Creatine Synthesis in the Perfused Liver of Normal and X-irradiated Rats* (27509)

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One of the metabolic disorders observed in animals after exposure to ionizing radiation is an intense creatinuria(1,2,3,4). Recent studies indicate that the depressed capacity of the skeletal muscle to utilize creatine is responsible for the creatinuria observed after irradiation(5,6). On the basis of *in vitro* studies with liver homogenates it has also been suggested that the synthesis of creatine from glycocyamine is increased after x-irradiation(7). However, previous experiments carried out in this laboratory on *in vivo* synthesis of creatine from glycine-1-C_{14} in rats exposed to x-irradiation(2) do not support the hypothesis of an increased creatine synthesis. To determine whether creatine synthesis is increased after exposure to radiation, the rate of creatine synthesis from glycocyamine-2-C_{14} in the isolated perfused liver of normal and x-irradiated rats has been studied and is reported here.

Methods. Four rats of Sprague-Dawley strain were exposed to 650 r of x-rays(4) and starved thereafter for 18 hours. At this time, a significant elevation of the creatine levels of blood and urine was observed. Four other rats of the same strain, also starved for 18 hours, served as controls.

The liver was removed from each rat and perfused with whole heparinized blood diluted with one-third volume of Ringer's solution containing 250 mg of glucose and 4 μc of glycocyamine-2-C_{14} (0.185 mg) (purchased from the California Biochemical Corp.). Blood donors were non-irradiated adult Sprague-Dawley rats fasted 18 to 20 hours before bleeding. Details of the perfusion technic and apparatus have been previously described(8,9). During the first hour of the experiment (the initial phase), the synthesis of creatine was determined using glycocyamine concentrations, approximating the levels ordinarily found in the blood. After one hour, 20 mg of non-radioactive glycocyamine and 35 mg of L-methionine were added to the perfusate and the perfusion continued for 5 hours (the final phase of the experiment). Thus, in the latter phase, the synthesis of creatine was determined at higher precursor concentrations than normally found in the blood. At regular intervals throughout the experiment 2- to 3-ml samples of blood perfusate were withdrawn and concentration and specific activities of creatine and glycocyamine were determined(10). Creatine concentration in the urine(11) and blood of the liver donors was also determined.

The rate of synthesis of creatine during the initial phase of the experiment was estimated from the increase in concentration and from the increase in specific activity of creatine in the perfusate. During the final phase of the experiment, creatine synthesis was estimated from the increase in concentration of creatine, the decrease in specific activity of creatine and decrease in concentration of glycocyamine. Details of the experimental procedure and methods of calculation of the results have been described(10).

Results and discussion. Data on the synthesis of creatine in the isolated perfused liver of normal and x-irradiated rats are presented in Table I. Average values for creatine synthesis during the initial and final phase of the experiment are also shown. No significant difference between normal and x-irradiated rats was detected in the rate of creatine synthesis expressed in any way (per total liver or per g liver). Our data are thus at variance with those of Nerurkar *et al.*(7) who found an increased synthesis of creatine in liver homogenates of irradiated, starved rats as compared with that of sham-irradiated, starved

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TABLE I. Creatine Synthesis in the Perfused Rat Liver.

Treatment	Liver wt (g)	Body wt (g)	Rate of creatine synthesis* (μg/hr): By increase in perfusate of creatine, Initial phase	By increase in perfusate of creatine, Final phase	By change of its specific activity, Initial phase	By change of its specific activity, Final phase	By decrease in perfusate of glycocyamine, Final phase	Creatine content of liver (mg)
Normal	5.85	221			750	820		.73
"	10.8	281	455	830	730	625	610	.90
"	5.21	222	510	430	960	630	355	.85
"	13.1	416	630	810	1200	770	565	1.15
X-irrad.	8.37	215	630	610	870	680	590	1.2
"	8.65	257	590	460	860	480	770	1.3
"	9.85	317	360	490	690	430	490	1.2
"	10.7	392	590	530	830	650	550	1.4

* The individual values for rate of creatine synthesis were pooled separately for the initial and final phases of the experiment and means and stand. errors computed. Avg value for creatine synthesis during initial phase was 745 ± 95 for normal rats, and 677 ± 63 for x-irradiated rats; avg value for creatine synthesis during final phase was 645 ± 55 for normal rats, and 560 ± 31 for x-irradiated rats.

rats. Although it is realized that no *in vitro* system can duplicate precisely conditions prevailing in the intact animals, the perfused organ probably approximates as closely as any experimental system the metabolic state in the intact animal. This contention is supported not only by numerous studies on metabolic functions of the perfused liver, but also by the fact that the amount of creatine synthesized by the perfused liver at concentrations of precursor in the physiological range can satisfy the need for creatine of the intact animal(10). Thus, since creatine synthesis in the liver appears to be unaffected after irradiation, the radiation-induced creatinuria is probably the result of changes in the creatine metabolism of other organs, *e.g.*, the muscle. Such a mechanism for creatinuria after irradiation, namely a decreased utilization of creatine by skeletal muscle had already been postulated on the basis of previous experiments (5,6).

Summary. The synthesis of creatine from glycocyamine was studied in the perfused liver of normal and whole body x-irradiated rats. No significant difference was found in the ability of the perfused liver to synthesize creatine when normal and x-irradiated rats were compared. It is postulated that radiation-induced creatinuria does not result from an increased synthesis of creatine by the liver, but rather from a decreased utilization of creatine by skeletal muscle.

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