FACTORS DETERMINING CESSATION OF CORPUS LUTEUM FUNCTION; THE POSSIBLE ROLE OF OESTRADIOL AND PROGESTERONE

By

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ABSTRACT

The role of oestrogen and progesterone utilization by the uterus was investigated with regard to the mechanism of cessation of corpus luteum function. After the administration of oestradiol benzoate with or without progesterone in various combinations to spayed, spayed-hysterectomized, or spayed traumatized rats it was found that:

1) there were no percentage differences in the oestrogenic effects in the vaginal smears of spayed or spayed-hysterectomized rats after the administration of oestradiol benzoate alone.

2) After treatment with combinations of oestradiol and progesterone, the oestrogenic effects were inhibited in spayed-hysterectomized animals as compared with similarly treated spayed controls. A still more marked inhibition was obtained after traumatizing the uterine endometrium.

3) Increasing doses of progesterone in combination with a fixed dose of oestradiol benzoate progressively delayed vaginal opening.

It is concluded that the uterus does not utilize any measurable amounts of oestrogen, but that, on the other hand, it does utilize considerable quantities of progesterone. Traumatization of the uterus may have a similar progesterone-sparing effect. The findings are discussed against the background of factors which determine cessation of corpus luteum function, while it is suggested that progesterone may be an important factor accounting for the effects of hysterectomy.

Since recent findings in this laboratory (Wolthuis 1963) have indicated that, in contrast to other pituitary hormones, prolactin secretion and release in the rat is not subject to a negative feed-back mechanism (see also Rothchild 1960 a
and 1960 b), an investigation was made into the factors determining the cessation of corpus luteum function.

Although the difference between cessation of function and anatomical disappearance is not always clear in the literature (for two excellent discussions see Everett (1961) and Perry & Rowlands (1961), the results obtained suggest that cessation of function is an active process, while it is not certain whether any active mechanisms are involved in the anatomical disappearance. The complexity of the problem is even greater than expected when the possibility is considered that some of the corpus luteum cells (e.g. those most sensitive to a luteolytic substance) can disappear, while those remaining become hyperactive. Moreover, the finding that in some zoological species a corpus luteum may be recognized as an anatomical entity for quite a long period after hypophysectomy (Smith 1927) would suggest that in the anatomical disappearance too active mechanisms may be involved.

The following factors responsible for cessation of corpus luteum function or anatomical disappearance have been put forward:

a) An endometrial (humoral?) factor. The results obtained after hysterectomy and retransplantation of uterine tissue, i.e. prolongation and normalization of corpus luteum function, point to a role of the uterine endometrium (see also Duncan et al. 1961; Anderson et al. 1961; Butcher et al. 1962; Butcher & Melampy 1962; Rowlands 1962).

b) Utilization of oestrogens by the uterus. The findings suggest that removal of the uterus may lead to greater amounts of circulating oestrogens, and it is known that oestrogens (indirectly) prolong corpus luteum function. This view is held and supported by Heckel (1942), Chu et al. (1945) and Tarztaglia & Tarozzi (1961), and is consistent with some results obtained in man (Cedard et al. 1962).

c) The effects of LH, to which the anatomical disappearance (Bunde & Greep 1936; Greep 1938; Desclin 1958; see also Greep et al. 1942) and the cessation of function (Rothchild, personal communication) are ascribed.

d) Neural mechanisms. According to Selye (1934), Moore & Nalbandov (1953) and Hill & Alpert (1961) neural mechanisms may also be involved.

The present paper may be considered as a first approach to the problem which is under investigation in this laboratory. It is concerned with the possibility mentioned above under b) as well as with the possibility that progesterone may play a part.

Although progesterone is not mentioned in the above discussion a–d and although to our knowledge it has never been mentioned in this connection so far, we have extended the experiments to progesterone, since we were struck by the fact that prolongation of corpus luteum function in the rat after hysterectomy occurs only in pseudo-pregnant animals and not in animals with a normal cycle. On the other hand, in the quinea-pig and the guilt (animals...
having so-called »functional« corpora lutea) prolongation of the normal cycle occurs.

**MATERIAL AND METHODS**

All rats used were from an inbred but impure laboratory stock.

**Experiment I.** Adult female rats (body weight 200–260 g) were spayed and half of the number of animals were also hysterectomized at the same time. A thermo-cautery was used to stop any bleeding; in the spayed controls the uterine mesentery was also coagulated in several places. To prevent the risk of explosion, a non-volatile anaesthetic, avertin, was used.

The spayed (a) and spayed-hysterectomized (b) animals were then divided into two subgroups each (a₁ and a₂, b₁ and b₂), so that simultaneous treatment with two dose levels of oestradiol benzoate could be given and crossing over could be repeatedly made. The treatment was started three weeks after the operations, according to the following scheme.

<table>
<thead>
<tr>
<th>Daily dose of oestradiol benzoate in µg</th>
<th>1st run</th>
<th>2nd run</th>
<th>3rd run</th>
<th>4th run</th>
<th>5th run</th>
<th>6th run</th>
</tr>
</thead>
<tbody>
<tr>
<td>a₁ + b₁</td>
<td>0.30</td>
<td>0.25</td>
<td>0.20</td>
<td>0.25</td>
<td>0.30</td>
<td>0.35</td>
</tr>
<tr>
<td>a₂ + b₂</td>
<td>0.35</td>
<td>0.30</td>
<td>0.25</td>
<td>0.20</td>
<td>0.25</td>
<td>0.30</td>
</tr>
</tbody>
</table>

The injections were given subcutaneously once daily, the solvent used was arachis oil and the volumes were kept constant at 0.1 ml. Each run consisted of eight consecutive days of injections and the taking of vaginal smears. This was followed by a resting-period of 21 days; during the last three days of this resting-period vaginal smears were taken in order to be absolutely sure that no traces of oestrogenic effects were present at the beginning of a new run of eight days. Preliminary experiments had already shown that the oestrogenic effects with this dose range wear off in a few days.

The oestrogenic effects were called positive if no leucocytes were present in the vaginal smear preparation.

Care was taken that the average body weights in different groups were approximately identical, so that the effects could not be influenced by a body weight factor. This was also the reason why adult animals were used, since their growth rate is comparatively low.

**Experiment II.** In this experiment immature female rats (30–40 g body weight) were used, since we had some previous experience of the dose levels of the steroids to be used (Wolthuis 1963). The animals were spayed or spayed-hysterectomized and on the following day were divided into three equal groups, which were injected subcutaneously with one of the following combinations (daily per animal):

- 0.2 µg oestradiol benzoate + 400 µg progesterone
- 0.2 µg oestradiol benzoate + 800 µg progesterone
- 0.2 µg oestradiol benzoate + 1600 µg progesterone

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Treatment was given once daily and lasted for 12 days. During the last eight days of the treatment smears were taken from the vaginae if they were open (see Results).

Five days before the end of the experiments, a silk thread was passed at least four times through the left uterine horn in half the number of spayed animals in each group; care was taken that the thread was passed through the uterine wall into the lumen. All operations in experiment II were performed under ether anaesthesia.

On the 13th day the animals were autopsied and the uteri and hypophyses dissected and weighed on a precision torsion balance. The uteri were examined histologically according to routine procedures (fixation in Bouin-Holland fluid, staining with haematoxylin-eosin).

RESULTS

Experiment I. Using the vaginal smear as a criterion, hardly any difference could be detected (see Table 1) after repeated cross-over administration of oestradiol benzoate to 8 spayed and 10 spayed-hysterectomized rats, either in the individual runs, or in the total findings.

The fact that 0.2 µg oestradiol caused a slightly higher percentage of oestrogenic effects than 0.25 µg oestradiol benzoate remains a biological enigma. However, since the higher incidence of oestrogenic effects was equally divided over spayed and spayed-hysterectomized animals, this did not prevent a comparison of the results obtained in the two groups.

Experiment II. It may be seen from Table 2, that increasing doses of progesterone cause a progressive decrease of the oestrogenic effects on the vaginal smears. On theoretical grounds it would be expected that the effect of hysterectomy, if any, will best be seen around the 50% level of inhibition

<table>
<thead>
<tr>
<th>daily doses of oestradiol benzoate</th>
<th>spayed</th>
<th>spayed-hysterectomized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no. of</td>
<td>total no. of smears</td>
</tr>
<tr>
<td>0.35 µg</td>
<td>8</td>
<td>64</td>
</tr>
<tr>
<td>0.30 µg</td>
<td>2 x 8</td>
<td>128</td>
</tr>
<tr>
<td>0.25 µg</td>
<td>2 x 8</td>
<td>128</td>
</tr>
<tr>
<td>0.20 µg</td>
<td>8</td>
<td>64</td>
</tr>
<tr>
<td>Totals</td>
<td>384</td>
<td>56 %</td>
</tr>
<tr>
<td></td>
<td>(absol.: 216)</td>
<td></td>
</tr>
</tbody>
</table>

* The groups of 8 or 10 animals were treated twice with equal doses (see methods).
Table 2.
The effect of combinations of oestradiol and progesterone on vaginal smears and vaginal opening in female rats subjected to various operations.

<table>
<thead>
<tr>
<th>body weight</th>
<th>spayed</th>
<th>hysterect.</th>
<th>traumat.</th>
<th>daily treatment (s. c.)</th>
<th>no. of animals</th>
<th>vaginal smears</th>
<th>autopsy data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>total no.</td>
<td>no. of oestrogenic effects (per cent)</td>
<td>total no. of days of closed vaginae</td>
</tr>
<tr>
<td>36 ± 1.9¹)</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>oestradiol</td>
<td>7</td>
<td>56 (92 %)</td>
<td>0</td>
</tr>
<tr>
<td>35 ± 2.3</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>benzoate 0.2 μg</td>
<td>7</td>
<td>54 (96 %)</td>
<td>2</td>
</tr>
<tr>
<td>37 ± 1.1</td>
<td>+</td>
<td>—</td>
<td>+</td>
<td>progest. 400 μg</td>
<td>8</td>
<td>64 (51 %)</td>
<td>0</td>
</tr>
<tr>
<td>35 ± 1.0</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>oestradiol</td>
<td>8</td>
<td>59³) (47 %)</td>
<td>3</td>
</tr>
<tr>
<td>35 ± 0.7</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>benzoate 0.2 μg</td>
<td>7</td>
<td>52³) (23 %)</td>
<td>3</td>
</tr>
<tr>
<td>36 ± 0.9</td>
<td>+</td>
<td>—</td>
<td>+</td>
<td>progest. 800 μg</td>
<td>8</td>
<td>57³) (21 %)</td>
<td>6</td>
</tr>
<tr>
<td>36 ± 0.9</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>oestradiol</td>
<td>8</td>
<td>55 (11 %)</td>
<td>9</td>
</tr>
<tr>
<td>35 ± 1.5</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>benzoate 0.2 μg</td>
<td>8</td>
<td>55 (2 %)</td>
<td>9</td>
</tr>
<tr>
<td>35 ± 1.0</td>
<td>+</td>
<td>—</td>
<td>+</td>
<td>progest. 1600 μg</td>
<td>7</td>
<td>52 (2 %)</td>
<td>4</td>
</tr>
</tbody>
</table>

¹) mean ± S. E. M.
²) two smears missing.
³) one smear missing.
of oestrogenic effects. This was indeed the case: thus when treated with a combination of oestradiol and the middle dose of progesterone (800 µg daily), the spayed-hysterectomized animals showed a markedly greater inhibition than the similarly treated spayed animals in the same group. Traumatization of the uterus brought about more marked effects similar to those produced by hysterectomy.

The significance of the differences were computed according to Moroney (1954), i.e. by calculating chi-square after applying Yates' correction.

The results are:
- for the comparison between traumatized and non-traumatized spayed rats treated with the combination of oestradiol and the lowest dose of progesterone (400 µg daily), \( P < 0.001 \);
- for the comparison between traumatized and non-traumatized spayed rats treated with oestradiol and the middle dose of progesterone (800 µg), \( P \leq 0.01 \);
- for the comparison between hysterectomized and non-hysterectomized spayed rats treated with oestradiol and the middle dose of progesterone \( P = 0.01 \).

Microscopic examination of this uteri revealed that no fully formed deciduomata could be detected; only pre-stages could be seen, confirming almost identical experiments carried out previously (Wolthuis 1963).

It was found that vaginal opening was delayed, to an extent related to the doses of progesterone administered.

**DISCUSSION**

*The effects of hysterectomy*

From the results obtained it can be concluded that no utilization of oestradiol by the uterus of a spayed rat could be detected, whereas a clearcut utilization of progesterone was found. In other words, extirpation of the uterus does not lead to any measurable change in the circulating oestrogens, while the amount of circulating progesterone increases. Increased levels of progesterone most probably contribute towards prolongation of the corpus luteum function by a positive feedback on prolactin (Rothchild 1960a and 1962; Wolthuis 1963) and at least delays processes concerned with the subsequent ovulation by its negative feedback on LH and FSH (van Rees 1959).

The negative feedback on LH by progesterone may still be involved in another way, since LH itself may promote the termination of corpus luteum function (Rothchild, see below). Moreover, direct progesterone effects on the vaginal smear pattern will be more marked and prolonged after hysterectomy,
which makes the vaginal smear pattern a misleading criterion for the assessment of the corpus luteum function under these conditions.

Hence it seems that many of the results obtained with hysterectomized rats can be explained in terms of an increased amount of progesterone circulating after hysterectomy. These effects can only occur spontaneously if sufficient progesterone is circulating, e.g. in the pseudo-pregnant rat, or during the sex cycle in guinea-pigs and guilts. However, even if we accept the co-existence of an endometrial factor, this does not give a complete explanation of the facts: for although the function of an active (i.e. progesterone-secreting) corpus luteum is prolonged after hysterectomy, its period of function is nevertheless limited. In the case of the pseudo-pregnant rat, hysterectomy prolongs corpus luteum function by a few days, but then it ceases to function. This cessation of function cannot be caused by the uterus (which has been removed) and cannot be an intrinsic property of the ovary itself, as has been pointed out by Everett (1961), for corpus luteum function in the hypophysectomized rat bearing a hypophysal transplant is maintained for at least several months. In other words, the primary cause of the cessation of corpus luteum function must lie in extra-ovarian and extra-uterine factors. The longer maintained corpus luteum function in the hypophysectomized animal bearing a hypophysal transplant cannot be caused by the greater amounts of prolactin secreted by the hypophysal transplant as compared with the hypophysis in situ (Wolthuis & de Jongh 1963), for in an intact animal bearing a hypophysal transplant (Mühlbock-Boot preparation, see Everett 1961) pseudo-pregnant cycles occur and the period of functioning of the corpora lutea is limited. This rather seems due to some unknown factor(s) absent in the hypophysectomized rat even when bearing a hypophysal transplant. It is very likely that at least one important factor is the deficiency of LH, since Rothchild (personal communication) only recently found that exogenous LH administered to such animals causes a substantial decrease in both the size of the corpora lutea and their rate of progesterone secretion.

The effects of traumatization

The fact that animals in which the uteri were traumatized also showed an increased inhibition of oestrogenic effects (as after hysterectomy) seems to indicate that progesterone may be an important factor. It has been found in the pseudo-pregnant rat (Ershoff & Deuel 1943; Velardo et al. 1953) and in the guinea-pig (Loeb 1927) that the induction of deciduomata prolongs corpus luteum function. These results could also be explained by a greater amount of circulating progesterone after traumatization.

Although our results point in this direction, it cannot be excluded (in contrast to the findings after hysterectomy) that following traumatization changes also occur in oestrogen utilization.
The effect of progesterone on vaginal opening

No explanation can be given for the inhibiting effect of progesterone on vaginal opening. Although the results are suggestive, a larger number of animals as well as repeated experiments would be required to evaluate the extent and importance of this effect.

REFERENCES

Rothchild I.: Endocrinology 67 (1960 a) 9.
Rothchild I.: Endocrinology 67 (1960 b) 54.