Effect of Serotonin upon Liver Cells of Young Rats.* (24840)

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Serotonin (5-hydroxytryptamine) has been studied in connection with many physiologic and disease states, and because patients with carcinoid tumor frequently have acquired valvular lesions of heart(1) there has been great interest in morphologic changes caused by this compound. Prolonged administration of 5-hydroxytryptamine or 5-hydroxytryptophane to experimental animals has resulted in tubular and cortical necrosis of kidneys(2), dermal fibrosis at injection sites(3), gastric mucosal erosions(4), necrosis of tip of tail and of digits, and lenticular opacities(3). There has been no report of morphologic changes in human or animal liver induced by serotonin. With the aid of autoradiographic technic, it has recently been found that in young rats, administration of large doses of serotonin resulted in increased formation of hepatic parenchymal cells, accompanied by minor histological changes. This communication reports these observations.

Materials and methods. Thirty-eight male Sprague Dawley rats‡ weighing 90 to 245 g were maintained on diet of Purina lab chow and water, and housed in individual cages in air-conditioned room. Twenty-five of the animals were given a single subcutaneous injection of serotonin creatinine sulphate§ in 3 ml isotonic saline, the doses ranging 0.009 to 62.8 mg/kg body weight (Table I). Twenty-four hours later, 1 millicurie tritiated thymidine‖/kg was administered by single intraperitoneal injection(5), and after another 4 hours, the rats were killed by decapitation. Thirteen control rats, all litter mates of experimental animals (Table I), were treated in the same way, except that serotonin was replaced by injection of saline. Serotonin and saline injections were performed at same times. The organs were sliced and fixed in cold 10% neutral buffered formalin or in Zenker's solution. After fixing for 24 hours, tissues were washed in running tap water for another 24 hours and then dehydrated, cleared and imbedded in paraffin. Sections of 6-8 μ thickness were prepared and stained as follows: Feulgen's method for desoxyribonucleic acid (DNA), methyl green-pyronin for ribonucleic acid (RNA), hematoxylin and eosin, phloxine methylene blue, periodic acid Schiff's reagent (PAS), diazo coupling reaction for alkaline phosphatase, and Oil Red O stain for neutral fat. For autoradiography, fine grain stripping film¶ was applied over unstained sections and over sections stained with hematoxylin or by Feulgen's method(5). The preparations were exposed in a light-tight box at 10°C for 3 to 4 weeks. Slides were developed in Kodak D-19 Developer 7 minutes, fixed in Kodak Acid Fixer 10 minutes, washed in cool running tap water one hour, and mounted in polyvinylpyrrolidone (PVP).** For histochemical studies, slices of liver were frozen and dried at time of autopsy, using Freed Tissue Dryer, and frozen sections were also cut from formalin fixed tissues. Estimation of mitotic activity and number of hepatic cell nuclei bearing the label was carried out as follows: for each liver the total number of hepatic cells present in 400 X field was determined in sections stained with hematoxylin and eosin. Fifty consecutive 400 X fields were then searched for mitotic figures and results expressed/100,000 hepatic cells. In autoradio-

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† Advanced Research Fellow, Am. Heart Assn.

‡ Purchased from Charles River Breeding Labs., Cambridge, Mass.

§ Supplied by Dr. George Berryman, Abbott Labs. Dosage is expressed in terms of serotonin creatinine sulphate compound.

‖ Schwarz Laboratories, Mount Vernon, N. Y. Specific activity, 0.360 curie/millimole.

¶ Kodak Ltd., London; film AR.10.

** Antara Chemical Co., N. Y.

TABLE I. Autoradiographic and Mitotic Observations in Livers of Rats Weighing Less Than 115 g and Given Single Subcutaneous Injection of Serotonin Creatinine Sulphate (SCS).

Wt, g	Age, days	Dose SCS, mg/kg	Labelled hepatic nuclei*	Hepatic mitoses†
7 control rats given saline (avg of determinations)				
102	36	0	565.7	16.6
10 rats given serotonin				
111	36	.009	581	10
110	36	.09	663	7
111	36	.09	153	4
100	36	1.0	617	5
100	38	2.0	473	27
		Avg	497.4	10.6
107	38	45.0	1,057	42
106	38	47.3	1,090	132
101	38	48.0	1,740	96
97	38	49.5	3,065	495
90	31	62.8	648	72
		Avg	1,520.0	167.4

* No. of tritium labelled hepatic cell nuclei in autoradiographs, expressed/100,000 hepatic cell nuclei.

† No. of hepatic cells in mitosis in hematoxylin and eosin sections, expressed/100,000 hepatic cell nuclei.

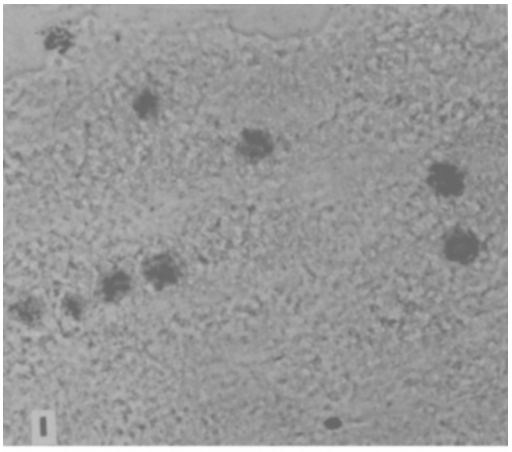
graphs, number of labelled liver cell nuclei present in 100 consecutive 400 X fields was counted, and results expressed/100,000 hepatic nuclei. In 7 additional male Sprague-Dawley rats weighing 73 to 98 g, the central lobe of liver was removed under ether anesthesia. Forty-one hours later 1 millicurie of P^{32}/kg body weight was injected by intraperitoneal route. Partial hepatectomy was performed to assure adequate incorporation of P^{32} into nucleic acids of the liver(6,7). Thirteen days later 4 rats were given a single intraperitoneal injection of 40 mg/kg serotonin creatinine sulphate, while the 3 other animals received an injection of saline alone. All 7 rats were killed 24 hours later and the liver homogenized(8). Nucleic acid phosphorus was estimated by method of Schneider(9) and radioactivity of a dried aliquot was determined in a window G.M. counter. Specific activity of nucleic acid phosphorus was expressed as "biological concentration coefficient," *i.e.*, counts/minute/millimole of phosphorus as percent of counts/minute injected/gram of body weight(10). All determinations were performed in duplicate.

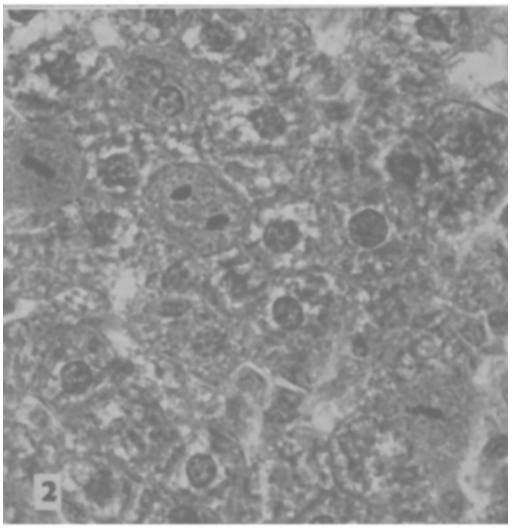
Results. The results obtained differed depending on weight of animals. Five rats weighing less than 115 g and treated with 40 mg kg or more of serotonin creatinine sulphate exhibited increase in number of both hepatic cell nuclei bearing the label and mitotic figures as compared with control rats given only saline (Table I, Figs. 1-3). In a similar group of 5 rats that received smaller doses of serotonin (0.009-2.0 mg/kg), no such difference was observed (Table I). In 15 rats weighing more than 115 g, a statistically significant increase in number of labelled cell nuclei and of mitotic figures could not be demonstrated, even though doses of serotonin exceeded 40 mg/kg.

The increase in labelling appeared to affect only hepatic parenchymal cells; nuclei of bile duct epithelium, of endothelial cells and of Kuppfer cells were labelled to the same extent in livers of treated and control animals. In rats receiving large doses of serotonin (Table I), histologic sections stained with hematoxylin and eosin or with phloxine methylene blue exhibited intact liver cells, but slight disorganization of architecture and slight clumping of cytoplasm was demonstrable. In no instance, however, was frank hepatic cell necrosis observed. In sections stained with Feulgen, PAS, alkaline phosphatase and fat stains, no histological differences were seen between serotonin treated and control animals.

In addition to the liver, the following organs were studied by autoradiography: brain, esophagus, stomach, small and large intestine, skin, heart, lung, spleen, tongue, striated muscle, testis, epididymis, thymus, adrenal, eye, and kidney. In these organs, no significant difference in number of labelled cell nuclei was observed between serotonin treated and control animals, except in the kidney. In rats of all weights given large doses of serotonin, this organ showed an increase in number of labelled nuclei of tubular cells associated with histologic evidence of tubular necrosis.

In 4 rats given P^{32} and serotonin, the "biological concentration coefficient" for nucleic acid phosphorus in liver was 171 (Range 148-189) whereas in 3 control rats that received only saline, the value was 218 (Range 200-237). This difference was significant.





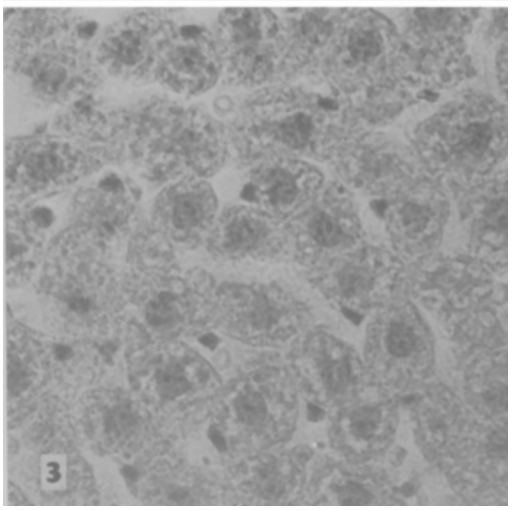


FIG. 1. Autoradiograph of liver of young rat given one subcut. inj. of 45 mg/kg serotonin creatinine sulphate 24 hr preceding inj. of tritiated thymidine. Unstained × 560.

FIG. 2. Liver of same animal as in Fig. 1, showing 3 mitotic figures, slight distortion of cords of liver cells, and clumping of cytoplasm. Hematoxylin and eosin × 560.

FIG. 3. Liver of control rat for comparison with Fig. 2. Hematoxylin and eosin × 490.

Comment. Incorporation of tritiated thymidine into cell nuclei is believed to reflect active nuclear synthesis of desoxyribonucleic acid(5). Thus, it appears that cells which exhibited label in the nucleus were preparing for mitotic division at time of thymidine injection(5). That livers of small rats, given 40 mg/kg or more of serotonin, had increased number of labelled parenchymal liver cells suggests that new liver cells were being formed at increased rate in response to serotonin injection. This is supported by histological observation that mitotic figures were more numerous in these livers than in those of control animals. Moreover, the decreased "biological concentration coefficient" for nucleic acid phosphorus in livers of rats treated with serotonin, suggests an increased turnover of nucleic acid, consistent with above autoradiographic and histologic observations.

The manner in which serotonin affects liver cell proliferation is not understood although the demonstrated dose dependency suggests a toxic effect, acting either directly upon hepatic cells or resulting from temporary ischemia. Furthermore, it is not known why this phenomenon could be demonstrated only in small rats. It is conceivable that serotonin may act by accentuating normal liver growth, which might explain why no significant effect was observed in larger animals.

Summary. In young rats weighing less than 115 g a single subcutaneous injection of 40 mg/kg or more of serotonin creatinine sulphate resulted in increased formation of hepatic parenchymal cells as demonstrated by autoradiographic technics employing tritiated thymidine. With histologic methods, livers showed an increase in mitotic figures. Slight cytologic changes in hepatic cells were observed, but in no instance was frank cellular necrosis found, even with extremely large doses of serotonin. The mechanism by which serotonin affects hepatic cell formation is not

known, although toxic or ischemic effects are considered to be the most probable explanation.

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Nature of Leukocyte Agglutinating Agent Occurring in Blood of Immunized Animals.* (24841)

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Agglutination of blood leukocytes occurs in a variety of apparently unrelated phenomena (1), among which are *in vivo*(2-4) and *in vitro*(5) hypersensitivity reactions. Such agglutination is probably a manifestation of cell injury, or at least an alteration at cell surface. In hypersensitivity reactions, agglutination would be due to a reaction of antigen with an agent probably antibody. Characterization of such an agent is a prerequisite to studying the mechanism by which an antigen-antibody reaction alters leukocytes so that they agglutinate. The work here presented was undertaken to determine whether the leukocyte agglutinating agent occurring in blood of immunized animals behaves in a manner characteristic of antibody and whether such antibody is cellular or humoral.

Material and methods. Male guinea pigs weighing 400-500 g were used. Animals in experimental group received a single intraperitoneal injection of either 1 mg crystalline egg albumin (Armour) in 1 ml normal saline or 1 ml heat killed brain-heart infusion broth culture of type III pneumococci (ATCC 6303) containing approximately 10^5 organisms/ml. Control animals received a single intraperitoneal injection of either 1 ml saline or 1 ml sterile brain-heart infusion broth. Animals which received egg albumin (EA) and their controls were used 6-10 weeks following injection. Animals which received pneumococcal vaccine and their controls were used after 8-12 weeks. Blood was obtained by cardiac puncture. Serum was stored at –30°C after separating from clotted blood which remained at room temperature not more than 2 hrs. Solutions of EA, pneumococcal capsular polysaccharide (S III)† and heparin (.3 mg/ml) were prepared with normal saline. Balanced salt solution contained isotonic concentrations of NaCl and KCl. All solutions in contact with leukocytes were pyrogen free. Leukocyte agglutination was quantitated by technic previously described(6) and degree of agglutination expressed as agglutination index. The *t* test was used for all statistical evaluations. Two methods were used to test for leukocyte

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