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Sensitivity of Irradiated Mice to Bacterial Endotoxin. (28489)

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In the course of studies on the mobilization of granulocytes with bacterial endotoxin it was observed that extremely small doses given on the third day after lethal irradiation killed mice that would otherwise have lived for several additional days(1). Greatly increased sensitivity to endotoxin has been reported in a number of circumstances, notably adrenalectomy(2), reticuloendothelial blockade(3), vaccination with BCG(4) serial zymosan injections(5), and elevated ambient temperature(6). The mechanisms of endotoxin hypersensitivity are not well understood. None of the suggested possibilities would have led us to anticipate the effect observed in irradiated mice.

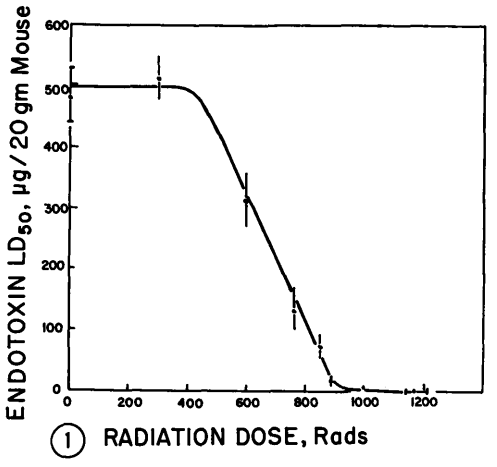
In the present studies we attempt to quantify and localize the response with respect to *a*, radiation dose; *b*, time after irradiation, and *c*, organ or system responsible for the hypersensitivity reaction.

Methods. The mice used were (BALB/c $\times$ DBA/2)F_1 females 12 to 20 weeks old, maintained during the experiment in individual cages. The temperature in the animal room ranged from 76 to 80°F. Except where shielding was involved, irradiation was from a Co^{60} source. In the shielding experiments radiation was delivered from 2 opposing Westinghouse Quadraconex tubes operating at 200 KV, 15 ma, and filtered with 0.25 mm Cu and 1 mm Al. Lead shields were ⅛ inch thick. A preparation of *S. typhosa* endotoxin* was used, injected intraperitoneally. The end point used was death in less than 72 hours after challenge. LD_{50} values, with 95% fiducial limits, were estimated by probit analysis and are given as μg per 20 g mouse.

Results. 1. *Effect of radiation dose on endotoxin LD_{50} 3 days after irradiation.* Counting the animals that died in less than 72 hours after challenge, the LD_{50} of the endotoxin preparation in control mice was 480 (440-530) μg per 20 g mouse. When the challenge was given on the third day after irradiation the endotoxin LD_{50} values shown in Fig. 1 were observed. There was no evidence of increased sensitivity following an exposure of 300 rads but after 600 rads the endotoxin LD_{50} dropped to 310 μg and to 80 μg after 820 rads. Between 400 and 900 rads the endotoxin LD_{50} decreased at the rate of about 100 μg per 100 rads. After a dose of 1000 rads the endotoxin LD_{50} was estimated to be 0.5 (0.3-1.1) μg per 20 g mouse. The radiation LD_{50} for death during the same interval, (*i.e.*, 6 days after irradiation) was 1170 (1140-1210) rads in mice given no endotoxin. This value is plotted in Fig. 1 to correspond with an endotoxin LD_{50} of zero.

2. *Effect of time of irradiation on endotoxin LD_{50}.* In our original mobilization experiments it was noted that sensitivity to endotoxin was greater 3 days than 2 days after irradiation(1). Using doses of 850 and 890 rads the effect on endotoxin toxicity was studied for challenges made 2, 4 or 5 days as compared to 3 days after irradiation. Results of these experiments are shown in Fig. 2. The third day was clearly the most criti-

* Prepared and generously supplied by Difco Laboratories, Detroit, Mich.



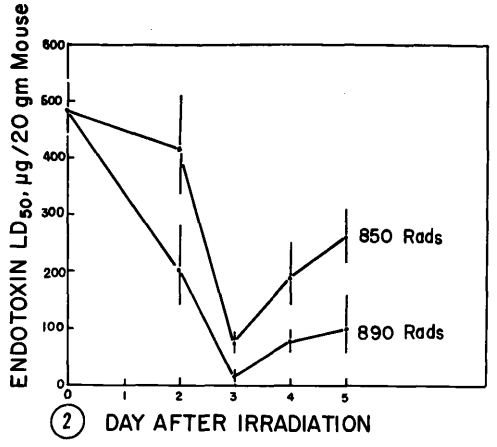


FIG. 1. Locus of treatment combinations leading to 50% mortality, with endotoxin given 3 days after irradiation.

FIG. 2. Effect of time of administration after irradiation on endotoxin LD_{50}.

cal for the hypersensitivity. Not only was the radiation effect on sensitivity relatively mild on day 2 but significant recovery was observed for days 4 and 5. Results of challenges given later than that would be obscured by radiation deaths.

3. *Effects of partial shielding on endotoxin LD_{50}.* Lead shields were applied as shown in Fig. 3 to mice anesthetized with nembutal, which were then exposed to 850 r 200 KV X-irradiation.† On the third day these mice, along with non-shielded irradiation controls, were challenged with appropriate doses of endotoxin. For non-shielded mice the endotoxin LD_{50} was 2.3 (1.3-4.3) µg per 20 g mouse. When the hind legs were shielded (*A*) LD_{50} remained essentially unchanged (2.4; 1.7-3.3). When the back was shielded as in *B* LD_{50} increased to 55 (35-90) µg. When the front of the animal was shielded but the back irradiated as in *C*, LD_{50} returned to 415 (360-475) µg, almost the normal value. When a shield 1/5 the size was placed on the abdomen as shown in *D* the endotoxin LD_{50} was 165 (90-290) µg, roughly 70 times the value observed when the hind legs were shielded.

4. *Effect of cortisone on endotoxin LD_{50} in irradiated mice.* Leg-shielded mice exposed to 850 r 200 KV X-irradiation were given an intramuscular injection of 2.5 mg of cortisone one day and again 2 hours before endotoxin challenge. With challenging doses of 20-40 µg per 20 g given 3 days after irradiation, 14 of 17 mice survived. This was essentially the same proportion observed in back-shielded groups given no cortisone (12 of 16 mice). In the same experiment a dose of 2.2 µg was survived by only one of 5 leg-shielded mice given no cortisone. Thus, both cortisone treatment and back shielding reduced to some extent the radiation-induced sensitivity to endotoxin.

Discussion. The shape of the curve in Fig. 1 differs from that usually found in studies of the joint effects of 2 or more toxic agents. In the case of 2 agents which contain the same toxic ingredient, but in different concentrations, the various combinations leading to a 50% kill will necessarily fall (except for experimental error) on the single straight

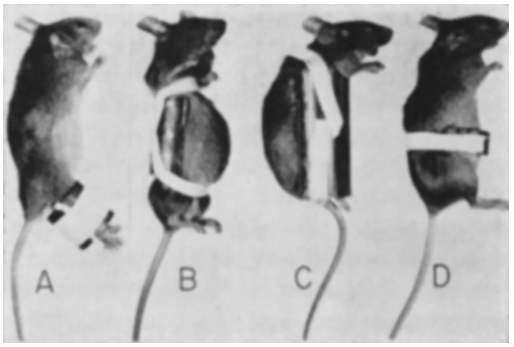


FIG. 3. Placement of lead shields for 850 r, 200 KV X-irradiation.

† Roughly equivalent to 1000 rads Co_{60} γ irradiation.

line obtained by connecting the point determined by the LD_{50} for agent *1* in the absence of agent *2* and that determined by the LD_{50} for agent *2* in the absence of agent *1*. Agents which show this behavior are called additive whether or not they contain the same toxic ingredient(7). Agents which are more than additive (potentiating) lead to 50% lethal treatment combinations which fall below the characteristic straight line. Agents which are less than additive (either synergistically or antagonistically) lead to treatment combinations which fall above the straight line.

The relationship for endotoxin and radiation is more complex. Below 400 rads the 2 agents are less than additive; between 400 and 900 rads they are additive; and above 900 rads they are more than additive. This suggests that the way endotoxin kills below 400 rads is qualitatively different from the way it kills above 900 rads. In fact, there appears to be a threshold at 400 rads below which radiation makes no contribution to the combined treatment.

Between 400 and 900 rads the curve behaves as if one treatment were dose-increasing with respect to the other. One might suspect depressed reticuloendothelial function as a cause of this, for there is much evidence showing that interference with normal RE function leaves the animal vulnerable to the toxic action of endotoxin. Benacerraf and his associates found carbon clearance in the mouse to be depressed a significant, though not spectacular amount, 2 days after 800-1200 r(8), but reported no tests for day 3, when endotoxin sensitivity is greatest. An explanation of increased sensitivity based largely upon depression of RE function does not appear to be in accord with the observation that considerable protection is afforded by shielding a small portion of the abdomen, but not by shielding the hind legs.

It has been amply demonstrated that adrenal function is involved both in normal sensitivity to endotoxin and in the hypersensitivity induced by BCG vaccination or zymosan injections(5,8,9). The extent to which irradiation affects adrenal function remains unclear (*e.g.*, 10-12). There is evidence for transistory adrenal hyperactivity following irradiation(12), and endotoxin LD_{50} values for low radiation doses may very well include a component of increased resistance resulting from this. Irradiation in the lethal range did not leave the mouse excessively sensitive to KCl or histamine(12). Nevertheless, since shielding the back afforded a certain amount of protection it seems likely that impairment of adrenal function was responsible for part of the increase in sensitivity to endotoxin. It should be emphasized, however, that when the adrenals were irradiated and the abdomen shielded there was little if any increase in sensitivity. The observation that cortisone treatment affords some protection conforms with what is known of its effect in normal mice treated with endotoxin(5,8,9).

An alternative hypothesis is that the intestinal tract damaged by radiation is exceedingly sensitive to endotoxin. This would be in accord with the independence of endotoxin sensitivity and radiation at low doses of radiation and the almost complete protection which was afforded by abdominal shielding. Although a remarkable amount of protection was obtained with the small abdominal shield of Fig. 3, D, comparable protection against death from acute intestinal radiation damage was observed in the rat by Swift and Taketa when a small segment of duodenum or ileum was shielded(13). The shape of the curve in Fig. 1 suggests that endotoxin has a potentiating effect at radiation doses above 900 rads as intestinal damage becomes a critical factor in survival. It seems likely also, that hypersensitivity related to the intestinal tract is responsible for the 3-day time of maximum sensitivity, although this would depend upon the manner in which the reaction would cause the animal to die.

It might be conjectured that absorption of bacterial endotoxins from the intestinal lumen influences the time of the so-called intestinal death following high doses of radiation. Therapy which included antibiotics prolonged survival in dogs(14), and in rats (15), having the gastrointestinal syndrome. Germ-free mice with this syndrome live a day or two longer than conventional mice(16,17). Demonstration of endotoxin in body fluids

at the time of acute intestinal damage is, of course, needed to give substance to this conjecture. The present studies also reopen the question of the extent to which endotoxins may contribute to death from the Gram-negative infections which occur during the first few weeks after irradiation.

Summary. Exposure to radiation, especially in the lethal range, caused a marked increase in sensitivity of mice to *S. typhosa* endotoxin. This was most pronounced on the third day after irradiation. At that time the endotoxin LD_{50} in mice given 1000 rads was 0.5 μg compared to a control value of 480 μg per 20 g mouse. Shielding the hind legs was without effect on endotoxin LD_{50} (2.3 μg for 850 r, 200 KV X-rays). Shielding the back, including the adrenals, was partially effective (LD_{50}, 55 μg). Treatment with cortisone had an effect comparable to that of shielding the back. Shielding the front of the animal, including practically all of the intestinal tract, was very effective in spite of the fact that the adrenals were irradiated (LD_{50}, 410 μg). Shielding a small portion of the abdomen was also quite effective in reducing endotoxin sensitivity in the irradiated mice (LD_{50}, 165 μg).

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Detoxification of Endotoxin by Splenic Extracts.* (28490)

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Bacterial endotoxin is extracted from the circulation by the reticuloendothelial system (1). This system also degrades endotoxin. Experiments reported previously demonstrated that *in vivo* perfusion of the normal dog's spleen with P^{32} labelled endotoxin results in almost immediate extraction and detoxification, with simultaneous release of dialyzable P^{32}(2). Later experiments demonstrated that detoxification also occurs within a few minutes on exposure of endotoxin to saline extracts of fresh spleen from the normal dog(3). Similar extracts of fresh liver from the normal dog are about one-fifth as potent as those from spleen, whereas extracts of kidney, heart muscle and skeletal muscle are inactive. Splenic extracts from animals exposed for some hours to traumatic shock are also inactive. Further data on

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