EFFECT OF CONTINUOUS GAMMA-RAY EXPOSURE ON PERFORMANCE OF LEARNED TASKS AND EFFECT OF SUBSEQUENT FRACTIONATED EXPOSURES ON BLOOD-FORMING TISSUE

J. F. Spalding, L. M. Holland, J. R. Prine, D. N. Farrer,* and R. G. Braun*

Biomedical Research Group, Los Alamos Scientific Laboratory,
University of California, Los Alamos, New Mexico 87544

Sixteen *Macaca mulatta* monkeys trained to perform continuous and discrete-avoidance and fixed-ratio tasks with visual and auditory cues were performance-tested before, during, and after 10-day gamma-ray exposures totaling 0, 500, 750, and 1000 rads. Approximately 14 months after the performance-test exposures, surviving animals were exposed to 100-rad gamma-ray fractions at 56-day intervals to observe injury and recovery patterns of blood-forming tissues.

The fixed-ratio, food-reward task performance showed a transient decline in all dose groups within 24 hours of the start of gamma-ray exposure, followed by recovery to normal food-consumption levels within 48 to 72 hours. Avoidance tasks were performed successfully by all groups during the 10-day exposure, but reaction times of the two higher dose-rate groups in which animals received 3 and 4 rads per hour or total doses of 750 and 1000 rads, respectively, were somewhat slower. Performance of reward and avoidance tasks was equal to control levels 60 days after termination of gamma-ray exposures, showing no residual neurological injury reflected as a decrement in motivation to perform learned tasks. Peripheral blood characteristics showed dose-dependent injury to blood-forming tissue. Radiation-induced injury in higher dose groups reached critical levels, resulting in death of three subjects. During a 60-day recovery period, peripheral blood elements returned to control levels.

During the fractionated gamma-ray exposure regime, initiated 414 days after the performance-test exposures, bone-marrow suppression was observed after each 100-rad exposure. Recovery to pre-exposure levels was observed in all groups during each 56-day recovery period between exposures. All animals in each of the four pre-test dose groups were vigorous, active, and aggressive after fifteen 100-rad fractions, and the bone marrow of each group was sufficiently resilient to maintain animals in a clinically healthy condition.

Using acute, fractionated, or protracted exposure techniques to vary dose rate, the effects of exposure to large and small doses of ionizing radiations have been studied extensively in rodents. Reference material on this subject is too voluminous to cover here. Insight gained concerning the mechanisms or kinetics of radiation injury, both transient and irreparable, has contributed significantly to the establishment of radiation standards suggested by national and international committees as being acceptable for present and future generations. Civil defense and military agencies continue to require total-dose and dose-rate effects data. Looking forward to the next decade and the future manned space program, we can expect larger numbers of space career personnel to probe deeper into space for longer periods of time. Future space missions of longer duration will entail increased risk of solar flares, greater accumulations of ambient radiation from the space environment, and possible radiation exposure from on-board nuclear power systems. In short, a new set of radiation standards, acceptable for space professions and earth-originated nuclear emergencies, is required.

Earlier investigations with mice demonstrated the following. Dose-rate affects mean survival time during continuous exposure to cobalt-60 gamma rays (ref. 1) but does not affect mean after-survival time following equal discrete gamma-ray exposures with dose rates ranging from 2.5 to 250 rads per hour (ref. 2); there is a relationship between age and radiation sensitivity with age response to high-dose-rate discrete exposure being more pronounced than to low-intensity protracted exposure (refs. 3 and 4); there is a genetic involvement in radiation-induced hematopoietic injury (refs. 5 and 6); the recovery rate of hematopoietic tissue

*6571st Aeromedical Research Laboratory, Holloman Air Force Base, New Mexico.

is independent of the size of the acute (sublethal) conditioning dose (ref. 7) or total accumulated dose from continuous exposure (ref. 8); and under given exposure conditions, it may be possible to predict residual radiation-induced injury from challenge exposure doses (ref. 9). Many of these findings may, in principle, be applicable to human response to radiation experience. Suggested guidelines for emergency radiation-exposure limits (ref. 10), based on small-animal research and human radiation-accident data, have proved overly optimistic for subhuman primates (ref. 11). Therefore, radiation-exposure experience anticipated from space and earth-bound emergency conditions must be studied on mammals more closely related to man. The rhesus monkey (*Macaca mulatta*) is more closely related to man than is the rodent and has been recommended as the best "all-purpose" laboratory primate (ref. 12). The following is a report on a continuing program to obtain radiation-exposure experience with the subhuman primate (*Macaca mulatta*).

METHODS

Sixteen rhesus (*Macaca mulatta*) monkeys, 7 males and 9 females, weighing 4.5 to 5.4 kg, were trained to perform four tasks while seated in specially designed restraint chairs (figure 1). The tasks were as follows. *Continuous avoidance.*--The monkeys were required to press a lever (on the right-hand side of the panel in figure 1) at least once every 5 seconds while a red light above the lever was on. A mild shock from the seat bars was administered automatically if the task was not performed properly. Four-minute performances were required at closely spaced intervals. *Discrete avoidance with sound cue.*--A 1000-hertz tone was emitted aperiodically from a speaker in the upper center of the performance panel (figure 1). To avoid a mild shock, the animal had to terminate the sound within 3 seconds by pressing the stimulus-response key directly below the speaker. *Discrete avoidance with visual cue.*--Seven blue lights on the performance panel were lit individually according to a preset random schedule. Monkeys were allowed 3 seconds to press the stimulus-response key to switch the light off to avoid a shock. *Fixed ratio.*--A food reward was obtained by pressing a lever at the lower left side of the panel (obscured by the monkey in figure 1) 50 times when a yellow light directly over the lever was on. One-gram banana-flavored food pellets obtained in this manner plus half an apple each day made up the total diet of the monkeys during the test period.

Twelve monkeys were positioned in performance chairs in an arc around a cobalt-60 point source at distances that would provide dose rates of 2.13, 3.19, and 4.26 rads per hour, respectively, to three groups of four animals (figure 2). Four more monkeys were positioned behind shielding to act as a control group (figure 3). Gamma-ray dose rates were mid-body air doses measured with high-energy Victoreen chambers. Thermoluminescent dosimeter implants on the front and back of each monkey were used to check total-body doses during the performance test.

One-hour work schedules included seven auditory and 28 visual discrete avoidance cues, eight 4-minute continuous avoidance sessions, and one 15-minute fixed-ratio food reward session. Six 1-hour work sessions were followed by a 6-hour rest period, during which the room was dimmed to near

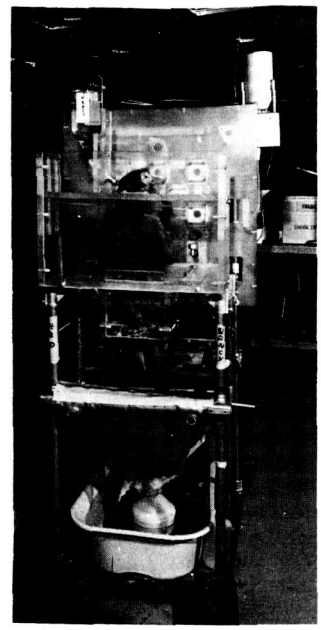

FIGURE 1.--Restraint chair showing pillory neck and waist plate. The performance panel with auditory and visual pressure disconnect plates is in front of the monkey. The continuous avoidance lever is at the lower right of the panel, but the food reward lever is obscured by the monkey. The foot rest exercise unit may be moved by foot and leg pressure.

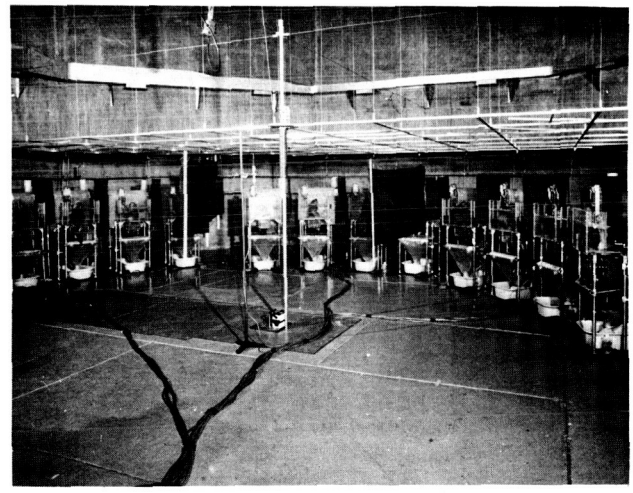

FIGURE 2.--Monkeys in performance chairs arranged in arcs around a cobalt-60 gamma-ray source (housed in tube in center of picture) at distances such that four monkeys in each of three groups would receive 2.13, 3.19, and 4.26 rads per hour, respectively.

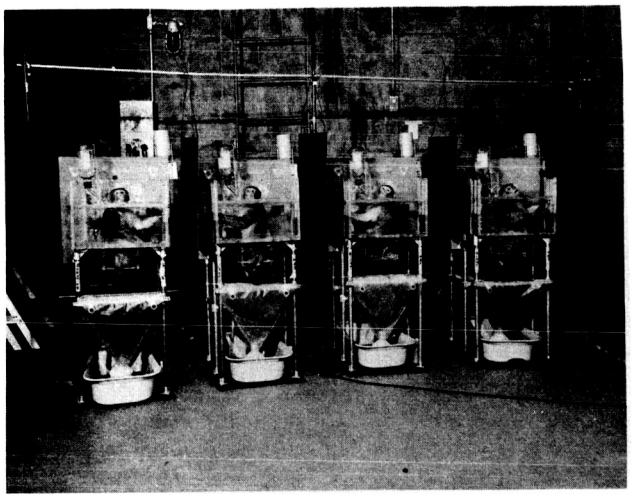

FIGURE 3.--Control monkeys arranged in performance chairs in shielded area of the source building.

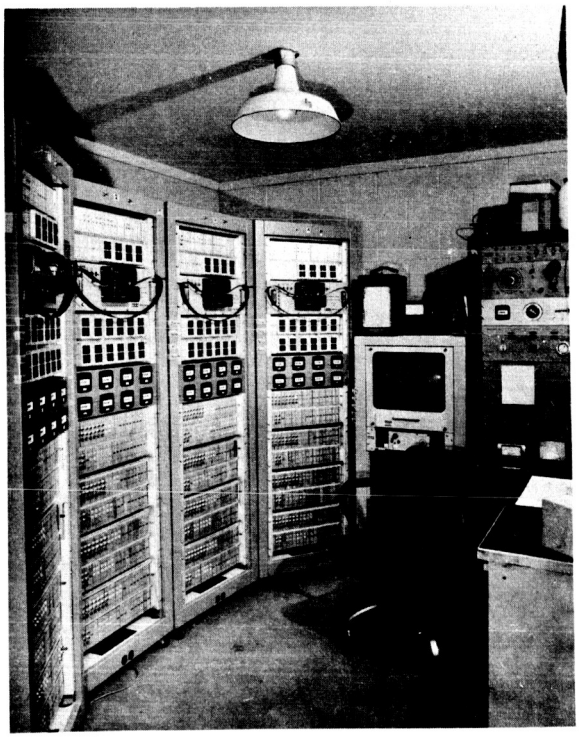

FIGURE 4.--Automated stimulus-presentation and data-collection equipment used to deliver task stimuli and to record performance data.

darkness. Animal care was performed daily during 30 minutes taken from one of the two 6-hour rest periods. Blood samples were also taken frequently. Automated stimulus-presentation and data-collection equipment was used (figure 4).

The performance test required 30 days: 10 before, 10 during, and 10 after exposure. Monkeys were transferred to comfortable cages after the test and allowed 60 days to recover from radiation injury. They were tested for possible residual effects during a second 30-day performance test without gamma-ray exposure.

Monkeys that survived the 500-, 750-, or 1000-rad gamma-ray exposure accumulated during the performance test were kept in comfortable monkey runs for 414 days to allow recovery from all repairable radiation-induced injury. After this extended recovery period, the animals in all the test groups and the control group were subjected to 100-rad whole-body gamma-ray exposures of approximately 40 rads per hour at 56-day intervals. Peripheral blood characteristics were observed before and between exposures.

RESULTS

A detailed analysis of the task-performance phase of this investigation will be published. Performance testing is discussed here primarily to show the radiation history of the animals used in the second, or fractionated-exposures, investigation. The three experimental groups performed learned tasks during the 30-day test (10 days before, 10 days during, and 10 days after gamma-ray exposure) in a like manner and did not differ from the control group. Control and irradiated groups performed all visual and auditory cue avoidance tasks with equal proficiency. The fixed-ratio, or food-reward, tasks showed a transient performance decrement beginning on the first day of gamma-ray exposure and lasting into the third or fourth day. The degree of this decrement was directly related to gamma-ray dose rate; the 2.13-rad per hour group showed the least effect, and the 4.26-rad per hour group showed the greatest.

One animal in the high-dose-rate group (1000 rads total dose) showed signs of serious radiation injury on the fifth day of the postexposure phase of the performance test and died on the eighth postexposure day. Although this animal was in a terminal stage of radiation injury, he showed no performance change until within 14 hours of death. During the 60-day recovery phase, two animals in the middle-dose-rate group (total accumulated dose of 750 rads in 10 days) died from radiation injury. The follow-up performance test after 60 days of recovery indicated that no animal surviving gamma-ray exposure had incurred irreparable radiation-induced injury measurable as a task-performance decrement. The irradiated monkeys performed all tasks at control level.

HEMATOLOGICAL OBSERVATIONS DURING AND AFTER LEARNED-TASK PERFORMANCE TESTING

Packed cell volume (PCV) values and white blood cell (WBC) counts of peripheral blood samples taken during and after the task-performance phase of this

investigation are shown in figures 5 to 8. A significant effect on PCV was apparent in all exposed groups 8 days after the cobalt-60 source was removed. The effects shown in figures 5 to 8 were dose-dependent and were greatest approximately 16 days after exposure. Recovery was rapid, and near-normal values were observed 37 days after the radiation stress was removed. The traumas of restraint and task-performance stimuli provoked an increase in WBC count in all groups during the 10-day pre-exposure test as shown in figures 5 to 8. White blood cell counts decreased within 2 days after whole-body gamma-ray exposure was started and dropped to critical levels in the two highest dose groups (750 and 1000 rads) within 8 days after the radiation source was removed (figures 7 and 8). Two animals in the 750-rad group and one in the 1000-rad group died of radiation sickness (the death of these animals was mentioned earlier). White blood cell recovery was evident in survivors by day 33 (13 days after end of exposure) and had returned to control levels 2 weeks later. Although the magnitude of injury was dose-dependent, the recovery rate appeared to be independent of dose (figures 6 to 8).

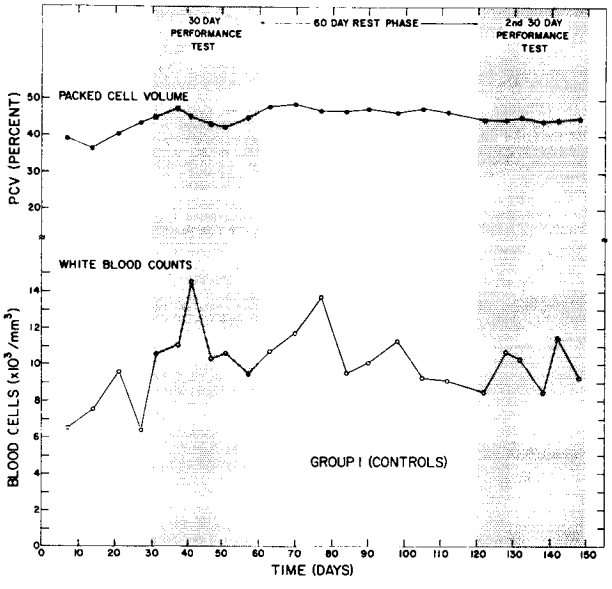

FIGURE 5.--Packed cell volume and white blood cell count plotted against time before, during, and after a 30-day task-performance test.

As might be expected, erythrocyte count (RBC) and hemoglobin (Hgb) levels paralleled PCV values. Lymphocytes, the most radiosensitive cellular element of peripheral blood, followed essentially the same injury-recovery pattern as did WBC. Lymphoid (monocytes and lymphocytes) and myeloid (neutrophils and eosinophils) ratios commonly ranged between 60 to 70 and 25 to 40, respectively. By the eighth day of exposure, these ratios were reversed with the degree of reversal being dose-dependent. Lymphoid and myeloid ratios stabilized at pre-exposure levels about 60 days after gamma-ray exposure.

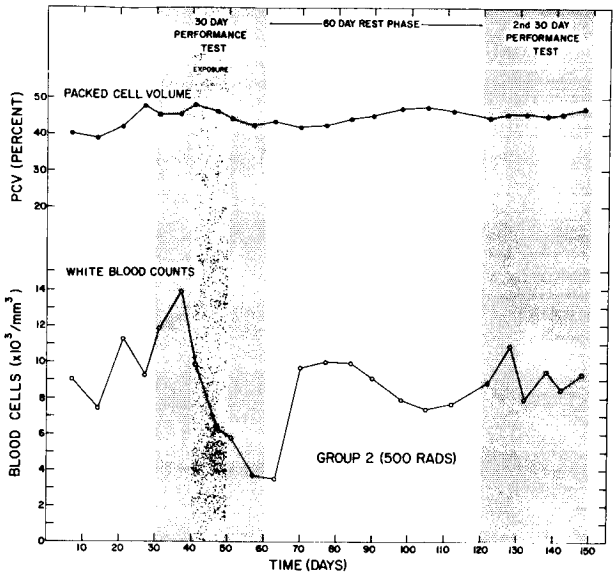

FIGURE 6.--Packed cell volume and white blood cell count plotted against time before, during, and after a 30-day task-performance test, including 10 days of gamma-ray exposure totaling 500 rads.

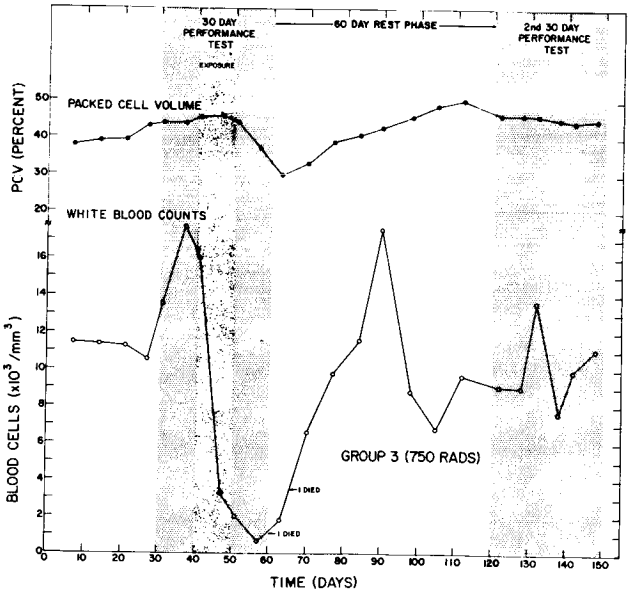

FIGURE 7.--Packed cell volume and white blood cell count plotted against time before, during, and after a 30-day task-performance test, including 10 days of gamma-ray exposure totaling 750 rads. Two animals died from radiation sickness at the times indicated on the WBC plot.

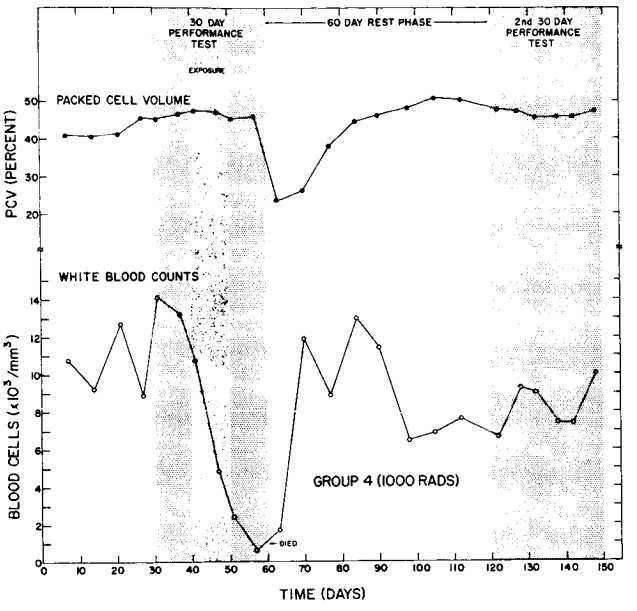

FIGURE 8.--Packed cell volume and white blood cell count plotted against time before, during, and after a 30-day task-performance test, including 10 days of gamma-ray exposure totaling 1000 rads. One animal died from radiation sickness at the time indicated on the WBC plot.

CLINICAL FINDINGS AND MORTALITY

Anorexia was present in varying degrees in all exposed animals during the first 2 or 3 days of exposure. However, all monkeys surviving the gamma-ray exposure had regained their appetites by the end of the 10-day exposure period. Exposed animals exhibited some diarrhea, and feces of those in the two higher dose groups were frequently bloody, indicating severe gastrointestinal damage. Several monkeys in the two higher dose groups showed petechiae and ecchymosis, which were most apparent on the conjuctivae and gingiva and at points of body contact with the restraining chairs. Menstrual-period cycling appeared to be unaffected by the 10-day gamma-ray stress. The three animals that died of radiation sickness suffered hematuria, and one of the two 750-rad monkeys exhibited paralysis of the right arm and left eyelid before death.

HEMATOLOGICAL RESPONSE OF MONKEYS TO 100-RAD GAMMA-RAY EXPOSURES DELIVERED AT 56-DAY INTERVALS

Peripheral blood samples taken from the saphenous vein at frequent intervals before and after each 100-rad gamma-ray exposure were analyzed for red blood cells (RBC), white blood cells (WBC), hemoglobin (Hgb), packed cell volume (PCV), and differential counts. Platelet counts were made several times but not routinely. Packed cell volume and WBC values of groups 1 through 4, respectively, are shown in figures 9 to 12. The response patterns of PCV, Hgb, and RBC to the 100-rad exposure regime were similar to each other, and lymphocytes followed the injury-recovery pattern of WBC values. Packed cell volumes ranged from 40 to 50% during the 840-day period in which the animals received fifteen 100-rad exposures. No significant differences in injury response to or recovery from the 100-rad fractions have been seen in PCV values of the four groups investigated. Thus, group 4 animals (figure 12) with 1000 rads of gamma-ray exposure 414 days before the start of the 100-rad fractionation regime appeared to have radiation resistance and recovery characteristics similar to those of group 1 (figure 9) which had no gamma-ray exposure before the fractionation study.

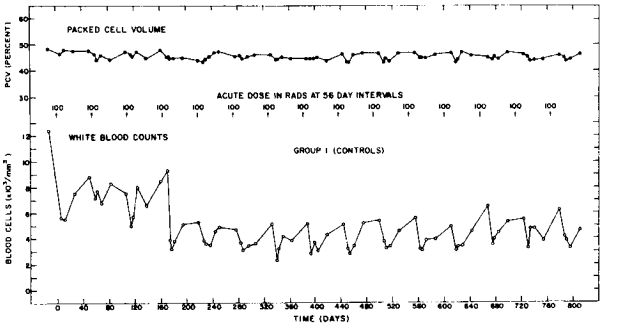

FIGURE 9.--Packed cell volume and white blood cell count of group 1 monkeys plotted against time during a fractionated gamma-ray exposure regime, including fifteen 100-rad exposures given at 56-day intervals. Group 1 monkeys had received no gamma-ray exposure prior to the fractionation series.

Lymphoid (monocytes and lymphocytes) and myeloid (neutrophils and eosinophils) ratios have fluctuated with the bone-marrow injury and recovery associated with each of the fifteen 100-rad gamma-ray fractions, but these ratios have not been scrutinized yet. White blood cells were depressed to somewhat lower levels after the first four 100-rad exposures and have maintained these lower levels (figures 9 to 12). Platelet counts done at irregular intervals on animals in all four groups were within the normal range (79,000 to 368,640) reported by Krise and Wald (ref. 13).

A major objective of this investigation is to determine the extent of radiation-induced "irreparable bone-marrow injury" using peripheral blood elements as recovery response indicators. Comparative PCV and WBC injury-recovery responses of peripheral blood from the four groups following the first and sixteenth 100-rad exposures are shown in figures 13 to 15. Packed cell volume and WBC responses from the first 100-rad exposure were extremely uniform for each of the four groups regardless of radiation history. Thus, if irreparable or residual injury remained from earlier exposures in the three exposed groups, it was not apparent in the cellular observation of peripheral blood after a single 100-rad exposure.

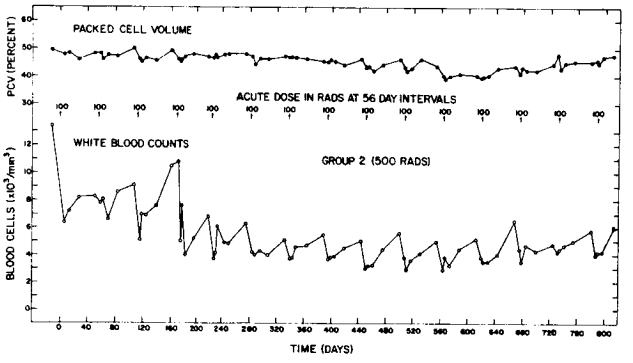

FIGURE 10.--Packed cell volume and white blood cell count of group 2 monkeys plotted against time during a fractionated gamma-ray exposure regime, including fifteen 100-rad exposures given at 56-day intervals. Group 2 monkeys had received 500 rads of gamma rays protracted over 10 days 414 days before the fractionation series.

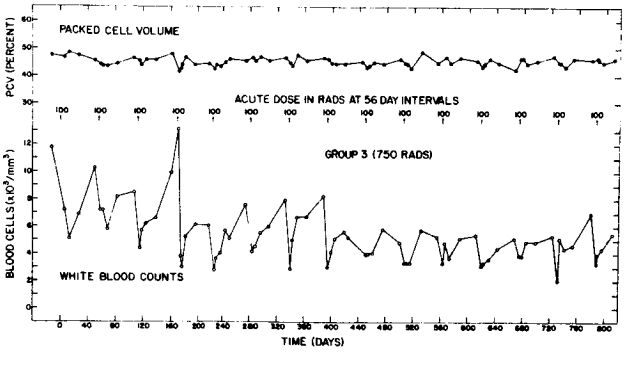

FIGURE 11.--Packed cell volume and white blood cell count of group 3 monkeys plotted against time during a fractionated gamma-ray exposure regime, including fifteen 100-rad exposures given at 56-day intervals. Group 3 monkeys had received 750 rads of gamma rays protracted over 10 days 414 days before the fractionation series.

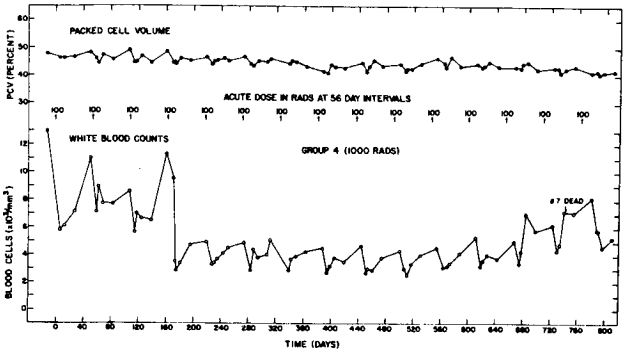

FIGURE 12.--Packed cell volume and white blood cell count of group 4 monkeys plotted against time during a fractionated gamma-ray exposure regime, including fifteen 100-rad exposures given at 56-day intervals. Group 4 monkeys had received 1000 rads of gamma rays protracted over 10 days 414 days before the fractionation series. One monkey (designated as #7) died from radiation sickness after the fourteenth 100-rad fraction.

Bone-marrow response from the sixteenth 100-rad exposure as reflected by PCV and WBC values is also shown in figures 13 to 15. The groups with radiation exposures of 500, 750, or 1000 rads 414 days before the fractionated gamma-ray study showed injury response and recovery capability comparable to that of group 1, which had not been exposed to gamma rays.

CLINICAL FINDINGS AND MORTALITY DURING FRACTIONATED GAMMA-RAY EXPOSURES

Two or three monkeys vomited during each of the 100-rad exposures, but there has been no increase in this number with additional exposures. Twenty-four days after the fourteenth 100-rad exposure, one animal in group 4 died. Symptoms were first observed only 2 days before death, and they were similar to the acute radiation death syndrome. Post-mortem examination of this monkey attributed death to generalized subcutaneous and visceral capillary hemorrhaging. The visceral organs exhibited focal hemorrhages much as would be expected in acute radiation death. The bone marrow was not depleted, and the myeloid-erythroid ratio appeared unchanged. The presence of many megakaryocytes in the marrow suggests that an adequate number of thrombocyte precursors was available. The mechanism that produced the capillary cellular permeability and resultant hemorrhage is not apparent from morphological findings.

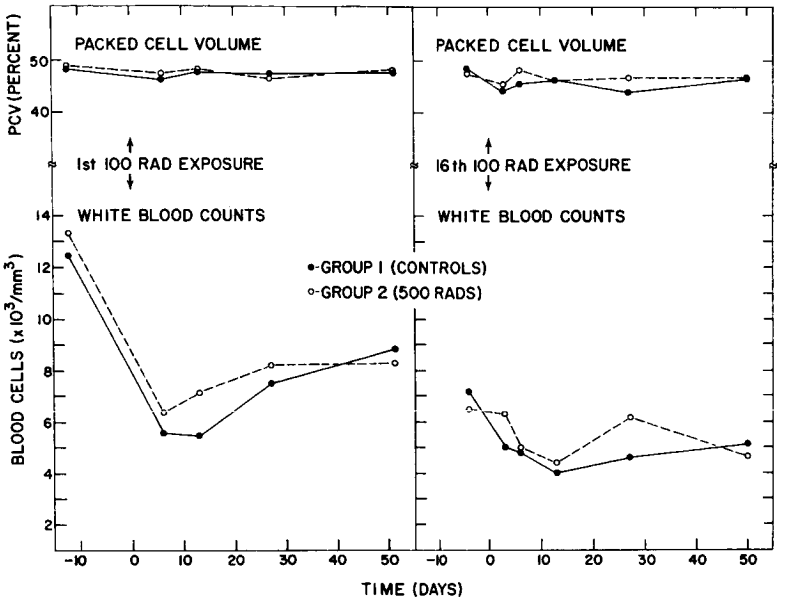

FIGURE 13.--Comparative packed cell volume and white blood cell count of group 1 (no gamma-ray exposure before fractionated exposures) and group 2 (500 rads of gamma rays before fractionated exposures) monkeys following the first and sixteenth 100-rad gamma-ray exposures.

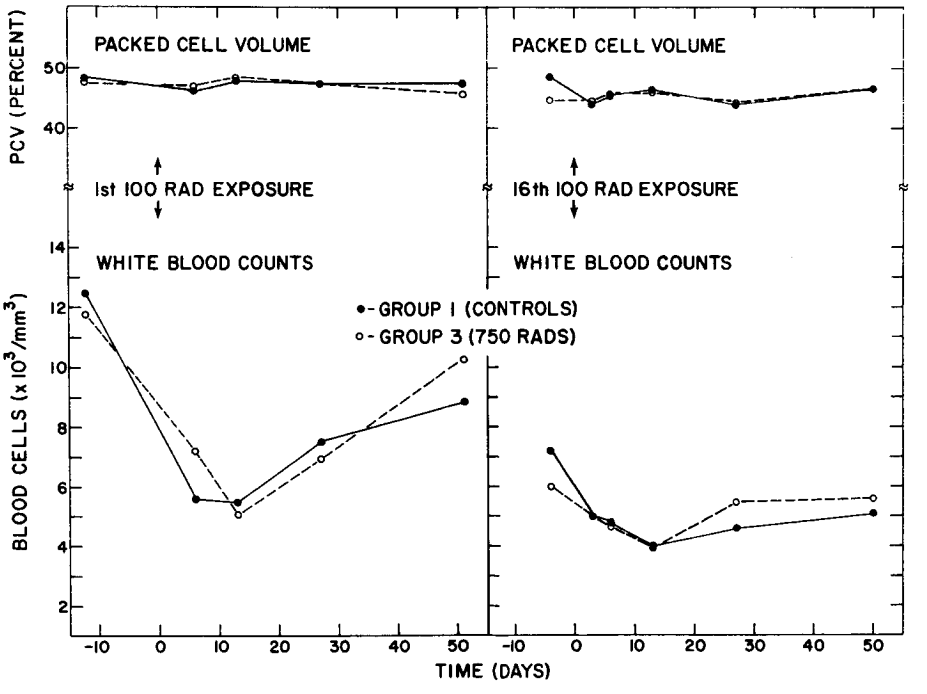

FIGURE 14.--Comparative packed cell volume and white blood cell count of group 1 (no gamma-ray exposure before fractionated exposures) and group 3 (750 rads of gamma rays before fractionated exposures) monkeys following the first and sixteenth 100-rad gamma-ray exposures.

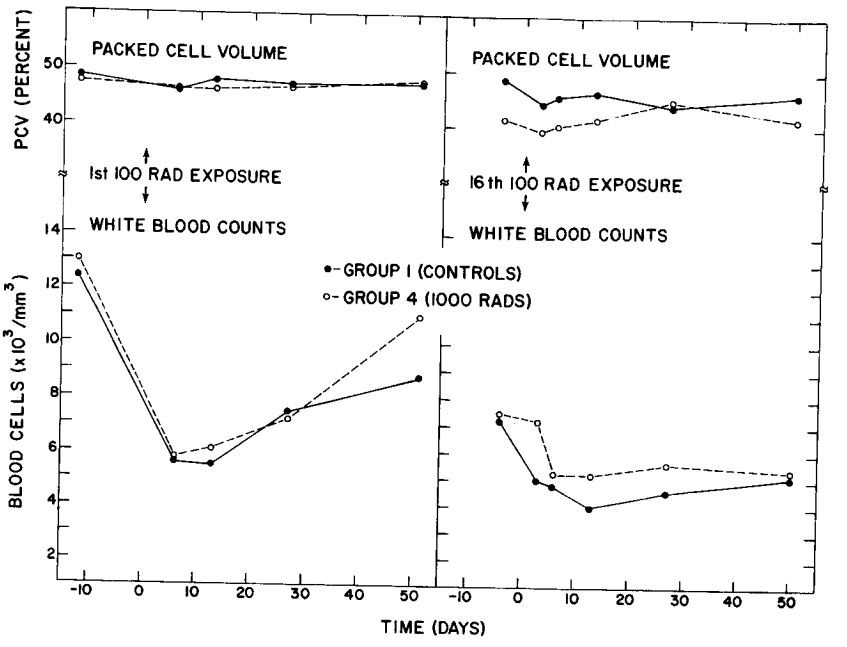

FIGURE 15.--Comparative packed cell volume and white blood cell count of group 1 (no gamma-ray exposure before fractionated exposures) and group 4 (1000 rads of gamma rays before fractionated exposures) monkeys following the first and sixteenth 100-rad gamma-ray exposures.

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