Effects of a Radioprotective Agent on Tissue Serotonin Levels in the X-Irradiated Rat.* (27573)

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It has been reported that exposure to x-irradiation results in a significant reduction in the serotonin (5-hydroxytryptamine) concentration of blood(1,2) and spleen(2). Further data are presented here on the effects of x-irradiation on tissue serotonin levels in the rat and the effects thereon of administering a radioprotective agent.

Procedure. Forty-eight male rats of the Holtzman strain which had been raised from weaning on a natural food stock ration were selected at an average body weight of 171 g (range 161 to 181 g) and were divided into 8 comparable groups of 6 each and treated as follows. Group I served as untreated controls; Groups II, III and IV were exposed to a single dose of 450 r total body x-irradiation; Groups V, VI and VII were exposed to a single dose of 900 r total body x-irradiation; Group VIII received 200 mg/kg of 2-mercaptoethylguanidine (MEG)† intraperitoneally 20 minutes before exposure to 900 r total body x-irradiation. Groups II and V, III and VI, and IV and VII were sacrificed 1, 3 and 6 days post x-irradiation respectively. Group I was sacrificed at the same time as Group II; and Group VIII at the same time as Group VII. Animals were kept in metal cages with raised screen bottoms (3 rats per cage) and food‡ and water were provided *ad libitum*. At time of sacrifice rats were mechanically stunned and after withdrawal of 1 ml of cardiac blood with a heparinized needle, killed by decapitation and the serotonin content of blood and tissues determined according to the method of Bogdanski *et al.*(3). Radiation factors employed were as follows: GE model Maximar 250; 250 kv; 15 ma; 0.5 mm Cu and 1 mm Al filters plus a Cu parabolic filter;§ HVL, 2.15 mm Cu; target distance to top of box, 78 cm; and exposure dose rate, 17.92 r/min (measured in air, at top of box). The animals to be irradiated were placed in a wooden box divided into 14 compartments 7 cm wide, 16 cm long and 10 cm deep. The partitions and top were made of ⅛-inch cellulose acetate sheeting; and the top, one side and bottom of each compartment were perforated with holes for purposes of ventilation. The container was rotated slowly on an electrically-driven turntable to insure equivalent irradiation.

Results. In agreement with earlier findings, exposure to total body x-irradiation caused a significant reduction in serotonin concentration of blood and spleen. A significant reduction also occurred in the serotonin content of brain and small intestine.‖ Serotonin levels were reduced as early as 24 hours after x-irradiation and in most cases were at their lowest 72 hours after x-irradiation ex-

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† MEG was prepared by dissolving weighed amounts of S-(2-aminoethyl)thiuronium bromide hydrobromide in pH 10.5 phosphate buffer (0.06M) and rapidly adding NaOH until pH 7.5 was reached. The solution was brought to a final volume (30 mg MEG per cc) by diluting with pH 7.5 phosphate buffer. The solution was covered and used within 2 hours of preparation.

‡ Rockland Rat Diet, A. E. Staley Manufacturing Co., Decatur, Ill.

§ A nonuniform filter which produces a flat isodose surface of x-ray intensity constructed by the method of Greenfield and Hand(9). The center of the filter had a thickness of 1.7 mm Cu; the edge, 0.5 mm Cu.

‖ The reduction in serotonin concentration of brain and small intestine is at variance with an earlier study(2) in which x-irradiation resulted in a slight but not significant reduction in brain serotonin levels and an increment in the serotonin concentration of the small intestine. Since rats in the former study were approximately 10 months of age at time of x-irradiation in contrast to 7 weeks of age in the present investigation, differences between the 2 experiments might indicate a differential response by these tissues to x-irradiation dependent on the age of the animal.

TABLE I. Effects of Radiation on Tissue Serotonin Levels.

Group	Body wt g at time of sacrifice	Serotonin content* of following tissues: Blood μg/ml	Spleen μg/g	Brain μg/g	Small intestines μg/g
Non-irradiated	177	.73 ± .06	2.99 ± .16	.54 ± .08	5.04 ± .23
450 r x-irradiation					
1 day post x-ray	168	.37 ± .02	1.42 ± .12	.39 ± .04	3.03 ± .10
3 " " "	167	.41 ± .06	1.29 ± .05	.23 ± .02	2.73 ± .07
6 " " "	177	.43 ± .04	1.58 ± .06	.24 ± .02	3.59 ± .11
900 r x-irradiation					
1 day post x-ray	167	.33 ± .05	1.33 ± .07	.47 ± .03	3.14 ± .03
3 " " "	167	.29 ± .05	.96 ± .08	.29 ± .04	2.26 ± .13
6 " " "	175	.26 ± .03	1.38 ± .06	.20 ± .02	3.24 ± .15
900 r x-irradiation plus 200 mg MEG per kg body wt					
6 days post x-ray	176	.62 ± .05	3.31 ± .28	.49 ± .03	6.31 ± .17

* Avg of 6 rats, including standard error of mean.

posure. In general comparable findings were obtained after 450 r and 900 r x-irradiation. In contrast to the above, no significant reduction in tissue serotonin levels occurred in rats exposed to 900 r total body x-irradiation and given the radioprotective agent MEG. Results are summarized in Table I.

No data are available as to the significance of reduced tissue serotonin levels¶ in the x-irradiated rat and what relationship these may have to manifestations of radiation injury. It is of interest that serotonin on a weight basis is one of the most potent radioprotective agents known(4-8) and that 2-mercaptoethylguanidine as shown above maintained normal serotonin levels in the x-irradiated rat. Findings suggest that determining the serotonin content of blood, spleen and other tissues may be a sensitive index of radiation injury and a useful tool for the rapid evaluation of radioprotective agents.

Summary. Young adult male rats exposed to a single dose of 450 r or 900 r total body x-irradiation exhibited a highly significant reduction 1, 3 and 6 days post x-irradiation in the serotonin concentration of the blood, spleen, brain and small intestines. No significant reduction in tissue serotonin levels occurred in rats exposed to 900 r total body x-irradiation and administered 2-mercaptoethylguanidine intraperitoneally at a level of 200 mg per kg of body weight 20 minutes prior to x-irradiation exposure.

¶ A reduction in tissue serotonin levels following x-irradiation might have been anticipated from Langendorff *et al.*'s suggestion(10,11) that radiation by causing a lack of high energy phosphate and damage to decarboxylase results in an impaired synthesis of 5-hydroxytryptamine from 5-hydroxytryptophan. These workers(12) failed, however, to detect a reduction in the serotonin content of brain, liver or spleen of mice sacrificed at graded periods up to 2 hours after exposure to 810 r total body x-irradiation. On the basis of present findings it would appear that 2 hours post irradiation was insufficient time for a reduction in tissue serotonin levels to become manifest. Royal *et al.*(13) reported, however, that one week after exposure to 900 r x-irradiation serotonin levels in brain, lung, kidney, spleen and liver of mice were reduced below that of nonirradiated controls.

Since serotonin in the blood is virtually all in the blood platelets and since the number of blood platelets is reduced following x-irradiation, the question arises whether the reduction in blood serotonin might not be a manifestation of reduced platelet count. Findings indicate, however, that blood serotonin levels were significantly reduced 24 hours after x-irradiation whereas a significant reduction in platelet count does not occur until several days later(14). The possibility that x-irradiation may have impaired the binding of serotonin not only to platelets but to various tissues as well has not, however, been excluded.

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Connective Tissue VII. Changes in Protein and Hexosamine Content of Bone and Cartilage of Rats at Different Ages.* (27574)

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Previously(1,2,3) we reported changes with age in the protein and hexosamine content of several soft tissues of the rat. In general, tissues rich in scleroprotein underwent greater changes in protein and hexosamine composition than did tissues low in scleroprotein. In the former, there was a decrease in nonscleroprotein and a concomitant increase in scleroprotein. Further, we found the hexosamine content to be increased with age in aorta and decreased with age in tendon and skin. Meyer (4) reported that 3 mucopolysaccharides have been isolated from bone, namely, hyaluronic acid, chondroitin sulfate C and keratosulfate. The keratosulfate content of human rib cartilage increased with an increase in age(5). To date, no data have been reported on the relationship between the mucopolysaccharide and the protein of bones and cartilage. If mucopolysaccharide is found in one or more of the several fractions of protein, prepared by standardized methods, it will clearly indicate a very intimate relationship with and a tight binding to the corresponding protein. For these reasons, in the present study hexosamine determinations were carried out for each protein fraction.

Our methods used for fractionation of soft tissue protein are not directly applicable to the fractionation of proteins in bone and cartilage, since the large amount of salts present alter solubility. Desalination is time-consuming and may be associated with a loss of soluble protein and soluble collagen. A combination of the methods of Lowry(6) and Fitch (7) afforded us a satisfactory procedure for fractionation of the various proteins found in the connective tissue of bone and cartilage. The present study is concerned with 1) fractionation of the proteins of bone and cartilage into 6 components and 2) variations with increasing age in the protein and hexosamine content of these moieties.

Method. Bone and cartilage from skull, femur, rib and vertebrae of female rats of Wistar strain, 5 weeks and 2½ years of age, respectively, were freeze dried and ground into fine powder in a Wiley electric mill.† 0.2 to 0.4 g of the powdered bone or cartilage were fractionated according to Schema I into 6 protein fractions, *i.e.*, trichloroacetic acid (TCA) supernatant of base soluble protein, nonscleroprotein, soluble collagen, insoluble

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† Purchased from Arthur H. Thomas Co., Philadelphia.