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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TABLE I. Fecal Sterol Excretion following the Feeding of H^3-β-Sitosterol. 7 rats/series.

Group*	Fecal sterols total, mg	Fecal H^3-β-sitosterol, mg	Fecal sterol other, mg	Specific activity† total fecal sterol, μc/mg
H^3-β-sitosterol	22.9 ± 3.5‡	11.8 ± 1.6	11.1 ± 2.5	.042 ± .004
Control	6.0 ± .4		6.0 ± .4	

* Represents 24-hr excretion period. † Each animal received 25 mg H^3-β-sitosterol with specific activity of .081 μc/mg. ‡ S.E. of mean.

excretion of cholesterol and related sterols. To overcome the difficulties of determining cholesterol in the presence of sitosterol, H^3-β-sitosterol was fed in a cholesterol-free mixture; total fecal sterols as well as the amount of H^3-β-sitosterol excreted were determined.

Methods and materials. The tritium labeled β-sitosterol was prepared by means of a platinum-catalyzed exchange reaction between β-sitosterol and a mixture of tritium acetic acid, tritium water and tritium gas as described by Fukushima and Gallagher(5). Two groups of male rats weighing 225-250 g, raised on Purina pellet chow, were used. One group, after being fasted 24 hours, received, by intubation, an emulsion containing 25 mg (2.04 μc) H^3-β-sitosterol, 50 mg egg albumin, 70 mg sodium taurocholate, and 75 mg oleic acid made up in 3 ml of saline. The other group received either the emulsion without H^3-β-sitosterol or a sterol-free diet prepared as described previously(3). The sterol excretion was the same in the control animals whether the sterol-free emulsion or diet was administered. At the end of 24 hours the animals were sacrificed, the intestinal tract removed, and washed with 50 ml of saline. The washings from the intestine were pooled with the feces. Non-saponifiable lipid extracts were prepared according to procedures described earlier(3). The sterol content was determined gravimetrically as the digitonide (3). The tritium activity of the fecal sterols was determined as follows: fecal sterol digitonides were combusted in oxygen to water and CO_2; the water was reduced to hydrogen gas by heating with Zn dust for two hours at 420°C. The hydrogen gas was liberated in an ionization chamber and its tritium activity determined. A galvanometer drift of 1 mm sec was equal to 2×10^{-3} μc of tritium with an error of ± .1 mm/sec or 2×10^{-4} μc.

Results. Table I shows that the feeding of 25 mg of H^3-β-sitosterol gave, during the following 24-hour period, 22.9 mg of total fecal sterol. Based on the specific activity of the sitosterol fed (.081 μc/mg) 11.8 mg of this amount was H^3-β-sitosterol and 11.1 mg other sterol. Calculation of the amount of β-sitosterol absorbed after subtracting total fecal sterol minus the control excretion gave a value of 8.1 mg or 32.4% of that fed. On the other hand, calculation based on the amount of fecal H^3-β-sitosterol showed 13.2 mg or 52.8% of that fed was unaccounted for and presumably absorbed. During the period of H^3-β-sitosterol administration there was a significant increase ($P<.05$) in fecal excretion of sterols other than β-sitosterol from 6.0 to 11 mg/day.

Discussion. It is evident from the data that after administration of β-sitosterol there was a considerable increase in the fecal excretion of cholesterol and other sterols which would not be apparent from chemical balance data alone. Likewise, there was a greater absorption of β-sitosterol than would be assumed from the chemical data. This confirms our earlier report(3) of significant plant sterol absorption under the optimum conditions formulated for cholesterol absorption. Plant sterol absorption ranging from 10 to 60% of that fed has also been reported by other investigators(6-9). The increased excretion of endogenous cholesterol when β-sitosterol is fed is in accord with the suggestion(1) that plant sterols inhibit the increase in blood cholesterol levels due to high cholesterol diets by competing with cholesterol for the sterol absorptive system of the intestinal wall. It should be pointed out that while β-sitosterol may inhibit the reabsorption of endogenous cholesterol, it does not necessarily follow that it will decrease normal blood cholesterol levels, since we have recently shown (10) that removal of reabsorbed endogenous

cholesterol *via* a thoracic lymph fistula produced an 8-10 fold increase in liver cholesterol synthesis from l-C^{14}-acetate.

Summary. An increased fecal excretion of cholesterol and related sterols followed the administration of H^3-β-sitosterol to rats receiving cholesterol-free emulsions containing bile salt and fatty acid. Approximately 53% of the administered labeled β-sitosterol was not recovered in the feces. Our study provides additional evidence that plant sterols are absorbed in the same manner as cholesterol and thereby compete with cholesterol for the sterol absorptive mechanism.

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Effects of Type of Restraint upon Heat Tolerance in Monkeys.* (23736)

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In the course of other experiments, monkeys were exposed to a warm environment while restrained on an animal board of the type ordinarily used for dogs and cats. Under these conditions, the rectal temperatures increased to an undesirable degree. The monkeys were able to reach equilibrium at an acceptable rectal temperature, however, with a different method of restraint; here the arms were held at the side of the body and the head was supported by a yoke around the neck. The observations are reported to call attention to the sensitivity of the temperature regulating mechanism of these animals to slight changes in the method of restraint.

Material and methods. Thirteen Macaca monkeys of both sexes ranging in weight from 3 to 6 kg, were used in 18 tests. Of the 6 exposed at 29°C ± 2°, the temperature of the animal quarters, 3 monkeys were tested with each type of restraint; heart rates were recorded on 2 of the 3. Two of the other monkeys were also exposed at 38°C, one with each type of restraint. The remaining 7 monkeys were exposed once at 38°C. This temperature was controlled to ± 0.5°C and the relative humidity was 45% ± 2%. The critical features of the 2 types of restraint, both with animal in prone position, are as follows: *Arms forward*: The animal rested with head free on a solid wooden platform, tied by wrists and ankles, with arms extended beyond the head. The knees and elbows had little freedom to bend. *Arms backward*: The animal rested on a platform of 0.5 in. wire mesh with an air space of 1.5 in. between it and the table. The body was held in position by yoke around the neck and by tying the arms at wrist and elbow along the side of the body. The legs were tied at the ankles but were only held loosely. At 29°C rectal temperatures were recorded with a Yellow Springs Tele-Thermometer thermistor and heart rate was obtained with a Burdick Electrocardiograph. At 38°C a cop-

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