Effect of Daily Exposure to 15 r γ Radiation on Susceptibility of Mice to Experimental Infection.* (25800)

CAROLYN W. HAMMOND, SONIA K. ANDERLE AND C. PHILLIP MILLER
Dept. of Medicine, University of Chicago, Chicago, Ill.

In earlier experiments(1) mice were exposed continuously to gamma radiation at 3 different levels of intensity: approximately 128, 69, and 34 r/day, respectively. After various periods of irradiation their susceptibility to bacterial infection was determined by intraperitoneal challenge with graded inocula (10-fold dilutions) of *Pseudomonas aeruginosa*. The results showed that increased susceptibility to this infection was related to dose rate rather than total amount of radiation accumulated. At the lowest rate, 34 r/day, no significant increase was demonstrated in 2 experiments on mice which had accumulated 2140 r during 9 weeks exposure. Despite that finding, dose rate in the present experiments was reduced to 15 r/day, but the challenge inocula (of the same test microorganism) were graded by less than 10-fold dilution in an atempt to detect smaller changes in susceptibility than were demonstrable in the previous experiments.

Materials and methods. Mice. A total of 895 CF-1 females were used, approximately half of them irradiated and half controls. In one experiment they were 4 weeks old, in all others 10 weeks old, when irradiation was begun. They were housed in groups of 10 in Lucite cages measuring 7½ x 12 x 7 inches covered with perforated aluminum tops. Food pellets were available at all times. Tap water was supplied in sterilized bottles which were changed every day. Cages were changed and disinfected once a week. *Irradiation.* The mice received approximately 15 r/day 6 days a week. Exposure time was 7 hours at first, gradually lengthened to 7 hours 21 minutes as the Co^{60} source decayed. Between exposure periods both irradiated and control mice were kept in the same room, one week in a room reserved for uninoculated mice, the next week in the exposure room with the source shielded. This was done so that irradiated and control mice should live as much of the time as possible in the same environment. No deaths occurred among irradiated or control mice before challenge. The longest exposure period was 15 weeks because it was necessary to terminate experiments at that time. *Exposure.* Mice were exposed in an air conditioned room in the sub-basement of the Argonne Cancer Research Hospital.† Cages were placed on a wooden rack with their long sides facing a nominal 10-curie Co^{60} source, their centers all 124 cm from it. The dose rate was based on the assumption that distribution of the mice was random within each cage and disregarded the shielding effect of the mice on each other. *Dose measurements* were made with a Victoreen ionization chamber (calibrated by the National Bureau of Standards) placed in the center of one of the Lucite mouse cages with its aluminum top in place. The ionization chamber was covered with a close-fitting 4-mm Lucite cap of sufficient thickness to provide electronic equilibrium for cobalt-60 gamma rays. Accuracy of these measurements was estimated to be $\pm$ 3%. *Challenge inoculations.* Each challenge inoculation included unirradiated controls from the same shipment of mice. The test microorganism was a streptomycin-resistant strain of *Pseudomonas aeruginosa* used in this laboratory for several years to challenge mice exposed to acute X-radiation(2,3) and to continuous gamma radiation(1). Virulence has been maintained by weekly passage through mice. *Preparation of inocula.* An 18-hour culture was washed off an agar plate, suspended in 5 ml saline and shaken 15 minutes on a rotator in a flask containing glass beads to disperse

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† Operated by Univ. of Chicago for U. S. Atomic Energy Commission.

TABLE I. Total Leucocyte Counts (Geometric Means) on Mice Exposed to 15 r/Day.

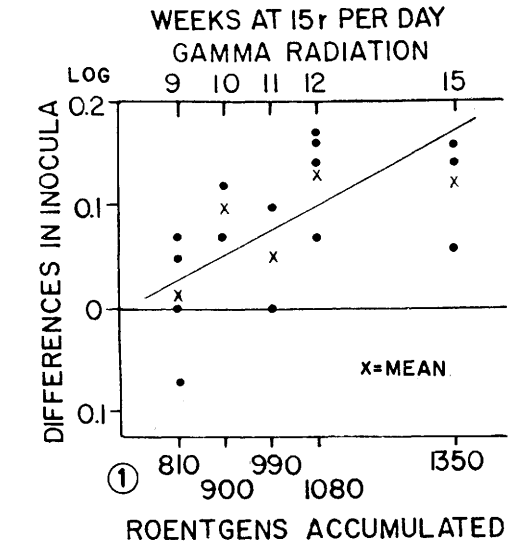
Exposure, wk	Radiation accumulated, r	Leucocytes/mm³
0	0	8150
1	90	6150
2	180	6000
3–4	270–360	5150
5–6	450–540	5100
7	630	4800
9–10	810–900	2700
11	990	2650
12–13	1080–1170	2800
15	1350	2200

any clumps of bacteria. The suspension was diluted to a standard density (10^9 per ml) by means of a Coleman colorimeter (filter 430 mm). Dilutions were made to provide inocula containing approximately 2.0, 1.2 and 1.0 x 10^8 (and in a few experiments 10^7) which were injected intraperitoneally in 0.5 ml volume into 5-20 mice each. The numbers were checked by plating in quadruplicate 0.1 ml samples of appropriate dilutions of the bacterial suspensions. *White blood counts.* Total leucocyte counts were made on 242 mice by customary methods on blood obtained from a tail vein. Their geometric means are shown in Table I.‡

Results. Almost all deaths occurred within 48 hours after challenge. Although 13 mice died later, autopsy cultures of heart's blood and spleen showed only 6 of them to have died with *Ps. aeruginosa* bacteremia. The other 7, therefore, were excluded from the mortality data.

From the results of each challenge inoculation, the LD_{50} for irradiated and control mice was determined by probit analysis. The difference between LD_{50} of each irradiated group and its controls is plotted (in tenths of a log) against the duration of their exposure, *i.e.*, accumulated radiation (Fig. 1). The mean of these differences (ratios) for each exposure time is also plotted. Points for the regression line were computed from the means by the method of least squares. It shows that a slight but progressive increase in susceptibility to experimental infection resulted from prolonged daily exposure to 15 r. Between 9th and 15th weeks, the period covered, this increase was calculated to be .029 log of inoculum per 100 r gamma radiation accumulated.

Comparison with effect of single exposure to X-radiation. In earlier experiments(3), mice were subjected to 300, 400, and 500 r X-radiation in a single acute exposure and challenged 5 days later by intraperitoneal inoculation with the same strain of *Ps. aeruginosa.* LD_{50}s



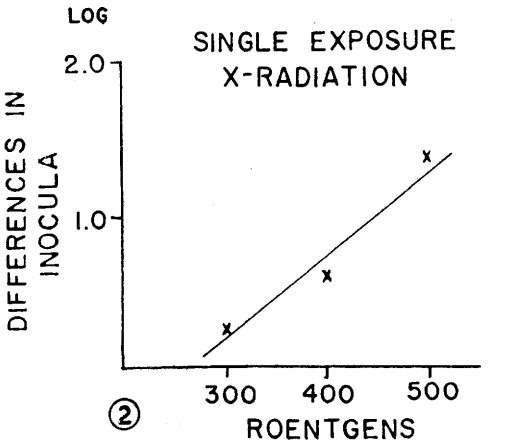


FIG. 1. Changes in susceptibility to challenge inoculation plotted as differences in LD_{50}'s of irradiated and control mice.

FIG. 2. For comparison: effect of single acute exposures to X-radiation. Challenge 5 days post-irradiation with same strain of *Pseudomonas aeruginosa.*

‡ Leucocyte counts were made by Miss Betty Wolfe.

were computed by the methods described above. The means of the differences between those of irradiated and control mice are plotted (as *whole* logs) in Fig. 2. The slope shows how much greater was the increase in susceptibility to the challenge inoculations produced by each increment of 100 r acute X-radiation, approximately .54 log of inoculum per 100 r.

Discussion. The differences in LD_{50}s of *Ps. aeruginosa* for irradiated and control mice in each challenge inoculation indicate a slight but progressive increase in susceptibility to this bacterial infection from the 9th through the 15th weeks of exposure to 15 r/day. The change was too small to have been demonstrable by use of 10-fold gradations of inocula as used in our previous experiments on mice exposed to 34 r/day(1). At 9 weeks, the shortest exposure time, the effect on susceptibility was so slight as to raise the question whether any change at all could have been detected earlier.

After 15 weeks' irradiation (total accumulation 1350 r) the increase in susceptibility, while still small, was clearly demonstrable. It is unfortunate that exposures could not have been prolonged beyond that time. Comparison of this effect of chronic γ irradiation with that produced by a single exposure shows that an accumulation of 1350 r (at 15 r a day) increased susceptibility to the challenge inoculations much less than did an acute exposure to 300 r X-radiation.

The effect on total leucocyte counts was about the same. The geometric mean of the white counts on 5th day following exposure to 300 r was 2400(1). After accumulating 1350 r (at 15 r a day) it was 2200.

Summary. 1) CF-1 female mice were exposed to approximately 15 r a day radiation for 9-15 weeks and challenged at intervals by intraperitoneal inoculation with graded doses of *Pseudomonas aeruginosa.* Comparison of LD_{50} of irradiated and control mice in each challenge showed that between 9th and 15th weeks a slight but demonstrable increase in susceptibility to experimental infection had occurred. 2) The increase caused by accumulation of 1350 r during 15 weeks exposure was, however, very much less than that resulting from a single acute exposure to 300 r X-radiation.

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Induction of Lactation and Mammary Growth by Pituitary Grafts in Intact and Hypophysectomized Rats.*† (25801)

JOSEPH MEITES AND T. F. HOPKINS

Dept. of Physiology and Pharmacology, Michigan State University, East Lansing

Recent investigations suggest that the hypothalamus normally inhibits prolactin secretion by the AP, and that severance of the pituitary from its cranial connections results in increased production of this hormone. Desclin(1-2) and Everett(3) reported that autotransplantation of the pituitary to the kidney capsule of the rat maintained functional corpora lutea for prolonged periods of time. Eckles *et al.*(4) noted lactation in several

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