Effect of Cobalt^{60} Gamma Radiation on Susceptibility and Immunity to Trichinosis.* (19674)

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Several sources of radiation such as radium (1), X-rays(2) and radioactive cobalt(3) have been used to study the lethal action of ionizing radiation upon the isolated larvae of *Trichinella spiralis* and the encysted larvae in trichinous meat. Research along this line has been directed largely towards a possible application of radiation to destroy the larvae in infected pork. Semrad(2) gave 1,000 to 2,250 r (roentgens) in daily exposures of 250 r to rats infected with Trichinella larvae 1 day prior to the initial exposure to X-radiation. He reported a reduction in the number of larvae produced in irradiated rats. There have been no reports on the effect of radiation upon susceptibility and immunity to infection with *Trichinella spiralis*. It is the purpose of this paper to report an increase in susceptibility and the destruction of active immunity to Trichinella infection in mice following cobalt^{60} gamma irradiation.

Materials and methods. The gamma radiation source was composed of 21 pieces of cobalt^{60} arranged to give a uniform field at 12 inches. The intensity of the radiation at the present time is approximately 600 rep (roentgen equivalent physical) per hour and the LD_{50} for 28-day-old mice 750 (rep). All gamma radiation referred to in these experiments are whole-body exposures. Swiss mice from our own colony were used in the present study. These mice are free of Salmonella and internal metazoan parasites. The Trichinella larvae used for test infections were isolated by pepsin digest from stock mice with infections of 6 weeks duration. Known infective doses were determined by the dilution count method of McCoy(4) and concentrated in conical centrifuge tubes. Using a rubber bulb, the larvae were drawn with about 1 cc of tap water into a tapered, small bore pipette. While under ether anesthesia, the infective dose was administered directly into the stomach by passage of the pipette down the esophagus. All mice received test infections on a larvae per gram of body weight (lpg) basis. The infective doses were given within a 1- to 3-hour post-irradiation period.

Experimental and results. Several experiments were carried out to determine the effect of sublethal irradiation of the host upon susceptibility to Trichinella infection. The results of sublethal radiation upon 25 and 50 (lpg) infections in Exp. 1 and 2 are presented in Table I. Sublethal gamma exposure resulted in a significant increase in susceptibility of 4-week-old mice infected with 25 (lpg). With a 50 (lpg) infection, sublethal radiation increased the susceptibility to Trichinella infection by 63%. Exp. 3, Table I, shows the effect of administering 650 (rep) exposure to mice given a 50 (lpg) infection. This level of radiation increased the susceptibility to a 50 (lpg) infection by over 85%. These data clearly indicate that gamma radiation of 550 to 650 (rep) increased the susceptibility to Trichinella infections in mice.

A high degree of acquired immunity to a challenge infection of *T. spiralis* may be demonstrated in mice actively immunized with a sublethal active infection. Immunity to reinfection with Trichinella was first reported by Ducas(5). An experiment was designed to test the effect of 600 (rep) gamma radiation on the ability of Trichinella-immune mice to withstand a challenge dose. Sublethal active infections of 100 larvae (8 lpg) were given to 4-week-old mice as an immunizing infection. A description of the experimental groups and the per cent survival is summarized in Table II. The challenge dose of 1,500 larvae (75 lpg) was administered 3 weeks following the initial immunizing infection. In the group of

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TABLE I. Effects of Gamma Radiation from a Cobalt^{60} Source upon Susceptibility of Mice to *Trichinella spiralis* Infection.

Exp.	Group description	Gamma radiation rep.	No. mice	No. died	% died
I	Irradiated control	580	35	0	0
	" and infected	580	35	9	25.7
	Infected control	(No radiation)	35	0	0
II	Irradiated control	550	38	0	0
	" and infected	550	40	32	80
	Infected control	(No radiation)	42	7	16.6
III	Irradiated control	650	30	4	13.3
	" and infected	650	33	33	100
	Infected control	(No radiation)	32	0	0

Exp. I—Infective dose 300 Trichinella larvae (25 lpg). Exp. I and II—Infective dose 625 Trichinella larvae (50 lpg).

TABLE II. Effect of Gamma Radiation from a Cobalt^{60} Source upon Immunity to *Trichinella spiralis* Infection in Mice.

Group description	Gamma radiation rep.	No. mice	No. died	% survival
Irradiated control (no immunization)	600	40	1	97.5
Trichinella challenge infection (no immunization)	None	39	39	0
Trichinella challenge infection (immunized)*	"	34	9	74
Irradiated and Trichinella challenge infection (immunized)*	600	37	37	0

* Immunized with an active infection 100 *Trichinella spiralis* larvae; challenge infection 1500 larvae (75 lpg).

irradiated control animals, which received 600 (rep), 1 mouse died and 97.5% survived. All the mice died in the non-immunized Trichinella challenge group. The challenge dose thus constituted an LD_{100}. Seventy-four per cent of the non-irradiated, Trichinella-immune controls survived the challenging infection, while in the last group which were immunized, irradiated and challenged there were no survivors, showing that this amount of radiation is sufficient to destroy the active immunity produced by a previous infection with 100 *T. spiralis* larvae.

Acquired immunity to Trichinella infections has been reported to be localized in the intestines by Ducas(5) and McCoy(6). Culbertson and Kaplan(7) reported immunity to Trichinosis as a generalized response, the antibody being directed against the larvae which are developing into sexually mature adults in the intestine. There appeared to be no evidence of cellular reaction in the intestinal wall of immune rats(6). We have found that gamma radiation does not reduce the level of influenza virus neutralizing antibody present at the time of irradiation. We have also determined that cobalt^{60} radiation does not decrease either the amount of or the effectiveness of tetanus antitoxin in mice present at the time of irradiation(8). Precipitin tests of serum from irradiated Trichinella-immune mice and serum from non-irradiated immune mice exhibited an equal level in the ability to precipitate dilutions of precipitinogen prepared from Trichinella larvae, indicating that irradiation does not decrease the precipitins for Trichinella larvae which are present at the time of radiation. The effects of 680 (rep) gamma radiation upon total leukocytes per cu mm of peripheral blood are shown in Fig. 1. Each point on the curve represents the average total white cell count for 5 mice drawn at random from 2 groups of 80 mice. The sharp reduction in the total number of white cells in the circulating blood following gamma radiation represents a serious

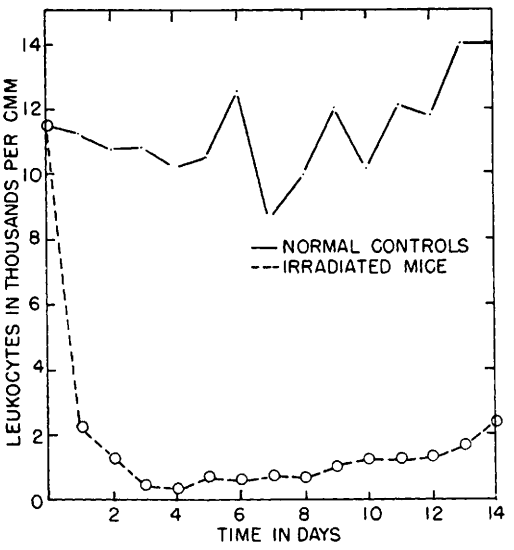

FIG. 1. Effect of gamma radiation 680 (rep.) upon level of leukocytes in peripheral blood of mice.

loss to the cellular defensive mechanism of an irradiated animal. It is indicated from these data that granular leukocytes and the lymphoid-macrophage system may be of greater importance in immunity to Trichinella infections than has formerly been considered.

Summary. 1. Cobalt^{60} gamma whole body irradiation of 550 to 650 (rep) increased the susceptibility of Swiss mice to infections with *Trichinella spiralis*. 2. Exposure of Trichinella-immune mice to 600 (rep) before administering a challenge infection destroyed their immunity to reinfection. 3. Data are presented on the effects of 680 (rep) radiation upon the total leukocyte count in the peripheral blood of mice. The sharp reduction in the number of circulating leukocytes may indicate that the role of cellular immunity should be emphasized more in acquired immunity to infections with *Trichinella spiralis*.

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Immunization of Rabbits with Type II Pneumococcal Polysaccharide.* (19675)

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The specific soluble substances of the pneumococci have been reported to be antigenic in man, mouse, horse, dog and cat(1), but all attempts to immunize rabbits with the type-specific polysaccharides have failed. For example, Avery and Morgan(2), Schiemann(3), and Avery and Goebel(4) reported attempts to immunize rabbits with pneumococcal polysaccharides, but they failed to detect type-specific precipitins, agglutinins, or protective antibodies in the sera of the treated rabbits. Schiemann(3) employed 1.0 to 1.5 mg of type II polysaccharide; Avery and Goebel(4) used as much as 18 mg and as little as 180 γ of type I pneumococcal polysaccharide in experiments to immunize rabbits. Although they failed to find type-specific antibodies, Avery and Goebel(4) were able to detect type I polysaccharide in the sera of their treated rabbits. It appeared likely that these investigators had employed "paralyzing" doses of the

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