SAM-TR-68-45

AD673352

EARLY HEMATOLOGIC CHANGES IN THE RHESUS MONKEY AFTER SUPERLETHAL MIXED NEUTRON-GAMMA IRRADIATION

DONALD R. ANDERSON, Ph.D.
ROBERT J. YOUNG, Lieutenant Colonel, USAF, VC

USAF School of Aerospace Medicine
Aerospace Medical Division (AFSC)
Brooks Air Force Base, Texas

May 1968

20050202057

14

Best Available Copy

Qualified requesters may obtain copies of this report from DDC. Orders will be expedited if placed through the librarian or other person designated to request documents from DDC.

When U. S. Government drawings, specifications, or other data are used for any purpose other than a definitely related Government procurement operation, the Government thereby incurs no responsibility nor any obligation whatsoever; and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

This document has been approved for public release and sale; its distribution is unlimited.

EARLY HEMATOLOGIC CHANGES IN THE RHESUS MONKEY AFTER SUPERLETHAL MIXED NEUTRON-GAMMA IRRADIATION

DONALD R. ANDERSON, Ph.D.
ROBERT J. YOUNG, Lieutenant Colonel, USAF, VC

FOREWORD

Research described in this paper was performed in the Radiobiology Division under task No. 571003, supported, in part, by the Defense Atomic Support Agency. The period covered by the study was from December 1966 to December 1967. The report was received for publication on 29 February 1968.

Robert J. Fuchs and Richard C. McNee of the Biometrics Division gave valuable suggestions and performed the statistical analyses presented in the tables. Staff Sergeant Harry R. Rithman, Jr., and Miss Nora Olivarez gave technical assistance in the conduct of the experiments.

The Fast Burst Reactor at White Sands Missile Range was used as the source of radiation. Dosimetry measurements were accomplished by Edgerton, Germeshausen and Grier, Inc., Santa Barbara, Calif.

The animals involved in this study were maintained in accordance with the "Guide for Laboratory Animal Facilities and Care" as published by the National Academy of Sciences–National Research Council.

This report has been reviewed and is approved.

GEORGE E. SCHAFER
Colonel, USAF, MC
Commander

ABSTRACT

To determine early hematologic response to mixed neutron-gamma irradiation, twenty *Macaca mulatta* monkeys were exposed to pulsed doses of 2,500, 3,750, and 5,000 rads.

Hematologic data at 4 hours postirradiation indicated a marked leukocytosis in all groups. The cellular increase was due to the increase of neutrophils contrasted to the lymphocytes, which decreased to less than half their baseline value. There was also a thrombocytosis at 4 hours postirradiation. Serum enzyme concentrations were determined with observed increases of lactic dehydrogenase and glutamic oxalacetic transaminase; however, the response was highly variable.

ABSTRACT

To determine early hematologic response to mixed neutron-gamma irradiation, twenty *Macaca mulatta* monkeys were exposed to pulsed doses of 2,500, 3,750, and 5,000 rads.

Hematologic data at 4 hours postirradiation indicated a marked leukocytosis in all groups. The cellular increase was due to the increase of neutrophils contrasted to the lymphocytes, which decreased to less than half their baseline value. There was also a thrombocytosis at 4 hours postirradiation. Serum enzyme concentrations were determined with observed increases of lactic dehydrogenase and glutamic oxalacetic transaminase; however, the response was highly variable.

EARLY HEMATOLOGIC CHANGES IN THE RHESUS MONKEY AFTER SUPERLETHAL MIXED NEUTRON-GAMMA IRRADIATION

I. INTRODUCTION

The classic work of Heineke (1) as early as 1903 demonstrated the marked radiosensitivity of the hematopoietic tissues to exposure to ionizing radiation. Since that time, a vast amount of data has accumulated on the hematologic responses of different species to various types of radiation. The reader may refer to a number of excellent reviews that summarize this earlier material (2-5).

This report presents data on early, 4-hour observations of the *Macaca mulatta* monkey following doses of mixed neutron-gamma irradiation ranging from 2,500 to 5,000 rads. It is believed this information is not available in the literature.

II. MATERIALS AND METHODS

Subjects were 20 rhesus *(Macaca mulatta)* monkeys weighing between 7 and 9 pounds. Animals of both sexes were used. The animals had been trained for four months on a multiple avoidance program designed to measure performance. The principal objective of the experiment was to obtain data concerning initial onset and duration of performance decrement resulting from exposure to the mixed fission source of radiation. The behavioral responses were monitored for an hour following the irradiation exposure. The subjects were exposed two at a time while seated in a Plexiglas restraining couch. The couch prevented gross movement except by the left arm and hand which were used in the performance of the learned task. The data obtained relative to the behavioral response will be published as a related report.

The neutron-gamma ratio of the radiation dose approximated 6/1 with a pulse width of 40 μsec. The animals were divided into three irradiation groups, 6 animals receiving 2,500 rads, 6 receiving 3,750 rads, and 8 animals receiving 5,000 rads from the pulsed reactor. Actual measured doses were: for the nominal *2,500 rads*—2,360, 2,450, 2,550, 2,580, 2,670, and 2,760; for *3,750 rads*—3,200, 3,280, 3,480, 3,500, 3,620, and 3,980; and for *5,000 rads*—4,510, 4,590, 4,600, 4,900, 4,930, 4,960, 5,750, and 6,410.

Each animal had been sampled three times before exposure for baseline hematologic determinations at approximately 2-week intervals. The last sample was drawn more than 2 weeks before exposure.

The animals had been implanted with cranial electrodes approximately 10 days prior to exposure, and immediately before exposure catheters for the physiologic observations were surgically inserted into the femoral artery.

At 1 hour after the pulsed exposure, the animals were removed from their exposure chairs and a 1-ml. sample of blood was taken from the catheter for later analysis of serum glucose. At 4 hours, a large sample was taken by venous puncture for blood counts and other serum chemistry determinations. After the blood preparations were made for hematocrit, hemoglobin, total white count, platelet count, and differentials, the serum clot was spun down and the remaining serum rapidly frozen in Dry Ice–acetone. Subsequently the sample was returned to the laboratory packed in Dry Ice for serum analyses. The blood determinations followed standard clinical methods (6)

and chemical assay procedures previously described (7).

Differences in the postirradiation values and the preirradiation values were analyzed, using analysis of variance technics. An appropriate test for difference between the preirradiation mean and the postirradiation mean for a given dose level or for the average dose is:

$$t = \frac{D}{S.E.}, \text{ with N-1 degrees of freedom}$$

where

t = Student's t-test.

N = Number of observations in each mean.

D = Mean difference.

S.E. = Standard error of the mean difference.

No significant difference in effects due to level of dosage was detected at the .05 probability level for any variable. Therefore, since there were apparently no differences in the preirradiation to postirradiation response for the different doses, this "average" test is an indication of irradiation effect, assuming the effect to be dose-independent over the range of doses tested.

III. RESULTS

Table I summarizes the experimental results of the white cell parameters showing for each nominal dose the preirradiation and postirradiation means, along with the mean difference (postirradiation value minus preirradiation value) and the standard error of the difference. The values for neutrophils and lymphocytes are the relative percentage of 100 cells counted.

TABLE I

Means of white cell parameters

Dose (rads)	N	Preirradiation	Postirradiation	Difference (post − pre)	S.E.
		WBC			
2,500	6	10,866.7	30,533.3	19,666.6	6,088.07
3,750	6	11,258.3	28,800.0	17,541.7	1,547.71
5,000	8	10,216.7	26,587.5	16,370.8	4,576.95
Av.	20	10,724.17	28,435.00	17,710.83	2,632.89
		$P < .001$			
		Neutrophils*			
2,500	6	42.6	90.5	47.9	3.19
3,750	6	45.9	90.8	44.9	1.03
5,000	8	47.1	92.0	44.9	3.80
Av.	20	45.37	91.20	45.83	1.86
		$P < .001$			
		Lymphocytes*			
2,500	6	55.8	7.0	− 48.8	2.35
3,750	6	52.6	7.8	− 44.8	1.58
5,000	8	50.4	6.8	− 43.6	4.06
Av.	20	52.65	7.15	− 45.50	1.85
		$P < .001$			

*Percent of 100 cells counted.

Table II shows that there is no significant change in hematocrit and reticulocyte counts but that the hemoglobin value is significant and the platelet count is highly significant.

Table III shows values for both serum glucose and total protein 1 hour and 4 hours postirradiation. It should be noted that the 4-hour postexposure values for glucose are not significantly different while those of the 1-hour sample are. The overall difference for total protein indicates a value of $P < .005$. The variability of the differences between the preirradiation and postirradiation values are significant for the three groups ($P < .005$). Since homogeneous group variability is an assumption in the analysis of variance, and this assumption was not met for total protein, the

TABLE II

Means of other blood values

Dose (rads)	N	Preirradiation	Postirradiation	Difference (post−pre)	S.E.
		Hematocrit			
2,500	6	37.4	37.3	− 0.1	1.30
3,750	6	37.3	38.0	0.7	1.04
5,000	8	35.5	37.6	2.1	0.57
Av.	20	36.61	37.65	1.04	0.54
		NS, $P > .05$			
		Hemoglobin*			
2,500	6	11.76	12.28	0.52	0.54
3,750	6	11.80	12.45	0.65	0.36
5,000	8	11.19	12.16	0.97	0.21
Av.	20	11.54	12.28	0.74	0.21
		$P < .005$			
		Reticulocytes†			
2,500	6	0.98	1.03	0.05	0.18
3,750	6	0.92	1.03	0.11	0.16
5,000	8	0.87	1.07	0.20	0.14
Av.	20	0.92	1.05	0.13	0.09
		NS, $P > .05$			
		Platelets			
2,500	6	270,111.1	457,166.7	187,055.6	25,522.60
3,750	6	267,611.1	413,666.7	146,055.6	15,616.57
5,000	8	261,750.0	439,125.0	177,375.0	33,288.09
Av.	20	266,016.67	436,900.00	170,883.33	16,171.13
		$P < .001$			

*Grams per 100 ml.

†Percent of 100 cells counted.

result of the analysis of variance for total protein is questionable. Making separate tests for the three groups did not reveal any differences in the preirradiation and postirradiation means in the 5,000-rad group, but did detect differences between these means in the 2,500-rad and the 3,750-rad groups ($P < .001$ and .02, respectively).

Table IV indicates serum enzyme values, with the changes in LDH and SGOT being significant, as indicated, while the SGPT change is not significant.

IV. DISCUSSION AND CONCLUSIONS

An increase in the polymorphonuclear neutrophilic leukocytes was reported in the monkey as early as 1919 by Taylor et al. (8). This primary elevation, from 24 to 48 hours after x-ray exposure, was followed by a decrease. Eldred and Eldred (9) also reported an early rise in the total white count made 1 to 5 hours after irradiation exposure in the rhesus monkey. Henshaw, in a series of reports (10-13), showed that a slight leukocytosis took place in mice during the second to fourth hours following exposure of 50 R, with a leukopenia evident at 24 hours. Again a leukocytosis was displayed within a few hours after treatment following 100 R. He reports that with small daily doses of 5 to 25 R per day, there was a fall in leukocyte level attributable mainly to the lymphocyte loss. In rabbits exposed to 25,000 R and 50,000 R the leukocytes were shown to drop to about one-third their normal value during the second hour.

TABLE III

Means of serum chemistry tests

Dose (rads)	N	Preirradiation	Postirradiation	Difference (post−pre)	S.E.
		Glucose* (1-hour)			
2,500	6	76.5	84.3	7.8	12.57
3,750	6	79.0	97.3	18.3	8.02
5,000	8	81.5	102.0	20.5	11.12
Av.	20	79.27	95.30	16.03	6.32
		$P < .025$			
		Glucose* (4-hour)			
2,500	6	76.5	72.0	− 4.5	10.06
3,750	6	79.0	99.5	20.5	12.55
5,000	8	81.5	82.8	1.3	8.97
Av.	20	79.27	84.55	5.28	6.01
		NS, $P > .05$			
		Total protein†			
2,500	6	7.76	7.15	− 0.61	0.07
3,750	6	7.61	7.15	− 0.46	0.13
5,000	8	7.35	6.87	− 0.48	0.34
Av.	20	7.55	7.04	− 0.51	0.14
		$P < .005$			

*Milligrams per 100 ml.

†Grams per 100 ml.

Nakahara and Murphy (14), by using a specially constructed low-frequency x-ray tube, were able to show an increase in lymphocytes in mice by the second day postirradiation. The present study confirms the evidence of an early leukocytosis at 4 hours after exposure but establishes the increased cellularity as being with the neutrophilic portion of the cells whereas the lymphocytes show a rather rapid decline in this short time period.

Duke (15) in 1915 reported that an intensive dose of roentgen rays causes a fall in platelet count, but in smaller doses it causes a rise in total platelets. Eldred and Eldred (9) reporting on the platelet count on irradiated monkeys stated that the daily counts indicated the number of platelets declined slowly until about the seventh day and then dropped rapidly. In the present study the 4-hour postirradiation sampling period indicates substantially a twofold increase in platelet counts observed at this time.

In comparing the baseline serum glucose values with the 1-hour and 4-hour postirradiation values, it can be seen that the 1-hour sample is generally elevated. In two of the three groups, however, the 4-hour sample is essentially normal. Earlier work in this laboratory (16) indicated that glucose elevation can occur very rapidly—in 15 to 30 minutes. By 4 hours after a lethal dose of 900 R of gamma radiation, the glucose concentrations can be reestablished at essentially a normal level. Baseline values and 4-hour postirradiation values for total serum protein show an overall change at $P < .005$, but because of

TABLE IV

Means of enzyme determinations

Dose (rads)	N	Preirradiation	Postirradiation	Difference (post − pre)	S.E.
		LDH*			
2,500	6	569.2	743.3	174.1	202.61
3,750	5	817.7	1,001.0	183.3	288.64
5,000	7	580.0	958.6	378.6	87.05
Av.	18	642.33	898.61	256.28	109.14
		$P < .05$			
		SGOT†			
2,500	6	48.3	81.2	32.9	19.26
3,750	5	55.7	81.4	25.7	20.24
5,000	7	44.6	79.4	34.8	13.73
Av.	18	48.89	80.56	31.67	10.04
		$P < .01$			
		SGPT‡			
2,500	6	52.3	44.8	− 7.5	11.35
3,750	5	47.5	50.4	2.9	3.79
5,000	7	50.0	53.7	3.7	9.15
Av.	18	50.10	49.83	− 0.27	5.33
		NS, $P > .05$			

*Determatube C-LDH; Worthington Biochemical Corp., Freehold, N. J.

†TransAc; Warner Chilcott, Morris Plains, N. J.

‡Reitman-Frankel method, as modified by Dade Reagents, Inc., Miami, Fla.

differences in variability, these changes require additional investigation.

Attempts have been made to assess the amount of radiation damage by measuring changes from normal levels of a number of enzymes found in serum (17). These included, particularly, lactic dehydrogenase (LDH), glutamic oxalacetic transaminase (SGOT), and glutamic pyruvic transaminase (SGPT). The postirradiation values shown for both LDH and SGOT are elevated when compared to the preirradiation baseline mean. Dalrymple et al. (18) comment that there is no satisfactory explanation for the highly variable response of different animals as demonstrated by the changed serum enzyme concentration but speculate there may well be a considerable variation as to the onset in respect to time following the tissue damage by the irradiation exposure. The SGPT values were essentially unchanged during this period. Further research should be directed toward study of the enzyme systems for a considerably longer period of time after the irradiation insult.

It is believed that these observations do show that hematologic changes can be very rapid and that additional time-response information should be obtained in order to develop a more meaningful interpretation of this altered cellularity with radiation damage.

REFERENCES

1. Heinecke, H. Ueber die Einwirkung der Roentgenstrahlen auf Tiere. München. Med. Wochnschr. 50:2090-2092 (1903).

2. Selling, L., and E. E. Osgood. Action of benzol, roentgen rays, and radioactive substances on the blood and blood-forming tissues. *In* Downey, Hal (ed.). Handbook of Hematology, vol. 4, pp. 2693-2801. New York: Hoeber, 1938.

3. Dunlap, C. E. III. Effects of radiation on the blood and the hemopoietic tissues, including the spleen, the thymus and the lymph nodes. Arch. Path. (Chicago) 34:562-608 (1942).

4. Cronkite, E. P., and G. Brecher. The protective effect of granulocytes in radiation injury. Ann. N. Y. Acad. Sci. 59:665-1070 (1955).

5. Clemedson, C. J., and A. Nelson. General biology: The adult organism. *In* Errera, M., and A. Forssberg (eds.). Mechanisms in radiobiology, vol. 2, pp. 95-205. New York: Academic Press, 1960.

6. Air Force Manual 160-51. Laboratory procedures in clinical hematology (1962).

7. Anderson, D. R. Normal values for clinical blood chemistry tests of the *Macacq mulatta* monkey. Amer. J. Vet. Res. 27:1484-1489 (1966).

8. Taylor, H. D., W. D. Witherbee, and J. B. Murphy. Studies on x-ray effects. J. Exp. Med. 29:53-73 (1919).

9. Eldred, E., and W. Eldred. Effects of total body x-irradiation on the peripheral blood of the monkey. Blood 8:262-269 (1953).

10. Henshaw, P. S. Experimental roentgen injury. I. Effects on the tissues and blood of C3H mice produced with single small whole-body exposures. J. Nat. Cancer Inst. 4:477-484 (1943-1944).

11. Henshaw, P. S. Experimental roentgen injury. II. Changes produced with intermediate-range doses and a comparison of the relative susceptibility of different kinds of animals, J. Nat. Cancer Inst. 4:485-501 (1943-1944).

12. Henshaw, P. S. Experimental roentgen injury. III. Tissue and cellular changes brought about with single massive doses of radiation. J. Nat. Cancer Inst. 4:503-512 (1943-1944).

13. Henshaw, P. S. Experimental roentgen injury. IV. Effects of repeated small doses of x-rays on blood picture, tissue morphology, and life span in mice. J. Nat. Cancer Inst. 4:513-522 (1943-1944).

14. Nakahara, W., and J. B. Murphy. Studies on x-ray effects. X. The biological action of small doses of low frequency x-rays. J. Exp. Med. 35:475-486 (1922).

15. Duke, W. W. The effect of the roentgen ray on the platelet count. J. A. M. A. 65:1602-1603 (1915).

16. Anderson, D. R., A. Hernandez-Diaz, and F. A. Quinn. Postirradiation hyperglycemia in the primate. SAM-TR-63-40, June 1963.

17. Albaum, H. G. Serum enzymes following whole-body radiation in the rabbit. Radiat. Res. 12: 186-194 (1960).

18. Dalrymple, G. V., I. R. Lindsay, J. J. Ghidoni, H. L. Kundel, and E. T. Still. The effect of massive doses of 32-Mev protons and Co^{60} gamma radiation on serum enzyme levels of whole-body irradiated primates. SAM-TR-65-22, May 1965.

Unclassified

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1 ORIGINATING ACTIVITY (Corporate author)

USAF School of Aerospace Medicine
Aerospace Medical Division (AFSC)
Brooks Air Force Base, Texas

2a. REPORT SECURITY CLASSIFICATION

Unclassified

2b. GROUP

3 REPORT TITLE

EARLY HEMATOLOGIC CHANGES IN THE RHESUS MONKEY AFTER SUPERLETHAL MIXED NEUTRON-GAMMA IRRADIATION

4 DESCRIPTIVE NOTES (Type of report and inclusive dates)

Dec. 1966 - Dec. 1967

5 AUTHOR(S) (First name, middle initial, last name)

Donald R. Anderson
Robert J. Young, Lieutenant Colonel, USAF, VC

6 REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
May 1968	7	18

8a. CONTRACT OR GRANT NO	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO	SAM-TR-68-45
c. Task No. 571003	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d.	

10 DISTRIBUTION STATEMENT

This document has been approved for public release and sale; its distribution is unlimited.

11 SUPPLEMENTARY NOTES	12 SPONSORING MILITARY ACTIVITY
	USAF School of Aerospace Medicine Aerospace Medical Division (AFSC) Brooks Air Force Base, Texas

13 ABSTRACT

To determine early hematologic response to mixed neutron-gamma irradiation, twenty Macaca mulatta monkeys were exposed to pulsed doses of 2,500, 3,750, and 5,000 rads.

Hematologic data at 4 hours postirradiation indicated a marked leukocytosis in all groups. The cellular increase was due to the increase of neutrophils contrasted to the lymphocytes, which decreased to less than half their baseline value. There was also a thrombocytosis at 4 hours postirradiation. Serum enzyme concentrations were determined with observed increases of lactic dehydrogenase and glutamic oxal-acetic transaminase; however, the response was highly variable.

DD FORM 1 NOV 65 1473

Unclassified

Security Classification

14 KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Radiobiology Primates, Macaca mulatta Radiation, ionizing Hematologic changes Serum enzyme changes						