PROLONGED VAGINAL OESTRUS
AND THE NORMAL OESTROUS CYCLE IN THE RAT

1. Morphological observations

By

G. P. Van Rees, J. J. Van der Werff ten Bosch
and O. L. Wolthuis

ABSTRACT

Morphological data are reported of 69 rats with prolonged vaginal oestrus following the placement of a basal lesion in the anterior hypothalamus, and of 137 rats with regular cycles which were grouped according to the stage of the vaginal smear cycle (early dioestrus, late dioestrus, pro-oestrus, oestrus). The groups of rats with regular cycles comprised 99 intact rats and 38 rats with hypothalamic lesions. Pituitary and uterine weights fluctuate during the cycle, with highest values at pro-oestrus and oestrus. In rats with prolonged vaginal oestrus, the weight of the uterus equals that of regularly cycling rats in oestrus, whilst the weight of the pituitary gland markedly exceeds the highest value found during the normal cycle. Some of the implications of these findings are discussed. Subsequent papers will contain data on pituitary and serum contents of ICSH and FSH in the various groups of control rats and in rats with prolonged vaginal oestrus.

Since the early experiments which revealed that hypothalamic lesions may cause genital hypertrophy and continuous vaginal opening in the guinea pig (Dey et al. 1941; Dey 1943) numerous workers have obtained comparable findings in the rat. Such animals with experimental lesions situated basally in the anterior hypothalamus display continuous vaginal oestrus and possess
relatively small ovaries which contain many ripe follicles but are devoid of corpora lutea.

In view of the fact that the block in the oestrous cycle is concurrent with an abolition of ovulation, attempts to clarify the mechanisms underlying this block have concentrated upon possible changes in the pattern of gonadotrophin secretion. Much of the work done, however, suffers from a lack of information about the control rats, which are usually treated as though they constituted a homogeneous group of animals. The present authors felt that much might be gained by grouping regularly cycling animals on the basis of the stage of the cycle found in the vaginal smear at the time of death. Thus four groups of control animals were formed: early dioestrus; late dioestrus; pro-oestrus and oestrus.

The present paper deals with data on organ weights, the histological findings in the ovaries and the histology of the hypothalamus of lesioned animals. Subsequent papers will be concerned with pituitary and serum contents of ICSH and FSH in the various groups of control rats and in rats with prolonged vaginal oestrus.

METHODS

The rats used were adult female rats of an inbred Great Wistar strain. They were fed a commercial standard diet and water *ad libitum*.

Operations were carried out under pentobarbitone sodium anaesthesia. Lesions were placed in the anterior part of the hypothalamus with the aid of a stereotaxic machine and a platinum-wire glass-insulated electrode with a 1 mm bare tip. Anodal lesions were made by passing 2 mA D.C. for one minute, using an indifferent electrode inserted into the rectum. Bilateral lesions were placed 0.5 mm on both sides of the midline.

Vaginal smears were started 6 to 8 weeks after the operation and continued for at least 3 weeks. The criterion for »prolonged oestrus« was the finding of continuous pro-oestrus or/and oestrous smears for at least 7 successive days immediately prior to death; 12 rats were killed after 7–14 days, 24 after 15–21 days and 33 after 22 or more days of continuous vaginal oestrus. Control animals were either lesioned animals which showed regular 4- or 5-day cycles or intact rats. At the time of death these animals were assigned to one of the following groups: *early dioestrus* (first dioestrous smear following oestrus); *late dioestrus* (second or third dioestrous smear following oestrus); *pro-oestrus* and *oestrus*. In view of the time involved in the hormone studies, the rats had to be killed in batches; great care was taken, however, to ensure that all groups (including the lesioned animals) were properly represented in each single batch.

The rats were anaesthetized with ether and killed by bleeding; adenohypophyses, uteri, ovaries and adrenal glands were removed and weighed fresh. Estimations of separate gonadotrophins were carried out in the pituitary glands and in the bloodsera. The brains of lesioned rats were removed, fixed in 10% formalin, embedded in low viscosity nitrocellulose, serially sectioned and stained with Luxol Fast Blue and cresyl violet (*Klüver & Barrera* 1958). Ovaries of a representative number of animals of each
group were fixed in Bouin-Holland, embedded in paraffin, sectioned at 8 µ and stained with haematoxylin and eosin.

The figures are represented as means ± standard error of the mean. Significance of differences was calculated with Student's t-test.

RESULTS AND DISCUSSION

Normal oestrous cycle

Table 1 contains the data on body and organ weights. The only significant differences between intact rats and lesioned rats with regular cycles were found in the body weights at early dioestrus and the ovarian weights at oestrus. The latter difference merely emphasizes a tendency, also notable at other stages of the cycle, for the ovaries of lesioned rats to be smaller. Although such animals had displayed regular vaginal smear cycles, the smaller size of the ovaries would justify a detailed study of their histological appearance.

The weights of the uterus and adenohypophysis show fluctuations during the oestrous cycle which are presumably correlated with the rhythmic processes of follicular maturation and ovulation in the ovary (Fig. 1). For both organs a minimum was recorded during early dioestrus; pituitary weight reached a peak during oestrus (earlier reported by Victor & Andersen 1936) whilst the weight of the uterus was highest at pro-oestrus and oestrus (which confirms previous work by Astwood 1939). The variations in weight of these organs probably reflect fluctuations in the amounts of sex hormones secreted by the ovary during the cycle.

Oestrogens may cause a weight increase of the uterus and the pituitary gland. It is known that the amount of oestrogen in the urine of women is lowest during menstruation and later reaches peak values, first at the time of ovulation and again in the middle of the luteal phase (Brown et al. 1958). Although there does not seem to be other than circumstantial evidence for the occurrence of cyclic variations in the amounts of oestrogen secreted by the rat ovary, it is possible that there is an analogous fluctuation, with highest levels during pro-oestrus and oestrus.

Progesterone secretion may reach peak values at about the time of ovulation. In women the excretion of urinary pregnanediol shows an elevation just before ovulation (Brown et al. 1958) and in the blood of monkeys a peak in progesterone content has been noted at about the time of ovulation (Forbes et al. 1950; Bryans 1951). Follicular fluid of sows and cows has been shown to contain progesterone (Hooker & Forbes 1942; Edgar 1952). Evidence for progesterone secretion by the rat ovary before ovulation was first adduced in an indirect way, by the demonstration that sexual receptivity, which begins before ovulation, largely depends upon the presence of some progesterone (Boling &
Table 1.
Data on rats with regular cycles (intact and lesioned) killed at different stages of the vaginal smear cycle, and on rats with prolonged vaginal oestrus. Number of rats per group appear in brackets.

<table>
<thead>
<tr>
<th></th>
<th>Group</th>
<th>regular cycles, vaginal smear on day at autopsy</th>
<th>prolonged vaginal oestrus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>early dioestrus</td>
<td>late dioestrus</td>
</tr>
<tr>
<td>body weight (g)</td>
<td>intact</td>
<td>178 ± 4 (26)</td>
<td>179 ± 3 (26)</td>
</tr>
<tr>
<td></td>
<td>lesion</td>
<td>191 ± 5 (7)</td>
<td>172 ± 6 (13)</td>
</tr>
<tr>
<td></td>
<td>all</td>
<td>181 ± 3 (33)</td>
<td>177 ± 3 (39)</td>
</tr>
<tr>
<td>adenohypophysis (mg)</td>
<td>intact</td>
<td>7.8 ± 0.3 (26)</td>
<td>7.9 ± 0.3 (26)</td>
</tr>
<tr>
<td></td>
<td>lesion</td>
<td>8.3 ± 0.5 (7)</td>
<td>8.1 ± 0.5 (13)</td>
</tr>
<tr>
<td></td>
<td>all</td>
<td>7.9 ± 0.3 (33)</td>
<td>8.0 ± 0.3 (39)</td>
</tr>
<tr>
<td>ovaries (mg)</td>
<td>intact</td>
<td>72.5 ± 2.4 (26)</td>
<td>71.5 ± 2.4 (26)</td>
</tr>
<tr>
<td></td>
<td>lesion</td>
<td>63.9 ± 5.4 (7)</td>
<td>63.5 ± 6.0 (13)</td>
</tr>
<tr>
<td></td>
<td>all</td>
<td>70.6 ± 2.2 (33)</td>
<td>68.8 ± 2.6 (39)</td>
</tr>
<tr>
<td>uterus (mg)</td>
<td>intact</td>
<td>238 ± 13 (26)</td>
<td>328 ± 16 (26)</td>
</tr>
<tr>
<td></td>
<td>lesion</td>
<td>250 ± 17 (7)</td>
<td>276 ± 27 (13)</td>
</tr>
<tr>
<td></td>
<td>all</td>
<td>240 ± 11 (33)</td>
<td>311 ± 14 (39)</td>
</tr>
<tr>
<td>adrenals (mg)</td>
<td>intact</td>
<td>46.9 ± 1.8 (26)</td>
<td>46.2 ± 1.5 (26)</td>
</tr>
<tr>
<td></td>
<td>lesion</td>
<td>49.7 ± 5.0 (7)</td>
<td>49.3 ± 3.0 (13)</td>
</tr>
<tr>
<td></td>
<td>all</td>
<td>47.5 ± 1.7 (33)</td>
<td>47.3 ± 1.4 (39)</td>
</tr>
</tbody>
</table>

a Significant difference between intact and lesioned rats of one stage (P < 0.05).
b Significantly different from regularly cycling rats in oestrus (P < 0.05).
To illustrate fluctuations in the weights of the adenohypophysis and the uterus during the oestrous cycle of the rat (combined data of all rats with regular cycles), and to show abnormally high adenohypophyseal weight with normal »oestrous« weight of the uterus of rats with prolonged vaginal oestrus.

Blandau 1939). Everett (1945) noted a sudden fall in the lipid content of corpora lutea, taken to be indicative of progesterone secretion, during pro-oestrum. Taylor (1961) has recently shown that plasma progesterone and urinary pregnanediol levels begin to rise during oestrus. These various findings indicate that the secretion of progesterone may fluctuate during the oestrous cycle, with a maximum at about the time of ovulation. Whilst it is possible that fluctuations in the rate of secretion of progesterone cause the fluctuation in uterine weight (Astwood 1939), the available evidence does not allow of an explanation for pituitary weight changes along such lines. Progesterone does not affect the pituitary weight of intact or spayed adult female rats unless a high dose is given; previous or simultaneous administration of oestradiol does not alter the response to progesterone (Van Rees 1959).

Ovarian weights seemed to be stable throughout the cycle. There was some fluctuation in adrenal weight with a maximum at oestrus, a finding which corroborates the work of Bourne & Zuckerman (1941).

It seems of interest that body weight was highest at the time of pro-oestrus; the subsequent fall at oestrus is statistically significant. This weight change
may be related to the findings that the maximum muscular activity and the least intake of food coincide with oestrus (Slonaker 1924, 1925).

**Histology of the hypothalamus**

The brains of 47 rats with prolonged vaginal oestrus and of 16 lesioned rats with regular cycles were studied.

The essential tissue to be destroyed for the production of prolonged vaginal oestrus appeared to be the midline area of the basal layer of hypothalamic tissue immediately above the caudal end of the optic chiasma. The lesions were usually symmetrical and confluent in the midline. Sometimes the hypothalamic tissue dorsal or caudal to the posterior border of the chiasma had also sustained damage.

In rats with regular cycles lesions were either predominantly unilateral, placed too far posteriorly – behind the optic chiasma – or located in the region between the chiasma and the anterior commissure.

**Prolonged vaginal oestrus**

Data appear in Table 1 and Figure 1. The ovaries of rats with prolonged vaginal oestrus were small, approximately half the size of the ovaries from rats with regular cycles. Their histological picture was typical: numerous small and large follicles but no corpora lutea. The interstitial tissue, though clearly defined, was poorly developed.

The pituitary weights of persistent oestrus rats were significantly higher than those of any of the control groups, which suggests that more oestrogen is secreted in these rats than in the controls. However, in the normal rat, oestrogen secretion probably varies during the cycle and a higher pituitary weight could result from the continuous secretion of relatively little oestrogen. Pituitary hypertrophy of the same magnitude as noted here has been obtained in spayed adult rats after the daily administration of 0.1 μg oestradiol benzoate for 28 days (Gans & Van Rees, unpublished).

An interesting feature is the weight of the uterus. It has repeatedly been reported that the uterine weight is increased in rats with prolonged vaginal oestrus due to anterior hypothalamic lesions (Van Dyke et al. 1957; Cook 1959; D’Angelo & Kravatz 1960; Flerkó & Bárdos 1960). In all these studies the values for normal rats had been obtained from pooled data without reference to the stage of the cycle at the time of death. The present study comprises 69 lesioned rats with an average uterine weight of 213 mg per 100 g body weight. When the figures for all 137 regularly cycling control animals are taken together, an average weight of 185 mg per 100 g body weight is obtained. The figures for the four subgroups of normal animals show, however, that great variations occur; comparison with the prolonged oestrus rats
demonstrates that their uterine weights are similar to those of rats with regular cycles which were in oestrus at the time of death. The same phenomenon may be seen when the data of Taleisnik & McCann (1961) are expressed per unit of body weight. These findings support the view that oestrogen secretion in the persistent oestrus rat may be approximately normal.

It is interesting that the association of a relatively high pituitary weight with a normal weight of the uterus was also found by Paesi & Van Soest (1954) when they studied the effect of various doses of oestrogen on the weights of these organs in spayed adult rats. Oestradiol was found to raise pituitary weight above that of untreated intact rats at doses which normalized the weight of the uterus; doses which normalized the weight of the pituitary gland were insufficient for obtaining normal uterine weights. It appears that oestrogen may not be the sole factor responsible for the fluctuations in weight of both pituitary gland and uterus during the normal oestrous cycle.

Everett (1948) has postulated a deficiency of progesterone in rats with spontaneously occurring persistent oestrus. In such rats exogenous progesterone may induce ovulation (Everett 1940) and may facilitate oestrogen-induced ovulation (Everett 1948). Similarly ovulation has been induced or facilitated by the administration of progesterone in rats with prolonged vaginal oestrus due to hypothalamic lesions (Greer 1953) or to prepuberal administration of testosterone (Barraclough & Gorski 1961). Although the mechanism and site of action of progesterone are obscure, these data suggest that the absence of ovulation and luteinisation in these conditions of prolonged vaginal oestrus may be due to a deficiency of progesterone.

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