Histological Changes in Interrenal Tissue of the Goldfish, 
Carassius auratus, following X-Irradiation*

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ABSTRACT

Goldfish, Carassius auratus, irradiated with 4 kR or more of X-rays and kept at 23°C invariably died around 10 days after irradiation, while those exposed to 1 kR or less survived for at least one month under the same temperature condition, without showing any harmful effects.

Interrenal cells located in the head kidney of the goldfish decreased in size after hypophysectomy and underwent a hypertrophy following administration of mammalian ACTH. Six to nine days after whole-body irradiation with 1-16 kR of X-rays, interrenal cells were definitely enlarged. In hypophysectomized fish a similar X-irradiation failed to cause interrenal cell hypertrophy. Irradiation of the head of goldfish resulted in a hypertrophy of interrenal cells, even if the posterior part of the body containing the head kidney was shielded from X-rays. These findings seem to suggest that, in the goldfish, secretion of ACTH from the hypophysis increases at least for a period following X-irradiation.

INTRODUCTION

In a variety of mammals, it seems to be well established that X-irradiation, like different kinds of stressors, causes changes in the adrenal cortex suggesting an increase in demand for adrenal hormones in the body and that the responses of the adrenal cortex to the whole body irradiation are mediated by the hypophysis. Furthermore, it has been pointed out that in mammals the hypophyseal adrenal system plays an important role in the recovery from radiation-induced injuries.

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In a previous paper, it was reported that in X-irradiated goldfish augmented secretion of adrenocorticotropic hormone (ACTH) by the hypophysis was responsible for an increase in melanization. Since the interrenal tissue of fish is generally believed to be homologous to mammalian adrenocortical tissue, it was attempted to study histological changes in the interrenal tissue of the goldfish following X-irradiation.

MATERIAL AND METHODS

The common goldfish, Carassius auratus L., 4-5 g in body weight, were used. Until the commencement of experiments, fish were kept in large tanks under natural conditions.

Procedures of hypophysectomy was essentially similar to those of Chavin. The completeness of operation was checked at the end of the experiment by carefully examining the hypophyseal region.

During whole body X-irradiation, a group of 2 to 6 unanesthetized fish was kept in a small cylindrical lucite vessel containing water about 1.5 cm in depth. In shielding experiments (Series 7), each fish was made immovable by placing it in a small sack made of gauze and the head region of the fish was directly exposed to X-rays, the rest of the body with interrenal tissue being shielded from the rays with a 4 mm lead plate. X-rays were generated at 200 kVP and 20 mA with 0.5 mm Cu and 0.5 mm Al filters. The distance between target and center of the vessel was 25 cm, dose rate in air being about 400 R/min. After irradiation, each group of fish was kept in a glass vessel containing about 1.5 liters of water, under constant illumination at 22-24°C. Water was renewed every 2 or 3 days during the course of the experiments.

At the close of the experiments, fish were sacrificed during the daytime by decapitation immediately after being taken out of water. Head kidneys were removed and fixed in Bouin’s solution. Sections, 8 μ thick, were stained with standard Mayer’s acid hemalum and eosin. In some cases, nuclear diameters of interrenal cells were measured by means of a micrometer. Further experimental procedures will be described, if necessary, under separate headings.

RESULTS

1. Lethal effects of different X-ray doses  This series of experiments was carried out with 1252 goldfish. Ten groups of fish were given a single irradiation with 1/4, 1/2, 1, 2, 4, 8, 16, 32, 64 and 128 kR of X-rays, respectively. Non-irradiated fish kept under similar conditions served as controls. After irradiation, the number of dead fish was recorded every day for a period of 30 days and the percentage of survivors was calculated for each dose-group.

The results shown in Fig. 1 indicate that (1) a single irradiation of fish with 1 kR or less produced no marked change in the mortality at least within 30 days, that (2) in the 2 kR-irradiated group, about 85 per cent of fish died by the 31st day,
that (3) in a majority of fish irradiated with 4 to 32 kR, the survival time was about 10 days (8-12 days) regardless of the dose, that (4) following irradiation with 64 kR some fish died shortly after irradiation while the others survived for 7 to 11 days, and that (5) all fish given 128 kR died immediately after irradiation.

The range of lethal doses in the present experiments is concordant with those reported by Shechmeister et al.\textsuperscript{5} and Egami et al.\textsuperscript{6}.

2. Observations on interrenal tissue in non-irradiated goldfish

Interrenal tissue from 10 non-irradiated goldfish kept at 23°C were studied histologically. The head kidney of intact fish consisted of lymphoid tissue containing reticular cells, blood cells (hematopoietic tissue), interrenal and chromaffin cells. In some cases, thyroid follicles were encountered among these cells. As reported by Chavin\textsuperscript{4} and Oguri\textsuperscript{7}, the interrenal cells formed compact masses around major branches of the posterior cardinal veins in the head kidney. Generally speaking, the interrenal cells were columnar in shape with acidophilic cytoplasm, each having a spherical nucleus. The nucleolus was usually well defined.

Two groups each consisting of 3 hypophysectomized goldfish were kept at 23°C. Head kidneys of the first group were fixed for histological study on the 9th day after operation and those of the second group on the 23rd day. There was no marked difference in histological characteristics of interrenal tissue between the first group and intact fish. However, interrenal cells of the second group decreased in size and were irregular in shape, each having a small nucleus located in its basal part.

A group of 5 intact fish and the other of 5 hypophysectomized ones were given intraperitoneal injections of 1 I.U. of a mammalian ACTH preparation (Daiichi Seiyaku Co.) dissolved in 0.05 ml of Ringer's solution for 4 successive days, the first

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Fig. 1 Survival curves for goldfish subjected to wholebody irradiation with various doses of X-rays. Numbers indicate doses of X-rays in kR.
injection being given 23 days after hypophysectomy. Fish were sacrificed 3 days after the last injection and head kidneys were dissected out and fixed for histological study.

The injections of ACTH resulted in a marked hypertrophy of interrenal cells in both intact and hypophysectomized fish. Nucleoli contained in enlarged nuclei located in the central part of the hypertrophied interrenal cells were usually much larger and more prominent than in cells of non-injected fish. These findings are in good agreement with those of Chavin4) and Oguri7).

3. Observations on interrenal tissue of X-irradiated goldfish In order to study histological changes in interrenal cells following X-irradiation, 7 series of experiments were carried out on fish from different colonies in different seasons of the year.

1) Eight groups of 3 intact fish were irradiated with 2 or 4 kR of X-rays and head kidneys of surviving fish were fixed 1, 3, 6 or 9 days after irradiation.

Generally speaking, in fish sacrificed 6 or 9 days after irradiation with 2 or 4 kR, interrenal cells were enlarged, although the hypertrophy of the cells was different considerably in degree among different individuals even in the same group.

2) Four groups each consisting of 5 fish were irradiated with 0, 2, 4 and 8 kR, respectively, and surviving fish were fixed on the 7th day after irradiation. Interrenal cells in the irradiated fish definitely increased in both width and height (Plate 1). Nuclei located in the central part of the cells were also enlarged. All these changes resembled those following treatment with ACTH. Effects of the 3 different doses of X-rays on interrenal cells were approximately the same in every respect. Hematopoietic tissue in the head kidney was severely damaged by the irradiation (Plate 1 B and C).

3) Three groups of 6 fish were irradiated with 0, 8 and 16 kR, respectively, and survivors were fixed on the 7th day. Diameters of nuclei of 1,000 interrenal cells were measured in 50 sections of head kidneys of 3 individuals from each group and the mean values and standard deviations were calculated. The results (Table 1, III, 1-3) clearly show that nuclei of interrenal cells increased in diameter in a majority of individuals given 8 or 16 kR irradiation.

4) Six groups of 5 fish irradiated with 0.5, 1 or 2 kR were fixed 1 or 3 weeks after irradiation (Table 1, IV, 1-6). Hypertrophy of interrenal cells took place only in those fish irradiated with 1 or 2 kR and fixed one week after irradiation.

5) Eight groups each consisting of 3-9 fish were fixed 3, 6 or 9 days after irradiation with 2, 4 or 8 kR (Table 1, V, 1-8) and the nuclear diameters of interrenal cells were measured as in the preceding series. The results showed that there was a marked increase in nuclear diameter of interrenal cells 6 or 9 days after irradiation. Moreover, the interrenal cells underwent hypertrophy like that following injections of ACTH. No marked difference was discernible in histological features of interrenal cells between groups exposed to lethal doses (4 and 8 kR).
and those irradiated with a sublethal dose (2 kR).

6) Four hypophysectomized fish were irradiated with 4 kR of X-rays on the 23rd day after operation. At 7 post-irradiation days only one of the four fish was alive. Four non-irradiated hypophysectomized fish kept under similar conditions served as controls. Interrenal cells of these two groups of fish were atrophic, showing no indication of enlargement.

7) Four groups of 3-9 fish were subjected to whole body irradiation with 0, 2, 4 and 8 kR of X-rays, respectively. In 4 other groups, only the head of the fish was
exposed to 0, 2, 4 and 8 kR of X-rays, respectively, the posterior part of the body being shielded from the rays. Histological studies revealed a significant increase in nuclear size in interrenal cells 6 days following irradiation in both groups of fish (Table 2 and Plate 1D). Therefore, it seems evident that changes brought about by irradiation in some organ in the head were responsible for the hypertrophy of interrenal cells.

**DISCUSSION**

Several workers\(^5,\ 4,\ 7,\ 8\) have studied histologically the interrenals of normal goldfish. Although Pickford\(^9\) reported that no histological changes took place in interrenals after hypophysectomy in the teleost, *Fundulus heteroclitus*, Chavin\(^4\) observed a marked decrease in size of interrenal cells in hypophysectomized gold-
fish. In the eel, Fontaine and Hatey\textsuperscript{10} reported a reduction in weight of the interrenal glands following hypophysectomy.

On the other hand, several workers\textsuperscript{7, 11} have shown that mammalian ACTH is effective in stimulating teleostean interrenal cells. Consistent with this finding are the results of the present experiments on both intact and hypophysectomized goldfish.

Interrenals of goldfish underwent a marked hypertrophy 6 days after whole body X-irradiation. Sublethal doses (1-2 kR) were as effective as lethal doses (4-16 kR) in this respect. Accordingly it seems likely that the interrenal hypertrophy does not directly relate to radiation injuries causing death in the irradiated fish. The histological responses of interrenals to whole body X-irradiation bore a striking likeness to changes in the tissue following ACTH administration. Similar changes occurred following exposure of the head of the fish to X-rays, even if interrenals were shielded from irradiation. However, interrenal cells no longer reacted to irradiation with hypertrophy if a fish had been hypophysectomized.

In view of these findings, it seems highly probable that the interrenal hypertrophy was mediated by the hypophysis and that ACTH secretion from the hypophysis was stimulated by X-irradiation. Egami \textit{et al.}\textsuperscript{1} reached similar conclusions regarding the mechanism involved in the production of melanophores in the skin of X-irradiated goldfish. Egami and Hyodo\textsuperscript{12} have reported in the teleost, \textit{Oryzias latipes}, that retrogression of the ovaries following irradiation with 2 kR of X-rays seems to be largely due to a reduction of the secretion of gonadotropins from the hypophysis. Therefore, it seems probable that in teleosts as in mammals the overall modification takes place in secretion pattern of hormones from the hypophysis following exposure to ionizing radiations.

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REFERENCES


