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PERFORMANCE OF THE MONKEY FOLLOWING TWO UNEQUAL PULSES OF RADIATION

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Atomic Support Agency
Bethesda, Maryland

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PERFORMANCE OF THE MONKEY FOLLOWING TWO UNEQUAL PULSES OF RADIATION

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FOREWORD
(Nontechnical summary)

Previous work in this laboratory indicates that a single 5000-rad pulse of radiation is more detrimental to the behavior of a monkey trained to perform a visual discrimination task than is the same dose delivered in two 2500-rad pulses separated by 6 hours. That is, in the 2 hours following delivery of the second 2500-rad pulse, performance was significantly better over this time period than that of the group receiving 5000 rads in a single pulse. The present experiment was initiated in order to see whether or not a 1700-rad pulse followed 6 hours later by a 3500-rad pulse would affect performance in the same manner as two 2500-rad pulses separated by 6 hours. The initial behavioral effects of the first 1700-rad pulse were equivalent to the initial effects of the first 2500-rad pulse, but performance in the first 20 minutes following the second pulse was as poor or poorer than in the first 20 minutes following the first pulse. This is different from the results of the 2500 + 2500 split dose group where the second pulse had less effect on performance than the first pulse. Beyond 20 minutes after the second pulse there was no significant difference between the two groups. Splitting the total dose into 1700-rad and 3500-rad pulses did have a less deleterious effect on performance both in the 2 hours following the second pulse and in the 20 minutes following the second pulse than giving the total dose in a single pulse. It is hypothesized that either there are slow and fast acting radioprotective or homeostatic mechanisms, with the fast acting not triggered by the 1700-rad pulse, or there is a single mechanism elicited by both the 2500- and 1700-rad pulses which does not persist in the latter case.

ABSTRACT

Ten monkeys housed in primate restraining chairs were trained to criterion performance on a shock avoidance visual discrimination problem. They were then exposed to a 1700-rad pulse of mixed gamma-neutron radiation followed in 6 hours by a 3500-rad pulse. The decrement in performance during the first 20 minutes following the first 1700-rad pulse was equivalent to the decrement in this time period following the first 2500-rad pulse in a previously reported 2500 + 2500-rad multiple exposure study. However, the decrement in performance during the first 20 minutes following the 3500-rad pulse was as great as that in the 20 minutes following the first pulse, in contrast to the 2500 + 2500 group where there was a smaller decrement following the second pulse than after the first pulse. Performance in the entire 2 hours after the second pulse of the 1700 + 3500-rad group was superior to that following a single 5000-rad pulse.

I. INTRODUCTION

Previous studies[5,6,8,9,11] on the effects of supralethal doses of pulsed gamma-neutron radiation on the performance of monkeys trained on a visual discrimination task indicate that most subjects will suffer a performance decrement within a few minutes after the pulse. Other studies in this laboratory suggest that giving the total radiation dose in two equal pulses separated by 40 minutes* to 6 hours[7] has effects on performance differing from a single pulse with the same total dose. These preliminary studies suggest that when the interval between the two 2500-rad pulses is 6 hours, performance following the second pulse is less debilitated than that following either the first 2500-rad pulse or a single 5000-rad pulse. In contrast, if the interval between the two 2500-rad pulses is shortened to 40 minutes, performance after the second pulse is more impaired than it was after either the first pulse for this group or the second pulse for the 6-hour group. The performance accuracy of the 40-minute group after the second exposure was approximately equivalent to that of the single 5000-rad group.

The question then arises as to whether similar differential effects after the second pulse could be obtained by making the dose levels of the two pulses unequal and by keeping the time interval between pulses constant. For example, with respect to performance after the second pulse, could a given ratio of the size of the first pulse to that of the second be found equivalent to a given time interval between equal pulses, where total dose is the same, and could a family of such equivalences be found? Answers to questions of this nature will provide clues as to the dose and/or

* Unpublished data

time-dependent factors in radiation damage. Questions raised by the INS* about the short-term effects on behavior of a 1700-rad pulse provided an opportunity to begin such an investigation.

II. PROCEDURE

Ten naive male Macaca mulatta monkeys weighing from 2.5 to 3.7 kg were used. The mean age of this group was approximately 2.5 years. The monkeys were maintained in primate chairs which were located in individual isolation cubicles.

The training procedure was a simplified version of that described in previous reports.[4,5] The monkey was required to make a visual discrimination between two stimuli, a circle and a square, presented simultaneously. Failure to choose the correct stimulus resulted in electrical shock to the monkey. Trials were presented every 10 seconds with each trial being initiated by simultaneous illumination of the two response keys and a 15-watt house light. If the animal failed to respond within 5 seconds of stimulus onset or responded to the incorrect stimulus the house light remained on, the stimuli extinguished, a tone was initiated and a brief electrical shock was delivered to the subject. If the animal responded correctly the house light and stimuli went out for the remainder of the 10-second trial interval. Trials were presented in blocks of 100 and each block was followed by a rest period. A block of 100 trials and the following rest period lasted 20 minutes.

Operant conditioning techniques were used to train each animal until a criterion of 90 percent correct responses for 2000 trials was reached. A base-line test under conditions similar to those of the radiation exposure was then conducted.

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* Institute of Nuclear Studies, U. S. Army Combat Developments Command, Fort Bliss, Texas

Two to three days after the base-line test the animals were placed in Exposure Room No. 2 of the AFRRI-TRIGA reactor for irradiation. The animals were positioned in the room where previous dose mapping had shown that they would receive a midline tissue dose (MTD) of 1700 rads from the first pulse and 3500 rads from the second pulse without changing the location of the monkey in the exposure room. The two dose levels were obtained by varying control rod insertions in the reactor core. The magnitude of each pulse was monitored through the activation of sulfur tablets located in a constant position near the subject prior to each exposure. The activation of these monitors was then related to the MTD and kerma from the data of previous dosimetry studies. The average dose was 1740 rads from the first pulse and 3520 rads from the second. See Table I for the MTD and tissue kerma, free-in-air, for each monkey.

Behavioral testing started with a block of 100 trials prior to the first exposure. The start of the second block of 100 trials coincided with the first pulse of 1700 rads and testing continued for 2 hours for a total of 700 trials. Subsequent testing then continued with blocks of 100 trials at 180, 240, and 300 minutes following the first pulse. Twenty minutes prior to the second pulse of 3500 rads, a second set of seven 100-trial blocks was initiated (at 340 minutes after the first pulse). The start of the second block of 100 trials in this set was simultaneous with the second pulse delivered 6 hours after the first pulse. At the end of the 700 trials the animals were removed from the exposure room, tested at 1-hour intervals for 6 hours and then at 2-hour intervals until death.

Table I. Radiation Doses Received by Monkeys in Two Pulses Separated by 6 Hours

Animal #	Kerma (rads)		Midline tissue dose* (rads)		Monkey weight (kg)	Approximate age (yrs)
	Pulse 1	Pulse 2	Pulse 1	Pulse 2		
213	2000	4300	1700	3700	2.9	2.5
214	2000	4200	1700	3600	3.3	2.5
216	2100	3900	1800	3300	2.5	2.5
217	2100	4200	1800	3600	3.6	2.5
222	2100	4200	1800	3600	3.4	2.5
226	2000	4300	1700	3700	2.7	2.5
227	2100	3900	1800	3300	3.6	2.5
230	2000	4200	1700	3600	3.4	2.5
240	2000	4000	1700	3400	3.5	1.5
244	2000	4000	1700	3400	3.7	2.0

* The midline tissue dose was obtained by multiplying the kerma value by a factor of 0.85

III. RESULTS

The data for the group of monkeys exposed to two 2500-rad pulses separated by 6 hours, the group of monkeys exposed to a single 5000-rad pulse, and the 1700 + 3500-rad group are presented in Figure 1 for direct comparison. Individual performance plots for the animals in the 1700 + 3500 group are presented in Appendix A.

1700 + 3500 intragroup comparisons. No statistically significant difference could be detected between mean correct responses during the base line and performance over the 2 hours following pulse 1, despite the initial dip in performance after the pulse. However, if a more detailed analysis is made immediately after pulse 1 (Figure 2), treating it in terms of 10-trial blocks, then a difference significant beyond the 2 percent level appears between the base line and the first 100 trials.* There is no significant difference between the base line and the second 100 trials after pulse 1. There were no statistically significant differences between the 600 trials following

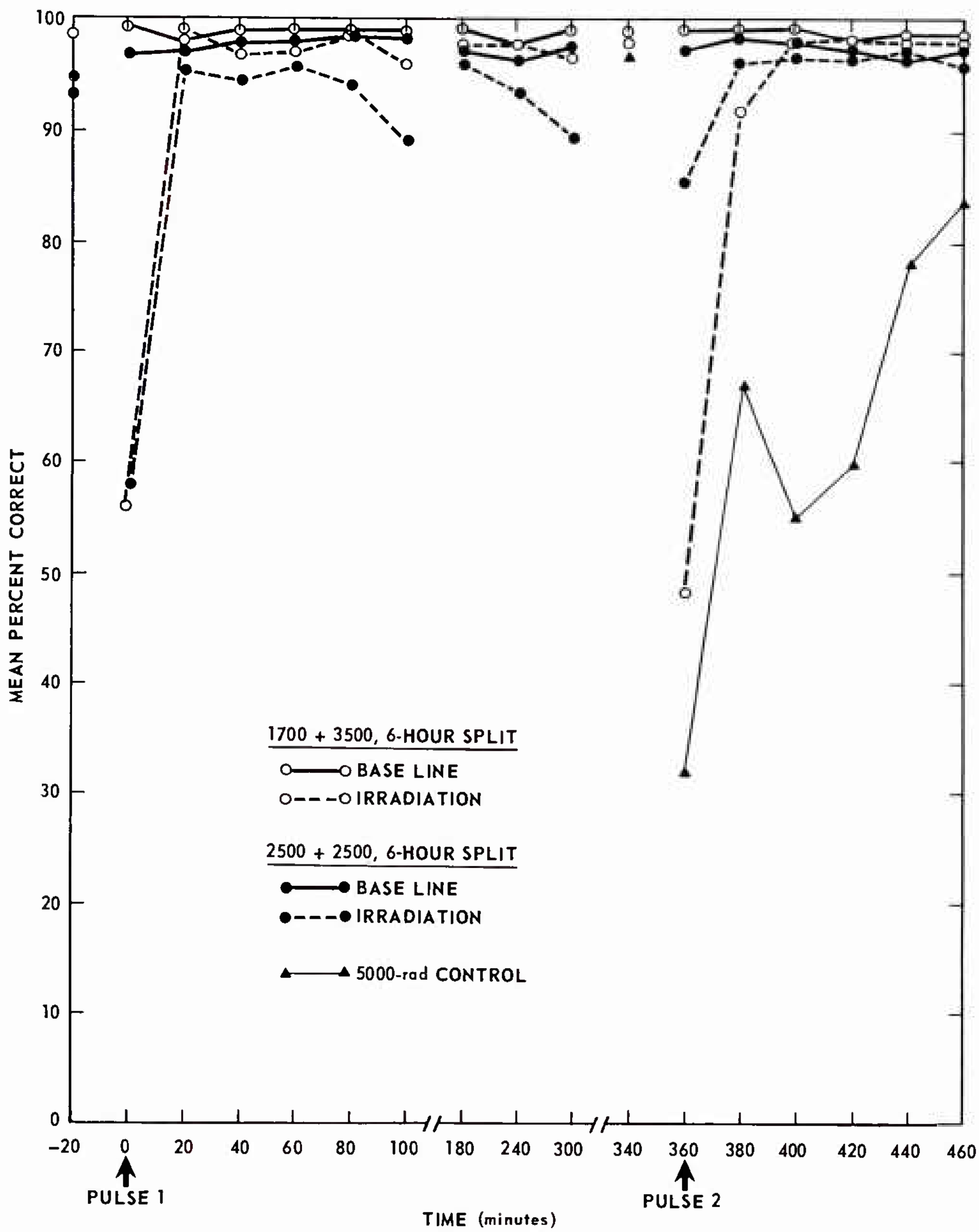

Figure 1. Postirradiation percentage correct performance for three groups of monkeys -- 1700 + 3500-rad 6-hour split, 2500 + 2500-rad 6-hour split, and 5000-rad single pulse control group

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* Either Wilcoxon or Mann-Whitney nonparametric tests

pulse 1 and the 600 trials after pulse 2, even though performance after the second pulse dropped to a low of 48 percent correct, compared with 56 percent correct after the first pulse (Figure 1). When compared with the base-line performance, there was a drop in mean correct responses significant at the 5 percent level after the second pulse of 3500 rads.

The effects on performance of a 1700-rad pulse and the interaction of this pulse with a subsequent 3500-rad pulse may be more subtle than the 2500-rad pulses of previous studies. For this reason, in Figure 3, the data are analyzed in the form of averages of percent correct response and latency to response over all 10 monkeys per block of 10 trials. The time period covers 20 minutes prior to each pulse to 2 hours following each pulse. First, in the top half of Figure 3, the mean percent correct

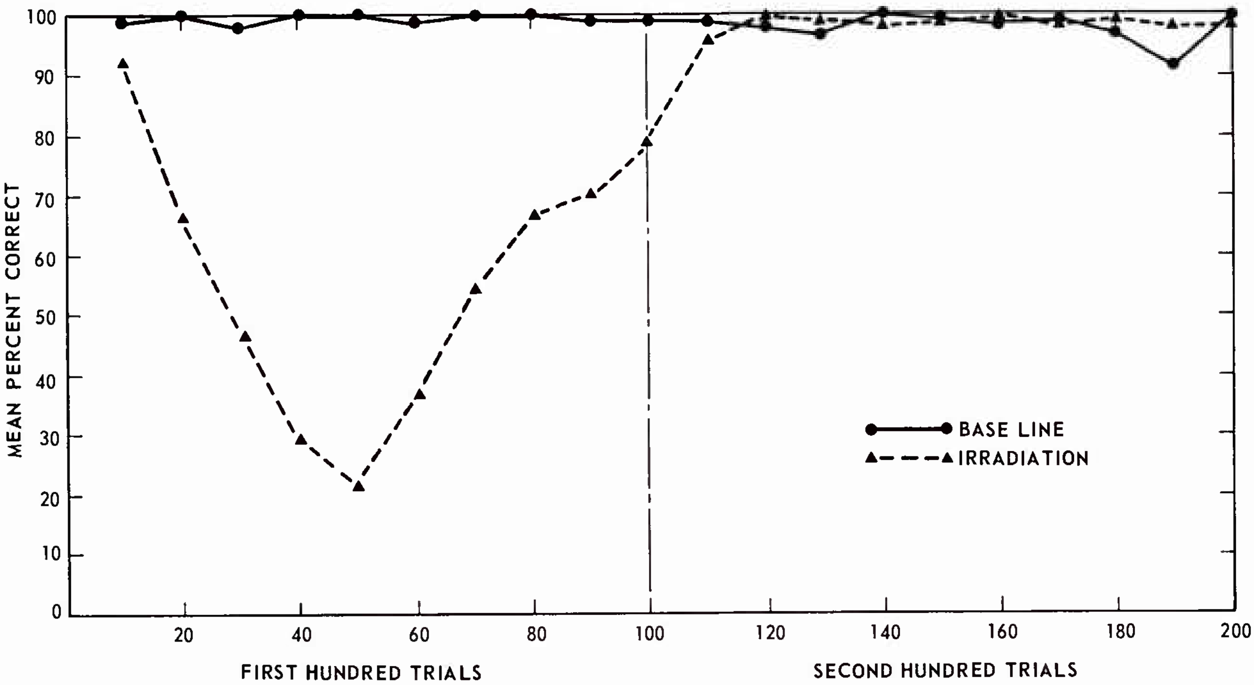

Figure 2. A detailed plot of the mean percent correct performance during the first 200 trials in 10-trial blocks after the 1700-rad pulse

responses are plotted. A least squares fit for a first order equation ($Y = a + bX$) has been calculated for the following sets of data points averaged over 10-trial blocks: (1) blocks 1-10 of the base line (B_{1-10}), corresponding to the 20 minutes prior to a sham pulse; (2) blocks 21-70 of the base line (B_{21-70}), corresponding to a period from 20 minutes to 2 hours after the sham pulse; (3) blocks 1-10 preirradiation (Pr_{1-10}), the 20-minute period preceding the radiation pulse; and (4) blocks 21-70

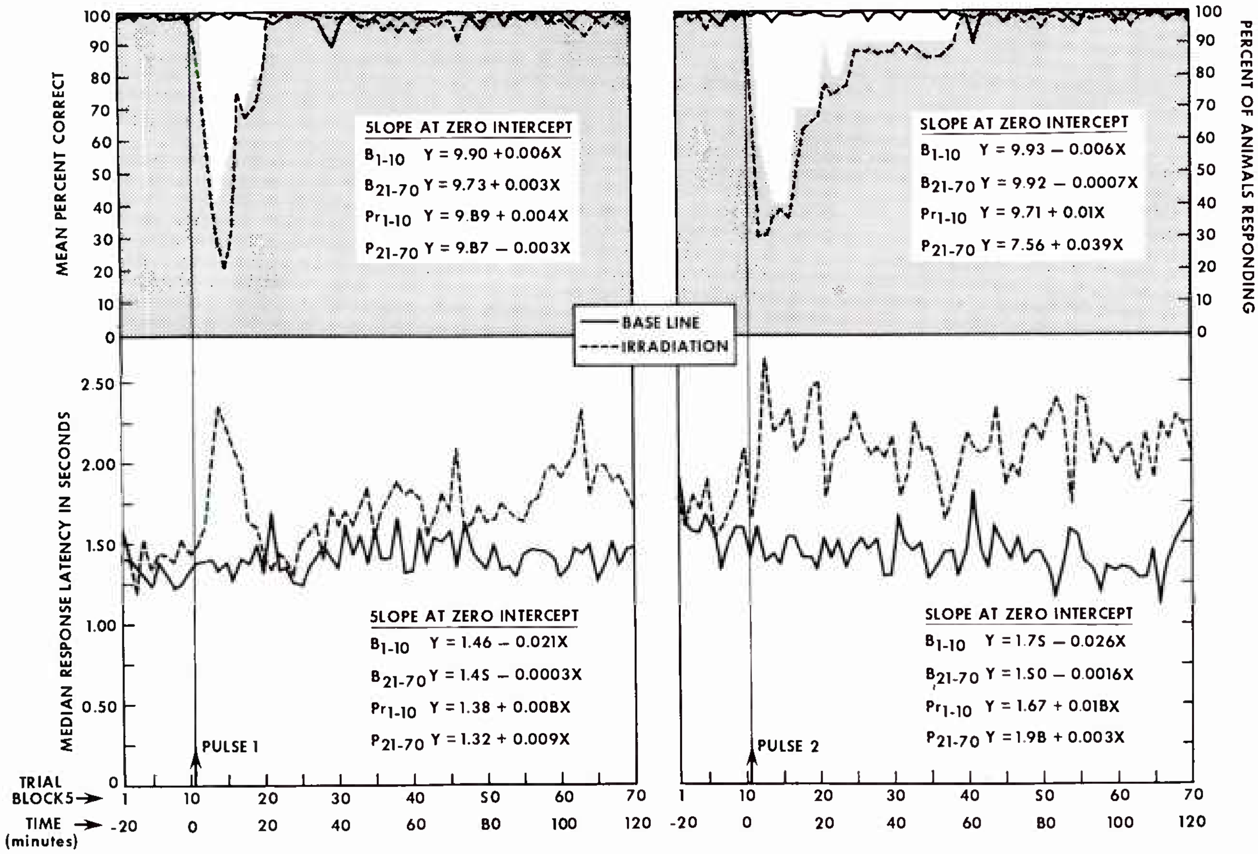

Figure 3. A plot of the performance of the 1700 + 3500-rad group in terms of 10-trial blocks from 20 minutes prior to each pulse to 2 hours after each pulse. The double abscissal scale is constructed so that one can locate data points in terms of either trial blocks or time relative to pulse. The upper curves are in terms of mean percent correct responses over all monkeys responding in the group. The ordinate scale for these curves is on the left. The shaded area indicates the percent of the population responding and the ordinate scale is on the right. The lower curves represent the median latency to response in seconds. Equations describing the zero intercept and slope of each curve are indicated.

postirradiation (P_{21-70}), the period 20 minutes after the pulse to 2 hours after the pulse. Blocks 11-20 (the 20 minutes immediately following sham or real pulse) cannot be fitted to a straight line in the case of the real pulse since pronounced changes are occurring after the pulse; therefore, these data were handled with nonparametric statistics and will be described later. It should be recalled that 6 hours elapsed between pulse 1 and pulse 2 and that intermediate data (seen in Figure 1) are not plotted in this figure. t-tests between the slopes of these lines of best fit through the percent correct data showed significant differences between PI_{21-70} and PII_{21-70}, BI_{21-70} and PII_{21-70}, BI_{21-70} and PI_{21-70}, and BII_{21-70} and PII_{21-70}.* t-tests between the data points of the curves indicated significant differences between BI_{21-70} and PI_{21-70}, BII_{21-70} and PII_{21-70}, PrI_{1-10} and $PrII_{1-10}$, and PI_{21-70} and PII_{21-70}. The height of the shaded area in the upper part of Figure 3 indicates the percent of the total population responding, whether correctly or not.

The lower half of Figure 3 is a plot of the median latency of responding over all 10 monkeys per 10-trial block. Medians were used rather than means since the distribution of latencies often tended to be skewed and the median is less affected than the mean by the presence of skewness. Straight lines were fitted to the latency data in the same manner as described for the mean correct response data. Within a 10-trial block, latency was calculated only for those monkeys actually responding (either correctly or incorrectly); those monkeys not responding within the 5-second limit set

* The Roman numerals I and II refer to pulse or base-line periods 1 or 2, respectively. For example, PI_{21-70} refers to the trial blocks 21-70 after the first pulse, PII_{21-70} refers to blocks 21-70 after the second pulse, BI_{21-70} refers to blocks 21-70 of base line 1, PrI_{1-10} refers to blocks 1-10 in the trials just preceding pulse 1, etc.

by the conditioning parameters, hence having a latency of 5 seconds or more, were not included. This means that one is looking at a radioresistant portion of the population of monkeys used in the study. t-tests between all possible pairs of interest indicated significantly different slopes in the latency data between BI_{21-70} and PI_{21-70}, PI_{21-70} and BII_{21-70}, and PI_{21-70} and PII_{21-70}. Additional t-tests between the data points of these latency curves showed significant differences between BI_{1-10} and BII_{1-10}, BI_{21-70} and PI_{21-70}, BII_{1-10} and $PrII_{1-10}$, BII_{21-70} and PII_{21-70}, PrI_{1-10} and $PrII_{1-10}$, and PI_{21-70} and PII_{21-70}.

As mentioned above, the 10-trial blocks from 11 to 20 were treated separately due to the curvilinear nature of the data in this region. The Mann-Whitney U test (one-tailed) for independent samples was used and indicated the following: in terms of percent correct, there are no significant differences between base lines 1 and 2 (BI_{11-20} and BII_{11-20}) nor between irradiations 1 and 2 (PI_{11-20} and PII_{11-20}); the differences between BI_{11-20} and PI_{11-20} and between BII_{11-20} and PII_{11-20} are both significant beyond the .001 level. In terms of median latency of response, there are differences significant at the .03 level or better between BI_{11-20} and BII_{11-20}, PI_{11-20} and PII_{11-20}, BI_{11-20} and PI_{11-20}, and BII_{11-20} and PII_{11-20}.

<u>1700 + 3500 - 2500 + 2500 intergroup comparisons</u>. When a series of U tests were run between appropriate subsets of data (percent correct) of the 1700 + 3500 and 2500 + 2500 groups the following was found: (1) in the first 20 minutes after pulse 2 there were significant differences between the 1700 + 3500 and 2500 + 2500 groups; in the 2500 + 2500 group there were significant differences in the first 20 minutes after pulses 1 and 2, in contrast to the 1700 + 3500 group where no significant

differences were detected in this time period; and (2) in the 20 - 100 minutes after each pulse there were significant differences between the 1700 + 3500 and 2500 + 2500 groups after pulse 1 and between pulses 1 and 2 of the 2500 + 2500 group. There were no significant differences in survival times between the 1700 + 3500 and 2500 + 2500 groups.

Control - multiple dose group comparisons. The single 5000-rad pulse control group is significantly more debilitated than either of the multiple dose groups in the 2 hours following delivery of the total dose.

IV. DISCUSSION

The study most pertinent to the present work is that of Germas and Shelton[7] which demonstrated a precipitous drop in performance in the first 100 trials after the first pulse of 2500 rads and a less severe deterioration 6 hours later after the second pulse of 2500 rads, as seen in Figure 1. The fact that performance over 600 trials after a pulse of either 1700 rads or 2500 rads did not quite reach a statistically significant difference indicates that, as far as this measure of radiation effects is concerned, the two doses in the time range of up to 20 minutes postpulse do not produce measurably different effects on behavior. However, from the second 20-minute block of trials after pulse 1 up to 5 hours postpulse, the 2500-rad group never quite achieves as high a performance level as the 1700-rad group, suggesting that while the initial "shock" effects of the two dose levels may be behaviorally equivalent, the higher dose has a slightly greater residual effect on performance. Both this study and that of Bogo et al.[1] show that in the period of 1 - 2 hours after a 1700- or 1500-rad pulse, percent correct response is near base-line levels, but there is a slightly increased latency of response. The necessity of taking measurements immediately after the pulse is indicated by our results in this case since the major behavioral changes occur within 20 minutes after the pulse.

Comparison of the effects of the second pulse are confounded by the fact that dose levels varied here also, one pulse being 2500 rads, the other 3500. It does seem clear that fractionating a 5000-rad total dose into the two unequal doses used here results in a greater deterioration of performance after the second pulse than equal dose fractionation. Whether this effect would hold up for other types of dose apportionment (a 3500 + 1700 split, for example) is to be determined. The possibility exists that the 2500-rad initial pulse triggered some radioprotective mechanism (as suggested by Thorp and Young[10]) and that the 1700-rad pulse did not trigger this mechanism. This hypothesis is confounded in this case, however, by the 1000-rad dose difference in the second pulses of the two groups. The data indicate that fractionating the total dose alleviates its effects on performance as shown by the depressed performance of the single 5000-rad group relative to the multiple dose groups.

Chaput and Kovacic[2] using pigs subjected to multiple doses ranging from 3400 + 3400 to 6500 + 6500 rads found, as did Germas and Shelton[7] in monkeys, that fractionating the dose had a radioprotective effect with regard to performance after the second pulse. This is similar to the results of this study relative to performance subsequent to 20 minutes after the second pulse, but differs relative to the first 20 minutes after the second pulse in the 1700 + 3500 group where the performance decrement was comparable to both the first 20 minutes after the first pulse and the first 20 minutes after the single 5000-rad pulse. However, a second study by Chaput and Kovacic[3] using pigs showed that the radioprotective effect of splitting the total dose into two equal pulses was lost when a 1700-rad pulse was followed in 1 hour by a 4800-rad pulse. These results are similar to our 1700 + 3500 group despite the differences in size of the second pulse and time interval between pulses. These data indicate there may be two recovery mechanisms at work, one involved in the fast "shock" effects immediately after the pulse and another slower acting one playing a role in longer acting phenomena. The 2500-rad first pulse was sufficient to activate protective or homeostatic mechanisms which persisted into the second pulse 6 hours later alleviating both initial "shock" effects of the second pulse and longer term effects, as indicated by the superior performance of this group relative to the control group. On the other hand, the 1700-rad pulse may have initiated only one mechanism which protected against long term effects but failed to protect against shock effects of the second pulse. An alternative hypothesis is that there is just one major radioprotective mechanism which is maintained in the 6-hour interval by the 2500-rad pulse, but not by the 1700-rad pulse.

The necessity of using finer measures of response decrement at relatively low doses such as the first pulse of 1700 rads is shown by the latency data of Figure 3. By about 20 minutes after the first pulse, group performance was back up to 90 percent correct or better, but the latency measure shows a continuing rise as demonstrated by the significant difference in slopes of these two portions of the curves. The greater sensitivity of the latency measure than the mean correct response measure in showing a behavioral alteration is clearly seen in blocks 11-20 where the latter measure revealed no differences between BI and BII and between PI and PII, but the latency measure does indicate significant changes in these regions. The value of multiple measures of performance is illustrated here in that there is no significant difference in slopes between latency curves for BII_{21-70} and PII_{21-70}, but the t-test. using the data points of the curves, shows a large difference between them. This points up the need to carefully define exactly what is meant by a "performance decrement" in animal studies and to delineate the aspects of any human task to which any animal study is to be extrapolated.

V. CONCLUSIONS

The results of this study and others in progress in this laboratory suggest that the behavioral effects of dose fractionation in the monkey are a function of the time interval between pulses and possibly also of the size of the first pulse relative to that of the second. The methodological value of using both a variety of measures of performance (percent correct response and latency of response), and several types of analysis (slopes of curves and data points of curves) of those measures is also illustrated.

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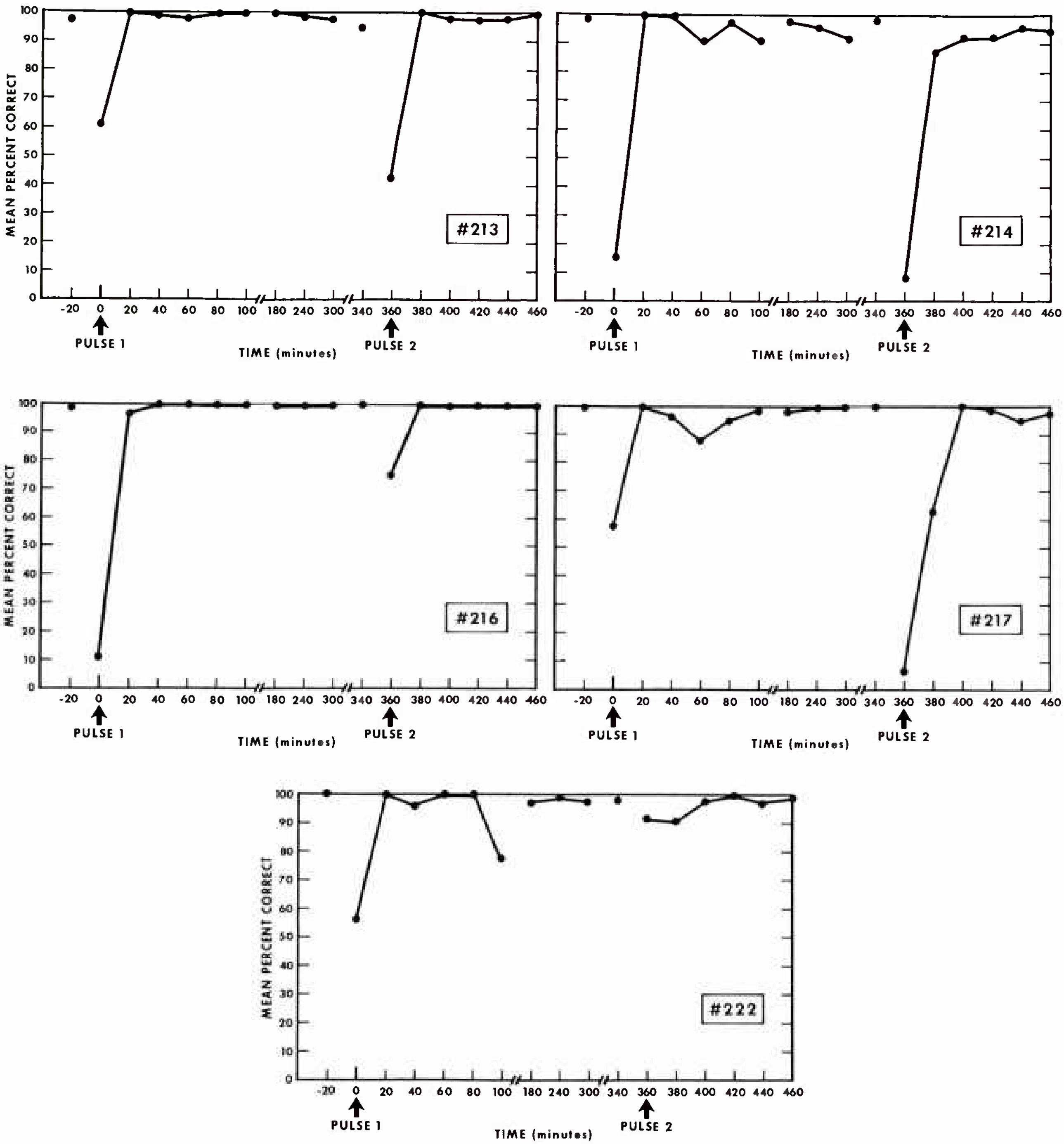

Figure A-1. Plots of the individual mean percent correct performances of the 1700 + 3500-rad 6-hour split animals

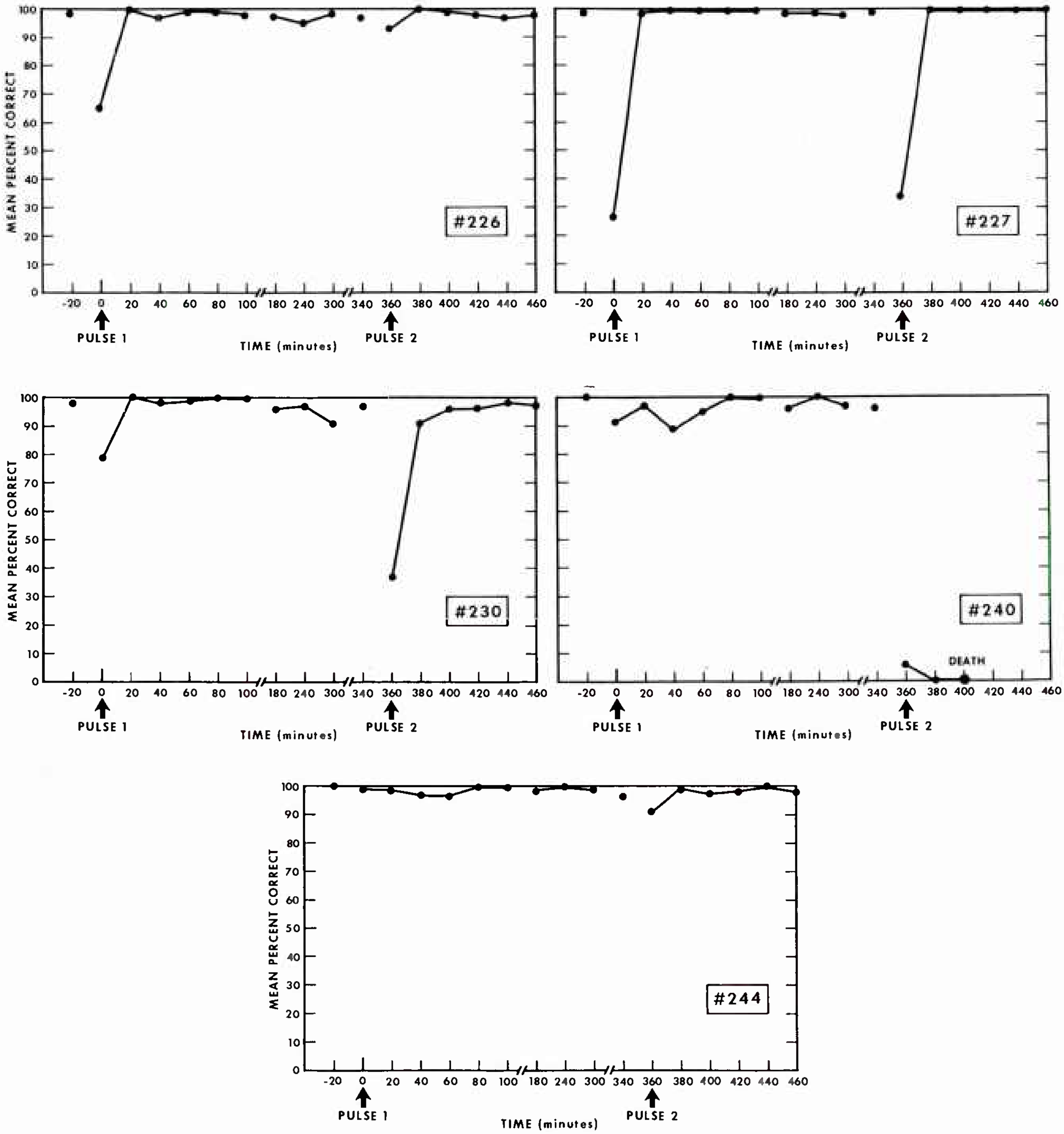

Figure A-1 (continued)

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13. ABSTRACT

Ten monkeys housed in primate restraining chairs were trained to criterion performance on a shock avoidance visual discrimination problem. They were then exposed to a 1700-rad pulse of mixed gamma-neutron radiation followed in 6 hours by a 3500-rad pulse. The decrement in performance during the first 20 minutes following the first 1700-rad pulse was equivalent to the decrement in this time period following the first 2500-rad pulse in a previously reported 2500 + 2500-rad multiple exposure study. However, the decrement in performance during the first 20 minutes following the 3500-rad pulse was as great as that in the 20 minutes following the first pulse, in contrast to the 2500 + 2500 group where there was a smaller decrement following the second pulse than after the first pulse. Performance in the entire 2 hours after the second pulse of the 1700 + 3500-rad group was superior to that following a single 5000-rad pulse.

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