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Effect of Thyroxine, Thyrotrophic and Somatotrophic Hormones on Skin of Dwarf Mice.* (25269)

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It is well known that thyroid hormone affects the composition of connective tissue. Histological studies have shown that the metachromatic mucopolysaccharide-containing ground substance decreases and mast cells decrease in size and granularity under the influence of thyroxine(1,2). The thyrotrophic pituitary hormone (TSH) exerts, besides stimulating the thyroid gland, a direct action on tissues which has been demonstrated in thyroidectomized animals(3,4,5). Injection of TSH into thyroidectomized guinea pigs is followed by mobilization of fat from normal depots. This tissue fat is replaced by a mucinous substance characterized by a content of acid mucopolysaccharides. The fat mobilizing principle is inhibited by thyroxine(6). Like TSH, somatotrophin (STH) has a direct effect on connective tissue. Various experiments have shown that STH stimulates fibroblast proliferation and formation of collagen (7). In the present work the effect of thyroxine, TSH and STH on number of mast cells and concentrations of hexosamine and hydroxyproline in the skin of dwarf mice has been studied. Hexosamine is an important constituent of acid mucopolysaccharides in the ground substance of connective tissue and was taken as a measure of concentration of these compounds. As collagen is the only protein in connective tissue which contains hydroxyproline, this amino acid was taken as an expression of amount of collagen.

Material and methods. Adult dwarf mice and their normal siblings were used. The dwarf mice are born with hypoplastic pituitary glands(8) which seem devoid of acidophil cells producing STH and basophil cells producing TSH(9). Dwarf mice are the only laboratory animals known to have pituitary (secondary) myxedema (with no or very little TSH and STH production). They were selected for the experiment because of this abnormality. In the first experiment, dwarf mice were divided into 5 groups with 5-16 animals in each group (no normal mice) (Fig. 1). The animals were injected intraperitoneally daily for 10 days. Group I: thyroxine (Roche) 0.1 ml (0.1%), II: TSH (Armour) 0.017 USP unit in 0.1 ml sterile water, III: STH (Armour) 0.5 mg in 0.1 ml sterile water, IV: Phyol‡ (Alfred Benzon) = STH 0.5 mg in 0.1 ml sterile water, V: physiological saline, 0.1 ml. On the 11th day the animals

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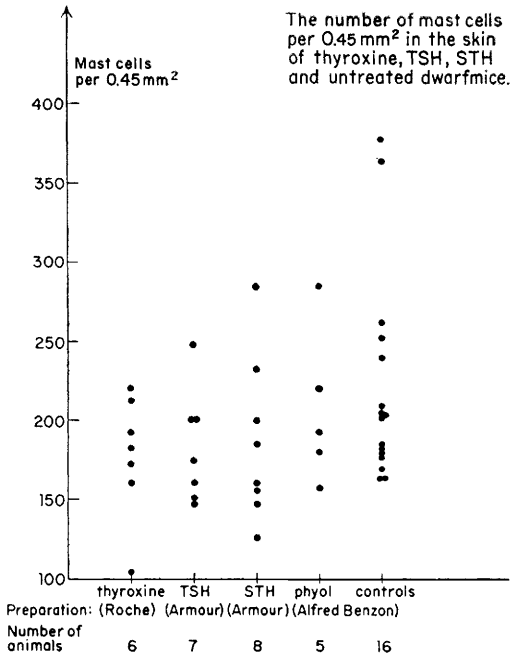

FIG. 1.

were killed and small pieces of skin from the dorsum removed and fixed in a 4% solution of lead subacetate. After dehydration and embedding in paraffin the tissue was cut into slices 10 μ thick (10^{-6}m) and stained for 30 minutes with toluidine blue (0.5% in water). The mast cells of the corium subjacent to the epithelial layer were then counted. An area of at least 0.45 mm^2 was counted. In the second experiment animals were divided into 3 groups of dwarf mice and 3 groups of their normal siblings with 7-10 animals in each group (Table I). Injections were carried out as in the first experiment except that STH preparations were not given. 100-200 mg of skin-samples were removed as before and dried. Hexosamine analyses were carried out by the method of Elson and Morgan(10), modified as previously described by Kirk and Dyrbye(11) and Dyrbye(12). Hydroxyproline content was determined by Martin and Axelrod's(13) modification of Neuman and Logan's(14) method.

Results. Mast cell countings showed a considerable scatter in all 5 groups (Fig. 1). Statistical examination was attempted. None of the treated groups differed significantly from the control group.

The results are listed in Table I. Statistical calculations showed that the values of hexosamine and hydroxyproline for control dwarf mice and control siblings did not differ significantly from each other.

Hexosamine values for the dwarf mice treated with TSH (Armour) were significantly higher than those of non-treated dwarf mice (t-value + 3.17). This accords with previous findings that TSH increases the quantity of metachromatic substances in the connective tissue. On the other hand, hexosamine values in the group of thyroxine-treated mice were significantly higher than in the group of control mice (t-value + 2.53). Hexosamine values in the other groups showed no significant difference. As regards hydroxyproline, none of the treated groups differed significantly from controls. Comparing dwarf mice treated with TSH with dwarf mice treated with thyroxine, however, we found that in the former group the skin showed a hydroxyproline content statistically higher than that of the latter (t-value + 3.11). These results suggest that thyrotrophic hormone stimulates and thyroxine inhibits formation of collagen fibrils.

Summary. Dwarf mice and their normal

TABLE I. Content of Hydroxyproline and Hexosamine in Skin of Untreated, Thyroxine (Roche) and TSH-Treated (Armour) Dwarf Mice and Their Normal Siblings.

Group	Hexosamine, mg/g dry tissue			Hydroxyproline, mg/g dry tissue		
	Mean value	Stand. dev.	No. of animals	Mean value	Stand. dev.	No. of animals
Dwarfs, thyroxine	3.7	.74	9	13.1	2.5	8
Mice, "	3.9	1.09	9	17.6	4.1	8
Dwarfs, thyrotrophic hormone	4.3	.85	10	18.2	3.9	8
Mice, " "	3.3	.87	8	19.5	6.5	7
Dwarfs, controls	3.2	.71	10	15.4	2.1	10
Mice, "	2.8	.80	10	15.0	3.7	9

siblings were given thyroxine, thyrotrophic and somatotrophic hormones or physiological saline intraperitoneally. In the skin the mast cells were counted and concentration of hexosamine and hydroxyproline were determined. The number of mast cells did not show any significant variation in the different groups. Concentration of hexosamine in the skin of thyrotrophin-treated dwarf mice was significantly higher than concentration in control dwarf mice. Hexosamine values in the group of thyroxine-treated mice were higher than in the group of control mice. Hydroxyproline content was significantly higher in the skin of thyrotrophin-treated dwarf mice than in thyroxine-treated dwarf mice.

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Influence of Exercise, Androgen Administration and "Atherogenic" Diet on Hematologic Changes in Pullets.* (25270)

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It is well known that the red blood cell count and hematocrit values of humans, mammals and avian species are much higher in males than in females. Juhn and Domm(1) showed that red blood cell differences between the sexes in chickens occurred after puberty and only in males in association with androgen secretion by testes. Gonadectomy of males prevented red blood cell count from differing significantly from that of females. Despite seasonal variations in the number of erythrocytes it was noted that adult male doves and pigeons had a higher count than adult females in all seasons(2). The present study was undertaken to determine the effects of exercise, androgen and an "atherogenic" diet on the erythrocytes, hematocrit and spleen size of pullets.

Methods. Six-week-old New Hampshire White Leghorn pullets were used in an experiment of approximately 11 weeks. The pullets were divided into 5 groups. Controls were fed plain mash. A second group was fed the same diet plus 2% cholesterol and 5% cottonseed oil. The third group, in addition to the diet of Group II, was exercised for one hour daily. Group IV received the same diet as II, was exercised, and injected with 1.25 mg testosterone propionate ("Oreton")‡ intramuscularly daily. We have found this dose of testosterone to be replacement therapy for castrated males. The last group was treated in the same fashion as the 4th, but no exer-

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