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RADIATION-INDUCED HEMOPOIETIC LESIONS IN FISH*

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INTRODUCTION

Fish radiobiology has not been studied comprehensively, but more work has been done on fish than on other poikilotherms. There have been recent reviews of fish radiobiology by Cosgrove and Blaylock,¹⁰ Donaldson and Foster,¹¹ Egami et al.,¹⁶ and Ulrikson.⁵⁴ This article will deal principally with radiation effects on the hemopoietic tissues of fish, omitting reference to such other aspects as LD⁵⁰, gonadal effects, growth and developmental effects, etc. Experimental studies utilizing radiation exposures in the dose range for hemopoietic effects in several species of fish will be presented, and much of the literature on such studies will be included in the references. In preparing this chapter we gained some familiarity with the literature on blood cells and sites of hemopoiesis in fish, and we felt that many of these references should be included here for essential background information.

MATERIALS AND METHODS

Our personal experience with changes in the hemopoietic organs of irradiated fish is a by-product of several different experiments (Table 1), which were performed at different times, with different radiation sources, and under different holding conditions. The only constant is the histopathology, which was all done in one laboratory by one individual. A brief summary of the experimental procedure follows.

In the experiments conducted at Oak Ridge National Laboratory on mature mosquito fish (Gambusia abbinis), the fish were collected at 2-3 cm in size from local creeks and ponds. In most experiments, "wild" fish were used; in one, however, F₁ offspring of wild

fish were used, which had been held in laboratory tanks until they attained maturity (Table 1). All fish were acclimated to the laboratory at 15 or 25°C for at least 2 weeks before the experiments.

Irradiation was performed with a Gammacell 200 (Atomic Energy Commission Limited of Canada) containing ^{60}Co . For the acute studies, dose rates of approximately 360 rad/min were delivered to fish in groups of five in 100 ml of water in a plastic container. Doses were checked with Toshiba fluoroglass dosimeters. For the chronic studies, plastic boxes were arranged in the source room at three distances from the ^{60}Co source. Radiation doses were measured by exposing Toshiba fluoroglass dosimeters (Type FGD-3B) in the water-filled boxes for 86 hr. The calculated dose rates ranged from 0.5 to 5.43 rads/hr. During irradiation, boxes at each dose were maintained at either 15 or 25°C ($\pm 1^\circ\text{C}$), with four fish per box. The fish were irradiated for 23.5 hr each day, with the other half hour used for observation, cleaning and feeding. The chronic experiment lasted 128 days.

After acute irradiation, the fish were returned to the appropriate environmental chambers, checked daily, and fed at least every other day. At selected times -- up to 36 days after the acute dose and 128 days after chronic irradiation -- fish were removed for histopathology; also, some fish that died were examined histologically.

In the experiments on young channel catfish (*Ictalurus punctatus*), the fish were approximately 3 inches long and were obtained from the Frankfort National Fish Hatchery, U.S. Dept. of Interior, Frankfort, Ky. They were acclimated for at

least 2 weeks in laboratory tanks at 25°C and then irradiated individually in plastic containers with acute doses of ^{60}Co gamma radiation from a Gammacell at a dose rate of about 305 rad/min, with total doses of 500–2500 rads. They were then returned to the laboratory tanks and maintained until they died or were killed.

The adult bluegills (Lepomis macrochirus) used in the study at the University of Michigan were obtained from Sugarloaf Lake, Washtenaw Co., Michigan, and were held at a fish hatchery in Saline, Michigan, in large metal holding tanks. After acclimation, they were transported by special container to the University of Michigan, where they were given 1000 or 3000 rads of whole-body radiation from a 6000-Ci ^{60}Co source at a dose rate of approximately 160 rad/min. They were then returned to the outdoor holding tanks. Temperature varied from about 13.5 to 20°C during the 12-day period of sampling. These fish were part of a more extensive study on LD₅₀, radiation sterilization, and blood and serum protein effects.⁵⁴

In the experiment at the Cooperative Science Education Center, six small species of aquarium-maintained fish were used. They were acclimated in balanced 5-gallon aquariums for several weeks at 23°C and then irradiated individually in small plastic bags in a Cesi-Radiator (American Nuclear Corp.) with ^{137}Ce gamma rays at a dose rate of about 1248 rad/min. They were then returned to the aquaria and sampled at weekly intervals for 7 weeks.

In most cases the small fish were fixed whole in Bouin's fixative, with a slit in the abdominal wall for better penetration. Larger fish were dissected, and their major viscera were preserved. Bouin's fixation produced decalcification in 7–10 days so the small fish could be sectioned whole in desired planes with a razor

blade. Tissues were processed according to the usual procedure and stained with hematoxylin and eosin.

RESULTS

Tissue sampling in the Gambusia experiment was more thorough than in the others. Findings were similar in all species, however. In the species examined, the chief site of hemopoiesis is the head kidney. Acute doses of 750 rads caused only slight depression of hemopoiesis in male Gambusia maintained at 25°C, and no depression in fish held at 15°C. After 1500 rads, effects in both sexes were mild and relatively late; more damage was seen in females at 15°C than at 25°C, while the reverse was true in males. At 2800 rads, kidney hemopoiesis was equally affected in both sexes, with slight depression in 8- and 16-day samples and moderate depression in 26- and 36-day samples. There was some disparity between the two temperature groups, similar to that seen at 1500 rads. Depression of kidney hemopoiesis at 5800 rads was rapid and complete, the fish at 25°C being affected more quickly than those held at 15°C. The spleens of control fish showed leukocyte accumulation and some hemopoiesis. The spleen was more sensitive to radiation than the kidney, with atrophy as early as 2 days after 750 rad and more severe responses after higher doses. No sex or temperature effect was noted for spleen damage.

In the first chronic radiation experiment, where doses of 5.43 rad/hr were accumulated in Gambusia for 37 days, there was no demonstrable damage to the hemopoietic organs. In the second chronic experiment, where doses as high as

3.0 rad/hr were accumulated for as long as 128 days to a total of more than 9000 rads, some fish in the two higher dose groups (3.0 and 1.5 rad/hr) showed hemopoietic atrophy of a relatively mild degree in kidney and spleen in the later days of the experiment. Other fish with equal total doses, however, did not show hemopoietic atrophy.

In the experiment with channel catfish, the initial dose of 2500 rad killed all the fish by 13 days, and severe hemopoietic atrophy was seen in all the samples, including the earliest at day 7 (Figs. 1 and 2). The radiation dose was then varied from 500 to 2000 rad at 500-rad intervals. Severe hemopoietic atrophy in kidney, spleen, and thymus was found in all the fish sampled after 500-2000 rads (Figs. 3 and 4), persisting until the last sampling at 32 days in the 1000-rad group. (The 2000-rad group all died well before 30 days.) However, in the 500-rad group, hemopoietic recovery was seen in samples taken after 29 days.

In the bluegill study, limited tissue samplings of fish that received 1000 rads indicated hemopoietic atrophy in the kidney by 3 days, which persisted until the end of the 13-day sampling period. Kidney findings were similar in the 3000-rad group. In addition, the spleen was sampled in the latter group and showed necrosis and hemopoietic cell debris at 1 day, disappearance of debris and hemopoietic cells at 3 days, and persistent atrophy thereafter.

In the study of six species of small aquarium fish given 2500 rads followed by weekly samplings, radiation effects were observed in hemopoietic organs only in the 7-day sample. Normal hemopoiesis was restored by 14 days, except for persistent thymic damage in the sampled Brachydanio (Figs. 5 and 6). These

F-1,2

F-3,4

F-5,6

findings seemed to indicate some unsolved dosimetry problems, so two more groups of fish were exposed, at two and three times the original dose. Tissues from these fish showed severe hemopoietic atrophy in kidney and spleen during the 3-week sampling period.

DISCUSSION

Table 2 lists various studies on the direct hemopoietic effects of radiation.

T-2

With the exception of Myxine and Petromyzon, all the fish listed in the table are teleosts (bony fishes); and in these, opinion is rather uniform that the head kidney is the chief site of hemopoiesis, showing early and rapid depletion of hemopoietic cells in proportion to the dose of radiation, with recovery from sublethal doses beginning in a few weeks. Depression of blood cells in the circulation reportedly lags somewhat behind the loss of cells in the irradiated head-kidney. The spleen and thymus likewise are radiosensitive sites of hemopoiesis, but they are thought to be relatively unimportant in the bony fishes. Radiation effects on hemopoietic organs in our experiments on several species of teleosts were similar to those previously reported in the literature.

The study using the hagfish (Myxine) and the lamprey (Petromyzon) is of special interest, because it shows the point in the vertebrate phylogenetic scale where hemopoietic cells change from very radioresistant (Myxine) to relatively radiosensitive (Petromyzon).²⁴

The temperature at which fish are held after irradiation is very important in determining the degree of hemopoietic damage. In general, cold is radioprotective,

but if it is too low (e.g. 4°C) it not only markedly slows the damage, but also prolongs or prevents the regenerative sequelae in irradiated cell populations.^{2,5,10,13,16,27,51}

Chemical protection studies are much used in higher vertebrates, but apparently few studies have been made of hemopoietic protection in fish. Egami¹⁴ and Egami and Etoh,¹⁵ using cysteamine and reserpine injections prior to irradiation, noted definite protective effects.

Administration of chronic or fractionated radiation doses is much less effective than acute irradiation in inducing hemopoietic damage, even though massive doses may be accumulated.^{5,10,16} In a study by Nakatani and Foster,³⁴ trout fed ^{90}Sr - ^{90}Y showed definite hemopoietic as well as growth and mortality effects proportional to dose.

Some studies indicate that fish with postirradiation hemopoietic damage are more susceptible to pathogens — e.g. Aeromonas or Gyrodactylus invasion in irradiated carp;^{50,55} however, irradiated plaice are still able to develop leucocytosis in response to bacteremia.³⁸ Depression of ^{59}Fe incorporation and of [^3H]thymidine uptake in irradiated kidneys of the tench (Tinca) are reported by Hevesy et al.²⁷ and Sletten et al.⁵¹

Table 3 lists 39 articles dealing with fish hematology and indicates the nature of the articles. There is a great deal of variation in the hemopoietic organs of different fish, related to their phylogenetic standing and anatomical and physiological peculiarities. As new species are used for research in hemopoietic radiobiology, their peculiarities will become apparent.

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TABLE 2

Studies on Hemopoietic Effects of Radiation in Fish*

Author(s)	Year	Ref. no.	Organism
Aoki	1963	2	<u>Carassius</u>
Aoki	1966	3	<u>Carassius</u>
Blaylock and Mitchell	1969	5	<u>Gambusia</u>
Bonham <u>et al.</u>	1948	6	Salmon fingerling
Egami	1969	13	<u>Oryzias</u>
Ellinger	1940	18	<u>Carassius</u>
Engel <u>et al.</u>	1966	19	<u>Lagodon</u>
Finstad <u>et al.</u>	1969	24	<u>Myxine, Petromyzon</u>
Hevesy <u>et al.</u>	1964	27	<u>Tinca</u>
Nakatani and Foster	1963	34	Rainbow trout
Preston	1959	38	Plaice
Ray	1970	40	<u>Campostoma</u>
Schechmeister <u>et al.</u>	1962	50	<u>Carassius</u>
Sletten <u>et al.</u>	1964	51	<u>Tinca</u>
Watson <u>et al.</u>	1963	55	<u>Carassius</u>
Welander <u>et al.</u>	1948	57	Salmon larvae
Welander <u>et al.</u>	1949	58	Rainbow trout

* Reviews and special articles are not included. Other articles are listed in the References.

TABLE 3
Articles on Fish Hematology

Author(s)	Year	Ref. no.	Fish species	Comment
Andrew	1959	1		Textbook
Babudieri	1930	4	50	
Boyar	1962	7	<u>Clupea</u>	
Bradley	1937	8	3	
Catton	1951	9	5	
Downey	1909	12	<u>Polyodon</u>	
Eisler	1965	17	15	
Fey	1963	20	<u>Carassius</u>	
Fey	1965	21	Several	Erythrocyte study
Fey	1966	22	Several	Granulocyte study
Fey	1966	23	Several	Other leucocytes
George	1941	25		Leucocyte study
Hafter	1952	26	<u>Astyanax</u>	Thymus changes with age
Hunn	1960	28		Bibliography
Jakowska	1956	29	11	Review
Jordan	1938	30		Review of phylogenetic levels
Kelenyi and Nemeth	1969	31	3	Eosinophils
Loewenthal	1928	32		Leucocytes
Loewenthal	1930	33	5	Leucocytes
Papermaster <u>et al.</u>	1964	35		Review of phylogenetic levels

(continued)

(Table 3, continued)

Patt and Patt	1969	36		Textbook
Piavis and Hiatt	1971	37	<u>Petromyzon</u>	
Rabalais	1938	39	3	
Reznikoff and Reznikoff	1936	41	<u>Mustelus</u>	Experimental
Romer	1962	42		Textbook
Sabnis and Rangnekar	1962	43	<u>Ophiocephalus</u>	
Saunders	1966-68	44-48	Many marine species	
Selye	1965	49		Mast cells
Slicher	1961	52	<u>Fundulus</u>	Experimental
Smith <u>et al.</u>	1967	53	<u>Lepomis</u>	
Weinreb	1958	56	Rainbow trout	Experimental
Werzberg	1911	59	16	Phylogenetic levels
Wintrobe	1933	60		Erythrocytes
Yoffey	1929	61		Spleen phylogenetic levels
Yokoyama	1960	62	<u>Perca</u>	

FIGURE LEGENDS

- Fig. 1. Kidney, unirradiated catfish. The intersitital tissue between the tubules is packed with nucleated hemopoietic cells.
- Fig. 2. Kidney, catfish 7 days after 2500 rads. The interstitial tissues is packed with nucleated erythrocytes, but there are very few hemopoietic cells.
- Fig. 3. Spleen, unirradiated catfish. The follicles are moderately cellular, and the sinusoids are packed with erythrocytes.
- Fig. 4. Spleen, catfish 7 days after 2500 rads. The follicles contain no lymphocytes and are dense and compact. The sinusoids are packed with erythrocytes.
- Fig. 5. Thymus, unirradiated Brachydanio rerio. The background stroma is obscured by dense collections of lymphocytes.
- Fig. 6. Thymus, Brachydanio rerio 7 days after 2496 rads. Most lymphocytes are gone and the stromal background is exposed.

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