CHAPTER II

EFFECT OF GRADED DOSES OF TESTOSTERONE PROPIONATE ON THE ADRENAL CORTEX OF YOUNG MALE RATS.
Mention has already been made about the contradictory reports regarding the response of the adrenal cortex to androgen administration (see Review of Literature). No satisfactory explanation is available for this equivocal response of the adrenal cortex to androgens. A critical consideration of the relevant observations reported in the literature along with the findings of the foregone studies (Chapter I, Part II) suggests that it may be possible that the differential response of the adrenal cortex to the androgens is related to different dose levels of the hormones. It thus seemed imperative to study the effect of graded doses of testosterone propionate on the adrenal cortex of young male rats.

The present study gives an account of the changes elicited in the adrenal cortex of young male rats by the administration of graded doses testosterone propionate.

**Experimental Procedure**

Fifty-six rats weighing 42.4 ± 4.1 gms. were divided into seven groups of eight each. Group A served as control and received intramuscular injections of 0.2 c.c. of sterile sesame oil on alternate days. To the remaining 6 groups (B, C, D, E, F & G) testosterone propionate was administered in six graded doses (25 µg., 50 µg., 100 µg., 200 µg., 400 µg. & 800 µg. respectively). The hormone
dissolved in 0.2 c.c. of sterile sesame oil was administered on alternate days. Altogether, 13 such injections were given over a period of 25 days.

The animals were sacrificed on the day following the last injection. The adrenals were dissected out and weighed after removing the adherent fat. The left adrenals were processed for the determination of ascorbic acid and the right ones for the cholesterol content. Six adrenals were allotted for each biochemical estimations. The rest of the glands were fixed for histological studies.

**Results**

Table 7 shows the absolute and relative weights, ascorbic acid and total cholesterol contents of the adrenals of control and testosterone treated animals. The mutual relationship of the responses of these to the influence of graded doses of testosterone is well illustrated in Fig. 1 (Plate V). It is seen that the adrenal weights, both absolute and relative are reduced at the lower dose levels (25 µg., 50 µg. & 100 µg.) of testosterone. But as the dose of the hormone is increased this effect is diminished and at certain dose levels (200 µg. and 400 µg.) there is no significant change in the weight of the glands. Testosterone in a dose (800 µg.) higher than these causes an increase in the weight of the adrenal.
Table 7. — The weight, ascorbic acid and total cholesterol contents of the adrenals in control and testosterone propionate (T.P.) treated animals.

<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 tissue)</th>
<th>Mean cholesterol (mg./100 gm. of tissue)</th>
<th>S.E. with final tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
<td>Control</td>
<td>44.5</td>
<td>74.6</td>
<td>17.3±0.3</td>
<td>372±8.3</td>
<td>22.4±1.5</td>
</tr>
<tr>
<td>B.</td>
<td>T.P. (25 µg.)</td>
<td>41.6</td>
<td>73.5</td>
<td>14.8±0.3</td>
<td>346±23.6</td>
<td>20.6±2.0</td>
</tr>
<tr>
<td>C.</td>
<td>T.P. (50 µg.)</td>
<td>41.6</td>
<td>74.2</td>
<td>15.2±0.5</td>
<td>364±19.5</td>
<td>21.5±2.5</td>
</tr>
<tr>
<td>D.</td>
<td>T.P. (100 µg.)</td>
<td>42.7</td>
<td>73.2</td>
<td>15.0±0.5</td>
<td>485±26.9</td>
<td>16.0±0.4</td>
</tr>
<tr>
<td>E.</td>
<td>T.P. (200 µg.)</td>
<td>42.2</td>
<td>69.0</td>
<td>16.2±0.5</td>
<td>447±18.8</td>
<td>14.3±1.3</td>
</tr>
<tr>
<td>F.</td>
<td>T.P. (400 µg.)</td>
<td>41.7</td>
<td>71.2</td>
<td>17.3±0.9</td>
<td>401±11.9</td>
<td>12.4±1.7</td>
</tr>
<tr>
<td>G.</td>
<td>T.P. (800 µg.)</td>
<td>42.0</td>
<td>69.9</td>
<td>21.8±0.8</td>
<td>372±15.3</td>
<td>9.9±0.3</td>
</tr>
</tbody>
</table>

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

Analysis

Adrenal weight

Absolute:

Group A vs. group B — t = 5.95; P < .001 (Significant)
Group A vs. group C — t = 3.62; P < .01 & > .001 (Significant)
Group A vs. group D — t = 3.96; P < .01 & > .001 (Significant)
Group A vs. group E — t = 1.89; P > .1 & > .05 (Insignificant)
Group A vs. group G — t = 5.29; P < .001 (Significant)

Relative:

Group A vs. group B — t = 3.22; P < .01 & > .001 (Significant)
Group A vs. group C — t = 2.77; P < .02 & > .01 (Significant)
Group A vs. group D — t = 2.39; P < .05 & > .02 (Significant)
Group A vs. group E — t = 0.38; P > .7 (Insignificant)
The changes in the relative weights of the adrenal run almost parallel to those of the absolute weights. The cholesterol content of the adrenals shows no significant change at 25 μg. and 50 μg. dose levels, whereas, at the doses higher than these there occurs a significant reduction. This depletion of the cholesterol content increased with the dose of the hormone used. The changes in the ascorbic acid content of the adrenals are, however, of quite different nature. With the lowest two doses there was no significant change and this is similar to the changes in the cholesterol content of the gland at the corresponding dose levels of the hormone. At 100 μg. dose level the ascorbic acid content of the adrenal rises significantly above that of the controls.
FIG. 1.— The figure shows the mutual relationship of the changes in the weight (absolute and relative), ascorbic acid content and total cholesterol concentration of the adrenals of rats at different dose levels of testosterone propionate.
FIG. 1.

- DOSE OF TESTOSTERONE PROPIONATE (mg)

1. ADRENAL ASCORBIC ACID
2. RELATIVE ADRENAL WEIGHT
3. ADRENAL CHOLESTEROL
4. ABSOLUTE ADRENAL WEIGHT
(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 testosterone propionate (25 µg.) treated rat. Note the narrowing of the cortex due to shrinkage of the cells of inner fasciculata. Compare with Fig. 1.

FIG. 3. — A section through the adrenal of a testosterone propionate (50 µg.) treated rat. Note more or less the same changes as in Fig. 2. Compare with Fig. 1.

FIG. 4. — A section through the adrenal of testosterone propionate (400 µg.) treated rat. No appreciable change from the normal is noted. Compare with Fig. 1.

FIG. 5. — A section through the adrenal of testosterone propionate (800 µg.) treated rat. Note the enlargement of the cortex due to hypertrophy of the zona fasciculata. Compare with Figs. 1, 2 & 3.
In subsequent higher doses also this value is higher than normal except in the highest dose level, where it is seen to be at the same level as that of the controls. It appears as if with the increase in dosage of the hormone this rise in the ascorbic acid content is checked.

The histological picture of the adrenals shows normal appearance of the cortical parenchyma in the control group of animals (Plate VI, Fig. 1). At the lower dose levels (25 µg., 50 µg. and 100 µg.) of testosterone there occurs some atrophic changes in the cortex due to shrinkage of the cells of inner fasciculata (see Plate VI, Figs. 2 & 3). This change is most evident in the lowest dose (25 µg.) level of the hormone. The adrenals at the intermediate dose levels (200 µg. & 400 µg.) of testosterone show no appreciable change from those of the controls (see Plate VI, Fig. 4). The glands at the highest dose level (800 µg.) show cortical enlargement due to hypertrophy and hyperplasia of the fascicular cells (Plate VI, Fig. 5).

Discussion

The stimulating influence of gonadotropic (LH) on the adrenal cortex has been reported by various investigators and has already been referred to (see Review of Literature). Stack Dunne (1953) has demonstrated that LH...
is effective in adrenal weight test while it has got very little activity in Sayer's test. It is known that testosterone depresses the secretion of LH from the pituitary (see Neiburgs, 1949; Greep and Jones, 1950a; Castellani, 1952a) and this effect increases with the dose of the hormone administered. Under such circumstances an inhibitory influence of testosterone on the adrenal weight due to depression of LH secretion may be envisaged. In a recent article, Jones (1955) also has tried to emphasize that the adrenal atrophic action of testosterone is due to inhibition LH secretion of the pituitary aided perhaps by increased storage of FSH which probably hinders the elaboration of ACTH in the hypophysis. On the other hand, it has been shown that testosterone in a relatively higher dose causes adrenocortical stimulation presumably by augmenting the ACTH secretion of the pituitary (Chapter I 'A', Part II).

In the light of the above observations it seems possible to give an explanation for the present findings. From the data presented above, it is evident that testosterone, at the lower dose levels causes a significant reduction in the weight of the adrenal glands. This can conceivably be due to reduction in the hypophyseal LH secretion. It is of interest, however, to note that as the dose of testosterone propionate is increased, this effect is reduced as if some neutralizing mechanism becomes operative. In fact at certain intermediate dose levels
there occurs no change in the weight of the adrenals, and
with a still higher dose, the weight of the gland rises
significantly above normal. This increase in adrenal weight
can be attributed to the possible enhanced ACTH secretion
of the pituitary. The reduction in adrenal atrophic action
of testosterone with the increase in dosage is probably
due to the influence of progressively increased rate of
ACTH secretion from the pituitary. Such an assumption
clarifies the adrenal cholesterol depletion which increases
with the dose of the hormone. This depletion of the cho-
lesterol content of the adrenal cannot justifiably be attri-
buted to any direct action of the hormone; because,
Montanari and Gualandi (1952) and Zizine et al. (1950) ob-
served that testosterone retarded the depletion of adre-
nal lipids in animals hypophysectomized for prolonged
periods.

A difficulty arises in explaining the changes in
the ascorbic acid concentration of the gland. However, a
plausible explanation can be given. At 100 µg. dose level
of testosterone the ascorbic acid content of the gland rose
much above the normal level whereas, the cholesterol con-
tent shows a significant decrease. This elevation in the
ascorbic acid level is not in contradiction to the assump-
tion that ACTH secretion is increased at higher dose levels
of testosterone. Probably, the magnitude of ACTH influence
with regard to ascorbic acid is not so high and the level
of the vitamin presents a picture similar to that of the resistant stage of the gland. With the increase in dosage of testosterone, the excitability of such stimulus is increased and the possibility of attaining the resistant state is probably diminished, and consequently the tendency for the adrenal ascorbic acid content to rise, is checked. In the highest dose level, the ascorbic acid content of the adrenals is at the same level as that of the controls. On the other hand, the weight of the gland is significantly increased, its cholesterol content is depleted and the histological examination shows hypertrophy of the cortex. These findings clearly indicate that the adrenal cortex is stimulated at this dose level of testosterone. During stimulation of the adrenal cortex there occurs either no change or an elevation in the ascorbic acid content of the gland in the resistant stage. Thus, these findings at the highest dose level lend further support to the assumption that the elevation in the ascorbic acid content of the gland at other dose levels also is similar to that observed in the resistant stage of the gland. The absence of any increase in the weight of the adrenal in association with such change in the ascorbic acid content of the gland is probably due to suppression of the secretion of LH, which has been shown (Stack-Dunne, 1953) to have mainly the adrenal weight maintaining property. It would, however, appear contradictory to note that the cholesterol content is depleted at these dose levels.
This is not surprising as it has been reported that the changes in these two chemical constituents of the adrenal may not always run parallel (Tepperman et al., 1947; Pinchot et al., 1949; Chapters I'B' & I 'C', Part II).

Finally, it may be recalled that cholesterol has been shown to be the precursor of adrenocortical steroids whereas, the specific role of ascorbic acid in the biogenesis of adrenocortical hormone is still obscure (Hechter and Pincus, 1954). Hence, for the evaluation of the state of adrenocortical activity the changes in the cholesterol content of the gland are probably more dependable than those in the vitamin content.

From the above it would appear that probably two principal opposing forces are at operation in mediating the influence of testosterone on the adrenal cortex. In the lower dose levels, the influence exerted through LH secretion of the pituitary probably predominates and the atrophy of the gland is encountered. As the dose of the hormone is increased gradually, the ACTH secreting ability of the hormone (from the pituitary) comes into force. This probably counterbalances the effect due to suppression of LH and when the forces balance each other no change in adrenal weight is encountered. In the doses above that level the ACTH secreting ability probably predominates and stimulation of the gland ensues.

The hypothesis presented above serves to explain various contradictory observations reported earlier.
Korenchevsky et al. (1937c) did not observe any significant change in the weight of the adrenal of female rats with a higher dose of testosterone, though there occurred a significant reduction in weight in the lower dose level of the hormone. Selye (1941) noted in male rats that testosterone was more effective in causing adrenal atrophy at the lower dose levels than at higher dose levels. Lewis et al. (1949) also reported similar observations in female rats. They also noted that the adrenal cholesterol depleting ability of testosterone increased with dose, while the adrenal atrophic effect decreased. All these can be explained by the above hypothesis.

Schilling and Laqueur (1942) noted that it is easier to produce adrenal atrophy at dioestrus than at oestrus. During oestrus the LH secretion of the pituitary is enhanced (Neiburgs, 1949). To suppress this increasing LH secretion, much greater amount of testosterone will be required and probably at such higher dose levels the ACTH stimulating ability of the hormone becomes manifest. This is perhaps the reason why it is easier to produce adrenal atrophy at dioestrus than at oestrus. Selye (1940a) reported that it is easier to suppress the growth of the adrenal in immature animals than to cause atrophy in a fully developed adrenal. This is probably due to alteration of the sensitiveness of adrenal cortex with age. Paulsen's (1950) observations seem to be pertinent in this connection. He noted that children under 7 years of age
are sometimes refractory to ACTH, but after pretreatment with gonadotrophic hormone they become responsive to normal ACTH doses. These observations suggest that perhaps pretreatment with gonadotrophic hormone can render the adrenal cortex more sensitive to ACTH than it would otherwise be at an early age. In the light of this observation it seems possible to give an explanation for Selye's (1940a) findings. Administration of testosterone at an early age suppresses the secretion of gonadotrophic hormone (LH) and thus in addition to the loss of the stimulating influence of LH, the normal sensitization of the adrenal is prevented and the gland probably does not respond to ACTH in the usual manner. This is possibly the reason why it is easier to suppress the growth of adrenal by testosterone at an early age than to cause atrophy in a fully developed gland.

Summary

Administration of testosterone propionate in graded doses to young male rats evoked varied responses in the adrenal gland. A tentative theory has been developed by utilizing the observations of the present study and this tends to explain all the contradictory reports on this problem.