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Effect of Cobalt-60 Irradiation on the Morphology of *Schistosoma Mansoni**

by

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For an understanding of the significance of ionizing radiation on *Schistosoma mansoni*, it is desirable to review briefly the life cycle and background of the disease produced by this parasite. *S. mansoni* produces a disease called schistosomiasis. In Africa and Arab countries it is better known as bilharziasis. The disease affects many vertebrate animals, including birds and mammals. Recent estimates have placed the number of persons infected with schistosomiasis between 150 to 200 millions.¹ Man may be affected by three different species of schistosoma worms which are found in different parts of the world: *Schistosoma haematobium* in Africa, and southern Asia, *S. japonicum* in China, Japan, Formosa and the Philippines, and *S. mansoni* in Africa, the West Indies and the more northern parts of South America. The parasite requires both a definitive host, such as man, in which the adult worms develop, and an intermediate host, a specific kind of snail, in which the larval form of the parasite develops. The adult form is a thread-like flatworm, between 10 and 25 mm. in length and 0.5 to 1.0 mm in width, depending on the species. The worms, sometimes called blood flukes, live in the blood vessels of the definitive host where the adult females lay eggs which eventually reach the tissue of the host.

When eggs from the body in the excreta of the host reach fresh water, they hatch into free-swimming forms known as miracidia, which penetrate a suitable snail. In the snail the miracidia reproduce many thousandfold. When they leave the snail, the larval forms are known as cercariae. These forms are also free-swimming, on or near the surface of fresh-water streams. They penetrate the skin of the adult host to reach the blood vessels. About 5 weeks after exposure, the adult female worms in the veins begin to deposit their eggs in the tissues, principally of the small intestine and liver. Some of the eggs are discharged with the feces, and reach water in which they hatch as miracidia. The miracidia then penetrate a suitable species of snail and the cycle is repeated.

The eggs are irritating and are deposited chiefly in the liver, intestines, or urinary bladder, but may also affect other vital organs such as the lungs and the brain. The eggs produce localized foci of damage,

¹McMullen, D., 1962. 27th Annual Chas. Franklin Craig Lecture, 11th meeting of Am. Soc. Trop. Med. & Hyg.

*Part of this study was carried out at The Puerto Rico Nuclear Center, University of Puerto Rico.

and are walled off at multiple sites. In man, if the infection in the liver is severe a cirrhosis or hardening of the liver results. The bladder involvement may produce severe urinary symptoms and lead to cancer of the bladder. Involvement of lungs may produce strain on the right side of the heart.^{2,3}

Irradiated cercariae have been used to induce resistance in mice and monkeys to subsequent experimental schistosomiasis.^{4,5,6,7} In order to induce such protection, the developing schistosomules (migrating cercarial form) must migrate some distance through the tissues of the host. Although some of the parasites may reach the portal-mesenteric circulation and develop to adult forms, they may be morphologically as well as physiologically rudimentary and produce few eggs or none at all.

The purpose of this paper is to report observations on: (1) the number of worms recovered from mice following their infection with irradiated cercariae, and (2) some gross morphologic changes occurring in the adult form of *S. mansoni* developed from gamma-irradiated cercariae.

Materials and Methods

Cercariae of *S. mansoni* were collected from pools of 50 or more snails of the Puerto Rican strain of *Australorbis glabratus*. The experimental animals consisted of 6 groups of 15 female CF1 mice weighing 18 to 20 grams and maintained with laboratory chow and water-bottles. Each mouse was inoculated percutaneously with 200 cercariae. Mice of group 1 were inoculated with non-irradiated cercariae; those of groups 2 to 5 were inoculated with cercariae which had been subjected to gamma radiation from Co⁶⁰ in doses ranging from 1000 to 3500 rad (radiation absorbed dose). The cercariae, while being irradiated, were suspended in 8 ml. of dechlorinated tap water in a plastic tube 50 mm. long by 21 mm. in diameter. The ir-

²Faust, E. C., and Russel, P. F., 1964. *Clinical Parasitology*. Phila., Pa., Lea & Febiger, pp. 530-566.

³Gould, S. E., 1962. Talk prepared for broadcast to Arab countries by Voice of America.

⁴Villella, J. B., Gomberg, H. J., and Gould, S. E., 1961. Immunization to *Schistosoma mansoni* in mice inoculated with radiated cercariae. *Science*, 134:1073-1074

⁵Hsu, H. F., Hsu, S. Y. Li, and Osborne, J. W., 1962. Immunization against *Schistosoma japonicum* in rhesus monkeys produced by irradiated cercariae. *Nature*, 194:98-99.

⁶Radke, M. G., and Sadun, E. H., 1963. Resistance produced in mice by exposure to irradiated *Schistosoma mansoni* cercariae. *Exper. Parasitol.*, 13: 134-142.

⁷Litchenberg, F. von, and Sadun, E. H., 1963. The parasite migration and host reaction in mice exposed to irradiated cercariae of *Schistosoma mansoni* *Exper. Parasitol.*, 13:256-265.

radiation was carried out in a 70-curie "gamma-cell" (cylindrical source) cobalt-60 unit which at the time was yielding 810 rad/min. in a reasonably uniform field. The radiation was measured by means of ferrous sulfate dosimeters, according to the method of Weiss⁸. From 12 to 16 weeks after inoculation with cercariae, the mice were killed by chloroform, after which their portal-mesenteric vessels were perfused by the technique of Moore *et al.*⁹ After perfusion, the liver and intestine of both experimental and control mice were cut into thin slices, pressed between glass slides, and searched for eggs with the aid of a dissecting microscope. The tissues were then ground in hypertonic saline, and the resulting homogenate was placed into a water-filled McMullen-Beaver side-arm flask to test for hatching of miracidia.

Schistosomes were collected and washed in a solution containing 1 percent sodium citrate and 0.85 percent sodium chloride. All of the recovered worms were relaxed at 4° C. for 12 to 15 hours, fixed in Bouin's solution, and stained with Delafield's hematoxylin. Measurements of the worms were made on a microscope fitted with a calibrated ocular micrometer. Some of the worms were used for morphologic studies.

Observations

The data in Table 1 shows the number of schistosomes recovered from mice which were alive 12 to 16 weeks after exposure to cercariae. In mice that were inoculated with cercariae irradiated at 1000 rad, no changes were noted in the adult worms that developed, but fewer worms were recovered than from the controls. Among animals that were inoculated with cercariae exposed to 3000 rad, all of the worms recovered were malformed, and the number of worms that developed was less than 3 percent of the number that matured among controls. Of the 15 mice that were inoculated with cercariae exposed to this level of radiation, only 2 yielded adult worms. The mice inoculated with cercariae which were exposed to 2000 or 2500 rad yielded 23 percent and 11 percent, respectively, of the number of worms obtained from the controls. Eggs were not found in the liver and intestine of mice exposed to cercariae irradiated with 2500 or 3000 rad, whereas considerable numbers were found among the controls.

The following morphologic changes in the worms occurred:

Retardation in growth: Examination of worms removed from mice between 12 and 16 weeks after inoculation with cercariae irradiated at levels from 2000 rad to 3000 rad showed retardation in their growth. Measurements of body length in Bouin-fixed specimens revealed an appreciable degree of stunting of worms of both sexes in comparison with non-irradiated controls.

⁸Weiss, J., 1952. Chemical dosimetry using ferrous and ceric sulfates. *Nucleonics*, 10:(7), 28-31.

⁹Moore, D. V., Yolles, T. K., and Meloney, H. E., 1945. A comparison of common laboratory animals as experimental hosts for *Schistosoma mansoni*. *J. Parasitol.*, 35:156-170

Ten male worms developing from cercariae exposed to 2500 rad and recovered 12 weeks after infection had an average length after fixation of 6.2 mm., whereas the average length of 10 fixed non-irradiated control worms was 10 mm. At 16 weeks, the average length of 10 male worms which had developed from cercariae exposed to 3000 rad was only 1.3 mm. At 15 weeks, the average length of 10 fixed specimens of female worms which had developed from cercariae exposed to 2000 rad was 7.4 mm. compared with 11.0 mm. for 10 fixed non-irradiated controls.

Malformation of the reproductive structures. Among 10 males developed from cercariae irradiated at 2000 rad, 8 had malformed testes, while another showed distal testicular displacement. At 12 to 16 weeks female worms developed from cercariae exposed to 2000 rad invariably showed one or more malformations. In a pair of worms recovered in copula from a mesenteric vein, the female lacked both gonad and accessory structures, while the testes of the male were rudimentary.

Among 5 female worms developed from cercariae exposed to 2500 rad, one or more reproductive structures were absent. Some female worms showed duplicate abortive ovarian development but no other structure of the reproductive complex. In another instance, examination revealed intestinal ceca and an empty ootype; the ovary, however, normally cradled between the intestinal crura, was apparently missing. Two of 5 females which had developed from cercariae exposed to 2500 rad displayed intestinal ceca but only a few scattered clusters of vitellaria.

Discussion

In the present study all live worms recovered after exposure to irradiated cercariae exhibited a drastic retardation in growth, especially at the higher radiation levels. Hsu *et al.*¹⁰, noted similar stunting effects in adult worms developed from irradiated *S. japonicum* cercariae, while Erickson and Caldwell¹¹ reported the recovery of 2 stunted worms in 1 of 15 mice exposed to *S. mansoni* cercariae irradiated with 4000 rad of gamma radiation. The above mentioned authors did not indicate the size of the irradiated worms. In this study, the average length of 10 male worms, which developed from cercariae irradiated with 3000 rad, was only 1.3 mm., whereas the average length of 10 non-irradiated males was 10.0 mm. One would expect 4000 rad, employed by Erickson, to produce considerably more stunting in males than I observed in the present experiments.

¹⁰Hsu, H. F., Hsu, S. Y. Li, and Osborne, J. W., 1962. Immunization against *Schistosoma japonicum* in rhesus monkeys produced by irradiated cercariae. *Nature*, 194:98-99.

¹¹Erickson, D. G., and Caldwell, W. L., 1965. Acquired resistance in rats after exposure to gamma-irradiated cercariae. *Am. J. Trop. Med. & Hyg.*, 14:566-573.

Body changes were seen more frequently in males developing from irradiated rather than from non-irradiated cercariae. In the present study, 5 of 10 males developing from cercariae irradiated with 2500 rad displayed granule-filled pouches, whereas only 1 of 286 non-irradiated males displayed a similar granule-filled pouch. It is of interest that pouches were not seen in either irradiated or non-irradiated female schistosomes. Standen¹², who in chemotherapeutic studies, observed similar malformations in the parenchyma of *S. mansoni*, suggested that the granular material within the affected area might be leucocytic congregations related to the process of phagocytosis.

Summary

Observations were made on morphologic changes occurring in adult *Schistosoma mansoni* which developed from cercariae exposed to gamma radiation from cobalt-60 at levels up to 3500 rad. No morphologic changes were evident in worms maturing from cercariae irradiated at 1000 rad, but adults of both sexes which developed from cercariae exposed to 2000 rad or more exhibited a variety of changes which include stunting and displacement or complete absence of reproductive structures.

Table 1

Number Of Schistosomes Recovered From Mice 12 To 16 Weeks After Percutaneous Exposure To 200 Cercariae Irradiated At Designated Doses

Group No.	No. of Mice Used	Radiation Dose To Cercariae (Rads)	No. of Worms	
			Mean	S. D.
1	12	0 (C)	46.3	19.02
2	12	1,000	27.4	8.81
3	14	2,000	11.0	4.86
4	13	2,500	5.2	3.67
5	15	3,000	1.4	1.18
6	11	3,500	0.0	

S. D., STANDARD DEVIATION.

C, CONTROLS. Worms from control mice were recovered 6 to 8 weeks after infection

¹²Standen, O. D., 1962. *Bilharziasis*. Boston, Mass., Little, Brown and Co., pp. 266-268.