CHAPTER III

EFFECT OF CHRONIC ADMINISTRATION OF A HIGH DOSE OF TESTOSTERONE PROPIONATE ON THE ADRENAL CORTEX OF YOUNG MALE RATS.
Selye and his associates (1950) subjected rats to chronic overdosage with methyl-testosterone and observed an atrophy of the adrenal cortex of the animals. They ascribed this adrenocortical atrophy to be due to the alleged inhibitory effect of testosterone on the ACTH secretion of the pituitary. A significant finding of this study was the appearance in the adrenal cortex of extraordinarily large vacuoles containing sudanophilic lipid substance. Often these lipid vacuoles distended the body of the cortical cells to such an extent as to push the nucleus to one side, giving rise to signet-ring appearance of fat cells of ordinary adipose tissue. It was further noted that such fatty metaplasia of the cortical cells failed to occur in hypophysectomized rats treated with testosterone. It became particularly more pronounced by simultaneous treatment with testosterone and a pituitary preparation containing a corticotrophic hormone. They, therefore, concluded that this fatty metaplasia of the cortical cells was due to the peripheral interaction of corticotrophin and the testoid hormones (see Selye, 1950).

It has been seen in the foregone studies that the response of the adrenal cortex to testosterone treatment may vary at different dose levels of the hormone administration. In the lower dose levels, testosterone causes adrenocortical atrophy but with increase in doses of the hormone hypertrophy of the gland is obtained (see Chapter...
This adrenocortical stimulation by the testosterone is presumably brought about by enhancement of ACTH secretion from the pituitary. A relatively higher dose of testosterone produced marked stimulation of the adrenal cortex along with fatty metaplasia of the cortical cells (see Chapter I 'A', Part II). It has further been observed (see Chapter I 'C', Part II) that such metaplasia of the cortical cells induced by testosterone became more pronounced following simultaneous treatment with thyroxine, which is known to increase the hypophyseal ACTH secretion (Eartly and Leblond, 1954).

In view of the above observations a question arose whether the interpretation given by Selye (1950) that the adrenocortical atrophy in rats chronically overdosed with methyl testosterone was due to alleged inhibitory influence of the testosterone on the pituitary ACTH secretion, was tenable with their finding of pronounced fatty metaplasia of the adrenocortical cells under such conditions. It was, therefore, thought worthwhile to investigate into the effect of chronic treatment with a high dose of testosterone propionate on the adrenal cortex of rats and the present study embodies the observations in this respect.

**Experimental Procedure**

Sixteen rats weighing 39 ± 2.9 gms. were divided into two groups of 8 each. One group received injections.
of testosterone propionate, in 0.25 c.c. of sterile sesame oil, on alternate days. The treatment was continued for 88 days. The dose of the hormone was 6 mg. for the first 56 days and was then raised to 9 mg. on the body weight basis. The latter dose was maintained for the rest of the experimental period. The other group served as a control and received similar treatment but with oil alone.

The animals were killed on the day following final hormone treatment. 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 contents. Six adrenals were allotted for each of the biochemical estimations. The rest of the adrenals were suitably fixed for studies of gross histology and sudanophilia.

**Results**

Table 8 shows the weight, ascorbic acid and total cholesterol contents of the adrenals of control and testosterone propionate treated rats. It will be seen that chronic administration of a high dose of testosterone propionate causes a significant increase in the weight of the gland. The ascorbic acid and total cholesterol contents of the gland record a significant fall.

Histological examination of the adrenal in the control
Table 3. — The weight, the ascorbic acid and total cholesterol contents of the adrenals in control and testosterone treated animals.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial (gm.)</th>
<th>Final (gm.)</th>
<th>Mean body weight (gm.)</th>
<th>Mean adrenal weight (gm.)</th>
<th>Mean ascorbic acid (mg./100 gm. adrenal)</th>
<th>Mean cholesterol (mg./100 gm. adrenal)</th>
<th>S.E. of mean adrenal weight (gm.)</th>
<th>S.E. of mean cholesterol (mg./100 gm. adrenal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Control</td>
<td>39.4</td>
<td>151.2</td>
<td>26.5 ± 0.80</td>
<td>324 ± 13.5</td>
<td>20.2 ± 0.35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. Testosterone</td>
<td>38.6</td>
<td>107.2</td>
<td>31.5 ± 0.47</td>
<td>241 ± 15.8</td>
<td>16.2 ± 0.35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>propionate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analysis

Adrenal weight: t = 5.43; P < .001 (Significant)
Adrenal ascorbic acid: t = 3.45; P < .01 & > .001 (Significant)
Adrenal cholesterol: t = 8.16; P < .001 (Significant)

group (Plate VII, Fig. 1) shows normal appearance of the cortical parenchyma with three clearly distinguishable zones. Medullary cells also present a normal picture. The adrenals in the testosterone treated group (Plate VII, Fig. 2) reveal considerable enlargement of the cortex due to hypertrophy and hyperplasia of the parenchymal cells. Marked fatty metaplasia of the cells (Plate VII, Figs. 2 & 3) are observed in the reticular as well as fascicular zones. The glomerular and the outermost fascicular cells are free from this change. The blood vessels of the medulla look...
(All the figures are photomicrographs. X 120)

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

FIG. 2.— A section through the adrenal of a rat chronically overdosed with testosterone propionate. Note the hypertrophy of the cortex and fatty metaplasia of the cells of zona fasciculata and zona reticularis. Compare with Fig. 1.

FIG. 3.— Another section through the adrenal of a rat chronically overdosed with testosterone propionate. In this section medulla is not seen. Note the pronounced fatty metaplasia of the cortical cell.
engorged.

Histochemical study reveals that there is an overall reduction in the sudanophilic lipids from the adrenal cortex of testosterone treated animals. The glomerular zone contained relatively greater amounts of lipid than the inner zones. The sudanophobic transition zone between glomerulosa and fasciculata, which is a constant feature in this species, is exhibited prominently in the control adrenals and appears to obliterated due to loss of lipid in those of the testosterone treated animals. The fat vacuoles noted in the inner two zones are, however, distinct from the usual sudanophilic materials of the adrenal cortex. These are of much larger size and they have clear and distinct outlines. Often a single large lipid vacuole distends the cytoplasm of the cell pushing the nucleus to one side.

Discussion

The data presented in this study show that chronic administration of a high dose of testosterone propionate produces a significant increase in the weight of the adrenal gland of rats. The ascorbic acid and total cholesterol contents of the gland show a decline. The histochemical examination also reveals depletion of sudanophilic lipids from the adrenal cortex following androgen administration.
Histological picture shows hypertrophy and hyperplasia of the cortical cells. All these findings signify a stimulation of the adrenal cortex. It thus appears that even chronic treatment with a high dose of testosterone produces adrenocortical stimulation, which is presumably due to enhancement of ACTH secretion of the pituitary (Chapter I 'A', Part II). Selye and Stone (1950), however, reported atrophy of the adrenal gland in rats chronically overdosed with methyl testosterone. There occurred significant reduction in the adrenal weight and histological picture showed atrophy of the cortical parenchyma. In their experiment the dose of testosterone was still higher (10 mg. per rat daily, for 90 days) in comparison to that used in the present case. Curiously, in another experiment (Selye et al., 1943), where the dose of the hormone was the same as above but the duration of treatment was shorter (10 mg. per rat daily, for 76 days) there occurred no statistically significant reduction in the weight of the adrenals. Moreover, the relative weight of the gland in the hormone treated group (16.3 mg.) was the same as that of the control (16.1 mg.). Histological picture showed involution of the cells of zona glomerulosa. The other two zones of the cortex did not show any atrophic change. It is pertinent to note here that the adrenocortical atrophy encountered with low doses testosterone propionate and methyl testosterone becomes manifest within much lesser time (in 20-25 days) (see Selye, 1941; Lewis et al., 1949; and Chapter II,
Part II). In view of these findings it appears surprising why there should not be any significant reduction in the weight of the gland inspite of such prolonged administration of methyl testosterone (see Selye et al., 1943), in case the influence of testosterone even in the higher dose levels is to inhibit the ACTH secretion of the pituitary (Selye, 1950). Added to this, there are evidences that testosterone administered in the same high dose or in a still higher dose for shorter length of time (20 days) produced adrenocortical hypertrophy (Selye, 1941). These observations coupled with those of the present study (also see Chapters I & II, Part II) clearly indicate that testosterone at higher dose levels causes increased secretion of ACTH from the pituitary and does not inhibit it. The atrophy encountered in the experiments of Selye and coworkers (1943, 1950) following prolonged administration of a comparatively higher dose of methyl testosterone is probably due to exhaustion as a result of prolonged over-stimulation of the gland. Such an explanation seems also to be more compatible with the occurrence of pronounced fatty metaplasia of the cortical cells in these cases. In the present study no atrophy was encountered even after prolonged administration of testosterone propionate probably because the dose in the present case was not so high as that used by Selye et al. (1943, 1950).
Summary

Chronic administration of a high dose of testosterone propionate to rats produces adrenocortical stimulation along with fatty metaplasia of the cortical cells. The ascorbic acid and total cholesterol contents of the gland are depleted. Histochemical observations with sudanophilic lipids agree with the biochemical alterations. It is thus seen that the crucial effect of testosterone at the higher dose level is the stimulation of the adrenal cortex presumably through increased pituitary ACTH secretion.