THE EFFECT OF 19-NORTESTOSTERONE ON THE ADRENAL CORTEX OF THE RAT

By

Amiya B. Kar, J. N. Karkun and N. N. De

A great deal of information was available on the influence of the anabolic androgen methylandrostenediol (MAD) on the adrenal cortex (Winter et al., 1953, Gaunt et al., 1953). The interest in this and other related compounds with reference to the adrenal cortex was stimulated by the earlier finding of Zizine et al. (1950) who demonstrated that testosterone brought about a significant repair of this gland in hypophysectomized animals. Subsequently, Zizine (1952), Winter et al. (1953) and Gaunt et al. (1953) confirmed this finding in principle but at the same time reported that a large number of other steroids including MAD, also possessed such adrenal-repairing property in varying degrees. These steroids were also found to be equally effective in preventing cortisone-induced changes in the adrenal cortex which resembled in many respects those seen after hypophysectomy. Though it was not possible to detect any correlation between the chemical structure of these steroids and their adrenal-maintaining capacity yet, in general, it was noted that the C-19 and C-20 compounds were more effective than C-21 steroids (Gaunt et al., 1953).

In view of a background with such informations, a study of the effects of 19-nortestosterone (19-NT) on the adrenal cortex became quite mandatory. Accordingly, an attempt was made to investigate (1) the effect of graded doses of this compound on the adrenal cortex. (2) its effectiveness in preventing atrophic changes in the adrenal cortex when administered along with cortisone and (3) its ability to repair ponderal and other manifestations in the gland, if any, following the cessation of cortisone treatment.

In brief, 19-NT like MAD was primarily an anabolic compound with low androgenic activity (Hershberger et al., 1953, Barnes et al., 1954, and Stafford et al., 1954). It was more than one half the myotrophic potency but less than one tenth of the androgenic activity of testosterone (Barnes et al., 1954). For detailed informations regarding 19-NT reference might be made to these work.
EXPERIMENTAL PROCEDURE

Animals. Young female albino rats of the Institute colony were used in this study. The details of grouping of the animals for different experiments are presented in Table 1. All of the animals were maintained under uniform laboratory conditions throughout the period of investigation.

19-noretestosterone. For studies on its effect on the adrenal cortex of normal animals three dosages of 19-NT were used. The compound was injected by the subcutaneous route at the rate of 0.5 mg., 1.5 mg., and 3 mg. (in sterile olive oil) daily for 10 days. This route of administration was followed in all the subsequent experiments. The control animals received sterile olive oil alone in a similar manner.

Cortisone. To test the effectiveness of 19-NT in preventing cortisone induced atrophy of the adrenal cortex the rats were injected with cortisone acetate ('Cortone' acetate, Merck Inc., N. Y.) in a dosage of 1.5 mg. daily for 10 days. One group received 1.5 mg. of 19-NT per animal/day conjointly with cortisone for the same period (Table 1).

For adrenal recovery experiments two groups of rats were injected first with 1.5 mg. of cortisone for 10 days and from the eleventh day one group received 1.5 mg. of 19-NT alone for a further period of 10 days. The other cortisone-treated group was left without any treatment for another 10 days. This experiments was a sequel of the prevention experiment and as such the two were initiated simultaneously.

Biochemical, histological and histochemical studies. All of the experimental animals were sacrificed 24 hours after the final treatments. The adrenals were carefully dissected out, weighed to the nearest mg., and finally processed for biochemical and histochemical studies.

The ascorbic acid content of the adrenal was estimated by a modification (Karkun et al., 1954) of the method of Bessey et al. (1947).

For histological and histochemical studies the adrenals were fixed either in 10 per cent neutral formalin or in chilled 80 per cent ethyl alcohol. For gross histology serial paraffin sections of formalin fixed tissue were stained with Ehrlich's hematoxylin followed by eosin. Sudanophilia was studied in frozen sections stained with sudan black B. Alkaline phosphatase was demonstrated in paraffin sections of ethyl alcohol fixed adrenals by the technique of Gomori (1941) as laid down by Glick (1949). The sections were incubated in the substrate for six hours and were counterstained lightly with eosin before mounting.

RESULTS

Experiment 1. The effect of graded doses of 19-NT on the adrenal cortex

Adrenal weight. It will be evident from Table 1 that the graded doses of 19-NT used in the present study were without any significant effect on the absolute and relative adrenal weights.

Ascorbic acid. There was no significant change in the ascorbic acid content of the adrenals after administration of 19-NT in different dosages (Table 1).

Gross histology. The histological appearance of the adrenal cortex of control animals was normal and the three familiar zones were clearly distinguishable. The cellular and vascular architecture of the different zones was typical but
Table 1.
The weight and ascorbic acid content of the adrenals of normal and experimental rats.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Treatment</th>
<th>Mean adrenal weight with S. E.</th>
<th>Mean adrenal ascorbic acid content (mg./100 gm. adrenal) with S. E.</th>
<th>Mean body weight (gm.) with S. E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Absolute (mg.)</td>
<td>Relative (mg./100 gm. body weight)</td>
<td>Initial</td>
</tr>
<tr>
<td>I</td>
<td>Controls (solvent only)</td>
<td>5.72 ± 0.26 (8)*</td>
<td>10.73 ± 0.71 (8)</td>
<td>380.1 ± 25.0 (6)</td>
</tr>
<tr>
<td></td>
<td>0.5 mg. 19-NT</td>
<td>6.22 ± 0.23 (8)</td>
<td>11.52 ± 0.68 (8)</td>
<td>367.0 ± 23.43 (6)</td>
</tr>
<tr>
<td></td>
<td>1.5 mg. 19-NT</td>
<td>6.35 ± 0.24 (8)</td>
<td>11.42 ± 0.52 (8)</td>
<td>341.0 ± 49.66 (6)</td>
</tr>
<tr>
<td></td>
<td>3 mg. 19-NT</td>
<td>5.67 ± 0.23 (8)</td>
<td>11.25 ± 0.55 (8)</td>
<td>365.0 ± 58.29 (6)</td>
</tr>
<tr>
<td>II</td>
<td>Controls (solvent only)</td>
<td>5.80 ± 0.35 (8)</td>
<td>11.92 ± 0.62 (8)</td>
<td>443.0 ± 12.50 (6)</td>
</tr>
<tr>
<td></td>
<td>Cortisone (prevention)</td>
<td>1.97 ± 0.25 (8)</td>
<td>5.41 ± 0.63 (8)</td>
<td>332.0 ± 1.00 (6)</td>
</tr>
<tr>
<td></td>
<td>Cortisone + 19-NT (prevention)</td>
<td>3.80 ± 0.29 (8)</td>
<td>8.43 ± 0.28 (8)</td>
<td>388.0 ± 13.94 (6)</td>
</tr>
<tr>
<td>III</td>
<td>Controls** (solvent only)</td>
<td>5.80 ± 0.35 (8)</td>
<td>11.92 ± 0.62 (8)</td>
<td>443.0 ± 12.50 (6)</td>
</tr>
<tr>
<td></td>
<td>Cortisone (recovery)</td>
<td>3.83 ± 0.40 (6)</td>
<td>8.33 ± 0.48 (6)</td>
<td>280.0 ± 10.02 (6)</td>
</tr>
<tr>
<td></td>
<td>Cortisone + 19-NT (recovery)</td>
<td>3.67 ± 0.17 (6)</td>
<td>8.81 ± 0.43 (6)</td>
<td>285.0 ± 16.03 (6)</td>
</tr>
</tbody>
</table>

* Figure in parenthesis indicates the number of animals. ** Data from experiment II.
the parenchyma of the glomerular zone tended to be somewhat more eosinophilic than that of the other areas (Fig. 1).

No alterations in the histological picture of the adrenal cortex was noticeable in the 19-NT treated animals.

Sudanophilia. The adrenal cortex of the control animals showed a heavy concentration of sudanophilic lipids in the zona glomerulosa. The sudanophobic zone, characteristic of this species, sharply demarcated the glomerulosa from the less reactive inner zones. The zona fasciculata contained relatively less sudanophilic lipids than the glomerular zone but the staining reaction of the zona reticularis was prominently weak (Fig. 5).

The distribution of sudanophilic lipids in the adrenal cortex of animals injected with 19-NT was essentially the same as in the controls.

Alkaline phosphatase. In agreement with the findings of previous workers (Kar et al., 1951, Bourne, 1955, and others) it was noted that alkaline phosphatase was principally demonstrable in the endothelium of the vascular sinusoids and the nucleus of the parenchymal cells. The cytoplasm was free of this enzyme. The zona glomerulosa showed a stronger reaction than the other zones and the nucleus of the capsular fibroblasts was also positive (Fig. 9).

The pattern of distribution and concentration of alkaline phosphatase in the adrenal cortex of 19-NT treated animals was similar to that of the controls.

Experiment II. The effectiveness of 19-NT in the prevention of cortisone-induced changes in the adrenal cortex

Adrenal weight. The absolute and relative adrenal weights of the cortisone-injected rats were significantly lower than that of the controls (P < .001). Simultaneous administration of 19-NT, however, tended to maintain the adrenal weights as these were significantly greater (P < .001) than those of the animals which received cortisone alone. Nevertheless, it would be clear from Table 1 that the adrenal-weight maintaining capacity of 19-NT was rather limited as the adrenals of control animals were still significantly heavier (both absolute and relative weights) than those of the combined treated group (P < .001).

Ascorbic acid. Cortisone treatment caused a significant fall in ascorbic acid content of the adrenals (P < .001) but simultaneous administration of 19-NT appeared to prevent this fall. This was evident from a comparison of the ascorbic acid level of the adrenals of cortisone and cortisone plus 19-NT treated groups. In the latter group the ascorbic acid concentration of the gland was significantly greater (P < .001) than that of the animals which received cortisone alone. It should, however, be noted that like the adrenal weight the maintenance of ascorbic acid level by 19-NT was not quite complete as the controls continued to show significantly greater amounts of adrenal ascorbic acid (P < .02) than the combined treated group.
**Gross histology.** Cortisone treatment was associated with a marked shrinkage of the parenchymal cells of the fascicular zone. The nucleus of the cells of this zone appeared small and closely packed. Nuclear pycnosis was particularly noticeable in the juxta-medullary portion of the reticularis and vacuolation of the cytoplasm was also not uncommon in the outer parts of this zone. The zona glomerulosa was somewhat thickened though the nucleus of the component cells appeared to be closely situated (Fig. 2).

Simultaneous administration of 19-NT with cortisone appeared to prevent the severe contraction of the cortex produced by cortisone alone. This was evident from the width of the cortex and virtual absence of any abnormality

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**EXPLANATION OF FIGURES**

*(All figures are photomicrographs and are of equal magnification. The cortico-medullary junction is indicated by the solid black line).*

**Fig. 1.** The adrenal cortex of a normal rat. H. & E.

**Fig. 2.** The adrenal cortex of a cortisone-treated rat. H. & E. Note severe contraction of the cortex and closely-packed nuclei. H. & E.

**Fig. 3.** The adrenal cortex of a rat injected simultaneously with cortisone and 19-NT. H. & E. Note the maintenance of the cortex. Compare with Fig. 1.

**Fig. 4.** The adrenal cortex of a rat pre-treated with cortisone and allowed to recover for 10 days. H. & E. Note some recovery but the glomerulosa is still thickened in appearance. Compare with Fig. 1.

**Fig. 5.** The adrenal cortex of a normal rat showing the distribution of sudanophilic lipids. Sudan black B.

**Fig. 6.** The adrenal cortex of a cortisone-treated rat. Sudan black B. Note the depletion of lipids from the inner zones but the glomerulosa continues to give intense reactions.

**Fig. 7.** The adrenal cortex of a rat injected simultaneously with cortisone and 19-NT. Sudan black B. Note the concentration of lipids in the fascicular zone. Compare with Figs. 1 and 6.

**Fig. 8.** The adrenal cortex of a rat pre-treated with cortisone and allowed to recover for 10 days. Sudan black B. Note reappearance of lipids in the inner zones but the glomerulosa gives an intense though diffuse reaction. Compare with Fig. 6.

**Fig. 9.** The adrenal cortex of a normal rat. Gomori technique. Note predominantly nuclear reaction for alkaline phosphatase. The glomerulosa is strongly positive.

**Fig. 10.** The adrenal cortex of a cortisone-treated rat. Gomori technique. Note intense nuclear reactions though patchy at certain places. The zona glomerulosa appears as a dark band. Compare with Fig. 9.

**Fig. 11.** The adrenal cortex of a rat injected simultaneously with cortisone and 19-NT. Gomori technique. The nuclear reactions are less intense as compared to Fig. 10. The zona glomerulosa appears almost similar to that of controls. Compare with Fig. 9.

**Fig. 12.** The adrenal cortex of a rat pre-treated with cortisone and allowed to recover for 10 days. Gomori technique. Note the concentration of the enzyme in glomerular zone but the inner zones are less reactive. Compare with Fig. 9.
in the parenchymal cells of the fascicular zone (Fig. 3). The glomerular zone presented a normal appearance and pycnosis of the nuclei in the reticularis was less extensive. The overall histological picture of the cortex was very much similar to that of the controls.

**Sudanophilia.** Cortisone administration caused a marked depletion of lipids from the inner zone of the cortex (Fig. 6). The sudanophobic zone appeared to be rather prominent but the glomerulosa continued to show the presence of large amounts of sudanophilic lipid as in the controls.

The pattern of distribution of sudanophilia in the adrenal cortex of animals treated with 19-NT concurrently with cortisone was more or less similar to that of the controls. The glomerular zone was strongly reactive and was demarcated from the inner zones by a sudanophobic area. The amount of sudanophilic lipids in the inner zones was greater than that of the cortisone treated animals (Fig. 7) though less as compared to that of the controls.

**Alkaline phosphatase.** Cortisone treatment favoured a strong mobilization of alkaline phosphatase in the glomerulosa and as a result this zone appeared as a discrete dark band (Fig. 10). The parenchymal cells and the endothelium of the vascular sinusoids gave a strong positive reaction for the enzyme though in some areas the staining was faint and patchy.

The overall pattern of distribution and concentration of alkaline phosphatase in the adrenal cortex of cortisone plus 19-NT treated animals approached that of the controls. The concentration of the enzyme in the glomerular zone was less and such was also the case with the rest of the cortex (Fig. 11).

**Experiment III. The effect of 19-NT on the recovery of the adrenal cortex following the cessation of cortisone treatment**

**Adrenal weight.** On cessation of cortisone treatment the adrenal weight showed some recovery during the subsequent ten day period though the control value was not reached. This was evident from a comparison of the two groups of cortisone treated animals (Table 1, cortisone-prevention vs. cortisone-recovery). The absolute and relative adrenal weights of the recovery group was greater (P < .001) than those of the prevention group though significantly less (P < .001) than those of the controls. 19-NT therapy in animals pre-treated with cortisone did not help in the recovery of the adrenals and this was clearly indicated by the almost similar weight of the adrenals (both absolute and relative) in the two cortisone pre-treated groups (Table 1, cortisone-recovery vs. cortisone plus 19-NT-recovery).

**Ascorbic acid.** The ascorbic acid content of the adrenals instead of showing signs of recovery, diminished even further on withdrawal of cortisone treatment. In fact, the adrenal ascorbic acid level of cortisone treated animals sacrificed on the eleventh day was significantly higher (P < .001) than that
of the group killed on the twenty-first day (Table 1, cortisone-prevention vs. cortisone-recovery).

19-NT therapy did not influence the ascorbic acid content of the adrenals of animals which were allowed to recover from the noxious effects of cortisone (Table 1).

Gross histology. On discontinuation of cortisone treatment the gross histological features of the adrenal cortex showed indications of recovery as the zonal demarcations became discernible again (Fig. 4). The entire cortex appeared to be wider and the parenchymal cells of the inner zones were less shrunken with normal nuclei. Nevertheless, the histological picture of the cortex was not quite comparable to that of the controls as the nuclei in some parts of the inner zones were still closely packed and the glomerulosa continued to be rather thickened in appearance.

19-NT therapy did not improve the histological features of the cortex of animals pre-treated with cortisone.

Sudanophilia. There was a reaccumulation of sudanophilic lipids in the inner zones of the cortex on cessation of cortisone administration but the overall concentration was still much less than in the controls. However, the zona glomerulosa continued to give an intense though somewhat diffuse reaction (Fig. 8).

The distribution and concentration of sudanophilic lipids in the adrenal cortex of animals pre-treated with cortisone but given 19-NT therapy subsequently, were similar to the above.

Alkaline phosphatase. There was an overall improvement in the pattern of distribution of alkaline phosphatase in the adrenal cortex of animals which were allowed to recover on cessation of cortisone treatment. The glomerular zone showed less enzyme as compared to the cortisone treated group sacrificed on the eleventh day but the concentration was still rather high as compared to the controls (Fig. 12). The distribution of phosphate in the inner zones, however, was more or less similar to that of the controls.

19-NT therapy to cortisone treated animals did not influence the distribution and concentration of alkaline phosphatase in the adrenal cortex.

**DISCUSSION**

One of the unwanted hormonal effects of cortisone which would be desirable to avoid in course of its therapeutic application in certain conditions was atrophy of the adrenal cortex. The magnitude of this untoward effect could be gauged from the recent observation that a sudden cessation of cortisone treatment left the patient in a state of adrenal insufficiency which could lead to collapse and even death (Astwood, 1955). It was therefore considered worth-
while to search for agents which would eliminate this undesirable effect and enlarge the range of usefulness of cortisone. This search was vigorously continued though in the meantime a number of steroidal compounds were reported to exert a protective action on the adrenal cortex of animals treated with cortisone (Pearson, 1951. Zizine, 1952. Winter et al., 1953, and Gaunt et al., 1953). The results of the present study indicated that 19-NT was yet another agent which could be added to the list of such compounds with capacity to protect the adrenal cortex from the ill-effects of cortisone.

A detailed consideration of the present data, however, revealed a number of interesting facts. Thus by the parameters of response considered in this study 19-NT had no influence on the adrenal cortex when administered alone in a dosage range varying from 0.5 to 3 mg. Nevertheless, concurrent administration of this compound with cortisone appeared to prevent the characteristic atrophic changes in the cortex consequent upon cortisone injection. However, such adrenal-maintaining capacity of 19-NT seemed to be somewhat limited (at least in the dosage in which it was used with cortisone) as some residual effects of cortisone treatment could still be detected in the cortex (see Table 1, experiment II, and Figs. 1–9). Whether the dosage used was rather inadequate for a full display of its sparing action on the adrenal cortex could not be said from the data at hand. But there was no doubt that 19-NT did not repair ponderal and other manifestations in the cortex on discontinuation of cortisone administration.

The mechanism whereby 19-NT exerted its adrenal-maintaining effect merited a consideration. Theoretically, it was possible that this compound antagonized the blockage of production or release of corticotrophin by the hypophysis which was known to occur invariably after cortisone administration (Sayers, 1950). However, the situation was not so simple because 19-NT failed to influence the adrenal cortex when administered independently of cortisone. Further, Winter et al. (1953) clearly demonstrated that MAD was equally effective against cortisone both in intact and hypophysectomized animals. This observation eliminated the possibility of an opposing action of MAD against the corticotrophin-suppressing action of cortisone and thereby complicated the issue to such an extent that it was not possible to arrive at any definite conclusion (Winter et al., 1953, and Gaunt et al., 1953). The non-responsiveness of the adrenal cortex of normal animals to 19-NT would also suggest that cortisone was antagonized by this compound at a site other than the hypophysis. Nevertheless, this point called for further elucidation by careful experimental analysis.

The recovery of the adrenal cortex on discontinuation of cortisone treatment revealed certain facts which should receive a passing comment. It was interesting that while morphological and histochemical features of the gland tended to show some recovery, the ascorbic acid level continued to drop even
further on cessation of cortisone administration (see Table 1). Bodansky & Money (1954) observed a similar fall in ascorbic acid concentration of the rat adrenal on discontinuation of cortisone treatment which, however, was followed by a gradual rise to attain the control level in course of time. This characteristic drop in adrenal ascorbic acid concentration during the post-treatment period perhaps indicated a release of corticotrophin at an accelerated rate ('rebound phenomenon?') in order to hasten the recovery of the cortex from the ill-effects of cortisone.

SUMMARY

1. 19-nortestosterone when injected alone in graded doses had no effect on the adrenal cortex of rats.
2. Simultaneous administration of this compound protected the adrenal cortex from the noxious effects of cortisone. However, the adrenal-sparing capacity of 19-NT in the dosage used seemed to be somewhat limited as some residual effects of cortisone treatment were still detectable in the cortex.
3. 19-NT therapy in animals pre-treated with cortisone did not hasten the repair of the adrenal cortex.

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REFERENCES