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THE ACUTE MORTALITY RESPONSE OF MONKEYS (MACACA MULATTA) TO PULSED MIXED GAMMA-NEUTRON RADIATIONS

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FOREWORD
(Nontechnical summary)

The acute mortality response to ionizing radiation has been studied in many laboratories. An extensive variety of animal species, radiation sources, and dose rates have been used. Studies of this type at the Armed Forces Radiobiology Research Institute (AFRRI) have emphasized the response of mammals to mixed gamma-neutron radiations.

The acute mortality response is characterized most often by the median lethal dose, or LD_{50}. This is the dose of radiation required to kill, within a specified period of time, 50 percent of the individuals in a large group of animals. Mammals vary in sensitivity to radiation, so that there is a unique LD_{50} for each species and often for variations or strains within a species. Furthermore, the LD_{50} is frequently a function of radiation type, energy, and/or dose rate.

LD_{50}'s are essential reference points for evaluation of shielding, changes in dose rate, changes in dose distribution, or other response modifying factors. Evaluation of different sources for biological effectiveness also requires a calibrated standard for comparison. An established LD_{50} for a given mammal can serve this purpose. In addition, study of the responses to lethal range doses in several larger mammals allows a more confident prediction of man's response to radiation.

The research reported herein determined the median lethality response of the monkey (Macaca mulatta) to a pulse (very high dose rate) of mixed gamma-neutron radiations from a TRIGA reactor. The $LD_{50/60}$ (60-day median lethal dose) was calculated to be 375 rads (midline tissue dose). Survival times, clinical responses, and pathological changes are also discussed.

ABSTRACT

The acute mortality response of the monkey (Macaca mulatta) to pulsed TRIGA reactor (mixed gamma-neutron) radiations was investigated to determine whether the $LD_{50/60}$ differed significantly from that established with the same source at a dose rate of 20 rads/min. Radiations were delivered unilaterally in a pulse with a width of less than 50 milliseconds at half height. Exposures were Class B, nonuniform. The $LD_{50/60}$ obtained was 375 rads, which did not differ significantly from the $LD_{50/60}$ of 381 rads for this source at a 20 rads/min dose rate. Clinical response, survival times, and hematological, pathological, and bacteriological changes are reported.

I. INTRODUCTION

Determination of median lethal doses (LD_{50}'s) for several species of mammals with respect to the different types, energies, and dose rates of radiations available is one research objective of the Armed Forces Radiobiology Research Institute (AFRRI). The LD_{50} is an important end point used frequently in radiobiological research as a reference parameter on which to base comparisons when modifying variables are introduced. It is used, for example, in evaluating the effect of dose rate changes, alterations in dose distribution, shielding specific organs or tissues, and protective agents. Several LD_{50} values have been determined at AFRRI.[4, 8-11]

The monkey (Macaca mulatta) has been an experimental animal of choice at AFRRI and elsewhere because of behavioral similarities, physiological similarities, and generally close biological relationship to man. Studies of the monkey's response to x and gamma radiations have been extensive.[1,2,7] Its response to mixtures of radiations, however, has only recently been investigated. Stanley et al.[9] reported the median lethal dose of the monkey exposed to mixed gamma-neutron radiations at a dose rate of approximately 20 rads/min, and Turbyfill et al.[12] studied monkeys exposed to pulsed (approximately 10^6 rads/min) mixed gamma-neutron radiations at two doses within the midlethal range. The present study determined the $LD_{50/60}$ for monkeys exposed to pulsed mixed gamma-neutron radiations.

II. MATERIALS AND METHODS

Animals

Young adult monkeys (Macaca mulatta) 2 to 4 years old and weighing from 3 to 6 kg were used. Age estimates were based on a dental formula[5] and on the

ratio of weight to crown-rump measurement (ponderal index).[13] The animals were nearly equally divided as to sex. These monkeys were captured wild, tested twice for tuberculosis, and conditioned by a commercial dealer before delivery to AFRRI.

Monkeys were held in quarantine for a minimum of 2 months at AFRRI. During this standardization period they received periodic examinations and indicated treatments, and had two successive negative tuberculin responses. No drugs or medicinals were administered from 2 weeks before exposure until the experiment was concluded. At least two preirradiation blood samples were collected as a check on each animal's condition and to establish base line hematological values.

Food and Water

Throughout the experiment, monkeys were fed twice daily with standard Purina monkey chow pellets and fresh apple. Water was available ad libitum through a standard gravity reservoir-tube apparatus.

Exposure Methods

Eighty-five animals were used, including 19 controls. The monkeys were irradiated in Exposure Room #1 of the AFRRI-TRIGA reactor. Seven different doses were scheduled from 400 to 650 rads (expressed as kerma, free-in-air, at the center of the exposure volume). The dose was delivered unilaterally in a single pulse having a width of less than 50 milliseconds at half height. Restraint chairs designed to minimize stress were used. The chair was made of plastic materials, with adjustable neck, arm, and leg restraints, and with a footrest adaptable to monkeys of different sizes. The torso was held firmly against the back of the chair by a curved

plastic part which applied a fairly even pressure to most of the abdominal surface of the monkey and held the monkey's torso in a rather firmly fixed position. The animals could rotate their heads and move their arms and legs to a limited degree. The chair is illustrated in Figure 1. It was mounted on a wooden pedestal during irradiation. All monkeys were subjected to two periods of 1 hour each of restraint in the chairs on successive days a few days before irradiation to introduce them to this form of restraint and reduce excessive stress on the day of irradiation, as experienced by some of the monkeys in the study by Turbyfill et al.[12]

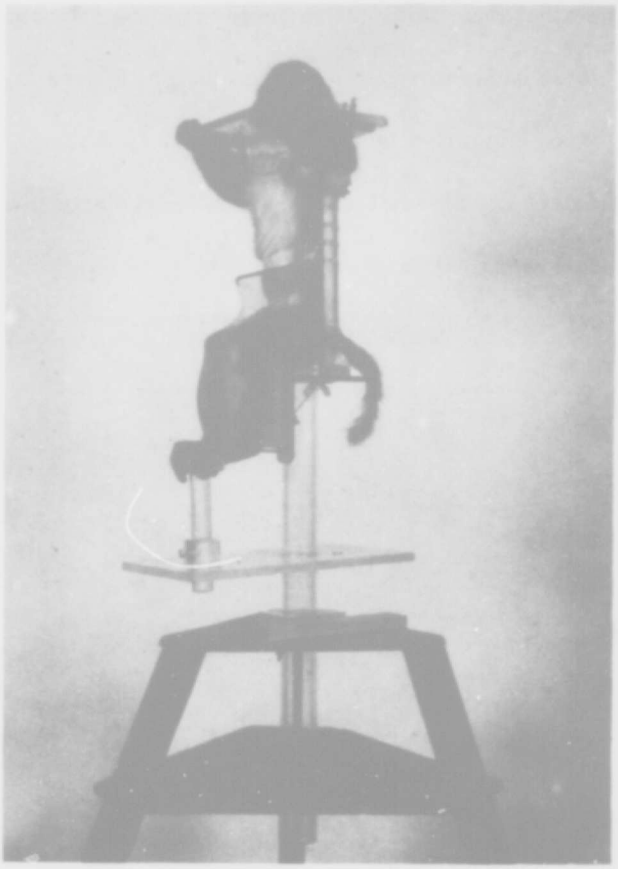

Figure 1. Monkey restraint chair

.The monkeys were provided food up to 1 hour before irradiation. They were put in the restraint chairs, moved from the animal room to AFRRI Exposure Room #1, and placed on the wooden stands.

Each dose group of monkeys was positioned in an arc within the exposure room so that their midlines were 400 cm from the reactor core vertical center line and their backs were toward the reactor (Figure 2). While in the exposure room, they were monitored by television to verify that proper positions were maintained, to detect any signs of undue stress before exposure, and to observe early postirradiation signs, such as vomiting.

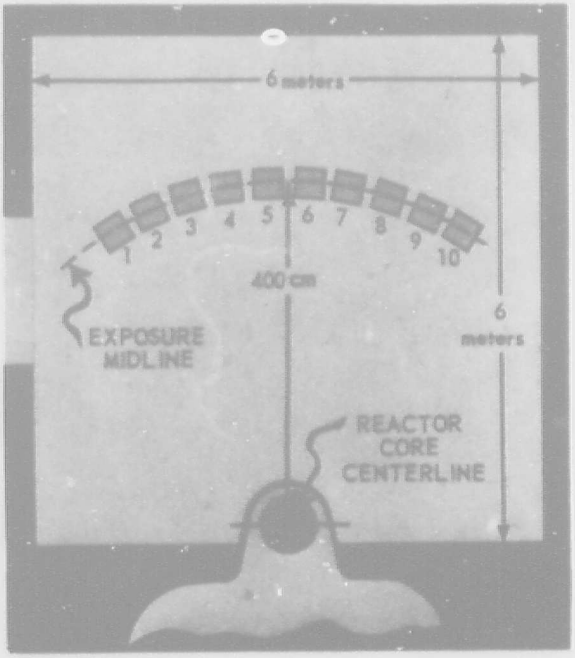

Figure 2. Plan view of reactor exposure arrangement

After irradiation, the monkeys were returned to their respective cages in the animal rooms. Their total time in the restraint chairs was approximately 65 minutes, with irradiation occurring at about the midpoint of this period. Control monkeys were restrained in the same manner and sham irradiated in the exposure room. Monkeys in one scheduled exposure group were in the restraining chairs for over 2 hours because of technical difficulties. These animals were excluded from the

experiment, since the added stress to w. h they were subjected probably influenced their response.

Radiation Environment and Dosimetry

Approximately 60 percent of the tissue kerma, free-in-air, was from gamma radiations having an effective energy between 1 and 2 MeV. Approximately 10 percent was from neutrons with energies greater than 3 MeV, 10 percent from neutrons with energies between 1.5 and 3 MeV, 10 percent from neutrons with energies between .01 and 1.5 MeV, and 10 percent from slower neutrons.

Midline tissue doses are reported. They were calculated from two factors. First, the ratio of absorbed dose at the midline of a tissue-equivalent mammal phantom to the tissue kerma, free-in-air, was calculated from miniature tissue-equivalent ionization chamber measurements in the phantom and 50 cm^3 cavity tissue-equivalent plastic-walled ionization chamber measurements at the center of the exposure volume. The value of this ratio was 0.8. Second, the tissue kerma, free-in-air, for each monkey exposure was calculated from the activation of sulfur tablets and fluorescence of silver activated phosphate glass rods. The product of these two factors provided the midline tissue dose. The variation in tissue kerma, free-in-air, from animal to animal for each exposure group varied less than 4 percent from the mean.

The dose profile illustrated in Figure 3 was obtained from measurements made in the tissue-equivalent mammal phantom. The ratio of the entrance dose to the exit dose was about 1.5. Exposures were, therefore, Class B, nonuniform, as defined in the International Commission on Radiological Units and Measurements Report 10e.[6]

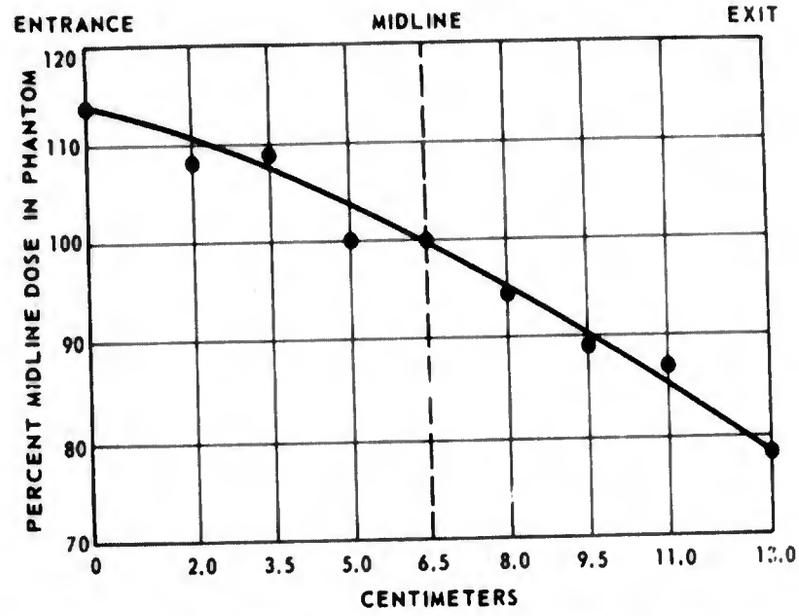

Figure 3. Dose profile -- unilateral mixed gamma-neutron radiations in tissue-equivalent phantom at 400 cm animal center line to core center line

Postirradiation Observations

The animals were observed continuously for 24 hours postirradiation, at 8-hour intervals during the next 18 days, and then once a day until the conclusion of the experiment 60 days after irradiation. Changes in activity and behavior, food intake, occurrence of vomiting and diarrhea, and any clinical responses which were apparent without handling the monkeys were recorded. Handling of the monkeys was avoided during the first 30 days to reduce the chance of altering the response by unnecessary stress.

Decedents were either necropsied shortly after death or were stored at $+2^{\circ}C$ until necropsies could be performed. Selected tissues of decedents were processed for histopathological examination and/or bacteriological culture.

Hematological studies were made of all survivors at 30 and 60 days postirradiation. Leukocyte, erythrocyte, and platelet counts were made with a Coulter counter, and routine methods were employed for hematocrits, hemoglobins, and differential leukocyte counts. Body weights of three dose groups were recorded before irradiation, at 30 and 60 days postirradiation for survivors, and at necropsy for decedents. The mortality data were analyzed by digital computer using a modified version of a United States Department of Agriculture probit analysis program, giving the maximum likelihood estimates of the parameters as described by Finney.[3]

III. RESULTS

Data on the acute mortality response of Macaca mulatta to pulsed mixed gamma-neutron radiations are presented in Table I. Over 90 percent of the monkeys which died, died between 10 and 21 days postirradiation. (One died on day 8, and three on day 9.)

Table I. Monkey Mortality Data

Midline tissue dose (rads)	Number of animals in group	Percent mortality	Mean survival time (days)	Survival time range (days)
336	10	30	17.3	13.7-20.7
360	10	40	16.4	13.7-19.3
360	8	37	14.9	14.3-16.0
384	8	75	13.7	11.0-16.3
448	10	70	14.0	8.3-17.0
504	10	100	12.2	7.3-15.7
528	10	80	13.2	10.0-17.7
SHAM (Controls)	19	0		

Controls remained healthy throughout the 60-day observation period. Mean survival times of the different dose groups ranged from 12.2 days for the 504-rad group to 17.3 days for the 336-rad group. Mean survival time for all decedents in the study was 13.9 days.

The $LD_{50/60}$ was calculated to be 375 rads (midline tissue dose), with a 95 percent confidence interval of 325 to 409 rads (Table II). No influence of sex on lethality was detected.

Table II. Results of Monkey Mortality Data Analysis

Percent mortality	Midline tissue dose (rads)	95% Confidence limits
10	269	162-315
30	327	248-362
50	375	325-409
70	429	394-498
90	522	464-744
Slope of Regression Line: 8.9		

Of the 66 monkeys irradiated, 79 percent vomited within 2 hours after irradiation; a majority of these episodes occurred between 30 and 60 minutes postirradiation. The incidence of vomiting during these first 2 hours did not vary greatly between eventual survivors (76 percent) and eventual decedents (80 percent). There was no significant difference among the seven dose groups in incidence of vomiting or time of vomiting.

Diarrhea occurred in 85 percent of the eventual decedents, and hemorrhagic diarrhea in 75 percent. Among the survivors, 88 percent experienced diarrhea, and 40 percent had hemorrhagic diarrhea. In two eventual decedents, neither diarrhea nor vomiting occurred.

Petechial hemorrhages of the integument in most of the irradiated monkeys occurred by day 11, signs of abdominal pains and malaise by day 4, onset of epilation by day 14, and edema of the face and neck around day 15.

Survivors from three dose groups, 360 rads, 448 rads, and 528 rads, were weighed at 30 days after irradiation. The mean weight losses of these groups expressed as percent of preirradiation body weights were 8 percent, 10.7 percent, and 17.3 percent, respectively. At 60 days, only a few monkeys had regained any of this loss. The mean weight loss of decedents in each dose group at time of necropsy ranged from 11.2 to 29 percent.

Appetites, judged by consumption of food, were depressed on the day of irradiation. This depression continued for 2 to 5 days in the lower four dose groups. Appetites were thereafter improved except that the eventual decedents became once again anoretic 2 or 3 days prior to death. In the 448-rad and higher dose groups, there was a temporary return of appetites on day 3 or 4, except that appetites of the 528-rad group remained depressed. Apple consumption frequently continued when no food pellets were eaten; over half of all eventual decedents continued to eat apples until the day of death.

Hematology

Blood samples collected from survivors on days 30 and 60 were examined, and the results are reported in Table III as percentages of preirradiation values.

Table III. Hematological Values of Survivors

	Percentage of preirradiation values (mean ± S. E.)	
	Day 30 (postirradiation)	Day 60 (postirradiation)
Leucocytes	73.4 ± 10.3	98.9 ± 12.1
Erythrocytes	70.9 ± 3.7	88.8 ± 2.4
Platelets	75.9 ± 7.8	96.7 ± 5.5
Hematocrit	72.3 ± 3.7	92.7 ± 2.7
Hemoglobin	75.9 ± 3.3	93.1 ± 2.7

Pathology

Prominent gross lesions found in decedents included hemorrhages in most organs and tissues, pneumonia, peritonitis, pleural adhesions, intestinal adhesions, ulcers and erosions of the large intestinal mucosa, and hyperemia of the kidneys. The ulcerations of the colon were usually deep into the muscle layers. Petechial hemorrhages were apparent in the skin of most decedents, as were petechial, ecchymotic, and suffusion hemorrhages under the epicardium and endocardium. The right heart was more frequently affected than the left heart. Diffuse myocardial hemorrhages were common (31 percent) in the three lower dose groups, and uncommon (4 percent) in the three higher dose groups. Hemorrhage of the gastrointestinal tract was commonly found with little or no dependency on dose. Other lesions of the gastrointestinal tract, including peritonitis, ulceration, and adhesions, were also seen

in all dose groups. Lesions of the respiratory tract were dependent upon dose, with pneumonia, consolidation, and adhesions common (62 percent) in the three lower dose groups and relatively uncommon (25 percent) in the three higher dose groups.

Preliminary histopathological studies demonstrated bacterial emboli in the vessels of the liver, lung, and kidneys of two decedents and in the vessels of the heart of one decedent which died suddenly while chewing an apple.

Bacteriology

Cultures were made from selected organs and tissues of 16 decedents. Enteric bacteria (E. coli, Pseudomonas sp., Proteus sp., and Aerogenes sp.) were demonstrated in heart blood, liver, spleen, and lymph nodes of 14 of these animals. Cultures from tissues of the other two animals were negative for any bacterial growth. The same combinations of bacteria were not found in all of the positive animals, but the bacterial species present in a given animal were consistent in most of the organs and tissues cultured.

IV. DISCUSSION

The $LD_{50/60}$ determined in this study for pulsed mixed gamma-neutron radiations, 375 rads (midline tissue dose), is not significantly different from the 381 rads (midline tissue dose) reported by Stanley et al.[9] for monkeys exposed bilaterally to the same radiations at a much lower dose rate (approximately 20 rads/min). The $LD_{50/60}$ for monkeys exposed to 250 kVp x rays reported by the same authors was 503 rads. The relative biological effectiveness (RBE) of AFRRI-TRIGA mixed gamma-neutron radiations for median lethality in the monkey was therefore calculated to be 1.3 for both pulsed and 20 rads/min irradiations. However, the low dose rate exposures were

Class A while those of this pulsed study were Class B, nonuniform. These results imply that the RBE for pulsed radiations of uniform distribution might be higher than those delivered at 20 rads/min, since the nonuniform distribution of the pulsed radiations might have spared a higher percentage of the hematopoietic stem cells than the lower dose rate uniform exposures for equal midline tissue doses.

The slope of the dose response curve indicates considerable response variation among individual monkeys. Nearly identical slopes were found in the low dose rate mixed gamma-neutron radiation and x ray studies.[9] Variability of response is attributed to the heterogeneous nature of wild caught monkeys.

The clinical responses were typical of those reported by others.[1,2,7,9]

The pathological changes seen at necropsy were primarily hemorrhagic lesions and lesions associated with bacterial infection. Both are consistent with a depressed hematopoietic system. Gross lesions of hemorrhage and infection which occurred more frequently in lower dose groups (diffuse myocardial hemorrhages, pneumonia, consolidation of the lungs, and pleural adhesions) probably were a result of longer survival times which increased the opportunity of these lesions to develop. The bacteriological cultures obtained from various organs and tissues shortly after death indicated that septicemia with enteric organisms was a common occurrence. The sudden death of one monkey while chewing an apple and in whose coronary arteries were found bacterial emboli emphasizes the many ultimate causes of death that are possible when such a septicemia exists. Although most deaths were attributed to septicemia and related toxemia, myocardial hemorrhages commonly found in the lower dose groups could have been responsible for some of the deaths.

V. CONCLUSIONS

The effects of whole-body exposure of the monkey (Macaca mulatta) to pulsed mixed gamma-neutron radiations were studied. The following conclusions were reached:

1. The $LD_{50/60}$ for the monkey (Macaca mulatta) was 375 rads (midline tissue dose).

2. Survival times were not dependent on dose in this range; nor did they differ from the survival times reported in studies using other radiation types, energies, and dose rates.

3. Vomiting was a prominent early effect, but was not found to be dose dependent.

4. The primary cause of death was septicemia, a complication of hematopoietic system damage.

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June 1968

MILITARY APPLICATION SUPPLEMENT
to
The Acute Mortality Response of Monkeys (Mucaca mulatta)
to Pulsed, Mixed Gamma-Neutron Radiations

This research report is a continuation of the overall effort to provide military commanders with sound command operational guidance relating to the effects of nuclear radiation on the human target. Although not conclusive in themselves, the findings from this experiment may contribute to the total data required for the construction or modification of tables and curves used by military commanders in predicting the effects of various doses of ionizing nuclear radiation on personnel. LD_{50} (lethal dose for 50% of the exposed population) studies of this type, performed on a number of species of animals, may result in extrapolation of dose-effect relationships to human responses following exposure to ionizing radiation.

Prepared by:
Military Analysis Department - AFRRI