3\beta$ -hydroxysteroid dehydrogenase activity coupled with the isomerase was somewhat enhanced by this treatment. By administration of human chorionic gonadotrophin to the locally X-irradiated rats, the enzyme activities related to testosterone formation except 17β -hydroxysteroid dehydrogenase were stimulated, in accordance with the increased weight of accessory organs by the gonadotrophin. From the results that the specific activities of the enzymes related to androgen production were higher in X-irradiated than in the control, and that those activities were stimulated by the gonadotrophin in parallel with histological observation on proliferation of interstitial cells, the enzymes related to testosterone production from pregnenolone were intercellularly located to the interstitial cells and intracellularly to their microsomes.

It was reported that X-irradiation to testes led to marked destruction of germinal cells in the seminiferous tubules with no apparent influence upon histological feature and function of the interstitial cells (Weiss, 1959). On the other hand, Christensen and Mason (1965) incubated ^{14}C -labeled progesterone, respectively with interstitial tissue and seminiferous tubules which were manually separated under microscope, and found that interstitial tissue was principally responsible for the biosynthesis of androgens. Ellis and Berliner

(1963), and Berliner and Ellis (1965a) indicated that the irradiated testes were less capable of transforming pregnenolone to testosterone, due to its reduced NADPH generating system or its availability. Schoen (1964) reported that the 17α -hydroxylase and 17α -hydroxypregnene- $C_{17}-C_{20}$ lyase activities were suppressed by local X-irradiation of testes of rats, and then the reduced activities were stimulated by gonadotrophin (HCG) which was administered immediately after X-irradiation.

Received for publication December 2, 1967.

* This work was supported in part by Grant (CA-07038) from National Cancer Institute, N.I.H., Bethesda, Md., U.S.A.

From this laboratory, it was reported (Shikita and Tamaoki, 1965a) that the $105,000\times g$ supernatant fluid obtained from X-irradiated testes contained the 20α -hydroxysteroid dehydrogenase in higher specific activity than the corresponding fraction of intact testes, indicating that the dehydrogenase was distributed locally to the radio-resistant cells such as interstitial cells. In this paper, the time course study of mouse testicular 17α -hydroxylase after whole body X-irradiation was conducted. Then, all the enzyme activities related to bioconversion of pregnenolone to testosterone were examined of locally X-irradiated testes of rat in the state of microsomal particle, in comparison to those of normal rats, and also the responsiveness of these enzyme activities of the irradiated testes to the administered gonadotrophin were quantitatively measured. With histological observation of X-irradiated and gonadotrophin treated testes, intercellular distribution of the enzymes in testicular tissue was discussed.

MATERIALS AND METHODS

Animals, X-irradiation and other treatments

Male mice of the ddY strain, aged 6 weeks, were used for the time course study on the influence of X-irradiation upon the bioconversion of steroids by testicular tissue. The whole bodies of mice were irradiated from their backs with a X-ray, which was generated at 200 KVp and 20 mA and filtered through 0.5 mm Cu and 0.5 mm Al. The dose rate measured under this condition was 95 R/min. in air and FSD was 50 cm. The mice were given a single irradiation with 200 R and 400 R of X-ray, respectively. Non-irradiated mice served as controls.

As the 2nd experiment, male rats of the Wistar strain bred in this Institute, aged 2 months, were used for examination of enzymic activities related to androgen biosynthesis in the locally X-irradiated testes, comparing with those of the normal testes. The rats were divided into the following 4 groups, consisting of about 10 animals per group; animals in the 1st group were irradiated locally to the testes, at total dose of 1,000 R. Then, after 16 days post-irradiation, the rats in this group were injected daily with 0.2 ml saline solution for 14 days. The rats in the 2nd group were irradiated at the same dose of X-ray, as that in the 1st group, but were injected with the purified human chorionic gonadotrophin 68 IU in 0.2 ml saline solution daily for 14 days, starting on the same day as mentioned of the 1st group. The rats of the remaining 2 groups were not irradiated. The 3rd group received the injection of saline solution, and the 4th group received the same amount of the gonadotrophin as mentioned of the 2nd group. The rats of the 1st and 2nd groups were fastened on their backs at plastic board without anesthesia, and then, the testes were locally irradiated by the same X-ray generator above stated, while the rest of the body was shielded with lead plate of 5 mm in thickness. Dose rate was 155 R/min. in air and FSD was 40 cm.

Tissue preparation and fractionation of the homogenate

Immediately after the animals were sacrificed, the testes were removed, weighed and chilled in twice their weight of ice-cold 0.25 M sucrose solution. The decapsulated glands were pooled per group and were homogenized with a loose-fitting glass-Teflon homogenizer, until few strips of the seminiferous tubules could be observed. The homogenates were then centrifuged at $800\times$

Trivial names of steroids, cofactor, enzymes and hormone used in the text are: pregnenolone, 3β -hydroxypregn-5-en-20-one; progesterone, pregn-4-ene-3, 20-dione; 17α -hydroxyprogesterone, 17α -hydroxypregn-4-ene-3,20-dione; androstenedione, androst-4-ene-3, 17-dione; 5α -androstenedione, 5α -androstane-3,17-dione; androsterone, 3α -hydroxy- 5α -androstan-17-one; NADPH, reduced form of nicotinamide adenine dinucleotide phosphate; the lyase, the enzyme which cleaves the side chain of 17α -hydroxyprogesterone at C_{17} - C_{20} bond, producing androstenedione; the isomerase, 3-ketosteroid Δ^4 - Δ^5 isomerase (EC 5.3.3.1) and HCG, human chorionic gonadotrophin.

g for 20 mins. The precipitate containing unbroken cells, cell wall, nuclei and other large particles were discarded and aliquot of the supernatant fluid was used for measurement of the testicular enzyme activities of mice. Then, an aliquot of the $800\times g$ supernatant fluid obtained from rat testicular homogenate was further centrifuged at $10,000\times g$ for 20 mins. to precipitate mitochondrial particles. The $10,000\times g$ supernatant fluid was then centrifuged at $105,000\times g$ for 60 mins. The precipitate was suspended in 50 times their weight of 0.25 M sucrose solution and sedimented again at $105,000\times g$. The precipitate thus obtained was used as the microsomal fraction of rat testes. The $105,000\times g$ supernatant fluid was centrifuged again at $105,000\times g$ for 60 mins. to remove the remaining microsomes, and thus obtained supernatant fluid was used as the soluble fraction. Most of these fractionation procedures were performed below 3°C , and the whole procedure required about 8 hrs.

Radioactive steroids

17α -Hydroxyprogesterone-4- ^{14}C (specific activity $113.6\ \mu\text{Ci/mg}$) was purchased from the Radiochemical Centre, Amersham, England. Pregnenolone-4- ^{14}C (specific activity $144.9\ \mu\text{Ci/mg}$), progesterone-4- ^{14}C (specific activity $146.5\ \mu\text{Ci/mg}$) and androstenedione-4- ^{14}C (specific activity $71.0\ \mu\text{Ci/mg}$) were purchased from New England Nuclear Corporation, Boston, Mass. All the radiochemical purities of these steroids were checked by thin-layer chromatography shortly before use. Radioactive steroids were diluted with corresponding non-radioactive steroids to appropriate specific activities as substrates for enzyme assays to insure the saturation of the enzymes with each substrate.

Incubation

The radioactive steroids dissolved in volatile solvents were transferred into incubation flasks, into which 2 drops of propylene glycol per flask were added. Shortly before the incubation, the solvent was removed under reduced pressure. Then, to each incubation flask, the mixture of testicular enzyme preparation in 0.25 M sucrose

solution, and NADPH in 0.25 M sucrose solution which contained Tris 0.05 M (pH 7.4) and MgCl_2 0.005 M was added, and finally, the volume of the mixture per flask was adjusted to be 5 ml and the concentration of the cofactor in it was $120\ \mu\text{M}$.

The 17α -hydroxylase activity in mouse testicular homogenate was measured as follows; progesterone-4- ^{14}C ($10\ \mu\text{g}$, 7×10^4 cpm) was incubated with the testicular homogenate (16 testes per flask) of mice irradiated at dose 200 and 400 R along with the control, at 37°C under a gas phase of $\text{O}_2 : \text{CO}_2$ (95 : 5) with continuous shaking for 30 mins.

The microsomal enzyme activities of rat testes were measured using the following amounts of the substrates per each flask; pregnenolone-4- ^{14}C ($30\ \mu\text{g}$, 12×10^4 cpm) for assay of the activity of the Δ^5 - 3β -hydroxysteroid dehydrogenase with the isomerase, progesterone-4- ^{14}C ($30\ \mu\text{g}$, 9×10^4 cpm) for 17α -hydroxylase assay, 17α -hydroxyprogesterone-4- ^{14}C ($30\ \mu\text{g}$, 12×10^4 cpm) for assay of the lyase, and androstenedione-4- ^{14}C ($30\ \mu\text{g}$, 10×10^4 cpm) for the measurement of the 17β -hydroxysteroid dehydrogenase. These steroid precursors were incubated with the testicular microsomal fraction (3.2 or 3.6 testes per flask) of rat for 30 mins. at 37°C under oxygen enriched atmosphere. On the other hand, for the measurement of the 20α -hydroxysteroid dehydrogenase activity, 17α -hydroxyprogesterone-4- ^{14}C ($10\ \mu\text{g}$, 5×10^4 cpm) was incubated as substrate, with the $105,000\times g$ supernatant fluid (2.1 or 2.4 testes per flask) of rat testicular homogenate for 60 mins. at 37°C with continuous shaking.

Assay of testicular enzyme activities related to steroid bioconversion

The enzyme activities of testes were expressed as follows; the Δ^5 - 3β -hydroxysteroid dehydrogenase with the isomerase, as the sum of progesterone, 17α -hydroxyprogesterone, androstenedione, testosterone and their derivatives originated from the administered pregnenolone; the 17α -hydroxylase, as the sum of 17α -hydroxyprogesterone, androstenedione, testosterone and their metabolites derived from progesterone used as the substrate; the lyase activity as the sum of

androstenedione, testosterone and other C-19 steroids formed from 17α -hydroxyprogesterone, which was used as the substrate; the 17β -hydroxysteroid dehydrogenase as the yield of testosterone from androstenedione used as the substrate; and the 20α -hydroxysteroid dehydrogenase activity, as the amount of $17\alpha,20\alpha$ -dihydroxypregn-4-en-3-one converted from the 17α -hydroxyprogesterone. Indices of the enzyme activities were calculated on the basis of the activity per gland or per mg protein involved.

Extraction, isolation and identification of products

Immediately after the incubation, enzymic reactions were arrested by addition of 15 ml of methylene dichloride and the mixture was vigorously shaken for extraction. Then, after centrifugation of the mixture at $2,000\times g$ for 10 mins., the lower layer was removed by a transfer pipette and reserved. This procedure was repeated 2 more times to complete extraction of the steroids, after non-radioactive progesterone, 17α -hydroxyprogesterone, androstenedione, testosterone and $17\alpha,21$ -dihydroxypregn-4-ene-3,20-dione were added as carriers (100 μg each per flask). The pooled extract was dried with anhydrous sodium sulfate, and evaporated to dryness under reduced pressure. At this stage, 90–98% of the initially added radioactivity was recovered as the extract. An aliquot of the radioactive steroid extract was chromatographed on thin layer plate coated with silica gel G and GF (4 : 1) (E. Merck, Darmstadt, Germany), using a benzene-acetone (8 : 2, v/v) and other systems at 15–20°C. After development of the thin layer chromatogram, the spots of the above mentioned 5 carrier steroids were detected under on ultraviolet light (wavelength, 253 m μ). The radioactive spots were detected by an autoradiographic method of exposing X-ray film to the coated phase of the chromatogram for several days, and also by a windowless flow-gas counter with a scanning device (Vanguard Autoscanner 880 and 885, Technical Measurement Corp., North Haven, Conn.). Detected steroid spots were scraped off from the glass plate and extracted by a solvent mixture of chloroform and

ethyl-alcohol (1 : 1, v/v) from the absorbent, after packing the silica gel powder as a small column. Final identification of the metabolites obtained was based on the following criteria: 1) Identical mobilities of the metabolite to the standard preparation on thin layer chromatograms developed with at least 3 different solvent systems. 2) Chromatographic behavior identical to the standard preparation, through chemical reactions such as oxidation and acetylation. 3) Constant specific activity after repeated crystallization of the radioactive metabolites with the corresponding authentic preparations, as stated in our previous paper (Inano and Tamaoki, 1966).

Quantitation of radioactivity of products

Suitable aliquots of the extracts were evaporated to dryness under nitrogen in 20 ml glass vials (Wheaton Glass Co., Millville, N. J.), and dissolved in 11 ml of toluene containing 2,5-diphenyloxazole, PPO (0.4%) and 1,4-bis-2-(5-phenyloxazolyl)-benzene, POPOP, (0.01%). The radioactivity was measured with a liquid scintillation spectrometer (System 725, Nuclear-Chicago, Des Plaines, Ill.) for a sufficient time to reduce the counting error below 5%. The efficiency of counting ^{14}C was about 70%.

Measurement of Protein

Protein concentrations were determined by the copper-Folin method, using a 1 cm light path at 750 m μ , after diluting the samples to suitable concentrations (Lowry *et al.*, 1951). A linear relationship between the concentration of protein and the absorbance was confirmed.

Histological examination

Specimens of testes obtained from the rats of the 4 groups were fixed in neutral formalin solution, cut at 5 μ in thickness and stained with hematoxylin and eosin.

RESULTS

Influence of X-irradiation on body and testes weights of mice

The mice which were irradiated at the dose,

0, 200 and 400 R were sacrificed after 1, 3, 7, 14 and 21 days post-irradiation. Body weights of 200 and 400 R irradiated animals did not show any significant difference in comparison with the control mice, which had not been irradiated ($p > 0.05$). On the other hand, the mean weights of the testicular glands became significantly smaller than those of intact mice on the 14th day post-irradiation ($p < 0.01$). On the 21st day after X-ray exposure, testicular size and weights of 200 and 400 R irradiated mice did not recover to the level of the control group (Fig. 1).

Testicular enzymic activity of converting progesterone in the X-irradiated mice

Change of the 17α -hydroxylase activity in testicular homogenate of the mice over the post-irradiation period was shown in Table 1. When the enzymic activity was expressed as μg products per mg protein, the 17α -hydroxylase activities at the 2nd and 3rd week after irradiation were found to be higher than those of the non-irradiated mice. One of the metabolites was located between the spots corresponding to 17α -hydroxyprogesterone and androstenedione on the thin-layer chromatogram which was developed in an acetone-benzene (2 : 8, v/v) system. The product obtained from this spot was found oxidizable with 0.5% CrO_3 in 90% acetic acid at room temperature, resulting a product identical with 5α -androstenedione. When an

Fig. 1. Change in mouse testicular weight during a 3-week period after X-irradiation.

aliquot of the radioactive substance was acetylated with the mixture of acetic anhydride and pyridine (1 : 1, v/v) at room temperature, the acetylated product was obtained judging from its reduced polarity after this reaction and showed the identical mobility

Table 1. Change of 17α -hydroxylase activity of X-irradiated mouse testicular homogenate

Dose (R)	Days Post-Irradiation					
	0	1	3	7	14	21
	Specific activity*					
0	17.3	20.6	14.7	12.8	12.3	14.1
200	—	17.8	12.9	16.9	18.4	23.2
400	—	15.4	17.5	13.2	19.6	27.9
	Enzyme activity per pair of testes**					
0	64.9	85.4	82.2	72.7	73.5	73.8
200	—	73.8	72.1	77.5	77.5	88.4
400	—	76.2	65.6	79.8	62.4	123.3

* μg of products $\times 10^{-2}/\text{mg}$ protein.

** μg of products $\times 10^{-2}$.

of androsterone acetate. Furthermore, crystallization of the radioactive substance with the authentic preparation of androsterone was repeated, and the specific activities of the crystal were found to be constant (Table 2).

Influence of local X-irradiation and HCG treatment upon the weight and histology of testes and sex accessory organs

The rats of the 4 groups treated as mentioned in Materials and Methods were sacrificed on the following day after the last injection or on the 30th day after local irradiation, and the weights of body and testes were

recorded. Furthermore, the weights of accessory organs such as seminal vesicles and ventral lobes of prostates were also measured as the indices for the produced and secreted androgens from X-irradiated testes in comparison with those of non-irradiated testes. As shown in Table 3, significant decrease of testicular weight was caused by X-irradiation, suggesting the destruction of relatively radio-sensitive cells, such as germinal cells in the seminiferous tubules, in accordance with the histological observation (Fig. 2a and c). But, administration of HCG did not increase testicular weight in either the irradiated or non-irradiated animals, even though prolifer-

Table 2. Identification of metabolites produced from substrate by X-irradiated testes

Recrystallization	Specific activities (counts/min./mg)*				
	Progesterone**	17 α -Hydroxy-progesterone**	Androsterone**	Testosterone**	Androsterone***
1st	989	718	369	150	106
2nd	969	713	376	145	111
3rd	972	715	374	145	107
4th	—	724	378	144	—

* Specific activities of crystals obtained.

** From pregnenolone-4-¹⁴C by X-irradiated and HCG treated rat testes.

*** From progesterone-4-¹⁴C by X-irradiated mouse testes.

Table 3. Biological effects of X-irradiation and human chorionic gonadotrophin upon rats

Group	Treatment		No. of animals	Initial body Weight (g)	Final body Weight (g)*	Testes (g)**	Seminal Vesicles (g)**	Ventral Prostates (g)**
	X-irradiation	HCG						
C	—	—	10	309.0±5.5***	318.5±19.8	2.736±0.113	0.996±0.051	0.367±0.024
CH	—	+	10	313.8±7.1	340.7± 9.1	2.841±0.072	1.772±0.051	0.643±0.030
X	+	—	10	312.0±6.1	348.0± 8.9	1.389±0.036	1.049±0.045	0.388±0.019
XH	+	+	9	304.1±6.8	329.2± 7.6	1.513±0.080	1.797±0.072	0.543±0.069
Difference				C-CH		NS****	p<0.01	p<0.01
				C-X		p<0.01	NS	NS
				C-XH		p<0.01	p<0.01	p<0.01
				X-XH		NS	p<0.01	p<0.05
				CH-X		p<0.01	p<0.01	p<0.01
				CH-XH		p<0.01	NS	NS

C, control; CH, control treated with HCG; X, X-irradiated and XH, X-irradiated; rats treated with HCG. Details of treatments, see Text.

* Killed on 30th day after 1,000 R of local X-irradiation.

** Bilateral weight.

*** Mean $\pm$ standard error.

**** p>0.05.

ation of interstitial cells was observed histologically by the treatment of gonadotrophin (Fig. 2b and d).

However, by administration of HCG, significant increase in weight of seminal vesicles and ventral lobes of prostates was observed of both intact and the X-irradiated rats. This result indicated that enhancement of production and secretion of androgens in the testicular glands of the rats caused by gonadotrophin was independent to the presence of germinal cells. In other words, the cells, such as interstitial cells, which remained still after irradiation retained the responsiveness to the gonadotrophin in producing androgens, while histologically only the interstitial cells among the radio-resistant cells showed the proliferation and stimulation against the gonadotrophin treatment. As there was no significant difference among the body weight increases of the rats of the 4 groups during the post-irradiation period, apparent somatic damage was able to be avoided by shielding the body with lead plate, in case of local irradiation to the testes at 1,000 R.

Influence of X-irradiation and HCG treatment upon testicular enzymic activities related to androgen production

The enzyme activities defined as stated previously were measured in the testes of normal, normal and HCG treated, X-irradiated, and X-irradiated and HCG treated rats, and were compared among them, on the basis of the activity per gland or per mg protein involved (Table 4). From this result, changes of the individual enzyme activity the to these treatment were further studied.

1. Δ^5 -3 β -Hydroxysteroid dehydrogenase

When the enzymic activities per testis were compared between the groups of C and X, and between the groups of CH and XH, this enzymic activity was reserved or somewhat enhanced after X-irradiation located to testes. By the treatment of HCG, the activities of both intact and X-irradiated testes per organ were increased in accordance with the increased secretion of androgen detected by the weights of seminal vesicles and ventral lobes of prostates. The enzymic activity per mg protein was increased by X-irradiation, and this indicates that the Δ^5 -3 β -hydroxysteroid dehydrogenase was located to the cells which remained after radiation or probably Leydig cells in interstitial tissue being in agreement with the results of histochemical observation on this enzyme (Baillie and Griffiths, 1964; Baillie and Mack, 1966).

Table 4. Testicular enzyme activities of the rats treated with X-irradiation and HCG

Group	Δ^5 -3 β -Hydroxysteroid dehydrogenase	17 α -Hydroxylase	Lyase	17 β -Hydroxysteroid dehydrogenase	20 α -Hydroxysteroid dehydrogenase
Specific activities*					
C	83.3	55.2	46.8	19.9	10.1
CH	77.5	64.5	704.2	7.0	7.1
X	185.8	64.0	51.0	24.0	16.9
XH	219.5	150.0	115.4	8.1	19.3
Enzyme activities per testis**					
C	625.0	414.2	350.8	149.2	252.9
CH	731.7	609.2	664.9	65.8	202.1
X	774.2	266.7	212.5	100.0	310.0
XH	891.6	609.4	468.8	32.8	327.8

C, control; CH, control treated with HCG; X, X-irradiated; XH, X-irradiated rats treated HCG. Details of treatments, see Text.

* μg of products $\times 10^{-2}$ /mg protein. ** μg of products $\times 10^{-2}$.

Fig. 2. Micrographic of testes (stained with hematoxylin and eosin, $\times 125$). (a) Normal rat. Spermatogenesis is active and some Leydig cells are seen in the small interstitial spaces between the seminiferous tubules. (b) Rat treated with gonadotrophin (HCG) for 2 weeks. Spermatogenesis is somewhat enhanced and increased number of Leydig cells is observed. (c) Rat locally irradiated to the testes (1,000 R). The seminiferous tubules showed marked atrophy, revealing severe destruction of germinal cells in the tubules. The Leydig cells are more apparent than those in the nonirradiated rats. (d) Rat locally irradiated to the testes, and then treated with HCG. Absence of germinal epithelium is observed, while hypertrophy of Leydig cells which lie between the atrophied tubules is noticeable.

2. 17α -Hydroxylase

After local irradiation to the testes, the total enzymic activity per organ was reduced to 64% of the activity of the intact one, but the enzyme activity per mg protein in X-irradiated testes is rather higher than the one of the intact testes. By administration of the gonadotropin, the 17α -hydroxylase activity in X-irradiated testes recovered to the level of the intact testes treated with HCG. From these results, the 17α -hydroxylase seemed to be also concentrated to interstitial cells, and the reduced activity by radiation was recovered by administration with HCG.

3. Lyase

The lyase activity per testis was reduced by local irradiation to about 61% of the

activity of the control testes, and the enzyme activity per mg protein remained unchanged or somewhat increased. This enzyme activity in the X-irradiated testes was remarkably increased by the gonadotropin beyond the level of the intact testes, but not enough to reach the level of the intact testes treated similarly with the gonadotropin. Therefore, the testicular lyase activity was reduced in the X-irradiated testis, but increased in response to the administered gonadotropin.

4. 17β -Hydroxysteroid dehydrogenase

The enzymic activity per testis of irradiated rats was reduced about 67% of the intact testes. By administration of HCG, the enzyme activity was particularly reduced both in the intact and X-irradiated animals, in respect

of both activity per gland or per mg protein.

5. *20 α -Hydroxysteroid dehydrogenase*

Due to the intracellular distribution of this enzyme, the activity was measured of the supernatant fluid at $105,000\times g$ entirely free from the microsomal fraction. The enzyme activity per gland was higher in the X-irradiated testes than the value of the intact testes, showing that this enzyme distributed mostly to the cells which remained still after irradiation. By HCG administration, the activity of the enzymes was not so remarkably influenced, being different to the other microsomal enzymes except 17 β -hydroxysteroid dehydrogenase above described.

DISCUSSION

Comparing the enzyme activities of testicular tissue of X-irradiated rats with those of normal animals, it is firstly necessary to consider the relationship between several testicular cells and their radio-sensitivities. Interstitial cells, which are responsible for androgen production and secretion, and Sertoli cells or sustentacular cells in seminiferous epithelium were accepted as resistant to X-ray from histological observation, while spermatogenic cells in seminiferous tubules were accepted as highly radio-sensitive (Schoen, 1964). Though the absolute population of Leydig cells in interstitial tissue of testicular gland is by no means easy to be determined, X-irradiation caused a small increase in the number of interstitial cells contrary to the cells in seminiferous tubules, and following administration of gonadotrophin, the interstitial cell count increased more than 3-fold in the irradiated animals (Schoen, 1964).

From the results in this report, the higher specific activities of the testicular enzymes of irradiated rats, *i. e.*, Δ^5 -3 β -hydroxysteroid dehydrogenase, 17 α -hydroxylase, the lyase and 17 β -hydroxysteroid dehydrogenase were caused by the fact that the microsomal fraction was obtained mainly from interstitial cells and other radio-resistant cells, being

not diluted with other microsomal fraction derived from radio-sensitive germinal cells which did not possess the activities.

However, the specific activities of these enzymes were not uniformly elevated by X-irradiation as shown in Table 4, (C vs. X), because of individually different radiosensitivities of the enzymic activities or these enzymes synthesis in the testicular glands. The radio-resistant cells in X-irradiated testes were capable to increase production and secretion of androgen in response to the administered HCG, which caused mostly the proliferation of interstitial cells among the radio-resistant cells, according to the present histological observation. Therefore, it was concluded that intercellular distribution of the enzymes related to biosynthesis of testosterone from pregnenolone was largely limited to the interstitial cells, and their intracellular distribution was restricted to the microsomal fraction (Shikita and Tamaoki, 1965b). Furthermore, as the higher specific activity of the 20 α -hydroxysteroid dehydrogenase was observed in X-irradiated testes, in accordance with the previous observation (Shikita and Tamaoki, 1965a), this dehydrogenase activity was also considered to be located to the interstitial cells. This was recently supported by the histological observation of the 20 α -hydroxysteroid dehydrogenase in human testes (Baillie *et al.*, 1966).

In the state of the homogenate (Schoen, 1964) of the irradiated rat testes, the 20 α -hydroxysteroid dehydrogenase activity was reported as increased by irradiation, and also it was observed (Berliner and Ellis, 1965b) that the production of 17 α ,20 α -dihydroxypregn-4-en-3-one by testicular mince of rat was enhanced by irradiation. Previously, the authors reported of the competition between the lyase in the microsomal fraction, and the 20 α -hydroxysteroid dehydrogenase activity in the soluble fraction, sharing 17 α -hydroxypregesterone as the common substrate, and discussed the decisive factor which determined the predominance of the two enzyme reactions (Inano *et al.*, 1967). In the present

study, the 20α -hydroxysteroid dehydrogenase activity in the $105,000\times g$ supernatant fluid which did not contain the lyase activity was found increased. Therefore, the increase of this dihydroxy compound was considered to be caused mainly by the increase of the dehydrogenase activity, and partly by the reduced activity of the lyase per gland, as observed in this experiment using the sub-cellular fraction.

By the time course study of the effect of radiation upon mice, the difference of testicular weight between normal and X-irradiated mice became significant on the 14th day post-irradiation.

Rats received daily injection of HCG starting on the 14th day post-irradiation for 16 days. Though any restorative effect by HCG could not be confirmed of spermatogenesis in X-irradiated testes at 1,000 R, the increases on the Δ^5 - 3β -hydroxysteroid dehydrogenase, the 17α -hydroxylase and the lyase activities by the gonadotrophin were observed among the enzymes examined, in accordance with the enhancement of over-all production and secretion of androgen detected by the weight of accessory organs. It was reported that the administration of the gonadotrophin caused increased androgen production *in vivo* (Brinck-Johnsen and Eik-Nes, 1957; Hall and Eik-Nes, 1963) and *in vitro* by increasing the 17α -hydroxylase, the lyase (Schoen, 1964; Schoen and Samuels, 1965) and the Δ^5 - 3β -hydroxysteroid dehydrogenase activities in testicular tissue (Samuels and Helmreich, 1956); it also accelerated testosterone production from acetate (Brady, 1951; Savard and Goldzieher, 1960; Mason and Samuels, 1961). On the other hand, the enzymes of side-chain cleavage of cholesterol was also enhanced by the administration of gonadotrophin to immature animals (Menon *et al.*, 1965). In this laboratory, it was reported that the administration of HCG to immature rats increased the Δ^5 - 3β -hydroxysteroid dehydrogenase activity and reduced the activity of the 17β -hydroxysteroid dehydrogenase in testicular microsomal frac-

tion in agreement with the present observation, while the 20α -hydroxysteroid dehydrogenase activity decreased (Inano and Tamaoki, 1966).

Due to X-irradiation to testes, over-all production and secretion of androgens were not significantly changed, but, in this investigation, the response of the individual enzyme activity related to steroid bioconversion against X-irradiation were examined, finding that 17α -hydroxylase and the lyase activities were relatively susceptible to radiation, and in this situation, the Δ^5 - 3β -hydroxysteroid dehydrogenase in testicular tissue seems to be an important rate-limiting factor for androgen production. On the other hand, it was demonstrated that the supply of NADPH was able to restore the androgen biosynthetic enzyme activities in testicular gland which was reduced by X-irradiation (Berliner *et al.*, 1964).

Steinberger and Wagner (1961) reported an elevation of endogenous respiration (QO_2) of testicular tissue after X-irradiation which was dependent upon pituitary function. Treatment of X-irradiated animal with testosterone propionate completely prevented the rise respiration (Steinberger, 1964).

ACKNOWLEDGEMENTS

The authors are indebted to Dr. M. Egusa, Department of Obstetrics and Gynecology, University of Tokyo Medical School, Miss. A. Machino and Miss. R. Oh, for their technical assistance, and to Dr. S. Matsushima, Teikoku Hormone Mfg. Co., for his kind supply of the purified HCG preparation for this study.

REFERENCES

- Baillie, A. H., M. M. Ferguson and D. McK. Hart, *Developments in Steroid Histochemistry*, Academic Press, London, p. 55 (1966).
- Baillie, A. H. and K. Griffiths (1964). *J. Endocrinol.* **31**, 63.
- Baillie, A. H. and W. S. Mack. (1966). *Ibid.*

- 35, 239.
- Berliner, D. L. and L. C. Ellis (1965a). *Radiation Res.* **24**, 572.
- Berliner, D. L. and L. C. Ellis (1965b). *Ibid.* **24**, 368.
- Berliner, D. L., L. C. Ellis and G. N. Taylor (1964). *Ibid.* **22**, 345.
- Brady, R. O. (1951). *J. Biol. Chem.* **193**, 145.
- Brinck-Johnsen, T. and K. Eik-Nes (1957). *Endocrinology* **61**, 676.
- Christensen, A. K. and N. R. Mason (1965). *Endocrinology* **76**, 646.
- Ellis, L. C. and D. L. Berliner (1963). *Radiation Res.* **20**, 549.
- Hall, P. F. and K. B. Eik-Nes (1963). *Biochim. Biophys. Acta* **71**, 438.
- Inano, H., H. Nakano, M. Shikita and B. Tamaoki (1967). *Biochim. Biophys. Acta* **137**, 540.
- Inano, H. and B. Tamaoki (1966). *Endocrinology* **79**, 579.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall (1951). *J. Biol. Chem.* **193**, 265.
- Mason, N. R. and L. T. Samuels (1961). *Endocrinology* **68**, 899.
- Menon, K. M. J., M. Drosowsky, R. I. Dorfman and E. Forchielli (1965). *Steroids* (Supplement) **1**, 95.
- Samuels, L. T. and M. L. Helmrich (1956). *Endocrinology* **58**, 435.
- Savard, K. and J. W. Goldzieher (1960). *Ibid.* **66**, 617.
- Schoen, E. J. (1964). *Ibid.* **75**, 56.
- Schoen, E. J. and L. T. Samuels (1965). *Acta Endocrinol.* (Kobenhavn) **50**, 365.
- Shikita, M. and B. Tamaoki (1965a). *Biochemistry (Wash)* **4**, 1189.
- Shikita, M. and B. Tamaoki (1965b). *Endocrinology* **76**, 563.
- Steinberger, E. (eds. Carlson W. D. and F. X. Gassner). Effect of Ionizing Radiation on the Reproductive System, Pergamon Press, Oxford, p. 213 (1964).
- Steinberger, E. and C. Wagner (1961). *Endocrinology* **69**, 305.
- Weiss, L. (1959). *J. Endocrinol.* **19**, 22.