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Attempts to Increase Resistance of Mice to Bacterial Infection by
Prolonged Low Dose γ Irradiation.* (25989)

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Previous experiments have shown that prolonged daily exposure to γ radiation increased susceptibility of mice to bacterial infection, as determined by challenge with *Pseudomonas aeruginosa*, and that the increase was proportional to dose rate rather than total amount of radiation accumulated(1). Even at the rate of 15 r/d a slight increase in susceptibility was detectable after 9-15 weeks exposure (2). The literature, however, contains reports(3,4) which suggest the possibility that at very low dose rates an opposite effect may result, *i.e.* that prolonged daily exposure to very small doses may actually increase resistance to bacterial infection. For if one assumes, as most investigators do, that the occasional death which occurs among stock laboratory animals is probably caused by intercurrent infection, one may reasonably conclude that any reduction in mortality among

irradiated animals below that of their controls may well be due to a lower incidence of death from intercurrent infection, hence to increased resistance to latent infection. Pertinent to this line of reasoning are the following observations: Lorenz *et al.* in an early attempt to establish limits of "permissible" daily doses of ionizing radiation exposed mature laboratory animals to 5 levels (8.8-.11 r) of γ radiation daily until death. Although the life span of those receiving the 3 highest doses was shortened, mortality of those receiving 1.1 r/day was slightly lower and of those receiving .11 r considerably lower than that of their controls during the first 23 months of exposure(3). Carlson, Scheyer, and Jackson found the mean survival time of rats exposed to 0.8 r/day to be longer than that of their unirradiated controls whether their environmental temperatures were maintained at 25° or 5°C(4). The present experiments were undertaken in an attempt to detect any increased resistance to an experimental bacterial

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TABLE I. Comparison of Mortalities of Irradiated and Control Mice in Each Challenge Inoculation.

	Dose rate, r/day		
	.5	1	2
	Wk of exposure		
No difference between irradiated and control mice	27	4	14
	30	5	22
	32	9	27
		17	30
		26	
Slightly lower among irradiated		27	
		30	
		39	
		23	
Slightly higher among irradiated		18	
		24	
		27	
		31	

infection among mice exposed to 0.5, 1.0 or 2.0 r per day for various lengths of time. Preliminary experiments did in fact give evidence of slightly increased resistance but additional experiments failed to confirm earlier results.

Materials and methods. *Mice.* CF-1 females 3 weeks old on arrival in the laboratory were kept under observation for one week, then randomly distributed into groups of 10, half of them to be irradiated and half kept as controls. They were housed in Lucite cages measuring $7\frac{1}{2} \times 12 \times 7$ inches covered with perforated aluminum tops. Rockland mouse 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* was carried out in an air conditioned room in the sub-basement of the Argonne Cancer Research Hospital.[†] Mice were exposed 6 days a week to γ radiation from a nominal 0.5 curie Co^{60} source at 3 dose rates: 0.5, 1.0 and 2.0 r/day. Cages were placed on curved wooden racks the center of each at its appropriate distance from the source. The daily exposure time varied from 5 hours 42 minutes to 8 hours depending on activity of the source. Duration of exposure varied from 4 to 39 weeks. *Calibration.* Dose measurements were made with

a Victoreen ionization chamber (calibrated by Nat. 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 γ rays. Accuracy of these measurements was estimated to be $\pm 3\%$. *Challenge inoculations.* The time of each, in terms of duration of exposure, is given in Table I. Most challenge inoculations contained 60 mice from the same shipment, approximately half irradiated and half unirradiated controls. Before inoculation, the mice were redistributed to separate cage mates. The test microorganism was a streptomycin-resistant strain of *Pseudomonas aeruginosa* used for a number of years to study resistance and susceptibility in irradiated mice (1,2,5). Virulence has been maintained by weekly passage through mice. *Preparations of inocula.* An 18 hour culture was washed off an agar plate in 5 ml saline and shaken in an Erlenmeyer flask containing glass beads for 15 minutes on a rotator to break up any clumps of bacteria. The suspension was diluted to contain approximately 10^9 microorganisms/ml by means of a Coleman spectrophotometer (filter 430 m μ). Its bacterial content was checked by plating in quadruplicate 0.1 ml of the 10^{-6} and 10^{-7} dilutions. Since the LD_{100} and LD_0 of *Ps. aeruginosa* for these mice was known from past experience, inocula were chosen within these limits in an attempt to detect small differences between irradiated and control mice. Approximately $2.0, 1.2$ and 1.0×10^8 in 0.5 ml were inoculated intraperitoneally into (usually) 10 mice each. *Leucocyte counts.*[‡] At approximately weekly intervals from the 3rd to 26th week of exposure white blood counts were made on 10-20 mice in each series, a total of 492 counts, about equally distributed among the 3 series. No mouse was used more than once for this purpose.

Results. In 14 of the 23 challenge inoculations mortality in the irradiated group was practically identical with that of its control group (Table I). Slightly lower mortalities

[†] Operated by Univ. of Chicago for U. S. Atomic Energy Comm.

[‡] Leucocyte counts were made by Betty Wolfe.

occurred among irradiated mice in 4 challenge inoculations in the 1 r series but were balanced by 4 which showed equally lower mortalities among controls. Overall, therefore, the experiments (involving 1344 mice) failed to demonstrate any consistent effect of irradiation on host resistance to experimental infection with *Ps. aeruginosa*. Nor was correlation found between duration of exposure and increased resistance or susceptibility in individual challenges. Omitted from Table I were 2 additional challenge inoculations on mice exposed 30 and 35 weeks to 0.5 r/day made with a beta hemolytic streptococcus of moderate virulence for mice (strain AD 242).§ Results with this test microorganism were inconclusive but suggestive of a very slight reduction in host resistance.

Leucocyte counts fluctuated considerably as did those reported by Lorenz *et al.* (3) and as we have found in other experiments. Their geometric means indicated occurrence of mild leucopenia (4800-5800) during prolonged exposure to 1.0 and 2.0 r/d (normal = 8500), but the leucopenia was not progressive as it was in mice exposed to 15 r/d (2). Individual counts rarely fell below 3000. Geometric means in the 0.5 r series varied from 5100 to 6400 during the 21st to 26th weeks of irradiation.

Discussion. These experiments failed to demonstrate any effect on host resistance to an experimental bacterial infection in mice exposed for many weeks to very low doses of γ radiation. The method employed, intraperitoneal inoculation with *Pseudomonas aeru-*

ginosa, is admittedly a drastic type of challenge. It was used because it had proved satisfactory in previous experiments to demonstrate changes in host resistance of mice exposed to higher doses of X or γ radiation (1,2,5). Two trials with a β hemolytic streptococcus of medium virulence showed it to be no more satisfactory as a test microorganism.

Summary. CF-1 female mice 4 weeks of age were exposed 6 days a week to 0.5, 1 or 2 r γ radiation/day. After 4 to 39 weeks exposure they were challenged by intraperitoneal inoculation of graded doses of *Pseudomonas aeruginosa*. No detectable effect on host resistance to this experimental bacterial infection was demonstrated.

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