CSF enzyme rises were also noted in occasional control patients with actively progressive cerebral degeneration. 3) The data suggest that nervous system necrosis incident to the lipidoses may be biochemically distinguished from other forms of neural degeneration by the analysis of multiple enzyme levels in serum and CSF.

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Dermal Fibrosis Following Subcutaneous Injections of Serotonin Creatinine Sulphate.* (23734)

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5-Hydroxytryptamine (serotonin) has been associated with many physiologic and disease states, but its primary function is at present unknown. One of its most dramatic associations is in patients with the "carcinoid syndrome," in which there are several or all of an unusual group of findings: metastatic carcinoid tumor (argentaffinoma) of the gastrointestinal tract, dyspnea, diarrhea, episodes of flushing and cyanosis, hepatomegaly, ascites and edema, peptic ulcers, and cardiac lesions, chiefly affecting the pulmonic and tricuspid valves. Administration of serotonin to experimental animals has been shown to reproduce the clinical features of the syndrome, and intraperitoneal injections of the serotonin precursor, 5-hydroxytryptophane, have been followed by gastric mucosal erosions(1). It has been hypothesized that the cardiac valvular lesions in the carcinoid syndrome, which consist chiefly of fibrous tissue, are caused by serotonin, and it has been postulated that serotonin elicits a fibrous proliferation of the cardiac valves(2). Up to the present time, however, there has been no experimental evidence to support this hypothesis. In the present study, twice daily subcutaneous injections of serotonin creatinine sulphate resulted in a collagenous and fibrous proliferation in the dermis of rats, with hyperplastic changes of the epidermis. Similar changes were not observed in control animals injected with physiologic saline and with carbon tetrachloride.

Materials and methods. Fifty-three rats of the Sprague-Dawley strain† were injected subcutaneously twice daily for periods of up to 342 consecutive days, using 8 mg doses of

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FIG. 1. The rat below received subcut. injections of serotonin creatinine sulphate 72 consecutive days; the rat above received similar injections of physiologic saline. Note focal scarring and loss of hair of skin of animal that received serotonin. Necrosis of digits and tip of tail is also apparent.

serotonin creatinine sulphate‡ dissolved in one milliliter of physiologic saline. The skin of the back was used as the injection site. Groups of animals were 59 and 67 days of age at the time injections were begun. Males and females were used in different experiments. Twenty-four control animals received twice daily injections of one milliliter of physiologic saline for periods of up to 342 consecutive days, and 12 additional control animals received 0.2 ml of carbon tetrachloride twice weekly for 5 months. Animals were sacrificed at approximately monthly intervals in order to study the developmental stages of the lesions. Fixation of tissues was in Zenker's solution, and in neutral buffered 10% formalin. Histologic sections were stained with phloxine methylene blue, hematoxylin and phloxine, periodic acid Schiff reagent, elastica-Van Gieson connective tissue stain, and a modification of Bielschowsky's silver stain for reticulum.

Results. After approximately 30 days the animals receiving serotonin lost most of their hair in the regions of the injections, (Fig. 1), and their skin became visibly thickened and later cornified. Diffuse and constant reddening of the scarred areas became apparent after approximately 4 months. With continuation of the injections the changes became progressively more marked. Skin in areas away from the injection sites was normal; in the affected areas, the histologic changes were as described below.

After approximately 30 days the dermal collagenous tissue in the regions about the injection sites became dense, was arranged in compact, closely packed bundles, and took an eosinophilic stain (Fig. 3). There was little or no inflammatory cell reaction, and no apparent increase in reticulum fibers as demonstrated with the reticulum stain. With the periodic acid Schiff reagent, many positively staining collagenous bands were evident within the dermis; similarly stained areas were not found in sections of skin from control animals. The staining with periodic acid Schiff reagent was interpreted to represent immature collagen fibers, probably due to new fiber formation. A marked increase in the number and

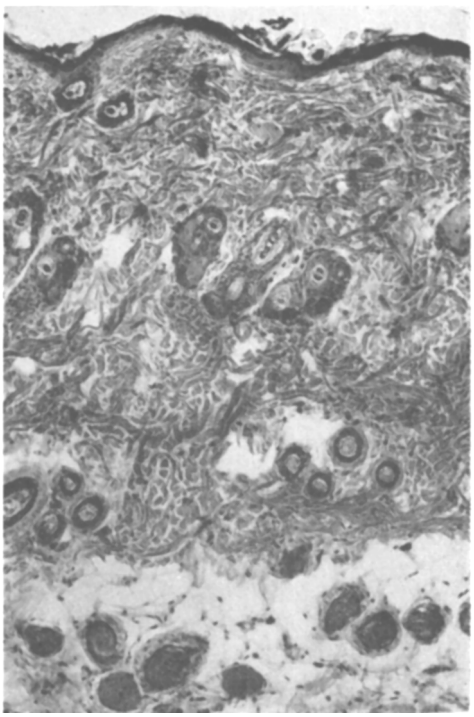

FIG. 2. Skin from an injection site of animal in Fig. 1 that received only physiologic saline 72 days. Note loose collagenous bundles in dermis, abundant hair follicles, and thin epidermis. PMB (phloxine methylene blue) × 67.

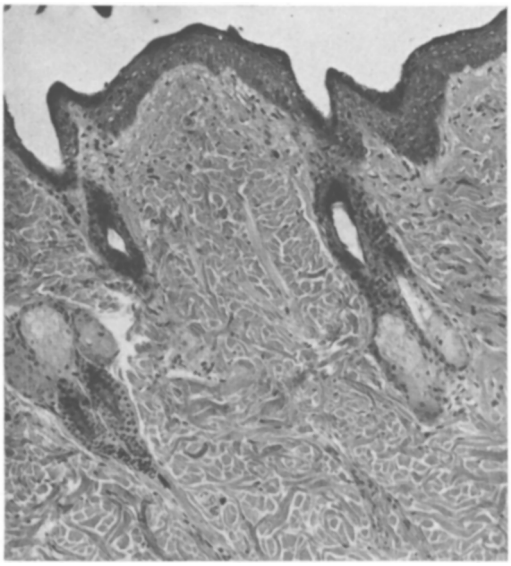

FIG. 3. Skin from injection site of animal in Fig. 1 that received serotonin creatinine sulphate 72 days. Note dense, closely packed collagen bundles in thickened dermis, decrease in hair follicles, and thickening of epidermis. PMB (phloxine methylene blue) × 67.

size of small blood vessels of capillary dimensions was noticeable within the dermis, and the epidermis showed hyperplasia, with thickening due to proliferation of cells (Fig. 3, 4). These changes became marked at approximately 60 days. Hair follicles decreased in number and sebaceous glands became larger than normal and were located deep within the dermis.

After approximately 6 months, the dermis became the site of fibroblastic proliferation and increased vascularity, and small arterioles in the basal regions of the dermis showed an eosinophilic staining with the phloxine methylene blue and hematoxylin and phloxine stains, resembling the changes found in small arterioles in benign nephrosclerosis. The basal layer of the epidermis frequently showed proliferation of basal cells to form small nests within the dermis, with anaplasia, suggesting "pre-cancerous" lesions (Fig. 4). Hair

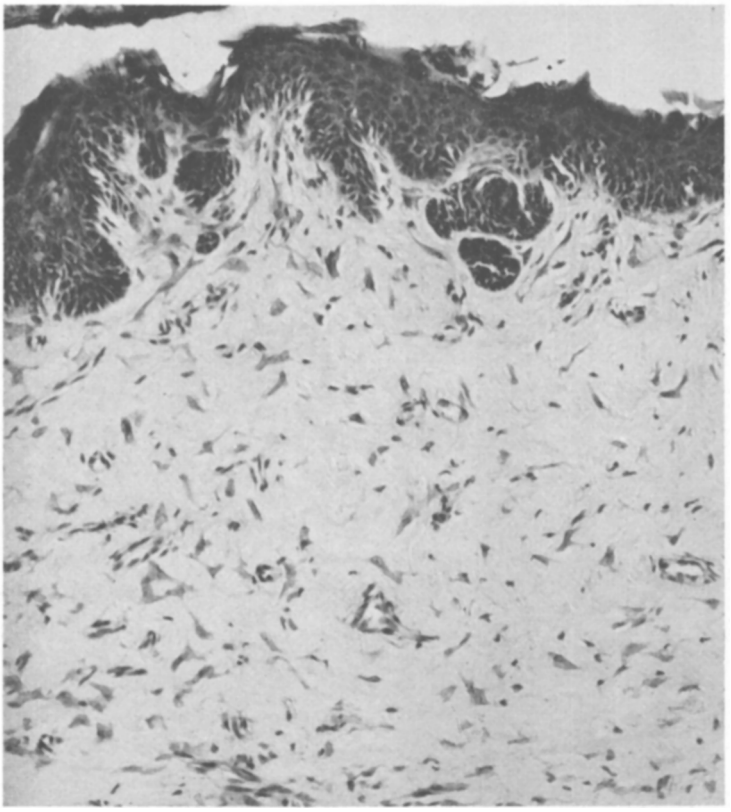

FIG. 4. Skin from injection site of animal that received serotonin creatinine sulphate 342 consecutive days. Note abundance of fibroblasts and increased vascular channels in thickened dermis, thickened epidermis with small islands of basal cells in adjacent dermis, and absence of hair follicles and sebaceous glands. PMB (phloxine methylene blue) × 400.

follicles and sebaceous glands became infrequent or absent in affected areas (Fig. 4). The above changes occurred in varying degree in all animals injected with serotonin, and no sex difference was noted. Control animals did not show similar changes after 342 days of saline injections or after 5 months of carbon tetrachloride injections.

Discussion. The present study indicates that serotonin creatinine sulphate stimulates a proliferation of collagenous and fibrous tissue within the dermis at the site of local injections. The manner in which this occurs is at present unknown, and further studies are in progress in an attempt to determine whether other substances closely related to serotonin can cause a similar fibrous proliferation of the dermis. The absence of significant inflammatory cell response preceding the collagenous and fibrous proliferation is a feature of interest and one that deserves further investigation. Fibrous proliferation was not observed in visceral and other organs of the animals, despite careful and complete autopsy search. This may have been due to a rapid inactivation of the injected serotonin by the mono amine oxidase system present in many tissues of the body(3). The hypothesis that serotonin causes cardiac valvular fibrous proliferation in patients with the carcinoid syndrome would appear to receive some support from the present study.

Summary. 1) Serotonin creatinine sulphate was injected subcutaneously twice daily into rats in 8 mg doses for periods to 342 consecutive days. After approximately 30 days of injections there was observed a progressive collagenous and fibrous proliferation within the dermis, an increased vascularity, hyperplasia of the epidermis, vascular changes in arterioles of the dermis, and diminution in skin appendages, all at sites of serotonin injections. Control injections of physiologic saline and carbon tetrachloride did not result in similar changes. 2) The present findings appear to lend some support to the hypothesis that serotonin causes the cardiac valvular fibrosis observed in patients with the carcinoid syndrome.

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Influence of H^3-β-Sitosterol on Sterol Excretion.* (23735)

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It was previously observed in these laboratories(1,2) that soybean sterols inhibit the increase in blood cholesterol of rats receiving a high cholesterol diet. Also, plant sterols were found to be esterified *in vitro* by pancreatic cholesterol esterase under the same conditions as cholesterol, albeit, at a slower rate than cholesterol. It was considered that soybean sterols inhibit cholesterol absorption by competing with cholesterol for the esterifying cholesterol esterase system. Bisset and Cook (4) fed humans 10 g of sitosterol a day and found that total fecal sterol increased, but only to about 60% of that fed. There was also an increased excretion of esterified sterol and a fraction rich in sitosterol esters was isolated by chromatography.

The experiment described below was designed to give more direct information on the absorption of sitosterol than previously obtained(3) and to allow measurement of the

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