The Mechanism of Radiation-Induced Creatinuria.* (27654)

G. B. GERBER, G. GERBER, AND K. I. ALTMAN (Introduced by H. A. Blair)
Department of Experimental Radiology, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

As a result of our earlier study(1) it was concluded that the abnormally large quantity of creatine excreted by rats after total body exposure to X-rays(2,3) was derived from newly synthesized creatine which the muscles failed to utilize. This conclusion was based on the finding that after administration of labeled creatine the ratio of specific activities of muscle creatine to "free" creatine decreased in irradiated rats as compared to that in sham-irradiated controls. These experiments permitted, however, only an indirect estimate of radiation-induced changes in rate of creatine synthesis or in the rate of efflux of creatine from muscle. To clarify this matter further, these factors were assessed directly in the present investigation by 2 different experimental technics.

Since newly synthesized creatine must appear either as creatine in the tissues or as creatine or creatinine in the urine and since creatinine is the sole end product of creatine metabolism, the rate of creatine synthesis after X-irradiation can be estimated by determining the quantities of each of the components in the body and in the urine(4,5). Also the contribution made by muscle creatine to the pool of "free" creatine† can be determined by irradiating rats several days after administration of labeled creatine. At this time muscle creatine is the major source of the C^{14} activity of "free" creatine. The dilution of creatine released from muscle by newly synthesized creatine can therefore be determined by comparing specific activities of urinary creatine and creatinine before and after exposure.

Methods. a) Measurement of rate of creatine synthesis after X-irradiation. Ten male rats of the Sprague-Dawley strain, each weighing 240 g, were divided into 2 equal groups. One group was exposed to 650 r of whole body X-irradiation and the other group was sham-irradiated. This dose of radiation was chosen because large amounts of creatine are excreted after this dose, whereas death as a result of the radiation damage occurs rarely (3). The conditions of irradiation have been described(3). After irradiation or sham-irradiation the animals were housed in individual metabolism cages with free access to food and water. The urine collected daily for 2 weeks was pooled for each animal over the whole period. Creatine and creatinine were determined in each of the pooled urine samples as described previously(6). The animals were sacrificed 14 days after exposure, the carcass homogenized after removal of skin and creatine determined as described previously(7). Normal values of body creatine and urinary creatine and creatinine were determined from 15 animals which had been used in another investigation(7).

b) Measurement of rate of efflux of creatine from muscle after X-irradiation. Each of ten rats of the Sprague-Dawley strain, weighing 180 g, was injected with 8 μc of creatine-2-C^{14}. Two groups of animals, each composed of 5 rats, were exposed to 650 r of whole body X-irradiation 7 days and 14 days respectively after injection of the labeled creatine. Urine was collected for 2 days before and 4 days after exposure to x-rays. Specific activities of the isolated creatine and creatinine were determined as described previously (1,7).

Results and discussion. Table I presents data concerning the amount of creatine synthesized in normal and X-irradiated rats. Whereas the quantity of creatine in the bodies of sham-irradiated rats is essentially the same as that of non-irradiated rats, the amounts of creatine in the bodies of X-irradiated rats is

* Supported in part by a grant from U. S. Dept. of H.E.W.

† The pools of creatine in blood urine and organs other than brain and muscle are intimately connected as evident from their metabolic behavior (7) and are referred to together as "free" creatine pool.

TABLE I. Synthesis of Creatine in Normal and X-irradiated Rats.

	Body creatine (μmole)*	Creatine excreted† (μmole)	Creatinine excreted† (μmole)	Creatine synthesized (μmole/day)*
Sham-irrad.	1780 ± 130‡	35 ± 12	400 ± 50	30
X-irrad.	1500 ± 160	180 ± 30	350 ± 40	17
Non-irrad.	1800 ± 110	25 ± 12	380 ± 50	29

* μmoles/100 g of body wt. † μmoles/100 g of body wt excreted during experimental period of 2 wk. ‡ Standard deviation.

diminished significantly. In the irradiated rats the total amount of creatine excreted during the 2-week periods was increased about 5-fold above normal, but only insignificant changes were observed in sham-irradiated as compared to non-irradiated rats. No alteration was observed in amount of creatinine excreted by the X-irradiated rats.

The amount of creatine synthesized during the experimental period can be computed from the difference in creatine content of the body before and after the experimental period plus amounts of creatine and creatinine excreted in the urine during the experimental period. The body creatine content before the experimental period is obtained from the value observed in normal animals. Since the amount of creatine in the body is much larger than the amount synthesized over the 2-week period, the error introduced into the computation is considerable. However, it is evident from the data that creatine synthesis is apparently not increased after exposure to radiation, but decreased.

The results of the experiment pertaining to release of creatine from the muscles of irradiated animals are presented in Table II. It is evident that the specific activity of creatine decreased after exposure concurrently with the increase in creatine excretion, and that this decrease was more marked when the interval between injection and exposure was longer. This decrease in specific activity of urinary creatine was considerably larger than that expected from the metabolic turnover in normal animals, which could result only in a decrease in specific activity of urinary creatine of about 4% per day(7). Since the specific activity of creatinine remains unaltered after exposure, the ratio of specific activities of creatine to creatinine is decreased after X-irradiation.

If an accelerated release of creatine from muscle of the irradiated animals had been the cause of the increased quantity of "free" creatine which after exceeding the renal threshold is excreted in the urine, the specific activity of "free" creatine, as reflected by urinary creatine, should approach that of muscle creatine as reflected by urinary creatinine. Consequently, the specific activity of urinary creatine should rise slightly to the level of specific activity of urinary creatinine. Suppose, however, that the pool of "free" creatine expanded at the expense of newly synthesized creatine, which the muscle had failed to utilize. Under these conditions the specific activity of "free" creatine should decrease after exposure because of the higher degree of dilution of labeled creatine from muscle by unlabeled creatine. The experimental results indicate that the latter alternative occurs after X-irradiation.

It is concluded, therefore, that exposure of rats to large doses of X-rays does not affect the biosynthesis of creatine and that muscle creatine does not represent the source for urinary creatine in irradiated creatinuric rats. The present study utilizing different experimental approaches supports our earlier conclusion(1) that radiation-induced creatinuria is caused by a failure of the muscle to utilize newly synthesized creatine at a normal rate.

Summary. Synthesis of creatine in X-irradiated, creatinuric rats was determined from the balance of creatine retained in the body and creatine and creatinine excreted in the urine. The rate of release of creatine from the muscle of irradiated rats was determined in irradiated rats which were injected with labeled creatine several days before exposure. The C^{14} activity of urinary creatine and creatinine reflecting the specific activity of "free"

TABLE II. Specific Activities and Quantities of Urinary Creatine and Creatinine after X-irradiation of Rats Previously Injected with Creatine-2-C^{14}.

Time interval between inj. and exposure (days)	Collection period†	Specific activity*		Ratio specific activity of creatine/ creatinine‡	Creatine excreted (μmole/day)
		Creatine	Creatinine		
7	−2	135	145	.94	2.0
"	−1	140	135	1.04	1.6
"	1	120	140	.87	5.6
"	2	75	120	.62	12
"	3	85	135	.63	15
"	4	70	140	.50	10
15	−2	90	110	.82	1.4
"	−1	86	85	1.1	1.1
"	1	70	95	.73	8.0
"	2	28	100	.28	17
"	3	21	80	.26	21
"	4	16	90	.19	16

* Specific activity is expressed as cpm/μmole adjusted to a standard dose of C^{14} injected/100 g of body wt. Values presented are avg values from 5 rats.

† Days before and after exposure.

‡ Difference between the ratios of specific activities of creatine/creatinine prior to and on second, third and fourth day after irradiation is significant at the $p \geq 0.01$ level for Series 1 and at the $p \geq 0.01$ level for Series 2.

and muscle creatine, respectively, was determined before and after exposure. No alterations in rate of creatine synthesis or in its release from muscle were observed after irradiation. In agreement with previous studies, it was therefore concluded that radiation-induced creatinuria results from a failure of the muscle to utilize creatine synthesized at a normal rate.

1. Gerber, G. B., Gerber, G., Altman, K. I., *Internat. J. Rad. Biol.,* 1960, v3, 17.

2. Haberland, G. L., Schreier, K., Bruns, F., Altman, K. I., Hempelmann, L. H., *Nature,* 1955, v175, 1039.

3. Gerber, G. B., Gertler, P., Altman, K. I., Hempelmann, L. H., *Rad. Res.,* 1961, v15, 307.

4. Peters, T. P., Van Slyke, D. D., *Quantitative Clinical Chem.,* Williams & Wilkins Co., Baltimore, 1946, v1, 897.

5. Van Pilsum, J. F., Wahman, R. E., *J. Biol. Chem.,* 1960, v235, 2092.

6. Gerber, G. B., Gerber, G., Altman, K. I., *Anal. Chem.,* 1961, v33, 852.

7. Gerber, G. B., Gerber, G., Koszalka, T. R., Emmel, V. M., *Am. J. Physiol.,* 1962, v202, 453.

Received June 18, 1962. P.S.E.B.M., 1962, v110.