181
16-9-74

DL-984

LA-5724-MS
Informal Report

UC-48
Reporting Date: August 1974
Issued: September 1974

**Recovery and Residual Injury in Monkeys
Exposed to Large Doses of
Radiation by Fractionation**

by

J. F. Spalding
L. M. Holland
O. S. Johnson
J. R. Prine
J. E. London
P. M. LaBauve

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

↓ ↓

UNITED STATES
ATOMIC ENERGY COMMISSION
CONTRACT W-7405-ENG. 36

MASTER

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

In the interest of prompt distribution, this LAMS report was not edited by the Technical Information staff.

Work supported by the Division of Biomedical and Environmental Research.

Printed in the United States of America. Available from
National Technical Information Service
U.S. Department of Commerce
5285 Port Royal Road
Springfield, Virginia 22151
Price: Printed Copy \$4.00 Microfiche \$1.45

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

RECOVERY AND RESIDUAL INJURY IN MONKEYS
EXPOSED TO LARGE DOSES OF RADIATION BY FRACTIONATION

by

J. F. Spalding, L. M. Holland, O. S. Johnson,

J. R. Prine, J. E. London, and P. M. LaBauve

ABSTRACT

The target site and nature of the long-term residual lesion (LTRL) caused by exposure to ionizing radiations are not well understood. Monkeys (*Macaca mulatta*) were given 100-rad conditioning doses of gamma rays at 56-day intervals to accumulate 2400 rad. Eighty-four days after the 24th conditioning exposure, the monkeys were challenged to death with continuous low-dose-rate gamma rays. The response of hematopoietic tissue was observed as the primary site of acute and long-term radiation injury. Hemograms were performed on monkeys during the fractionation radiation regime used to administer conditioning doses and during the subsequent continuously delivered challenge exposures. The hematopoietic recovery rate from radiation injury was found to be independent of total accumulated gamma-ray dose. The LTRL from gamma-ray conditioning doses totaling 2400 rad did not significantly decrease the mean survival time (MST) in a radiation field of 24 rad/23.5-hr day.

I. INTRODUCTION

Reasonable estimates of the magnitude of the radiation-injury component called the "irreparable," or long-term, residual lesion (LTRL) are difficult to make because there are few experimental data on large mammals, and reports on rodents are frequently conflicting. The primary site and nature of the LTRL have not been identified; however, two theories about the nature of the lesion have been proposed. Furth⁽¹⁾ reported that repeated x-ray exposures caused a progressive decline in recovery rate in dogs. Storer⁽²⁾ reported a relationship between the number of radiation doses delivered and mean rate at which mice recover from ultimately fatal radiation injury. He theorized that the life shortening that may result from nonlethal radiation insults may be due to an "irreversible decrease in the rate at which subsequent damage can be repaired," thus rendering the radiation-conditioned animal more susceptible to all subsequent life-shortening insults. Blair⁽³⁻⁶⁾ proposed the concept of repairable and irreparable components of radiation injury with a constant repair

rate for the repairable component. Thus, the equivalent-residual-dose (ERD) concept of predicting radiation injury and recovery evolved.⁽⁷⁾ Sacher⁽⁸⁾ showed the LTRL from gamma-ray exposure to be insignificant during attempts to quantitate it by subsequent acute exposures, but he did observe a significant lesion when radiation-conditioned mice were later challenged to death with daily irradiation. Spalding *et al.*⁽⁹⁾ demonstrated a LTRL proportional to the amount of the conditioning dose of both gamma rays and 1.4-MV neutrons when conditioned mice were irradiated to death in a continuous gamma-ray field.

Thus, two basic theories about the nature of the LTRL persist in the confusion of conflicting reports [the LTRL is expressed as (a) dose-related irreparable injury that does not affect the recovery or repair mechanism or rate, or as (b) dose-related injury to the repair mechanism only], and much more must be done before reliable predictions of long-term effects in man can be expected. The present investigation was done to obtain information on the

ameliorating effects on the prompt and long-term injury of dose protraction by fractionation in the monkey and to evaluate the two theories mentioned above in practical terms.

II. METHODS

We used 16 monkeys (*Macaca mulatta*), 6 males and 10 females, approximately 6-8 yr old. We gave 10 of them [12 monkeys were started on this experiment; one male died after the 14th 100-rad exposure and one female after the 23rd exposure (data from these monkeys not included in this report)], 3 males and 7 females, 24 separate 100-rad doses of gamma rays at approximately 35 rad/hr administered 56 days apart. Starting 84 days after the 24th exposure, we irradiated these monkeys, together with 6 nonirradiated controls (3 males and 3 females), to death during continuous gamma-ray exposures at 24 rad/23.5-hr day. Dose rates for these investigations were midbody air doses determined using 0.25-, 1.0-, and 2.5-R, high-energy, Victoreen chambers cross-calibrated by the National Bureau of Standards.

To test the hypothesis that the IIRL may decrease the recovery rate and be related to total exposure, we determined hematopoietic response to the 24 successive acute 100-rad gamma-ray doses at 3, 6, 13, 27, and 51 days after each exposure. Blood samples were obtained by venipuncture. We took 2-ml or smaller samples to avoid hemorrhagic stimulation of the bone marrow. We tested the hypothesis that the irreparable component of the two-component concept of radiation injury (reparable injury with a recovery halftime of 28 days and 10% of any given injury remaining as an "irreparable lesion")⁽⁷⁾ produces a decrement in mean survival time and hematopoietic response during continuous gamma-ray exposure at 24 rad/23.5-hr day, starting 84 days after the 24th 100-rad exposure [three suggested repair halftimes for man,⁽⁷⁾ at which time a theoretical lesion equivalent to 255 rad should have been induced]. Hematopoietic values of control and conditioned animals were compared at 7-day intervals during these terminal exposures.

III. RESULTS AND DISCUSSION

Mean packed-cell volume (PCV), lymphocyte, and neutrophil values observed 51 days after each

Fig. 1. Packed-cell volume, lymphocyte, and neutrophil values for 10 monkeys (percentage of pre-exposure levels) 51 days after each of 24 gamma-ray doses of 100 rad.

of 24 separate 100-rad gamma-ray exposures are plotted as percentages of pre-exposure levels in Fig. 1. The PCV remained within 80% of the pre-exposure level throughout the fractionated radiation regime. After the first 5 exposures, lymphocytes and neutrophils dropped to approximately 50 and 30%, respectively, of pre-exposure levels.

We performed hemograms 3, 6, 13, 27, and 51 days after each of the 24 successive 100-rad exposures. Injury and recovery from exposures 2-24 were compared with those from the first 100-rad exposure for indications of residual injury accumulating with dose. Figure 2 shows the comparative PCV response to the first 100-rad exposure and to the 6th, 12th, 18th, and 24th exposures. The same comparisons for white-blood-cell (WBC) response are shown in Fig. 3.

Fig. 2. Packed-cell volumes of 10 monkeys 3, 6, 13, 27, and 51 days after the 1st, 6th, 12th, 18th, and 24th 100-rad gamma-ray exposures. The zero value is actually that of the 51-day bleeding from the previous exposure.

Fig. 3. White-blood-cell values of 10 monkeys 3, 6, 13, 27, and 51 days after the 1st, 6th, 12th, 18th, and 24th 100-rad gamma-ray exposures. The zero value is actually that of the 51-day bleeding from the previous exposure.

The maximum cellular depression of leukocytic and erythrocytic components generally occurred 6-13 days after exposure. Recovery of the erythrocytic series occurred somewhat sooner (approximately day 13) than that of the leukocytic series (approximately day 51). Packed-cell volumes dropped and recovered to varying degrees between exposures, but the maximum depression and recovery did not appear to be related to the number of exposures or total accumulated dose (Fig. 2). Although the white-cell counts were depressed below pre-exposure levels, the recovery slopes following each exposure appeared similar (Fig. 3).

We investigated the extent and nature of the hematopoietic LTRL using PCV and WBC analysis 6 and 51 days after each exposure. The mean PCV plots are shown in Fig. 4, and similar WBC plots are presented in Fig. 5. A straight-line fit through 6th day PCV values revealed a slope expressed by the function $Y = 45.6 - 0.085x$. The t-test ($t = 1.86$) showed the slope to be significant at the 10%, but not at the 5%, level. The slope for 51st day PCVs ($Y = 47.3 - 0.141x$) was significant ($t = 2.69$) at the 5% level. Thus, PCV did appear to be decreasing significantly with each successive exposure or with increasing dose (Fig. 4).

Fig. 4. Fitted-line plots through PCV values of 10 monkeys 6 and 51 days after each of 24 gamma-ray doses of 100 rad.

Fig. 5. Fitted-line plots through WBC counts of 10 monkeys 6 and 51 days after each of 24 gamma-ray doses of 100 rad.

To determine the relationship between decreasing PCV and successive 100-rad exposures at 56-day intervals, we determined the magnitude of recovery from the 6th to the 51st day after each exposure by fitting a straight line to the data. The regression function representing these data was $Y = 1.04 - 0.0012x$, and the slope was not significantly different from zero ($t = -1.15$). The two observations stated above, that (a) a significant residual injury as shown by a progressive decrease in PCV values on the 51st day after each of 24 successive 100-rad exposures and that (b) no significant difference in percentage of recovery occurs between the 6th and

51st days after each of the exposures, suggest that some sort of dose-related residual lesion is provoked in monkeys by injury from ionizing radiation but that it is not expressed as a decreased recovery rate. The white-blood-cell numbers plotted 6 and 51 days after each 100-rad exposure are shown in Fig. 5. There was an obvious drop after the first four exposures and a plateau thereafter; therefore, we fitted a line to exposures 4-24. The equation for the 6th day values was $Y = 3572.2 + 26.19x$. Neither the 6th nor the 51st day slope differed significantly from zero. Thus, after the striking effect following the first four separate 100-rad exposures, the leukocytic series showed no further residual injury with increasing dose.

The fractionation part of this study showed hematopoietic injury and recovery characteristics more consistent with Blair's hypothesis (i.e., a radiation-induced irreparable-injury component with a constant repair rate)⁽³⁻⁶⁾ than with the thesis that an irreparable lesion adversely affects the repair mechanism and thus changes the recovery rate.^(1,2)

The monkeys we studied showed few signs of radiation sickness during the course of the 24 100-rad exposures. We observed isolated cases of vomiting during or immediately after the first and subsequent exposures, but the incidence did not increase with accumulating dose.

According to the ERD concept of radiation-injury kinetics recommended for man,⁽⁷⁾ monkeys accumulating 2400 rad of gamma rays from 24 100-rad exposures at 56-day intervals would have the equivalent of 360 rad of injury immediately after the 24th exposure and approximately 255 rad [three suggested repair halftimes for man⁽⁷⁾] 84 days later. This 255-rad theoretical ERD would be a significant part (39%) of the acute LD_{50}^{30} of the monkey and would be expected to reduce the mean survival time (MST) in a radiation field intense enough to stress continuously the hematopoietic system.

We subjected the gamma-ray-conditioned and the nonconditioned monkeys to continuous 24 rad/23.5-hr day gamma-ray exposure until they died. The MST values for both groups are shown in Table I, and survival-time distribution is plotted in Fig. 6 along with the best fitting cumulative normal distribution. Mean survival times (Table I) for

TABLE I
MEAN SURVIVAL TIMES (MSTs) OF MONKEYS WITH AND WITHOUT RADIATION CONDITIONING EXPOSURES

Treatment	MST (in hr)
No conditioning dose	2261 ± 260
Conditioning dose totaling 2400 rad	2438 ± 267
MST reduction in conditioned monkeys	- 177 ± 372 ^a
Residual injury (LTRL) in conditioned monkeys	- 7.8 ± 16.5%

^aThe t-test for equality of means showed no significant difference in mean values.

Fig. 6. Survival-time distributions of conditioned and nonconditioned monkeys continuously exposed to gamma rays at 24 rad/23.5-hr day.

conditioned (2438 ± 267 hr) and nonconditioned (2261 ± 260 hr) monkeys did not differ significantly. The curve-fits of survival-time data showed essentially the same distribution for both groups (see Fig. 6). These data failed to reveal the "irreparable," or "long-term," residual lesion predicted by the theoretical ERD.⁽⁷⁾

Paterson *et al.*,⁽¹⁰⁾ using the split-dose and LD_{50}^{30} technique, reported complete recovery in monkeys within 20 days after acutely delivered 260-R conditioning doses. However, Eltringham,⁽¹¹⁾ using similar techniques but 400-rad conditioning doses on monkeys, reported very slow recovery and large

residual-injury levels beyond the 28th day of recovery.

We took blood samples from conditioned and nonconditioned monkeys at 7-day intervals during the continuous-challenge gamma-ray exposure. Mean PCV and WBC values are shown in Figs. 7 and 8, respectively. The PCV and WBC values of conditioned monkeys were lower than those of nonconditioned monkeys at the onset of this exposure. However, hematopoietic response to continuous gamma-ray stress was essentially the same for both groups.

We made differential counts to compare the response of the leukocytic elements originating in bone marrow and lymphatic tissue. Lymphocytes and neutrophils, respectively, of conditioned and nonconditioned groups are plotted in Figs. 9 and 10 as percentages of the group values observed just before the continuous gamma-ray stress. The relative response of neutrophils and lymphocytes to continuous gamma-ray exposure was similar in conditioned and nonconditioned monkeys. Monkeys conditioned with 2400 rad of gamma rays were able to maintain the same relative cellular-injury level (percentage

Fig. 7. Packed-cell volumes of conditioned and nonconditioned monkeys during continuous gamma-ray exposure (numbers indicate surviving monkeys included in the mean value at time of exposure).

Fig. 8. White-blood-cell values of conditioned and nonconditioned monkeys during continuous gamma-ray exposure (numbers indicate surviving monkeys included in the mean value at time of exposure).

Fig. 9. Neutrophils of conditioned and nonconditioned monkeys (percentages of the pre-challenge exposure and the preconditioning dose values) during continuous gamma-ray exposure (numbers indicate surviving monkeys included in the mean value at time of exposure).

Fig. 10. Lymphocytes of conditioned and nonconditioned monkeys (percentages of the pre-challenge exposure and the preconditioning dose values) during continuous gamma-ray exposure (numbers indicate surviving monkeys included in the mean value at time of exposure).

of the group's pre-challenge value) as those not previously exposed.

Peterson *et al.*⁽¹⁰⁾ reported similar findings using acute dose rates. Neutrophils and lymphocytes remained at low levels 7 and 20 days after 260-rad conditioning doses but showed recovery patterns like those of nonconditioned monkeys following subsequent challenge doses. When neutrophils and lymphocytes of our conditioned animals are plotted as percentages of pre-conditioning exposure values (Figs. 9 and 10), a more dramatic picture of hematopoietic resilience is revealed. Lymphocytes and neutrophils in conditioned monkeys had dropped to approximately 15 and 23%, respectively, of their observed pre-conditioning exposure values. The persistent reduction in leukocytic elements following 24 individual 100-rad gamma-ray exposures might be considered as an expression of the LTRL in the hematopoietic system. However, this possible LTRL does not impair the ability of the animal to resist

continuous low-dose-rate radiation exposure beyond that of control animals without the lesion.

Necropsies were performed on the monkeys as they succumbed to the lethal effects of continuous gamma-ray exposure. Females apparently had normal menstrual cycles during conditioning and challenge exposures. At death, the ovaries of all females appeared normal and showed all stages of follicular and oocytic development. Without exception, the male gonads were devoid of germinal activity. The seminiferous tubules contained only Sertoli cells. We found no areas of epithelial atrophy in the gastrointestinal tract, and the crypts of Lieberkuhn contained cells with mitotic figures of a normal frequency and pattern for animals of this age. Occasional petechial hemorrhages were found at necropsy; however, we found no frank hemorrhagic suffusions. The death of all monkeys, both conditioned and nonconditioned, was attributed to hematopoietic hypoplasia.

Casarett⁽¹²⁾ has reviewed factors that may influence patterns of recovery from nearly lethal injury caused by large single-dose exposures to ionizing radiation and has reported that recovery rate is related to size of the conditioning dose. However, Spalding *et al.*,⁽¹³⁾ using the split-dose technique, reported that the recovery half-time in mice was independent of size of the conditioning dose and that⁽¹⁴⁾ recovery rate of the hematopoietic tissue was independent of total accumulated dose from continuous exposure, which is consistent with these observations.

Conditions under which any segment of the human population would accumulate sublethal radiation doses of the magnitude of the conditioning doses given to subhuman primates in this investigation are highly unlikely. However, these doses (approximately four times the acute LD₅₀ dose) failed to produce a LTRL large enough to be measurable as a decrement in hematopoietic response or reduced survival time in a continuous radiation-stress environment. The sample size, of course, is suspect, but we can consistently measure residual injury from smaller doses in an equal number of mice. Thus, this is not to say that a persistent lesion was not provoked by these large conditioning exposures. However, if such a dose-related lesion

is provoked by exposure to ionizing radiation, its nature is such that its expression probably can be measured only with a much larger sample or by using a more sensitive end point.

ACKNOWLEDGMENTS

We are grateful to R. F. Archuleta for facility design and fabrication, to E. A. Vigil and J. S. Martinez for technical assistance, and to G. L. Tietjen of the LASL Theoretical Division for statistical services and data analysis.

REFERENCES

1. F. W. Furth, M. P. Coulter, and J. W. Howland, "Effect of a Second Large Dose of X-Radiation on Dogs Surviving One Dose of X-Radiation," in University of Rochester report UR-152 (1951), pp. 47-53.
2. J. B. Storer, "Rate of Recovery from Radiation Damage and Its Possible Relationship to Life Shortening in Mice," *Radiation Res.* 10, 180-196 (1959).
3. H. A. Blair, "A Formulation of the Injury, Life Span, Dose Relations for Ionizing Radiations. I. Application to the Mouse," University of Rochester report UR-206 (1952).
4. H. A. Blair, "A Formulation of the Injury, Life Span, Dose Relations for Ionizing Radiations. II. Application to the Guinea Pig, Rat and Dog," University of Rochester report UR-207 (1952).
5. H. A. Blair, "Some Properties of Reparable and Irreparable Radiation Injury," University of Rochester report UR-602 (1961).
6. H. A. Blair, "A Formulation of the Relation between Radiation Dose and Shortening of Life Span," *Proc. Internat. Conf. Peaceful Uses of Atomic Energy*, Geneva (1955) 11, 118-120 (1956).
7. National Committee on Radiation Protection and Measurements, "Exposure to Radiation in an Emergency," National Bureau of Standards report 29 (Washington, D. C., 27 October 1961).
8. G. A. Sacher, "A New Procedure for the Investigation of X-Ray Injury and Recovery and Some Preliminary Results," in Argonne National Laboratory report ANL-4163 (1948), pp. 85-87.
9. J. F. Spalding, V. G. Strang, and F. C. V. Worman, "Effect of Graded Acute Exposures of Gamma Rays or Fission Neutrons on Survival in Subsequent Protracted Gamma-Ray Exposures," *Radiation Res.* 13, 415-423 (1960).
10. E. Paterson, C. W. Gilbert, and M. W. Haigh, "Effects of Paired Doses of Whole Body Irradiation in the Rhesus Monkey," *Brit. J. Radiol.* 29, 218-226 (1956).

11. J. R. Eltringham, "Recovery of the Rhesus Monkey from an Acute Radiation Exposure as Evaluated by the Split-Dose Technique: Preliminary Results," *Radiation Res.* 31, 533 (1967), abstract.
12. G. W. Casarett, "Patterns of Recovery from Large Single-Dose Exposure to Radiation. . . Comparative Cellular and Species Radiosensitivity;" (Igaku Shoin Ltd., Tokyo, Japan, 1969), pp. 40-52.
13. J. F. Spalding, T. T. Trujillo, and W. L. LeSturgeon, "Dependence of Rate of Recovery from Acute Gamma-Ray Exposure on Size of the Conditioning Dose," *Radiation Res.* 15, 378-389 (1961).
14. J. F. Spalding and L. M. Holland, "Species Recovery from Radiation Injury," in *Proceedings of a Symposium on Survival of Food Crops and Livestock in the Event of Nuclear War*, U. S. Atomic Energy Commission report CONF-700909 (1971), pp. 245-258.