Radiation Effects on Pneumococcal Infection Produced by Subcutaneous Injections into White Mice.* (21118)

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X-irradiation in doses of 400 to 600 r has been shown to produce a fatal bacteremia in white mice(1,2). This is apparently due to an impairment of the blood clearing mechanisms which normally destroy bacteria that occasionally pass through the intestinal wall. A recent review of the physiological changes brought about by irradiation has been made by Taliaferro(3). It has also been shown that irradiation decreases the resistance of animals to experimental(4-9) and natural infections(10,11), and that resistance is related to the post-irradiation time of the infection. The relation of this to changes observed in the blood and certain organs of irradiated animals is not entirely clear(5,8).

One approach to the evaluation of the effect of irradiation on the local defenses, as well as on the systemic defenses, is the determination of the time required for bacteria to reach the blood stream from a subcutaneous injection. Furthermore, this procedure simulates conditions accompanying an atomic blast, in which infectious agents could be carried through the skin with flying debris, and may indicate whether an increase or decrease in the severity of such infections could be expected. For this purpose the experiments reported in this paper were designed.

Methods. Female white mice, 6 to 11 weeks old, average weight 20 g, obtained from the Rockland Farms, were used. They were observed for at least 5 days after receiving, in order to have all test mice in a good nutritional state and free from disease. Small groups of 10 to 20 mice were injected subcutaneously with 0.2 ml of a suspension of Pneumococcus Type III made in pH 7 buffer from a 15- to 18-hour growth on a blood agar slant. The turbidity read 55 on a Klett Summerson colorimeter and the suspension contained approximately 2,000,000 bacteria per ml. The dosage used had been found to kill most of the mice in a 72-hour period. One-half of each group were subjected to total body x-irradiation in a single exposure delivered for 19 minutes at 220 Kvp, 15 ma., at a distance of 85 cm using ½ mm copper and one mm aluminum filters. This dosage was 350 r delivered on the skin as measured by a Victoreen r meter. Twelve mice at a time were rotated slowly through the field during exposure. The apparatus used for this purpose was essentially the same as that described by Miller *et al.*(1). This dose was sublethal for mice as determined by many other investigators and reaffirmed in our laboratory. Twice this dosage was 100% lethal. The injection of organisms was made with different groups of mice at zero time, 3 days and 6 days after irradiation. In order to study the time from injection to the appearance of bacteria in the blood, and also the time from bacteremia to death, tails were clipped and 3 drops of blood from each inoculated onto blood agar plates at intervals of 15 minutes for the first hour, at 2-hour intervals for the first 8 hours, and at 6- to 12-hour intervals thereafter until death. Plates were incubated for 48 hours and examined both grossly and by staining smears from each colony. Each mouse was marked so it could be studied individually. The pneumococcus was used because it produced a readily identifiable bacteremia in mice.

Results. Injection given immediately after irradiation. Each of 46 mice were studied individually in groups of 10 to 12 as outlined above. One-half of the group were irradiated and the other half served as controls. All were injected with pneumococci immediately

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TABLE I. Effect of Irradiation Given 3 and 6 Days before Infection of Mice with Pneumococci.

	% with bacteremia					% dead after bacteremia			
	I		II			III		IV	
	3 days		6 days			3 days		6 days	
Hr	Non-irrad. (23)	Irrad. (23)	Non-irrad. (28)	Irrad. (27)	Hr	Non-irrad. (23)	Irrad. (23)	Non-irrad. (28)	Irrad. (27)
1	13	43	19	15	10*	9	13	3.5	18.5
4	35	52	19	15	15	17	17	7	44.4
8	43	65	22	15	20	30	35	11	52
24	65	95	42	37	25	65	70	38	63
48	87	100	56	73	30	87	87	61	85

No. in parentheses are total animals used.
* Refers to the hours from the time bacteremia was established until death.

after irradiation. There were rather wide variations within both groups in the time of death, and the time that bacteria appeared in the blood. However, the time from the appearance of an extensive bacteremia until death occurred was quite constant (about 25 hours) in both the control and irradiated groups. The time from injection to the appearance of either a few or many bacteria in the blood was about the same in the irradiated as in the control mice.

Injection given 3 days after irradiation. A total of 60 mice in 5 groups of 12 each were studied as before, but the injection was given 3 days after irradiation of one-half of the group. Again it was found that time from inoculation to death and to the finding of bacteria in the blood varied widely in individual mice, while the time from extensive bacteremia to death was more constant. A greater percentage of the irradiated mice were dead at any given time than were the controls. Table I, which includes only a portion of the observations made, indicates that irradiation decreases the time for inoculation of the organisms under the skin until they appear in the blood in large numbers. More of the irradiated mice showed extensive bacteremia at any given time than did the non-irradiated. The statistical assurance for a real, rather than apparent, reduction in the time to extensive bacteremia was afforded by using the one-sided Wilcoxon test(12). (This test was utilized since its validity is not dependent upon the form of the distribution.) A value, $P = 0.012$, was found for the data in Column I. Column III indicates that the time from extensive bacteremia to death was little influenced by irradiation given 3 days before infection.

Injection given 6 days after irradiation. Fifty-five mice were studied under these conditions. They were divided into 4 groups. One-half of each group had been irradiated 6 days before infection. Column II, of Table I, shows that irradiation given 6 days before inoculation had little effect on the time for bacteremia to occur. The most striking difference is shown by the values in Column IV. The time from bacteremia to death was significantly decreased in the irradiated mice ($P < 0.005$ by the test noted above). Fifty-two per cent of the irradiated mice died in 20 hours or less after an extensive bacteremia was observed. Only 11% of the controls died within this time period. At almost any given time after bacteremia was established there was a considerably greater percentage of the irradiated mice dead.

Discussion. Irradiation at the time of infection had little effect on the susceptibility of mice to the pneumococcal infection. However, it increased their susceptibility when the infection was produced 3 and 6 days after irradiation. Similar observations have been made with other bacteria introduced into mice by other routes(5,8). At 3 days, pneumococci reached the blood stream in a shorter time in the irradiated animals, but the time from bacteremia to death remained the same. This could mean that at this period the local defenses are lowered, but the blood clearing mechanisms have not been effected. Six days after irradiation there was no difference in the time required for the establishment of bacteremia, but the time from bacteremia to

death was decreased in the irradiated animals. This indicates possible repair of the local defenses, but an impairment of the bactericidal and/or filtering mechanisms of the blood. It is recognized that the irradiated animals may have had less ability to withstand the bacteremia at this time because of other changes induced by the irradiation.

Irradiation 48 hours prior to inoculation has been reported to damage lymphatics so that bacteria reached the blood stream more rapidly(8). The induced bacteremia was no more lethal in the irradiated animals. This agrees with our results, at 3 days, with a more fulminating infection. The increased lethality of the bacteremia at 6 days may possibly be explained by recent reports of a lowered bactericidal activity of the blood in rabbits(13) and rats(14), 6 days after irradiation.

The present data imply altered responses of resistance to infection as a function of post-irradiation time. However, the type of animal, bacteria, and irradiation involved, as well as the dose used, may materially alter the character of the phenomena—at least the temporal relationship.

Summary. Whole body x-irradiation of white mice with 350 r influenced an infection with pneumococci induced by subcutaneous inoculation as follows: 1. Little or no effect was observed when radiation exposure was given at the same time as the inoculation of the bacteria. 2. The time from inoculation to death, and the time required for bacteremia to become established, was reduced when radiation exposure was given 3 days prior to inoculation. The time from bacteremia to death was not changed. 3. The time from inoculation to death, and the time from bacteremia to death, was reduced when radiation exposure was given 6 days prior to inoculation. The time required for bacteremia to become established was not changed.

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