AFRRI SR69-23
DECEMBER 1969

AFRRI
SCIENTIFIC
REPORT

AFRRI SR69-23

PLASMA CHEMISTRY VALUES FOR THE MONKEY (MACACA MULATTA) AFTER A SUPRALETHAL DOSE OF PULSED MIXED GAMMA-NEUTRON RADIATIONS

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

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PLASMA CHEMISTRY VALUES FOR THE MONKEY (MACACA MULATTA) AFTER A SUPRALETHAL DOSE OF PULSED MIXED GAMMA-NEUTRON RADIATIONS

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ACKNOWLEDGMENT

The author acknowledges the assistance of many individuals at AFRRI who contributed to this study. In particular, W. A. Dewes and V. A. Kieffer are acknowledged for their work in chemistry and the preparation and collection of data. Recognition is also extended to D. Wise and E. L. Barron for their assistance with the surgery.

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FOREWORD
(Nontechnical summary)

When mammalian organs or systems are injured, chemical components of the damaged tissue are released into the blood. The presence or changes in the concentration of these components can be measured and in many instances they are specific to the injured organ or system. These changes in the chemical components of blood after irradiation have been thought to be due to increased permeability of cell membranes or to cell death.

In the present study, the concentrations of 11 constituents of the blood plasma of monkeys were determined at approximately 1 hour preirradiation and at 1, 6, and 12 hours after a 4-krad midline tissue dose of pulsed mixed gamma-neutron radiations. After irradiation significant increases occurred in the plasma concentrations of glutamic-oxalacetic transaminase, total lactic dehydrogenase, urea nitrogen, creatinine, and creatine.

Some of the constituents of plasma which were found to increase after irradiation, such as glutamic-oxalacetic transaminase and total lactic dehydrogenase, were probably from radiosensitive tissues such as the intestine, bone marrow, spleen, and lymph nodes. The increase in the plasma concentration of urea nitrogen was probably due to a release of amino acids from radiosensitive tissues and conversion to urea nitrogen by the liver. The increase in the plasma concentrations of creatinine and creatine suggests some injury to muscle tissue, not usually considered sensitive to irradiation, causing an increased rate of release and conversion of these constituents.

ABSTRACT

Monkeys (Macaca mulatta) were given a 4-krad midline tissue dose of pulsed mixed gamma-neutron radiations. Chemical analyses of 11 different constituents of plasma were made before irradiation and at 1, 6, and 12 hours postirradiation to evaluate the extent of radiation injury as indicated by changes in the composition of the plasma. A significant increase in the plasma concentration of glutamic-oxalacetic transaminase, total lactic dehydrogenase, creatinine, creatine, and urea nitrogen was found postirradiation. Although some of the constituents of plasma which were found to increase after irradiation were probably from radiosensitive tissue, other tissues such as muscle, not considered radiosensitive, appear to have been also injured when subjected to this dose of radiation.

I. INTRODUCTION

Changes in the concentrations of chemical constituents of blood after supralethal doses of radiation have been found by several investigators.[3,4,9,17] These changes could result from either cell death, altered cell permeability, or other cell dysfunction. A study of the chemical constituents of blood should assist in the identification and evaluation of the more prominent sites of radiation injury.

The objective of this study is to evaluate the extent of radiation injury indicated by changes in the blood plasma from monkeys which have received a supralethal dose of pulsed mixed gamma-neutron radiations. A dose of 4 krads was chosen for this study. At this dose, the classical central nervous system syndrome predominates [16] but the average survival time is sufficient to permit the detection of constituents in the blood which may be altered after several hours but indicate injury that occurred at the time of irradiation.

II. MATERIALS AND METHODS

Seventeen young adult monkeys (Macaca mulatta) of both sexes were used in this investigation. The animals ranged from 2 to 4 years in age[11] and weighed from 2.5 to 5.0 kg at the time of irradiation. They were conditioned and fed as previously reported.[18]

Each animal was surgically implanted with a femoral artery catheter.* Following surgery the subjects were maintained in restraining chairs for a 2-week recovery period. At the end of this time they were employed either as unirradiated controls

* Polyvinyl tubing -- .044 in. i.d., .065 in. o.d., Becton, Dickinson and Company, Rutherford, New Jersey

(6 animals) or were subjected to a 4-krad midline tissue dose (MTD) of pulsed mixed gamma-neutron radiations (11 animals). The animals were fasted for 16 hours before and until 12 hours after irradiation or sham irradiation. Water was available ad libitum before and after irradiation. Six-milliliter blood samples were collected via the femoral catheter approximately 1 hour before irradiation and at 1, 6, and 12 hours postirradiation. The same blood sampling times were used with the control animals. The blood was placed in test tubes containing sodium heparin, centrifuged, and the plasma decanted. Blood storage and analysis were in accordance with previously reported procedures.[15]

At the time of irradiation the animals were transported to the exposure room of the AFRRI-TRIGA reactor and positioned to receive a calculated dose of 4 krads of pulsed mixed gamma-neutron radiations. Midline tissue doses are reported. They were obtained by determining the tissue kerma, free-in-air, at the midline exposure volume, and multiplying this value by an experimentally derived factor of 0.8. For these exposures the operation of the reactor and the characteristics of the radiation field were as previously described.[5,12]

III. RESULTS

The animals were irradiated individually and received an average midline tissue dose of 3900 rads with a range of 3500 to 4300 rads.

The results of the chemical analyses of the blood plasma from the control and irradiated animals are presented in Table I.

As would be expected, no significant differences were observed between the plasma constituents of control and irradiated animals during the preirradiation period.

Table I. Plasma Chemistry Values for Control and Irradiated Monkeys (Macaca mulatta)

Bleeding times		Preirradiation		Postirradiation									
				1 h			6 h			12 h			
Group		Control	Experimental	Control	Experimental		Control	Experimental		Control	Experimental		
Number of animals		6	11	6	11		6	8		6	4		
Plasma constituent	Units	Mean ± S. E.*	Mean ± S. E.*	Mean ± S. E.*	Mean ± S. E.*	p†	Mean ± S. E.*	Mean ± S. E.*	p†	Mean ± S. E.*	Mean ± S. E.*	p†
Glutamic-Oxalacetic Transaminase	Sigma-Frankel units/ml	25.5 ± 7.7	21.7 ± 2.1	23.8 ± 4.9	38.5 ± 7.5		24.5 ± 5.3	101.3 ± 26.0	<.05	28.2 ± 4.4	120.0 ± 22.5	<.05
Glutamic-Pyruvic Transaminase	Sigma-Frankel units/ml	24.5 ± 6.0	15.7 ± 1.3	24.2 ± 5.2	17.1 ± 1.1		25.7 ± 5.4	19.9 ± 1.7		27.3 ± 5.9	32.2 ± 5.1	
Total Lactic Dehydrogenase	Berger-Broida units/ml	470 ± 67	523 ± 65	463 ± 92	685 ± 72		510 ± 59	1308 ± 191	<.05	490 ± 90	1830 ± 261	<.05
Total Protein	g/100 ml	7.1 ± 0.2	7.2 ± 0.2	6.8 ± 0.2	7.6 ± 0.3		6.8 ± 0.2	6.8 ± 0.2		6.7 ± 0.2	7.0 ± 0.5	
Urea Nitrogen	mg/100 ml	18.6 ± 1.5	15.1 ± 1.0	18.8 ± 1.6	18.6 ± 1.1		20.0 ± 1.5	27.3 ± 2.3	<.05	20.0 ± 1.3	40.7 ± 2.3	<.05
Creatinine	mg/100 ml	0.87 ± 0.09	0.92 ± 0.06	0.88 ± 0.07	1.22 ± 0.08	<.05	0.90 ± 0.06	1.75 ± 0.25	<.05	0.97 ± 0.08	3.01 ± 0.48	<.05
Creatine	mg/100 ml	0.28 ± 0.04	0.20 ± 0.05	0.25 ± 0.04	0.30 ± 0.06		0.28 ± 0.05	0.66 ± 0.20		0.34 ± 0.07	1.68 ± 0.53	<.05
Cholesterol	mg/100 ml	155.0 ± 10.2	170.0 ± 10.4	148.3 ± 9.3	163.6 ± 9.5		145.0 ± 7.6	159.4 ± 11.6		145.0 ± 10.2	173.8 ± 4.3	
Sodium	meq/liter	143.7 ± 0.6	145.0 ± 1.2	142.7 ± 0.7	146.4 ± 1.2		144.2 ± 0.6	147.5 ± 1.7		144.2 ± 1.1	148.5 ± 1.8	
Potassium	meq/liter	3.6 ± 0.1	3.9 ± 0.1	3.6 ± 0.1	3.5 ± 0.2		3.7 ± 0.1	4.5 ± 0.3		3.9 ± 0.1	5.6 ± 0.8	<.05
Chloride	meq/liter	113.2 ± 1.7	111.6 ± 0.8	115.3 ± 1.6	112.9 ± 1.0		115.2 ± 2.5	114.4 ± 1.1		114.8 ± 23	111.3 ± 1.0	
Hematocrit	Volume percent	35.0 ± 1.4	36.0 ± 0.6	33.3 ± 1.4	36.5 ± 0.8	<.05	31.8 ± 1.5	35.5 ± 0.8	<.05	31.0 ± 1.1	34.2 ± 0.8	

* Standard error of the mean

† Probability that the two means are members of the same population, as determined by Student's "t" test. No values listed for comparisons in which p >.05.

Furthermore, after irradiation the plasma concentrations of glutamic pyruvic transaminase (GPT), total protein, cholesterol, sodium, and chloride were not statistically different from control values.

At 1 hour postirradiation a decrease in the hematocrit of the control animals was observed while the corresponding value for the irradiated animals remained relatively constant. This difference between the hematocrits of the irradiated and control animals remained evident until the 12th hour postirradiation.

The concentrations of glutamic oxalacetic transaminase (GOT), total lactic dehydrogenase (LDH), urea nitrogen, and creatinine were found to be significantly increased over the control values at the 6th and 12th hour postirradiation. Differences between the control and irradiated animals with respect to creatine and potassium were not statistically significant until the 12th hour postirradiation.

IV. DISCUSSION

The use of restraint and indwelling catheters in themselves resulted in the plasma concentrations of several components differing from normal values. The hematocrits of restrained catheterized monkeys were significantly less than those of monkeys maintained in regular cages.[14] The sodium and potassium concentrations of the plasma of restrained catheterized monkeys were also less than those of unrestrained monkeys.[15] In a preliminary investigation these changes did not occur in uncatheterized monkeys maintained in restraining chairs. Ellinwood et al.[6] found a decrease of potassium in the plasma of dogs with catheters surgically implanted in the heart. A decreased plasma concentration of GOT and creatinine and an increased concentration of urea nitrogen were found in the restrained catheterized monkeys of

this study as compared to those previously reported on unrestrained monkeys.[15] The sodium, potassium, and hematocrit changes have been generally associated with surgery. The changes in GOT, creatinine, and urea nitrogen were probably due to the inactivity of the restrained animal.[13]

From Table I it can be noted that the hematocrits of the control animals decreased with each blood sampling while the hematocrits of irradiated animals remained relatively unchanged and significantly different from those of the control animals at 1 and 6 hours postirradiation. The former was probably the result of blood sampling since each specimen represented approximately 2 to 5 percent of the donor's total blood volume; the latter could indicate a decrease in the plasma volumes of the irradiated subjects.

A significant increase in the plasma concentration of total LDH and GOT occurred by 6 hours postirradiation. These changes at 6 hours were similar to those found by Dalrymple et al.[4] at 24 hours postirradiation when the monkeys were exposed to 1000 rads of 400-MeV protons. Highman et al.[9] found similar changes in the plasma GOT concentrations of the rat 6 hours after an 800 R x-ray exposure and attributed the increase to damaged lymphoid and other radiosensitive tissues. Hawrylewicz and Blair[7,8] and Blair,[2] using the monkey, studied the effects of gamma and proton irradiation on the serum and tissue LDH isoenzyme concentration. They found a significant increase in the M-type LDH isoenzyme after irradiation. Hori et al.[10] found significant decreases in the LDH concentrations of the spleen and thymus of mice after 600 R of x irradiation but found no change in the LDH level of liver, kidney, or testis.

It has been well established that creatinine is a waste product derived from creatine. Creatine is contained almost entirely intracellularly in muscle and is mainly bound as phosphocreatine. Though the site of the creatine to creatinine conversion has not been clearly established, it probably occurs mainly in the muscles.[1] The significant increase of the creatinine plasma concentration by 1 hour postirradiation probably indicates that the creatine to creatinine conversion rate has increased. By 6 hours postirradiation the concentration of creatine also had increased; the increase was significant by 12 hours postirradiation. This result implies that the release rate of creatine had surpassed the conversion rate to creatinine, a condition known to occur with increased catabolism of muscle tissue.[1]

The predominant site of urea production is known to be the liver. The greater proportion of nitrogen released by catabolism of amino acids is through urea.[1] When monkeys received 1000 rads of mixed gamma-neutron radiations, the free ninhydrin positive substance of plasma had increased by 12 hours postirradiation.* An increased hepatic production of urea nitrogen from amino acids could be expected due to hyperaminoacidemia.

The significant increase in plasma potassium concentration by 12 hours was largely due to high values for two of the animals and the response was not uniform as shown by the large standard error.

Early injury is well documented histologically for radiosensitive tissues such as the intestine, bone marrow, spleen, and lymph nodes. These tissues were probably

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* Unpublished: Chaput, R. L. and Turbyfill, C. L., Armed Forces Radiobiology Research Institute, Bethesda, Maryland 20014

the major contributors to the increased plasma concentrations of GOT and LDH, although much of the increase in LDH could have been due to an increase in the M-type isoenzyme found largely in such tissues as skeletal muscle. The increase in the plasma concentration of urea nitrogen was probably due to a release of amino acids from radiosensitive tissues and conversion to urea nitrogen by the liver. The increase in the plasma concentrations of creatinine and creatine suggests some injury or alteration in muscle tissue causing an increased rate of release and conversion of these constituents. Although some of the constituents of plasma which were found to increase after irradiation were probably from radiosensitive tissue, other tissues such as muscle, not considered radiosensitive, appear to have been injured when subjected to a 4-krad dose of irradiation.

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UNCLASSIFIED
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DOCUMENT CONTROL DATA - R&D

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1. ORIGINATING ACTIVITY *(Corporate author)*
Armed Forces Radiobiology Research Institute
Defense Atomic Support Agency
Bethesda, Maryland 20014

2a. REPORT SECURITY CLASSIFICATION
UNCLASSIFIED

2b GROUP
N/A

3. REPORT TITLE
PLASMA CHEMISTRY VALUES FOR THE MONKEY (MACACA MULATTA) AFTER A SUPRALETHAL DOSE OF PULSED MIXED GAMMA-NEUTRON RADIATIONS

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*

5. AUTHOR(S) *(Last name, first name, initial)*
Turbyfill, C. L.

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
December 1969	16	18

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO.	AFRRI SR69-23
c. R MD 3 9034	9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*
d.	

10. AVAILABILITY/LIMITATION NOTICES
Distribution of this document is unlimited

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	Defense Atomic Support Agency Washington, D. C. 20305

13. ABSTRACT

Monkeys (Macaca mulatta) were given a 4-krad midline tissue dose of pulsed mixed gamma-neutron radiations. Chemical analyses of 11 different constituents of plasma were made before irradiation and at 1, 6, and 12 hours postirradiation to evaluate the extent of radiation injury as indicated by changes in the composition of the plasma. A significant increase in the plasma concentration of glutamic-oxalacetic transaminase, total lactic dehydrogenase, creatinine, creatine, and urea nitrogen was found postirradiation. Although some of the constituents of plasma which were found to increase after irradiation were probably from radiosensitive tissue, other tissues such as muscle, not considered radiosensitive, appear to have been also injured when subjected to this dose of radiation.

DD FORM 1 JAN 64 1473

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14. KEY WORDS	LINK A		LINK B		LINK C	
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