EFFECTS OF OESTRADIOL BENZOATE ON CORPORA LUTEA IN RATS BEARING PITUITARY AUTOGRAFTS

By

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ABSTRACT

Female rats received pituitary autotransplants beneath the kidney capsule at 11 to 12 weeks of age or were hypophysectomized only at the metoestrous stage of the cycle. Subcutaneous injections of oestradiol benzoate (OB) were started 30 to 40 days following surgery in the first 3 experiments. In Experiments 1 and 2, Series 1 injections consisted of 50, 50 and 25 μg of OB given subcutaneously on days 0, 3 and 5. Series 2 injections were the same as Series 1 but given on days 16, 19 and 21. Pituitary grafts were removed from half of the rats on day 15 in Experiment 1. Ovarian weights were obtained on day 28. In Experiment 3, the dose of OB was raised to 100 μg per injection giving a total of 300 μg for each series. In Experiment 4, hypophysectomized rats without pituitary autotransplants were given Series 1 and 2 OB injections at the level of 125 μg per series. In Experiment 5, subcutaneous injections of OB were started 5 to 7 days following pituitary autotransplant. Rats were injected daily with 50 μg for 5, 10, 20, 40 and 80 days, with autopsies following 4 or 5 days after the last injection. In Experiment 6, 50 μg was injected daily in hypophysectomized rats without pituitary transplants for 5 and 20 days.

The immediate effect of OB injections into rats bearing pituitary autografts was a significant (P < 0.01) increase in ovarian weight. Long term treatment (> 40 days) caused a significant (P < 0.05) decrease in ovarian weight. Short term treatment followed by a 23 or 35 day period of no treatment gave an even greater decrease in ovarian weight (P < 0.01). Hypophysectomized rats showed no effect on ovarian weights with similar OB treatments, indicating the importance of the pituitary gland in this response. Removal of the autotransplanted pituitary gland 10 days after...
the first series was completed, had no apparent effect on regression of the corpora lutea. There was no effect on adrenal weight in any of the experiments.

It is suggested that oestrogens initiate a process which ultimately results in luteal regression in rats bearing pituitary autografts.

When the anterior lobe of the pituitary gland of the mature female rat is separated from hypothalamic control such as by transplantation beneath the kidney capsule or to other regions (Everett 1954, 1956) or by severance of the pituitary stalk (Nikitovitch-Winer 1965), corpora lutea can be maintained and may remain functional for months. Follicles are few and small in size, interstitial tissue becomes scarce and degenerate shortly after pituitary transplantation. Thus, ovarian weight predominantly reflects the weight of corpora lutea. Uterine traumatization results in a decidual response (Everett 1954; Nikitovitch-Winer 1965) whereas treatment with an oestrogen causes a copious discharge of vaginal mucus and vaginal smears in response to oestrogen treatment are predominantly leucocytic (Desclin 1950; Everett 1956; Nikitovitch-Winer 1965). These effects all indicate progesterone secretion by functional corpora lutea.

In addition to a vaginal mucification and hypertrophy following a series of three oestradiol benzoate injections over a period of five days, a marked increase in ovarian weight has been observed in rats bearing pituitary autografts (Everett 1956; Rothchild 1965). Repetition of the oestrogen test following removal of the pituitary graft provoked persistent vaginal cornification and at autopsy, the corpora lutea were in a state of advanced regression (Everett 1956).

We repeated the experiments of Everett (1956) in the hope of setting up model conditions whereby corpora lutea would be in a state of rapid regression. In our initial studies, it became apparent that after a sufficient length of time following OB treatment, the corpora lutea were in a state of regression even if the pituitary graft was not removed. The present study was directed at investigating both the increase in size of corpora lutea and their subsequent regression following treatment with oestradiol benzoate in rats bearing pituitary autotransplants.

MATERIALS AND METHODS

Normal cycling 11–12 week old rats\(^a\), maintained in 14 hours of light daily, received pituitary autotransplants at metoestrus in a single stage operation. The hypophysis, removed by the parapharyngeal approach, was placed beneath the lower pole of the

\(^a\) CD strain of Charles River, Inc., North Wilmington, Massachusetts.
left kidney. Other rats of similar age were hypophysectomized only. Following surgery, all animals received 5% glucose in their drinking water for the duration of the experiment.

During the course of these experiments, vaginal smears obtained by lavage, without staining, were followed each morning for 5–7 days per week. At the termination of each experiment, all animals were killed by exposure to CO₂. Ovarian, adrenal and uterine weights were obtained after the organs were freed of fat and connective tissue and blotted dry. Tissues were fixed in Bouin’s solution, 6 μ sections were mounted on slides and stained with eosin-haematoxylin. Pituitary grafts were fixed in 4% formaldehyde solution and sectioned at 4 μ. Slices of grafts mounted on slides were stained with periodic acid Schiff’s (McManus 1946) and counterstained with orange G.

At each autopsy, the sella turcica was examined with the aid of an illuminated magnifier for pituitary remnants and in doubtful cases serial sections of the sella turcica decalcified in 5% trichloroacetic acid and stained with eosin-haematoxylin, were examined histologically. All animals with pituitary remnants were excluded from the results.

**Experiments 1–4**

**Graft Ablation and Oestradiol Benzoate (OB) Treatments**

Subcutaneous injections of OB were started 30–40 days following surgery in accordance with the schedule outlined in Table 1. Series 1 injections consisted of 50 μg contained in 0.2 ml sesame oil administered on days 0 and 3, and 25 μg on day 5. Series 2 injections were the same as Series 1 but administered on days 16, 19 and 21, respectively. Controls received similar volumes of sesame oil subcutaneously. In Experiment 1 where pituitary grafts were removed, ablation was on day 15. A graft was removed together with a thin strip of adhering kidney and capsular tissue. Control rats received sham surgery and similar damage to the kidney. On day 28, adrenal, ovarian and uterine weights were obtained and tissues were preserved for histological processing.

The schedule of treatment and the design of Experiment 3 was the same as that of Experiments 1 and 2. Only the dosage was increased to 100 μg per injection to give a total of 300 μg for each series of injections. In Experiment 4, hypophysectomized rats without pituitary grafts received OB in accordance with the schedule and dosage in Experiments 1 and 2.

| Table 1. |
| Schedule of treatments. |
| Experiments 1–4 |
| Day of treatment |

<table>
<thead>
<tr>
<th>0</th>
<th>3</th>
<th>5</th>
<th>15</th>
<th>16</th>
<th>19</th>
<th>21</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Series one injections</td>
<td>Graft ablation</td>
<td>Series two injections</td>
<td>Autopsy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Treatments were started 30–40 days after pituitary autotransplantation.

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Daily Injection of OB for 5, 10, 20, 40 and 80 Days

Treatments were initiated at a considerably earlier period after pituitary transplantation. In our earlier experiments, there was a greater variation in ovarian weights between animals because of spontaneous luteal regression that occurred in some control animals. In Experiment 5, treatments were started 5–7 days after pituitary transplantation because by this time vascularization of the surviving portion of the graft is extensive. All animals with any signs of infection or disease were excluded from the experiment. A control sesame oil treated rat was paired with each treated rat, both of which were operated on the same day, or in a few cases, a day apart.

In Experiment 5, rats bearing pituitary autografts were subcutaneously injected each morning with 50 µg of OB in 0.1 ml sesame oil for a period of 5 to 80 days. Autopsy followed 4 or 5 days after the last injection. In one group, rats were injected for only 10 days and autopsy was performed 35 days after the last injection.

In Experiment 6, rats that were hypophysectomized without receiving pituitary transplants, were injected each morning with 50 µg for 5 or 20 days. Treatment was started 2 days after hypophysectomy with those that were injected for 5 days or on the day of hypophysectomy with those that were injected for 20 days. Autopsies were performed 4 or 5 days following the last injection.

RESULTS

Tables 2 to 7 present the mean values of organ weights and standard errors of the means. Statistical significance was determined by analysis of variance. In Tables 2 to 4 organ weights were compared for main effects and interactions using $2 \times 2$ factorial arrangements of treatments.

Experiments 1–4

Graft Ablation and OB Treatment

In Experiment 1, there was a highly significant ($P < 0.01$) decrease in ovarian weight due to the treatment (see Table 2). Under the conditions of this experiments, there was no effect of graft ablation on ovarian weight and no interaction between graft ablation and OB treatment. As expected, OB caused a highly significant ($P < 0.01$) increase in uterine weight, whereas graft ablation had no effect on uterine weight. Neither of the two treatments had an effect on adrenal weight.

In Experiment 2, an attempt was made to establish whether the effect of OB was due to Series 1 or Series 2 injections. It should be recalled that autopsy followed Series 1 injections by 23 days, Series 2 injections by 7 days. There was no graft removal or sham surgery involved in this study. Table 3 indicates that there was a highly significant ($P < 0.01$) decrease in ovarian weight due to Series 1 injections but no effect of Series 2 injections. There was no inter-
### Table 2.
Effect of OB injections and ablation of the pituitary graft on organ weights.

#### Experiment 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of animals per group</th>
<th>Mean organ weight (mg) ± standard errors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection</td>
<td></td>
<td>Ovary</td>
</tr>
<tr>
<td>Control</td>
<td>Sham</td>
<td>13</td>
</tr>
<tr>
<td>Control</td>
<td>Graft ablation</td>
<td>13</td>
</tr>
<tr>
<td>OB</td>
<td>Sham</td>
<td>13</td>
</tr>
<tr>
<td>OB</td>
<td>Graft ablation</td>
<td>14</td>
</tr>
</tbody>
</table>

Total OB injected was 125 µg/Series. All animals on experiment received Series 1 and 2 injections.

### Table 3.
Effect of Series 1 and Series 2 OB injections on organ weights in rats bearing pituitary autografts.

#### Experiment 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of animals per group</th>
<th>Mean organ weight (mg) ± standard errors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ovary</td>
</tr>
<tr>
<td>Series 1</td>
<td>17</td>
<td>22.8 ± 1.7</td>
</tr>
<tr>
<td>Series 2</td>
<td>16</td>
<td>40.1 ± 4.6</td>
</tr>
<tr>
<td>Series 1 and 2</td>
<td>17</td>
<td>24.7 ± 3.7</td>
</tr>
<tr>
<td>Control</td>
<td>17</td>
<td>35.8 ± 2.5</td>
</tr>
</tbody>
</table>

action between Series 1 and Series 2 injections. As expected, uterine weights were significantly higher \((P < 0.01)\) due to Series 2 treatment. A residual effect due to Series 1 injections of this long acting oestrogen on uterine weight was apparent and the difference due to Series 1 treatment was statistically significant \((P < 0.01)\). There was no interaction between Series 1 and 2 treatments on uterine weight. None of the treatments had an effect on adrenal weights.

Because we failed to obtain an increase in ovarian weight due to OB treatment, the dose was raised to 100 µg for each injection to give a total of 300 µg for each series in Experiment 3. As Table 4 indicates, Series 1 injections again caused a highly significant \((P < 0.01)\) decrease in ovarian weight. On the
Table 4.
Effect of Series 1 and Series 2 OB injections on organ weights in rats bearing pituitary autografts.
Total OB = 300 µg/Series
Experiment 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of animals per group</th>
<th>Mean organ weight (mg) ± standard errors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ovary</td>
</tr>
<tr>
<td>Series 1</td>
<td>9</td>
<td>21.9 ± 2.9</td>
</tr>
<tr>
<td>Series 2</td>
<td>9</td>
<td>59.7 ± 5.7</td>
</tr>
<tr>
<td>Series 1 and 2</td>
<td>9</td>
<td>36.2 ± 8.4</td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>42.1 ± 1.6</td>
</tr>
</tbody>
</table>

other hand, Series 2 injections this time caused an increase in ovarian weight which was significant (P < 0.05). The combination of Series 1 and 2 injections tended to give ovarian weights intermediate to that attained by the two separate treatments. Uterine weights were highly elevated due to Series 2 treatment (P < 0.01). There was a large residual effect on uterine weight due to Series 1 treatment which was highly significant (P < 0.01). Again, there was no interaction between Series 1 and 2 injections. Even at this high level of oestrogen treatment, there were no statistically significant differences in adrenal weights.

In Experiment 4, the effect of a combined treatment of Series 1 and 2 OB injections on hypophysectomized rats without pituitary grafts was tested. Treatment with OB was started immediately following hypophysectomy because

Table 5.
Effect of Series 1 and Series 2 OB injections on organ weights in hypophysectomized rats.
Experiment 4

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Number of animals per group</th>
<th>Mean organ weight (mg) ± standard errors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ovary</td>
</tr>
<tr>
<td>OB</td>
<td>9</td>
<td>27.2 ± 1.0</td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>32.0 ± 2.5</td>
</tr>
</tbody>
</table>

* Treatment started immediately following hypophysectomy. Total OB injected was 125 µg/Series. All animals on experiment received Series 1 and 2 injections.
** Significantly different from control, P < 0.01.
luteal regression would be expected to commence following removal of the pituitary. As seen in Table 5, there were no statistically significant effects on ovarian or adrenal weights. There was the expected increase in uterine weight due to oestrogen treatment which was highly significant ($P < 0.01$).

**Experiment 5**

*Daily Injection of OB for 5, 10, 20, 40 and 80 Days in Rats with Pituitary Autotransplants*

As indicated in Table 6, peak ovarian weight was obtained after 5 or 10 days of treatment with 50 $\mu$g per day. Up to 20 days, the increase in ovarian weight over control was highly significant ($P < 0.01$). At 40 days, ovarian weight of the treated group fell below that of control but this difference was not statistically significant. At 80 days, ovarian weight of the treated group fell below that of the control group and the difference was statistically significant ($P < 0.05$). Every rat in the 80 day group had ovaries with corpora lutea in a stage of complete regression. The pituitary grafts in the 80 day rats appeared highly enlarged but no weights were taken of the grafts at autopsy.

In numerous experiments involving a total of 3 to 6 injections, it appeared that sesame oil was without effect on ovarian weight and histology. Therefore, there were no untreated control groups in Experiment 5. It is obvious that there was a progressive decline in ovarian weight with time (Table 6) which may have been caused by continuous long term treatment with sesame oil. Everett (1956) observed no apparent decline in ovarian weight nor in function of corpora lutea 90 days or more following pituitary transplantation.

In group 6 of Table 6, OB was injected for 10 days because such treatment appeared to result in peak ovarian weights. Autopsy was performed 35 days after the last injection because it corresponded temporally to group 4 which received 40 days of injections and sacrificed 4 days after the last injection. The results indicate that this treatment resulted in a highly significant ($P < 0.01$) decrease in ovarian weight. Yet, as indicated in Table 6 for group 4, there was no statistically significant difference in ovarian weight compared to control when the oestrogen was continuously administered for 40 days and autopsy followed 4 days later. Thus, it appears that continued treatment beyond the 10 day period may have moderated the regression of corpora lutea. This essentially agrees with results in Table 4 where at the 100 $\mu$g dose, Series 2 injections appeared to modify the regression pattern of corpora lutea due to Series 1 injections.

As expected in Experiment 5, uterine weights were significantly higher ($P < 0.01$) in all cases due to OB treatment. There were no effects on adrenal weights. Although body weight differences between treated and control groups
Table 6.
Effect of continuous injection of 50 µg OB per day on organ weights and body weight increase in rats bearing pituitary autografts.
Experiment 5

<table>
<thead>
<tr>
<th>Group</th>
<th>Days on OB treatment</th>
<th>Days from last treatment to autopsy</th>
<th>Number of animals per group</th>
<th>Mean organ weight ± standard error</th>
<th>Mean body weight increase ± standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ovary</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OB Treated</td>
<td>Control</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>4-5</td>
<td>6</td>
<td>89.8 ± 8.2**</td>
<td>50.3 ± 3.0</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>4-5</td>
<td>5</td>
<td>97.6 ± 9.9**</td>
<td>49.0 ± 3.6</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>4-5</td>
<td>6</td>
<td>71.6 ± 6.5**</td>
<td>43.5 ± 3.2</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>4-5</td>
<td>6</td>
<td>29.0 ± 4.5</td>
<td>36.7 ± 3.9</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
<td>4-5</td>
<td>5</td>
<td>10.4 ± 1.3*</td>
<td>24.0 ± 5.0</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>35</td>
<td>6</td>
<td>17.6 ± 1.8**</td>
<td>37.7 ± 3.5</td>
</tr>
</tbody>
</table>

* Significantly different from corresponding control, $P < 0.05$.
** Significantly different from corresponding control, $P < 0.01$. 
were not statistically significant after 5 and 10 days, these differences were significant at 20 \( (P < 0.05) \), 40 \( (P < 0.01) \) and 80 \( (P < 0.01) \) days of treatment. Thus, the oestrogen seemed to impair the gradual increase in body weight of rats with pituitary autotransplants under our conditions.

Continuous daily injections of 50 \( \mu \)g OB into hypophysectomized rats without pituitary autotransplants had no effect on ovarian or adrenal weights (see Table 7), although uterine weight was increased to a large extent \( (P < 0.01) \). Prolonged treatment appeared toxic to hypophysectomized rats. There was a sharp loss in body weight \( (P < 0.01) \) and a 33\% mortality (4 of 12 rats) due to treatment for 20 days. Apparently, a pituitary transplant protects a hypophysectomized rat from the toxic effects of moderately high doses of OB.

**Vaginal Smears**

Cell types in vaginal smears resulting from OB treatment in the first two experiments was generally similar to that reported by Everett (1956). The continuous dioestrous smears characteristic of rats bearing pituitary autografts were occasionally interrupted by the appearance of squamous and cornified epithelium for a day or two regardless of whether the rats received Series 1 or Series 2 injections or the combination of both Series 1 and 2 injections. This was accompanied by a copious discharge of vaginal mucus even when vaginal smears displayed squamous and cornified epithelium. After removal of the graft, the treatment resulted in vaginal cornification within 2 or 3 days which usually persisted to autopsy. When the level of OB was increased to 100 \( \mu \)g per injection in Experiment 3, cell types in vaginal smears were similar to that in the preceding experiments in groups that received Series 1 or Series 2 injections only. In the group that received both Series 1 and 2 injections, 5 of 9 rats (56\%) displayed continuous vaginal cornification for 5 or more consecutive days during and after Series 2 injections. However, this did not impair the copious flow of vaginal mucus.

During the first 25–30 days of continuous treatment in Experiment 5, the continuous dioestrous smears were also occasionally interrupted by the appearance of squamous and cornified cells but this reverted to the dioestrous type of smear within 1–4 days. During the first 30 days, vaginas tended to be mucified. Afterward, however, the vaginal smears were almost entirely of the cornified type up to 80 days of oestrogen treatment, indicating a withdrawal of progesterone secretion. Occasionally, the continuously cornified vaginal cells were interrupted by a day or two during which the smears were characteristic of the dioestrous state, usually between 30–40 days of treatment.

**Ovarian Histology**

The increase in size or degeneration of corpora lutea observed histologically generally corresponded with ovarian weight. Degenerating luteal cells were
Table 7.
Effect of continuous injection of 50 µg OB per day on organ weights and body weight increase in hypophysectomized rat. Experiment 6

<table>
<thead>
<tr>
<th>Days on OB treatment</th>
<th>Days after hypophysectomy treatment started</th>
<th>Days after final injection autopsy performed</th>
<th>Mean organ weight ± standard error</th>
<th>Mean body weight increase ± standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ovary</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OB Treated</td>
<td>OB Control</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>553 ± 439**</td>
<td>162 ± 14.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(5)</td>
<td>(4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>32.5 ± 1.7</td>
<td>33.3 ± 1.6</td>
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<td></td>
<td>(5)</td>
<td>(4)</td>
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<td></td>
<td></td>
<td></td>
<td>-10.8 ± 4.5</td>
<td>-5.2 ± 2.3</td>
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<td></td>
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<td></td>
<td>(5)</td>
<td>(4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>34.3 ± 5.4**</td>
<td>+3.2 ± 1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(8)</td>
<td>(11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>45.2 ± 2.5</td>
<td>47.3 ± 2.0</td>
</tr>
<tr>
<td></td>
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<td>33.3 ± 2.2</td>
<td>35.9 ± 1.2</td>
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<td>(8)</td>
<td>(11)</td>
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<td></td>
<td></td>
<td></td>
<td>491 ± 38.3**</td>
<td>108 ± 4.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(8)</td>
<td>(11)</td>
</tr>
</tbody>
</table>

** Significantly different from corresponding control, P < 0.01.
* Number of animals per group.
characterized by pyknotic nuclei which ranged in shape from round to elongated. Nuclear and cytoplasmic volumes were diminished, cell boundaries were less distinct, cells seemed more variable in size and shape; there was cytoplasmic vacuolization and infiltration by connective tissue. The stages of degeneration of corpora lutea based on appearance of cells varied within the same ovary and appeared more advanced in some corpora lutea than in others. In fact, within the same corpus luteum, various degrees of degeneration among luteal cells could be seen in different regions. In the 80 days treated rats of Experiment 5, luteal regression was so advanced that only remnants of previously existing luteal bodies were found. In the 5, 10 and 20 day treated rats of Experiment 5, the diameter of corpora lutea as well as the volumes of luteal cells appeared enlarged. In all other respects, the luteal cells appeared normal and similar to controls. In all cases, follicles became small after pituitary transplantation and interstitial cells degenerated. OB treatment appeared to have no influence on the size or appearance of follicles or on the interstitial cells.

Pituitary Graft Histology

Pituitary grafts of OB treated rats appeared to show an increase in vascularity which was most apparent in the 80 day treated rats. There were no apparent differences in granulation of acidophils between the treated and control groups. There was the usual paucity of PAS-positive cells in all pituitary grafts regardless of treatment, which is characteristic of pituitary grafts in rats. A very conspicuous increase in size of the pituitary graft was observed at autopsy in the 80 day treated rats. Experiments are being planned to further investigate this observation and to measure the increase in volume of pituitary graft caused by OB treatment. Everett (1956) observed a notable increase in numbers of cells with hypertrophied Golgi zones and in frequency of mitotic figures due to OB treatment among the pituitary grafts in his experiments. It may thus be possible to attribute the apparent increase in volume of pituitary grafts in the 80 day treated rats to an increase in cell numbers.

DISCUSSION

The foregoing experiments demonstrate that corpora lutea in rats bearing pituitary autografts regress after an initial increase in size caused by OB treatment. The continued long term stimulus of OB (> 10 days) appears to have modified the regression pattern of corpora lutea when compared to those that were treated for only 10 days. Furthermore, with continuous treatment, up to 80 days, the ovaries became less and less responsive to the luteotrophic effect of OB and finally regressed. Corpora lutea in hypophysectomized rats without
pituitary grafts were not responsive to oestrogen treatment in our experiments and in others previously reported (Everett 1956; Rothchild & Schwartz 1965). Many reports indicate that oestrogens have been associated with a luteotrophic action in cycling, pregnant, pseudopregnant and lactating rats (Everett 1961; Amoroso & Finn 1962; Bogdanove 1966; Rothchild & Schwartz 1965) as well as in rats bearing pituitary autografts (Everett 1956; Rothchild & Schwartz 1965). Oestrogens have been reported to enhance the luteotrophic action of prolactin in the hypophysectomized rat (Desclin 1949).

In the intact rat, oestrogens are known to influence adenohypophysial cyto-
logy, size and secretion pattern (Bogdanove 1964; Everett 1964). In pseudo-
pregnant rats, oestradiol was found to depress LH secretion as judged by
growth of follicles and depressed hypophysial LH content (Rothchild & Schwartz 1965). Yet, under appropriate conditions, oestrogens may stimulate
the release of LH (Bogdanove 1964). Systemic oestrogen administration has been observed to increase pituitary prolactin content and initiate lactation (Meites & Nicoll 1965), whereas implantation of oestrogen into the anterior
pituitary increased prolactin release (Ramirez & McCann 1964).

In ectopic pituitary grafts, the secretion rate of prolactin is considered to be
markedly enhanced. Although not readily detectable, a number of reports indi-
cate that there is a residual secretion of GH, TSH, ACTH and gonado-
trophins (Everett & Nikitovitch-Winer 1963; McCann & Dhariwal 1966). Oestrogens have been reported to elevate the prolactin content of pituitary
autografts (Desclin & Koulischer 1960). Oestradiol has been found to increase
the release of prolactin by rat pituitary glands incubated in vitro (Nicoll & Meites 1964).

The increased size of corpora lutea in oestrogenized rats with pituitary auto-
transplants was initially interpreted as an effect of synergy between luteo-
trophic hormone and oestrogen (Everett 1956) similar to that observed by
Desclin (1949) in the hypophysectomized rat. However, it was later suggested
that this increase in size could have resulted from an increased production of
luteotrophic hormone by the pituitary grafts stimulated by the administration
of oestrogen (Everett & Nikitovitch-Winer 1963).

Testosterone has been reported to prevent a decrease in FSH content in
pituitary homografts which results after a period of time following trans-
plantation. Oestrogen partially counteracted this effect by testosterone (Van
Rees & Wolthuis 1962). LH has been reported to increase the size of corpora
lutea (Macdonald et al. 1966) as well as to be luteolytic (Rothchild 1965) in rats
bearing pituitary autografts. It was suggested that the increase in size of cor-
pora lutea observed one day after a series of LH injections was due to the
stimulation of oestrogen secretion (Macdonald et al. 1966). We have observed
both an immediate increase in size of corpora lutea followed by their re-
gression when examined 35 days after LH treatment. Both effects are attributed
at least in part to the stimulation of oestrogen secretion by the ovaries (unpublished). Rothchild (1965) observed luteal regression 15 days after LH treatment which probably accounts for the differences in results between his experiments and those reported by Macdonald et al. (1966).

It is suggested that under the conditions of our experiments, OB modified the secretion pattern by the pituitary autotransplant in a manner that would ultimately favour luteal regression. The presence of a pituitary graft was essential to obtain this effect since the regression rate of corpora lutea was not affected in hypophysectomized rats without pituitary autotransplants that were similarly treated with OB. However, the possibility that luteal regression was initiated by a direct action of OB on the ovaries which can occur only in the presence of secretions from a pituitary transplant cannot be excluded. In fact, experiments are now in progress to determine if this luteal regression is caused by a direct action on the ovaries, the pituitary transplant, or both.

If it is presumed that the luteal regression in our experiments was due to an effect of OB on the pituitary graft, it may be reasoned that OB enhanced the synthesis and release of prolactin. Furthermore, it may have suppressed the secretion of other gonadotrophins, particularly LH. Although the immediate effect was luteotrophic as indicated by an increase in luteal weight and evidence of enhanced progesterone secretion, it may have set up conditions whereby luteal regression would result following depletion of oestrogen stores. It has been reported that under appropriate conditions prolactin will hasten luteal regression in the hypophysectomized rat (Malven & Sawyer 1966). On the other hand, if it is found that LH is luteolytic by a direct effect on the ovaries as suggested by Rothchild (1965) and that this effect is not necessarily mediated by an enhancement of oestrogen secretion, it may be reasoned that OB treatment caused a temporary surge of LH secretion by pituitary transplants. It is possible that any of these effects on pituitary transplants may have been mediated by an action of the oestrogen on the hypothalamus. There is evidence for hypothalamic influence on pituitary grafts presumably mediated by trophic hormone-releasing factors carried by the vascular system to the site of the transplant (McCann & Dhariwal 1966; Piacsek & Meites 1967; Smith & Davidson 1967).

No effect of OB treatment on adrenal weight was observed in any of our experiments. Oestrogens administered systemically or by hypothalamic implant have caused increases in adrenal weight in male but not in female intact rats (Kitay 1963; Chowers & McCann 1967). On the other hand, oestrogens administered by hypothalamic or pituitary implant (Richard 1965/1966) or by subcutaneous injection (Fonzo et al. 1967) have enhanced ACTH secretion rates in intact female rats. Ectopic pituitary glands are capable of increased secretion of ACTH due to a wide variety of stimuli including physical stresses, blood loss, epinephrine, vasopressin, histamine and urethane administration as well as

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ether inhalation (Cheng et al. 1949; Fortier & Selye 1949; Fortier 1951; Goldberg & Knobil 1957; Martini et al. 1959; Purves & Siret 1967; Stolzenberg et al. 1968).

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