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I. G. PETERS, M.A.
H. E. HAMILTON, B.S.

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FOREWORD

This report was prepared in the Radiobiology Branch under task No. 775702. The revised paper was submitted for publication on 2 June 1966. The work was accomplished between June 1965 and January 1966.

The experiments reported herein were conducted according to the "Principles of Laboratory Animal Care" established by the National Society for Medical Research.

This report has been reviewed and is approved.

James B. Nuttall

JAMES B. NUTTALL
Colonel, USAF, MC
Commander

ABSTRACT

Macaca mulatta primates irradiated by Co^{60} gamma rays in three groups of four animals each to dose levels of 2,000, 4,000, and 6,000 rads showed marked creatinuria.

Refinement of the fluorometric determination of creatine based on the reaction of ninhydrine with creatine in alkaline media has been achieved. Interfering guanido compounds calculated on a creatine equivalence constituted from unmeasurable amounts to approximately 10% of the total creatine in samples containing a small amount of creatine.

POSTIRRADIATION CREATINURIA IN MACACA MULATTA PRIMATES

I. INTRODUCTION

An earlier study from this laboratory established that *Macaca mulatta* primates exhibit a postirradiation creatinuria (1). Four *Macaca mulatta* primates on a creatine-free diet showed a marked increase in urine creatine after 1,000 rep of filtered 250 kvp x-ray.

The purpose of the present study was to extend the initial observations to higher irradiation dose levels with Co^{60} gamma rays.

II. MATERIALS AND METHODS

Twelve normal adult *Macaca mulatta* primates, composed equally of males and females, were randomly divided into three equal-number groups. They were fed a normal diet. One group was irradiated with 4,000 rads (415 rads/min.); the second group was irradiated with 6,000 rads (415 rads/min.); and the third group was irradiated with 2,000 rads (200 rads/min.) after serving as a baseline control for two days. To supplement the two-day baseline control group, a baseline was established for an additional group of two males and two females for four and one-half days. Also, four animals were sham-irradiated.

The irradiation was given in two equal doses of ventrodorsal and dorsoventral exposure to give a more uniform dose-depth distribution. A one-minute interval interruption of the irradiation was required for repositioning the primates. The irradiation dosages are on an animal midline dose basis.

Urine samples were collected twice a day except for a single two-day period when they were collected once a day (table I). The animals were confined in metabolic cages throughout the course of the experiment.

Samples were refrigerated or frozen immediately after collection when determinations were not made immediately. The frozen samples were thawed just prior to use, and the remaining solution was refrozen for a duplicate determination.

Creatine was determined by the fluorometric method of Conn (2). A model 110 Turner fluorometer equipped with the general purpose primary and secondary filter pair (<4000 A. primary; >4100 A. secondary) was used for all of the analyses. Quinine sulfate and creatine standards were run daily. All measurements were corrected for reagent blank fluorescence.

In four determinations on urine from postirradiated animals, the order of mixing the reagents was reversed. The diluted urine was added more than five minutes after the admixture of the alcoholic potassium hydroxide with the ninhydrin solution.

In four other determinations, 0.1 ml. of concentrated hydrochloric acid was added to 1 ml. of the urine, and the mixture was digested in a water bath at about 100° C. for three hours. No pH adjustment was made before proceeding with the Conn method on the acid-treated samples; however, an equal volume of acid was incorporated into the reagent blank.

III. RESULTS AND DISCUSSION

The results agree with previous observations on *Macaca mulatta* primates; the animals at all three dose levels exhibited a general

TABLE I

Creatinuria in Macaca mulatta *primates irradiated with cobalt-60 gamma rays*

Day	2,000 rads								
	Animal T43		Animal V92		Animal V42		Animal V66		Av. rate
	Conc.*	Rate†	Conc.	Rate	Conc.	Rate	Conc.	Rate	
1	0.04	5.22	0.06	0.32	0.30	1.15	0.18	1.02	0.65
	0.00	0.00	0.04	0.22	———		0.88	3.40	1.21
2	1.68	8.19	0.42	1.78	1.46	9.87	1.48	6.06	6.48
	2.60	11.03	0.65	6.25	1.80	13.71	1.40	3.85	8.71
3	2.00	6.05	0.40	1.95	1.04	3.20	1.86	5.34	4.14
4	———	‡	———		———		———		
5	———		0.44	5.43	1.30	5.36	1.56	4.56	5.12
	0.36	5.54	0.94	6.51	1.00	4.62	1.60	6.40	5.77
6	1.30	4.38	0.98	3.30	2.60	4.19	1.16	1.66	3.38
	0.01	0.02	1.00	3.49	0.28	0.75	1.80	3.64	1.97
7			2.20	1.04	———		0.66	4.94	
			———		———		3.90	5.72	
8			———						
	4,000 rads								
	Animal T45		Animal T92		Animal T83		Animal S71		Av. rate
	Conc.	Rate	Conc.	Rate	Conc.	Rate	Conc.	Rate	
1	0.68	3.85	0.42	2.47	0.70	3.66	0.52	5.34	3.83
	1.25	6.36	0.65	4.77	0.34	0.91	0.44	2.08	3.53
2	1.46	7.10	———		0.55	3.16	0.19	0.42	3.56
	1.41	5.67	1.95	5.73	0.68	4.80	0.09	0.22	4.10
3	1.12	3.10	1.30	5.39	0.70	6.23	0.04	0.09	3.70
	1.20	4.76	0.35	3.79	0.48	5.62	0.05	0.15	3.58
4	1.38	5.25	0.90	5.11	0.70	5.45	0.01	0.03	3.96
	1.60	11.89	1.40	8.53	1.20	5.66	———		8.69
5	0.80	2.71	0.80	2.71	0.74	3.68	0.16	0.45	2.39
6			———		———		———		
7							1.22	2.15	
	6,000 rads								
	Animal V90		Animal T90		Animal S47		Animal S41		Av. rate
	Conc.	Rate	Conc.	Rate	Conc.	Rate	Conc.	Rate	
1	0.22	1.44	0.12	0.57	0.68	1.74	0 27	1.26	1.25
	0.00	0.00	0.00	0.00	0.00	0.00			
2	0.70	2.79	0.00	0.00	———				
	0.92	2.88	0.00	0.00	———				
3	1.38	2.82							
	1.80	5.61							
4	1.90	3.06							
	0.00	0.00							
5	1.04	2.13							
6	———								

*Concentration of creatine is expressed in milligrams per milliliter of urine.
†Rate of creatine excretion in the urine is expressed in milligrams of creatine per hour.
‡A urine sample was taken, but no analysis was run on the sample.

creatinuria (fig. 1 and table I). The control animals excreted at a rate (mean rate of 0.40 mg. creatine/hr.) well below that of the irradiated animals (table II). Sham-irradiation, in general, had no effect (table III).

One marked difference between the results of Krise et al. (1) and the data reported here is that approximately a tenfold difference in the postirradiation creatine level is evident between the two experiments. This difference may be interpreted on the basis that a creatine-free diet was used in the previous study, whereas a normal diet was utilized in the present study.

The creatine procedure of Conn entails the conversion of ninhydrin (1,2,3-indantrione hydrate) to o-carboxyphenylglyoxal (2, 3). This α,β-dicarbonyl compound is unstable in alkaline media and undergoes an internal Cannizzaro reaction to give o-carboxymandelic acid (3). The alkali-catalyzed opening of the five-membered ring of ninhydrin is rapid. The o-carboxyphenylglyoxal conversion to o-carboxymandelic acid is relatively slow, requiring about five minutes.

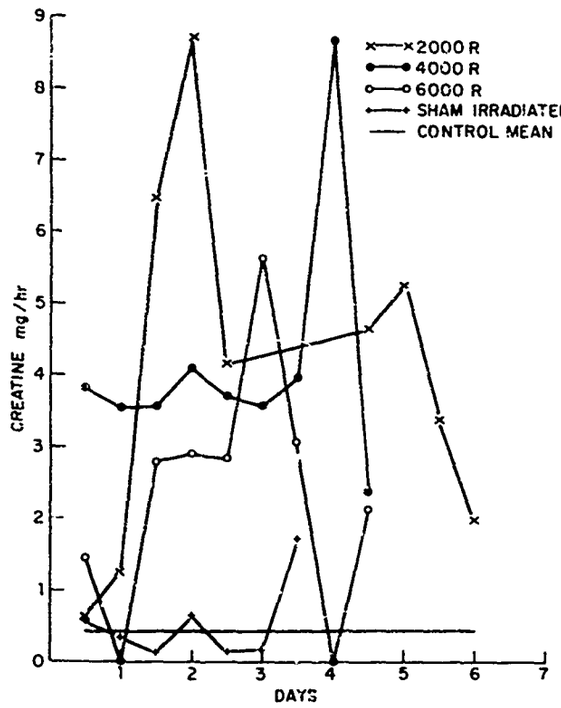

FIGURE 1

The rate of creatine excretion in the urine after irradiation.

The molecular species reacting with creatine is presumed to be o-carboxyphenylglyoxal. Because of the transient nature of o-carboxyphenylglyoxal, fluorescence is not developed unless the guanido compounds are present from the initiation of the reaction sequence. We have taken advantage of this fact in preparing a urine blank. Diluted urine samples (1:20,000) added five minutes after the admixture of the alcoholic potassium hydroxide with the ninhydrin solution gave no evidence of fluorescence over that of the reagent blanks. This observation, in addition to the fact that the preirradiation control samples all exhibited negligible fluorescence, indicates that the only fluorescent materials being detected by the procedure are active guanido compounds.

To estimate possible interference with the creatine determination by other active guanido compounds, several urine samples were pretreated with hydrochloric acid on a water bath for three hours. According to Folin (4), creatine is converted quantitatively to creatinine by heat and acid. Creatinine is a N,N'-substituted guanido compound and does not yield a fluorescent species with ninhydrin in strong alkaline solution. Other suspected interfering guanido compounds such as arginine are relatively stable to the acid treatment, thus permitting an estimate of the amount of their interference. Interfering guanido compounds calculated on a creatine equivalence constituted from unmeasurable amounts to approximately 10% of the total creatine in samples containing a small amount of creatine. No correction was made for this interference.

Duplicate determinations separated by a time interval of several weeks revealed no marked change in creatine content of the samples with time. Also, control creatine samples show that creatine is stable in frozen aqueous mixture for a period of several weeks.

TABLE II

Creatine control values

Day	Animal T43		Animal V92		Animal V42		Animal V66		Av. Rate
	Conc.*	Rate†	Conc.	Rate	Conc.	Rate	Conc.	Rate	
1	0.20	1.33	0.08	1.24	0.13	1.27	0.02	0.28	1.03
	0.08	0.27	0.05	0.77	0.05	1.83	0.02	0.88	0.94
2	0.02	0.09	0.02	0.29	0.06	0.71	0.05	0.54	0.41
	0.00	0.00	0.02	0.16	0.00	0.00	0.01	0.70	0.22
	Animal 8B8		Animal 7B4		Animal W59		Animal V41		
	Conc.	Rate	Conc.	Rate	Conc.	Rate	Conc.	Rate	
1	0.12	0.75	0.05	0.25	0.08	1.27	0.03	0.22	0.62
	0.00	0.00	0.07	1.60	0.01	0.25	0.00	0.00	0.46
2	0.06	0.20	0.05	0.34	0.10	0.63	0.04	0.24	0.35
	0.09	0.02	0.03	0.15	0.11	0.08	0.06	0.38	0.16
3	0.01	0.04	0.09	0.37	0.19	0.68	0.09	0.25	0.33
	0.06	0.17	0.00	0.00	0.03	0.49	0.07	0.20	0.21
4	0.01	0.02	0.00	0.00	0.04	0.39	0.02	0.10	0.13
	0.00	0.00	0.05	0.44	0.03	0.13	0.05	0.13	0.17
5	0.02	0.21	0.03	0.19	0.02	0.18	0.03	0.09	0.17

*Concentration of creatine is expressed in milligrams per milliliter of urine.

†Rate of creatine excretion in the urine is expressed in milligrams of creatine per hour.

TABLE III

Effect of sham-irradiation on creatine excretion

Day	Animal 0B3		Animal 0B7		Animal W83		Animal 8D2		Av. rate
	Conc.*	Rate†	Conc.	Rate	Conc.	Rate	Conc.	Rate	
1	0.26	1.58	0.22	0.92	0.11	0.64	0.01	0.07	0.80
	0.01	0.32	0.00	0.12	0.01	0.69	0.00	0.26	0.35
2	0.01	0.04	0.16	0.21	0.17	0.51	No urine output		0.19
	0.01	0.12	0.08	0.25	0.11	0.71	0.01	0.30	0.34
3	0.01	0.23	0.05	0.47	0.12	1.26	0.01	0.11	0.52
	0.01	0.29	0.01	0.41	0.03	0.93	0.00	0.08	0.43
4	0.06	0.46	0.06	0.23	0.46	1.62	0.01	0.02	0.58
	0.01	0.39	0.02	0.17	0.10	0.66	0.01	0.10	0.33
5	0.02	0.19	0.08	0.09	0.02	0.14	0.01	0.07	0.12
	0.01	0.80	0.00	0.65	0.01	0.75	0.00	0.31	0.64
6	0.04	0.26	0.07	0.14	0.04	0.07	0.02	0.06	0.13
	0.01	0.36	0.03	0.20	0.00	0.12	0.01	0.05	0.18
7	0.69	2.38	0.38	3.42	0.23	0.98	0.02	0.11	1.72

Double line indicates sham-irradiation.

*Concentration of creatine is expressed in milligrams per milliliter of urine.

†Rate of creatine excretion in the urine is expressed in milligrams of creatine per hour.

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