Effects of Radiation on Tissue Serotonin Levels in the Rat.* (26879)

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It has been demonstrated by a number of workers that serotonin (5-hydroxytryptamine) has marked radioprotective activity when injected prior to x-irradiation and on a weight basis is one of the most potent radioprotective agents known(1-5). Since serotonin is a normal constituent of body tissues it was of interest in view of the above to inquire whether the serotonin content of an animal's tissues was related to its capacity to withstand radiation injury and further what effect exposure to radiation would have on tissue serotonin levels. Data are presented here on the effects of total body x-irradiation and gamma radiation on tissue serotonin levels in the rat.

Procedure. Thirty-two male rats of the Holtzman strain which had been raised from weaning on a natural food stock ration with an average body weight of 451 (range 411 to 530) g were used. Animals were placed in individual metal cages with raised screen bottoms and were divided into 4 comparable groups of 8 each. Group I was exposed to a single dose of 900 r total body x-irradiation; Group II was administered continuous total body gamma radiation at a level of 500 r per week; Groups III and IV served as untreated controls. Rats in Groups I, II and III were fed *ad libitum*‡ with food consumption determined daily for animals in Groups I and III. Those in Group IV were pair-fed daily an amount of food equal to the average of that ingested the previous day by animals in Group I. Water was provided *ad libitum* to all groups. Six days after x-irradiation rats in Groups I, III and IV were mechanically stunned and after withdrawal of 1 ml of cardiac blood with a heparinized needle, killed by decapitation and serotonin content of blood and tissues determined according to the method of Bogdanski *et al.*(6). Radiation factors employed for Group I 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 gamma radiation administered to rats in Group II was supplied by a Cesium-137/Barium-137 source mounted in a camera.‖ Metal cages with raised screen bottoms were grouped around the camera so that each cage received an average exposure of approximately 500 r/wk. Animals were continuously exposed to radiation except for a short period (approximately 10 minutes) each day at which time they were fed and watered. Rats in Group II were sacrificed after 9 days of gamma radiation and serotonin content of blood and tissues determined as indicated above.

Results. Findings (Table I) indicate that a single dose of 900 r total body x-irradiation resulted in a highly significant reduction in serotonin concentration of the blood and spleen and a marked increment in serotonin concentration of the small intestine compared to values obtained in untreated *ad libitum-*

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‡ 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(11). The center of the filter had a thickness of 1.7 mm Cu; the edge, 0.5 mm Cu.

‖ The gamma radiation facilities were designed and constructed by Isotopes Specialties Co., Burbank, Calif.

TABLE I. Effects of Radiation on Tissue Serotonin Levels (8 Rats per Group).

Group	Body wt, g Initial	Final	Serotonin content of following tissues: Blood, μg/ml*	Spleen, μg/g*	Small intestines, μg/g*	Brain, μg/g*	Spleen wt, mg	Total serotonin content of spleen, μg
I (X-irradiated)	453.6	391.9	.21 ±.03	1.21 ±.11	5.47 ±.35	.59 ±.06	516	624
II (Gamma-irrad.)	450.4	416.4	.89 ±.10	1.57 ±.19	3.03 ±.17	.58 ±.05	767	1204
III (Untreated, fed *ad lib*)	449.7	454.5	.87 ±.07	2.63 ±.19	3.38 ±.25	.81 ±.05	1187	3122
IV (Untreated, pair-fed to Group I)	449.4	392.0	.68 ±.17	2.13 ±.16	3.55 ±.15	.58 ±.04	824	1755

* Including stand. error of mean.

fed and pair-fed controls. Since x-irradiation also caused a highly significant reduction in average weight of the spleen, total serotonin content of this organ relative to that of the untreated controls was even more depressed than was indicated by data on the serotonin concentration per gram of tissue alone. A significant reduction in both concentration and absolute amount of serotonin in the spleen was also observed in rats exposed to continuous gamma radiation but to a lesser degree than in the x-irradiation series. In contrast to findings in the latter group, serotonin concentration in the blood and small intestine of rats exposed to gamma radiation did not differ significantly from that of untreated controls. Brain serotonin content was also reduced in x-irradiated and gamma-irradiated rats compared to values in untreated *ad libitum*-fed controls.¶ A similar reduction was also observed, however, in untreated, pair-fed controls.

Discussion. A number of investigators have reported that the spleen plays an important role in resistance to x-irradiation (see review of literature by Jacobson and coworkers(7,8)). Shielding of the spleen during irradiation, the intraabdominal implantation of spleen from normal animals into irradiated animals after exposure, intraperitoneal injections of isologous spleen cells or spleen homogenate or of homologous and heterologous cell-free spleen extracts have all been reported to reduce mortality following exposure to a lethal dose of total body x-irradiation in various species of animals including the mouse, guinea pig, rabbit and dog although there is some evidence that such procedures are less protective in the rat(9). The marked reduction in serotonin content of the spleen of both x-irradiated and gamma-irradiated rats in the present experiment might possibly be related to the findings indicated above. It is well established that the serotonin in blood is present primarily in the platelets and that the latter are significantly reduced following exposure to doses of x-irradiation comparable to that employed in the present experiment(10). A reduction in blood serotonin concentration might have been anticipated, therefore, on the basis of the reduced platelet count and absence of binding sites for serotonin in the blood. The increased concentration of serotonin in the small intestine of x-irradiated rats might reflect a defect in the transport mechanism of serotonin from the small intestine related possibly to the reduction in blood platelet count. No data are available as to the significance of the changes noted above. It is possible that reduction of tissue serotonin levels in the blood and spleen following x-irradiation is directly

¶ Values for brain serotonin concentration in this study which were obtained on animals approximately 10 months of age at time of sacrifice were higher than those previously reported in younger rats of another strain(12). To determine the effects of age on brain serotonin levels, determinations were made of brain serotonin concentration of 8 male rats of the Holtzman strain obtained from the same breeding colony as the older animals and raised from weaning on the same stock diet. Animals were sacrificed at 33 days of age and an average body weight of 134 g and brain serotonin levels determined by the same procedure as was employed for the older animals. Serotonin concentration in the brain of the younger animals averaged .28 ± .02 μg/g. It would appear that brain serotonin concentration increases with age, this agrees with findings previously reported by Kato(13).

related to the pathological manifestations of radiation injury. If so, determining serotonin content of blood or spleen may be a sensitive index of radiation injury and a useful tool for rapid evaluation of radioprotective agents. Further studies are indicated to determine the time-dose relationship of tissue serotonin levels following radiation and the effects of radioprotective agents thereon.

Summary. Rats exposed to a single dose of 900 r total body x-irradiation exhibited a highly significant reduction 6 days after x-irradiation in the serotonin concentration of the blood and spleen and a marked increment in serotonin concentration of the small intestine compared to values obtained in untreated *ad libitum*-fed and pair-fed controls. A significant reduction in serotonin concentration in the spleen was also observed in rats exposed to gamma radiation. No significant difference in brain serotonin concentration was observed between x-irradiated and gamma-irradiated rats and untreated pair-fed controls with values for all 3 groups, however, reduced below that of untreated, *ad libitum*-fed controls.

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Nicotine Oxidation by *Arthrobacter*.* (26880)

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The metabolism of nicotine by several micro-organisms has been investigated and a number of oxidation products of the alkaloid have been isolated and identified. These products include 6-hydroxynicotine, 6-hydroxypseudooxynicotine, pseudooxynicotine and a group of derivatives in which the pyrrolidine ring of nicotine has been opened and degraded. Rittenberg and co-workers(1,2,3) have obtained cell-free preparations of a soil bacterium, designated as P-34, which catalyzed the oxidation of nicotine successively to 6-hydroxynicotine, 6-hydroxypseudooxynicotine and 2,6-dihydroxypseudooxynicotine. Wada(4,5) reported that a Pseudomonas species, also isolated from soil, could oxidize nicotine to some derivatives in which either the pyridine or pyrrolidine ring or both were oxidized. Frankenberg and Vaitekunas(6) have reported similar nicotine metabolites to be produced by unidentified organisms derived from the surface of tobacco seeds. Wadsworth(7) has shown that a Cory-

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