THE RESPONSE OF HAIRLESS MICE TO LONG-TERM SEROTONIN ADMINISTRATION*

DENNY L. TUFFANELLI, M.D.

The effects of long-term serotonin administration in animals are not well-known. The increasing interest in the function of serotonin, and its possible relationship to connective tissue disorders, necessitates study of such effects. One of the many theories of serotonin function is that it is a fibrosing agent. MacDonald, Robbins and Mallory (4) found that long-term subcutaneous injections of serotinin resulted in a collagenous and fibrous proliferation in the dermis of the rat, with hyperplastic changes of the epidermis. Scherbel, McKittrick and Hawk (7), studying the response to subcutaneously implanted Ivalon sponges in rats, reported that large and unphysiological doses of serotinin increased inflammation, but impeded rather than stimulated connective tissue growth. Lapiere (3) also studied the connective tissue response around implanted Ivalon sponges, following repeated local injections of serotonin. He found an increase in the soluble forms of collagen and felt that this indicated an increased activity of fibroblasts. There are only a few reports concerning the systemic effects of injected serotonin. Waugh and Beschel (8) studied the effects on rat kidney. They found that serotonin injected intraperitoneally produced variable degrees of necrosis of the distal and convoluted tubules, and concluded that the nephropathic effects were due to vasospastic ischemia. The purpose of the present study was to study fully the effects of long-term administration of serotonin to hairless mice, both at the site of administration and systemically.

METHODS

The HR/CH strain of hairless mice were used†. They were evenly divided between sexes and averaged 30 gm. in weight. The age varied from one to 14 months. They were fed on unrestricted quantities of Wayne Lab Blox and allowed drinking water ad libitum. The temperature of the room was 70° ± 1° F. Injections were given intradermally with a 1 cc. sterile tuberculin syringe and a 26 gauge needle. Mice were injected only over the right side of the body. Serotonin purchased from California Corporation for Biochemical Research was injected in doses ranging from 1 to 6 mg. daily into 60 mice. After the effects of varying the dose were noted, the animals were injected with 4 mg. daily. Ten animals were sacrificed and studied each month over a period of six months. Saline and water injections were used as controls in 30 mice and again the mice were sacrificed at monthly intervals. Necropsies were performed at death. The abdomen and thorax were opened and the skin, lungs, heart, liver, spleen and intestines removed and examined grossly. Sections were made of these organs. Hematoxylin and eosin stains were used routinely. Other stains used were Giemsa, Van Gieson and Verhoeff's elastic fiber stain.

RESULTS

Within two to six minutes following the injections of serotonin, the animals developed an anaphylactic-like response with tachycardia, respiratory embarrassment and cyanosis. Next noted were diminished muscle tone and sluggishness. Locally edema and blanching occurred. Following repeated doses, infarction and local necrosis occurred at the site of injection. Soon the injected area of the animal became covered with multiple ulcerations which slowly healed. After long-term injections the animals appeared smaller and hyperpigmented in comparison with the controls. In addition the areas of injection had either formed chronic ulcerations or appeared thickened and fibrous. Histologically, one first saw hemorrhage, ulceration and necrosis (Fig. 2). Numerous mast cells with deeply metachromatic granules were scattered in the dermis. Later granulation tissue formed and the ulcers healed via fibroplasia and epithelialization (Fig. 3). Typical stages of connective tissue repair with edema, proliferation of fibroblasts and deposition of fibrils and collagen were seen. In all, it followed quite closely the formation of normal scar tissue. Multiple biopsy specimens of the opposite non-injected side were studied and appeared entirely normal. The lungs, heart, spleen, and liver were examined and appeared entirely normal, both

* From the Division of Dermatology, Department of Medicine, University of Southern California School of Medicine, Los Angeles, California.
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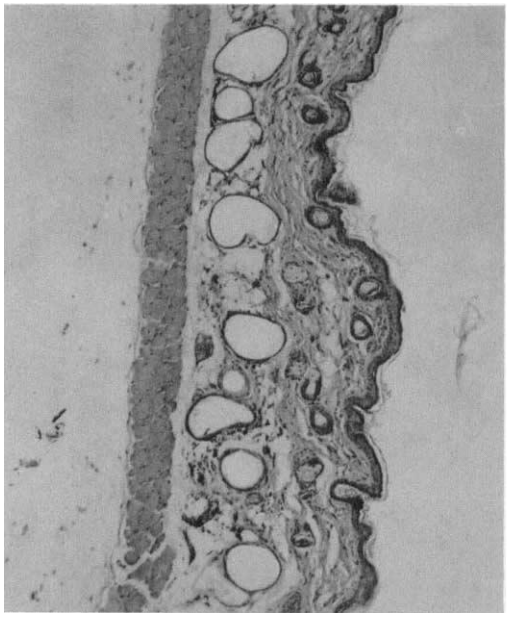

FIG. 1: Photomicrograph of a section of normal skin of the hairless mouse. Hematoxylin and eosin stain, × 54.

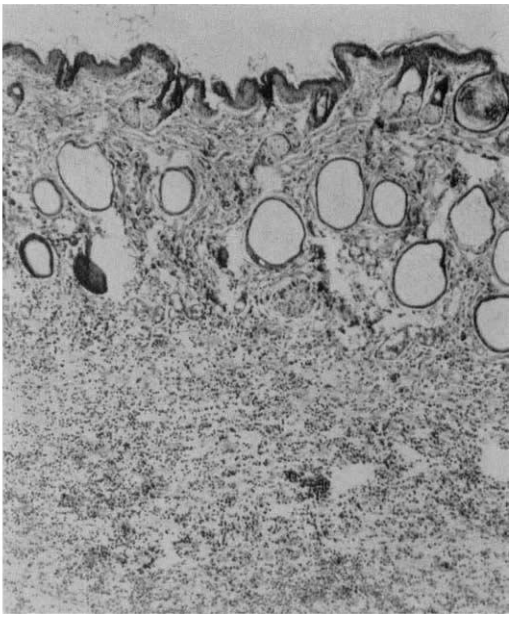

FIG. 3: Same as Figure 1. Healed ulcer with granulation tissue, fibroplasia and epitheliazation. × 54.

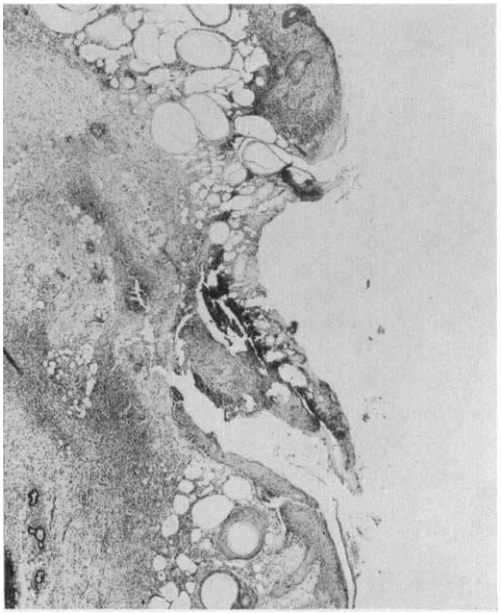

FIG. 2: Same as Figure 1 showing ulceration and inflammation above site of serotonin injection. × 22.

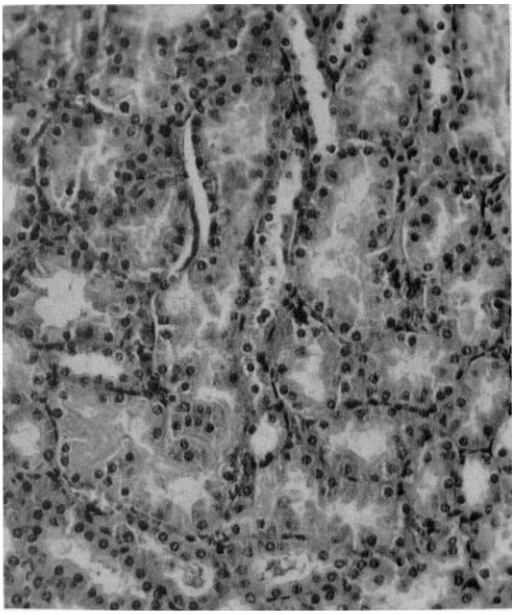

FIG. 4: Photomicrograph of hairless mouse kidney demonstrating tubular necrosis following long-term serotonin administration. Hematoxylin and eosin stain. × 180.

grossly and histologically. No increase in fibrosis was noted. Histologically, the kidneys demonstrated necrosis of the distal and proximal tubules (Fig. 4).

DISCUSSION

There is ample evidence that serotonin is liberated in the antigen-antibody reaction and participates in the inflammatory reaction (2). In low concentrations, it increases capillary permeability and edema (5). Its presence in adequate amounts in inflammatory exudates suggests that it may mediate some of the earliest changes of the inflammation (6). The anaphylactic reaction in mice is prevented by serotonin antagonists. The long-term effects of serotonin administration are less well-understood. There is increasing circumstantial evidence that serotonin may play a role in the etiology of the connective tissue diseases. It has been especially implicated in scleroderma (9). Some authors have thought it to be a "sclerosing agent" clinically and experimentally (4). In the present study the immediate effects of injected serotonin closely resembled the hypersensitivity reaction or anaphylactic shock, though the effects may have been pharmacological rather than physiological. In addition, it was attempted to determine if serotonin truly was a "fibrosing agent" and if serotonin-injected mice would form a possible laboratory model for the study of systemic sclerosis. While small amounts of serotonin cause vasodilation and increased permeability, the amounts used in this experiment seemed to cause local vasoconstriction. Repeated injections lead to the formation of infarctive ulcers which slowly healed. Thus, the only sclerosis noted in our studies occurred at the site of the injection of the serotonin, and seemed secondary to the vasoconstriction and local necrosis. The skin of the uninjected side appeared normal. Furthermore, except for the tubular damages of the kidneys also felt to be secondary to vasoconstriction, there was no evidence of "fibrosis" of internal organs. The renal changes did not resemble those seen in scleroderma.

SUMMARY

1. Serotonin was injected subcutaneously into hairless mice daily over a six-month period.

2. The immediate response resembled the hypersensitivity reaction. Later chronic cutaneous ulcerations occurred near the sites of injection.

3. Sclerosis of skin and internal organs was not noted.

4. There was no evidence that serotonin-injected mice form a laboratory model for the study of systemic sclerosis.

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