Factors Affecting Metabolism of Muscle Collagen. II. Effect of Radiation Exposure.* (24924)

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It has been shown(1) that turnover rates of both protein and its associated polysaccharide moiety of collagen are a function of age of rats studied. By use of glycine-2-C^{14} and glucose-U-C^{14} it was possible to show that the turnover rate of the protein component increased moderately with age, whereas that of the polysaccharide moiety decreased markedly. Using these experiments on normal animals as a basis for comparison, the present study deals with the effect of x-irradiation on collagen metabolism. Since radiation exposure appears to accelerate aging processes(2), a preliminary attempt has been made to determine whether or not x-irradiation simulates aging in its effect on metabolism of muscle collagen.

Methods. Two experiments were carried out using glycine-2-C^{14} (specific activity, 1.9 x 10^7 counts/minute (cpm)/mg and uniformly labeled glucose-U-C^{14} (specific activity, 2.5 x 10^7 cpm/mg). In each experiment, 2 groups of rats were used: one group was injected with glycine-2-C^{14}, the other with glucose-U-C^{14}. In the first experiment there were 4 animals/group; in the second experiment there were 6 animals/group. Total C^{14} dose stated in Table I was injected intraperitoneally in 3 equal doses on consecutive days. During period of isotope administration, rats used in first experiment were fed a synthetic diet (normal protein test diet fortified with vitamin supplements as obtained from Nutritional Biochemicals Corp.) whereas animals in second experiment were given Purina Fox Chow. The difference in protein content of the 2 diets probably accounts for the difference in specific C^{14}-activity of muscle collagen isolated in the 2 experiments involving glycine-2-C^{14} administration. Food and water were allowed *ad lib.* until 48 hours after last injection of C^{14}-labeled material. At this time, half the animals in each group were exposed to 756 r of 250 kv x-rays at 19.4 r/minute, using filters of 0.42 mm Al (parabolic) and 0.5 mm Cu. This dose represents approximately an LD 25 for 30-day survival. While test animals were exposed to x-rays, control rats were sham-irradiated. After irradiation, all animals were housed in individual cages without food until sacrificed 48 hours later. Collagen was extracted from skeletal muscle and purified according to the combined procedures of Neuberger *et al.*(3) and Fitch *et al.*(4) employed in that order. The hydroxyproline content of collagen so isolated was approximately 13%. Samples were plated as infinitely thin layers on polyethylene planchets and C^{14}-activity was determined using a gas flow counter in the Geiger region with a mixture of helium-isobutane as the quenching gas. The counting error in all instances was 3% or less. In the first experiment, each animal was processed individually, whereas in the second experiment, equal quantities of muscle from x-irradiated and sham-irradiated rats of each group were pooled separately prior to processing.

Results. Table I summarizes all pertinent data in both experiments. In experiments employing glycine-2-C^{14}, C^{14}-activity/gram collagen (or percent of injected dose) in irradiated rats is a little more than half the corresponding value for non-irradiated rats. This confirms an observation previously reported (6) using slightly different experimental conditions. When glucose-U-C^{14} was given, however, the reverse was observed, namely, C^{14}-activity/gram collagen in irradiated rats was almost twice that of controls.

Discussion. C^{14}-activity of glycine is incorporated into certain amino acid residues of the protein component of collagen, whereas

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TABLE I. Effect of Irradiation on C^{14}-Activity of Muscle Collagen.

No. of animals	Treatment	C^{14}-labeled compound inj.	Wt of animal (g)	C^{14}-activity inj./100 g body wt (cpm)	C^{14}-activity/g collagen (cpm)	% of inj. activity/g/collagen/100 g body wt
			Exp. 1			
1	X-irradiated	Glycine-2-C^{14}	199	2.2×10^6	7.9×10^3	.4
1	"	"	197	2.2 "	7.5 "	.3
1	Sham-irradiated	"	201	2.1 "	13.2 "	.6
1	"	"	203	2.1 "	13.0 "	.6
1	X-irradiated	Glucose-U-C^{14}	205	1.4 "	8.4 "	.6
1	"	"	206	" "	9.7 "	.7
1	Sham-irradiated	"	205	" "	5.7 "	.4
1	"	"	207	" "	5.9 "	.4
			Exp. 2			
3	X-irradiated	Glycine-2-C^{14}	164 ± 5	9.7×10^6	1.6×10^5	1.7
3	Sham-irradiated	"	166 ± 2	9.6 "	2.9 "	3.0
3	X-irradiated	Glucose-U-C^{14}	169 ± 2	4.4 "	21.3×10^3	.5
3	Sham-irradiated	"	167 ± 2	" "	9.1 "	.2

that of glucose contributes to the polysaccharide moiety(5). Therefore, irradiation caused a reduction in isotope concentration in the protein fraction of collagen and an increase in isotope concentration in the polysaccharide moiety as compared with control. From comparison with previous studies(5) of turnover rates of these components, it can be inferred that x-irradiation accelerates rate of turnover of the protein moiety and decelerates the rate of turnover of the polysaccharide component. In terms of its effect on metabolism of muscle collagen, one may conclude that exposure of rats to a dose of radiation in the lethal range produces acute changes similar to those occurring naturally as a result of aging.

Summary. 1. C^{14}-activity of muscle collagen was measured in x-irradiated and sham-irradiated rats given glycine-2-C^{14} and glucose-U-C^{14} prior to exposure. 2. Whole body x-irradiation resulted in reduction of isotope concentration of collagen in animals given glycine-2-C^{14} but caused an increase in isotope concentration when glucose-U-C^{14} had been administered. 3. It is concluded that x-irradiation and aging have similar over-all effects on collagen metabolism when studied with above-mentioned C^{14}-labeled precursors.

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