Influence of Gonadotrophic Hormones on 5-Hydroxytryptophan-Induced Renal Necrosis.* (31145)

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The injection of serotonin (5-hydroxytryptamine; 5-HT) into rats causes a characteristic lesion in the kidneys(1-3). The lesion is distinguished by fragmentation of the epithelium of the proximal convoluted tubules and deposits of mucous substance in the lumina of tubules. Pyknotic nuclei are found in both proximal and distal tubules(4). The immediate metabolic precursor of 5-HT, 5-hydroxytryptophan (5-HTP), will also cause the same type of lesion with more marked pathologic changes than that caused by 5-HT (5).

Recently, during the postmortem examination of rats in which the effect of serotonin on ovulation and luteinization was being investigated(6,7), a conspicuous difference in the occurrence of gross kidney lesions was observed between control and experimental animals. The degree of gross kidney damage appeared to be influenced by the treatment of the rats with gonadotrophic hormones and serotonin. Evidence is herein reported to show that the severity of kidney lesions, produced by injection of 5-hydroxytryptophan, is influenced by treatment of rats with gonadotrophic hormones.

Methods. Immature female rats, Holtzman descendants (Cheek Jones Co., Houston), age 22 days, were housed in a constant temperature room with controlled lighting (14 hours light and 10 hours darkness) during the course of the experiment. The animals were divided into 4 groups and treated with pregnant mares' serum gonadotrophin (PMS, *Equinex* Ayerst) and 5-dl-hydroxytryptophan (5-HTP; Sigma Chemical Co.) as follows: a) 10 intact rats, 5-HTP; b) 10 intact rats, PMS and 5-HTP; c) 21 ovariectomized rats, PMS and 5-HTP; d) 21 sham-operated rats, PMS and 5-HTP. PMS (25 IU) was given as a single subcutaneous injection in 0.25 ml saline on the 25th day of age and 5-HTP (50 mg/kg) was given as a single subcutaneous injection in 0.25 ml distilled water on the 29th day of age.

Ovariectomy was performed at 23 days of age. A sham operation, which consisted of bilateral incisions through the dorsal skin and musculature into the coelomic cavity, without ovariectomy, was performed as a control procedure.

All rats were autopsied at 30 days of age (24 hours after the injection of 5-HTP), and kidneys were examined for gross lesions, weighed, and fixed in Bouin's or 10% neutral formalin solution for 48 hours. Sections of kidney were stained with hematoxylin and eosin for general histology, periodic acid-Schiff reagent (PAS) for mucopolysaccharides, Perl's ferrocyanide reaction for hemosiderins and mineral iron, and von Kossa's silver nitrate method for calcium salts. Perl's and von Kossa's reactions were controlled by processing adjacent sections after extraction of iron and calcium from the tissues.

Results and discussion. Initial results in this series of experiments were from rats that had been treated with either 5-HTP or a combination of PMS and 5-HTP in an attempt to determine the drug effects on ovulation, as mentioned in the introduction. The kidneys of animals that received PMS and 5-HTP were obviously more damaged as judged by gross lesions (4 kidneys with small lesions, 10 with large lesions over 5 mm in diameter, and 6 normal kidneys) than those of rats that had been treated with 5-HTP only (8 kidneys with small lesions, 2 with large lesions, 10 normal kidneys). In this experiment the determination of percentage of kidney damage was not attempted, but degree of damage was always proportionate to kidney weight. This relationship could be determined easily in kidneys that were completely free of lesions as compared to those in which

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TABLE I. Comparison of Kidney Lesions in Sham-Operated and Ovariectomized Rats After Treatment with Pregnant Mares' Serum Gonadotrophin and 5-Hydroxytryptophan. Kidney damage is expressed in a range from unaffected (0) to kidneys with lesions involving 75 to 100% of surface area. Mean kidney weight is calculated in g/100 g body weight ± S.E.

Extent of lesion	No. of kidneys involved	
	Sham-op.	Ovariectomy
0	2	25
5– 20%	0	10
25– 75%	3	0
75–100%	37	7
Kidney wt	1.25 ± .02*	1.11 ± .03

* $p < .001$ (t test).

almost all of the cortical surface was damaged and ischemic. Most of the lesions were very irregularly shaped and more difficult to assess quantitatively than either completely damaged or unaffected organs. Kidney weights in rats that were given gonadotrophin and 5-HTP were 1.38 ± 0.03 g/100 g body weight; those of rats receiving 5-HTP only were 1.19 ± 0.03 g/100 g body weight ($P < 0.001$). Since PMS has a follicle stimulating and some luteinizing activity, and since this gonadotrophic hormone stimulates the production of ovarian hormone, the severity of kidney lesions in this group of animals was apparently related either to PMS or to ovarian function initiated by injection of this hormone.

Two groups of rats, one of them sham-operated and the other ovariectomized, were treated with PMS and 5-HTP to determine the essentiality of the ovary in the development of kidney lesions. In these experiments an attempt was made to evaluate the results (Table I) by expressing the area of the lesion as a percentage of the surface of each kidney. These results indicate that ovarian response, and not simply the presence of systemic gonadotrophins, is definitely related to the severity of kidney lesions in immature rats.

The histology of the lesion has been described previously(5) as a renal cortical necrosis involving the proximal convoluted tubules. Injections of the renal artery with China ink showed that areas of hyperemia alternated with anemic zones. Renal tubules in the damaged areas were greatly dilated and contained hyaline substance and desquamated tubular epithelium. Histochemical techniques, applied to sections from the presently described experiments, identified this substance as a mucoprotein or neutral mucopolysaccharide (PAS-positive). In the most severe lesions this hyaline material contained calcium carbonate and phosphate deposits, which ranged in size from small granules to large amorphous masses which were visible macroscopically.

Deposition of inorganic iron also was demonstrated in the mucopolysaccharide matrix. Its presence probably can be associated with the liberation of ferric ions from combinations with protein, such as hemosiderin(8).

These results demonstrate that (a) the kidney lesions caused by 5-HTP are more extensive, and involve more kidneys if rats also receive gonadotrophic hormone; (b) the kidneys of rats receiving 5-HTP and gonadotrophins are significantly larger than those in animals injected with 5-HTP only; (c) the presence of the ovary is important in determining the number of involved kidneys and the severity of lesions in each kidney; (d) and the response of the ovary to gonadotrophic hormone, and not the hormone itself, is definitely related to the severity of the lesion, since the lesions were more extensive in hormone-treated, intact rats than in animals treated with 5-HTP only, or in ovariectomized rats treated with PMS and 5-HTP. However, the occurrence of lesions is not strictly dependent on ovarian response since some (17 of 42) of the kidneys from ovariectomized rats did have lesions (Table I). Renal cortical necrosis in these animals may be associated with differences in biological response to the drug.

Summary. Treatment of immature female rats with pregnant mares' serum gonadotrophin (PMS) increases the incidence and severity of kidney lesions produced by 5-hydroxytryptophan. This influence of PMS on kidney lesions is associated with ovarian response to gonadotrophic hormones.

1. Erspamer, V., Asero, B., J. Biol. Chem., 1953, v200, 311.

2. Erspamer, V., Correale, P., Fiori-Donati, L., Arch. int. Pharmacodyn., 1956, v106, 122.

3. Salgado, E., Green, D. M., Am. J. Physiol., 1955, v183, 657.
4. Murray, S. M., J. Path. Bact., 1965, v90, 409.
5. Csötörtök, L., Földes, I., Beregi, E., Arch. int. Pharmacodyn., 1964, v148, 419.
6. O'Steen, W. K., Endocrinology, 1964, v74, 885.
7. ———, ibid., 1965, v77, 937.
8. Lillie, R. D., Histopathologic Technique, 2nd Ed., Blakiston, Co., 1954, p243.

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Inhibition of Erythropoietic Stimulation by Testosterone in Polycythemic Mice Receiving Anti-Erythropoietin.* (31146)

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Erythropoiesis in the normal animal can be stimulated by erythropoietin and various other hormones. In the mouse made polycythemic by transfusion, erythropoiesis is virtually abolished. A single injection of erythropoietin into such mice initiates an orderly wave of erythropoiesis in the hematopoietic tissue which can be detected easily 3 days later either by measurement of the blood reticulocyte level or the incorporation of Fe^{59} into the red blood cells(1,2). In the polycythemic mouse, in contrast to the normal mouse, the injection of ACTH, thyroxin(3), triiodothyronine, or testosterone(4,5) does not initiate a wave of erythropoiesis comparable to that produced by erythropoietin. The injection of testosterone, however, is followed by a wave of erythropoietic activity in the polycythemic mouse observable several days later than that caused by injection of erythropoietin(6). It has been further demonstrated by several groups of investigators(7-10) that plasma taken from several animal species after injections of testosterone stimulates erythropoiesis in the polycythemic mouse. This stimulation has been generally assumed to be due to the presence of erythropoietin in the plasma taken from the testosterone injected animals; however, Fried and Gurney (9) have stated that it is equally "possible that this erythropoietic activity is due to a metabolic product of testosterone which is slow to be formed, reaches high concentrations in the plasma in 4 days, and is active in much smaller titers than testosterone alone."

The present paper demonstrates that the stimulation of erythropoiesis in polycythemic mice following testosterone does not occur in the presence of an immune serum against erythropoietin.

Methods. Eight- to 10-week-old C_3H female mice weighing about 25 g were transfused by intraperitoneal injection of isologous red blood cells on 2 consecutive days. The red blood cells were washed 3 times with saline before use.

On the fifth and sixth day after the last transfusion 25 mg of testosterone propionate in 0.1 ml of sesame oil was injected subcutaneously. Control mice received similar injections of sesame oil alone. Ninety-six or 56 hours after the second testosterone injection, 0.5 μc of Fe^{59} as iron citrate (specific activity approximately 10 $\mu c/\mu g$) was injected intravenously and 72 hours later the incorporation of the injected Fe^{59} in the circulating blood was determined. The blood sample was washed once with saline before counting. The blood volume of the polycythemic mice was assumed to be 7% of the body weight. Mice having hematocrits below 55% at the time of sampling were discarded.

A group of mice receiving testosterone injections also received intraperitoneal injections of 0.1 ml of immune serum capable of neutralizing the erythropoietic activity of erythropoietin. The immune serum was injected daily commencing on the day of the second testosterone injection and continuing until the day before the Fe^{59} injection. The preparation and properties of the immune

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