CHAPTER IV

EFFECT OF GRADED DOSES OF TESTOSTERONE PROPIONATE ON THE ADRENAL CORTEX OF PREPUBERTALLY CASTRATED MALE MICE.
It has been shown that the response of the adrenal cortex of young male rats to testosterone propionate may vary depending upon the dose of hormone (see Chapter II, Part II). Basing on those observations a theory has been developed and according to that the influence of the androgen on the adrenal cortex is exerted through several pathways. The main pathways involve the LH and ACTH secretions of the pituitary. The influence of testosterone mediated through the former is inhibitory, whereas, that through the latter is a stimulating one. They are thus opposite in nature. At lower dose levels of testosterone, where its influence is probably exerted predominantly through its effect on the LH secretion of the pituitary, atrophy of the adrenal gland is encountered. At higher dose levels probably the ability of testosterone to augment ACTH secretion from the pituitary is predominantly manifested and hypertrophy of the gland is obtained. Intermediate to these low and high dose levels there occurs dosage range of testosterone where probably the two opposing forces balance each other and there occurs no significant change in the weight of the adrenals.

In the adrenal cortex of immature mouse there are two distinct zones upon which LH and ACTH of the pituitary exert trophic influences separately (Jones, 1949b, 1949c). In view of this it was thought worthwhile to test the validity of the above theory, by studying the effect of graded doses of testosterone on the adrenal gland of pre-
pubertally castrated male mice. In the male mouse the X-zone normally disappears at puberty. If, however, the immature mouse is castrated the X-zone continues to develop and comes to occupy a large portion of the cortex. For this reason, prepubertally castrated male mice were employed in this study.

**Experimental Procedure**

Thirty-six male albino mice were taken. Thirty mice were castrated at 21st or 22nd day of age and left for 10 days (during which time the adrenal X-zone persists and increases in size). The remaining six animals were left as normal controls (Group A). At the 11th post-operative day the castrated animals were divided into five groups of six each. One group from these castrated animals was left as castrated control (Group B). The remaining four groups (C, D, E & F) received daily subcutaneous injections of testosterone propionate in doses of 0.25 mg., 1 mg. and 3 mg. respectively. The hormone, dissolved in 0.1 c.c. of sterile sesame oil, was administered for ten days. The control animals were treated similarly with an equal volume of oil alone. The animals were kept under uniform husbandry conditions.

The mice were sacrificed on the day following the last injection. The adrenals were dissected out, freed
carefully from the adhering fat and weighed on a Torsion balance. The left adrenals were fixed in alcoholic Bouin's fluid for histological studies. Serial paraffin sections of the gland were stained with Ehrlich's haematoxylin followed by alcoholic eosin.

Results

Table 9 shows the weight of the adrenals of the control and testosterone treated mice. It will be seen that prepubertal castration causes a significant increase in the weight of the adrenals of mice. Testosterone administration causes a significant decrease in the adrenal weight of castrated mice in the lower dose levels (groups C and D). The adrenal weight in group D appears to be lesser than that of even the normal controls. In the highest dose level (group F) the weight of the adrenal is virtually at the same level as that of the castrated controls. The adrenals in the groups E and F are heavier than those of the normal controls. The difference in the case of group E, however, fails to be statistically significant. It is noteworthy that the adrenals of the animals at the higher dose levels (groups E and F) are significantly heavier than those at the lower dose levels (groups C and D).

Histological examination of the adrenals in the non-castrated control mice (group A) reveals normal appearance
Table 9. The body weight and the adrenal weight in the control and testosterone propionate treated mice.

<table>
<thead>
<tr>
<th>Group and Treatment</th>
<th>Mean Final body weight (gm.)</th>
<th>Mean adrenal weight (gm.) with S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Normal control</td>
<td>14.0</td>
<td>2.2 ± 0.13</td>
</tr>
<tr>
<td>B. Castrated control</td>
<td>13.5</td>
<td>3.5 ± 0.23</td>
</tr>
<tr>
<td>C. Testosterone propionate (0.25 mg.)</td>
<td>13.3</td>
<td>1.9 ± 0.23</td>
</tr>
<tr>
<td>D. Testosterone propionate (1 mg.)</td>
<td>13.6</td>
<td>1.7 ± 0.17</td>
</tr>
<tr>
<td>E. Testosterone propionate (2 mg.)</td>
<td>14.2</td>
<td>2.8 ± 0.32</td>
</tr>
<tr>
<td>F. Testosterone propionate (3 mg.)</td>
<td>13.8</td>
<td>3.1 ± 0.12</td>
</tr>
</tbody>
</table>

Analysis

(Adrenal weight)

- Group A vs. group B: \( t = 5.00; P < .001 \) (Significant)
- Group B vs. group C: \( t = 5.00; P < .001 \) (Significant)
- Group B vs. group D: \( t = 6.43; P < .001 \) (Significant)
- Group B vs. group E: \( t = 1.79; P > .1 \) (Insignificant)
- Group B vs. group F: \( t = 1.60; P > .1 \) (Insignificant)
- Group E vs. group A: \( t = 1.76; P > .1 \) (Insignificant)
- Group E vs. group C: \( t = 2.30; P < .05 & > .01 \) (Significant)
- Group E vs. group D: \( t = 3.22; P < .01 & > .001 \) (Significant)
- Group F vs. group A: \( t = 5.29; P < .01 & > .001 \) (Significant)
- Group F vs. group C: \( t = 4.61; P < .001 \) (Significant)
- Group F vs. group D: \( t = 7.00; P < .001 \) (Significant)
of the cortical parenchyma (Plate VIII, Fig. 1). The cortex shows zona glomerulosa, fasciculata and a very narrow X-zone, a distinctive layer surrounding the medulla. The cells of this layer have somewhat dense cytoplasm with deeply stained nuclei. The medulla shows normal appearance.

Histological picture of the adrenals in the castrated animals shows considerable enlargement of the cortex (Plate VIII, Fig. 2). This appears to be due to a very great development of the X-zone. The zona glomerulosa and zona fasciculata present normal appearance.

In group C, the X-zone of the adrenal collapses under the influence of testosterone and a very narrow layer of degenerated cells is noticed. The other two zones are more or less normal (Plate VIII, Fig. 3).

In group D, the X-zone of the adrenal disappears completely and forms a thin connective tissue capsule surrounding the medulla. The zona fasciculata appears to be slightly narrower than that of the controls (Plate VIII, Fig. 4).

In the last two groups (i.e., groups E and F) the change in the X-zone is similar to that of the preceding one, but zona fasciculata presents a different picture (Plate VIII, Figs. 5 & 6). The cells of this layer show marked hypertrophy and hyperplasia. It is noteworthy that inspite of the total disappearance of the X-zone, the adrenal cortex in these groups is much wider than that observed in group D. This appears to be due to the hyper-
(All the figures are photomicrographs. X 90)

FIG. 1.—A section through the adrenal of a normal male mouse. Note the different zones of the cortex—zona glomerulosa, zona fasciculata and a deeply stained narrow X-zone surrounding the medulla.

FIG. 2.—A section through the adrenal of a pre-pubertally castrated mouse. Note the enlargement of the cortex due to marked hypertrophy of the X-zone which takes somewhat deeper stain. Compare with Fig. 1.

FIG. 3.—A section through the adrenal of a castrated and testosterone propionate (0.25 mg.) treated mouse. Note the marked degeneration of the X-zone which is represented by a very narrow band collapsed cells. Compare with Figs. 1 & 2.

FIG. 4.—A section through the adrenal of a castrated and testosterone propionate (1 mg.) treated mouse. Note the total disappearance of the X-zone which is replaced by a very narrow band of connective tissue capsule surrounding the medulla. Zona fasciculata appears to be narrower than normal. Compare with Figs. 1 & 2.

FIG. 5.—A section through the adrenal of a castrated and testosterone propionate (2 mg.) treated mouse. Note the total disappearance of the X-zone which is replaced by a band of connective tissue capsule surrounding the medulla. Zona fasciculata is hypertrophied. Compare with Figs. 2, 4 & 1.

FIG. 6.—A section through the adrenal of a castrated and testosterone propionate (3 mg.) treated mouse. The changes are similar to those noted in Fig. 5, but here the fascicular hypertrophy is much greater. Compare with Figs. 2, 1 & 4.
Discussion

From the data presented above it is seen that castration of immature mice increases the weight of the adrenal gland. This seems to be due to the great development of the X-zone in the castrated mice. Testosterone propionate decreases the weight of the adrenals of the castrated mice in the lower dose levels. This effect appears to be due to the degeneration of the X-zone. In group C the X-zone collapses and is seen as a very narrow band of degenerated cells. In group D the X-zone disappears totally and forms a thin connective tissue capsule surrounding the medulla. In the higher dose levels of testosterone the weight of the adrenals is not much diminished, inspite of total disappearance of the X-zone. In fact, the weight of the adrenals in group F is virtually at the same level as that of the castrated control. It is of interest to note that the adrenals in the highest dose level of the testoid are significantly heavier than those of the normal controls. This effect appears to be due to marked hypertrophy of the fascicular zone.

It has been shown by different investigators
(Dumesly and Parkes, 1937; Jones, 1949a) that prepuberal castration of mice causes persistence and development of the X-zone of the adrenal cortex. In the adult male mice there is no X-zone, but post-puberal castration allows a new zone to arise from the inner fascicular cells. This new cortical zone has been termed the secondary X-zone (Howard, 1939). The persistence and the development of the X-zone is under the trophic influence of the gonado-trophic hormone, presumably LH secretion of the pituitary and ACTH has no influence on it (Jones, 1949b, 1949c). Rapid involution of this zone under the influence of the androgens has been demonstrated by various workers (Martin, 1930; Poll, 1933; Cramer and Horning, 1937; Howard, 1940; Starkey and Schimidt, 1938; Jones, 1949a). It is well-known that testosterone lessens the secretion of pituitary LH (Neibuhrs, 1949; Greep and Jones, 1950a)—the X-zone maintaining factor. Of course, testosterone has also been shown to exert a direct degenerative effect on this zone in the absence of the X-zone maintaining principle of the pituitary (Jones, 1949a). Thus, it is seen that the destruction of the X-zone is brought about by a direct action of testosterone aided perhaps by lessening of the secretion of LH—the X-zone maintaining factor.

At the higher dose levels of testosterone the zona fasciculata hypertrophies and this is conceivably due to augmentation of pituitary ACTH secretion. The extent of the fascicular hypertrophy may be such as to counterbalance
the decrease in the adrenal weight due to the degeneration of the X-zone. It is interesting to note that the weight of the adrenals in the highest dose level of the hormone is significantly greater than that of the normal animals and is almost similar to that of the castrated controls.

The above observations clearly demonstrate that the influence of testosterone on the adrenal cortex of mice is exerted through various pathways. Depending upon the dose of testosterone the influence may be predominantly manifested through one or the other path/s and the response of the adrenal is correspondingly determined.

The results of this study strongly support the theory developed on the observations of the previous study with rats (Chapter II, Part II). It would, however, appear that the conditions in the adrenals of rat are not strictly similar to those of the mouse. Rat adrenal has no separate zone like X-zone upon which gonadotrophin exerts a specific trophic effect. However, post-pubertal castration of the adult male mouse gives a very clear example of the gonadotrophins to act upon the inner fasciculata thus evoking a wide secondary X-zone. This demonstrates a potentiality in one species which may be present in others, as has been supposed in the case of rats (Jones, 1955; Chapter II, Part II). Another point of difference is that in the mouse adrenal, testosterone exerts a direct depressing influence on the X-zone (Jones, 1949a), a fact which is lacking in the case of rats. It is to be noted, however,
that testosterone is capable of decreasing the secretion of pituitary LH (Nelburgs, 1949; Greep and Jones, 1950a; Castellani, 1952a), which has been proved to be the trophic factor for the X-zone. Hence, the testoid would have been able to depress the X-zone even the androgen had no direct effect on it.

**Summary**

Prepubertal castration of mice causes an increase in the weight of the adrenal gland due to a great development of the X-zone which is under the trophic control of pituitary LH. Treatment of castrated mice with testosterone propionate causes disappearance of the X-zone at the lower dose levels and decreases the weight of the adrenals. Inspite of the total disappearance of the X-zone at the higher dose levels of androgen, the weight of the adrenal is not much diminished due to marked hypertrophy of the fascicular zone which is under the influence of pituitary ACTH. In fact the adrenal weight at the highest dose level is greater than that of normal animals and is nearly at the same level as that of the castrated controls.

The results of the present study clearly demonstrate that the influence of testosterone on the adrenal cortex is exerted through several pathways. Depending upon the dose of the hormone the influence is predominantly
exerted through one or the other pathway/s and the response of the adrenal is accordingly determined.
In view of the fact that the thyroid hormone stimulates the adrenal cortex, any possible role of the thyroid stimulating action of testosterone propionate (Part I) in the response of the adrenal cortex of rats to the androgens was studied. Testosterone propionate in a dose which had been shown to stimulate the thyroid (Chapter I, Part I) caused a stimulation of the adrenocortical activity (Chapter I 'A', Part II) and, as per expectation, this was associated with some hypertrophy of the thyroid gland. This hormone also stimulated the adrenal gland of thiourea-induced hypothyroid rats. The extent of adrenal stimulation was, however, reduced in those animals where hypothyroidism was of longer duration (Chapter I 'B', Part II). In a subsequent study (Chapter I 'C', Part II), where the thyroid stimulating action of the androgen was blocked by simultaneous administration of thyroxine, it was observed that thyroxine could not prevent the adrenocortical stimulation by androgen.
the contrary, an additive effect of the influences of the two hormones (thyroxine and testosterone propionate) was observed. These latter observations clearly demonstrated that the adrenocortical stimulation by testosterone propionate did not require the mediation of thyroid gland. The comparatively lesser stimulation of the adrenal gland in hypothyroidism (thiourea-induced) of longer duration was probably due to some other factors. 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. Such a view is reminiscent of Selye's 'Pituitary shift' theory.

A stimulation of the adrenal cortex by testosterone was also reported by Selye (1941) in rats and Castellani (1952b) in guinea-pigs. The former investigator attributed this stimulation to the non-specific damaging action of testosterone while the latter ascribed this to the potassium retaining property and the general anabolic action of androgen. In the present study, however, the adrenocortical stimulation by testosterone could not be attributed to any of the above factors (Chapter I 'A', Part II). Indirect evidence suggested that this action of the androgen was due to increased secretion of pituitary ACTH.

Another significant observation in these studies
was the occurrence of fatty metaplasia of the adrenocortical cells in the hormone-treated animals (Chapters I 'A', I 'B' & I 'C', Part II). The fatty metaplasia of the cortical cells induced by testosterone became more prominent after simultaneous administration of thyroxine. This was probably due to a further enhancement of ACTH secretion brought about by thyroxine, as it is known that this fatty metaplasia is due to a peripheral interaction between testosterone and the adrenocorticotropic hormone (see Selye, 1950).

The present work also demonstrated that the overt effect of testosterone propionate on the adrenal gland might vary depending upon the dose of the hormone administered (Chapter II, Part II). At the lower dose level it caused atrophy of the adrenal cortex while at the higher levels it caused stimulation. In between the two there was a dosage range which caused no change in the weight of the gland.

These observations were utilized to construct a theory which tended to explain and accommodate all the contradictory reports on this problem (see Chapter II, Part II). According to this, the influence of androgen on the adrenal cortex has mediated through several pathways (Plate IX, Fig. 1), the major ones were through the pituitary LH and ACTH. The influence exerted through LH has an inhibitory one, whereas that mediated through ACTH was a stimulating one. The two were, therefore, opposed to each other.
FIG. 1.— This is a diagramatic representation of the probable pathways of influence of androgen on the adrenal cortex.
PITUITARY

ACTH

LH

ADRENAL

TESTIS

ANDROGEN

STIMULATING

DEPRESSING

STIMULATING OR DEPRESSING

FIG. 1
Depending upon the dose, the influence of androgen was predominantly manifested through either of these pathways. When the influence through LH predominated (in the lower dose levels) an atrophy of the adrenal gland was encountered, but when the action was preponderably mediated through ACTH (in the higher dose levels) a hyper trophy of the gland ensued. Lastly, when the opposing forces balanced each other no change occurred in the weight of the gland.

Selye and coworkers (1950) reported that chronic overdosage with methyl testosterone caused adrenocortical atrophy in rats. A pronounced fatty metaplasia of the cortical cells was also encountered. The adrenal atrophy was attributed to an inhibitory influence of testosterone on the ACTH secretion of the pituitary. This would appear to go against the theory presented above. The present data on chronic overdosage with testosterone (Chapter III, Part II) clearly demonstrated that the crucial action of testosterone at the higher dose level was to augment ACTH secretion and not to inhibit it. If any atrophy of the gland was encountered, it was most probably due to exhaustion following prolonged overstimulation of the gland. A critical analysis of the data presented by Selye and coworkers (1943, 1950) against the background of the findings of the present study (Chapters I, II & III, Part II) tacitly pointed to such a possibility. The occurrence of pronounced fatty metaplasia of the adrenal cortical cells...
after chronic overdosage with methyl testosterone (Selye, 1943, 1950) was more in keeping with the above view, because it was shown (see Selye, 1950) that this fatty metaplasia by testosterone did not occur in the absence of ACTH in hypophysectomized animals, but became particularly more pronounced in those animals treated simultaneously with a corticotrophin preparation.

The validity of the above theory was also adequately demonstrated by the experiment on prepubertally castrated mice (Chapter IV, Part II). In these animals there are two distinct zones in the adrenal cortex upon which LH and ACTH exert trophic effects separately. Testosterone in lower doses caused a decrease in the adrenal weight due to degeneration of the X-zone while in the higher doses this effect was counteracted by a hypertrophy of zona fasciculata as a result of increased secretion of ACTH. A direct effect of the androgen on the adrenal cortex might have some modifying influence on this generalized response.

In the light of this theory, therefore, it becomes possible to explain (see Chapter II, Part II) why in the female rats a low dose of testosterone caused a reduction in the weight of the adrenal while a higher dose was ineffective (Korenchevsky et al., 1937c); why smaller doses of testosterone are more effective in producing adrenal atrophy than the higher doses (Selye et al., 1941; Lewis et al., 1949); why it was easier to produce adrenal atrophy at dioestrus than at oestrus (Schilling and Laqueur, 1942).
why treatment of castrated rats with testosterone for prolonged period was found less effective to cause adrenal atrophy than the treatment for shorter period (Korenchevsky et al., 1939). This theory also explains why the adrenal cholesterol depleting action of testosterone increases with the dosage while the adrenal atrophic effect decreases. It is also possible to give an explanation as to why it was easier to suppress the growth of the adrenal in the immature animals by testosterone than to cause atrophy in the adrenal of mature ones (Selye, 1940a).

The present work has indicated the nature and pathways of action of testosterone propionate on the adrenal cortex in a rational manner. Several contributory factors hitherto unrecognized have been shown to have played a part in this phenomena.

Among the various steroid hormones, testosterone propionate enjoys a unique position in the clinician's armamentarium. It is used in a wide range of disorders in varying dosages and for varying periods of time. It is, therefore, necessary to know the nature and pathways of its influence on vital endocrine glands like the adrenal cortex and the thyroid. The present work might prove to be of considerable help to the clinician in evaluating its therapeutic effects in a more logical manner. In this connection the use of testosterone in Cushing's disease may be cited as an interesting example (see Williams, 1955). In this disease testosterone is very often used
for counteracting the catabolic effect of the corticoids, elaborated in massive amounts under the influence of pituitary ACTH. Another reason for the use of this hormone in Cushing’s disease may be to suppress the characteristic increase in pituitary ACTH output (see Selye, 1950, 1951; Bartter et al., 1949). This is based on the assumption of the earlier workers that the androgens have the ability to suppress pituitary corticotrophic activity. Whatever may be the reason, the data provided in the present work warrants that caution should be exercised in prescribing the dosage and duration of treatment of testosterone in Cushing’s disease.