CHAPTER II

EFFECT OF TESTOSTERONE PROPIONATE ON THE THYROID GLAND OF THIOUREA TREATED MALE RATS.
It has been reported that testosterone stimulates the thyroid gland of normal animals. This stimulation is brought about presumably by an enhancement of TTH secretion from the pituitary and the androgen seems to have no direct stimulating action on this gland (Chapter I, Part I). It has also been claimed by certain authors that testosterone exerts a beneficial influence on the thyroid of goitrogen treated animals. Voitkevich (1950) reported that methyl testosterone caused a considerable decrease of hypertrophy of the thyroid and prevented the complete loss of activity of the gland of thiouracil treated guinea-pigs and cocks. Kar and Sur (1953) also noted that the typical hyperplastic changes in the thyroid of thiourea treated rats were prevented by simultaneous treatment with testosterone propionate. On the other hand, Leathem (1947) observed that testosterone propionate had no effect on the thyroid hypertrophy of thiourea treated rats. Segaloff (1944) and Leathem (1948) failed to note any change in the weight of the thyroid of thiouracil treated rats after androgen administration.

It is seen from the above that the same anomaly regarding action of testosterone on the thyroid of normal rats has crept in the studies concerned with the thyroid’s response to testosterone in goitrogen treated rats. The true nature and mechanism of influence of the testoid on the thyroid in goitrogen treated animals is also not clear. As the conditions, under which testosterone has to act, are
expected to be different in the normal and thiourea treated animals, it was thought worthwhile to reinvestigate the above problem in a more detailed manner.

Thiourea was administered to rats for varying periods with the idea of producing different degrees of thyroid insufficiency. Testosterone propionate was administered to such rats and the response of the thyroid was studied.

**Experimental Procedure**

The present study comprises of three separate experiments.

**Experiment 1.** — Twenty four rats weighing 59.7 ± 2.8 gms. were divided into three groups of 8 each. To group B, thiourea (dissolved in 0.4 c.c. distilled water) was administered for 21 days. The dose of thiourea was 4 mg. per rat daily, to start with; it was gradually raised to 10 mg. within 5 days and the maximum dose was maintained for the rest of the experimental period. Group C, in addition to thiourea in the above dose schedule, received simultaneous treatment with testosterone propionate (4 mg. in 0.2 c.c. of sesame oil per rat on alternate days). The group A, served as a control and only the diluents were injected to them.

The animals were sacrificed on the day following the last treatment. The thyroid glands were carefully
dissected out, weighed and suitably fixed for histological
studies.

Experiment 2. — Twenty seven rats weighing 52.0 ±
5.9 gms. were divided into three groups of 9 each. To
groups B and C, thiourea was administered daily for 38 days.
The dose of thiourea was gradually raised from 4 mg. to
8 mg. within the period of first 14 days, subsequently the
maximum dose was maintained for the rest of the experimental
period. The animals of group C received, in addition,
injections of testosterone propionate (6 mg. in 0.2 c.c. of
sterile sesame oil per rat, on alternate days) from the
19th day of thiourea treatment. Ten such injections were
given over the period of 19 days. Group A served as the
control, receiving only the diluents.

The rest of the experimental procedures were the
same as those of the previous one.

Experiment 3. — Twenty-four rats weighing 40.0 ±
4 gms. were divided into three groups of 8 each. Groups B
and C received subcutaneous injections of thiourea (in 0.5
c.c. of distilled water) for 88 days. The dose of the
toiterogen was gradually increased from 4 mg. to 20 mg.
within the period of first 20 days and the maximum dose
was maintained for the rest of the experimental period.
The animals of group C received, in addition, injections
of testosterone (8 mg. in 0.25 c.c. of sterile sesame oil,
on alternate days) from the 66th day of thiourea treatment.
Altogether twelve injections were given in 23 days. Group A
served as a control and received the injections of the diluents.

The remaining procedures were the same as those of the other two experiments.

**Results**

The data on thyroid weight and body weight of the animals in the three experiments are shown in Table 2. For convenience, the results of each experiment are described separately.

**Experiment 1.** It will be seen that the weight of the thyroid is increased following thiourea administration. The weight of the gland in the combined treated group does not show any appreciable difference from that of the goitrogen treated ones.

Histological picture of the thyroid of the animals in the control group presents a normal appearance (Plate II, 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.

Microscopical examination of the thyroid in the thiourea treated animals reveals considerable changes in the gland (Plate II, Fig. 2). The acinar cells show marked hypertrophy and some hyperplasia. The acinar colloid is lesser than that of the controls and it is devoid of
Table 2. — The thyroid weight and body weight of the control, thiourea treated and thiourea plus testosterone propionate treated animals.

<table>
<thead>
<tr>
<th>Group and treatment</th>
<th>Mean body weight (gm.)</th>
<th>Mean thyroid weight (mgm.) with S.E.</th>
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<td></td>
<td>Initial</td>
<td>Final</td>
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<tr>
<td>Experiment 1:</td>
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<tr>
<td>A. Control</td>
<td>60.2</td>
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<tr>
<td>B. Thiourea</td>
<td>60.2</td>
<td>79.5</td>
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<td>C. Thiourea plus</td>
<td>59.1</td>
<td>73.5</td>
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<td>testosterone propionate</td>
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<td>Experiment 2:</td>
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<tr>
<td>A. Control</td>
<td>49.7</td>
<td>97.9</td>
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<td>89.0</td>
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<td>C. Thiourea plus</td>
<td>56.8</td>
<td>98.0</td>
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<td>Experiment 3:</td>
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<tr>
<td>A. Control</td>
<td>40.1</td>
<td>147.7</td>
</tr>
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<td>B. Thiourea</td>
<td>40.0</td>
<td>125.5</td>
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<td>C. Thiourea plus</td>
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<td>120.8</td>
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<tr>
<td>testosterone propionate</td>
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Analysis (Thyroid weight)

Experiment 1:
- Group A vs. group B: \( t = 6.20; P < .001 \) (Significant)
- Group A vs. group C: \( t = 9.10; P < .001 \) (Significant)
- Group B vs. group C: \( t = 1.81; P < .1 & > .05 \) (Insignificant)

Experiment 2:
- Group A vs. group B: \( t = 8.86; P < .001 \) (Significant)
- Group A vs. group C: \( t = 3.92; P < .01 & > .001 \) (Significant)
- Group B vs. group C: \( t = 6.06; P < .001 \) (Significant)

Experiment 3:
- Group A vs. group B: \( t = 3.83; P < .01 & > .001 \) (Significant)
- Group A vs. group C: \( t = 3.83; P < .01 & > .001 \) (Significant)
- Group B vs. group C: \( t = 5.04; P < .001 \) (Significant)
vacuoles. The vascularity of the gland is increased.

The histological picture of the thyroid in the combined treated group is more or less similar to that of the thiourea treated ones except that the hyperplasia of the cells seems to be somewhat marked in this case (Plate II, Fig. 3).

**Experiment 2.** — The weight of the thyroid is increased in the hypothyroid rats. In the hormone treated group it is significantly decreased in comparison to that of goitrogen treated animals. Compared to normal values it is slightly heavier.

The thyroid in the control animals presents a normal histological picture. Considerable hypertrophy and hyperplasia of the acinar cells are observed in the thyroid of thiourea treated animals (Plate IIA, Fig. 4). The acinar colloid is lesser than that of the controls. Vascularity of the gland is increased.

In the combined treated group the hypertrophic and hyperplastic changes in the acinar cells of the thyroid, as noted in the thiourea treated animals are absent (Plate IIA, Fig. 5). The picture of the gland is more or less similar to that of the controls. The acinar colloid content is more or less similar to that of the controls.

**Experiment 3.** — The weight of the thyroids in the thiourea treated animals like that in the previous experiments is increased (Table 2). The weight of the thyroids
PLATE II
FIG. 1.—A section through the thyroid of a normal rat.

FIG. 2.—A section through the thyroid of thiourea treated rat of 'Experiment I'. Note the hyperplastic changes in the acinar cells and the acinar colloid content. Compare with Fig. 1.

FIG. 3.—A section through the thyroid of thiourea plus testosterone propionate treated rat of 'Experiment I'. Note the hyperplastic changes in the acinar cells and the acinar colloid content. Compare with Figs. 1 & 2.
(All the figures are photomicrographs. X 230)

FIG. 4.— A section through the thyroid of thiourea treated rat of 'Experiment 2'. Note the hyperplastic changes in the acinar cells and the acinar colloid content. Compare with Fig. 1.

FIG. 5.— A section through the thyroid of thiourea plus testosterone propionate treated rat of 'Experiment 2'. Note the absence of the hyperplastic change and the acinar colloid. Compare with Figs. 4 & 1.

FIG. 6.— A section through the thyroid of thiourea treated rat of 'Experiment 3'. Note the marked hyperplasia of the acinar cells resulting in squeezing out of acinar colloid. Compare with Figs. 1, 2 & 4.

FIG. 7.— A section through the thyroid of thiourea plus testosterone propionate treated rat of 'Experiment 3'. Note the marked atrophy of the acinar cells and the acinar colloid. Compare with Figs. 6 & 1.
in the hormone treated animals is, however, decreased even below the normal level. But the difference from the normal fails to be statistically significant.

Histological examination reveals very marked hyper trophy and hyperplasia of the acinar cells of the thyroid in the goiterogen treated animals (Plate IIA, Fig. 6). At some places no colloid is noticeable in the follicular space and is practically replaced by the hypertrophic and proliferating acinar cells. Vascularity of the gland is very much increased.

The picture of the thyroid in the combined treated group shows marked atrophy of the gland (Plate IIA, Fig. 7). The acinar cells are flattened and are hardly discernible at some places. The nuclei are flattened and deeply stained. The acini are distended with colloid which is devoid of any vacuole. Vascularity of the gland is very poor.

Discussion

It is well-known that thiourea depresses the thyroxine secretion probably by inhibiting the peroxidase activity of the thyroid (see Williams, 1955) and the TTH output of the pituitary is increased as a compensatory mechanism producing hypertrophy and hyperplasia of the thyroid gland. The probable ways by which the testoid can influence the activity of such thyroid can be considered
along the following lines. Testosterone has been shown to stimulate the normal thyroid gland (Selye, 1939; Mathanson et al., 1940; Money, 1950; Castellani, 1952c; Burris et al., 1953) and this is due to augmentation TTH output of the pituitary (Chapter I, Part I). That such a stimulation of the thyroid gland is associated with an increase in the thyroid hormone secretion becomes relevant from the fact that testosterone elevates B.M.R. (Thompson, 1941; Kenyon, 1942). Due to this ability of the testoid to enhance the elaboration of TTH from the pituitary, there will be a tendency for it to increase the overall TTH secretion in the thiourea treated animal. Thyrotrophic hormone, in its turn, has been shown (Bastenie and Kowaleski, 1950) to increase the oxygen consumption of the thyroid in the thiourea treated animals, though to a lesser extent than it can do in normal ones. Moreover, it has also been demonstrated that TTH increases the peroxidase activity of the thyroid gland (Villamil and Mancini, 1947). From these observations it seems possible that testosterone might improve the secretory activity of the thyroid of goiterogen treated animals by increasing the TTH secretion from the pituitary. In that case, an enhanced secretion of thyroid hormone will tend to inhibit the output of TTH, which was previously geared up as a result of lack of the thyroid hormone induced by thiourea administration.

It is not known whether testosterone can influence directly the peroxidase activity of the thyroid gland. If
it possessed any such property it could be expected to counteract the action of thiourea and prevent any hyperplastic change caused by the latter.

It is pertinent to note that testosterone has been reported to stimulate the adrenal cortex (Rennels et al., 1953; Castellani, 1952b; Roy et al., 1957). The adrenal cortical hormone has been shown to inhibit the thyroid gland producing depression of the acinar epithelium with corresponding rise in the acinar colloid content (Marthi and Rasanen, 1954). Such depressing action of corticoid on the thyroid activity has been reported by other investigators as well (Anapol, 1950; Money et al., 1950; Hill et al., 1960; Wolfson et al., 1950; Fredrickson et al., 1952 and others). It may, therefore, be commented that any increased adrenocortical activity induced by testosterone is likely to influence the thyroid.

From the present data it is seen that the administration of thiourea invariably causes an increase in the weight of the thyroid and produces hypertrophy and hyperplasia of the acinar cells. The changes induced by testosterone propionate are, however, different in the three experiments. In experiment 1, there is no significant difference in the weight of the thyroids in thiourea treated and combined treated animals. From the histological study of the thyroids it appears that following simultaneous treatment with testosterone the hyperplastic changes in the thyroid are somewhat increased. This tendency of increasing the thyroid
hyperplasia can be explained by the ability of testosterone to augment the TTH secretion from the pituitary. The absence of any significant change in the weight or histology of the thyroid is probably due to the simultaneous suppressing action of the increasingly secreted thyroid hormone and of the hypertrophied adrenal gland, which was caused by the testoid influence.

In experiment 2, the weight of the thyroid is diminished and the hyperplastic changes in the thyroid are absent following treatment with testosterone and the thyroid presents more or less a normal picture. This change in the thyroid is difficult to explain. The improved hormonal secretion of the thyroid of the goitrogen treated animals under the androgen influence may depress the hypophyseal TTH secretion and thus tend to diminish the hyperplastic changes in the thyroid. However, it is to be considered whether it would be possible for the goitrogen treated thyroid to secrete such an amount of thyroid hormone in the absence of any hyperplastic change, in case the androgen has no direct stimulating effect on the peroxidase activity of the gland. From the results of the third experiment it appears that testosterone has no such direct effect (vide infra). It is noteworthy, however, that the adrenal glands of the hormone treated animals were very much hypertrophied**

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*Mean adrenal weight (mgm.) with S.E. { Group B : 16.0 ± 0.76, Group C : 22.1 ± 1.07.

**Mean adrenal weight (mgm.) with S.E. { Group B : 16.3 ± 0.47, Group C : 28.0 ± 0.91.
Probably the increased secretion of the corticoid hormone from the hypertrophied adrenals plays an important part in depressing the thyroid hyperplasia. Such change was not observed in the first experiment, probably because the dose of testosterone was low and the adrenal enlargement was not marked in that case.

In experiment 3, the weight of the thyroid in the hormone treated animals is very much diminished and the histological examination reveals marked atrophy of the acinar cells. In this experiment where the goitrogen was administered in higher doses and for longer period, the thyroid showed very marked hyperplastic changes. Further stimulation by testosterone probably leads to exhaustion with eventual atrophy of the gland. Of course, the possibility of exhaustion at the pituitary level cannot be ruled out. Had the testoid any direct beneficial effect on the peroxidase activity of the thyroid, the gland should not have atrophied.

Any probable suppressing influence of the cortical hormone cannot be thought of in this case as the adrenals were not hypertrophied in comparison to those of the controls.

*Mean adrenal weight (mgm.) with S.E.

<table>
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<tr>
<th>Group</th>
<th>Weight</th>
<th>S.E.</th>
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<td>A</td>
<td>22.7</td>
<td>1.05</td>
</tr>
<tr>
<td>B</td>
<td>17.5</td>
<td>0.75</td>
</tr>
<tr>
<td>C</td>
<td>23.4</td>
<td>1.21</td>
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Summary

Administration of thiourea causes an increase in the weight of the thyroid gland and produces hypertrophy and hyperplasia of the acinar cells, the degree of hyperplasia varying roughly with the duration of treatment. Testosterone administration produces varied changes in such thyroids. Various factors like, duration of thiourea administration, stage of thyroid hyperplasia at which the hormone therapy is instituted, the dose of testosterone and the state of adrenocortical activity as influenced by the testoid, probably determine the ultimate response of the thyroid of goitrogen treated rats to testosterone treatment.
Data presented in Part I show that testosterone propionate stimulates the thyroid gland of rats irrespective of the dosage level used in this study (Chapter I). Indirect evidence has been provided to indicate that the androgen stimulates the thyroid by augmenting the secretion of thyrotrophic hormone of the pituitary. Such a stimulation seems to be independent of gonadotrophic activity of the pituitary. Testosterone has got no direct stimulating action on the thyroid.

The stimulating influence of testosterone propionate in normal animals prompted us to study the effect of the androgen on the thyroid of goitrogen treated rats (Chapter II). This condition is characterized by an increased outpouring of pituitary TTH due to a suppression of thyroxine production. It is interesting that under this pathological (experimentally produced) condition the nature of response of the thyroid to testosterone propionate is determined by a number of variable factors, which have hitherto not been recognised. The androgen caused either (i) no appreciable change, or (ii) an alleviation of hyperplastic picture, or (iii) a pronounced atrophy of the acinar cells. These differential responses of the thyroid probably depend upon factors like the duration of goitrogen administration, the extent of thyroid hyperplasia at...
the time when testosterone administration is instituted, the dose of the hormone and the functional status of the adrenal cortex as influenced by the androgen. Indirect evidence suggests that testosterone has no direct stimulating influence on the enzyme peroxidase of the thyroid, which is believed to be depressed by thiourea. One is therefore, led to the inevitable conclusion that, as in normal animals, the major pathway of influence of testosterone propionate on the thyroid of goitrogen treated rats is through the TSH activity of the pituitary.

Hitherto, most of the work relating to the influence of androgens on the thyroid were of an isolated nature in the sense that thyroid was considered per se and not as a possible link in the chain of interactions which might contribute to shaping the overall effects of androgens on the thyroid—adrenal—pituitary axes. To our knowledge no work, comparable to that presented here, has been reported in the literature where thyroid, adrenal cortex and pituitary have been implicated simultaneously and relevant data deduced both from normal (also see Chapter I, Part II) and pathological (experimentally produced) conditions. The experiments on thyroid have tended to establish clearly that testosterone propionate does have a stimulating effect on the thyroid.

The bearing of the above conclusion on the influence of testosterone propionate on the adrenal cortex will be dealt with in Part II.