PART II
ADRENAL CORTEX

INFLUENCE OF ANDROGEN ON THE ADRENAL CORTEX. ELUCIDATION OF THE PRECISE NATURE AND MECHANISM OF ACTION.
CHAPTER I

EFFECT OF TESTOSTERONE PROPIONATE ON THE ADRENAL CORTEX OF RATS—THE POSSIBLE ROLE OF THE THYROID IN THE RESPONSE OF THE ADRENAL CORTEX TO TESTOSTERONE.
Chapter I 'A'

Effect of Testosterone Propionate on the Adrenal Cortex of Young Male Rats.
The varied responses of the adrenal cortex to androgen treatment have been reviewed in detail (see Review of Literature). Majority of the investigators, however, reported an inhibitory effect of the androgen on the adrenal cortex and this has contributed largely to the general belief that androgen depresses the elaboration and secretion of ACTH from the pituitary. There are, however, a few observations which furnish direct or indirect evidences to show that the androgen may have a stimulating influence on the adrenal cortex. Thus, Selye (1941) and Rennels et al. (1953) observed adrenocortical stimulation in rats and Castellani (1952b) in guinea-pigs following testosterone administration. Various explanations have been given for such stimulation. Selye (1941) ascribed this to the non-specific damaging action of the hormone, while Castellani (1952b) attributed this effect to the potassium retaining property and general anabolic effects of testosterone.

It has been shown that testosterone exerts a stimulating action on the thyroid (see Part I). That such an effect results in an enhanced rate of secretion of the thyroid hormone is also suggested by the increase in B.M.R. after testosterone administration (Thompson, 1941; Kenyon, 1942). The thyroid hormone is known to stimulate the adrenal cortex by augmenting pituitary ACTH output (Smith, 1930; Eartly and Leblond, 1954). In view of these observations, it seemed of interest to study whether the thyroid stimulating action of testosterone plays any role in determining
the nature of response of the adrenal cortex to testosterone.

To clinch this issue in a satisfactory manner, it is mandatory that the dosage of testosterone propionate has to be chosen on the basis of its ability to stimulate the thyroid. Accordingly, a dosage of 6 mg. of testosterone propionate every alternate day was used in the present experiment, as this was previously noted (Chapter I, Part I) to have the desired effect on the thyroid. Besides, because of the alleged action of testosterone on the potassium metabolism and the body weight, which in their turn may influence the adrenal gland, the serum potassium level and the body weight changes were also considered when evaluating the effect of this particular dose of the hormone on the adrenal cortex.

**Experimental Procedure**

Eighteen rats, weighing 40.1 ± 3.6 gms., were divided into two groups of 9 each. One group was injected with testosterone propionate (6 mg., dissolved in 0.25 c.c. of sesame oil, per rat on alternate days). The other group served as a control and received the solvent alone. In all, ten injections were given over a period of 19 days. The initial and terminal body weights of the rats were recorded.

The animals were sacrificed on the day following the last injection. The blood was collected carefully avoiding
haemolysis and the potassium content of the serum was determined. The adrenals were dissected out and weighed after removing the adhering fat. The left adrenals were processed for the determination of ascorbic acid and the right ones for the total cholesterol content. Six adrenals were allotted for each biochemical estimation. The rest of the adrenals and a few thyroid glands were suitably fixed for histological examinations.

Results

Table 3 shows the weight (both absolute and relative), ascorbic acid and cholesterol contents of the adrenals of control and testosterone treated rats. The body weight of the animals and the serum potassium content are also included in the same table. It will be seen that testosterone administration causes a significant increase in the weight (both absolute and relative) of the glands. The adrenal ascorbic acid and cholesterol contents record a significant fall and so also the serum potassium level. The body weight changes of the animal are, however, virtually the same in the experimental and the control groups.

Histological examination of the adrenal in the control group (Plate III, Fig. 1) shows normal appearance of the cortex and three zones are clearly distinguishable. The parenchymal cells present normal morphology. The cyto-
Table 3.—The weight, ascorbic acid and cholesterol content of the adrenals and the potassium content of the serum in control and testosterone treated animals.

<table>
<thead>
<tr>
<th>Group and Treatment</th>
<th>Mean body weight (gm.)</th>
<th>Mean adrenal weight (mg.)</th>
<th>Mean ascorbic acid content with S.E. (mg./100 gm. adrenal tissue)</th>
<th>Mean cholesterol content of adrenal tissue (mg./gm. of adrenal tissue) c.c.</th>
<th>Mean potassium content of serum (mg./100 gm. of body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Control</td>
<td>40.0</td>
<td>62.1</td>
<td>12.9 ± 0.46</td>
<td>466.0 ± 21.6</td>
<td>17.8 ± 1.20</td>
</tr>
<tr>
<td></td>
<td>*21.2 ± 0.67</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. Testosterone propionate treated</td>
<td>40.2</td>
<td>62.9</td>
<td>23.4 ± 0.67</td>
<td>309.0 ± 9.2</td>
<td>11.4 ± 1.21</td>
</tr>
<tr>
<td></td>
<td>*37.0 ± 1.06</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
FIG. 1. — Photomicrograph of a section through the adrenal of a normal rat. Note the different zones of the normal cortex. (X 70)

FIG. 2. — Photomicrograph of a section through the adrenal of testosterone propionate treated rat. Note the hypertrophy of the cortex and the fatty metaplasia of the cells of inner fasciculata and zona reticularis. Compare with Fig. 1. (X 70)

FIG. 3. — Photomicrograph of the same section as in Fig. 2 with greater magnification (X 230) to show the inner cortical zones. Note the appearance of fat vacuoles in the cytoplasm of cortical cells, and the eccentric position of the nuclei.
plasm of the cells of the fasciculata and reticularis shows the usual lipid vacuoles. Sinusoids are noticeable in between the columns of cortical cells. Medullary cells also present a normal appearance.

The adrenals in testosterone-treated group (Plate III, Fig. 2) reveal considerable stimulation of the cortex. Some hypertrophy is noticeable in the cells of glomerular zone. The zona fasciculata, however, shows marked hypertrophy and hyperplasia. Mitotic figures are relatively more abundant than in the controls and some of the cells are binucleated. Many of the cells of inner fasciculata and zona reticularis show "fatty metaplasia" (Plate III, Figs. 2 and 3). Fat vacuoles are seen in the cytoplasm of these cells and the nuclei are pushed to one side giving the cells a typical 'signet-ring' appearance. Increased vascularity of the cortex is evident. Medullary cells present normal appearance. Blood vessels of medulla look engorged.

Discussion

The data presented in this study show that the adrenal gland increases significantly in weight following testosterone treatment. There is also depletion of ascorbic acid and cholesterol which signifies a stimulation of the gland. Histological observations support this view and indicate that such stimulation is confined to the cortical portion.
of the gland.

A stimulation of adrenal gland in guinea-pigs following testosterone treatment was observed by Castellani (1952b). He attributed this change to the general anabolic action and potassium retaining property of testosterone. Selye (1941) also obtained such hypertrophy of the rat adrenal with a massive dose of testosterone and suggested that this might be due to a non-specific damaging action of the hormone. Variations in body weight gain between control and hormone injected animals provide a simple criterion by which the possible damaging action of steroid hormone might be estimated (Greep and Jones, 1950a). In the present study, the body weight gain of the testosterone-treated animals is virtually the same as that of the controls. The serum potassium content is not raised but on the contrary it is significantly lowered. It is interesting that Butler et al. (1942) reported a similar fall in serum potassium level in patients treated with methyl testosterone. The hypertrophy of the adrenal gland observed in the present experiment cannot, therefore, be attributed to any of the above factors.

Roy et al. (1956) observed a stimulation of the thyroid in young rats following androgen treatment at the dosage employed in the present study. A somewhat similar stimulation of the thyroid was also noticed in the present experiment. It has been reported previously that thyroid hormone can exert a stimulating influence on the adrenal cortex (McQueen-Williams, 1934; Hartman and Brownell, 1949).
Abelin, 1944; Dean and Greep, 1947; Kar et al., 1955b; and others) and this is mediated through the pituitary (Smith, 1930; Eartly and Leblond, 1954). This interesting association between the activity of the two glands in response to testosterone treatment suggests that the adrenocortical stimulation might be dependent on a prior stimulation of the thyroid. However, other possible mechanisms should also be considered.

It has been claimed that testosterone exerts a direct stimulating action on the adrenal (Cutuly et al., 1938; Leonard, 1942; Zizine et al., 1950; and others). However, Lewis et al. (1949) reported on the contrary. The problem was critically reviewed by Rennels et al. (1953), who found that testosterone had no significant effect on the relative adrenal weight of hypophysectomized rats. When, however, the absolute weight of the gland was taken into consideration, the authors noticed a slight maintenance effect of testosterone on the (diminishing) weight of the adrenal and ascribed it to be due to the tendency of androgen to prevent the loss in body weight of the animals seen after hypophysectomy. It may be recalled that the increase in adrenal weight following testosterone treatment in the present study is not only absolute, but also relative. The changes in the weight of animals are more or less same in both the groups. In view of these, one is inclined to believe that the direct effect of androgen does not play a very signifi-
It follows from the above that the androgen stimulated the adrenal cortex mainly through indirect means, presumably through augmentation of ACTH secretion from the pituitary. The observations such as the increase in size of the adrenal, depletion of adrenal constituents and hypertrophy of the cortex bear eloquent testimony to an enhanced release of pituitary ACTH in response to androgen treatment. However, it remains to be seen whether such enhancement of ACTH secretion is brought about through thyroid hyperactivation or by a direct action of testosterone on the adeno-hypophysis without the mediation of the thyroid.

Another interesting observation of the present study is the occurrence of fatty metaplasia of the cells of the adrenal cortex of rats treated with testosterone propionate. This will be referred to again and discussed in a detailed manner (see Chapters I'C' & III, Part II).

Summary

Administration of testosterone propionate to young male rats in a dose which stimulates the thyroid causes adrenocortical hypertrophy along with fatty metaplasia of the cortical cells. The ascorbic acid as well as the total cholesterol content of the adrenal are depleted following the hormone treatment. All these findings denote a stimula-
tion of the adrenocortical activity by testosterone. The probable mechanisms of such influence of testosterone on the adrenal cortex are discussed. Any possible role of the thyroid in such androgen action requires further elucidation.
Chapter I 'B'

Effect of Testosterone Propionate on the Adrenal Cortex of Thiourea-Induced Hypothyroid Rats.
It will be evident from the results of the foregone study (Chapter I 'A', Part II) that testosterone propionate caused a stimulation of the adrenocortical activity of young male rats. This was also accompanied by a hypertrophy of the thyroid gland as per expectation. It is well-known that thyroxine stimulates the adrenal cortex through the pituitary (Smith, 1930; Eartly and Leblond, 1954). It was, therefore, suggested that the adrenocortical stimulation by testosterone is due to increased hypophyseal ACTH secretion, which might have been evoked through thyroid hyperactivation. Alternately, testosterone might act per se on the pituitary causing increased elaboration of ACTH without the mediation of the thyroid gland. In view of these considerations, it seemed mandatory to study whether testosterone influenced the adrenocortical activity in animals which have been rendered hypothyroid by goitrogen treatment.

The present study, therefore, gives an account of the changes elicited in the adrenal cortex of young male rats at different degrees of thiourea-induced hypothyroidism and of the influence of testosterone propionate on such changes.

**Experimental Procedure**

Hypothyroidism was induced by the administration of thiourea to rats for shorter and longer durations with an idea of producing different degrees of thyroid insufficiency.
Short term experiment. Twenty-seven rats weighing 52.0 ± 5.9 gms. were divided into three groups of 9 each. The animals of groups B and C were injected daily with 0.4 c.c. of an aqueous solution of thiourea. The dose of thiourea was gradually raised from 4 mg. to 8 mg. per rat daily, within the period of the first 14 days. The maximum dose was maintained subsequently up to the 38th day. 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. Altogether 10 such injections were given in a period of 19 days. The group B received similar treatment with oil alone. The animals of group A served as controls and were injected with the solvents only.

The animals were sacrificed on the 39th day. The adrenals were dissected out, freed from adhering fat and weighed in a microbalance. The left adrenals were processed for the determination of ascorbic acid and the right ones for the total cholesterol content. Six adrenals were allotted for each biochemical estimation. The rest of the adrenals along with the thyroid glands were suitably fixed for histological studies.

Long term experiment. Twenty-four rats weighing 40.0 ± 4.0 gm. were divided as before into three equal groups. To groups B and C thiourea was administered for 86 days. The dose of the goitrogen was gradually raised from 4 mg. to 20 mg. per rat, daily within a period of the
first 20 days and the latter dose level was maintained subsequently. Group C received injections of testosterone propionate (8 mg. per rat on alternate days) from the 65th day of thiourea treatment. Altogether 12 injections were given in a period of 23 days. The group A served as a control. The other associated procedures were identical with those of the short-term experiment.

The animals were killed on the 89th day and processed as before.

Results

The data on the weight, ascorbic acid and total cholesterol contents of the adrenals in the short-term experiment are presented in Table 4. It will be seen that thiourea treatment causes a reduction in the weight of the adrenal gland. The change, however, fails to be statistically significant when the weights of the glands are expressed in terms of the body weights of the respective animals. A reduction in the adrenal ascorbic acid concentration is observed following the development of hypothyroidism. The cholesterol content of the gland of the hypothyroid animals is, however, raised above that of the normal level.

In the testosterone treated group, the adrenals are much heavier than those of either the control or the thiourea treated group. The adrenal ascorbic acid content of
Table 4. The weight, the ascorbic acid and total cholesterol contents of the adrenals in the short-term experiment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean body weight (gm.)</th>
<th>Mean adrenal weight (gm.)</th>
<th>Mean ascorbic acid (mg./100 gm. of tissue)</th>
<th>Mean cholesterol (mg./100 gm. of tissue)</th>
<th>S.E. with S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, Control</td>
<td></td>
<td>49.7</td>
<td>97.9</td>
<td>19.7 ± 0.49</td>
<td>467.5 ± 14.0</td>
<td>15.7 ± 0.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*19.4 ± 0.87</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B, Thiourea</td>
<td></td>
<td>50.2</td>
<td>89.0</td>
<td>16.3 ± 0.47</td>
<td>255.7 ± 22.3</td>
<td>20.0 ± 0.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*18.1 ± 0.70</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C, Thiourea</td>
<td>plus test-</td>
<td>56.8</td>
<td>98.0</td>
<td>28.0 ± 0.91</td>
<td>225.7 ± 13.1</td>
<td>15.9 ± 0.39</td>
</tr>
<tr>
<td></td>
<td>tosterone</td>
<td></td>
<td></td>
<td>*32.1 ± 1.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>propionate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Relative adrenal weight (mgm./100 gm. of body wt.)

Analysis

Adrenal weight:

(a) Absolute

Group A vs. group B \(-t = 5.074; \ P < .001\) (Significant)
Group A vs. group C \(-t = 8.058; \ P < .001\) (Significant)
Group B vs. group C \(-t = 8.137; \ P < .001\) (Significant)

(b) Relative

Group A vs. group B \(-t = 5.256; \ P < .001\) (Significant)
Group A vs. group C \(-t = 9.217; \ P < .001\) (Significant)
Group B vs. group C \(-t = 10.949; \ P < .001\) (Significant)

Adrenal ascorbic acid:

Group A vs. group B \(-t = 8.022; \ P < .001\) (Significant)
Group A vs. group C \(-t = 9.133; \ P < .001\) (Significant)
Group B vs. group C \(-t = 1.162; \ P > .2\) (Insignificant)

Adrenal cholesterol:

Group A vs. group B \(-t = 4.074; \ P < .01 & > .001\) (Significant)
Group A vs. group C \(-t = 0.217; \ P > .8\) (Insignificant)
Group B vs. group C \(-t = 5.256; \ P < .001\) (Significant)
the hormone treated animals shows a tendency to fall below the corresponding values of the goitrogen treated ones, but the difference is not statistically significant. The cholesterol concentration is, however, significantly lower than that of the hypothyroid animals.

Histological examination of the adrenal reveals normal appearance of the cortical parenchyma in the control animals. The adrenals of the hypothyroid rats show some shrinkage of cells in the zona fasciculata. The fascicular cells show more lipid vacuoles than normal. In the testosterone treated animals the adrenocortical enlargement is well marked. The zona fasciculata shows hypertrophy and hyperplasia of the cells. Fatty metaplasia occurs in the cells of zona reticularis and a few cells of zona fasciculata. Lipid vacuoles are more or less same as noted in the controls.

Results of the long-term experiment are presented in Table 5. Here too, the goitrogen causes a reduction in adrenal weight. The change in the relative adrenal weight, however, fails to be statistically significant. The ascorbic acid content of the adrenal is lowered following the development of hypothyroidism. The cholesterol content also, unlike that observed in the short-term experiment, is definitely lower than that of the normal control.

In the androgen treated group, the adrenals have become heavier than those of the hypothyroid animals, but the increase in weight is of much lesser extent than that obtained
Table 5. The weight, the ascorbic acid and total cholesterol contents of the adrenals in the long-term experiment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean body weight (gm.)</th>
<th>Mean adrenal weight (mgm.)</th>
<th>Mean ascorbic acid (mg./100 gm. of adrenal tissue)</th>
<th>Mean cholesterol (mg./100 gm. of adrenal tissue)</th>
<th>S.E.</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Control</td>
<td></td>
<td>40.1</td>
<td>147.7</td>
<td>22.7 ± 1.06</td>
<td>475.8 ± 16.3</td>
<td>21.1 ± 0.99</td>
<td>0.98</td>
</tr>
<tr>
<td>B. Thiourea</td>
<td></td>
<td>40.0</td>
<td>125.5</td>
<td>17.5 ± 0.75</td>
<td>284.2 ± 17.3</td>
<td>17.1 ± 0.34</td>
<td>0.76</td>
</tr>
<tr>
<td>C. Thiourea</td>
<td>plus testosterone propionate</td>
<td>39.9</td>
<td>120.8</td>
<td>23.4 ± 1.21</td>
<td>274.8 ± 16.6</td>
<td>11.1 ± 1.95</td>
<td>0.95</td>
</tr>
</tbody>
</table>

*Relative adrenal weight (mgm./100 gm. of body wt.)*

Analysis

Adrenal weight

(a) Absolute:

- Group A vs. group B: $t = 4.031$; $P < .01$ & $>.001$ (Significant)
- Group A vs. group C: $t = 0.437$; $P < .6$ (Insignificant)
- Group B vs. group C: $t = 4.157$; $P < .01$ & $>.001$ (Significant)

(b) Relative:

- Group A vs. group B: $t = 1.371$; $P > .1$ (Insignificant)
- Group A vs. group C: $t = 2.111$; $P < .1$ & $>.05$ (Insignificant)
- Group B vs. group C: $t = 4.217$; $P < .001$ (Significant)

Adrenal ascorbic acid

- Group A vs. group B: $t = 8.077$; $P < .001$ (Significant)
- Group A vs. group C: $t = 8.646$; $P < .001$ (Significant)
- Group B vs. group C: $t = 0.391$; $P > .7$ (Insignificant)

Adrenal cholesterol

- Group A vs. group B: $t = 3.077$; $P < .01$ & $>.001$ (Significant)
- Group A vs. group C: $t = 4.587$; $P < .001$ (Significant)
- Group B vs. group C: $t = 3.003$; $P < .01$ & $>.001$ (Significant)
in the short-term experiment. The adrenal ascorbic acid remained at the same level as that found in the goitrogen treated group. In the case of cholesterol content, a further reduction occurs following the hormone treatment.

Histological examination of the adrenals reveals considerable involution of the cortex in the goitrogen treated animals. The inner two zones show shrinkage of cells, the change in the fascicular zone being more marked. Lipid vacuoles in these two zones are lesser than normal. The cortical shrinkage of the hypothyroid group seems to have been corrected in the testosterone treated animals. Fatty metaplasia occurs in the cells of zona reticularis and some of the cells of zona fasciculata in this hormone treated group. Lipid vacuoles are much less in comparison to that of the controls.

Histological examination of the thyroid in the short-term experiment shows considerable hypertrophy and hyperplasia of the acinar cells in the goitrogen treated rats. Such changes are absent in the thyroids of the animals receiving combined treatment with thiourea and testosterone. In the long-term experiment, the glands of the hypothyroid group are found to be very markedly hyperplastic. Testosterone treatment in such cases brings about marked atrophic changes in the gland.
Discussion

The data presented above show that in-hypothyroidism of shorter and longer duration the absolute weight of the adrenal is reduced. This change, however, becomes insignificant when the adrenal weight is expressed as relative to the body weight. Thiourea and its derivatives are considered to be potent stressor agents (see Selye, 1950; Roy et al., 1953). Such an effect of thiourea will tend to counteract the adrenal atrophic effect due to induced hypothyroidism, whereas, it will intensify the inhibitory effect of hypothyroidism on the body weight gain. In that case such actions should have modifying influence on the adrenal weight, particularly the relative one, of the goiterogen induced hypothyroid animals. Nevertheless, the histological examination reveals shrinkage of the cells of the adrenal cortex in the hypothyroid animals. In the short-term experiment such change occurs only in the fascicular zone whereas, in the long-term experiment similar changes include zona fascicularis and the zona reticulata.

Such adrenal atrophy in the hypothyroid animals might be due to a decrease in the secretion of pituitary ACTH (Selye, 1950) and secondarily due to the diminished utilization of the cortical hormone under the hypometabolic state of the hypothyroid animals (Freedman and Gordon, 1955).

The changes observed in the adrenal constituents of the hypothyroid rats are interesting. The rise in the
cholesterol content of the adrenal found in hypothyroidism of shorter duration probably indicates a reduced pace of steroidogenesis. Cholesterol is not utilized in the biogenetic process at its normal rate and is, therefore, accumulated. It is probable that due to continued hypofunction of the adrenal resulting from prolongation of the hypothyroid condition, the adrenal constituents which accumulate at first due to diminished steroidogenesis, try to adjust themselves at comparatively lower levels. The fall obtained in the cholesterol content of the adrenal in the hypothyroid animals of the long-term experiment are in accord with such a viewpoint. It is pertinent to note that after hypophysectomy there is an initial rise in the lipid content of the adrenal; but with the prolonged absence of ACTH a fall in the concentration of lipids invariably occurs (see Selye, 1950).

An apparent difficulty is presented by the adrenal ascorbic acid values which record a significant fall below the normal levels in both short and long-term experiments. Sayers and Sayers (1948) noted that during the hyperactivation of the adrenal gland, the initial fall and the subsequent increase in adrenal ascorbic acid definitely precede the analogous changes in the cholesterol concentration of the gland. It is probable that in the regressive phase of the adrenocortical activity also, the initial rise in the adrenal ascorbic acid occurs earlier than the corresponding rise of adrenal cholesterol content and is thus
missed even in the short-term experiment. The observations of certain investigators (Freedman and Gordon, 1950; Pekkarinen et al., 1951; Redaelli, 1950) that in rats treated with thiouracil there is an initial transitory rise in the adrenal ascorbic acid content gradually followed by a return to normal values, point to such a possibility.

It is evident from the data on the adrenal weight and histology, that testosterone corrects the atrophic changes of the adrenal cortex produced by hypothyroidism in both the experiments. In the short-term experiment, the weight of the adrenal is considerably increased above the normal value after testosterone treatment (Table 4). In the long-term experiment the adrenal atrophy is corrected by the hormone treatment and the weight of the adrenal comes back to the normal level (Table 5). The cholesterol content of the adrenals of the hypothyroid animals is depleted following the hormone treatment in both the occasions. The changes indicate that testosterone stimulates the adrenal cortex of goitrogen induced hypothyroid rats. It is to be noted, however, that the adrenal ascorbic values remain more or less unchanged after testosterone treatment, but that does not exclude a possible stimulation of the adrenal cortex. Whenever, the gland is stimulated by any agent for a certain period of time, its ascorbic acid content tends to rise subsequent to an initial fall and these changes always precede the analogous changes in the cholesterol concentration of the gland (Sayers and Sayers,
In the present experiments, the adrenal ascorbic acid is probably trying to recover after the preliminary fall as a result of androgenic stimulation, whereas, the cholesterol concentration is still low.

This adrenocortical stimulation by testosterone propionate is presumably due to increased secretion of ACTH. The fatty metaplasia of the cortical cells is probably due to interaction between ACTH and testosterone (Selye, 1950; Roy et al., 1957).

In the short-term experiment the testosterone increases the weight of the adrenal of goitrogen treated rats much above the normal level but the changes induced in the thyroid, in no way indicate that the gland is secreting hormone in a greater rate than normal. From this it will appear that the adrenal stimulating action of testosterone propionate is exerted mainly, if not wholly, without the mediation of the thyroid gland. It is interesting to note, however, that although the doses of androgen on the body weight bases, are approximately identical in the two experiments and the duration of hormone treatment is slightly greater in the long-term experiment, the degree of adrenal stimulation by the testoid is lesser in the latter case. The real cause of it is not clear. The thyroid gland of the testosterone treated animals in the latter experiment shows marked atrophic changes whereas, that in the first experiment show more or less normal picture. The difference noted in the thyroid picture of
the two testosterone treated groups may be a factor to account for the discrepancy in the adrenal enlargement in the two hormone treated groups. **The role of thyroid in such testoid action, therefore, needs further elucidation.**

Another question which may arise is, whether the responsiveness of the adrenal cortex is diminished in hypothyroidism of longer duration. From the observations of some investigators it is noted that the adrenal of thiouracil treated rats responds to exogenous ACTH with usual depletion of ascorbic acid as the normal ones (Zarrow and Zarrow, 1961; Perry, 1952). The duration of hypothyroidism in the experiment of Zarrow and Zarrow (1951) was as long as that of the present study. Added to this, some investigators (Hess and Finerty, 1952; Freedman and Gordon, 1955) claim that the depletion of ascorbic acid from the adrenal, caused by a given amount of exogenous ACTH is manifested in a more pronounced manner in the hypothyroid animals than in the normal ones. Though the experimental conditions and the criteria used for evaluation of the adrenocortical activity in these experiments are not strictly comparable to those of the present study, it seems that any diminution of the sensitivity of adrenal cortex of the hypothyroid animals is probably not responsible for the difference in the degree of stimulation obtained in the present experiment.

The other alternate explanation is that probably
the pituitary fails to elaborate sufficient ACTH in response to testosterone treatment, in case the duration of hypothyroidism is prolonged. However, it has also been reported that the adrenals of thiourea treated rats respond to epinephrine by secreting endogenous ACTH equally well as those of the normal ones (Gabrilove and Soffer, 1950; Perry, 1952). These findings tend to indicate that there occurs no defect in the production and release of ACTH from the pituitary of the hypothyroid animals. But in these experiments duration of stimulation (by epinephrine) was very short. It has been reported by some workers (Halmi and Bogdanove, 1951; Hess and Finerty, 1952) that, although the release of ACTH in hypothyroid animals may be lesser than normal, the storage in the pituitary shows no difference from that of normal. In such a situation the release of ACTH from the pituitary of hypothyroid animals, evoked by certain stimuli acting for a short period may not reflect the true picture of the secreting capacity of the pituitary. In the present experiment, where the stimulus was acting over a longer period of time probably brought out the hypophyseal incapacity which was otherwise not revealed in those cases where the stimulation period was shorter. It might be possible that due to a prolonged preoccupation of the pituitary in the elaboration of TTH at an unusually high rate in the hypothyroid animals, there was a compensatory loss of ability (of the pituitary) to form ACTH.
Hypothyroidism of shorter and longer duration, induced by the administration of thiourea leads to adrenocortical atrophy. The cholesterol content of the adrenal rises above the normal level in case of hypothyroidism of shorter duration, whereas in prolonged hypothyroidism it shows a lower resting level than normal. The ascorbic acid content shows a decline in both the occasions.

Treatment with testosterone propionate stimulates the adrenal cortex of thiourea treated rats in both the occasions, causing an increase in adrenal weight. The cholesterol content of the gland records a fall after testosterone treatment while the ascorbic acid content does not show much change. The degree of adrenal stimulation, as judged by the increase in the adrenal weight after testosterone administration is, however, much less in hypothyroidism of longer duration. Thus the role of thyroid in the androgen action requires further elucidation.
Chapter I

Effect of Testosterone Propionate on the Adrenal Cortex of Thyroxine Treated Rats.
A stimulation of the adrenal cortex in association with a hypertrophy of the thyroid was noted in rats treated with testosterone propionate (Chapter I 'A', Part II). Thyroid hormone plays an important role in regulating the adrenocortical activity (see Selye, 1950; Williams, 1950) and it stimulates the adrenal cortex by augmenting the secretion of ACTH from the pituitary (Smith, 1930; Balty and Leblond, 1954). The above observations, therefore, raised a question whether the adrenal stimulating action of testosterone requires the mediation of thyroid or not. Such a question becomes pertinent in view of the finding that while the androgen can stimulate the adrenals of experimental hypothyroid rats, the degree of stimulation is lessened when the hypothyroidism is of longer duration (Chapter I 'B', Part II).

It has been shown (Chapter I, Part I) that thyroxine blocks the thyroid stimulating action of the androgen. A study of the influence of testosterone propionate on the adrenal cortex of thyroxine treated animals was, therefore, undertaken with a view to elucidate further the role of thyroid gland in the action of androgen on adrenal cortex.
Experimental Procedure

Thirty-two rats weighing 41.1 ± 4.1 gms. were divided into four groups of 8 each. To one group thyroxine (15 µg., dissolved in 0.4 c.c. of distilled water per rat daily) was administered orally for 13 days. Another group received injections of testosterone propionate (6 mg. dissolved in 0.2 c.c. of sesame oil per rat on alternate days) over the same period of time. The third group had the combined treatment with thyroxine and testosterone propionate in the above dose schedule. The remaining group served as a control receiving only the diluents.

The animals were sacrificed on the day following the final hormone treatment. The adrenals were carefully dissected out, freed from adhering fat and weighed in a microbalance. The left adrenals were processed for the determination of ascorbic acid and the right ones for the total cholesterol contents. Six adrenals were allotted for each biochemical estimations. The rest of the adrenals and a few thyroid glands were suitably fixed for histological studies.

Results

Table 6 shows the weight, ascorbic acid and total cholesterol contents of the adrenals of different groups of animals. It is seen that testosterone propionate
Table 6. The weight, ascorbic acid and cholesterol content of the adrenals of control and experimental rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial Mean body weight (gm.)</th>
<th>Mean adrenal weight (mgm.) with S.E.</th>
<th>Mean ascorbic acid (mg./100 gm. tissue) with S.E.</th>
<th>Mean cholesterol (mg./gm. tissue) with S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Control</td>
<td>41.1</td>
<td>58.5</td>
<td>15.6 ± 0.97</td>
<td>680.0 ± 6.5</td>
</tr>
<tr>
<td>B. Testosterone propionate</td>
<td>41.0</td>
<td>57.1</td>
<td>22.4 ± 0.61</td>
<td>296.6 ± 10.5</td>
</tr>
<tr>
<td>C. Thyroxine</td>
<td>41.5</td>
<td>50.6</td>
<td>17.9 ± 0.64</td>
<td>509.2 ± 16.4</td>
</tr>
<tr>
<td>D. Testosterone propionate plus thyroxine</td>
<td>41.8</td>
<td>54.4</td>
<td>24.7 ± 0.77</td>
<td>300.0 ± 0</td>
</tr>
</tbody>
</table>

Analysis

Adrenal weight:
- Group A vs. group B: $t = 5.933$, $P < .001$ (Significant)
- Group A vs. group C: $t = 1.982$, $P < .01$ & $>.05$ (Insignificant)
- Group A vs. group D: $t = 7.250$, $P < .001$ (Significant)
- Group B vs. group D: $t = 2.363$, $P < .05$ & $>.02$ (Significant)
- Group C vs. group D: $t = 6.833$, $P < .001$ (Significant)

Adrenal ascorbic acid:
- Group A vs. group B: $t = 23.453$, $P < .001$ (Significant)
- Group A vs. group C: $t = 4.036$, $P < .01$ & $>.001$ (Significant)
- Group A vs. group D: $t = 43.076$, $P < .001$ (Significant)
- Group B vs. group D: $t = 0.323$, $P > .7$ (Insignificant)
- Group C vs. group D: $t = 12.334$, $P < .001$ (Significant)

Adrenal cholesterol:
- Group A vs. group B: $t = 2.459$, $P < .05$ & $>.02$ (Significant)
- Group A vs. group C: $t = 2.318$, $P < .05$ & $>.02$ (Significant)
- Group A vs. group D: $t = 3.797$, $P < .01$ & $>.001$ (Significant)
- Group B vs. group D: $t = 1.879$, $P < .1$ & $>.05$ (Insignificant)
- Group C vs. group D: $t = 1.844$, $P < .1$ & $>.05$ (Insignificant)
increases the weight of the adrenal and the ascorbic acid and total cholesterol contents of the gland record a fall. Thyroxine treatment seems to bring about an increase in adrenal weight but the change fails to become statistically significant. The ascorbic acid and total cholesterol contents of the adrenals in this group are, however, significantly lowered from those of the control. The adrenals of the combined treated group show marked enlargement and they are significantly heavier than those of the animals treated with testosterone alone. The cholesterol content too, shows a greater decline. The ascorbic acid content, however, though significantly lower than that of the control, is virtually at the same level as that observed in the testosterone treated animals.

Histological picture of the adrenals of the control animals presents a normal appearance of the cortical parenchyma. The sinusoids are noticeable in between the columns of cortical cells. Medullary cells present a normal appearance. (Plate IV, Fig. 1).

The adrenals in the testosterone treated group shows considerable enlargement of the cortex. Cells of the zona glomerulosa show hypertrophy. Considerable hypertrophy and hyperplasia of the cells are observed in the zona fasciculata and increased mitosis is noticeable. Some of the cells of zona reticularis and a few cells of the zona fasciculata show fatty metaplasia. Fat vacuoles are seen in the cytoplasm of the cells and the nuclei are pushed
FIG. 1.— A section through the adrenal of a normal rat.

FIG. 2.— A section through the adrenal of thyroxine treated rat. Note the hypertrophy of the cortex. Compare with Fig. 1.

FIG. 3.— A section through the adrenal of testosterone propionate treated rat. Note the hypertrophy of the cortex and fatty metaplasia of some of the cells of the inner cortical zones. Compare with Fig. 1.

FIG. 4.— A section through the adrenal of thyroxine plus testosterone treated rats. Note greater hypertrophy of the cortex and greater extent of fatty metaplasia of the cortical cells in comparison with those noted in testosterone treated group. Compare with Figs. 1 & 3.
one side giving the cells a typical signet-ring appearance. But this change is not consistently noted in all the adrenals. Vascularity of the gland is increased. Medullary cells present a normal picture. (Plate IV, Fig. 3).

The adrenals in the thyroxine treated animals show some enlargement of the cortex due to hypertrophy and hyperplasia of the cells of zona fasciculata (Plate IV, Fig. 2).

Histological picture of the adrenal in the combined treated group is more or less same as that observed in the adrenals of testosterone treated animals. But the fatty metaplasia of the cortical cells are found consistently and seem to be more pronounced in this group. This change occurs in some of the cells of zona reticularis and zona fasciculata. Increased vascularity of the gland is noticeable. (Plate IV, Fig. 4).

Microscopical examination of the thyroid reveals that testosterone causes hypertrophy and hyperplasia of the acinar cells. Thyroxine treatment produces atrophy of the acinar cells both in normal and testosterone treated animals. It thus becomes quite evident that the former hormone has blocked the thyroid stimulating action of the latter.
Discussion

The data presented in this study show that testosterone stimulates the adrenal cortex of the rat. It produces hypertrophy of the adrenal and lowers the ascorbic acid and total cholesterol contents of the gland. These findings corroborate the previous observations (Chapter I 'A', Part II). But here, the fatty metaplasia is not observed consistently and is of lesser degree in comparison to that observed in the previous study. It may be due to the fact that the duration of treatment with the testoid in the present experiment was less than that of the previous one.

Changes in the weight of the adrenal along with those in the chemical constituents indicate that thyroxine also stimulates the adrenal cortex. These observations are in agreement with those by previous investigators (see Salve, 1950; Kar et al., 1955b). The weight of the adrenals in the combined treated group is greater not only than that of the controls but also than that of either thyroxine or testosterone treated animals. In fact, the enlargement of the adrenals reflects the additive effect of the influences of the two hormones. Degree of depletion of the cholesterol content too, in this combined treated group, appears to point out the same additive effect. The change in the vitamin content is, however, the same as noted in the animals treated with testosterone alone. The reason for the dis
Discrepancy in the behaviour of the two chemical constituents is not clear. Of course, it has been reported that the changes in these two constituents of the adrenal do not always run parallel (Tepperman et al., 1947; Pinchot et al., 1949).

It is thus seen that testosterone exerts its stimulating influence on the adrenal cortex in spite of the fact that the thyroid stimulating action of the sex hormone is blocked by thyroxine. Moreover, it appears that the additive effect of the two hormones is obtained in the combined treated group. The changes in the adrenal weight and the cholesterol content of the gland at least support such a viewpoint. It seems, therefore, quite reasonable to conclude that this adrenal stimulating action of testosterone propionate does not mediate through the thyroid gland.

The fatty metaplasia of the adrenal cortical cells as noted in the testosterone treated animals require some comment. According to Selye (1950) this fatty change is due to peripheral interactions between testosterone and the adrenocorticotropic hormone. It fails to develop in the absence of the latter hormone but becomes particularly more pronounced in those cases where the animals were simultaneously treated with a corticotrophic preparation in addition to testosterone. The present observations seem to be significant in this respect. Though fatty metaplasia of some of the adrenal cortical cells inconsis-
Recently occurs in the rats treated with testosterone alone, this change becomes consistent and more pronounced in the animals treated with thyroxine in addition. Probably, the increased amount of ACTH secreted by thyroxine (Eartly and Leblond, 1954) intensifies the fatty metaplasia of the adrenocortical cells brought about by testosterone treatment.

**Summary**

Administration of testosterone propionate causes stimulation of the adrenal cortex of normal and thyroxine treated rats producing hypertrophy and hyperplasia of the cells and causing depletion of the total cholesterol and ascorbic acid contents of the gland. Thyroxine, which is seen to block the thyroid stimulating action of testosterone is not able to prevent the adrenal stimulation by the androgen. It is thus concluded that the adrenal stimulating action of testosterone does not mediate through the thyroid gland.

Fatty metaplasia of the cortical cells brought about by testosterone seems to be intensified following simultaneous treatment with thyroxine. The significance of this finding in the elucidation of the mechanism of action of testosterone is discussed.