THE EFFECTS OF OVARIAN STEROIDS
ON FOOD AND WATER INTAKE AND BODY WEIGHT
IN THE FEMALE RAT

By

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ABSTRACT

The influence of ovarian steroids on food intake (FI), water intake (WI) and body weight (BWt) was measured under various conditions. Ovariectomy results in an increase in FI and BWt, which plateaued around one month after surgery. Daily injection of 1.5 μg oestradiol benzoate (OB) initiated at this time significantly reduced both FI and BWt. This effect of daily OB treatment on FI is only transitory since the FI returns to normal during OB treatment although the effect on BWt is maintained throughout and beyond OB treatment. Following ovariectomy, WI gradually falls, but is returned to normal by daily OB treatment. When oestrogen treatment is initiated at the time of ovariectomy, the increase in FI and BWt is prevented. In additional ovariectomized rats, 3 μg OB was injected every fifth day with either progesterone or oil administered on the intervening days. Although no influence of progesterone injection (either with OB or alone) was detected, the intermittent injection of OB induced cyclic suppression of FI, and the pattern of FI approached that of the intact cycling female. Adaptation to the intermittent injection of OB was not observed. Finally, OB treatment was found to decrease the increased FI seen during pseudopregnancy by a proportion similar to the effect of oestrogen in the long-term ovariectomized animal. These results suggest that oestrogen, but not progesterone, is the ovarian hormone active in the regulation of intake parameters and body weight in the female rat.

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Fluctuations in food and water intake during the rat oestrous cycle have been described and related temporally with known neuroendocrine correlates of the reproductive cycle (Tarttelin & Gorski 1971). Plasma oestrogen, which peaks shortly before the afternoon of proestrus (Brown-Grant et al. 1970) may provide the stimulus to inhibit food intake during the following dark period. Several investigators have demonstrated changes in intake parameters and in body weight (BWt) following ovariectomy in the female rat. Bogart et al. (1944) described postovariectomy increases in BWt which could be reduced with oestrogen treatment and also reported BWt increases after treatment with progesterone (P), both in the intact and in the ovariectomized rat. Galetti & Klopper (1964) confirmed that ovariectomy increased BWt and demonstrated that larger doses of P would also increase BWt of intact rats, but they found that P treatment was without effect on the BWt of ovariectomized rats. Hervey & Hervey (1964, 1965a) confirmed that P treatment was without effect on the growth of ovariectomized rats and also demonstrated that P treatment in intact animals caused the same changes in BWt as seen in the ovariectomized rat. Hervey & Hervey (1965b) showed further that P treatment would increase BWt in castrated males bearing ovarian grafts, whereas P treatment in intact or castrated male rats was without effect. This work suggests that P has a weight-promoting effect only in the presence of the ovary. The study by Bogart et al. (1944) is a variance with this finding, but recently Rodier (1971) has reconfirmed the work of Hervey & Hervey (1965b) and Galetti & Klopper (1964), although he used very large amounts of P.

The effects of ovariectomy on food intake (FI) are not well represented in the literature. Vallenstein et al. (1969) demonstrated that treatment with oestrogen could reduce the increased FI seen after ovariectomy. The present series of experiments reinvestigates more fully the influence of ovarian steroids on FI, water intake (WI) and BWt in rats housed in a strictly controlled environment so that an accurate estimate could be made of the voluntary daily FI and WI. The experiments were designed to look critically at the effects of various treatments of oestradiol benzoate (OB) and P, either alone or in combination, on FI, WI, and BWt and were conducted mainly on ovariectomized rats to eliminate the possibility of interaction from endogenous ovarian steroid production. These experiments are presented together in an attempt to elucidate whether changes in intake and BWt at oestrus, following ovariectomy, and in pseudopregnancy are due to changes in the levels of oestrogen or P or interactions between these two steroids.

MATERIALS AND METHODS

A total of 74 Sprague-Dawley rats (350–450 g) derived from a closed colony bred at UCLA were housed in individual cages in a light- and temperature-controlled room.
This room was strictly isolated at all times from personnel other than the senior author who made daily visits for one hour during the end of the light period (14 h light, 10 h darkness, lights off at 10 a.m.) to record intake data, BWt and to clean cages weekly. The room was on reversed lighting to facilitate this work schedule. Rats were habituated to this room for several weeks before measurements were made.

The rats were provided with an excess of ground Purina Rat Chow which was weighed out daily to the nearest 0.1 g. Fresh tap water was provided daily in glass bottles calibrated in 2-ml intervals; WI was determined by direct reading of the water levels. Rats were accustomed to being handled daily for subcutaneous injections given at the nape of the neck in a volume of 0.025 ml of sesame oil.

Since the rats varied in their BWt and in the amount of food they consumed individually, a data transformation was used to obtain a more reliable estimate of group behaviour. A sample of data, usually a week or two before a treatment or surgical procedure was performed, was taken as baseline and all subsequent data were expressed as a percentage deviation, positive or negative, from this pretreatment or control period. The authors believe this transformation is more valid than other possibilities such as selection on the basis of similar BWt or growth pattern which could introduce experimental bias at the outset. All rats were assigned to their experimental groups on a random basis. Tests of significance were made using Student's t-test on the transformed data.

Since four different experiments were carried out, specific experimental procedures will be described with the results. Also, for clarity, a brief discussion of the results will be presented for each experiment.

RESULTS AND DISCUSSION

Experiment 1. Effect of oestrogen replacement on food and water intake and body weight in the ovariectomized rat

Sixteen rats initially in the weight range 260–300 g were used in this study. Seven were normal unoperated controls. Nine rats were ovariectomized by a conventional flank approach under ether anaesthesia and their intakes studied for 32 days prior to treatment with oestrogen. Tarttelin & Gorski (1971) have shown that by this time the FI of the ovariectomized rat has plateaued. The ovariectomized rats were divided into three groups of three subjects each which received sesame oil vehicle, daily injections of 1.5 µg OB, or a single injection of 3 µg OB.

Although daily measurements were made of intake parameters and BWt, in order to condense the time scale, the arithmetic mean value was calculated for eight-day consecutive periods. The FI and BWt changes are illustrated in Fig. 1, and Table 1 presents the WI data which are not illustrated because of great variability. The data are expressed as percentage deviations from the 16-day period prior to ovariectomy. Since this preoperative period by definition is the zero baseline, it is not illustrated. The FI of the control intact rats fluctuated about the 10% level and this increase above the baseline is due to the growth of these rats which continued throughout the experiment.

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The influence of ovariectomy and daily injection of 1.5 µg oestradiol benzoate (OB) on 8-day mean food intake and body weight expressed as the mean percentage deviation (± standard error) from a 16-day preoperative period (not shown). The shaded area indicates the period of OB (continuous lines) or oil (dashed lines) treatment of ovariectomized rats. The lines interrupted by the standard errors indicate data obtained from control intact rats given no treatment. On days 66 and 71 after ovariectomy the dose of OB injected was increased to 12 µg.

Fluctuations of the FI of these rats is caused by normal oestrous cycles. At the time of oestrus, there is a depression in FI (Tarttelin & Gorski 1971) and since the oestrous cycles of these rats were not synchronized, FI would be at a minimum when several rats were in oestrus at the same time.

During the initial 32-day postoperative period, the FI of the nine ovariectomized rats increased significantly \( (P < 0.001) \) compared to that of the controls. These data are not presented collectively since this group was subdivided during treatment. The FI of the oil-treated ovariectomized rats is still significantly \( (P < 0.005) \) higher than that of the unoperated controls between 41–48 days after ovariectomy, but after this there is no longer a significant difference between these two groups. One injection of 3 µg OB depressed FI
Table 1.

Effect of ovariectomy and oestrogen replacement on water intake expressed as the percentage deviation from a 16-day preoperative period. The oestrogen treatment began on day 32.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. rats</th>
<th>% Deviation from preoperative period</th>
<th>Pretreatment period (days)</th>
<th>Treatment period (days)</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1–8</td>
<td>33–40</td>
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<tr>
<td></td>
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<td>9–16</td>
<td>41–48</td>
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<td>17–24</td>
<td>49–56</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>25–32</td>
<td>57–64</td>
</tr>
<tr>
<td><strong>Intact controls</strong></td>
<td>7</td>
<td>15.9 ± 5.0</td>
<td>19.8 ± 4.1</td>
<td>26.5 ± 3.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>34.0 ± 5.9</td>
<td>28.0 ± 4.3</td>
<td>25.0 ± 6.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18.7 ± 1.1</td>
<td>28.0 ± 4.3</td>
<td>30.3 ± 3.8</td>
</tr>
<tr>
<td><strong>Ovariectomized rats</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily oil</td>
<td>3</td>
<td>38.0 ± 2.3</td>
<td>25.7 ± 2.4</td>
<td>26.3 ± 4.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18.0 ± 4.7</td>
<td>8.0 ± 2.6</td>
<td>5.7 ± 1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.3 ± 4.5</td>
<td>77 ± 3.8</td>
<td></td>
</tr>
<tr>
<td>Daily 1.5 µg OB</td>
<td>3</td>
<td>34.3 ± 1.2</td>
<td>21.0 ± 2.6</td>
<td>18.0 ± 4.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21.3 ± 2.6</td>
<td>24.7 ± 15.7</td>
<td>20.3 ± 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0 ± 3.2</td>
<td>36.0 ± 5.0</td>
<td></td>
</tr>
<tr>
<td>1 injection 3 µg OB</td>
<td>3</td>
<td>36.7 ± 9.5</td>
<td>30.0 ± 5.5</td>
<td>37.0 ± 9.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27.3 ± 11.5</td>
<td>14.7 ± 10.3</td>
<td>22.0 ± 10.0</td>
</tr>
</tbody>
</table>
below that of the oil-treated rats during the next eight days, but this difference only approached significance. However, when the mean FI of the first four days after treatment with 3 µg OB was compared with the oil controls, there was a significant depression ($P < 0.001$). Daily injection of 1.5 µg OB produced a more prolonged and significantly greater fall in FI compared with the oil-treated controls ($P < 0.005$). During the second eight-day period after initiation of OB treatment, FI was reduced 30% in comparison to that of the oil-treated ovariectomized controls. Following this nadir, a gradual increase of FI was observed in spite of continuing treatment. To test the apparent recovery from oestrogen suppression, doses of 12 µg OB were injected at 34 and 39 days after the onset of oestrogen treatment (injections of 1.5 µg OB were continued on the intervening days). In spite of this treatment with increased doses of oestrogen, the FI of these rats was not significantly different from either the oil-treated ovariectomized rats or the intact control rats.

Water intake results were different from those of FI. Following an initial postovariectomy rise in WI lasting about 16 days, the WI of intact and ovariectomized rats was the same. Following the onset of daily OB treatment, WI fell markedly but after eight days of treatment, the WI had risen to the level of the intact rats. The WI of the oil-treated ovariectomized rats fell significantly ($P < 0.001$) below the levels of the intact rats at the time oil treatment started. This fall in WI was partly due to the mild stress of the onset of daily injections but was also due to a coincidence of the start of oil injections with the time that the WI of ovariectomized rats spontaneously begins to fall (see Experiment III). The WI of these rats remained below the levels of intact rats and after 32 days of oil treatment was still significantly lower ($P < 0.02$). The WI of the rats given a single injection of 3 µg OB was between the levels of the oil-treated and OB-treated rats but was not significantly different from either.

The BWt of the ovariectomized rats rapidly increased above the intact rats and remained significantly ($P < 0.001$) greater even at 108 days postoperatively in the oil-injected group (see Fig. 1 for percentage changes). A single injection of 3 µg OB produced a slight fall in BWt which was not significantly different from that of the oil-treated animals. However, daily treatment with 1.5 µg OB caused a steady and profound fall in BWt and eight days after the initiation of OB treatment the BWt was no longer significantly different from that of the intact rats. The mean BWt of the OB-treated rats remained at the level of the intact rats but did not fall any lower. Oestradiol benzoate treatment was terminated after 40 days and even 36 days later the BWt of these was not significantly different from that of the intact rats.
Discussion. – This experiment establishes that the raised level of FI seen in the ovariectomized rat is only transitory since 50 days following ovariectomy FI is not different from that of controls. Oestrogen treatment in the ovariectomized rat has a rapid and profound effect on FI. A single injection of 3 µg OB can reduce FI below control levels but daily injections of a smaller amount (1.5 µg OB) reduces FI by 30% of the level of control oil-treated ovariectomized rats. Zucker (1969) described a depression of FI following chronic daily treatment with OB. The depression in FI in experiment I, however, is only transitory because after 30 days of chronic treatment FI returned to the control level. During this period even larger amounts of OB (12 µg) had no effect. When daily treatment with 1.5 µg OB was discontinued for periods up to three weeks and then resumed, a similar depression in FI was produced as illustrated in Fig. 1 (data not presented). Thus, the FI response to OB is only temporary and some form of adaptation occurs. This adaptation apparently is not dose-dependent since larger doses were not effective in depressing FI. Recovery from the adaptive response follows a period of cessation of OB treatment.

Body weight following ovariectomy rises and remains higher than that of the intact rat. Daily treatment with OB will depress the BWt of the ovariectomized rats to intact rat levels. Note that during OB treatment BWt never fell below the level of the intact rats. No reduced response of BWt to OB treatment has been seen even after 40 days of daily treatment. Selye (1940) described an adaptation phenomenon when he found that chronic treatment with oestradiol or stilboestrol depressed BWt with recovery after 14 days. However, his experiments were performed on intact male and female rats and used large amounts of oestrogen. Sullivan & Smith (1957) reported data similar to the present results with no recovery of BWt after prolonged treatment of male rats with oestrogen.

It would appear that the WI is also oestrogen-dependent since following ovariectomy WI peaks, but then falls significantly below control levels and remains at this lower level. Following OB treatment WI falls transiently but then rises to control levels. These results suggest that oestrogen has only a transient effect on FI but a more sustained and long-term effect on WI and BWt. The immediate fall in BWt after OB treatment can be explained in part by the fall in food intake as shown by the paired feeding experiments of Sullivan & Smith (1957), but an explanation based on food intake only will not account for the maintenance of BWt at intact rat levels when the FI recovers from the OB depression and reaches the level of the oil-injected ovariectomized rats whose BWt is maintained 25–30% higher. Clearly there are some profound metabolic readjustments in the ovariectomized rat permitting maintenance of an increased BWt with reduced daily food intake. Oestrogen appears to correct the metabolism to normal levels.

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Experiment II. Effect of single injections of oestradiol benzoate, either alone or in combination with progesterone, on food intake, water intake and body weight

Fourteen rats were used in this experiment 30 days after ovariectomy when their FI had plateaued. The rats were divided into four groups. Two groups were controls: one group of three rats received daily injections of oil, the second group of three rats received 3 µg OB daily. A third group of four rats received 3 µg OB every fifth day with oil injections on intervening days and a fourth group of four rats were given 3 µg OB every fifth day with 0.625 mg P on the intervening days. Treatment was continued for 30 days.

Fig. 2.
Actual daily mean food intake ± standard errors for 3 groups of ovariectomized rats. The lower curve represents data from two groups of 4 rats in which either progesterone or oil was injected daily except for every fifth day, at which time 3 µg oestradiol benzoate (OB) was injected (vertical arrows). There were no differences between these 2 groups and the data have been combined.
As a control procedure, an additional group of six ovariectomized rats were given daily injections of 0.625 mg P and a group of three rats treated were given oil injections, both for a 20-day period. All rats were treated daily with oil for 14 days to acustom them to the injection procedure. The P treatment did not significantly affect the FI, WI, or BWt during the 20-day period or following this period which confirms previously published data (Galetti & Klopper 1964; Hervey & Hervey 1965a; Zucker 1969; Rodier 1971) that P alone given to ovariectomized rats has no effect on intake parameters or BWt.

Fig. 2 presents untransformed daily FI data from Experiment II. The FI of the oil-treated ovariectomized rats gradually falls as reported in Experiment I. The FI of the rats treated with OB daily also shows the abrupt fall with subsequent recovery as described in Experiment I. There was no difference in the FI response between OB given every fifth day with oil or with P injected on the intervening days, so these data have been combined. Cyclic injections of OB produced a depression of FI with recovery prior to the next injection. The

![Fig. 3.](image)

Comparison of the cyclic fluctuations in food intake during the oestrous cycle (left side; from Tarttelin & Gorski 1971) and following the injection of 3 µg oestradiol benzoate (OB) every fifth day in the ovariectomized rat (right side). Food intake is expressed as the percentage deviation from the mean intake over the period of analysis. In the intact rats this period extended over 8 days from the day of vaginal pro-oestrus (P) to the day of metoestrus (M) of the following cycle. In the case of the ovariectomized rats the period of analysis was 9 days and encompassed the day before 1 injection of OB through the 2 days following the subsequent injection. A total of 24 induced cycles (8 rats) were analyzed in this manner to produce the average curve.
depression characteristically occurred on the day following the OB injection and there is evidence of a carry-over effect to the next day. Fig. 3 shows the average FI cycle calculated from 24 cycles (eight rats) induced by intermittent OB injection and plotted as daily percentages from the mean intake level of the nine days plotted and includes data transformed in the same way from seven normally cycling females for comparison. Food intake on the day following OB treatment was significantly ($P < 0.005$) depressed but then increased until three days later it was significantly ($P < 0.005$) higher than the FI during the day of depression. There were no clear changes in daily WI following cyclical treatment with OB. Therefore, the WI data have been reduced to means of 5-day periods and are presented together with the FI and BWt similarly transformed to percentages of a pretreatment ten-day period (Table 2). There are no differences between the WI of the groups given OB plus oil or OB plus P on intervening days. Nor are these two groups different from the oil-treated group. However, the group treated daily with OB showed a significant ($P < 0.001$) fall as compared with the oil-treated group during the first ten days but then rose above the levels of the oil-treated groups, although without reaching statistical significance at this time.

The transformed FI data showed (as in Experiment I) that daily treatment with OB depressed the FI for ten days ($P < 0.02$) with subsequent recovery to the levels of the oil-injected rats. There was no difference between the FI of the OB-plus-oil or the OB-plus-P treated groups. The FI of these groups remained significantly below that of the oil controls, achieving the greatest levels of significance at the 16–20 days period ($P < 0.01$ for the OB plus oil; $P < 0.02$ for the OB plus P). These groups, therefore, showed no tolerance or adaptation to the oestrogen treatment. During the 16–20-day period the FI of the groups treated cyclically with OB were both significantly below the levels of the daily OB-treated groups ($P < 0.005$ for the OB plus oil; $P < 0.02$ for the OB plus P) which had shown adaptation.

As reported in Experiment I, the control oil-injected ovariectomized rats were significantly heavier than any of the groups receiving OB. After the tenth day of treatment, the BWt of the daily OB-treated rats was significantly ($P < 0.01$) depressed below the levels of the rats treated cyclically with OB and given oil or P on the intervening days. A surprising finding was that the BWt of the OB-plus-P-treated groups was depressed significantly below the levels of the OB-plus-oil-treated groups after the fifteenth day of cyclical treatment ($P < 0.05$); the BWt of these groups continued to diverge and after the thirty-fifth day of treatment (data not shown), achieved greater levels of significance ($P < 0.005$).
The influence of the cyclic injection of 3 μg estradiol benzoate (OB) with progesterone (P) or oil given on intervening days on intake parameters and body weight as expressed as a percentage deviation from a 10-day control period.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>No.</th>
<th>Day 0-5</th>
<th>Day 11-15</th>
<th>Day 16-20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake</td>
<td>Oil daily</td>
<td>3</td>
<td>-8.0 ± 2.6</td>
<td>-7.0 ± 2.4</td>
<td>-7.0 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>OB daily</td>
<td>3</td>
<td>-18.0 ± 3.4</td>
<td>-15.0 ± 2.2</td>
<td>-10.0 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>OB 5th day + oil</td>
<td>4</td>
<td>-21.0 ± 1.8</td>
<td>-16.0 ± 3.4</td>
<td>-18.0 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>OB 5th day + P</td>
<td>4</td>
<td>-22.0 ± 2.5</td>
<td>-20.0 ± 2.8</td>
<td>-18.0 ± 0.9</td>
</tr>
<tr>
<td>Water intake</td>
<td>Oil daily</td>
<td>3</td>
<td>-15.0 ± 0.7</td>
<td>-15.0 ± 6.4</td>
<td>-5.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>OB daily</td>
<td>3</td>
<td>-20.0 ± 1.7</td>
<td>-20.0 ± 1.7</td>
<td>10.6 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>OB 5th day + oil</td>
<td>3</td>
<td>-10.0 ± 1.8</td>
<td>-4.0 ± 8.1</td>
<td>0.3 ± 4.4</td>
</tr>
<tr>
<td></td>
<td>OB 5th day + P</td>
<td>4</td>
<td>-15.0 ± 2.5</td>
<td>-8.0 ± 3.3</td>
<td>-1.3 ± 3.3</td>
</tr>
<tr>
<td>Body weight</td>
<td>Oil daily</td>
<td>3</td>
<td>0.0 ± 0.5</td>
<td>3.0 ± 0.5</td>
<td>7.0 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>OB daily</td>
<td>3</td>
<td>-1.0 ± 0.7</td>
<td>0.0 ± 0.3</td>
<td>-6.0 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>OB 5th day + oil</td>
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<td>-0.5 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>OB 5th day + P</td>
<td>4</td>
<td>-5.0 ± 0.8</td>
<td>-0.5 ± 0.8</td>
<td>-3.0 ± 0.5</td>
</tr>
</tbody>
</table>

*Table 2.*

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Discussion. – This experiment indicates that the cyclic injection of OB can produce cyclical changes in FI but not in WI. In contrast to the rats treated with daily OB, even after 40 days of cyclic treatment, tolerance or adaptation to the OB had not developed. The cyclic depression of FI was independent of any action of P. There was no effect of P treatment on WI. The only difference between cyclical OB plus oil or OB plus P was in the BWt where a depressed BWt was seen in the OB-plus-P-treated rats as compared with the OB-plus-oil-treated rats. This finding is in contrast with the data of Rodier (1971) who found that combinations of larger doses of P (12–15 mg) with 17 oestradiol cyclopentane, a long-acting oestrogen, caused an increase in BWt.

Experiment III. Effects of oestrogen in the acutely ovariectomized rat

This experiment utilized 12 rats divided among four groups. Two of the groups were ovariectomized; one received daily oil injections and the other received 3 µg OB daily commencing on the day of surgery. The remaining groups were intact, one received daily oil injections and the other 3 µg OB daily for an equivalent period.

Food intake and BWt are given in Fig. 4 and WI is given in Table 3. Treatment with OB from the time of surgery prevented the rise in FI and BWt seen in the ovariectomized oil-treated rat. The FI in the intact OB-treated rats was variable and the reduction seen on the 10–15th day in Fig. 4 was not significant. OB treatment in the intact rats seemed to retard the rate of growth as compared with the intact oil-treated rats but this was not statistically significant. As in Experiment I, there was a transient increase in WI following ovariectomy. WI in the daily OB-treated group fell initially but soon began to increase and by day 26 had risen above that of the oil-treated ovariectomized rats whose WI was falling. The size of the groups and the variability of the WI data makes tests of significance of doubtful value, but reference to Table 3 shows the trends clearly. There were no differences between the WI of the intact rats receiving OB or oil. The intact oil-treated rats continued to show normal vaginal cycles with FI depressions at oestrus. The intact rats given daily OB injections ceased their normal oestrous cycles and showed cornified vaginal smears.

Discussion. – This experiment, conducted with adequate controls, indicates that oestrogen treatment can prevent the rise seen in FI, WI and BWt following ovariectomy. Oestrogen treatment in the intact female interrupted normal vaginal cycles but did not have a significant effect on intake parameters or on BWt. Bogart et al. (1944) showed that BWt was not increased following ovariectomy and oestrogen treatment.
The influence of daily treatment with oestradiol benzoate (OB) on the 5-day mean (± standard error) food intake and body weight expressed as a percentage deviation from the mean of an 8-day preoperative period. On day zero the rats were ovariectomized or sham operated (intact) and the injections of OB or oil initiated and continued for the period indicated by the length of the horizontal bar.

Experiment IV. The effects of oestrogen injections on food and water intake in the pseudopregnant rat

Fourteen rats were used in this study. Eight normally cycling rats were stimulated per vaginam with a glass rod on the days of pro-oestrus and oestrus. Six out of these eight rats developed pseudopregnancy as evidenced by a persistent dioestrous vaginal smear and typical BWt changes as previously described (Tarttelin & Gorski 1971). After the sixth day of pseudopregnancy, daily injections of 3 µg OB were given for six days. The same OB treatment was given to two control groups of three ovariectomized rats and three normal intact rats. The FI data are illustrated in Fig. 5. Oestrogen treatment caused significant depression in FI in the ovariectomized rats (as reported in Ex-
Table 3.
The influence of daily treatment with 3 μg oestradiol benzoate (OB) on 5-day mean water intake of intact or acutely ovariectomized (Ovary-X) rats expressed as a percentage deviation from the mean of an 8-day preoperative period.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No.</th>
<th>% Deviation from mean preoperative and pretreatment level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovary-X</td>
<td>Oil</td>
<td>3</td>
</tr>
<tr>
<td>Ovary-X</td>
<td>OB</td>
<td>3</td>
</tr>
<tr>
<td>Normal</td>
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<tr>
<td>Normal OB</td>
<td>OB</td>
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</tbody>
</table>
The influence of daily oestradiol benzoate (OB) treatment during days 8–13 of pseudopregnancy on food intake expressed as a percentage deviation (± standard error) of the mean food intake for the 10-day period prior to vaginal stimulation. Control data include intact and ovariectomized rats given OB over a 6-day period, and non-treated pseudopregnant rats. The intervals chosen for data sampling are irregular due to the odd length of pseudopregnancy (13 days) and to the fact that the control rats exhibited 5-day oestrous cycles prior to pseudopregnancy and then 4-day cycles. Data from the 3 other groups were reduced to the same intervals for direct comparison.

periment I) and also a depression in the FI of the pseudopregnant rats which was of the same order of magnitude (20%/o) as that of the ovariectomized rats. No change in intake was seen in the intact rats during treatment, but immediately following the treatment, increases were seen in FI and BWt due possibly to the rats entering a state of pseudopregnancy induced by the oestrogen treatment. FI data from nine spontaneously pseudopregnant rats from a previous study (Tarttelin & Gorski 1971) are included and a direct comparison can be made with the OB-treated pseudopregnant rats. It can be seen that OB treatment depressed FI significantly (P < 0.01). The BWt of pseudopregnant rats was not changed by OB treatment, probably because the six-day period of treatment was too short. WI showed a dramatic fall during OB treatment, but again, the treatment period was too short to establish a pattern.

Discussion. — Although progesterone secretion is increased during pseudopregnancy (Hashimoto et al. 1968), the plasma concentration of oestrogen varies during this period. From initially low levels, a brief surge of oestrogen secretion occurs on the fourth day of pseudopregnancy (Shaikh & Abraham 1969), and late in pseudopregnancy oestrogen levels increase gradually (Hori-
In the present study the pseudopregnant rat was chosen to test whether or not oestrogen could exert a depressant effect in a naturally occurring state of increased FI and BWt. Since OB was effective, it may be argued that the increase in FI and BWt seen during pseudopregnancy is not due to a direct action of progesterone but rather is due to a prolonged period of reduced oestrogen secretion.

**GENERAL DISCUSSION**

The present literature on the subject of hormones and FI and BWt is sparse and is confused by experiments performed in both male and female rats and which have not always taken into account the sexual state of the animal. Previous experiments have used naturally occurring oestrogens with different biological activity (oestradiol and oestrone) or synthetic oestrogenic substances such as stilboestrol, with doses varying from supraphysiological to grossly pharmacological. In the present study we attempted to elucidate the action of small doses of oestradiol, in a few well-controlled experiments, on FI, WI and BWt. It is clear that oestrogen has potent effects on FI and WI in the ovariectomized rat but not in the intact rat in the doses employed. The effects of oestrogen in the ovariectomized rat can be summarized as correcting the water intake and the BWt back to intact rat levels. Oestrogen has potent effects on FI and depresses both the high levels seen in the acutely ovariectomized rat and the lower levels seen in the chronically ovariectomized rat, but its effect is only transient, and with continued treatment oestrogen is no longer effective and FI returns to control levels. The data reported in Experiment II show that oestrogen treatment on an intermittent basis can induce cyclic changes but overall maintains FI at a constant level. These experiments support the hypothesis that in conditions such as ovariectomy or pseudopregnancy, when oestrogen levels are absent or low, the displacement of FI, WI and BWt from normal levels is caused by oestrogen deficiency.

How and where oestrogen acts is uncertain. *Wade & Zucker* (1970) claim, on the basis of direct crystalline implants into the hypothalamus, that the ventromedial nucleus (VMH) is the site of action. In this laboratory we have been unable to demonstrate alterations in food intake following crystalline OB implantation into the VMH region (unpublished observations). *Reynolds* (1968) described experiments in which oestrogen would depress the raised BWt of ovariectomized rats in which the VMH had been destroyed by lesions;

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* During final preparation of this manuscript a concise theoretical review of this topic was published by *Wade* (1972).
we have also confirmed these observations by demonstrating that both FI and BWt in the same preparation can be depressed with OB treatment (unpublished observations). Simpson (1970) suggests that oestrogen might have a site of action in the anterior hypothalamus. Clearly, the experimental data available at present are not sufficient to define the site of action of oestrogen. Care must be taken in interpreting data from experiments in which FI, WI and BWt are not simultaneously measured.

The present data are consistent with the view that oestrogen has a rapid, though transient, effect on FI but a longer-acting and more marked effect on BWt. The initial effect on FI must be by action somewhere in the central nervous system but the long-term BWt and WI effects indicate that oestrogen might have some profound effect on body metabolism which need not be dependent on direct CNS participation, but rather may depend on other hormones with a peripheral action such as thyroid hormones, growth hormones or adrenal corticosteroids, although oestrogen could affect secretion of these hormones at the hypothalamic level. The ovarian/adrenal axis has been implicated in the growth changes following castration in the rat. Grunt (1964) showed that concomitant ovariectomy and adrenalectomy inhibited the post-ovariectomy rise in BWt and these data have been reconfirmed and discussed more recently by Mook (1971). Recently, unpublished experiments in this laboratory have shown that daily corticosteroid injections in the ovariectomized-adrenalectomized rat will permit the normal BWt gain seen in the control ovariectomized rats but progesterone has no such action. Such interactions demonstrate the problems which arise when a hormonal imbalance is created by selective removal of endocrine glands and caution against the premature assessment of sites and mechanisms of action of hormones administered in an attempt to correct the imbalance.

At the present time, however, although the site of action and the interrelationships with other hormones are not fully realized, it seems clear that oestrogen is important as a BWt determinator and is concerned with the regulation of WI in the normal rat and is responsible for the cyclic depressions in FI seen in the female rat showing normal oestrous cycles. We have been unable to determine any direct role of progesterone in FI, WI or BWt regulation. Weight-promoting effects attributed to progesterone may be due to the action of progesterone in inhibiting the production or action of oestrogen.

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