Role of Host Defenses in Streptomycin Treatment of X-Irradiated Mice.* (20337)

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The complementing action of natural defenses in the control of various infections with sulfonamides and antibiotics has been demonstrated in several ways. Wood and his colleagues(1) showed the importance of surface phagocytosis in sulfonamide treatment of pneumonia; Skinsness and Woolridge(2) found the effectiveness of penicillin against pneumococcal infections to be reduced in protein-depleted rats; and Kaplan and his associates(3) showed aureomycin to be relatively less effective against β hemolytic streptococcal infection in irradiated than in non-irradiated mice.

In view of these and related findings one may predict that the effectiveness of prophylactic antibiotic treatment after whole-body X-irradiation will depend to a considerable degree upon the residual level of the severely injured host defenses. The probability that such is the case is apparent in our results with streptomycin treatment of natural and experimental infections in irradiated mice(4-6), where deficiencies in streptomycin effectiveness were not adequately explained by the drug-resistance of invading or injected organisms or by inadequacies in dose or duration of treatment. Jacobson(7) and Lorenz(8) demonstrated the increased hematopoietic recovery and increased survival which results from spleen transplants or bone marrow injection after irradiation. Cole and his associates(9) obtained similar results with the administration of spleen homogenate from immature mice. Our group has demonstrated a close correlation between the degree of leucocyte recovery, with and without the injection of spleen homogenate, and resistance to experimental infections(10). Results with streptomycin treatment of experimental infection in irradiated mice whose recovery is accelerated by the administration of spleen homogenate are reported here.

Procedures. Male mice of the N.I.H. strain were caged individually and distributed by litter into treatment and irradiation groups as previously described(4). Each irradiated infected control group contained 20 mice and each of the other groups 36 to 40 mice. At 12 weeks of age they were given 475r (LD about 5%) with an X-ray unit operated at 200 KV and 20 ma with 0.25 mm Cu and 0.51 mm Al added filtration. The target distance was 50 cm. Spleen homogenates were prepared by a method similar to that of Cole *et al.*(9). Spleens from decapitated 2- to 3-week-old mice were removed, freed from adhering adipose tissue, placed in Earle's buffer(11) and ground, in batches of about 30, in a Potter-Elvehjem type homogenizer. More buffer was added to give a final dilution equivalent to 2 spleens per ml. The suspension was allowed to stand a few minutes until large clots appeared and was then filtered twice through thin layers of glass wool. Within a few hours after irradiation each mouse was given a single injection of 0.5 ml into a tail vein through a No. 24 needle. Mice from each treatment group were given the injections in rotation to minimize variations in spleen preparation and in time of injection. *Proteus Kf7* (Kaufmann), an organism foreign to our mice, was used for infection. A lyophilized culture was inoculated into horse meat infusion broth and incubated at 37°C for 24 hours. A 24-hour subculture was centrifuged and the organism resuspended in sterile saline to give a 1:10 dilution of the subculture for immediate injection. Each mouse was given 0.2 ml subcutaneously in the region of the groin. Injections of 1.25 mg streptomycin sulfate in 0.2 ml were given under the dorsal skin daily beginning 5 hours after the injection of *Proteus*. This dose was

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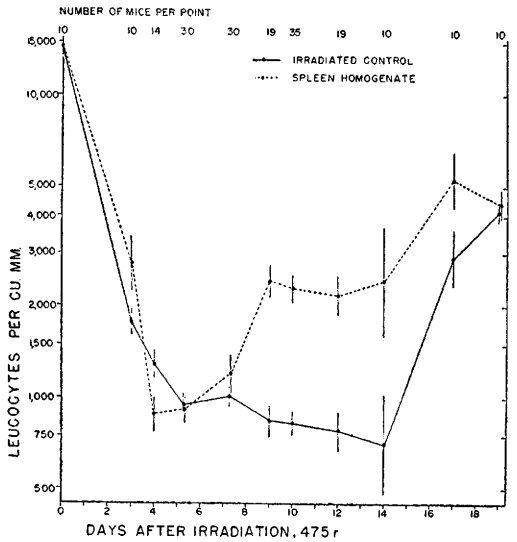

FIG. 1. Geometric means and stand. errors of leucocyte counts with and without inj. of spleen homogenate within a few hr after irradiation.

half of that previously found to be approximately optimal for the treatment of experimental *Proteus* infection(6). Leucocyte counts were made from tail blood. The values are shown as geometric means with standard errors and are derived from 5 experiments in which irradiation and the injection of spleen homogenate were essentially the same as in the experiment reported in detail here.

Results. A composite graph showing the effect of spleen homogenate on leucocyte count is given in Fig. 1. The start of recovery is usually apparent by the 7th day, while in the irradiated controls the counts usually remain below 1000 per cu mm for 14 days.

Survival curves following inoculation with *Proteus* are shown in Fig. 2. The 4 groups in the first block had been irradiated 5 days previously and parallel groups in the second and third blocks 7 and 10 days prior to infection. Most of the deaths occurred in less than 2 days after infection. Of 19 non-irradiated mice 2 died following the *Proteus* inoculation. In each untreated irradiated group 90 to 100% died after infection. In the spleen-treated group that was infected on the 5th day there was little improvement in survival, but with infection on the 7th or 10th day almost half of the mice survived. Similarly, results with streptomycin were better in mice infected 7 or 10 days after irradiation.

Although the injection of spleen homogenate caused no perceptible increase either in leucocyte count or in resistance to infection by the 5th day after irradiation, it had, at that time, caused considerable improvement in the efficacy of streptomycin treatment, increasing survival from 8% to 47%. The leucocyte count on the 7th day was not much higher in spleen-treated than in non-treated mice, but it was increasing rapidly and the efficacy of streptomycin in the spleen-treated group at that time, as well as 3 days later, was improved from a level of about 45% to approximately 75%.

Discussion. From the studies carried out in Miller's laboratory(12) and in our own (4,5) it is clear that, in mice, infection is a determining factor in postirradiation death or survival. Further experiments(10) strongly suggest that in this species the efficacy of post-irradiation injection with spleen homogenate is largely dependent upon its action in restoring defenses against bacterial invasion. Although such treatment accelerates recovery of all hemopoietic elements, phagocytosis stands out as the logical factor of critical importance in the experimental conditions employed here. By giving sub-lethal irradiation and injecting the organism subcutaneously, intestinal epithelial barriers were by-passed; when infection was given on day 5 or 7 the experiment was essentially complete before anemia developed; and by injecting an organism foreign to our mice which kills in less than 48 hours, the possible contribution of specific antibodies was almost certainly excluded. The extent to which preformed nonspecific antibodies may influence resistance to infection under the conditions of these experiments is not known.

The results here demonstrate a dependence of streptomycin therapy upon the level of host defenses, both in natural recovery and in recovery under the influence of injected spleen homogenate. The extent of this dependence is particularly critical for the irradiated mouse during the first 2 weeks when the leucocyte count is very low and antibody formation has ceased. This depleted state of the host defenses may reasonably account for many of the undramatic results frequently observed

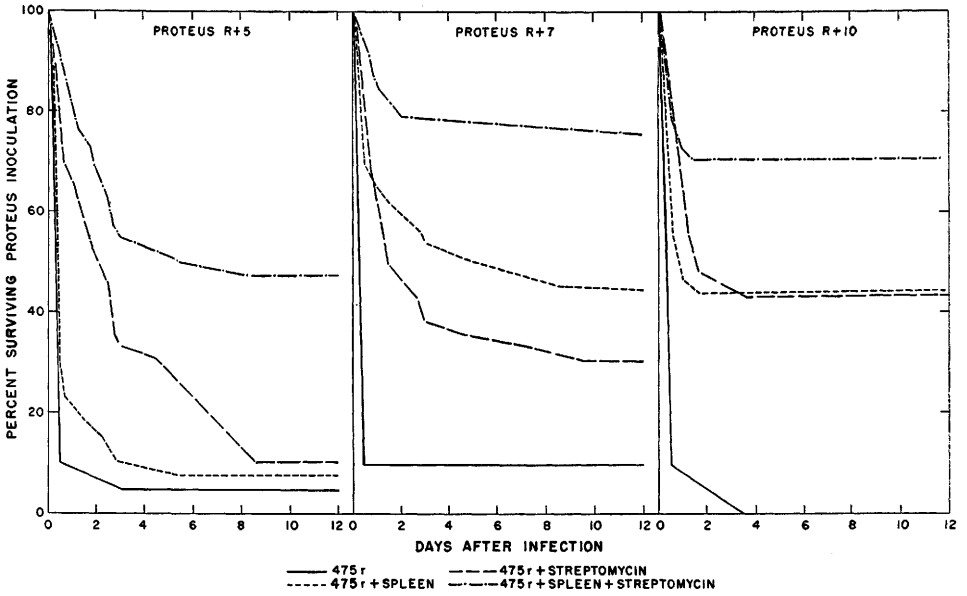

FIG. 2. Cumulative survival curves following subcutaneous inj. of *Proteus Kf7* 5, 7, and 10 days after irradiation.

with antibiotic therapy after whole-body X-irradiation(4,12).

Summary. The intravenous injection of a homogenate of immature spleen increases the resistance of sub-lethally irradiated mice to *Proteus* infection and significantly complements the action of streptomycin.

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