THE EFFECT OF PRE-PUBERTAL GONADECTOMY ON THE ADRENAL GLANDS

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SUMMARY

In the female but not the male rat, the effect which gonadectomy has on the weight of the adrenal glands is conditioned by the functional state of the gonads at the time of their removal.

Recent studies of the influence of sex hormones on the adrenal glands of rats show that oestrogens may increase and androgens decrease the response of the adrenal glands to ACTH [Carter, 1956; Gompertz, 1958]. These findings suggest that the gonadal hormones are responsible for the difference that is observed in the size of the adrenal glands of the two sexes. If this inference is correct, and given that the secretion of sex hormones before puberty is negligible, it would follow that there would be no difference in the size of the adrenal glands of adult male and female rats whose gonads had been removed before puberty.

Contrary to expectation, a preliminary study showed that the adrenal glands of female rats which had been spayed when immature and killed 3 months later were not smaller than those of intact litter-mates. The present experiments were therefore designed to check the conclusion that gonadectomy does not have the same effect on the adrenal gland in immature as in adult female rats, and to see whether a corresponding difference in response to pre- and post-pubertal gonadectomy also occurs in the male.

The gonads were removed from animals of both sexes before and after puberty, and the weights of the adrenal glands compared, at different post-operative periods, with those of control animals. An attempt was also made to induce ‘artificial puberty’ by giving ovarian hormones to pre-pubertally spayed female rats, and to compare the effect on the adrenal glands of stopping the treatment with that associated with ovariectomy.

MATERIALS AND METHODS

Animals

Two hundred and sixty-nine female rats derived from seventy-seven litters and one hundred and twenty-eight male rats derived from thirty litters (Birmingham Strain) were used. At the start of the experiment, the females varied between 18 and 57 days in age, and between 22 and 128 g in weight; the males varied between 25 and 70 days in age, and between 29 and 189 g in weight.
Arrangement of experiments

Female rats. The arrangement of the experiments is shown in Tables 1 and 2.

The object of Expt. 1 (Table 1) was to check the preliminary observation that the adrenal glands of animals spayed when immature (aged 3 weeks) and killed when adult (3 months after operation) do not involute.

The purpose of Expt. 2 (Table 1) was to compare the growth of the adrenal glands of animals that had been spayed at the age of 3–3½ weeks with that of litter-mates which had been ‘control-operated’ (see below). The ages at which the animals were killed varied between 35 and 75 days. (The age at which animals of this strain undergo puberty, as judged by the breakdown of the vaginal closure membrane, is 41 days [Mandl & Zuckerman, 1952].)

An attempt was next made (Expt. 3, Table 1) to discover whether the removal of the ovaries a few days after the onset of puberty has the same effect on the adrenal glands as has ovariection in fully mature animals; and correspondingly, whether ovariection immediately before the onset of puberty would be followed by the changes observed in animals spayed at the age of 3 weeks. Eleven litter-groups of four or more females were used. At least one rat in each litter was spayed and a second ‘control-operated’ shortly before the onset of puberty. A third rat from each litter was spayed and a fourth ‘control-operated’ shortly after the breakdown of the vaginal closure membrane (see Table 1). All the animals were killed 12 weeks after operation, the rats that were operated on before puberty being about a fortnight younger at autopsy than those operated on after puberty. (Carter's [1956] observations on the involution of the adrenal glands after ovariection suggest that a difference of a fortnight in age would contribute less to the variability of the results than a corresponding difference in the experimental period.)

Expt. 4 (Table 1) was designed to determine the effect of oestrogen on the size of the adrenal glands in animals which had been spayed at the age of 3 weeks. The dose of oestradiol dipropionate and the length of treatment were the same as in Carter's [1956] study on adult rats.

In the remaining three experiments (5–7, Table 2), ‘artificial puberty’ was induced by means of ovarian hormones administered at about the age when puberty occurs naturally. Control animals were injected with arachis oil (Expts. 5 and 6, Table 2). The treatment was stopped after a variable period. In Expt. 5 the dose of oestrone was constant and injections were given for 12 days. In Expt. 6 the dose was gradually increased and then reduced. In the final experiment, a constant dose of oestriol was given daily, and progesterone on every 4th day (Expt. 7, Table 2).

Male rats. The animals were arranged in litter-pairs; one animal was castrated and the other ‘control-operated’ (see below). The ages at which the operations were performed were 25, 30, 40, 45, 55 and 70 days. The post-operative period varied between 5 and 75 days, and the animals were killed in groups at the ages of 30, 35, 40, 45, 50, 60, 70, 80, 90 and 100 days.

Experimental procedures

Anaesthesia. All operations were performed under anaesthesia induced by means of tribromoethanol (‘Avertin’).
Table 1. Arrangement and results of Experiments 1–4

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Age at operation (days)</th>
<th>Post-operative period</th>
<th>No. of rats</th>
<th>Hormones given</th>
<th>Wt. of adrenal glands (mg)</th>
<th>Wt. of adrenal glands (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18–21</td>
<td>12 weeks</td>
<td>21</td>
<td>None</td>
<td>59.4</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>19–24</td>
<td>11–56 days</td>
<td>33</td>
<td>None</td>
<td>See Fig. 1</td>
<td>27</td>
</tr>
<tr>
<td>3</td>
<td>35–38</td>
<td>12 weeks</td>
<td>14</td>
<td>None</td>
<td>53.6</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>48–57</td>
<td>12 weeks</td>
<td>13</td>
<td>None</td>
<td>47.2</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>12 weeks + 12 days'</td>
<td>injections</td>
<td>31</td>
<td>10 µg ODP</td>
<td>56.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>26</td>
<td>arachis oil</td>
<td>54.4</td>
<td></td>
</tr>
</tbody>
</table>

ODP: oestradiol dipropionate.

Table 2. Arrangement and results of Experiments 5–7

<table>
<thead>
<tr>
<th>Expt.</th>
<th>No. of rats</th>
<th>Age at operation (days)</th>
<th>Hormones given</th>
<th>Daily dose (µg)</th>
<th>Length of treatment (days)</th>
<th>Age at start of treatment (days)</th>
<th>Age at autopsy (days)</th>
<th>Vaginal closure membrane</th>
<th>Wt. of adrenal glands (mg)</th>
<th>Wt. of adrenal glands (mg)</th>
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</thead>
<tbody>
<tr>
<td>5</td>
<td>23</td>
<td>21–25</td>
<td>Oestrone</td>
<td>5</td>
<td>12</td>
<td>38–42</td>
<td>105–109</td>
<td>Opened at 42–46 days</td>
<td>60.3</td>
<td>Closed in 8/12; pin-hole opening in 4/12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Oil)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>23</td>
<td>21–25</td>
<td>Oestrone</td>
<td>1</td>
<td>10</td>
<td>35–37</td>
<td>124–126</td>
<td>Opened at 49–51 days</td>
<td>62.6</td>
<td>Closed in 8/10; pin-hole opening in 2/10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Oil)</td>
<td>2</td>
<td>4</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10</td>
<td>4</td>
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<td></td>
<td>1</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>14</td>
<td>21–25</td>
<td>Oestriol + pro-gesterone (2.5 µg) every 4th day</td>
<td>10</td>
<td>24</td>
<td>36–37</td>
<td>128–129</td>
<td>Opened at 40–41 days</td>
<td>57.8</td>
<td></td>
</tr>
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</table>
Ovariectomy and 'control-operation'. The ovaries were removed through two dorso-lumbar incisions. In the control animals, dorso-lumbar incisions were made as for ovariectomy. The ovaries were touched with the forceps but not removed, and the incisions closed in the usual way.

Castration and 'control-operation'. The testes were removed through bilateral incisions in the scrotum. Similar incisions were made in the control animals, the testes were touched with forceps but neither damaged nor removed, and the incisions closed.

Vaginal smears. The animals were examined daily, and a record made of the date when the vaginal closure membrane broke down. In the case of animals injected with ovarian hormones in order to induce 'artificial puberty', vaginal smears were taken daily for part of the experimental period (Expts. 5–7).

Injection of hormones. The hormone preparations were dissolved in arachis oil (oestril was dissolved in propylene glycol) so that the daily dose was contained in 0.1 ml. Control animals were injected with 0.1 ml of the solvent.

Autopsy. The animals were weighed and then killed by chloroform vapour. The adrenal glands and the uterus (or accessory sex organs in the male) were dissected from adjacent tissue and weighed.

RESULTS

Female rats

The adrenal glands of the animals that had been ovariectomized at the age of 18–21 days and killed 3 months later (Expt. 1, Table 1) weighed about the same as those of the operated controls (means 59.4 and 57.2 mg respectively; \( P = 0.4 - 0.3 \)). Ovariectomy in the adult, on the other hand, is known to be followed by a significant decrease in the weight of the adrenal glands. In two separate experiments on adults belonging to the same strain and also killed 3 months after operation, the adrenal glands of the spayed animals weighed 40.9 and 45.2 mg; those of the corresponding controls weighed 51.6 and 51.9 mg respectively (total of fifty-four animals [Mandl & Zuckerman, 1956]). The means of 40.9 and 45.2 mg were significantly lower than the mean for the spayed animals (59.4 mg) in the present experiment (\( P < 0.001 \)).

Of the eleven litter-pairs (one ovariectomized; one control) that were killed before the vaginal closure membrane had broken down, the adrenal glands of the spayed animals were larger in seven and smaller in the remaining four. Of the sixteen pairs of animals killed after the control partner had become sexually mature, the adrenal glands of the spayed animal were larger in nine (Fig. 1). Estimates were made of the growth-rates of the adrenal glands of the spayed and control animals, and the slopes were found not to differ significantly from each other (spayed: \( b = 123.4; \) controls: \( b = 138.0; P = 0.2 - 0.1; \) Expt. 2).

The mean weight of the adrenal glands of the animals which were ovariectomized shortly before puberty (Table 1, Expt. 3; age at operation 35–38 days) was 53.6 mg; that for their operated controls was 53.5 mg. The mean weight of the adrenal glands of animals ovariectomized soon after puberty was 47.2 mg. This differed significantly from the corresponding figure of 54.0 mg for their operated controls (\( P = 0.01 - 0.001 \)). The difference between the means for the animals spayed before and after puberty (53.6 and 47.2 mg respectively) was also significant (\( P = 0.01 - 0.001 \)).

The results of the first three experiments thus imply that the effect which gonad-
ectomy has upon the size of the adrenal glands of female rats is influenced by the age at which the operation is performed, and that the response changes at puberty.

Carter [1956] observed that the adrenal glands of spayed rats receiving oestrogen were consistently heavier than those of spayed controls injected with oil (means 55.6 and 48.8 mg respectively; \( P < 0.001 \)). Under the same experimental conditions, and using the same strain of animals, treatment with oestrogen had no effect on the adrenal glands of rats spayed before puberty (Table 1, Expt. 4; mean weights for oestrogen- and oil-injected animals were 56.6 and 54.4 mg respectively). However, the dose of oestrogen was sufficient to cause a consistent increase in the weight of the uterus and pituitary, and a decrease in body weight.

The doses of ovarian hormones used to induce ‘artificial puberty’ were sufficient to bring about the breakdown of the vaginal closure membrane in all animals at ages ranging from 40 to 51 days (Expts. 5–7, Table 2). They were also sufficient to cause an increase in the weight of the uterus and the pituitary. They had no effect, however, on the size of the adrenal glands (autopsy some 10–12 weeks after the end of treatment; Table 2). In none of the experimental animals given ovarian hormones were the adrenal glands as small as those of female rats of the same strain which had been spayed after puberty and killed 3 months later.

**Male rats**

As judged by the growth of the accessory sex organs, the onset of puberty and the production of androgen in the male occurs at the age of 50–55 days.

The adrenal glands of castrated animals which were killed before reaching the age of 50 days weighed about the same as those of control litter-mates (\( P > 0.4 \)). In contrast, castrated animals which were more than 60 days old at autopsy had consistently larger adrenal glands than their operated controls, whether they were operated on before or after the onset of puberty (\( P < 0.001 \); Fig. 2).
ADRENAL GLANDS AFTER GONADECTOMY

Fig. 2. Growth of adrenal glands in gonadectomized and control male rats.

DISCUSSION

The present results indicate that the effect of gonadectomy on the adrenal glands of female rats is conditioned by the functional state of the ovaries at the time of their removal. The observation that the adrenal glands of animals spayed before puberty grow at the same rate as those of control litter-mates, whereas post-pubertal ovariectomy is followed by adrenal involution, is of particular interest.

The difference between the two sexes in the size of the adrenal glands, which first becomes manifest as puberty approaches, was originally accounted for on the hypothesis that oestrogens secreted by the ovaries after puberty stimulate adrenal growth either directly, by increasing adrenocortical sensitivity to adrenocorticotropic hormone (ACTH), or indirectly, by increasing the output of ACTH from the anterior pituitary. It was correspondingly assumed that the growth of the adrenal glands of the male was at least partially controlled by testicular secretions. These hypotheses alone cannot account for the present observation that the adrenal glands grow more quickly in gonadectomized females than in gonadectomized males of corresponding ages (slopes: spayed females, 123.4; castrated males, 102.8; $P \approx 0.05$; cf. Figs. 1 and 2). This latter finding would imply that the adrenal glands of the female are inherently larger than those of the male, even after elimination of any possible effect exerted by sex hormones.

The present experiments also show that the response of the adrenal glands of spayed animals to injected oestrogen depends upon the functional state of the ovaries at the time of their removal. In adult animals, adrenal glands which had previously undergone atrophy after ovariectomy may be restored to their normal size by the administration of oestrogen. In animals spayed when immature, on the other hand, oestrogen exerts no effect on the size of the adrenal glands.
Finally, the present observations indicate that the withdrawal of ovarian hormones, given to induce ‘artificial puberty’ in previously ovariectomized rats, does not lead to involution of the adrenal glands which normally follows ovariectomy in the adult. This observation is not surprising, since it is hardly likely that the injection of oestrogen alone would reproduce the complex physiological process of puberty which is primarily initiated by changes in pituitary function.

The present results thus lead to the following tentative conclusions: (i) at the time of puberty some substance(s), presumably of ovarian origin, irreversibly change(s) the reaction of the adrenal glands to gonadectomy; (ii) the process makes the adrenal glands more reactive to ACTH and/or stimulates the pituitary to increase its production of ACTH; (iii) only after this ‘pubertal’ stimulus has taken effect does the withdrawal of ovarian hormones from the circulation bring about a reduction in the adrenocortical responsiveness to ACTH and/or the hypophysial output of ACTH.

In contrast, the adrenal effects of gonadectomy in the male do not appear to depend on the secretory activity of the testes at the time of their removal. The present observations may be accounted for on the basis of the hypothesis, postulated by Gompertz [1958], that androgens decrease the size of the adrenal glands by impairing adrenocortical sensitivity to ACTH and/or the output of ACTH by the pituitary. The adrenal glands of castrated males are thus either stimulated by more ACTH, or able to react to ACTH more vigorously than those of their non-castrated controls which, after the onset of puberty, are subjected to the influence of testicular secretions.

We are indebted to Prof. Sir Solly Zuckerman and Prof. P. L. Krohn for their valuable criticism and help. We would also like to thank Dr W. J. Tindall, Organon Laboratories Ltd, for the supply of oestradiol dipropionate and oestriol.

REFERENCES