21-75
102-1133
LA-5813-MS
Informal Report

UC-48

Reporting Date: December 1974

Issued: January 1975

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by

J. F. Spalding

O. S. Johnson

G. L. Tietjen

los alamos
scientific laboratory
of the University of California
LOS ALAMOS, NEW MEXICO 87544

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Price: Printed Copy \$4.00 Microfiche \$2.25

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AN INVESTIGATION OF THE TWO-COMPONENT RADIATION
INJURY CONCEPT USING EQUIVALENT RESIDUAL DOSE FACTORS

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J. F. Spalding, O. S. Johnson, and G. L. Tietjen

ABSTRACT

An investigation of the two-component concept of radiation injury assuming a 7-day RT_{50} and 5% long-term injury was carried out with mice. The results suggest that this concept is useful only under a limited range of exposure conditions. In this study, the incidence of neoplasia (primarily thymomas) appeared to be dependent on the radiation regime used, with possible therapeutic effects changing the death patterns in the largest dose group. No progressive bone-marrow lesion was seen with the dose levels used and, in this sense, the recovery rate was not affected by radiation history. This and other investigations suggest that, if the effects of a wide range of total exposures and dose rates are to be anticipated, ERD factors in the two-component concept of radiation injury recommended for man in emergency conditions should be modified downward.

I. INTRODUCTION

Models to describe the kinetics of radiation-induced biological injury and repair with reasonable accuracy have been sought for over a decade. Although most investigators and theorists introduce slightly different concepts into their various models,¹⁻⁸ two factors (namely, short-term repairable and irreparable or long-term injury) and the uncertainties revolving around them are basic to all models. That a reliable model is the hope of future research is evident from Report No. 29⁹ in which the National Committee on Radiation Protection and Measurements bases its suggested allowable exposure to radiation in an emergency on one of the earliest concepts formulated [namely, Blair's equivalent residual dose (ERD) hypothesis].⁶ Their proposed recommendations⁹ are made with cognizance of the fact that experimental proof of the reliability of this concept is not yet available. The only test of the basic ERD concept known to have been done with dogs and monkeys¹⁰ caused death in 7 of 8 monkeys at theoretical ERD values assumed to be safe for man.⁹ However, attempts to measure the long-term component

of the radiation-induced lesion as a reduction in mean survival time during continuous low-dose-rate exposure in dogs and monkeys have not shown the magnitude of the lesion anticipated.¹¹⁻¹³ This investigation was done to observe the comparative effects of three levels of gamma-ray exposure administered by the fractionation method with assumed ERD factors for the two-compartment radiation-injury concept to establish ERD exposure limits.

II. MATERIALS AND METHODS

Two hundred RF strain virgin female mice 90 $\pm$ 7 days of age were randomly assigned to four groups of 50 mice each. Groups I, II, and III were assigned to ERD test conditions such that they would receive (by discrete gamma-ray exposure) but never exceed theoretical ERD levels of 100, 200, and 300 rad, respectively, throughout their entire lives. Group IV was a sham-irradiated control. The ERD levels were established with the assumptions that a repairable component of radiation injury repairs with a half time (RT_{50}) of 7 days,¹⁴ that an irreparable component (or long-term lesion) of radiation injury

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is equivalent to 5% of any given dose,¹¹ that the repair rate is unaffected by size of the dose,¹⁴ and that the irreparable or long-term component is independent of radiation history. The ERD function, assuming an exposure is given at time t , is:

$$A_i = L \left[\gamma_i + (1 - \gamma_i) e^{-\lambda(t_i - t_j)} \right],$$

where A_i = ERD at time t

L = MP ERD

γ_i = accumulated irreparable component at time t (fraction)

$\lambda = \ln 2 / RT_{50}$

t_i = time t

t_j = time of last exposure ($t_i - t_j$)

The irreparable component of radiation injury in this function accumulates as follows:

$$\gamma_i = p + p(L - A_1) + p(L - A_2) + \dots + p(L - A_i),$$

where p = the irreparable fraction

$k_i = \frac{L - A_i}{L}$, fraction of injury repaired at time t_i (i.e., the fraction of dose given at t_i)

Each of the three irradiated groups was started on its exposure schedule with a single gamma-ray exposure equal to its assigned maximum permissible equivalent residual dose (MP ERD). Group I received 100 rad, Group II 200 rad, and Group III 300 rad. After randomly chosen time intervals (allowed for repair) of between 1 and 14 days (one-half to two RT_{50} values),¹⁴ additional gamma-ray exposures were given to each group to bring its biological dose level back to its assigned MP ERD. This gamma-ray exposure regime was continued throughout the life of the animals involved.

The mice were checked daily to obtain survival data for the three experimental groups. Blood samples were obtained from a few animals in each group in advance of each exposure to determine the red blood cell (RBC) counts and the mean cell volume (MCV) as indicators of a possible progressive bone-marrow lesion. Body weight and voluntary activity were observed through the time span required for Groups I, II, and III to accumulate the equivalent

one, two, and three acute LD_{50} doses, respectively.

A cobalt-60 gamma-ray source of approximately 1 Ci was used, and the dose rate (27 rad/h at the start of the investigation) was determined with 25-cm Victoreen chambers calibrated by the National Bureau of Standards for cobalt-60 high-energy gamma rays. The animals were exposed in individual ventilated plastic tubes and were housed 10 animals per cage on wood shavings between exposures.

III. RESULTS AND DISCUSSION

Results of the blood studies through the first 190 days of exposure are shown in Fig. 1. The RBC numbers were generally depressed from the control values throughout the life of the experimental groups, and, with a few exceptions, the depression was dependent on dose (ERD) level assigned. The MCV values increased with each recovery phase, as reticulocytes were found in relatively greater numbers in the peripheral blood a few days after each exposure. The relationship between the RBC and MCV values can be seen in Fig. 1. This relationship between MCV, RBC, and control values remained about the same through slightly over one year of the exposure regime.

No progressive bone-marrow lesion or impairment in the recovery process of the bone marrow was apparent through 175 days of scheduled exposure in any of the dose levels studied. The remarkable resilience of the bone marrow demonstrated here is not a characteristic unique to the mouse. We have seen similar bone-marrow resilience in dogs and monkeys when subjected to gamma-ray dose protraction by fractionation.^{11,12} Thus, response of the mouse bone marrow to this gamma-ray stress regime may be indicative of the bone-marrow resilience.

Fig. 1. Total RBC count and MCV distribution with respect to ERD level and accumulated dose (expressed as percent of control values).

in nature if not in degree, to be anticipated in man under similar exposure conditions.

During this period of time (190 days), over 50% of the mice in each ERD group succumbed, and survivors in Groups I, II, and III had accumulated approximately 1450, 2900, and 4350 rad of gamma rays, respectively. The recovery rate, in terms of erythrocyte repopulation in peripheral blood, appeared to be unaffected by radiation history in terms of progressive accumulation of the gamma-ray dose (see Fig. 1) -- an observation consistent with earlier studies¹⁴⁻¹⁶ and a basic assumption of Blair^{6,7} but not supportive of the postulation of a diminution of recovery rate in proportion to the number of doses received¹⁷ or a lowering of recovery rate with increased level of injury.³

Voluntary activity, as measured by the total number of revolutions in activity wheels during 24-h test periods, was investigated at chronologic ages of 241, 305, and 403 days to test for reduced vigor in the irradiated groups. Results of these tests are shown in Table I. Groups I, II, and III had accumulated the approximate equivalent of one, two, and three LD₅₀ doses of gamma rays, respectively, at age 241 days, and lethality to that age was light (less than 10% in any group, see Fig. 1). At this age and point in time of the exposure regime, general vigor (as measured by activity) in all three ERD groups was significantly less than that of control mice, but there were no significant differences among the three ERD groups. Groups I, II, and III had accumulated additional gamma-ray doses of approximately 200, 400, and 600 rad, respectively, at 305 days of age, but the activity picture in all

groups, including controls, was not different from that observed at 241 days of age. A change in general vigor with increased age from 305 to 403 days was apparent in control animals (an observation consistent with earlier studies)¹⁸ and was also seen in ERD Groups I and III. Only Group III (the 300-rad ERD level) showed significantly less voluntary activity than the control group (Table I). The 200-rad ERD level failed to show a decline in vigor during this age interim. Thus, voluntary activity tests failed to show any correlation between the accumulated dose and vigor but did show that the gamma-ray dose *per se* caused a significant reduction in vigor.

The body weights from 186 through 400 days are shown in Table II. At 186 days of age when Groups I, II, and III had accumulated total gamma-ray doses of approximately 593, 1166, and 1779 rad, respectively, the body weights did not differ significantly from the controls. From age 200 days, significant differences in body weights were observed among the four groups. It is interesting to note, however, that at no age tested did body weight in the highest ERD level (Group III) differ from that of the control mice; at 238 days of age and above, the lowest ERD level (Group I) had mean body weights greater than all other groups, including the control. The shape of the weight vs age curve for all groups was consistent with that obtained earlier in a lifetime body-weight study done on the same strain of mice.¹⁹

Necropsies showed that thymic lymphomas, tumors, etc., as described by Cosgrove²⁰ and Leshner et al.,²¹ were the cause of most early mortality.

TABLE I

VOLUNTARY ACTIVITY AS MEASURED IN ACTIVITY WHEEL REVOLUTIONS PER 24-H PERIOD (AVERAGE PER GROUP TESTED)

Age (days)	Voluntary Activity ^a and Accumulated Gamma-Ray Dose								Test of Significance (5% level)
	Control		Group I (ERD = 100)		Group II (ERD = 200)		Group III (ERD = 300)		
	Dose (rad)	Activity	Dose (rad)	Activity	Dose (rad)	Activity	Dose (rad)	Activity	
241	0	11 506 ± 573	824	8756 ± 451	1648	7329 ± 679	2472	8803 ± 538	+
305	0	11 510 ± 573	1043	8591 ± 684	2080	7722 ± 648	3120	8822 ± 685	+
403	0	8310 ± 893	1301	6845 ± 796	2596	7481 ± 537	3894	5778 ± 822	+

^aMean of activity (wheel revolutions per 24-h period) ± standard error.

TABLE II

BODY WEIGHTS (GROUP AVERAGES) FOR CONTROL AND ERD GROUPS FROM AGES 185 THROUGH 400 DAYS

Age (days)	Accumulated Gamma-Ray Dose ^a								Test of Significance (5% level)
	Control		Group I (ERD = 100)		Group II (ERD = 200)		Group III (ERD = 300)		
	Dose (rad)	Mean Weight	Dose (rad)	Mean Weight	Dose (rad)	Mean Weight	Dose (rad)	Mean Weight	
186	0	33.0 ± 0.32	593	33.6 ± 0.49	1186	33.4 ± 0.40	1779	33.0 ± 0.42	-
200	0	32.2 ± 0.29	665	33.7 ± 0.45	1331	33.3 ± 0.40	1997	32.0 ± 0.45	+
213	0	33.3 ± 0.26	710	34.7 ± 0.39	1420	33.7 ± 0.31	2130	32.5 ± 0.42	+
224	0	33.1 ± 0.26	766	34.6 ± 0.45	1534	35.0 ± 0.40	2300	33.8 ± 0.41	+
238	0	34.2 ± 0.36	824	35.9 ± 0.44	1648	35.0 ± 0.42	2472	34.0 ± 0.45	+
251	0	34.4 ± 0.35	867	36.1 ± 0.46	1733	34.8 ± 0.48	2600	33.9 ± 0.45	+
258	0	35.0 ± 0.34	895	36.6 ± 0.46	1790	35.3 ± 0.53	2685	34.6 ± 0.51	+
272	0	35.0 ± 0.45	930	37.2 ± 0.48	1859	35.7 ± 0.52	2789	34.9 ± 0.43	+
294	0	35.0 ± 0.46	1018	37.8 ± 0.57	2031	36.2 ± 0.57	3046	35.1 ± 0.50	+
341	0	37.9 ± 0.55	1148	40.3 ± 0.93	2294	38.3 ± 0.85	3440	37.4 ± 0.68	+
400	0	40.5 ± 0.55	1289	43.4 ± 0.95	2572	39.6 ± 1.46	3859	38.2 ± 1.27	+

^aColumn entries represent mean values ± their standard error.

Body weight appeared to be more closely associated with tumor incidence (animals with tumors were larger) than the ERD radiation level.

Survival data for animals in the control and exposed groups are plotted as percent survival vs time in days at 25-day intervals in Fig. 2. Mean survival times, ± standard error of the mean, for control and experimental Groups I, II, and III were 581 ± 24 , 420 ± 18 , 374 ± 16 , and 402 ± 15 days,

Fig. 2. Survival data for control and ERD groups, plotted as percent survival vs time at 25-day intervals.

respectively. A one-way analysis of variance, followed by the Duncan-Kramer "multiple range test," showed mean survival times for all three dose-level groups to be significantly less than that of the control group but not significantly different from each other. The mean accumulated dose (MAD) values for Groups I, II, and III were approximately 1300, 2400, and 2800 rad, respectively.

There is no generally accepted factor for obtaining the reduction in life span caused by a given dose of ionizing radiation. It is quite generally agreed, however, that radiation does have a life-shortening effect in terms of advancing the physiological clock of certain neoplasia and non-neoplastic diseases associated with aging. One study by Trujillo *et al.*²² reported a "radiation aging" factor of 0.093 day/rad of whole-body gamma rays. Assuming this value to be a reasonable estimate of radiation-induced life shortening for gamma-ray exposures delivered by this method, we may predict theoretical mean after-survival (MAS) times for the three experimental groups investigated. The age at day zero (start of exposure series) is added to

Fig. 3. Theoretical, anticipated, and actual observed mean effective ages at ERD exposure levels of 100, 200, and 300 rad.

an exposure period which will accumulate a gamma-ray dose that, when multiplied by the life-shortening factor (0.093) and added to the exposure period, will yield an effective age equal to the mean survival time of control mice. Theoretical predictions, based on the above, and observed values are shown in Fig. 3. ERD Group I (100-rad level) fell somewhat short of the predicted mean effective age, Group II (200-rad ERD level) exceeded the predicted mean effective age slightly, and Group III (300-rad ERD level) outlived its predicted mean effective age by a wide margin.

If a portion of the biological damage induced by gamma-ray exposure is "irreparable" or long-term in nature, it follows that this component of radiation injury is responsible for life shortening from causes other than acute response. In this investigation, 5% of each exposure was assumed to result in this long-term injury.¹³ This injury would be equivalent to 66, 122, and 193 rad (5% of the MAD, see Fig. 3) for Groups I, II, and III at their respective MAS times. When the portion of the mean accumulated dose assumed to be irreparable (0.05 x MAD) is divided by the life-shortening effect of each radiation regime, the life-shortening effect attributable to the irreparable lesion is 24.4, 17.0, and 9.30 days/rad for ERD levels of 100, 200, and 300 rad, respectively. To be consistent with the assumption that the factor for determining the long-term lesion is a constant, the life-shortening factor above should be equal.

It is evident from these observations that a dose-dependent irreparable damage factor does not

fit this rather narrow range of exposure conditions. The induction of neoplastic diseases by gamma-ray exposure was not responsible for the difference between the theoretical and observed life-shortening values because the differences were due to greater than expected survival times in the two higher radiation regimes. The life-shortening effect per rad declined with the increase in exposure level or dose. The reason for this is not clear; however, the higher radiation levels appeared to have a "therapeutic" effect or were less carcinogenic than the lower ERD level. This observation was made earlier by Leshner *et al.*²¹

If the two-component concept of radiation injury were valid and the ERD repair rate and irreparable injury factors used here were correct, the radiation regime used in this investigation should be tolerated until experimental animals involved died of life-shortening effects of the long-term component of radiation injury. In fact, life-shortening did not fit this concept in degree or kind. A dependence of repair rate on radiation history was also not seen in this investigation or in earlier studies.¹⁴ Thus, the hematopoietic repair rate may be considered as a constant, at least within the limits of any practical exposure regime. The two-component injury concept as tested in this investigation did not produce the MAS range anticipated for the conditions used; however, use of the ERD factors in fractionation exposure did permit the accumulation of doses by acute increments equal to the continuous exposure method. When no consideration was given to ERD factors, frequent discrete exposures were reportedly about twice as damaging as the same dose of continuous exposure.^{23,24} In an earlier investigation using continuous exposure at a dose rate of 12 rad/24 h,²⁵ mice were able to accumulate 3540 rad with a MAS time of 295 days. In this investigation, at a 300-rad ERD level, the MAD was approximately 3800 rad with a MAS time of 312 days. The average daily dose at this ERD level would be very close to 12 rad/day.

The basic premise of the two-compartment and ERD concept of radiation injury is of fundamental interest in studying the kinetics of radiation injury and repair and, under certain experimental conditions, may predict radiation effects with reasonable accuracy.¹⁵ However, the influence of dose and

dose rate on long-term effects such as neoplasia and non-neoplastic diseases in different species and strains of mammals is an inherent variable which restricts the usefulness of fixed ERD factors or any other concept to a limited range of exposure conditions. Considerable work with large animals, primates in particular, is essential to establish the various parameters required for a reliable radiation injury and recovery model.

ACKNOWLEDGMENT

Mr. R. F. Archuleta is gratefully acknowledged for his technical assistance in this investigation.

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