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Antibody Production in Mice Exposed Intermittently to Radium Gamma Rays. (23772)

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Recovery processes taking place between successive irradiations are thought to account for the increased LD_{50} and prolonged survival time reported for mice(1,2) and for rats(3) when the radiation was administered in 2 or more exposures made at varying time intervals. Mortality and survival times following radiation exposures in the lethal range and given as divided doses have been considered theoretically to be measurements of the summed injury to several physiological processes(4). Some of these processes, such as hematopoiesis, blood coagulation, defense against infection and antibody production following acute exposures to ionizing radiations have been studied in great detail. Less attention has been given to the study of injury to any of these processes following intermittent irradiation. Observations on the immune response following divided-dose exposures to ionizing radiation are of interest because measurement of injury to this physiological process may be made following sublethal exposures with good accuracy by procedures now available. Furthermore, studies of antibody production following intermittent exposure to radiation provide some information on injury and recovery processes involving the cellular mechanisms of protein synthesis. In the experiments here described antibody production and total circulating leucocyte counts were studied in mice following their intermittent exposure to radium gamma rays.

Materials and methods. Male NIH mice used throughout these experiments were 5 to 8 weeks of age at the beginning of the radiation exposure. Litters were represented in each of the treatment groups and mice exposed to radiation were confined in wood boxes containing 5 to 8 animals each. Control animals were housed in steel cages in the animal rooms and at the end of the exposure both the irradiated and control mice were transferred to individual cages. Mice of parallel groups were bled from the tail for circulating leucocyte counts on the second day after the end of exposure. Immunization was done on the third day and the mice were sacrificed for serums on the 8th day after the end of the irradiation. Mice of another experiment were given acute exposure to X-rays 4 days after the end of the "chronic" accumulation of 450 r from the radium source described below in order to see whether the prior exposures would be additive to the acute irradiation in their effect on antibody production. Immunization was done one week and leucocyte counts 6 days after the acute exposure. Serological procedures followed those previously described(5) except that the serums were frozen and stored at -20°C until titration within 3 to 4 weeks after sacrifice of the mice. Briefly, the serum hemolysin content was estimated after a single intravenous inoculation of a 2.5% suspension of washed sheep erythrocytes. Approximately 4.85 x 10^6 erythrocytes were given per gram of body weight. Serums were titrated by the procedure described by Taliaferro(6) and the hemolysin titers are expressed as the negative log of the serum dilution providing 50% hemolysis in a standard suspension of sheep erythrocytes.

The arrangement for exposing the mice to the radium source was described in detail by Lorenz(7) and provided 8.8 r or 2.2 r per 8 hour day. In the experiments described in this paper the total exposure dose to the mice

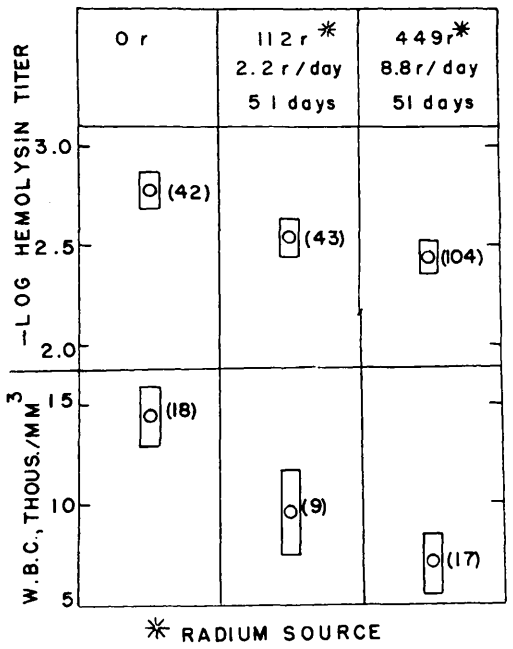

FIG. 1. Avg hemolysin titers on 5th day after immunization, above, and total circulating leucocyte counts, below, in NIH mice exposed to radium gamma rays at rate of 2.2 r or 8.8 r/8 hr day for 51 days. Immunization was done 3 days after end of radium exposure. Numbers in parentheses indicate numbers of animals/group and vertical bars represent 95% confidence limits of the means.

was about 112 r to one group while to the others about 450 r and these exposures were accumulated over a period of 51 days. The results of film dosimeter measurements* done on one occasion were in reasonable agreement with the more frequent ionization chamber measurements of the exposures. Acute exposures were made with a therapy X-ray unit operated at 200 Kvp and 20 ma with 0.51 mm Al and 0.25 mm Cu additional filtration and HVL = 0.76 mm Cu. The radiation was measured in air at 50 cm target distance to the center of the body.

Results. Average hemolysin titer (Fig. 1) in serums of mice of 2 experiments (n = 47 and 57) exposed to 8.8 r per 8 hour day for 51 days was −2.445 compared to −2.787 for their age controls (n = 20 and 22). The difference between control and experimental pooled means is significant at $p = <0.001$ while for the individual experiments the differences were significant at $p = <0.01$ and <0.05 respectively. It is of interest to note also in Fig. 1 that exposure of the mice to a total dose of 112 r resulted in a significantly lowered average hemolysin titer compared to the controls.

Average circulating leucocyte counts for parallel groups of mice simultaneously exposed to the radium dropped from 9.6 x 10^3 cells mm^3 in the 2.2 r/day group to 7.1 x 10^3 cells mm^3 in mice exposed to 8.8 r/day as compared to 14.4 x 10^3 cells/mm^3 for control mice as shown in the Figure. Mean leucocyte counts for the treated mice are significantly below, $p = <0.05$, the control mean. Comparison of the data presented in Fig. 1 shows that there is a tendency for the highest radiation dose to be associated with the lowest mean values both for the hemolysin titers and total leucocyte counts.

Impairment of the antibody producing capacity of the mice resulting from the radium gamma ray exposures in the experiments just described was small though highly significant. Table I summarizes the results of an experiment to test whether the exposure of mice to a dose of 450 r accumulated over a period of 51 days at the rate of 8.8 r per 8 hour day would have an additive effect if followed by an acute X-ray exposure. Four days after their exposure to 450 r in the radium field

TABLE I. Hemolysin Titer and Total Leucocyte Counts in NIH Mice after an Accumulated Exposure to 450 r Radium Gamma Rays followed by an Acute X-ray Exposure.

Treatment			
Radium	X-ray	Avg hemolysin titer ± S.E.*	Avg total WBC × 10^3 ± S.E.†
0	0	−2.83 ± .08	10.67 ± 1.36
450 r	0	−2.72 ± .13	9.15 ± .84
0	150 r	−2.34 ± .08	3.73 ± .40
450 r	"	−2.26 ± .09	3.18 ± .60
0	250 r	−1.70 ± .14	2.15 ± .32
450 r	"	−1.41 ± .17	2.04 ± .34
0	350 r	<−1.0	1.33 ± .23
450 r	"	"	.86 ± .08

* Mice, 18-20/group, were immunized 7 days after indicated acute exposure and serum samples obtained on 5th day after immunization.

† Leucocyte (WBC) counts were made on 9th day after end of exposure to radium, 9-10 mice/group.

* The authors are grateful to Mr. Walter Cool of the Plant Safety Branch, Nat. Inst. Health, for conducting the film measurements.

groups of 18 to 20 mice were exposed to 150 r, 250 r or 350 r of X-rays (Table I). Average hemolysin titers for the mice exposed to the X-rays alone are only slightly higher than for the mice given a preliminary exposure of 450 r radium gamma rays. Mean titer for the 20 control mice was only slightly higher than the average titer for the mice receiving the radium exposure alone. It should be noted that the mice of this experiment were immunized 11 days later than those of the experiments described earlier in this paper. Total circulating leucocyte counts decreased as the acute exposures increased and in the mice given a preliminary exposure to radium the average counts were somewhat lower than in the mice given acute exposures only.

Discussion. It must be assumed from the results shown in Fig. 1 that recovery of the immune mechanism in the mice did not take place during the 16-hour interval which elapsed between successive exposures to as little as 2.2 r per 8 hour day. However, any residual injury to either antibody or leucocyte production which may have remained following the "chronic" exposure to 450 r of radium gamma rays was insufficient to alter significantly the response of the mice to a subsequent acute exposure to X-rays.

Unrecovered injury to the production of leucocytes following the radium exposure was also detectable from the lowered average counts observed and the values are in agreement with the leucocyte counts in LAF_1 mice following similar exposures(7). These results differ from the results of an acute X-ray exposure to partially shielded mice described previously(5) in which the total circulating leucocyte count was decreased without a reduction in the antibody producing capacity of the mice. In other experiments(8) antibody production recovered 3 to 4 weeks later than recovery of the leucocyte count in mice given an acute exposure to 450 r of X-rays. In mice, therefore, hematopoiesis and antibody production do not appear to be closely dependent processes during the recovery from exposure to ionizing radiation.

Summary. Exposure of groups of mice to a radium source providing 8.8 r or 2.2 r per 8 hour day for 51 days resulted in significantly lowered antibody titers. In these experiments the mice were immunized on the third day after the end of the radiation exposure and the serums were sampled on the 5th day after immunization. Antibody titers and leucocyte counts following a single exposure of the mice to an acute X-ray dose 4 days after the end of an intermittent exposure to 450 r of radium gamma rays provided no evidence that the prior exposure produced an altered radiation sensitivity to either the hematopoietic process or the process of hemolysin production. The possibility that antibody synthesis in response to sheep erythrocyte antigen and leucocyte production are independent processes during the recovery from irradiation is discussed.

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