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ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE

ANNUAL RESEARCH REPORT

1 JULY 1970 — 30 JUNE 1971

Defense Nuclear Agency, Bethesda, Maryland

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13 ABSTRACT

This report contains a summary of the research projects of the Armed Forces Radiobiology Research Institute for the period 1 July 1970 to 30 June 1971.

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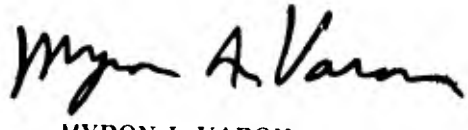
FOREWORD

This report describes, in brief summary, the scientific accomplishments of the Armed Forces Radiobiology Research Institute (AFRRI) for the period 1 July 1970 to 30 June 1971.

During this period, the Institute's two new radiation sources, the electron linear accelerator (LINAC) and the cobalt-60 facility, were utilized in support of not only the AFRRI research program but also the scientific programs of neighboring research laboratories, such as the National Institutes of Health, the Naval Medical Research Institute, the National Bureau of Standards and the Harry Diamond Laboratories. With the support of the Director, Defense Nuclear Agency, the laboratory facilities of the AFRRI, in addition to the radiation sources, will be made available to other military and civilian research institutions in the Washington area for collaborative research investigations.

The Radiation Study Section of the National Institutes of Health, Public Health Service, Department of Health, Education and Welfare, met at AFRRI 19-21 April 1971.

Eleven technical papers by members of the AFRRI scientific staff were presented at the Joint Oak Ridge Associated Universities-Defense Nuclear Agency Information Exchange Program on 29-30 March 1971.



MYRON I. VARON
Captain MC USN
Director

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INVESTIGATION OF INCAPACITATING DOSES OF RADIATION IN THE LARGER MAMMALS

Principal Investigators: *R. L. Chaput and R. T. Kovacic*
Technical Assistance: *E. L. Barron, W. W. Wolfe, J. K. Warrenfeltz, M. E. Flynn, N. L. Fleming and T. K. Dalton*

In the evaluation of incapacitation and performance decrement response of several mammalian species to pulsed mixed gamma-neutron radiation, the performance of trained beagles[2] and miniature pigs[1] was investigated.

Trained monkeys, miniature pigs and rats experience a period of transient performance decrement shortly after a rather broad range of supralethal doses of radiation. Untrained beagles did not show this early decrement. Since the dog's performance had only been subjectively evaluated, it was possible that the animal's ability to perform a learned task would, as in the other species, fall off temporarily almost immediately after irradiation. To test this possibility, nine beagles were trained by shock avoidance conditioning to work a discrimination problem cued by visual and auditory stimuli. The trained dogs were exposed unilaterally to the left side to a pulse of mixed gamma-neutron radiation. Midline tissue doses to the head were 5200 to 17,600 rads.

Postirradiation performance data for each of the nine dogs are illustrated in Figure 1. Dogs that received doses of 11,900 rads or less generally performed well during the initial 60 minutes after irradiation. Animals that received 17,600 rads experienced a severe irreversible performance decrement within 1 hour after irradiation. At doses below 10,000 rads dogs performed at or near preirradiation levels for several days after irradiation. Later, a rapid decline in performance led to complete incapacitation and death. At doses of 10,000 to 12,000 rads most animals performed at or near preirradiation levels for only a few hours. Thereafter, a rapid, irreversible decline in performance led to complete incapacitation and death.

These results indicate that the response of trained dogs to supralethal doses of radiation is different from that of monkeys, miniature pigs and rats. Sustained periods of transient performance decrement did not occur in trained dogs shortly after irradiation.

Trained miniature pigs were studied to determine if the task is a significant factor in postirradiation performance accuracy. The pigs were trained to work a shuttlebox problem and their performance postirradiation was compared to the postirradiation performance of pigs trained to work a visual discrimination problem. The pigs were trained by shock avoidance conditioning. When presented with auditory and visual stimuli pigs performing the shuttlebox task had to cross to the opposite chamber of the two-chambered shuttlebox; whereas pigs performing the discrimination task had to touch, with their snouts, the brighter of two lighted panels.

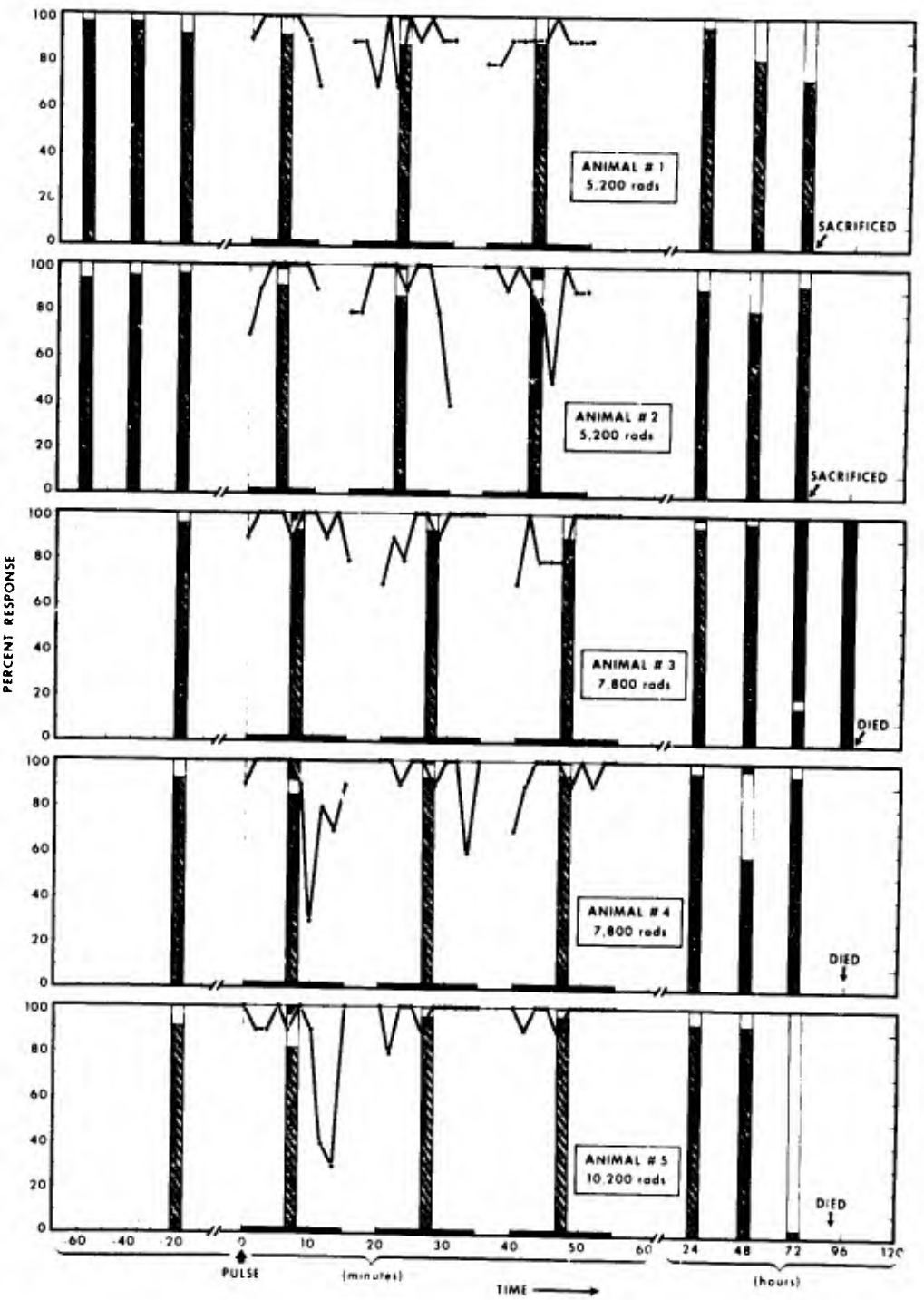

Figure 1. Beagle performance after irradiation. Each vertical bar represents the average performance for each test session. ▨ Correct and ▯ incorrect responses; ▮ omissions. Each data point is an average of a 10-trial session. The standard deviation around the mean of the preirradiation base-line performance is indicated by the shaded area.

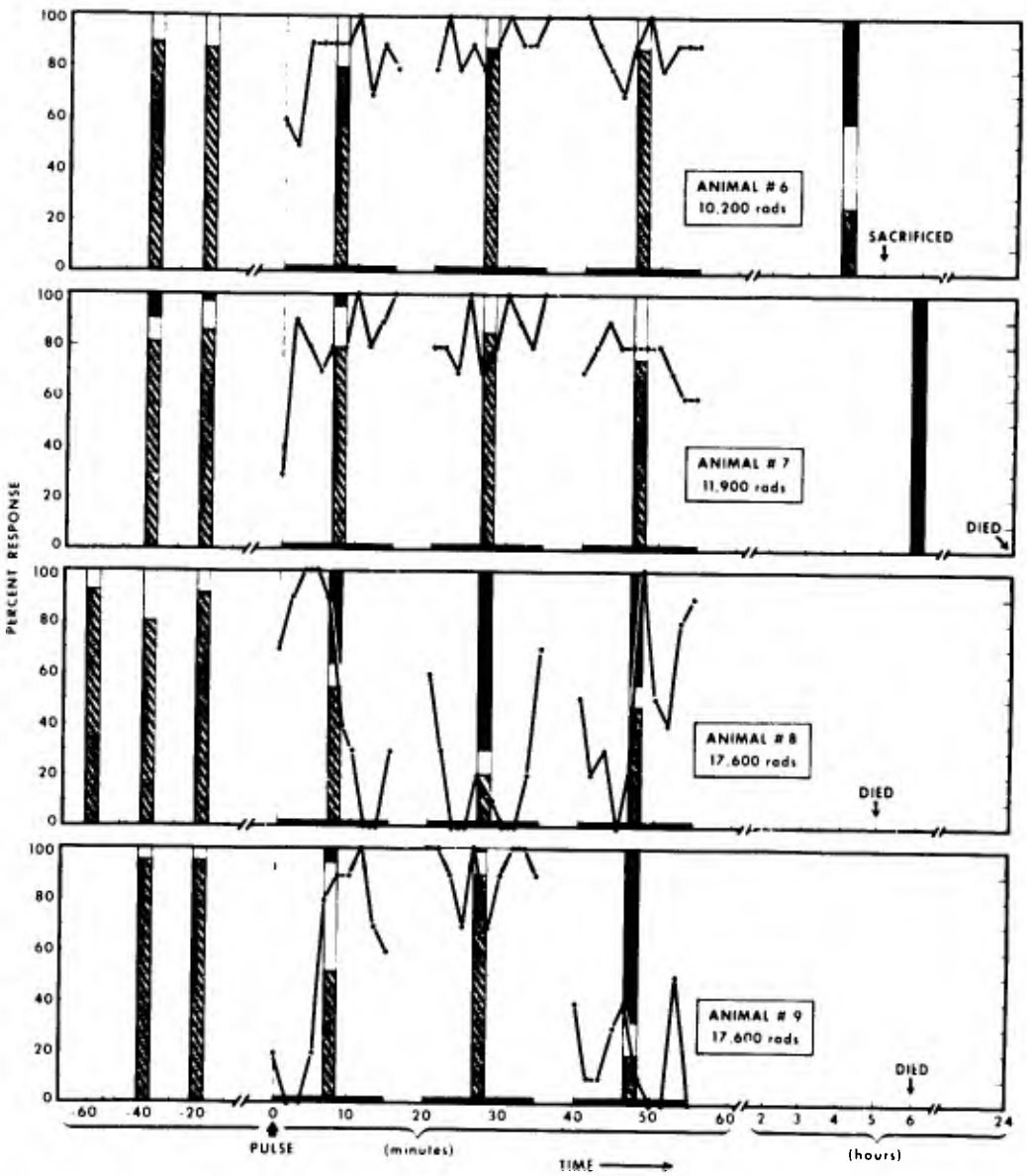

Figure 1 (continued)

Sixty-one trained pigs were exposed unilaterally to the left side to a pulse of radiation from a gamma ray field (incident neutron to gamma ratio of 0.06). Midline tissue doses to the brain were 1200 to 3300 rads.

A comparison of the overall postirradiation performance of pigs trained to the shuttlebox and discrimination tasks is shown in Figure 2. The major difference in performance occurred during the first 30 minutes after irradiation. At the doses used in this study, pigs trained to the discrimination task experienced a more severe

and prolonged period of early temporary performance decrement than did the pigs trained to the shuttlebox task. The ED_{50} or 50 percentile midbrain dose for early performance decrement was calculated to be 1870 and 2380 rads for the pigs trained to the discrimination and shuttlebox tasks, respectively.

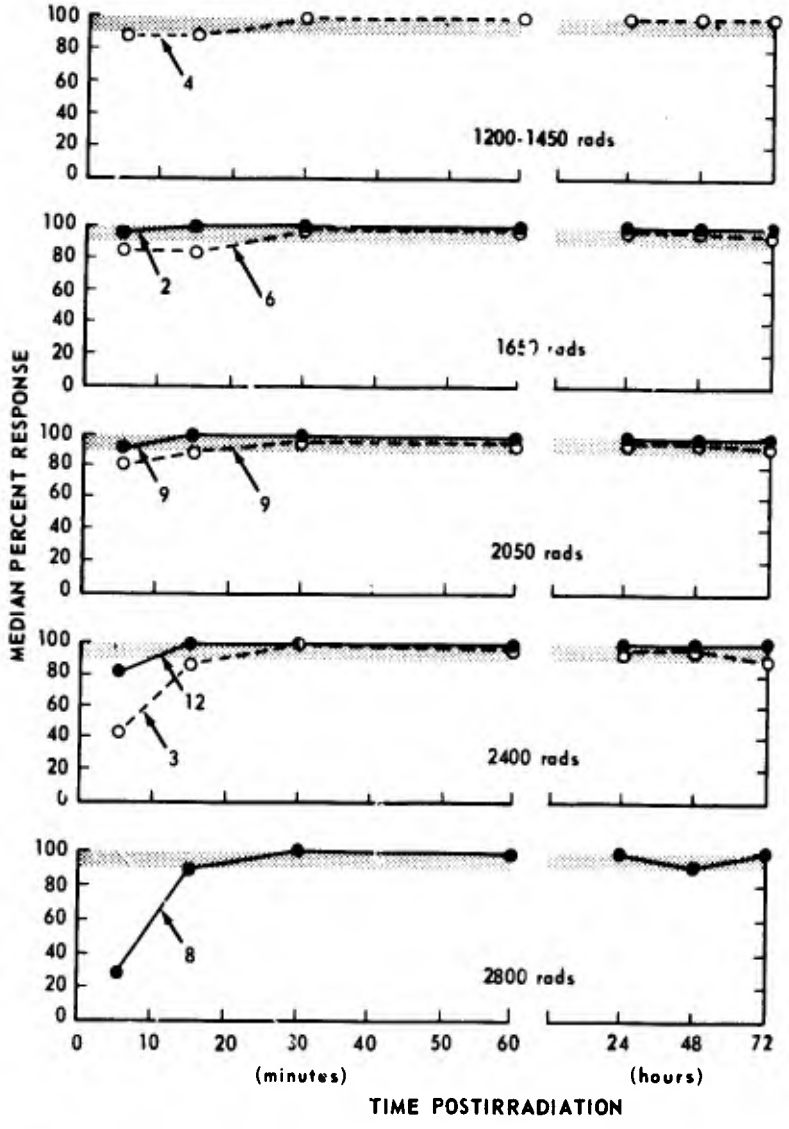

Figure 2. Postirradiation performance of shuttlebox-trained ●—● and discrimination-trained O---O miniature pigs (average over 10-minute increments during the first 30 minutes). The number of pigs in each group is indicated. Satisfactory performance is indicated by the shaded area.

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THE RELATIVE EFFECTIVENESS OF FISSION NEUTRONS FOR INCAPACITATION

Principal Investigators: *J. W. Thorp, R. W. Young, R. E. George, R. L. Chaput and D. M. Verrelli*

Technical Assistance: *T. K. Dalton, J. R. Harrison, G. G. Kessell and P. Mannon*

The objective of this research was to evaluate the relative effectiveness of neutron irradiation for causing early transient incapacitation in monkeys.[2]

Monkeys, trained by shock avoidance conditioning to work a simultaneous visual discrimination problem, were irradiated in a nuclear reactor-produced neutron field (incident neutron to gamma ray ratio of about 10). Their postirradiation performance was compared to that of monkeys similarly irradiated in a gamma ray field (incident neutron to gamma ray ratio of about 0.1) from the same reactor. The dose rate at the midline of the brain was approximately 2000 rads/minute for all irradiations. The midline tissue doses to the brain ranged from 1600 to 2700 rads for the gamma ray field and from 2200 to 4400 rads for the neutron field.

The performances of the irradiated monkeys are summarized in Tables I and II. There was no apparent difference between the early transient incapacitation (ETI) observed after gamma ray exposures and that which occurred in the neutron field. Among the neutron-irradiated animals, the lowest dose at which ETI occurred was 2600 rads, and that was in only one of four monkeys (Table I). By contrast, ETI occurred in one monkey at a gamma ray dose of 1800 rads and ETI always occurred in monkeys that received more than 2500 rads in the gamma ray field (Table II).

Table I. Performance of Neutron-Irradiated Monkeys

Dose group allotment* † (rads)‡						Postirradiation performance			Midbrain dose (rads)‡
2200	2500	2900	3300	3800	4400	Correct	Incorrect	Omission	
		+				581	7	13	2700
			+			584	14	2	3400
				0		525	16	59	3800
			+			592	1	7	3300
				0		576	4	27	3800
			+			594	1	5	3100
				0		447	1	152	3900
			0			551	20	30	3400
		+				594	2	4	3000
			+			601	1	6	3300
				+		592	2	7	3800
					0	20	1	579	4200
				0		389	4	207	3700
			0			274	61	266	3300
		0				575	2	24	2900
	+					589	4	7	2600
		+				574	17	9	2800
			+			591	4	5	3200
				0		485	1	114	3900
			0			563	24	14	3300
		0				403	19	178	3000
	+					584	6	10	2600
		0				561	29	10	3000
	0					305	4	291	2600
+						590	4	6	2100
	+					572	3	26	2600
		+				293	3	4	3100
			+			392	0	8	3300

* Each succeeding animal was tested at the dose level one step below the dose used in the preceding test if it resulted in ETI (three or more consecutive omissions during first 30 minutes), or at the dose level one step higher if performance was satisfactory.

† Satisfactory performance is indicated by "+", ETI is indicated by "0".

‡ The midbrain dose reported is the dose derived from measurements during the exposure. The "dose group" to which an animal was assigned indicated the dose the animal should have received according to experimental design. The dose measurements have an estimated accuracy of better than ± 5 percent.

The relative effectiveness of the neutron field was 0.68 using the reactor-produced gamma ray field as the reference radiation and the 50 percentile midbrain dose (ED_{50}) for early performance decrement as the biological end point. The relative effectiveness of neutrons for causing ETI was lower for miniature pigs (0.23)[1] than for monkeys (0.68). The fact that the value for neutron relative effectiveness

was significantly less than one in both monkey and miniature pig experiments supports the conclusion that gamma rays are more effective than neutrons for causing early postirradiation central nervous system disturbances.

Table II. Performance of Gamma-Irradiated Monkeys

Dose group allotment[*,†] (rads)[‡]					Postirradiation performance			Midbrain dose (rads)[‡]
1600	1800	2100	2400	2700	Correct	Incorrect	Omission	
	+				583	9	8	1700
			0		502	7	91	2400
		0			545	1	55	2000
	+				595	0	5	1900
		0			276	4	24	2200
	+				590	2	8	1900
		+			546	0	58	2000
			0		558	8	34	2400
		0			591	3	6	2200
	+				598	1	1	1900
		+			597	3	0	2100
			+		494	20	26	2400
				0	563	4	33	2600
			+		535	2	3	2400
				0	565	7	28	2600
			0		465	11	124	2500
		0			589	1	10	1900
	0				542	6	52	1800
+					574	20	6	1700
	+				594	0	6	1900
		0			391	16	93	2100
	+				572	28	0	1900
		+			588	3	9	2300
			0		186	25	389	2500
		+			560	34	6	2300
			0		563	1	36	2500
		+			394	1	6	2300
			0		514	1	36	2400
		+			583	12	5	2100
			0		19	1	380	2300

* Each succeeding animal was tested at the dose level one step below the dose used in the preceding test if it resulted in ETI (three or more consecutive omissions during first 30 minutes), or at the dose level one step higher if performance was satisfactory except for the second animal.

† Satisfactory performance is indicated by "+", ETI is indicated by "0".

‡ The midbrain dose reported is the dose derived from measurements during the exposure. The "dose group" to which an animal was assigned indicated the dose the animal should have received according to experimental design. The dose measurements have an estimated accuracy of better than ± 5 percent.

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ACUTE MORTALITY RESPONSE OF LARGER MAMMALS TO IONIZING RADIATION

Principal Investigators: *D. Wise and R. E. George*
Technical Assistance: *E. L. Barron, T. K. Dalton, W. W. Wolfe, M. E. Flynn, N. L. Fleming and J. K. Warrenfeltz*

The objective of this research was to evaluate postirradiation treatment and clinical management in an effort to modify the lethality response of dogs to mixed gamma-neutron radiation. Use of the beagle was based on its status as a well standardized and easily handled experimental animal which resembles man in many of its hematological parameters.

The effectiveness of a combination of readily available therapeutic measures in the treatment of beagles irradiated with doses known to cause grave injury to the hematopoietic system was studied.[2]

The therapeutic regimen chosen included prophylactic administration of antibiotics, biologicals and other agents, followed by further treatment based on clinical judgment. To minimize the development of antibiotic resistant microorganisms, large doses and combinations of antibiotics were used. The group of dogs receiving these treatments was designated Group A. Another group of beagles (Group B) received leukocyte and platelet transfusions in addition to the antibiotics, biologicals, etc.

The effectiveness of the medical care was judged by the mortality response. The $LD_{50/60}$ of the Group A beagles was calculated to be 276 rads (midline tissue dose)

with a 95 percent confidence interval of 236 to 295 rads (Table III). The $LD_{50/60}$ for similarly irradiated beagles receiving no medical treatment was 218 rads.[1]

Table III. Probit Analysis Results of Group A Beagle Mortality Data

Percent mortality	Midline tissue dose (rads)	95 percent confidence limits (rads)
10	234	134-259
30	250	189-277
50	276	236-295
70	295	275-337
90	325	302-461
Slope of regression line - 17.9		

The Group B irradiated beagles receiving medical care plus intravenously administered leukocyte and platelet concentrates responded favorably to treatment following doses as high as 330 rads.

Table IV lists the mortality response from beagle studies at this laboratory using essentially the same radiation parameters. Figure 3 illustrates the dose response regression lines from two studies.

Table IV. Mortality Response of Beagles with and without Postirradiation Treatment

Untreated*		Antibiotics (Group A)		Antibiotics plus blood elements (Group B)	
Midline tissue dose (rads)	Mortality (percent)	Midline tissue dose (rads)	Mortality (percent)	Midline tissue dose (rads)	Mortality (percent)
235	100	250	0		
263	100	261	50	270	33
284	100	285	75		
292	100	290	75		
		306	75	300	50
		325	75	330	50

* George et al.[1]

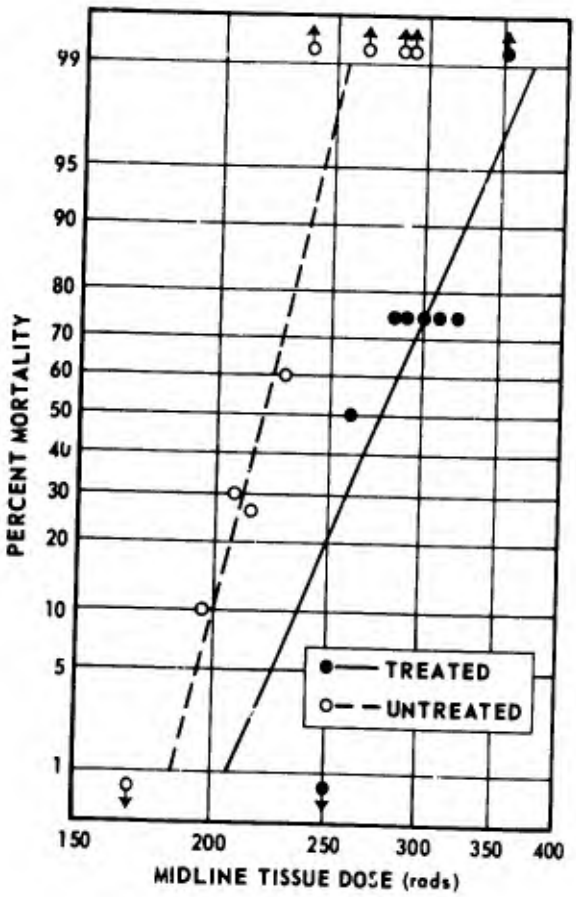

Figure 3. Dose response regression lines for beagles bilaterally exposed to mixed gamma-neutron radiation

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DRUG RESPONSIVENESS IN THE POSTIRRADIATION ANIMAL

Principal Investigators: *T. A. Strike, T. F. Doyle and J. E. Turns*
Collaborator: *C. R. Curran*
Technical Assistance: *S. L. Bradley, R. L. Brubaker, W. G. Ewald, C. G. Franz and L. Clark*

The effects of norepinephrine,[2] a vasoconstrictor, and chlorpheniramine maleate, an antihistamine, on radiation-induced performance decrement in the monkey were investigated. Research was also conducted on the radiation inactivation of histamine in water and plasma.[1]

Monkeys, trained to perform a discrete trial, cued avoidance task, were used to measure the effectiveness of intravenously infused norepinephrine in preventing the hypotension and performance decrement which usually follows 3000- to 30,000-rad doses of radiation. After a 4000-rad dose of mixed gamma-neutron radiation, 10 animals were infused with norepinephrine at a rate designed to maintain mean arterial blood pressure at approximately 100 mm Hg; for comparison 10 control animals were infused with only isotonic saline after similar irradiation. Typical of monkeys receiving a 4000-rad dose of radiation, severe hypotension generally occurred very soon after irradiation in the saline-treated animals (Figure 4). In most instances the hypotension was accompanied or followed by a period of performance decrement of at least moderate severity (less than 75 percent correct responses). Blood pressures of the norepinephrine-treated animals generally remained near preirradiation values; however, at least moderately severe performance decrement occurred in six of the ten animals within 4 minutes postirradiation. The difference between the average performance of the two groups of monkeys was not significant at any time during the first 30 minutes postirradiation (Figure 5).

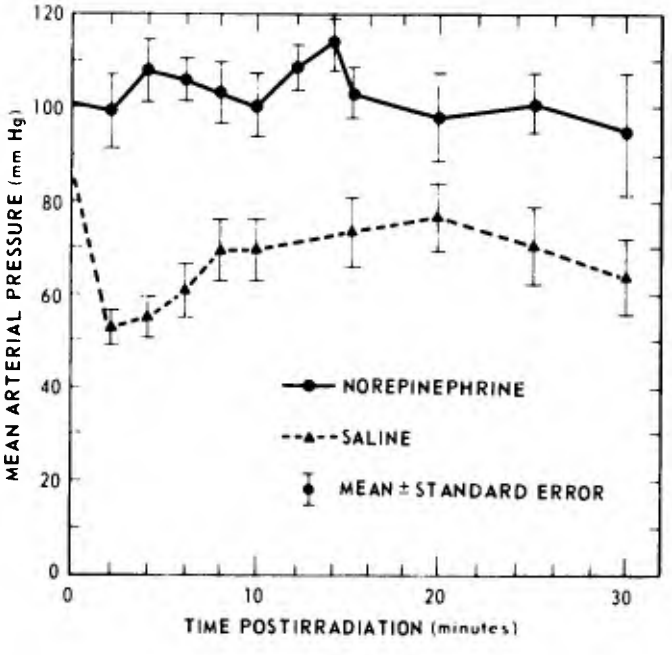

Figure 4.
Average mean arterial blood pressure of monkeys infused with isotonic saline or norepinephrine following 4000 rads of mixed gamma-neutron radiation. Each group was composed of 10 animals.

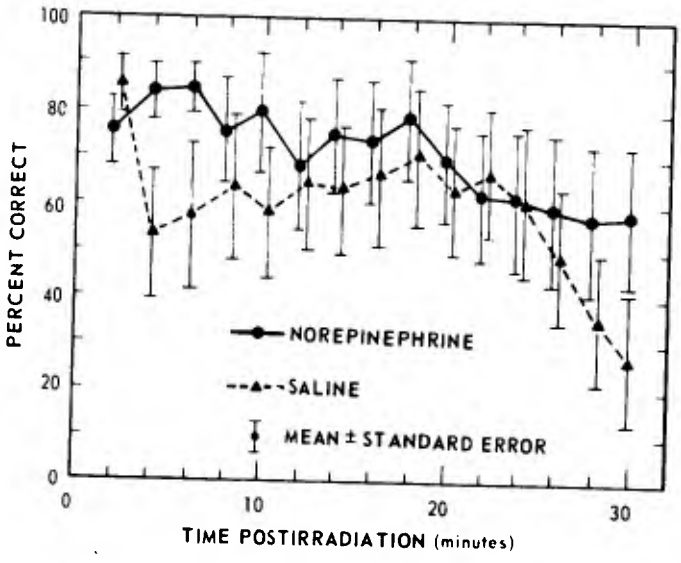

Figure 5.
Average performance of monkeys infused with isotonic saline or norepinephrine following 4000 rads of mixed gamma-neutron radiation. Each group was composed of 10 animals.

The effectiveness of the antihistamine chlorpheniramine maleate in preventing the early performance decrement in monkeys following a supralethal dose of ionizing radiation was investigated. Monkeys, trained to perform a discrete trial, cued avoidance task, were divided into four groups and injected intravenously with isotonic saline or chlorpheniramine as shown in Table V. The monkeys were tested and their

Table V. Groups of Monkeys as Treated with Saline or Chlorpheniramine

GROUP A	CONTROL, 10ml OF ISOTONIC SALINE 30 MINUTES BEFORE IRRADIATION.
GROUP B	10 mg OF CHLORPHENIRAMINE (2 mg/ml OF ISOTONIC SALINE) 60 MINUTES BEFORE IRRADIATION AND ANOTHER 10 mg OF CHLORPHENIRAMINE 30 MINUTES BEFORE IRRADIATION.
GROUP C	20 mg OF CHLORPHENIRAMINE 24 HOURS BEFORE IRRADIATION, 10 mg OF CHLORPHENIRAMINE 60 MINUTES AND ANOTHER 10 mg OF CHLORPHENIRAMINE 30 MINUTES BEFORE IRRADIATION. TOTAL OF 40 mg OF CHLORPHENIRAMINE.
GROUP D	INJECTED WITH 20 mg OF CHLORPHENIRAMINE IN A SUPERFICIAL LEG VEIN 60 MINUTES BEFORE IRRADIATION. THIS GROUP DID NOT UNDERGO SURGERY FOR THE PLACEMENT OF INDWELLING CATHETERS.

performance recorded for 2 hours following a 4000-rad dose of mixed gamma-neutron radiation; blood pressure was monitored in all monkeys with the exception of the monkeys in Group D. The average blood pressure of the chlorpheniramine-treated monkeys was higher than that of the saline-treated monkeys throughout the observation period (Figure 6). The most beneficial response was observed in those animals that received the drug during the 60 minutes prior to exposure (Group B). A beneficial response is also indicated for those animals which received 40 mg during the 24 hours preceding irradiation (Group C). Performance of the chlorpheniramine-treated monkeys, Groups B, C and D (Figure 7), was significantly better for the first 40 minutes postirradiation than that of the monkeys which received saline (Group A). After

44 minutes there was no significant difference in performance between the monkeys which received 40 mg of chlorpheniramine during the 24 hours preirradiation (Group C) and the saline-treated animals. The performance of the monkeys that received 20 mg of chlorpheniramine during the 60 minutes preirradiation (Groups B and D) continued to be significantly better than the saline-treated monkeys for the entire observation period.

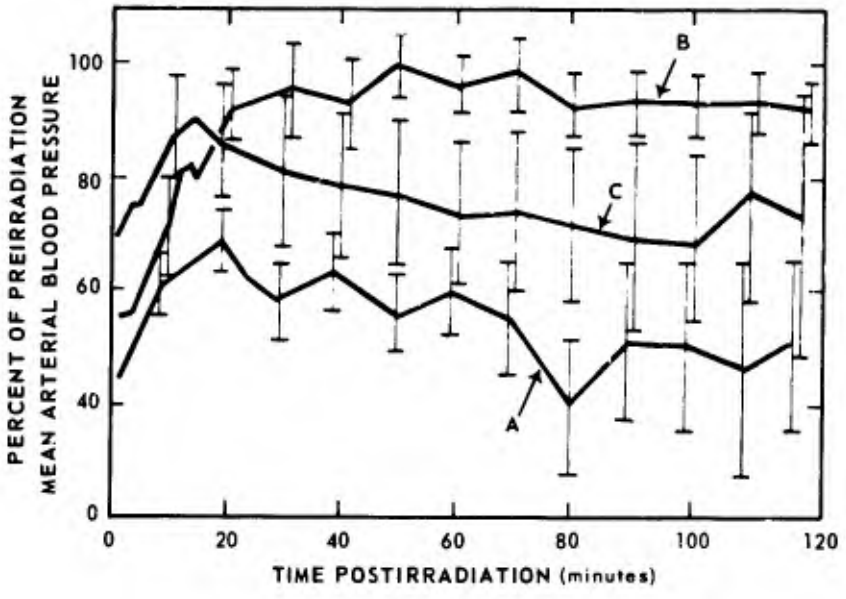

Figure 6. Average mean arterial blood pressure of monkeys treated with saline or chlorpheniramine following 4000 rads of mixed gamma-neutron radiation. (Treatment groups are given in Table V.)

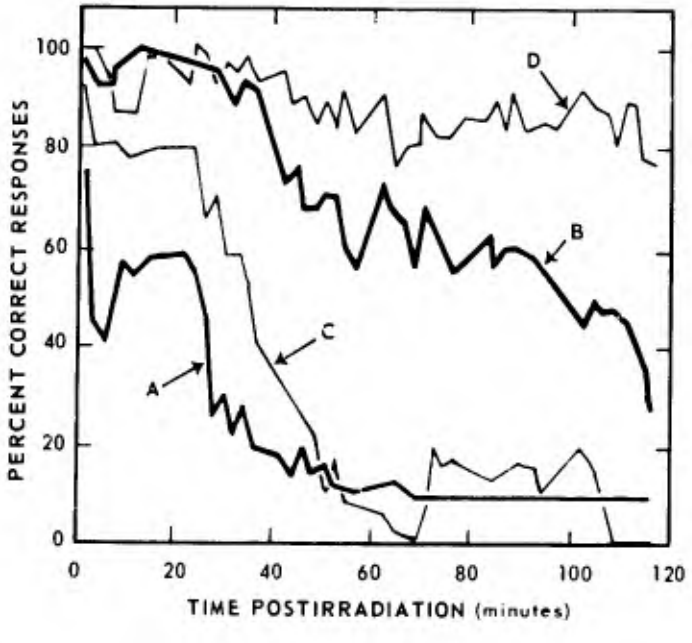

Figure 7. Average performance of monkeys treated with saline or chlorpheniramine following 4000 rads of mixed gamma-neutron radiation. (Treatment groups are given in Table V.)

Research was conducted to determine in vitro whether pulsed mixed gamma-neutron radiation could inactivate histamine to an extent that the less pronounced hemodynamic effects observed in monkeys following very high doses of radiation might be explained. Dilute solutions of histamine in water and plasma were given 140- to 38,000-rad doses of pulsed mixed gamma-neutron radiation. Histamine activity of the solutions was bioassayed using guinea pig ileum. Figure 8 shows that inactivation of the histamine in water was apparent at doses of 2000 rads or more; 1.5 μg/ml of histamine in water were completely inactivated by 17,300 rads, 3.0 μg/ml required 33,000 rads. Different concentrations of histamine (1.5 to 2.7 μg/ml) receiving the same dose of radiation (17,300 rads) clearly showed an inverse relation between concentration and inactivation (Figure 9). Histamine in plasma, however, showed less than 10 percent inactivation even at the highest dose used, 38,000 rads. Thus histamine inactivation in vivo probably does not play an important role in hemodynamic effects from very high doses of radiation.

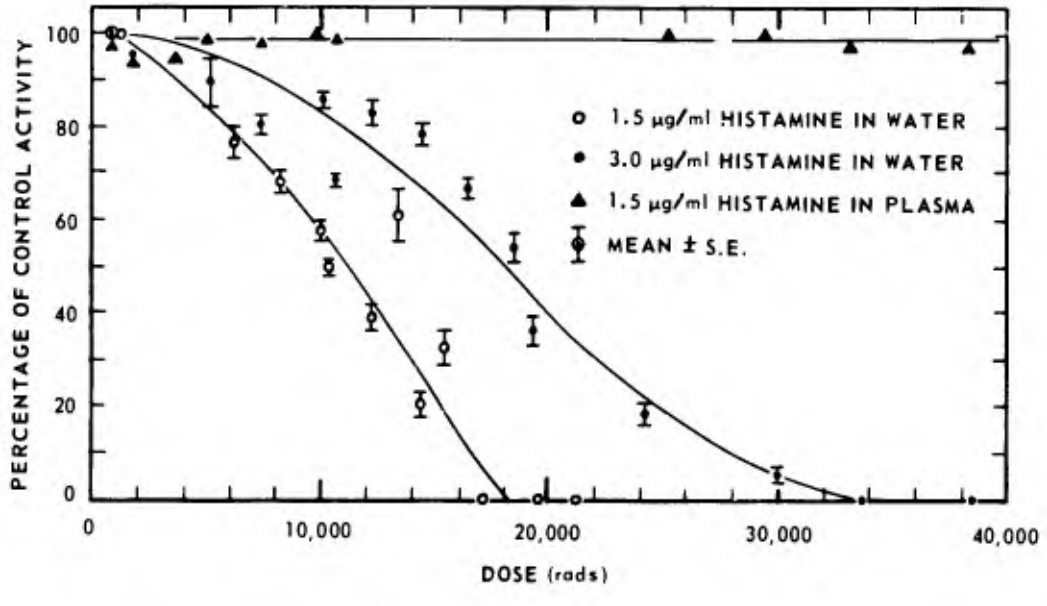

Figure 8. Histamine inactivation in water and plasma solutions after mixed gamma-neutron irradiation

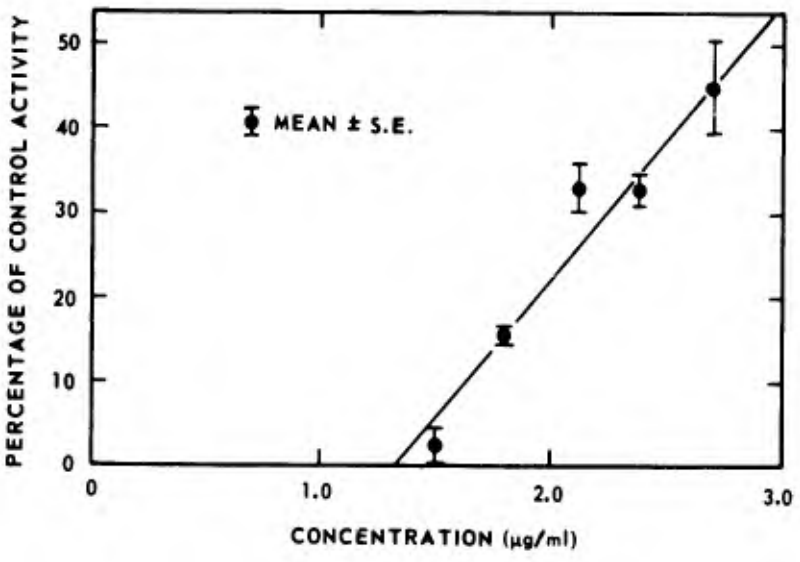

Figure 9. Activity of aqueous histamine solutions after 17,300 rads of mixed gamma-neutron irradiation

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THE RELATIVE EFFECTIVENESS OF FISSION NEUTRONS FOR GASTROINTESTINAL DAMAGE

Principal Investigators: *G. H. Zeman, S. R. Jones and R. E. George*
Collaborators: *J. E. West and S. G. Levin*
Technical Assistance: *G. D. Lee and D. F. Trainor*

Characterization of the relative effectiveness of fission neutrons for intestinal damage has continued. Following the study of miniature swine,[1] the relative effectiveness of fission neutrons for gastrointestinal lethality and jejunal crypt destruction in mice was investigated.

Mice were unilaterally irradiated in either a neutron field (incident neutron to gamma ratio of 5) or a gamma ray field (incident gamma to neutron ratio of 9). In the study of gastrointestinal lethality, the radiation was delivered in a single pulse of approximately 20 milliseconds duration. In the study of the jejunal crypts and crypt stem cells, the radiation was delivered in a single pulse or at 40 or 250 rads/minute.

Median lethal doses for gastrointestinal lethality ($LD_{50/6}$) were found to be 350 rads and 882 rads for the neutron and gamma ray fields, respectively. Thus the relative effectiveness of the neutron field at the $LD_{50/6}$ is 2.5. The percentage of jejunal crypts destroyed at various gamma and neutron doses and dose rates was determined. The relative effectiveness of the neutron field ranged from 3.1 at 10 percent crypt destruction to 2.1 at 90 percent. The pulsed and 250 rads/minute gamma rays were approximately 20 and 10 percent more effective for 90 percent crypt destruction, respectively, than the 40 rads/minute gamma rays. However, the pulsed neutrons appeared to be about 10 percent less effective than the 250 and 40 rads/minute neutrons at the same level of crypt destruction.

Figure 10 illustrates the relation between gastrointestinal lethality and jejunal crypt destruction. The data points derived from probit analysis of gastrointestinal lethality versus dose and jejunal crypt destruction versus dose lie on two straight lines, one for pulsed neutron irradiated animals and the other for pulsed gamma irradiated animals. The lines have essentially the same slope and are only slightly displaced. Median lethality occurred in association with destruction of 65 and 72 percent of the jejunal crypts following neutron and gamma irradiation, respectively.

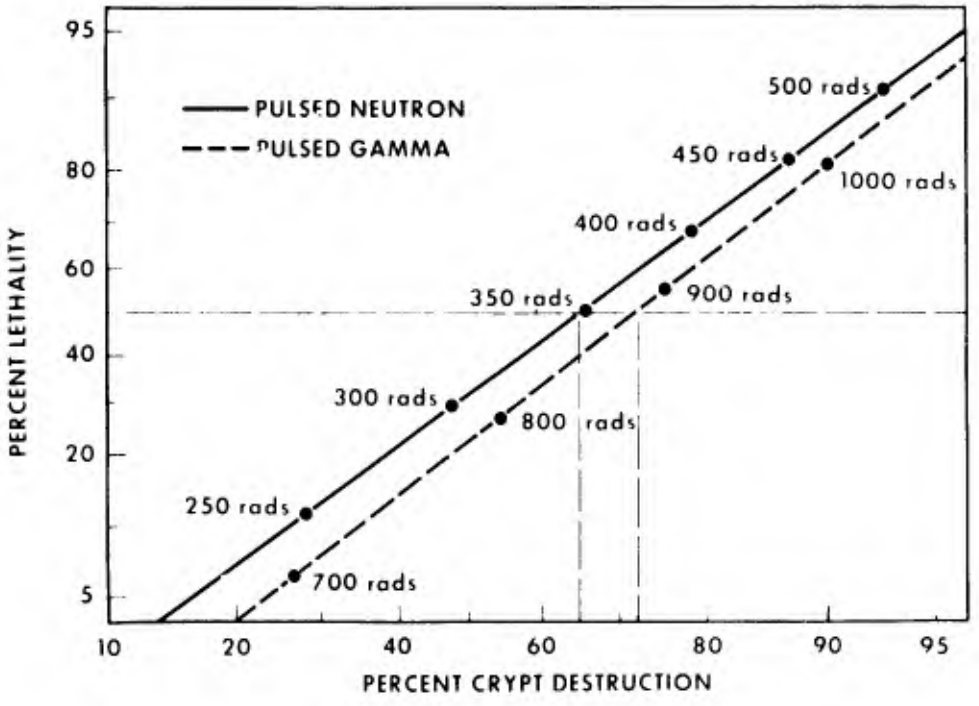

Figure 10. Relation between gastrointestinal lethality and jejunal crypt destruction

REFERENCE

1. Jones, S. R., George, R. E., West, J. E. and Verrelli, D. M. The relative effectiveness of fission neutrons for gastrointestinal death in miniature pigs. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR71-17, 1971.

BLOOD PO_2 AND pH IN MONKEYS AFTER INCAPACITATING DOSES OF IONIZING RADIATION

Principal Investigator: *J. W. Thorp*
Technical Assistance: *T. K. Dalton*

The objective of this research was to measure oxygen levels in aortic and internal jugular blood of irradiated monkeys to evaluate the possibility that brain hypoxia is associated with early transient incapacitation.[1]

Female monkeys were surgically prepared so that blood oxygen content (PO_2) and hydrogen ion concentration (pH) could be measured continuously. PO_2 and pH of arterial blood which supplied the brain and PO_2 of venous blood from the brain were monitored before and after each monkey received a 3000-rad midline tissue dose of pulsed mixed gamma-neutron radiation.

Figures 11 - 13 present the arterial PO_2 and pH and venous PO_2 of three monkeys. In addition, arterial PO_2 and pH, arterial PO_2 or venous PO_2 was measured in three other monkeys (not shown). Venous PO_2 did not markedly change after irradiation. Arterial PO_2 generally was between 60 and 80 torr before irradiation. After irradiation the arterial PO_2 usually increased slightly (5 to 10 torr) within 2 or 3 minutes. Within 6 to 10 minutes postirradiation the arterial PO_2 had declined to about its preirradiation level, and no other sudden changes were recorded. Before irradiation the arterial pH was about 7.5. With all monkeys, pH decreased immediately after irradiation. In one monkey the pH increased to its preirradiation level within a few minutes and no other change was observed. In three monkeys (Figures 11-13) the pH increased to slightly higher than the preirradiation level (about 0.05 pH units); later the pH returned to its preirradiation level. The arterial PO_2 and pH increases occurred simultaneously.

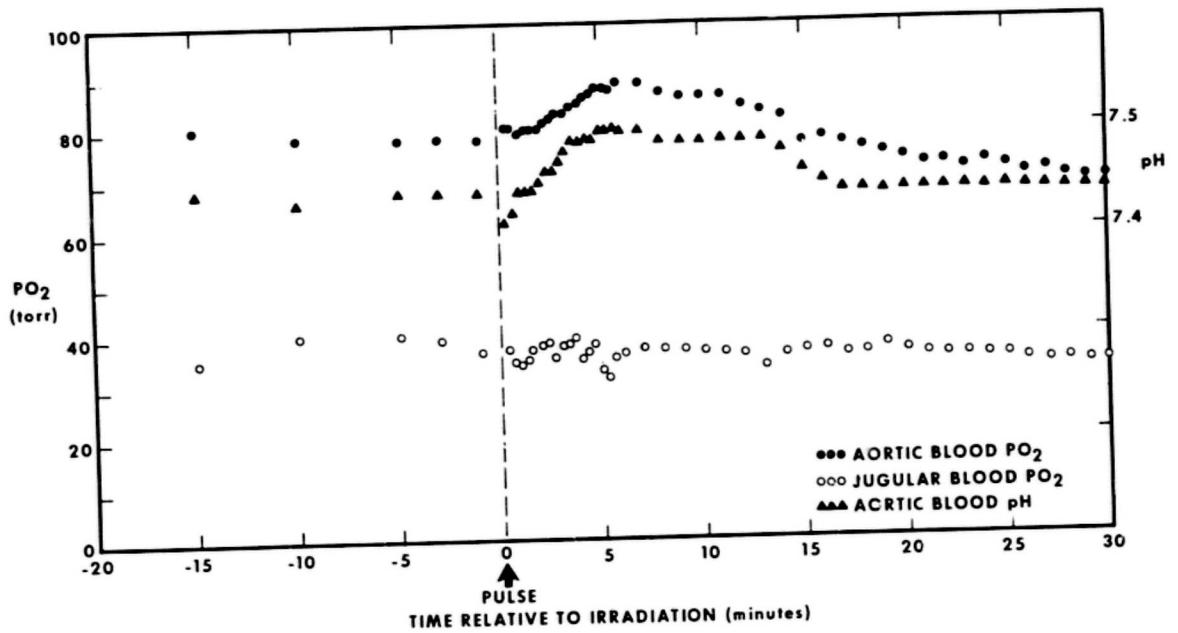

Figure 11. Postirradiation blood PO_2 and pH (monkey B-78)

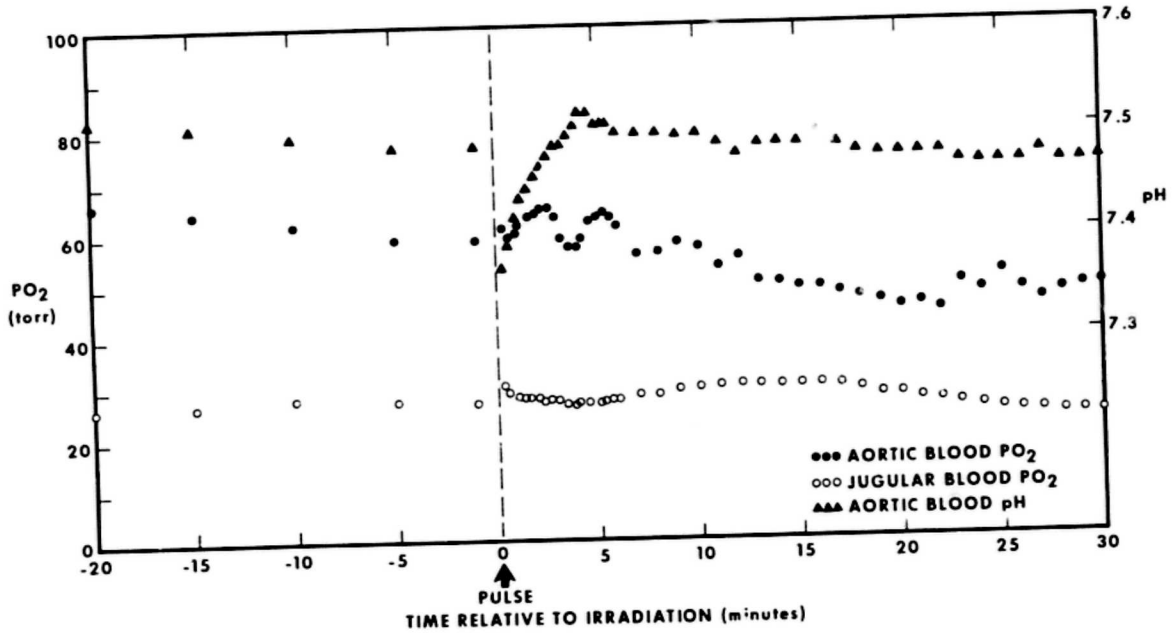

Figure 12. Postirradiation blood PO_2 and pH (monkey B-64)

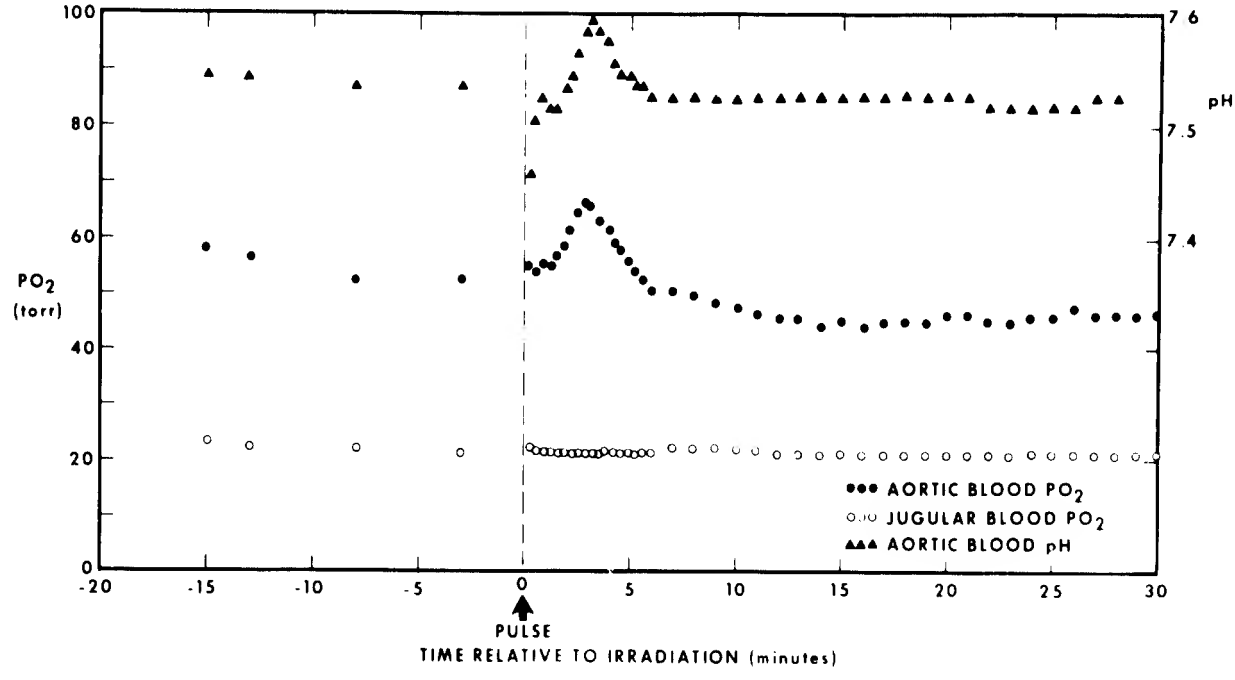

Figure 13. Postirradiation blood PO_2 and pH (monkey A-71)

The results of this study indicate that there was no substantial decrease in the blood PO_2 during the period when early transient incapacitation usually occurs.

REFERENCE

1. Thorp, J. W. Blood PO_2 and pH in monkeys after incapacitating doses of ionizing radiation. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Technical Note TN72-3, 1972 (in press).

IDENTIFICATION OF PROMINENT SITES OF RADIATION INJURY AND THEIR RELATIONSHIP TO BEHAVIOR

Principal Investigators: *C. L. Turbyfill and R. M. Roudon*
Collaborator: *R. W. Young*
Technical Assistance: *V. A. Kieffer and B. A. Dennison*

The objective of this research is to evaluate physiological and behavioral indices of monkeys following injection of radioprotective agents prior to irradiation.

The physiological and behavioral changes in monkeys following irradiation were previously investigated[1] and compared to give a better insight into the mechanisms involved in radiation injury. In the present study the same parameters were studied following injection of 2-(n-decylamino) ethanethiosulfuric acid (WR1607)[2] or S-2-(3-aminopropyl) aminoethyl phosphorothioic acid (WR2721AF) prior to irradiation.

Trained and untrained monkeys were given 10 mg/kg of WR1607, 30 minutes prior to irradiation, to investigate its effects on behavior and physiology during the early transient incapacitation period. Catheters were surgically implanted in the untrained monkeys to monitor aortic and venous pressures, heart rate and respiratory rate. Following the injection of WR1607 a significant increase occurred in aortic pressure with a significant decrease in heart rate (Figures 14-17). The untrained injected animals were irradiated with a pulse of 2500, 4000 or 15,000 rads of mixed gamma-neutron radiation. After irradiation a significant decrease in aortic pressure occurred from 2-4 minutes in the 2500-rad group and from 1-2 minutes in the 15,000-rad group. After irradiation the respiratory rate increased significantly in all injected groups.

The trained monkeys were trained to perform a shock motivated visual discrimination task. No significant changes in percent correct trials or latency occurred following injection (Figure 18). Trained injected monkeys irradiated with a pulse of 2500 or 4000 rads of mixed gamma-neutron radiation did not display a decrease in performance during the early incapacitation period (0-20 minutes postirradiation). Trained monkeys not receiving the drug displayed a decrease in performance below a 90 percent correct response level in both the 2500- and 4000-rad groups. The physiology and behavior of treated and untreated animals were not significantly different after the first 20-minute postirradiation period.

Trained monkeys were injected via the saphenous vein with 150 mg/kg of WR2721AF prior to irradiation with 4000 rads of pulsed mixed gamma-neutron radiation. The animals' performances were monitored for 2 hours following irradiation. The percent correct trials decreased to below acceptable levels (less than 90 percent correct) in the control group during the first 20 minutes following irradiation. A significant improvement in performance was observed in the group injected with WR2721AF.

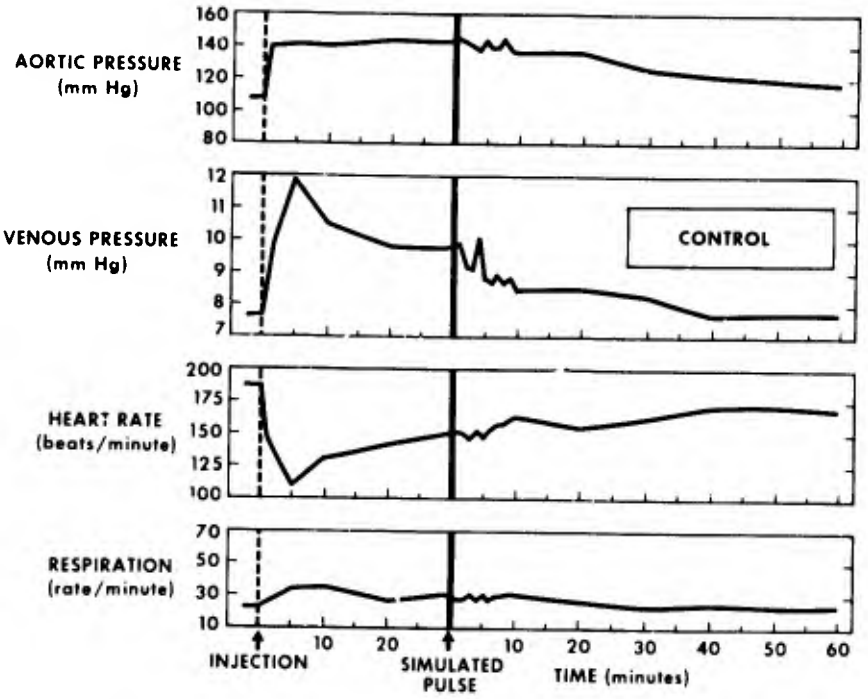

Figure 14. Physiology of control animals injected with 2-(n-decylamino) ethanethiosulfuric acid

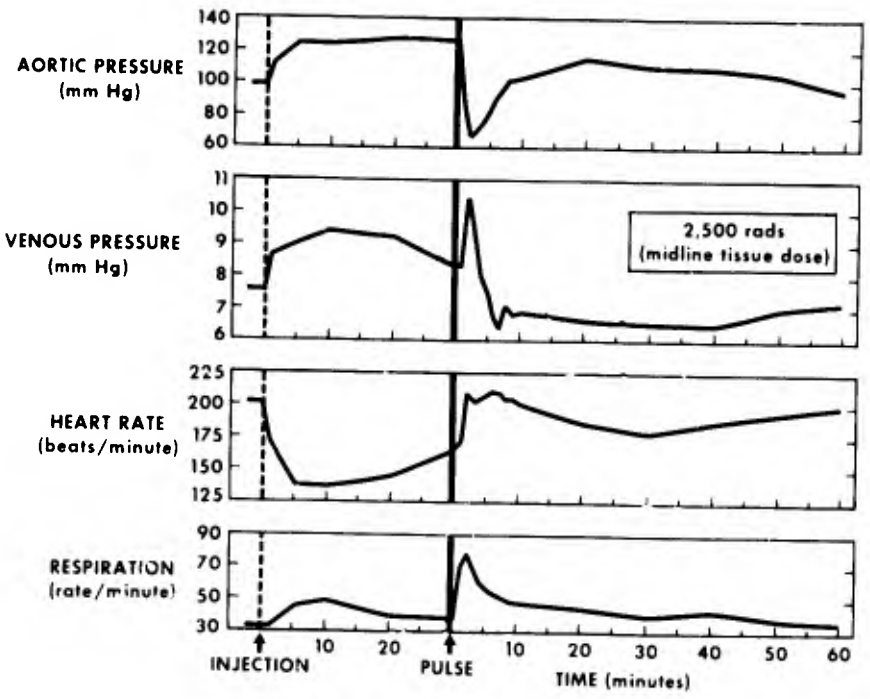

Figure 15. Physiology of animals injected with 2-(n-decylamino) ethanethiosulfuric acid and irradiated

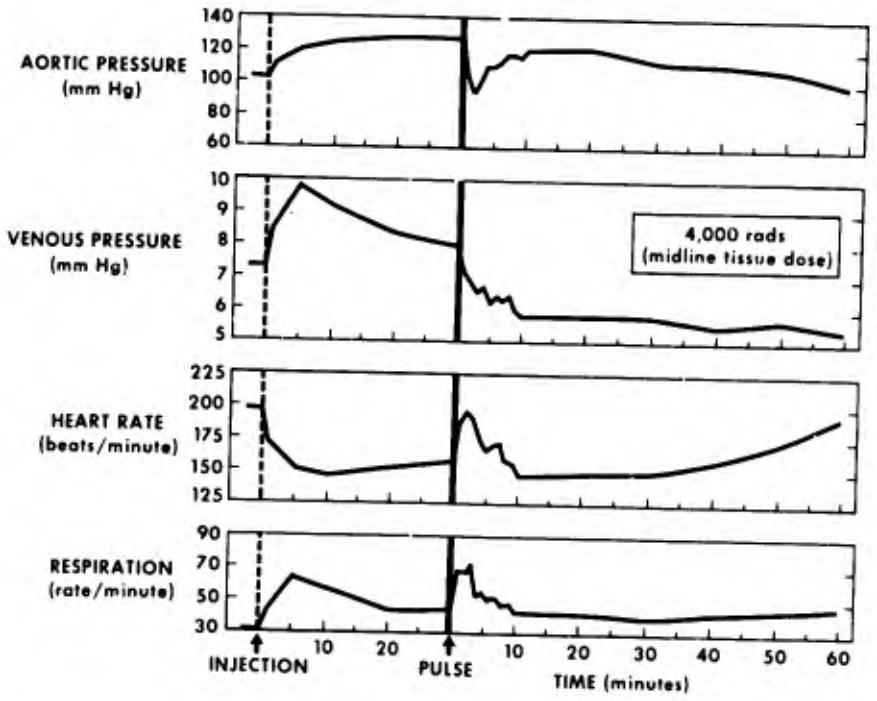

Figure 16. Physiology of animals injected with 2-(n-decylamino) ethanethiosulfuric acid and irradiated

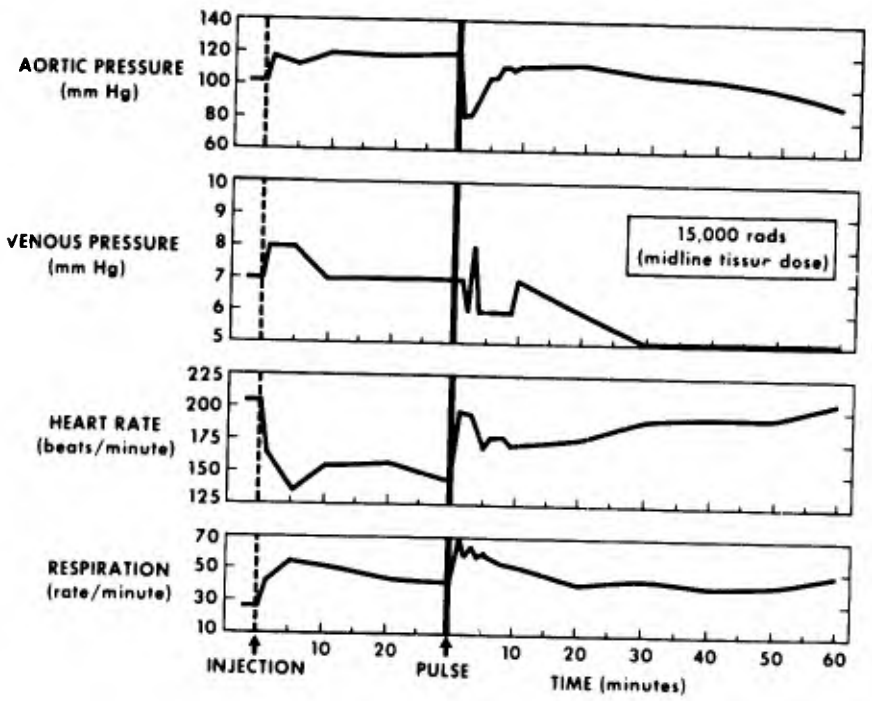

Figure 17. Physiology of animals injected with 2-(n-decylamino) ethanethiosulfuric acid and irradiated

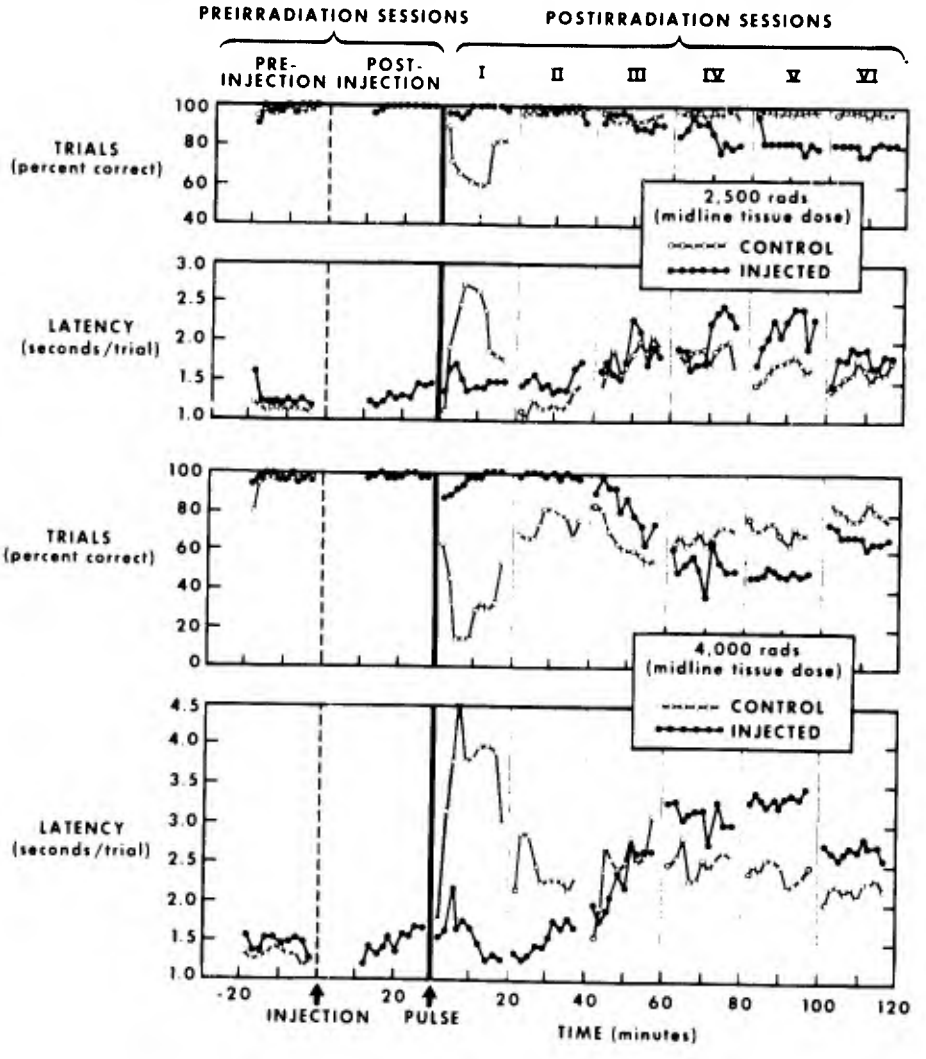

Figure 18. Behavioral response of animals injected with 2-(n-decylamino) ethanethiosulfuric acid and irradiated with 2500 or 4000 rads of mixed gamma-neutron radiation

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1. Turbyfill, C. L., Roudon, R. M. and Kieffer, V. A. Behavior and physiology of the monkey (Macaca mulatta) following 2500 rads of pulsed mixed gamma-neutron radiation. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR71-10, 1971.

2. Turbyfill, C. L., Roudon, R. M., Young, R. W. and Kieffer, V. A. Alteration of radiation effects by 2-(n-decylamino) ethanethiosulfuric acid (WR1607) in the monkey. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR72-3, 1972 (in press).

BEHAVIORAL INCAPACITATION STUDIES OF THE RESTRAINED MONKEY (MACACA MULATTA)

Principal Investigators: *W. L. McFarland and R. W. Young*
Technical Assistance: *J. R. Harrison, G. G. Kessell and P. Mannon*

The study of behavioral incapacitation of restrained monkeys after fractionated doses of mixed gamma-neutron radiation was continued. Following the study of two unequal pulses of radiation,[1] the effect of changes in the time interval between two equal dose fractions was investigated.

Monkeys housed in primate chairs were trained to criterion performance on a shock avoidance visual discrimination problem. They were then exposed to two 2500-rad pulses of mixed gamma-neutron radiation. The total midline tissue dose was approximately 5000 rads. The two pulses were separated by 20, 30, 40, 60, 180, 270 or 360 minutes. The controls received the total dose (5000 rads) in a single exposure. The postirradiation performance data are presented in Table VI.

Table VI. Average Postirradiation Performance (percent correct response*) after Pulse 1 (P1) and Pulse 2 (P2)

TIME POSTIRRADIATION (MINUTES)	TIME INTERVAL BETWEEN EXPOSURES (MINUTES)													
	CONTROLS	20		30		40		60		180		270		360	
	P1	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
0-20	34	58	55	58	33	58	41	58	69	58	67	58	75	58	86
21-40	71		72		56	91	59	77	82	97	86	80	80	95	96
41-60	59		79		81		53	70	89	88	96	80	80	95	97
61-80	58		83		81		52		93	85	97	80	80	96	97
81-100	60		84		100		58		89	94	96	80	80	94	97
101-120	58		86		100		65		84	95	95	80	76	89	96
AVERAGE	56		77		75		55		84		89		79		95

* THESE PERFORMANCE DATA HAVE BEEN NORMALIZED TO THE GROUP MEAN FOR THE FIRST 20 MINUTES AFTER THE INITIAL EXPOSURE.

In the group of monkeys irradiated with two dose fractions separated by 20 minutes, 7 of the 10 subjects exhibited early performance decrements (less than 90 percent correct responses) during the first 20 minutes after receiving the total dose of 5000 rads. The response of this group after the second fraction is substantially different from that observed for the 40-minute group. In the 40-minute group all the subjects exhibited a performance decrement after the second exposure and group performance declined to 41 percent for the 100 trials immediately after the pulse. Statistically, the performance of the 20-minute group after the second exposure was significantly better than the 40-minute group and the single dose (5000 rads) control group.

In the group of monkeys irradiated with two dose fractions separated by 30 minutes, 9 of the 10 subjects exhibited performance decrement during the first 20 minutes after receiving the total dose. Performance for this group during the first 20 minutes after the second pulse was highly comparable to that observed for subjects receiving the same total dose either in a single pulse or an equally fractionated dose at the 40-minute interval. While the performance of the 30-minute group was equivalent to that of the 40-minute group, it was substantially less than that observed for either the 20-minute group or the 60-minute group. Subsequent to the first 20 minutes of post-irradiation testing, the recovery of the animals in the 30-minute group was comparable to that exhibited by the 20- and 60-minute groups in contrast to that of the 40-minute group whose subsequent behavior remained depressed.

In the group irradiated with two pulses separated by 60 minutes, the performance after the second pulse was not measurably different from their performance prior to this fraction of radiation.

In the group irradiated with two pulses separated by 180 minutes, 8 of the 10 animals exhibited performance decrement during the first 20 minutes after receiving the total dose of 5000 rads. The number of decrements, the magnitude of the decrement and the pattern of recovery for this group make the response of this group most comparable to the 30-minute group after the second exposure.

In the group irradiated with two pulses separated by 270 minutes, performance decrements were observed in only 2 of the 10 subjects following the second pulse. These decrements were slight and of short duration.

This study indicates that, with the exception of a 40-minute interval between fractions, a 5000-rad dose of radiation delivered in two equal fractions is less detrimental to behavior than if the total dose is delivered in a single pulse. The two pulses are not additive with respect to behavioral effects.

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1. McFarland, W. L. and Young, R. W. Performance of the monkey following two unequal pulses of radiation. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR71-6, 1971.

NEUROCHEMICAL CHANGES IN THE HIPPOCAMPUS ASSOCIATED WITH CONDITIONED LOCOMOTION IN THE RAT

Principal Investigators: *W. L. McFarland, G. N. Catravas and C. G. McHale*
Collaborator: *H. Teitelbaum, University of Maryland*
Technical Assistance: *W. N. Fry*

The objective of this research was to determine the biochemical changes in the brain of rats which may be associated with changes in behavior.

Water deprived adult rats were trained for 2 weeks to run back and forth in a testing chamber for water reward. The activities of the enzymes RNA polymerase, choline acetyl transferase, monoamine oxidase and acetylcholinesterase were determined in the dorsal hippocampus in the above-mentioned group as well as in water deprived naive controls. It was found that the activities of RNA polymerase and choline acetyl transferase showed a twofold to threefold increase over controls whereas monoamine oxidase and acetylcholinesterase were not appreciably changed (Table VII). When a group of rats was trained and then the training was extinguished, RNA polymerase was further increased whereas choline acetyl transferase activity decreased. The activities of monoamine oxidase and acetylcholinesterase remained relatively unchanged. Other brain areas, namely cerebral cortex, cerebellum and anterior and posterior hypothalamus were also studied.

Table VII. Activities of Rat Brain Enzymes

	RNA polymerase	Choline acetyl transferase	Mono-amine oxidase	Acetylcholin-esterase
Controls	100	100	100	100
Hippocampus				
Naive (W.D.)*	110	118	137	100
Trained (W.D.)	175	362	118	108
Trained extinguished	615	248	94	116
Cerebellum				
Naive (W.D.)	126	115	85	87
Trained (W.D.)	104	495	64	100
Trained extinguished	94	182	65	127
Cerebrum				
Naive (W.D.)	86	93	103	93
Trained (W.D.)	79	320	107	106
Trained extinguished	127	195	98	134

* W.D. water deprived

EFFECTS OF PULSED GAMMA-NEUTRON IRRADIATION ON THE EEG AND BEHAVIOR OF THE MONKEY

Principal Investigator: *W. L. McFarland*
Collaborator: *S. G. Levin*
Technical Assistance: *J. F. Lee*

The objective of this research was to correlate electroencephalographic changes and behavioral impairment in monkeys following pulsed mixed gamma-neutron radiation.[1]

Cortical EEG recording leads were implanted in seven monkeys (Macaca mulatta). The monkeys were then trained to perform a visual discrimination task requiring both response and inhibition of response. In this task the monkey is presented with a green, red, white or yellow light. When the green light ("go" cue) appears the subject has 2 seconds to respond to avoid a brief electrical shock. If any of the other lights ("no go" cues) appear the subject must refrain from responding. If he does respond he is given a brief electrical shock.

After the animals had been trained to acceptable levels of performance, blood pressure measuring devices were surgically implanted. Two weeks after surgery the monkeys were irradiated with a single 2500-rad whole-body pulse of mixed gamma-neutron radiation. EEG, behavioral and cardiovascular parameters were continuously monitored and recorded on magnetic tape. The EEG was analyzed by a power spectral density procedure, and various measures, including first and second moments of the spectra, were obtained. These measures included the antilog of the first moment of the log power density spectrum (AM1L), the antilog of the standard deviation of the log power density spectrum (ASDL), and total power.

Relationships between these EEG measures, behavioral changes and cardiovascular alterations were studied both preirradiation and postirradiation. Figure 19A-G presents data for the measures used in this study with Figure 19B-G giving the data for the animals separated into two categories, those showing a significant early transient incapacitation (ETI) within 15 minutes after the pulse and those not exhibiting such an ETI.

Shifts in values of the EEG measures indicating an alteration to high amplitude slow waves occurred in about the same time period as the behavioral deterioration of the ETI period. Cardiovascular changes were not as closely related to behavioral changes. In the recovery period following the ETI, performance of the monkeys returned to near normal levels of accuracy and then usually deteriorated in the last part of the session. There was no clear relationship between EEG measures and accuracy of response in this period following ETI.

Figure 19.

A. Median percent correct response for successive 5-minute intervals for all seven monkeys for 30 minutes prior to the pulse and 60 minutes after the pulse. The solid line represents the performance on the "go" (before the green cue) condition, and the dashed line that on the "no go" (before the nongreen cue) condition. Standard errors are not indicated on this or subsequent plots in this figure since the statistical assumptions prerequisite for use of this measure have not yet been verified in this study.

B. Median latency of response from cue onset for consecutive 5-minute intervals plotted separately for those monkeys (four) showing a significant ETI within 15 minutes after the pulse and for those monkeys (three) not showing such an ETI. All monkeys showed a secondary decrement toward the end of the session.

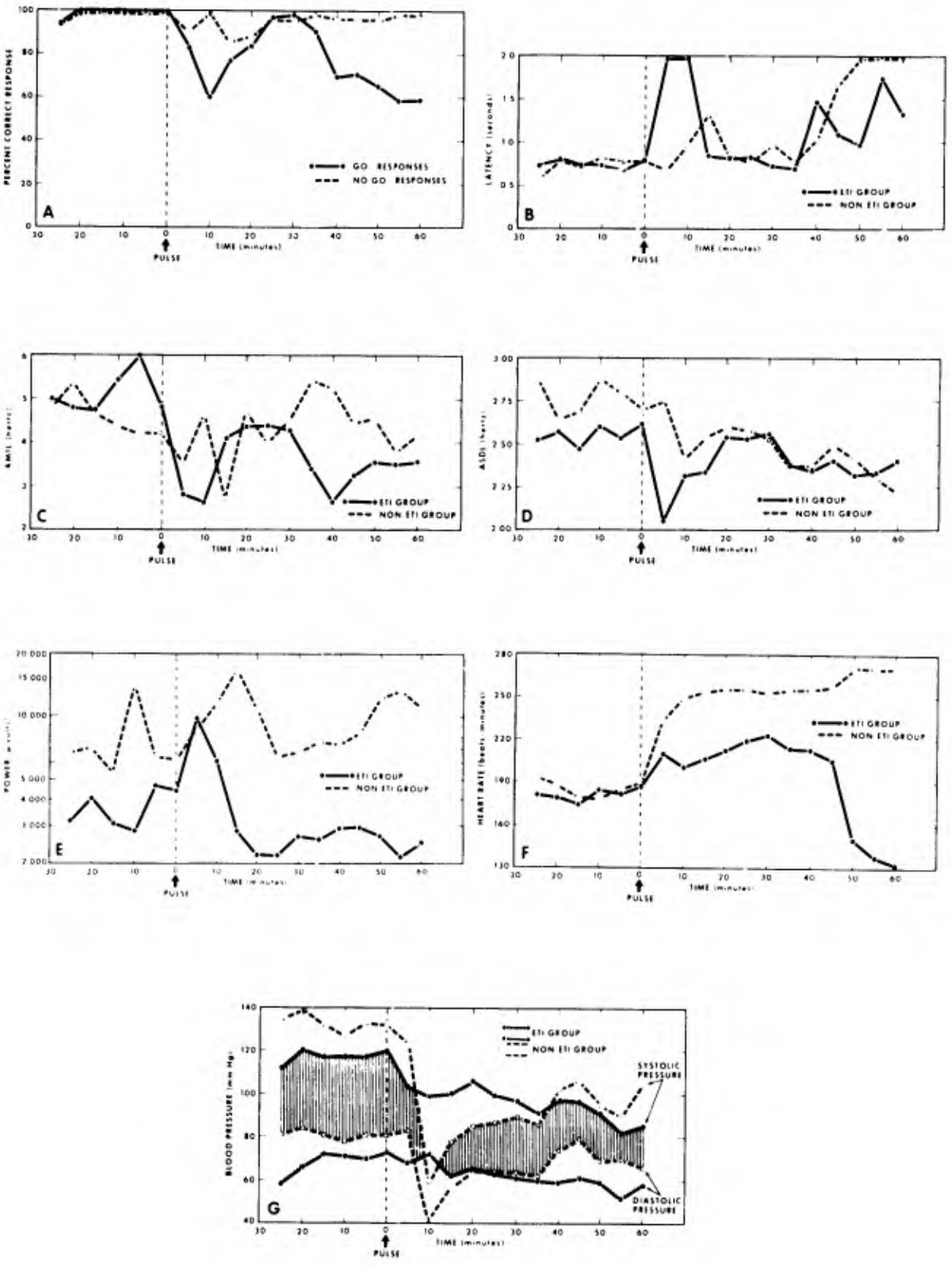
C. Median AM1L values of EEG epochs plotted in successive 5-minute intervals for those monkeys showing an ETI soon after the pulse and for those not demonstrating such an ETI. See text for derivation of this and subsequent EEG measures.

D. Median ASDL values of EEG epochs plotted at successive 5-minute intervals for the group of monkeys showing an ETI within 15 minutes after the pulse and those not demonstrating such an ETI.

E. Median power values of EEG epochs for ETI and non-ETI groups of monkeys plotted in 5-minute increments. The peaks at minus 10 minutes and plus 15 minutes are due to aberrantly large values from a single monkey.

F. Median heart rate in beats per minute plotted over 5-minute intervals for two monkeys showing an ETI and two monkeys not showing an ETI within 15 minutes after the pulse. Only four of the seven monkeys in this study were instrumented for cardiovascular measurements.

G. Average systemic systolic and diastolic blood pressures plotted separately for two monkeys showing an ETI and two not showing an ETI soon after the pulse.


A
PERCENT CORRECT RESPONSE
GO RESPONSES
NO GO RESPONSES
TIME (minutes)
PULSE
B
LATENCY (seconds)
ETI GROUP
NON ETI GROUP
C
AMSE
D
ASDE
E
POWER
F
HEART RATE (beats/minute)
G
BLOOD PRESSURE (mm Hg)
SYSTOLIC PRESSURE
DIASTOLIC PRESSURE

REFERENCE

1. McFarland, W. L. and Levin, S. G. Effects of pulsed gamma-neutron irradiation on the EEG and behavior of the monkey. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR72-8, 1972 (in press).

DETERMINATION OF L-FUCOSE IN GLYCOPROTEINS

Principal Investigator: *P. Z. Sobocinski*
Technical Assistance: *W. J. Canterbury and K. M. Hartley*

The determination of the methylpentose L-fucose (6-deoxy-L-galactose) in glycoproteins is usually performed by Winzler's[4] modification of the cysteine-sulfuric acid reaction (CyR) described by Dische and Shettles.[1] However, it has been demonstrated that nonfucose moieties of glycoproteins contribute spurious chromogens to the reaction. The fucose level obtained by Winzler's method, although a measure of methylpentose content, is not the true level when assaying samples containing large amounts of neutral hexoses (galactose and mannose).[2] The objective of this investigation was to determine the reliability of the fucose levels obtained by these methods since an elevated level of the serum glycoprotein fucose has recently been suggested as a diagnostic aid for the detection of malignancy.

Our study[2] of the effect of nonfucose moieties of serum glycoproteins demonstrated that the nonspecificity of the previous method is attributable to the erroneous use of the CyR3 absorption increment (AI) between the two wavelengths 396 nm and 430 nm as a measure of the absorption due specifically to fucose. This absorption increment is linearly related to both fucose and hexose concentrations, i.e., AI is positive for hexoses in the absence of fucose and not zero as generally assumed from the work of Dische and Shettles. In fact, it was pointed out by Dische and Shettles that the wavelength (W) at which absorption of CyR3 due to hexoses equals absorption at 396 nm must be experimentally determined when fucose analyses are performed in the presence of hexose. Our results indicate that the value for W depends on the concentration of hexoses present in the CyR3 which in turn is dependent on the protein-bound hexose content of the serum glycoprotein when the analytical method proposed by Winzler is used. It is easy to see how false-positive results, e.g., elevated fucose levels in benign disease, could be found.

The effect of various concentrations of equimolar mixtures of galactose and mannose on the OD of 5 and 10 μg/ml fucose standards when the CyR3 procedure is used and measurements are made at 430 nm and 396 nm is shown in Figure 20. These results indicate that there is a linear relationship between OD increment and the amount of galactose and mannose in the reaction mixture and that the chromogens produced by these hexoses add to the fucose chromogens. An empirical relationship was derived from the data presented in Figure 20 to correct the observed increment in OD in the presence of nonfucose sugars to an increment in OD due to fucose alone. The relationship may be expressed as:

$$A_F = Y - 0.452\ (X)$$

where A_F is the corrected increment (396 nm - 430 nm) in OD due to fucose, Y is the observed increment, and X is the amount (mg) of hexose present as galactose-mannose in the reaction mixture.

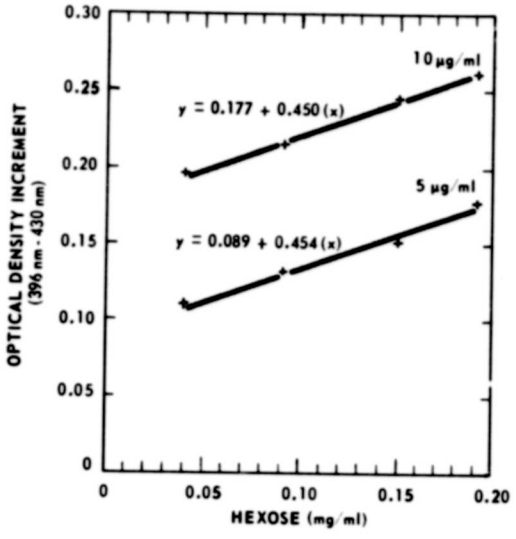


Figure 20.
Effect of various concentrations of hexoses on the absorption increment obtained for fucose standards in the CyR3. Equimolar solutions of galactose-mannose were used. Lines drawn through experimental points (+) are best fit. Equations for each line are shown.

The role of tryptophan in the CyR3 appears to be the production of chromogens in the presence of hexoses with maximum absorption at 510 nm. The finding that 50 μg or 100 μg of tryptophan decreases the absorption increment to the same extent (5 percent) suggests that the absorption at 510 nm is not the result of the conversion of much of the hexose-cysteine chromogen at 400 nm to a new chromogen with an absorption maximum at 510 nm but rather that degradation products of hexoses and tryptophan interact to form new chromogens whose absorption at 510 nm is independent of the absorption of chromogens between 396 nm and 430 nm. In our studies, 50 μg of tryptophan in the CyR3 is within the range that would be expected to be present in 50 μl of normal serum. In view of this, higher levels of protein-tryptophan encountered in serum samples from patients with neoplasms would be expected to produce increased absorption at 510 nm with the CyR3.

Another area of concern in interpreting fucose analyses performed by investigators using CyR is that the citation of Dische and Shettles as a reference could mean either the CyR3 or CyR10 procedure, two methods which do not produce identical results with glycoprotein material, i.e., the fucose value obtained by CyR10 > CyR3.

It is believed that prior acid hydrolysis of terminal fucose residues of glycoproteins followed by application of CyR3 appears to be the best practical method available for fucose determinations since it eliminates a large amount of interfering hexoses. We have automated this procedure[3] to provide (1) a better approximation of "true" fucose levels, and (2) a rapid method for screening large numbers of samples.

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2. Sobocinski, P. Z., Canterbury, W. J. and Hartley, K. M. Determination of L-fucose in glycoproteins. I. Effect of nonfucose moieties of serum glycoproteins. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR72-6, 1972 (in press).

3. Sobocinski, P. Z., Canterbury, W. J. and Hartley, K. M. Determination of L-fucose in glycoproteins. II. An automated method for the determination of L-fucose in serum glycoprotein hydrolysates. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR72-7, 1972 (in press).

4. Winzler, R. J. Determination of serum glycoproteins. Meth. Biochem. Anal. 2:279-311, 1955.

STRUCTURAL ABNORMALITIES OF HEPATIC GLYCOGENS ISOLATED FROM GAMMA IRRADIATED RATS

Principal Investigator: *P. Z. Sobocinski*
Technical Assistance: *K. M. Hartley*

Recent work[1] indicated that whole-body exposure to ionizing radiation alters the relative in vitro activities of glycogen synthesizing enzymes. Because of the

The effect of various concentrations of equimolar mixtures of galactose and mannose on the OD of 5 and 10 μg/ml fucose standards when the CyR3 procedure is used and measurements are made at 430 nm and 396 nm is shown in Figure 20. These results indicate that there is a linear relationship between OD increment and the amount of galactose and mannose in the reaction mixture and that the chromogens produced by these hexoses add to the fucose chromogens. An empirical relationship was derived from the data presented in Figure 20 to correct the observed increment in OD in the presence of nonfucose sugars to an increment in OD due to fucose alone. The relationship may be expressed as:

$$A_F = Y - 0.452\ (X)$$

where A_F is the corrected increment (396 nm - 430 nm) in OD due to fucose, Y is the observed increment, and X is the amount (mg) of hexose present as galactose-mannose in the reaction mixture.

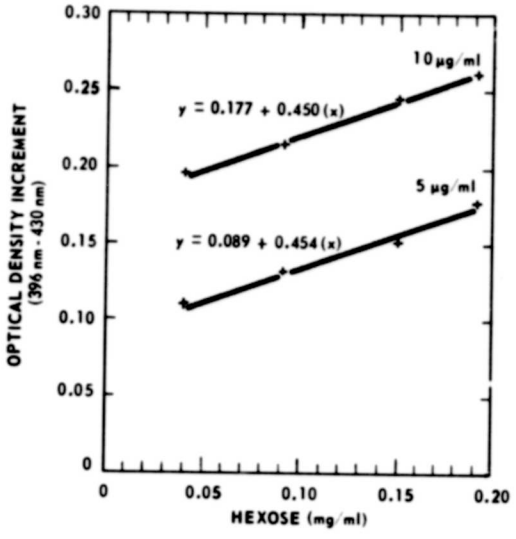

Figure 20.
Effect of various concentrations of hexoses on the absorption increment obtained for fucose standards in the CyR3. Equimolar solutions of galactose-mannose were used. Lines drawn through experimental points (+) are best fit. Equations for each line are shown.

The role of tryptophan in the CyR3 appears to be the production of chromogens in the presence of hexoses with maximum absorption at 510 nm. The finding that 50 μg or 100 μg of tryptophan decreases the absorption increment to the same extent (5 percent) suggests that the absorption at 510 nm is not the result of the conversion of much of the hexose-cysteine chromogen at 400 nm to a new chromogen with an absorption maximum at 510 nm but rather that degradation products of hexoses and tryptophan interact to form new chromogens whose absorption at 510 nm is independent of the absorption of chromogens between 396 nm and 430 nm. In our studies, 50 μg of tryptophan in the CyR3 is within the range that would be expected to be present in 50 μl of normal serum. In view of this, higher levels of protein-tryptophan encountered in serum samples from patients with neoplasms would be expected to produce increased absorption at 510 nm with the CyR3.

I_2-KI absorption spectra for both glycogen preparations are shown in Figure 22. Peak absorption occurs at approximately 480 nm for both preparations. Curve C in Figure 22 was obtained with amylodextrin and illustrates the absorption at longer wavelengths for polysaccharides containing long glycosyl chains. There is no apparent difference between the I_2-KI spectra obtained with glycogens from irradiated and nonirradiated rats.

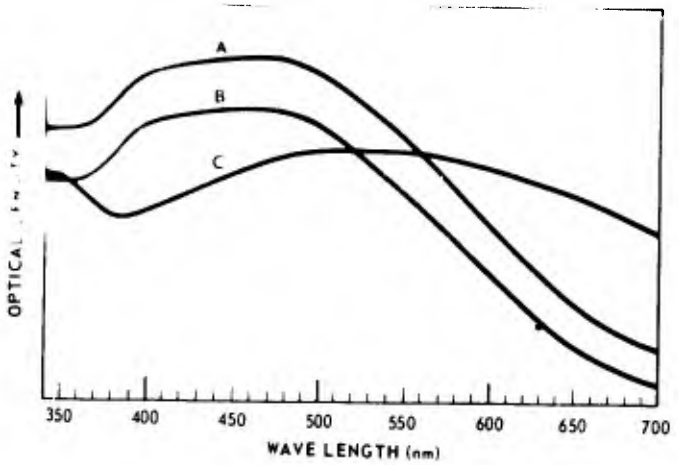

Figure 22. Absorption spectra of glycogen-iodine complexes obtained with glycogens from irradiated (A) and nonirradiated (B) rats. Curve C was obtained with a sample of amylodextrin which was used as a control for polysaccharide-iodine complex formation. Each of the reaction mixtures contained the recommended concentrations of polysaccharide, I_2, KI, and $CaCl_2$ described by Krisman.[3]

Some of the structural characteristics of glycogens isolated from irradiated and nonirradiated rats are presented in Table IX. The data indicate that glycogens isolated from livers of irradiated rats have an abnormal structure when compared to glycogens of nonirradiated rats fed ad libitum. The structural abnormalities, increased average chain length and decreased degree of branching, are consistent with the radiation-induced alterations in activities of glycogen synthesizing enzymes.

Table IX. Structural Characteristics of Isolated Glycogens

Source	Average chain length	Periodate end group (percent)	β-amylolysis (percent)	Glucose residues*	
				Outer	Inner
Irradiated	121.7	0.8	45.9	59.4	62.3
Nonirradiated	15.2	6.5	51.6	9.9	5.3

* Average number of glucose residues calculated by the method of Illingworth et al.[2]

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AN IMPROVED AUTOMATED METHOD FOR PLASMA GLUCOSE ANALYSIS WITH HIGH SPECIFICITY AND SENSITIVITY IN THE 5 TO 50 MICROGRAM RANGE

Principal Investigator: *P. Z. Sobocinski*
Technical Assistance: *K. M. Hartley and W. J. Canterbury*

Studies of blood glucose changes after radiation exposure of small laboratory animals produced a requirement for a specific and highly sensitive procedure for glucose analysis in ultramicro sample volumes. A highly sensitive automated method employing an extract of gum guaiac as the chromogenic oxygen acceptor has been suggested by Hochella and Hill.[1] We have described[2] modifications in manifold construction and reagent composition which are required to make the Hochella and Hill procedure usable for assaying plasma glucose in the 5 to 50 microgram range normally encountered in the ultramicro sample volumes obtained from small laboratory animals. The high sensitivity of the chromogen eliminates the need for recorder range expansion.

The sensitivity of the method is 0.210 O.D. units per 10 μg glucose. The optimum pH for the reaction mixture was found to be approximately 5.2. However the sensitivity is not appreciably affected within the pH range 5.0 to 5.6.

Results of replicate analyses performed on two commercial control sera shown in Table X indicate that the dilution procedure does not affect the precision of the method. The standard deviation for the procedure is 2 mg/100 ml. Neither ascorbic acid, uric acid, nor reduced glutathione in physiological concentrations was found to interfere with the procedure.

Table X. Replicate Glucose Analyses of Two Commercial Control Sera

Assay* #	Glucose (mg. 100 ml)	
	Control A+	Control B‡
1	97.5	220.0
2	100.0	225.0
3	100.0	225.0
4	97.5	225.0
5	95.0	225.0
6	100.0	225.0
7	97.5	220.0
8	95.0	225.0
9	100.0	223.0
10	97.5	225.0
11	97.5	220.0
12	97.5	223.0
13	97.5	223.0
14	97.5	223.0
Mean	97.9	223.4
S.D.	1.7	2.0
Manufacturer's assay (method)	103 ± 5 (glucose oxidase, Hyland)	228 ± 10 (Nelson-Somogyi)

* Separate 1 to 50 aqueous dilutions

\+ Hyland Lot 0369D040A1, Hyland Division Travenol Laboratories, Inc., Los Angeles, California

‡ Moni-trol II Lot PTD-21 C.D., Dade Division, American Hospital Supply Corporation, Miami, Florida

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RECOVERY AND RESIDUAL INJURY OF THE HEMATOPOIETIC SYSTEM IN IRRADIATED MAMMALS

Principal Investigators: *A. G. Pratt and S. J. Baum*
Technical Assistance: *R. J. Emerson*

The objective of this research was to test the hypothesis that a combination of erythropoietic suppression by preirradiation induction of transfusion polycythemia and granulopoietic stimulation with endotoxin administered after irradiation might result in enhancement of postirradiation granulocyte recovery in dogs.[1]

Red cell production was suppressed by transfusion of blood from donor dogs on three occasions during the week prior to irradiation with 150 rads of total-body x rays. Endotoxin was given 24 hours after irradiation. This treatment combination was compared to a control group receiving no treatment and to the effect obtained with each treatment used alone.

During the middle of the 2nd week following radiation exposure, the group receiving both transfusions and endotoxin had significantly higher numbers of granulocytes in their blood compared to the other three treatment groups (Figure 23). No treatment combination appeared to affect the two periods of low counts at the beginning and end of the 2nd week. Recovery during the 3rd and 4th weeks was accelerated by endotoxin and retarded by transfusion, with the net effect being no difference between the group receiving both treatments and the group which was untreated.

Platelet concentration in the blood was reduced in all groups during the 2nd and 3rd weeks following radiation exposure (Figure 24). Endotoxin did not appear to modify this pattern while transfusion seemed to produce a more marked drop in platelet numbers. This decrease in platelets appeared to be associated with an increase in the size of the spleen, an organ which has been described previously as a reservoir for the platelet, particularly when enlarged.

It is felt that the data support the concept of interdependence between the various cell lines of hematopoiesis in an experimental model not previously described.

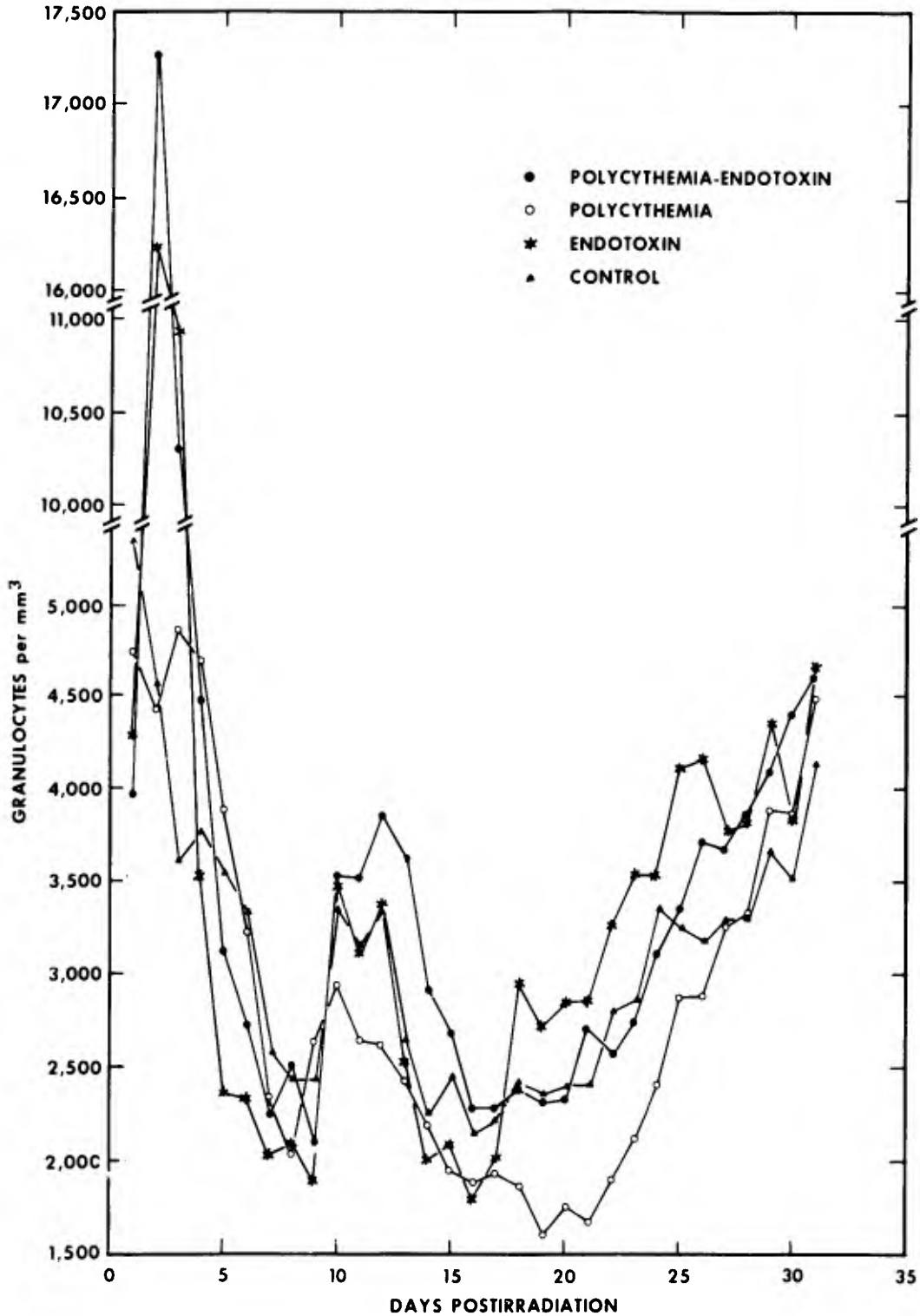

Figure 23. Postirradiation granulocyte values in dogs exposed to 150 rads of x rays

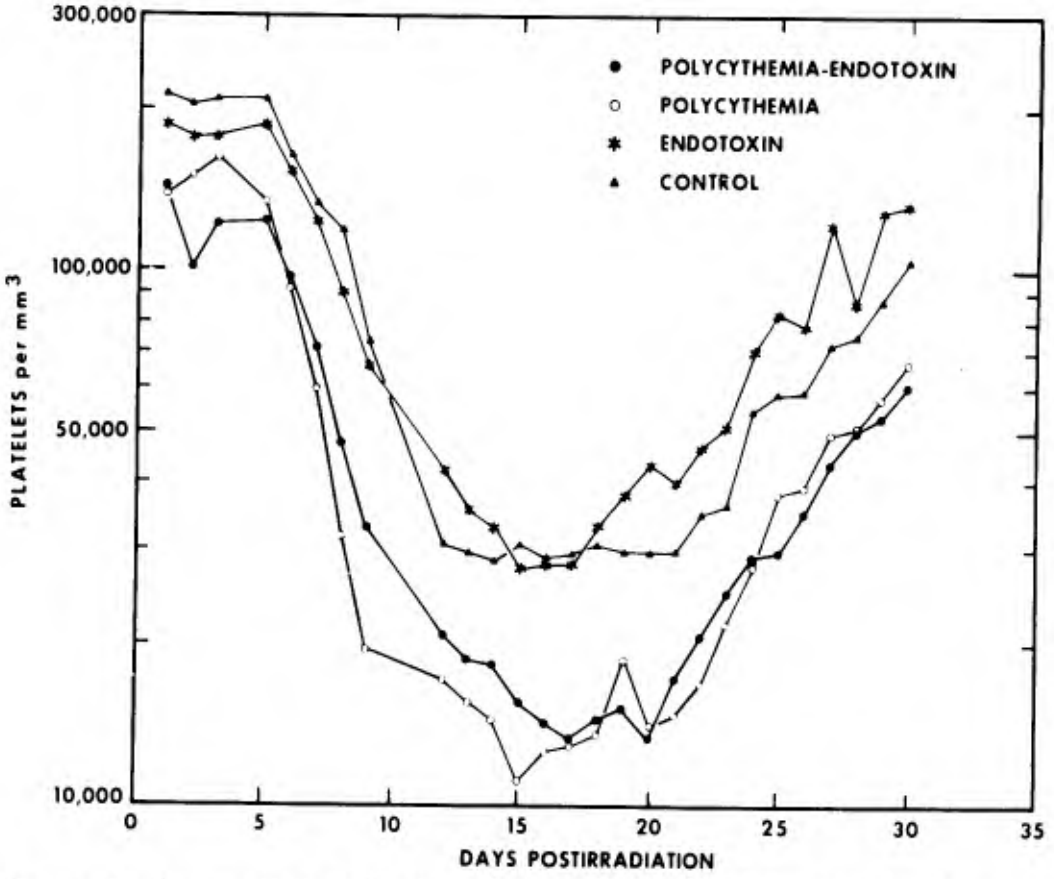

Figure 24. Postirradiation platelet values in dogs exposed to 150 rads of x rays

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MOLECULAR STUDIES OF CELLULAR AND SUBCELLULAR DAMAGE IN THE IRRADIATED ANIMAL

Principal Investigator: *G. N. Catravas*
Technical Assistance: *C. G. McHale*

Lipogenin is a substance which plays a regulatory role in the biosynthesis of fatty acids. It is heat stable and has been isolated from mammalian liver and also from yeast. When lipogenin is added in catalytic amounts to cell-free liver homogenates from starved rats it restores the rate of fatty acid synthesis to normal levels. Similar results were obtained when the material was injected into the intact animal. The objective of this research is to investigate the effect which lipogenin may have on the activity of the fatty acid synthesizing liver enzyme system in the irradiated rat and to determine if and to what extent the endogenous liver lipogenin is affected by irradiation of the animals.

Experiments were performed in which groups of rats were exposed to 1200 rads of x rays or of mixed neutron-gamma radiation (neutron to gamma ratio of 7 to 1) and sacrificed 2 or 24 hours postirradiation.[1] The livers were excised and cell-free homogenates containing the fatty acid synthesizing enzyme system were prepared. The synthesized fatty acids were chemically isolated and their radioactivity was determined in a liquid scintillation counter. The amount of radioactivity found in the isolated fatty acids, which is a measure of the activity of the enzyme system under study, was calculated. Liver homogenates from starved rats which were used for the determinations of lipogenin activity were prepared as were also liver extracts containing endogenous lipogenin and lipogenin extracted from yeast.

The effect of the addition of catalytic amounts of yeast lipogenin to liver homogenates from irradiated rats on the fatty acid synthesizing liver enzyme system is shown in Table XI. The addition of 1.2 μg of yeast lipogenin to liver homogenates from x irradiated rats has the tendency to restore the activity of the enzyme system under study to levels corresponding to those of control unirradiated animals. Addition of lipogenin to liver homogenates from rats exposed to mixed neutron-gamma radiation resulted in a relatively small increase of activity. Table XII shows the results obtained when endogenous liver lipogenin from irradiated rats was assayed for activity. The exposure of the animals to x rays did not result in any appreciable change in the activity of the endogenous liver lipogenin compared to that in the livers of fed unirradiated rats, whether the animals were sacrificed at 2 or 24 hours after irradiation. In contrast lipogenin extracted from livers of rats exposed to mixed neutron-gamma radiation had a pronouncedly decreased activity.

Table XI. Effect of Yeast Lipogenin on the Activity of the Fatty Acid Synthesizing Enzyme System in the Liver of Irradiated and Control Rats

	^{14}C-acetate incorporation into fatty acids*
Controls (starved)	100
Controls (starved) + 1.2 μg lipogenin	582 ± 46.8+
Controls (fed)	486 ± 27.9
Controls (fed) + 1.2 μg lipogenin	197 ± 31.7
x irradiated, 24 hours postirradiation	535 ± 39.3
x irradiated, 24 hours postirradiation + 1.2 μg lipogenin	179 ± 34.6
n-γ irradiated, 24 hours postirradiation	62 ± 6.4
n-γ irradiated, 24 hours postirradiation + 1.2 μg lipogenin	89 ± 7.1

Average of four experiments
* Relative isotope concentration (counts/min per mg fatty acids)
+ Standard errors

Table XII. Effect of Ionizing Radiation on the Activity of Endogenous Liver Lipogenin

	^{14}C-acetate incorporation into fatty acids*
Controls starved (unirradiated)	100
Controls starved + 1.2 μg yeast lipogenin	582 ± 46.8+
Controls starved + fed liver lipogenin (0.15 ml extract)	284 ± 20.4
Controls starved + 2 hours x irradiated liver lipogenin (0.15 ml extract)	274 ± 31.5
Controls starved + 24 hours x irradiated liver lipogenin (0.15 ml extract)	252 ± 26.0
Controls starved + 2 hours n-γ irradiated liver lipogenin (0.15 ml extract)	86 ± 9.2
Controls starved + 24 hours n-γ irradiated liver lipogenin (0.15 ml extract)	80 ± 6.8

Average of four experiments
* Relative isotope concentration (counts/min per mg fatty acids)
+ Standard errors

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1. Catravas, G. N. and McHaie, C. G. Effect of lipogenin on fatty acid synthesis in the liver of irradiated rats. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR72-9, 1972 (in press).

EFFECT OF IONIZING RADIATIONS ON BIOLOGICAL OXIDATION IN MAMMALIAN CELL MEMBRANES

Principal Investigators: *W. D. Skidmore and G. N. Catravas*
Technical Assistance: *C. G. McHale, E. E. Ricks and O. Z. Williams*

The effects of whole-body irradiation on structural and functional integrity of rat liver mitochondria were further investigated.

The study[1] was designed to approach the objective by comparing effects of ethyl linoleate in the diet, starvation, intraperitoneal glucose, and whole-body x irradiation on oxidative swelling and oxidative phosphorylation. Iron-induced mitochondrial swelling associated with lipid oxidation was used as an index of injury to the structural integrity of the outer membranes. Oxidative phosphorylation with succinate as substrate was selected as an index of the functional integrity of the inner membranes.

In rats exposed to 1000 R x rays, the lag time for oxidative swelling, calculated as t50 (time required for a 50 percent decrease in absorbancy when the light scattering was measured at 520 nm (A520)) values, was found to be decreased below that for controls 24 hours postexposure (Figure 25A). The amount of malondialdehyde (MA) produced at t50 (MAt50) for irradiated and unirradiated rats indicates a concomitant nature of mitochondrial swelling and MA production (Figure 25B).

X irradiation affected five parameters of functional integrity of mitochondria during oxidation of succinate. These parameters were % Op (percentage of total oxygen utilized that is coupled to phosphorylation of added ADP), P/O (number of g atoms phosphorus forming ATP per g atom oxygen utilized), SA III (specific activity of oxygen utilization, oxygen/min per mg protein, during phosphorylation of added ADP), SA IV (specific activity of oxygen utilization after the added ADP has been converted to ATP), and RC (respiratory control value calculated as the ratio of specific activities, SA III/SA IV). The effect of x irradiation on those parameters is shown in

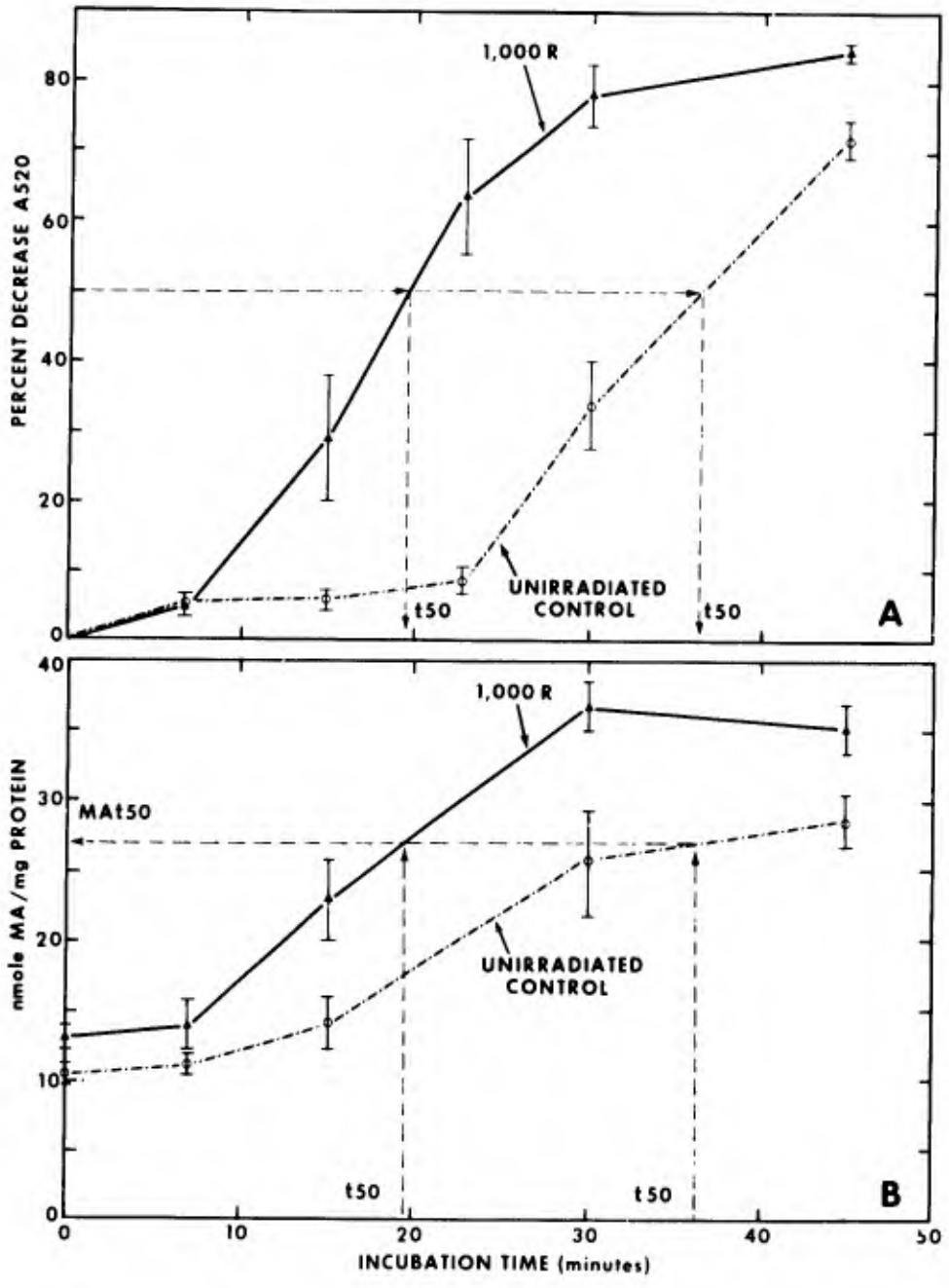
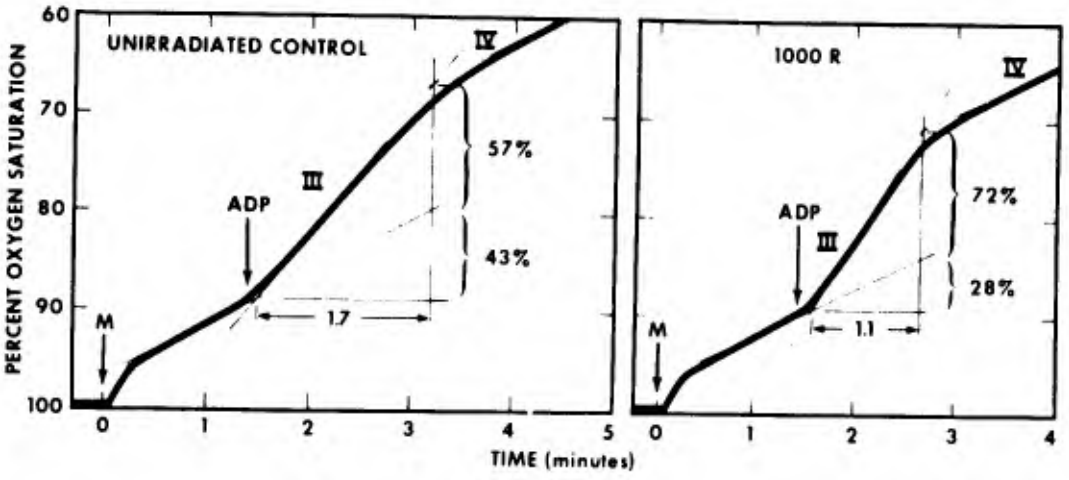


Figure 25. (A) Swelling assay of mitochondria from liver of rats 24 hours postexposure to x rays. (B) Lipid oxidation assay during the mitochondrial swelling. The data points represent mean values with their associated standard errors for eight fat-free, fed rats.

typical tracings (Figure 26). Less time is required to utilize the added ADP in the 1000 R sample than in the unirradiated control. Thus the efficiency of oxidative phosphorylation was increased above the controls.



	%Op*	P/O*	SA III*	SA IV*	RC*
CONTROL	57	1.26	79	33	2.4
1000 R	72	1.51	106	29	3.6

*DEFINED ABOVE

Figure 26. Polarographic assay of oxidative phosphorylation in liver mitochondria (M) from a fat-free, fed rat 24 hours postexposure to x rays and its unirradiated control

In comparing diets, results with rats fed a fat-free diet indicated that the lag times for the oxidative swelling were increased above those for rats fed a diet supplemented with 5 percent ethyl linoleate, whereas the efficiency of oxidative phosphorylation was lower for the fat-free group than for the supplemented one. More MA was formed in mitochondria from x irradiated rats fed a fat-free diet than in unirradiated controls at a comparable time during oxidative swelling. Shorter lag times for oxidative swelling and lower oxidative phosphorylation values were observed for starved rats than for fed rats. Also, intraperitoneally injected glucose ameliorated some effects of x irradiation-induced starvation.

The results indicate that x irradiation, starvation, a fat-free diet supplemented with ethyl linoleate or intraperitoneally injected glucose significantly affects both oxidative swelling and oxidative phosphorylation. The structural alteration observed as a decrease in lag time for oxidative swelling depends on radiation-induced starvation. The functional alteration observed as a radiation-induced stimulation of oxidative phosphorylation is independent of radiation-induced starvation. It is concluded that whole-body x irradiation induces a functional enhancement of phosphorylation in mitochondria. It is possible that a structural alteration of the outer membrane allows increased diffusion of substrates to the site of phosphorylation in the inner membrane and enhances its activity.

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EFFECTS OF IONIZING RADIATION ON THE ULTRASTRUCTURE OF MAMMALIAN TISSUES

Principal Investigators: *A. A. René and J. H. Darden*
Technical Assistance: *J. L. Parker and M. L. Guimond*

Acetylcholinesterase activity in synaptic vesicles of the medulla of rats exposed to high doses of gamma-neutron radiation was studied. Acetylcholinesterase (ACHE), an enzyme found along the external membrane of synaptic vesicles, hydrolyzes acetylcholine (ACH). This hydrolysis ends a previous impulse transmission and allows the start of subsequent impulses. Under normal conditions the balance of the transmitter substance, ACH, and the hydrolyzing agent, ACHE, is such that impulses are under complete control during nerve stimulation. The absence of ACHE or the reduction of normal ACHE activity could be catastrophic, resulting in a neurosynaptic block. This condition, which can be potentiated by drugs such as eserine, can be functionally incapacitating resulting in numerous physiological problems leading to death.

In an investigation of ACH and ACHE activity,[1] Sprague-Dawley rats were subjected to a whole-body dose of 15 krads of mixed gamma-neutron radiation. At 2 and 24 hours following irradiation, 14 animals from the irradiated group and 14 nonirradiated controls were sacrificed by replacing the blood in the brain circulation by perfusion with a sucrose-eserine solution. The medulla section of the brain was removed, homogenized and prepared for ultracentrifugation and sucrose density gradient separation. The separated synaptic vesicles were assayed for protein and ACHE. A decrease in protein was found 2 hours after irradiation with a greater decrease 22 hours later. A decrease in ACHE activity was also seen 2 hours after irradiation but there was no further decrease at 24 hours (Table XIII).

Table XIII. Enzyme Activity (ACHE) in Samples of Synaptic Vesicles of Medulla Homogenates of Control and Irradiated Rats (15 krads, sacrificed 2 and 24 hours after exposure)

	P_2*		P_3*	
	Rate+	Percent decrease	Rate+	Percent decrease
Control	4.02		3.24	
2 hours	1.31‡	67	1.20‡	62
24 hours	1.28‡	68	1.16‡	62

* P_2 and P_3 represent two stages of purity of the synaptic vesicles in the separation procedure

+ μmoles/min per g

‡ p value is < .01

These data suggest that at supralethal doses of radiation there is probably complete alteration of the synaptic vesicle membrane to the point of total destruction, resulting in a decrease in the activity of ACHE in the synaptic vesicles of the irradiated rats' medulla. The present data also suggest that the decreased ACHE activity accompanied by increased ACH activity might play a considerable role in the alteration of permeability in the blood-brain barrier or the vascular system or both. Finally, it is suggested that these physiological disturbances are involved in producing early incapacitation in animals subjected to high doses of ionizing radiation.

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CANINE CARDIOVASCULAR HOMEOSTASIS IN THE GASTROINTESTINAL RADIATION SYNDROME

Principal Investigators: *J. Kabal and S. J. Baum*
Technical Assistance: *L. J. Parkhurst*

The gastrointestinal radiation syndrome terminates in cardiovascular collapse. The initiating and one of the continuing factors during the 4-day survival period undoubtedly is the structural breakdown of the small intestine. However the progressive intestinal morphological deterioration has not been directly correlated with the progress of this clinical picture. The objective of this investigation[1] was to obtain information about the involvement of the cardiovascular system in the development of the gastrointestinal radiation syndrome. Since overt cardiovascular disturbances are rarely observed until the terminal stage, a cardiovascular stress (epinephrine) was employed to test the response of irradiated animals.

Beagles were subjected to 1500 rads of whole-body pulsed gamma-neutron radiation. The animals were anesthetized 48 and 72 hours later and the major cardiovascular parameters were obtained. At 48 hours postirradiation the major cardiovascular parameters were unchanged (Figure 27). After 72 hours, except for mean blood pressure, significant hemodynamic deterioration developed. The average stroke volume and stroke work decreased 50 percent as compared with the nonirradiated control average values. The total peripheral resistance progressively increased in the same magnitude.

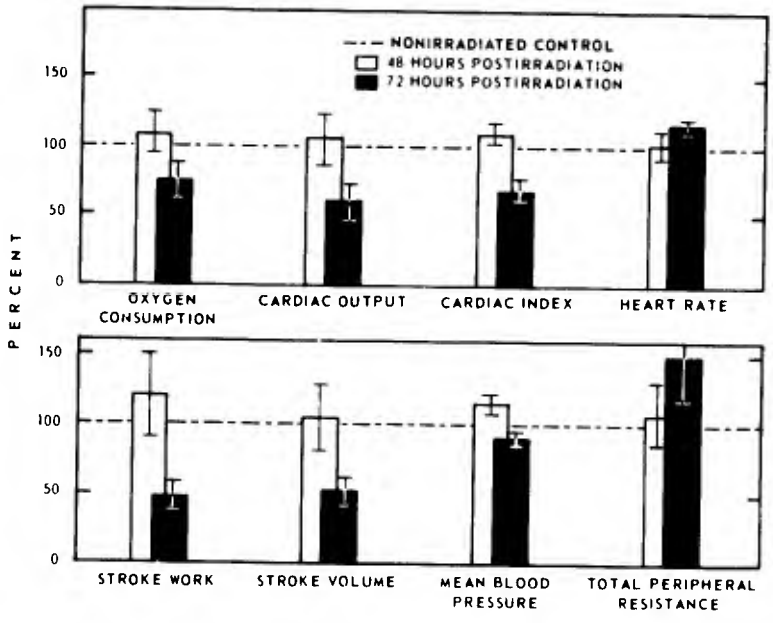

Figure 27. Percent hemodynamic alterations induced by 1500 rads pulsed gamma-neutron whole-body irradiation in beagles

After intravenous administration of 5.0 μg/kg epinephrine, the blood pressure response was significantly higher in the 48-hour group and the vagal reflex bradycardia became less expressed (Figure 28). After 72 hours postirradiation, the epinephrine-induced cardiovascular regulation was further deteriorated.

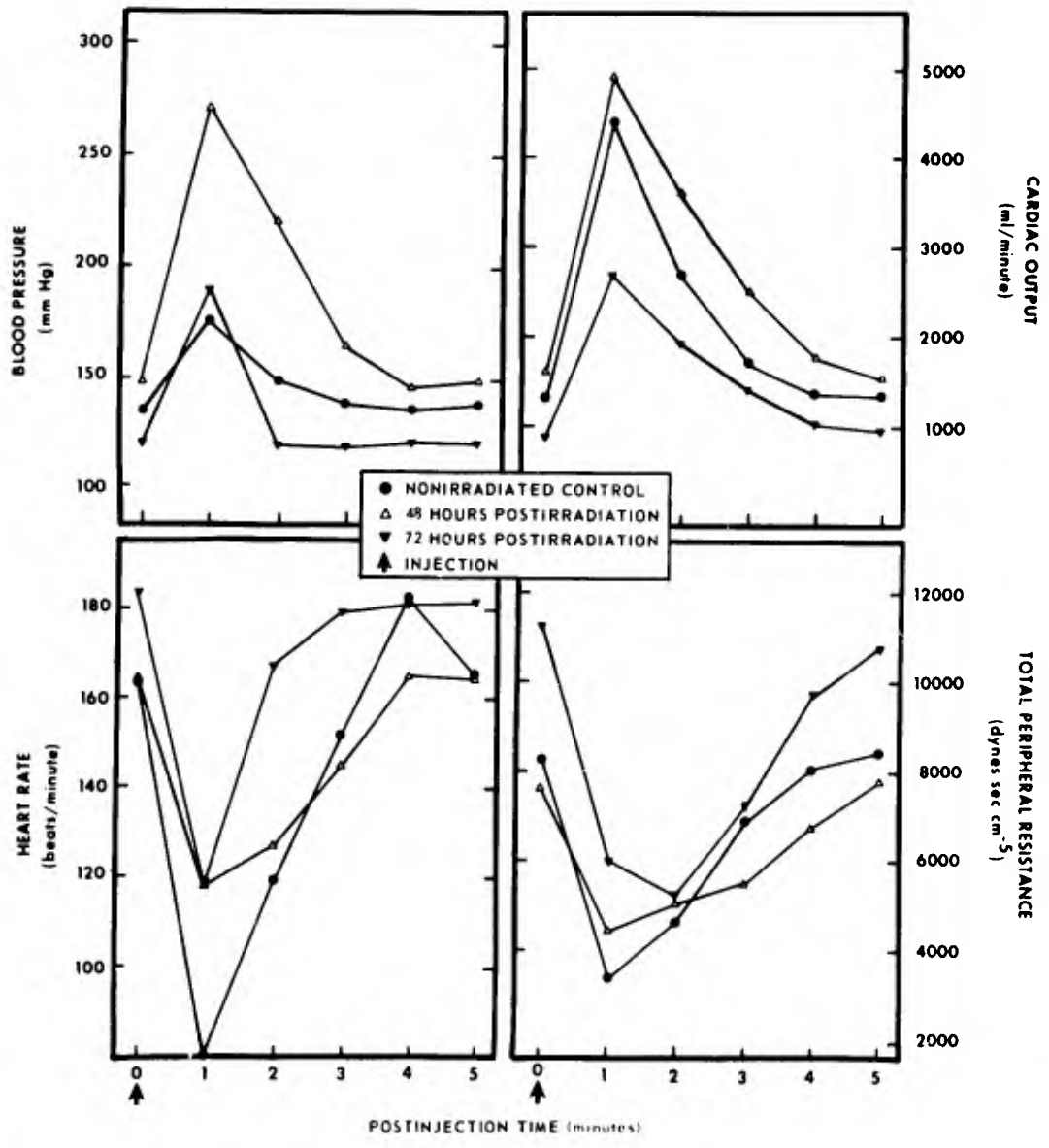

Figure 28. Median hemodynamic responses induced by 5.0 μg/kg epinephrine in beagles

The findings of the present study support the concept that functional vascular alterations underlie the development of the gastrointestinal radiation syndrome.

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THE MEASUREMENT OF CAPILLARY PERMEABILITY CHANGES IN THE IRRADIATED RAT USING A DOUBLE ISOTOPE TECHNIQUE

Principal Investigator: *M. M. Graham*

The objective of this research was to investigate changes in capillary permeability after irradiation[1] because of the possible connection with radiation-induced incapacitation and with the generalized capillary fragility that may occur several days after irradiation.

Capillary permeability was measured using ^{131}I albumin and ^{125}I albumin to assay the movement of albumin out of the bloodstream into the interstitial fluid of small intestine and muscle of the irradiated rat. One hour after 800 rads 30 MeV electron whole-body irradiation, small intestine capillary permeability increased significantly, returned to normal levels in a few hours, and then after 8 hours increased and remained at 65 percent above control levels for several days (Figure 29). Muscle capillary permeability increased within 2 hours after irradiation and remained elevated for several days (Figure 30).

The effect of cyproheptadine on capillary permeability was also investigated. This drug is a powerful antagonist of histamine and serotonin, two substances that are known to increase capillary permeability and to dilate blood vessels. Cyproheptadine injected 30 minutes prior to irradiation completely blocked a 45 percent increase in small intestine capillary permeability seen immediately after 10,000 rads ^{60}Co irradiation but had little effect at 48 hours after irradiation (Figure 31). These results indicate that histamine or serotonin may be responsible for the initial increase in capillary permeability.

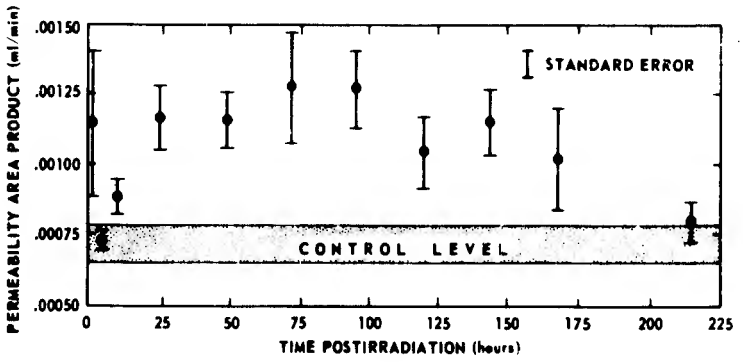

Figure 29. Capillary permeability area of rat small intestine following 800 rads fast electron irradiation. All points excepting the ones at 4, 168 and 216 hours are significantly above control.

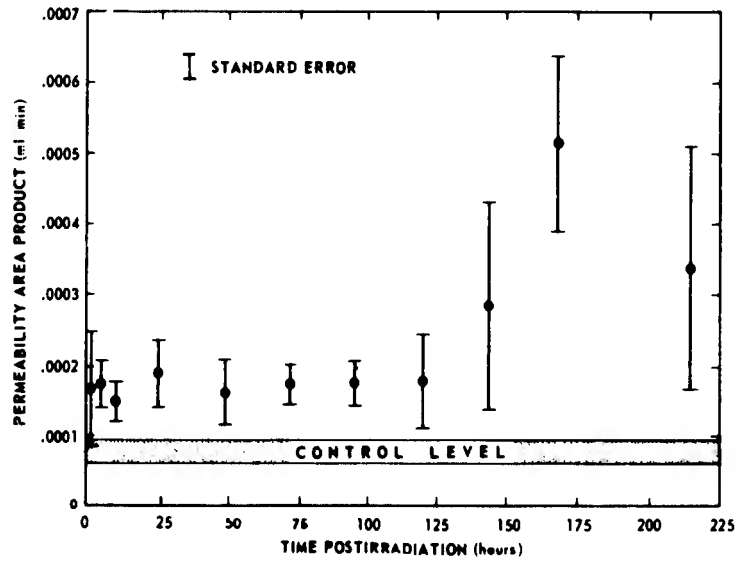

Figure 30. Capillary permeability area of rat muscle following 800 rads fast electron irradiation. All points excepting the ones at 1, 120, 144 and 216 hours are significantly above control.

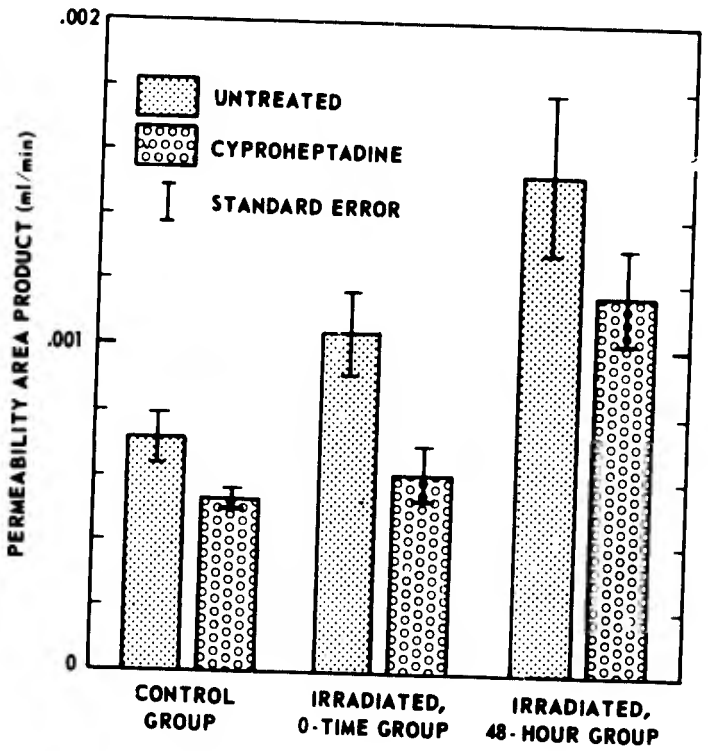

Figure 31. Capillary permeability surface area product of rat small intestine after 10,000 rads cobalt-60 irradiation. The treated groups were injected subcutaneously with 0.1 mg/kg cyproheptadine 30 minutes prior to the beginning of the measurement of the capillary permeability area.

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CHEMICAL RESPONSE OF BIOLOGICAL SYSTEMS TO IONIZING RADIATION: FREE RADICAL INTERACTIONS

Principal Investigator: *G. M. Meaburn*
Collaborator: *G. W. Donaldson*
Technical Assistance: *P. J. Ferry*

There is a current strong interest in the basic mechanisms of radiation-induced reactions in biological systems. Radiation chemical studies of model aqueous systems have provided much of the existing information on the nature and extent of the reactions between radiolytic products of water, including free radicals and ions, and a great variety of biological substrates. With the development of pulse radiolysis techniques[1,2] it is now possible to study directly many of the individual molecular processes which contribute to the overall chemical change in irradiated material.

A pulse radiolysis program has been established at the AFRRI for the purpose of obtaining a more complete understanding of the early stages of radiation-induced damage in key biological molecules, particularly deoxyribonucleic acid (DNA) and related macromolecules. The equipment required for observing and measuring the rapid chemical changes occurring in irradiated solutions is being used with the AFRRI electron linear accelerator (LINAC) as the pulsed source of radiation.

The effects of high intensity pulses of electrons on dilute aqueous solutions of DNA are currently being investigated using kinetic optical absorption spectroscopy as a detection method for following the formation and disappearance of unstable intermediates in the induced chemistry. A complex transient absorption has been recorded in the near ultraviolet region of the spectrum. Two typical spectra resulting from reactions of (1) hydroxyl radicals and (2) hydrated electrons with DNA are shown in Figure 32. They were recorded 50 μsec after DNA solutions had been subjected to a 100 nsec pulse of 40 MeV electrons, corresponding to an absorbed dose of 960 rads.

The kinetic behavior of these transient spectra is being extensively studied in carefully defined solvent matrices. Measurements of the rate of formation of the transient ultraviolet absorption at different wavelengths indicate that the band is made up of at least two distinct components. The rate of formation at 340 nm is considerably lower than that at 310 nm. This is probably a reflection of the differing rates of attack of free radicals on the various nitrogenous bases in the DNA.

The transient species responsible for the optical absorption are extremely long-lived and do not decay by a single mechanism. They are clearly visible for many milliseconds after their initial formation with a rate of decay which is insensitive to changes in such solution parameters as DNA concentration and pH.

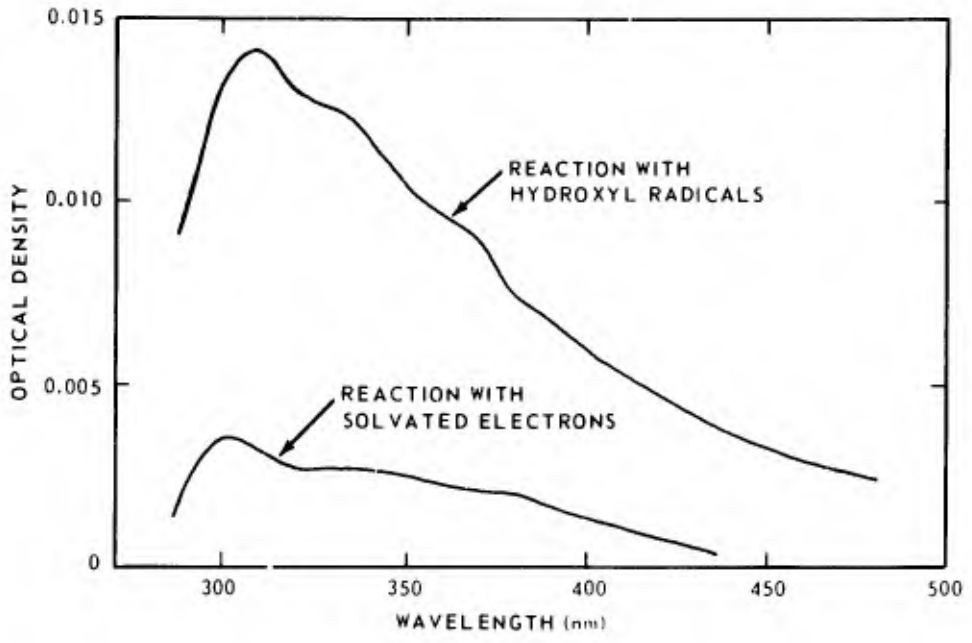

Figure 32. Transient absorption spectra observed in irradiated calf thymus DNA solutions, pH = 7, |DNA| = 70 mg/l, dose = 960 rads

The equipment used in the pulse radiolysis technique provides the high time resolution necessary for detailed study of the kinetic behavior of chemical intermediates which often have lifetimes of only a few microseconds. In using optical absorption spectroscopy as a detection method, changes in the intensity of light transmitted at a predetermined wavelength by an irradiated sample are detected photoelectrically. After suitable amplification, the signal is usually displayed on an oscilloscope and the resulting trace photographed. A detailed analysis of the raw kinetic data necessitates reading the oscilloscope traces in a quantitative manner. This step is a time-consuming and error-prone procedure when performed manually. To overcome this difficulty a compact and relatively inexpensive data retrieval and reduction system has been designed for use in pulse radiolysis studies of this type.[3] The equipment is built around a Biomation Model 610 transient recorder to take advantage of the fast response of this instrument's analog to digital converter. The wide frequency range of the system (dc to 2.5 MHz) permits digital recording of a rapidly changing non-recurrent signal with storage on punched paper tape as an intermediate step prior to further analysis.

Data resulting from the operation of this equipment are illustrated in Figure 33. This shows a computer plot of a raw data tape for a decaying optical absorption at 600 nm due to the hydrated electron (e^-_{aq}) produced by radiolysis of water. The corresponding oscilloscope trace, recorded simultaneously with the Biomation input operation, is shown for comparison purposes. Further analysis of the 120 data points with conversion of the digital information into appropriate kinetic form is now a relatively easy task.

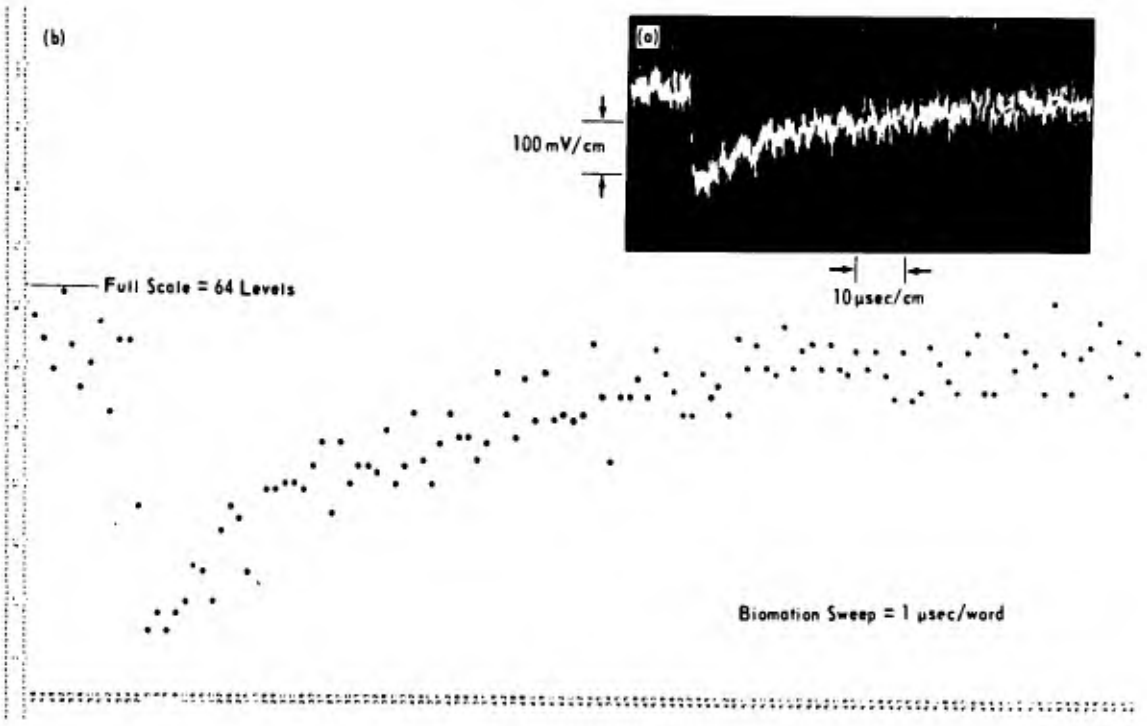

Figure 33. Decay of e^-_{aq} absorption at 600 nm; (a) typical oscillogram, (b) paper tape output after A-D conversion

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PAIRED MINIATURE IONIZATION CHAMBERS FOR REACTOR DOSIMETRY

Principal Investigators: *D. W. Shosa and D. M. Verrelli*
Technical Assistance: *C. Carter and J. T. Istock*

The objective of this research was to study paired miniature ionization chambers for reactor dosimetry.[4]

When biological specimens are exposed to the radiations of a nuclear reactor, there is a minimum amount of information necessary to characterize the exposure. Because the biological effects of neutron and gamma ray interactions with tissue may be different, the specification of total kerma at a point in the field, or even total absorbed dose at points of interest within the specimens, provides only an incomplete picture. To correlate data from different exposures, information on the separate neutron and gamma components of the total absorbed dose at points within the specimen is usually essential.

At this Institute the paired chamber technique is used for separation of neutron and gamma absorbed dose components. The basic principle is that the radiation-induced charge in an ionization chamber is made up of one component which is linear with the neutron tissue dose and another component which is linear with the gamma tissue dose. The response equation is then:

$$Q = \mathfrak{Z}kN + \eta a\Gamma$$

where Q is the charge produced by radiation within the chamber when exposed to a neutron dose of N rads and a gamma dose of Γ rads, k and a are neutron and gamma ray sensitivity coefficients and $\mathfrak{Z}$ and η are neutron and gamma wall attenuation corrections.

For free-in-air tissue kerma measurements, 50 cm^3 ionization chambers are employed. The first of the two chambers is fabricated from tissue-equivalent (TE) plastic and is filled with tissue-equivalent gas. The second chamber is fabricated from graphite and is filled with CO_2 gas.

Depth-dose dosimetry is performed with miniature 0.05 cm^3 ionization chambers.[3] The materials of the first miniature chamber are the same as those of the 50 cm^3 chamber. The second miniature chamber is machined of magnesium and is filled with CO_2 gas.

The neutron sensitivity of the tissue and the graphite chambers has been determined by application of the Bragg-Gray cavity chamber theory for homogeneous chambers.[1] The neutron sensitivity of the magnesium chamber has been determined by comparison with the 50 cm^3 graphite-CO_2 chamber in a variety of mixed neutron-gamma ray fields. Neutron attenuation corrections for the chambers have been

calculated in a manner similar to that used by Neary et al.[2] The neutron sensitivity coefficients and the neutron attenuation corrections are summarized in Table XIV.

Chamber	ℨ	η	k
TE	0.90	0.96	0.98
Graphite	0.99	0.96	0.08
Magnesium	0.95	0.98	0.07

Table XIV. Paired Chamber Equation Coefficients

Estimates of the systematic errors associated with the measurement of charges and estimates of sensitivity coefficients are shown in Figure 34. These arise from the following estimates of systematic error components:

(a) An estimated uncertainty of ± 3 percent for the neutron sensitivity coefficient of the TE chamber arises as a result of an energy dependence of W for heavy recoils.

(b) An estimated uncertainty of ± 10-15 percent for the Mg chamber neutron sensitivity arises as a result of the energy dependence of W for heavy recoils and also as a result of an energy dependence of the mass energy transfer coefficients of Mg and CO_2 for neutrons relative to that of tissue.

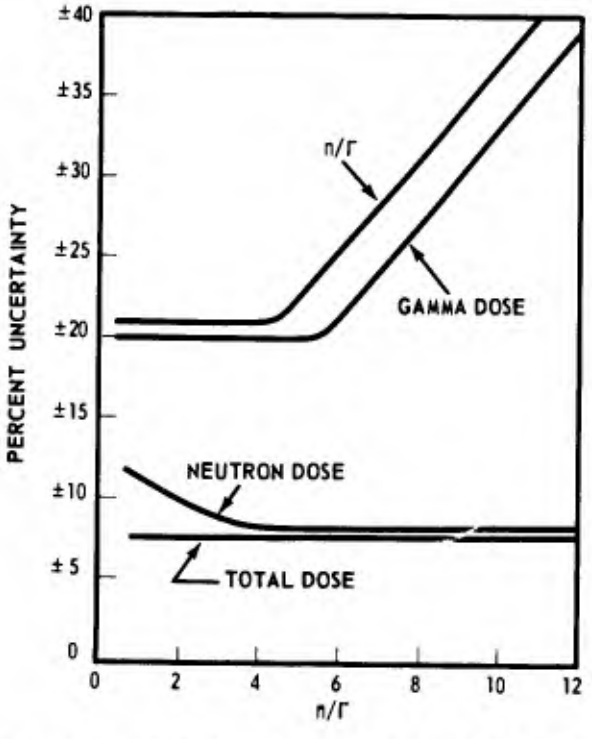

Figure 34. Uncertainty in separated neutron and gamma doses

(c) An estimated uncertainty of ± 5 percent of the gamma sensitivity of the Mg chamber arises as a result of a low-energy component in the gamma component of the mixed field.

(d) An estimated uncertainty of ± 1 percent is associated with the charge measurement process.

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IRRADIATION TECHNIQUES AND DOSIMETRY AT THE AFRRI LINAC

Principal Investigators: *P. A. Berardo and R. T. Devine*
Collaborators: *D. W. Shosa, D. M. Verrelli and T. W. Hinz*
Technical Assistance: *C. L. Bransford*

The AFRRI linear accelerator (LINAC) became available for radiation biology experimentation during this report period. Three types of radiation (electrons, x rays and neutrons) can be produced by this machine; experimentation to date has been limited to electron irradiation.

The AFRRI LINAC has an effective energy range from 5 to 50 MeV, with two modes of operation, providing maximum instantaneous currents of approximately 3 amperes at 12 MeV and 0.75 amperes at 37 MeV. The LINAC is capable of a 0.001 duty factor, but has been routinely operated with 4 μsec pulses at 60 Hz, or at about one-fourth maximum output.

Irradiation experiments to date include partially shielded and unshielded rat arrays, head only irradiation of miniature pigs, sterilization of tissue samples and irradiation of transistors and diodes. Monkey irradiations (whole body, partial body and retina) are presently being planned.

Absorbed dose rates have ranged from 10 rads/pulse to 15,000 rads/pulse. The high doses per pulse present special dosimetry problems. Dosimetry at the LINAC relies routinely on thermoluminescent dosimeters (TLD's) in cadavers and phantoms. The main motivations for using TLD's are their small size, handling ease, and freedom from external electronics during irradiation. LiF extruded rods supplied by Harshaw as TLD 700's are currently being used. The primary reasons for selecting LiF were its insensitivity to neutrons, its nearly flat energy response, and the hope that problems associated with supralinearity would not be too severe. The TLD's are calibrated at AFRRI's 30,000 curie ^{60}Co facility, with dose rates determined with a 3 cc ion chamber calibrated at the National Bureau of Standards.

The accuracy of TLD dosimetry, according to our experience, is only about 5 percent. This is illustrated in Table XV which shows the mean and standard deviation in charge collected for successive irradiations of 69 TLD's to 1000 ^{60}Co rads. Also shown are the results in a subset of 10 TLD's. This subset had the least deviation from the mean after the fourth and fifth exposures.

Table XV. Exposure of TLD 700 to Cobalt Gamma Rays

Exposure #	Mean charge per 1000 rads (microcoulomb)	
	69 TLD's	10 TLD's
1	Not read	
2	3.10 ± 6.2%	3.09 ± 5.0%
3	3.32 ± 4.6%	3.34 ± 2.9%
4	2.90 ± 6.0%	2.84 ± 4.6%
5	2.83 ± 6.1%	2.84 ± 4.6%
6	Calibration:	3.06 ± 3.6%
7	10 TLD check:	3.06 ± 6.8%

If there were systematic errors associated with the response of individual TLD's, it was anticipated that the standard deviation could be reduced by eliminating those TLD's which had a large deviation from the mean. However, as depicted in Figure 35 this does not help a great deal. Shown is the change in deviation from the mean for each TLD, between the second and third exposures. This distribution has a standard deviation of about 5.7 percent and illustrates a random nature in TLD response. Since the reader maintains a precision of about 0.6 percent, based upon its internal calibration source, the random response is attributed to the individual TLD's. This phenomenon is being analyzed in more detail.

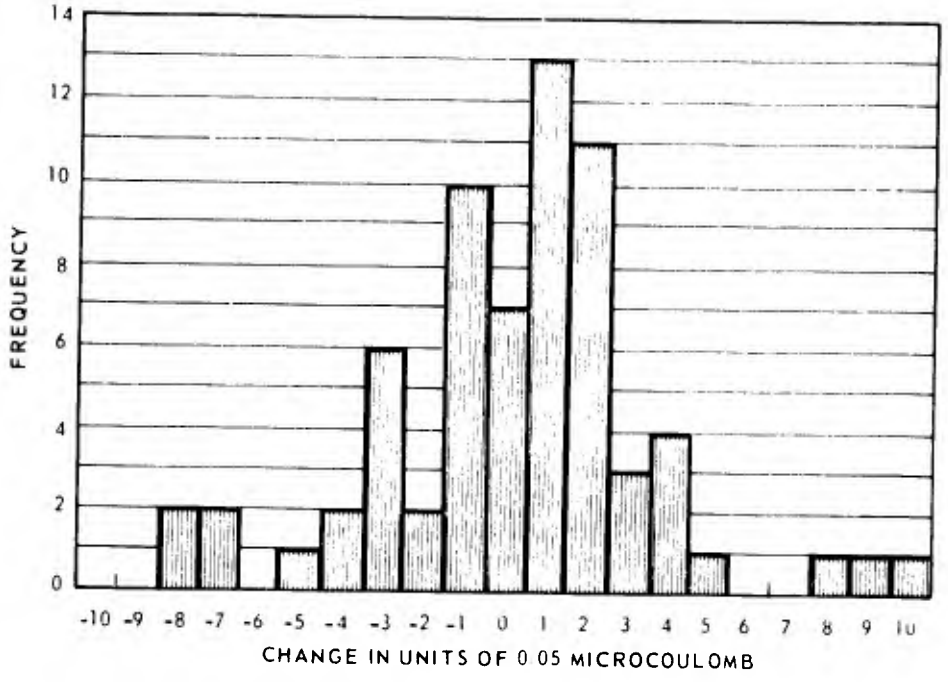

Figure 35. Frequency distribution of change in deviation from mean for exposure 3 - exposure 2

Since most of the LINAC exposures are on the order of 10,000-20,000 rads, which would rapidly extend the accumulated dose in TLD's beyond acceptable limits, diodes are used to monitor the electron field. In this approach the dose rate per pulse is determined with TLD's, and the diode response per pulse is noted and then scaled for the desired number of pulses. The precision of the diodes is at the 1 percent level, with high linearity, so that the dosimetry is still limited by the TLD's.

Of particular interest is the relative response of TLD's to other dosimetry systems. Figure 36 shows a relative depth-dose distribution in water as determined by TLD 700 rods, TLD 700 ribbons, Fricke solution, and diodes. The field had a diameter of 30 cm, and the water phantom was 30 cm square. Each depth-dose distribution is independently normalized to its maximum response. The agreement is worth noting, even though only relative response is shown.

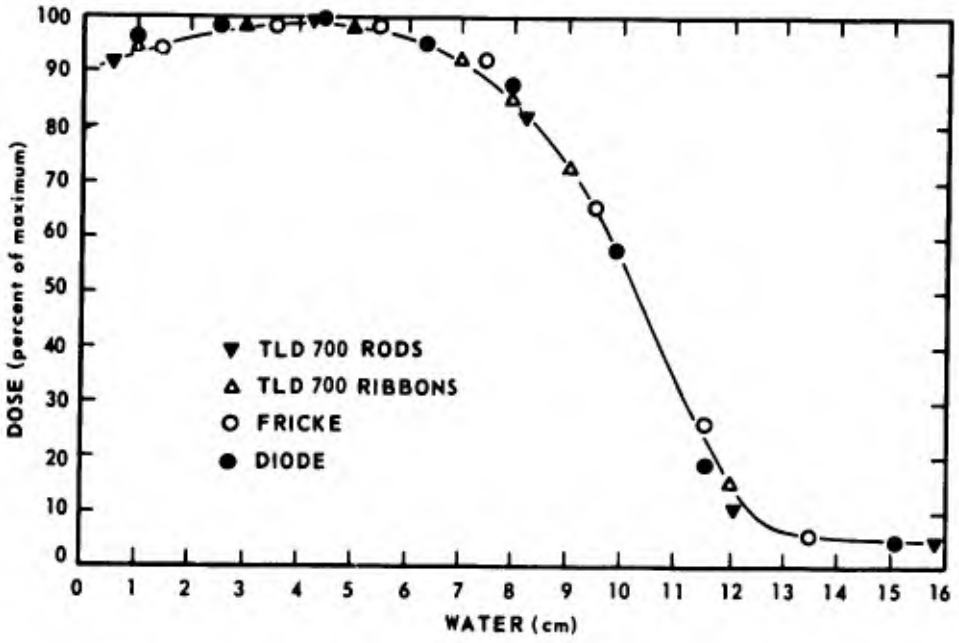

Figure 36. Depth-dose distribution in water, 27 MeV

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