Alkaline Phosphatase in the Mouse Thyroid Following Testosterone Propionate, Thiouracil and Thyroglobulin.* (17385)

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The BMR of patients is increased by testosterone propionate and suggests that this androgen might influence the thyroid gland.[1] In animals, testosterone propionate has been reported to increase mouse thyroid weight[2] and to increase mitotic figures in rat thyroids.[3] It should be noted, however, that other investigators have failed to find an effect of testosterone on thyroid weight in mice[4] and rats[5] as well as in rats fed thiouracil.[5,6] Since weight was the essential criterion of effect and this factor is variable, it seemed more plausible to reinvestigate this problem on a histochemical basis and the alkaline glycerophosphatase technic was chosen.[7] This enzyme was not found in the rat thyroid in initial studies[7-9] but Dempsey and Singer[10] obtained positive results with longer incubation times. Alkaline phosphatase of the mouse thyroid has not been described but our preliminary studies revealed its presence making possible the androgen study in this species.

In addition to the effect of testosterone propionate on the normal thyroid it was of interest to determine whether the androgen would influence a thyroid gland altered by thiouracil or thyroglobulin feeding. Changes in physiological state may alter the alkaline phosphatase concentration. For example, stress induced by cold reduced enzyme concentrations[11] but no definite conclusions were drawn from thyroids of 2 rats fed thiouracil.[10] Kroon[12] observed an increased alkaline phosphatase in the distended thyroid blood vessels following thiouracil feeding to rats. Hypophysectomy did not effectively alter the alkaline phosphatase of the rat thyroid follicle.[13] The influence of thiouracil or thyroglobulin feeding alone on the mouse thyroid is presented.

Twenty-two day old Swiss albino female mice were used and maintained on Purina Fox Chow. Groups of 7-11 animals were given testosterone propionate (Perandren, Ciba†) in a single injection of 0.05 mg, 0.10 mg, 0.20 mg, or 0.50 mg when 32 days old. One group of 5 animals received a single injection of 2.5 mg of the androgen. The mice were sacrificed 7 days later. Control mice were autopsied at 32 and 39 days of age.

To study the influence of hypo- and hyperthyroidism alone and with testosterone propionate, 0.25% thiouracil or 0.1% thyroglobulin (Proloid) was incorporated in the Fox Chow diet. The drug feeding was started

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† Testosterone propionate (Perandren, Ciba) was supplied by Dr. E. Oppenheimer, Ciba Pharmaceutical Products, Summit, N. J., Thiouracil (Deravet) was supplied by Dr. Mark Welsh, Lederle Laboratories, Pearl River, N. Y., and Thyroglobulin (Proloid) was provided by Dr. Robert Kroc, The Maltine Company, Morris Plains, N. J.

TABLE I.
Effect of a Single Injection of Testosterone Propionate on Alkaline Phosphatase Content of Mouse Thyroid Gland.

Testosterone propionate dosage, mg	Mice fed fox chow only	Mice fed fox chow plus 0.25% thiouracil	Mice fed fox chow plus 0.10% thyroglobulin
0	+	0	+
.05	+	0	+
.10	+	0	+
.20	+	0	+
.50	++	0	+
2.50	+++		

0 = An overall negative phosphatase content.
+ = Nuclear and slight cytoplasmic content.
++ = Nuclear and moderate cytoplasmic content.
+++ = Fairly heavy content in the nuclei and cytoplasm of the acinar cells; slight content in the nuclei of the interfollicular cells.

at 22 days of age with autopsy at 32 and 39 days of age. The androgen, when administered, was injected on the 32nd day of age and permitted to act over 7 days thus a preliminary drug feeding period of 10 days preceded injection and in all cases drug feeding was continued after hormone administration.

Mice were killed by cervical dislocation. The right thyroid fixed in cold 80% alcohol and the left thyroid in Bouin's fluid. Alkaline glycerophosphatase was checked on sections cut at 7 μ and prepared by the Gomori technic[14] with the addition of a 2% $MgSO_4$ solution to the incubation mixture to enhance the stain. Incubation was carried out at 37° for 7 hours at pH 9.2-9.4. The Bouin's fixed material was sectioned at 10 μ and stained with hematoxylin and eosin.

The thyroid gland of immature female mice has alkaline glycerophosphatase essentially concentrated in the nuclei of the follicular cells with a tinge appearing in the cytoplasm. This condition was not altered by testosterone propionate until a dosage of 0.5 mg was reached when, however, the enzyme concentration exhibited an increase in the cytoplasm in all 8 mice studied (Table I). This proved a critical level, but an even more striking effect on the cytoplasmic phosphatase could be seen with a 2.5 mg dosage. Routine sections stained with hematoxylin and eosin failed to reveal an androgen effect.

Thiouracil feeding resulted in the anticipated hypertrophy of the thyroid epithelium and loss of colloid. Alkaline phosphatase was essentially lacking as the enzyme was not present in the cytoplasm and was reduced or absent in the nucleus of the follicular cells. Only the endothelial lining of the blood vessels contained alkaline phosphatase to any degree. Administration of testosterone propionate failed to alter this essentially negative enzyme picture even at the 0.5 mg dose which was effective in the normal mouse (Table I). The general histology also was not altered.

Following thyroglobulin feeding the thyroid histology was that of an atrophic gland as the follicular epithelium was now low cuboidal or squamous. Alkaline phosphatase was definitely present in the nucleus and the cytoplasmic staining concentrated so that the follicular epithelium frequently appeared as a line of alkaline phosphatase. Essentially the same histology has been described for the rat thyroid following hypophysectomy.[13] Testosterone propionate in the dosages used failed to alter the alkaline phosphatase histology and provided no evidence of an effect in our routine preparations (Table I).

Increased activity of the thyroid gland is correlated with a loss in alkaline phosphatase whether induced by stress or thiouracil feeding. Furthermore, pituitary activation of the thyroid or disuse atrophy due to thyroid administration plays a primary role in the control of enzyme concentration since testosterone propionate can not override their effects. Nevertheless, in a normal female mouse, androgen can increase the alkaline

[14] Gomori, G., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 23.

phosphatase concentration suggesting the use of histochemical technics to evaluate steroid hormone actions on the thyroid and at the same time emphasizing the importance of the physiological state of the end organ and its effect on anticipated results.

Summary. Testosterone propionate definitely increased the cytoplasmic alkaline phosphatase in the follicular cells of the mouse thyroid when dosages of 0.5 mg or more were used. Thiouracil (0.25%) induced marked hyperplasia in 17 days and virtually eliminated the alkaline phosphatase in the follicular epithelium whereas thyroglobulin caused a follicular cell atrophy without loss of the enzyme. Testosterone propionate did not influence the thyroid alkaline phosphatase in the presence of thiouracil or thyroglobulin.

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Lysozyme Titres in Regional Enteritis, Miscellaneous Tissues, Microorganisms, and Excreta.* (17386)

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(Introduced by Karl Meyer.)

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The apparent increase in the lysozyme content of the stools in regional enteritis has already been reported.[1] At that time the proposition that lysozyme is a local initiating agent in the pathogenesis of this disease (as well as in chronic ulcerative colitis) was advanced. The circumstantial evidence supporting this conclusion was reported there and in an accompanying article discussing the probable etiologic role of lysozyme in peptic ulcer.[2]

Only 6 cases of regional enteritis were reported at that time; it therefore appears desirable to supplement this evidence with that subsequently obtained.

The values in the additional cases studied appear in the table. All were regional ileitis with involvement of varying lengths of bowel. The disease process ceased at the ileo-cecal valve in every instance. The assays were done by the viscosimetric technic of Meyer.[3]

The results were markedly uniform except for 2 low titres and one quite high one. The former were from constipated patients and the latter individual had marked diarrhea. It has already been noted that the rate of fecal expulsion influences the stool titre, particularly when the disease process does not extend to the descending colon. This depends upon the gradual inactivation of fecal lysozyme re-

TABLE I.

Case	Lysozyme concentration in units/g of stool (wet wt)	Lysozyme output per day in units
1	17.8	1,727
2	3.6	—
3	14.8	17,538
4	18.2	1,170
5	32.9	3,258
6	21.3	—
7	1.7	1,120
8	22.4	2,910
9	15.4	—
10	22.2	—
11	14.3	—
12	17.0	—
13	20.4	—
14	55.0	15,817
Means	19.8	6,220
Avg stool lysozyme values in normal individuals (1) 2.7		158
Avg stool lysozyme values in purged individuals (1) 1.6		1,064
Avg stool lysozyme values in chronic ulcerative colitis (1) 73.6		26,568

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