

Effect of Gamma-Ray Irradiation on Adrenocortical Activity of Albino Rats

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1. The biological effects of Co^{60} γ -ray irradiation in the dose of 900 r on albino rats were similar to those of X-irradiation in the same dose, with special reference to the body weight and survival time of the irradiated albino rats.
2. The adrenal weight of albino rats irradiated by 900 r of Co^{60} γ -rays or X-rays increased from the 4th day of exposure.
3. In the albino rats irradiated by 900 r of X-rays, urinary formaldehydogenic steroids (FGS), determined in successive days after the X-irradiation, increased on the 1st and 3rd day of the exposure and decreased gradually until the animal died. A preterminal increase of urinary FGS was recognized in some of the irradiated albino rats.
4. The response of urinary FGS to ACTH-Z injected on the 2nd and 5th day of the irradiation by 900 r of Co^{60} γ -rays or X-ray revealed low adrenocortical activity of the irradiated albino rats.
5. Some of the Co^{60} γ -ray irradiated albino rats were sacrificed 4 hours, two and five days after the lethal exposure, respectively, and steroid production of the removed adrenals of the rats was determined by the in vitro method, according to Saffran et al and Eisenstein. Steroid production of the adrenals in the irradiated albino rats showed a tendency to decrease on the 2nd day of the exposure.
6. The albino rats irradiated by 900 r of X-rays or Co^{60} γ -rays could survive longer by injection of ACTH-Z in a dose of four units on the 2nd day of the exposure than those not injected or injected with ACTH-Z on the 5th day of the exposure.

INTRODUCTION

The considerable pathological changes of the adrenal glands have been confirmed in the victims of the atomic bombing^{16,21,26,29}. Latent adrenal insufficiency was noticed in some atomic bomb survivors who had been severely injured by the explosion^{10,12,14,22} and in some sufferers at Bikini Atoll¹¹. There have been many clinical^{6,13,19} and experimental^{1,2,3,9,17} reports on the relation between adrenocortical activity and radiation injuries. The authors intend to report in this paper the influence of the Co^{60} γ -ray and X-ray irradiation on the adrenocortical activity of albino rats.

MATERIALS AND METHODS

Male albino rats of Wistar strain, weighing 140-240 g, were used and kept

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in individual metabolic cages. They were fed ad libitum on Oriental compressed diets and tap water.

Co^{60} γ -ray irradiation was performed with the Co^{60} irradiator at the Institute for Chemical Research, Kyoto University. The rats received single total body irradiation of Co^{60} γ -rays in the dose of 900 r from the source of Co^{60} arranged in a shape of a cylinder. The dose rate was about 3370 r per minute in air and the exposure time for 900 r was sixteen seconds.

Single total body irradiation of X-rays was performed in each dose of 900 r and 600 r respectively, with the Hakuai deep therapy apparatus at the Department of Radiology, Kyoto University Hospital. The irradiation factors were as follows: 160 kv, 3 mA, 0.6 mm Cu plus 0.5 mm Al filter, 30 cm target-skin distance and the dose rate of 16.0 r per minute.

The body weight was indicated by "index number", calculated in comparison with the initial value of 100.

Urine specimens collected during one day were used to determine form-aldehydogenic steroids (FGS) with the author's method¹⁵⁾. On the 2nd, 5th, 10th and 28th day of the irradiation of Co^{60} γ -rays or X-rays, respectively, the albino rats were injected subcutaneously with 4 units of ACTH-Z (Organon, Daiich Seiyaku) and their urinary FGS were determined before and after the injection.

Some of the albino rats irradiated by Co^{60} γ -rays were used for the in vitro steroidogenesis study, according to the method which was introduced by Saf-ran and Bayliss²³⁾ and was modified by Eisenstein⁵⁾. The adrenal glands were removed from the albino rats under intraperitoneal nembutan anesthesia. The wet weight of the adrenal glands was measured by a torsion balance. The adrenal glands were dissected and incubated aerobically in 2 ml of Krebs-Ringer-phosphate-glucose solution with 1 unit of ACTH. Corticosteroids in the incubation medium were extracted with chloroform and determined by spectrophotometric procedure.

EXPERIMENTAL RESULTS

1) Body Weight of the Irradiated Rats

In the rats irradiated by 900 r of Co^{60} γ -rays, the body weight decreased rather rapidly till the 4th day of the exposure, and then it decreased gradually and the rats died. The same tendency was found in those irradiated by 900 r of X-rays (Figs. 1 and 2).

2) Period of Survival in the Irradiated Rats

Following the irradiation of 900 r of Co^{60} γ -rays, the rats not injected with ACTH-Z survived 8.3 days in average, but those injected with 4 units of ACTH-Z on the 2nd day of the exposure lived 12.3 days long, while those injected with ACTH-Z on the 5th day of the exposure survived only 9.3 days (Fig. 1). The rats irradiated by 900 r of X-rays also survived 8.2 days in average. One of them, which was injected with ACTH-Z on the 2nd and 15th day, lived till

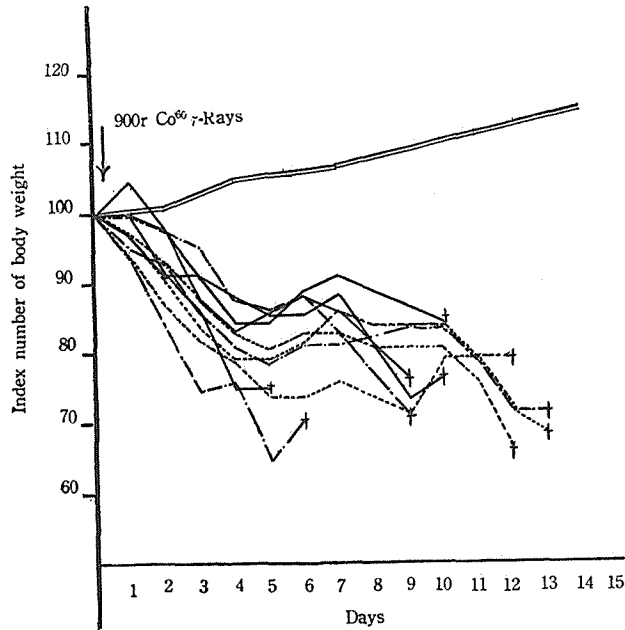


Fig. 1. Change of body weight and the survival time in the Co⁶⁰ γ-ray irradiated rats.

— non-irradiated, average of five rats ; — irradiated, without ACTH-Z injection ; irradiated, with ACTH injection on 2nd day ; - · - · - irradiated, with ACTH injection on 5th day.

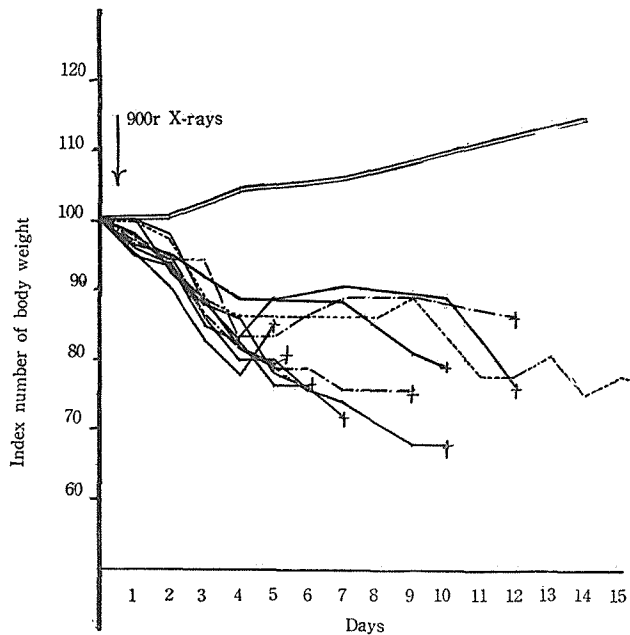


Fig. 2. Change of body weight and the survival time in the X-ray irradiated rats.

— non-irradiated, average of five rats ; — irradiated, without ACTH-Z injection ; irradiated, with ACTH-Z injection on 2nd ; - · - · - irradiated, with ACTH-Z injection on 5th day.

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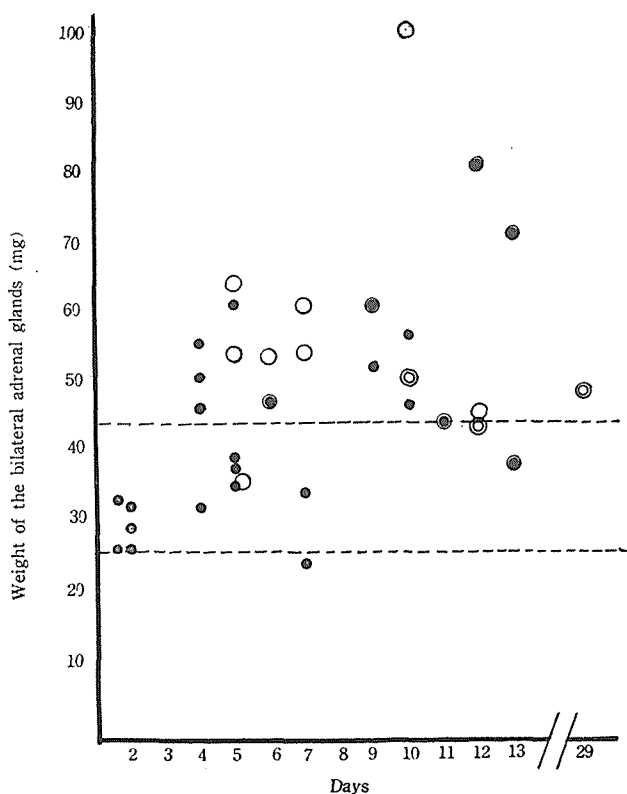


Fig. 3. Variations of the weight of the bilateral adrenal glands in the 900 r irradiated rats after the exposure. ○ X-irradiated rats ; ● Co⁶⁰ γ -rays irradiated rats ; ⊙ ⊙ irradiated rats injected with ACTH-Z

the 29th day of the exposure (Fig. 2).

3) Adrenal Weight of the Irradiated Rats

The adrenal weight of the rats, which were irradiated by 900 r of Co⁶⁰ γ -rays or X-rays, tended to increase after the exposure as shown in Fig. 3. The adrenal weight of the irradiated rats was yet in a normal range on the 2nd day of the exposure, but increased from the 4th day of the exposure.

4) *In vitro* Steroidogenesis of the Adrenals in the Irradiated Rats by Co⁶⁰ γ -rays

Table 1 indicates the results of the *in vitro* steroidogenesis of the adrenals in the rats irradiated by 900 r of Co⁶⁰ γ -rays. In the irradiated rats, the production of steroids by the adrenals was larger after 4 hours of the exposure and was lesser on the 2nd day of the exposure than in normal rats, but it was approximately equal on the 5th day of the exposure to that in normal rats.

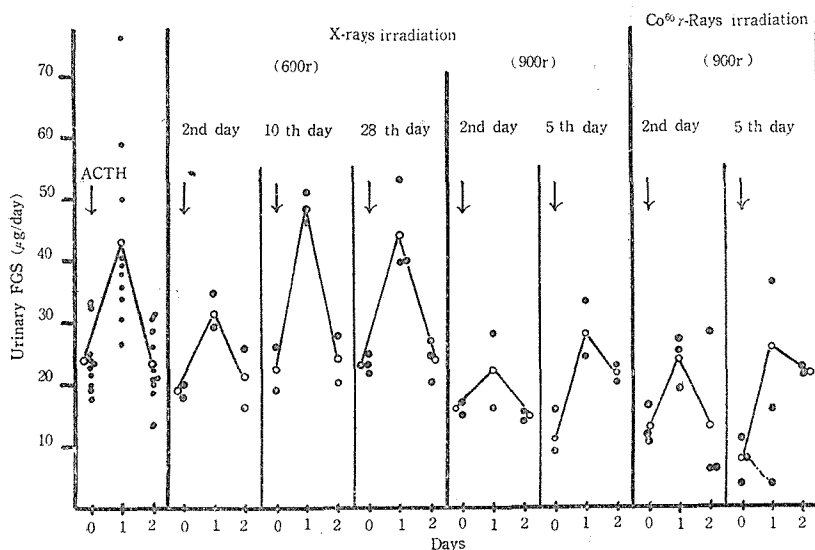
5) Urinary FGS in Response to ACTH-Z Injection in the Irradiated Rats

The irradiated rats were injected subcutaneously with 4 units of ACTH-Z in order to know the reserve function of the adrenal glands. The urinary FGS-

Table 1. *In vitro* steroidogenesis in the normal and irradiated rats.

	Body Weight (gm)	Adrenal Weight (mg)	Actual Steroid secretion/Adrenal (μ g)	Steroid Secretion per 100mg Adrenal Wt. (μ g)
Control (10)*	160~240	18.7 $\pm$ 3.81**	8.6 $\pm$ 2.61	46.2 $\pm$ 7.57
after Exposure				
Co ⁶⁰ γ -rays (900r)				
4 hrs. (5)	145~165	13.8 $\pm$ 1.72	7.2 $\pm$ 3.63	54.5 $\pm$ 8.26
2nd day (5)	125~145	16.7 $\pm$ 1.44	5.6 $\pm$ 1.96	33.7 $\pm$ 3.55
5th day (5)	120~130	20.6 $\pm$ 3.68	8.9 $\pm$ 2.18	44.7 $\pm$ 4.17

*() Number of rats.

** Mean Value $\pm$ S. D.Fig. 4. Urinary formaldehydogenic steroids in response to ACTH-Z injected in the rats irradiated by X-rays and Co⁶⁰ γ -rays.

response to ACTH-Z in the rats irradiated by 600 r of X-rays was nearly equal on 10th and 28th day of the exposure to that in normal rats, but the response was rather low on the 2nd day of the exposure.

The urinary FGS-response to ACTH-Z in the rats irradiated by 900 r of X-rays or Co⁶⁰ γ -rays was apparently lower on the 2nd and 5th day of the exposure than that in normal rats, as seen in Fig. 4.

9) Urinary FGS determined in Successive Days after the Exposure in the Rats irradiated by 900 r of X-rays

In the rats irradiated by 900 r of X-rays, the urinary FGS showed a transient slight increase on the 1st day of the exposure, and it increased again on the 3rd day. From the 3rd day of the exposure to the death of the rats, the urinary FGS decreased gradually. A remarkable high value of urinary FGS was recognized preterminally in some irradiated rats (Fig. 5).

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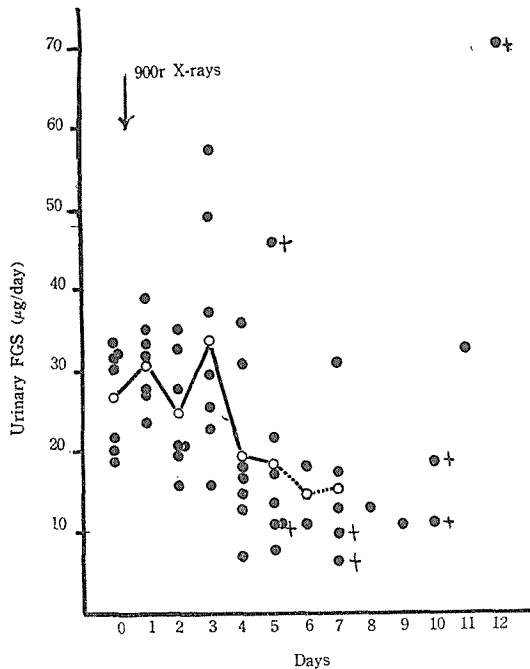


Fig. 5 : Urinary FGS determined in successive days after the exposure in the rats irradiated by 900 r of X-rays.

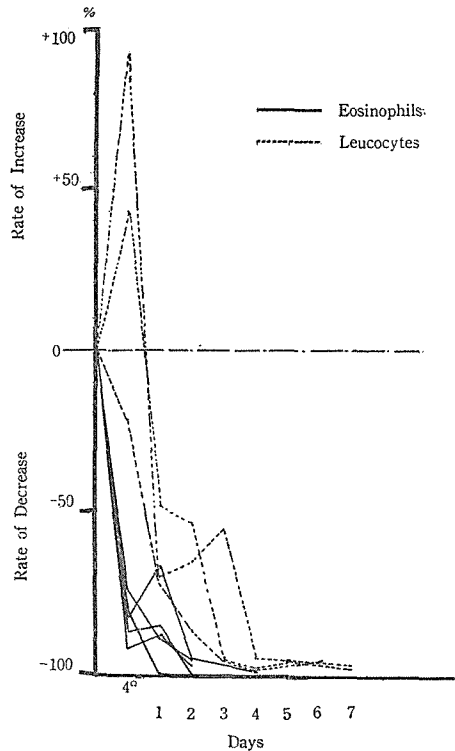


Fig. 6. Variations of the rate of increase or decrease in number of the leucocytes and eosinophils in the rats irradiated by 900 r of X-rays.

7) The Counts of Peripheral Leucocytes and Eosinophils in the Rats irradiated by 900 r of X-rays

After the 900 r irradiation of X-rays, the leucocyte count in the irradiated rats increased 4 hours after the exposure, and thereafter it decreased to minus 90 percent on the 4th day of the exposure. It never increased again until the rats died. The eosinophil leucocyte count in the rats irradiated by 900 r of X-rays decreased immediately after the exposure, and it almost disappeared from the peripheral blood of the irradiated rats during the period from the 2nd to the 3rd day after the exposure (Fig. 6).

DISCUSSION

It has been confirmed by us that the Co^{60} γ -ray irradiation has approximately the same biological effects as the X-ray irradiation, with special reference to the body weight and survival time of the irradiated rats.

Patt *et al.*¹⁷⁾ described in their report that the adrenal weight of the X-irradiated rats was increased by 20-40 percent on the 2nd day after the exposure of 650 r or 900 r, and a marked increase of the adrenal weight was observed on the 3rd or 4th day after the irradiation of 900 r. In the report

of Rosenfeld²⁰, the adrenal weight of a calf irradiated by 600 r of Co⁶⁰ γ -rays was increased on about the 7th day of the exposure. It was revealed by us that the adrenal weight was increased in the irradiated rats.

The initial increase of corticosteroids in the blood⁹, the adrenals²¹, or urine^{1,3} after irradiation has been observed by many researchers. The authors found a transient increase of urinary FGS in the initial stage of irradiation. This is probably due to an indirect effect of irradiation mediated by the pituitary gland^{18,28}. Patt *et al.*¹⁸ mentioned that hypophysectomy prevented the initial decrease of the adrenal cholesterol level after X-irradiation. The change of urinary FGS determined in successive days after the irradiation suggests the decrease of the adrenocortical activity of the rats irradiated by lethal dose. This was confirmed by the urinary FGS-response to ACTH-Z in the irradiated rats. Therefore, the increase of the adrenal weight in the irradiated rats does not always indicate the adrenocortical hyperfunction. As for the direct effect of irradiation on the adrenal gland, Ungar *et al.*²⁷ carried out the study of the in vitro steroidogenesis by perfusion of the calf adrenals under irradiation of Co⁶⁰ γ -rays, and they concluded that the steroidogenic activity of the adrenals, as radiosensitive organ, could be reduced directly by the irradiation. The dose of Co⁶⁰ γ -rays used in their experiment was about 1800-3000 r, which was too high to be compared with the dose of total body irradiation.

The results of our in vitro study did not indicate low adrenocortical activity in the irradiated rats, because the adrenocortical steroids produced in vitro after the exposure were nearly normal. The same results were obtained by Rosenfeld²⁰ in his perfusion experiment of the adrenals of the calves whose total body was irradiated by 600 r of Co⁶⁰ γ -rays. His findings demonstrated that the function of the adrenal cortex was far from "exhausted" at any time after a lethal total body irradiation. It is difficult to explain the difference of the results between the in vivo and in vitro experiment. However, it is considered that urinary corticosteroids may be influenced not only by the activity of the adrenals themselves but also by various general body conditions.

Edelman and Katsh⁴ found that the shorter the interval between the X-ray exposure and adrenalectomy was, the shorter the survival was. They mentioned that the demand for adrenal hormone was greatest during or immediately after the exposure and was reduced during 3 to 5 days after the exposure. Ellinger⁶ reported that desoxycorticosterone acetate (DCA) was clinically effective for radiation sickness presumably due to secondary adrenocortical insufficiency as the result of adaptation syndrome and that administration of DCA prevented the diminution of the liquids of the adrenal cortex in the X-ray irradiated mice. Ellinger *et al.*⁷ made a clinical investigation on 50 patients with radiation sickness treated with DCA and they obtained good results. On the other hand, Ellinger⁸ found that the administration of cortisone produced a shortening of the period between the irradiation and the onset of death, and resulted in a definite increase of the mortality rate. Smith *et al.*²⁴ failed to increase the survival time or the number of irradiated mice surviving by means of injection of cortisone or ACTH. Taber²⁶ was able to prolong the

lives of mice irradiated by 600 r of X-ray with ACTH treatment of ten days period. It was an interesting one of our experimental results that the lethal dose irradiated rats injected with 4 units of ACTH-Z on the 2nd day of the exposure survived longer, while those injected with ACTH-Z on the 5th day of the exposure did not survive so long. These results suggest that it is important to administer ACTH-Z as early as possible after the exposure for prevention of radiation injuries.

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