PART I

THYROID

INFLUENCE OF ANDROGEN ON THE THYROID GLAND. ELUCIDATION OF THE PRECISE NATURE AND MECHANISM OF ACTION.
CHAPTER I

EFFECT OF TESTOSTERONE PROPIONATE ON THE THYROID OF YOUNG MALE RATS.
While reviewing the literature on thyroid it was noted that a number of investigators reported a thyroid stimulating action of testosterone in different species of animals; others failed to note any change following testosterone administration. Another group of workers reported a depression of thyroid activity. It is thus evident that the response of the thyroid to the androgen is still a debatable point and needs proper elucidation. The precise mechanism of action of testosterone on the thyroid is also not properly understood.

Testosterone in lower doses depresses the gonadotrophin (FSH) secretion of the pituitary (Burrows; 1949), whereas in higher doses it may enhance the gonadotrophin secretion (Neiburgs, 1949; Castellani, 1952a). Gonadotrophin (FSH) in its turn has got a thyroid stimulating action (Riddle et al., 1933; Fluhmann, 1934; Kar et al., 1955a). Nathanson and coworkers (1940, 1941) observed that thyroid stimulating effect of testosterone ran closely parallel to the ovarian changes, which had been shown to be stimulated through hypophysis (Nathanson et al., 1938). A question, therefore, arises as to whether the thyroid stimulating action of testosterone requires the mediation of hypophyseal gonadotrophin and the reported discrepancy in the response of the thyroid to testosterone is anyway related to differential influence of the testoid on the gonadotrophin secretion from the pituitary.

Kar and Roy (1955) observed that in young rats, a
low dose (0.16 mg. on alternate days) of testosterone propionate inhibited the testicular activity, suggesting a reduction of gonadotrophin secretion from the pituitary, whereas a high dose (6 mg. on alternate days) had a stimulating effect on the organ, thus pointing to a possible enhancement of gonadotrophin output.

It is well known that thyroxine exerts an inhibitory influence on the thyroid by blocking the elaboration of hypophyseal thyrotrophic hormone (Sillers, 1950).

The differential effects of testosterone on the pituitary gonadotrophin secretion, as well as the blocking effect of thyroxine on the hypophyseal TTH output, have been utilized in the present investigation to elucidate the mechanism of action of testosterone on the thyroid gland.

Experimental Procedure

Young male albino rats, weighing 41.5 ± 3.6 gms., were used in this study. Thirty-six rats were divided into six groups of 6 each. One group was treated with testosterone propionate, 0.16 mg. in 0.25 c.c. of sesame oil on alternate days (low dose) and another group with the same hormone in the dosage of 6 mg. in 0.25 c.c. of sesame oil at the same intervals (high dose). Altogether eight intramuscular injections of this hormone were given over a period of 15 days. The control group received 0.25 c.c. of sesame oil alone on alternate days. Thyroxine (12.5 µg. of L-thyroxine sodium,
dissolved in 0.5 c.c. of distilled water daily) was administered orally to the fourth group for 15 days. Fifth group received thyroxine in the above dosage along with the low dose of testoid, while the sixth group received thyroxine along with the high dose of the sex hormone.

The animals were sacrificed on the day following the final hormone treatment. The thyroids were carefully dissected out and suitably fixed for histological studies. Thyroid activity was evaluated by microhistometric measurement of acinar cell height according to the method of Rawson and Salter (1940).

Testes were carefully dissected out and weighed. For histological examination pieces of testis were fixed in Bouin's fluid and serial paraffin sections were stained with Mallory's trichrome stain. The testis weight and histology were studied in order to verify whether it responded differentially to low and high doses of testosterone in the present investigation.

**Results**

**Thyroid.** Microscopical picture of the thyroid of the control group of animals presents a normal appearance (Plate I, Fig. 1). The follicles are filled with acidophilic colloid which shows a few vacuoles. The cells lining the acini are of cuboidal type with rounded nuclei. The mean height of the acinar cells is 8.2±0.8 μ (Table 1) and the
Table 1. — The thyroid acinar cell height in normal and experimental rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean height of acinar cells with S.E. (μ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control</td>
<td>8.2 ± .09</td>
</tr>
<tr>
<td>2.</td>
<td>Low testosterone propionate</td>
<td>12.2 ± .19</td>
</tr>
<tr>
<td>3.</td>
<td>High testosterone propionate</td>
<td>16.6 ± .20</td>
</tr>
<tr>
<td>4.</td>
<td>Thytoxine</td>
<td>4.1 ± .09</td>
</tr>
<tr>
<td>5.</td>
<td>Thyroxine plus low testosterone propionate</td>
<td>4.3 ± .07</td>
</tr>
<tr>
<td>6.</td>
<td>Thyroxine plus high testosterone propionate</td>
<td>4.8 ± .08</td>
</tr>
</tbody>
</table>

Analysis

Group 1 vs. group 2: t = 19.05; P < .001 (Significant)
Group 1 vs. group 3: t = 38.36; P < .001 (Significant)
Group 1 vs. group 4: t = 32.28; P < .001 (Significant)
Group 1 vs. group 5: t = 34.21; P < .001 (Significant)
Group 1 vs. group 6: t = 28.33; P < .001 (Significant)
Group 4 vs. group 5: t = 1.75; P > .1 (Insignificant)
Group 4 vs. group 6: t = 5.83; P < .001 (Significant)
Group 2 vs. group 5: t = 39.11; P < .001 (Significant)
Group 2 vs. group 6: t = 54.88; P < .001 (Significant)

The cytoplasm of the cells contains a few colloid droplets. The capillaries can be seen in the interfollicular tissue.

The picture of thyroid in the low-dose testosterone treated group indicates considerable stimulation of the gland (Plate I, Fig. 2). The acinar cells show great degree of hypertrophy and some amount of hyperplasia. The follicular cells are of columnar type and the mean height of the cells is 12.2 ± 0.19 μ (Table 1). The acinar colloid is thin.
small in amount and shows vacuolations. Increased vascularity of the gland is noticeable.

Histological examination of the thyroid in the high thyroxinoid group reveals marked degree of stimulation of the gland (Plate I, Fig. 3). The acinar cells show marked hypertrophy and hyperplasia and are of columnar shape. The mean height of the follicular cells is 16.6 ± 0.20 μ (Table 1) and the cytoplasm of the cells are filled with deeply stained colloid droplets. The acinar colloid content is much less compared to that of normal. It is comparatively thin and shows increased vacuolization. At certain places it is practically replaced by the hyperplastic epithelial cells. Vascularization of the gland is very conspicuous and engorged blood vessels are visible.

Atrophic nature of the thyroid is the prominent feature in the thyroxin treated group and points towards the inhibitory influence of the hormone on the gland (Plate IA, Fig. 4). The follicles are distended with deeply stained colloid which is devoid of vacuoles and the lining epithelia are flattened, showing no colloid droplets in the cytoplasm. At certain places, the cytoplasm of the cells is hardly noticeable. The nuclei are small, deeply stained and have become flattened. The mean acinar cell height is 4.1 ± 0.09 μ (Table 1). The vascularity of the gland is poor.

Appearance of the thyroid in thyroxin plus low androgen treated animals resembles closely that of thyroxin
(All the figures are photomicrographs. X 560)

FIG. 1.— A section through the thyroid of a normal rat.

FIG. 2.— A section through the thyroid of testosterone propionate (low dose) treated rat. Note the changes in the acinar cells and acinar colloid content. Compare with Fig. 1.

FIG. 3.— A section through the thyroid of testosterone propionate (high dose) treated rat. Note the marked hyperplastic changes in the acinar cells. Compare with Figs. 1 & 2.
(All the figures are photomicrographs. X 560)

FIG. 4.— A section through the thyroid of thyroxine treated rats. Note the distended follicles and the atrophic changes in their lining cells. Compare with Fig. 1.

FIG. 5.— A section through the thyroid of thyroxine plus testosterone propionate (low dose) treated rat. Note the atrophic changes in the acinar cells and the distension of follicles. Compare with Figs. 1 & 4.

FIG. 6.— A section through the thyroid of thyroxine plus testosterone propionate (high dose) treated rat. Note the atrophic changes in the follicles. Compare with Figs. 1 & 4.
treated glands. (Plate IA, Fig. 5). Dense colloid dis-
tends the follicles, the lining cells of which are flatten-
ed and the cytoplasm of the cells is not discernible at
certain places. The nuclei are small, flattened and deeply
stained. The mean height of the acinar cells is 4.3 ± 0.07μ
(Table 1). Vascularization of the gland is poor.

The histological picture of the thyroid in thyroxine
plus high androgen treated group is more or less of the
similar nature as is seen in the previous two groups
(Plate IA, Fig. 6). The mean acinar cell height is 4.8 ±
0.08 μ (Table 1).

Testis.— Observation of the testis weight and
histology shows that it responded differentially to low and
high dose of testosterone. The atrophy of the seminiferous
epithelium with marked disorganization of the cellular
elements and arrest of spermatogenesis are prominent features
of the testis of rats treated with low dose of androgen.
On the other hand, an overall stimulation of spermatogene-
sis is evident in the testis of rats treated with high dose
of testosterone propionate. The observations corroborate
the previous findings of Kar and Roy (1955).

Discussion

The results of the present study reveal that testos-
terone propionate exerts a stimulating influence on the
thyroid in both low and high doses. The histological picture and the micro-histometric data on the acinar cell height clearly indicate that the glands are in hyperactive stage. The degree of stimulation of the glands, as reflected by acinar cell height and hyperplastic changes, is, however, considerably marked in the group treated with high dose of androgen.

From the findings on testis it will be clear that the organ responded differentially to the low and high doses of androgen and this observation is in accordance with those by other workers (Moore and Price, 1932, 1937, 1938; Shay et al., 1941; Selye and Friedman, 1941).

It is evident from the above that low dose of androgen stimulated thyroid activity inspite of its depressant effect on the testis. As this inhibitory effect on the testis is due to reduction of gonadotrophin secretion from the pituitary (Moore, 1930; Bottomley and Folley, 1938a), it may be concluded that androgen in low dose stimulated the thyroid quite independent of hypophyseal gonadotrophin. In view of this finding it would not be unreasonable to presume that in the higher dose level also, such an independent influence is exerted causing stimulation of the thyroid gland. But it cannot be said whether this is the only mode of action of testosterone in the higher dose level, for at this dose level there occurs stimulation of the testis also. This testicular stimulation might be due to augmentation of pituitary gonadotrophin output, as it is known that androgen in higher doses causes increased secretion of FSH (Hebrews...
Comparable observation has been made in female rats too, by Nathanson et al. (1938), who has noted that testosterone causes ovarian stimulation through the mediation of pituitary. Gonadotrophin in its turn has got a thyroid stimulating action (Riddle et al., 1933; Fluhmann, 1934) and this effect is believed to be exerted through an enhancement of TTH production by the adenohypophysis (Kar et al., 1955a). It would not, however, be illogical to presume that the enhanced hypophyseal gonadotrophin output may have a contributory effect to the "independent" thyroid stimulating action of the androgen at this higher dose level, by increasing the overall TTH secretion from the pituitary.

The results of the combined thyroxine plus androgen treatment clearly show that thyroxine inhibits the thyroid stimulating effect of testosterone propionate. The extent of such inhibition, however, appears to be quite marked as the histological picture of the thyroid and the microhistometric data on the acinar cell height in the combined treated groups closely resemble those of the animals treated with thyroxine alone. Besides, it would appear that, in the presence of thyroxine, testosterone could not much alter, by its stimulating influence, the adverse changes in the thyroid produced by the former (Plate IA, Fig. 6). Though, it is noted that in the high-testoid group, the acinar cell height is slightly greater than that of thyroxine treated group, the overall atrophic changes are almost similar in the two groups.
It is now well established that thyroxine depresses the function of thyroid mainly by blocking the elaboration of TTH (Salter, 1950). Maqsood (1952) has pointed out that thyroxine when administered in a dose equal to what is secreted in a normal animal, blocks the normal TTH secretion from the pituitary. Bartly and Leblond (1954) ascertained that the physiological dose of thyroxine in young adult rats weighing about 150 gms. is between 3 µg. to 6 µg. daily. The dosage employed in the present investigation was, therefore, much higher than the physiological amount, specially considering the fact that the animals used in this experiment were of considerably lesser weight than those by Bartly and Leblond (1954). So this dose of thyroxine is expected to inhibit the action of much greater amount of pituitary TTH than normal. Thyroxine exerts no direct inhibitory effect on the thyroid as is revealed by the experiment of Aron et al. (1947), where introduction of crystalline thyroxine into the thyroid is shown to produce no adverse effect on the gland. In the light of the above observations, it may be concluded that thyroxine prevents the thyroid stimulating effect of testosterone propionate by its inhibitory influence on the thyrotrophic hormone of the pituitary.

This finding furnishes evidence to the theory that testosterone influences the thyroid ultimately through enhanced output of pituitary thyrotrophic hormone. Otherwise, had testosterone any direct influence on the thyroid,
Summary

Intramuscular injections of testosterone propionate, in a low dose which causes depression of testicular activity as well as in a high dose which stimulates the testis cause a stimulation of the thyroid in young male rats. This thyroid stimulating action of testosterone is exerted, mainly, independent of hypophyseal gonadotrophin secretion. Simultaneous administration of thyroxin blocks the thyroid stimulating action of the androgen, presumably by its inhibitory effect on the thyrotrophic hormone of the pituitary. It is concluded that testosterone has got no direct stimulating action on the thyroid gland; it stimulates the thyroid through increased elaboration of thyrotrophic hormone of the pituitary.