Transient Increase in Resistance of Mice to Experimental Infection Following a Small Dose of X-Radiation.* (26885)

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The literature on the effects of total body exposure to ionizing radiation deals largely with its deleterious effects. A few publications, however, have reported that previous exposure to a small dose of radiation may reduce the 30-day mortality from a mid-lethal dose of similar radiation administered a few weeks later. This phenomenon has been designated "acquired radio-resistance."

Dacquisto(1) has included an excellent critical review of the literature in his report of his observation that the LD_{50} of adult female mice exposed to whole body X-radiation was increased from 487 r to 617 r by a "conditioning dose" of 50 r given 17 days previously. Hagen and Simmons(2) showed that the LD_{50} of rats was raised by 50 r if they had been exposed to 300 r 3 weeks (but not 1 or 2 weeks) earlier, and Sacher(3) has recently found that lower mortalities resulted from a mid-lethal dose of γ radiation among mice conditioned by previous exposure to .6 r per day for 2 or 3 months.

Acquired radio-resistance has not been adequately explained. It seemed possible that one effect of a "conditioning dose" of radiation might be a temporary increase in resistance to bacterial infection which is an important cause of death in mice during the first few weeks following exposure to mid-lethal doses of radiation(4,5). To test this supposition, mice were irradiated with 50, 75, or 100 r (approximately 1/10, 1/7, and 1/5 their $LD_{50/30}$) and challenged at intervals thereafter by intraperitoneal inoculation with graded doses of *Pseudomonas aeruginosa,* one of the enteric microorganisms which not infrequently causes fatal bacteremia in irradiated mice.

Mice. CF-1 female mice, 9-10 weeks old were kept under observation for at least a week before use. Rockland mouse pellets were available at all times. Tap water was supplied in sterile bottles which were changed every day. Cages were changed and autoclaved once a week. All mice in each experiment, irradiated and controls, came from the same shipment.

Irradiation.‡ The mice were irradiated with a 250 kv, 30 ma maxitron 250 (General Electric) machine using 0.25 mm copper and 1 mm aluminum filters at a target distance of 79 cm and a dose rate of approximately 69 r/min. For details of method of exposure, see Hammond, Ruml, Cooper, and Miller(6).

The test microorganism was a streptomycin resistant strain of *Pseudomonas aeruginosa* used for a number of years in this laboratory to determine the effect of irradiation on susceptibility to bacterial infection(7,8,9,10). This strain was chosen because the infection it produces is rapidly fatal in susceptible animals; *i.e.,* inoculated mice either die within 18-36 hours or survive the observation period of 30 days. Virulence has been maintained by weekly passage in CF-1 mice. An 18-hour culture was washed off an agar plate in 5 ml saline, and any clumps of bacteria were dispersed by shaking in an Erlenmeyer flask containing a few glass beads on an electric rotator for 15 minutes. The suspension was then diluted to provide the desired inocula. The actual numbers of bacteria inoculated were determined from quadruplicate platings of appropriate dilutions of the suspension used. Three groups of 10 irradiated mice and an equal number of unirradiated controls from the same shipment, were inoculated intraperitoneally with approximately 2.0, 1.2 and 1.0×10^8 *Ps. aeruginosa.* Before inoculation, the mice were randomized to separate cage mates.

Results. From the resulting mortalities (30

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‡ Mice were irradiated at the Argonne Cancer Research Hospital, operated by Univ. of Chicago for U. S. Atomic Energy Commission.

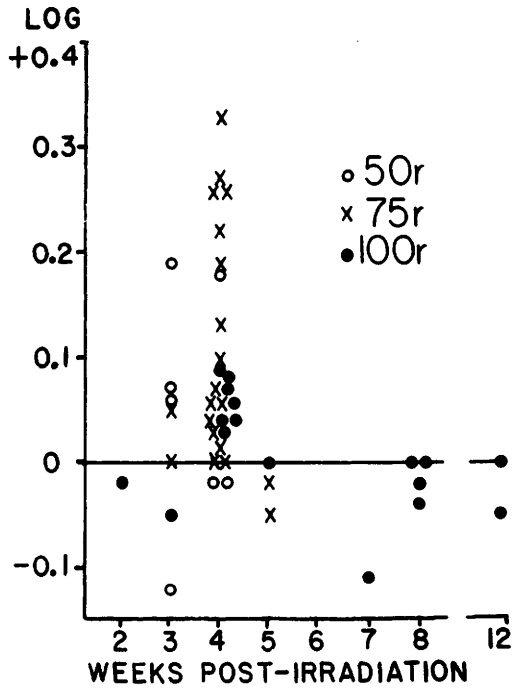

FIG. 1. Increase in log LD_{50} of challenge microorganism for irradiated mice compared with their unirradiated controls.

days) log LD_{50}'s were determined by probit analysis. The differences between those of irradiated and unirradiated mice in each experiment are plotted in Fig. 1. In 16 groups of mice challenged 4 weeks after exposure to 75 r, the LD_{50}'s exceeded those of their controls by an average of 0.129 log. This increase, though not large, is statistically significant at the level $P < 0.001$. The 95% confidence interval is from 0.070 to 0.187. Exposure to 100 r resulted in a smaller (0.059 log) but still significant ($P < .001$) increase in resistance of mice challenged after the same interval, *i.e.*, 4 weeks post-irradiation. From the results on mice challenged after 50 r, it seems improbable that any effect resulted from exposure to that dose.

Leucocyte counts.§ Total white counts made by customary methods at intervals on mice exposed to 75 r showed a reduction to 6550 (geometric mean) on the 17th day with gradual return to normal (9-10,000) by the 30th day post-irradiation. This finding suggests that when challenged at the 4th week post-irradiation, their circulating phagocytes probably contained a larger proportion of young cells than did those of their unirradiated controls. Differential counts were not made, nor were bone marrows examined for evidence of regeneration of hematopoietic elements.

Discussion. The results show that by the methods employed a small but significant increase in resistance to an experimental bacterial infection was demonstable during the fourth week following exposure to 75 or 100 r X-radiation. This transient effect is regarded as a manifestation of overcompensation in the course of the natural process of recovery and repair, which for a short time may exceed or "overshoot" the normal level.

The increased resistance to infection following a small dose of X-radiation, though not marked nor of long duration, nevertheless suggests a possible explanation for the phenomenon of acquired radio-resistance since endogenous bacterial infection plays an important role in the death of small laboratory animals exposed to mid-lethal doses of ionizing radiation.

Summary. CF-1 female mice, 9-10 weeks old, were exposed to a small dose of whole body X-radiation (50, 75, or 100 r) and together with unirradiated controls were challenged at various intervals by intraperitoneal injection of graded inocula of *Pseudomonas aeruginosa*. Comparison of the LD_{50}'s (30 days) of irradiated and control mice in each challenge showed a small but significant increase in resistance to the experimental infection during the 4th week following irradiation with 75 or 100 r. Exposure to 75 r was more effective than 100 r. The results suggest a possible explanation of "acquired radio-resistance."

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§ Leucocyte counts were made by Miss Sally Akan.

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Some Biochemical and Physical Changes Occurring in Experimentally-Inflicted Poultry Bruises.* (26886)

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In previous publications on bruised tissue (9) evidence was presented that the characteristic color alterations in bruised tissue represent definite stages in the various physical and metabolic changes which occur during healing. Furthermore, it was shown that when cattle(10) or chickens(12) were bruised upon a second or third occasion, each successive bruise healed more rapidly than the preceding one. Serological and electrophoretical analyses of rabbit serum following bruising have shown that a humoral factor may be responsible for the accelerated healing of secondary bruises(8) and that injection of streptokinase or trypsin enzymes at site of a bruise enhanced the rate of healing in rabbit bruises(13). Zamecnik, Stephenson, and Cope(21) demonstrated the presence of an amino exopeptidase enzyme in normal lymph, serum, muscle, skin, subcutaneous tissue and erythrocytes of dogs and established that the level of this enzyme rises abruptly in the lymph draining a burned or traumatized tissue. The changes that occur in the concentration of a number of compounds of biochemical interest in the plasma and the damaged tissue following onset of an acute inflammatory response(5), trauma(7,14), or wound(18,20) have been investigated.

The present study was initiated to investigate the proteolytic activities and physical changes that occur at specific time intervals following infliction of bruises in poultry. Some of the factors which affect these changes were also examined.

Materials and methods. Bruising and sampling. Chickens 8 to 10 weeks old and weighing approximately 3 lb were kept in constant temperature houses at 7.2, 21 and 30° C for 5-7 days prior to bruising. The area to be bruised and control (non-bruised areas) were clipped and the birds bruised using a standard technique on the breast or the thigh muscles(12). Control and bruised birds were sacrificed at specific intervals following bruising, and both the bruised and control tissues were immediately excised, sliced, minced, and aliquants used for analyses. *Assay of proteolytic activity.* A homogenate of the bruised or control tissue was prepared at 5° C by blending 25 g of the minced tissue with 75 ml of chilled 0.9% NaCl and removing the insoluble residue (blood cells, nuclei and mitochondria) by centrifugation at about 3° C at 6,000 × g for 20 minutes. The supernatant solutions were assayed for proteinases by the method of Kunitz(16) with 0.5% buffered casein as substrate. The proteolytic activity is expressed herein as optical density (O.D.) increment of the incubated solution for 1 hour minus that of the corresponding blank at zero time. Specific activity was defined as Δ O.D. at 280 mμ per 1 hour per

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