THE EFFECT OF NOR-ANDROSTENOLONE-PHENYL-
PROPIONATE ON THE ADRENAL CORTEX
AND BODY WEIGHT OF MALE RATS

By

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Sex hormones have been observed to have considerable effect not only on the secondary sexual characteristics but also on the metabolism and on the endocrine glands. There has also been interest in the changes occurring in the adrenal cortex.

By injecting oestrogens into laboratory animals a marked increase in the weight of the suprarenal glands and a hypertrophy of the cortex have been brought about (e.g. Albert, 1942, Knobil, 1953, Merklin, 1955, Paesi & Hoogstra, 1955, Perry, 1955). On the other hand, previous studies have shown that certain male hormones cause a loss in weight and atrophy of the adrenals (e.g. Selye, 1941 a, 1941 b); this result, however, has been produced more easily in female than in male animals (e.g. Selye, 1941 a, 1941 b). This is in agreement with the observations that female laboratory animals have larger adrenals than males (e.g. Bennet, 1940, Cole & Harned, 1942). Nevertheless, androgens have also been observed to induce enlargement of the adrenal cortex when administered in sufficiently large doses, and it has been suggested that this is a result of a marked non-specific stress (Selye, 1941 b).

On the other hand, it has been observed that methylandrostenediol (Winter et al., 1953, Gaunt et al., 1953) and certain other androgens (Gaunt et al., 1953) do not appreciably affect the adrenal cortex of male rats after treatment for 7–18 days.

Among the anabolic hormones, androgens have been observed to cause N-retention and an increase in body weight, which, however, slows down considerably with time and after about 50 days' treatment falls again to the level observed at the beginning of the experiment (e.g. Kochakian, 1951). When

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various doses of hormone have been used better results have been achieved with regard to body weight with small rather than with large doses (Selye, 1941 b).

Thus previous studies have clearly revealed that the effect of male sex hormones on the adrenal cortex and body weight is dependent to a large extent not only on the dose but also on the type of steroid used. It was therefore interesting to investigate what changes could be induced in the adrenal cortex and the body weight by different doses of an new compound belonging to the androgen group, nor-androstenolonephenylpropionate (19-nor-17-beta-hydroxy-3-keto-androst-4-ene-17-phenylpropionate), with a strong anabolic (Overbeek & de Visser, 1957) but weak virilizing effect (Hartenbach, 1956).

MATERIAL AND METHODS

The material consisted of 40 young male rats of the same strain, which at the beginning of the experiment weighed between 100 and 150 gm.

Ten animals were kept as controls, and received daily injections of 0.2 ml. oil. The remaining animals were divided into three groups of ten, those in the first group receiving 0.2 mg. (0.2 ml.), those in the second 2 mg. (0.2 ml.) and those in the third 4 mg. (0.2 ml.) nor-androstenolonephenylpropionate («Durabolin», Organon) (abbreviated N–A. P. P.).

All the abovementioned doses were given daily for a period of 14 days and injected under the skin of the back of the animal. The rats in each group were weighed each day at the same hour immediately after the injections.

During the period of the experiment the animals were handled in the customary manner and were given the usual food and water ad libitum.

The laboratory animals were killed 24 hours after the final injection. The organs to be examined were removed immediately and weighed on a torsion balance. The adrenals were fixed for 24 hours in 10 % neutralized formalin. One of the adrenals of each animal was treated in the customary manner, embedded in paraffin and stained with haematoxylin-cosin. The other adrenal was cut with a freezing microtome and some of the sections stained with Sudan III and some with Sudan III + Ehrlich's haematoxylin.

In the statistical treatment of the results, the standard error of the mean \( b_X \) was determined by means of the formula: 

\[
b_X = \sqrt{\frac{\sum (X_i - \bar{X})^2}{n(n-1)}}
\]

where \( X_i \) = individual observation, \( \bar{X} \) = mean and \( n \) = number of observations. The difference between two means was regarded as significant if the value \( P \), obtained by the t-test, was 0.05.

RESULTS

Macroscopical

Adrenals: Table 1 shows the mean weights of the adrenals as calculated per 100 gm. body weight. As can be seen 0.2 mg. N–A. P. P. dose caused a decrease
Table 1.
Body and relative organ weights (mg./100 gm. body weight) in control and test animals.

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Treatment</th>
<th>Body weight - gm.</th>
<th>Adrenals mg./100 gm. body weight</th>
<th>Thymus mg./100 gm. body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>% Gain</td>
</tr>
<tr>
<td>10</td>
<td>Controls</td>
<td>128</td>
<td>168</td>
<td>+31.3</td>
</tr>
<tr>
<td>10</td>
<td>N-A. P. P. 0.2 mg.</td>
<td>100</td>
<td>150</td>
<td>+50.0</td>
</tr>
<tr>
<td>10</td>
<td>N-A. P. P. 2 mg.</td>
<td>147</td>
<td>186</td>
<td>+26.5</td>
</tr>
<tr>
<td>10</td>
<td>N-A. P. P. 4 mg.</td>
<td>120</td>
<td>151</td>
<td>+25.8</td>
</tr>
</tbody>
</table>

* Standard error.

Fig. 1.
Daily changes in the body weight of laboratory animals (in percentages of the weight at the beginning of the experiment). I = controls; II = rats receiving 0.2 mg. N-A. P. P. doses daily; III = rats receiving 2 mg; IV = rats receiving 4 mg.

in the relative weight of the adrenals, the difference in comparison with the controls being quite significant (P < 0.001). The relative weight of the adrenals of the rats receiving 2 mg. N-A. P. P. was slightly greater than that of the control animals, although there was no significant difference between the

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groups (P > 0.05). On the other hand, a 4 mg. N-A. P. P. dose induced a notable increase in the relative weight, the difference in comparison with the controls being very significant (P < 0.01).

**Body weight:** Fig. 1 presents the changes taking place daily in the body weight of the laboratory animals (in percentages of original weight). An increase in weight occurred in all the groups during the period of the experiment, the controls growing during this period by approximately 31%. The rats receiving a dose of 0.2 mg. N-A. P. P. showed a greater increase in weight than the controls after treatment for 5–6 days, the body weight rising during the period of the experiment by 50%. On the other hand, after 8–10 days' treatment the increase in the body weight of the animals of the groups receiving 2 and 4 mg. N-A. P. P. began to slow down as compared with the controls, and the changes induced by the larger dosage appeared to be the most marked.

Table 1 also makes it clear that N-A. P. P. brought about atrophy of the thymus, the extent of which increased with the size of the dose.

**Microscopical**

**Controls:** (Fig. 2). The cells of the glomerular zone, which formed in clusters like grapes, were extremely eosinophilic. The transitional zone separating the former from the zona fasciculata was clearly discernible by virtue of its denser cytoplasm and densely situated nuclei. Arranged in fairly straight cords with capillaries between them, the fascicular cells were polyhedral in shape, and numerous vacuoles were to be observed in them. The zona reticularis contained many distended capillaries, sinusoids, and its cells were generally smaller than those in the fascicular zone.

The zona glomerulosa contained an abundance of sudanophilic drops of fat. In the outer part of the fascicular zone there was intense sudanophilia, but within, the quantity of fat drops diminished toward the reticular zone, where, in contrast, there was usually only a scattering of sudanophilic fat drops. The pale sudanophobic layer between the glomerular and fascicular zones corresponded to the transitional zone.

**Treated animals:** The adrenal cortex of the animals receiving 0.2 mg. N-A. P. P. (Fig. 3) appeared to have narrowed slightly as compared with the controls. This narrowing occurred in the fascicular and reticular zones, in the cell sizes of which a slight decrease could be seen. On the other hand, the zona glomerulosa and the transitional zone remained almost normal. No significant changes were seen in the sudanophilia of the adrenal cortex as compared with the controls.

No microscopic changes were observed in the adrenal cortex of the animals treated with N-A. P. P. in 2 mg. daily doses (Fig. 4).

The 4 mg. N-A. P. P. dose (Fig. 5) induced a slight broadening of the
Figs. 2-5.

2. The adrenal cortex of a control rat. Sudan III. × 48.
3. The adrenal cortex of a rat after daily doses of 0.2 mg. N-A. P. P. for 14 days. Sudan III. × 48.
reticular and fascicular zones, in the cells of the outer part of which hypertrophy could be observed. On the other hand, the zona glomerulosa appeared normal or, in some cases, slightly narrowed, as was true of the transitional zone. The glomerular zone contained about the same amount of fat drops as that of the controls, whereas the sudanophilia of the fascicular zone appeared to be somewhat subnormal.

**DISCUSSION**

The present study made it evident that with regard to N-A. P. P. too the effect on the adrenal cortex depends on the amount administered, especially when relative weights are used as a criterion.

0.2 mg. N-A. P. P. induced a significant decrease in the relative weight of the adrenals; nevertheless, it should be noted that in the group concerned, the body weight increased substantially more than in the controls. If, on the other hand, the body weight of the animals in question had increased only as much as that of the controls, or about 31 %, and the adrenals had continued to increase during the course of the experiment, it could be calculated that the relative weight would in that case have remained within normal limits. Thus, in fact, the adrenals of the rats in this group grew to an extent corresponding to the controls, whereas an extra increase in the body weight as a whole occurred. Previously it had been demonstrated that the catabolism as well as anabolism brought about by hormones might be extremely different and even opposite in different tissues (e.g. Kochakian et al., 1951). Therefore it would appear that the decrease in the relative weight of the adrenals of the animals receiving 0.2 mg. N-A. P. P. was actually due to the fact that the anabolic effect on the adrenals was not as marked as on the body as a whole. Nevertheless, a slight involution of the adrenal cortex may be detected in the histological picture, indicating the presence of a slight direct inhibiting influence. Further studies are necessary in order to find out whether this acts via the pituitary or the adrenal gland.

On the other hand, N-A. P. P. administered in 2 mg. doses did not induce significant changes in the adrenal cortex, while 4 mg. brought about a slight hypertrophy of the cortex, possibly due to the resulting systematic stress, or to a direct stimulation with a sufficiently large amount of the drug.

Thus the present study has, in part, confirmed the results obtained previously with testosterone (e.g. Selye, 1941 b).

Androgens have been observed to bring about atrophy of the thymus (Selye, 1941 b, Greep & Jones, 1950, Winter et al., 1953, Hartenbach, 1956), and the N-A. P. P. used in the present study also produced the same effect, the degree of involution being in direct proportion to the size of the dose. Since the largest
The dose of N-A. P. P. caused hypertrophy of the adrenal cortex, the atrophy of the thymus might be considered to result from an increase in the quantity of corticosteroids. However, since a 0.2 mg. dose also induced a reduction in the weight of the thymus, and the adrenal cortex in that particular group was not, at any rate, hyperactive, it would appear that N-A. P. P. has to large extent a direct effect on the thymus, in the same way as other androgens (e.g. Greep & Jones, 1950).

With regard to the changes in body weight the present study is also in agreement with previous observations, according to which small doses of male sex hormones augment and large doses retard the increase in body weight (Selye, 1941 b). Previous studies have clearly brought out the fact that testosterone propionate (1 mg./day) causes a retardation in the growth of laboratory animals after treatment for about 16 days (e.g. Kochakian, 1951). But the N-A. P. P. used in the present investigation appears to bring about a discernible retardation in the growth after as short a period as 8-10 days, when administered in doses of 2 and 4 mg. Notwithstanding the fact that 0.2 mg. induced a continuous rise in weight during the period of the study, there is nevertheless reason to assume, taking into consideration the results previously obtained with anabolic androgens (Kochakian, 1951), that a slowing down in the rate of growth would also have occurred in this group had the experiment been continued for a sufficiently long period.

**SUMMARY**

The present study was carried out in order to determine the effect of norandrostenolonephenylpropionate on young male rats when administered in various doses.

A daily dose of 0.2 mg. N-A. P. P. caused a considerable decrease in the relative weight of the adrenals after 14 days' treatment. This appeared to be largely due to an extra increase in body weight, since only a slight atrophy could be observed in the histological picture. A 2 mg. dose did not have any significant effect on the adrenals, whereas 4 mg. brought about a significant increase in the relative weight of the adrenals, together with a slight hypertrophy of the cortex.

During the period of the investigation the smallest dose of N-A. P. P. used induced a greater increase in body weight than occurred in the controls. Daily doses of 2 and 4 mg., again, induced a discernible retardation in growth, as compared with the controls, after 8-10 days' treatment.

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1. The concept of growth is used in the established sense, when referring to young animals, as a synonym for an increase in body weight.
ACKNOWLEDGMENT

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REFERENCES

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